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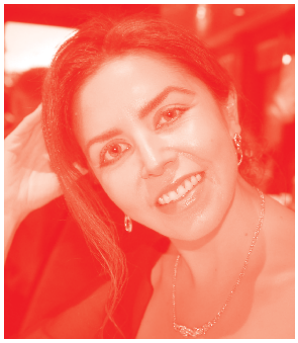

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Meet the editor

Dr. Muhammad Akram is currently working as an Assistant Professor in the Department of Eastern Medicine at Government College University Faisalabad, Pakistan. He received his Ph.D. from Hamdard University Karachi-Pakistan in 2013. Dr. Akram was a Chairman in the Department of Eastern Medicine and Surgery, University of Poonch, Rawalakot Azad Kashmir from 2015-2017. He received many honors and awards during his carrier. He serves as Editor and invited Reviewer of several national and international Journals. He has numerous publications and presentations and he is acting as an active member of several professional societies. Dr. Muhammad Akram's research interest includes Hyperuricemia, Xanthine oxidase inhibition by some selected medicinal plants, Enzyme Inhibition, Study of traditional system medicine (Indusyunic Medicine), Phytochemistry, and poisonous plants. Bioactivity and phytopharmaceutical evaluation of herb drugs and their natural products, Medicinal plants and Clinical trial, Biochemistry, Bioinformatics.

Contents

Preface

This Edited Volume is a collection of reviewed and relevant research chapters, concerning the developments within the "Alternative Medicine - Update" field of study. The book includes scholarly contributions by various authors and edited by a group of experts pertinent to alternative medicine. Each contribution comes as a separate chapter complete in itself but directly related to the book's topics and objectives.

The book is divided in 8 sections: "Pain", "Immunity", "COVID19", "Nanomedicine", "Cancer", "Homeopathy", "Diabetes" and "Medicinal Plants".

The section "Pain" includes a chapter dealing with the topic: *Alternative Treatments for Pain through Dhikr, Hijamah and Herbal Medicine as Integrative Medicine*. The section "Immunity" includes *Immunomodulator in Traditional Healthcare System*. The section "COVID19" includes *Some Igbo Indigenous Plants with Anti-COVID-19 Properties*. The section "Nanomedicine" includes *Nanomedicines: Nano based Drug Delivery Systems Challenges and Opportunities*. The section "Cancer" includes *Prevalence, Symptomatology and Herbal Management of Polycystic Ovarian Syndrome*. The section "Homeopathy" includes *Alternative Medicine: A Recent Overview, Thermodynamic Aspects of Homeopathy, Acupuncture, Yoga, Homeopathy, and Apitherapy under the Vibrational Point of View, Natural Compounds in the Modulation of the Intestinal Microbiota: Implications in Human Physiology and Pathology* and *Non-Pharmacological Interventions in Preventive, Rehabilitative and Restorative Medicine*. The section "Diabetes" includes *Antidiabetic Activities of Terminalia Species in Nigeria, Some Folk Antidiabetic Medicinal Herb of Himachal Pradesh* and *Red Wine and Yacon as a Source of Bioactive Compounds with Antidiabetic and Antioxidant Potential*.

Chapters *Collection, Storage and Market of Medicinal Plants: A Case in Peru, Case Study of Bacterial Decontamination of an Aromatic and Medicinal Plant: Decontamination of Thymus Satureioides by Gamma Radiationat Low Doses and Impact on Hygienic and Physicochemical Quality, Plant-Based Drugs as an Adjuvant to Cancer Chemotherapy, Impact of Shodhana on Semecarpus anacardium Nuts and Safety of Herbal Medicines in* Children are included in the last section of this book, "Medicinal Plants".

The target audience comprises scholars and specialists in the field.

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Section 1

Pain

Chapter 1

Alternative Treatments for Pain through Dhikr, Hijamah and Herbal Medicine as Integrative Medicine

Meity Elvina, Wahyudi Widada, Joko Suwito and Mansur Ibrahim

Abstract

Most people suffer pain at some stage of their lives. Therefore, pain is the most common reason to seeking therapeutic alternative or conventional medicine. Pain management particularly in chronic pain due to postoperative is systematically have been developed by various great recommendations that assist the health care practitioner and patient in making decisions about health care. In recent years, we have developed a better understanding of the pathomechanisms that link inflammation and peripheral sensitization about pain which can be overcome through an alternative treatment. This approach is known as complementary and alternative medicine (CAM). The development of the combined health approach has lately been known as Integrative Medicine, which seeks to restore and maintain health by understanding a series of patients' unique circumstances and overcoming their various physical, psychological, environmental, and spiritual influences. Through "whole system" treatment will be to elaborate an integrated approach, by dhikr, hijamah, and herbal medicine for facilitating the transition from viewing "pain" only as a matter of specific bodily functions (specific) to a more holistic view at the systemic level through integrative medicine to health care with a broader holistic approach.

Keywords: pain, integrative medicine, dhikr, hijamah, herbal medicine

1. Introduction

The term "alternative therapy" is generally used to describe any medical treatment or intervention that is used in place of conventional medicine. When alternative therapies are used in conjunction with conventional medicine, it is called complementary medicine. Alternative therapy covers a wide variety of disciplines. In the last decade, many evidences have been gathered for the benefits of mind-body therapy [1]. This approach has the potential to reduce pain in some cases. Mind-body therapy is a treatment intended to help the mind's ability to influence bodily functions and symptoms. Mind-body therapy uses a variety of approaches, including relaxation techniques, such as dhikr meditation, hijamah (wet cupping),

and herbal remedies approaches. These techniques can relieve the discomfort associated with chronic pain [2].

Pain is when the body's normal reaction to injury or illness, a warning that something is wrong. When the body heals, it usually stop getting sick. But for many people, pain persists long after the cause has gone away. If it lasts 3 to 6 months or more, it is called chronic pain [3]. When the body gets hurt day after day, it can affect the emotional and physical health. About 25% of people with chronic pain will continue to have a condition called chronic pain syndrome. That's when people have symptoms beyond just pain, such as depression and anxiety, which interfere with their daily life [3, 4].

Integrative medicine is defined as medicine that "reaffirms the importance of the relationship between practitioner and patient, focuses on the whole person, is informed by evidence, and makes use of all appropriate therapeutic and lifestyle approaches, healthcare professionals, and disciplines to achieve optimal health and healing" [5]. Integrative medicine incorporates all appropriate therapeutic approaches by all healthcare providers from both, conventional and complementary medicine, that are likely to improve an individual patient's health status [6, 7].

Integrative health care for pain management often brings together conventional and complementary approaches in a coordinated manner. It emphasizes a holistic patient-focused approach to health and wellness care - often covering mental, emotional, functional, spiritual, social, and community aspects - and treating as a whole bodily function. Thus, more research is needed to see how the complementary treatment for pain compares to conventional treatments [8, 9].

2. Dhikr meditation for pain management

Pain is a subjective experience involving biological, psychological, social and spiritual variables. Perceived pain is highly dependent on biological, psychological, social and spiritual variables [10]. One of non-pharmacological management method is meditation. It's focusing patient attention and easy to do anywhere, and does not require special equipment. Meditation is one of the most important non-pharmacological practices that promote relaxation. This is because through meditation, relaxation can provide an integrated response a psychophysiological response originating in the hypothalamus in the central nervous system [11, 12].

Meditation has physiological, mental, and spiritual benefits for the patients suffering from pain. In addition, meditation is an effective practice for pain management [13]. This can reduce the use of drugs or tranquilizers. Meditation is an activity undertaken to enter the state of unconsciousness and can strengthen soul, body, and mind and thus can reduce the sensation of pain. When someone meditates, the perception of pain can be minimized [14].

During practicing meditation, it was found that the brain produced large quantities of the pleasure-causing neuro-chemicals such as endorphins (as measured by EEG brainwave biofeedback machines) which made people a whole experience pleasurable, reduced pain, alleviated stress and gave an overall feeling of well-being. In addition, the alpha rhythm of the brainwaves was recorded during practicing meditation which leaded the patient to relaxation state [15]. The gate control theory for pain proposed by Melzack and Wall [16]. This theory explains the physical and psychological aspects of pain. It deals with nerve impulses that are released from every part of the body and modified in the spinal cord before being sent to the brain. It involves the spinal gate mechanism, central system, central biasing system, and action system. This correlative system works together rapidly when pain is felt [16, 17].

This theory proposes that the experience of pain is modulated by integrated emotional reactions, a relaxed state, and a meditative state in the higher centers of the brain (**Figure 1**), they modulate a noxipus input, reduces the perception of pain, and stimulates action to relieve it. Activation of the higher centers of the brain can cause the spinal cord gates to close. Closing the gate prevents pain input from reaching the higher centers of the brain, and translates into a total pain experience. The patient's reaction to pain includes the perception of pain intensity and physiological changes as a result of activation of the complex nervous system [17, 18].

To better understand this pain, a psychoneuroimmunological approach is used to explain the mechanism of acute pain, chronic pain and the working mechanism of pain control efforts including dhikr from the perspective of spiritual variables [19]. The mechanism of action of pain is not only played by the peripheral nervous system by involving neurotransmitters and cytokines, but also by the central nervous system [20]. The pain process from stimulation to perception, includes the role of modulation, where the modulation process occurs at the spinal level as well as at the brain level. At this modulation stage there are opportunities for therapeutic modalities, including dhikr, to control acute pain and chronic pain [21, 22].

Chronic pain mechanisms include the role of Long Term Potentiation (LTP), so that even though the cause of the pain is not found, the mechanism for generating pain stimulants can still occur, so that therapeutic modalities other than drugs are needed [23]; and from many research results show that the effectiveness of dhikr for controlling acute pain and chronic pain. Dhikr is one of the Islamic ritual forms, and used to concentrate the mind in order to experience calm inner feelings. Some of the research results of dhikr and the like which are effective in controlling pain and especially chronic pain, along with their biological mechanisms will be described in this section [24].

2.1 Dhikr through affirmation-tapping for pain management

Affirmation tapping interventions have been shown to reduce pain complaints in postoperative patients who have completed conventional treatment. This is thought to be due to serotonin's performance. The aim was to compare the mean perceived pain

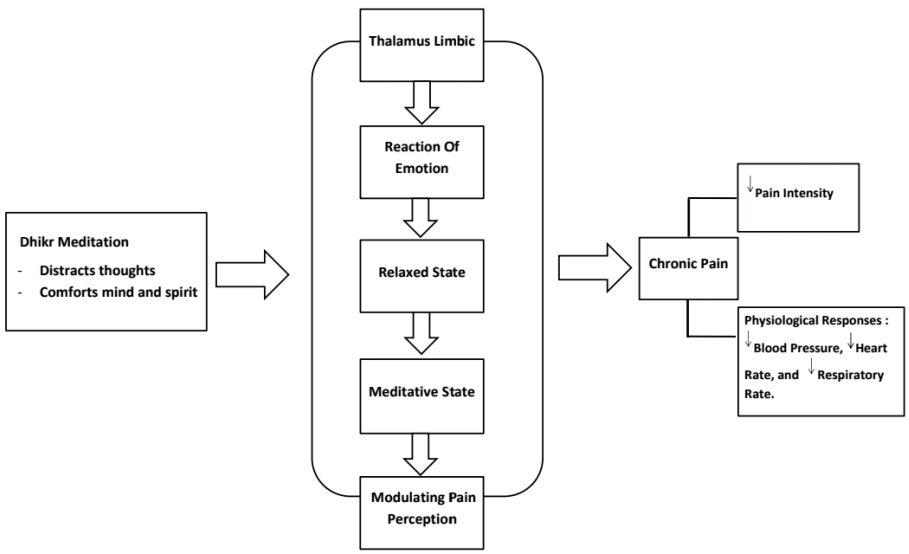

Figure 1.
Study framework effect of Dhikr for pain management among Muslim patients.

reported by postoperative patients who were given tapping treatment with the other treatments as complementary interventions. This study used a randomized post-test only control group design carried out in parallel with post-cesarean section patients. The sample consisted of 40 patients divided into four groups (10 in affirmation, 10 in tapping, 10 in affirmation-tapping and 10 in control). They were obtained through simple random sampling. The instruments include the tapping affirmation guide, the Elisa kit and McGill - Melzack Pain Questionnaire short-form (MPQsf) [25, 26]. The independent variable was affirmation-tapping intervention and the dependent variable was pain perception and serotonin levels. Data analysis included affirmation-tapping intervention, pain perception, and serotonin serum levels using simple linear regression. The Results showed the variation in the mean serotonin levels in the affirmation tapping treatment group was higher and significantly different from the other groups [26].

Therefore, affirmation-tapping as a complementary intervention can increase serum serotonin levels in post-cesarean patients by complementing conventional treatment. The lowest participant pain complaints were in the affirmation group with the highest serotonin levels and this differed significantly from the other groups [26]. Affirmation-tapping is recommended as a complementary intervention in postoperative patients that complements conventional medicine [26, 27].

Affirmation-tapping switches the activation of the pain process to inhibition of the pain process that occurs in the Pre Frontal Cortex (PFC) and Amygdala. In this state, through the performance of the descending pain modulator pathway, Peri Aquaductal Gray (PAG) modulated ON cells in the Rostral Ventromedial Medulla (RVM) to become inactive while the OFF cells in the RVM became active; and results in inhibition of the transmission of pain from the periphery to the central nervous system. The performance impact of PFC and PAG is supported by the perception of non-threatening, will stimulate peripheral inflammatory cells to secrete Serotonin, stimulate the secretion of Endorphins, which then modulate the transmission of pain in the Dorsal Horn spinal cord so that the perception of pain is not felt. Tapping on the acupoint will modulate peripheral inflammatory cells so as to reduce the secretion of IL6 and Glutamate, so that there is no inhibition of Serotonin and Endorphin secretion, and then inhibition of pain transmission in secondary neurons. Stimulation of Feishu (BL13), Hegu (L14), and Zusanli (ST36) accupoints resulted in lower interleukin-6 (IL6) and Glutamate [28]. Inhibition modulation in the Amygdala will increase the performance of the descendent pain inhibitor system through RVM and PAG so that pain transmission from the periphery in the spinothalamic pathway will be inhibited, so the overall pain perception is lower [26, 27, 29, 30].

2.2 Standard Operating Procedure for the implementation of affirmation-tapping

2.2.1 Affirmations

Affirmation of saying and or listening to and following the utterances of prayer, with a focused mind (only listening to and following prayer) and driven by the belief and hope that Allah will grant his prayers starting with istighfar 7 (seven) times, then ta'awudz and the Qur'an surah Al-Fatihah from verse 1 (one) to verse 7 (seven), slowly, the meaning is understood word for word. Prayers said and or heard, are repeated in the same order for up to 10 (ten) minutes [26].

2.2.2 Tapping

Tapping is performed by tapping the fingertips of another person's hand or tapping the tips of one's own fingers on acupuncture points. With a frequency of 2

(two) beats per second; With maximum power you can feel the beats, sequentially from GV 20 (Baihui), B1 (Tongziliao) right, BL 2 (Zanshu) right, B1 (Tongziliao) left, BL 2 (Zanshu) left, each 10 (ten) times and move; and back again starting from GV 20 (Baihui), and so on till time 5 (five) minutes [26].

2.2.3 Affirmation-tapping

Affirmation-tapping is done by saying or listening to and following the words of prayer, with a focused mind (only listening to and following prayer) and driven by the belief and hope of the fulfillment of prayer by Allah subhanallahu wa ta'ala, and coupled with tap the fingertips of another person or tap the tips of one's own fingers on acupuncture points. Prayers are said and or heard, repeated in the same order until 10 (ten) minutes, and at the 5th minute it is continued and added by tapping the tips of other people's fingers or tapping the tips of one's own fingers on acupuncture points, with a frequency of 2 (two) beats per second; with maximum power can be felt, sequentially from GV 20 (Baihui), B1 (Tongziliao) right, BL 2 (Zanshu) right, B1 (Tongziliao) left, BL 2 (Zanshu) left, each 10 (ten) times and moving; and back again starting from GV 20 (Baihui), and so on until a total time of 10 (ten) minutes [26].

2.3 Decreased pain perception through affirmation-tapping

Pain perception in all treatment groups was lower than the positive control group, and the lowest was found in the affirmation-tapping treatment group, which was accompanied by low levels of IL 6, Glutamate and high levels of Serotonin compared to the control group. The performance of brain in the pain process in the Pre Frontal Cortex (PFC), Amygdala can be modulated with the performance of the mind through affirmations, thereby increasing the performance of the decendent pain inhibition pathway, so that Serotonin secretion increases, and is still strengthened by tapping which results in decreased IL6 secretion which has an impact on decreasing glutamate secretion. and consequently the inhibition of Serotonin secretion is also reduced; thus Serotonin secretion is increased. The effect of serotonin on the decendent pain inhibition pathway is the release of endorphins which inhibit substance-P in the dorsal horn of the spinal cord, resulting in blockade of pain signal transduction to the brain. The Pain perception in all treatment groups was also lower than that of the positive control group. This is in line with Svensson's research, that IL-6 has a major influence in the induction and maintenance of pain as well as a regulator of emerging pathological pain [26, 27, 29, 30].

Decreased postoperative serum IL6 levels, reduced pain perception and reduced postoperative inflammatory response [30, 31]. Affirmation-tapping decreases pain perception more effectively than just affirmations, tapping and standard treatment. A process of awareness and focus of attention involves the performance of the pre frontal cortex (PFC) which will modulate the performance of the amygdala, resulting in adaptive performance. Research with experimental animals, it was found that the pain stimulation given would affect the performance of the amygdala, thus inhibiting the performance of PFC, where in the physiological condition, PFC could strengthen the performance of the Descendent-Pain Inhibition System so that the pain at the threshold value will not be felt [28].

A randomized controlled clinical study in a group of depressed women with Mindfulness exercise (which involved the performance of the Pre Frontal Cortex) and a control group with just a meeting, found that the Mindfullness group had lower levels of IL6 in saliva than the control group. This proves that the empowerment of PFC performance has an impact on reducing IL6 levels in the periphery.

The results of this study showed that in the Affirmation and Affirmation-tapping group, serum IL-6 levels were also lower than the control group. This finding is also supported by the results of a study in which groups specially trained in Mind Body Training also experienced decreased levels of IL6 [28].

Referring to the concept research model, affirmation-tapping, besides involving the performance of the Pre Frontal Cortex and Amygdala, also involves the performance of Inflammatory cells (Keratinocyte and Lymhocyte) so as to stimulate Pro-opiomelanocortin (POMC) to release endorphins, which are known to inhibit the transmission of pain signals [32]. Acupoint stimulation in Tapping treatment, can stimulate target cells so that it has an effect on modulation of target cell function in secreting certain cytokines. In experimental animal studies, with stimulation including the GV-20 acupoint (Bai Hui) which was also used in the Tapping treatment, it was found that a significant decrease in IL6 levels in plasma was found [33]. A study of Tu et al., also concluded that in a mouse model of depression with high glutamate and the acupuncture method was carried out, it could modulate glutamate to be low and the depressive effect due to high glutamate could be restored [34]. By extrapolating from these findings, it can be concluded that acupoint stimulation can make gutamate level was lower.

According to Garland's research, the "attention network" is one of the brain circuits most influenced by the practice of Affirmations. Accumulation of research suggests that affirmations are associated with beneficial neuroplastic changes in the "attention network" in particular, the Pre Frontal Cortex (PFC) and Anterior Cingulate Cortex (ACC) areas relevant to cognitive control, as a dimension of pain perception. Meta-analyzes indicated that participation in affirmations was associated with greater activation and thickness of PFC and ACC. Change of broad neuroplastics in the "attention network" are associated with a number of specific results relevant to the construction of ongoing threats. For example, it was associated with increased dorsal activity of ACC during conflict detection and attention orientation. The Pre Frontal Cortex (PFC) and Anterior Cingulate Cortex (ACC) are involved in top-down regulation of negative emotional reactions to stressful and threatening stimuli used in the amygdala. Given the central role of the Amygdala in processing fear, anxiety, and negative emotions for the better and positive after affirmation, it is probably a function of the changes that are in the Amygdala. In affirmations that involve training activities to focus on the power and mercy of the Creator and emotional control have been repeatedly associated with wholesome structural and functional changes in the amygdala, including a decrease in gray matter and a decrease in the amygdala's response to negative emotional stimuli [35].

Furthermore, Affirmation transforms the functional connection between Amygdala and PFC in useful ways. Affirmative activity shows a significant reduction in resting functional connectivity of the Amygdala-subgenual ACC status which correlates with decreased biomarkers of chronic HPA axis activation, suggesting that affirmation can reduce molecular mediators of chronic stress by decreasing amygdala reactivity. This approach can be explained that affirmation-tapping, which combines prayer techniques and acupoint stimulation, works to reduce pain perception in two ways, namely increasing Serotonin by utilizing the performance of PFC, Amygdala, PAG, RVM and ITC (Descendent - Pain Inhibition System) as well. Reduced peripheral IL6 output and another way is to take advantage of the acupoint's performance so that it stimulates the POMC to release Endorphins and stimulates the Amygdala to free the Pre Frontal Cortex barrier to improve the performance of the Descendent-Pain Inhibition System [32–38].

3. Hijamah (wet cupping) for pain management

Traditionally, Cupping Therapy has been practiced and practiced in various cultures around the world. The Arabic name for cupping therapy is Al-Hijamah which means to reduce the size that is to return the body back to its original state. Al-Hijamah has been a part of Middle Eastern cultural practices for thousands of years (400 BC). From the western world, the first that perform cupping therapy was ancient Egypt, and the oldest medical recorded on a textbook, the Ebers Papyrus, was written since 1550 BC in Egypt. Anthropologists have also found evidence in China of cupping dating back to 1000 BC. Cupping has been known since ancient times, namely the Sumerian kingdom, then continued to expand to Babylon, Ancient Egypt, Saba, and Persia. At the time of the Prophet Muhammad, he used buffalo or cow horns, camel bones, elephant tusks [39].

Cupping is a simple technique of fast, strong, rhythmic movements to stimulate muscles and is very helpful in the treatment of aches and pains associated with various ailments. Thus, cupping has the potential to improve quality of life. There are two types of cupping. Dry cupping pulls the skin into the cup without bleeding. In wet cupping, the skin is torn off so that the blood enters the cup. The wet cupping technique (Al Hijamah) used in the Middle East is different from the technique used in Asia [40].

A hijamah application takes about 20 minutes and is done in five steps. The first step is primary desludging. In this phase, the therapist determines a specific point or area to be covered and then disinfects the area. The cup is sized according to the selected location and the therapist sucks the air inside the cup by manual suction technique. Then the cup is left on the surface of the skin and left for 3 to 5 minutes. The second step is about scarification or stabbing. A superficial incision is made into the skin using surgical knife No. 15 to 21, or pricking with a needle, or auto-lancing tool. The third step is suction and discharge of blood. The cup is placed back on the skin using the same procedure in the previous step and then left in the cup for 3 to 5 minutes. The forth step involves removing the cup, followed by the fifth step which involves drying the area from the blood and then attempting to clean it with a disinfectant. It can also be covered with a plaster strip on the scarified area for 1–2 days [41].

People get it for a variety of purposes, including to pain management, inflammation, stagnant of blood flow, relaxation, and deep tissue massage. Wet cupping therapy is becoming widespread and tends to be more prevalent in the Far East and in the Middle East and Eastern Europe. Wet cupping has been used as an alternative therapy in the management of patients with chronic pain [42]. However, high-quality trials are needed to produce stronger evidence.

Many published studies have shown that both techniques appear to be effective for patients with pain compared to an inactive control group but they were not compared in previous clinical studies. Wet cupping therapy also stimulates the inflammatory responses [43, 44]. The Middle East technique uses a three-step technique (a sequence of steps into cupping, stabbing, and cupping) in a continuous manner. General, where a sharp scalpel is used for scarification, and the nomenclature of the cupping place is also different. Asian techniques use a two-step technique. Cupping is applied only after piercing (piercing followed by cupping), which uses an automatic lancet needle rather than a scalpel. In certain countries or protocols they are guided by acupuncture points as cupping sites [45, 46].

The main difference is that in the Middle East, cupping is applied before and after skin scarification. Both techniques are currently used in cupping training programs in Saudi Arabia. However, traditional healers in Muslim countries prefer

local techniques such as those used during the time of the Prophet of Islam. Wet Cupping is indicated for blood disorders, pain relief, inflammatory conditions, mental and physical relaxation, tissue massage. Cupping is done to extract blood believed to be harmful from the body which in turn removes the potential harm from the body's symptoms leading to health problems [47]. The main goals of this therapy are to accelerate blood circulation and to remove blood-stasis and waste from the body [48]. Benefits of cupping by creating a suction through negative pressure on the surface of the skin is known to affect body up to four inches into the tissue. Cupping can also be used to treat muscle pain and spasms, draining away excess fluids and toxins, loosens adhesions, connective tissue and hard knots in soft tissue, stimulates circulation and promotes stagnant blood flow to muscle tissue and skin, thereby increasing energy flow, stimulates the peripheral nervous system, and activates the lymphatic flow system (**Figure 2**) [40, 47].

The mechanism of wet cupping therapy (Al Hijamah) is started from the clogged blood that will be removed from the skin, thereby improving blood and lymphatic circulation and relieving painful muscle spasms, and this will have the desired effect. Wet cupping can cause the production of endogenous nitric oxide (which is considered a vasodilator) or removes oxidants, which reduces oxidative stress. In addition, lacerations of the skin can induce pervasive inhibitory control, which in turn acts as a nociceptive stimulus. The mechanism has been explained by Pain-Gate Theory (PGT).

This theory is one of the most influential theories on pain reduction introduced by Melzack and Wall since 1965 [49]. They explain that injuries due to touch, pressure, and vibration to the surface of the skin carry pain signals from the site of injury to two destinations in the dorsal marrow, the spine, and transmitting pain signals to the brain. While interneurons work as an inhibitor of cell transmission activity. The theory comprehensively explains how pain is transmitted from the point of origin of pain to the brain, and how it is processed in the brain, then sends signals back to the injured efferent area. The wound inflicts local damage to the skin and capillaries acting as a nociceptive stimulus. This Theory was origin from the neuronal hypothesis that cupping can treat chronic pain by altering signal delivery at the level of nociceptors in both the spinal cord and brain [49].

The clinical manifestations of cupping in a randomized control trial report that cupping can be an effective therapy for pain management. The activity in thin and large diameter fibers supports the work of the transmission cell. Thin fiber activity inhibits interneuron cells (tends to allow transmission cells) and large diameter

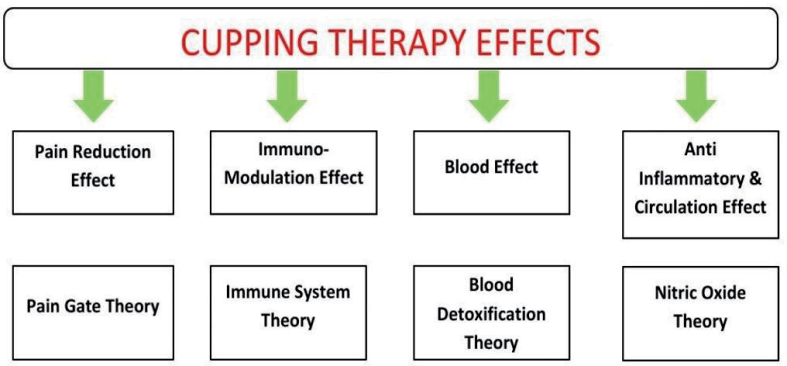

Figure 2.
Possible mechanism of cupping action theories.

fiber activity stimulates interneuron cells (tends to inhibit transmission cell activity). It can be concluded that the greater the activity of touch, pressure, and vibration on the skin surface, the less pain is felt. Thus, it is hoped that activation of nociceptors through cupping can stimulate "A" and "C" fibers with the involvement of the spino-thalamo-cortical pain pathway. The peripheral nociceptors can be sensitized by metabolic factors such as lactate, adenosine triphosphate, and cytokines. When a stimulus is applied to the surface of the skin, it increases the number of fiber-receptor units that are active to receive the stimulus that will be transmitted to the brain. Cupping therapy can reduce pain through its anti-nociceptive effects by fighting irritation. So it is believed that cupping therapy stimulates pain receptors by increasing the frequency of impulses, which in turn closes the pain gate [49].

4. Herbal Medicine for Pain Management

4.1 Cytokine, inflammation and pain

Cytokines are small amounts of proteins that mediate the relationship between cells, cytokines are the link between immune cells and nerve cells. Cytokines play a role in the process of immunity, inflammation and hematopoesis. Both pro-inflammatory and anti-inflammatory cytokines are involved in pain process. There is significant evidence that certain cytokines are involved in not only the initiation but also the persistence of pathologic pain by directory activating nociceptive sensory neurons. Based on its effect on immune cells, especially lymphocytes, cytokines are divided into 2, namely pro-inflammatory cytokines, including: TNF α, IL-1β (Interleukin) and IL-6 and anti-inflammatory cytokines, including: IL-4 and IL-10. Pro-inflammatory cytokines have algesic properties whereas anti-inflammatory cytokines are analgesic. Pro-inflammatory cytokines are produced in the early phase while anti-inflammatory cytokines in the late phase of inflammation [50].

The three pro-inflammatory cytokines can each induce production of one another and work synergistically. Meanwhile, anti-inflammatory cytokines suppress the gene encoding IL-1, TNF α and chemokine. A research in the role of cytokines in the pathogenesis of pain has been extensively investigated. In animal models, peripheral nerve lesions will immediately be followed by changes in cytokine expression continuously where cytokine levels increase in both the peripheral and central nervous system. A study proposed the Law of Pain hypothesis which states that all pain originates from inflammation and inflammatory responses, regardless of whether the pain is acute or chronic, peripheral or central and nociceptive or neuropathic pain [50, 51].

Neutrophils and macrophages both produce and secrete inflammatory mediators such as TNF-α, Prostaglandin E2 (PGE2), bradykinin, serotonin, histamine and so on. These inflammatory mediators directly activate nociceptors or cause sensitization of nociceptors, causing both spontaneous inflammatory pain and primary hyperalgesia. Neural lesions also cause Schwann cells to de-differentiate and release several algesic mediators such as: pro-inflammatory cytokines [50].

There is also the recruitment of T lymphocytes which can secrete various cytokines depending on the subtypes. These mediator cytokines play a role in the inflammatory response due to primary afferent nerve lesions and contribute to neuropathic pain. A study also explained that the cytokines Interferon-gamma (IFN-γ) can induce hyperexcitability of dorsal horn neurons in vitro, suggesting that pro-inflammatory cytokines play a role in central sensitization [50, 51].

4.2 Natural compounds for inflammation therapy and pain management

Pain is a nociceptive sensation in which perception causes unpleasant emotions. It is accepted that sensitization of primary sensory neurons is essential for inflammatory pain. Nonetheless, this nociceptor sensitization was, for a long time, thought to be the result of the stimulatory action of various inflammatory mediators i.e. cytokine released at the site of inflamed or damaged tissue. Cytokines generally function as intermediate mediators, releasing other cytokines and end mediators. In some cases, however, cytokines can act as late hyperalgesic mediators and several studies have described the involvement of cytokines in the development of nerve cell damage that will contribute to the onset of pain symptoms that are felt differently than expected by the type of stimulus applied (touch, for example, can produce stabbing pain). In one review, an expression used the term "hyper-nociception" to describe increased pain sensitivity by primary nociceptor neurons in an animal model [52]. Both in vitro and in vivo clinical evidence showed that cytokines play an important role in the mechanism of neuropathic pain can guide alternative therapeutic strategies that target humoral signal molecules and provide opportunities for the development of herbal drugs that can prevent or treat neuropathic pain. There have been studies explaining the evidence supporting the role of cytokines in pain manifestation. It discusses possible therapeutic approaches for pain control using therapies that target cytokines such as dissolved receptors, receptor antagonists, neutralizing antibodies, as well as herbal therapeutic approaches aimed at inhibiting cytokine production [53].

Many significant side effects of taking steroids and nonsteroidal anti-inflammatory drugs (NSAIDs) have led to greater interest in natural compounds, as dietary supplements and as herbal remedies, which have been used for centuries to reduce pain and inflammation. Natural compounds derived from plants and animals have been used for hundreds or even thousands of years to find effective pain relievers. Herbal medicine is becoming popular because of its relatively few side effects. However their use still requires knowledge of their biological action, clinical studies and potential interactions with other prescription drug products. Many researchers will reinforce the positive findings on the use of natural compounds to treat diseases, including pain management [54].

Recently, several herbal studies have shown that blends in various extracts from various herbs are predicted to be more effective than a single purified natural ingredient. A new study reports on the potential for polyherbal extracts to reduce inflammation. The novelty polyherbal used in this study is EMSA ERITIN. EMSA ERITIN contains soybeans, brown rice and coconut water. The EMSA ERITIN polyherbal composition has been shown to be effective in suppressing inflammatory transcription factors. EMSA ERITIN consists of soybean extract [53], coconut water extract [54] and brown rice extract. As we know soybeans contain many active compounds. One of the active compounds is genistein. Genistein is an isoflavone isolated from soy, is a powerful antioxidant with good anti-inflammatory effects. The results of this study indicate the effect of poly-herbal EMSA ERITIN can suppress pro inflammatory cytokines, TNF-α and IFN-γ in vivo. The study also looked at transcription expression of inflammatory factor (NF-κB). EMSA ERITIN exhibits anti-inflammatory activity when administered to lymphocytes in BALB/c mice after radiation [55]. EMSA ERITIN can decrease proinflammatory cytokine TNF-α and IFN-γ, also transcription factor of NF-κB. The increasing of dose in EMSA ERITIN showed more effective than lower doses. So, EMSA ERITIN is potential polyherbal plant used as anti-inflammatory agent [55] and further it can be studied for used as herbal medicine for pain relief.

The hypothesis that all pain processes originate from inflammation and inflammatory responses, as well as the hypothesis that pain occurs due to an imbalance between pro and anti-inflammatory cytokines needs to be studied more deeply,

so that if the hypothesis is proven correct, thus the therapeutic strategy can inhibit pro-inflammatory cytokines. or increasing anti-inflammatory cytokines would hopefully have more promising results.

5. Conclusion

Complementary and alternative medicine has grown in the past decades with many of its systems worldwide. Integrative approaches in pain control methods for Muslims patients in addition to: preventing cell damage, reducing the effect of chemical mediators of pain can be managed by dhikr, hijamah and herbal medicine. It was recomended that dhikr therapy through affirmation-tapping either singly or in combination with hijamah and herbal medicine can signifantly reduce pain intensity. Cupping therapy (Al hijamah) was cited by Muhammad (PBUH), Wet cupping (Al-hijamah) is a therapeutic approach that is believed to combat illness and promote well-being. Also the herbal medicine for healing purposes predates human history and forms the origin of much modern medicine. These approaches will be a promise therapeutic as integrative care for pain management.

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Conflict of interest

The authors declare no conflict of interest.

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Section 2

Immunity

Chapter 2

Immunomodulator in Traditional Healthcare System

Shweta Saboo

Abstract

In all over world the importance of traditional medicine for the use as treatment against of life threatening diseases is acceptable. Due to heavy potency and availability of the natural sources it's easy to utilize this traditional knowledge for treatment, prevention or mitigation against diseases. In this chapter we discuss about different potent Immuno-modulating drugs which majorly act as immune stimulant. From the ancient time these drugs having potent active constituent which shown prominent effect in treatment of diseases. Considering efficiency with safety plant derived drugs having very global market this review discusses immunomodulating plant with their active constituent.

Keywords: traditional herbs, immunomodulators, medicinal plants

1. Introduction

The Indian traditional system of medicine (Ayurveda) had important role in prevention and treatment of disease. Many of the plants showing effect as an immunomodulator. Though conventional immunomodulatory chemotherapy is available but it is costlier and is not usually affordable to ordinary people with loweconomic status [1]. Therefore, the modulation of immune system by traditional medicinal plant products has become a subject matter for current scientific investigations worldwide. A huge number of medicinal plants have been indicated in Siddha and Ayurveda classical literature towards the management of several diseases that results in immune deficiency. The present study summarized that the Ayurveda, siddha used as Immunomodulator in Health care system.

2. Immunity

Immunity is the capability of the Multicellular organism to resist harmful microorganism .these microorganism act as foreign body for living cell, and it try to kill that microorganism. It is also called defense mechanism of the organism to protect from the external factor. Immunity involves both specific and nonspecific components. The nonspecific components act as fighter or barriers to the number of pathogens with various genetic makeup and its nature while specific type of immunity is produce only when specific type of Pathogen produce specific type of Infection and to resist it specific type of immune cell become active and produce resistant to that pathogens [2].

Immunity can be defined as a complex biological system of the body which having capacity to identify whatever belongs to cell or which is beneficial to body and resist to foreign or harmful material with the capacity to recognize and tolerate whatever belongs to the self, and to recognize and reject what is foreign (non-self) [2].

3. Immune cells has two distinct

The basic architecture of the immune system is multi layered, with the Defense on several levels. First barrier is the skin, against the infection. Another is physiological conditions, where conditions like temperature and pH of the body provide in appropriate living condition for foreign organisms. Once pathogens have successfully entered in the body, they are addressed by the innate or acquired or adaptive immune system.

The immune system has two components: innate and adaptive immunity. The innate immunity is present in all metazoans, while the adaptive immunity only occurs in vertebrates.

The innate system after the stimulation of foreign material body produces Inflammation and phagocytosis. On the other side on adaptive system is composed with Lymphatic cell, these cells have ability to distinguish between self cell and non self cell [3].

The inflammation reaction is produce when foreign material enters into body and cells able to find out its foreign material.while The non-reaction to self substances is described as immunity - meaning to exempt.

These two action produce dynamic biological environment and it called health. Disease can occur when foreign material not eliminated or what is self is not spared [3].

4. Immuno modulators

These are natural or synthetic compound or its combination which alter or modify both types of immune system either adaptive or innate of the body.

5. Classification of an immunomodulators

Imunoadjuants are used to enhance the efficiency of vaccines and therefore could be considered a specific immune stimulant. Imunoadjuants hold the promise of being the true modulator of the immune response. It has been proposed that they be exploited as selectors between cellular and humoral helpers [4].

Immunostimulants are inherently non specific as they are envisaged as enhancement to the resistance to the inspection. They act through the intent as well as adaptive immune responses. In healthy individuals, the immunostimulants are play a role as proteolytic or promoter for formation of immune cell. Drugs which impart in production of immune cells are called Immunomodulator [4].

Immunosuppressant is structurally and functionally heterogeneous group of drugs which are often, simultaneously administered in a combination of Regiment to treat the various types of organ transplant rejection and autoimmune disease.

In clinical practice, both aspects of immunomodulation, i.e. immunostimulation and immunosuppression are equally important. Immunostimulation may require during conventional chemotherapy when the host defense mechanisms are to be activated under conditions of impaired immune responsiveness.

The mechanism ofImmunomodulation activity occurs mainly via the stimulation of phagocytes, macrophages, lymphoid cells, increasing circulating total white cell counts and interleukin-2 levels. Immunological defense is a constant interplay between nonspecific and specific, cellular and humoral immune responses, stimulation and suppression of immunocompetent cells, and the influence of endocrine and other mechanisms. The Primary targets of the Immunostimulant are T or B lymphocytes and it plays a central role an immunostimulation. The second most important role in the stimulation of T lymphocytes is Activation of macrophages, which can be achieved either directly or indirectly, via macrophages [5].

6. Ayurveda system and immunomodulation

The primary objective of Ayurveda, the Indian traditional system of medicine is the prevention of the disease. The different health care measures to be adopted by an individual are grouped together under the heading of "Rasayana." Rasayana meaning in sanskrit that literally implies to the circulation of rasa means Nutrients and these nutrients reach to the various parts of body, reaches upto tissues and cell. In that nutrients some are micro and macro nutrients which help in proper functioning of the body and resistant to diseases. Sushruta was more specific, describing a Rasayana as one, which is antiaging, increases the life-span, promotes intelligence and memory, and increases resistance to diseases [6].

7. Siddha system and immunomodulation

The great Tamil saint 'Thiruvalluvar' has discuss the significance of diet as the cause of disease. According to Siddha system, the Term "Food is medicine and medicine is food" indicated that proper diet and healthy lifestyle containing medicinal herbs has intrinsic elements allowing the body to remain healthy. The ultimate essence of our consumed food acts as an essential component to strengthen the seven UdalkattugalSaaram (Plasma), Seneer(Blood), Oon (Muscles), Kozhuppu, Enbu, Moolai, Sukkilam, Suronitham thereby stimulating immunomodulation [6].

8. Indian medicinal plant

An in India wide range of traditional medicine that is from ancient time use for medicinal purpose. India is heritage for wide range of medicinal plants. These medicinal plants show various pharmacological action like anti-inflammatory, Anti diabetic, Antiulcer, Stimulant, Nervine tonic and many more.

In that some plants contain some micronutrients present in form of secondary metabolite and produce action of immunomodulation [7].

Some of the indigenous plants investigated for immunomodulatory effects.

8.1 Garlic (*Allium sativam*)

Allium sativam is also known as Lahsuna. It consists of bulb belonging to family.

Liliaceae. It grows 1.5 to 2.5 cm with having Characteristic and Aromatic Odor.

Bulb are whit to pink in color. Garlic contain volatile oil, alliin and allicin.Garlic used as Carminative, expectorant, Antibacterial, disinfectant and also used in treatment ofpulmonary infection [7].

Immunomodulatory effect

- Inhibit growth of cancer cells.
- Modulates activity of chemical carcinogens.
- Enhances capillary skin perfusion.
- Enhances macrophage (oxidative burst) and T lymphocytes.

8.2 Ghriti Kumari (*Aloe vera*)

It consists of dried juice of leaves of *Aloe vera* belonging to family Liliaceae.

Aloe is indigenous to eastern and southern Africa and grown in cape colony, Zanzibar and island of Socotra. In India it is available in almost all the houses. The genos Aloe consists of about 200 species, theseplants have rosettes of subulate, succulent large leaves. These leaves are sessile and have a strong spine at apex and also no. of spines in margins. Aloe mainly contains Barbaloin and alosin. Barbaloin is C- glycosides. Aloe used as a purgative, Antiseptic, cooling agent and also in many of the cosmetic preparation [7].

Immunomodulatory effect

- Prevents UV-induced suppression of DTH.
- Polysaccharides from it show adjuvant activity for antibody (Ghrit-kumari) production and DTH.
- Inhibits inflammation.
- Improves wound healing.
- Serves as oxygen radical scavenger, acts synergistically with NO.
- Causes regression of tumor.

8.3 Kalmegh (*Andrographis paniculate*)

It consists of dried leaves and tender shoots of plant *Andrographis paniculate* belonging to family Acanthaceae. Kalmegh is an annual herb distributed in shri lanka and throughout india, specially in Maharashtra, Karnataka, Utter Pradesh, Tamilnadu, Andhra Pradesh and madhyapradesh. It is cultivated in some extent in Assam a west Bengal. Leaves are dark green, whit flowers and rose colored. It is odorless and intensely bitter in taste. It is used as Immunostimulant [7].

- Stimulates macrophage migration,
- Phagocytosis of *E. coli*; Induces paniculata stimulation of antibody and DTH response to SRBC in mice.
- Proliferation of splenic lymphocytes.
- Inhibits NO synthase.

8.4 Shatavari (*Asparagus racemosus*)

It is also called as Shatmuli. It consists of dried roots and the leaves of the plant *Asparagus racemosus* belonging to family Liliaceae. The roots are silver white or ash color externally and white internally. Spindle shaped and having slightly bitter followed by sweet taste. Shatavari roots is used as Galactagogue, tonic, diuretic. Shatavari I having ant oxytocic property. Shatavari also used as anti stress, Anticancer, Antiseptic, Antiaging and immunostimulant [8].

Immunomodulatory effect

- Stimulates RE System and PMN cell.
- Induces lag in tumor development.
- Prevents leucopoenia induced by cyclophosphamide.
- Inhibits ochratoxin A induced suppression of IL-1, TNF-α – Antiaging and macrophage chemotaxis.

8.5 Neem (Azadirectaindica)

It is also known as Margosa. It is non edible fixed oil obtained from fully matured seeds of *Azadiractaindica* belonging to family Meliaceae.Neem plant found throughout India and other tropical countries. It mainly contains Glycerides saturated and unsaturated fatty acids. It having yellow colored oil with specific odor and bitter in taste. It contains Nimbin, nimbidin and other related compound possess antiviral activity. It is also used for making soap and manufacturing of oleic and steric acids. It also acts as pesticides and spermicides.

- Stimulates IL-1, INF-γ, TNF-α production, enhances proliferative response of spleen cells to Con A and tetanus toxoid.
- Inhibits both complement pathways as well as activates PMN cells.
- Enhances macrophage phagocytosis and expression of MHC II antigen.
- Enhances anti-ovalbumin antibody response, DTH response, macrophage migration inhibition.
- Attenuation of stress and xenobiotic induced suppression of humoral and cell-mediated immunity.
- Enhances PMN leucocytes and cell-mediated immunity.
- Induces production of interferons.
- Reduces mortality induced by Tacaribe viral encephalitis.
- Inhibits intracellular multiplication of Chlamydia and cytopathic effects of herpes.
- In a clinical study reduced erythema desquamation and infiltration of psoriatic lesions.

8.6 Amla (*Emblica officinalis*)

Amla consist of dried and fresh of plant *Embellica officinalis* belonging to family Euphorbiaceae. It is small in size tree found in all deciduous forest in India. It is also found in Shrilanka and Myanmar. The Fruits are Green in color, after maturity it is converted into yellow and brick red. Having sore and astringent taste. It is used as a Antibacterial, Rejuvanate and it improve vitality.

Immunomodulatory effect

- Protects against pancreatitis
- Induce positive nitrofgen balance
- Protects against toxic effect of metals
- Enhance NK cell and Antibody dependant cellular toxicity against Dalton lymphoma ascites Tumor [9].

8.7 Tulsi (*Ocimum sanctum*)

It is also called as Holy basil. Tulsi consists of fresh and dried leaves of plant *Ocimum sanctum* belonging to family Lamiaceae. It is herbaceous, branched, small herb annual plant found throughout India. The plant considered as sacred by Hindus. It is commonly cultivated in garden and grown in temples. It mainly contains volatile oil and eugenol is the main chemical constituent. Generally, all part of tulsi is used as a medicine.it act as an Anti-inflammatory, antiseptic, Antifungal, antiviral, Antiasthematic [10].

- Increases colony forming unit in spleen and protects mice after irradiation.
- Enhances survival of viral encephalitis patients.
- Enhances humoral immunity; inhibits histamine release from sensitized mast cells and antagonizes tissue responses to histamine [10].

8.8 Haldi (*Curcuma longa*)

I also known as Haridra, Indian Saffron, Turmeric, Curcuma.

Turmeric consist of dried and also fresh Rhizomes of plant *Curcuma longa* belonging to family Zingiberaceae. Externally it is having yellowish color with characteristic odor and bitter in taste. Curcumin is the main chemical constituents. It used as a spice, colorings agent, antiseptic, anti-inflammatory [11].

- Increases mitogenic response of lymphocytes
- Inhibits NO production and scavenges reactive oxygen species.
- Enhanced IgG level but did not affect DTH and NK cell activity.
- Helps in rheumatoid arthritis.
- Chemoprotective agent against cancer.

8.9 Ginseng (*Panax pesudoginseng*)

It consist of dried roots 0f various species of Panax like P. *pesudoginseng, P. japonica, p.notoginseng.* Belonging to family Araliaceae. It is an important immunomodulatory drug. It generally used as health tonic and Adaptogen.

Immunomodulatory effect

- Stimulates macrophage migration;
- Enhances circulating antibody and antibody forming cells to SRBC in mice.

It is also called Withania root, Winter cherry. Withania consists of dried roots and stem bases of *Withaniasomnifera* belonging to family Solanaceae.it mainly contain, Withaferin A, Withaferin, Anaferin, Withanolides. It acts as a Immunostimulant, Antirheumatic, Antistress [11].

Immunomodulatory effect

- Stimulates RE system and PMN cells.
- Inhibits tumor development.
- Increases WBC counts in irradiated mice.
- Prevents myelosuppression induced by azathioprine, cyclophosphamide and prednisolone.
- Inhibits Ochratoxin A induced suppression of IL-I, TNF-α and macrophage chemotaxis.
- Enhances spleen colony forming units.
- Enhances Radio sensitization for V97 Chinese hamster cell.

8.10 Kutki (*Picrorrhizakurroa*)

It used as Immunostimulant and antioxidant.
Immunomodulatory effect

- Enhances antibody and DTH response to SRBC in mice.
- Inhibits ochratoxin A induced suppression of IL-1, TNF-α and macrophage chemotaxis.
- Protects animals against leishmania and filarial infections.
- Enhances phagocytosis, stimulates PHA, ConA and LPS induced lymphocyte proliferation, macrophage migration, enhances antibody response against SRBC [12].

Murrayakoenigii is commonly known as curry leaves, belonging to family Rutaceae. Because of its aromatic value mainly used as spice throughout India. Leaves are green in color. The bark, leaves and root are used as tonic, stomachache, stimulant and carminative Plant identified as Rsayanas in Indian ayurvedic system

of medicine have various pharmacological properties such as immunostimulant, tonic, neurostimulator, antiaging, antibacterial, antiviral, antirheumatic, anticancer, apoptogenic, ant stress.

Methanolic extract of *Murrayakoenigii* leaves gives cellular and humoral immune response [1].

8.11 *Tinosporacordifolia*

It is commonly called as Guduchi. It is large, glabrous, perennial, climbing shrub of weak and fleshy stem found throughout India. It is used as an antidiabetic, anti-pyretic, antimalarial, anti-inflammatory, hepatoprotectives, immunomodulatory, antispasmodic, antineoplastic activity.

8.12 *Bauhinia variegate linn*

The plant commonly found in moist waste ground and open plantations.it is cultivated throughout India. Its family is Caesalpiniaceae. The bark powder of plant is ingredients of the herbal tonic Kanchanar guggul,is an Ayurvedic remedy used to increases white blood cells. The plant also used as tonic for liver, in treatment of leprosy, menorrhagia, impurities of blood, wounds, ulcer, asthma.The effect of the ethanolic extract of the stem bark of *Bauhinia variegate* on the primary and secondary antibody responses was evaluated by the humoral antibody response for a specific immune response. Phagocytic cells, such as macrophages and neutrophils, barriers such as skin and a variety of antimicrobial compounds synthesized by the host, all play important roles in innate immunity [13].

8.13 *Abutilon indicumlinn*

Abutilon indicumlinn commonly known as Atibala, it is a stronger diuretic and heart tonic. It is also used for the remedy of the jaundice, piles, ulcer, leprosy, rakt-tapittadosha and blood purifier. The ethanolic extract of *Abutilon indicum* leaves beneficial for the treatment of impaired immunity.

8.14 *Terminia arjuna Roxb*

It is commonly called as Arjuna bark and Arjun. It consists of dried stembark of the plant known as *Terminia Arjuna Roxb*. Belonging to family Combretaceae. It is common tree in Indian peninsula. Bark having Astringent taste. Bark is used as acardiotonic it also possesses diuretic and tonic properties. Diuretic properties are due to Arjunolic acid. The drug exhibit hypotension action with vasodilation and decrease heart rate [13].

9. Conclusion

Besides of allopathic diagnosis system, we can use Traditional system for the treatment of various diseases and which can be affordable to the common communities. Ayurveda and siddha system involved the medicinal plants for the treatment of diagnosis and it is available easily in Indian forest. So, the medicinal plant or Indian traditional system is best way for treatment or Immunomodulator.

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Section 3

COVID 19

Chapter 3

Some Igbo Indigenous Plants with Anti-COVID-19 Properties

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Abstract

Coronavirus (COVID-19) has shaken the world not minding the strength of the global health system leading to over 824, 000 deaths amidst the search of a cure and total prevention. The Igbo states in Nigeria has the average prevalence of 711 cases of COVID-19 with the highest 1096 (Enugu) and least 207 (Anambra) as at 26th August, 2020. This chapter studied some Igbo indigenous plants in use since the outbreak and presents Bitter kola, Garlic, Giloy, Ginger, Lime, and Turmeric which are having anti-COVID-19 properties. The authors suggest that these plants have the properties that alter the PH on the interface between the virus spike proteins and the human respiratory surfaces causing a brake on the interaction with human ACE-2 and where interaction has taken place, the replication and translation stages are disrupted. The plants thus are potential modifiers of this milieu and inhibitor of the main protease and endoribonuclease via epigenetics and homeostasis. These plants consumption should be encouraged as prophylactic or curative measures pending the discovery of a definitive cure. The chapter recommends that the search for COVID-19 cure should not be limited to conventional medicines, rather should be extended to some indigenous plants in Igbo land.

Keywords: Igbo plants, anti-COVID-19, bitter kola, garlic, giloy, ginger, lime, turmeric

1. Introduction

COVID-19 pandemic is a global threat without any confirmed treatment regimen. The global community is on the trail to get preventive and treatment measures. The pandemic burden has increased the effort of traditional/herbal medicine practitioners across the globe and, Nigeria in particular towards arriving at a cure for the novel virus. Coronavirus (SARS-COV-2), a zoonotic disease

and the seventh member of coronaviruses was named COVID-19 and officially declared a pandemic on 11th February, 2020 and 11th March, 2020 respectively [1, 2]. There is no doubt that COVID-19 has posed a challenge to the best healthcare systems around the world; giving a leeway to herbal/plant experts to demonstrate their capacity.

On 26th August 2020, using Corona Scanner Realtime coronavirus statistics App (Free online dashboard solution), the Coronavirus has infected 215 countries of the world. Global Infections are 24,092,885 with 824,194 deaths showing 3.42% death rate and 69.04% survival rate (16,634,272 recovery) with daily infection rate at 223,070 persons. Nigeria is ranking 50 on the global list of infections with 52,800 reported cases, 1007 deaths showing a 1.91% death rate, and 75.69% survival rate (39,964 recoveries) as at 1.10 pm with a daily infection rate of 460 persons.

The COVID-19 pandemic is currently recording a higher number of infections on a daily bases because many Nigerian researchers had advocated stepping up of medical laboratory testing. The increased prevalence of COVID-19 is noted because of increased testing capabilities in various countries and, Nigeria, in particular [3, 4]. Already, the community transmission of the virus has increased while spread could be attributed to the zoonotic nature [5, 6] of the COVID-19. All approaches be it, surveillance, molecular testing, immunomodulation, conventional, or phytomedicine towards stopping the COVID-19 pandemic that needs to be adopted [7, 8].

In Africa, the use of herbs and concoctions in the management of various ailments has been recorded [9], and COVID-19 cannot be exempted. The index case as announced in Nigeria by FMOH [6, 10] left many herbal medicine practitioners to go back to their various products that could be used to contain the pandemic. The first line of herbs in consideration are edible and nutritious vegetables that could be readily approved by the scientific world have been consumed without toxic effects or have been scientifically approved in the past. This is where Bitter kola (*Garcinia kola*) Garlic (*Allium sativum*), Giloy (*Tinospora cordifolia*), Ginger (*Zingiber officinale*), lime (*Citrus limonum*), and Turmeric (*Curcuma longa*) belong and can be easily assessed and verified.

2. Igbo land in the time of COVID-19

Igbo land "Ala Igbo" is the South Eastern zone of Nigeria located at 1000 m (3,300 ft) above sea level and covers the area 41,440 km^2.

South-Eastern Nigeria (Igbo land) is part of the old eastern region that was part of Biafra. This geopolitical region is made up of five (5) states- Abia, Anambra, Ebonyi, Enugu, and the Imo States, as highlighted in **Figure 1**.

The language spoken in southeastern Nigeria is the Igbo language.

As of 26th August 2020 based on NCDC COVID-19 Situation Report (situation report 180), shows that Igbo land had 3553 cases out of 53,021 cases recorded in Nigeria with lowest case fatality of 8.32% (84 out of 1010 death cases in Nigeria) as derived from **Figure 1** [11].

Though there may be other factors that may be responsible for the fatality cases, the authors observed that the indigenous people of Igbo land both home and in diaspora made use of some of their indigenous plants during the COVID-19 not minding their places of abode.

STATES	CONFIRMED CASES		DISCHARGED CASES		DEATHS		TOTAL ACTIVE CASES	DAYS SINCE LAST REPORTED CASE
	TOTAL	NEW	TOTAL	NEW	TOTAL	NEW		
Lagos	18,035	17	15,227	13	202	0	2,606	0
FCT	5,079	33	1,468	18	50	2	3,561	0
Oyo	3,060	2	1,819	87	37	0	1,204	0
Edo	2,555	2	2,263	11	100	0	192	0
Plateau	2,245	60	1,187	19	29	0	1,029	0
Rivers	2,108	18	1,910	9	57	0	141	0
Kaduna	2,085	26	1,862	30	12	0	211	0
Kano	1,722	1	1,507	0	54	0	161	0
Delta	1,719	4	1,540	22	46	0	133	0
Ogun	1,633	2	1,462	15	26	0	145	0
Ondo	1,524	9	1,305	0	31	0	188	0
Enugu	1,096	9	852	0	21	0	223	0
Ebonyi	965	0	921	0	27	0	17	1
Kwara	945	9	740	0	25	0	180	0
Katsina	771	0	457	0	24	0	290	4
Osun	771	2	670	22	16	0	85	0
Abia	759	4	669	26	7	0	83	0
Borno	740	0	663	17	36	0	41	3
Gombe	719	5	609	4	23	0	87	0
Bauchi	645	1	547	2	14	0	84	0
Imo	526	3	192	5	11	1	323	0
Benue	451	0	141	0	9	0	301	2
Nasarawa	427	6	298	0	12	0	117	0
Bayelsa	378	0	331	5	21	0	26	1
Jigawa	322	0	308	0	11	0	3	41
Akwa Ibom	271	0	220	0	8	0	43	7
Niger	239	0	168	0	12	0	59	2
Ekiti	238	3	130	5	4	0	104	0
Adamawa	217	0	159	0	15	0	43	2
Anambra	207	5	159	0	18	0	30	0
Sokoto	158	0	138	0	16	0	4	3
Kebbi	92	0	82	0	8	0	2	2
Taraba	87	0	73	0	5	0	9	1
Cross River	82	0	70	7	8	0	4	2
Zamfara	78	0	72	0	5	0	1	6
Yobe	67	0	59	0	8	0	0	27
Kogi	5	0	3	0	2	0	0	54
Total	53,021	221	40,281	317	1010	3	11,730	

Figure 1.
Nigerian states COVID-19 status as at 26th august 2020 by 11.59 pm as accessed from NCDC [11].

3. Some Igbo indigenous plants

Replace Conventional medicine utilizes active compounds mostly isolated from some medicinal plants to the extent that about 80% of the active ingredients indicate a positive correlation between their modern therapeutic uses and herbal or traditional use depending on where the plants are found [12]. Igbo indigenous plants and herbs have been used for treating and preventing several diseases, including respiratory viral infections in the past. The current novel virus also poses a challenge to which conventional or herbal medicine would be useful and no wonder some Igbo indigenous plants and herbs are listed for consideration in a bid to discover the COVID-19 management drugs and cure. Such plants understudy in this chapter is bitter kola, garlic, giloy, ginger, lime, and turmeric.

3.1 Bitter kola

Plant name: Bitter kola.
Pictorial exposition (**Figure 2**):
Botanical name: Bitter kola – *Garcinia kola.*
Indigenous (local) name: Aku ilu.
Taxonomical classification:
Kingdom: Plantae.
Division: Magnoliophyta.
Class: Magnoliopsida.
Order: Theales.
Family: Clusiaceae.
Genus: Garcinia.
Species: kola.
Part of the plant in Use: Seeds.
Phytochemical components:

Several studies have been carried out on the phytochemical components of *Garcinia kola*. Such studies have shown that *Garcinia kola* contains alkaloids, saponins, tannins, flavonoids, glycosides, sterols, and phenols. The major constituents of the plant are kolaviron, garcinia biflavonoid (GB-1a-glucoside, GB-1a, GB-1, GB-2), kolaflavonone, benzophenone, xanthone, coumarin, apigenin, quercetin, and garcinoic acid [13–17].

Hexadecanoic acid, 9-octadecanoic acid, methyl ester, linoleic acid, heptadecane-(8)-carbonic acid, formaldehyde, N, N-Diethyl, n-tetradecanoic acid amide; 3,4,8-trimethyl-2-nonenal were gotten from the seed of *Garcinia kola*. Carbohydrates were separated from the seed. The mineral composition of *G. kola* seeds extracts was also reported [18]. The seed contains an enormous amount of potassium and phosphorus. Other constituents include ash, crude protein, crude fiber, crude lipid, water-soluble oxalate, terpenoids, and fat [19].

Gas–liquid chromatography and High-Performance Liquid Chromatography was used to study these phytochemical contents of *Garcinia kola*. The seed oil was found to contain fatty acid and amino acid derivatives, namely meristic, pentadecanoic, margaric, trans-palmitoleic, cis-vaccenic, cis-oleic, cis-linoleic, α-linolenic, threonine, tyrosine, methionine, serine, histidine, and alanine [20].

Figure 2.
Bitter kola fruits on the plant and bitter kola seeds.

Based on dietary properties, the following have been found: moisture (7.2%–92.7%); crude protein (0.58%–7.8%); ash (0.33%–5.9%); crude fiber (1.23%–20.51%); crude fat (0.19%–14.5%); and NFE (10.85%–91.35%). The dominant fatty acids in the seed are oleic (38 mg/kg), linoleic (36 mg/kg), and palmitic acid (32 mg/kg). The prevalent essential amino acids are lysine (2.4 g/kg), leucine (1.9 g/kg), and valine (1.7 g/kg), and nonessential amino acids are glutamic acid (6.8 g/kg) and arginine (5.5 g/kg). The bitter kola seeds are low in anti-nutrients such as phytate or oxalate. However, high amounts of vitamin C have been recorded up to 23.1 mg/100 g. Potassium (722 mg/kg) and phosphorus (3.3–720 mg/kg) were recorded in bitter kola as the most abundant minerals in bitter kola seeds [21].

Health benefits:

The medicinal importance of bitter kola is based mainly on the photochemical components of the plants. Some of these components isolated include oleoresin, tannin, saponins, and alkaloids. Other components isolated from bitter kola seeds are bioflavonoids such as kola flavanone, and hydroxy flavonoids. Bitter kola is highly valued in African ethnic medicine because of its varied and numerous social and medicinal uses, thus making it an essential ingredient in folk medicine. Herbal Medicine has been advocated as a major contributor to the cure of many ailments [9, 22] and Bitter kola is believed to be an important source of flavonoids and chemical substances with potential therapeutic benefits especially in the treatment of diabetes [23].

Omeh et al. posit that bitter kola is cardioprotective because of the lipid reduction ability [24].

The bitter kola is commonly chewed by both rural and urban dwellers for treating gastric problems or for their typical astringent taste. The bioflavonoid kolaviron complex content of bitter kola is anti-inflammatory, neuroprotective, and antimicrobial. Kolaviron possesses anti-malarial and wound healing effects. Kolaviron is useful in the treatment of benign prostatic hyperplasia, multiple sclerosis, and AIDS. It has shown the capacity to stop Ebola virus growth in medical laboratory trials [21].

Kolaviron possesses antinociceptive (sedative) and anti-inflammatory activities, both centrally and peripherally, which justifies its folkloric use to relieve pain and inflammation. Moreover, Abarikwu [25] revealed that kolaviron could block signaling pathways implicated in lipopolysaccharide-induced inflammatory genes and equally prevent oxidative stress. It also helps in demyelination and neurotoxicity. It aids in the treatment of multiple sclerosis that can be clinically viable against ischemia/reperfusion injuries.

Bitter kola has hepatoprotective properties, promotes survival of hepatocytes, and prevents liver injuries and intoxication. In rat models, bitter kola is anti-diabetic and protecting against hyperglycemia-induced apoptosis, attenuate the level of lipid peroxidation. Bitter kola has anti-malarial activities.

Nworu et al. [26] discovered its immunomodulatory and immuno-restorative effects, making it useful in fighting immune-destructive diseases such as acquired immunodeficiency syndrome (AIDS) and other viruses which may include COVID-19.

Bitter kola wether as alcoholic or aqueous extracts has antimicrobial properties against many multidrug-resistant bacteria (gram-positive and gram-negative) and fungi [21].

Possible Toxicology:

There is speculation that only high doses of Kolavirons (400 mg/kg) can cause liver damage but its ability to inhibit cyclooxygenase (COX-2) and inducible nitric oxide synthase (iNOS) expression through downregulation of nuclear factor kappa

B (NF-κB) and activator protein-1 (AP-1) DNA binding activities shows the hepatoprotective properties of Kolaviron content of bitter kola [21].

Anti-COVID-19 Properties:

Guttiferones are polyisoprenylated benzophenone derivatives of bitter kola that can inhibit the cytopathic effects of the virus responsible for HIV infection [27].

Garcinol has the same or even very similar structure to that of Guttiferone F that has similar properties. However, relatively few studies have been reported describing the design and performance of bitter kola constituents and drug delivery systems on its proposed antiviral properties. [28]. Reports of use during COVID-19 [29] are helpful in bitter kola effects against the pandemic.

3.2 Garlic

Plant name: Garlic- *Allium sativum.*
Pictorial exposition (**Figure 3**):
Botanical name: *Allium sativum.*
Indigenous (local) name: Yabasi Hausa.
Taxonomical classification:
Kingdom: Plantae.
Division: Magnoliophyta.
Class: Monocotyledonae.
Order: Liliales.
Family: Liliaceae.
Genus: Allium.
Species: Sativium.
Part of the plant in Use: Bulb.
Phytochemical components:

Garlic contains carbohydrates, glycosides, and proteins in high concentrations; alkaloids, saponins, reducing sugars, oils, and steroids in medium concentrations, while flavonoids and acidic compounds were present in low amounts [30].

Phytochemicals screening revealed the results that alkaloids, reducing sugar, flavonoids, glycosides, cardiac glycosides, tannin and phenolic compounds, saponins, amino acid & triterpenoids in aqueous extract but methanolic extract shows the absence of reducing sugar [31].

Figure 3.
Garlic plants and the bulbs.

Health benefits:

Garlic is useful in chronic cough, arthritis, and constipation. Garlic has also been mentioned to protect from epidemic diseases, and no wonder it is always reckoned during COVID-19. Many studies revealed that garlic has antioxidant, anti-inflammatory, immune-modulating, antibiotic, bacteriostatic, antifungal, antiviral, antihelminthic, antithrombic, hypotensive, hypoglycemic, and hypocholesterolemic properties [32].

Garlic has shown a virucidal effect on human rhinovirus-2, parainfluenza virus-3, HSV-1, HSV- 2, and vesicular stomatitis virus during in vitro study by Weber et al. [33].

Kang et al. [34] explored the antioxidant and reactive oxygen species scavenging property of saponins produced by garlic while Naji et al. [35] demonstrated the hepatoprotective and antioxidant property of single clove garlic in rabbits' models.

Garlic used for management of abdominal discomfort, diarrhea, otitis media, and respiratory tract infections in the eastern part of Nigeria [36] and treatment of common colds, hay fever, and asthma in Europe and India [37].

The garlic has immunomodulation, anti-inflammatory, and antioxidant with cardioprotective, and pulmonary protective properties [32].

Possible Toxicology:

Fowotade et al., [37] reported a dose-dependent increase in levels of liver enzymes AST, ALT, and ALP) as well as an increase in serum creatinine levels and dose-dependent histologic alterations in hepatic, renal, and cardiac tissues in rat models indicating toxicity at higher dozes to the liver, heart, and kidney.

Anti-COVID-19 Properties:

Garlic modulates cytokine expression in lipopolysaccharide activates human blood and inhibits NF-κB from which makes it immune-modulatory. It activates macrophages and promotes immunoglobulins. Garlic extract reduces the migration of polymorphonuclear cells through endothelial cell layers. Garlic inhibits the production of nitric oxide and prostaglandin-E2, suppresses the inducible form of nitric oxide synthase and COX-2 expression, and decreases the production of inflammatory cytokines like TNF-α, interleukin six, and interferon γ. It improves lung function in smokers and reduces tracheal exudates in horses. It is useful in inflammatory and asthma-like conditions of the lungs [37].

The exploits in pharmacologic actions in handling respiratory diseases and other viruses are possible in handling COVID-19.

3.3 Giloy

Plant name: Giloy - *Tinospora cordifolia.*
Pictorial exposition (**Figure 4**):
Botanical name: *Tinospora cordifolia.*
Indigenous (local) name: Udo akpu enyi.
Taxonomical classification:
Kingdom: Plantae.
Division: Magnoliophyta.
Class: Magnoliopsida,
Order: Ranunculaceae.
Family: Menispermeaceae.
Genus: Tinospora.
Species: cordifolia.

Part of the plant in Use: Root, Stem, Back, Leaves, and Fruits. This implies that all the parts of the plant are useful as an herbal remedy of one disease or the other depending on the preparation.

Figure 4.
Giloy leaves on the plant and Giloy stems.

Phytochemical components:

Giloy contains polysaccharides, phenolics, diterpenoids, steroids, and sesquiterpenoids [38]. Singh and Chaudhuri [39] reported that giloy has the following components:

Alkaloids – Giloy has 13 alkaloids of isoquinoline and aporphine skeletons, amine, and amide with main alkaloids that are protoberberine alkaloids berberine, corydine, magnoflorine, and palmatine.

Terpenoids – Giloy has 32 diterpenoids and their glycosides of clerodane and norclerodane skeleton, 5 sesquiterpenoids, 1 monoterpenoids, and 1 triterpenoid cycloeuphordenol. There is also a bicyclic diterpenoid (C21H24O7) from the whole plant identified as tinosporin.

Phenolics – Giloy has 4 phenylpropanoids, 3 lignans, 2 flavonoids, and 2 benzenoid derivatives.

Steroids – Giloy has 4 steroids along with δ-sitosterol and 2,3,14,20,22,25-hexahydroxyl-5-cholest-7-en-6-one.

Essential oil and aliphatic compounds – Giloy hydro distilled essential oil of fresh leaves showed the presence of alcohols (32.1%), phenols (16.6%), aldehydes (16.2%), fatty acids (15.7%), alkanes (8.3%), esters (3.2%), and terpenes (1.2%), along with hydroquinone (16.6%), 2-hexenal (14.2%), palmitic acid (14.1%) and phytol (11.4%). Also, hexane extract of giloy stems revealed methyl-9,12-octadecadienoate (23.2%), methyl 9-octadecenoate (19.7%), methyl hexadecanoate (16.3%), and methyl octadecanoic (5.5%) but Heptacosanol, octacosanol, nonacosan-15-one and cyclohexyl-11-heneicosanone were seen in the whole plant extract.

Polysaccharide – Giloy stems polysaccharide shows glucose 98.0%, arabinose 0.5%, galactose 0.3%, mannose 0.2%, rhamnose 0.2%, and xylose 0.8% units.

Others – Other compounds seen in giloy are giloinsterol, a bitter glucoside giloin, a non-glucoside bitter substance giloinin, gilo-sterol, tinosporan acetate, tinosporic acid, tinosporal acetate, tinosporone, and tinosporal. Bitter compounds found in giloy are tinosporide and cordifolide. There are also 3 furanolactone diterpenoids -C20H20O6, C20H22O8 and C26H34O11.

The giloy leaves are also rich in protein, calcium and phosphorus [40].

Health benefits:

Chemical components and products from giloy have been found to have free radical scavenging properties and decrease the activities of superoxide dismutase and glutathione peroxidase in rats [41].

Anti-inflammatory properties like that of indomethacin and nonsteroidal drugs have been found in giloy. Giloy reduces histamine-induced bronchospasm in animals. 1, 4-alpha-D-glucan derived from giloy activates macrophages, NFκB translocation, and cytokine production, and hence activates the immune system. Giloy favors HIV positive patients. Giloy helps young chicks under infectious bursal disease during conventional antiviral treatment. Vedavanthy et al. [42] demonstrated antipyretic and Gupta et al. [43] observed its antimicrobial effects. Researcher [39] analyzed the parts thus:

Leaves: Powdered leaves preparations help in treating gout, ulcers, jaundice, fever, and wounds, and to control blood glucose.

Stem: The mixture of stem extract alone or with honey is useful in treating jaundice, skin diseases, and fever. The stem starch (Sativa) can also be used as a tonic and as an antidote to snakebite and scorpion sting.

Bark: In India, the root and stem bark of the plant is used along with milk to treat cancer.

Fruits: Are used in the treatment of jaundice and rheumatism.

Roots: Roots are used as an emetic for visceral obstructions, leprosy, diarrhea, and dysentery.

Possible Toxicology:

Giloy has been described as a safe drug to use. There is no available report on its toxicity on humans though regular use of giloy in high doses can cause constipation [39].

Anti-COVID-19 Properties:

Giloy is the source of various types of bioactive compounds, including alkaloids, steroids, glycosides, and aliphatics. Giloy may have inhibitor potential as a drug for SARS-CoV-2 otherwise called COVID-19 with phytochemicals such as berberine, ß-sitosterol, octacosanol, tetrahydropalmatine, and choline with 3CL pro targets I, II of protease enzymes.

3.4 Ginger

Plant name: Ginger - *Zingiber officinale.*
Pictorial exposition (**Figure 5**):
Botanical name: *Zingiber officinale.*
Indigenous (local) name: Jinja.
Taxonomical classification.
Kingdom: Plantae.
Division: Angiosperms.
Class: Monocots.
Order: Zingiberales.
Family: Zingiberaceae.
Genus: Zingiber.
Species: officinale.
Part of plant in Use: Rhizomes.
Phytochemical components:

The phytochemical components reveal: citronellal, linalool, borneol, 10-dehydrogingerdione, 6- et 4,6, 8 ou 10-gingerdione, limonene; [6]-methyl gingediol, le [4]-gingediacetate, le [6]- gingediacetate, and le [6]-methyl-gingediacetate.

Figure 5.
Uprooted ginger plants and rhizomes.

Constituents of essential oils are: curcumene, farrnesene, gingerols, zingiberene, zingerone, car-3-ene, α-terpinene, shogaols, paradols, αterpineol, neurol, 1, 8-cineole, neral, geranial, geraniol et geranyl acetate, isovaleraldehyde, nonanol, ethylpinene, α-Pinene, α-sesquiphellandrene, β-bisabolene myrecene, β-pinene, β sequithujene, sesquiphellandrene, camphene, sabinene, cis-sequisabinene hydrate, zingiberol, gingerone, and citral (geranial et neral).

Macronutrients found are carbohydrates, fiber, and proteins (with amino acids such as cystine, phenylalanine, histidine, isoleucine, methionine, tyrosine, threonine, leucine, lysine, tryptophan, valine), lipids (including omega 3, 6 and 9 fatty acids).

Micronutrients found are sodium, magnesium, phosphorus, potassium, calcium, magnesium, manganese, phosphorus, potassium, sodium, selenium, iron, copper, zinc, selenium, iodine, vitamins A (thiamine), B1 (thiamine), B2 (Riboflavin), B3 or PP or niacin, B5, B6 (pyridoxine), B9 (folic acid), C, D, E, K1, and K2.

Other Compounds present are: Flavonoids (Flavan-3-ol, flavone, flavonol, flavanone, tannins, quercetin, rutin, fisetin, morine, gallic acid, ferulic acid, vanillic acid, hexahydrocurcumin and desmethyl-hexahydro curcumin, 3S,5S)-3,5-diacetoxy-1,7-bis(4-hydroxy-3-methoxyphenyl), allicin, alliin, ajoene, galanolactone, gingerenones, and gingediones [44].

Health benefits:

Considering many bioactive ingredients like gingerol, zingiberine, shogaol, gingerdione, hexahydrocurcumin, paradol and gingerenone A, ginger has antioxidants that help reduce oxidative stress and inhibit superoxide production.

Dried ginger possesses potent anti-inflammatory and analgesic activities.

Ginger has a better effect against swine flu (H1N1), and human respiratory syncytial virus in human respiratory tract cell lines.

There are antiretroviral sesquiterpenes in ginger.

Ginger provides a bronchodilatory effect, prevents severe damage to the lungs due to inflammation, and ameliorates allergic asthma.

Gingerol can be used to prevent and treat cancer and chronic inflammatory diseases. It has an antiparasitic effect, especially against *Ichthyophthirius multifiliis* (ciliate parasite of freshwater fish). The bioactive bitter and pungent component of ginger and its derivatives, reduced heavy menstrual bleeding in women of reproductive age; and protected rat fetuses against Gabapentin-induced hepatotoxicity. Ginger improved concentration of blood lipid and reduced body overweight, obese, and have an anti-diabetic effect [45].

Possible Toxicology:

In rats, 2500 mg/kg gave a toxic effect leading to severe hypotension and bradycardia with the induction of prenecrotic changes in cardiac tissues, but 50 mg/kg given for 28 days gave bradycardia to the rats with waviness in cardiac muscle fibers [46]. Idang et al. [47] describe the effect of ginger on some organ's histology and biochemical parameters as reversal when the administration is discontinued in rat models.

Anti-COVID-19 Properties:

The anti-inflammatory, analgesic, and antiviral activities of ginger provide the positive property that COVID-19 can be managed by ginger.

3.5 Lime

Plant name: Key Lime - *Citrus aurantifolia.*
Pictorial exposition (**Figure 6**):
Botanical name: *Citrus aurantifolia.*
Indigenous (local) name: Oroma nkirisi.
Taxonomical classification:
Kingdom: Plantae.
Division: Streptophyta.
Class: Magnoliopsida.
Order: Sapindales.
Family: Rutaceae.
Genus: Citrus.
Species: aurantifolia.
Part of the plant in Use: Fruits.
Phytochemical components:

The aqueous extracts of lime pulp revealed the presence of carbohydrates, reducing sugars, proteins, alkaloids, tannins, fixed oils, cardiac glycosides, phytosterols, phenols, steroids, and flavonoids [48].

The ethanolic pulp extracts showed only the presence of fixed oils, reducing sugars, cardiac glycosides, steroids, phytosterols, flavonoids, and amino acids.

The aqueous peel extracts showed the presence of carbohydrates, alkaloids, tannins, fixed oils, proteins, cardiac glycosides, steroids, phenols and flavonoids, and amino acids [49].

Health benefits:

It is known that due to the presence of various phytochemicals such as alkaloids, flavonoids, glycosides, saponins, steroids, anthraquinones, phenols, resins, fatty acids, and gums present in the plant extracts are responsible for the antibacterial

Figure 6.
Lime fruits on the plant and plucked fruits.

properties. The total phenolic content values have evidence of antimicrobial activity, just like the presence of steroids, flavonoids, alkaloids, tannic acid, and phenolics against both gram-positive and gram-negative bacteria [50].

Lime is popular as antioxidant activity, immunomodulation, and antibacterial activities [48].

Possible Toxicology:

It may cause burns in the mouth, throat, and stomach. It may not be perfect for chronic ulcer patients. However, it is generally less toxic [51].

Anti-COVID-19 Properties:

There is evidence that suggests that lemon essential oils have shown potent antiviral activity to other coronaviruses, such as SARS-CoV-1, and could also be the same in respect of COVID-19 by inhibition of viral infection and replication [52].

3.6 Turmeric

Plant name: Turmeric - *Curcuma longa*.
Pictorial exposition (**Figure 7**):
Botanical name: *Curcuma longa*.
Indigenous (local) name: Tumerik.
Taxonomical classification.
Kingdom: Plantae.
Division: Angiosperms.
Class: Monocots.
Order: Zingiberales.
Family: Zingiberaceae.
Genus: Curcuma.
Species: longa.
Part of the plant in Use: Rhizomes.
Phytochemical components:

The components reveal α- and β-turmerones, ar-turmerone, atlantone, cineole, d-phallandrene, α-phellandrene, curlone, zingiberene, ar-curcumene, d-sabinene, borneol, terpinolene, 1, 8-cineole, undecanol, and p-cymene [53].

Micronutrients contents are Calcium, Phosphorus, Zinc, Magnesium, Manganese, Copper, Iron, Potassium, Vitamins A, B1 (Thiamine), B2 (Riboflavin), B3 (Niacin), B5, B6, B9, Folate, C (Ascorbic Acid), E, K,

Figure 7.
Turmeric plants and rhizomes in plate.

Macronutrients found are: carbohydrates, fiber, lipids (omega 3, omega 6, and omega 9), and proteins.

Other Compounds present includes alkaloids, anthraquinones, curcumin, cyclocurcumin, cardiac glycosides, demethoxycurcumin, bis-demethoxycurcumin, tannins, terpenes, steroids, saponins, anthocyans, leucoanthocyans, saponins, quinones, and flavonoids [54].

Health benefits:

Turmeric has tremendous medicinal benefits ranging from been used as phlegmagogue, anti-inflammatory, analgesic, antipyretic, blood purifier, to healing properties.

Turmeric is antioxidant, immunomodulating, anti-inflammatory, antimicrobial, and has anticancer activities [55].

The important ingredients of *Curcuma longa* are curcumin, dihydro curcumin, and hexahydrocurcumin. Some volatile compounds like cinol, α-phellandrene, borneol, zingiberine, and different sesquiterpenes have been found in turmeric. Curcumin has been observed to be extremely effective in acute respiratory distress syndrome, COPD's, acute lung injury, and pulmonary fibrosis.

Turmeric suppresses NF-α and inhibits NF-κB, in this way acts as a potent anti-inflammatory agent. Curcuma extract acts against various pathogenic bacteria, including *Streptococcus, Staphylococcus, Klebsiella pneumonia, Helicobacter pylori, Bacillus subtilis*, and *Vibrio cholera* [55].

The revolutionary potential of turmeric is against viruses like H1N1, H6N1, respiratory syncytial virus, herpes simplex virus, parainfluenza virus type-3, coxsackievirus B3, Japanese encephalitis, hepatitis B virus, hepatitis C virus, human papillomavirus-16, and – 18. It has also been found to inhibit HIV-1 long terminal repeat directed gene expression [55].

Possible Toxicology:

Balaji and Chempakam [56] posit that curcumin and its derivatives may cause dose-dependent hepatotoxicity. They equally put that, in contrast to curcumin, other compounds in turmeric which are non-mutagenic, non-carcinogenic, non-hepatotoxic, and do not have any side-effects.

National Toxicology Program (USA) the toxic and carcinogenic properties of an organic extract of turmeric, called turmeric oleoresin from animal models after a very long time feeding. For example, rats and mice were fed diets containing several concentrations of turmeric oleoresin for three months and two years, and the possible toxic and carcinogenic effects were evaluated. In the 2-year feeding studies, turmeric oleoresin ingestion was associated with increased incidences of ulcers, hyperplasia, and inflammation of the forestomach, cecum, and colon in male rats and of the cecum in female rats. In female mice, the ingestion of diets containing turmeric oleoresin was associated with an increased incidence of thyroid gland follicular cell hyperplasia. These negative effects of curcumin were said to be mediated by several possible mechanisms showing reactive oxygen species (ROS) such as superoxide anion and hydrogen peroxide-based on the facts that:

i. ROS can induce cell malignant transformation,

ii. Cancer cells commonly have increased levels of ROS,

iii. The malignant phenotype of cancer cells can be reversed by reducing the cellular levels of ROS.

Experimental studies have demonstrated that, although low concentrations of curcumin induce antioxidant effects, while higher concentrations of this compound increase the cellular levels of ROS [57].

However, Aggarwal et al. [55] gave a counter opinion on turmeric when they worked on Curcuminoid-essential oil complex (CEC) and found non-mutagenic effects in all three mutagenic investigations studied. Therefore, following investigations of acute toxicity, repeated dose toxicity, and mutagenicity, CEC was deemed a safe, non-toxic pharmacological formulation.

Anti-COVID-19 Properties:

Soheil et al. [53] compiled other studies on the role of turmeric on some viral infections, thus:

For human immunodeficiency virus (HIV), the presence of curcumin; caused inhibition of HIV-1 LTR-directed gene expression, inhibited Tat-mediated transactivation of HIV-1 LTR and Tat protein acetylation, inhibited HIV-1 integrase while curcumin boron complexes inhibited HIV-1 and HIV-2 proteases though reported no antiviral effect in a clinical trial for HIV.

For Influenza, Curcumin inhibited hemagglutination.

For herpes simplex virus-1 (HSV-1), Curcumin, gallium-curcumin, Cu-curcumin reduced to HSV-1 replication.

For herpes simplex virus-2 (HSV-2), Curcumin gave significant protection in mouse models.

For Coxsackievirus, Curcumin caused replication inhibition through UPS dysregulation.

For hepatitis B virus (HBV), the aqueous extract suppressed HBV replication by increasing the p53 level.

For hepatitis C virus (HCV), Curcumin decreased HCV replication by suppressing the Akt-SREBP-1 pathway.

For human papillomavirus (HPV), Curcumin inhibits expression of viral oncoproteins of E6 and E7 and downregulation effect on the transcription of HPV-18.

For Japanese encephalitis virus (JEV), Curcumin reduced the production of infective viral particles.

For human T-lymphotropic virus-1 or human T-cell leukemia-lymphoma virus-1 (HTLV-1), Curcumin causes downregulation of JunD protein in HTLV-1-infected T-cell lines.

Therefore, if all these viruses can be managed by turmeric, COVID-19 management is possible with this indigenous plant through inhibition, reduced replication, and dysregulation.

4. Possible mechanisms of action of the Igbo indigenous plants against COVID-19

The SARS-CoV-2, otherwise called COVID-19, possess some protein structures by which it enters into the host cells, which is called spike (S) glycoprotein, and forms homotrimers protruding from the viral surfaces. The spike protein interacts strongly with the human ACE2 (angiotensin-converting enzyme 2) receptor [58]. Such interaction is followed by replication through some cyclic processes and translating its genomic RNA (gRNA). There is proteolysis in the presence of viral 3C-like proteinase, with more replication of gRNA. There is viral replication complex formed which consists of RNA dependent RNA polymerase (RdRp), helicase, 30-to-50 exonuclease, endoRNAse, and 20-O-ribose methyltransferase and followed by the assembly of viral components [59, 60].

The proteins (S) which are associated with replication, are the primary targets of post-entry treatment and these Igbo indigenous plants have viral proteinase targeting based on literature in this chapter on the anti-coronavirus properties thereby giving a hint on the inhibition of the viral replication process.

Though there is no empirical evidence in this chapter, the authors suggest that Bitter kola, Garlic, Giloy, Ginger, Lime, and Turmeric has the properties that alter the PH on the interface between the virus and the human respiratory surfaces causing a brake on the interaction with human ACE2 and where interaction has taken place, the replication and translation stages are disrupted. The plants, thus, are potential modifiers of this milieu and inhibitor of the main protease and endoribonuclease via epigenetics and homeostasis.

5. Preparations from Igbo indigenous plants towards COVID-19 eradication

The world awaits the declaration of a possible medicinal cure for COVID-19. WHO has confirmed no official treatment regimen for the COVID-19 cure [61]. The global community is searching from post to post for useful medicine in the management or treatment of COVID-19 and the related symptoms. The search could not neglect medicinal plants and herbs which has been used for the treatment of related or difficult to cure infections; the herbal remedy [62] as opined by Omer and lauds Tadepalli who tags alkaloids preparations of Indian herbal medicine as novel remedial approaches [32]. Jahan and Onay demonstrated the antiviral potentials medicinal plants that inhibit human coronaviruses [63] just as Mirzaie *et al*. [64] described medicinal plants as options for treatment of Coronavirus.

The preparations of the Igbo indigenous plants towards COVID-19 management and treatment may not be different from the way they are prepared for other ailments. Some studies mostly mention ginger, garlic, and lime as having antiviral properties and immune system boosting capacities [62–70]. Some prefer to describe them as part of nutrition that aid immunity towards the management of coronaviruses [71, 72].

Figure 8.
Water-based preparation containing ginger, turmeric, garlic, bitter kola, lime, and cinnamon.

Figure 9.
Alcohol-based preparation containing ginger, turmeric, bitter kola, lime, and garlic.

There are various methods of preparations available in Igbo land towards the use of these Vitamin C enriched plants [29] in the management of COVID-19 depending on the person that is making the preparation or the status of the subject who shall be taking the preparation. There are two major methods- The water-based method (**Figure 8**) or the Alcohol-based method (**Figure 9**) and the measurement of consumption. For those who are not used to alcoholic products, the Water-based one is preferred.

The preparations could also be made using a single plant or combination depending on the availability or the need. The preparations may be using whole plant parts or a particular part. Take, for instance, Giloy can be prepared using leaves, stem, roots at the same time, or just by using the ground dry leaves.

This chapter may not be enough to explain the ways of preparations available in Igbo land in the Eastern part of Nigeria but may convince someone of some selected indigenous plants capable of managing COVID-19. Though there is an exposition on this plant here, be it conventional or herbal medicine, self-medication is dangerous, and WHO has also not confirmed an acceptable treatment of COVID-19.

6. Conclusions

There are numerous indigenous plants in Igbo land, South Eastern Nigeria that is capable of managing coronavirus called COVID-19. Such plants include but not limited to Bitter kola (Aku ilu), Garlic (Yabasi Hausa), Giloy (Udo akpu enyi), Ginger (Jinja), Lime (Oroma nkirisi), and Turmeric (Tumerik).

The preparations for those plants differ depending on the Herbalist or the patient (subject) who needs the products, and there is always an important need to consult your Indigenous Herbal Doctor for advice and preparation.

The side effects and toxicity of plants under review in this chapter are at very high doses, especially in animal models, and mostly go away after withdrawal. It should be noted that every drug has side effects, especially when abused. These effects by natural plants and products can also be compared with synthetic and conventional drugs.

Bitter kola, Ginger, Garlic, Giloy, Turmeric, and Lime could be considered for use in the management and treatment of COVID-19 symptoms starting from Igbo land in South-Eastern Nigeria to across the globe towards ensuring that Coronavirus is eliminated before the targeted two years.

While encouraging the use of Igbo indigenous plants as a single or in a combination of at least four of them depending on the availability as they have anti-COVID-19 activities, which causes inhibition on the virus proteases within the milieu and via epigenetics and homeostasis, though there is no confirmed cure for the pandemic.

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Conflict of interest

The authors declare no competing interests.

Notes/Thanks/Other declarations

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Section 4

Nanomedicine

Chapter 4

Nanomedicines: Nano based Drug Delivery Systems Challenges and Opportunities

Rabia Hamid and Ifrah Manzoor

Abstract

Nanomedicine and nano delivery systems, although relatively recent but fast-developing technology is one where nanoscale materials are used to function as diagnostic tools or to deliver therapeutic agents to specifically targeted sites in a controlled manner. It also provides many advantages in the management of human diseases. Recently, there has been a range of excellent uses of nanomedicine as chemotherapeutic agents, biological agents, immunotherapeutic agents, etc., for treatment of different diseases. In this chapter we discuss the recent developments and insights obtained in the field of nanomedicine. It provides a review of the numerous nano-based drug delivery systems that enhance the efficacy of new and old drugs. The new opportunities and challenges arising in the area of nanomedicine from therapeutic viewpoint are also addressed.

Keywords: nanomedicines, nanoparticles, drug delivery systems, drug targeting, natural products added

1. Introduction

Human beings have been widely utilizing plant-based natural products as medicines against various diseases since ancient times. Many medicines are derived primarily from herbs, based on traditional knowledge and practices. Currently about 25% of the available therapeutic compounds and their derivatives are derived from natural resources [1, 2]. Natural compounds have impressive characteristics, such as exceptional chemical versatility, chemical and biological properties of macromolecular specificities and less toxicity. These thus constitute them as leads in the discovery of novel drugs [3]. In spite of several advantages, pharmaceutical companies are hesitant to commit to more in drug discovery and drug delivery systems based on natural compounds due to concerns associated with biocompatibility, toxicity, large size and targeted delivery, etc., and many natural compounds not even clearing the clinical trial phases [4, 5]. Hence, this presents a greater challenge of using them as medicine. Thus alternatively available libraries of chemical compounds are being explored to discover novel medicines. Various techniques like nanotechnology play substantial role in advancing drug formulations, targeting, efficient release and delivery with immense success. Nanotechnology bridges the barrier between physical and biological sciences by providing nanostructures with potential to fill the lacunae existing in various fields of sciences and in particular in the field of medicine.

The use of nanotechnology in the production of efficient medicines has been recognized as a key enabling technology, capable of delivering fresh and creative therapeutic approaches to address unmet medical demands [6]. The use of nanotechnology for medical purposes is referred to as nanomedicine [7] and nanomaterials are used for prevention, early diagnosis or treatment of a wide range of diseases with high specificity, efficacy, and personalization, to improve quality of life of patients. Owing to their small scale, nanomaterials have novel physicochemical properties, distinct from those of their traditional bulk chemical counterparts. Such properties significantly improve a range of opportunities in drug development. These physicochemical properties of nanoformulations can lead to pharmacokinetics/pharmacodynamics being changed, namely the delivery, absorption, removal and metabolism, the potential for more easily breaching biological barriers and their persistence in the environment and the human body.

The key component of nanomedicines are nanoparticles (NPs) and currently wide range of nanoparticle types exist depending on their structural features such as spheres [8], rods [9], wires [10], stars [11], sheets [12], multipodes [13], cages [14], etc. These particles can efficiently carry and deliver therapeutic agents as well as imaging and sensing agents to targeted sites. Nanoparticle carriers or nanocarriers have many advantages in medicine. First, they allow stable aqueous dispersions of active but poorly water-soluble therapeutic agents for delivery into the biological environment. Second, their structure, scale, shape and surface properties can be finely designed to protect the encapsulated agent when incorporated into the biological world and prevent it from degradation by various endogenous defense mechanisms including, immunodegradation, enzymatic degradation, reticuloendothelial system sequestration (RES) in the bloodstream, acid hydrolysis, lung mucociliary clearance, etc.

2. Delivery system of nanoformulations

Delivery of nanomedicines can be by intracellular transport, epileptic transport and other types. Intercellular transport is facilitated and regulated through intracellularization, transporter mediated endocytosis, and permeation by interactions through particle size and/or cell surface [15, 16]. In addition, a smaller nanomedicine particle size improves intercellular transport which facilitates cell permeation and affects nanomedicine absorption, dissemination, and excretion. In fact, cell internalization by transporter-mediated endocytosis depends on the size of the nanomedicine molecule. Similarly in large particle sized nanomedicine, opsonization occurs quickly and its removal from the blood is facilitated by endothelial macrophages. The susceptibility of nanomedicinal cell surface transporters to nanomedicinal products has been reported to vary depending on the particle size of nanomedicinal products, and this can also impact the effective removal by macrophages of large particles from the blood. Nanomedicines composed of non-charged polymers, surfactants, or polymer coatings that degrade *in vivo*, associate with cell surface receptors or ligands because of their hydrophilicity to increase permeability or promote internalization of nanomedicines. In addition, through interacting with bioadhesive polymers or chelates, nanomedicines improve the intracellular transport of active pharmaceutical ingredients. Improved intracellular movement of active pharmaceutical ingredients coupled with various proteins, antibodies and other *in vivo* polymers is due to the opening of tight junctions and/or improved membrane permeability. In particular, the incorporation of anti-cancer agents with such a role would increase the effectiveness of chemotherapy, including the treatment of brain tumors that are immune to drugs associated with close junctions, the targeting of tumor cells and the routine targeting of cells. Using such a strategy

for nanomedicines, cytotoxicity against normal cells can be minimized and greater anti-cancer efficacy will be achieved. Decrease in intake of nanomedicines in the lungs through inhalation results in an improvement attributable to decreased deterioration and absorption by lung mucosa or macrophages, resulting in improved product processing period and product transfer to goal. The enhanced permeability and retention (EPR) effect improves anti-cancer efficacy by enhancing tumor permeation and retention time. The effect of the EPR also makes it possible to directly transmit nanomedicines to target tissue by combining an antigen, enzyme, peptide, or polysaccharide that can be used to modify the delivery of nanomedicines to target tissues via receptor/ligand interactions or other physiologically sensitive cell regulation interactions, drug efficacy modification or adverse reactions. There is improved longevity of hydrophilic-coated nanomedicines, preventing their opsonization or accumulation in the mucus. Nanomedicines can be retained *in vivo*, e.g. in the lung tissue for extended periods of time by particle size, by inhibiting macrophage-induced or mucosal disturbance and escape elimination by mucus ciliates, which may lead to deterioration or macroscopic consequences of lung mucosa [17]. Thus, a number of formulations have been designed that use delivery pathways that can regulate the pharmacokinetics and pharmacodynamics of nanomedicines.

3. Nanomaterial based delivery system

Nanotechnology in drug delivery has the potential to overturn the treatment of various diseases such as cancer, diabetes, neurodegenerative diseases, vascular diseases, etc. [18]. In the market for sale, nanotechnology based formulations are largely parenteral, with some intended for oral administration [19]. It is hoped that a significant number of preclinical and clinical trials would lead to the production of novel nanotherapeutics intended for non-parenteral delivery routes, such as pulmonary, nasal, vaginal, ocular, and dermal delivery routes. Of special concern to drug delivery systems (European Commission/ETP) [20] is the option of delivery and the obstacles to be addressed. Over time, various formulations based on nanoparticles have been developed to enhance the delivery mechanism of drugs, such as discussed below:

3.1 Polymeric nanoparticles

The most widely used chemical nanoparticles are constructed from synthetic polymers as natural polymers result in low reproducibility and controlled release actions for the trapped products, leading to variability in purity and batch-to-batch quality. At the other side, synthetic polymers with good to batch reproducibility and purity are available which facilitates the modification of the pattern of drug release from polymeric nanoparticles [21]. Nanoparticles formulated with synthetic polymers have been widely studied for drug distribution/delivery. In double emulsion methods hydrophilic moieties will encapsulate onto synthetic polymer-based nanoparticles, as it is not easy to maintain activity in unfavorable environment. Various synthetic polymers reported for drug delivery with biodegradable aliphatic polymers such as polylactide (PLA), poly lactide-co-glycolide, copolymers (PLGA) and poly (ε-carpolactone), as well as non-biodegradable polymers like polyacrylates and poly (methyl methacrylate) are used widely [22]. Polymer nanoparticles can efficiently shield unstable drugs from deterioration/degradation, thus avoiding the side effects of toxic medications. Natural polymeric nanoparticles consist of polymers of natural products like alginate, chitosan, albumin and gelatin [22]. Application of polymeric nanoparticles with therapeutic drugs such as dexamethasone or alpha-tocopheryl succinate can be used to avoid the cisplatin ototoxicity

due to treatment with chemotherapy. Nanoparticles, trapping, transporting and ultimately spreading dexamethasone or alpha-tocopheryl succinate are capable of partially preventing large-dose ototoxicity of CDDP [23]. However, when administered systemically for long periods of time, these least soluble drugs have serious side effects. In the hydrophobic cavity of nanoparticles, the integration of such pharmaceutical products provides the requisite results *in vitro* and *in vivo.* Few popularly marketed formulations of the polymeric nanoparticles are Decapeptyl®, Gonapeptyl Depot®, Enantone Depot®, and Abraxane [24, 25].

3.2 Lipid nanoparticles

Lipid nanoparticles that are prepared with a solid matrix are called solid lipid nanoparticles (SLNs). These are constructed from nanoemulsions of oil in water with the utilization of a solid lipid. The first generations of SLNs were formed in the early 1990's [26]. The benefits associated with SLNs include cheap raw materials, usage of physiological lipids, avoidance of organic solvents, ease of scale-up, strong biocompatibility, enhancement of bioavailability, safety of vulnerable molds from environmental hazards and regulated drug release [27]. Using ultrasonic melt emulsification [28], ciprofloxacin (CIP)-loaded SLNs have recently been formulated with powerful antibacterial action. These were produced with a scale ranging from 165 to 320 nm and a polydispersity index with high trapping efficiency falling between 0.18 and 0.33. A controlled-release pattern of different lipids was shown by CIP release showing the full burst reaction, which contributes to the drug's rapid release. For 120 days this composition of CIPSTE was found to be stable at room temperature. SLNs for different routes of delivery, such as oral [29], dermal [30], pulmonary [31], ocular [32] and rectal [33], have been extensively tested *in vitro* and *in vivo*. Nano base and nano pearl are marketable SLN formulations [34].

3.3 Dendrimers

Dendrimers are special three-dimensional, hyper-branched, globular nanopolymeric structures. Attractive features such as water solubility, nano scaled size, narrow polydispersity index, modifiable molecular structure, internal cavity and several peripheral functional groups separate these from other nano systems. Terminal functionality serves as a platform for the conjugation and targeting of drugs. Such peripheral functional groups also provide them with tailor-made properties which improve their versatility [35]. The most commonly studied dendrimer for drug delivery is polyamidoamine. It's synthesis starts with the amine group, which interacts with methyl acrylate and contributes to the formation of two new branches of dendrimer terminated by ester. The amine-terminated dendrimer 'Full-generation' may be formed by subsequent amidation of the methyl ester with ethylene diamine. PAMAM dendrimers are non-immunogenic, biocompatible and water-soluble, and have functional terminal amine groups that can be altered to targeting drugs [35]. Dendrimers have been widely investigated for biodelivery via transdermal, nasal, ocular, and pulmonary pathways, in addition to improving solubility. Many of the synthetic cationic polymers such as amidised acid-labile allow different cargo delivery [36]. Changing their structure could solve toxicity-related problems [35]. A recent study showed that arginine terminated peptide dendrimers, along with sonophoresis, can significantly increase ketoprofen's transdermal penetration [37]. The findings revealed that the use of peptide dendrimer and application of ultrasound has worked synergistically. *In vitro* experiments have found that dendrimer and ultrasound-mediated drug permeation contributes to higher active drug plasma concentration as opposed to passive diffusion. Transdermal

administration of ketoprofen with A8 dendrimer demonstrated similar drug absorption and oral path plasma concentration [37]. Commercially available dendrimers of poly-propylenemine (PPI, AstromolR, DAB) [38] and polyamidoamine (PAMAM; Starburstk) have been the most usually explored for pharmacological use [38, 39].

3.4 Nanoemulsion

Nanoemulsions are a fascinating colloidal drug delivery mechanism, thermodynamically stable and filtration-sterilizable [40, 41]. There are heterogeneous mixtures of oil droplets in aqueous media resulting in nano droplets with a small scale distribution. The resultant nanoemulsions are analyzed as translucent or clear, isotropic and supported by the suitable surfactant [42]. Three types of nanoemulsions can be developed:

a. water in oil nanoemulsion

b. oil in water nanoemulsion

c. bi-continuous nanoemulsion

The most detailed function of nanoemulsions is to mask the unpleasant taste of oily liquids. These also provide long-term drug action and prevention from hydrolysis and oxidation. These nanoformulations can therefore be identified as an efficient and impregnable delivery option with high bioavailability. Nanoemulsions are currently being explored extensively to target different photosensitizers, anticancer drugs, or therapeutic agents. Such nanoformulations propose a number of applications such as drug delivery, biologic diagnostics and chemical agents [43]. In 2016, Simion et al. developed targeted dexamethasone-loaded P-selectin lipid nanoemulsions to minimize vascular inflammation [44]. Prepared formulations have been described for physicochemical assays. In their study, nanoformulation was found to be efficient in both *in vitro* and *in vivo* experiments. It reduces the function of the endothelium activation selectively and thereby the inflitration of monocytes, resulting in a substantial reduction in inflammation of the lungs in a model animal mouse. Examples of nano-emulsion formulations are Norvir (Ritonavir), Restasis, Gengraf (Cyclosporin A), Etomidat-Lipuro (Etomidate), Ropion (Flurbiprofenaxtil), Diprivan, Troypofol (Propofol), Limethason (Dexamethasone) and Liple (Alprostadil palmitate) [45].

3.5 Nonstructured lipid carriers (NLC)

Nonstructured lipid carriers comprise the nanosystems of the second generation, consisting of solid lipid embedded into liquid lipids [46]. These nano carriers allow for a strong immobilization of therapeutic agents and avoid particle coalition of particles relative to emulsions [47, 48]. Therefore, because of the liquid oil droplets in a solid matrix, their drug loading potential is increased relative to SLNs. Biodegradability, lower toxicity, controlled release, drug tolerance and avoidance of organic solvents during manufacturing are among the beneficial effects of NLC on polymeric nanoparticles. NLCs have been extensively studied for hydrophobic and hydrophilic drug transport in recent years. The NLCs are developed to satisfy industrial specifications related to certification and registration, basic infrastructure, scale-up and low cost criteria [49]. The presence of multiple consumer goods reflects the carrier's success story. Numerous other NLC products, including NLC repair cream and NLC restoration cream, are commercially available. For the treatment of different diseases, NLCs

were explored through various routes of administration viz. oral, nasal, and parenteral [50]. Fluconazole-loaded NLCs were constructed using probe ultrasonication method and studied for antifungal activity on various *Candida species*. A substantial decrease in maximum inhibitory concentration (MIC) for all classes of *Candida* was observed using fluconazole NLCs. It is also mentioned that *Candida albicans* is more susceptible to fluconazole loaded NLCs than *Candida Parapsylosis, Candida glabrata* [51].

3.6 Nanogel

Nanogels, comprised of flexible hydrophilic polymers, can be prepared as plain gels [52]. Upon swelling, the drug can be randomly inserted into the nanogel. As a result, the gel collapses, resulting in the creation of solid, compact nanoparticles with reduced solvent amount. Nanogels provide novel applications for polymer-based drug carrier systems due to their biocompatibility, high moisture content and suitable mechanical properties. These gels have expanded polyvalent bioconjugation surface area and an internal network for biomolecule trapping. Physical encapsulation of bioactive compounds in the polymeric interlock along with their releasing pattern has been widely explored as a targeted mode of drug delivery [53]. Several approaches for the preparation of nanogels include micro-molding and photolithographic methods, continuous micro fluidics, modification of biopolymers, and heterogeneous living/controlled radical and free radical polymerizations [54]. Several criteria are required for designing and manufacturing of an efficient nanogel drug carrier system for therapeutic application. The consistency of nanogels for long-lasting blood circulation is one significant criterion. Another extraordinary novel feature that can detect receptors on infected cells is the bioconjugation of nanogel surfaces with particular ligands. Eventually, the biodegradability of nanogels should not only control the release of the drug for the required amount of time, but also make it possible to eliminate the empty system after the release of the drug [54]. In a recent study, topical delivery of chitin nanogel loaded with clobetasol is reported. This nanogel demonstrated exceptional toxicity against THP-1 and HaCaT cell lines by MTT assay. Nanoformulation demonstrated significant anti-inflammatory ability with an average inhibition of LOX and COX activities in THP-1 cells of 70 percent and 65 percent. Increased transdermal flux has been obtained from permeation studies of *in vitro* skin. Antipsoriatic activity conducted *in vivo* on imiquimod model demonstrated the value of nanogel for the topical application of clobetasol for psoriasis. Some selected and marketed nanogels are Sane Care Nanogel, Zyflex Nanogel, Augen Nanogel Eye-care Gel, Skin Beautiful Brightening Nanogel [55], and Oxalgin.

3.7 Nanocapsule

Nanocapsule consists of either liquid or solid core in which drug is loaded and encapsulated by membrane of synthetic or natural polymers [56, 57, 58]. Lipid core nanocapsules are prepared by the precipitation method. Prepared nanoparticles have been tested for physical, chemical and biological characteristics. The most important characteristics to note during their synthesis are particle size and distribution. This can be calculated through multi-angle laser light scattering in a superconducting quantum interference instrument through X-ray diffraction, X-ray photoelectron spectroscopy, Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) [57]. Chemically stable, biocompatible and readily reproducible are industrial bioactive nanocapsules. Because of their coating, which protects the encapsulated material from unenviable effects, such as dissolving the liquid and avoiding the release of active components, they have captured the attention of research groups. In biomedical research, agrochemicals, sanitizing materials, cosmetics and water treatment, nanocapsules have

a wide range of biomedical applications. In addition, the effectiveness of such medications has also been studied for cancer treatment [59], radiotherapy [60], self-healing, contagion [78] and for use in food and agriculture. New developed nanocapsules will open new avenues of research and development for the delivery of bioactive compounds to target tissues in the future [57, 58]. Due to their ability to destroy colon cancer cells, resveratrol-charged lipid-core-nanocapsules (RSV-LNC) were developed and characterized. Constant and controlled drug release has been confirmed by the RSV-LNC. Increased anticancer activity in HT29 cancer cells compared to free RSV resulted in RSV incorporated in the nanocapsule. RSV-loaded nanocapsules have a promising potential for enhancing therapeutic effectiveness in colon cancer cells based on *in vitro* evaluation. In order to authenticate the improved behavior of RSV nanoformulations, more experiments on animal models are nevertheless proposed. SOLUDOTS-PTX (Lipid Nanocapsules of Paclitaxel) is currently in clinical trials.

3.8 Nanosponges

Nanosponges have drawn the interest of drug delivery scientists in pharmaceutical science as they have the capacity to load both hydrophilic and lipophilic moieties [61, 62]. These are thin, non-toxic, porous colloidal structures of scaffolds that have multiple cavities where drug molecules can be stuck. In the processing of these nanocarriers, α-cyclodextrins are the most commonly used. It is possible to investigate different crosslinkers in their development, such as hexamethylene di-isocyanate, carbonyl di-imidazole, pyromellitic dianhydride, diphenyl carbonate, etc. In water as well as in organic solvents, these structures are insoluble [63], self-sterile [64, 65] and stable up to 300° C and pH range of 2–11. Using ultrasound-assisted synthesis techniques, Trotta and colleagues produced cyclodextrin nanosponges [86] and examined them for anti-tumor drugs [66]. Efavirenz is a class II drug, a non-nucleoside reverse transcriptase inhibitor widely used for HIV [67]. This medicine, however, exhibits less solubility and reduced bioavailability. Beta-cyclodextrin cross linking with carbonates in variable ratios was performed to increase the solubility and dissolution of this compound. Some of the advertised formulations of nanosponge are Glymasason, Prostavastin, Brexin and Mena-gargle [68, 69].

3.9 Inorganic nanoparticles

Silver, gold, iron oxide and silica are included in inorganic nanoparticles. Nevertheless, only a few nanoparticles have been approved for clinical use, while most of them are still in the clinical trial stage. Metal nanoparticles, silver and gold, have different properties such as SPR (surface plasmon resonance) that liposomes, dendrimers, micelles do not exhibit. They show a variety of benefits when it comes to surface durability, such as decent biocompatibility and flexibility. Studies of their delivery-based actions have not been able to establish whether their toxicity is based to the particulate or ionized form; and while two mechanisms, such as paracellular transport and transcytosis, have been suggested, there is inadequate evidence on their *in vivo* transmission and uptake mechanisms [70]. Drugs can be conjugated by ionic or covalent bonding and physical absorption to gold nanoparticles (AuNPs) surfaces and can be transmitted and regulated by biological stimulation or light activation [71]. Silver nanoparticles display antimicrobial activity however, as far as drug distribution is concerned, very few experiments have been performed, e.g. Prusty and Swain [72] synthesized a spongy polyacrylamide/dextran nano-hydrogel hybrid structures with covalently attached silver nanoparticles for ornidazole production, resulting in an *in vitro* release of 98.5 percent [72]. Likewise, in another study, iron oxide nanoparticles were synthesized using a laser pyrolysis process and

protected by Violamycin B1 and antracycline antibiotics and tested against MCF-7 cells for their cytotoxicity and anti-proliferation properties, compared with commercially available iron oxide nanoparticle and showing promising results [73].

3.10 Quantum dots

Quantum dots (QDs) are regarded as semiconductor nanocrystals with a diameter ranging from 2 to 10 nm with their optical characteristics, such as absorbance and photoluminescence being size-dependent [74]. QDs have received significant interest in the field of nanomedicine, because, unlike traditional organic dyes, QDs pose emissions in the near-infrared region (< 650 nm), a very advantageous phenomenon in the field of biomedical imaging, due to low tissue absorption and decreased light dispersion [75]. Furthermore, the same light source can excite QDs with different sizes and/or compositions resulting in separate emission colors over a wide spectral range [76, 77]. In this way, QDs are quite attractive to multiplex imagery. QDs have been extensively studied in the field of medicine as targeted delivery of drugs, sensoring and imaging agents. A large number of studies on the use of QDs as contrast agents for *in vivo* imaging are currently available in the literature [78, 79]. Han et al. [80] have produced a novel fluorophore for intravital cytometric imaging based on QD conjugate antibodies coated with norborne-displaying polyimidazole ligands. This fluorophore has been used for the *in vivo* marking of bone marrow cells. The investigators found that fluorophore has been able to diffuse across the bone marrow and mark rare cell types, such as hematopoietic stem and progenitor cells [80]. Shi et al. (2015) [79] have produced a multifunctional biocompatible graphene oxide quantum dot protected by a magnetic nanoplatform for the detection/diagnosis of specific tumor cells of liver cancer (glypican-3-expressing hep G2). According to the scientists, the adhesion of an anti-GPC3 antibody to the nanoplatform resulted in a systematic isolation of hepG2 hepatocellular carcinoma cells from blood samples [79]. The continuous and/or controlled release of therapeutic agents can also have benefits from QDs. This behavior can be achieved by active stimuli by light, wind, radio frequency or magnetic fields [81, 82] as far as controlled release is concerned. Olerile et al. [83] have developed a theranostic framework as a multi-functional parenteral system that focuses on the co-loading of QDs and anti-cancer drugs in nanostructured lipid carriers. The nanoparticles were spherical with a higher paclitaxel encapsulation potential (80.7 ± 2.11 percent) and a 77.85 percent tumor growth inhibition score. The authors observed that the device was able to monitor and identify H22 tumor cells precisely [83]. Cai et al. [84] have produced pH-responsive quantum dots based on ZnO quantum dots coated with PEG and hyaluronic acid to be stable under physiological conditions and for targeting specific HA-receptor CD44 cells. This nanocarrier was also assessed for doxorubicin's (DOX) sustained release. At physiological pH, these carriers were stable and DOX was loaded into the carrier and the complex form of Zn^{2+} ions or PEG conjugation. DOX was only released from the tumor cells under acidic intracellular conditions due to disturbance of ZnO QDs. The investigators found that the combination of DOX and ZnO QD [84] enhanced the anticancer function.

4. Natural product based drug delivery system

Natural product-based materials are currently considered to be the key ingredients in the preparation and processing of new nanoformulations as they have interesting features such as biodegradability, biocompatibility, availability, renewability and low toxicity [85–87]. In addition to the aforementioned properties, biomaterials are largely capable of undergoing chemical modifications, ensuring unique and desirable

properties for potential nanomedicine uses [88, 89]. For example, nanoparticles of metals, metal oxide and sulfides have been recorded to be synthesized using different microorganisms, including bacteria, fungi, algae, yeast, etc., [90] or plant extracts. Microorganism that assists the synthesis process is prepared in the adequate growth medium and then mixed with a metal precursor and left for incubation to form the nanoparticles either intracellularly or extracellularly [91–93]. Similarly, plant extracts are used for synthesis in which the extract is mixed with the metal precursor and incubated further at room temperature or boiling temperature for a definite time or exposed to light as an external stimulus [94]. Currently, natural product-based materials are considered essential ingredients in the preparation and production of nanoformulations as they have fascinating characteristics such as biodegradability, biocompatibility, sustainability, renewable energy and low toxicity [85, 86, 95]. In addition to the above mentioned properties, biomaterials are, for the most part, capable of undergoing chemical modifications, guaranteeing them special and attractive properties for future applications in the field of nanomedicine [89, 96, 97]. Nanoparticles, especially the silver nanoparticles have been prolifically studied *in vitro* for their antibacterial, antifungal, and cytotoxicity potential [98, 99]. Nanocarriers such as crystal nanoparticles, liposomes, micelles, polymeric nanoparticles, solid lipid nanoparticles, superparamagnetic iron oxide nanoparticles and dendrimers are formulated for natural product based drug delivery. Gupta et al. [100] synthesized chitosan-based nanoparticles loaded with Paclitaxel (Taxol) extracted from *Taxus brevifolia* for cancer therapy applications, and used them to treat various forms of cancer. The drug loaded with nanomedicines exhibited better efficacy with sustained release, high cell absorption and decreased hemolytic toxicity compared to pure Paclitaxel [100]. Chang et al. [101] developed a heparin/berberine conjugate to improve the suppressive development of *Helicobacter pylori*, thus reducing cytotoxic effects in infected cells. In a study conducted by Dian et al. [102], polymeric micelles were used to deliver Quercetin (polyphenol) and the results showed that these micelles could provide continuous release for up to 10 days *in vitro*, with continuous plasma levels and increased complete *in vivo* drug accessibility. Spillmann et al. proposed a multifunctional liquid crystal nanoparticles device as intracellular fluorescent imaging and doxorubicin distribution in which nanoparticles were functionalized with transferrin. Daunorubicin is a natural product extracted from a number of wild strains of *Streptomyces,* doxorubicin (DOX) is a hydrolated version of it used in chemotherapy [103]. Within the endocytic vesicles of HEK 293 T/17 cells, cellular uptake and continuous liberation have been achieved. For intracellular transport, perylene was used as a chromophore to chase particles and encapsulated compounds [104]. Liposomes are studied mostly, and have been used in various formulations for the delivery of natural products like Resveratrol, Curcumin, etc. [105, 106].

In addition, it can be seen that the sustained release mechanisms of naturally occurring therapeutic agents are a crucial method for increasing the biological efficacy of these agents and addressing their drawbacks by introducing new options for chronic and terminal disease management [107–110].

The global demand for plant-derived pharmaceuticals will rise from $29.4 billion (as in 2017) to around $39.6 billion in 2022 with a compound annual growth rate (CAGR) of 6.15% in this timeframe (BCC-Data), according to BBC Report. Any of the nanostructure-based materials included in this section have already obtained FDA clearance.

5. Challenges and opportunities

While there have been a large number of nanomedicine-related studies and tests, only a handful have advanced to market-related review and once again a

smaller handful have earned final clearance. The conversion of fundamental science into clinical practice was less than 10 percent, based on some reports [111, 112]. Thus, drugs that travel through what is known as the 'valley of death' do not seem convenient. This will lead to a time-consuming, lengthy, futile series of reviews, escalating the expense of health care as a whole [113]. Perhaps the reasons for such an undesirable state of affairs lie in multiple fields and procedure facets. One of the key problems involves nanoparticles' *in vivo* behavior, which is expected to be somewhat different from their *in vitro* behavior. The key problems that need to be extensively explored using various animal *(in vivo*) models are cellular interactions, tissue transfer, diffusion, and biocompatibility. It is not simple or cheap to perform such tests to provide adequate proof of effectiveness and protection. Another obstacle for tumor-targeted nanoformulations in particular is the heterogeneity and heterogeneous nature of tumors. Different gene expression profiles, molecular patterns and degree of drug resistance between different tumors may impede penetration and decrease the efficacy of tumor-targeted NPs [114, 115]. This challenge could lead to an unsuccessful clinical trial (despite promising animal preclinical data) and to rejection of the nanoformulations examined. Relevant drug penetration into tumors, the efficacy of the release of drugs into the target cells, and the quality of the drug loaded nanoparticles, are other factors that involve a precise professional experimentation [114]. Owing to time and money problems, this comprehensive research may not be possible in all biomedical laboratories which itself is another concern. The multifunctional structure and operation of some nanoformulations could be another obstacle on the road to nanoformulation acceptance. Many investigative nanoformulations have a hybrid structure and contain separate diagnostic and therapeutic components. Different experiments are required to demonstrate the protection of such systems, and the long-term biocompatibility of such systems is not yet clear [116, 117]. Regarding this issue, the regulatory authorities have different restrictions, and it will take time-consuming and costly regulatory studies to be sure of the long-term safety of these theranostic nanoformulations. Many of the classical approaches for nanoformulation synthesis are already incomplete and need to be more developed and optimized. Batch-to-batch variance is another problem that can hamper the development of enough stocks of nanoformulations for market approval to be achieved. The updating of production methods and the highly accurate characterization of nanoformulations are expected to be laborious, time-consuming and expensive [116, 118].

In spite of all the above-mentioned obstacles, the demand for nanopharmaceuticals and nanomedicines will continue to expand over the next few years, primarily thanks to developments in bionanotechnology and nanoengineering, the implementation of explicit guidelines on new nanotechnology-based products, more support from government organizations, more consensus on environmental issues and the creation of collaborations between nanomedicines startups and leading pharmaceutical companies [119]. In other words, in order to convince investors about the value of nanopharmaceuticals and to improve the overall health and well-being of society, intellectual property and regulatory agencies need to change their approach to meeting the specific needs of nanomedicine and shorten their time to regulatory approval. However, in the case of nanodrugs, it is particularly important to consider the risks to health and the environment *vis a vis* it's short-term gains. Another similarly significant aspect that has drawn researchers and companies' interest is the increasing role that cancer plays in mortality and morbidity statistics worldwide As in 2018, FDA earned the most approvals for oncology drugs [120, 121]. This, and the large number of other cancer-related pharmaceuticals licensed over the past few years, shows not just the patients' desperate need for better cancer treatment, but also the massive cancer care market. There is still tremendous hope for the application of chemotherapy and photothermal or photodynamic treatment. These, though, are variations of products, which could be

easier to progress to complete clearance. Diseases affecting the immune system are also very important for the pharmaceutical industry. These applications include stimulating the immune system to combat infections and cancer, but also to down-regulate the immune system to combat autoimmune diseases and allergies. Overall, it is believed that the rising rate of cancer-related deaths will be driving the anticipated increase in the size of the global nanomedicine market in the coming years.

6. Conclusion

Initially, the use of nanotechnology was mostly based on improving the solubility, absorption, bioavailability and controlled release of drugs, but now a wide range of nanodimensional tools are included that can be used to diagnose, precisely deliver at target, sense or activate material in the living system. By using nanocarriers formulated with gold, silver, cadmium sulphide, and titanium dioxide polymeric nanoparticles along with solid lipid nanoparticles, nanogels, liposomes, micelles, iron oxide nanoparticles, and dendrimers, the efficacy of the natural products has greatly improved. One of the major interests in the advancement of nanomedicine in recent years is the convergence of therapy and diagnosis (theranostic) as an example of cancer as a disease model. Since the 1990s, there has been a remarkable growth in the number of FDA-approved nanotechnology-based products and clinical trials, including synthetic polymer particles; liposome formulations; micellar nanoparticles; nanocrystals and many others frequently associated with drugs or biologics. Although regulatory frameworks for nanomedicines along with safety/toxicity tests will be the focus of further research in the future, the way we discover and deliver drugs in biological systems has already revolutionized nanomedicine. Thanks to advances in nanomedicine, the ability to deliver, and even targeted delivery, has also become a reality.

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Section 5

Cancer

Chapter 5

Prevalence, Symptomatology and Herbal Management of Polycystic Ovarian Syndrome

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Abstract

Polycystic Ovarian Syndrome (PCOS) is multi-organ syndrome that affects 6–25% of females during reproductive age. It disrupts normal hormone levels of females and affects adrenal hormone and sex hormones along with pituitary hormones including adrenocorticotropic, growth hormone and gonadotropins hormones. It results in several secondary characteristics in females such as infertility, hormonal imbalance, oligomenorrhea, amenorrhea, obesity and hirsutism. Polycystic ovarian syndrome is associated with mental and reproductive disorder and almost 4–18% mature female students are affected by polycystic ovarian syndrome. Female affected by polycystic ovarian syndrome have increased risk of infertility, ovarian cancer, skin problems and psychological problems such as anxiety, depression and changes in sleep along with mood swings. This chapter discusses the Prevalence, Symptomatology and Management of Polycystic Ovarian Syndrome. For the management of PCOS, the role of some medicinal plants including *Asparagus racemosus*, *Tinospora cordifolia, Foeniculum vulgare, Ocimum tenuiflorum, Actaea racemosa*, and *Lepidium meyenii* have also been discussed in addition to other treatment modalities. The literature data was searched out and compiled using relevant original research articles and reviews published and indexed in Google Scholar, Scientific Information Database, Elsevier, PubMed and Science Direct.

Keywords: polycystic ovarian syndrome, prevalence, management, medicinal plants

1. Introduction

Polycystic Ovarian Syndrome (PCOS) is a multi-organ syndrome that disturbs the normal hormone levels of females and affects the sex and adrenal hormones as well as pituitary hormones including growth hormone, adrenocorticotropic hormone and gonadotropins [1]. Female illness PCOS commonly results in hormonal imbalance, infertility, amenorrhea, oligomenorrhea, hirsutism and obesity. This study will contribute to the knowledge of prevalence, awareness, practices and attitudes of female university students towards polycystic ovarian syndrome and will enables us to understand the new approaches to control this issue for further strengthening as well as precautions and better treatment methods.

The most common female syndrome is known as PCOS. PCOS is a state of illness that mostly disturbs the hormonal levels in women [1]. Polycystic ovarian syndrome can affect the pituitary hormones including growth hormone, gonadotropin hormone, adrenocorticotropic hormone (ACTH) and can also affect the adrenal and sex hormones, that's why this syndrome can be called as multi organ syndrome [2].

The female reproductive organs consist of gonads known as the ovaries. The function of ovaries is to release mature and properly developed ovum and increase the secretion of steroid hormones. Pituitary gland secretes hormones LH and FSH which are controlling the functions of ovaries [3].

To diagnose PCOS, recently introduced criteria called Rotterdam Criteria is being practiced. According to this criterion, the diagnosis of PCOS depends upon the presence of hyperandrogenemia, oligo-ovulation and presence of inflamed ovaries on ultrasonography. Polycystic ovarian syndrome is classified as a set II ovulation sickness according to World Health Organization (WHO). About 4 to 18 percent women of child bearing age are suffering from polycystic ovarian syndrome. It is associated with metabolic, psychological and reproductive dysfunctions [4]. Those women that are suffering from polycystic ovarian syndrome are at risk of endometrial hyperplasia or cancer, infertility, obstructive sleep apnoea or inability to sleep, abnormal glucose metabolism, anxiety and depression [5].

The exact cause of polycystic ovarian syndrome is not known. Different studies demonstrate that there are some inborn abnormalities like follicular development and ovarian steroidogenesis which are important in the inflamed ovaries disorder. This syndrome is also associated with an excess of luteinizing hormone (LH), decreased secretion of follicle stimulating hormone (FSH) and continuous increase in gonadotropin-releasing hormone (GnRH) which contribute to ovulatory dysfunction and overproduction of androgen hormones [5].

A transmutation in polycystic ovarian syndrome proteins also correlates with multiple environmental factors and inherited factors. There are multiple genes which are responsible for the incidence of polycystic ovaries disorder [6]. Most common endocrine complaint in female is PCOS. PCO has highly variable incidence such as 2.2 to 26% [7, 8]. The indications of polycystic ovarian syndrome may occur in adolescence. But it mostly diagnosed in adulthood. It is thought that different life style and genetic factors take part in the occurrence of PCOS. It has been hypothesized that the earlier predisposing gestational factors of polycystic ovarian syndrome constitute the disturbed foetal programming in the uterus and birth weight [9].

Premature adrenarche has 15 to 20% risk of developing polycystic ovarian syndrome [10]. There was genetic predisposition in a female to developing polycystic ovarian syndrome [11, 12]. Alteration in the life style including high caloric diet and reduced exercise ultimately leads toward insulin resistance and obesity which has been considered as aggravating factors for polycystic ovarian syndrome [13].

It has been found that there is an association between polycystic ovarian syndromes and stress. But in other studies, stress is not considered as a causative factor of polycystic ovarian syndrome. The rate of incidence of polycystic ovarian syndrome depends upon the criteria used to define this disorder. In 1980s the ultrasound imaging showed that inflamed ovaries were mostly associated with hyperandrogenemia and hirsutism in the females with continuous ovulatory cycle [14, 15]. It was also revealed in different studies, that there are some similar share of biochemical features in female with PCOS and regular rotations [16].

The incidence of premenstrual syndrome appears to vary in different countries. In the U.S., according to NIH criteria two studies have showed 4% prevalence rate in a 400 population [17]. About 6.6% prevalence rate have been documented in

females of south-eastern university [18]. From other races the incidence among females seems to be similar. The similar incidence rates of 6.5% also found in a study on 154 Caucasian females in Madrid, Spain [19].

Thus, the incidence of clinically evident PCOS in childbearing female in America and Europe ranges from 6.5 to 8.0% if the 1990 NIH criteria are applied. The incidence raises about two to three folds if the Rotterdam criteria are applied. The prevalence of polycystic ovarian syndrome seems to be lower in Asian countries with a reported incidence rate of 2.4% in China [20]. The prevalence rate reported in Sri Lanka is 6.3% according to Rotterdam criteria [21].

Common endocrine system sickness that affects women of childbearing age is known as PCOS And can also be mentioned as Stein-Leventhal syndrome or hyper androgenic involution (HA). In 1935 Stein and Leventhal described that polycystic ovarian syndrome represents a state in which approximately 10 cysts are made on ovaries and have diameter 2 to 9 mm. The ovarian volume exceeds about 10 ml in at least one ovary [22].

Almost, 7 million females in U.S. have been affected with polycystic ovarian syndrome from which more than half females are aware about polycystic ovarian syndrome and remaining are unaware about PCOS. Polycystic ovarian syndrome awareness not only concern to treat a disease in spite of it, PCOS concern is to improve the female health and make easier their living style [23].

Polycystic ovarian syndrome is associated with mental and reproductive disorder and almost 4–18% mature female students are affected by polycystic ovarian syndrome [24]. Females effected by polycystic ovarian syndrome have increased risk of infertility, ovarian cancer, skin problems and psychological problems such as anxiety, depression and changes in sleep along with mood swings. Polycystic ovarian syndrome is still unknown aetiology but in several studies it is assumed that polycystic ovarian syndrome is inherited and also associated with follicle stimulating hormone (FSH) and luteinizing hormone (LH). Excessive secretion of LH and decreased formation of FSH lead to excessive release of gonadotropin hormone, which causes ovulatory disturbance [25].

Polycystic ovarian syndrome has various symptoms such as weight gain, acne, excessive hair growth on unwanted body parts, irregular menses, and psychological problems like anxiety, depression, sleep & voice changes, and also include dysmenorrhea. Hirsutism is defined as excessive hair growth on unwanted parts of the body in 10% of females caused by excessive production of male hormone androgen which is significantly associated with polycystic ovarian syndrome. Amenorrhea is defined as the lack of menstrual cycle in female of reproductive age, it can be primary (due to hormonal imbalance) and secondary (absence of menstrual cycle for more than 6 month) [26].

Major complications of polycystic ovarian syndrome are hypertension, diabetes mellitus, obesity, cardiovascular disorder, ovarian cancer, skin problems, liver diseases and psychological disorders [27]. The combination of hirsutism, infertility, oligomenorrhea and two sided enlargement of polycystic ovaries was recognized as reported in an article by Stein and Leventhal in 1935 [28]. Syndrome for some time period was known by their name. The term PCOS seem to be looked in 1960s and progressively substituted by the name of Stein-Leventhal syndrome. A major histopathological study conducted in 1982 [29] revealed a comprehensive detail about ovaries, which are inflamed and contain large number of small fluid filled cysts of 10 mm diameter.

The sac of the ovary may be similar with a bulge, so the presence of cystic imaging in the ovary must be deliberated as a normal event. It is only the excessive number of ovarian follicle that necessarily be considered as a disease condition associated by means of polycystic ovarian syndrome. Consequently it is correct

and precise to state ovaries containing large number of follicle instead of polycysts. After Initiation of ultrasound, the follicular surplus became the main feature in the morphology of polycystic ovaries.

An increase in ovarian area (OA) and ovarian volume (OV) are being considered as the exact and accurate markers of polycystic ovarian morphology (PCOM). These two markers provided the dimensions that are voted for the middle segment of the berries. It is confirmed from histopathological studies that both ovarian area (OA) and ovarian volume (OV) are infect good indications of follicular excess and stromal hypertrophy which are the structural or anatomical symbols of the polycystic ovarian morphology (PCOM) [30].

The most common cause of menstrual disruption such as anovulation, infertility, oligo menorrhea and menorrhagia, is the polycystic ovarian syndrome [31]. In the USA, Spain and Greece, the incidence of polycystic ovarian syndrome was assessed to be 4 to 8%. The prevalence of polycystic ovarian syndrome is increasing all over the world and is presenting great increase in parallel with type 2 diabetes mellitus (T2DM) [32].

There were about 116 million females affected by polycystic ovarian syndrome in all over the world. In 2009, about 5 to 10% women were affected by polycystic ovarian syndrome in Pakistan [33]. Polycystic ovarian syndrome is heterogeneous disorder that is associated with polycystic ovarian morphology, hyperandrogenism and ovulatory dysfunction regarding to the pathophysiology. It's distinctive neuroendocrine feature include increased ratio of luteinizing hormone and follicular stimulating hormone (LH/FSH), increased serum level of luteinizing hormone (LH) and increased frequency and rate of LH secretion [34].

Gonadotropin releasing hormone (GnRH) secreted from hypothalamus binds to its receptors that are present on secretory cells of adenohypophysis. Gonadotrophs create LH and FSH in reply to GnRH, and these two hormones (LH, FSH) regulate reproductive processes in the body and regulate the pubertal growth, maturation and development [35]. Follicular stimulating hormone and luteinizing hormone stimulate the ovaries to produce oestrogen hormone in females and inhibit to regulate the menstrual cycle. By inhibiting the production of gonadotropin releasing hormone (GnRH), oestrogen forms a loop of negative feedback mechanism [36].

In polycystic ovarian syndrome, the level of progesterone hormone is decreased which cannot reduce the frequency of GnRH/LH pulse. Hence, the increased secretion of oestrogen hormone may lead towards the formation of autoantibodies in the body. Recently, there are two commonly recognised investigative criteria of polycystic ovarian syndrome and both suggest the presence of two out of three signs to be characterized as polycystic ovarian syndrome (PCOS) [37].

Obesity aggravates different types of comorbidities of polycystic ovarian syndrome for example diabetes, heart disease, hypertension (HYN) and hypercholesterolemia. An ovulation in polycystic ovarian syndrome leads towards unrestricted oestrogen secretion which is a risk factor for carcinoma and endometrial hyperplasia. Quality of life reduces by depression, obesity, hirsutism and infertility caused by polycystic ovarian syndrome (PCOS). Kerchner *et al*. identified the depression in 40% of females of polycystic ovarian syndrome (PCOS). The incidence rate of suicide is increased by seven folds in polycystic ovarian syndrome [38].

In polycystic ovarian syndrome, there is an excessive production of oestrogen hormone which has been related to different types of autoimmune diseases. Oestrogen hormone increases the production of different interleukins like IL-1, IL-6, IL-4 and interferon-*y.* In polycystic ovarian syndrome, the level of progesterone hormone is decreased and it triggers the immune system that ultimately leads towards the production of autoantibodies. Therefore, polycystic ovarian syndrome can be characterized as an autoimmune disorder [39].

Females with PCOS had high risk to develop endometrial cancer while their risks of developing ovarian and breast cancer are same to those of females in overall common population. There are some health significances of polycystic ovarian syndrome including overweight, null parity and continued unrestrained increase of oestrogen that are highly liked with cancer. A study conducted by Barry found an important increase of about three folds for endometrial cancer among females having polycystic ovarian syndrome. But there is no prominent increase in risk factor of ovarian or breast cancer [40].

Women of polycystic ovarian syndrome (PCOS) have a common feature of infertility. In general, infertility is considered as an ovulatory complaint. These types of patients are mostly treated with conservative therapies such as ovulation-induction medications. These medications include clomiphene citrate (CC) or gonadotropins, and considered as first line treatment. These medications increase the incidence of multiple pregnancies. Due to the use of gonadotropins there is an excessive risk of ovarian hyper stimulation syndrome. These types of drug modalities increase the risk of formation of different types of ovarian cysts, pain in ovaries and torsion of ovaries. For pregnancy and infertility problems, most commonly used drug is known as metformin [41].

A heterogeneous condition which has different phenotypic expression is known as polycystic ovarian syndrome (PCOS). Due to its phenotypic forms it becomes a controversial issue which is based upon the diagnostic criteria. The incidence of polycystic ovarian syndrome is about 6 to 15% and it depends upon the use of diagnostic criteria. Every patient experience the severity of the components of polycystic ovarian syndrome and its management is based upon the preferences of the patient. The most prominent endocrinopathy in females is known as polycystic ovarian syndrome (PCOS) with the incidence rate of 6.5 to 6.7% among those women who are in menopausal stage [42].

In 1990, this disorder was well known as the combination of biological and clinical increase in androgen hormones, chronic anovulation and oligomenorrhoea as presented in the NIH conference. The investigative criteria revised in 2003 by Rotterdam consensus [43], with the presence of either two or three following preconditions including clinical chronic anovulation or oligomenorrhoea. Various possible pathophysiological consequences of polycystic ovarian morphology (PCOS), detected in 75% of females of polycystic ovarian syndrome (PCOS), has been comprehensively discussed. Further, it has been discussed that there is no association between the metabolic irregularities of the typical polycystic ovarian syndrome phenotypes and the phenotype of anovulation combined with the morphology of polycystic ovaries [44].

2. Prevalence

Due to increase in endometrial and follicular activity level almost 100% polycystic ovarian syndrome patient suffered from amenorrhea, anovulation and infertility, its occurrence is also due to irregularity in their cyclic menstrual cycle. Almost 60% women's had menstrual problems (amenorrhea, anovulation, infrequent bleeding) in a study conducted on 400 women of general population [45].

Sanchez *et al.* assessed the frequency of this syndrome in females in US (age 18–45 years) is almost 6.6%. According to present study on polycystic ovarian syndrome, occurrence of PCOS is 6% because 6% were diagnosed as having PCOS while 16% girls were refer to doctor and 9% girls did ultrasonography and blood reports [46].

Joshi *et al.* reported that the prevalence of PCOS was extremely inconstant worldwide; fluctuating from 2.2–26%. They found 22.5% prevalence of PCOS by Rotterdam and 10.7% according to Androgen Excess Society criteria [47].

Joshi B and her group evaluated that PCOS is an evolving illness during puberty and screening to avoid illnesses and psychological problems [48]. Shetty D assess that around 10% Indian women was having PCOS symptoms like excessive hair growth in unwanted parts of the body, obesity and acne [49]. Choudhary et al. assessed 9.13% occurrence of PCOS in Indian youths [50]. Vaidya R considered that according to WHO, there was 116 million women throughout world were affected with PCOS in 2012 [51]. Lakshmi KS reported 32% occurrence of PCOS at a tertiary care hospital [52].

Radha P assessed that Indian woman had high risk of PCOS compared to their others counterparts, with an expected incidence of 9.13% in Indian youths [53]. Radha P assessed that 20% of contributors were diagnosed with PCOS. The percentage of PCOS was found increased in city area as compared to rural areas, due to their unhealthy life style [54]. Almost 9.5% girls consulted dermatologist for excessive hair growth on abnormal body parts or acne (major sign of PCOS), 4.5% female referred to gynaecologist for abnormality of periods, and 1% girls used herbal treatment while 1% used homeopathy.

Sills ES assessed that regardless of age, physicians are the most common provider of PCOS information for all (rather than other sources like paper, family and books) [55].

A research about the occurrence of PCOS was conducted at Taibah University Al Madinah Al Munawara on adult unmarried female students, aged 18–28 years. From 201 contributors, 108 (53.7%) were diagnosed to have disorder with a mean age of 21.3 ± 2.1 years, the demographic facts, menstrual irregularities and acne seen in 108 students [56].

Polycystic ovarian syndrome is disturbing 5–10% population because it can cause infertility and endocrine diseases in women's of child bearing age. Symptoms of polycystic ovarian syndrome usually arise during adolescence at the start of menstruation and disturbed the females health and mental state, symptoms can be mild or severe. PCOS is a common endocrinology sickness in young females. Occurrence of PCOS in child bearing age women is 7 to 10% [57]. Study on the prevalence of polycystic ovarian syndrome was conducted in Karachi, Pakistan, to assess the ratio of affected females in Pakistan especially in Karachi [58].

In 2011 a study conducted in Rawalpindi to assess the prevalence of PCOS [43]. In India studies related to occurrence of PCOS was conducted at Thandalam, all these studies indicated that prevalence of polycystic ovarian syndrome is increasing with time and most of our females are affected by PCOS [59]. A study was conducted in Karachi to evaluate the information and awareness of PCOS in urban Pakistani women at 2014 [60].

In present studies maximum adolescent and young girls (almost 33%) got knowledge about polycystic ovarian syndrome from teachers, 19% girls got knowledge from friends, 11.5% females are diagnosed and informed by doctor, 3.5% female got knowledge by reading newspaper and 5% got knowledge from internet resources. Almost 28% young and adolescent girls are unaware about polycystic ovarian syndrome and 72% females have knowledge about polycystic ovarian syndrome symptoms, prevalence and severity.

According to Sunanda B *et al.* 76% females had average knowledge about polycystic ovarian syndrome and 10.7% females had good information related to PCOS [61]. Sills ES assessed more than 97% females were aware with PCOS, while 1.9% had not been aware about PCOS, and < 1% females were unclear [62].

3. Pathophysiology and aetiology of PCOS

The exact cause of polycystic ovarian syndrome is still unknown. PCOS is possibly a mixture of factors such as inheritances, ecological factors as well as fetal contact to high androgen in uterus. The polycystic ovarian syndrome has been noted a familial disorder which runs in families. Those who have PCOS positive family history in mothers or sisters have greater chances to develop the syndrome [63].

The current studies showed that polycystic ovarian syndrome run in families but it is not sex chromosomes disorder, it is of autosomal dominant pattern. A number of factors of polycystic ovarian syndrome have been known, that are associated with PCOS. Maternal causes include low or high birth weight in girls borne from obese females [64].

There are various risk factors like acanthus's Nigerians (skin disorder), metabolic, precocious puberty (puberty in the age of 7 to 9 years) and pubarche [65]. Weight gain sometimes leads toward the development of syndrome. PCOS is diagnosed in adolescent due to their irregular menstrual cycles. Maximum researchers evaluated those natural defects in steroid formation in ovaries and development of follicle play an important role in the etiology of PCOS. The syndrome is also related with insistently quick pulses of GnRH, an excessive production of LH, and inadequate production of FSH, which plays role in extreme ovarian androgen formation and loss of ovulatory function [66].

There are multiple familial genes which are responsible for the development of polycystic ovarian syndrome, many environmental as well as inherited factors which are the main cause in the mutation of polycystic ovarian syndrome protein. The result of transformation and scientific ascertainment reveals in everyday life and it completely altered our lifestyle. Consumption of diet is concentrated increasingly on sugar, fast food, and soft juices and decrease from fresh vegetables and balanced diet. This unhealthy eating behaviors and lack of workout leads toward polycystic ovarian syndrome (PCOS) as well [67].

Causes of many symptoms are treated or become less dangerous because hormonal level become normal due to weight loss. Weight gain can be treated through exercise and balanced food.in last few years 30% Polycystic Ovaries Syndrome cases rise in India. This may occur due to unhealthy life style and lack of information about PCOS [68].

3.1 Pathophysiology

In the women of polycystic ovarian syndrome the gonadotropin releasing hormone (GnRH) level is increases or decreases in frequency with time. Due to decreased gonadotropin releasing hormone level, production of LH is increased resulting in decreased formation of FSH.

Increased level of luteinizing hormone stimulates the theca cells of ovaries to release androgenic hormone and may also disturb the menstruation. Moreover, this low level of follicle stimulating hormone cannot stimulate the granulosa cells of ovaries to convert androgen to estrogen, and ovulation cannot occur in the absence of estrogen.

Normally after ovulation, corpus lutein releases the progesterone hormone. Due to increase in progesterone level, the gonadotropin hormone level is decreased through feedback mechanism. Some readings have explored the role of the controlling genes of cytochrome P450 (CYP) 11A, FST, IVSR, 3-HSDL and CYP 17 enzyme in connection with PCOS.

In the ovaries of PCOS theca cells changed whose cytochrome P450 (CYP) 11A, 3- HSDL and CYP 17 gene display raised intensities. And there is practical anomaly of the 17 -hydroxylase which control androgen biosynthesis. Hereditary studies revealed a link among PCOS and hyper-insulinaemia. Insulin resistance at the margin and to irregular pancreatic ^{2} cell function is secondary to hyper-insulinaemia. 50–70% of females effected with PCOS due to insulin conflict. Increased insulin levels may affects the gonadotropin which acts on ovarian function. Ovarian function can be regulated with insulin, and excessive insulin stimulates the ovaries to produce gonadotropin. Increased insulin also subdue generation of sex hormone binding globulin (a carrier protein) which as a result increases androgenicity [69].

A scientific hallmark of PCOS is hyperandogenism, causing reserve development of sac, micro cyst materialization in ovaries and anovulation [70]. Increase level of androgen and insulin affect menstruation and prevent ovulation.

3.2 Mechanisms of anovulation

There is prominent difference between follicle development as well as steroid genesis. One thinkable justification for this puzzle may be a marvel that is assumed to happen usually at the onset of the mid cycle of luteinizing hormone release. This syndrome is considered through excessive secretion of LH and insulin. Many patients have normal blood concentrations of LH has raised serum insulin level [71].

Insulin cooperates with LH in increasing steroidogenesis by granulosa cells of ovaries. Elevated stimulant intensities of LH and its augmentation of action on follicle by hyperinsulinemia had explanation of detention of follicle development and improvement of estradiol release [72].

The “control” granulosa cells were differentiated and follicle is released which is then activated at the onset of mid cycle of LH release by intracellular cAMP. Production of cAMP was affected by insulin; it assumed that insulin increases the LH production due to which LH receptors are increased on growing granulosa cells. Excessive secretion of androgens from ovaries can affect follicle growth. Within granulosa cells, androgens enlarge gonadotropin-induced cAMP release [73].

4. Clinical features

Polycystic ovarian syndromes has various symptoms such as weight gain, acne, excessive hair development on unwanted body parts, irregular menses, and psychological problems like anxiety, depression and sleep and voice changes and also include dysmenorrhea.

Hirsutism is defined as excessive hair growth on unwanted parts of the body in 10% of females caused by excessive production of male hormone androgen which is significantly associated with polycystic ovarian syndrome. Amenorrhea is defined as menstrual cycle is absent in female of reproductive age, it can be primary (due to hormonal imbalance) and secondary (absence of menstrual cycle for more than 6 months). Major complications of polycystic ovarian syndrome are hypertension, diabetes mellitus, obesity, cardiovascular disorder, ovarian cancer, skin problems, liver diseases and psychological disorders [74].

The combination of hirsutism, infertility, oligo menorrhea and two sided enlargement of polycystic ovaries was recognized by Stein and Leventhal. For some time period, syndrome use their name. The word polycystic ovarian syndrome (PCOS) seems to be looked in the 1960s and progressively changed by label of

Stein-Leventhal syndrome. Last main histopathological study was conducted in 1982 [75].

And it delivers a comprehensive explanation of the polycystic form of the ovaries and the number of follicles that are 10 mm in diameter. Cyst may resemble with ovarian follicle, so the existence of cyst within ovary must be deliberated as a usual experience. Extreme number of ovarian follicle that considered as disease ailment is associated by polycystic ovarian syndrome. Therefore it is correct and precise to express as ovaries containing large number of follicle instead of polycyss. A rise in ovarian area and increased volume was considered the exact and accurate markers of polycystic ovarian morphology (PCOM). These two markersprovided the dimension that was passed out on the middle segment of the ovaries.

It is confirmed from histopathological studies that both ovarian area (OA) and ovarian volume (OV) are infect good indications of follicular excess and stromal hypertrophy which are the structural or anatomical symbols of the polycystic ovarian morphology (PCOM). Hyperandrogenism (Acne, hirsutism, alopecia); 60% PCOS females are suffered with hirsutisim on chin, face upper lips, buttocks [76].

Irregular menstrual cycle means cycle repeat much time within month or may absent for several months. Almost 85% - 90% of women of oligomenarrhoea have PCOS worldwide, while 30–40% of amenorrhea women also affected with this syndrome. Heavy or flimsy menstrual flow indicates polymenorrhea and oligo menorrhea. Lack of ovulation may lead toward infertility and subfertility/70% of pregnancy problem due to anovulation are connected to inflamed ovaries [77]. Fatness with BMI > 35 kg/m^2.

Some other features are:

- Weakening and falling of hair.
- Membranes label below the armpits, neck and below knees.
- Temper complaints such as hirsuitism are common in teenage. Most of females often feel unsatisfied and depressed if they expected difficulty to loose weight, cure acne, abnormal body hair and menstrual problems.
- Cardiac abnormalities.
- Pre diabetes and diabetes
- High total cholesterol and low good cholesterol and high LDL
- Almost 25% females with PCOS had elevated prolactin levels [78].

5. Diagnostic criteria of PCOS

European Society for Human Reproduction and Embryology/American Society for reproductive medicine – funded PCOS workshops in 2004. definition of this syndrome must include two out of the subsequent three conditions:

1. Absence of menstrual cycle for 6 months.
2. Medical or organic symbols of increase androgen product.
3. Inflamed ovaries on ultrasound.

Polycystic ovary was redefined, ovary with more than 12 follicles of size 2–9 mm in diameter.

These standards are not proper for all females, because some PCOS girls had no cyst and experience regular periods.

- Insulin resistance is most common in all PCOS females. 70–95% obese and 30–75% lean PCOS females are affected. This can lead to diabetes and cardiovascular disease in PCOS females.

- It also responsible to raised plasminogen activator inhibitor –1 (PAI-1) in patients with PCOS. Elevated PAI-1 can cause intravascular thrombosis.

- Endometrial hyperplasia and endometrial cancer are thinkable, due to over gathering of uterine lining, due to deficiency of progesterone hormone causing stimulation of uterine cells by oestrogen hormone.

- Breast cancer, obesity and infertility are common in PCOS patients [79].

PCOS mostly affect the obese female's then lean built. Moreover pregnant women suffered with PCOS should be informed about miscarriage, gestational diabetes, pre-eclampsia, eclampsia and premature delivery. Barry found significant rise of three folds for endometrial cancer among females suffered with polycystic ovarian syndrome. But there is no prominent increase in it [80].

6. Investigations

To eradicate all other illnesses that may cause menstrual problems, breast cancer and infertility, the most common diagnostic tests include ultrasound, FNAC and reproductive hormones like estrogen and progesterone.

7. Management

The vital treatment option for polycystic ovarian syndrome is the lifestyle modifications in those cases in which different risk factors are correlated to stimulate the presentations of polycystic ovarian syndrome (PCOS). Treatment modalities are included in other types of intermediations. The main objective of these types of modifications is to decrease the weight and maintain reduced body weight for longer time. Improvement of insulin resistance through weight loss with the help of exercise and diet seems to contribute to the development of metabolic, clinical, and hormonal factors of polycystic ovarian syndrome (PCOS). Though even the uncertain weight loss has been revealed to increase the incidence of ovulation, improvement of hormonal factors and decrease metabolic defects in the females affected with polycystic ovarian syndrome.

Life style modification is very essential in overweight patients. Every female with polycystic ovaries disease can take advantage from a proper balanced diet and consistent exercise. In most of programmes of dietary involvement, the energy restriction has been forefront while excellence of diet may also play a major role in polycystic ovarian syndrome.

To accomplish long term reduction in weight is the major difficulty in polycystic ovarian syndrome. Additional medical therapies are also necessary. In the

management of PCOS, there was a stimulating pathophysiological background to sustain the therapeutic benefits of insulin sensitizers including hyperandrogenaemia, metabolic characteristics and ovulatory functions. Accessible literature delivers acceptance to this whole concept and inspires the addition of insulin sensitizers in the therapeutic management of polycystic ovarian syndrome. There are two main and most beneficial insulin sensitizers including metformin and thiazolineodiones.

Moreover, different traditional drugs such as clomiphene, oral contraceptives, and antiandrogens drugs are used in the management of polycystic ovarian syndrome. These medications have been considered as supplementation by original pharmacological modalities including insulin sensitizers. Lifestyle modification is still considered beneficial therapeutics. The most commonly studied insulin sensitizer is known as metformin due to its comforting safety profile.

Metformin aids to reduce visceral adiposity, weight loss and decrease insulin resistance commonly at the muscle and hepatic tissue level and it contribute to a more approving hormonal as well as metabolic profile. The metabolic actions of metformin are well recognised in other patients like obese and slim women presenting with PCOS. In a meta-analysis of different people, such as 13 controlled studies of females with polycystic ovarian syndrome it established that metformin has essential role to decrease fasting insulin levels [81].

PCOS is still unknown disorder and has no specific treatment. But it can be treated and managed in several ways. Regularity of menstrual cycle, improve skin disorders and reduce insulin and androgen level are the aims of management for young women. Initial treatment for women with PCOS is life style modification like:

1. Take protein, keep low carbohydrate.

2. Workout.

3. Loss weight.

4. Pharmacological treatments are used.

   i. Overweight:

BMI > 30 kg/m^2 considered as overweight and should be encouraged to loose weight.

   ii. Menstrual abnormality:

Use low dose combined oral contraceptive to manage menstrual cycle.

   iii. Metformin become common medicine selected for this population.

Spironolactone or Flutamide are androgen lowering medication, but shows their effect slowly. Metformin is an anti-diabetic drug that can be used to recover fertility, decline insulin conflict, regulate menses, and recover cardiovascular health in teenagers with PCOS. Aromatase inhibitors are suitable for females of clomiphene resistance. Some girls with PCOS develop depression, therefor they need mental health professional [82]. Cancerous cysts are removed surgically. Ovarian drilling is the best surgery to cure infertility. It is necessary for PCOS girls to consult their doctor often and use proper medication and precaution to regulate periods and decrease the chances to develop diabetes, CVS and skin issues.

8. Medicinal plants

8.1 *Asparagus racemosus* (Shatavari)

In traditional ayruvedic medicine *Asparagus racemosus* (Asparagaceae) is used which aids in regulation of menstrual cycle, stimulating the normal growth of ovarian follicles and strengthening the female reproductive system mostly due to phytoestrogen (known as natural plant consisted of estrogen). It also helps in resisting the hyperinsulinemia [83].

A. racemosus has many other pharmacological effects in addition to above, like tumors, inflammation, neuropathy, nervous disorders, dyspepsia, neuropathy, and hepatopathy. It also acts as antiulcer, antioxidant and anti-diarrheal. *A. racemosus* also helps in preventing aging, increase the endurance of life as well as improves mental function and immune modulatory activities [84].

8.2. *Tinospora cordifolia* (Guduchi)

Tinospora cordifolia, (Menispermaceae) is a well-recognized medicinal plant which has hypoglycemic effect [82]. It is a powerful and effective inflammatory herb. The basic cause of insulin imbalance and ovarian cysts is the chronic inflammation in tissues. *Tinospora Cordifolia* aids in strengthening all the body tissues, increasing metabolism and lowing the insulin resistance [85].

8.3. *Foeniculum vulgare* (Shatapushpa)

The seeds of *Foeniculum vulgare, (Apiaceae)* are used as an excellent supplement for managing the polycystic ovarian syndrome (PCOS). Seeds are the richest source of phytoestrogsns. In fennel, these phytoestrogens helps in decreasing insulin resistance and in lowering the inflammation in PCOS. It is also thought that it helps in lowering the cellular imbalance which ultimately leads towards the metabolic disturbances in PCOS [86].

Currently, various parts of this medicinal plant are used in the management and treatment of many disorders, specifically digestive system disorders. Moreover, it is highly useful in the treatment of inflammation of bronchioles, chronic cough, diabetes mellitus, kidney stones as well as in nausea and vomiting [87].

8.4. *Ocimum tenuiflorum* (Holy Basil)

A traditional herbal medicine *Ocimum tenuiflorum L. (*Lamiaceae) generally known as Tulsi*. Ocimum tenuiflorum* is very effective for polycystic ovarian syndrome. It lowers the production of androgen because it has tremendous anti-androgenic properties [88].

8.5. *Actaea racemosa* (Black Cohosh)

In many disorders of female reproductive system like anvolution, hormonal imbalance, infertility, the *Actaea racemosa* (Ranunculanae) is used because these are important issues in PCOS. Black Cohosh has the capability to stimulate the ovulation in females with PCOS [89].

8.6. *Lepidium meyenii* (Maca)

A traditional herbal medicine *Lepidium meyenii* from Brassicaceae family is used in treating the menopausal symptoms, stimulates the endocrine system. It acts as

natural hormonal balancer without any adverse effects. Progesterone and estrogen hormones help in encouraging the healthy menstrual cycle in the body. It is an implausible fertility super food as well as an adaptogen. Maca also restores the level of testosterone in males [90].

9. Conclusion

The incidence of polycystic ovarian syndrome is rising day by day but students were not aware and conscious of polycystic ovarian syndrome (PCOS) even though the different features regarding to this syndrome were present in many students. Therefore, few teaching projects or schemes have to be prepared to make available information related to these types of disorders in women. Moreover, survey shows many women do not discuss with gynaecologist until there is rigorous or difficult situation occurred. Consequently, women must have to talk to any gynaecologist or doctor for health and fitness. The last prominent matter was about the contemplation of the society about polycystic ovarian syndrome. The most common answer suggested that the people needed awareness concerning the polycystic ovarian syndrome and also most people said that it is significant issue to be discussed because there were misconceptions about polycystic ovarian syndrome.

Conflict of interest

We declare no conflict of interest.

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Section 6

Homeopathy

Chapter 6

Alternative Medicine: A Recent Overview

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Abstract

Alternative medicine has renewed its growing public interest in recent times due to inequality of patients and healthcare professionals' ratios with increased workload for the latter, various side effects of modern medicine, lack of complete remission from chronic diseases, high cost of new drugs, and emerging new diseases. Hence, people have become more dependent on treatment systems replying on alternative medicine or herbal medicine from traditional medicinal practitioners. Alternative medicine has grown substantially over time and encompasses several millennia of therapeutic systems. The significant areas of alternative medicine include mind–body therapies, body manipulation, and the therapies based on biological systems. Natural products based biological treatment is the most popular of them as nature has endowed us with abundance of effective pharmacologically active phytochemicals. These phytochemicals possess numerous specific clinical health benefits including antioxidant, antidiabetic, anti-inflammatory, anticancer, anti-infectious and analgesic effects. In addition, alternative medicine is easily accessible, affordable, most often noninvasive, and provides favorable benefits during terminal periods of some diseases. However, due to the lack of well-designed clinical trials, the safety and effectiveness of many alternative medicines/therapies remains elusive. This chapter will critically discuss major areas, uses, safety and regulation, current challenges & future perspectives of alternative medicine.

Keywords: alternative medicine, health benefit, safety & regulation, challenges & future perspective of AM

1. Introduction

Alternative medicine (AM) is a holistic approach recognized as a heterogeneous set of medicinal products and practices with potent healing effects. The practices remain as the most ancient yet traditions for treating different ailments continues [1, 2]. It is estimated that two-thirds of the world's population seek health care support and services from alternative sources over modern medicine. Recent statistics show that half of the global population are dependent on AM, including the USA 42%, Australia 48%, Canada 70%, and France 49% (**Figure 1**) [3, 4]. Despite current progress in modern medicine, the use of AM has been found to be radically focused on treatment of deadly pandemic diseases e.g., novel coronavirus disease 2019 (COVID 19) when there is no approved systematic targeted therapy yet [5].

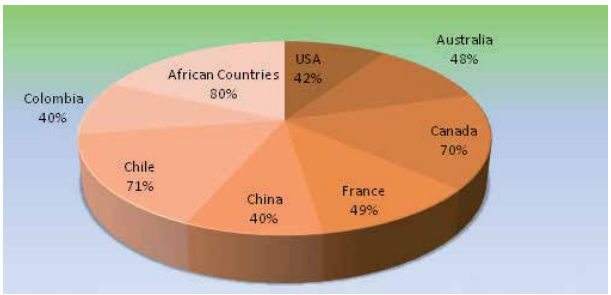

Figure 1.
Utilization of alternative medicine up to 2019.

The renewed public interest has revitalized due to the lack of curative treatment for several emerging and chronic diseases, high cost of modern drugs, time constrain from both patients and healthcare providers, microbial resistance and side effects of modern medicine [5, 6]. The most common treatments of AM are self-medication, traditional healing practices, indigenous systems of medicine particularly ayurveda, herbal preparations, yunani, homeopathy, acupuncture, naturopathy, chiropractic manipulation, etc. which have made AM more popular [1, 7]. In comparison, modern medicine focuses on symptom- related care, often utilizing pharmacological or invasive elimination procedures [8]. Although AM is not guaranteed to be safe, effective and biologically plausible [9], there is still a debate about which method can be proven as useful and secure. Old records encourage alternative modes whereas comprehensive clinical trials support conventional modes based on modern approaches [10]. However, today, many physicians accept the benefits of all forms of medicine, incorporating effective complementary and modern approaches in terms of patients, symptoms, and circumstances [11].

This scenario has necessitated development of knowledge bridge among physicians, traditional practitioners, pharmacist, and patients about AM treatments, safety use, and toxicity or contraindications. In addition, advancement of research efforts, minimizing publication bias, protecting intellectual property rights, and policymaker's contribution are required to make decisions about the future of alternative medical practice to provide cost-effective treatments. This would strengthen the position of AM industry and increase public acceptance in future [12, 13]. This chapter primarily discusses the different areas of AM, its uses, safety and regulation, current challenges and future perspectives.

2. Areas of AM

The National Center for Complementary and Alternative Medicine (NCCAM) has characterized the complementary and alternative medicine (CAM) as a community of various medical practices, methods and products currently excluded from modern medicine [14]. NCCAM has also categorized AM branches into five main groups: (1) traditional medical techniques, such as whole medical systems; (2) mind–body therapy; (3) biological substance-based treatment; (4) manipulative and body-based treatment; and (5) energy medicine [15].

2.1 Whole medical systems

A whole medical system is a complete system of theory and practice works independently or along with modern medicine. The methods contain various groups of

therapies or treatments that are practiced in diverse communities across the globe. Indian ayurveda and traditional Chinese medicine (TCM) are mainly practiced in the Eastern part of the world while homeopathy and naturopathy are predominantly used in the western region [16].

2.1.1 Ayurvedic medicine

Ayurveda is an extensive medical system that contemplates the body, mind, and soul essential to maintain the individual's wellbeing. Its fundamental purpose is to maintain good health instead of struggling against the illness. Various ayurvedic herbs or medicinal plants like turmeric, ashwagandha, amla, black cumin, rhubarb root, triphala, and kumanjam have medicinal properties for treatment of various diseases or health complications like cardiovascular conditions, cancer, neurological disorders, and diabetes [17, 18]. To determine the efficacy of the ayurvedic therapies, appropriate research with rigorous investigation is required [19].

2.1.2 Traditional Chinese medicine

Traditional Chinese medicine (TCM) originated thousands of years ago from ancient China and has flourished over time. Japan, Korea, and Vietnam have also developed similar systems for treatment of ailments [20]. TCM consists of several different techniques such as acupuncture, moxibustion, Chinese herbal medicines, nutrition, t'ai chi, qi gong and massage. However, the most frequent therapies are Chinese herbal medicine, t'ai chi and acupuncture [21].

2.1.2.1 Chinese herbal medicine

Chinese herbal medicine restores the balance of the whole body and equilibrates the forces of qi, yin and yang, which are basic elements of human body. Qi describes as a vital force energy which is carried throughout the body via meridians. Yin shows slow, cold and passive strength, where yang shows excited, hot, and active strength [22]. Chinese herbal formulas are known to have an advantage with regard to body regulation [23]. Several herbs tonify qi to treat patients with qi deficiency syndrome; some herbs promote yin to treat patients with yin deficiency syndrome and some reduce phlegm to treat patients with Phlegm syndrome. The medications related to Chinese herbal medicine are given in different ways like powders, tablets, and teas. Botanical extracts or plants are typically familiar to treat different diseases such as Chinese herbal medicine is often used as defensive care and improves health by stimulating an immune response before diseases arise [24].

2.1.2.2 Acupuncture

Acupuncture is a form of AM originated in China more than 2000 years ago. It is commonly used to alleviate pain or stress by inserting hair-thin needles through the skin at specific points on the body. Traditional Chinese medicine explains acupuncture as a technique for balancing the flow of energy or life force followed the principle of Yin and Yang. Acupuncture practitioners believe the human body has more than 2,000 acupuncture points connected by 12 pathways or meridians that interact with various organs such as heart, liver and kidneys [25]. Along these meridians, the energy flow rebalances by inserting the needles into specific points. In our contemporary lifestyle, numerous physical challenges arise due to the lack of proper physical activity, unbalanced food habits and lifestyle. Acupuncture has numerous positive effects against metabolic diseases, inflammation, digestive

issues, respiratory and nervous system problems [26]. In addition, releasing neurotransmitters and hormones also regulates neurochemistry, thus influencing the sensing and cognitive functions.

2.1.2.3 T'ai Chi

T'ai Chi is another type of AM of traditional Chinese medicine initiated during the 13th century in China. It is a movement technique that facilitates recovery through breathing and gradual movements of the body. The advantages of t'ai chi are improved mobility and balance, and reduced tension and anxieties [27]. It has been found to improve the quality of life, particularly those who are suffering from chronic diseases [28]. Many controlled and uncontrolled trials showed the effects of t'ai chi on various health conditions and diseases such as cardiovascular disorders [29], diabetes, osteoarthritis [30], anxiety, insomnia, functional mobility and fall prevention [31, 32]. The benefits of t'ai chi are generally most significant before developing a chronic illness or functional limitations. Tai chi is very safe, and no costly equipment is needed for the practice.

2.1.3 Naturopathy

Naturopathy is an integrating division of AM by combining traditional practices and health care approaches, and became popular in Europe during the 19th century. This medication system provides a unique way of treating patients, which maintains the homeostatic principle of the body, identifies the source as well as treats the diseases. Although many other allopathic or holistic therapy fields offer specific therapies to specific conditions, naturopathic practitioners tend to employ the self-healing process by maintaining healthier lifestyles, diet and nutrition [33]. Popular naturopathic therapies include physical treatments (light therapy, ultrasound and electric currents), dietary supplements, homeopathy, medical counseling, hormone therapy and personalized treatment modalities to relieve mental and emotional stress [34, 35].

2.1.4 Homeopathy

Homeopathy is another type of AM system discovered in the 19th century. Homeopathy comes from the Greek word in which homoios means 'similar' and pathos indicates 'suffering'. Homeopathic drugs treat diseases by triggering the body's natural defenses instead of fighting against them. The underlying principle of homeopathy is "like cures like". In other words, when a substance is capable of inducing a series of symptoms in a healthy living system, low doses of the same substance can cure these symptoms under certain circumstances ('similia similibus curentur') [36]. Hahnemann stated that treatments for a specific disorder could cause undesirable effects identical for the disease itself to stimulate a homeostatic or complementary reaction to correct these disorders [37]. This medicine industry solely depends on a "minimum dose law," in which dosage concentrations are inversely related to the active potency. Many homeopathic medicines contain active substances overly diluted and minimal amounts of active substances throughout the resulting dosages.

2.2 Mind–body therapy

Mind can control physical and biological processes, and the mind–body modality regulates the connections between mind, body, spirit, and attitude.

Many of the treatments involved in the mind and body's stimulation aim to maintain sound health and heal diseases. Mind–body therapies include relaxation, meditation, yoga, breathing hypnotherapy, cognitive behavioral therapy, and visualization. Music, movement, and dance therapy have shown to have beneficial roles for patients with anxiety [38, 39], while hypnosis, acupuncture, and music therapy serve as a successful therapy for depression and anxiety in cancer patients [40].

2.3 Biology-based therapy

Natural and biological-based practices refer to the substances made from nature or living things, such as herbs, special dietary and orthomolecular substances to improve, control, and regulate human health. Among these, herbal preparations, are the most common variety of CAM in the United States [9]. The mechanism of this therapy is to stimulate the immune system of the body and help to fight against cancer, infection, and other diseases. Common supplements used for biology-based therapy are botanicals, nutritional supplements, such as vitamins and minerals, probiotics, prebiotics, fatty acids, proteins, amino acids, and functional foods [41].

2.4 Manipulative and body-based therapy

Manipulative and body-based practices rely on structures and systems of the body, such as bones and joints, the soft tissues and the circulatory as well as lymphatic systems. It is one of integral tools of alternative medicine in which body can regulate and heal itself [35]. Various manipulative and body-based techniques are currently used – such as massage (normalizes the soft tissues), reflexology, craniosacral therapy, chiropractic (affiliated between spinal structure and role), rolfing, and osteopathic manipulation [42]. These therapies are thought to stimulate the body's energy and enables toxins to leave the body.

2.5 Energy therapy

Energy therapies are based on the belief that vital life energy flows through the body. The goal of energy therapy is to restore energy balance in the body by unblocking flow of energy. The ancient Chinese healing traditions, energy therapies were well-established as a technique for easing pain, reducing anxiety, and mitigating side effects of cancer treatment. Energy therapies focus either on energy field originating into the body (biofields) or from other sources (electromagnetic fields). There are different energy medicine techniques, including hands up and down and remote therapies [43]. Biofield therapy aims to trigger the energy that covers and penetrates the body and has not been experimentally proven to exist. Certain types of energy treatment control biofields by putting pressure or controlling the body by bringing the hands in or through therapies touch [44]. Different ancient Chinese arts like qigong, which put together subtle physical action, deeper breath, and mental intensity, regulate the human body. The approach integrates body and soul efficiently and productively [45]. Another type of energy therapy denoted as bioelectromagnetic therapy is based on an electromagnetic field used to treat or prevent diseases, and promote health and longevity. It may be given singly or in combination with many other methods. This therapy involves different magnetic fields, pulsed fields, direct or altered electric sources to treat many kinds of ailments like asthma, cancer and migraine pain [46].

3. Uses of AM in different ailments

Alternative medicine became much popular over the past several decades. The use of this medicine has always been commonly seen among Chinese and other Asian patients in the countries such as Korea, Taiwan, Singapore, India and Hong Kong. A number of AM has been often used to manage some chronic diseases namely diabetes, cancer, cardiovascular diseases (CVD), asthma, menopause, rehabilitation, autism spectrum disorder etc. [47–53]. Patient characteristics, socio-demographic status, and gender are the predominant determinants of AM use. The following describes the multiple uses of AM in different fields.

3.1 Control of blood glucose

Diabetes mellitus (DM) is the most prevalent and chronic metabolic disorder. The worldwide prevalence of diabetes has risen approximately from 4.7% to 8.5% over the last 34 years [54]. To control blood glucose levels, numerous modern antidiabetic drugs have been discovered and introduced in the market. However, most of the drugs may have some drawbacks when it is used for long time, such as drug resistance, drug addiction, adverse side effects and so on [55]. In addition, the therapeutic expenditures and dissatisfaction with mainstream have prompted the search for alternatives [47]. Intriguingly, the treatment strategies of diabetes is in favor of alternative practices. Along with conventional drugs, diabetes patients are treated by diet and exercise [56]. Therefore, it has drawn much attention as the effects of AM particularly herbal medicine has been found effective in diabetes prevention, management and/or delay its complications.

The alternative treatment of diabetes is mainly accomplished by non-pharmacological ways including diet therapy, relaxation, kinesitherapy, acupuncture therapy, psychotherapy, hydrotherapy, yoga etc. [57]. Natural Health Products (NHP) based therapy including vitamins and minerals, herbal remedies, homeopathic medicines, traditional medicines, such as traditional Chinese medicines, probiotics, and other products like amino acids and essential fatty acids are also commonly used for the effective management of diabetes [47, 58]. All are used in both type 1 and type 2 diabetes and found to improve diabetic condition significantly, or even, it can revert from prediabetes to normal stage [59, 62]. For example, the traditional Chinese medicine Shenzhu Tiaopi granule (SZTP) decreased the conversion rate of 8.52% from impaired glucose tolerance (IGT) to type 2 diabetes and 15.28% from with placebo, and normalized blood glucose from patients with IGT [59]. NHP have also been shown to improve diabetes complications by reducing 0.5% glycated hemoglobin within 3 months [47]. The following products are used in the treatment, and prevention of diabetes and its complications:

- Ayurveda polyherbal formulation, *Citrullus colocynthis*, *Coccinia cordifolia*, Eicosapentaenoic acid, *Ganoderma lucidum*, Ginger (*Zingiber officinale*), *Gynostemma pentaphyllum*, *Hintonia latiflora*, lichen genus *Cladonia* BAFS "Yagel-Detox", marine collagen peptides, soybean extract etc. are used for T2DM management [60]

- Traditional Chinese medicine herbs are also used for DM treatment like, fructus mume, gegenqinlian decoction (GQD), jianyutangkang (JYTK) with metformin, jinlida with metformin, sancaijiangtang, shen-qi-formula (SQF) with insulin, tang-min-ling-wan (TM81), xiaoke (contains glyburide), zishentongluo (ZSTL) and *Trigonella foenum-graecum* (fenugreek) [61]

- A few products, such as vitamin D, vitamin E, L-carnitine, cinnamon, gymnema, green tea, fibre, bitter melon, momordica, chromium, and vanadium have been the subjects of special interest in diabetes [62].

- *Panex ginseng* and *P. quiquefolius* (ginseng) play significant role in controlling diabetes by altering hepatic glucose metabolism, however, evidence of its clinical use in patients with diabetes is scarce [63]

For decreasing stress-related hyperglycemia, mind–body medicine, such as yoga, reflexology, chiropractic or osteopathic manipulation, homeopathy, shiatsu, registered massage therapy or craniosacral therapy have been shown short term significant benefits in clinical use, however, specific mind–body interventions and long-term improvements in glycemic control have not been found in larger randomized controlled trials (RCTs) [64].

3.2 Management of inflammation

The body naturally responses to various stresses including infection, irradiation, chemical, or physical injury [65]. Short term inflammation protects the body, while long term inflammatory response in the body damages healthy cells, tissues, and organs leading to the development of some diseases, such as arthritis, alzheimer's disease, and even cancer [65, 66]. The common treatment option for inflammatory diseases have been limited to nonsteroidal anti-inflammatory (NSAIDs) medications such as COX-2 inhibitors or steroid hormones (e.g., corticosteroids). Although most of the NSAIDs are considered to be safe however, it may aggravate other diseases such as stomach ulcer, hemorrhage, liver or kidney impairments for long term use [67]. The National Kidney Foundation reported that in each year, approximately 10% of kidney failures are directly associated with the substantial overuse of NSAIDs [68]. AM has been used for hundreds and even thousands of years in the management of chronic inflammation through antioxidative alternative medicine-based therapies, mainly diet- and natural products based therapies [69]. Strong scientific evidence supports the use of some products such as omega-3 essential fatty acids (EFAs) (ω-3) as an alternative and/or complementary agent to NSAIDs [70]. Capsaicin, oil of camphor, is commonly being used for muscle soreness and it has also local application for painful traumatic injuries [68]. Epidemiological studies and associated meta-analyses strongly suggest that long term consumption of diets rich in plant polyphenols (red and blueberries, green and black tea) protects the body from cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases [71, 72].

Other alternative practices namely exercise, mind–body treatments like t'ai chi, qigong, yoga, meditations, massage, acupuncture, and moxibustion may decrease pain intensity by reducing circulatory concentrations of proinflammatory cytokines like IL-6, IL-18, C-reactive protein and other circulatory inflammatory cytokines like IL-1α, and TNF-α, through controlling the expression of these proinflammatory and inflammatory marker genes [73, 74].

Music therapy plays an important role in alleviating pain of various etiology. A systematic review of 42 RCTs documented the effect of music therapy on relieving preoperative anxiety and stress as well as postoperative pain in cardiac surgery [75]. A Cochrane database of systemic review of 51 studies concluded that listening to music reduced pain intensity and opioids requirements [76]. Thus, the above findings strongly support the importance of different alternative approaches to pain and inflammation management, and better understanding on the mechanism and function associated with AM may provide new insights to treat inflammatory diseases.

3.3 Asthma treatment

Asthma is a common, multifactorial respiratory disease with chronic inflammation of the respiratory system affecting more than 300 million people world-wide and 25 million people in the United States, including 1 in 10 US children (10%) [77]. Common symptoms of asthma include: wheeze, cough, shortness of breath, and chest tightness. Despite advancement of modern medicine and its treatment modalities, many people are turning to alternative medicine as an another option for treating respiratory diseases.

Several types of alternative medicine are used in asthma treatment such as herbs and supplements, yoga, relaxation therapy, and biofeedback [50, 78]. Herbal products and dietary supplements have been used for thousands of years to treat lung problems. Ethnobotanical Survey in Nigeria found 87 local medicinal plant species from 39 families and these plant species are being used for treating cough associated respiratory diseases [79]. Whole plants, leaf, roots, fruit etc. are preferentially used to combat the diseases [9]. Korean ginseng root extract has potential role for treating lung inflammatory disorders. Some Chinese herbs, like ding-chan tang, may decrease inflammation and relieve bronchospasm [80]. The fruits of *Momordica charantia* L. are commonly used for cold, cough, tuberculosis, and asthma [81]. Again, caffeine is a natural and mild bronchodilator, which can improve airway function in people with asthma. Further, supplements like magnesium and fish oil (omega-3 fatty acids), vitamin C, D, and E may reduce inflammation and alleviate asthma symptoms. Moreover, both breathing exercises in yoga and massage therapy can control breathing and relieve stress [50, 82]. Although much of the research is currently under investigation or found to elicit significant improvements of the diseased conditions yet some findings indicate that many natural and over-the-counter products have potential side effects.

3.4 Management of cancer

The most common modern treatment modalities for cancer are surgery with radiation and/or chemotherapy, and immunotherapy. However, these therapies possess severe side effects including fatigue, skin problems, hair loss and low blood count [83]. Thus, many cancer patients and health care practitioners prefer AM as a potential therapeutic management [84]. AM may provide numerous health benefits by managing disease symptoms, preventing illness, or improving immune function [85]. The widely accepted and safe alternative practices are acupuncture, aromatherapy, massage therapy, exercise, hypnosis, meditation, music therapy, relaxation techniques, tai chi and yoga [86].

The uses of AM vary among different cancers. The highest uses of AM are found in breast cancer patients (93%), followed by colorectal cancer (83%), prostate cancer (77%), and lung cancer (77%). Each of the 4 cancer types, dietary supplements were the prominent alternative modality (52% to 82%), followed by energy medicine (39% to 55%), mind–body medicine (16% to 52%), and body-based therapy (14% to 42%) [87–88]. Although AM is not powerful enough to replace modern medicine, it may be used parallelly with modern medicine for better management in cancer patients. The following alternative practices are commonly used in different symptoms related care

- Hypnosis, massage, meditation, prayer, relaxation techniques are predominantly used to relief patients from anxiety [89]
- Exercise, message, relaxation techniques and yoga reduce fatigue and improve quality of life in cancer patients [90]

- Acupuncture, aromatherapy, hypnosis and music therapy prevent nausea as well as vomiting

- Acupuncture, aromatherapy, hypnosis, massage and music therapy are helpful in relieving pain [84, 86]

- Exercise, prayer, relaxation techniques and yoga may help cancer patients to sleep better [86, 91].

There are also some alternative modalities used in cancer patient's treatment, which are outlined as

- Dietary treatments including gerson, ketogenic, peskin, budwig, alkaline, paleo, vitamins and minerals, and herbalism [92].

- Biologic products-based therapy including different kinds of tea (e.g., green, medicinal, chaga mushroom, Essiac), natural health products such as ginger, curcumin, flaxseed oil; and miscellaneous products like pancreatic enzyme therapy, medicinal cannabis, laetrile B17, and probiotic foods and supplements [48].

- Energy therapies based on therapeutic touch and reiki, which use surrounding subtle known energy field and penetrate the human body [93]

- Alternative medical systems that are mainly traditional Chinese medicine, Indian medicine, homeopathy, chiropractic etc. used in different cancers [23, 48].

- Improvements in physical and psychosocial well-being and increasing hope to the cancer patients e.g., osteopathy, and Aboriginal medicine.

- Certain natural products (taxol, vinca alkaloids) are also much famous [94].

3.5 Management of blood pressure and CVD

CVDs are the leading cause of deaths all over the world. The recent advances in modern western medicine have been made available for treating CVDs, however, the complications and disease recurrence still occur, which compromise quality of life. Noticeably, AM has drawn great attention to treat such chronic CVDs for long term benefits by relieving symptoms, rehabilitation, and even in preventing these diseases.

Many of the natural products can act more directly on cardiovascular homeostasis by improving lipid profiles and vascular reactivity, and reducing the undesirable immune response [95, 96]. Diet should be regarded as a cornerstone of preventive medicine and, at least in part, as a viable treatment for blood pressure (BP), CVD and other chronic diseases [49]. Certain dietary supplements like fish oil, multivitamins, and coenzyme Q10 are considered the best preventive medications [97].

Beyond dietary strategies, certain additional non-pharmacological treatments have been shown to lower BP. These alternative approaches can be broadly classified into three categories: behavioral therapies, including meditation, yoga, biofeedback, and relaxation or stress-reduction programs; noninvasive procedures or devices, including device-guided breathing modulation and acupuncture; and

exercise-based regimens, including aerobic, resistance, and isometric exercise methods [98].

Traditional medicine methods, including acupuncture, electroacupuncture, and transcutaneous electrical acupoint stimulation, have been increasingly adopted by health-care professionals despite the lack of evidence on its effects on CVDs [99].

Medicinal herbs namely *Allium sativum*, *Ginseng, Aesculus hippocastanum*, *Ginkgo biloba*, *Salvia miltiorrhiza* have been used in patients with atherosclerosis, hyperlipidemia, systolic hypertension, cerebral and venous insufficiency, angina pectoris, and congestive heart failure [100–102]. In fact, numerous bioactive compounds present in the herbs can prevent vascular smooth muscle cell phenotypic switching, endothelial dysfunction, platelet activation, lipid peroxidation, ROS production, and macrophage atherogenicity, and thus, it may have the ability to modulate the CVD incidence [49, 103]. However, the role of these herbs in CVDs still needs more clinical evidence and elucidation of definite mechanism of actions.

A Cochrane database of systematic review of 23 RCT's concluded that listening to music has beneficial effects on BP, heart rate and respiratory rate, and also on anxiety and pain in persons with coronary heart disease [104].

Therefore, alternative medicine use in patients with CVDs seems to be common, however, a more patient-physician communication about the use of AMs and evidence-based research are required.

3.6 Alternative medicine for management of anxiety or sleep disorders

Anxiety disorders are the most common psychiatric disorder, with an estimated lifetime prevalence is 29% in the general population [105]. The high prevalence and complex comorbidity of anxiety or sleeping problems such as insomnia makes a concern particularly in elder people because it affects physical and mental health, and worse the quality of life by relating with significant clinical implications in obesity, diabetes, hypertension, cardiovascular and neurological diseases [106]. It is well documented that treatment of anxiety or insomnia may provide positive effects, not only by alleviating comorbidity but also by preventing new incidents.

Insomnia patients can be treated by alternative therapies namely herbs, supplements, relaxation and meditation, acupuncture, and exercise. Among these therapies, biologically-based products such as herbal or nutritional medicine, and mind–body therapies, are the most commonly used interventions. For instance, herbal supplements particularly Valerian root and Chamomile may help to fall asleep faster and boost the quality of sleep however, more research is needed for the safety and efficacy [107]. Melatonin is the key natural hormone in the sleep–wake cycle produced by the pineal gland, regulates numerous biological functions including circadian rhythm, sleep, stress response, aging, and immunity [108]. Aromatherapy with lavender (*Lavandula angustifolia*) increases serum melatonin levels as evident from nonrandomized clinical trials on older adults [109]. Furthermore, acupuncture, relaxation and meditation, and regular exercise may improve sleep quality, sleep onset latency, total sleep time, and insomnia severity [110–112]. Lately, high levels of anxiety, fear, depression, panic, emotional outburst and sleep disturbances has been observed in COVID-19 positive patient, particularly from isolation and quarantine events [113]. Some sedatives and antianxiety drugs are used to manage these symptoms however, it may inhibit the respiratory system and worsen the condition [114]. Evidence suggests that progressive muscle relaxation technique can reduce anxiety and improve sleep quality in patients with COVID-19 beyond the side effects [115].

Music has a powerful effect on our emotions, therapy such as mind–body medicine has a direct influence in antianxiety effect. Listening to music may have an immediate positive impact on stress-related physiological, cognitive, and emotional processes [116]. It enhances parasympathetic activity, increase physiological coherence, reduce the cortisol ratio, and boost immunity. A systematic review of 23 studies on physiological parameters, the anxiolytic effect of music therapy is proved in all the studies [117]. Moreover, it has been shown that music can increase comfort, decrease anxiety thereby can improve sleep disorder [118]. Hence, healthy and safe alternative practices may at least in part, replace the strong anti-anxiety medications thereby improving the quality of life in these patients.

3.7 Treatment of menopause

Hormone therapy is one of the most effective treatment for managing symptoms of menopause. However, many women need to avoid hormone therapy due to health risks from stroke, heart attack and cancer. In these cases, AM is preferred for symptom management [51].

There are various alternative interventions for the treatment of menopause. They fall into two main categories: a) mind–body practices that combines mental focus, controlled breathing, and body movements resulting in relaxation of body and mind. It has significant health benefits by reducing pain, stress, anxiety, and mood. Some common mind–body practices are meditation, hypnosis, cognitive behavioral therapy, biofeedback, yoga, and tai chi, and b) natural products-based intervention by using herbs, vitamins, minerals, and dietary supplements [51, 119]. Apart from these categories, some interventions based on system-wide AM have been commonly used such as traditional Chinese medicine, reflexology, acupuncture, and homeopathy [120]. Several studies indicate that mind–body practices such as hypnotherapy, meditation, relaxation etc. are beneficial in reducing problematic menopausal symptoms [119, 121]. Therefore, AM may improve the quality of life particularly, the women who are transitioning to menopause. Finally, though natural products, such as herbs, vitamins, minerals etc. are commonly used for remedy of symptoms related menopause, consistent evidence to support their safety and efficacy still remains elusive [122].

3.8 Management of rehabilitation

Patients in rehabilitation of musculoskeletal conditions often use alternative medicine treatments. Commonly used treatments including massage therapy, acupuncture, manipulation medicine, yoga and pilates, mind–body medicine, effleurage, petrissage, friction, tapotement, and vibration [52, 123, 124].

Massage therapy is one of the most commonly used therapies for athletes to enhance recovery and performance, particularly postexercise [123]. The benefits from therapeutic massage are enormous such as relieve of muscle tension and stiffness, healing of strains and sprains; reduce muscle pain, swelling and spasm; improve flexibility and motion, enhance blood flow and so on [125, 126].

3.9 Treatment of autism spectrum disorder

Autism spectrum disorder (ASD) is a heterogeneous group of neurodevelopmental conditions, which is characterized by impaired social interactions and communications, restricted, repetitive, and stereotyped patterns of behavior and interests [127]. It is assumed that both genetic and environmental factors play a key role in ASD etiology, but no clear pathogenesis has been identified yet [128]. Although autism is a lifelong disorder and there is no causal treatment currently

known, AM may stand as an therapeutic option for alleviating symptoms of patients with autism spectrum disorder.

Biologically based therapy including dietary supplement (vitamins and minerals), and herbal medication (meadowsweet, calendula, chamomile, marshmallow root and lemon balm etc.) can be used for treating ASD. In addition, mind–body medicine (i.e., prayer, yoga, music, dance, and art in general), manipulative and body-based practices (i.e., massage, chiropractic care, and acupuncture), and energy medicine (i.e., reiki or homeopathy) are useful for treating ASD [129]. Music therapy may have strong impact in autistic children. Cochrane meta-analysis showed that listening music significantly improved the cooperation and communication ability in autistic children [130]. Another study remarked that music therapy might provide a basic and supportive therapy for children with delayed speech development [131].

Though some trials demonstrated the importance of chosen alternative therapies (e.g., equine therapy) and have gained attention by the scientific community, there is insufficient evidence to assess the safety and efficacy of AM [9, 132]. Therefore, combination of standard medical therapies along with safe alternative approaches like diet, exercise and lifestyle modification might benefit patients from functional disorder like autism.

3.10 Prevention or treatment of COVID-19

COVID-19 is considered as a life-threatening disease, which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [133]. To date, it has been accounted as a global public health emergency and declared as a pandemic by World Health Organization (WHO) as there is no specific antiviral treatment available in the modern medicine system [5, 19]. Although several attempts have been initiated after the disease onset, truly effective vaccine is still unavailable [134, 135]. A few vaccines exist in the market but the safety and efficacy need further scrutiny using multi-site clinical data [134]. Under this circumstance, a more rational phytotherapeutic choice to the disease may be a cheaper option for prophylaxis or treatment against this virus [136]. Strikingly, the phytocompounds of *Momordica charantia* L. and *Azadirachta indica* have been recently shown adequate inhibitory potential aganist SARS-CoV-2 when compared with FDA reference drugs such as ribavirin, remdesivir and hydroxychloroquine [137]. In China itself, the total number of confirmed cases treated by TCM has reached 60,107 [138]. Indian government ministry of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy (AYUSH) recommended homeopathy and ayurveda for prophylaxis and unani medicines for symptomatic management of COVID-19 [139, 140]. In Bangladesh, herbal and fruit extracts have been used to get relief from COVID-19. Infected people are advised to drink masala tea, ginger tea, and lemon with hot water for recovery [141].

It has been shown that Chinese, Indian and Iranian herbal medicine with 1000 years' experience in the prevention of pandemic and endemic infectious diseases are worth learning, and provide alternative candidates for controlling patients with COVID-19 infection [19, 142]. As there are no effective treatments for COVID-19, it provides one of the biggest opportunities to test different plants and discover new targeted bioactive compounds for therapeutic management of this disease.

4. Safety and regulatory issue of AM

Though approximately 80% ailing people in developing countries rely on AM as a source of primary healthcare or traditional medical practice [143], there is limited scientific evidence regarding the potential toxicity of a variety of AM [9].

In fact, most of the AM are untested and the safety and efficacy are either poorly or not even monitored at all [144]. Moreover, doctors and nurses are not trained enough to describe the potential side effects and contraindications to their patients [145]. Again, not all health professionals favor the concept of integrative health approaches, neither do they have the confidence in dealing with CAM due to lack of knowledge on standardization of practices and overall benefits of holistic approaches. In contrast, most of the patients who use CAM are hesitant to inform their primary health care provider about the methods for fear of disapproval [145]. Further more, biological substances are not tested rigorously to ensure their safety and efficacy in the context of pharmaceutical products because FDA approval is not mandatory in case of a new therapeutic component [146]. Manufacturer only need to attest as a dietary supplement's safety, purity, and contents by expressing on the label before marketing. However, some medicinal plants might be inherently toxic. Herbal products may also cause adverse side effects including hypersensitivity reactions, cardiovascular events, neurologic dysfunction, hepatic and renal failures, and the development of malignant disease due to the presence of mercury, lead, arsenic, corticosteroids and poisonous organic substances [147, 148]. Adverse events may also arise from the lack of knowledge by selecting wrong species of medicinal plants, incorrect dosing, interactions with other drugs and error in the use of herbal medicines [144]. For example, the herb arnica, black seed and feverfew stimulate uterine contractions and possible miscarriage in pregnant women [149, 150]. In addition, ginkgo Ginkgo biloba and chamomile (Matricaria chamomilla) may increase the risk of bleeding in patients taking nonsteroidal anti-inflammatory drugs like aspirin, and anticoagulant-warfarin. Even many forms of AM are rejected by orthodox medicine as the safety and efficacy of the drugs have not been confirmed in clinical trials [144].

The regulation on AM varies widely from country to country because each country has their own regulations policy. In most countries, the AM market is poorly regulated, and the medical products are often neither registered nor controlled [151]. However, relatively few countries have developed policies and regulations on TM/AM. Among the 194 Member States of WHO, only 98 countries have a national policy on TM/AM, and 109 countries regulate herbal products. The WHO African and South-East Asian countries (>80%) have the highest percentage of national or state level laws and regulations for traditional and AM whereas, European (40%) and American (43%) region have the lowest percentage [152]. In the United States, TM/AM legislation is the responsibility of state, provinces or territorial jurisdictions, and regulation varies from jurisdiction to jurisdiction.

In the United Kingdom (UK), there is no regulation that restricts the practice of AM except of chiropractic and osteopathy. However, now the UK Government has gradually acknowledged the need for extensive regulation of AM. Chiropractic and osteopathy have adopted statutory self-regulation, though this has proved expensive for individual members of these professions. A recent House of Lords has recommended that the herbal medicine and acupuncture professions should also develop a system of statutory regulation. Some occupations, such as aromatherapists, are in the process of forming a common professional body as a first step towards self-regulation [153].

Hence, it would be helpful to increase training opportunities for health care professionals and share information to their patients about potential interactions of AM with modern treatments. Finally, the knowledge of the usage, safety and efficacy of AM as well as the evolution of awareness may increase the ability of health care providers to follow the legislation.

5. The current marketplace of AM

The global demand for AMs was reported at USD 69.2 billion in 2019 and is rising every day. Different energy healing therapy comprises reflexology, reiki, and havening techniques are increasingly used in anxiety and mental disorder patients in different countries. Nowadays, many magnetic therapies such as bioflex magnets, mattresses, and magnabloc for pain reduction are used. Other alternative therapies like yoga, meditation, and spa have been well attributed globally due to their popularity, which led to development in the number of yoga studios, meditation centers, spas, and complementary healing facilities institutes in the particular communities [154–156]. Moreover, some alternative medicine services are now offered as benefits in state Medicaid programs, Medicare, and private health insurance plans [157, 158]. Study shows that at least 50% American medical schools are currently offering courses in alternative medicine to their medical students. Among which 25.0% of the courses referenced personal growth or self-care through alternative practices, while only 11.0% referenced inter-professional education activities involve interaction with alternative medicine providers [159]. In the promotion of CAM, a governmental initiative can play a crucial role. In India "Ministry of Ayush" has been set up by national authorities to govern research, development, increased funding opportunities, education, and other facilities pertaining to ayurveda, yoga, naturopathy, and homeopathy [154]. Therefore, the expensive existence of mainstream treatment and governmental facilities devoted to alternative therapies may encourage companies to invest in alternative medicine markets.

6. Major challenges for AM

Alternative treatment has improved our awareness and centered our view of medical treatment, but it still faces tremendous challenges. After two eras of robust efforts by the NCCAM at the National Institute of Health (NIH) on behalf of AM research, it remains an extreme challenge for scientists to analyze thousands of years' worth of clinical research issues to demonstrate the safety as well as efficacy of AM [160]. The complex and complicated, multivariate and multifaceted factors of AM systems require continual innovations for comprehensive and well-designed studies. The control trends of existing biomedicine restrict alternative treatment research, which must be expanded and extended [161]. The exploration and eventual discovery of plausible scientific mechanisms, theoretical and historical investigations are essential to further and fully understand the holistic role of alternative medicine and claim it within the realm of modern medicine [162]. In many cases, alternative therapies are commonly documented as false cases due to proper public awareness [124, 146]. Many modern medical practitioners and physicians are reluctant to discuss the importance of new effective AM with patients. A study showed 89% of patients were self-referred to an alternative practitioner and 72% did not inform to their physicians about their AM use [163]. In addition, alternative treatments defy the scientific procedures in terms of objectivity, measurement, codification, and classification because it comprises physical and spiritual realms, that cannot be subjected to scientific analysis [154]. So, it is essential to generate important insights into comparative clinical efficacy trials to improve patients' treatments, especially for long-term results.

7. Conclusion

AM has been practiced in numerous countries before the advent of modern medical science but its usage is not supported by the medical community due to lack

of evidence-based safety and effectiveness evaluation. Despite the promising results reported with various natural and biologic products, the clinical efficacy of such alternative therapies is yet to be determined. More than half of the world's population does not have access to modern medicine where most funding for healthcare in the developing world goes to 20% of the population and it can certainly be presumed that healthcare costs will be expected to double over the next decade. Low-cost intervention, such as lifestyle modifications, diet, supplement therapy and behavioral medication, can be used as a replacement for prescribed high-cost medications and technological innovation. More research of AM treatments in humans are needed to elucidate whether alternative treatments can have beneficial effects when they are used alone or have additional benefit while used with modern treatment methods. As a result, its usage requires exploration and eventual discovery of plausible scientific mechanisms, theoretical and historical investigations, continual innovations, comprehensive and well-designed studies in order to validate, advance and fully understand the holistic roles of AM and position it appropriately within the context of modern medicine. It is imperative that medical practitioners and physicians need to be aware about potential alternative therapies and discuss benefits and potential adverse effects or limitations with patients. With concerted efforts involving different relevant stakeholders including medical and research councils in different countries, systematic approaches could be developed and incorporation of standardized procedures, awareness of validated, authenticated and easily accessible scientific resources can substantially improve the current scenario of AM and meet the increasing healthcare needs of global population.

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Conflict of interests

The authors declare that they have no conflict of interests.

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Chapter 7

Thermodynamic Aspects of Homeopathy

Mihael Drofenik

Abstract

The well-known definition of disease, which Samuel Hahnemann presented in a tentative theory for his new science and art of healing, is used as the starting point for the thermodynamic model of homeopathy. The *Le Chatelier principle* was applied to the biochemical equilibrium compartmentalized in the individual human cells of an ill person to explain the curing based on the re-establishment of the starting equilibrium of a healthy person when using a remedy. It is revealed that a high dilution accompanied by succession is required to release the remedies to their constituent molecular species in order to increase their activity when taking part in the biochemical equilibrium that is essential for healing. In addition, a single remedy reaction-product species, when it is in excess, as well as satisfying the kinetic equilibrium, is a necessary and sufficient condition to force the new biochemical equilibrium in the direction of the basic original equilibrium associated with a healthy state. In addition, homeopathic aggravation is considered on the basis of the Law of Mass Action and the role of the small remedy concentration in some high-profile models is revisited. The second elementary law of homeopathy, the Law of the Infinitesimals, was explained based on a kinetic model. When a remedy occurs in the human cell of a healthy person and forms a reaction product (Simillimum) that induces the finest medical symptoms of an ill person, then remedies entering the cell of the ill person will form identical Simillimum molecules and re-establish the initial equilibrium of the healthy state and cure the ill person. However, this will also induce a molecular crowding in the cells of the ill person. For kinetic reasons, this will aggravate the re-establishment of the initial equilibrium and consequently worsen or even interrupt the medical treatment. At a low remedy concentration, the molecular crowding becomes negligible while the formation of the Simillimum and the re-establishment of the initial equilibrium will take place continuously and cure the person who is ill. The final understanding of the Simillimum in the thermodynamic model was illuminated and wide-opened its duality with the ill person's key compound.

Keywords: homeopathy, thermodynamics, aggravation, Le Chatelier, Simillimum

1. Introduction

Homeopathy has been a medical phenomenon since the beginning of human history. It was positively considered during different periods of our development in a non-optimal form when the therapy had not yet been optimized. After Samuel Hahnemann [1] described the routine of homeopathy in the late 18th century, this activity has continued in an optimized way up to the present day. Today it works as

a result of Hahnemann's ideas and experiments developed in the late 18th and early 19th centuries and spread to different continents and was preserved in the same form for about 200 years. Despite the great advances in instrumental engineering and the basic science available in the field of biochemical research, there is still no scientific explanation for homeopathy.

On the other hand, due to the increasing use of homeopathy (it is now used by over 30 million people in Europe), studies of its medical effectiveness and the theoretical basis of its action are important. Not least because it is central to the social and economic development of society [2].

The entire scientific development of homeopathy is related to the ancient empirical observation that a disease can be cured by a substance (known as the remedy) that produces similar medical symptoms in a healthy person. The amount and the delivery of the medicine can be varied; however, its fundamental importance to homeopathy is critical.

The empirical observation of homeopathy (in Greek "homeo pathos") is the way of considering "like cures like"; the phenomenon that a sickness can be healed by an ingredient that produces similar symptoms in a healthy person. This is the primary axiom of homeopathy, often referred to as the "Law of Similars." It dates back to Hippocrates (460–377 BC) and even as far back as mankind's early development and was accepted very early by the ancients and perhaps even before those times. In other words, this axiom is a phenomenon that has been part of human history from the very beginning and was used in various periods of our development, and continues in a similar form to the present day [3].

Today, the literature covers a lot of research related to the scientific validation of homeopathy and to technical issues related to a comparison of this discipline with pharmacology. In particular, the physicochemical nature of homeopathic medicinal products obtained by the sequential dilution method is often the subject of research.

Investigators have produced many explanations related to the mechanism of homeopathy and its impact on treatment, depending on the concentration of low doses of the drug in the human body. Among them, the clarification of "water memory" has often been the topic of publications [4–12]. In addition to these evaluations, several models have been published: quantum mechanical entanglement [13, 14], quartz crystals and the structural concept of glass [15], electromagnetic activities [16], biological signaling [17], the nonlinear dynamics of complex systems [18, 19] the stress-effect model and hormesis [20, 21], the biopsychosocial model [22] and the thermodynamic model [23–25]. Bell and Koithan recently published an extensive article reviewing the results of key publications [26], and Vithoulkas published a book [27] covering scientific explanations and the practical application of homeopathy.

2. Law of Similars

We will begin with the well-known explanation of disease presented by Samuel Hahnemann when he tried to make a preliminary theory available for his new science and the art of healing. He said, "Illness is the destruction of the life force." On this basis, a cure would be "to return the reduced life force to its original state of tuning" [1]. Here, we will interpret this statement with the basic principles of chemical thermodynamics and use the term "basic biochemical equilibrium," associated with a healthy state, and the term "new biochemical equilibrium," with an unhealthy state. So, curing would be the return of the new biochemical equilibrium to the original biochemical equilibrium.

In other words, he was of the opinion that the disease in patients is a retreat from the tuned state and healing is associated with the establishment of the basic state. This idea is upgraded here with the basics of chemical thermodynamics, which was not available in his time. La Chatelier's (1850–1936) principle appeared after Hahnemann's (1755–1843) scientific work.

So, this thermodynamic model, which explains in detail what is happening in homeopathy and explains the core of homeopathy, the "Law of Similars," using the chemical equilibrium, is the result of scientific development created after Hahnemann's research work in the field of homeopathy. Thus, the basis of the Law of Similars is associated with the thesis "when it is possible to establish a healing process in which a drug that causes the same symptoms of disease in a healthy person can cure a sick person, then the only possible explanation for such a treatment (in today's understanding of the matter) is the presence of a suitable biochemical equilibrium." As a rule, all biochemical equilibria are subjected to the basic thermodynamic principle, the so-called Le Chatelier principle, which has been known in chemistry for more than a century and is thermodynamically well founded: if a chemical system experiences a change in concentration, temperature or even pressure, equilibrium will shift so that these changes are minimized.

This principle makes it possible to predict the shift of any equilibrium in a chemical reaction. However, it is much more general and can be extended to all processes in which a kinetic equilibrium is the essence of the process. According to this thermodynamic principle, biochemical equilibrium will alleviate any disturbance by moving the equilibrium in the direction that it will alleviate. So, when a remedy that causes the same disease symptoms (disturbance) as the original disturbance that moves the equilibrium from the initial equilibrium to the new one that causes the disease, enters that system (the human body), it will be, in light of the Le Chatelier principle, the new biochemical equilibrium that alleviated this disturbance and shifted the equilibrium in the direction of the initial equilibrium related with the healthy state. This is the basic thermodynamically grounded mechanism of the healing principle of homeopathy and covers the "Law of Similars" and follows Hahnemann's definition that the "detuned state" will return to the initial "tuned state," representing a healthy person.

When we apply this phenomenon in clinical homeopathy and consider homeopathic curing in the above concept we are confronted with three mutually connected and clinically supported processes: (i) the appearance of the specific illness symptoms of the targeted disease in an ill person, (ii) the appearance of identical illness symptoms when a healthy person digests a remedy (a remedy is a substance that induces identical illness symptoms in a healthy person) and (iii) the disappearance of similar illness symptoms in the course of curing when an ill person digests a remedy. In particular, the appearance and disappearance of illness symptoms, i.e., between (i) and (iii) processes are decisive. Namely, when we equalized the illness symptoms with characteristic biochemical reactions, then the appearance and disappearance of the illness symptoms mean direct evidence for the re-establishment of the initial biochemical equilibrium when consuming the remedy, which must take place, as the basic natural principle, the Le Chatelier principle, and demonstrates the Law of Similars, which acts as the basic natural law.

The Le Chatelier principle can be considered in a more quantitative way and interpreted using basic thermodynamic values. Thus, in an ill patient, the overall biochemical equilibrium deviates from the healthy state. This new equilibrium state then regulates the status of the ill patient; however, in such cases, visible signs of the patient's behavior and appearance indicate that the expected medicine is a deviation from the norm and/or a healthy condition. Thus, the patient's behavior is indirectly related to the important biochemical reactions and/or reaction products

of the new equilibrium state. The same status can be achieved if a healthy person takes the remedy.

We will consider the biochemical equilibrium between the healthy condition $[A] = \Sigma\nu_{na}A_n$ (which determines the status of the healthy patient) and the diseased condition $[B] = \Sigma\nu_{nb}B_n$ (which determines the status of the ill patient). Here, ν_{na} represents the number of vital molecules A_n maintaining the healthy state in the human body before the onset of the disease. On the other hand, ν_{nb} represents the number of molecules of B_n reaction products formed during the disease's progression. We can present the biochemical equilibrium for the time when it is considered in the form:

$$\textbf{vital molecules} \leftrightarrow \textbf{reaction products}$$

$$[\mathbf{A}] \leftrightarrow [\mathbf{B}]$$

and the corresponding equilibrium constant: $K = [B]/[A]$

When a disease-related biochemical reaction (the driving force) takes place, the equilibrium shifts to the right due to the formation of reaction products that cause disease-related deterioration. When an ill person digests the remedy, the concentration of the total molecules of the reaction products [B] increases.

The above considerations (equilibrium constant) can be connected to the Law of Mass Action, which implements the restoration of the equilibrium. In chemistry, the Law of Mass Action is the proposition that the rate of a chemical reaction is directly proportional to the product of the concentration of the reactants present in the system, i.e., the cells of the human body. Precisely, it indicates that for a chemical reaction mixture that is at equilibrium, the ratio (K) between the concentration of the reaction products [B] and the vital molecules [A] is constant. Therefore, the Law of Mass Action when the equilibrium constant is considered, i.e., $K = [B]/[A]$, literally means that an increase in the concentration of the reaction products induced by the uptake of the remedy [B] will increase the percentage of vital molecules [A] in the system. So, at biochemical equilibrium the percentage of vital molecules [A], which are the holders of the healthy state, increases at the expense of the digested remedy. So, for an increase in [B] there will be a strong increase in [A]. This is tightly connected with homeopathic aggravation, as explained below.

3. Homeopathic aggravation in light of the Law of Mass Action

When Hahnemann began using drugs with the accuracy required by his method, he found that the usual doses worked strongly and greatly aggravated the symptoms before the treatment could occur. He then reduced the doses in steps until he found that he could achieve a healing effect without aggravating the medical symptoms. In some cases, he found that the attenuating process had actually increased the healing power of the remedy. The method he introduced was to dilute tinctures in the ratio of 1 to 99 rectified spirits and grind insoluble substances with sugar of milk in the same proportions. For the higher attenuations the process was repeated, and with the same proportions being observed at each stage. This all began because he wanted to decrease the aggravation of the patient's symptoms, obtaining the remedy in ordinary doses. In Hahnemann's centesimal scale, each step of the process divides the original quantity by 100, and hence each higher number represents a higher degree of attenuation. He also pointed out the phenomenon of the asymptotic

dilution when he stated: "But the attenuation is so progressed that, no matter to what extent it is carried out, something of the original substance must remain, though it may be beyond the power of chemistry to detect its presence" [28].

Here we will explain the origin of the aggravation of medical symptoms in homeopathy when the patient digests the prescribed remedy [B]. Now we must be aware that in reality the essential molecule when treating the thermodynamic models of homeopathy is Simillimum. This is the reaction product induced by remedy (B) in a healthy person. However, we manipulate, in a clinic, homeopathy exclusively with the remedy, we dilute end successes the remedy, due to that we must also use the (B) remedy in the formal equilibrium equations. Simillimum is a component that occurs as the reaction product in human cells and we cannot physically manipulate with it. However, it is the key component in the considered chemical equilibria. Here, during the presentation of the phenomenon we use (B) "representing" the Simillimum, which is in fact the reaction product of the remedy. The actual role of Simillimum is considered in the last paragraph of the paper.

In the body of an ill person the equilibrium [A] ↔ [B] governs the illness. The starting composition of the system in the human body is then [A] + [B]. When we add to the system (human body) ordinary doses of remedies, for example 2[B], then we obtain a composition in the system [A] + 3[B]. This is then the composition before the re-establishment of the initial equilibrium. Here, we increase the amount of remedy and accordingly its reaction products, Simillimum, which increases the symptoms of the illness. So, this stage, due to the increase of (disease-making remedy reaction product) Simillimum before the action of the Law of Mass Action, causes homeopathic aggravation, the intensity of which depends on the remedy dose. This was Hahnemann's original concern when he started to "dilute tinctures in the proportion of 1 to 99 of rectified spirit."

After homogenization of the Simillimum in the human body, the chemical equilibrium starts to work, associated with the operation of the Law of Mass Action. Here, one [B] changes to [A], with respect to the constant K, and we obtain the final chemical composition 2 [A] + 2[B] in the system. Thus, after the working of the Law of Mass Action, during the re-establishment of the initial equilibrium associated with the healthy state, the true previous composition of the [A] + 3[B] system changes to the final composition of 2 [A] + 2[B]. So, in system [A] it increases by 100% relative to [A] → 2[A], while [B] decreases by 60% 3[B] → 2[B].

This is the confirmation of the strong increase in vital molecules [A] and the strong decrease of [B] in the system at the expense of the added remedy [B]. And consequently, this is associated with improving the health or the disappearance of adverse medical symptoms. We can see that the thermodynamic model accurately forecasts the aggravation before the healing starts. This aggravation can be severe, as first reported by Hahnemann, who started the process of dilution when he, by degrees, reduced his doses until he found he could obtain the curative effect without aggravating the illness.

What is also interesting is that he noted that even at huge dilution, to some degree the original substance remains and cannot be detected with chemical analyses. This observation is also relevant in today's reconsideration of some models, i.e., the water memory and the silent communication, also mentioned in this paper.

4. The role of the small remedy concentration in most exposed models

When Hahnemann's optimizations reach very low therapeutic concentrations, researchers believed that they had pure water as a drug-free solvent, and suggested different models that would clarify the treatment process under these conditions.

During the development of homeopathic remedies, the preparation process involves very strong mechanical mixing (succession) with lactose and serial dilution in an ethanol-water solution, usually in glass containers. The concrete significance of this process is to break the physical bonds of the molecular aggregates and to chemically activate them. At high dilutions, these molecules will not be able to re-associate, but will physically associate with the sugar-lactose molecules, which are in large excess. The sugar molecules will be digested in the human body and the chemically activated remedy molecules will remain suitable for targeted biochemical reactions in the healing process, i.e., the formation of Simillimum and re-establishing the initial equilibrium associated with the healthy state.

When Hahnemann's optimizations reached very low therapeutic concentrations, researchers believed that they had pure water as a remedy-free solvent, and suggested different models that would clarify the medical treatment under these conditions.

They suggested curing in the absence of healing substances in the frame of the model called the "memory effect of water," which was very popular in the 1980s [3–11]. In some cases, homeopaths were convinced that, after successive dilution, the solution/water no longer contained the active compound (remedy) molecules; however, the effects were still observed. Based on such examples, the "memory effect of water" was proposed, according to which water "remembers" the properties of the substance originally contained and retains the healing effect of the solution, even when it supposedly no longer contains the active substance. Recent studies, which showed the substance molecules in extremely diluted medical preparations [29, 30], have excluded this model [4].

One of the models addressed today by homeopaths concerns the use of the concept of the "Vital Force," as Hahnemann called it. According to this model, at very high dilutions combined with succession, we can no longer speak of "substances" in solution, but only about "fields of forces." In the same way we cannot talk about the organism that the remedy acts upon on a biochemical basis, but only about the energy part of the organism, i.e., the "Vital Force." So, we have an interaction of forces, not biochemical agitations. The "fields of forces" (electromagnetic wave interactions, $h\nu$) is an extensive property not dependent on the mass of the remedy. On the other hand, the amplitude is an intensive property and is dependent on the mass. So, a zero-remedy concentration must exhibit zero amplitude and cannot upgrade anything concerning common electromagnetic effects, i.e., resonance, synchronization, reinforcement and interference, and cannot take part in the interaction of the "fields of forces." On the basis of the above-mentioned facts, it is to be expected that homeopaths will always deal with a tiny amount of substance.

How difficult it is to eliminate the impurities and/or clean a contaminated solution was also observed by Hahnemann, and which we also stated when considering homeopathic aggravation.

5. The origin of the Law of Infinitesimals

Here we will start from the thesis that if it is possible to establish a healing fact in which the success of a therapeutic treatment is impaired by increasing the content of the added drug (remedy), then the most likely explanation for such a treatment (in today's understanding of the situation) is that this phenomenon is related to the kinetics of biochemical reactions in human cells.

Processes involving homeopathy take place at the molecular level, which divides in the human cell, where equilibrium regulates the biochemical status of the cell in an ill person. In the human body there are biochemical reactions in human cells,

where a huge number of highly sensitive, fine-tuned and regulated biochemical reactions take place at any time in a single cell. To begin the healing process, the remedies must flow into the cells of the ill person.

After increasing the initial drug concentration at the entrance to the cell, the drug molecules use passive diffusion and cross the cell membrane in the direction of the concentration gradient. The remedy creates a reaction product in the cells of a healthy person, Simillimum, which will cause a complex of symptoms that are almost close to the cure for the disease in question. These Simillimum molecules are already present in the cells of the affected person and cause the disease in question (as will be explained in the last paragraph). When the remedies go into the cell of an infected person, they will form the same reaction products, i.e., Simillimum molecules, as in the cell of a healthy person and increase the concentration of Simillimum molecules in the cell of the infected person. After the Simillimum enters the cell equilibrium of the diseased person, it will restore the initial equilibrium of the healthy state and heal the ill person in accordance with the "Law of Similars" described above. The remedy as a molecule is not in itself a critical issue, but the reaction pattern developed by each individual remedy molecule that enters the diseased person's cell and forms reaction products during the formation of Simillimum molecules that enter and shift the chemical equilibrium and heal the ill person.

However, the healing is associated with molecular crowding, due to the formation of reaction products that accompany the Simillimum formation, which aggravates the re-establishment of the initial equilibrium and consequently curing for kinetic reasons, as will be considered below. The chemical reactions in human cells are in the steady state under the suitable conditions and given enough time, distinct biochemical reactions carried out in a test tube will sooner or later reach equilibrium. Within cells, however, many reactions are related to pathways in which a product of one reaction serves as a reactant in another pathway, or is driven out of the cell. In this more complex situation, when the rate of formation of a substance (the reaction products) is equal to the rate of its consumption, the concentration of the substance remains constant, and the system of linked reactions for producing and consuming that substance is said to be in a steady state. One consequence of such linked reactions is that they prevent the accumulation of excess intermediates, caring cells from the harmful effects of intermediates that have the potential to be toxic at high concentrations [31, 32]. The remedies that enter the cells of an ill person are determined to synthesize the reaction product, i.e., Simillimum, after an identical procedure occurring in the cells of healthy people. So, there cannot be any linked reactions that might prevent the accumulation of excess intermediates formed during biochemical reactions in the cells of the ill person. Therefore, the remedy's influx will cause biochemical reaction products and induce molecular over-crowding. For this reason, the kinetics of the biochemical reactions taking place after the entry of the remedy into the cells of an ill person will be heightened. From the kinetic point of view [33], in a human cell, molecules move and collide, and their bonds vibrate and rotate. When molecules collide, there is the possibility of a reaction taking place, but only if the colliding molecules have enough energy and are aligned correctly.

Collisions in a liquid solution are regulated by diffusion instead of direct collisions, so diffusion takes control of the frequency of collisions. Direct collisions between two target molecules no longer predominate, as each molecule must collide with a large number of cytoplasmic molecules and other molecules before it can find a suitable molecule with which to react. Instead of a direct collision between the target molecules, we must use a diffusion-controlled collision frequency [34]. A larger influx of drugs increases the concentration of the reaction products and, consequently, molecular over-crowding occurs. The effects of molecular crowding

strongly reduce the rate of intercellular diffusion and the reaction. The cytoplasmic network leads to a decrease in the mobility of the molecules [35]. Before a successful collision, there will be a series of collisions (ineffective collisions) with other molecules, and the concentration will increase with each arrival of the remedy molecule. Each molecule of the remedy makes a number of molecules of reaction products that can be considered as obstacles to effective reaction collisions and will interfere with the reaction kinetics and aggravate the curing, since the re-establishment of the initial equilibrium of the healthy state will be strongly delayed. To prevent such over-crowding and enhance the healing process, we need to reduce the concentration of the remedy. To address the "Law of Infinitesimals" at very low concentrations, we need to consider two key statements.

(i) It has been shown that medicinal products diluted and hand-successed to 30 or even 200 C retain the original materials [30] and that the drug component in the solution decreases asymptotically during serial dilution [31], mainly due to the fact that the air-liquid phase boundary formed during succession behaves as an impurity snare and retains the diluent molecular constituent species, so that contamination of the solution with the remedy can be expected, regardless of the dilution protocol, and second, (ii) in theory every single molecular constituent species (Simillimum) being in excess while re-establishing the equilibrium is a necessary and sufficient condition to trigger the equilibrium restoration. Considering the above, in extreme dilution, we are convinced that a typical homeopathic remedy does not guarantee that any of the remedy molecules are present (in potentiated and diluted solutions). A small number of remedy molecules can, through the process of "infinite dilution," form a small number of Simillimum molecules and restore the equilibrium and allow the patient to heal, which is observed in clinical homeopathy. In this case, the status of the medicinal solutions in question can be taken as "infinitely diluted" and thus the minimum concentration level in the concentration range covered by the "Law of Infinitesimals," i.e., "infinitesimal dose." This is the most characteristic feature of modern homeopathy.

The increase of the curing efficiency with the strong decrease of the remedy concentration is to be expected, and is also confirmed in clinical homeopathy.

On the other hand, as already pointed out, a relatively high concentration of remedy can even block the healing process. In homeopathy, we have two concentration extremes, i.e., the high and low concentrations, and in between there remains a constant increase in curing efficiency.

In pharmacy, the drug is a conventional solution of an active ingredient that cures and is compatible with a dissolved and diluted chemical drug in bulk in a true solution that can only act pharmacologically with a linear dose-response relation diametrically different from homeopathy.

6. The role and significance of Simillimum in the thermodynamic model of homeopathy

Simillimum is a compound that forms when a substance (remedy) is given to a healthy person that triggers the most similar symptoms of the disease that is being considered. A large number of substances are usually tested in order to find a suitable remedy, whose reaction product in a healthy person has the properties of the Simillimum.

Simillimum is a compound that acts as a medicine when it enters the ill person's cells. Its mechanism of action is connected with the re-establishment of the initial chemical equilibrium associated with a healthy state during the action

of the Law of mass action. Simillimum is the key equilibrium molecule included through (B) in the equilibrium constant. With increasing the remedy (B) and consequently Simillimum concentration we induce the well-known homeopathic aggravation. After the onset of the working of the Law of Mass Action and the shift of equilibrium, the healing begins as described in the section on homeopathic aggravation.

Namely, for the application of the Law of Mass Action the compound that increases the "mass" (concentration) in the equilibrium's re-establishment must exhibit the same chemical composition as that governing the ill person's equilibrium, otherwise the thermodynamic model is out of functioning and the homeopathy cannot be explained in the frame of this model. So, this is the main argument, supported by the natural law, the Law of Mass Action, that both compounds must exhibit the same chemical composition.

In essence, Simillimum must always occur in pairs for every disease and Simillimum has its "shadow," i.e., parallel compound in the ill person, which is the driving force of the current disease and governs the "new equilibrium" which Hahnemann described as the "detuned state."

According to the same symptoms, the Simillimum "recognizes" its contra part, the key compound in the ill person, which must exhibit the same chemical composition. However, the same chemical composition is associated with the same electromagnetic resonance spectra. Resonance response spectra in diseased individuals and in provers must exhibit the same resonance frequency. Since the spectra are confined to the chemical composition it can be taken that there are same chemical compounds in diseased individuals and in provers. This is the crowning proof that both compounds have the same composition.

Besides, we must not overlook the reinforcement of the electromagnetic spectra of both considered compounds, i.e., the remedy (Simillimum) and the disease-forming compound.

The electrodynamic field is the interrelationship of particles that are affecting each other through charge and movement, which are definable in terms of the oscillation and movement. So, the compounds must have their own resonance spectrum, which is compatible with its composition, i.e., the same composition exhibits the same resonance spectrum.

As stated above, both compounds, Simillimum and his contra part, in diseased individuals must have the same composition in order to make possible the equilibrium restoration via the activation of the Law of Mass Action. The same composition makes possible the electromagnetic reinforcement of the spectrum of the ill person when adding the remedy (Simillimum) as a medicine to cure the patient.

One of the basic properties of electromagnetic spectra lies in its principles of synchronization resonance, harmony, reinforcement and interference. So, this would be a possibility to identify an electromagnetic reinforced response of key molecular species in ill persons with the addition of a remedy (Simillimum). When we add to the ill person, exhibiting normal electromagnetic spectra induced by the disease-making compound, a defined amount of remedy (Simillimum), then the amplitude of the spectra should increase due to the reinforcement of the spectra or, in other words, by increasing the concentration of the "common compound" (exhibiting the same composition) the intensity of the spectra must increase.

This agrees with the statement of Vithoulkas [27], i.e., "If a substance is capable of producing a similar symptom picture in a healthy organism, then the likelihood of its vibration rate being very close to the resultant frequency of the diseased organism is good, and powerful strengthening of the defense mechanism can occur through the principle of resonance." This statement supports the conclusions of

this model, i.e., the same frequency, same composition and optimal healing ability. Namely, the similar spectra demand a similar composition and the thermodynamic model covers the above statement.

After the addition of the remedy (Simillimum), the therapeutic agent, the intensity of the electromagnetic spectra must increase due to the increase in the concentration of the electromagnetic-spectra-emitting compound (reinforcement of the both signals). Thus, the sudden increase in the electromagnetic signal is associated with homeopathic aggravation.

After the operation of the Law of Mass Action and the decrease of the concentration of the remedy (Simillimum), the amplitude of the spectra must have decreased, revealing the healing. So, the characteristic spectra of the remedy and that in the ill person's body must be identical and subjected to reinforcement, which would be a direct proof of this thermodynamic model and is also in agreement with statement made by Vithoulkas [27]., i.e., "In this sense, the "shape" of the remedy and of the disease can be understood as having the same "resonant frequency" and further "the resonant frequency of a particular pattern of symptoms in diseased individuals and in provers. It is this matching of symptom-pictures that is the primary task of the homeopath in prescribing a remedy."

The definition of Simillimum in the thermodynamic model lies in its duality with the ill person's key compound, i.e., the same composition, the same medical symptoms, the same electromagnetic response and the same biological origin (the human body). This duality can be considered as one of the most relevant properties of homeopathic science.

7. Conclusions

The thermodynamic approach to explaining the most important phenomenon in homeopathy reveals that the key condition for its successful interpretation must be the assumption that the chemical composition of Simillimum in healthy persons must have the same chemical composition as in diseased individuals. Namely, this assumption is supported by all key homeopathic phenomena that are clinically sustained and are also consistent with common electromagnetic spectra induced in diseased individuals and in healthy persons.

The most certain proof that the thermodynamic model works is the Law of Similars and the homeopathic aggravation. Both phenomena are essential in healing and can be precisely explained by the thermodynamic model. In addition, the same electromagnetic spectra and the same composition also support the results of the outcome of the thermodynamic model.

So, the utmost exclusive statement of homeopathy, that similar medical symptoms are associated with the similar composition of Simillimum, can be confirmed by these phenomena and is the realm of homeopathic science.

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Chapter 8

Acupuncture, Yoga, Homeopathy, and Apitherapy under the Vibrational Point of View

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Abstract

Albert Einstein was one of the greatest physicists in recent history and his contributions changed the paradigm of science in the 20th century. From this, it was proposed the understanding that all matter is energy, and we can assume the understanding that the human body is a dynamic energy system. Energy and matter are two different manifestations of the same universal substance from which we are all formed, atoms. It is known that the movement between the constituent parts of the atom (electrons, neutrons, protons and smaller particles) and the forces that connect them, generates energy. Since the atoms are in constant movement, what can be measured is vibration. Therefore, in this chapter, we propose to present some millenary therapies like acupuncture, yoga, apitherapy and, more recently, Homeopathy, under the point of view of vibrational integrative therapies, exploring the hypothesis that through these therapies we can adapt the waves generated in the bodies to transform them into personalized therapeutic agents.

Keywords: acupuncture, yoga, homeopathy, apitherapy, vibrational medicine

1. Introduction

In 1948, the World Health Organization (WHO) defined health as 'a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity'. WHO supports the idea about the integration of conventional and complementary practices to reach the best results for the patient and society [1] Traditional, Integrative, and Complementary Medicine practices are being adopted in several countries' members of WHO. Acupuncture was the most common form of practice, closely followed by herbal medicines and indigenous traditional medicine. Homeopathy and traditional Chinese medicine came in next, each used by 100 Member States [2].

In 2006, Brazil the Health Ministry launched the National Policy of Integrative and Complementary Practices (PNPIC, MS ruling no. 971/2006) [3] with ensures to the population partial access to Complementary and Alternative Medicine. Under PNPIC, patients are provided with traditional Chinese medicine/acupuncture, homeopathy, botanicals, and herbal medicine cares free-of-charge, among others at Health Basic Units (HBU) and Family Health Support Units (NASF). Yoga was included in 2017 [4], and finally, apitherapy with the other 9 practices was included

in 2018 [5]. According to the Health Ministry from Brazil, in 2019, 1,4 million of individual queries were done in these practices, with acupuncture in the leadership. Integrative practices are present in 9350 services and 3173 cities, from this 88% of the public health care system.

Considered the last frontier to be crossed in Hippocratic medicine, vibrational medicine aims to shed light on the understanding of the bodily system in its most subtle aspect of energy transference and systemic functioning regarding the physical-etheric interface of bodies. The Cartesian and mechanistic division of science and the world of the 20th century, which undoubtedly drove the great technological advance that the world finds today, as postulated by Einstein, Heisenberg, and colleagues, overcame this Newtonian fragmentation and led us back to the idea of unity, expressed in ancient Greece and Eastern philosophies [6].

Albert Einstein was one of the greatest physicists in recent history and, from him, there was the recognition that all matter is energy, and one can assume the understanding that the human body is a dynamic energy system. Energy and matter are two different manifestations of the same universal substance from which we are all formed, atoms. It is known that the movement between the constituent parts of the atom (electrons, neutrons, protons and smaller particles) forces that lead them, to generate energy. As the atoms are in constant motion, what can be measured is vibration. Each atom is unique because the distribution of positive and negative charges, combined with the speed of rotation, creates a specific vibration and a personalized frequency pattern [7]. The biophysical aspects of some therapies such as acupuncture, yoga, homeopathy among others, in general, are not many in publications in the area, due to the limited instrumental validation available.

The electromagnetic force is the fundamental reason for life. Atoms are sets of electrons and protons, molecules are sets of atoms, biopolymers are groups of macromolecules, and in the same way, life is an interactive congregation of biopolymers and macromolecules. The living being can then be considered an electromagnetic entity, which responds to a given electric or magnetic signal, as an expected result based on the laws of physics. Experiments have shown a level of electromagnetic organization in living organisms; intrinsic electrical characteristics and their sensitivity to external electromagnetic fields [8]. In this way, the human body can be considered a "resonance box", capable of responding to various sources of stimuli (mechanical, sound, light, olfactory and tactile). Thus, everything that acts in its electromagnetic field or that comes into contact in the form of vibration in its physiological ensemble is liable to mobilize and be transformed into energy. It is from understanding the connection between the subatomic universe and physiology that we can understand how acupuncture, yoga, homeopathy, and why not apitherapy, that are capable of triggering various biochemical activities already mapped by science with their purely vibrational aspect. Considering the importance of the integrative and complementary medicine for health and aiming to contribute to the data already available in the scientific literature, this chapter aims to present some concepts about acupuncture, yoga, homeopathy, and apitherapy from the vibrational point of view, an important approach to understand the functioning of these impacting traditional medicines in human life.

2. Talking about some integrative therapies

2.1 Acupuncture

Acupuncture, one of the tools of Traditional Chinese Medicine (TCM), in its more than 3000 years of existence, appeared intuitively in China, however it has

had progressive scientific validation since the middle of the 18th century and, until today, it proposes to do the integration of the physical, psychic and spiritual bodies through the management of vital energy (*Qi*) on tracks arranged in the physical body called meridians. There are evidences that these tracks are arranged in the connective tissue, which permeates the body in its multidimensions. What is proposed is to connect the ancestral knowledge of these tracks that lead the electromagnetic energy from the external environment to the internal environment (addressed to each body system) to the production of homeostasis, the fundamental principle of health. Brito et al. recovered, through the originals of the emeritus physiologist Claud Bernad, the concept of homeostasis, where the highly developed living being is an open system that has many relationships with its surroundings - in the respiratory and alimentary tracts, and through receptors of surface, neuromuscular organs and bone levers. Changes in the surroundings excite reactions in this system, or affect it directly, so that internal disturbances of the system are produced. Such disturbances are normally kept within narrow limits, as automatic adjustments within the system are put into action, and therefore, large oscillations are prevented and internal conditions are kept virtually constant [9]. In its acupuncture meridian system, which is an interface of energy exchanges between the various body tissues with the Central Nervous System (CNS), the connective tissue is stimulated inserting needles for the transduction of energy until the outbreak of energetic phenomena, physiological factors that influence cellular electrophysiology [10].

According to Lipton, the behavior of energy waves is important for biomedicine because vibrational frequencies can change the chemical and physical properties of an atom [11]. The knowledge of the links between the material body and its subtle form can enable a greater understanding of how it is possible to expand health in a preventive way in a global, affordable, and personalized environment [12]. We will not stick to the descriptions of the meridians paths, treatments, or explanation of the syndromes that are treated by acupuncture, but the biophysical events as a link that provides the effectiveness of the treatments of this ancient method.

2.1.1 Chinese philosophical thinking

According to Chinese philosophical thought, there is a concept of energy that permeates everything around us. It influences from the simplest form of life and even the movement of the planets, in a constant flux of renewal and expansion. This force/energy is part of the essence of the universe and involves all the entities that exist in it are called TAO [13]. In this philosophy there is a very particular view that correlates all the movements of the universe, macrocosm with the microcosm that represents everyone, in this way, everything is part of a great set. In this logic, the body is a macrocosm with countless microcosms, transforming and expanding with each movement of the individual. With this position, when contemplating a garden or a beautiful landscape, human being knows that the same energy that sustains and nourishes nature also exists within him; it manages to amplify its capacity for self-understanding and to value the importance of life cycles, confirms that it can establish itself in the face of the elements of existence [14]. From this point of view, the individual relates better and more respectfully to everything around him, perceives and contemplates the beauty of cycles, such as the arrival and the end of a season, harmoniously moving creation and destruction, and above all, he perceives himself to be actively acting in a macrocosm full of microcosms. Energy flows can be compared to the movement of the oceans. The currents have different characteristics, one is hot and fast, the other cold and slow, they vary according to the depth, and when they move they generate a lot of energy in this ecosystem [6].

In the millennial Huang Ti Nei Jing, known as Principles of Internal Medicine of the Yellow Emperor, we find the following statement: "the whole universe is an oscillation of *Yin* and *Yang* forces". Through centuries of observation, from the era of the Yellow Emperor (2704–2100 BC), many masters noticed these correlations and found that this knowledge could be used to understand the functioning of the human body and, consequently, its regulation and harmonization for maintaining health [13]. These energies are always dual. The duality it is identified as *Yin* and *Yang*, opposites that complement each other, demonstrating that in nature there is always, even if in small quantities, the influence of one polarity within the other.

Yin and *Yang* in an eternal movement of mutation determine the entire balance between pairs, constituting a polarized unity, such as female and male, body and soul, conscious and unconscious, right and left, hot and cold, day and night. Each pole is associated with an energy identical in value to the other, but of opposite signs, one exists only because the other does not exist; they are complementary and need each other. However, when this harmony of the parts is disturbed, each has its destructive side. Therefore, nature does not encourage one pole to dominate/overcome the other, there is no better or worse pole, both are important in maintaining this delicate and intricate balance for the benefit of the whole, or the TAO [13].

For the quality of life and health maintenance, acupuncture treatments provide information for understanding the circulation of vital energy (*Qi*) to harmonize the flow of energy that flows through the body. A blocked or uncontrolled/excessive internal flow can cause illness, feelings of regret, and loss of vitality. If the movement of these energies is properly tuned, the harmony is resumed, and the stagnations are undone. Understanding the functioning of vital energy in all systems of the universe and, especially in its relations with the human body, allows a conscious and respectful posture of the vital cycles that everyone will go through. The movements of creation and destruction that are witnessed at every moment outside and inside each one keeps this flow constant. The TAO keeps macro and microcosms connected and within the same energetic web [15].

In Oriental philosophy, the absence of health is seen as a momentary resource of the body in the search to reorganize itself, being a system of adaptation of this organism to anomalous environmental stimuli, pollutants, toxic agents, conflicts, and a decrease in vital energy.

2.1.2 Classical meridian theory and the School of the Five Elements

The oldest written reference on acupuncture meridians is present in the book Huang Ti Nei Jing and contains precise descriptions of its principles and we can assume that it is the result of observing the beginnings of Chinese medicine. It contains information that, when stimulating the points on the map associated with certain organs or viscera (*Zang Fu*), a clear sensation of heat and paresthesia is triggered along the paths described from the acupoint [16]. These points, modernly we know that they belong to equal dermatomes. Dermatomes are determined areas of the body innervated by a nerve that exits the spine, the spine is composed of 33 vertebrae from which 31 pairs of nerves and 2 coccygeal vestigial vertebrae come out that are distributed throughout the body in an organized manner. Each nerve that leaves the spine is responsible for giving sensitivity and strength to a certain area of the body [11]. When these lines were drawn connecting the various analogous points, they obtained the longitudinal trajectories, called *Tin* (meridians), and horizontal trajectories called *Lo* (communications) [13].

Exploring the developments resulting from the relationships between them is not the goal of this chapter but understanding how the dermatomes connect with the meridians serves to complete the understanding of the invisible systemic tracks

that run through the organism. To understand the vibrational vision in acupuncture, and which supports it scientifically, the first step is to know the studies that can validate this specialty over time, through the embryogenesis of acupuncture meridians.

2.1.3 The embryogenesis of acupuncture meridians

It is known that meridians are a distinct morphological pathway. This finding was possible in humans through the findings of Frenchman Pierre de Vernejoul and his collaborators, who injecting metastable radioactive technetium 99 (99mTC) into the acupuncture points, observed the progression of the isotope in the mapped meridian lines, covering a distance of 30 cm in 4 to 6 minutes. When 99mTC was injected at random points on the skin, there was no similar result compared to the injection in the cuff [10].

Vernejoul based the work of Korean Kim Bong Han in the 1960s. By visualizing the path of the isotope phosphorus 32 (P32) injected into a rabbit's acupuncture point, Kim observed the absorption of the isotope in a tubular system with about 0,5 to 1.5 μ (microns) in diameter with the use of micro auto-radiography; this path corresponded to the acupuncture meridian tracing of a given point; little or no activity was noticed in the meridional line when the isotope was injected into a vessel adjacent to the point. Through the histological study of these tubules, Kim's group found that there were superficial and deep ducts that "floats" freely over the inside of the lymphatic vessels and vascular tissue, penetrating the vessel walls at specific points of entry and exit. The fluid present in these tubules moved in the same direction of blood and lymph flow and also in the opposite direction to them, which suggests that its origin is probably independent and previous chronologically to the embryogenesis of blood and lymphatic vessels.

According to him, as long as blood vessels develop, they grow around the meridians. The sequence of these findings was followed by the description of other tubular systems: The Intra-external Ducts, which appear to be arranged along the surface of Organs internal organs and flow independently from the blood, lymph, and nervous vessels. Superficial Ducts are found on the skin; and, finally, the Neural Ducts, distributed in the central and peripheral nervous system. All Duct systems are interconnected by Terminal Ducts of the various ductal systems and these reach the nucleus of the cell. Kim has also done numerous experiments that confirm the continuous flow of these ducts and the fluid contained in them. Within these ducts were found high concentrations of DNA, RNA, amino acids, nucleic acids, sixteen types of free nucleotides, adrenaline, corticosteroids, estrogen, and other hormonal substances at levels different from those found in the bloodstream. This indicated the path of understanding the interrelationship of acupuncture meridians and the regulation of the endocrine system [10]. Kim's findings, according to Richard Gerber (2007), were associated with those of Yale's neuroanatomist Harold S. Burr. Burr deeply studied the energy fields surrounding plants and living animals. He mapped the electric fields in the salamanders at an early stage of embryogenesis and found that the electrical axis that was aligned with the animal's brain and spine originated in the unfertilized egg. Kim, consulting Dr. Burr's findings, found that in the chicken embryo, the meridian ducts formed within 15 hours of fertilization. At that time, not even the most rudimentary organs were formed. With this, he can suggest that the functioning of the acupuncture meridian system influences the migration and the spatial orientation of internal organs. Burr and Kim proposed that meridians form a physical-etheric interface, where bioenergetic information and vital energy flow from the etheric body to the cellular level through these tracks, nourishing the bodily systems [10].

We can connect these studies with those of molecular biologist Bruce Lipton (2007), who, through a quantum perspective, reveals that the universe is a set of integrated and interdependent energy fields. The physical part and the energy fields that make up matter describes a non-linear or holistic flow of permanent emanation. According to him, the specific frequencies and patterns of electromagnetic radiation in the environment can regulate DNA, RNA, protein synthesis, alter the function and shape of proteins, control genes, cell division, their differentiation, morphogenesis (a process in which cells group together to form organs and tissues), hormonal secretion, growth and nerve functions [8]. In acupuncture treatment, the insertion of needles into acupoints, known in Chinese literature as the arrival of the sensation of *Qi*[Ch'i], is the source of vibration that triggers a tingling sensation called deqi energy when this sensation extends to the along the channel in which the insertion is carried out, we can say that a PSC (Propagated Sensation along the Channel) phenomenon is established [17]. There are countless methods of insertion, ways to manipulate the needles - how to press, pull or rotate them - however as soon as the patient feels the sensation of the de*Qi*[deCh'i], it is known that the neural trigger was given away. This information contributes to the foundation of acupuncture as a vibrational therapy in its essence.

2.1.4 What can be behind deQi

We have already seen that the meridians are not like a vascular system and neither are nerves, but they can be the neural and neurohormonal links between the central nervous system (CNS) and the peripheral nervous system, influencing it through the connective tissue when stimulated through their paths. In the nervous system, communication takes place through electrical action potentials. Information is transmitted through changes in the frequencies of action potential discharges. The rate of nerve electrical discharge per second generates a code, which will have a certain reading depending on the nerve that is communicating with the brain sensory region. The discovery that the systems made up of glial and Schwann cells - which previously only served to nourish the surrounding nerves also has the function of an electrical nature [10], is an important link for understanding this communication system physical-etheric. The most recent research indicates that the glial cell network can transmit information through slow changes in direct current potentials. It is possible that with the vibration of acupoint stimulation, which is characterized by a location on the skin with low electrical potential, an input is created in the nervous system, influencing the direct-current potentials of the glial cell network, which follows the path of the nerves [10]. When an action potential discharge begins in a nerve cell, a sequence of events is triggered, which passes through the entire sensory nerve fiber until reaching its synaptic ends. The electrical impulse, which carries a message to the brain, undergoes an energetic transformation in the synaptic cleft, which is converted into the release of neurotransmitters. The electrical potential in the cell membrane determines the reaction of each neuron to release neurotransmitter packages. Each nerve cell is in contact with many others forming a network, thus "spreading" the information for the modulation of the CNS and, consequently, the harmonization of regions that are unbalanced in the body system. It can be assumed in this way that the dual wave-particle behavior of subatomic particles is producing a set of "information" that, in essence, generates vibrations (waves) that seem to make the connection between the material and immaterial systems of bodies [12]. The acupuncture meridians and the nervous system operate in a complementary manner and, when adequately supplied with information and nutrition, they will promote higher energetic phenomena translated into harmonic cellular physiological patterns and

also immunity, organized by the individual and unequivocal model of functioning present in each individual in the pituitary.

With the change in the energy environment of glial cells, the meridian system becomes capable of influencing the bioelectronic systems of growth and regeneration. We conclude with this that the effects of neurochemical releases, associated with changes in direct currents that are slowly transmitted along the perineural pathways, are not primary, but a secondary effect of the fluctuation of energy fields located in the vicinity of nerves and glial cells. Surrounding them from the vibrational stimulation of the needles in the dermis.

It is common knowledge that vibration triggers the action potential (AP) in neural cells, which originates through a disturbance of the resting state of the cellular membrane, with a consequent flow of ions, through the membrane and alteration of the ionic concentration intracellular and extracellular media [18]. Thus, the main cause of the resting potential would be the unequal distribution of ions in solution on both sides of the membrane, compartmented actively or passively by the selective mechanisms of transmembrane ion transport. The membrane, therefore, acts with a capacitor, storing energy in this spatial distribution of electrically charged ions; this potential electrical energy is available to be recovered quickly, in addition to stabilizing the membrane preventing this system from being disturbed by any minor factor. The aqueous medium fills most of the intracellular and extracellular spaces, and it is where almost all molecules (soluble, of course) interact to animate the intermediate metabolism, the mobilization of energy and nutrient sources, and the maintenance processes are suspended, and molecular and cellular repair [19]. The important fact is that all living cells have some differences in electrical potential between the cytoplasm and the extracellular space, being generally negative on the inside (resting potential of the cells). Some cells, however, can leave this situation of rest, propagating, throughout their membranes, disturbances that cause transmembrane ionic currents throughout the cell, and that can even invert the electrical profile concerning rest, even leaving it for some time the cytoplasm positive concerning the exterior: these are called excitable cells, and include neurons, muscle cells, and endocrine secreting cells. Some of these nerve endings release outgoing transmitters that tend to trigger an impulse; others are inhibitors and reduce the nerve's tendency to fire. The impulse trigger will depend on the balance between the exciting and inhibiting influences of hundreds of synapses [15]. This mechanism and all known developments in the Western are responsible for the vibrational potential produced by acupuncture (**Figure 1**).

The trigger above explains the *Yin-Yang* concept as polarities; these are the ignition of the "boiler" flame of substance transformation. However, for this boiler to remain in operation, a permanent energy generation process is necessary.

It is in the transformation of *Qi* into various nutritive and humectant fluids, in the transmission and processing of stimuli from the medium into neural signals, and in the maintenance of metabolism for the continuous production of energy that is obtained *Qi* strong and circulating, free from stagnation, it is health in the systemic and vibrational point of view of acupuncture.

The greater the understanding of the biophysical aspects of the energy generation, information, nutrition, and defense production processes in the body system, the better and more comprehensive the direction of integrative and complementary therapies in current and future integrative medicine will be.

2.2 Yoga

Yoga is one of the six most important philosophies in India and to understand it from a vibrational point of view, first we will present some important concepts. As

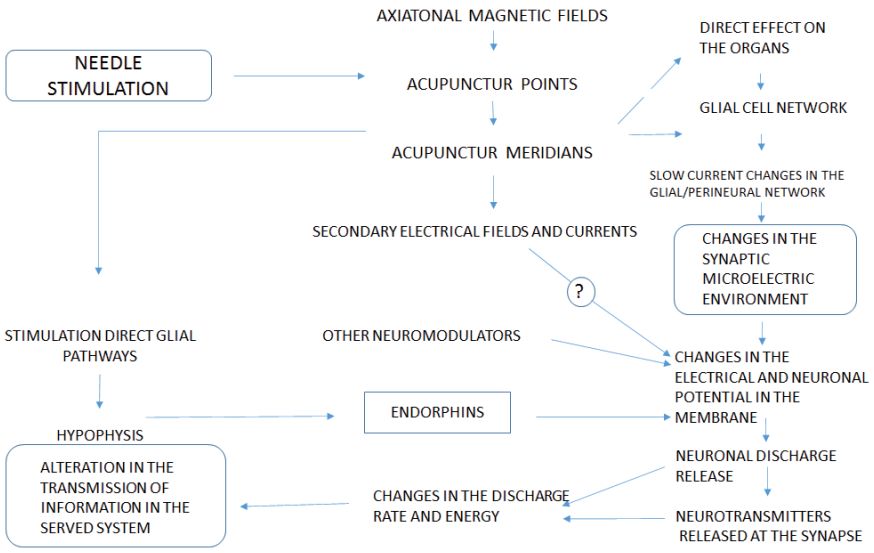

Figure 1.
Neuroendocrine modulation from acupuncture stimulus (Source: Gerber, 2007).

well as several other holistic knowledges, there are indications that Yoga has existed for at least 3000 years. It is a timeless philosophy that comprises a set of moral and ethical values, as well as some techniques to harmonize body, mind, and spirit. And although it is based on teachings contained in the "Vedas", texts that form the basis of Hinduism, they have no religious connection. In Sanskrit, a language considered sacred in India, Yoga means to "unite". It refers to the union of the being with the whole, through the study of oneself (self-knowledge) and the understanding that mind, body, and spirit do not separate.

For centuries, the teachings of Yoga were passed on orally, directly from master to disciple. Until, around 200 BC, a great Indian philosopher, "Patañjali", codified them in 196 aphorisms, constituting what is known today as one of the most important texts of Yoga, the "Yoga Sutras" [20]. It should be noted that among these aphorisms, two of them, the III-53 and IV-33 deal with theories where the nature of time and matter would be discontinued, in line with current discoveries of quantum physics, scientifically proven, that irradiation of light is not continuous [21]. Patañjali describes eight steps that the Yoga practitioner must follow. The first two stages, *Yamas* and *Nyamas*, comprise an ethical and moral conduct that everyone must observe and practice to live well in society and with themselves. *Yamas* deals with behaviors that we should avoid, while *Nyamas* deals with behaviors that we should adopt. The third stage deals with the practice of *Asanas* - Yoga postures - where the practitioner must remain stable and comfortable in each one. The fourth stage deals with the practice of *Pranayama*, that is, breathing exercises that aim at mastering vital energy. *Pratyahara* is the fifth step, where one must abstain from external senses. The sixth stage explains *Dharana*, the directing of attention, which is essential to reach the next step, which is *Dhyana*, where the goal is to keep your attention on the object of meditation. Finally, the eighth step is Samadhi, when the practitioner reaches the state of equanimity, understanding of existence and communion with the universe.

The "Yogis" believe that the physical body is only one of the five different bodies (layers), which constitute the being and that must be in perfect harmony to reach the state of totality. The five bodies, or *kosas* as they are called in Sanskrit would be: the physical body *annamaya kosa*, the energetic body *pranamaya kosa*,

the body mental *manomaya kosa*, the intellectual body *vijnanamaya kosa* and the spiritual body *anandamaya kosa* [22]. According to them, there are no tangible boundaries between these bodies, and their interactions cause everyone to emit a unique frequency. Following this concept, the individual's health is also related to the balance between these bodies and not just to the proper functioning of the physical body.

According to this philosophy, there is an energy originated in the Sun, which penetrates the Earth's atmosphere through the sun's rays and is responsible for all life on the planet. This energy is called Prana and has been reported by different peoples around the world for millennia. They gave these energy different names, but it is the same vital energy. In China, it is known as *chi* or *qi*, in Egypt as *ka*, in Polynesia *mana*, in Japan *ki* and in Jewish mysticism they call it *ruach*.

The sacred texts also speak of 72,000 *nadis* that would be the channels through which vital energy flows [23]. According to the "Yogis", there are three main channels: *Ida nadi* that starts on the left side of the base of the spine, *Pingala nadi* that starts on the right side and *Sushumna nadi* that rises right in the center of the base of the column, the latter being considered the main channel among the three. Everyone moves in an upward flow, reaching the top of the head [24]. *Ida* would be related to the negative pole representing the feminine and lunar energy and *Pingala* to the positive pole, representing the masculine and solar energy. While *Sushumna* represents the free spiritual manifestation released by the action of *Ida* and *Pingala*. Making an analogy with anatomy, *Ida* and *Pingala* would correspond to the trunks of the sympathetic nerve and *Sushumna* to the spinal cord.

Nadis are also responsible for transporting *Prana* to the energy centers: the *Chakras*. These energy centers would be like circular vortexes, or swirls of energy that rotate at high speed, corresponding to the seven musical notes and the seven colors of the prism. Seven are considered *Chakras* main, arranged vertically from the base of the spine to the top of the head. They are *Muladhara, Svadhisthana, Manipura, Anahata, Vishudha, Ajna*, and *Sahasrara*. The earliest records on the *Chakras* appear to have appeared in the ancient scriptures "*Upanishads*" around 600 BC [25]. The description of *Chakras* in ancient scriptures is also very similar to the anatomy of the nervous system described by modern science [26]. The *Chakras* act as transforming elements of higher energies, reducing their shape and frequency to suit the energy pattern of the physical body. This energy is translated into hormonal, physiological, and cellular changes. The *Chakras* also seem to establish a relationship with some psychic aspects.

The analogy between these two different structures, one based on neurophysiology and the other on a tradition of ancestral wisdom, has been the focus of several scientific studies. They aim to understand how this complex and extensive energy network is related to the nervous system, endocrine glands and blood and its relationship with the health-disease process [27–32].

Neuropsychiatrist S. Karagulla deepened her research on the interaction of the etheric body and *Chakras* and their effects on the endocrine glands, where she observed more than 200 cases respecting the rigor of scientific methodology. Her findings indicated that severe abnormalities in the color, rhythm, direction of movement, luminosity or shape of the *Chakras* indicated illness in the respective endocrine gland or in the region of the body to which that *Chakra* supplied energy. With that, it was possible to predict which area this disease would be installed even before the appearance of the first symptoms. In his research he used information about etheric abnormalities identified in the *Chakras* through clairvoyance, comparing them with the medical records available. The word clairvoyance has a French origin (clairvoyance) and means "to see clearly". Clairvoyants have an open etheric or astral vision, being able to see at corresponding levels [33]. This ability is related to the

energy center located on the forehead, the *Ajna Chakra*. Therefore, in the research in question, the clairvoyant was oriented to concentrate on the etheric level, so it would be possible to observe the function of the endocrine glands and the *Chakras* corresponding. The clairvoyant never spoke to any patient, nor did she have contact with medical reports and examinations. She was about twenty feet from the patient and did not even see their faces. It usually took two to three hours to complete your observation on the seven *Chakras* main. Then she filled in the kind of a map with her observations and the researcher made her comparison with the clinical map of the respective patient [34]. Finally, he identified the following relationships between the *Chakras* and the endocrine glands as shown in the table below.

It is also worth mentioning the research carried out by Motoyama, Ph.D. in Philosophy and Clinical Psychology, which tends to confirm the existence of *Chakras* and their possible bioenergetic and or bioelectric action. That is because he believed that although the nature of the energy in these energy centers was subtle, there could be secondary reverberations that could be measurable due to their electrostatic nature. These secondary energies would be in a lower harmonic octave, like the electrostatic fields. For such studies, he developed two instruments, the "AMI" that serves to measure the functional states of the energy channels and their internal counterparts and the "*Chakra* instrument" to discover the energy produced and expelled by the body, in terms of physical variables. The latter was developed to detect the smallest energy variations of a patient. The detectors are installed in a light-proof container, with walls covered with ground wire and the internal surfaces covered by a thin aluminum sheet. The patient is 12 to 20 centimeters at distance and a disc-shaped copper electrode and a photoelectric cell are positioned towards the supposed location of the *Chakra* to be studied. A magnetic field fluctuation detector is placed on the floor, in front of or beside the patient. Thus, they record all signals that are then amplified and analyzed by a processor, a force spectrum analyzer and other similar equipment [35].

Another relevant study was carried out by Dr. Valerie Hunt, which were used electrodes electromyography devices connected to telemetry equipment, to capture changes in the skin, in the region corresponding to the location of the *Chakra*. And it also counted on with the clairvoyant works, to identify changes in the individual's energy field (aura). The electrical impulses were sent to a recording booth where different types of oscillographs recorded energy fluctuations in those regions. The clairvoyant did not have access to this information during the period of her observations. The results obtained in this study demonstrated a perfect correlation between the clairvoyant's observations about the color changes in the individuals' energy fields and the electrical records presented by the electromyogram electrode [10].

According to the Yoga philosophy, the principle of everything that exists in the universe is propagated through vibrations. Thus, it is believed that thoughts, words, feelings, food, and everything else that exists on this planet, has a vibratory pattern that can affect people's health in a positive or negative way.

The balance between the five bodies and the healthy functioning of the *Nadis* and the *Chakras* are of the utmost importance for the "Yogis" since their dissonances can breed several diseases. This balance can be achieved through the correct performance of psychophysical postures (*asanas*), breathing exercises (*pranayama*), chanting of *mantras*, contraction of some muscles of the body (*bandhas*), gestures (*mudras*) and meditation (*dhyana*).

Recently, scientific studies have been published to assess the benefits of *Bhramari Pranayama*, in which the practitioner is instructed to make a bee-like tinnitus. One of the studies suggests that the performance of this *Pranayama*, increases the expression of nitric oxide, increasing carbon dioxide by prolonged

exhalation and alkaline pH, avoiding coagulopathies and morbidity due to Covid-19, due to the oscillating sound wave [36]. It is estimated that tinnitus increases the endogenous generation of the nitric oxide level by 15 times compared to the total exhalation [37]. Studies carried out with adolescents have identified that *Bhramari Pranayama* promotes the improvement of cardiac cognitive and autonomic function for this population [38, 39]. In another study conducted with patients with essential hypertension, there was a significant increase in parasympathetic tone in the first session [40].

The vibrational benefits of Yoga are also promoted by chanting *mantras* which, when repeated, make the *Chakras* spin faster. Each *mantra* has a different vibration that can attract energies of peace, happiness, harmony, love, etc. A study carried out with college students, concluded that the sound vibrations improved the participants' general joy and clarity of mind [41]. Another study looked at the effects of the *mantra "OM"*, the most important of the *mantras*, according to the Yoga philosophy in participants who were instructed to chant the *mantra "OM"* for 30 minutes aloud. The *mantra "OM"* is considered the sound of the universe, it means the beginning and the end of everything. In this study, the results showed a significant increase in theta power when the average of all regions of the brain after the intonation was calculated, confirming its high relaxing power [42]. The vibrational power from chanting *mantras* has also been proven to improve perceived stress, sleep, mood and osteoarthritis in the knee of elderly people [43].

Asanas, psychophysical postures, gained prominence and became a binding element when it comes to Yoga in the West. However, they represent much more than health for the body, because when performed correctly, they also stimulate the practitioner's psychological action. According to the psychoanalyst Georg Groddeck, there is no separation between body and psyche, but both would be facets of the same whole [44]. Through *Asanas*, the functions of internal organs, muscles, and the nervous system are toned and stabilized. But the aim is to control energy flows. When performed correctly, with permanence and adequate breathing, *Asanas* massage internal organs, endocrine glands, nervous system, and muscular system. There are three groups of trunks, the first of which consists of increasing the assimilation of *Prana*, stimulating its circulation through the *Nadis*. The second group aims to strengthen and regularize *Sushumna*, the main energy channel, according to this philosophy. And the third group, strengthen the concentration on the energy centers. However, it is noted that the performance of *Asanas* needs to follow a progress, where *Prana* and *Nadis* are worked to then work the *Chakras* [35]. Knowing this concept performing *Asanas* in Yoga has a purpose that goes far beyond the physical aspects of the practice. In the scientific literature, there are thousands of studies on the benefits of *Asanas* in several diseases, but the lack of clear and common methodological criteria among the researchers makes it difficult to prove its benefits.

Another technique widely used in Yoga, are the *Bandhas*. It can be said that they are contractions made consciously in some regions of the body that subtly influence the *Chakras.* The *Jalandhara Bandha* whose concentration is on the *Vishuddha Chakra*, the *Mula Bandha* that focuses on the *Muladhara Chakra*, and the *Uddiyana Bandha* with a concentration on the *Manipura Chakra*. Both help to release the flow of *Prana* in the channel *Sushumna*. At first, this technique can be performed alone so that the practitioner becomes aware of it, however, the ideal is that it is performed with breath-holding and *Mudras*.

Mudras are gestures usually performed with the fingers. At first not its effects are visible, but the truth is that the performance of these gestures has power dressing helps in the balance of the five elements in the human body and stimulates the circulation of vital energy in the energy channels that will eventually lead to the *Chakras* [45].

The five fingers of the hands represent the five elements. Fire (*Agni*) is represented by the thumb, air (*Vayu*) by the index finger, ether (*Akash*) by the middle finger, earth (*Prithvi*) by a ring, and water (*Jal*) at a minimum. Some *Mudras* act within minutes, but others can take up to 45 minutes to work.

There are several reports on the "powers" acquired through the practice of Yoga. Motoyama recounts his experiences in the book "Theory of the *Chakras*". This book contains rich details about his process and how to perform each technique he used. In the Yoga philosophy, these powers are called *Siddhis* and are also described in the "Yoga Sutras". According to Patañjali, the individual can either be born or acquire these powers using some medicinal herbs, through the chanting of *Mantras*, through the effort on oneself, or through the attainment of *Samadhi* (understanding of existence and communion with the universe). Some of the *Siddhis* that can be obtained through the practice of Yoga and meditation are: knowledge of the past, present, and future; tolerance to cold and heat; self-control; knowledge of what goes on in other people's minds; insensitivity to the sun, fire, water and poisons.

However, Yoga is a philosophy that uses techniques to manipulate and harmonize vital energies, unlock the channels and energy centers, encouraging the practitioner to (re)connect with the vibration that exists in everything. Induces the practitioner to disconnect from the external agitation and dive into the internal vibration, becoming aware of the energetic reality emerging in their bodies and minds, and thus, letting go of *Maya*, the illusion, the primary cause of all human suffering and its greatest prison in this world.

2.3 Homeopathy

Homeopathic therapy was created by the German physician Samuel Hahnemann in 1796, due to his dissatisfaction with the therapy used at that time. Hahnemann made his proposal for this new therapy after seeing that the symptomatic picture of malaria, which had quina bark (*Cinchona officinalis*) as standard treatment at the time, had the same intermittent fever and other symptoms that were characteristic of malaria, in healthy individuals. Based on this observation, Hahnemann proposed the thesis that institutes homeopathic therapy that is based on the Law of Similars or *Similia similibus curentur*, that is, "be the similars healed by similars" [46].

This concept had already been presented by Hippocrates, considered the father of Medicine, who describes that "the disease is produced by the similar and, through the similar, the patient returns to health". Although this proposal was previously presented, it was Hahnemann who was responsible for demonstrating it clinically, based on his fundamentals, endowed with information in the Experimental Medical Matter and with an exclusive Pharmacotechnics [47].

In addition to the presented principle that "the similar heals other similar", Hahnemann introduced the concept of minimum doses, explaining that these were chosen after their effectiveness was noticed, since, by reducing the concentration of substances through their dilution, the same continued to have a therapeutic effect, without harming the symptoms of intoxication [48].

The art of studying the toxic effect of substances on animals already existed, but it was necessary to make a similar study in man, so that Hahnemann developed this experimentation, elaborating the homeopathic medical materials from these observations. To explain this fundament, the father of Homeopathy quotes in his book considered being the greatest work [49], *"There is, therefore, no safer or more natural way to infallibly discover the proper effects of medicines on the man Health, than try them separately and in moderate doses in healthy individuals and observe the changes that result in physical and moral states"*.

Another basis of homeopathy presents the proposal of using a single medicine, capable of covering the entire symptomatic of the individual, which is, a medicine whose pathogenesis best coincides with the manifestations presented by the patient, this being his simillimum [47], that is, a medicine that when used, will act considering the organism as a whole, having all its integral parts, in order to re-establish the harmony of the system.

From his experiences, Hahnemann proposed a therapy based on the vital force, explained by him in the 6th edition of Organon [50] by the words *"In the individual's state of health, the vital force of the non-material type reigns absolutely that animates the material body like Dynamis, keeping all its parts in a vital process admirably harmonious in its sensations and functions, so that our rational spirit that inhabits it can freely use this living and healthy instrument for a higher objective of our existence"*.

And then, according to the principle of Vital Force, under the Law of Similars proposal, and following the fundamentals of experimentation in a healthy man, minimum doses and single medicine, it was published by Hahnemann, in 1976, in the Journal of Practical Medicine, an article in which states that "disease can be cured by drugs that cause symptoms similar to the disease" [47].

To exemplify the law of similars, Bellavite et al. [49], presented some schematic representations of two examples of the application of the law of similars and of diluted and dynamized substances, in inflammatory models. The example presented was obtained with the medicine *Apis mellifera* and *Pulmo histaminum*, which not only represent the law of similars, since both cause the same symptomatic condition that they intend to heal, but also represent the concept of 'iso' therapies, where a substance that causes the problem is streamlined to address it, as can be seen below.

The scheme presented represents the results of Poitevin et al. [50] and were based on the use of bee venom (*Apis mellifera*) and *Pulmo histaminum* (pulmonary histamine), since these substances present, respectively, melittin and histamine, substances capable of stimulating basophils and triggering an inflammatory reaction. It is known that histamine is produced by the decarboxylation of histidine, stored in granules of basophils and mast cells, and are released within seconds after its activation. Histamine in tissues exerts vasodilation and increased permeability, and thus causes the formation of edema and papules. On the other hand, *Apis mellifera* and *Pulmo histaminum*, are used in homeopathic medicine to treat allergic and inflammatory processes [49], with the same symptoms, such as edema, heat, pain, and allergy.

As shown in **Figure 2a** and **b**, the sources of the medical material *Apis mellífera* and *Pulmo histaminum*, when diluted and dynamized (homeopathic medicines) are able to prevent the effects of the stimulation triggered by the anti-IgE antibody (which has the same effect a melithin and histamine). In other words, it was demonstrated in "in vitro" experiments by measuring the degranulation of basophils, the law of similar, where a substance that triggers an effect, when diluted and dynamized, was able to inactivate this effect. In clinical practice, the symptomatic picture of allergies and acute inflammations is eliminated.

The mechanisms by which Hahnemann's proposal is based is not yet clearly demonstrated, and most likely due to the technological limitation for such energetic measures involved to be made. A look from a vibrational point of view may shed light on a possible way in which homeopathic medicines act, especially when you think about the concept of a single medicine and its fundaments that the similar heals other similar.

Homeopathic medicines come from a solution or mother tincture produced from products of plant, animal, or mineral origin. All of these have atoms, protons, electrons, neutrons and smallest particles, and for that reason, they are in constant vibration, and the energy/matter duality, just like humans, having the

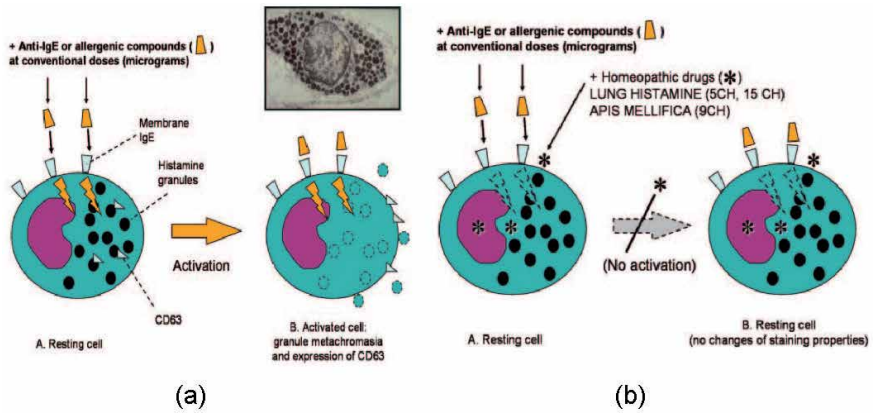

Figure 2.
a - Activation of normal basophil degranulation caused by IgE antibodies. b - Inhibition of basophil degranulation by the use of homeopathic drugs (ultra-diluted and dynamized) such as Apis mellifera *and* Pulmo histaminum. *Source: Immunology and Homeopathy. 2. Cells of the Immune System and Inflammation [51].*

premise of Einstein's theory, in which matter is composed of particles, which ultimately consist on light/energy, in the wave/particle duality. Analyzing the Hahnemannian point of view on the vital energy, someone could suggest that the vital energy is the vibration that needs to be preponderant for the state of health. When diseases change this vibrational frequency, they need something that offers the dosage of energy/frequency necessary for this vibration return the original vital energy.

In the process of health and illness, it could be said that the individual's energetic/vibrational state has been altered, and in order to restore the original energy, a vibrational energy medication compatible with that necessary for the restoration of the individual's vital energy, is necessary. Thus, and taking as a reference the homeopathic process of dynamization of the medicines, which follows a dilution and a succussion (rhythmic shaking of the bottle containing a medicine), aiming to awaken the medicinal energy of the substance, in water, and considering that the raw materials of departure have their own vibration, it is possible to suggest that homeopathic medicines, already without quantifiable matter by chemical methods, considering the number of Avogadro, have only the vibration of the original substance, the subtle energy, which will exercise its function of the ethereal subtle energy of the sick individual.

One hypothesis is that the drug energy of a substance that causes a symptomatic picture identical to that provoked by the disease, presents the same frequency as it, and thus, the frequencies cancel each other out, returning the vibration of the normal individual to preponderate. It is not uncommon in the homeopathic healing process, the individual presents an aggravation of the symptoms, and this could be the consequence of the initial summation effect of the medication vibration, added to the energetic picture of the disease, which highlights the symptomatic picture to stimulate the immune response to restore the individual's energetic/vibrational state of balance.

2.4 Apitherapy

After the Albert Einstein presentation about the revolutionary theory of energy and matter, using the very famous equation: $E = mc^2$, which establishes the quantitative equivalence of the matter's transformation into energy or vice versa, and the comprehension of each atom is composed by electrons, neutrons and protons that

are under constant vibration, emitting energies with different frequencies, and that humans are, in the last level, composed by atoms (equivalent to energy), it is much easier to understand that each individual possesses your own vibrational standards. When this vibrational standard state of 'normal' level is modified by one disease, something needs to happen to re-establish the energy to the 'normal level' again (Vital Energy, *Prana, Qi*) [10].

To clarify this statement, imagine the electrons that are in specific space regions known as orbitals. Each orbital presents a specific characteristic of energy and frequency, according to the atom and its molecular weight. In order to change the position of one electron to another next superior orbital, it is necessary to transmit the energy of a certain frequency. Only one quantum of exact energy necessary will stimulate the jump of the electron to the next superior orbital. Through the resonance process, the energy of appropriate frequency will excite the electron and cause it to pass to the highest energy level in its orbit around the nucleus. Maybe human behavior could be compared to the electron change of energy level, if we consider (as an analogy) the existence of 'orbitals' of health and disease, for example with different vibrational levels (vibration/frequency). In the case of human beings whose energetic systems are in a disease orbit, only a subtle energy dose with the appropriate frequency can be accepted and cause the body to move into a new orbit or steady state of health. Vibrational remedies could inject the quantity necessary of subtle energy in the human system through the induction of resonance. This etheric energy changes the vibrational level of disease to the orbital of health [10].

The energy that gives life is called 'vital energy' in homeopathy, 'prana' in yoga and *"Qi"* in acupuncture. This etheric energy can dictate the cellular physical information of the matter, on the health, and in the disease. And consequently, vibrational medicines that can modify these frequencies, can change the disease's vibration into a health steady-state.

During this chapter, it was possible to present until now, 3 different types of treatments, all based on vibrational medicine, with different techniques: acupuncture, yoga, and homeopathy. Considering all this information, now it is time to talk about apitherapy and how bees can help during the health process.

Apitherapy or "bee therapy", from Latin *"Apis",* which means bee, is the medicinal use of products produced by honeybees, like honey, propolis, bee venom, bee pollen, royal jelly, beeswax, besides others, as therapy for some diseases. If we think in a more complete and philosophy way, the definition proposed by Stangaciu (2015), can be more suitable to define all levels reached by this therapy, *'the art and science of treatment and holistic healing through the honeybee and her products for the benefit of mankind and all the animal kingdom'* [52].

Ancient civilizations with their millennial therapies recognize and use the bee products as valuable resources in their medical practices. The history of medicines of the Chinese, Tibetan, Egyptian and also the Greco-Roman civilizations are quite rich, containing in its records, dated for a long time (more than 6000 years in ancient Egypt), hundreds of recipes, including among the main ingredients, honey, propolis, bee larvae and eventually the bees themselves to cure or prevent diseases [53].

Hippocrates (460–370 BC), Aristotle (384–332 BC), and Galen (130–200 AD) prescribed the use of honey and bee venom as a cure for baldness. Hippocrates recognized the healing virtues of bee venom for treating arthritis and other joint problems, also used propolis for healing sores and ulcers internally and externally. Today growing scientific evidence suggests that various bee products promote healing by improving circulation, decreasing inflammation, and stimulating a healthy immune response [51].

Nowadays, apitherapy is adopted in some countries as Complementary and Integrative Medicine with a specific regulation, as is the case of Brazil for example [5], but some others deserve special attention since they possess an important tradition in the apitherapy use and/or Organizations of Apitherapy running some job in each country in this field. In Brazil, honey and propolis possess widespread use by the population. Apis Flora Company introduced propolis extract in the Brazilian market at the beginning of the 80s, and from this moment, several other innovations appeared as the natural syrups based on mixtures of honey with propolis and others bee products, and/or with herbal extract and/or essential oils, vaporizers with the same type of mixtures. Extracts, lozenges, tablets, capsules, and others can be found in European, American and Asiatic countries, and they can be found in pharmacies, drugstores, natural product houses, supermarkets, etc. [54]. Besides honey and propolis, royal jelly, bee pollen, and apitoxin can also be found, however with less expression than the firsts.

For understanding the apitherapy importance as integrative medicine and in order to propose some connection with vibrational medicines, it could be useful to present some small information about what each bee product is, its traditional use, composition, and benefits according to clinical trial and/or systematic review available until now. As the literature about each bee product is quite large, and the deeper description about "in vitro" and "in vivo" protocols about mechanisms involved already done, are not our focus in this chapter, only some information will be provided in order to try to propose an innovative way about thinking apitherapy with vibrational medicine information. But first, let's know something about the vibrational communication between bees.

2.4.1 Vibration signal and communication

"Cooperative activities in honey bee colonies involve the coordinated interactions of multiple workers that perform different, but interrelated tasks. The vibration signal functions as a type of 'modulatory communication signal'. It is directed toward diverse recipients, causes a non-specific increase in activity that may alter responsiveness to a wide array of stimuli, and thus may influence the performance of many different tasks simultaneously. These signals are involved in coordinating at least three colony-level activities: food collection and foraging-dependent tasks, queen behavior during swarming and queen replacement, and house hunting by honey bee swarms. Signals that function like the vibration signal may be widespread in highly social insects and social animals in general and may help to fine-tune the collective decision-making processes that underlie cooperative actions in a wide array of species" [55].

As could be seen in the text published by Scheider & Lewis [55] the vibration signal produced by bees is used as a modulatory communication signal, as it is involved in several actions executed in the beehive. The frequencies, high or low, the pulses short or long, besides other characteristics in the vibration not only activate but also are involved in the stop of some functions. Von Frisch [56] compared this warning sounds with the vibrations produced by honeybees during "buzzing dances" and the buzzing tones. Stop signals are short, high-frequency signals that occur under rather different conditions. Nieh [57] described that short pulses of body vibrations were performed by foraging bees, offering a warning to the others about a dangerous food experience. The literature available proposes that different signals communicate different things, activating, or stopping actions. Not surprisingly, many different terms have been used to characterize these different short pulses (e.g., piping, begging, buzzing, shaking, whooping).

Besides the communication actions developed, Schneider, Huang & Lewis discovered that the juvenile hormone titers of workers 15–30 min after receiving

vibration signals are slightly, but significantly higher than those of non-vibrated control bees that are matched for age, location in the nest and initial activity levels [55]. One parallel that it is possible to do in this topic is that the evidence already described the higher concentrations of some hormones like adrenalin, estrogens, corticosteroids, besides others, in the meridians used as key points in acupuncture, in comparison with the concentration in the blood, show evidence about the connection of these points with the body endocrines glands [10]. So, the vibration modified by the application with the needles in the acupuncture in these meridians can modify the endocrine glands' behavior, affecting some physical behaviors in the body. Here it is possible to meet some convergence with what vibration provoked by bees can do in the production of some hormones of other bees.

It let us consider that vibration produced by bees can stimulate higher production of some compounds in the bee organism. And the question that one could do is: Can vibration produced by bees energize bee products produced in the beehive as royal jelly, bee venom, honey, propolis, etc.? Is there some characteristic vibration of bee products that can affect the etheric vibration of the humans? Of course, this hypothesis needs to be validated, but let's do an exercise with bee products in order to evaluate this hypothesis.

2.4.2 Honey

Honey has been used in the human diet since 25,000 years ago when the first tangible demonstration was found [58]. Besides its use for nutrition and mainly as a sweetener, its use as a traditional medicine in several cultures worldwide was very well documented [59–61]. The use of honey for therapeutic purposes dates back to Egyptian papyrus, and it was also mentioned in Chinese medicine and Hindu documents [62].

The chemical composition of honey is based on a supersaturated sugar solution, especially rich in fructose and glucose, which also present more than 181 substances containing minor quantities of minerals, vitamins, phenolic acids, flavonoids, enzymes, organic and amino acids, proteins, and a huge variety of essential oils according to the different flowers visited by honey bees [63, 64]. The honey composition will never be the same since it depends on the source-type, soil, climate, and genetic factors and procedures methods involved. In this sense, the feeling and sensation promoted by honey as colors, flavors, smells, and tastes difficult will be the same [65, 66].

Several biological properties were already demonstrated for honey as antioxidant action that contributes to the prevention of several diseases as cardiovascular problems, cancer, diabetes, and others, especially by the protection against free radicals and other oxidative substances [67–69]. Besides antioxidant action, antimicrobial and wound healing are other two properties very well recognized in the literature and it is mainly attributed to high osmolality and sugar concentration, low pH, aromatic acid, volatile substances, and peroxides composition [70–73]. Antiviral and fungicide activities were also demonstrated and linked to some flavonoids found in honey as chrysin, acacetin, and apigenin [74].

Riera et al. evaluated the Cochrane systematic reviews and protocols for complementary medicine in order to check several new therapies approved in Brazilian regulation. These studies demonstrated that honey dressings for partial healing of wound burns and honey to reduce coughing among children with acute coughs were effectively able to demonstrate scientifically and with the scrutiny of rigor science, the important safety and efficacy of honey (26 randomized clinical trials, with around 3100 individuals), supporting apitherapy introduction in the therapies proposed by the Brazilian Government [75].

Although several biological actions under Newtonian methods were already demonstrated, to think about the influence of the vibrations produced by bees when they are communicating with each other ("buzz") and its impact on the bee products under vibrational level, the question could be: could honey be 'carrying' the energies of the flowers that were visited by bees during the collection of nectar and pollination? Flowers as the humans are composed of atoms, protons, electrons, and neutrons, and also, vital energy, and consequently, also possess their own frequencies. Besides it, flowers are rich in essential oils, volatile compounds that can be energized during the flights of the bees with the vibrational movements and sounds. Could honey be acting in the healthy restoration by modifying the etheric Humans Vibration? (in the level of Vital Energy, *Prana,* and *Ch'i*? Here are good questions to be investigated.

2.4.3 Propolis

The word propolis means 'in defense of the city', and it is derived from Greek *"Pro"* and *"polis".* Propolis is a resin collected and transformed by *Apis mellifera* bees to use it for sealing up their hives. Propolis is a sticky filler material with aroma, smell, and taste particular and dependent on the botanical source [76]. In Europe and Asiatic countries, propolis mainly botanical source is poplar (*Populus spp*.); in Brazil, it was already related to 12 types of propolis, according to Park et al. [77].

The literature already demonstrated several important biological activities for propolis as an antioxidant, anti-inflammatory, immune modulator, antibacterial, antifungal [76–80] and several many others [76]. Propolis was evaluated in some clinical trials with important good results in preventing respiratory tract infections in children in a randomized, double-blind, placebo-controlled, multicenter study [81], as a supplement as an adjuvant in asthmatic patients [82] and as a prophylactic for immune stimulation [83].

As it happens in the case of honey, propolis also is variable according to the region, botanical sources, climate, etc. that bees collect the exudates to transform into propolis. This was an important remarkable point for regulatory agencies such as the European Medicines Agency (EMA) to criticize the approval of propolis as a medicine, due to the lack of standardization [84]. In this sense, it was presented in 2012, a propolis standardized extract called EPP-AF® [79]. Propolis EPP-AF® was already studied according to its safety and efficacy in animal and human protocols for several applications [79, 85–89]. Of remarkable interest was the clinical trial done with EPP-AF® (500 mg/kg/day) in kidneys damage protocol during 12 months of research and monitoring of elderly patients, with impacting results in the reduction of proteinuria biomarkers [90]. Also, and not less impacting was the demonstration of the lack of interaction evaluated in a clinical study with hepatic enzymes CYPs and the transport protein PgP. Propolis EPP-AF® demonstrated safety at this level using WHO protocols [91]. Finally, Berretta et al. [80] published a review article supporting the use of propolis to reduce the damages and aggressiveness of SARS-CoV-2 in the COVID-19 patients. But, besides the Cartesian mechanisms already demonstrated above to propolis effects, could propolis also act by vibrational medicine? After looking forward to finding some toxicity data in animal and clinical trials for propolis, no impacting results were found, except by sparse cases of an allergic reaction [92, 93]. Some patients can be allergic to propolis due to a topical application, inhalation, or ingestion. Curiously, diluted propolis applied by inhalation reduced allergic airway inflammation system [94], besides the several studies demonstrating that propolis possesses anti-inflammatory results in several models with a huge range of dosages. In this example about allergy, Does the small dosage inhaled, could be acting following

the principle of homeopathy? Could propolis be "energized" by the vibration produced by bees? This is another hypothesis to be tested if we can attribute vibrational effects to apitherapy.

But, if we consider that propolis was produced by bees after the collection of plant exudates, rich in chemical substances as flavonoids, phenolics, essential oils, etc., that possess vital energy and a vibrational standard, and suffer vibration during this process, could we postulate the hypothesis that propolis could be acting in the frequency modification on an etheric level as a vibrational medicine? Newtonian evidence already demonstrated the pharmacological effects, but could something more be happening?

2.4.4 Apirespiration

Some European countries as Germany and Slovenia are using as apitherapy beehive air inhalation ("apirespiration") to promote the health of respiratory systems. The inhaled aroma produced in beehives has been suggested to be beneficial to the health of human beings [95]. The best results usually were related to immune disorders such as allergies, chronic sinusitis, and susceptibility to infections, for patients of all ages [96].

In the beehive environment, the air is constantly circulating due to the vibration of the wings of thousands of bees. The air is saturated with water vapor, particles of isoprene, terpene, essential oils, hormones, feromones, liquid wax, alcohol, bee saliva secretions, propolis, trace elements, enzymes, choline, phytohormones, etc. By natural respiration, these substances are applied in the human body, having a positive effect on various diseases, whether as prevention, cure, or stabilization [51]. "Apirespiration" sum the benefits of the substances produced in the beehive with the vibrational energy of the flight of the bees, associated with the breathing of the pleasantly warm bee air (around 36°C). Excellent results of apirespiration have been proven with bronchitis, asthma, allergies, diphtheria, chronic inflammation, impaired immunity, migraines, and depression [51].

Several systems were proposed to this type of apitherapy, as a respiratory system connected directly to the honeycomb (**Figure 3**) and others completer and more planned in well-structured rooms were already suggested, the ApiHouses.

The inhaled aroma produced in beehives has been suggested to be beneficial to the health of human beings. This integrative medicine is still an incipient field of study, and research projects in rich countries have improving its current results despite the inherent difficulties by the high number of bee species and consequently

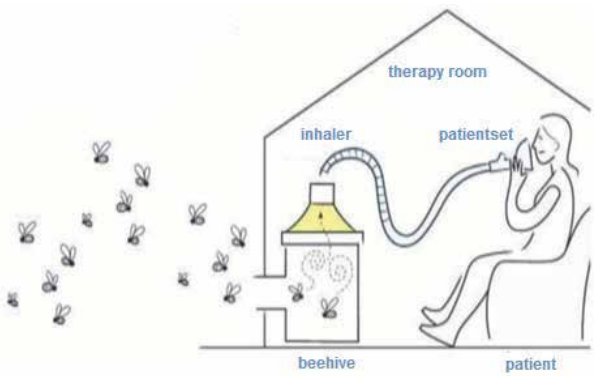

Figure 3.
Apirespiration system.

Figure 4.
Some ApiHouses proposed by [51].

the quantitative and qualitative differences in the chemical composition of the honeybees and the concentration of their by-products (**Figure 4**).

The different models and classifications used in each case for ApiHouses were related to different kinds of experience. Some more related to the api respiration properly said, the inhalation of the volatile substances and the vibration offered by bees, but also the interaction and peace obtained with the vibration of nature and its sounds.

In the cases of ApiHouse, the question could be if the vibration/sounds produced by bees were affecting the etheric vibration of the patient modifying it to the 'normal' level, or if the vibration was "energizing" the volatile substances emitted by the beehive, or both.

2.4.5 Bee Venom

Bee venom importance was highlighted with the publication of the First scientific paper by Desjardins, a French physician on the successful treatment and curative properties of bee venom for rheumatic disease. Filip Terc (1888) was considered as the "Father of Apitherapy", he applied around 39,000 bee stings to over 500 rheumatic patients, was the first one to use systematically this type of therapy culminating with the publication "Report about a peculiar connection between bee stings and rheumatism" [96].

Bee venom is produced by bees for two glands (acid and alkaline glands) associated with the sting apparatus of worker bees [96]. It is a complex mixture from honey bees, which possesses various peptides including melittin, apamin, adolapin, apamin and mast cell degranulation peptide, enzymes (PLA2, hyaluronidase, acid phosphomonesterase, α-D-glucosidase, and lysophospholipase), biologically activity amines, and non-peptide components such as histamine, dopamine, and norepinephrine [97].

Bee venom therapy can be applied in some different ways, (i) live bee sting, (ii) bee venom injection, and (iii) bee venom acupuncture. The scientific data suggests that bee venom acupuncture is more effective than the application of live bee sting, bee venom injection, and acupuncture alone [96, 97]. The sum of bee venom and acupuncture offers the benefits of the chemical and pharmacological effects of the substances found in the bee venom and the mechanical application in the acupoints [97].

The number of studies already published with bee venom is too large. In order to show some application of its bee product, some interesting results will be present. Curiously, bee venom and acupuncture are very connected. Khalil et al. [98] demonstrated that bee venom acupuncture at Yanglingquan acupoint (GB34) improved

locomotor behavior significantly, reduced several central amines, reduced pro-inflammatory cytokines, and neuronal apoptosis, demonstrating strong evidence of a neuroprotective effect of this therapy. Bee venom and acupuncture also demonstrated good results in idiopathic Parkinson's disease [99]. In rheumatoid arthritis, the effects occur beginning with an anti-inflammatory action, reducing the expression of some cytokines and the level of glucocorticoids, protease activity, and reactive oxygen species (ROS) [97].

Besides the innumerous animal studies with bee venom, only some clinical trials were already performed, however, with a relatively small number of patients, for the lumbar disc disease treatments, knee osteoarthritis, rheumatoid arthritis, adhesive capsulitis, lateral epicondylitis, neuropathic pain, Parkinson disease, stroke, depression. An interesting mini-review about the use of bee venom under the point of view of safety and efficacy in acupuncture puts the question "to bee or not to be" in this topic, since the risks about the allergic reactions can be very high and so, a good evaluation of the 'costs' and benefits needs to be done in order to take this decision - "To bee or not to be, is that question" [100].

Now, under the point of view of vibrational therapy, definitely, the tiny quantities of the bee venom necessary to produce the effect let us know about the effect of homeopathic medicine, especially thinking about the potential toxicological effects. Would the Bees vibration offer a homeopathic characteristic to this product? The high incidence of successful results applying bee venom in the acupoints really calls attention. The substances present in bee venom, in a tiny concentration, can offer different pharmacological types of effects, including important damages. The mechanical application of needles in specific acupoints can stimulate vital energy *"Qi"*. Could bee venom present some frequency able to offer a more intense stimulus to the vital energy of *Qi?* Curiously, bee venom is recommended to be applied intradermally and not intravenous. It can remind the conjunctive route tissue explained in the acupuncture topic.

Other bee products could be presented here as royal jelly, bee wax, bee pollen, however, with the examples presented above it was possible to know something about apitherapy and its importance for humanity, and open the mind to think about new possibilities for the influence of bee products in living beings. If these hypotheses are true, we will only know if appropriate techniques are used. So far, it is only speculation to be tested.

3. Conclusion

It is noted that, although more comprehensive, the new concept of health adopted by the World Health Organization is still incomplete, not considering the "individual" in its entirety, which includes the vibrational and energetic aspects addressed in this chapter. Biologists have long been impressed with the ability of living things to maintain their own stability. The idea that a disease is cured by natural powers, by "*a vis medicatrix naturae*", an idea that was supported by Hippocrates, implies the existence of entities ready to act corrective when the normal state of the organism is disturbed [9]. The most important principle of vibrational medicine is the concept that human beings are dynamic energy systems that reflect evolutionary patterns [10]. The assumptions we formulate throughout this chapter are not new. Since the publication of the General Systems Theory, proposed by Austrian biologist Ludwig von Bertalanffy in 1950, the basic assumptions point to a clear tendency towards integration between the natural and social sciences, allowing, in a comprehensive way, to study the

non-physical fields of scientific knowledge, promoting the multidisciplinarity so necessary to science.

Starting from the essential unit of matter, these authors presented the biophysical links in common and specific methods for the search for homeostasis. The search for the integral man, full of its capacity for self-regulation, goes through the path of effective and progressively more natural preventive health.

Launching a vibrational look at ancient therapies (acupuncture, yoga and apitherapy), as well as for the youngest among them, homeopathy, represents contemplating and renewing the ancestral bonds that connect the three parts of the human being: body, mind and spirit. An innovative way of think in this sense were presented for homeopathy and some new hypotheses were formulated, especially regarding the function of bees as a vibrational instrument for each bee product known.

It is possible that many readers will be concerned, because the larger the body of evidence, the more it will be possible to provoke a closer look within the countless therapies presented in this book. The greater the understanding of the biophysical aspects of the energy generation process, the distribution of information, the use of nutrition for the production of defense in the body system, the more accessible and comprehensive the integrative and complementary therapies in current and future medicine will be.

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Conflict of interest

The authors declare that they have no conflict of interests.

Appendices and nomenclature

WHO	World Health Organization
PNPIC	National Policy of Integrative and Complementary Practices
MS	Ministério da Saúde/Health Ministery
HBU	Health Basics Units
NASF	Family Health Support Units
TCM	Traditional Chinese Medicine
CNS	Central Nervous System

AP	Action Potential
SP	Spleen and Pancreas meridian
LU	Lung meridian
K	Kidney meridian
L	Liver meridian
HT	Heart meridian
TE	Triple Burner meridian
SI	Small Intestine meridian
LI	Large Intestine meridian
EMA	European Medicines Agency
EPP-AF	Propolis Standardized Extract – Apis Flora®
CYPs	Cytochromes
PgP	Glicoproteína-P
PLA2	Phospholipase A2
SARS-CoV-2	Severe acute respiratory syndrome
COVID-19	Coronaviruses

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Chapter 9

Natural Compounds in the Modulation of the Intestinal Microbiota: Implications in Human Physiology and Pathology

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Abstract

Clinical interest in the human gut microbiota has increased considerably, because of the increasing number of studies linking the human intestinal microbiota and microbiome to an ever increasing number of non-communicable diseases. Many attempts at modulating the gut microbiota have been made using probiotics and prebiotics. However, there are other avenues that are still little explored from a clinical point of view that appear promising to obtain modifications of the microbial ecology and biological activities connected to the microbiome. This chapter summarizes all *in vitro*, *in vivo* and clinical studies demonstrating the possibility to positively modulate the intestinal microbiota by using probiotics, foods (and prebiotics), essential oils, fungus and officinal plants. For the future, clinical studies investigating the ability to modify the intestinal microbiota especially by using foods, officinal and aromatic plants or their extracts are required. More knowledge in this field is likely to be of clinical benefit since modulation of the microbiome might support the therapy of most non-communicable diseases in the future.

Keywords: microbiota, microbiome, probiotics, essential oils, phytotherapy

1. Introduction: The pivotal role of gut microorganisms in human health

Our knowledge of the relationship between human beings and the microorganisms we harbor in our gut has greatly increased in the past years, even if we are still far from having understood all their functions. We no longer consider these living entities as simply commensal, and we start to realize that humans are "super organisms" governed also by the microorganisms living inside us. There are approximately 100 trillion cells in the human body, and more than 90% of them are microbes. They make up the human microbiota, consisting of bacteria, fungi and even viruses, mainly located in the intestine where they are referred to as the intestinal microbiota.

The terms currently employed in this field are the following:

Microbiota, which refers to the communities of living microorganisms residing in a defined ecological niche.

Microbiome, which indicates the whole of the microorganisms, *i.e.* their genes (genome), their proteins (proteome) and their metabolites (metabolome) (even if the terms microbiota and microbiome are often used interchangeably).

Metagenomics, which is the analysis usually performed by next-generation sequencing techniques, of the genetic material of microorganisms obtained from a sample of the environment that is being studied, such as for example feces for the profiling of the fecal microbiota.

Dysbiosis, which is an alteration in the microbiota structure (as opposed to *eubiosis*), with negative implications for microbial metabolism and host physiology.

The first consideration that we have to do is that the microbial ecosystem of the intestine called gut microbiota, is one of the most dense communities that we know, surpassing for complexity those present in soil, subsoil and also oceans [1].

The second consideration is that the microbiota does not represent an inheritance dependent on our species or genes, but rather an environmental inheritance, mainly due to the type of environment to which we have been exposed in the first 3–4 years of our life [2]. This also implies that we can act during life with the aim of improving our microbiota (**Figure 1**).

The last one is that our gut microbiota and microbiome are strictly connected with our state of health or illness and, together with genetics and environment, certainly represent a discriminating point in predisposing us to the onset of some particular diseases rather than that of others. The gut microbiota is closely related to our metabolic balance as well as to the development and functioning of our immune system, as studies on germ-free animals have clearly shown. It is also closely connected with the intestinal and systemic endocrine system, and indirectly with the central nervous system, via the enteric nervous system, within what is commonly called the gut-brain axis [3].

These considerations must not make us think of the microbiota and microbiome as something fixed and stable in the course of our life. The aging of our organism physiologically leads to a change in the gut microbiota with a decrease in some specific populations, such as the short-chain fatty acid (SCFA)-producing families *Lachnospiraceae* and *Ruminococcaceae*, and an increase in potential or opportunistic pathogens, as enterobacteria. Aging is also accompanied by an increase in low-grade, chronic inflammation. This so-called "Inflammaging" impacts gut integrity and can be causally linked to age-related changes in the microbiota [4]. Inflammation and dysbiosis are always closely connected to each other and inevitably end up entering

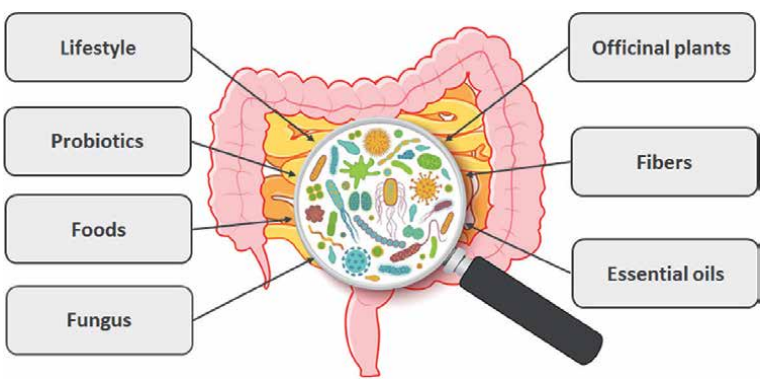

Figure 1.
The microbiota present in human gut is strictly connected with the whole organism state of health or illness. Major factors capable of modulating the gut microbiota in adults are represented in this picture.

into loops of which it is difficult or impossible to establish which of the two is the cause and which the consequence. Aging is also accompanied by increased intestinal permeability, impaired digestion, and disrupted nutrient absorption, each of which exhibits bidirectional interactions with the gut microbiome [5].

In addition to the physiological and irreversible increase in our biological age, there are other conditions that have a decisive impact on the composition and function of the intestinal microbiota. The first for importance and for the daily life with which it is implemented, is certainly our diet, which can cause, as we will see in the next paragraph, positive or negative changes in the microbiota. Another, often overlooked, condition is our lifestyle. Smoking and alcohol, for example, can negatively alter the microbiome [6], while regular physical activity seems to be capable of significantly improving it [7].

Finally, as we will analyze in the following paragraphs, there are many different pathologies, and consequent therapies, that can alter our intestinal microbiota, sometimes irreversibly. The most illuminating example concerns the transmissible pathologies of bacterial origin, encountered at an early age. The antibiotic therapies that often become necessary can, in the first 3 years of life, irreversibly alter the developmental trajectory of the intestinal microbiota leading, in the adult age, to a microbiota substantially different from that which would have developed in the absence of broad-spectrum antibiotic therapies [8]. On the contrary, antibiotic therapy in adults only reversibly alters the intestinal microbiota, which returns exactly to the starting point after the end of the therapy [9] Other intestinal pathogens, such as *Campylobacter jejuni*, can cause dysbiosis that persists even after the infection has been resolved, as it happens for example in the case of patients who develop post-infectious Irritable Bowel Syndrome (IBS) after enteritis caused by this bacterium [10].

However, we must not think that the pathologies correlated to alterations of the microbiota are essentially limited to the gastro-intestinal or metabolic ones. In recent years, many studies have linked alterations in the gut microbiome with a plethora of various diseases, including the neurodegenerative ones, such as Alzheimer's or Parkinson's [11]. Despite our limited mechanistic understanding of how the microbiota can predispose to neurodegenerative diseases, efforts to manipulate the microbiota through fecal microbiota transplantation, probiotic treatment, or other nutritional strategies, highlight the potential for microbial improvement in successfully preventing or decreasing the symptoms of these diseases, at least in laboratory animals [12]. It is therefore not surprising that some studies today are explicitly aimed at microbiome-targeted interventions for the prevention or treatment of neurodegenerative diseases.

To conclude this paragraph of premises, we can state that while conventional medicine aimed at maximum specialization, with branches such as organ and cellular medicine, on the other side of the pond the role of the intestinal microbiota has gradually assumed more and more importance, to remind us that our "super organism" is unique and that alterations of our gut microbial component, that is not even part of our cellular pool, can have a broad-spectrum negative impact on many if not all the organs and apparatuses that make up our organism. The microbiota well represents the complex relationships that exist between our health and the environment in which we are born and spend the first years of our life. A compromised environment, due to excessive sterilization or pollution, certainly has a strong impact on the structure of our microbiota in adulthood and, consequently, also on our state of health and well-being. Although fecal microbiota transplantation has opened new frontiers on the prevention and treatment of many pathologies, it is indisputably true that this community of microorganisms represents a central node in the functioning of all our organs and systems, and at the same time

it denotes a fundamental point of interaction between us and the environment in which we spend our lives.

2. Intestinal dysbiosis, immune system and related human pathologies

Intestinal dysbiosis is mainly characterized by lower bacterial diversity and it is often associated with an increase in bacterial species with pathogenic potential (*i.e.*, pathobionts) to the detriment of health-associated symbiotic commensals. Dysbiosis has been associated with several human pathologies, as demonstrated by several preclinical and clinical studies (**Figure 2**). Despite this evidence, it is almost never clear whether dysbiosis is a consequence of these diseases or if it is directly involved in their pathogenesis. Nevertheless, it is widely accepted that an imbalance of the intestinal microbiota may impact on diseases development and their clinical outcome. A consequence of intestinal dysbiosis is the loss of the barrier effect, followed by an impairment of the gut-associated immune system function. Mucosal barrier disruption leads to the release of pathogen-associated molecular patterns (PAMPs), triggers epithelial release of damage-associated molecular patterns (DAMPs), and finally causes the release of bacterial lipopolysaccharides (LPS) into the systemic circulation (metabolic endotoxaemia) [13]. The consequent activation of inflammatory pathways links intestinal dysbiosis to chronic inflammatory pathologies, autoimmune disorders and also cancer.

Moreover, together with the dysbiosis-related inflammation, the depletion of specific bacterial taxa involved in endocrine signaling may directly affect the function of different organs, and for these reasons dysbiosis has also been linked to metabolic, endocrine (e.g. thyroid-related) and also psychiatric disorders [14].

2.1 Dysbiosis in gastrointestinal disorders

A marked dysbiosis has been found to be associated with the main intestinal disorders, such as Inflammatory Bowel Diseases (IBD), Irritable Bowel Syndrome (IBS) and coeliac disease (CD). IBD are chronic inflammatory disorders characterized by the chronic activation of the immune system with an unbalanced production of inflammatory cytokines. Despite the pathogenesis of these diseases is unclear, there is evidence that, other than genetic and environmental factors,

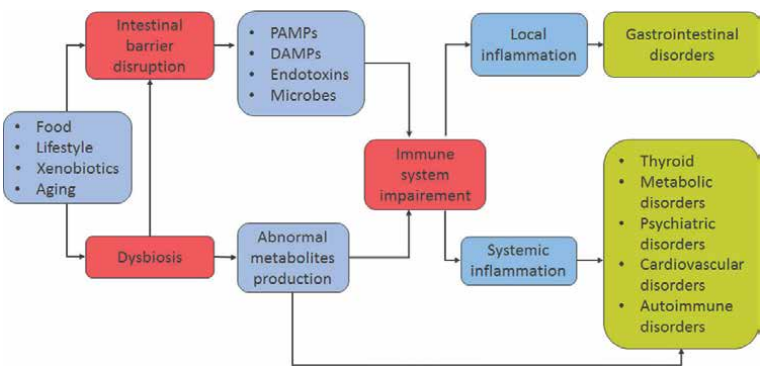

Figure 2.
Food, lifestyle, xenobiotics and aging are the main causes that can lead to dysbiosis and consequently to an alteration of the intestinal barrier function. These two conditions are linked immune system impairment and to the possible onset of many pathologies. PAMPs, pathogen-associated molecular patterns; DAMPs damage-associated molecular patterns (DAMPs).

an abnormal immune response against the microbial component of the gut may be involved in inflammation development and maintenance. It has been supposed that dysbiosis could trigger an aberrant activation of immune system in IBD patients, resulting in an unbalanced inflammatory cytokine production. In particular, compared to controls, the anti-inflammatory butyrate-producing species *Faecalibacterium prausnitzii* has been found to be reduced in both Crohn's disease and ulcerative colitis patients, with the latter also showing an increase in *Clostridium perfringens* and a decrease of *Eubacterium rectale*. Overall, members of the Proteobacteria phylum, such as *Enterobacteriaceae*, including *Escherichia coli*, are increased in patients with IBD compared to healthy individuals [15]. Also pouchitis, an inflammation involving the transition tissue (pouch) in patients with IBD who underwent proctocolectomy, is characterized by severe chronic dysbiosis [16, 17]. The involvement of the gut microbial component in these disorders is also supported by the efficacy of antibiotic therapy and probiotics, which are often used in order to manage inflammatory flares, especially in pouchitis, despite specific bacterial pathogens have never been found in these patients [18].

IBS is characterized by recurrent abdominal pain associated with a change in the bowel habits. IBS patients are divided into four subtypes: diarrhea-predominant (IBS-D), constipation-predominant (IBS-C), mixed diarrhea and constipation (IBS-M), and patients with non classifiable IBS symptoms (IBS-U) [19]. These patients are characterized by a lower microbial diversity compared to the healthy population, and also by increased proportions of Proteobacteria and Firmicutes members, such as *Veillonella*, *Lactobacillus* and *Ruminococcus*, and decreased relative abundance of *Bifidobacterium*, *Faecalibacterium*, *Erysipelotrichaceae*, and methanogens. Despite the etiology of this disease is unknown, the role of the gut microbiota component is supported by experiments on animals with induced dysbiosis, which showed abnormal intestinal behaviors similar to those typical of IBS patients [20]. Moreover, a number of studies have shown some improvement in IBS symptoms with antibiotic therapy, including rifaximin [21].

Coeliac disease (CD) is a well-characterized gut autoimmune disorder triggered by the interaction between the gut-associated lymphoid immune system and the undigested gluten peptides that translocate through the epithelial barrier into the lamina propria. About 30% of the world population is genetically predisposed to develop CD, but only a small amount (about 1% in developed countries) develops the disease, so a multifactorial etiology is supposed for this disorder. CD patient microbiota is characterized by an increased relative abundance of *Bacteroides*, *Prevotella* and *Escherichia*, and reduced amounts of bifidobacteria and lactobacilli. It has been supposed that this dysbiotic profile may contribute to the disease development by influencing the gluten peptide digestion, by stimulating dendritic cells and Treg lymphocytes and also by increasing intestinal permeability [22].

2.2 Dysbiosis in thyroid and autoimmune disorders

There is rising evidence that the intestinal microbiota compositional structure may impact on thyroid function, since microbial components can regulate iodine, selenium, iron and zinc uptake, and also enterohepatic cycling of thyroid hormones. Moreover, the microbiota may also impact on the bioavailability and metabolism of L-thyroxine and the anti-hyperthyroid drug propylthiouracil (PTU) [23]. The gut microbiota influences the synthesis of neurotransmitters, such as dopamine, which can inhibit thyroid-stimulating hormone (TSH) and modulate hypothalamus-pituary axis. It is therefore reasonable to affirm that intestinal dysbiosis may contribute to the abnormal immune activation in Hashimoto's thyroiditis (HT) [24] but also

in Grave's disease (GD), which is the second leading autoimmune thyroid disease. Studies on animals showed that microbiota transplant may increase the susceptibility to HT in rats. A proposed mechanism of action, is that *Lactobacillus* spp. and *Bifidobacterium* spp. may affect the synthesis of antibodies cross-reacting with thyreoperoxidase and thyroglobulin [25]. Notably, it has also been supposed that dysbiosis in HT patients may affect Treg cells modulation, a common feature shared with CD, which is often associated with thyroid disorders [26].

HT and GD evolve, respectively, in hypothyroidism and hyperthyroidism, with two distinct immunological patterns. HT is characterized by antibodies against thyreoperoxidase and thyroglobulin while GD is characterized by the presence of antibodies against TSH receptor. Nevertheless, in both disorders, anti-gliadin, anti-transglutaminase and anti-*Saccharomyces cerevisiae* antibodies have been detected, and both disorders are characterized by intestinal dysbiosis. A study conducted on 28 HT patients and 16 healthy controls showed an increase in the proportions of *Blautia*, *Roseburia*, *Ruminococcus*, *Romboutsia*, *Dorea*, *Fusicatenibacter* and *Eubacterium* group genera, and a decrease in *Fecalibacterium*, *Bacteroides*, *Prevotella* and *Lachnoclostridium* in HT [24]. Another study on 27 GD patients showed an increase of *Prevotellaceae* and *Pasteurellaceae* and a decreased amount of *Enterobacteriaceae*, *Veillonellaceae* and *Rikenellaceae* compared to healthy subjects [27].

2.3 Dysbiosis in metabolic disorders

Obesity, type-2 diabetes, metabolic syndrome and nonalcoholic fatty liver disease (NAFLD) are all metabolic disorders that manifest in comorbidity, and lead to an exacerbation of atherosclerosis and cardiovascular diseases [28]. These disorders are characterized by different microbial signatures, which may contribute to their chronicization. The intestinal microbiota has an active role in regulating host metabolism, indeed experiments on mice showed that conventionally raised mice had more total body fat than mice raised in germ-free condition, and that a fecal transplant in these mice was able to restore nutrient adsorption, metabolic function and body fat [29].

In obese subjects, a lower bacterial richness was detected, along with a predominance of "pro-inflammatory" taxa, such as *Ruminococcus gnavus* and *Bacteroides*, over the "anti-inflammatory" species *F. prausnitzii* [30]. Microbiome analysis in type 2 diabetes patients showed an altered pattern enriched in membrane transport of sugars and branched-chain amino acid transport, while depleted in butyrate synthesis, with a decrease of *Roseburia intestinalis* and *F. prausnitzii* [31]. Moreover, fecal transplant from lean donors to patients with metabolic syndrome showed to ameliorate their insulin resistance condition [32]. There is also evidence that overgrowth of SCFA-producing bacteria is directly correlated to an improvement of glycemic control, through regulation of glucagon-like peptide 1 [33]. In NAFLD patients, a microbial signature characterized by higher relative abundance of proteobacteria was detected, moreover there is a correlation between the microbiota composition and the degree of liver fibrosis. Patients with an advanced liver fibrosis showed a further increase of proteobacteria, particularly *E. coli*, and a decrease of Firmicutes [34].

Intestinal dysbiosis has also been found in subjects with a high risk for cardiovascular diseases compared to subjects with low risk. In particular, some bacterial genera, such as *Prevotella* and *Klebsiella*, seem to correlate with hypertension [35] Fecal transplantation from patients with overrepresentation of these two genera to germ-free mice led to an increase of blood pressure in experimental animals [36].

2.4 Dysbiosis in cancer

Intestinal microbiota disruption has been linked to the development of cancer, and different specific strains have been linked to the development of different tumors. In colorectal cancer (CRC) a particular strain of *Fusobacterium nucleatum* seems to be involved in CRC initiation and progression by activating different pathways leading to a rise of pro-inflammatory cytokines, such as IL-6, IL-8 and TNF-α, and to the development of an immunosuppressive microenvironment and also to the induction of chemoresistance to 5-fluorouracil [37].

In hepatocellular cancer, the translocation of gut microbiota and its products via the portal vein seems to be a condition able to trigger inflammation and chronic liver disease that predisposes patients to the development of cancer [38].

Leukemia patients showed a marked dysbiosis. In acute lymphoblastic leukemia (ALL) patients, a lower microbial diversity has been found, along with an enrichment in *Enterococcaceae*, *Porphyromonadaceae* and other Bacteroidetes members, and a depletion in *Blautia*, Erysipelotrichales, *Lachnospiraceae* and Clostridiales members. In acute myeloid leukemia (AML), the abundance of *Staphylococcaceae* and *Streptococcaceae* represents a typical signature [39]. In lung cancer, a dysbiosis characterized by increased relative abundance in *Enterococcus*, *Bacteroides* and *Fusobacterium*, and a depletion in *Bifidobacterium* and other Actinobacteria components, *Dialister*, *Enterobacter*, *Escherichia-Shigella*, *Fecalibacterium* and *Kluyvera* has been detected [40].

In non-small cell lung cancer (NSCLC) patients, a depletion of butyrate producers such as *F. prausnitzii*, *Clostridium leptum*, Clostridial cluster I, *Ruminococcus* spp., Clostridial cluster XIVa, and *Roseburia* spp., has been described [41, 42].

2.5 Psychiatric disorders

There is evidence that psychiatric disorders such as schizophrenia (SCZ), autism spectrum disorders, mood disorders, and anxiety are linked to gut inflammation and that inflammatory status could be sustained by gut microbiota eubiosis breakdown [43]. Epidemiological studies link autoimmune and atopic disorders such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and ankylosing spondylitis (AS) to affective, personality, and neurotic disorders [44].

A study conducted on Danish population demonstrated that individuals with SCZ have a 50% lifetime prevalence of autoimmune disorders. On the other hand, given a history of autoimmune disorders, the relative risk for SCZ increased by 45% [45].

An association between SCZ and RA, autoimmune thyroiditis, type 1 diabetes mellitus (T1DM), SLE, Guillain-Barre´ syndrome, psoriasis, multiple sclerosis (MS) and autoimmune hepatitis has been described [46]. Interestingly, all these diseases have been associated with CD and non–celiac gluten sensitivity, with a higher prevalence of immunological markers of CD among these patients [47].

Clinical and animal preclinical studies support the relationship between gut inflammation and mental disorders. Indeed, high levels of pro-inflammatory circulating cytokines such as IL-1b, IL-6, and TNF-α, have been found in patients suffering from SCZ. Moreover, immunomodulatory drugs have been used to effectively treat psychosis [43]. In patients with a high risk of psychosis, Clostridiales, Lactobacillales and Bacteroidales were found to be significantly higher than in healthy controls [48].

It has been hypnotized that the excessive rise of SCFA synthesis could be one of the causes of microglia activation. Studies on SCZ patients showed heterogeneous

results on the microbiota dysbiosis so, despite such a dysbiosis was always confirmed in these patients, it is difficult to link specific taxa to this disorder [43]. Anyway, fecal transplantation from SCZ patients to germ-free mice resulted in the development of SCZ-like behaviors in receiving mice, providing final evidence of the gut microbiota involvement in SCZ. An unbalanced microbiota was also detected in bipolar disorders and autism spectrum disorders, to underline that our gut microbiome may contribute, probably with varying importance, to most mental and stress-related disorders [43].

2.6 Neurodegenerative disorders

The implication of gut microbiota in neurodegenerative disorders has been widely investigated. Several clinical studies in Parkinson's disease (PD) patients showed modifications in the gut microbiota, characterized by a rise in the relative abundance of *Bifidobacterium*, *Lactobacillus* and *Verrucomicrobiaceae*, and a decrease in *Blautia*, *Coprococcus* and *Prevotellaceae* [49]. Interestingly, microbiota modifications are stable after the disease onset and some of these changes correlate with alterations in microbial metabolism of tryptophan and beta-glucuronide [50]. In addition to altered microbial metabolism, intestinal dysbiosis could be involved in PD development through immune-mediate pathways, since there is evidence that links GI inflammation to PD, maybe since inflammation may enhance alpha-synuclein aggregation [49].

For what concerns Alzheimer's disease (AD), animal experiments on mice with induced dysbiosis and on germ-free mice showed that microbiota manipulation can impact on disease severity and cognitive impairments. LPS seems to be involved in fibrillogenesis of β-Amiloid (Aβ), and some bacterial species, such as *E. coli*, *Bacillus subtilis*, *Salmonella* Typhimurium, *Salmonella enterica*, *Mycobacterium tuberculosis*, and *Staphylococcus aureus* may generate functional amyloid, contributing to the pathogenesis of AD through the accumulation of proteinaceous misfolded Aβ oligomers and fibrils [51].

3. Probiotics for the modulation of human microbiota: Effectiveness and limits

Since the second half of the 19th century, with Metchnikoff's studies on the possibility of using lactic acid bacteria to decelerate the process of self-intoxication and infection by intestinal microbes [52] probiotics have been recognized as a tool to modulate the gut microbiota while conferring benefits to health. Their economic value was recognized shortly thereafter, and their global market is estimated to reach USD 69.3 billion by 2023 [https://www.marketsandmarkets.com/PressReleases/probiotics.asp]. Nowadays, probiotics represent one of the most commonly consumed food supplements worldwide, being present in yogurt, cheese, ice cream, snacks and nutritional bars, breakfast cereals, infant formulas and more recently also added to cosmetic products. They are also marketed as lyophilized pills, and their consumption is widely supported by physicians, particularly gastroenterologists [53]. The administration of probiotics is indeed a more than feasible approach in clinical practice, compared for example to diet, despite its recognized role as a pivotal determinant of the structure and function of the gut microbiota, able to support homeostasis or *vice versa* to contribute to the susceptibility to disease [54], due to the sometimes modest effects of nutritional interventions and the difficult of enforcing and monitoring patient compliance. The mainstay of commercial supply is represented by *Lactobacillus* and *Bifidobacterium* species, along with *E.*

coli Nissle 1917, *Streptococcus thermophilus* and *Saccharomyces boulardii*, all with a long history of use, having Generally Recognized as Safe (GRAS) status in the US or being granted Qualified Presumption of Safety status by the European Food Safety Authority (EFSA). Being sourced from the gut or traditional fermented foods, they have been selected, in large part, for their technological properties, *i.e.* the ability to survive processing and to retain viability during the shelf-life of the product.

According to the International Scientific Association for Probiotics and Prebiotics consensus meeting in October 2013 [55], the framework "probiotics" must include microbial species that have been shown in properly controlled studies to confer health benefits. Probiotics are also new commensals and consortia that include defined strains from human samples, for which adequate evidence of safety and efficacy exists. On the other hand, live cultures, traditionally associated with fermented foods (with no evidence of health benefits), and undefined, fecal microbiota transplants must be kept outside this framework.

Probiotics may have several effects on the host, including certainly the modulation of the gut microbiota but also the metabolism of lactose with improved digestion or bile salts with various systemic effects, vitamin synthesis, direct and indirect pathogen antagonism, regulation of intestinal transit and alleviation of visceral pain, strengthening of the gut barrier, production of specific bioactives and neurological, immunological and endocrinological effects. As expected, some underlying mechanisms are observed across taxonomic groups, such as the inhibition of potential enteropathogens or the production of useful metabolites or enzymes, while others, especially those at the extra-intestinal level, are more likely to be strain specific. These effects can be contact-dependent and/or mediated by surface molecules, e.g. lipoteichoic acid, peptidoglycan, cell surface proteins, exopolysaccharide, pili or other appendages, or by secreted molecules, e.g. SCFAs and bacteriocins [56]. In light of this, it is not surprising that paraprobiotics and postbiotics have recently been proposed as an alternative with a longer shelf-life and enhanced safety, especially for compromised individuals, with the former being non-viable (intact or broken) microbial cells or crude cell extracts [57] and the latter microbial cell constituents and metabolites, which act as bioactive compounds with local and systemic effects [58].

With specific regard to the gut microbiota, probiotics may impact resident communities through at least three different mechanisms: trophic interactions (*i.e.* by stimulating growth through the supply of metabolites such as lactate, acetate or propionate, growth factors such as vitamins or exopolysaccharide, or other substrates), a direct alteration of fitness, through a decrease in pH, niche competition or bacteriocin production, or an indirect one via host, through changes in the gut environment (*i.e.* by stimulating the production of mucins, increasing the levels of secretory IgAs and inducing the secretion of defensins, which represent the first line of defense of the intestinal epithelium against microbial invasion) [59]. As expected, while these effects may be relevant in the context of dysbiosis, *i.e.* when the blooming of potential opportunistic pathogens and/or the depletion of health-associated (mainly SCFA-producing, oxidative stress-sensitive) taxa occur, there is no convincing evidence of consistent effects of probiotics on the gut microbiota of healthy subjects, *i.e.* on an eubiotic and resilient microbial ecosystem [60].

Among the main (although sometimes only suggested) prophylactic and therapeutic indications and claims of probiotics, we can certainly mention gastrointestinal diseases, including the prevention or treatment of acute, antibiotic-associated and *Clostridium difficile*–associated diarrhea, and the amelioration of IBD and IBS [56]. Recent reviews have also cautiously suggested a beneficial role of probiotics in preterm infants, especially in terms of preventing necrotizing enterocolitis and reducing the risk of late-onset sepsis [61]. Bearing in mind that probiotics efficacy

is mostly strain-dependent and generalizations are highly inappropriate, moderate to strong evidence is also available for the eradication of *Helicobacter pylori* and the prevention of adverse reactions to its therapy or post-surgical infections [62]. Contrasting data have instead been reported in the context of many other disorders, including for example respiratory infections and metabolic syndrome [63]. This confusing situation may arise from the heterogeneity of probiotic agents, dosage, duration and mode of administration, but also from other issues related to study design and reporting of results (not always transparent, easy to assess and rigorous), to the population (e.g. demographic characteristics) and environmental variables (e.g. habitual diet).

In this regard, the awareness that one size does not fit all is rapidly gaining ground. It is now a fact that distinct baseline features of the host (e.g. age and underlying medical condition) and its microbiota (taxa represented and functions performed), including varying environmental exposure (mainly diet), can actually lead to differing outcomes even with the same probiotic preparation. As discussed recently, this could for example be due to the fact that the individual configuration of the gut microbiota may be permissive or resistant to even transient colonization of probiotics [64]. Moreover, it has been shown that probiotics could even perturb rather than aid in the recovery process of the gut microbiota after antibiotic treatment [9]. It is therefore now clear not only that their validity is not to be considered absolute but also that, if not tailored, probiotic-based interventions could not be entirely risk-free.

Future directions will be the adoption of a mechanism-based approach, in which probiotic strategies are designed *ad hoc*, taking into account a series of "precision" aspects related to the host and its microbiota, *i.e.* with careful consideration of the subject to be treated and the medical goal to be achieved. It is also expected that future human trials will overcome other current caveats in the probiotics field, by ensuring an adequate sample size (based on power analysis), clearly defining endpoints, accounting for placebo effects and reporting adverse events, while ensuring strain-level resolution [56].

Alongside traditional probiotics, it should be mentioned that novel candidate microorganisms with potential health benefits have been discovered thanks to recent research on the composition and function of the gut microbiota, deeply accelerated by massive sequencing. These microorganisms are referred to as next-generation probiotics or live biotherapeutics [65], as they fit well within the US Food and Drug Administration definition of live biotherapeutic as "a biological product that contains live organisms, such as bacteria, is applicable to the prevention, treatment or cure of a disease or condition of human being and is not a vaccine". Unlike currently used probiotics, they are generally strict anaerobes and therefore present a number of manufacturing challenges, and they should undergo a formal regulatory approval process similar to drugs or any other medical intervention. Among them, we can list SCFA producers, e.g. *F. prausnitzii*, proposed for the treatment of inflammatory bowel disease and other inflammation-based disorders [66], or the mucus degrader, *Akkermansia muciniphila*, identified as a promising candidate for the treatment of obesity and related complications [67]. Interestingly, a very recent proof-of-concept exploratory study has demonstrated that 3-month daily oral administration of live or pasteurized *A. muciniphila* to overweight/obese insulin-resistant volunteers was safe and well tolerated, and associated with numerous metabolic improvements [68].

Alternatively, it has been thought to engineer GRAS organisms or commensals as a delivery vehicle for bioactive molecules or to express certain functionality. In this approach, the bacterial vehicle is known not to produce any virulence factors, it will be tolerated by the host and, if chosen carefully, may not even colonize the host.

As an example, some researchers have used *Lactococcus lactis* strains (normally not considered probiotics but GRAS food-derived bacteria) as vehicle to deliver a range of anti-inflammatory molecules, e.g. elafin, a serine protease inhibitor, to reduce colitis-related inflammation [69], trefoil factor 1, for the treatment of oral mucositis [70] and IL-10 to control allergen sensitivity [71]. Other research groups have used the common intestinal bacterium *Bacteroides ovatus* to express IL-2 [72] or TGF-beta 1 [73], and *E. coli* Nissle 1917, which was engineered to bind to the surface of cancer cells and secrete myrosinase, to convert dietary glucosinolates into isothiocyanates, such as sulforaphane, a well-known anti-cancer compound [74].

However, in addition to the limitations discussed above, it should be emphasized that for most of these next-generation probiotic candidates, the available evidence is currently mostly preclinical, *in vitro* or on an animal model. Therefore, rigorously planned large-scale randomized controlled trials together with *in vivo* and *in vitro* experimentation are strongly needed for efficacy and long-term safety assessment, and data-driven explanation of the mechanisms of action.

In the future it is expected that overcoming all these challenges in the probiotics field will improve the state of evidence, regulation of use and, finally yet importantly, public awareness, for a precision, informed use. The current limitations in the field and future strategies to be undertaken to overcome them are summarized in **Figure 3**.

4. Foods and their prebiotic activities for the modulation of the gut microbiota

Food is a primordial need for our survival and well-being. However, diet is not only essential to maintain human growth, reproduction and health, but it also modulates and supports the symbiotic microbial communities that colonize the digestive tract, the gut microbiota. Type, quality and origin of our food shape our gut microbes and affect their composition and function, impacting on host–microbe interactions. Macronutrients (fat, protein, carbohydrate) and micronutrients (vitamins, minerals, polyphenols) directly interact with gut microbes and are involved in the production of key metabolites such as SCFAs and vitamins. Moreover, dietary fiber impacts on gut microbial ecology, host physiology, and health.

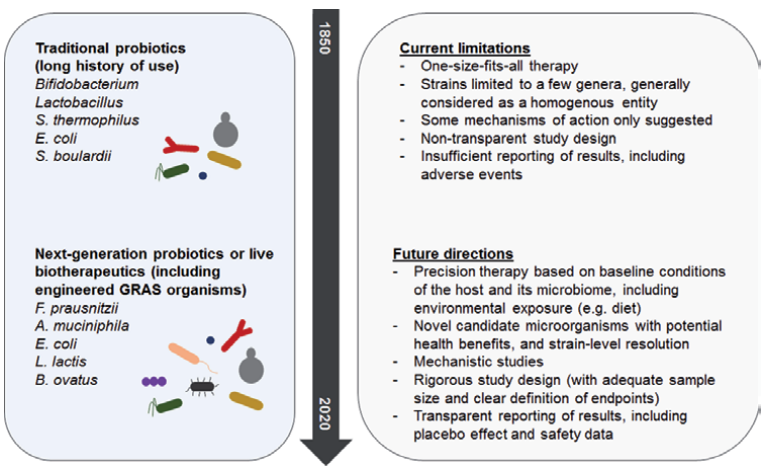

Figure 3.
From traditional to next-generation probiotics: Current limitations and future directions.

During or shortly after birth, the human gut is colonized by microbes. The fact that babies born spontaneously have higher bacterial counts in the gut at 1 month of age than those born by the cesarean section indicates that colonization of the gut by microbes starts and is improved during natural birth [75]. The growth and maintenance of a healthy gut microbiota is essential for the development of the immune system and continues during breastfeeding, a stage that seems essential to the individual's long-term health. Oligosaccharides found in breast milk encourage the growth of *Lactobacillus* and *Bifidobacterium*, which control the infant intestine, and this may improve or facilitate immune system development and help to prevent pathological conditions such as eczema and asthma. Functional maturation of the human microbiota, including the capacity to produce vitamins, increases during the early years of life [76].

A standard Western style diet offers about 50 g daily of potentially fermentable substrate, primarily dietary fiber, to the colonic microbiota. Non-starch polysaccharides are major components of dietary fiber and constitute 20–45% of the dry matter supplied to the colon. Simple sugars and oligosaccharides also account for another 10%, whereas starch (and starch hydrolysis products) supply less than 8% of dry matter. Some sugar alcohols also avoid the absorption of the small intestine and are minor dietary substrates for colonic microbiota [77]. Approximately 90% of dietary polyphenols (approximately 1 g/day) avoid digestion and absorption in the small bowel and can have a major effect on microbial composition and activities.

About 5–15 g of proteins and 5–10 g of lipids, mainly of dietary origin, pass daily through the proximal colon. Various other minor dietary constituents, including catechins, lignin, tannins and others, also nourish colonic microbes [78]. The action of all these macro and micronutrients is certainly synergistic and complex at the level of the intestinal microbiota, however in the following paragraphs we will analyze separately the effects of individual macro and micronutrients, trying not to lose the overall vision that is fundamental when it comes to microbial ecology.

4.1 Macronutrients and microbiota

Fats. The increase in dietary fats greatly changes the composition of the gut microbiota. Mice fed with high-fat diets (HFD, 40–80% of total caloric intake from fat) display phylum-level changes, with a decrease in Bacteroidetes and an increase in Firmicutes and Proteobacteria.

These changes have also been observed in weight gain-resistant mice, which implies a direct effect of dietary lipids on the microbiota. It has recently been found that microbes in the small intestine are highly susceptible to fat load and are essential for lipid digestion and absorption. These data suggest that the regional microbiota composition may have significant functional implications, and highlight the need for distinct microbiota and microbiome analysis along the gastrointestinal tract [79]. The lipid-mediated effects on the microbiota depend on the form and source of lipids. For example, mice fed with an isocaloric diet rich in long-chain saturated fats derived primarily from meat products showed greater insulin resistance and inflammation of the adipose tissue compared to mice fed with a high-fish oil diet. In addition, transgenic mice that constitutively generate n-3 polyunsaturated fatty acids (PUFAs) have higher phylogenetic diversity of the microbiome, which provides protection against the metabolic consequences of a high-saturated, high-sugar diet. One mechanism by which gut microbes can mediate the negative metabolic effects of high-fat intake could be by translocating LPS, a membrane toxin of gram-negative bacteria. An increase in circulating LPS after a high-fat meal has also been documented in humans, with amplified effects in obese people. Once in circulation, LPS induces a powerful inflammatory response by activating

Toll-like 4 receptor signaling, which has been involved in cardiovascular and metabolic disease development [80].

Inflammation appears to be the common denominator among the seemingly unrelated biological negative effects of fats on the gut microbiome, involving the immune system and n-3 PUFAs. It is currently accepted that inflammation plays a key role in the progression of several chronic diseases, such as atherosclerosis, inflammatory bowel disease, cancer, diabetes, and neurodegenerative syndromes [81]. Moreover, as described above, several evidence supports the role of n-3 PUFAs on the microbiota and on the regulation of inflammation and the immune system [82]. In addition, dietary n-3 PUFAs have been shown to reduce clinical colitis in IBD patients [83]. In clinical human studies, n-3 PUFA administration resulted in decreased Firmicutes/Bacteroidetes ratio, reduced relative abundance of Coprococcus and Facecalibacterium, and increased proportions of health-associated genera, i.e., Bifidobacterium, Lachnospira, Roseburia and Lactobacillus [84]. These data were consistent with those obtained in a subsequent study in which the authors also found a significant correlation between the plasma levels of n-3 PUFAs and the relative abundance of SCFA producers [85]. In addition, a diet supplemented with n-3 PUFAs has been able to prevent neuropsychiatric disorders and dysbiosis caused by social instability stress during adolescence, and these effects have been maintained through adulthood, supporting the concept that a healthy diet enriched in fish or n-3 PUFAs can have beneficial long-lasting effects and may help to prevent neuropsychiatric disorders [86]. Taken together, all these data allow us to hypothesize the existence of a strong link between n-3 PUFA intake, gut microbiome shaping and modulation of the immune system, with the ultimate objective of hampering the existing loop between bowel inflammation and gut dysbiosis.

In the fat dietary component, n-3 PUFAs can rightly be considered prebiotics. Therefore, the consumption of an n-3-rich diet is currently thought to be beneficial for microbiota health, even if the gut microbiome changes induced in humans by n-3 PUFA supplementation deserve further clinical investigations.

What we can conclude for the fat dietary component is that the lipid excess present in HFD diet is dangerous for the microbiota and, on the other hand, that a diet enriched in n-3 PUFAs protects the microbiota from possible alterations. However, n-3 PUFA sources, mainly fish, should not considered completely safe, considering the pollution of the sea and the growing presence of microplastics and xenobiotics in the trophic chain of marine animals. In particular, scientific data suggest that shellfish and other small marine organisms consumed with their intestine pose particular concern because they accumulate and retain microplastics. The biological effects of microplastics in human gut are poorly understood, but it has been supposed that in high amounts they could cause an alteration of the gut microbiome, with cascading effects on host physiology [87].

Proteins. That dietary proteins may affect the gut microbiota was first described in 1977. A pioneering study showed lower counts of *Bifidobacterium* and increased counts of *Bacteroides* and Clostridia in subjects eating a diet enriched in beef meat, compared with those eating a vegetarian diet [88]. With advances in metagenomics analysis, several studies have been able to investigate in depth the effects of dietary protein on the gut microbiota. These studies have evidenced a different effect depending on the protein source: animal or vegetarian. While the intake of animal meat proteins has been associated with a general worsening of the microbiota profile [89], vegetarian protein intake is overall positively associated with microbial diversity. For example, intake of pea protein extract has been reported to increase the proportions of the gut commensals *Bifidobacterium* and *Lactobacillus*. Pea protein intake was also observed to increase the levels of intestinal SCFAs, considered

to be important for several metabolic and immunological aspects, including the maintenance of the intestinal barrier. In contrast, counts of bile-tolerant anaerobes, such as *Bacteroides, Alistipes* and *Bilophila*, increase with animal-based protein intake [90]. Notably, different studies comparing high-animal protein diets and high-carbohydrate/fiber plant-based diets reported that the first dietary pattern can be effective for rapid weight loss but detrimental to microbiota health. In particular, the research showed that subjects following a high-protein/low-carbohydrate diet were depleted in *Roseburia* and *E. rectal*e in their gut microbiota with decreased butyrate levels in their feces [91]. Other studies confirmed decreased fecal SCFAs in Italian subjects eating a protein-rich diet. It has been proposed that high total protein intake, especially animal protein, could be associated with a significantly increased risk of IBD [89]. In addition, many microbial genera promoted by consumption of red meat have been related to increased levels of blood trimethylamine-N-oxide (TMAO), considered a pro-atherogenic marker of cardiovascular disease [92]. Finally, it is important to note that animal products-based diets are often high both in protein and fat, with potential synergistic negative effects on the human microbiota.

Carbohydrates. The effects of dietary carbohydrates on the gut microbiota are complex, since they can be classified based on three major components that are simple sugars, starches and fiber. Simple sugars such as sucrose, both alone and as part of a high-fat/high-sugar Western-style diet, can induce rapid remodeling of microbiota and metabolic dysfunction in laboratory animals and also in humans. Fibers should be considered as human indigestible carbohydrates. In a healthy microbiota, different bacterial genera possess fiber-degrading enzymes and thus use these indigestible carbohydrates as a primary source of energy. The term fibbers is widely used to classify such indigestible polymers, although this classification is problematic given that certain fibers are only partially degraded by intestinal microbes (such as cellulose), whereas other are readily fermentable (soluble fibers such as inulin). The metabolic effects of fiber are shown in **Figure 4**. Sonnenburg and his colleagues recently proposed the term 'microbiota-accessible carbohydrates' (MACs), to identify carbohydrates that are metabolically available to gut microbes [94]. MACs provide energy and a source of carbon for bacteria but also to the host. They can modify the microbiota structure by increasing the populations of fiber-degrading bacteria. This property of fibers warrants their additional classification as prebiotics, which by nature are non-digestible components of the diet that support selective growth of certain health-associated microbial populations, such as bifidobacteria. Examples of prebiotics include inulin, fructans, fructooligosaccharides, galactoligosaccharides, xyloligosaccharides and arabinoligosaccharides [95]. A diet low in prebiotic substances has been shown to reduce total abundance and diversity of gut bacteria. In particular, a diet rich in non-digestible carbohydrates most consistently increases intestinal bifidobacteria and lactic acid bacteria [96]. Other non-digestible carbohydrates, such as resistant starch and whole grain barley, also tend to increase the proportions of health-promoting SCFA-producing bacteria such as *Ruminococcus* and *Roseburia.*

Digestible carbohydrates are enzymatically degraded in the small intestine and contain starches and sugars such as glucose, fructose, sucrose and lactose. All these compounds release glucose into the bloodstream upon degradation, triggering an insulin response. Human subjects fed high levels of glucose, fructose and sucrose in the form of fruit, had increased relative abundance of bifidobacteria, with reduced *Bacteroides* [97]. Also, lactose supplementation was found to raise the fecal amounts of beneficial SCFA-producing bacteria in non-intolerant subjects [98].

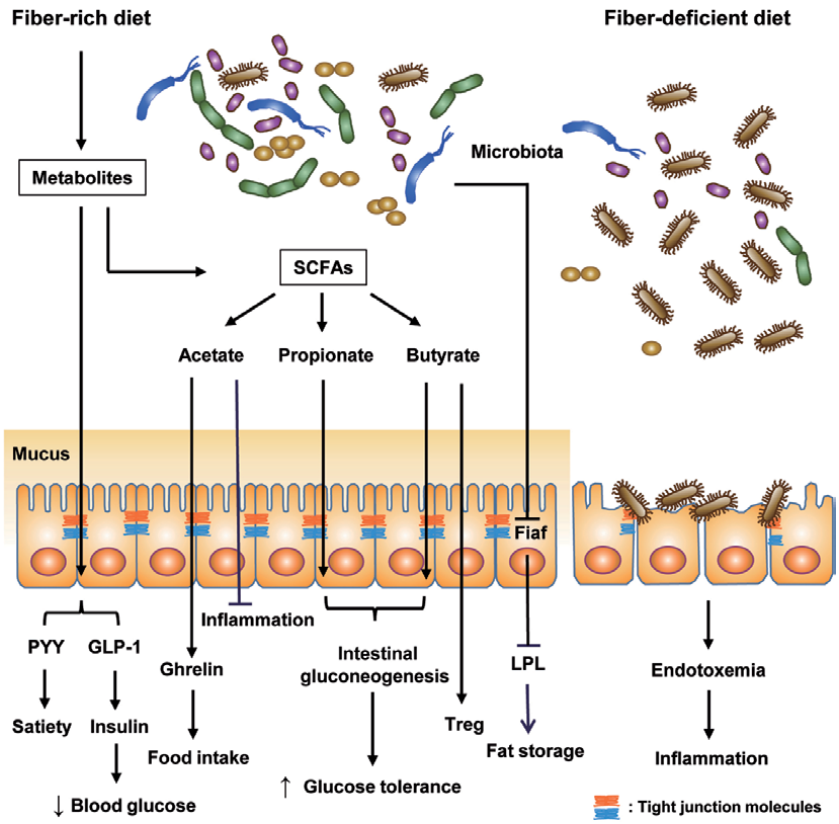

Figure 4.
Fiber intake impacts on host metabolism and immunity by affecting the gut microbiota. Under a fiber-rich diet, the gut microbiota metabolizes undigested dietary fiber into SCFAs (acetate, propionate, and butyrate), affecting host metabolism and immunity. Microbial metabolites from this process improve host metabolism. In particular, the secretion of peptide hormones, such as PYY and GLP-1, is promoted by microbial metabolites: PYY decreases appetite and GLP-1 lowers blood glucose level via promotion of insulin secretion. Among SCFAs, butyrate and propionate activate intestinal gluconeogenesis and improve systemic glucose profiles. Meanwhile, acetate promotes secretion of ghrelin, a hunger hormone, and increases food intake, consequently causing hyperphagia and obesity. Nevertheless, acetate has anti-inflammatory function like butyrate. Butyrate enhances gut barrier function of intestinal epithelial cells and increases regulatory T (Treg) cells. In addition, the gut microbiota suppresses expression of fasting-induced adipose factor (Fiaf), an inhibitor of LPL, promoting fat storage in adipocytes. Under fiber-deficient diet, mucus-degrading bacteria expand and impair the integrity of the mucus layer. Thereby, endotoxemia-induced metabolic inflammation ensues. SCFAs, short-chain fatty acids; PYY, peptide YY; GLP-1, glucagon-like peptide-1; LPL, lipoprotein lipase. From [93].

4.2 Micronutrients and microbiota

Vitamins. Diet is the primary source of vitamins, because our bodies cannot synthesize them to meet our daily needs, but certain vitamins, especially vitamin K and the B-group vitamins, are synthesized by some intestinal microbes. As a consequence, the abundance and diversity of intestinal microbiota components can modulate the metabolism and absorption of vitamins in the upper intestine [99].

The administration of retinoic acid (physiologically active vitamin A metabolite) in patients with norovirus infection significantly increased the abundance of *Lactobacillus* spp. Since *Lactobacillus* showed antiviral activity *in vitro*, it has been hypothesized that the intake of vitamin A and the consequent increase in the amount of *Lactobacillus* in the gut were partially responsible for norovirus inhibition [100]. Retinoic acid administration has also been shown to increase the relative abundance of *Allobaculum, Aggregatibacter, Bifidobacterium, Dialister* and

Enhydrobacter. Epidemiological studies have shown that norovirus infection rate and clinical symptoms decrease significantly with a sufficient supplementation of vitamin A [101]. In infants, supplementation of vitamin A showed to improve the Bacteroidetes/Bacteroidales population, and increase the relative abundance of *Bifidobacterium* and *Akkermansia* in feces [102].

Vitamin C is the most important antioxidant agent, and it must be obtained from dietary sources (mainly fruits and vegetables). This vitamin regulates the redox state and can considerably modulate the gut microbiota. In weaned piglets, vitamin C levels correlated positively with Firmicutes and negatively with Bacteroidetes relative abundances [103]. Vitamin D is thought to be a multifunctional vitamin involved in calcium homeostasis and in a list of systemic physiological functions that include the modulation of gut microbiota [104]. A randomized controlled trial showed that weekly vitamin D supplementation (50,000 ergocalciferol IU) over 12 months increased SCFA fecal levels and the relative abundance of SCFA-producing genera such as *Ruminococcus*, *Fecalibacterium* and *Dialister* [105].

Some vitamins of the B group have been shown to promote bacterial colonization of the gut, modulate bacterial virulence and participate in pathogen clearance [106]. However, they may also have a role in the growth of enteropathogens, such as *Salmonella* Typhimurium [107]. For example, different gut bacteria can synthesize vitamin B6, but dysbiosis could reduce the luminal level of vitamin B6 and facilitate gut colonization by enteropathogenic strains.

It is evident that there is a high and complex interaction between vitamins and the gut microbiota: some vitamins are produced by the microbiota itself and others, particularly liposoluble vitamins, are responsible for its modulation. On the other hand, some of these vitamins may also contribute to enhanced virulence and colonization of potential pathogenic microbes. These studies together suggest that vitamin supplementation could modulate the gut microbiota, but its effects depend on the level of vitamin in the host and the microbiota status. Further clinical trials should be carried out to understand the effects of multivitamin supplementation, in order to evaluate possible effects linked to over-supplementation.

Polyphenols. Dietary polyphenols are studied for their antioxidant properties. Popular foods with a rich content of polyphenols include fruits, nuts, vegetables, tea, cocoa, and wine. For example, the relative abundance of *Bacteroides* was reported to increase in subjects consuming pomegranate [108]. The consumption of cocoa-derived polyphenols has been associated with significant changes in the gut microbiome [109]. Fruit seed, wine and tea polyphenols were capable to positively modulate the human fecal microbiota by affecting the levels of pathogenic *Clostridium* species (*C. perfringens and C. histolyticum*) [110].

Conceptually, it is difficult to isolate the activity of polyphenols from the overall activity of the foods that contain them. Nevertheless, overall we can conclude that a diet rich in foods with high polyphenol content, can have positive effects on the human intestinal microbiota.

Food additives and xenobiotics. Another poorly understood area with potential implications for the human gut microbiota health is the impact of food additives and xenobiotics on the microbial ecology and intestinal homeostasis. Although Western diets typically attribute microbial and health consequences to macronutrient composition, several studies suggest that food additives may be driving the detrimental effects of these diets on the microbiota. For example, in the absence of other dietary manipulations in mice, two dietary emulsifiers, polysorbate-80 and carboxymethyl cellulose, induced obesity, intestinal inflammation, metabolic dysfunction and dysbiosis. The microbiota was both necessary and sufficient to explain all these effects as germ-free mice were protected from these detrimental effects, and the transfer of microbiota from emulsifier-treated mice was sufficient to

recapitulate the metabolic disruptions [111]. These results are particularly striking considering the wide range of foods containing emulsifiers (for example gluten-free and reduced-fat products, ice cream, and pickles), and that the doses used in this study reflect the human intake. Non-nutrient sweeteners (NNSs) have been linked to gut-associated metabolic alterations, in addition to emulsifiers. In experiments conducted in rodents and humans, NNS consumption induced glucose intolerance in a microbiota-dependent manner [112]. Nevertheless, literature data on the effects of NNSs on intestinal microbiota and microbiome sometimes are divergent, and this is also dependent on the fact that NNSs are a broad class of substances with high structural and functional variability. Additional human intervention studies examining the impact of individual NNSs on microbiota and microbiome are certainly needed.

Artificial sweeteners such as saccharin, sucralose and aspartame have been considered as options that might be used to replace natural sugar to prevent and control glucose dysmetabolism. However, recent evidence suggests that consumption of all types of artificial sweeteners may induce glucose intolerance. It is important to note that artificial sweeteners are thought to mediate this effect also by altering the gut microbiota. For example, it was noted that saccharin-fed mice had intestinal dysbiosis with increased relative abundance of *Bacteroides* and reduced *Lactobacillus reuteri* [112].

Even on the large category of xenobiotics it is very difficult if not impossible to generalize. Just as an example, analysis of the microbiome of children with Crohn's disease developed at a very young age showed that the most altered metabolic patterns in the gut microbiome were those related to xenobiotic metabolism [113].

4.3 Dietary patterns

Several popular diets have been studied for their ability to modulate the intestinal microbiota, including Western, ketogenic, omnivore, vegetarian, vegan and Mediterranean diets. The Western diet (high in animal protein and fat, low in fiber) has led in several studies to a marked decrease in microbial diversity and in some beneficial genera, such as *Bifidobacterium* and *Eubacterium* [114].

Ketogenic diets are characterized by a very low consumption of carbohydrates (5 to 10 percent of total caloric intake), sufficient to increase the production of ketone bodies. They were originally developed as a treatment for refractory childhood epilepsy, and the gut microbiota responses to a ketogenic diet seem to play a role in the effectiveness of this intervention in epileptic infants [41, 42]. In recent years, these diets are commonly adopted in order to obtain rapid weight loss and in some studies, they have been shown to improve longevity and reduce the onset of disease in experimental animals. Conversely, some human studies in which ketogenic diets were examined, suggest negative impacts on microbial ecology and gut health. These studies, however, were carried out in small cohorts with specific metabolic conditions, limiting the generalization to larger populations [115].

Vegan/vegetarian diets are both plant-rich diets associated with positive health outcomes and reduced risk of some diseases [116]. The beneficial effects of these diets on human health could also be linked to intestinal microbiota modulation. Plant-based foods are the primary source of dietary MACs, and it has been found that individuals who consume vegetarian or predominantly plant-based diets have a microbiota metabolically optimized for MAC fermentation. However, some intervention and cross-sectional studies have found only modest differences in microbiota composition between omnivores and vegetarians, and suggest that the effects of dietary patterns on the microbiota are greatest at the level of genus and species, but relatively minimal on broader compositional features such as diversity [117].

Despite the absence of a wide microbiota compositional shift, the species-level changes appear to be sufficient to alter metabolic outputs as SCFA production, which in vegetarians is typically increased. It is still unclear to what extent these microbiota-dependent metabolic outputs can mediate the beneficial effects of vegetarian diets.

Plant-based foods, in addition to supplying MACs, provide a diverse source of vitamins, polyphenols and other biologically active phytochemicals. Many phytochemicals may often reach the lower intestinal tract and have direct antimicrobial and anti-inflammatory effects in the intestine. Furthermore, microbial enzymes can modify phytochemicals into metabolites with increased bioactivity [118, 119]. So, microbiome-mediated changes in phytochemical bioavailability can be an additional mechanism underlying the beneficial effects of plant-based diets.

Several studies classify the Mediterranean diet as the most healthy and balanced human diet. It is characterized by a beneficial fatty acid profile, rich in both monounsaturated and polyunsaturated fatty acids, high polyphenols and other antioxidants and high fiber intake. Fruits, vegetables, cereals, legumes and nuts are at the basis of this diet, as well as consumption of fish and red wine [120]. The potential benefits of Mediterranean diet on the gut microbiota are linked to the increased levels of fecal SCFAs together with an increase of *Prevotella, Lactobacillus* and *Bifidobacterium*, and a decrease in *Clostridium* [92].

Even if there are different types of Mediterranean diet, as well as several ketogenic diets (e.g. normo- or iper-proteic) and even vegetarian diets (with or without eggs, with or without fish), what can be concluded in general about the effects of dietary patterns on the intestinal microbiota is that all those patterns which, for various reasons, tend to restrict the amount of vegetables, seem to be inadvisable. Thus the Western diet, which is poor in fruit and vegetables, and the ketogenic diets, which necessarily eliminate fruit for its carbohydrate content, appear to be diets with a probable negative impact on the intestinal microbial ecology. Despite this, comparative controlled clinical trials are needed to fully evaluate the possible short-term and long-term effects of these dietary patterns on the gut microbiome.

5. Use of fungus and officinal plants for the modulation of the intestinal microbiota and immune system

Microbiota and its multiple connections, already described in the previous paragraphs, remind us that every human being is an unrepeatable and unique Psycho-Neuro-Endocrine-Immuno-Somatic-Environmental unit that is constantly dynamic and interactive in its parts [121]. From this perspective, the gastrointestinal system should be evaluated and treated as a neuro-immuno-endocrine-visceral-microbial interface of the human body. The modulation of the gut microbiota and, consequently, of the immune system is a key function of this complex network. Any disorder of the gastrointestinal tract, be it functional or with organic inflammatory basis, involves cells belonging to multiple tissues, including the sphere of the microbiota, and is therefore continuously reflected at the systemic level.

Consequently, even medicinal plants can, indeed should, act at multiple levels of the organism through direct and indirect actions that certainly, with various types of mechanisms, involve the Intestinal Immune System (IIS) and the intestinal microbiota. The action of fungi and medicinal plants is exerted on the gastrointestinal system through the immunomodulating, antioxidant and protective properties of the microbiota. Furthermore, the protection of the biofilm and the intestinal barrier, in the structuring of which the microbiota directly and actively participates,

also fall within these therapeutic actions. These effects on the intestinal barrier and on the gastrointestinal system can obviously also have systemic consequences.

Several medicinal plants and fungi are described in the scientific literature as being able to act positively on various acute and chronic inflammatory disorders of the gastrointestinal system, most of these are also part of the medical tradition of one or more regions of the world.

Medicinal mushrooms that have been used in most preclinical and clinical studies are *Hericium erinaceus*, *Inonotus obliquus* (called Chaga), *Ganoderma lucidum* (called Reischi), and *Auricularia auricula*. Instead, the most used medicinal plants for the gut are *Boswellia serrata*, *Pistacia lenticus*, and *Aloe* in its various species. With a mainly European traditional use, we find *Olea europea* (olive tree), *Angelica arcangelica*, *Achillea millefolia*, *Cichorium intybus* and finally *Cetraria islandica*, which belongs to the lichen species.

In this brief discussion, we will limit ourselves to analyzing the scientific literature supporting possible therapeutic use of some of these fungi and these plants, in the modulation of intestinal inflammation and dysbiosis, the two components that are always associated in almost all pathologies of the gastrointestinal tract.

5.1 Microbiota-modulating fungi

Hericium erinaceus represents the most used fungus for all the disorders of the gastrointestinal system. Also known as Lion's Mane Mushroom, is an edible fungus, which has a long history of usage in traditional Chinese medicine for the protection of mucous membranes, gastric ulcers, acute and chronic gastritis and nervous degeneration [122–124].

The drugs used are the fruiting body and/or the mycelium in aqueous or hydroalcoholic or alcoholic extracts titrated and standardized in one or more of the following components: polysaccharides and beta-glucans (with anti-inflammatory and antibacterial action), alpha-glucans, diterpenes and triterpenes and polyphenols [125, 126].

The most studied activities of this fungus relate to its immunomodulatory effects on the gut, its anti-inflammatory systemic activity, but also its prebiotic activities on the intestinal microbiota [127].

A single protein, called HEP3, isolated from *H. erinaceus* and administered to rats treated with trinitrobenzenesulfonic acid (TNBS) to induce experimental colitis similar to IBD, was capable of restoring the microbiota diversity in treated rats. In particular, treatment with *H. erinaceus* single protein increased the amounts of Actinobacteria and Tenericutes, reduced those of Bacteroidetes and Firmicutes, and was able to restore a healthy-like ecological structure [128, 129]. The effectiveness of HEP3 was also confirmed in other animal models of colitis [130]. We would like to underline the general concept that it is always advisable to use titrated extracts with greater complexity than single proteins, as these extracts can keep the phytocomplexes and mycocomplex intact, and *in vivo* could have synergistic actions on multiple targets. Also, raw extracts of *H. erinaceus* were tested in an IBD animal model as whole polysaccharide, alcoholic extract or whole extract. Results indicate that all these formulations were capable of positively modulating the microbiota, but while the polysaccharide extract seems to play a major prebiotic role, the alcoholic extract and whole extract showed major bactericidin-like effects [128, 129].

Similar results were obtained in a model of dextran sulfate sodium (DSS)-induced colitis in mice. DSS treatment resulted in increased relative abundances of Verrucomicrobia and Actinobacteria and decreased amounts of Bacteroidetes in fecal samples, compared to the control group. Treatment of colitic mice with dry power of fermented *H. erinaceus* mycelium reversed most changes, including the increased levels of *A. muciniphila*. Collectively, these results showed that *H.*

erinaceus effectively modulate the gut microbiota of colitic animals, restoring a microbiota composition similar to that of healthy mice [131].

Inonotus obliquus commonly known as Chaga is a parasitic fungus mainly of Birch (*Betulaceae*) trees with numerous biological properties [132, 133]. Commonly used as a folk remedy in Russia and other northern European countries for various disorders affecting the digestive system, it is now widely studied for its numerous potential applications in the medical field. The most used formulations are powder, aqueous extract and hydroalcoholic extract. These can be titrated in polysaccharides, beta-glucans, alpha-glucans and polyphenols. Even the fungus *I. obliquus* has been successfully used to counteract the effects of DSS-induced colitis in mice [134]. Its major effects seem to be the modulation of IIS [Won et al., 2011]. Nevertheless, its polysaccharides showed a positive regulatory effect on the microbiota in animal models of colitis. In a chronic pancreatitis mice model, the compromised microbiota profile was partially restored by *I. obliquus* polysaccharides administration, which was able to increase microbiota diversity and richness and also to improve mouse clinical conditions [93].

Ganoderma *lucidum* (in Japanese called Reishi) is considered, in the Far East, the mushroom of immortality due to the countless biological activities it would be able to promote. It is a mushroom with a woody texture and a bitter taste, which grows preferably on oaks and chestnut trees. The main traditional use in China and Japan is aimed to counteract allergic and inflammatory status [136] but its traditional use also covers hypertension, liver and cardiovascular problems. Recent studies have identified more than 400 bioactive molecules present in this mushroom. Some of these were identified for the first time in this specie, and consequently named as ganoderiol, ganolucidinic acids, and ganodermantriol [137].

The most studied activities of this mushroom are the immunostimulatory effect exerted on the gut but also at systemic level. However, there is also evidence of prebiotic activity on the microbiota, although this could be secondary to a direct effect on immune system components. Its powerful immunomodulatory effects led to extend its field of use also to the therapy of tumors, a topic which, however, goes beyond the themes of this chapter [138]. In DSS-induced colitis in rats, *G. lucidum* β-glucans increased SCFA-producing bacteria such as *Ruminococcus*, and reduced pathobionts such as *Escherichia* and *Shigella* in both the small intestine and cecum [139]. In mice fed a HFD, which showed increased body weight, gut and systemic inflammation and insulin resistance, treatment with *G. lucidum* mycelium reversed the HFD-induced gut dysbiosis, decreasing the Firmicutes-to-Bacteroidetes ratio and the Proteobacteria levels. Moreover, Reishi treatment reduced metabolic endotoxemia by restoring the intestinal barrier integrity. These anti-obesity effects were transmissible via fecal transfer from *G. lucidum*-treated mice to untreated HFD-fed mice, demonstrating that the leading mechanism of action of *G. lucidum* was linked to the modulation of the microbiota. High molecular weight polysaccharides (>300 kDa) present in *G. lucidum* were capable of producing similar microbiota modulation and anti-obesity effects [140]. Similar results were obtained in a rat model of type 2 diabetes, in which *G. lucidum* treatment reduced the relative abundance of harmful bacteria, such as *Aerococcus*, *Ruminococcus*, *Corynebacterium* and *Proteus*, and increased the levels of *Blautia*, *Dehalobacterium*, *Parabacteroides* and *Bacteroides*. Microbiome analysis indicated that Reishi treatment could also restore the microbial metabolism of amino acids, carbohydrates, inflammatory substances and nucleic acids, altered by the obesity status [141, 142]. Taken together, these results indicate that *G. lucidum* and particularly its high molecular weight polysaccharides may be effectively used as prebiotic agents to prevent gut dysbiosis and obesity-related metabolic disorders, at least in obese rodents.

In a mouse model of pancreatitis, induced by diethyldithiocarbamate (DDC), polysaccharides from *G. lucidum* were capable of positively modulating the gut microbiota, by decreasing the relative abundance of Bacteroidetes and increasing that of Firmicutes. At the genus level, supplementation of Reishi polysaccharides increased the relative abundance of beneficial bacteria, such as Lactobacillales, *Roseburia* and *Lachnospiraceae*. These results confirmed that also the therapeutic mechanism on chronic pancreatitis might be dependent on the restoration of a eubiotic intestinal microbiota layout [16, 17].

Finally, it should be emphasized that even if all these mushroom preparations can be easily found for free sale, and even if they do not seem to have side effects, it is a good practice to never use them in self-prescriptions as their direct interactions with drugs, or their effects on detoxifying enzymes such as CYP, have not yet been studied or poorly known. For example, Chaga extract inhibited platelet aggregation in mice. It may also have synergistic effects when used with anticoagulant/antiplatelet drugs, but the clinical relevance in humans is not known [143]. Chaga may also interact with hypoglycemic agents drugs, since it has demonstrated to possess hypoglycemic activity in animals [144, 145]. A single case-report described oxalate nephropathy as a side effect associated with the ingestion of Chaga mushroom powder (4–5 teaspoons daily for 6 months), in a 72-year-old Japanese woman with liver cancer [146].

Chaga effects on detoxifying enzymes such as CYP have not yet been studied. Reishi may increase the risk of bleeding, interfering with anticoagulants/antiplatelets drugs [147]. Reishi can also enhance immune response and this effect should be taken into account in patients on immunosuppressive therapy. Finally, at least *in vitro*, Reishi polysaccharides inhibited many different CYP enzymes [148].

5.2 Microbiota-modulating plants

Cichorium intybus is a perennial herbaceous plant whose rhizome and roots are traditionally used in Europe to treat gastrointestinal disorders [149]. It has been tested in farmed broiler chickens in order to improve productive performance, and showed to induce changes in ileal microbiota consisting of lower counts of *E. coli* and higher counts of *Lactobacillus*. These effects were associated with improved growth performance. Dietary chicory powder supported ileal microbiota ecology probably by acting as a prebiotic, since it has a high content in soluble fibers, particularly inulin [150]. In mice fed with different chicory genotypes, all preparations were capable of modifying the fecal microbiota by modulating the Firmicutes/Bacteroidetes ratio and some bacterial genera, such as *Alloprevotella*, *Blautia*, *Alistipes*, and *Oscillibacter*, with a variable effect depending on the chicory genotype. In addition to microbiota changes, some modifications in the release of satiety hormones, and as a consequence appetite, were also observed in mice treated with *C. intybus* [151].

Boswellia serrata is an arboreal plant that forms an aromatic resin also known as frankincense. The *B. serrata* resin has been used as supplementation in rabbit diets at different dosages to observe variations in the cecal microbiota. Substantial changes in microbial cecal populations were found in rabbits treated with *B. serrata*, with a significant decrease of total bacterial counts and in particular a decrease of *Salmonella enteritidis* and *E. coli* if compared to the control untreated rabbit group. These results could be ascribed to the high polyphenol content in *B. serrata* and to the presence of Boswellic acid that holds a powerful anti-microbial effect [152, 153].

Pistacia lentiscus is a shrub or small evergreen tree that produces a resin called Chios mastic gum, used as a natural food supplement. The effect of *P. lentiscus* was investigated in mice with obesity, nonalcoholic steatohepatitis (NASH), and liver

fibrosis induced by HFD. Treatment with *P. lentiscus* promoted a partial but significant recovery of microbiota diversity associated with a decrease in Bacteroidia and an increase in Proteobacteria relative abundance [154].

Olea europaea is an evergreen fruit tree found traditionally in the Mediterranean area. *O. europaea* extra virgin oil (EVO) obtained from its fruits, olives, is able to induce higher gut microbiota biodiversity and promote the growth of beneficial commensal bacteria, as showed by studies on humans and animals [155]. Nevertheless, the traditional use of *O. europaea* as officinal plants is limited to its leaf preparations. *O. europaea* leaf extract administered to obese mice was capable of improving the gut microbiota by partially restoring the amounts of Actinobacteria, Bacteroidetes and Verrumicrobia. Also, the relative abundance of *Akkermansia* spp. was restored, suggesting a possible effect on intestinal barrier function in treated mice [156].

As for *Angelica arcangelica*, *Achillea millefolia*, *and Cetraria islandica,* officinal plants that are traditionally used to treat intestinal dysbiosis and inflammation, there are no scientific studies so far published to support their positive action on the intestinal microbiota and microbiome. This does not mean that these medicinal plants are not effective in modulating the gut microbial ecology, but only that the documented scientific evidence of their supposed therapeutic activities are still very poor.

6. Aromatic plants end essential oils (EOs) as bowel "Eubiotics"

6.1 Intestinal microbiota modulation exerted by essential oils and aromatic plants

Aromatic plants are a wide group of herbs with characteristics aroma due to the presence of high amounts of volatile compounds known as EOs. Consequently, aromatic plants have always constituted a characteristic aspect of the gastronomic traditions. In recent years, the use of these aromatic plants has been replaced, especially in countries with high per capita income, with artificial flavors that allow the elaboration of more sophisticated aromas that in many cases are kept secret by the food industry, to avoid plagiarism. This replacement is certainly part of the transition from the traditional cuisines to the so-called western diet, the process called westernization of the diet that has taken place in many countries, parallel to the increase in the incidence of many intestinal diseases related to alterations of the gut microbioma, such as Inflammatory Bowel Diseases (IBD) [157]. EOs have multitarget effects on the intestine due to their antioxidant, anti-inflammatory but also antimicrobial properties directed on the bacterial, yeasts, fungi and viruses components of the human microbioma [158]. The antibacterial activity of EOs depends on the concentration that they reach into the gut, but also on the species of bacteria that they encounter. In fact, some OEs have more marked effects (i.e. lower Minimum Inhibitory Concentrations or MICs) for bacterial species considered pathogenic, while showing less activity (i.e. higher MICs) towards components of the microbiota such as bifidobacteria and lactobacilli [159]. This multitarget positive effects of EOs on the intestinal microbiota, different from those obtained with the use of probiotics and prebiotics, has not found a definition in the literature yet. Hence, we propose here for the first time the term "eubiotic" activity since EOs restore the intestinal microbiota back to a physiological state of eubiosis, when a dysbiosis has been established into the gut.

6.2 Eubiotic proprieties of EOs on gut microbiota of animals and humans

There is no doubt that EOs are able to modulate the intestinal microbioma for their antimicrobial activities, which is one of the reasons why nature has selected these complex mixtures of active molecules with evolution. EOs may have "eubiotic" effects thanks to their capability to control and modulate bacterial growth, acting both as bacteriostatic or bactericidal agents [160]. In fact, due to their lipophilic properties, EOs can penetrate membranes, and damage bacterial cells structure making their membranes more unstable and permeable. Membrane disruption may also lead to bacterial death caused by the significant leak of ions and other essential cytosolic components. These EO effects are generally more pronounced on Gram positive bacteria respect to Gram negative ones [161]. However, it has been demonstrated that EOs can also affect bacterial cell wall in Gram-negative bacterial strains [162]. Despite this, there are very few clinical studies of their eubiotic activity on humans, while the scientific data obtained on animals bred for human consumption or on experimental animal models are numerous and really convincing.

In broiler chickens, EOs have been widely adopted to improve intestinal microbiota and, as a consequence, to boost the growth performances of farmed animals. For example, the effects of liquidambar essential oils (LEO) isolated from Turkish sweet gum leaves (*Liquidambar orientalis* Mill.) were a decreased *Escherichia coli* counts in jejunum, associated with an increased weight of chickens after 42 days of treatment [163]. Feeding broilers with a trade mark EO mixture containing thymol (*Thymus vulgaris*), eugenol (*Cinnamomum* spp.), and piperine (*Piper* spp.) resulted in an increase in Lactobacillus and a decrease in *E. coli* counts in ileal microbiota, associated with an increased food conversion ratio [164]. In another study, the administration of a commercial EO mixture, containing cinnamaldehyde, isophorone and eugenol significantly increased the relative abundance of phyla Bacteroidetes and decreased the abundance of phyla Firmicutes in cecal microbiota of chickens, with an increase in the relative abundance of genus of *Alistipes*, *Rikenellaceae, Roseburia,* and *Anaeroplasma*. This microbioma changes was confirmed by more than hundred different metabolites detected in cecum of EO treated animals, probably linked to their improved growth performances [141, 142].

Broiler chicken is not the only farmed animal treated with EOs for the purpose of modifying microbiota and reduce the susceptibility to infection by pathogenic bacteria. In farmed rainbow trout, the treatment with a mixed EO (containing eucalyptus, oregano, thyme and sweet orange EOs) caused significant microbiota changes in alpha and beta diversity, increasing also their growth performance and the final product quality. [165]. In farmed pigs, oral administration of a EO mixture (containing cinnamon and oregano EOs) caused a significant decrease of infections caused by two porcine diarrhetic enterotoxigenic *E. coli* strains [166].

Two different essential oils were tested on farmed ducks, again in order to improve their growth performance and also to replace the use of antibiotics in animal farming. One consisted of oregano oil, the second of thyme and cinnamon oil. Both of these EO preparations were able to decrease the cecal populations of coliforms and lactose-negative enterobacteria, demonstrating also in these animals an eubiotic effect of these OE on the gut microbiota [167].

Even on farmed crustaceans, a blend of organic acids and essential oils was tested for the improvement gut microbiota and disease resistance of Pacific white shrimps. Results demonstrated that this mixture was capable to enhance microbiota diversity and richness, increasing the abundance of Firmicutes and reducing the abundance of Proteobacteria. Also, a significant increase in the abundance of *Lactobacillus* was observed in shrimp gut [125, 126].

All these studies as a whole demonstrate without doubt the eubiotic potential of orally administered EOs. Furthermore, they clearly demonstrate that dosages effective for modulating the microbiota are free of toxic effects on animals. Nevertheless, it remains rather difficult to understand which components of EOs are most active for modulating the microbiota, because of their natural complexity and their use in mixtures. For these reasons, several studies have explored the eubiotic properties of EO single molecules. The most studied was certainly geraniol, for its interesting antimicrobial potential. Geraniol antibacterial activity seems to be linked to his property to destabilize bacterial cell wall and damage transmembrane efflux pumps [168]. Despite being absorbed very quickly and in an active manner by the small intestine mucosa, geraniol is reported to positively modulate the colitis-associated dysbiosis when administered by oral route by using a controlled delivery system based on microencapsulation [169]. In mice but also in humans, geraniol has demonstrated to act as an excellent modulator of intestinal microbiota, capable to boost populations of butyrate-producer bacteria such as *Collinsella* and *Faecalibacterium*, normally reduced in the dysbiotic human intestinal flora of IBS patients [157]. It is interesting to note how geraniol antibacterial activities is quite selective for pathogenic bacteria and do not involve commensal species [159]. For these reasons, geraniol can be considered as an efficient eubiotic for the human gut microbiota.

Another interesting EO molecule with antibacterial activities is eugenol (2-Methoxy-4-(prop-2-en-1-yl) phenol), the major compound present in clove oil, but also found in many other EOs. Eugenol has demonstrated antimicrobial activities based on a non-specific permeabilization of the bacterial membrane with depletion of adenosin triphosphate (ATP), an energy moiety necessary for bacterial metabolism and survival [170]. This effect has been observed against gut pathobionts such as *E. coli*, *Listeria monocytogenes* and *Lactobacillus sakei* at the relatively low concentration of 10 mM [171]. In mice, orally administrated eugenol improved the secretion of the intestinal mucus, creating a thicker intestinal layer associated with positive changes of the mucosal-microbiota ecology. In particular, eugenol inhibited the intestinal adherence of *Citrobacter rodentium*, a mice pathogen that shares several biochemical features with *Clostridium difficile* in humans [172]. It would be really interesting to use eugenol in a clinical study aimed at the eradication of *C. difficile*: the results could be surprising.

Cinnamaldehyde (2E-3-Phenylprop-2-enal) is a phenylpropanoid naturally present in the plant of the genus *Cinnamon*. Cinnamaldehyde is one of the most studied EO molecule and it has been already approved as antimicrobial food preservative [173]. Antibacterial effects of cinnamaldehyde have been demonstrated by using many different bacterial models, but only few studies evaluated its impact on the whole intestinal microbiota. *In vitro*, cinnamaldehyde was capable to inhibit the growth of potentially pathogenic bacteria such as *S. aureus, E. cloacae, A. baumannii* and *L. monocytogenes* [174] and it was able to kill a pathogenic strand of *E. coli* at very low concentrations (0,05% v/v) [175]. One of the proposed antibacterial mechanisms of cinnamaldehyde inhibition of *E. coli* growth was the inactivation of its acetyl-CoA carboxylase enzyme [176]. Other studies showed that cinnamaldehyde antimicrobial activity has a broad spectrum of action, being effective against many different intestinal pathobionts such as *Enterococcus faecalis, Enterococcus faecium, E. aerogenes Salmonella enterica* and *Clostridium perfringens* [177]. *In vivo*, only few studies have been conducted on cinnamaldehyde, perhaps because of its strong aggressiveness towards the mucosal epithelia. Nevertheless, in animal experimental colitis, the oral administration of cinnamon EO (approx. 70% in Cinnamaldehyde) at 10 mg/Kg or 15 mg/Kg lead to an improvement of the ecological biodiversity of the intestinal microbiota. Short-chain fatty acids (SCFA)-producing bacteria

family, such as Bacteroidaceae, were increased while intestinal *Helicobacter* and *Bacteroides* were reduced [178].

Other molecules, such as thymol do not seem to show eubiotic effects in the gut, being non-selective and affecting all the intestinal bacteria and therefore behaving like a broad-spectrum antibiotics, depleting the microbiota even when administered at low dosages with a negative impact also on commensal bacteria [159].

Carvacrol, a major component of oregano EO, showed to inhibit bacterial adhesion, invasion and biofilm development in cultured intestinal cells [144, 145]. In farmed broiler, treatment with carvacrol-rich EO was tested to control the pathogenic bacteria spreading inside the farms. Results of these studies demonstrated that carvacrol reduced the microbial counts of *E. coli* and different *Salmonella* species in the small intestine of farmed chicken [144, 145]. Moreover, carvacrol administration to broiler chickens, was capable to eliminate the intestinal presence of *Campylobacter spp*. after 21 days of oral daily administration at 120–300 mg/Kg. This effect was associated to the enhanced growth of *Lactobacillus*, that were found to be increased in chicken microbiota, after carvacrol administration. For its eubiotic activity, this molecule is today the most used in organic chicken farming [179].

Limonene (1-Methyl-4-(prop-1-en-2-yl) cyclohex-1-ene) is a cyclic monoterpene present in high amount in EO of citrus fruit peels that has widely demonstrated antimicrobial and eubiotic effects *in vivo*. In mice, daily oral administration of 160 mg (8.000 mg/Kg) of limonene-rich orange EO modulated the gut microbiota by enhancing the relative abundance of *Lactobacillus* genus and of *Bifidobacterium* population [180]. Despite the low toxicity of limonene, it should be noted that these eubiotic effects were obtained only with high dosages of this EO.

Eucalyptol (1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octane) is a cyclic ether and a monoterpenoid. It is the major compound in *Eucalyptus* EO, but it can be also found in many other officinal plants. *Eucalyptus* EO has extraordinary antimicrobial activities and has shown to be effective against a plethora of bacteria species and among them *S. aureus*, *E. coli, Bacillus subtilis, Klebsiella pneumonia, Salmonella enteritidis* and *P. aeruginosa* [181]. Nevertheless, *Eucalyptus* EO also contains high amounts of other antimicrobial components besides eucalyptol, therefore not all of *Eucalyptus* EO antibacterial activity can be ascribed to the presence of eucalyptol. However, literature data regarding eucalyptol eubiotic activity are very limited, and new studies focused on this interesting compound are needed.

Menthol (5-Methyl-2-(propan-2-yl)cyclohexan-1-ol) is a chiral alcohol and the main molecule present in cornmint and peppermint EOs. It has been well known for its use in foods as a cooling and minty-smell aroma. Many *in vitro* studies focused on its antibacterial activities [182]. Nevertheless, studies on the use *in vivo* of menthol alone, to modulate the gut microbiota, are lacking.

6.3 EOs in the modulation of gut mycobiome

Fungi were reported to represent about 0,1% of all the microorganisms present in the gastrointestinal tracts. Maybe also for this reason, despite the presence of fungi in the intestine has been known for many years, in depth studies of the human mycobiome were only recently performed [183]. Together with bacteria, fungi contribute to the modulation of the intestinal immune system [184]. Many of them have a clear pathogenic potential even if, physiologically, they are commensals in our bodies. Only in some specific conditions their overgrowth can lead to well-known mycosis. The best known fungal pathogen of humans is certainly *Candida albicans,* which is a normal component of the gut mycobiota but may causes candidiasis in case of its intestinal and vaginal overgrowth [185]. An altered intestinal

mycobiota has also been observed in other human pathological conditions, such as IBS [186], inflammatory bowel disease (IBD) [187] and also autism-spectrum disorders and Rett syndrome [188].

EOs antimycotic activities are characterized by a broad spectrum of actions [189]. *C. albicans* has been one of the main target for studies focusing the antifungal effect of EOs and their single molecules. The antifungal activities of EO obtained from *Thymus vulgaris*, *Citrus limonum*, *Pelargonium graveolens*, *Cinnamomum cassia*, *Ocimum basilicum*, and *Eugenia caryophyllus* have been evaluated against clinical isolates of *C. albicans* and *C. glabrata.* All of these EOs exhibited both fungistatic and fungicidal activity towards these two *Candida* species, but cinnamon oil demonstrated the highest activity [190]. Since the most represented active compounds of *Cinnamomum* EO is cinnamaldehyde, many studies have been addressed to analyze in depth its activity against *C. albicans* [191].

Limonene has shown to possess strong antifungal properties [192] and in particular an excellent anti-*Candida* activity. A recent study analyzed the efficacy of this compound against the growth of *C. albicans* isolates, whose growth was completely inhibited at doses ranging between 5 mM and 20 mM [193].

Mentha EOs have demonstrated good antimycotic activities against different fungi genus, including *Candida* [194]. Menthol and (+)-carvone are the major components of peppermint EO and both exhibited strong antifungal activity *in vitro* [195] and mycobiome modulation activities *in vivo* [196].

Thymus vulgaris EO has also shown to be effective against fungi pathobionts capable to infect humans. A study on Dermatophyte, fungi that can cause superficial infections of the skin, and on *Aspergillus*, fungus genera that can cause respiratory infections, reported MIC values for *Thymus vulgaris* EO ranging from 0.16 to 0.32 µl/ml. Higher MIC values, between 0.32 and 0.64 µl/ml, were reported for *Candida spp*. The antifungal activity of this EO has been attributed to its two major components: thymol and carvacrol, that accounted respectively 26% and 21% of *Thymus vulgaris* EO [196]. Both these phenolic compounds seem to act by disrupting the fungal cell membranes [161].

Clove EO has been traditionally used in dentistry for its anesthetic and antimicrobial activities [197]. Its anti-fungal action has been attributed to eugenol, the major clove oil molecule. A recent study indicated that Clove EO, at concentrations that ranged between 0,03% and 0.25% (v/v), inhibited the biofilm formation in many *Candida* species, grown on different substrates [198]. For what the mechanism of action concerns, eugenol was able to cause permanent injury to the cell membranes of *C. albicans* and morphological alterations to its cell wall [161, 199]. Although these studies suggest that the eubiotic effect of EOs and their individual chemical compounds may also be extended to the mycobiome, there is currently no conclusive evidence showing that the improvement in microbial ecology linked to the use of these compounds can also involve the fungal component of the human microbiota.

EOs have also been shown to have strong antiviral activities, which could affect the gut virome, which is an integral part of the human microbiota [200]. To date, no study has been performed to understand the impact of EOs on the intestinal virome. The main physiological viral component of the gastrointestinal tract is represented by prophages or phages [201]. The bacteriophage component is mainly composed by temperate virus of the Caudovirales order, but most of the detected viral sequences in human gut virome could not be attributed to known viruses [202] and to date it is estimated that the number of virus in human stools is up to 10^9 per gram [203]. Despite it is clear that EOs may impact on the intestinal virome composition by modulating all the microbiota components, it could be really difficult to understand the direct impact of EOs on the intestinal viruses and the consequences of this modulation on the intestinal ecology.

7. Conclusions

The scientific data present in the literature undoubtedly demonstrate that some EOs and some of their components are able to positively modulate the human intestinal microbiota, acting in a differentiated way on pathobiontic microorganisms, without altering or even improving the component of microorganisms defined as healthier commensals. This selective antimicrobial activity is certain for the bacterial component of the intestinal microbiota, conceivable for fungi, but at the moment completely unknown for viruses. It is therefore possible to define with certainty an eubiotic activity for some EOs and some of their components, such as for example geraniol, eugenol, cinnamic aldehyde and limonene, which can properly be considered as eubiotics. Finally, it is interesting to note that the antibacterial activities of these compounds are always multitarget and that for this reason the bacteria are unable to develop resistance. These data associated with the low toxicity of these compounds (by oral administration), suggests that these EOs may be part of a long-term therapy aimed at restoring an eubiotic and resilient microbial ecosystem.

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Conflicts of interest

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Chapter 10

Non-Pharmacological Interventions in Preventive, Rehabilitative and Restorative Medicine

Andrés J. Ursa Herguedas

Abstract

Non-pharmacological interventions (NI) have been known since before modern pharmacology was developed. They occupy a prominent place in the healthcare sciences. The aim of this chapter is to show the role of NPI in medicine today. The reasons for implementing NPI, both in the scope of prevention and cure, are due to the fact that there are many diseases for which we still do not have a cure, such as Alzheimer's dementia, multiple sclerosis or fibromyalgia. By selecting those NPI that have more scientific evidence and applied by health or non-health personnel, it is intended to improve quality of life, slow down deterioration, relieve pain or restore health at a lower economic and environmental cost whilst complying with the Hippocratic maxim "first, do no harm". There are many NPI currently managed, which are used in most known conditions, to support specific treatment or as a single therapy. Further studies on NPI to improve their safety and efficacy are advisable.

Keywords: iatrogenesis, integrative medicine, non-pharmacological interventions, non-pharmacological treatments

1. Introduction

Non-pharmacological interventions (NPI) are part of the chapter on therapeutics in the health sciences. Together with pharmacotherapy, ionising or non-ionising radiation, surgery and rehabilitative medicine they comprise the procedures used to prevent and treat diseases.

Non-pharmacological treatments (NPT) are used for many unconventional treatments in integrative medicine.

The need to use NPI is justified because it is a valid option if indicated as a preventive or curative measure. Side effects of medications are avoided, health costs are brought down and there is no significant environmental impact.

The use of medicines has entailed an important change for humankind. No one doubts the benefits of antimicrobials, vaccines, anti-inflammatories, analgesics, opotherapy and specific medicines for each health problem. Modern surgery has been possible thanks to the development of anaesthetics, anticoagulants and a large

number of medicines that make it possible for each intervention to be performed. Many material and human resources have been devoted to the study of numerous drugs and a powerful pharmaceutical industry (PI) has developed which occupies the highest echelons in the economy of developed countries.

Although there are many benefits provided by PI and they continue to contribute to the health of humankind, a series of problems that have arisen due to the so-called medicalization of life must be taken into account [1].

Prescribing is far from being totally scientific and suffers from serious shortcomings for various reasons such as commercial interests, deficiencies in clinical trials and regulatory bodies, ethics and environmental problems. Sometimes as many medicines are prescribed as the client has symptoms, whereby it invites a follow-up for possible drug interactions and side effects [2].

Greater prescription of medicines (polypharmacy) is associated with poorer quality of life and higher morbidity. In some developed countries, iatrogenic drugs have displaced accidents as the third or fourth leading cause of death after cardiovascular disease and cancer [3].

The criteria of the prescribing physician, whether primary or specialised care, is important to avoid interactions, overdose, duplicates and other problems that may contribute to the onset of side effects. In addition, the criteria must avoid pressures from the PI and act with a cost criterion; effectiveness, safety and environmental

PI performance	**Consequences**	**Solutions**
Most clinical trials (CT) are sponsored by the PI	Large percentage of positive results	Legislation and greater control
Negative results are not published	The scientific community is deprived of important information	It was approved that all clinical trials had to be registered (WHO, 2004) and/or Latin American Registry of CT in progress (Latinrec)
PI sometimes manipulates CT results	The user of the medicine is harmed by side effects, etc.	Greater control of clinical trials (legislation)
CT results are not always replicable	Concern	Legislation
Bioethics committees and regulatory bodies are not always up to the task	They evaluate efficacy, quality and safety but not the medicine's therapeutic value	Need for analysis by independent bodies
Sometimes medicines for adults are prescribed at paediatric age	Greater chance of side effects	Further information from the laboratory
CT are performed with the most disadvantaged population groups (homeless, illegal immigrants, Latin Americans, etc.)	Selection bias and uncertainty Ethics issues	Adequate legislation
There are conflicts of interest in the study	New medicines are approved without sufficient knowledge of side effects	Greater control by regulatory bodies
The PI distorts clinicians' beliefs and substitutes marketing for testing	Increase in pharmaceutical spending due to inappropriate medication	Information transparency
Criteria for approval of a new medicine are often ineffective	The user and health system are harmed	Adequate legislation

Table 1.
Irregular pharmaceutical industry practices (taken from Goldacre and completed by A. Ursa).

sustainability. Due to this latter aspect, NPI should be taken into account since, in principle, they are more environmentally sustainable than medicines.

Evidence-based medicine (EBM) is the current benchmark when it comes to performing a healthcare intervention. Its influence also extends to the design of clinical trials and their reporting.

Since the onset of the 21st century, independent scientists from multinational pharmaceutical companies have denounced the inappropriate practices of the PI. **Table 1** shows some of the irregular practices carried out by the PI for financial purposes, their consequences and solutions according to Ben Goldacre [4].

During the medical procedure, all health professionals when prescribing within the scope of their competence, must choose the best therapeutic option for their client, always bearing in mind the NPI. The reality, however, is different because the future doctor is educated in the prescription of drugs. Because the current medical paradigm requires rapid, accurate and symptomatic actions. However, the side effects of the medicines also need to be tackled. Because of this, a powerful PI has been developed with major economic interests, the medicine has been overvalued and research, development and innovation (RDI) are targeted at these interests and not at NPI [5].

The PI generally spends more on marketing and marketing of medicines than on research [6].

Although it is true that the whole process that entails the launch of a new medicine on the market is lengthy and expensive, the PI often opts for a false innovation. That is how "me too" drugs arrive on the market [7], molecules similar to others in use, enantiomers, racemic mixtures, etc. The PI brings out "me too" drugs when the end of their drug patents approaches. These novelties that are not such, are usually expensive, not superior to the old drugs and are a source of major revenues for the PI [8].

Table 2 shows some methods used by researchers to obtain favourable results in clinical trials according to Sackett, Oxman, Smith, Peiró and Peralta [9].

Methods used by the PI to obtain favourable results in CT
Contrast the effects of the drug with placebo and not with other drugs
Conduct a CT against a treatment known to be inferior
Compare the new drug with doses that favour the study drug over the reference drug
Not apply true uncertainty criteria
Perform CT too small to reveal differences from the competitor
Use multiple variables in the CT and select only those that provided favourable results for publication
Perform multicentre trials and select only those from sites that obtained favourable results for publication
Perform subgroup analyses and select only those that are favourable for publication
Present results most likely to impress. Relative risk reduction is often used instead of absolute risk
Selection of the population participating in the study inappropriately
Using inappropriate routes of administration of the reference drug
Use surrogate instead of clinically relevant variables
Delay publication and retention of data
Establish the primary endpoint at the end of the study
Mask side effects
Highlight data favourable to the funder and not publish unfavourable data

Table 2.
Methods used to obtain favourable results in clinical trials [9].

The PI only finances research projects most likely to yield positive results. This breaches the uncertainty principle that establishes that the patient should be included in a CT only if there is substantial uncertainty about which treatment will benefit them the most [9].

The publishers that own the medical journals where the CT are published depend on the PI, since drug advertising, special issues, reprints, etc. are a source of revenue [10]. If an author publishes an unfavourable criticism against a drug or PI, he runs the risk of not receiving income for the above concepts [9].

The fact that certain medicines are included in a clinical practice guide (CPG) is of major interest to the PI, since these guidelines are drawn up by experts for their use [11]. A study published in JAMA in 2002 found a high number of financial relationships between CPG experts and PI. Serious omissions were found in the declarations of conflicts of interest [9].

Conflicts of interest and potential biases in the publication of scientific-medical research have cast doubt on the credibility of the PI [9].

According to Peter Gotzsche, from the University of Gopenhage and director of the Nordic Cochrane Centre, the PI "does not work to improve health, but to obtain the maximum benefits" and to do this "extorts, commits fraud, breaches legislation and lies" [3].

2. Non-pharmacological interventions in the health sciences

2.1 Concept and generalities

Non-pharmacological interventions (NPI) or non-pharmacological therapies (NPT) are defined as any non-chemical intervention, which is theoretically supported, targeted and replicable, performed on a patient or caregiver and potentially capable of obtaining a relevant benefit [12].

The adoption of a healthy lifestyle is perhaps the best NPI as it will contribute to better health, more life enjoyment and reduce, except for contingencies, health costs. Thus, the ideal place to recommend NPI, as a preventive and/or curative measure is Primary Health Care, in line with the Declaration of Alma Ata of 1978 [13] and ratified 40 years later in Astaná in 2018 [14].

A large number of the techniques used in physiotherapy such as massage, kinesitherapy, etc., manual techniques (joint manipulations, chiropractic, etc.), various techniques used in psychotherapy, yoga, meditation, and others framed under the term non- conventional medical therapies (NCMT) such as acupuncture, moxibustion, homoeopathy, etc., belong to the NPT chapter. Many act by stimulating the body's healing power, sometimes because they stimulate the production of biogenic amines, neuropeptides, stimulate natural defences, produce neuroprotection, etc., which contributes to homeostasis [15].

Although herbal medicine or treatment with medicinal plants forms part of the treatments used in NCMT, it is not included in this section since it deals with chemical substances. This does not mean that they should not be used but rather that it would be desirable to supplement NPT with medicinal plants of proven efficacy and safety. Homoeopathic preparations, however, do fall under the NPT heading, since after several dilutions the original substance is not observed.

NPT seek to relieve symptoms and improve quality of life, which is why they are widely used in the management of dementias, especially Alzheimer's dementia, both in institutions and at home. Applied exclusively or in combination with drugs, they aim to slow down the course of the disease [16].

NPT should meet safety and efficacy standards [17] and for this, studies and meta-analyses have had to be performed for scientific validation, as required by evidence-based medicine according to Sackett et al. [18].

Unfortunately, there is not always a company or entity that finances many of these studies. Therefore, there are fewer studies published than those offered by the PI.

Although NPT are used above all in the field of Gerontology, in many other disciplines they also have both preventive and curative applications, either alone or in combination with other therapies.

2.2 NPT in the field of social and healthcare assistance

NPT began to be applied systematically for dementia, both in institutionalised patients (nursing homes, day care centres) and in their homes. The aim was to, alleviate these processes, since there is no curative treatment [19]. NPT in the field of social and health care are called psychosocial interventions (PSI).

In the 1980s, support programmes for caregivers of dementia patients, whether they were family members or individuals, needed to be performed. In recent years there has been a need to extend these programmes to professional caregivers [19].

Since the last century there have been several attempts to classify PSI. With regard to validating PSI in Alzheimer's dementia, experts recommend basing their actions on systematic reviews and meta-analyses.

Alzheimer's relatives' patient associations consider the areas of intervention in terms of cognitive, functional, emotional and comprehensive aspects.

Some intervention programmes (IP) used in the field of Alzheimer's disease are listed in **Table 3** according to Gárate Olazábal [20].

These interventions can be performed either individually or in groups. Those carried out individually are more effective.

Intervention programmes (IP)	**Techniques**	**Person who applies it**
IP focused on behaviour	Behavioural training Cognitive behavioural therapy	Psychologist
Environmental IP	Adaptation of physical space Adaptation of the social environment	Clinical assistants
IP focused on emotion	Montessori method Validation therapy Reminiscence Orientation to reality	Psychogeriatricians Occupational therapist Physiotherapist Nursing assistant
Cognitive stimulation programme	Art therapy Music therapy Aromatherapy Physical exercise Light therapy	Psychogeriatrician Occupational therapist Physiotherapist Nursing assistant
IP focused on stimulation	Massage Therapeutic touch	Physiotherapist
Other IP	Relaxation, acupuncture, animal therapy, etc.	Psychologist, doctor

Table 3.
Intervention programmes focused on Alzheimer's disease (taken from Gárate Olazábal and completed by A. Ursa).

The Montessori-Based Dementia Programming (MBDP) method enables adults with dementia to be given tasks initially designed solely for children. Dr. Cameron Camp and the Myers Research Institute are pioneers in the MBDP system, which began to be used in the late 1990s. It is applied at advanced stages and consists of performing scheduled activities based on activities of daily life (ADL). To achieve this, he uses cognitive rehabilitation techniques such as task division, guided repetition, progression from simple to complex, and progression from concrete to abstract. When applied properly, it improves motor skills and basic functional abilities within a reasonable period of time (included in the Barthel index) [21].

Many other NPI can be performed in the social health field and as a first choice, for common pathologies such as insomnia [22], anxiety and stress [23], etc.

Support groups, education techniques and cognitive-behavioural training, counselling and case management, and prevention of physical and/or chemical restraints have been devised among other interventions to reduce the morbidity associated with caring for these patients [24]. This is for the caregiver, whether family or non-family, due to the major burden that falls upon them.

2.3 NPT in the cardiovascular system

Cardiovascular diseases (CVD) are the most common cause of mortality in Western countries and involve high health costs. Arteriosclerosis develops insidiously over many years and its clinical manifestations appear when the disease is advanced. The CVD burden has grown in recent decades, in parallel to an increased prevalence of risk factors such as obesity, smoking, type 2 diabetes mellitus and high blood pressure [25]. Prevention of CVD involves adopting a healthy lifestyle and intervening on biochemical modifiable factors, etc., by means of pharmacological and/or non-pharmacological treatments.

In recent years, a preventive strategy has been developed in clinical practice based on what is known as cardiovascular rehabilitation (CVR), which is defined according to the World Health Organisation as "the set of activities necessary to ensure people with cardiovascular diseases, an optimal physical, mental and social condition that allows them to occupy by their own means as normal a place as possible in society" [26]. A team of professionals is required to perform CVR, it has relatively little implementation and according to cost-effectiveness studies it is favourable [27].

The prevention of such common pathologies as arterial hypertension is based on dietary advice, practice of physical exercise appropriate to each situation [28], stress control, emotional management and avoiding both legal and illegal drugs.

Many other cardiovascular diseases can be treated as first intention with NPT or as an accompaniment to pharmacological treatment. **Table 4** shows some of these pathologies, NPT and the healthcare professional who applies this.

It would be desirable to implement cardiovascular pathology NPI in health systems to reduce the side effects of medication, polypharmacy, improve quality of life and reduce health costs.

2.4 NPT in the respiratory system

Chronic obstructive pulmonary disease (COPD) and asthma are common respiratory diseases and in many cases, they go undiagnosed, reduce quality of life and represent a high health cost.

NPT is essential in COPD patients. However, this treatment is sometimes not given adequate importance. Patients diagnosed with COPD should benefit from

Pathology	NPT	Professional who applies/ supervises this
Hypertension	Physical exercise (Briones Arteaga)	General practitioner/specialist
Acute heart failure [29]	Ventilation, ultrafiltration, mechanical circulatory support, myocardial revascularization, etc. [29]	Cardiologist and nursing staff
Venous insufficiency of the lower limbs [30]	Dietary advice, hydrotherapy, physical exercise (Schneider)	Physician/nurse/physical therapist
Primary arterial hypotension [30]	Dietary advice, hydrotherapy, physical exercise (Schneider)	Physician/nurse/physical therapist

Table 4.
Some CVD and their non-pharmacological approach.

comprehensive care services (CCS), which are an articulated set of standardised actions aimed at meeting the COPD patient's health needs, considering the environment and particular circumstances. Pulmonary rehabilitation (PR) is one of the essential components of non-pharmacological treatment in COPD. NPT is used as an adjunct to drug therapy [31] and has been shown to improve functionality [32].

Table 5 shows the pulmonary rehabilitation plan according to the National Heart, Lung, and Blood Institute (INCPS) [33].

Many other actions have been published for asthma (therapeutic education, massage, music therapy, etc.). However, results are not conclusive.

2.5 NPT in the digestive system

Gastrointestinal tract diseases are numerous, due to different causes and many are related to an inappropriate lifestyle. In addition to the pharmacological and/or surgical, dietary and psychological treatment from which a benefit can be derived, some are susceptible to improvement with physical treatments such as different applications of hydrotherapy (washes, damp cloths plus drug substance, jets, etc.), physical exercise, relaxation techniques, etc., within the context of personalised medicine.

Table 6 shows some NPT applied in the most common digestive tract disorders (taken from Schneider and Pizzorno et al. [34, 35]).

In the section on hepatobiliary diseases, there are many accompanying measures to pharmacological, hygienic and dietary treatments that can be performed. Given the characteristics of the book, it is not possible to elaborate in this context.

2.6 NPT in endocrine-metabolic disorders

Obesity and diabetes mellitus are among the most common of the many endocrine-metabolic disorders in Western countries. Both constitute a public health problem since they cause major morbidity and mortality, which increases the country's health expenditure. The first measure in tackling obesity consists of adopting a healthy lifestyle that enables maintaining an optimal weight. Diet, physical exercise and medical advice should not be lacking when the body mass index is higher than 30. Individualised treatment should take precedence over guidelines or protocols. In the case of type 2 diabetes mellitus, the most common, hygienic-dietary advice needs to be strengthened as an aid to pharmacological treatment if needed [36].

For dyslipidaemia, good results have been achieved with the application of cardio-healthy diets, especially for secondary dyslipidaemia [37].

Procedure	Purpose	Resources/professionals
Exercise training	Improve muscular endurance and strength	Treadmill, exercise bike, weights
Nutritional advice	Eating to achieve a healthy weight	Periodic supervision by the nutritionist
Health education	Knowledge of the disease, proposals for a healthy life, recognition of flare-ups, drug management, etc.	Specialist doctor/nursing team
Tackling fatigue	Advice on how to perform daily tasks, stress management, sleep, etc.	Specialist doctor/nursing team
Tips on breathing	Improve the quality of breathing and oxygenation	Specialist doctor/nursing team
Psychological advice	Individual or group approach. Avoid anxiety/depression	Psychologist

Table 5.
Pulmonary rehabilitation plan according to the INCPS.

Condition	NPT	Effects
Caries and periodontal disease	Mechanical cleaning of teeth with dental floss	Removes the bacterial plaque causing the disease
Gastroesophageal reflux esophagitis (from hiatus hernia, etc.)	Postural when lying down (head elevated) Physical exercise	Prevents passage of acid from the stomach
Chronic gastritis	Compresses, damp cloths plus drug substance, wraps, jets, etc. according to disease stage	Reduce discomfort, improve functionality
Gastrointestinal ulcer	Flax seed/clay plasters on abdomen, wraps and compresses for the first 4 weeks. After dry brushing of the skin, jets at alternate temperatures, etc.	Shortens course, relieves symptoms (pain, etc.) and reduces medication
Irritable bowel syndrome	Diet (fibre, etc.) Stress reduction (yoga, meditation) Physical exercise	Improves annoying symptoms (pain, etc.)
Functional constipation (no organic cause)	Diet, physical exercise, hydration Warm sitz baths. Chamomile enema. Belly massage. Abdominal wraps, etc.	Adoption of a healthy lifestyle improves the frequency of defecation and avoids associated diseases (haemorrhoids, etc.)
Haemorrhoids (internal and/or external)	Depending on scope they can benefit from a sitz bath at an alternating temperature, homoeopathy, etc.	Reduce congestion, relieve discomfort, etc.

Table 6.
NPT in some of the most common digestive tract diseases (taken from the book health by nature and natural medicine manual).

Physical exercise is the first indication in metabolic syndrome with the aim of reducing abdominal fat deposition and adverse cardiovascular effects. The remaining associated conditions are managed with medical advice, drug therapy, and a correct diet [38].

Bone mineral density (BMD) gradually decreases with age and is more evident in women when menopause begins. Physical exercise in conjunction with dietary and hygiene advice has been shown to improve BMD in postmenopausal women [39].

2.7 NPT in musculoskeletal disorders

Rehabilitation medicine and physiotherapy as members of the health sciences are the paradigm of NPT, since a large part of their actions are based on physical procedures.

Some symptoms and signs that accompany many osteoarticular, neurological, psychiatric and other diseases are the usually associated inflammation and pain. **Table 7** includes some procedures used in rehabilitation medicine and physiotherapy taken from Miranda Mayordomo [40].

The choice between heat and cold treatment is governed by principles and is sometimes applied empirically.

Heat provides transient relief in subacute and chronic inflammatory and traumatic disorders, such as sprains, muscle strains, fibrositis, tenosynovitis, muscle spasms, myositis, lower back pain, neck injuries, various forms of arthritis, arthralgia, neuralgia, etc. Heat increases blood flow, and helps relieve inflammation, oedema and exudates from connective tissue injuries. Heat can be applied either superficially (infrared, hot compresses, paraffin bath, hydrotherapy) or deep (ultrasound). The intensity and duration of physiological effects depend primarily on the temperature of the tissue, the rate of temperature rise, and the area treated [40].

Cold can help relieve muscle spasms, myofascial or traumatic pain and acute inflammation (sprain, low back pain, etc). As of a certain temperature, cold induces

Technique/procedure	Effect	Indications
Kinesitherapy in its different variants	Gain in strength and mobility	Various injuries of the locomotor system, neurological, etc.
Therapeutic exercise (active kinesitherapy)	Improved proprioception	Indicated in many osteoarticular processes/ injuries
Heat/Cold	Analgesia, etc.	See text below
Transcutaneous electrical stimulation (TENS)	Analgesia	Many musculoskeletal and other conditions (oncology, etc.)
Cervical traction	Analgesia	Cervical spondylosis, disc prolapse, cervical injuries, torticollis, etc.
Massage	Mobilises contracted tissues, relieves pain, reduces inflammation and induration in trauma	Sprains, muscle strain, contusion, peripheral nerve injuries, lower back pain, arthritis, periarthritis, bursitis, fibromyalgia, hemiplegia, paraplegia, tetraplegia, multiple sclerosis, cerebral palsy and amputation
Acupuncture	Analgesia	Conditions that present with acute or chronic pain
Homoeopathy	Analgesia, reduces inflammation and oedema in trauma	Sprain, painful shoulder, osteoarthritis, bursitis, epicondylitis, carpal tunnel syndrome, etc.

Table 7.
Some physical therapies used in rehabilitation/physiotherapy (taken from Miranda Mayordomo's book, Medical Rehabilitation and completed by A. Ursa).

a certain local anaesthesia (cryotherapy). Cold is usually used for a few hours after a muscle or tendon injury, up until evaluation [40].

Hydrotherapy in rehabilitative medicine is used in many conditions. Stirred hot water stimulates blood flow and debrides burns and wounds. This treatment is performed in a Hubbar tank with water between 35.5°C and 37.7°C. Full immersion in water heated to between 37.7°C and 40°C can also help relax muscles and relieve pain. Hydrotherapy is particularly useful for range-of-motion exercises [41, 42].

Electrotherapy in rehabilitative medicine plays an important role in many locomotor system disorders, either exclusively or as a complement to other techniques [43].

The various areas of physiotherapy, such as paediatric, respiratory, pelvic floor, neurological or sports - with their preventive, curative and rehabilitative approach – tackle numerous conditions that I do not address given the characteristics of this chapter.

2.8 NPT in neuropsychiatry

Although pharmacological therapy has played an important role in psychiatric conditions since its introduction, sometimes it is difficult to comply with the therapy due to the disease itself, due to side effects or due to access to medication, either during hospitalisation or domiciliary care. Because of this, a series of non-pharmacological techniques and procedures to treat the most common neuropsychiatric pathologies have been developed. NPT in psychiatry should generally be used before drug treatment. However, the reality is usually different. **Table 8** reports some of the most frequent techniques and procedures used in the most common neuropsychiatric conditions, taken from various authors.

There are NPT for neurological conditions such as migraine, multiple sclerosis, Parkinson's disease, etc., which have been implemented in recent years. These require further studies for their validation.

2.9 NPT in sense organ conditions

Among the eyeball conditions, the Bates method for improvement of vision without glasses is notable. This work was published for the first time in 1919 in the USA [53].

After several years of observation, Dr. William H. Bates (1860–1931), an American ophthalmologist, devised some exercises to restore normal vision in some eye problems and dispense with using glasses. He started from the hypothesis that

Condition	Technique/procedure	Author(s)
Anxiety	Cognitive-behavioural therapy [44], relaxation techniques [44], yoga [44], meditation [45], contact with nature [46]	Galve, Ursa Herguedas
Insomnia	Cognitive-behavioural therapy, physical exercise during the day, etc. [47, 48]	Díez González, et al., Baides Noriega et al.
Depression	Physical exercise [49], phototherapy [50]	Alonso López et al., Tuunainen et al
Cerebral palsy	Equestrian therapy [51]	Jiménez de la Fuente
Equestrian therapy	Music therapy [52]	Acebes de Pablo et al

Table 8.
Most common neuropsychiatric pathologies and non-pharmacological approach (compiled by A. Ursa).

Technique	Procedure	Effect
Oscillations	Rotate the trunk with the feet on tiptoes. The opposite heel lifts on every turn	?
Palming	Cover the eyes with the palms of the hands so that no light penetrates	Facilitates eye relaxation
Sunning	Look at the sun with closed eyes, alternating light and shadow	?
Neck rotation/ flexion-extension	Rotate the neck to both sides alternately and cervical flexion and extension	Activation of muscle chains
Shoulder movement	Roll shoulders in a clockwise and anticlockwise direction	Activation of muscle chains
Targeting exercise	Fix vision alternately at a near (outstretched arm) and distant point	The lens ligaments are exercised
Eyeball rotation back and forth	Clockwise and anticlockwise rotation	The eye muscles are exercised

Table 9.
Some Bates method exercises (taken from Roselló's book see well without glasses).

the tension caused by certain visual habits were the main cause of poor eye vision. This method helps patients become aware of use of their visual organ by means of a series of eye and non-eye exercises. **Table 9** shows some of these exercises according to Roselló [54].

The Bates method is indicated for all vision refractive problems such as myopia, astigmatism, hyperopia and presbyopia. It is contraindicated in the event of macular degeneration, eye infection or eyeball tumour [53].

In the last few years, the Bates method has been taught on postgraduate courses at some European universities and recommended by some ophthalmologists. However, there are detractors of the method [55].

3. Summary and conclusions

Although we cannot dispense with medicines, medical protocols and guidelines must be urgently reviewed. This is because most are based on medicines as a first line treatment option.

Bioethics committees in clinical trials should be comprised of independent staff. Conflicts of interest in scientific publications should be more closely monitored.

The acquisition of a healthy lifestyle must be promoted through Primary Healthcare, as part of a primary prevention programme.

Non-pharmacological treatments (NPT) are especially indicated for chronic diseases. However, many acute conditions can also benefit.

Numerous conditions of most bodily systems can be treated with NPT. Implementing this modality would contribute to reducing the adverse effects of medicines, bring healthcare expenditure down and lead to environmental sustainability.

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Section 7

Diabetes

Chapter 11

Antidiabetic Activities of *Terminalia* Species in Nigeria

Franklyn Nonso Iheagwam, Omoremime Elizabeth Dania, Happiness Chijioke Michael-Onuoha, Olubanke Olujoke Ogunlana and Shalom Nwodo Chinedu

Abstract

Terminalia species are well recognised in traditional medicine. They are known for producing fruits and nuts which are edible and possess pharmacotherapeutic properties. They also have ornamental purposes in urban areas where they are found. These species are used by traditional healers in the treatment and management of diabetes mellitus, its complications and other related ailments that are involved in the pathophysiological process of this disease. Research has been extensively done to validate these antidiabetic claims scientifically as well as understand the mechanism and mode of antidiabetic action. This chapter proposes to highlight the antidiabetic activities of *Terminalia* species found in Nigeria.

Keywords: *Terminalia* species, antidiabetic, Nigeria, diabetes mellitus, mode of action, mechanism, traditional medicine

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder that is not only affecting various populations worldwide but also poised on affecting the developing nations of the world much more than developed countries [1, 2]. The International Diabetes Foundation (IDF) reported a diagnosis of over 400 million people living with diabetes and postulated an estimated increase to over 600 million people by the year 2040 in a worldwide survey [3, 4]. The report also shows that diabetes accounts for a death every 6 seconds [3]. In a recent study, it was observed that the total reported cases of people affected by DM had increased by 10 million in the subsequent survey carried out by IDF over the next year [5].

DM is a heterogeneous metabolic disorder and is difficult to classify. However, DM has been categorised into three major types based on the pathologic process. Type 1 diabetes mellitus (T1DM), also known as childhood/early-onset diabetes or insulin-dependent DM, is characterised by insulin deficiency as a result of β-cell dysfunction, degeneration and degradation by the immune system [6]. Type 2 diabetes mellitus (T2DM), also known as adult/late-onset diabetes or non-insulin-dependent DM has insulin secretion and insulin resistance (IR) as its major characteristics [7]. Gestational diabetes mellitus (GDM) has glucose intolerance in pregnant women as its major characteristic. It is as a result of the β-cells inability to meet up with the insulin demand in pregnant women without a previous diagnosis of diabetes [8].

Diabetologists have a few other categories, such as tropical DM and Type 3 diabetes mellitus (T3DM). The former is thought to have a relationship with malnutrition [8], while the latter is a suggested mechanistic link to Alzheimer's disease via inflammatory response and other mechanisms resulting in the pathophysiologic changes relating diabetes to dementia [3]. However, there is little information on the rarer forms of diabetes, such as secondary diabetes, mitochondrial diabetes, maturity-onset diabetes of the young, and latent autoimmune diabetes of adults [9].

1.1 Risk factors

Physical inactivity or sedentary lifestyle, excessive alcohol, overweight, obesity and unhealthy diet intake are modifiable DM risk factors [10]. Family history, hypertension, history of previously impaired glucose tolerance (IGT) or impaired fasting glucose (IFG), advancing age, history of GDM, ethnicity and genetic makeup are some unmodifiable risk factors. However, various researchers have reported that novel risk factors such as short sleep duration [11], noise pollution [12] and environmental toxins [13] contribute to the causal pathways which lead to diabetes. Trade and agricultural production policies are thought to contribute to both individual and societal level risk factors [14].

2. Diabetes mellitus in Nigeria

2.1 Epidemiology

The transition from infectious diseases to non-communicable diseases as leading causes of death is fast becoming a growing epidemiological trend and public health dichotomy in Sub-Saharan African countries [15]. In Africa, there is a 1% estimated prevalence of diabetes in rural areas while in urban areas, the range is from 5–7% [16]. Nigeria accounts for about one-sixth of Africa's population [1]. The national prevalence of diabetes, which was less than 1% between 1960 and 1990, has risen from 2.2% in 1997 to 5% in 2013 [17]. However, the current prevalence may currently be as high as between 8 and 10% [9], with 4.83% recorded for patients aged 20 and above, accounting for over 3 million people currently living with this condition [18]. This observation makes her the country with the highest number of people living with diabetes and IFG in Africa [19]. Epidemiological statistics show that Nigeria is responsible for one in every five reported sub-Saharan case of diabetes, with a steep increase in the prevalence of this disease from the rural areas to members of the high socio-economic population [9]. Continuous urbanisation, the increasing population and poor economy, will further drive the incidence and burden of diabetes upwards in Nigeria [1, 2, 20]. T2DM appears to be the majority of the DM burden in Nigeria with T1DM accounting for less than 10% of DM cases [21], while tropical DM makes up less than 1% [8]. Lifestyle factors such as sedentary lifestyle, cigarette smoking and generous consumption of alcohol are known risk factors linked to the development of T2DM. Obesity has been reported to be a major contributor to approximately 55% of diagnosed cases of T2DM, with a prevalence of 3.3 to 18% [2]. It has also been associated with several life-threatening diseases such as cardiovascular disease (CVD), several cancer types, as well as reduced quality of life [22, 23]. Diabetes-related morbidity and mortality have been reported to be high in different locations in Nigeria with 105,091 diabetes-related deaths recorded as at 2013 and most patients reported to have been suffering from T2DM [10].

2.2 Management

Given the current DM epidemic and its projected consequences, effective population-based intervention identification has become a priority public health strategy in Sub-Saharan Africa [24]. In Nigeria, insulin, oral glucose-lowering drugs, diet and exercise are used in the management of DM. Complementary and alternative medicine such as concoctions, infusions, tinctures and herbal supplement is also used [1]. Inability to use insulin syringe, the high cost of therapy, few options in the Nigerian market and poor policies on DM management are a few challenges affecting insulin treatment [25]. The medications used in the management of diabetes become less effective over time as most patients do not achieve normal glycaemic control with their use [26], and thus have resulted to possible second-line medications to achieve the normal glycaemic target [27]. Despite the high cost of medication as well as the inability to maintain normal glycaemic control for an extended period, the use of polytherapy to achieve sufficient glucose control is a common feature in Nigeria [28]. Challenges such as needle phobia, hypoglycaemia, drug-associated side effect and cost of medication have made over 46% of diabetic patients opt for complementary and alternative medicine, with *Vernonia amygdalina* which is also known as "bitter leaf" being most utilised [29]. The school of thought that diabetics should abstain from carbohydrate rich meals has led to the intake of monotonous food like unripe plantain, beans and wheat rich diet [1, 30]. This challenge occurs due to the absence of a taste-appealing standardised diet for diabetics as well as their dietary requirements influenced by economic status, religious and cultural beliefs [1].

3. *Terminalia* species as medicinal plants

Medicinal plants (MPs) are a rich source of natural products with potential medical interest. There is an increased interest in the use of medicinal plants and their products as a result of their reported wide range application. Asides their application, they are the richest bioresource of modern medicines, nutraceuticals, food supplements, chemical entities for synthetic drugs, pharmaceutical intermediates, folk medicines and drugs of traditional systems of medicine [31]. These plants are also known to contain different plant secondary metabolites such as tannins, flavonoids, saponins alkaloids, terpenoids and phenols, which are responsible for numerous characteristics such as colour, flavour, smell and texture in various parts of these plants. These plant metabolites are also known for their pharmacological mechanism of actions in the treatment, management and prevention of diseases [32].

Terminalia genus has about 250 flowering tree species which belong to the Combretaceae family. They are found in the tropics of Australia, Asia, Africa and South America. The bark of many *Terminalia* species appear to be cracked from the stem, the branches are arranged in a stepwise manner with the leaves appearing large and leathery on the tips of shoots. The appearance of the leaves is responsible for the genus nomenclature *Terminalia* which is a derivative of the Latin word Terminus. The fruits of most *Terminalia* species are edible with deep red, yellow or black pulp colouration and hard nuts [33]. Extensive research has shown that *Terminalia* species are a rich source of phytocompounds ranging from flavonoids (gallic acid, ellagic acid, quercetin, hesperetin), steroids (β-sitosterol, terminic acid), tannins (punicallin, terchebulin, castalagin), vitamins (α-tocopherol), carotenoids (lutein) and others [33–35]. The various reported pharmacological activities

such as antimalarial, antioxidant, antibacterial, antifungal, cardiovascular effects, antidiarrhoeal, analgesic, anti-inflammatory, hypolipidaemic, hypoglycaemic, antiprotozoal, antiviral, wound healing, antimutagenic and anticancer properties have been attributed to these compounds [33].

3.1 *Terminalia* species in Nigeria

There are about ten species of *Terminalia* found in Nigeria, namely; *Terminalia altissima* (Synonym: *superba*), *Terminalia avicennioides, Terminalia brownii, Terminalia catappa, Terminalia glaucescens, Terminalia ivorensis, Terminalia laxiflora, Terminalia macroptera, Terminalia mollis and Terminalia schimperiana* [33, 36, 37]. These species have been reported to be pharmacologically active with antimicrobial, antimycobacterial, wound healing, gastroprotective, antimalarial, antioxidant, antifungal, anthelmintic, antibacterial, antifungal, antiviral, analgesic, radical scavenging, hepatoprotective, anticancer, antimutagenic, antiaging, aldose inhibitory, antiplasmodial, cytotoxic, antipsychotic, sedative, analgesic, anti-inflammatory, trypanocidal, hypolipidaemic, antioxidant, antimycoplasmal and androgenic, properties as shown in **Table 1** [34, 35, 38–42].

Terminalia species in Nigeria, have numerous application in the treatment and management of ailments among the various traditional medicine systems of different ethnic groups. Different parts are utilised by traditional healers to treat cholera, malaria, typhoid, hepatitis, stomach ache, tuberculosis, leprosy, diarrhoea, skin diseases, gastritis, hyperglycaemia, diabetes, gonorrhoea, wounds, epilepsy and catarrh [56–58]. They are also used as tonic, laxative and chewing sticks [26, 59, 60].

Several reports have highlighted some pharmacological properties of *Terminalia* species in Nigeria, such as its antimicrobial properties, antibacterial property, anti-inflammatory action, anti-HIV, hypoglycaemic, modulatory properties, analgesic, wound healing, antioxidant and radical scavenging activity, hepatoprotective, anticancer, anti-trypanocidal, antimutagenic and antiaging properties.

Nigeria's vegetation is made up of forests, savannahs and montane land. All others but the latter are further divided into three parts which have ensured the wide distribution of these species across the country. This variation in the country's vegetation has not only made these *Terminalia* species specific to Nigeria and West Africa, but accounts for the difference in their evolutionary relationship, development and pharmacologic activity. Upon assessment of the phylogenetic relationship on www.phylogeny.fr [61], using the available nucleic acid sequence of the *Terminalia* species that were deposited in National Center for Biotechnology Information (NCBI) GenBank, it was observed that species that were closely related such as *T. catappa* and *T. glaucescens* as well as *T. superba* and *T. avicennioides* were located in the same vegetative region of the country (**Figure 1**). Irrespective of their evolutionary differences, it was observed that there were conserved regions that were similar in the deposited genetic sequence of the *Terminalia* species in Nigeria showing over 94% sequence similarity (**Figure 2**).

3.2 Pharmacologic antidiabetic activities of Nigerian *Terminalia* species

The pharmacologic antidiabetic activity of *Terminalia* species have been reported in different climes using various *in vitro, in vivo* and *in silico* techniques in mice, rat, rabbit and humans to elucidate them. Nonetheless, in Nigeria, there is a paucity of data on the antidiabetic mode of action and mechanisms of *Terminalia* spp. despite its abundance. However, there are antidiabetic reports of these species from neighbouring countries with similar vegetation.

Name of specie	Location in Africa	Common name	Pharmacological activity	References
Terminalia altissima (Synonym: *superba*)	Tropical west Africa, Sierra Leone, Congo, Nigeria, Cameroon	White afara, Limba	Antimicrobial, α-glucosidase inhibitory properties	[37, 43, 44]
Terminalia avicennioides	West Africa	Kpace, Kpayi, Baushe, Idi	Antimycobacterial, wound healing, gastroprotective, antimalarial, antioxidant, antifungal, anthelmentic activities	[45, 46]
Terminalia brownii	Nigeria, Congo, Sudan, Tanzania, Kenya, and Sudano-Sahelian Africa	Different names based on location	Antibacterial, antifungal, antiviral activities	[47]
Terminalia catappa	Africa	Indian almond, Tropical almond	Analgesic, wound healing, antioxidant, radical scavenging, hepatoprotective, anticancer, antimutagenic, antiaging properties	[41]
Terminalia glaucescens	Tropical Africa	Different names based on location	Antimicrobial, aldose inhibitory, antiplasmodial, cytotoxic properties	[48, 49]
Terminalia ivorensis	Western Africa	Idigbo, Black Afara, Blackbark	Antibacterial, antipsychotic, sedative, analgesic, anti-inflammatory, trypanocidal properties	[50, 51]
Terminalia laxiflora	Sudano-Sahelian Africa	Idi, Baushe	Antimycoplasmal activitiy	[37]
Terminalia macroptera	Tropical West Africa	Orin idi, kwandare	Antimicrobial, antimalarial, hypolipidaemic, antioxidant, antimycoplasmal properties	[52, 53]
Terminalia mollis	Tropical Africa	Bush willow, baúshin giíwaá	Antimycoplasmal, antimalaria activitiy	[33, 54]
Terminalia schimperiana	Tropical West Africa, Uganda, Ethiopia	Idi, Tuit plant, Kwuegh, Buashe	Androgenic, antioxidant, antimicrobial properties	[55]

Table 1.
List of Terminalia *species found in Nigeria and their reported ethnopharmacological activity.*

3.2.1 In vitro assessments

The crude aqueous and hydroethanolic leaf extracts of *T. catappa* from Nigeria have been reported to inhibit both α-glucosidase and α-amylase effectively. Mixed and non-competitive mode of inhibition were the mechanisms of action elucidated for the extracts [35]. This finding was further corroborated by *in silico* studies, in which the identified bioactives showed preferential binding to the active site than the allosteric site of α-glucosidase and α-amylase [35]. The α-amylase inhibitory property of crude methanol extract and solvent fractions of *T. brownii* stem bark was lower than that of acarbose as reported in [62]. When compared with some

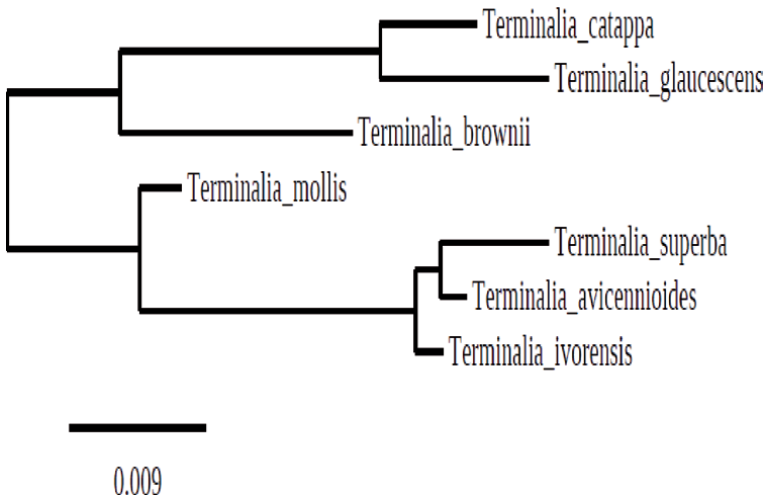

Figure 1.
Phylogenetic tree of some selected Terminalia *species in Nigeria.*

other medicinal plants, crude ethanol, aqueous and hydroethanolic extracts of *T. superba* root exhibited better inhibitory action on α-amylase activity than their respective counterparts [63]. For α-glucosidase and lipoxygenase inhibitory activity, the potency of dichloromethane, methanol and solvent fractions of *T. macroptera* leaves have been established to be more potent than acarbose and quercetin respectively [40].

High-throughput techniques were used to identify isolated bioactive compounds (gallic acid and methyl gallate) from *T. superba* stem bark dichloromethane extract, which exhibited very high inhibitory property on α-glucosidase activity [64]. Other isolates such as arjunic acid and glaucinoic acid from *T. glaucescens* stem barks and chebulagic acid, corilagin and narcissin from *T. macroptera* leaves are reported to exhibit significant β-glucuronidase, α-glucosidase and 15-lipoxygenase inhibitory activity respectively [40, 65].

3.2.2 In vivo assessments

The pre-administration of methanol-methylene chloride extract of *T. glaucescens* leaves have been reported to confer protective properties in mice against streptozotocin-induced diabetes effects [66]. *T. schimperiana* root bark extracts have been reported to be effective in reducing blood glucose and excess body lipids in alloxan-induced diabetic rats [67, 68]. The hypoglycaemic activity of *T. catappa* leaves has also been recorded [69]. The leaves have also been associated with a significant decrease of C-reactive protein, interleukin-6, fibrinogen and inflammatory markers associated with diabetes in rats when compared with other non-steroidal anti-inflammatory drugs [70]. In male rats fed with *T. catappa* drupe and seeds supplemented-diets for fourteen days, they were found to have exhibited enhanced sexual behaviour and biomarkers relevant to erectile dysfunction that were initially suppressed by streptozotocin-induced diabetic state [71]. Most research on the antidiabetic assessment of *Terminalia* species in Nigeria have reported the beneficial effect in rats and mice. Interestingly, in Ref. [72], *T. catappa* intake was found to illicit negative herb-drug effect by increasing the activity of transaminases concomitantly enhancing the adverse hepatic effects of antidiabetic drugs such as pioglitazone and atorvastatin.

Figure 2.
Multiple sequence alignment of some selected Terminalia *species in Nigeria.*

4. Conclusion

The Nigerian *Terminalia* genus is made up of species that possess antidiabetic principles. This activity has been related to the presence and synergistic action of phytochemicals such as tannins, phenolics, terpenoids, flavonoids and other active bioconstituents. The species of this genus in Nigeria can provide great medicinal value to the country and its populace. However, most of the antidiabetic

pharmacological assessment has been done only on *Terminalia catappa*, *Terminalia glaucescens* and *Terminalia schimperiana*. Moreso, high throughput analytical techniques and equipment can be utilised to identify and isolate novel phytocompounds that may be of therapeutic value in the management and treatment of diabetes. It is also imperative to identify the sequence of all Nigerian *Terminalia* species to understand better the genetic relationship, genetic variability, intraspecific variability and traits heritability in vegetative and floral characters of these species.

It was also observed that the majority of antidiabetic assessments of these *Terminalia* species were done *in vitro*, in rats and mice. Nonetheless, more *in vivo* studies should be carried out to identify the molecular mechanisms involved in its antidiabetic activity. Nigeria is the most challenged sub-Saharan nation with diabetes, a public health issue that needs to be tackled urgently. Hence, there is a need to increase translational research and explore the antidiabetic assessment of these *Terminalia* species directly on patients to extrapolate results that will be beneficial to the Nigerian public health system.

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Conflict of interest

The authors declare no conflict of interest.

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Chapter 12

Some Folk Antidiabetic Medicinal Herb of Himachal Pradesh

Monika Rana and Meenakshi Rana

Abstract

The Prevalence of Diabetes Mellitus (DM) is increasing day by day at an alarming worldwide. As per the statics of International Diabetic Federation, currently worldwide approximately 463 million adults (20–79 years) affected with diabetes that is expected to increase rise to 700 million by 2045. Diabetes and its complications imposes an economic loss to people with diabetes and their families, and to health systems and national economy. Diabetes is a complex disease which link with multiple of factors. Present reviewdocument the information of traditional used Antidiabetic plants by the inhabitants of Nadaun, District Hamirpur, Himachal Pradesh, India. During the survey 31 Medicinal Plants have been documented on the basis of information collected from the respondents of the study area.

Keywords: diabetes, ayurveda, Himachal Pradesh, traditional medicines

1. Introduction

Diabetes mellitus (DM) is a serious lifelong disease characterized by elevation of blood glucose level in the body resulting from the defects in insulin secretion and insulin resistance [1, 2]. The global diabetes prevalence for all age-groups was estimated to be 9.3% in 2019 rising to 10.2% in 2030. The prevalence is lower in rural than the urban areas [3]. The total number of people with diabetes is estimated to rise from 171 million in 2000 to 366 million in 2030. Among DM, about 90 percent of population affected with Type 2 DM [4]. In addition to hyperglycemia, diabetes also associated with various vascular complications, which are the major causes of morbidity and death in diabetic Patients [5].

In Ayurveda Diabetes Mellitus (DM) is referred to as Madhumeha (means sweet urine disease). Madhumeha consists of two words-'madhu and meha' where 'madhu' denotes sweetness and 'meha' stands for urination. In Ayurveda, plants are known to be excellent source of drugs. Plant based drugs have been in use against various diseases since time immemorial. There is large number of drugs of herbal origin mentioned in Ayurveda texts, which were advised for treatment of Madhumeha [6]. Even today a huge number of population in the world used the medicinal plants for the treatment of Diabetes Mellitus [7]. As the incident and severity of Diabetes is increasing worldwide, it imposes an huge economic loss to people with diabetes and their families, and to health systems and national economy [8]. The importance of traditional plant medicines from the last decade goes on increasing with both medical and economic implications [9]. On the other hand the chemically synthetic hypoglycaemic agents used for the treatment of diabetes are not only expensive but also cause various complications and side effects to the health [10].

2. Materials and methods

Nadaun is a small town in Hamirpur district, located in central Himachal Pradesh, India, right near Beas River. The Townis situated between 76°18′ –76°49′ East longitude and 31°52′30″ North Latitudes. The track is hilly covered by Shivalik range and the elevation varies from 450 to 11,000 meters. As per the census of India 2011, it has a population of 4430. The Climate is characterized by an intensely hot summer, a pleasant cold season. The summer season from March to about middle of June is followed by the south-west monsoon season from mid-June to the end of September. October and first half of November constitute the post-monsoon period. The cold season is from mid-November to February. The minimum and maximum day time temperature varies between 20° and 42°. People in this region can easily understand Hindi and can communicated in that language.

In order to documentation of the record frequent field surveys were conducted many time (**Figure 1**). A questionnaire contains the details of the plants, parts used, medicinal uses and mode of preparation of remedies is structured and informal talks were employed to gain the information about the use of plants as Antidiabetic. Any statistical survey is not used in the given study.

Figure 1.
Field survey.

3. Result and discussion

Ethnobotany may be defined as the scientific study of the dynamic relationship between various plant and people. The present study highlighted the traditional herbal medicine used for the treatment of diabetes in the particular selected study area. During the survey, around 50 people mostly old aged person selected randomly for the study. Information of plants along with their common name, useful part, time of availability, mode of preparation and consuming is documented (**Table 1**). All the plants are photographed as a record (**Figure 2**). Most of the recorded plants are available from the wild growth, and some are cultivated by the local villagers. Mostly plants materials are preserved in dry powder form as these are available only in a particular season. Various methods of preparation of these herbal remedies were recorded during the study. The preparation were also consumed by the people in the form of juice, churna, chutney and chapattis. In the present study we found that people have a close relationship with the nature for their health care. As the importance of ethnobotanical studies goes on increase day

S. No	Botanical name	Family	Local name	Part of plant	Antidiabetic use
1	*Acacia catechu*	Fabaceae	Khair	Heartwood	Katha (extract of heartwood)
2	*Aegle marmelos*	Rutacese	Bil	Fruit, leaf	Fruits eaten as a powder, leaf consumed empty stomach early in morning
3	*Allium cepa*	Liliaceae	Pyaz	Bulb	As salad, and as chutney
4	*Allium sativum*	Liliaceae	Lehsun	Bulb	Bulbs are consumed empty stomach in early morning.
5	*Aloe vera*	Liliaceae	Kwar	Leaf	Juice is consumed
6	*Azadirachta indica*	Meliaceae	Neem	Leaf	Tendor leaves are used
7	Berberis aristata	Berberidaceae	Kashmal	Bark	Decoction of bark with water
8	*Carica papaya*	Caricaceae	Papita	Fruit	Ripe fruit is consumed
9	Carissa spinarum	Apocynaceae	Garnu	Fruit	Ripe fruit is consumed
10	*Colocasia esculenta*	Araceae	Arbi	Leaf	Cooked
11	*Curcuma longa*	Zingiberaceae	Haldi	Rhizomes	Dry rhizomes powder consumed with milk
12	Gymnea sylvestre	Apocynaceae	Gudmar	Leaf	Consumed as powder
13	*Lagenaria siceraria*	Cucurbitaceae	Lauki	Fruit	Juice of fruit is used with amla juice and also cooked as vegetable
14	*Momordica charantia*	cucurbitaceae	Karela	Fruit	Used as vegetable and juice
15	*Mentha arvensis*	Lamiaceae	Pudina	Leaf	As juice, and chutney
16	*Murraya koenigii*	Rutaceae	Gandhla	Leaf	Fresh leaves consumed empty stomach in morning
17	*Ocimum sanctum*	Lamiaceae	Tulsi	Leaf	Decoction of leaf with water
18	*Phyllanthus emblica*	Euphorbiaceae	Amla	Fruit	Ripe fruit consumed, and as dry fruit powder
19	Pogostemon benghalensis	Lamiaceae	Kali Basuti	Leave	Fresh leaves are used
20	*Prunus persica*	Rosaceae	Aadu	Fruit	Ripe fruits are consumed
21	*Psidium guajava*	Myrtaceae	Amrood	Fruit	Ripe fruit is consumed
22	Syzium cumini	Myrtaceae	Jamun	Fruit	Fresh fruits is consumed, seed used as powder

S. No	Botanical name	Family	Local name	Part of plant	Antidiabetic use
23	Terminalia ballerica	Combretaceae	Bahera	Fruit	As powder
24	*Terminalia chebula*	Combretaceae	Harda	Fruit	As powder
25	Tinospora cardifolia	Menispermaceae	Giloe	Stem	Powder is consumed in empty stomach
26	Trigonella foeumgraceum	Fabaceae	Methi	Seed	Seeds are consumed as powder with water in empty stomach and overnight soaked with water
27	*Triticum aestivum*	Poaceae	Kanak	Plant	Plant juice is used, ans consumed as sprouted
28	*Vinca rosea*	Apocynaceae	Sadavahar	Leaf	Fresh leaves and leaves powder used as treatment
29	*Vitex negundo*	Lamiaceae	Vana	Leaf	Tendor leaves used
30	Zingiber officinalis	Zingiberaceae	Sonth, adrak	Rhizomes	As such, and as pickle
31	*Ziziphus jujuba*	Rhamnaceae	Ber	Fruit	Ripe fruit used

Table 1.
Antidiabetic plants recorded from Nadaun, Hamirpur District.

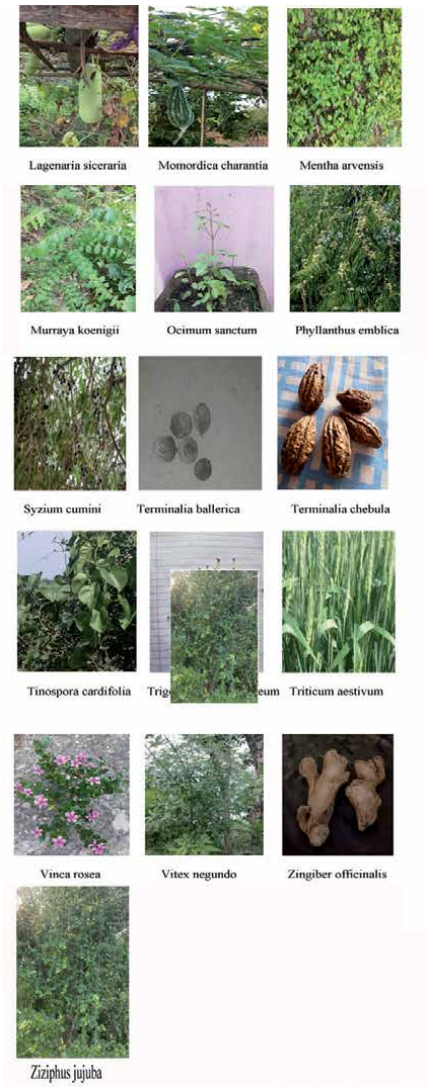

Figure 2.
Plants used traditionally for the treatment of diabetes.

by day, it is mandatory preserve the information's about the knowledge of folklore medicinal plants which is use by local communities before it is permanently disappear.

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Chapter 13

Red Wine and Yacon as a Source of Bioactive Compounds with Antidiabetic and Antioxidant Potential

Mariia Nagalievska, Mariya Sabadashka and Nataliia Sybirna

Abstract

Phytochemicals derived from different plants are promising therapeutic agents. Herbal compounds can be used under diseases, etiological causes of which are alterations of carbohydrate, protein, and lipid metabolisms, along with increased oxidative stress and chronic low-grade inflammation. Potential sources of biologically active substances may be grape wine, rich in phenolic compounds. Well-studied examples of polyphenols are phenolic acids, catechins, anthocyanins, and flavonoids, etc. Another source of biologically active compounds is yacon (*Smallanthus sonchifolius* Poepp. & Endl.). The aboveground part of yacon is rich in phenolic compounds and terpenes. Main biologically active substances from tuberous roots of yacon are fructooligosaccharides and phenolic compounds. The section will be devoted to the analysis of hypoglycemic and antioxidant effects, and molecular targets of the complex of biologically active substances derived from red wine and yacon.

Keywords: polyphenols, phenolic compounds, flavonoids, fructooligosaccharides, red wine, yacon, diabetes mellitus, antioxidant

1. Introduction

1.1 Red wine as a source of bioactive compounds with antidiabetic and antioxidant potential

Plant foods contain vitamins, phytosterols, sulfur compounds, carotenoids and organic acids that are healthy for human. However, the most effective protective agents are phenolic compounds that are secondary metabolites found in fruits, vegetables, and cereals. It is known that 100 g of apples, pears and cherries fruit contain 200–300 mg of polyphenols [1–4]. Grapes are rich in phenols. 10% of the total phenolic compounds of grapes are contained in the pulp, 60–65% in the seeds, and 20–35% in the peel. The content of phenolic compounds in grapes depends on the plant variety, climatic and other geographical conditions, as well as the degree of maturity [5]. These healthy components are stored in drinks made from grapes. When grape wine is produced, almost 63% of all phenolic substances from grape

seeds and berry peel are extracted into wine. So provided that the optimal dose is consumed, wine can be considered one of the most effective natural remedies.

It is important that in the process of obtaining wort (fermentation) and maturation of wine, phenolic compounds undergo structural changes, which determines characteristics of the drink. The most intense reactions during the maturation of wine are the polymerization and oxidation of catechins. The products of these reactions give a pleasant taste and golden-brown color of different intensity of wine, so that aged wines are easy to distinguish from young [6, 7].

Another group of substances that are extracted into wine during fermentation is procyanidins. Procyanidins are contained mainly in grape seeds, so they are virtually absent in grape juice. Initially, the wort contains a small amount of procyanidins, as these substances started to extract from the seeds during fermentation when the alcohol content is 6%. As the alcohol concentration increases during fermentation, procyanidins are extracted into the wine. Young wine rich in procyanidins has a tart taste. In the aging process procyanidins react with each other and form longer polymers - condensed tannins. As the wine ages, these chains become very long and difficult to dissolve, so they precipitate [6–8].

Because grape peel and seeds float on the surface, the more often they are immersed in the fermenting wort, the process of extraction of procyanidins better proceed. After fermentation, many wines also insist on the pomance to enhance the color, taste and extract the tannins. Therefore, the highest content of procyanidins and tannins is characteristic of wines that have been infused for three weeks or more. Thus, the consumption of grapes, grape juice and wine has different effects on the body [6].

Numerous researchers pay much attention to the study of the effects of red wine consumption on the organism since the discovery of the "French paradox". Although the father of medicine Hippocrates emphasized the benefits of "moderate wine consumption" [9]. As a result of large-scale studies involving almost 300 thousand people, it was shown that the consumption of 150–400 ml of dry red wine daily significantly reduces the risk of cardiovascular and neurological pathologies, diabetes, many types of cancer, and dysfunction of gastrointestinal tract. These positive effects are associated with the action of grape wine polyphenols [10]. Despite this, the molecular mechanisms of the protective action of wine remain insufficiently studied.

The pharmacological, medical, and biochemical properties of phenols are widely studied. Antioxidant, vasodilating, anti-oncological, anti-inflammatory, immunostimulatory, anti-allergic, antiviral and estrogenic effects are shown. Wine polyphenols inhibit phospholipase A2, cyclooxygenase, lipoxygenase, glutathione reductase, and xanthine oxidase, and chelate metal ions [9, 11–16]. *In vitro* wine polyphenols scavenge free radicals, including superoxide anion radical ($O_2^{\bullet -}$), hydroxide radical ($OH^{\bullet}$), and inhibit lipid peroxidation [9, 12, 17, 18].

In cells incubated with phenols, the expression of genes encoding proteins involved in antioxidant detoxification is induced. These genes are regulated by a specific enhancer, the antioxidant response element (ARE). Red wine polyphenols can alter Nitric oxide synthase (NOS) activity due to the effect on cellular concentration of Ca^{2+} and the phosphorylation of key proteins of the phosphatidylinositol-3′ kinase/Akt pathway after short incubation with cells. After long-term incubation, polyphenols alter NOS activity by regulating the expression of the genes of the constitutive isoforms of NOS enzyme [12, 17, 19, 20].

In vivo studies are less convincing; some indicate the effect of wine on the antioxidant system of blood cells and the oxidation of low-density lipoproteins [17, 21, 22].

Most dietary polyphenols are absorbed in the intestine by passive transport, intensively metabolized in the small and large intestine and liver, where they are converted into metabolites with higher antioxidant and estrogenic activity.

Sulfated, glucuronidated, and methylated polyphenols were found in blood plasma. Moreover, a large part of polyphenols undergo hydrolysis and degradation under the action of intestinal microflora to simple phenolic compounds [12, 14, 16, 21, 23]. Metabolites of polyphenols circulate in the blood in a protein-bound form, in particular with albumin, which plays an important role in regulating the bioavailability of polyphenols. The affinity of polyphenols to albumin varies depending on their chemical structure. Albumin binding determines the rate at which metabolites are delivered to cells and tissues or excreted. The accumulation of polyphenols in tissues is the most important stage of polyphenol metabolism because it preserves the necessary concentration for the biological effects of polyphenols. Polyphenols easily penetrate tissues, especially the intestines and liver. Polyphenols excretion and their derivatives occur in urine and bile. In this case, large conjugated metabolites are more likely to be excreted in the bile, while small conjugates, such as monosulfate, are preferably excreted in the urine. The amount of metabolites excreted in the urine correlates with the maximum concentration in plasma [24].

1.2 Composition of phenols in grape wine

Phenols include more than 8000 natural compounds. Their molecule contains phenol (aromatic ring with at least one hydroxyl group). Phenols are classified into polyphenols and simple phenols, depending on the number of phenolic rings in their molecules. Simple phenols include phenolic acids. The group of polyphenols, i.e. phenols that contain at least two phenolic rings, includes flavonoids, stilbenes, and tannins (containing three or more phenolic rings) [11, 13–16, 18, 24].

Flavonoids are a large group of low molecular weight polyphenolic compounds. According to the degree of oxidation of the pyranose ring, hydroxylation of the nucleus and properties of the substituent at the third Carbon atom, flavonoids are divided into subclasses: flavones, isoflavones, flavanols (catechins), flavonols, flavanones, anthocyanins and proanthocyanidins [11, 14, 24–26].

Flavonoids have a vasodilating effect. They cause vascular smooth muscle relaxation, probably mediated by inhibition of protein kinase C or decreased Ca^{2+} uptake by cells [14].

Flavan-3-ols, in particular (−) - epicatechins, (+) - catechins, gallates, and products of their methylation, decarboxylation, and dehydroxylation, as well as quercetin (3,5,7,3′,4′-pentahydroxyflavone), activate antioxidant enzymes. Herewith, quercetin is effective at lower concentrations (5–20 μM) than catechins (500 μM - 1 mM) [27].

Catechins affect cell apoptosis by altering the expression of antiapoptotic or proapoptotic genes. Epicatechins inhibit apoptosis by activating genes of Bcl family proteins and inhibiting caspase-6 activity and Bax, Bad, and Mdm2 gene expression. These compounds also ensure cell survival by activating protein kinase C. It should be noted that at low concentrations flavan-3-ols have an antiapoptotic effect, and at high concentrations (50–500 mM) they promote cell death by the mechanism of apoptosis [28].

Grape wine anthocyanins (malvidin, delphinidin, peonidin, petunidin, and cyanidin) are most often identified in the glycosylated form. It has long been thought that glycosylation is the only pathway for anthocyanin metabolism, but glucuronides and sulfates of these polyphenols have recently been identified [2]. Plasma concentrations of anthocyanins are too low to capture reactive oxygen species (ROS) and reactive nitrogen species (RNS). But anthocyanins are potent antioxidants because they can affect NO content and its stable metabolites. Consumption of 16–500 μM of anthocyanins reduces NO production by more than 50%, mainly due to inhibition of inducible NOS. In this case, anthocyanins do not

cause cytotoxicity [3]. Like other flavonoids, anthocyanins and anthocyanidins poses antioxidant properties. Anthocyanins act as donors of electron or to transfer a hydrogen atom of hydroxyl groups to free radicals [29]. Isolated anthocyanins and a suspension of flavonoids enriched with anthocyanins prevent the disruption of DNA molecule, the development of hormone-dependent pathologies (affect estrogen secretion), regulate immune response by preventing excessive production of cytokines [30]. Anthocyanins exhibit also an anti-inflammatory activity by inhibiting transcription factor NF-κβ. The content of several NF-κβ-dependent chemokines, cytokines, and inflammatory mediators decreases in the plasma and monocytes of healthy people after consumption of anthocyanins [30, 31].

In plants are also synthesized other phenols – non-flavonoids (phenolic acids, tannins, and stilbene), which are also present in grapes and wine.

Phenols, which include one functional group of carboxylic acid called phenolic acids. There are two groups of phenolic acids – hydroxycinnamic and hydroxybenzoic acids. To hydroxycinnamic acids belong *p*-coumaric, caffeic, ferulic, caftaric, coutaric, and coumaric acids. The example of hydroxybenzoic acids is gallic acid [11, 14, 21, 24].

Gallic acid (3,4,5-trihydroxybenzoic acid) is the phenol that is best absorbed into cells and exhibits various biological properties [2, 32]. Gallic acid and its derivatives (unconjugated and conjugated 4-O-methylgallic acid, 2-O-methylgallic acid, pyrogallol, 4-O-methylpyrogallol, resorcinol) in a dose-dependent manner inhibit tyrosine kinases, inhibit P-selectin exposure on the surface of blood cells, affect the release of Ca^{2+} into the cytoplasm, free radicals formation and thus modify cellular signaling pathways [33, 34]. Gallic acid and (−) -epicatechins inhibit NO formation by inhibiting the formation of mRNA of iNOS in immunocompetent cells [30].

Hydroxycinnamic acids cause an increase in the activity of cellular antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase) by activating the transcription of their genes [5, 35].

The family of stilbenes includes resveratrol, pterostilbene, and piceatannol, which are characterized by the presence of a double bond connecting phenolic rings [14, 24]. Resveratrol has anti-infectious, antioxidant, cardioprotective, anti-proliferative, and pro-apoptotic activities. It induces apoptosis by activating signaling pathways mediated by phosphorylation of p53 proteins, protein kinase C, MAPK or through the death receptor Fas/CD95/APO-1 [36].

Tannins are polymer compounds, divided into two groups (condensed and hydrolyzed). Condensed tannins are polymeric flavonoids. Hydrolyzed tannins include gallotannins, that are gallic acid polymers, and similar in structure esterified compounds [14]. These plant polyphenols are powerful antioxidants that protect against free radical damage and, as a result, reduce the risk of skin cancer and premature aging [15].

It is known that the consumption of white or red wine causes various effects. The reason for this is the differences in the quantity and quality of polyphenols in different varieties of grape wines. The bioavailability of phenolic compounds also plays a crucial role [18]. For example, data on the absorption and the kinetics of disproportion of quercetin indicate that a glass of red wine is a much poorer source of this compound than a cup of black tea and onions [37].

It should be taken into account that excessive consumption of wine has a toxic effect on the body. Using concentrated preparations of natural polyphenol complex of grape wine can be promising, as it will allow to obtaining the required useful dose of phenolic compounds and reduce wine consumption.

Today, a large number of methods to obtain a concentrate of phenolic compounds of grape wine have been developed. The technique of lyophilization, which consists in drying polyphenolic compounds in a vacuum with pre-freezing of wine,

is most often uses in the industry. The method of isolating polyphenols through a column and their subsequent drying by spraying is quite common. Although these methods prevent the loss of phenolic compounds, however, the obtained dry preparations are poorly soluble in water, which reduces their value [38]. To obtain a polyphenol concentrate, we chose the method of evaporation of dry red grape wine, in the optimal conditions for the preservation of polyphenolic compounds present in the raw material. Obtained concentrate contained also monomeric polyphenols, which were found in wine [39].

The following substances were detected in the obtained concentrate: anthocyanins (malvidin, delphinidin, peonidin, petunidin, cyanidin), flavones (quercetin, quercitin-3-O-glycoside), flavan-3-ols ((+)-catechins, (−)-epicatechins), phenolic acids (gallic, caftaric, coutaric, syringic). This spectrum of polyphenols probably determines the antioxidant and antidiabetic properties of the obtained concentrate [40, 41].

1.3 Antidiabetic action of red wine polyphenols

Chronic hyperglycemia in diabetes mellitus causes chronic inflammation, which are accompanied by relapses and are difficult to treat. Diabetes mellitus causes damage, dysfunction, or insufficiency of various organs and systems, including eyes, kidneys, nervous system, heart, and blood vessels.

In recent years, there has been growing evidence that plant polyphenols, due to their biological properties, can be an unique dietary supplement and additional treatment for various aspects of diabetes. Natural polyphenols are potential multifunctional agents that reduce the risk of developing diabetes and diabetic complications [42]. Red wine polyphenols significantly increase the sensitivity of peripheral tissue cells to insulin in diabetes [25].

Decreased insulin secretion in diabetes is often combined with reduced sensitivity to this hormone in peripheral tissues. The lower sensitivity of tissues to insulin can be diagnosed using a glucose tolerance test. It allows to obtain information about the dynamics and degree of assimilation of carbohydrates and identify possible violations of this process [43, 44].

When administered polyphenol complex to animals with diabetes mellitus during 14 days, fasting blood glucose was 9.8 mmol/l (in control this index was 4.9 mmol/l). 15 min after *per os* glucose administration, the level of glucose was increased and reached a maximum after 60 minutes (13.9 mmol/l). The concentration of glucose in the blood was 1.5 times lower than the values at the same time point in animals with diabetes [45].

Hypoglycemic effects of polyphenolic compounds may be associated with inhibition of carbohydrate digestion. Polyphenols inhibit α-amylase and α-glucosidase activity, slowing glucose absorption in intestine, stimulate insulin secretion, and protect pancreatic β-cells against glucose toxicity. Polyphenols can inhibit the release of glucose by liver cells by affecting hepatic glucose homeostasis, in particular glycolysis, glycogenesis, and gluconeogenesis, which are impaired under diabetes mellitus. Polyphenols also activate insulin receptors or stimulate glucose uptake into insulin-sensitive tissues [46–48]. In addition, some polyphenols, including resveratrol and quercetin, contribute to the preservation of the integrity of pancreatic β-cells in rats with streptozotocin-induced diabetes against oxidative stress damage, thus help maintain normal insulin levels [48].

During carbohydrates metabolism glucose, fructose, or glucose-6-phosphate can non-enzymatically bound to proteins, including hemoglobin. This is a glycation reaction, the essence of which is the non-enzymatic addition of free aldehyde groups to free amino groups of proteins. Under hyperglycemia, excessive glycation

is observed. The structure and function of glycated proteins change, which leads to cell damage and various diabetic complications [49, 50].

Glycated hemoglobin (HbA1c) reflects the average glucose level for the previous 2–3 months and is one of the reliable diagnostic criteria for diabetes [51]. Accordingly, this indicator has become one of the main standard methods for assessing the level of glycemia and the effectiveness of its correction, as well as the most important way of long-term metabolic control over the course of diabetes [52].

It was found that the content of glycated hemoglobin increases in the blood of rats with diabetes mellitus compared with control. In the condition of polyphenolic complex concentrate administration to animals with diabetes, we observed the normalization of glycated hemoglobin content [53]. The decrease in the level of glycated hemoglobin under the administration of polyphenolic complex to animals with diabetes mellitus indicates a stable long-term hypoglycemic effect of the studied concentrate.

Revealed properties to regulate glucose tolerance and reduce the level of glycated hemoglobin justify the possibility of using polyphenolic compounds of wine as a basis for the development of new adjuvant antidiabetic therapeutic agents or to prevent the development of diabetic complications.

1.4 Antioxidant potential of polyphenols of red wine

Due to the peculiarities of the chemical structure, all phenols are able to neutralize the electron of free radicals and form relatively stable phenoxyl radicals and thus stop oxidative chain reactions in cells [24]. Polyphenols can scavenge ROS and RNS, lipoperoxide radicals, and can chelate metal ions such as iron and copper, which play an important role in initiating free radical reactions [15]. Thus, these compounds realize antioxidant and anti-inflammatory activity. There are data in the literature on the ability of some polyphenols to affect cellular signal transduction [30], to modulate the functioning of the endocrine system, and hence the action of hormones on various physiological processes, as these compounds react with metal ions and enzymatic cofactors [11].

It is noted that the obtained concentrate of natural polyphenol complex of red wine showed antioxidant properties, at the level of individual tissues and organs and at the level of the whole organism under low level irradiation and experimental diabetes mellitus [45, 53–55]. The use of polyphenolic complex concentrate helped to prevent the accumulation of lipoperoxidation products, which indicates the powerful antioxidant properties of polyphenolic components of red grape wine. Polyphenols react with ROS and convert them into products with much lower reactivity. It is believed that the most effective protection of the lipid bilayer is provided by more hydrophobic polyphenols. Epicatechin gallate has been shown to be soluble in the membrane lipid bilayer and is a highly effective protector under excessive lipid peroxidation [56, 57].

The level of ROS in the cell is controlled by the endogenous system of antioxidant protection. However, under pathological conditions, the production of ROS increases, and, at the same time, the mechanisms of antioxidant protection are disrupted [58–60]. Polyphenolic compounds cause a decrease of ROS level by normalizing the activities of antioxidant enzymes. The ability to affect the endogenous antioxidant system has a large number of phenolic compounds present in grape wine. In particular, flavan-3-ols, ((−) - epicatechins, (+) - catechins, gallates and products of their methylation, decarboxylation and dehydroxylation), quercetin, hydroxycinnamic acids (caftaric, coutaric and coumaric acids) activate transcription of genes of the enzymes.

It is known that red wine polyphenols increase the antioxidant capacity of plasma and other tissues of animals and humans. This effect is associated with the stimulation of the activity of superoxide dismutase, catalase and glutathione peroxidase and with an increase in the content of both reduced and oxidized glutathione [10, 14, 38, 61].

It was established a decrease of NOS total activity in peripheral blood, leukocytes, aorta and kidneys of rats after low doses irradiation on the background of polyphenolic complex concentrate consumption. The same effect was found in leukocytes, erythrocytes, pancreas and heart of rats with streptozotocin-induced diabetes mellitus [62]. It was detected a lowering in the total content of nitrites and nitrates in the case of X-ray irradiation in peripheral blood, leukocytes, aortic and renal tissues [40, 45, 63–66]. Under conditions of streptozotocin-induced diabetes mellitus, it was observed a significant decrease in the content of nitrite and nitrate in leukocytes, in peritoneal macrophages and in pancreas in the case of polyphenol complex concentrate consumption.

It is known that polyphenolic compounds of grape wine have the ability to capture and neutralize NO and its metabolites. Due to this, polyphenols can also prevent the development of oxidative-nitrative stress.

Grape wine anthocyanins (malvidin, delphinidin, peonidin, petunidin and cyanidin) are potent antioxidant because they can affect NO content. One of the possible mechanisms of polyphenols influence on the level of NO is the regulation of the activity of NO synthases. It is known that phenolic compounds show diverse effects on the activity of various isoforms of the enzyme: they inhibit neuronal NOS (nNOS) and inducible NOS (iNOS) and increase the activity of endothelial NOS (eNOS). In blood cells has been detected inhibition of mRNA translation of iNOS, the synthesis of which is induced by lipopolysaccharides, interleukin-1 or tumor necrosis factor α (TNF-α) [28, 31]. Catechins scavenge NO and peroxynitrite, inhibit the activity of neuronal and inducible NOS by inhibiting the binding of nuclear factor NF-κβ to the NOS gene promoter. For example, catechins activate endothelial NOS in rats aorta by binding to the antioxidant response element (ARE) of the promoter of the eNOS gene [17, 19, 21, 28, 30, 31, 67].

This effect on NOS activity is offset by an increase in Ca^{2+} concentration due to release into the cytoplasm from intracellular depots or a receptor-dependent mechanism, the key event of which is an increase in guanylate cyclase activity in cells. As a result, the activity of eNOS increase, as this isoform of the enzyme is calcium-dependent [12]. A number of authors describe the ability of catechins, anthocyanins, quercetin, and other wine polyphenols to activate eNOS by phosphorylation mediated by activation of the Src/PI3′-kinase/Akt signaling pathway. This mechanism is dependent on the intracellular generation of ROS.

However, much more attention today is paid to the role of peroxynitrite ($ONOO^-$). Peroxynitrite is a powerful prooxidant and cytotoxin, interacting with lipids, DNA and proteins in oxidation, nitration and nitrosylation reactions cause cell damage and cell death [68–73].

Modern strategies aimed at limiting the formation of cytotoxins are the use of various herbal compounds with the ability to neutralize RNS *in vitro*, in particular, $ONOO^-$, for example, phenolic compounds of grapes and grape wine [69]. It was shown that polyphenolic compounds from grape wine regulate the intensity of protein nitration processes in leukocytes, aorta and kidney cortical layer of irradiated rats and in erythrocytes of animals with diabetes mellitus [40, 63, 64]. The decrease in the content of 3′-nitrotyrosine-modified proteins may be due to the ability of grape polyphenolic compounds to detoxify NO, $ONOO^-$, and other RNS. Similar effects of grape wine polyphenols on the level of nitrated proteins have been described in the literature [74, 75].

Our results open up prospects for the use of drugs, the main active ingredients of which are phenolic compounds, as adjuncts in complex therapy and prevention of damage to the blood system, cardiovascular and excretory systems caused by ionizing radiation. Drugs of complex action, which will inhibit the development of oxidative-nitrative stress, will be effective treatment of different diseases, including diabetes mellitus and radiation sickness.

2. Yacon as a source of bioactive compounds with antidiabetic and antioxidant potential

Today there is an urgent need for effective drugs for the treatment of metabolic disorders, the etiological cause of which is a violation of the redox status of cells. A successful strategy for finding such substances is to search for them among agents of natural origin due to their lower generation of side effects and the availability in obtaining material.

One of the promising plant is yacon, which has been discovered antidiabetic and antioxidant properties [76].

Yacon (*Smallanthus sonchifolius* (Poepp. and Hendl.) H. Robinson) is also known by its old name *Polymnia sonchifolia* Poepp. & Endl. Such a discrepancy in the classification of this plant is due to the fact that it was described for the first time as *Polymnia sonchifolia* Poepp. by Eduard Friedrich Poeppig in 1845. Afterward in 1978 genus *Smallanthus* (Asteraceae, Heliantheae) was rediscovered by Harold Ernest Robinson, who established the *Smallanthus* gender by separating *Polymnia* [76, 77]. Yacon is known to mankind for centuries, it was found in burial grounds from centuries before the Incas. This plant was represented on textile and ceramics in a littoral archeological deposit Nazca (500–1200 A.C.). 1653 is a year of first written allusion on yacon comes from the chronicler Padre Bernabé Cobo [77]. Until the 1980s, the scientific community paid little attention to yacon. The plant itself originates from the Andean region, from where it spread to New Zealand, Japan, and Brazil. Today it is cultivated in many countries around the world, including Ukraine.

2.1 Phytochemical profile of *Smallanthus sonchifolius* tuberous roots

Yacon is a perennial plant with underground tubers that are grouped in clump. Average tuber weight fluctuates from 100 to 500 g, and rarely reaches more than 1 kg [77]. Yacon root tubers have great nutritional potential due to its sweet taste and lower energy content (619–937 kJ/kg of fresh matter) provided by its 70% water composition [78].

The underground storage organs of yacon accumulate mainly low molecular mass oligomeric (GF2–GF16) inulin-type β(2 → 1) fructans (over 60% on a dry basis). The main *fructooligosaccharides (FOS)* are nystose, 1-kestose, and 1-fructofuranosyl nystose [79, 80]. Storage compounds of yacon tubers are low in glucose content and by the structure are of the inulin type, i.e. β(2 → 1) fructofuranosyl saccharose or fructooligosaccharides [77]. These FOS are mainly represented by oligosaccharides from trisaccharide to decasaccharide with terminal saccharose [81]. FOS derived from yacon are linear fructooligosaccharides containing almost exclusively (2 → 1)-linked β-fructofuranosyl units, with terminal α-glucopyranosyl and β-fructofuranosyl units [82].

β(2 → 1) fructans of the inulin-type are considered to be dietary fiber or the indigestible residues of plant origin due to lack of enzymes in humans body capable to hydrolyze the β(2 → 1) bond in such compounds. Because FOS do not digest in

the human gastrointestinal tract and they transported to the colon they recently been classified as prebiotics. In the colon they undergo fermentation into short-chain fatty acids (acetate, propionate, and butyrate), lactic acid, carbon dioxide, and hydrogen by selected species of gut microbiota, especially *Bifidobacterium* and *Lactobacillus* [77, 82].

FOS except as prebiotics can be used as non-specific immunostimulators. Mechanisms of such effect can be indirect by shifting the composition of the intestinal flora and enhanced production of immunoregulatory short-chain fatty acids. On the other hand, it was suggested that fructooligosaccharides can possess direct effects on the intestinal epithelial cells and immune cells through binding to carbohydrate receptors [82].

S. sonchifolius roots are also rich in *fructose, glucose, sucrose*, amount of which fluctuates during the growing cycle, and harvest [77, 78, 80].

Other important biologically active substances in the composition of yacon root tubers are *phenolic compounds*. The phenols group include chlorogenic acid, protocatechuic acid, p-coumaric acid, ferulic acid, and caffeic acid derivatives [77–79]. Phenolic acids have a high antioxidant potential result from the presence of an aromatic ring, a carboxyl group, and one or more hydroxyl and/or methoxyl groups in the molecule [83]. Yacon tubers have been identified as a source of flavonoids including quercetin and some unidentified flavonoids [77, 84]. Flavonoids can be found only in acid-hydrolyzed yacon tubers and have an influence on lipid peroxidation, acetylcholinesterase, and butyrylcholinesterase [80].

The most abundant *amino acid* in this part of the plant is tryptophan known as a precursor of serotonin and melatonin. This compound eliminates free radicals from the oxidative damage low-density lipoprotein [80]. Chlorogenic acid and tryptophan were identified as two of the major antioxidants of yacon roots [85].

From yacon tuber have been isolated 4′-hydroxyacetophenone, 4′-hydroxy-3′-(3-methylbutanoyl) acetophenone, 4′-hydroxy-3′-(3-methylbutenyl) acetophenone, and 5-acetyl-2-(1-hydroxy-1-methylethyl) benzofurane which are related antifungal *phytoalexins* that possess antimicrobial activity [77, 80].

Also, *S. sonchifolius* root contains small amounts of *vitamin C and potassium* [80].

2.2 Phytochemical profile of *Smallanthus sonchifolius* leaves

Smallanthus sonchifolius stems can reach 2 m in height are densely foliaged with dark green leaves and covered by violet-colored trichomes. The inflorescence grows at the top of the main stem is small (30 mm in diameter), with a yellow or orange color. The fruits are black, about 2 mm small [77]. Yacon leaves, next to the root tubers, also have a high potential, both in nutritional purposes and in medical practice. It can be consumed as a tea, also can be used as a material for raw and organic extracts [78].

One group of compounds that can play an essential role in antioxidant and antidiabetic properties of yacon leaves is *phenolic compounds*. The compounds were identified as chlorogenic, caffeic, ferulic, gallic, and gentisic acids [77], also protocatechuic, rosmarinic, vanillic and gentisic acids, as well as 3,4- dicaffeoylquinic, 3,5-dicaffeoylquinic and 4,5- dicaffeoylquinic isomers of dicaffeoylquinic acid [86].

S. sonchifolius leaves are rich in flavonoids, such as luteolin 3′,7-O-diglucoside and luteolin 7-O-glucoside together with apigenin and luteolin [86], 5, 7-dihydroxy-4′-methoxyflavonol, 5, 7, 3′-trihydroxy-4′-methoxyflavonol, 5-hydroxy-4′-methoxy-7-O-glycosilflavone and 7,4′-dihydroxy-3,5′-dimethoxyflavone [87]. Presence of polyphenols in yacon leaves predetermine its acrid and astringent flavor and characteristic odor. Due to the high antioxidant capacity of polyphenols these

compounds may play an important role in lowering the risk of cancer, cardiovascular disease, atherosclerosis, and diabetes [80]. It can be concluded that yacon is a very rich source of antioxidants. Regarding the content of phenolics and phenolcarboxylic acids, the yacon parts could be arranged in such an order: rhizomes >leaves > stems > tuberous roots [88]. The fact that not only yacon leaves but also its root part are rich in antioxidants is valuable for search of effective means against hyperglycemia.

Terpenes in yacon leaves determine its antifungal properties. To substances with such properties belong *ent*-kaurenoic acid, and related diterpenoid substances *ent*-kaur-16-en-19-oic acid 15-angeloyloxy ester, 18-angeloyloxy-*ent*-kaur-16-en-19-oic acid and 15-angeloyloxy-*ent*-kauren-19-oic acid 16-epoxide, as well as 4-hydroxystyrene and 3,4-dihydroxystyrene. Antifungal properties also possess melampolide-type sesquiterpene lactones sonchifolin, polymatin, uvedalin, and enhydrin from leaves [77], as well as smallanthaditerpenic acids A, B, C and D [89]. Recently were identified propionate and butyrate analogs of sonchifolin, tiglate analog on C8 of polymatin B, fluctuadin, polymatin C and the aldehyde derivative on C14 of uvedalin. Some of above-mentioned terpenes (sonchifolin, uvedalin, enhydrin, and related compounds fluctuanin, 8β-tigloyloxymelampolid-14-oic acid methyl ester and 8β-methacryloyloxymelampolid- 4-oic acid methyl ester) are also reported to be antibacterial compounds [77, 79, 80]. The main lactone of yacon leaves is enhydrin that has antidiabetic properties, so much so that it was included in a patented anti-diabetic pharmaceutical formulation [90]. Melampolide-type sesquiterpene lactones shown to inhibit NO production in LPS-stimulated murine macrophage RAW 264.7 cells [91]. Sesquiterpene lactones also can regulate the immune response. Enhydrin and uvedalin, inhibit the NF-κB, a transcriptional factor that has a central role in the transcription of the genes related to the inflammatory process [92]. Enhydrin, uvedalin and sonchifolin inhibited cell proliferation and induced apoptosis in cervical cancer cells. Their apoptotic effect is associated with caspase-3/7 activation and NF-κB inhibition [93].

Major unsaturated *fatty acids* found in yacon leaves extracts were gamma-linolenic (ω-6), eicosapentaenoic (ω-3), and linoleic (ω-6) acids. In addition, in this part of the plant were found lauric, myristic, pentadecanoic, palmitic, palmitoleic, margaric, stearic, oleic, arachidic, eicosatrienoic, di-homo-alpha-linolenic, heneicosanoic, behenic, eicosadienoic, and docosahexadienoic acids [94]. Polyunsaturated fatty acids in addition to their anti-inflammatory, vasodilator, antihypertensive, and immunosuppressive effects can cause the reduction of plasma lipids amount and normalize hyperglycemia [95].

Yacon leaves contain a wide range of *essential oils* such as beta-pinene, caryophyllene, y-cadinene, β-phellandrene, β-cubebene, β-caryophyllene and β-bourbonene [86].

2.3 *Smallanthus sonchifolius* antidiabetic potential

The possibilities of innovative technologies in the pharmaceutical industry make it possible to expand the range of search for effective natural substances as a form of additional or substitution therapy of different pathological conditions. Natural substances affect not only carbohydrates but also lipids metabolism, regulate water balance, and normalize the functional state of the kidneys and liver. Herbal preparations support the state of long-term compensation for diabetes mellitus. In folk medicine around the world for the treatment of diabetes, aqueous extracts of yacon are widely used.

One of the biochemical methods for diagnosing carbohydrate metabolism disorders, in particular, in diabetes mellitus, is a glucose tolerance test. This approach

allows checking the dynamics and degree of glucose absorption in the body and identifying possible violations of this process. The rate of decrease in glucose levels after oral administration depends mainly on the function of the cells of the islets of Langerhans of the pancreas.

Glucose tolerance test is a convenient tool for analyzing not only changes in the efficiency of carbohydrate absorption but also can be a convenient tool for assessing the effectiveness of treatment aimed at reducing postprandial hyperglycemia. This approach is often used to assess the antidiabetic potential of medicinal plants.

A screening study showed that under conditions of glucose load in healthy animals, different parts of the aboveground part (leaves, petioles, stems) of yacon have different hypoglycemic effects. A comparative analysis of the hypoglycemic effect of aqueous extracts of the aboveground part of yacon showed that the highest and longest hypoglycemic effect after single oral administration possesses yacon leaves extract. It should be noted, that in control animals, the hypoglycemic effect was achieved at a dose of 0.07 g per kg of weight of the animal [96]. No such pronounced hypoglycemic effect was found while administering a similar dose of yacon leave extract to animals with experimental diabetes mellitus. However, increasing the dose of the extract to 0.5 g per kg of animal weight led to a significant improvement in the absorption of exogenous glucose by animals with experimental diabetes mellitus [97]. In addition to convincing data on the hypoglycemic effect of the aqueous extract of yacon leaves obtained by the glucose tolerance test, a pronounced hypoglycemic effect of this extract was also demonstrated when administered it to rats with diabetes for 14 days [98]**.** This study confirmed that an effective hypoglycemic effect has an aqueous extract of yacon leaves at a dose of 0.5 g per kg of body weight. When using the extract at this dose, a significant decrease in both plasma glucose and glycosylated hemoglobin was shown [98].

Some authors attribute the hypoglycemic effect of yacon leaves to the presence of a number of biologically active substances, among which polyphenols play an important role [77, 99]. Chlorogenic acid has been shown to inhibit the enzyme glucose-6-phosphatase, thus, affecting the metabolism of carbohydrates (glycolysis, glycogenolysis, and gluconeogenesis). Some studies have shown that polyphenols derived from aqueous extracts of yacon leaves inhibit alpha-amylase and sucrose. They also inhibit glucose transport through gastrointestinal cells by inhibiting the functioning of the sodium glucose co-transporter (S-GLUT-1) [86]. The yacon leaves contain enhydrin, which increases the number of β-cells and the level of insulin mRNA in the pancreatic islets of rats with streptozotocin-induced diabetes [90]. Enhydrin also inhibits α-glucosidase activity, a similar inhibitory effect possesses smallanthaditerpenic acids A, B, C, and D isolated, which are also contained in the leaves of yacon [89, 90].

The uniqueness of yacon as a source of biologically active substances for the treatment of diabetes is that their source can be not only the aboveground part of the plant but also the root tubers. A comparative analysis of the hypoglycemic effect of water extracts of yacon roots in healthy animals in a dose of 0.07 g per kg of weight of the animal suggests that a more pronounced hypoglycemic effect has an extract of yacon root tubers, while the extract of root tubers peels possess much less pronounce effect [96]. However, another study showed that a dose of 0.07 g per kg of body weight of water extract of root tubers is insufficient for hyperglycemia compensation. Only the use of the extract at a dose of 0.5 g per kg leads to significant changes in the dynamics of exogenous glucose uptake under conditions of streptozotocin-induced experimental diabetes mellitus [100]. An additional approach in the creation of drugs based on plant raw materials is their use in the form of suspensions. The advantages of this form of medicines include the production of medicines of prolonged action by regulation of duration of their action by

changing in the size of medicinal raw materials particles, simultaneously usage of soluble and insoluble medicinal substances, allow mask unpleasant taste and smell of medicines. Suspensions prepared by mixing homogeneous powdered root tubers with water (at a dose of 0.5 g per kg) significantly affect the intensity of glucose uptake in animals with experimental diabetes mellitus. Interestingly, the use of surfactants to stabilize the physical properties of the suspension increases its hypoglycemic effect. Comparing all forms of yacon underground part administration, yacon root tubers when they are used in the form of stabilized suspension possesses the best hypoglycemic effect [100, 101]. Long-term use (within 14 days) of the extract and suspensions of yacon root tubers in diabetes has shown a pronounced hypoglycemic effect. The use of water extract in doses 0.07 and 0.5 g per kg of body weight causes a significant reduction of plasma glucose level. However, only the use of the extract in a higher dose caused a significant decrease in the level of glycosylated hemoglobin. Suspensions of yacon root tubers stabilized with surface-active substances of biogenic origin at fourteen days of use also caused a significant decrease in both glucose and glycosylated hemoglobin in diabetic animals. Non-stabilized form of the suspension had a less pronounced hypoglycemic effect. The authors attribute this to the fact that the addition of surfactants to the suspension increases its stability and bioavailability of biologically active substances [98].

The hypoglycemic effect of yacon root tubers is less studied. The sugar-lowering effect of this part of the plant may be due to presence in its composition a high FOS content that can change the kinetics of carbohydrates absorption. As mentioned above FOS do not decompose in the gastrointestinal tract, can absorb a great amount of exogenous glucose. High absorption ability of FOS interferes with glucose transportation into blood, which causes a decrease in the level of blood sugar after meals. The stable decrease in the level of glucose causes normalization of insulin production by pancreatic cells. Intestine microorganisms hydrolyze FOS into smaller fragments and free fructose. Short fragments of FOS molecules facilitate the transportation of glucose into the cell by inserting into the cell membrane [102]. FOS also modulates concentrations of GIP (glucose-dependent insulinotropic polypeptide) and GLP-1 (glucagon-like peptide 1) - peptides that regulate insulin release after meals [103]. Some yacon root tubers' hypoglycemic effect can be attributed to essential amino acid L-tryptophan. It is known that this amino acid normalizes tolerance to carbohydrates and elevates the insulin level. In hepatocytes, L-tryptophan increased activity of glucokinase, hexokinase, and glucose-6-phosphate dehydrogenase that are the key enzymes of the carbohydrate exchange [85].

Hypoglycemic effect of yacon leaves and root tubers is very valuable in the development of antidiabetic medicines in terms of counteracting the harmful effects of hyperglycemia as an etiological cause of chronic diabetic complications.

2.4 *Smallanthus sonchifolius* antioxidant potential

The advantages of yacon as a source for the creation of effective antidiabetic medicine is that it has a high content of antioxidant compounds. Extract of the yacon leaves possesses the free radical scavenging activity and inhibitory effects on lipid peroxidation in rat brain and liver [104, 105]. *S. sonchifolius* leaves extracts show antioxidant activity in 1,1-diphenyl-2-picrylhydrazyl and xanthine/xanthine oxidase superoxide radical scavenging tests [106]. Similar effects perform extracts from yacon tuberous roots [85, 107].

Red blood cells are one of the most suitable models for the investigation of the antioxidant effect of plant material. During their circulatory life span, erythrocytes are continuously exposed to glucose. The glucose concentration in the erythrocyte

cytosol is close to that in the plasma because is ensured by passive transport through GLUT1 (insulin-independent glucose transporter) [108, 109].

Hyperglycemia induced generation of free radicals is a plausible contributing factor of lipid peroxidation the intensity of which is reliably evidenced by the level of thiobarbituric acid reactive substances (TBARS). Water extracts of *S. sonchifolius* leaves in doses of 0.07 and 0.5 g per kg of body weight cause a significant reduction of TBARS in erythrocytes of diabetic rats. Extract in higher concentrations had a more pronounced protective effect on the peroxidation of erythrocyte lipids. The extract causes a similar effect on healthy animals. The development of oxidative stress in diabetes has a destructive effect not only on cell lipids but also significantly damages the structure of proteins. The level of such damage is indicated by protein carbonyl content (PCC). Yacon water extract cause PCC reduction on the condition of diabetes mellitus and such change was not dose-dependent [110].

One of the mechanisms of this antioxidant effect of yacon leaves extract may be its effect on antioxidant enzymes of cells. Indeed, it was established that the administration of yacon extract to diabetic rats (at a dose of 0.07 and 0.5 g/kg) causes increased activity of SOD in a dose-dependent manner. Interestingly, the extract in a lower concentration caused a more pronounced increase in CAT activity compared to its higher dose [110]. An additional mechanism of the antioxidant effect of yacon is its ability to inhibit the synthesis of myeloperoxidase that can cause causes oxidative damage of proteins and DNA [111].

The antioxidant effect of leaves extract may be caused by the presence of phenolic compounds. Chlorogenic and caffeic acid effectively scavenge N_2O_3, organic free radicals, HOCl, $O_2^{\bullet -}$, $OH^{\bullet}$, $ONOO^-$ and peroxyl radical. After the reaction of chlorogenic or caffeic acid with free radicals products that are formed rapidly broken down to the products which are unable to generate more free radicals. Thus, no other antioxidants are necessary for the reduction of such oxidation products [112]. Flavonoids by which leaves of yacon are rich in can reduce enhancement of transition metal oxidation by donating a $H^{\bullet}$ to them, rendering them less prooxidative. In addition, flavones and some flavanones can preferentially bind metals at the 5-hydroxyl and 4-oxo groups [113].

In the condition of diabetes, yacon root tubers in the form of water extract or suspensions cause a significant reduction of TBARS and PCC levels. Water extract of root tuber at a dose of 0.5 g per kg body weight causes a remarkable increase in SOD, CAT, and glutathione peroxidase activities, while in 0.07 g per kg body weight dose its effect was less pronounced. In comparison with the water extract, the suspensions obtained from the powder of yacon root tubers caused a smaller increase in the activity of the antioxidant enzymes of erythrocytes. However, the surfactant-stabilized suspension had a slightly higher antioxidant potential compared to non-stabilized one [110].

The antioxidant potential of the root part of yacon may be due to the high content of FOS. It was confirmed the significant antioxidant activity of inulin [114]. The ability of FOS to enhance the absorption of copper can reduce the deficiency of this element under the conditions of diabetes and as a result, might be one of the reasons for increased SOD activity in diabetes animals that were treated with yacon root tuber extract and suspension [115]. Similar to the leaves, yacon root tubers contain a number of phenolic compounds that have a pronounced antioxidant effect. In addition, yacon roots contain tryptophan, an antioxidant compound that scavenged hydroxyl radicals [116].

Effect of leaves and root tubers on the state of prooxidant-antioxidant balance of red blood cells may predetermine yacon as a promising source of biologically active substances that can be used for treatment and prevention of chronic diseases involving oxidative stress, among which diabetes mellitus is present [110].

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Section 8

Medicinal Plants

Chapter 14

Collection, Storage and Market of Medicinal Plants: A Case in Peru

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Abstract

There are few studies on the collection and market of medicinal plants in South America and particularly in Peru. Through a review of secondary sources, information is gathered on the use and market of medicinal plants, and information is provided on the collection, collection and market of medicinal plants in the department of Cajamarca, focused on a chain of value of fresh plants (57 species) and another of dry plants (37 species), which mainly supply coastal markets. It is also reported on the collection in the first months of the COVID19 pandemic. The species come mainly from the Quechua region (2300 to 3500 masl), 51% are wild and the others are cultivated or weeds. Its main threats are mining, agricultural expansion, overgrazing, burning of natural vegetation, and over-harvesting. The monetary value of these plants is approximately US $ 804,333.64/year. In the first months of COVI19, the demand for eucalyptus, matico, chamomile and husk increased, mainly, and prices rose by more than 200%. Value chains are informal, they add minimal value to products, with the predominance of the interest of wholesale collectors.

Keywords: traditional medicine, medicinal plant market, value chain, high mountains, Peru, COVID19

1. Introduction

In Peru 80% of the population uses phytotherapy as a medicinal resource, 76% of population insured by EsSalud (a health system for state employees) are willing to receive treatment with medicinal plants, and about 90,000 insured people per year, use complementary medicine (CM) services in 83 CM care centers In addition, despite of being a multi-diverse country, no attention has been paid to the development of medicinal plants value chain, pointing out as some of the greatest challenges, the proper registration of these plants, the protection of their biodiversity, investment in research and the guarantee of quality and safety when being used [1].

On the other hand, the growing demand of medicinal plants has generated problems regarding their future availability, because most of them are collected from nature (minimal cultivation), without any type of adequate management guaranteeing the long-term sustainability of the extraction and the *in-situ* species preservation [2].

Cajamarca region's land (Peru) is an important area for the collection, production, use and market of medicinal plants with pre-Inca heritage and is part of the

Northern Peruvian *shaft of health* extending towards Ecuador and Bolivia [3, 4]. In this report, based on the South American context, we approach the study of medicinal plants in Peruvian markets, particularly in Cajamarca, describing collection and stockpiling of fresh and dried medicinal plants in two provinces capital, giving place to two informal value chains. The results of an observation on the collection of medicinal plants during the first months of the COVID-19 pandemic in these two markets are included.

2. Use and market of medicinal plants in South America

Inventories of medicinal species by countries in South America are insipient and the estimates indicate about 23,403 species (**Table 1**), from 50,000 to 80,000 medicinal species (flowered) estimated for the world [14]. These approaches leave many questions to be resolved in the future. For example, it is estimated that Peru has 1400 medicinal species [15]. Then, the estimate was more than 3000 species with this use, and 774 medicinal species in current use were described with 343 pharmacological actions [5]. In South America, Colombia is the country that has best documented the medicinal species used in their pharmacopeia and has established strategies and guidelines for their knowledge, conservation and sustainable use [6].

South America cities continue to use medicinal plants, as their first alternative in health treatment; however, in few cases complete studies were done on markets supply (**Table 2**). Exits a lack of studies on the subject in most of South American cities. A comparative study in three countries (Colombia, Peru and Bolivia) indicates that only 4% of species and 1% of genera in the markets are shared [16].

3. Studies on medicinal plants in the markets of the cities of Peru

We do not have a complete vision of the agribusiness for medicinal plants in Peru because the studies in these cities' markets are still incomplete (**Table 3**). The few studies are focused mainly to identify the species marketed and to establish their use.

Country	No. species	No. families	Source
Peru	> 3000	nd	[5]
Colombia	2404	202	[6]
Ecuador	3118	206	[7]
Bolivia	3000	102	[8]
Chile	781$^{\Delta}$	nd	[9]
Brazil	1500$^{\bullet}$	148	[10]
Argentina	9000$^{\lozenge}$	nd	[11]
Paraguay	600	nd	[12]
Uruguay	249$^{\blacksquare}$	nd	[13]

nd: no data.
$^{\Delta}$*It refers to edible wild plants only, which are also medicinal. Detailed information exists for 120 species.*
$^{\bullet}$*Includes species which there is information on use and pharmacology.*
$^{\lozenge}$*Chemical, pharmacological and toxicological studies: 121.*
$^{\blacksquare}$*Species on sale in authorized stores. The informal sale of these herbs is prohibited in this country.*

Table 1.
Number of medicinal species and families from south American countries.

City/country	No. species	No. families	Source
Valencia, Carabobo, Venezuela	84	45	[16]
Caracas Venezuela	164	72	[17]
Pernambuco, Brazil	169	70	[18]
Cuarto Río, Córdova, Argentina	125	48	[19]
Rio de Janeiro, Brazil	376	94	[20]
La Paz and El Alto, Bolivia	163	58	[21]
Loja, Ecuador*	160	57	[22]
Bogota, Colombia	409	nd	[23]

nd: no data.
**33 traditional markets in the province of Loja, southern Ecuador were included.*

Table 2.
Number of medicinal species and families in markets in South America cities.

City	No. species	No. families	Source
Corongo	70	32	[24]
Jauja	45	nd	[25]
Cusco	152	45	[26]
Ayacucho	66	30	[27]
Huaraz, Yungay and Carhuaz	178*	44	[28]
Cajabamba	123	49	[29]
Trujillo and Chiclayo	400	118	[30]
Cajamarca	470	129	[31, 32]

nd: no data.
**incluyó pequeñas comunidades.*

Table 3.
Registries of medicinal species and families in markets of cities in Peru.

The issue has not been addressed from the perspective and concept of the value chain [33, 34]. Commercialization of medicinal plants in Quero and Masma Chicche communities (approximately 3500 to 4800 masl), Masma Chicche district, province of Jauja, department of Junín was studied following the route of the gatherers - growers and the other actors in the chain to the cities of Jauja and Lima. Through interviews and direct observation, it was determined the links in the chain that includes gatherers-producers, intermediaries, processors (small, medium and large), retailers and consumers. In addition, it was found that the chain has added a little value, it mainly offers primary transformation products (creams, extracts, flours and syrups) made with artisanal methods. There are no formal relationships between the actors in the chain, most of the plants are collected and cultivation is incipient [25].

Studies of medicinal plants, carried out with semi-structured interviews and direct observation, in the commercial town of Ayacucho (highlands of Peru, approximately 240,000 inhabitants) highlight the presence of 66 species, between cultivated and wild. The most important volume sales corresponded to matico, manzanilla and ortiga. The species with the highest cultural value were ruda macho (*Ruta graveolens* L.), ruda hembra (*Ruta chalepensis* L.) and honojo (*Foeniculum vulgare* Mill.); those with the highest economic value were muña (*Satureja brevicalyx* Epling), manayupa and ruda hembra. The estimated total volume was 163.6 t/ year, which represents a monetary value of 409,602.7 soles/ year (US $ 117,361.8/year) [27].

The most in-depth studies on medicinal plants in the Peruvian markets were carried out in the north of Peru and correspond to the RW Bussmann group. In one of them [35], through collections made in the fields, markets and the house of healers in northern Peru (Piura, Lambayeque, La Libertad, Cajamarca and San Marín), 510 species from 126 families (83% native) of medicinal plants used to treat 20 groups of diseases were identified. This study not only shows a 2000-year timeline of healing culture in this region, but also the importance of the mountains of Cajamarca and particularly the surroundings of the city of Cajamarca as a supplier of medicinal plants for these markets. The following studies aimed to establish the relationship between the plants found in the Trujillo and Chiclayo markets and the collection and storage sites [36, 37], as well as the relationship between collection and cultivation of medicinal plants and the supply of these markets [38].

Subsequent research analyzes the relationship between the market, healers, vendors and collectors and the sustainability of the use of medicinal plants in northern Peru [30]. A meticulous review of the species used in northern Peru and southern Ecuador, with all their changes since the colonial era, was also made [4]. After more than a decade of work, one of the latest studies [39] points out that the increase in the demand for phytosanitary products does not increase their cultivation. On the contrary, its greatest threat is the disturbance of high ecosystems and calls into question the sustainability of the cultivation, use and market of medicinal species. Likewise, it is estimated that the value of medicinal species in the markets of northern Peru is 1.2 million USD/year. Recently, other studies on this topic were summarized in Cajamarca, focused on specific geographic areas and the ethnobotany of species [40, 41].

4. The market for medicinal plants in the city of Cajamarca

Cajamarca is located in the north of Peru, in South America. It is the center of a large region, made up of the departments of Tumbes, Piura, Lambayeque, La Libertad, Amazonas and San Martín. This region is considered a *shaft of health* (**Figure 1**, shaded area), which, since antiquity, share traditions, knowledge, natural resources, particularly medicinal plants, healers and shamans. At the same time, this center has close ancestral relationships with Ecuadorian and Bolivian populations regarding diagnosis, treatment and cure of diseases [3, 4]. Most of the Cajamarca land (with 13 provinces) is located in the high mountains, another part in upper rainforest (Jaen and San Ignacio) and a small area on the coast (Contumazá). Two informal value chains for medicinal plants are being developed in this area. One in the city of Cajamarca (**Figure 1(1)**), for fresh plants and the other in the city of San Marcos (**Figure 1(2)**) for dry plants. Both, supply the markets of eight cities located in the coast (**Figure 1(3)–(8)**) and one in upper rainforest - Nueva Cajamarca- (**Figure 1(9)**).

Cajamarca city, capital of the department, is located at 2700 masl and 859 km from Lima, the capital of Peru. It was important since Inca times and here the meeting between European and Andean culture took place in 1532. Currently it has a population of 225,800 inhabitants and its immediate surroundings are made up of 12 eminently rural districts. The city of San Marcos is the capital of the province of the same name (with seven rural districts), located 62 km from the city of Cajamarca, it has an approximate population of 9500 inhabitants [42].

Our team carries out research on medicinal species in the Cajamarca city market since 2003 and we have observed the changes that have occurred in the last two decades [31, 40, 41, 43, 44]. Among other aspects, the market for medicinal plants has grown significantly. Thus, in 2003 the city was supplied with 305 species (94

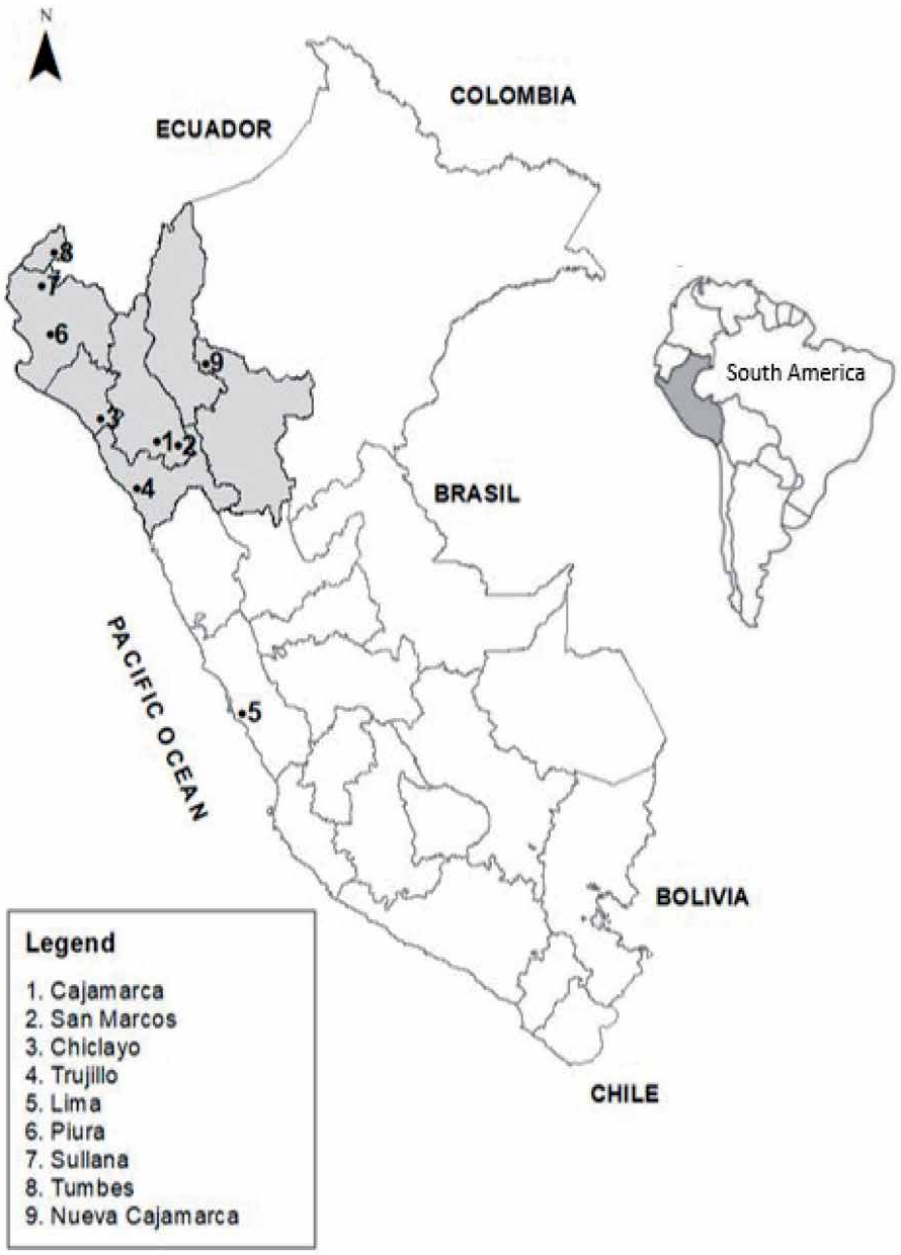

Figure 1.
Shaft of health *area of northern Peru ([3] modified) indicating the collection and storage centers and targeting markets of the medicinal plants stockpiled in Cajamarca, Peru.*

botanical families) and in 2018, 470 medicinal species were on market (129 botanical families). Here it is included eight new medicinal species incorporated into the market over the past two decades: *Estevia rebaudiana* Bertoni (estevia), *Salvia hispánica* L. (chía), *Moringa oleífera* Lam.(moringa), *Morinda citrifolia* L. (noni), *Azadirachta indica A.Juss.* (nin), *Hibiscus sabdariffa* L. (Jamaica), *Vaccinium corimbosum* L.(arándano) y *Phalaris canariensis* L. (alpiste). The number of herbal stalls (from 8 to 27) and stores of processed products (from 3 to 29) also grew. The collection points fell from 8 to 4, but the number of stockers increased from 8 to 18 and the stockpiling days, from two (Monday and Friday) to three (Monday, Thursday and Friday).

The city receives fresh, dried and processed medicinal plants from the mountains, coast and rainforest. A significant part of the fresh plants come from the communities of the surrounding districts. In this report we refer in a special way to this part of plants, with an emphasis on stockpiling to supply the coastal markets. The value chain (**Figure 2**) of these plants collected on the heights of Cajamarca, until it reaches local consumers or other cities is informal, with actors loosely connected and which, prevailing interest from the wholesale stockers of the Coast. The links involved are described below.

The **gatherer-producer** is the one who collects wild medicinal plants and *arvenses* (weeds) or grows and harvests them in their gardens. *Arvenses* are plants that grow spontaneously in crops (and in fallow land), are tolerated and encouraged in their first stage and then are used as fodder, food or medicine. Collection is carried out mainly by women and children and is encouraged by the growing demand for consumption in the cities and by the need of families to increase their economic income. It is a secondary activity carried out while grazing animals or while doing work on the crops. After harvesting, bundles or thirds that have an average weight of 0.350 kg are formed, placed in cloths or plastic containers and transferred to the market. Most of the gatherers sell their product to local stockers and others are gatherers-stockers, who collect and buy from their neighbors and take the product to the coastal markets.

No technical criteria related to the conservation and sustained use of the species are applied, nor is it necessary to have a State collection permit. The collectors say that "it was always like this." Even when the promotion and sustainable use of medicinal plants, in harmony with the environmental, social, health and economic interests of the Nation, is legally regulated [45]; so far, the State has no direct intervention in this matter.

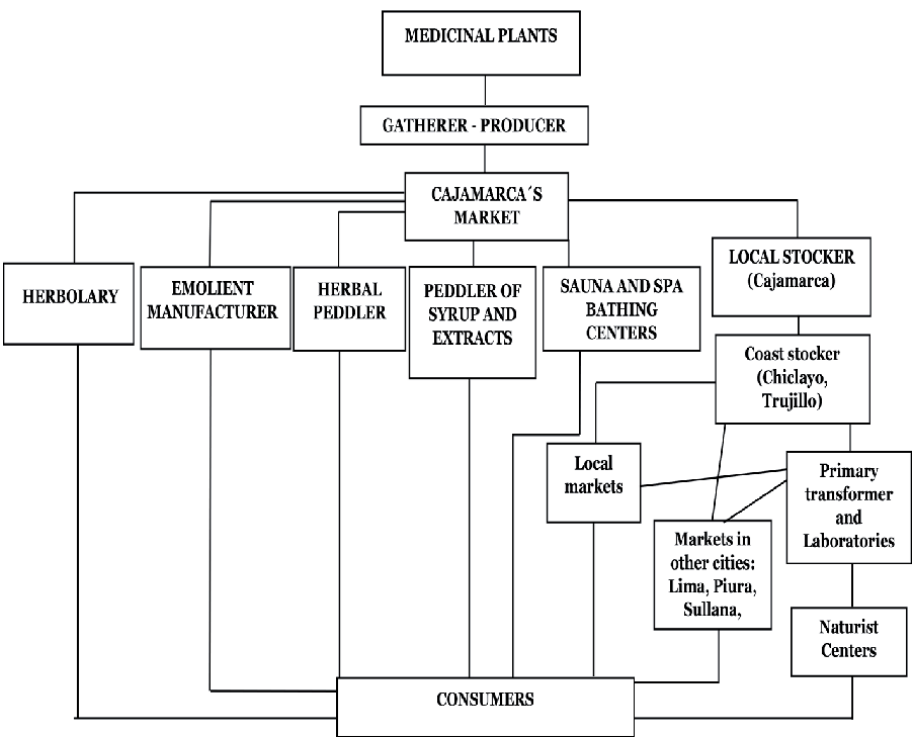

Figure 2.
Scheme of the informal value chain of medicinal plants collected in the city of Cajamarca, Peru.

The **intermediates** are the link between the gatherer-producer and the user, and there are several types: herbalist, emollient manufacturer, peddlers of aloe extract, syrups and other extracts, peddlers of fresh and dried plants, sauna and spa bathing centers, gyms with sauna bath service and the local stocker. The first three have been reported in detail in previous investigations [41, 43, 44]. Next, we will refer to the last four.

- **The peddler of syrups and extracts.** This is a businessman that emerged in the city of Cajamarca in the last two decades [40]. The vendor equipped with a wheelbarrow or cart travels the area surrounding the city market, offering handcrafted preparations to heal illnesses. Customers approach the cart and ask for a preparation (which usually includes a pharmacy product) according to their condition. During one day, this vendor can serve 122 clients, with 14 different illnesses. In this group, sellers of *tocosh* are included, which emerged in the city in the last decade. Tocosh is a traditional product that is obtained by hand, through the bacterial fermentation of potatoes (or corn) in tanks, placed in streams or pools of water, until it becomes a mass, from which the water and the remains of the tuber (skin) are removed and, dried in the sun. This product is highly nutritious and rich in penicillin, recommended against infections, gastritis and ulcers. Animal laboratory studies have shown to possess antioxidant activity, cytoprotective to and regenerative gastric mucosa [46, 47].

- **The peddler of fresh and dried herbs.** It is an outpatient herbalist, with no fixed location point offering fresh and dried herbs, preferably species of the eastern Peru (Amazon, San Martin, Loreto, Ucayali), combined with Cajamarca species. It also offers artisan preparations of plants, "made at the moment" [31, 40].

- **The sauna baths and the** *spa.* These centers consume as yet undetermined amounts of medicinal and aromatic plants on a daily basis. In the cities of Cajamarca and Baños del Inca (located 5 km from the city of Cajamarca), there are 18 *spa* centers, 14 sauna and *spa* bathing centers and 4 gyms with sauna bathing service. Some are part of the hotel service and others are independent. The largest, which provides services (thermal bath, sauna, hydromassage) to significant numbers of tourists is the Baños del Inca Tourist Center, administered by the district Municipality of Baños del Inca. This center is a main point of the tourist circuit of the city of Cajamarca, which, in 2018, received more than 900,000 tourists [48]. In a telephone interview with nine administrators of these centers, it was found that the species used are eucalipto (*Eucalyptus globulus*), manzanilla, romero (*Rosmarinus officinalis*), rosa (*Rosa* sp.), toronjil (*Melissa officinalis* L.), hierba luisa (*Cymbopogon citratus* (DC.) Stapf.), jengibre o kión (*Zingiber officinale* Rosc.), canela (*Cinnamomum verum* J. Presl), ruda (*Ruta graveolens* L.), molle (*Schinus molle* L.), sauce (*Salix* sp.), geranio (*Geranium* sp.), té verde (*Camellia sinensis* (L.) Kuntze) y sábila (*Aloe vera* (L.) Burm f.). On average, they buy 1.3 soles day from one to two species and in three cases they partially supply themselves from their own garden.

- **The local stocker of medicinal plants.** This character is an intermediary who collects fresh medicinal plants in the city for sale in markets along the coast. Other agents, including primary transformers and laboratories, depend on this link until the plant reaches the final consumer (**Figure 2**, right). They are mostly women between 40 and 55 years old, all with experience in the business of more than 10 years. They are located in certain streets and points of the city

and wait for the gatherers-producers who come from the surrounding communities (Monday, Thursday and Friday). They buy until they collect the largest possible volume, place the herbs in bags of approximately 50 kg and ship them by truck to the markets of Chiclayo and Trujillo (**Figure 1(3, 4)**). In these cities there are wholesale stockers that collect large volumes of each species, dehydrate them and distribute them to various destinations: local markets in the city and its districts; laboratories that process medicinal plants in the same city and in Lima; markets in other cities such as Lima, Piura, Sullana and Tumbes (**Figure 1(5)–(8)**) and healers and shamans as well.

5. Cajamarca as a supply center of medicinal plants for the market

5.1 The collection of fresh medicinal plants in the market of the city of Cajamarca

5.1.1 Scope of gather and storage

The gatherers-producers come from the Cajamarca district and districts surrounding the city of Cajamarca such as Baños del Inca, Llacanora, Jesús, Chetilla, Magdalena, Llacanora, Namora and La Encañada; belonging the same province. The main communities where the collection is carried out are Pariamarca, Huambocancha, La Laguna, Jesús, San Juan, Chotén, Chigdén, Higuerón, Yumagual, Gavilán, Huayllapampa, Agocucho, Cumbico, El Cumbe, La Encañada (several communities), Huanico, Baños del Inca (several communities), Cerrillo, Chetilla (several communities), Otuzco (several communities), Miraflores, Huacataz, La Colpa, Cruz Blanca, Llacanora (several communities), Porcón, Porconcillo, Chamis, Aylambo, Choropunta, Secsemayo and Tual. These communities are included in the Yunga Alta, Quechua and Jalca regions, between 2200 to 4200 masl (with a predominance in Quechua, 2300–3500 masl). The gatherers-stockers, who bring medicinal plants directly to the coastal markets, from certain communities, are excluded. For example, those that are on the coastal route such as San Juan, Chigdén, Yumagual, Higuerón, Chotén. This collection and direct shipment also occur in the capitals of districts like Jesus, Llacanora, Namora and Matara.

Although collection and stockpiling of medicinal plants has been developed for several decades in these areas, currently its main threat is metallic and non-metallic mining, since 66% of the territory of this province is under concession to this activity [49]. For now, three major centers of collection and storage (Combayo, La Encañada and Chanta Alta) stopped working, because they are mining areas. Other factors are the expansion of the agricultural frontier, overcollection, overgrazing and the burning of natural vegetation (an ancestral practice of the peasants that has the purpose of promoting the regrowth of the vegetation that serves as pasture).

The first study on the gather of fresh medicinal plants in the market town of Cajamarca was made early in the decade of 2000 [43] when the city had 125,000 inhabitants and began a stage characterized by revaluation of traditional medicine and the consumption of organic products. Sixty-six species were collected for the local and coastal market, coming from the communities near the city, with an average of 5702 kg/month.

Five years later, the ambulatory marketing of medicinal plants was observed on Revilla Pérez de Cajamarca street and two types of vendors were identified; the unskilled who arrived every day and sold, in addition to medicinal plants, other plants (spices, vegetables, ornamentals and fodder) and; the specialized ones, who arrived

on Mondays and Thursdays and only bought and sold medicinal plants. The latter were the stockers who collected large quantities to send to the coastal markets. They observed 42 stored species (34 native), 26 collected, not cultivated. The most common were manzanilla, orégano, culantro (*Coriandrum sativum* L.), ruda y perejil [38].

A recent evaluation of the collection indicates that in the city of Cajamarca 57 medicinal species are stored, in the fresh state (28 families) (**Table 4**), which are then transported to Chiclayo and Trujillo. Sixty five percent of the species are native to the Andes, there was prevalence (46%) of leaves as part used; 25 are cultivated and 14 are wild; the others share wild-weed and cultivated-weed states, so that most are collected [31, 40].

Species of the Quechua region (2300–3500 masl) are predominant (47%), only four are exclusive to Jalca. List does not include Jalca species as chinchimalí (*Gentianella* graminea (H.B.K.) Fabris), trenza (*Hupersia* sp.), estrella (*Phyllactis rigida* (Ruiz & Pav.) Pers. maqui maqui (*Loricaria ferrugínea* (Ruiz & pav.) Wedd.), lengua de ciervo (*Campiloneurum angustifolium* (Sw) Fée.), pachachancua (*Satureja nubigena* (Kunth) Briq.), carnero (*Puya hamata* L.B.Sm.), condor (*Huperzia crasa* (H. & B. ex Willd. Rothm.) purunrosa (*Bejaria aestuans*), órnamo (*Valeriana* sp.), andacushma (*Geranium sessiliflorum* Cav.) y romero de jalca (*Clinopodium sericeum* (C.Presl ex Benth.) Govaerts) and; other lowland species as san pedro (*Echinopsis pachanoi* (Briton & Rose) Friedrich and G. Rowley), sábila (*Aloe vera* (L.) Burm. f.) y flor blanca (*Iresine weberbaueri* Suess), which were identified in high demand in previous studies [50]. These species are collected and shipped, at strategic points, directly to the markets of the coast.

5.1.2 Main species according to the volume of collection.

If we take into account the 10 most collected species, during a day, a gather can collect 180 bundles of manzanilla, 80 bundles of valeriana, 60 bundles of pie de perro and so on, up to a minimum of 35 bundles of menta [31, 40]. So, in order to gather significant quantities, a buying and selling system is established, between gatherers, where each one seeks his objective at the end of the day. The first two species have not changed in their relative importance compared to 2003 (**Table 5**). However, the following eight species have varied in their relative importance and none is repeated. This situation is explained by the changes in the demand for the species and also by the new collection and shipping points for the coast. Other studies are not comparable, as they paid more attention to the most common species that are sold daily for local consumption, in which, prevail food species as perejil, ruda, paico (*Dysphania ambrosoides* L.) and chancua and; aromatic herbs such as cedrón, culantro, hierba luisa, menta, romero, orégano y toronjil. The formers are used to make the *green broth,* a traditional plat of frequent consumption in the city [38].

5.1.3 Volume and economic value of the collected plants

An evaluation carried out between 2017 and 2018, in the 18 local stockers, indicated that the total volume of 57 collected species, means 407.03 t/ year of fresh product, more than five times of the collection in 2003. The gather-producer sold at 0.43 ± 0.14 soles / bundle and the Cajamarca stocker sold in Chiclayo at 1.22 ± 0.26 soles / bundle, obtaining a gross profit of 0.79 ± 0.18 soles /bundle. It is to say, the monetary value in the gather-producer of Cajamarca is 500145.93 soles/ year (US $ 143308.29/year) and the monetary value in the wholesale of Chiclayo is 1419018.67 soles (US $. 406595.60. Therefore, the value of fresh medicinal plants collected in Cajamarca, means 33.9% of the total trade, estimated by Bussmann (2013) in the markets of Trujillo and Chiclayo.

Species	Vulgar name	Family	Origin	Biological state	Used part	Region
Artemisia absinthium L	Ajenjo	Asteraceae	I	S, C	Leaf	Q
Cynara scolymus L.	Alcachofa	Asteraceae	N	C	Leaf, Inflorescence	Y, Q
Scabiosa atropurpurea L.	Ambarina	Caprifoliaceae	I	C	Inflorescence	Q
Tagetes filifolia Lag.	Anis del campo	Asteraceae	N	S, C	Foliage	Q
Apium graveolens L.	Apio	Apiaceae	I	C	Leaf	Y, Q
Nasturtium officinale R. Br.	Berro	Brassicaceae	I	S	Foliage	Q, J
Capsella bursa-pastoris (L.) Medik.	Bolsa de pastor	Brassicaceae	N	S, A	Foliage	Q
Borago officinalis L.	Borraja	Boraginaceae	I	C, A	Leaf, flower	Q
Baccharis genistelloides (Lam.) Pers.	Carqueja	Asteraceae	N	C	Leaf	Q
Aloysia citrodora Palau	Cedrón	Verbenaceae	I	C	Leaf	Q
Minthostachys mollis (Benth.) Griseb.	Chamcua/ muña	Lamiaceae	N	S, C	Leaf, stem	Q, J
Fascicularia bicolor (Ruiz & Pav.) Menz	Chochocón	Bromeliaceae	N	S, C	Leaf, stem	Q
Cupressus macrocarpa L.	Ciprés	Cupresaceae	I	C	Foliage	Q, J
Equisetum giganteum L.	Cola de caballo	Equisoceae	N	S	Foliage	Q, J
Oreocallis grandiflora (Lam.) R. Br.	Cucharilla	Proteaceae	N	S	Leaf	Y, Q
Adiantum poiretii Wikstr.	Culantrillo	Pteridaceae	N	S	Leaf	Q,
Otholobium glandulosum (L.) JW Grimes	Culen	Fabaceae	N	S, C	Leaf	Q
Asplenium peruvianum Dev.	Cuti cuti	Aspleniaceae	N	S	Plant	Q
Cheilanthes pruinata Kaul.	Cuti cuti blanco	Pteridaceae	N	S, C	Leaf	Q
Taraxacum campylodes G. E. Haglund	Diente de león	Asteraceae	I	C, A	Foliage	Q
Perezia multiflora (Humb. & Bonpl.) Less.	Escorzonera*	Asteraceae	N	C	Flower	Q, J
Eucalyptus globulus Labill	Eucalipto	Myrtaceae	N	C	Leaf	Q

Species	Vulgar name	Family	Origin	Biological state	Used part	Region
Brugmansia sanguinea (Ruiz & Pav.) D. Don	Floripondio rojo	Solanaceae	N	C	Flower, leaf	Q
Cymbopogon citratus (DC.) Stapf.	Hierba luisa	Poaceae	I	C	Leaf	Y, Q
Foeniculum vulgare Mill.	Hinojo	Apiaceae	I	C	Leaf, stem	Q
Achyrocline alata (Kunth) DC.	Ishpingo amarillo	Asteraceae	N	S	Leaf, stem	Q, J
Gnaphalium dombeyanun DC.	Ishpingo blanco	Asteraceae	N	S	Leaf, stem	Q, J
Plantago major L.	Llanten	Plantaginaceae	I	S, A	Leaf	Y, Q
Alcea rosea (L.)	Malva grande	Malvaceae	N	C	Foliage	Q
Matricaria chamomilla L.	Manzanilla	Asteraceae	I	C	Foliage	Q
Piper aduncum L.	Matico	Piperaceae	N	S, C	Leaf	Q
Origanum majorana L.	Mejorana	Lamiaceae	I	C	Leaf	Q
Mentha x piperita L.	Menta	Lamiaceae	I	C	Leaf	Q, Y
Alternanthera porrigens (Jacq.) Kuntze	Moradilla	Amaranthaceae	N	C	Foliage	Q
Juglans neotropica Diels	Nogal	Juglandaceae	N	C	Leaf	Q
Origanum vulgare L.	Orégano	Lamiaceae	I	C	Leaf	Q
Urtica urens L.	Ortiga*	Urticaceae	N	S, A	Foliage	Q
Plantago sericea Ruiz & Pav.	Paja blanca	Plantaginaceae	N	S, A	Leaf	Q
Clinopodium pulchellum (Kunth) Govaerts	Panizara	Lamiaceae	N	S, A	Plant	Q
Geranium ruizii Hieron .	Pasuchaca	Geraniaceae	N	S	Leaf	J
Petroselinum crispum (Mill.) Fuss	Perejil	Apiaceae	I	C	Leaf, stem	Q, Y
Desmodium molliculum (Kunth) DC.	Pie de perro	Fabaceae	N	S	Foliage	Q
Sanguisorba officinalis L.	Pimpinella	Rosaceae	I	C, A	Foliage	Q
Calceolaria argentea Kunth	Romerito	Calceolariaceae	N	S	Leaf	J

Species	Vulgar name	Family	Origin	Biological state	Used part	Region
Rosmarinus officinalis L.	Romero	Lamiaceae	I	C	Leaf	Q
Ruta graveolens L.	Ruda	Rutaceae	I	C	Leaf, flower	Q, Y
Myrcianthes myrsinoides (Kunth) Griffin	Rumilanche	Myrtaceae	N	S	Leaf	Q
Linum prostatum Dombey ex Lam.	Sangrenaria	Linaceae	I	C, A	Seed	Q
Sambucus peruviana Kunth	Sauco	Adoxaceae	N	C	Leaf	Q, J
Tillandsia sp	Siempre viva	Bromeliaceae	N	S	Leaf	Q
Stachys arvensis L.	Supiquewa	Lamiaceae	N	S, A	Foliage	Q
Caesalpinia spinosa (Molina) Kuntze	Taya	Fabaceae	N	S, C	Fruit, leaf	Y, Q
Melissa officinalis L.	Toronjil	Lamiaceae	I	C	Leaf	Y, Q
Mauria heterophylla Kunth	Trinidad	Anacardiaceae	N	S	Leaf	Q
Valeriana pilosa Ruiz & Pav.	Valeriana*	Caprifoliaceae	N	S	Root-rhizome	J
Culcitium canescens Humb. & Bonpl	Vira vira*	Asteraceae	N	C	Foliage	J
Smallanthus sonchifolius (Poepp.) H. Rob.	Yacón	Asteraceae	N	C	Leaf, root	Q

**C: cultivated; S: wild; A: arvense. N: native, I: Introduced. Y: Yunga, Q: Quechua, J: Jalca.*

Table 4.
List of medicinal plants collected in the city of Cajamarca, Peru: 2017–2018.

2003	2018
Manzanilla	Manzanilla
Valeriana	Valeriana
San pedro	Pie de perro
Eucalipto	Ciprés
Berro	Escorzonera
Laurel	Apio
Flor blanca	Supiquehua
Manzanilla macho (*Tanacetum* sp.)	Alcachofa
Chinchimalí	Nogal
Andacushma	Menta

Source: [31, 43].

Table 5.
Main species, according to the volume of collection in the Cajamarca market: 2003 and 2018.

In this value chain, the least favored agent is the gather-producer, who in some cases is also a local gather. However, their tenure in business for more than 10 years indicates that these agents have attractive incomes. Our inquiries through friendly conversations with gatherers in the city of Cajamarca, indicate that the stockers put into play various strategies that ensure profitable economic income, every time they take or send their products to the coastal markets: they arrange orders from buyers interested in certain species, they direct their attention to the species with the highest demand and whose prices remain more stable, without neglecting to offer diversity; they do not store and do not travel if the market is not good, due to the entry of plants from other areas; they are attentive to the rise in demand and prices of certain species.

This perception is different from that reported by other authors [37], who followed a gather – stocker (Julia) from Chigdén (San Juan district), 37 kilometers from the city of Cajamarca, to the Chiclayo market (Moshoqueque) and estimated that after working for 3 days (from gather and purchase), in the best case (it sold everything) it earned 6.45 US dollars (22.51 soles) and in the worst case scenario (it sold below the average) it earned 2.80 US dollars (9.77 soles). Two questions arise here: Is this a representative case of what happens regularly to gatherers-stockers? And, would it make sense to Julia and her 20 colleagues who did the same, to continue in this business when their earnings are at the indicated level?

Species that achieved best prices (2017–2018) were valeriana and taya, followed by ambarina, bolsa de pastor, alcachofa, apio, manzanilla, orégano, hierba luisa y culén. The last five have dual purpose, are medicinal and both aromatic and, used in teas. The species that generally have the lowest price are eucalipto, ciprés, rumilanche and trinidad. The average sale price of each bundle may be temporarily distorted, due to the rise in the price of a certain product. For example, at one point in the evaluation, the price of the bundle of fresh yacon leaf, paid to the producer, reached S/. 10 and the coast stocker sold it by S/. 25/bundle.

5.1.4 The other links

If the plants are already in the warehouses of the wholesale stockers of the coast one part goes to supply the local markets of the same cities and another, to the markets of other cities such as Lima, Piura, Sullana and Tumbes. A third part is directed to primary transformers and laboratories.

Transformation is one of the fastest growing areas in the country in recent decades. It can be small formal companies that do primary artisanal transformation, such as dehydration, crushing, grinding, juices, extracts and syrups; including packaging and labeling, with minimal quality control. There are cases where, the raw material (medicinal plants) is transported from Cajamarca for 6 to 7 hours to Chiclayo or 18 hours to Lima and then returns billet or shredded, bagged and labeled and, reenters the market the city. There are also medium and large pharmaceutical and organic products companies, with recognized laboratories, located in Lima, which have a sanitary registry and are the ones that process most of the natural products found on the market (syrups, extracts, pills, capsules, gels, ointments, etc.). These companies have grown in number and their presence in the conventional market and online is remarkable. They offer in addition, dried plants and seeds. However, they face the problem of adulteration and counterfeiting of their products. The products of laboratories (formal and informal) in its various presentations, is targeting processed products stores nationwide, known as generically *Naturist centers.* An evaluation in 10 centers (34% of the total) of Cajamarca indicated that there are 202 processed products, which include 170 species (according to label).

In this activity there are two types of small businesses: One sells only laboratory processed products and the other, sells processed products and additionally offers artisan preparations, according to the condition indicated by the client. Processed products can also enter markets through the herbalist's posts and peddlers. The sale of processed products has grown enormously and there are new ventures in distribution and retail sale, and even courses are offered for local entrepreneurs for this purpose.

In Peru, the DIGEMID (General Directorate of Medicines, Supplies and Drugs) is the entity in charge of registering, re-registering, modifying, denying, suspending or canceling the sanitary registration of pharmaceutical products, as well as carrying out the control and sanitary surveillance of the same; in application of Law No. 29459 (2009) on Pharmaceutical Products, Medical Devices and Health Products. Supreme Decree 016-2011-SA (2011) contains its rules. The DIGEMID grants the registration as medicine, to the processed products, which are attributed, at least, a proven therapeutic property, which must appear on the label, in addition to the professional (pharmaceutical chemist) responsible. On the other hand, according to Peruvian legislation [51], natural therapeutic resources have been classified in (1) **Natural Resource for Health Use** (Art. 70)**:** any natural resource (plant, animal or mineral) that has not been processed, chopped, dehydrated or ground, and constitutes the raw material for prepared natural products or preparations. It is admitted that, if the sale label does not contain therapeutic indications, it does not require a health registration. For example, the uña de gato bark, valeriana root, boldo leaves, clay and maca powder. Natural resources are sold without prescription, both in pharmaceutical and commercial establishments, as authorized by the health registry and; (2) **Natural Product for Use in Health** (Art. 71): Simple or complex industrial elaboration, based on one or several natural resources, that uses the isolated or synergistic virtues of said resources, which have an ancestral history of recognition and use among the indigenous populations of one or more national or international cultures. Natural products can be sold with or without prescription, as authorized in the health registry.

The consumer as the final link in the chain has variable behavior, according to their economic power, social position, culture and knowledge. The main type is the one that, when faced with a condition, resorts, in the first instance, to herbs and natural products in general. Buy fresh or dried herbs to prepare at home, guided by his own knowledge or following the recommendations of a neighbor, family member or herbalist. The second type is one that, when faced with a condition, buys and consumes syrups or extracts of artisan preparation, in a peddler or health food store. This guy is increasing, due to the way of life in the city: lack of time to prepare his remedy

(and their food) and because these preparations are cheap. The third type is one who cares for his health and prevent disease through natural products processed with a certain level of assurance, which shows through the brand or label of formal laboratories. He is the typical middle-class client, who goes to naturist centers. The fourth type is the one who, when faced with a condition, for which he has not found a solution, goes to the healer or shaman. He may or may not participate in an allowance, but receives a prescription, which must be applied in the time and in the indicated way.

5.2 Collection of dry medicinal plants in the city of San Marcos (Cajamarca)

5.2.1 Collection and storage

The market for medicinal plants in the city of San Marcos, unlike the market in the city of Cajamarca, is a center for the collection of plants dried under natural environmental conditions (approximately 13–14% humidity). This was studied in two stages: The first between June and December 2017 [31], through semi-structured interviews with four wholesale stockers, direct observation of the collection process in two stockers and, dialogs with gatherers, stockers and a transporter of medicinal plants.

The second stage was carried out between October 2018 and January 2019 in 15 communities of the four most important districts, where the collection is carried out; located between 2250 and 3423 masl (Yunga Alta and Quechua regions). The districts of Chancay, Ichocán and Eduardo Villanueva were not included (it is estimated that in these districts there are at least 10 more communities where collection is carried out). Twenty-two gatherers –12 women- (out of an estimated total of 45) and 11 local stockers –6 women- were included. Semi-structured interviews and direct observation were conducted with two wholesale stockers, and two gatherers were accompanied in their work of collecting and drying plants. More than 50% of the gatherers and stockers were over 50 years old 77% of gatherers had complete or incomplete primary education and; 59% of the stockers had between 4 and more than 6 years in this activity [52].

Collection is carried out in wild areas (which are also grazing), except in three cases (eucalipto, nogal y aliso) it is carried out around the cultivated fields. This dependence on wild areas and the lack of technical criteria on the rates and good collection practices mean that the main threats to the sustainability of the supply are overgrazing, the burning of natural vegetation, the overcollection, expansion of the agricultural frontier and mining [34, 53] --21% of the province's territory was under concession -- [49].

The collected plants are taken home, where they are dried in the shade, until the humidity is balanced with the ambient humidity. In tests carried out with eucalipto y cola de caballo, it was found that the weight losses in drying are 60% and 70%, respectively. In the informal value chain described by the species collected in this market (**Figure 3**), a new destination is added, in relation to the plants collected in the city of Cajamarca. This is the market of the city of Nueva Cajamarca, in the department of San Martín (**Figure 1(9)**). Its agents act with minimal coordination and the interest of the wholesale stockers of San Marcos and the Coast prevails. In all cases, the collection and sale of medicinal plants is a secondary activity in the generation of economic income (the main activity is agriculture-livestock). The income for the gatherers, for this concept, can vary from 100 soles (US $ .29)/year to more than 1000 soles (US $. 287)/year.

5.2.2 The collected species

In this market 37 species belonging to 20 botanical families are collected. Three are trees and the rest are herbs (18) and shrubs (16). Five are introduced from other continents and the rest are native to the Andes. Three are cultivated and the

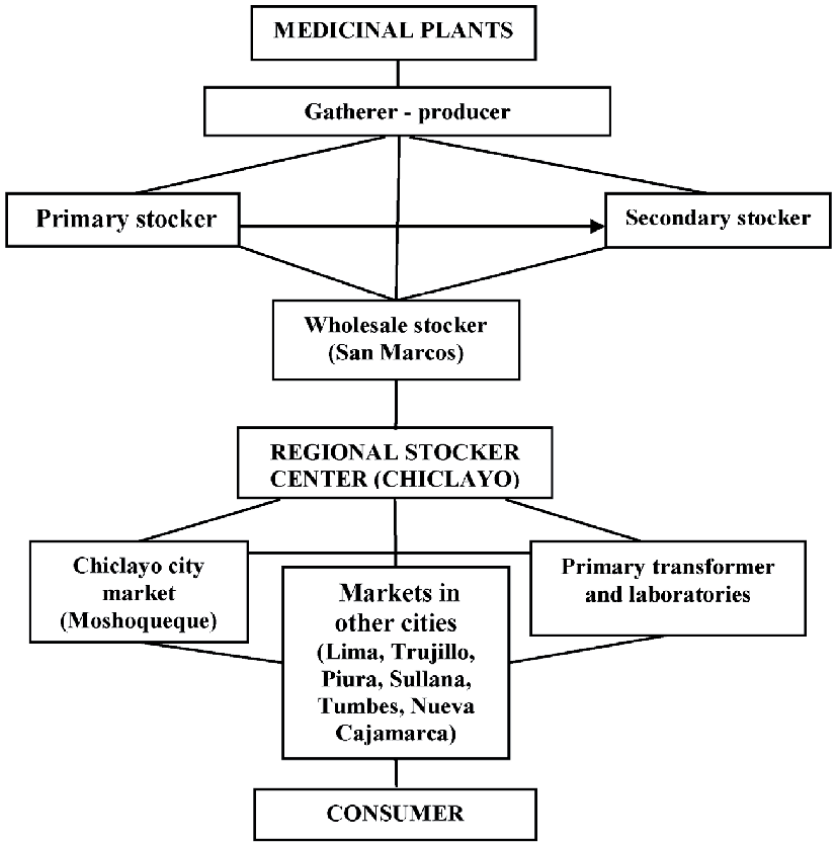

Figure 3.
Diagram of the informal value chain of medicinal plants collected in the city of San Marcos, Cajamarca, Peru.

difference, wild. In 65% of cases the leaf is used. All, except one, come from the Quechua region (**Table 6**). Fifteen of these species are the same as those collected in the city of Cajamarca, but in a fresh state.

5.2.3 Volume and economic value of medicinal plants collected in San Marcos

In the first stage of the study, the total collected volume was calculated by direct observation and the daily purchase records of the four wholesale stockers. In the second stage, we worked with two wholesale stockers and the volume collected by the other two was estimated, based on shipments to the coast. The four stockers compile 510.33 tons / year, of the 37 species. The ten species with the highest collection volumes (t/year) were: pul (83.8), flor blanca (76.9), purenrosa (62.5), lanche (42.8), pie de perro (39.5), chancua (39.0), romero blanco (35.7), zarcilleja (15.0), ishpingo (14.2) y chilca (12.4). However, the species with the best market prices (S./kg) were té de indio (12.6), flor blanca (6.4), pul (3.0), pie de perro (2.0), rumilanche (1.6), escoba amarga (1.6), lanche (1.5) y; chancua, chilca y purenrosa (1.4). Price distortions were also presented, for example, in February - 2018, the price paid to the gatherer of pie de perro rose from 2 to 12 soles/kg, due to the demand for a company of Lima.

The volumes of medicinal plants collected in San Marcos (510.33 t/year), at the gatherer - producer level, mean 724672.86 soles (US $. 207642.65), if sold to a primary stocker and 882876.09 soles (US $. 252973.09), if sold directly to the wholesale stocker of San Marcos. On the other hand, at the level of the wholesale stocker

Species	Vulgar name	Family	Origin	Biological state	Part used	Region
Alnus acuminata kunth	Aliso	Betulaceae	N	C	Leaf	Q
Chuquiraga weberbaueri Tovar	Amaro o Amarro	Asteraceae	N	S	Leaf	Q
Tagetes filifolia Lag*	Anís	Asteraceae	I	S	Foliage	Q
Equisetum giganteum L.*	Cola de caballo	Equisoceae	N	S	Leaf	Q
Otholobium glandulosum (L.) JW Grimes*	Culén	Fabaceae	N	S	Leaf	Q
Minthostachys mollis (Benth.) Griseb.*	Chancua	Lamiaceae	N	S	Leaf	Q
Aristeguietia discolor (DC.) R. M. King & H.Rob	Chilca, Chilca negra	Asteraceae	N	S	Leaf	Q
Cheilanthes myriophylla Desv	Dominio	A diantaceae	N	S	Leaf	Q
Schkuhria pinnata (Lam.) Kuntze ex Thell	Escoba amarga	Asteraceae	N	S	Foliage	Q
Eucalyptus globulus Labill*	Eucalipto	Myrtaceae	I	C	Leaf	Q
Iresine weberbaueri Suess	Flor blanca	Amaranthaceae	N	S	Flower	Y
Achyrocline alata (Kunth) DC.*	Ishpingo Amarillo	Asteraceae	N	S	Foliage	Q
Gnaphalium dombeyanum DC.*	Ishpingo blanco	Asteraceae	N	S	Plant	Q
Myrcianthes discolor (Kunth) McVaugh	Lanche	Myrtaceae	N	S	Leaf	Q
Morella sp.	Laurel de campo	Myricaceae	N	S	Leaf	Q
Niphidium crassifolium (L.) Lellinger	Lengua del ciervo, calaguala	Polypodiaceae	N	S	Stem	Q
Myrsine sp.	Mangle	Primulaceae	N	S	Leaf	Q
Oreopanax eriocephalus Harms	Maqui maqui	Araliaceae	N	S	Leaf	Q
Piper aduncum L.*	Matico	Piperaceae	N	S	Leaf	Q
Piper barbatum Kunth	Mogo mogo	Piperaceae	N	S	Leaf	Q
Dendrophthora peruviana Kuijt	Muca, popa amarilla	Viscaceae	N	S	Leaf	Q
Juglans neotropica Diels*	Nogal	Juglandaceae	N	C	Leaf	Q

Species	Vulgar name	Family	Origin	Biological state	Part used	Region
Clethra fimbriata Kunth	Olvido	Clethraceae	N	S	Leaf	Q
Clinopodium pulchellum (Kunth) Govaerts*	Panizara	Lamiaceae	N	S	Leaf	Q
Desmodium molliculum (Kunth) DC.*	Pie de perro	Fabaceae	N	S	Foliage	Q
Coreopsis senaria S. F. Blake & Sherff	Pul	Asteraceae	N	S	Leaf	Q
Bejaria resinosa Mutis ex Lf	Purenrosa	Ericaceae	N	S	Flower, leaf	Q
Clinopodium sericeum (C.Presl ex Benth.) Govaerts	Romero blanco	Lamiaceae	N	S	Leaf	Q
Myrcianthes myrsinoides (Kunth) Griffin*	Rumilanche	Myrtaceae	N	S	Leaf	Q
Ephedra americana Humb. & Bonpl. Ex Willd.	Suelda con suelda	Ephedraceae	N	S	Foliage	Q
Clinopodium sp.	Te de indio, romero de jalca	Lamiaceae	N	S	Leaf	Q
Lycopodium clavatum L.	Trencilla	Lycopodiaceae	N	S	Plant	Q
Mauria heterophylla Kunth*	Trinidad, tres hojas	Anacardiaceae	I	S	Leaf	Q
Valeriana pilosa Ruiz & Pav.*	Valeriana	Caprifoliaceae	N	S	Root-rhizome	Q
Culcitium canescens Humb. & Bonpl.*	Vira vira	Asteraceae	N	S	Foliage	Q
Brachyotum radula Triana	Zarcilleja hoja grande	Melastomataceae	I	S	Leaf	Q
Brachyotum quinquenerve (Ruiz & Pav.) Triana	Zarcilleja hoja chica	Melastomataceae	I	S	Leaf	Q

**Also collected as fresh plants in the market town of Cajamarca.*

Table 6.
List of medicinal plants collected in the province of San Marcos (Cajamarca): 2019.

of the Coast (when he sells to the other agents of the chain) means 1388105.76 soles (US $. 397738.04). This implies 33.1% of the value of medicinal plants in the Trujillo and Chiclayo markets [39].

6. The collection of medicinal plants, in Cajamarca, in times of the COVID-19 pandemic

The observation made in the cities of Cajamarca and San Marcos, between June and July 2020, at a time when the pandemic has not ceased, indicated the following: In the city of Cajamarca, the stockpiling for shipments to the coast was paralyzed, but the gatherers worked during the quarantine to supply the city, which increased demand and achieved better prices. The weight of each bundle fell by approximately 50% and the price paid to the gatherer-producer rose from 0.35 to 1.00 soles. Also, the retail sale price rose from 0.5 to 1.50 soles/bundle. The species with the highest demand were eucalipto, matico and cascarilla –leaf-- (*Cinchona* spp.). The latter comes from Jaén (174 km from the city of Cajamarca) and the bundle was sold for 5 soles. Other species with high demand were ortiga, ciprés, manzanilla, romero, kión, limón (*Citrus limon* (L.) Osbeck) and ajo (*Allium sativum* L.). In San Marcos, collection and stockpiling were stopped for 3 months (March–June). For a stocker, this stoppage meant a loss of 40,000 soles (US $ .11641.3), approximately. When stockpiling was restarted, the main demand was for matico, eucalipto y manzanila. The latter species is not normally stockpiled in this market. Prices paid to gatherers rose from 0.95 to 2.6 soles/kg in matico and from 1.04 to 2.17 in eucalipto.

7. Conclusions

Inventories of medicinal plants in the countries of South America are incomplete. Similarly, studies on species that supply markets and their commercialization are incomplete.

The department of Cajamarca (Peru) is an important center for the collection, production, use and commercialization of medicinal plants of pre-Inca origins. Currently, the capital city (Cajamarca) is supplied with 470 medicinal species, most of them wild and native to the Andes. In this city there are various intermediaries in the market for medicinal plants, of which one of the most important is the stocker that supplies the markets of the Coast.

The two informal value chains that are developed in this area make minimal quality control, add little value to the products and the predominant actors are the local and coastal wholesale stockers (Chiclayo and Trujillo). Gatherer-producers are informal, without organization, for whom the sale of medicinal plants is a secondary activity in the generation of economic income. The transformation is a growing area and the transforming companies and formal laboratories face the problem of the adulteration and counterfeiting of their products. The consumer of the cities uses products with little security and guarantee.

Most medicinal plants are collected from the Quechua region, few are cultivated, and the main threat in Cajamarca is mining. Others are agricultural expansion, overgrazing, burning of natural vegetation, and overcollection. In San Marcos, the threats in order of importance are overgrazing, burning of natural vegetation, agricultural expansion, and overharvesting and mining.

The commercial value of the plants collected in Cajamarca (fresh) and in San Marcos (dry) is equivalent to US $ 804,333.64 (67% of the market value of the plants sold in the Trujillo and Chiclayo markets).

In the first 3 months of the COVID-19 pandemic, there were no shipments to the coast, from both markets. Demand increased in the city of Cajamarca, especially for eucalipto, matico y cascarilla. Other species with high demand were ortiga, ciprés, manzanilla, romero, kion, limón y ajo. The weight of bundles fell by 50% and prices rose by more than 200%. In San Marcos, when the stockpile was restarted, the main demand was matico, eucalipto y manzanilla.

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Chapter 15

Case Study of Bacterial Decontamination of an Aromatic and Medicinal Plant: Decontamination of Thymus Satureioides by Gamma Radiation at Low Doses and Impact on Hygienic and Physicochemical Quality

Mahrouz Mostafa, Lahnine Lamyae, Mghazli Safa, Mourad Ouhammou, Mohammed Mouhib and Moulay Ali Misdaq

Abstract

The purpose of our study is to verify the usefulness of gamma irradiation treatment at low doses (0.25, 0.5 and 1 kGy) combined to vacuum packaging on commercial teas of Thymus satureioides deliberately contaminated with *Escherichia coli*. The efficiency and the influence of the process on contamination level and the shelf life of the product were studied. The phenolic composition and concentration were identified in the unirradiated and irradiated thyme. The total phenolic content (TPC) was assayed by the Folin-Ciocalteu method, the individual phenolic compounds were determined by high liquid chromatography (HPLC) and the essential oil was characterized by gas chromatography coupled to mass spectroscopy (GC-MS). The plant was observed by scanning electrons microscopy and the radioactivity effect was analyzed. The results show a complete decontamination of thyme depending to the dose and the storage time. Privileged hygienic quality was found in the irradiated thyme with the highest concentrations of polyphenols. The process showed the conservation of thyme quality without any alteration of its characteristics or radioactivity effect.

Keywords: decontamination, gamma irradiation, polyphenol, radioactivity, thymus satureioides, quality

1. Introduction to the ionization process

The irradiation of food (also called ionization) is a process using radiation (photons, electrons, X-rays) of sufficient energy. It ensures optimal hygiene quality

or prolongs the shelf life and marketing of food, reduces losses during storage (destruction of insects) or substitutes for chemical substances, to present an alternative solution to autoclaving for sterile feeding (cosmonauts, immunocompromised patients, ...) or other applications for example of a technological nature (improvement of extraction yield in fruit juice ...). The term "radapertisation" is used to describe the ionization applied to prepackaged foods whose enzymes have been inactivated, at specified doses so that no alteration or toxicity occurs regardless of the conditions and duration of storage (in the absence post-trial contamination) [1, 2].

The interest of ionization in agribusiness is twofold. It essentially lies in the penetration of radiation into the heart of the foodstuff through the packaging (thus avoiding any recontamination) and without raising the temperature (fresh or frozen products). Unfortunately this process is poorly known and is considered with suspicion by some consumers in whom it evokes in a completely unfounded and irrational way a "radioactive danger" and its possible risks [3–8].

To treat foodstuffs by ionizing radiation consists in subjecting them to the direct action of certain electromagnetic, electronic or photonic radiations, of sufficient energy, so that they can be preserved more or less long while keeping to their best qualities organoleptic, hygienic and nutritional.

Ionization is now the term used, on the one hand to distinguish it from other irradiation treatments and on the other hand, because the term "irradiation" is misleading, in the sense that it inevitably leads to false associations of ideas such as: "irradiated products are radioactive" [9].

For the decontamination of food powders, three ionizing sources are used:

- The γ radiation corresponds to the spontaneous emission of photons by the nucleus of a radioactive isotope (γ-rays of cobalt-60, more rarely those of cesium-137 (for research) (authorized dose between 2 and 10 kGy) [1, 10].

- X-rays are electromagnetic waves emitted by the deep electronic layers of atoms. Their wavelength is between 0.01 and 10 nm (authorized dose: 5 MeV) [1].

- Accelerated electron beams or beta radiation refer to a displacement of electrons created from a source (authorized dose: 10 MeV).

These types of radiation have been chosen because they produce the desired effects on the food, without inducing radioactivity within the product or packaging and allow industrial use in terms of quantity and cost of operation [11].

The irradiation technology has undeniable advantages for the decontamination treatments of heat-sensitive products, rich in active ingredients such as herbs and spices.

In agrifood, the most used irradiation is ionization by gamma radiation. Gamma treatment has been approved by international bodies, namely FAO, IAEA and WHO. The report of the Joint Expert Committee, published in 1980, recommended irradiation as a safe process to achieve hygienization without causing nutritional, microbiological or toxicological concerns [12–14].

Gamma emission is produced by the decay of radioactive nuclides. The isotopes 60 of cobalt and 137 of cesium were chosen because the energy of the radiations they emit is 10 times lower than the minimum threshold allowed. As a result, they are not responsible for any induced radioactivity.

For this type of radiation, the dose is usually expressed in Gray. This dose is equivalent to the absorption per kg of ionized food, with an energy of 1 Joule (1 J/kg). The dose of radiation must be adapted to each case:

- The minimum radiation dose is the amount needed to achieve the desired effect on the food product [10].

- The maximum dose is given by the regulations in force and depends on the food.

- The key parameter of irradiation is the absorbed dose, which determines disinfection (<1 kGy), pasteurization (1–10 kGy) or sterilization (>10 kGy) [15].

The Directive 1999/3/EC [16] has declared a list of foods and food ingredients that can be processed by gamma radiation. The maximum overall average dose that can be absorbed is 10 kGy for dried aromatic herbs, spices and vegetable seasonings. The Food and Drug Administration (FDA) has limited the dose to 30 kGy for culinary herbs, seeds, spices, vegetable seasoning, and mixtures of these aromatic vegetable substances [17].

For aromatic plants, an ionization between 1 and 10 kGy provides a total decontamination of germs. However, some species just require low doses of the order of 1–2 kGy [12, 15].

These types of radiation have been chosen because they produce the desired effects on the food, without inducing radioactivity within the product or packaging and allow industrial use in terms of quantity and cost of operation [18]. Thus, irradiation is used in agri-food for various applications such as: inhibition of germination, disinsectisation, lengthening the shelf life of perishable foods, delaying the maturation and aging of fruits and vegetables, the destruction of parasites and the fight against food poisoning [2, 3, 8, 13, 14, 18].

The effects of ionizing radiation result from a transfer of energy to the material that ionizes or excites molecules and atoms. Among the molecules that undergo these effects, two have a particular importance: the water molecule, given its abundance, and the DNA molecule (deoxyribonucleic acid), because of its major biological function (expression of the genes that govern the functioning of the cell and duplication of the entire genetic heritage during cell division) and its unique presence in the nucleus of each cell. Ionizing radiation can induce various modifications especially in the structure of DNA:

- Oxidation of deoxyribose and release of a base by hydrolysis,

- Bridging between bases of the same strand or between bases belonging to two different strands,

- Hydroxylation of bases and in particular of thymine, with formation of peroxides in the presence of oxygen,

- Single-strand breaks between the base and the sugar of a nucleotide, essentially produced by the action of hydroxyl radicals,

- Alterations of bases and destruction of sugars.

The action of ionizing radiation corresponds to a damage to the membranes and nucleic acids of the microorganisms while preserving the integrity of the constituent atoms molecules of living products [19]. Ionization virtually eliminates pathogenic microorganisms (including sporulated forms) and does not alter the nutritional properties and organoleptic qualities of ionized products [18, 20].

Gram- bacteria are even more sensitive than Gram + and spores ever more resistant than vegetative cells, although some species have proved very resistant in their vegetative form. Viruses are very resistant to radiation [21]. A log reduction of 2 units on mold was observed on coffee beans with a dose of 5 kGy. Thayer et al. [22] determined that a treatment of 2.8 kGy on alfalfa seed with a source of Cesium 137 at a dose of 0.1 kGy/min allowed a reduction of more than 3 log cycles of Salmonella Mbandaka. They evaluate a destruction of 5 logarithmic cycles for a dose of 4.05 kGy. Many factors strongly influence the effectiveness of these treatments: the composition of the medium, the activity of the water, the temperature during the treatment, the presence of oxygen (which increases the sensitivity) and the freezing (the dose necessary for inactivation of 90% of the population is 2–3 times higher in frozen foods than in fresh foods). Doses of 5–7 kGy have been shown to be effective against yeasts, molds and spores [23].

Researchers have shown the effect of gamma radiation on some plants, especially the effect on the phenol composition. This research has approved the increase in phenols in plants after irradiation which leads to the improvement of the phenolic compounds content of these plants and therefore the increase of antioxidant power [2, 3, 9, 14, 20, 24].

Researchers have shown a significant quantitative and non-qualitative increase in the composition of the polyphenols of *Thymus vulgaris* and Menthapulegium compared with a non-irradiated control, following the application of relatively high doses of 10, 20 and 30 kGy [25]. However, other researchers have confirmed a slight increase in total polyphenol content in cumin seeds by applying doses of 1, 3 and 5 kGy. Thus, they showed no significant change in phenolic content by comparing non-irradiated Nigellastaiva seeds with those irradiated at doses of 2–16 kGy [26]. Mahrouz et al. observed a significant increase in the phenolic compounds of Citrus clementina and this is due to the maximal activity and stimulation of phenylalanine ammonia-lyase (PAL) following the application of a low dose of 0.3 kGy [27].

Often ionization alone cannot produce the desired results [18, 27–29]. In particular, the necessary dose is unsupportable by the product, the cost is very high and limits the ionization [30]. Thus, researchers have combined irradiation with other preservation methods:

a. **Ionization and refrigeration**: The protocol is based on the use of medium doses combined with a low temperature preservation of 0–5°C. This process is used for the preservation of fresh fruits and vegetables. Mahrouz et al. Improved the storage conditions of Moroccan clementine by combining low doses and cold at 5°C [6].

b. **Ionization and treatment in heat**: she allows to reduce the high doses of ionization harmful to the organoleptic quality of the product and to reduce the cost. The inactivation of enzymes autolytiques by the ionization of the meat requires a high dose of 200 kGy while associated with heat in 80°C, only a dose? 10 kGy is sufficient [15].

c. **Ionization and conditioning** (packaging): generally, the ionization is combined (organized) in the vacuum packing to avoid the contact between the food and the oxygen. Specially, food rich in fat is very sensitive (perceptible) to the oxygen. However, the study elaborated by Mahrouz et al. emphasized the preservation of the aromatic and medicinal plants " Menthapiperita " by gamma radiations in small doses of 1 kGy combined (organized) in the vacuum packing [31].

d. **Ionization and chemical treatment** (processing): the use of the chemical conservatives (curators) can be reduced by combination (overall) with the ionization. Nitrites used in the delicatessen, to warn (prevent) Clostridium Botulinium and which being able to be carcinogenic if they are transformed nitrosamines there, can be so reduced by the use combined (organized) by the ionization Gamma.

However, the ionization of certain foods may affect the chemical profile of these foods, either an improvement or a degradation [6, 27]. Mahrouz et al. [6] noted that irradiation at low doses of 1 kGy order of Moroccan clementine improved the organoleptic quality: sweeter taste, orange color more intense, phenolic content significantly higher than the control, ... and this during a live shelf of more than 4 weeks.

Radiation degradation products such as formaldehyde, butanone and cyclobutanone are released and can be attributed to chemical reactions responsible for the development of bad flavors and flavors during storage. The addition of essential oils of aromatic plants (rosemary, oregano, thyme, ...) or spices, combined with ionization, can reduce the dose to the extent that the active ingredients of these natural plants have an antimicrobial effect therefore improve the radiosensitivity of pathogenic bacteria and can also help improve the flavor of food. However, ionization decontamination has three major disadvantages:

- Ionization requires the implementation of very restrictive safety rules and therefore very expensive (lead envelope, security lock ...) and purchase costs are high.

- In contact with oxygen in the air, ionizing treatments cause the formation of very reactive free radicals that can cause the oxidation or hydroxylation of certain essential oils and aromatic compounds most often responsible for the organoleptic qualities of spices.

- Despite its proven effectiveness, irradiation faces the reluctance of consumers who see only a complicated and abstract technique involving nuclear energy. Faced with this psychological barrier and the obligation to label, manufacturers of powdered foods, especially spices and herbs, have chosen to opt for other techniques, which today limits the use of irradiation for the decontamination of food products [1, 19, 28].

2. Case study of bacterial decontamination of an aromatic and medicinal plant

2.1 Decontamination of thymus satureioides by gamma radiation at low doses and impact on hygienic and physicochemical quality

In the agro-food industry, the type of conservation treatment to be adopted is conditioned by the intended use of the product. Generally, Thymus satureioides is traditionally used in Morocco as herbal tea to prepare tea, which decreases the risk of contamination given the high temperature applied. However, in the industry, we cannot limit production to this single use, but we should prevent its application as flavoring already prepared foods such as salads or others. This operation could introduce cross-contamination by aromatic plants. As a result, the treatment and decontamination of the product has become an obligation based on its use.

The decontamination processes of aromatic and medicinal plants are essentially based on more or less destructive thermal processes. In addition, plants are a delicate product to handle during storage because of their richness in polyphenols. Several decontamination procedures have been developed and their effectiveness is most often at the expense of the overall quality of the product. As a result, plant conservation is ensured by athermal treatments such as fumigation, UV irradiation and hydrostatic pressures [12, 32, 33]. However, radiation decontamination remains the most used process for its bactericidal efficacy. Gamma irradiation is known as a process of decontaminating plants, materials and food products. This process is widely used in the world for the decontamination of aromatic plants, condiments and dried vegetables with a rate of 46% [34]. It allows the reduction of micro-organisms and ensures the stability of the product [18, 35].

The interest of ionization in agribusiness is twofold. It essentially lies in the penetration of radiation into the heart of the foodstuff through the packaging (thus avoiding any recontamination) and without raising the temperature (fresh or frozen products) [1, 18, 36].

The aim of our study is to evaluate the effect of low-dose gamma radiation storage combined with vacuum packaging on the hygienic and physicochemical quality of Thymus satureioides. Thus, we focused on the application of doses below 1 kGy. The treatment was carried out at the National Institute for Agronomic Research (INRA), Regional Center for Agricultural Research in Tangier, Research Unit on Nuclear Techniques Environment and Quality (URTNEQ). First, we tested the efficacy of a low dose (1, 0.5 and 0.25 kGy) on microbiological quality by studying the abatement of microorganisms after voluntary *E. coli* contamination as major contaminant of plants [12]. Second, we analyzed the impact of the process on the physico-chemical quality specifically of bioactive substances such as essential oils and phenolic compounds. In addition, we evaluated the impact of the dose on the overall quality of thyme: color, phenolic content by UV/visible spectrophotometry and HPLC, composition of essential oils by GC/MS and radioactivity. The quality of irradiated thyme was monitored during storage.

The plant lot was divided into three types of samples according to the analyzes to be carried out:

- Physico-chemical and therapeutic analyzes: preparation of 60 sachets of 25 g for each dose of irradiation
- Microorganism abatement study after deliberate contamination: preparation of 60 sachets of 25 g for each irradiation dose (3 different doses 0.25, 0.5 and 1 kGy) in addition to contaminated but unirradiated samples to identify the load bacterial.
- Uncontaminated and non-irradiated samples are used as a control for the analyzes.

2.2 Ionization protocol

The irradiation was carried out by gamma radiation with a source of cobalt (60Co) in the Boukhalef ionization station (INRA) of Tangier. After dosimetry determination, the flow rate is 1.05 Gy/min. We treated the non-contaminated samples and the contaminated samples in different doses. For each irradiation, we placed a batch of 20 samples in the irradiator. The doses chosen for the treatment are 1, 0.5 and 0.25 kGy. The treatment time was set according to the desired dose (**Table 1**).

Debit (Gy/min)	Dose (kGy)	Duration (min)
1.05	0.25	238 = 3 h 58 min
	0.5	476 = 7 h 56 min
	1	952 = 15 h 52 min

Table 1.
Ionization treatment conditions.

2.3 Observation by scanning electron microscope after irradiation

Samples were prepared for SEM observation either for unirradiated or irradiated thyme at 1 kGy. The samples are placed in the scanning electron microscope chamber (JEOL JSM 5500 LV), observed at 5 kV and photographed at different amplitudes.

2.4 Measurement of radioactivity of thyme

Les échantillons de thym irradiés ont été mis séparément dans une capsule en plastique cylindrique bien fermée en contact direct avec les deux détecteurs solides de traces nucléaires utilisés (**Figure 1**).

Since the irradiation cell is well closed, there is no escape of radon and thoron gases and the exposure time is 30 days. A secular radioactive equilibrium is established between uranium-238, thorium-232 and each of their respective progeny. The global trace densities ($cm^{-2}\ s^{-1}$) due to the alpha particles emitted by the uranium-238 and thorium-232 series recorded on the CR-39 and LR-115 type II detectors are given respectively by the following expressions:

$$\rho_G^{CR} = \frac{\pi q^2}{2\,S_d} C(U)\, d_s \left[A_U \sum_{j=1}^{8} k_j \varepsilon_j^{CR} R_j + \frac{C(Th)}{C(U)} A_{Th} \sum_{j=1}^{7} k'_j \varepsilon_j'^{CR} R'_j \right] \quad (1)$$

and

$$\rho_G^{LR} = \frac{\pi q^2}{2\,S'_d} C(U)\, d_s \left[A_U \sum_{j=1}^{8} k_j \varepsilon_j^{LR} R_j + \frac{C(Th)}{C(U)} A_{Th} \sum_{j=1}^{7} k'_j \varepsilon_j'^{LR} R'_j \right] \quad (2)$$

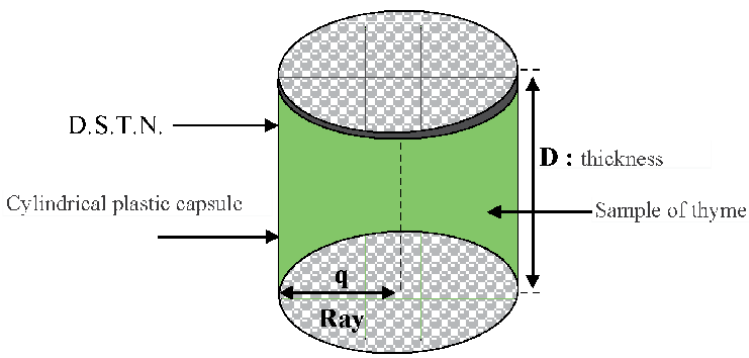

Figure 1.
Experimental device of the irradiation cell.

where:

- A_U ($Bq.g^{-1}$) et A_{Th} ($Bq.g^{-1}$) are the sample-specific activities for 1 ppm uranium-238 and 1 ppm thorium-232 respectively.
- d_s is the sample density of thyme studied ($g\ cm^{-3}$).
- S_d and S'_d are the scanned surfaces of detectors CR-39 and LR-115 type II respectively.
- R_j and R'_j are the alpha particle pathways within the studied thyme sample emitted by the families of l'^{238}U and of ^{232}Th.
- q is the radius of the capsule from which the films are irradiated.
- k_j et k'_j respectively, are the branching ratios corresponding respectively to the decay of the radionuclides of the series of l'^{238}U and of ^{232}Th.
- ε_j^{CR}, $\varepsilon_j'^{CR}$, ε_j^{LR} and $\varepsilon_j'^{LR}$ are the film detection efficiencies CR-39 and LR-115 type II for the alpha particles emitted by the two families of ^{238}U and of ^{232}Th, respectively.
- Pathways of α-particles in thyme and DSTN were evaluated using TRIM software.

By combining Eqs. (1) and (2), we obtain a relationship between the overall trace densities and the ratio of thorium contents to that of uranium, for a sample of thyme. Thus, we have:

$$\frac{C(Th)}{C(U)} = \frac{A_U}{A_{Th}} \frac{\left(\frac{S'_d}{S_d}\right)\sum_{j=1}^{8} k_j\ \varepsilon_j^{CR} R_j - \left(\frac{\rho_G^{CR}}{\rho_G^{LR}}\right)\sum_{j=1}^{8} k_j\ \varepsilon_j^{LR} R'_j}{\left(\frac{\rho_G^{CR}}{\rho_G^{LR}}\right)\sum_{j=1}^{7} k_j\ \varepsilon_j^{CR} R_j - \left(\frac{S'_d}{S_d}\right)\sum_{j=1}^{7} k_j\ \varepsilon_j^{LR} R'_j} \tag{3}$$

et

$$C(U) = \frac{2\ S'_d\ \ \rho_G^{LR}}{d_s \pi q^2\ \left[A_U \sum_{j=1}^{8} k_j\ \ \varepsilon_j^{LR} R'_j + \left(\frac{C(Th)}{C(U)}\right)\ \ A_{Th} \sum_{j=1}^{7} k_j \varepsilon_j^{LR} R'_j\right]} \tag{4}$$

By calculating the detection efficiencies of solid nuclear track detectors CR-39 (ε_j^{CR}, $\varepsilon_j'^{CR}$) and LR-115 type II (ε_j^{LR}, $\varepsilon_j'^{LR}$) using the code "SSNTDEαM", and counting the densities of traces recorded on (ρ_G^{CR}, ρ_G^{LR}), we can determine the ratio $C(Th)/C(U)$ and then the uranium and thorium contents contained in the samples of thyme studied [37].

3. Results and discussion

3.1 Microbiological or hygienic quality

Analysis of food safety indicator germs "total aerobic mesophilic flora FMAT, yeasts and molds, *E. coli*, ASR anaerobic sulfiturizer and salmonella" revealed that our lot is not contaminated and can be used to our study [38–43].

In our study, we applied low doses on a bacterial load of the order of 1.1106 CFU/g. The monitoring of the microbiological quality of the irradiated thyme (**Figure 2**) demonstrates a decontamination of the order of 98, 96 and 90% after irradiation with the doses of 1, 0.5 and 0.25 kGy respectively and these percentages tend to 100% during storage. Following this treatment, we obtained a complete elimination of *E. coli* bacteria in our samples. Such results show the effectiveness of ionization even at low doses on the debacterization of thyme contaminated with *E. coli*.

In addition, the irradiation dose of 1 kGy was more lethal with a reduction rate of 98% just after irradiation and 100% after 7 days of storage. This dose appears to be the lowest effective dose for E decontamination. Coli. These results show the effectiveness of ionization, even at low doses, on *E. coli*-contaminated thyme.

Similar effects have been observed [28, 44]. The study on freshly cut celery showed a decrease in the number of bacteria and fungi of the order of 102 and 101, respectively, with 1 kGy of irradiation and the number of *E. coli* decreased to less than 30 CFU/g [13]. Surviving colony analysis (**Figure 3**) shows the effect of irradiation doses on thyme compared with unirradiated inoculated thyme and control thyme. It is remarkable that the contamination decreases during storage even for non-irradiated inoculated thyme. This result can be explained by a synergy of effects between the irradiation treatment and the plant itself. Right after treatment, there is a direct influence of the process on the bacteria. During storage, on the one hand there is the effect of the plant's compounds including the essential oil and its compounds could be in contact with bacteria showing a bactericidal effect. On the other hand, the influence of irradiation on the metabolism of the plant by the activation of its compounds during storage.

Each point is the average of three repetitions. The analysis of the differences is made by the bidirectional analysis of the variances (ANOVA and Tukey), the main effects are the time and the dose. The same letters indicate a lack of significance at $P > 0.05$.

In the literature, the effect of irradiation has been attributed to ionizing radiation acting directly or indirectly on DNA and inducing local modifications of the double helix. The interaction with the DNA leads to ionizations and excitations that produce direct modifications of the molecule. In addition, the interaction of ionizing radiation with water molecules causes the radiolysis of water which results in the formation of free radicals. These products, very unstable, in turn, form other radical species or molecules (H_2O_2) that have a high reactivity with any nearby biological molecule

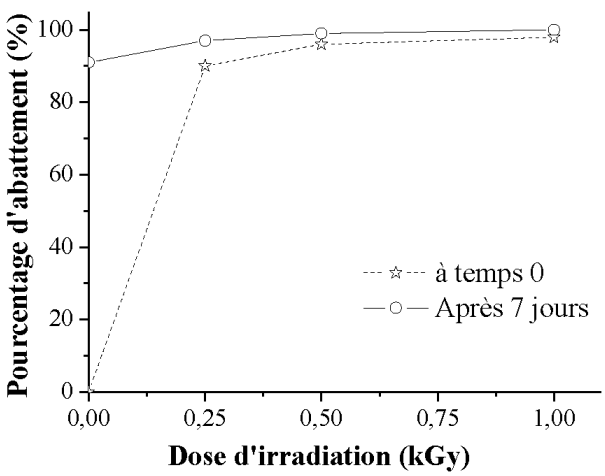

Figure 2.
Abatement of E. coli *as a function of ionization dose and storage duration.*

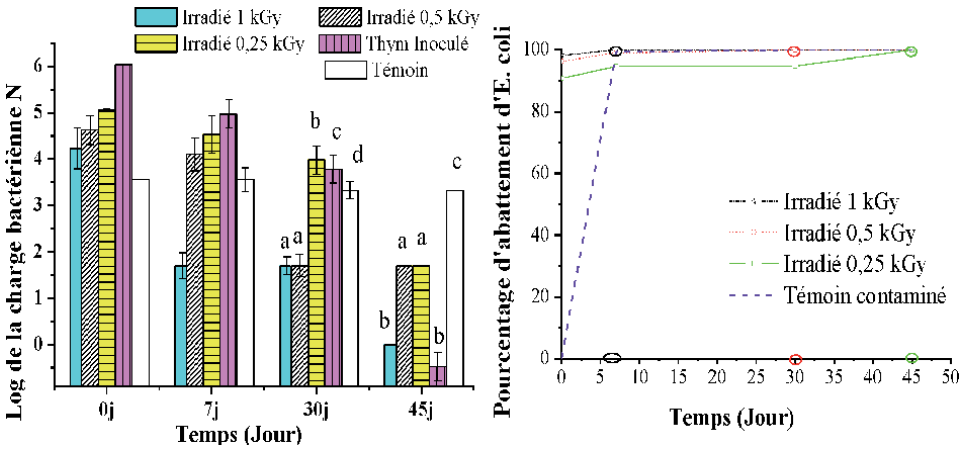

Figure 3.
Monitoring of the bacterial load according to irradiation doses and storage.

(water, protein, DNA). Their interactions with DNA produce chemical modifications of the polymer, such as oxidation [18, 45]. In addition, the increase in the treatment dose causes the decontamination effect. Similar results are presented in the case of plant decontamination (Menthaepip., Cynaraescol., Valerianae rad., Lepidiumsativum, Brassicanigra L. Koch and lemon leaves) [28, 46]. In addition, the microbicidal activity, induced by polyphenols and stressful conditions due to vacuum storage, is observed in unirradiated inoculated control thyme. Burt has demonstrated the effect of the essential oil on the cell membrane of bacteria. Due to its hydrophobic character, it penetrates cell membranes and mitochondria making them permeable and leads to leakage of cell contents [47].

3.2 Organoleptic quality: color analysis

Plant color analysis by UV/Visible spectrophotometry demonstrated maximum absorbance of thyme pigments in the wavelength of 340 nm and a slight absorbance at 630 nm and 664 nm from the absorbance of chlorophyll (**Figure 4**). The control

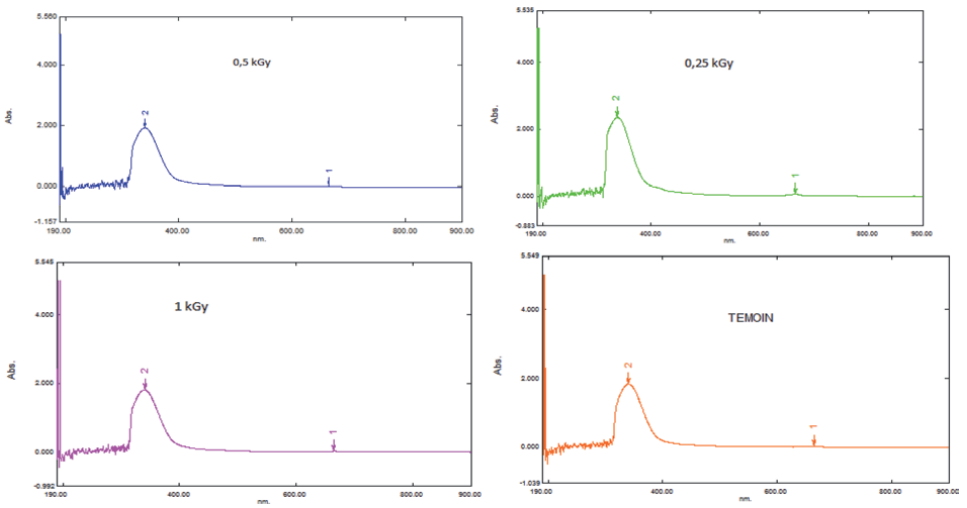

Figure 4.
Absorption spectrum of control and irradiated thyme pigments.

and irradiation thyroid absorption spectra have the same pace. Thus, we find that athermal treatment with low dose gamma radiation has no impact on the degradation of total pigments.

Similar results were reported and correlated the effect observed on the pigments at the irradiation dose. Machhour et al. [31] illustrated the absence of difference between the pigment spectra of peppermint before and after irradiation at 1 kGy. Similarly, Pinela et al. [48] have shown that gamma irradiation treatment at a dose of 1–10 kGy has no influence on the T. lignosa color parameter. Next door, Koseki et al. [35] showed that there is no evidence that radiation caused significant degradation of total β-carotene in basil, artichoke and rosemary samples irradiated at 0, 10, 20 and 30 kGy However, the irradiated Vignaradiata (L.) study of 10–100 Gy showed that gamma irradiation significantly affects photosynthetic pigments. Thus, the chlorophyll a and b content decreases with increasing gamma exposure [49].

3.3 Morphological observation of the texture by the MEB

The surface of thyme leaves before and after irradiation is shown in **Figure 5**. The glands observed on the surface of non-irradiated thyme are modified after treatment. Irradiation can cause structural abnormalities in the cell wall.

Some studies have linked this effect to sample preparation. Hammond and Mahlberg [50] associated the modification and structural alteration of *Cannabis sativa* on the one hand with fixation and dehydration. On the other hand, they noted the effect of freeze-sublimation treatment for the difference between fresh and prepared samples. Similarly, Nayak et al. [51] demonstrated a correlation between irradiation exposure of 3–12 kGy and increased cell wall permeability and changes in histological properties.

4. Quality of antioxidants: characterization of phenolic compounds

The analysis of the essential oil and phenolic compounds is used as an indicator of the quality of aromatic plants. Therefore, the composition of thyme before and after irradiation has been studied. The determination of total polyphenols (PPT) is shown in **Figure 6**. At the beginning of the treatment, the total phenol compounds content in the irradiated plant remains similar to that of the control. However, PPTs increase during storage as a function of radiation dose. This increase may be related to the effect of irradiation, which affects the chemical bonds and therefore induces the discharge of soluble phenols of low molecular weight.

Values are expressed as mean ± standard deviation (n = 3). Vertical bars represent standard deviations. Values with the same letters are not significantly different ($P > 0.05$).

The effect of irradiation has been reported for gamma irradiated plants at different doses. Khattak [5] showed a significant increase in the composition of the total polyphenols of Fagonia arabica compared to the non-irradiated control after doses between 1 and 10 kGy. Similar results were confirmed by Cheng and Breen and Mahrouz et al. [6, 52]. Similarly, Mahrouz et al. [28] correlated this change to the activation of phenylalanineammoniac lyase (PAL) biosynthesis as the first enzyme involved in the synthesis of phenolic compounds. However, Pinela et al. [29] showed a decrease in the concentration of phenolic compounds in irradiated versus non-irradiated samples. Also, Banerjee et al. [3] demonstrated the inhibition of PAL activity in irradiated shriveled cabbage. In the case of this product, the PAL enzyme influences the inhibition of browning. This finding was confirmed by a decrease in PAL gene expression just after irradiation.

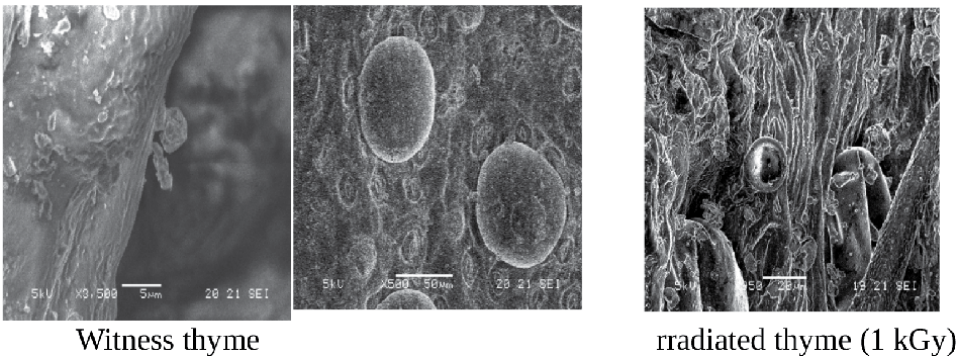

Figure 5.
Observation by scanning electron microscope of control and irradiated thyme.

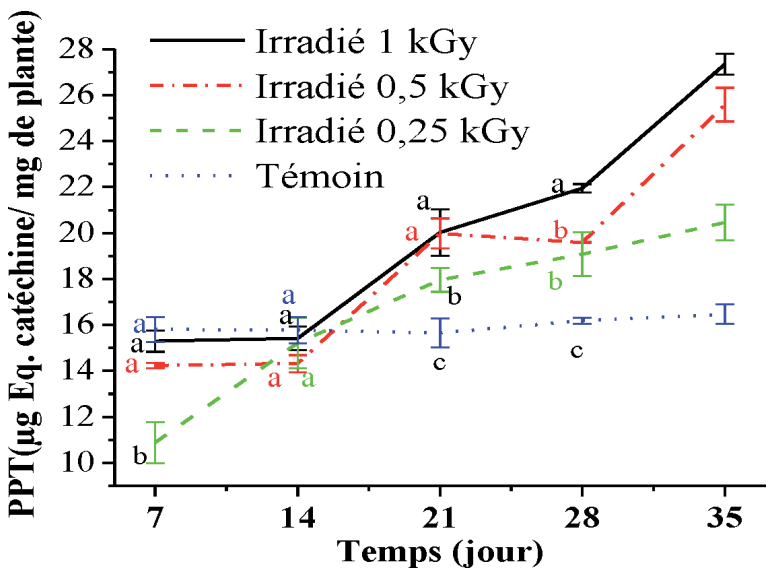

Figure 6.
The total polyphenols of thyme control and irradiated during storage.

The phenolic material extracted was analyzed by HPLC. The chromatographs of the control and irradiated thyme have been illustrated at various wavelengths. The samples showed similar profiles where at least seven peaks are observed. Rosmarinic acid, luteolin 7-O-glucoside and luteolin 3-O-glucoside predominate in all samples. In addition, caffeic acid and catechin were identified before and after irradiation. However, the other peaks are not identified. Nevertheless, previous research has reported the presence of other phenolic compounds. Ramchoun et al. [53] identified caffeic acid and catechin in Thymus satureioides and flavonoids such as rosmarinic acid, luteolin 7-O-glucoside, apigenin-7-glycoside and hesperetin. Similarly, Ismaili et al. [54] illustrated the high level of total phenols in the polar fraction of the methanolic extract of T. satureioides and correlated with the main isolated compounds: rosmarinic acid, luteolin aglycones, eriodictyol, thymonine and three flavonoids which are luteolin-3'-O-glucuronide, luteolin-7-O-glucoside and eriodictyol-7-O-glucoside. In the Van Den Broucke [55] study, three flavones were reported: cirsilineol, 8-methoxycirsilineol and thymonine. In addition, the composition obtained after irradiation is approximately similar for the different

doses compared to the control. This demonstrates that storage by gamma irradiation has no significant change in the qualitative phenolic composition.

4.1 Physico-chemical quality: analysis of the essential oil of thyme by CPG-MS

The essential oil of Thymus satureioides is characterized by its variable composition (**Table 2**). The plant studied is characterized by its richness in carvacrol (45.86%), borneol (9.89%), thymol (2.14%) and β-caryophyllene (6.88%) and a low concentration of camphene (0.28%), terpineol α- (1.87%), α-pinene (0.12%) and linalool (0.43%). This composition is quite different from the composition of the essential oils previously reported. The change in thyme composition is a function of harvest period, extraction technique and environmental conditions, including area, season and fertilization [56–58]. Some studies have shown a concentration of borneol as a stronger characteristic compound than carvacrol. As a result, the amounts of the compounds became different: borneol (23–32%), thymol (4–16%),

N°	TR	IR	Compound	Percentage (%)			
				Witness	1 kGy	0.5 kGy	0.25 kGy
1	5.58	1060	α-Thujene	0.04	0.03	0.04	0.04
2	5.75	1072	α-Pinène	0.12	0.12	0.18	0.28
3	6	1089	Camphène	0.28	0.26	0.4	0.62
4	7	1055	α-terpinènes	0.02	0.02	0.02	0.03
5	7.13	1064	*p*-cymène	0.21	0.16	0.19	0.44
6	7.53	1090	Limonène	0.04	0.04	0.03	0.05
7	7.65	1098	γ-Terpinènes	0.12	0.09	0.09	0.17
8	7.8	1008	(E)-Sabinene hydrate	0.08	0.06	0.06	0.07
9	8.23	1038	Linalol	0.43	0.51	0.53	0.47
10	9.03	1092	Camphor	1.74	0.4	0.53	0.24
11	9.38	1017	Bornéol	9.89	15.02	14.01	8.92
12	9.68	1039	α-Terpinéol	1.87	2.52	0.25	2.32
13	10.1	1067	Bornyl acétate	0.01	0.01	2.58	0.05
14	10.5	1097	NI	10.3	1.66	0.21	0.25
15	11	1038	Thymol	2.14	2.48	2.33	2.3
16	11.2	1053	Carvacrol	45.86	40.81	42.17	36.05
17	11.4	1067	NI	4.53	6.45	7.09	6.38
18	12.4	1042	Copaène	0.23	0.36	0.29	0.48
19	13.1	1000	β-caryophyllène	6.88	8.59	8.09	6.85
20	13.4	1021	NI	0.09	0.21	0.22	0.26
21	14.2	1087	NI	1.2	1.43	1.7	1.39
22	15	1062	Caryophyllène oxyde	0.64	0.93	0.95	1.03
23	15.6	1017	NI	1.47	2.84	2.36	3.76
			Total	88.19	85	84.32	72.45

NI: non identified; TR: retention time (min); IR: Kovats retention index.

Table 2.
Composition of essential oils extracted from control and irradiated thyme.

Samples	ρ_G^{LR} (10^{-5} cm^{-2} s^{-1})	ρ_G^{CR} (10^{-5} cm^{-2} s^{-1})	C (^{238}U) (10^{-6} g/g)	C (^{232}Th) (10^{-6} g/g)
Non irradiated	1.3 ± 0.05	5.62 ± 0.22	0.36 ± 0.01	0.63 ± 0.03
Irradiated at 1 kGy	1.35 ± 0.07	5.84 ± 0.29	0.41 ± 0.02	0.72 ± 0.04
Irradiated at 0.5 kGy	1.32 ± 0.08	5.73 ± 0.34	0.39 ± 0.02	0.68 ± 0.04

Table 3.
Concentration of uranium C (238U) and thorium (232Th) of control and irradiated thyme.

camphene (7–27.4%), α-terpineol (6–11%), α-pinenes (17.5%) and linalool (6.3%). In addition, the concentration of certain compounds is modified after treatment with gamma irradiation. This effect is observed depending on the dose applied specifically 1 and 0.5 kGy. The effect of irradiation is investigated by the increase of borneol (15.02%) and β-caryophyllene (8.59%). A slight effect was observed on thymol (2.48%) and caryophyllene oxide (0.93%). However, the amount of some compounds decreased, especially carvacrol (40.81%), p-cymene (0.16%) and camphor (0.4%). The results showed that the content of borneol increased significantly while carvacrol decreased. Similarly, the influence of irradiation has been observed on other unidentified compounds.

By analogy, the investigation of the biosynthesis of phenolic compounds in fresh coriander seedlings (culantro) showed a significant increase of flavonoids, anthocyanins and flavonone in the plant irradiated at a dose of 40 Gy compared to the control. However, flavonols decreased by increasing the dose of irradiation [29]. Similarly, an analysis by Farag et al. [59] demonstrated the change of monoterpene hydrocarbons to terpene alcohol in the essential oil of irradiated black pepper.

4.2 Radioactive quality: analysis of thyme radioactivity

The results of the radioactivity are shown in **Table 3**. These results show that there was no difference in concentration of uranium (238U) and thorium (232Th) in unirradiated and irradiated thyme. As a result, there was no radioactivity following gamma radiation, except natural radioactivity.

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Chapter 16

Plant-Based Drugs as an Adjuvant to Cancer Chemotherapy

Lakshmi Mohan

Abstract

Humans have turned to natural products, obtained from plants, animals and aquatic life for treating diseases since time immemorial. Modern medicine is based on ancient wisdom transferred over generations. Drug development relies mainly on natural sources. Herbal medicines are making a comeback due to lower side effects, and positive results in the long term when compared to synthetic drugs. The current drug discovery process relies on identifying traditional medicines followed by Bioactivity-guided fractionation to isolate significant lead molecules. Plants have a history of long-term use by humans and hence it can be presumed that the bioactive compounds obtained from plants will have low human toxicity. There exists a huge potential for discovering new antitumor drug leads by screening natural products either in the form of crude extracts purified phytochemicals which have already been described in the literature. The fact that phytochemicals like paclitaxel, vinblastine, vincristine and camptothecin are being successfully used in clinical practice and several others like combretastatin and noscapine are in different stages of clinical trials implies the importance of plants in cancer chemotherapy.

Keywords: plant, medicinal plants, cancer, alternative therapy, synergy

1. Introduction

> *"Until man duplicates a blade of grass, nature can laugh at his so-called scientific knowledge. Remedies from chemicals will never stand in favourable comparison with the products of nature, the living cell of the plant, the final result of the rays of the sun, the mother of all life." - Thomas Alva Edison.*

The global cancer burden has escalated to 9.6 million deaths and 18.1 million new cases in 2018. It is a fact that one in 5 men and one in 6 women around the world develop cancer during their lifetime, and it kills one in 8 men and one in 11 women [1]. Cancer continues to be the second leading cause of death globally, the first being cardiovascular diseases. Patients with cancer normally have a poor prognosis in low and middle-income countries such as India, due to a lack of awareness about the disease, delayed diagnosis, and inadequate or no access to affordable therapeutic services when compared with patients in high-income countries. The number of incident cancer cases in India is estimated to be 1 069 000 in 2016. Access to critical cancer treatment is also very low in the country. It is the need of the day to find a natural, affordable treatment strategy for cancer [2].

The conventional modality for cancer treatment involves the use of surgery, radiation and chemotherapy either alone or in combination with the others. Each of the treatment modality offers its risks and benefits. Although chemotherapeutic medicines are toxic and have a very narrow therapeutic index, they offer a transient relief from symptoms and help prolong life especially in the case of cancers where surgery and radiation is not a feasible mode of treatment like leukaemia and lymphomas. Chemotherapy is a systemic treatment because it can be used to treat cancer anywhere in the body when compared to local treatment approaches. The chemotherapeutic agents that are currently used in clinical practice lack specificity to the cancer cells and could damage the healthy cells causing adverse side effects. Toxicity and severe side effects continue to be significant setbacks involved in chemotherapeutic approaches to the treatment of cancer. To overcome the limitations, scientists across the globe are searching for new anticancer agents with more specificity and fewer side effects. Many recent studies have found that an extensive array of natural substances exert selective toxicity against cancer cells by selectively eliminating them causing less harm to the normal cells [3].

Cragg and Newman et al. have identified that nearly 5% out of the 1031 new chemical entities approved for use as drug between 1981 and 2002, by the US FDA (Food and Drug Administration) were natural products and 23% were derived from natural products [4]. Some well-known plant secondary metabolites used as medicine are paclitaxel, vinblastine, vincristine, artemisinin, atropine, inulin, digoxin, morphine and codeine, and quinine [5, 6]. Normal metabolism in plants produces a variety of chemical compounds. The primary metabolites are found ubiquitously like fats and sugars whereas the secondary metabolites are more specific to a particular genus or species. An advantage of plant metabolites is that apart from serving as functional drugs, they can be used as lead molecules for the synthesis of derivatives or synthetic molecules with the active pharmacophore.

2. Cancer and metastasis

The term 'cancer' refers to a range of diseases in which abnormal cells proliferate and spread uncontrollably in the body [7]. Under normal conditions, cells grow and multiply systematically to form organs and tissues that have a specific function. Occasionally, however, they multiply in an uncontrolled manner after developing a random genetic mutation or due to the influence of a carcinogen and form a mass known as a tumour or neoplasm that has no physiological function. It was Hippocrates (460–370 B.C.), the Greek physician who used the names 'carcinos' and 'carcinoma' to define non-ulcer forming and ulcer-forming tumours. In Greek, carcinos and carcinoma mean 'crab'; and the disease was named so because the finger-like projections extending from cancer resemble a crab in shape. Carcinomas, a type of cancer which arises from epithelial cells, is the most common type of cancer affecting people today. The first abnormality concerning cell maturation to be evident microscopically is known as dysplasia. This, in turn, leads to architectural chaos, irregularity in the nucleus, augmented and abnormal mitoses, and an increase in the number of apoptotic cells.

Tumours in the body can be benign or malignant. Benign tumours are those which do not invade other tissues or spread to other parts of the body. Malignant tumours, however, can grow in an uncontrolled way and by a process known as metastasis, can spread within the body. Even though all tumours are diverse and heterogeneous, they share the capacity to proliferate beyond the constraints that limit the growth of healthy tissue [7]. They can spread by direct local invasion,

vascular spread, cerebrospinal fluid (CSF) spread, transcoelomic (peritoneal or pleural) spread or lymphatic spread.

Modifications in the regulation of some crucial pathways that control cell proliferation (cell cycle) and survival (apoptosis) are responsible for creating all tumours [8]. The modifications include the loss of function of the tumour suppressor gene, oncogenic transformations, as well as modifications in the signal transduction pathways which leads to an augmented proliferation in response to external/mitogenic signals. As such, tumour-associated mutations in many of these pathways result in the alteration of the necessary regulatory mechanisms that control the mammalian cell cycle.

3. Cancer therapy

Surgery remains one of the foremost treatments for cancer. It has been mentioned by Roman doctor Gallien as a means of treating cancer as early as the 2nd century. It was followed by radiation therapy using radium and other diagnostic machines using relatively less voltage. Although the present methodology and the equipment for delivery of radiation therapy have improved allowing the obliteration of malignant tumours with great precision, this mode of therapy is limited by severe side effects and a restricted capacity to distinguish between healthy and tumour cells. Furthermore, both radiation and surgery are not beneficial in cases of advanced metastatic cancers.

Traditional treatments for cancer such as chemotherapy (e.g. anti-metabolites, alkylating agents, topoisomerase inhibitors) and radiation therapy were developed based upon the observation that transformed cells multiply at a higher rate when compared to normal cells. For example, ionising radiation results in DNA damage which, after multiple cell divisions, leads to errors in transcription and translation, eventually resulting in cell death [9]. In the same way, cytotoxic chemotherapy interferes with microtubule organisation, which is essential for mitosis and in due course, affects cell survival [10]. The same is true for various haematopoietic malignancies, however, as little as 5% of some solid tumours consist of rapidly proliferating, and therefore, susceptible cells. Hence, only a small subset of cancers such as Hodgkin's lymphoma, testicular cancer, acute lymphoid leukaemia and non-Hodgkin's lymphoma are routinely cured using these agents [11]. This is largely because therapies that are targeted against rapidly proliferating cells cause the death of normal tissues which also show enhanced proliferation rates, such as the gastrointestinal (GI) tract, bone marrow and the hair follicles [12].

4. Drug resistance

The development of drug resistance is also a major obstacle in patients receiving prolonged chemotherapeutic treatment. Clinical resistance to anticancer agents can occur at the time of drug introduction, as well as during treatment and following relapse [13]. Although various resistance mechanisms have been described, such as insufficient activation of the drug, utilisation of alternate metabolic pathways, mutations in the p53 gene and overexpression of the Bcl-2 gene family, the most intensely studied has been the decreased accumulation of drugs in cells, which is the leading cause of multi-drug resistance [14]. Such resistance is indicated by a failure to respond to a range of chemotherapeutic agents, many of which are structurally dissimilar and do not share a common intracellular target. The

mechanism responsible for Multidrug resistance in mammalian cells involves the overexpression of a 170 kDa cell surface, energy-dependent plasma membrane glycoprotein (P-gp) encoded on the MDR1 gene [15]. The physiological role of P-gp is the protection of cells against environmental toxins and works by exporting drugs outside of mammalian cells, thereby lowering the intracellular drug concentration less than the toxic threshold [16]. However, the chemotherapy of cancer, as compared with that of bacterial disease, poses a critical problem. Microorganisms are quantitatively and qualitatively different from human cells, while, cancer cells and normal cells are so similar that it has proved difficult to find general, exploitable biochemical differences between them. This is exemplified by the number of drugs selected for preclinical or clinical testing, based on their activity in experimental animal systems, which do not become clinically useful agents due to their severe or unpredictable toxicity towards normal cells, or because they lack any therapeutic advantage. The prevalence of MDR and systemic toxicity associated with currently administered cancer chemotherapies therefore suggest the need for alternative possibilities to be investigated to find new and worthy therapeutic agents.

5. Apoptosis and the need for apoptotic inducers

The process of homeostasis in multicellular organisms is strongly regulated by a process known as PCD (programmed cell death) or apoptosis. When cells obtain diverse indications for growth they generally die. This happens when certain developmental processes call for cell division but there are no external growth signals when a growth-related gene, e.g. c-myc gets highly expressed but the cellular environment lacks nutrient content, and in the presence of a toxic xenobiotic and the cell dies by a process termed apoptosis. The term 'apoptosis' was used for the first time in 1972 in literature, to describe a structurally-distinctive method of cell death which caused the loss of cells within live tissues [17].

There are inherent cellular programs that direct a cell into self-destruction. Several occurrences helped establish this; e.g. in the nematode, *Caenorhabditis elegans*, it has been discovered observed that a set of 113 cells is destined for programmed cell death in the hermaphrodite form of the worm during embryogenesis, and a different set of 18 cells later in life, forming a total of 131 cells [18].

The key features include blebbing and shrinkage of the cytoplasm, conservation of cellular organelle structure, involving the mitochondria and the condensation and margination of chromatin, although all cell types do not show all of these characteristics. These changes are a consequence of a developmental program for cell death which is activated by the deficiency of a growth factor, or by the presence of a xenobiotic compound such as a therapeutic anticancer drug. The morphological criteria are still the most important when complex cell populations, such as tissues, are examined, and overall cell shrinkage and nuclear condensation are the easiest to recognise [19].

The discovery of about 30 novel molecules whose functions are completely related to the initiation or control of apoptosis has been made over the last decade. Another 20 molecules, associated with essential roles in cell signalling and DNA transcription, replication or repair, have been established as effectors of apoptosis regulation. The rate of apoptosis influences the lifespan of cells in the human body, both healthy and cancer cells. Thus, the modulation of apoptosis is useful in the deterrence, management and therapy of cancer. Synthesis of novel compounds based on existing templates continues to be an indispensable aspect of research. Natural products are capable of providing such templates. Latest studies on tumour inhibitory compounds originating from plants have given rise to a remarkable group

of unique structures. Moreover, epidemiological findings confirm the theory that following a diet containing plenty of fruits and vegetables which are key sources of micronutrients and phytochemicals, reduces the risk of acquiring cancer [20]. It has also been reported that some products from plants bring about apoptosis in neoplastic cells alone and not in normal cells [21].

There have been reports confirming the role of apoptosis as an essential mode of action for several anti-tumour agents, such as alkylating agents including the widely used cisplatin and 1,3- bis (2-chloroethyl)-1-nitrosourea (BCNU) [22], ionising radiation [23], topoisomerase inhibitor etoposide, taxol [24], the tumour necrosis factor (TNF) [25], and N-substituted benzamides like 3-chloroprocainamide and metoclopramide [26].

6. Plant secondary metabolites used IN conventional medicine

The WHO (World Health Organisation) defines a medicinal plant as a plant whose one or more parts, has constituents which can be applied for therapeutic purposes, or can act as precursors for chemical or pharmacological semi-synthesis. The parts of these medicinal plants such as the roots, tubers, barks, stems, leaves, flowers, seeds and fruits/grains, contains metabolites that are therapeutically active and are used to control or treat a disease condition.

Such non-nutritional chemical compounds or bioactive components in plants are called phytochemicals, the word –'phyto'- from Greek, meaning 'plant'. These phytoconstituents are responsible for protecting the plant against pest infestation or microbial infections [27]. A large variety of phytochemicals have been isolated and characterised from familiar sources including vegetables such as onion and broccoli, fruits like apples and grapes, spices such as nutmeg, pepper and turmeric, brews such as green tea, oolong tea and red wine [28], which possess strong antioxidant properties. These antioxidants in chemoprevention and treatment of cancer and many more diseases by defending cells from damage by highly reactive oxygen compounds called 'free radicals'. The common classes of plant secondary metabolites which have found their way into traditional and modern medicine include.

Quercetin, quercitrin and kaempferol are common flavonoids present in approximately seventy per cent of all plants. Flavonoids vary notably in their anti-proliferative effectiveness depending on their structure. Kuntz et al. discovered that baicalein and myricetin induced apoptosis in Caco-2 and HT-29 cells [29]. Flavone, flavanone, flavonol, and isoflavone classes of flavonoids possess anti-proliferative effects in various cancer cell lines. Tangeretin found in the peel of tangerine is used extensively in Kampo medicines in Japan for treating cancer [21].

Solamargine, derived from a Chinese herb, *Solanum incanum*, has been noted to bring about apoptosis in human hepatocyte (Hep-3B) cells and normal skin fibroblast cells [30]. The alkaloids isolated from *Tiliacora racemosa* root with several bis-benzylisoquinoline alkaloids induced apoptosis in K-562 cells. Camptothecin (CPT), extracted from the stem wood of a Chinese tree, *Camptotheca acuminata Decsne, Nyssaceae*, works as a topoisomerase I inhibitor and induces apoptosis in PLB-985 (a human leukaemia cell line) cells [31]. The widely popular alkaloids Vinblastine and vincristine are obtained from the Madagascar periwinkle, *Catharanthus roseus* (previously called *Vinca rosea*).

A new cytotoxic proteoglycan, which is related to the family of arabinogalactan proteins, isolated from the saffron plant (*Crocus sativus L.*) exhibited induction of apoptosis in cultured macrophages with a lesser non-cytotoxic concentration increasing the DNA laddering effect in apoptotic cells [32].

A phytopreparation made from *Viscum album L.*, is currently being used as an adjuvant in cancer therapy and is found to stimulate the immune system by improving the number and activity of neutrophils and NK cells [33]. The formulation has various toxic proteins including viscotoxins (VT) and mistletoe lectins (ML); induces the synthesis of cytokines such as IFN-g,TNF-a, IL-6 and 1 L-1 and exhibits cytostatic and cytotoxic effects on human lymphocytes and cultured tumour cells alike.

7. Why use plant-based drugs?

Plants are an important part of nature's reservoir of medicinal agents and it is safe to say that they are nearly devoid of the side effects generally caused by synthetic drugs and chemical agents [34]. The WHO (World Health Organisation) reports that traditional medicine remains the chief mode of the treatment availed by 75–80% of the world's total population for primary health care, particularly in developing countries. This can be attributed to improved compatibility with the human body, better cultural acceptability, and reduced or practically no side-effects [35, 36].

Although several compounds isolated from plants are in the process of being thoroughly tested for their anticancer properties, it is becoming acknowledged that the medicinal effects of plants are due to a complex interaction of the combination of compounds present in the whole plant (additive/synergistic and/or antagonistic) rather than the single constituents [27].

The review of the literature reveals that phytochemicals present in normal fruit and vegetables have harmonising and overlapping mechanisms of action, such as the modulation of detoxification enzymes, stimulation of the immune system, scavenging of free radicals, regulation of gene expression, hormone metabolism, antibacterial and antiviral properties. Bioactive plant extracts are valuable resources which aid in the development of less toxic, more efficient drugs to manage the progression of cancer.

A major problem concerning cancer chemotherapy is the development of resistance to cytotoxic agents. Overcoming multidrug resistance requires research into new antineoplastic agents. In this regard, natural products acquired from plants have shown to have high potential as drug reservoirs [37]. According to the WHO, around 80% of the population in developing countries rely on traditional medicines, mostly derived from plants for primary health care. The modern pharmacopoeia contains a minimum of 25% drugs which are derived from plants and several others which are synthetic analogues [38]. Hence, fighting cancers with natural compounds derived from plants present a very favourable alternative.

Phytochemicals display structural diversity and contain scaffolds tailored to bind and inhibit the functions of several key proteins. They have more chiral centres and varied ring systems when compared to synthetic drugs. This complexity is responsible for increasing its target selectivity thereby reducing non-specific binding and adverse side effects [39].

8. Drug combinations and synergy

Drug combinations are widely used to treat deadly diseases such as AIDS and cancer. The main intention is to accomplish a reduction in dose and toxicity, synergistic therapeutic effect and lessen or delay the induction of drug resistance.

Plant secondary metabolite	Synergy with anti-cancer agents	Experimental models	References
Apigenin	TRAIL	HeLa cervical cancer cell line	[42]
		Human acute lymphoblastic leukemic cell line Jurkat, human prostate cancer cell line DU145, human colon cancer cell line DLD-1	[43]
	Tamoxifen	MCF7 human breast cancer cells	[44]
	Fulvestrant		
Berberine	Cisplatin	Ovarian cancer cell line VCAR3	[45]
	Doxorubicin	Murine melanoma B16F10 cells	[46]
	Epirubicin	T24 bladder cancer cells	[47]
	Evodiamine	Human breast cancer MCF-7 cells	[48]
	Tamoxifen	Human breast cancer MCF-7 cells	[49]
Curcumin	Cisplatin, etoposide, camptothecin, doxorubicin	Human and rat glioblastoma cell lines	[50]
	Cisplatin, oxaliplatin	Human ovarian carcinoma cell lines (2008 and C13)	[51]
	5-fluorouracil, a combination of 5-fluorouracil and oxaliplatin	Human colon cancer cell line (HT-29)	[52]
Genistein	Cisplatin	Human pancreatic carcinoma cell line (BxPC-3), murine xenograft model of BxPC-3 cells	[53]
		Human pancreatic carcinoma cell lines (COLO-357 and L3.6pl)	[54]
	Camptothecin	Human cervical cancer cell line (HeLa), human ovarian carcinoma line (OAW-42)	[55]
	Doxorubicin	Hormone-independent human breast cancer cell line (MDA-MB-231)	[56]
(-)-epigallocatechin 3-gallate (EGCG)	Cisplatin	Human ovarian cancer cell lines (SKOV3, CAOV3, and C200)	[57]
	doxorubicin	Murine xenografts of human carcinoma DOX-resistant cells (KB-A-1)	[58]

Plant secondary metabolite	Synergy with anti-cancer agents	Experimental models	References
Eupatin	Mitoxantrone	NCI-H460 human lung non-small-cell carcinoma cells	[59]
Indirubin	Arylidene derivatives	Human non-small cell lung carcinoma cells	[60]
	TRAIL	Breast cancer and bladder carcinoma cell lines	[61]
	Vinblastine	HeLa cells	[62]
Kaempferol	TRAIL	Human glioblastoma cell lines U87, U251, and U373	[63]
	Vinblastine	Cervical carcinoma cell lines (KB-V1, KB-3-1)	[64]
	Paclitaxel		
	Mitoxantrone	K562, LLC, K562, and KB Cell Lines	[65]
Luteolin	Cisplatin	Human liver cancer cells HepG2and Hep3B and human colorectal cancer cells HT29 and HCT116	[66]
	Doxorubicin	4T1 and MCF-7 cells	[67]
	Rapamycin	Human breast and ovarian cancer cell lines MDA-MB-453, AU565, SKOV3.ip1, HBL100, and MCF-7. MCF-7 and AU56	[68]
Quercetin	Cisplatin	Human laryngeal carcinoma cell line (Hep-2)	[69]
	Doxorubicin	Neuroblastoma and Ewing's sarcoma cell lines	[70]
	Vinblastine Paclitaxel	Cervical carcinoma cell lines (KB-V1, KB-3-1)	[64]
	Gemcitabine Topotecan	Murine fibrosarcoma cell lines	[71]
Resveratrol	Cisplatin	Wistar rats	[72, 73]
	Doxorubicin	Human acute myeloid leukemia cell lines (ML-2/DX30, AML-2/DX100, and AML-2/DX300)	[74]
Silybin	Cisplatin Carboplatin	Human prostate carcinoma cell line DU145	[75]
	SN-38 Mitoxantrone	K562, LLC, K562, and KB Cell Lines	[65]
	Paclitaxel TRIAL	Human ovarian carcinoma line A2780	[76]

Table 1.
Combinations studies with anti-cancer drugs in clinical practice.

Synergistic interactions are essential in phytomedicine and explain the effectiveness of extremely low doses of active constituents in herbal formulations. Traditional medicine works on the idea that a whole or partly purified plant extract offers improvements over a single isolated ingredient. Synergism also leads to toxicity reduction and minimization of resistance. Though vinblastine is successful clinically by itself [40, 41], its use in combination with other anticancer agents is now under evaluation, mostly for the management of recurrent or advanced cancers that are resistant to conventional chemotherapy. The occurrence of clinical drug resistance has emphasised the need to search for novel chemotherapeutic drugs and better combinations among these agents. Typically, synergy is considered to be greater than additive therapeutic effects when compared with the efficacy of each drug by itself. Recently, combination therapies being tested make use of drugs with different mechanisms of action, under the rationale that targeting two separate pathways will result in improved cytotoxicity, whether additive or synergistic.

Several researchers have tried to enhance the potential of known anti-cancer agents like vinblastine and paclitaxel by the virtue of combination therapy with cisplatin, etoposide and doxorubicin. **Table 1** gives a list of the combination studies performed on plant secondary metabolites with anti-cancer drugs in clinical practice which showed synergistic activity.

9. Future perspectives

Cancer cells have evolved multiple mechanisms to evade apoptosis and escape to other sites. Phytomedicine and ethnopharmacology have proved to be very effective in the prevention and treatment of human ailments. Plant extracts have several components with diverse possible intracellular targets. From literature, it is evident that plants have a long history of oral use in traditional medicine and hence, are considered safe and non-toxic and there lies a huge potential in developing crude whole plant extracts for the treatment of cancer, alone or in a combination with other drugs in clinical practice. It is also advisable to explore the potential of these plants as chemopreventive agents because of their antioxidant and free radical scavenging activity. However, before these plant metabolites can be used for cancer prevention or therapy, they must be subject to further testing which should include in vivo studies in animal models and clinical trials (randomised double-blind) in human subjects.

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Chapter 17

Impact of Shodhana on *Semecarpus anacardium* Nuts

Pratap Kumar Sahu and Prashant Tiwari

Abstract

Semecarpus anacardium is classified in Ayurveda under the category of toxic plants. However, this toxic plant is reported to possess anti-inflammatory activity, anti-arthritic effect, antioxidant activity, antimicrobial activity, anti- carcinogenic activity, hypoglycemic activity, cardioprotective, hepatoprotective, neuroprotective, and hypolipidemic activity etc. All these activities are attributed to its various constituents like phenolic compounds, flavonoids, carbohydrates, alkaloids, steroids, etc. In Ayurveda, a series of pharmaceutical procedures which converts a poisonous drug into a safe and therapeutically effective medicine is termed as Shodhana. Shodhana improves the yield, decreases the phenolic and flavonoid content; and converts toxic urushiol into nontoxic anacardol derivative thereby reducing toxicity of nuts of *Semecarpus anacardium*. There are reports of alteration in pharmacology and phytochemistry of nuts of *Semecarpus anacardium* due to Shodhana.

Keywords: Shodhana, *Semecarpus anacardium*, nuts, ayurvedic, toxic, urushiol, anacardol

1. Introduction

Ayurveda is proven to be the ancient traditional way of treatment in India, which is fully based on philosophical, experimental and practical concepts. It includes the use of indigenous drugs which have been preferred by many pharmaceutical industries towards a novel strategy for natural drug discovery. Ayurvedic proven concepts signifies more on human health and disease that recommend the use of herbal enriched compounds as special diets. However, some herbal compounds may have toxicity besides their therapeutic potential if used improperly [1].

There are so many plants which are identified as poisonous and semi-poisonous in Ayurveda. Plants like Atsanabha (Aconitum species), nux-vomica, *Acorus calamus*, *Semecarpus anacardium*, Strychnos, *Abrus precatorius* etc., are the most known examples of toxic plants. These plants are known for their hidden medicinal values and broadly accepted by the Indian Ayurvedic system of medicine. These plants are still used in Indian system of development of medicine for treatment. Aconite, strychnine, β–asarone, bhilawanols, abrin are some of the toxic components present in these plants [2].

Shodhana is the purificatory measure used in Ayurveda to purify toxic medicinal plants (*upavishadravyas*), by various pharmaceutical procedures like soaking, rubbing and washing etc. with specific medias like *gomutra* (cow's urine), *godugdha* (cow's milk) etc. Poisonous plants are subjected to *shodhanasanskara* (purification

process), before their therapeutic use. This process reduces toxicity of poisonous plant considerably and keeps it at required optimum level. Physico-chemical changes and reduction of the toxic chemicals from the poisonous plants like strychnine, brucine in *kupilu* and scopolamine in *dhattura* have been reported [3].

Bhallataka (*Semecarpus anacardium* Linn; Anacardiaceae) fruit is one of the *upavishadravyas* (semi poisonous drugs). Its importance and utility is increasing day by day because of its therapeutic significance in many a diseases including cancer. Though the fruits of *Bhallataka* has many therapeutic values, pharmacies are scared to use this drug because of its irritant vesicating nature. If juice of *Bhallataka* (even in traces) comes in contact with body, produces severe *daha* (burning sensation), and *Vrana* (ulcer). When it comes in contact with face, it produces acute burning sensation with *shotha* (inflammation) and *Visarpa* (skin disease). The fruit contains tarry oil which causes contact dermatitis. Medically it is very well recognized as Urushiol induced contact dermatitis because the chemical Urushiol is responsible for dermatitis. If this vesicant nature is removed, the drug could be a good source for pharmaceutical industries in manufacturing many formulations containing *Bhallataka* as an ingredient [2, 4].

Ayurveda advocates *bhallataka* after *shodhana* (purificatory procedures). Though there are different *shodhana* methods mentioned in Ayurveda, the *shodhana* method mentioned in the text *Rasamrutam* was adopted and quoted in (The Ayurvedic Pharmacopeia of India) (API) and the Ayurvedic formularly of India (AFD). The procedure is soaking the fruits in cow's urine, cow's milk and rubbing it in brick powder [5]. It is reported that R_f values change in *shodhita* samples of *Bhallataka* when compared to raw *bhallataka* [3].

2. *Semecarpus anacardium*

This is a native of India. It is known as bhallatak in India and "marking nut" by Europeans. *Semecarpus anacardium* plant (**Figure 1**) is widely available in sub-Himalayan province, tropical and central part of our country India. It is known as a deciduous tree; medium in size. Height of the tree is normally 12–15 m. Leaves are large and simple about 60 cm long and 30 cm wide. The color of bark is deep brown and is quite rough in structure. The flowers are dull greenish in color [6].

Figure 1.
Semecarpus anacardium *plant and its nuts.*

Figure 2.
Semecarpus anacardium *(Bhallatak) nuts.*

Abundantly the plant is found in Odisha, Chittagong, central India and Northern Australia [7]. The color of fruit is black when ripe as well as smooth and shiny in texture (**Figure 2**). The fruit is generally categorized as toxic and the integral part of the fruit i.e. nut is about 1 inch long in size [8].

3. Active principles of *Semecarpus anacardium*

The active principles present in *S. anacardium* Linn. are given in **Table 1** and their structures are presented in **Table 2**. Bhilawanols, phenolic compounds, [9, 10] biflavonoids, sterols and glycosides [11] are proven to be the most significant components of *S. anacardium* Linn. An alkaloid, Bhilawanol, has been identified as isolated from oil and seeds of *S. anacardium*. Bhilawanol is a mixture of cis and trans isomers of urushiol. Bhilawanol is isolated from oil of nuts. It is a mixture of phenolic compounds like 1, 2-dihydroxy-3 (pentadecadienyl-8, 11) benzene and 1, 2- dihydroxy-3 (pentadecadienyl-8′, 11′) –benzene [10]. Bhilawanol on catalytic reduction absorbs one mole of hydrogen to give hydrourushiol (3-pentadecyl-catechol) [12, 13]. When the phenolic compounds are exposed to the air, then they get oxidized to Quinones. When the oil is kept under nitrogenoxidation process can be prevented. Nut shells contain several biflavones [14], jeediflavanone [15, 16], semecarpuflavan and gulluflavone [17–19] (**Table 1**).

4. Uses of *Semecarpus anacardium*

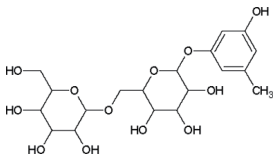
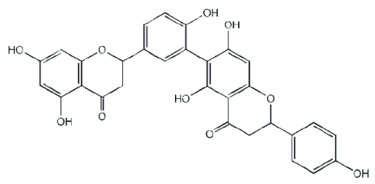
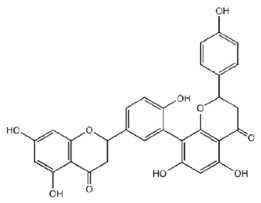
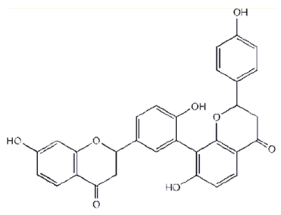
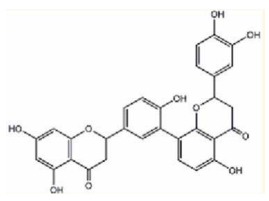
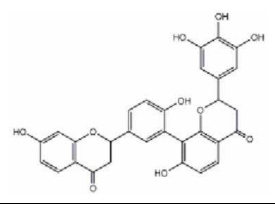
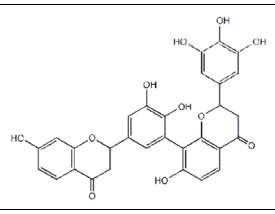
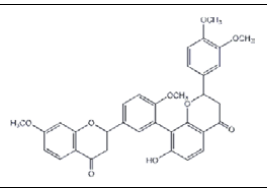
It has been reported for wide arena of ethno-pharmacological activities. Researchers have identified SA nuts extracts for potent pharmacological actions. Most of these studies are pre-clinical studies. Their clinical efficacy is yet to be reported. The list of health disorders against which *Semecarpus anacardium* has a potential to be used is given in **Table 3**. The possible mechanism of action is also described.

4.1 Analgesic and anti-inflammatory effect

There are reports of analgesic [20] and anti-inflammatory [21, 22] activity by *Semecarpus anacardium*. Biflavonoid like tetrahydroamentoflavone (THA) showed significant COX-1 and COX-2 inhibition *in vitro.* THA may be responsible for its

Phytoconstituents	Name
Glycoside	Anacardoside
Alkaloid	Bhilawanol/urushiol
	Urshenol
Phenolic compounds	1,2-dihydroxy-3 (penta decadienyl-8, 11) benzene
	1,2-dihydroxy-3 (penta decadienyl-8′, 11′) benzene
	Bhilavanol A (monoenepentadecyl catechol I)
	Bhilavanol B (dienepentadecyl catechol II)
Biflavonoids	Biflavones A, C, A1, A2
	Tetrahydrorobustaflavone
	Tetrahydromentoflavone
	Jeediflavanone
	Semicarpuflavonone
	Galluflavone
	Nallaflavanone
	Semicarpetin
	Anacarduflavanone
	O-trimethylbiflavanone A1
	O-trimethylbiflavanone A2
	O-tetramethylbiflavanone A1
	O-hexamethylbichaleone A
	O-dimethyl biflavanone B
	O-heptamethylbichaleone B1
	O-hexamethylbichaleone B2
	O-tetramethylbiflavanone C
Other components	Anacardic acid
	Cardol
	Catechol
	Fixed oil
	Anacardol
	Anacardoside
	Semecarpol
	Oleic acid
	Linoleic acid
	Palmitic acid
	Stearic acid
	Arachidic acid

Table 1.
Phytoconstituents present in Semecarpus anacardium.

Active compounds	Chemical formulae
Anacardoside	
Tetrahydrorobustaflavone	
Tetrahydromentoflavone	
Biflavanone C	
Biflavanone A	
Semicarpuflavonone	
Galluflavone	
Semicarpetin	

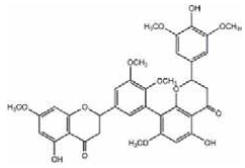
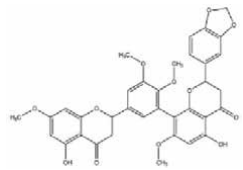
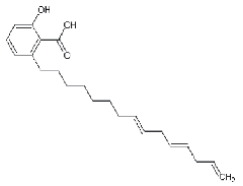
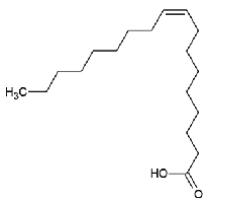
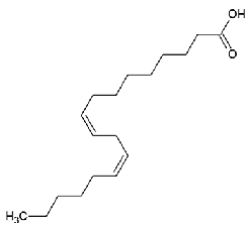
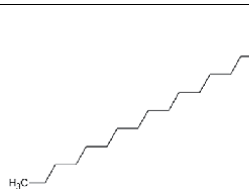
Active compounds	Chemical formulae
Nallaflavanone	
Anacarduflavanone	
Anacardic acid	
Oleic acid	
Linoleic acid	
Palmitic acid	
Stearic acid	

Table 2.
Chemical formulae of the active principles of Semecarpus anacardium.

analgesic and anti-inflammatory activity [23]. SA extracts were studied for their anti-inflammatory activities *in vitro* using synovial fluid and blood of healthy individuals and rheumatoid arthritis patients. SA inhibited proinflammatory cytokine production like IL-1 beta and IL-12P40 without affecting IL-6 and TNF-alpha production [24].

Potential use/activity	Efficacy proved in	Possible mechanism of action
Analgesic, anti-inflammatory, anti-arthritic	Animal models (pre-clinical)	Inhibition of cyclooxygenase (COX 1 and COX 2), inhibit pro-inflammatory cytokine production
Anti-cancer (breast cancer, hepato cellular carcinoma, leukemia)	Cell lines and animal models (pre-clinical)	Cytotoxicity by inducing apoptosis following caspase 3 pathway
Cardioprotective (anti atherogenic, lipid lowering)	Animal models (pre-clinical)	Anti-oxidant, decrease cholesterol, increase HDL
Nootropic (memory enhancer)	Animal models (pre-clinical)	Inhibit acetylcholine esterase, increase cholinergic activity
Hepatoprotective	Animal models (pre-clinical)	Anti-oxidant
Anti-fungal and Anti-bacterial (Gram +ve, Gram −ve, tuberculosis)	Microbial culture (in-vitro)	Inhibit microbial growth
Aphrodisiac (increase sex desire) in male but spermicidal	Animal models (pre-clinical)	Increase mounting and mating performance, cause spermatogenic arrest (decrease motility and density of sperm)
Anthelmintic	Indian earthworm (*Pheretima posthuma*)	Muscle paralysis

Table 3.
Potential uses of Semecarpus anacardium *with possible mechanism of action.*

4.2 Anticancer activity

Nut extracts of *Semecarpus anacardium* showed efficacy against human breast cancer cell line (T47D) [25] and mammary carcinoma in rats [26]. It also showed efficacy against leukemic cells in mice [27]. SA extracts have energy restoration, tumor marker regulation and membrane stabilization effect against hepato-cellular carcinoma [28]. *Semecarpus anacardium* may have a protective as well as therapeutic contribution against Mitomycin-C induced mutagenicity [29]. *Semecarpus anacardium* showed significant cytotoxicity having LC50 29.5 μg in brine shrimp lethality test [30]. The mechanism of cytotoxicity is by inducing apoptosis following caspase 3 pathway [31].

4.3 Cardioprotective effect

S. anacardium nuts prevented isoproterenol (ISO) induced myocardial damage in rats [32]. *S. anacardium* (1 mg/100 g body weight) reduced serum cholesterol levels and raised HDL levels in rats fed with atherogenic diet [33]. The process of atherogenesis triggered by lipid peroxidation can be inhibited by *Semecarpus anacardium* [34].

4.4 Nootropic effect

Semecarpus anacardium effectively inhibits acetyl choline esterase which in turn prolongs the half-life of acetylcholine. Hence, SA has been shown to be useful in improving cognitive ability [35–37].

4.5 Hepatoprotective effect

S. anacardium decreased the levels of the marker enzymes induced by lead acetate in liver [38]. This hepatoprotective action may be attributed to its anti-oxidant action [39].

4.6 Antimicrobial activity

The flavonoid present in *S. anacardium* showed antifungal activity at 400 mg/ml concentration [40]. Furthermore, the oil possessed anti-microbial activity against both Gram positive (*B. subtilis, S. aureus*) and Gram negative (*P. vulgaris, E. coli*) organisms [41]. The petroleum ether and aqueous extracts of SA inhibit the growth of *Staphylococcus aureus* and *Shigella flexneri*. However, chloroform and ethanol extracts showed inhibition against *Bacillus licheniformis* and *Pseudomonas aeruginosa* respectively [42]. The alcoholic extract of SA was found to be bactericidal against Gram positive (*E. coli*, S. Typhi and *P. vulgaris*) and Gram negative (S aureus and C diphtheria) strains [43]. Water extract showed potential with MIC 6.25 μg/ml against M. tuberculosis during in vitro bioassay [44].

4.7 Aphrodisiac and spermicidal activity

Semecarpus anacardium significantly improved both mounting and mating performance of male mice [45]. However, there are reports of spermicidal activity including spermatogenic arrest in male rats. There is also decrease in density and motility of sperms [36, 46, 47].

4.8 Anthelmintic activity

Petroleum ether, chloroform extract of nuts of *S. anacardium* showed anthelmintic activities against adult Indian earthworm (Pheretima posthuma) [48].

4.9 Hypoglycemic effect

Ethanolic extract of SA (100 mg/kg) reduced blood glucose level in normoglycemic rats. However, no effect was observed in case of hyperglycemic rats [49, 50].

5. Toxicity of *Semecarpus anacardium*

Use of Bhallataka needs adequate precaution due to its extreme hot and sharp attributes. It should be kept away from pregnant women, old aged person and also children. Individual persons showing allergic reactions like rash, itching and swelling to it should avoid its use. Furthermore, it is highly recommended to keep away from direct exposure to sunlight, heat and extreme sex during the course of Bhallataka treatment. The oily portion of nut should be removed for its safe use which can lead to nephropathy. Fewer antidotes like coconut oil, coriander leaves pulp and ghee is useful in case of allergic reactions [51]. The traditional way of administration with peanut oil was proven to be safe up to 25 mg/kg/day for 9 day [52].

Bhallataka nut oil extracts in male albino rats is reported to decrease hemoglobin count as well as erythrocytes indicating anemia. It exhibited an alteration in kidney enzyme level leading to nephrotoxicity during acute and subchronic toxicity [53].

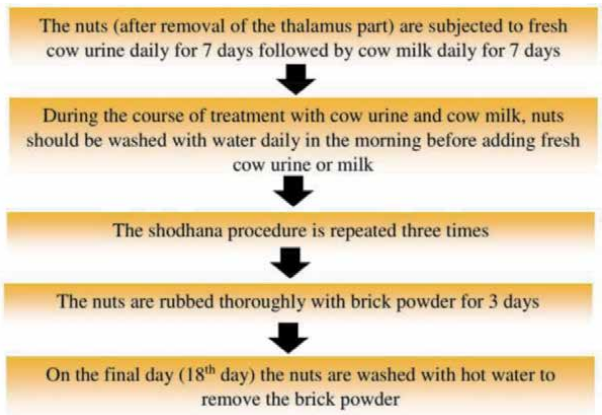


Figure 3.
Flow chart of Shodhana of Semecarpus anacardium *nuts.*

Hence, it is necessary to undertake Shodhana sanskara of Bhallataka with precaution before using it in medicine to avoid toxic effects of Ashuddha (impure) Bhallataka [54].

6. Shodhana of *Semecarpus anacardium* nuts

The process Shodhana, which is also known as detoxification or purification process signifies the conversion of any poisonous drug into beneficial, non-poisonous/nontoxic drug. *Shodhana* process involves sequential steps to purify and reduce the extreme toxicity levels/principles and also sometimes may result in enhancing the therapeutic efficacy. Shodhana is essential because higher concentrated chemicals may contribute towards adverse episodes on human body. There are 2 types of Shodhana i.e. Samanyashodhana and visheshshodhana which purifies toxic drugs. Furthermore, shodhana limits toxicity by removing the visible and invisible impurities, heterogeneous substances and toxic substances [55].

As per Ayurvedic texts shodhana can be done for SA nuts (**Figure 3**). The thalamus part of the fruit is removed with a steel knife. Then, the nuts are subjected to fresh cow urine daily for 7 days followed by cow milk daily for 7 days followed by rubbing thoroughly with brick powder for 3 days. During the treatment with cow urine and cow milk, the nuts are washed with water before adding fresh cow urine or milk. On the final day (18th day), the nuts are washed with hot water to remove the brick powder. This shodhana procedure is repeated three times [35, 56–58].

7. Effect of Shodhana

Shodhana helps in conversion of toxic urushiol into nontoxic anacardol [56]. Our studies on GC-MS which elucidate the presence of anacardol derivative (Anacardol, tetrahydro-; retention time 51.538 in GC-MS) in shodhit extract and urushiol derivative in pre-shodhit extract (1,2-Benzenediol, 3-(8,11,14-pentadecatrienyl)-, (*Z*,*Z*)-, retention time 56.270 in GC-MS) further confirms that shodhana helps in removal of toxic principle urushiol [59].

Shodhana improves the yield in methanolic extract, but decreases the phenolic and flavonoid content [31]. Shodhana decreases cytotoxicity without affecting anticancer activity significantly. The reduction in cytotoxicity may be attributed to reduction in oxidative stress [59]. Shodhana of the nuts reduce nootropic activity

[35]. So shodhana not only reduces toxicity but also alters its pharmacological activities.

8. Conclusion

Semecarpus anacardium is classified in Ayurveda under the category of toxic plants. There are reports of anti-inflammatory activity, anti-arthritic effect, antioxidant activity, antimicrobial activity, anti- carcinogenic activity, hypoglycemic activity, cardioprotective, hepatoprotective, neuroprotective, and hypolipidemic activity etc. shown by *Semecarpus anacardium.* Shodhana of nuts of *Semecarpus anacardium* can be done as per method given in Ayurvedic Pharmacopeia of India. Shodhana improves the yield, decreases the phenolic and flavonoid content; and converts toxic urushiol into nontoxic anacardol derivative thereby reducing toxicity. Shodhana not only reduces toxicity but also alters its pharmacological activities. Shodhana decreases cytotoxicity without affecting anticancer activity significantly. Shodhana also reduces nootropic activity.

9. Future scope

The effect of Shodhana on other pharmacological activities of *Semecarpus anacardium* can be studied in future. This work can also be extended to other poisonous and semi poisonous plants for which shodhana method is described in Ayurvedic Pharmacopeia of India.

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Conflict of interest

The authors declare that they have no conflict of interest.

Abbreviations

API	The Ayurvedic Pharmacopeia of India
SA	*Semecarpus anacardium*
THA	tetrahydroamentoflavone
COX-1	cyclooxygenase 1
COX-2	cyclooxygenase 2
HDL	high density lipoprotein
MIC	minimum inhibitory concentration
GC-MS	gas chromatography-mass spectrometry

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Chapter 18

Safety of Herbal Medicines in Children

Sevinç Polat and Ayşe Gürol

Abstract

Herbal medicine is used by individuals of all ages, including children. Herbal medicine includes herbs, herbal materials and preparations, and finished herbal products. Herbal medicine or herbal products' use for all ages have increased in recent years. Based on the data of the World Health Organization, almost 80% of the population in developing countries trust herbal medicines to meet their health needs. Herbal medicines use unconsciously as though these products are harmless. The use of herbal products in children is a concern because little information is available concerning the benefits and risks of these products in the pediatric population. This creates a serious problem in the treatment of children, and reveals a serious and under-recognized hazard in clinical care. The safety of most herbal medicinal products is absent since lack of suitable quality controls and not available of appropriate patient information. Owing to the possibility of serious health complications arising from the use of herbal products, it is mandatory to understand their use in the general population in order for appropriate measures to be put into place.

Keywords: children, herbal medicine, nursing, use of herbs, toxicity

1. Introduction

Herbal medicine is the use of only plants for medicinal and therapeutic purpose to treat the diseases and to improve human health [1–4]. Herbal medicine is the most commonly used complementary and alternative medicine (CAM) [5]. World Health Organization (WHO) has defined herbal medicines as last labeled medicinal product that contain an active ingredient, aerial, or underground parts of the plant or other plant material or combinations [4, 6]. Herbal medicine is classified into three groups: 1) herbal drugs have proven efficacies and known active compounds and doses, 2) have expected efficacies, active compound needs to be standardized, and 3) uncertain efficacies [4, 6].

Herbal medicine includes herbs, herbal materials, and last herbal products. In some countries, herbal medicine may contain natural organic or inorganic active ingredients [7]. The number of medicinal plants as medicine is around 20.000 and they are also used for adding taste, odor and color to food since the ancient times [8].

In the 21st century, herbal medicine has been considered as a promising future medicine for people health [4]. During long time before the modern medicine, herbs had been the mainstream remedies for nearly all illness [1, 4]. People had commonly diagnosed illnesses themselves, prepared and prescribed their own herbal medicines for thousand years ago [1]. Herbal remedies, nowadays, it still

plays an important role in the health care and most of people rely on herbal medicine for their primary health care [9, 10].

Herbal plants for medicine are easily obtainable without prescription or prescribed by herbal practitioners. Due to drug resistance and difficulties in finding accessible and reliable drugs, herbal medicines have become an alternative option in our healthcare system [11].

Herbal medicines have been used either alone or in combination with conventional medicines [11]. But even though herbal products are frequently used and regarded as 'natural', they can also cause adverse drug reactions [9, 12, 13] as well as adverse interactions with other medicines [9, 14, 15].

Herbal remedies are generally considered as safe, though their efficacies are unclear and their adverse effects may vary from human to human [2]. Despite of increasing popularity of herbal medicine, their safety and effectiveness have not been scientifically proven [1]. According to their wide use, many herbal products used frequently have not undergone complex scientific analyses via clinical experiments [9]. Though its reported that some of these plants have medicinal properties in the literature it has also been showed that other plants could be not safe for consumption as being toxic and adverse effects in the human body [4, 16]. Therefore, the aim of this review is to understand the current status of the herbal medicine used on the children and adolescents.

2. Prevalence of herbal medicinal product use

The use of herbal remedies has increased in recent years. It is predicted that this rate will be greater for the COVID-19 pandemic process. WHO has been reported nearly ~80% of the world's population uses and trust herbal products for treatment [17–20].

About 2.9 million American children and teenagers have used herbs or their supplements [21]. In China, the use of herbal medicine is about changing from 30–50% of the total drug consumption. It is estimated that in other developed countries, more than 50% of the population use herbal products at least once in their life. The herbal medicines account for 60% of treatment at home in developing countries [4, 22]. In the children with a chronic illness or among inpatients and outpatients are higher use of the herbal medicine [9, 23].

The using herbal drugs among children are 85.5% in Germany [23]. Children with neuropsychiatric diseases use herbal medicine about 35.4% [24]. The elementary school-age children in South Korea have epileptic problems ~17.2% and they use herbal medicine at high range varying from 65.2% to 67.8% [25]. In Turkey, the prevalence of pediatric use of herbal drugs was 58.6% [26]. Bülbül et al. [27] reported that 27% of parents, who used herbal products for their children within 1 year, used them without a doctor's recommendation [27].

3. Areas of herbal medicinal product use

The use of at least one herbal or food product has been commonplace during episodes of acute illness among African American communities [28]. Families with children who have chronic medical conditions, such as autism, cystic fibrosis, rheumatoid arthritis, respiratory tract infections or asthma use to herbal remedies as part of their treatment [1, 10, 29–31]. The majority of herbal remedies are used to treat coughs, colds, and intestinal disorders [9].

The herbal medicine use in children with respiratory illnesses was 59.3% [32]. Parents of children with asthma reported using a range of herbal products (12.8%) for self-care [33]. The most common used herbal medicine for pediatric asthmatic patients were linden (21.6%) and ginger (21.2%) [34]. Herbal medicine has traditionally been used in the treatment of symptoms for nocturnal enuresis or urinary incontinence [35–37].

The ginger, chamomile, mint, cardamom, garlic and onion were used to prevent and treat nausea caused by chemotherapy [38]. It has been reported some herbal products are effective in the management of ear pain in Otitis Media [2]. Children with Attention Deficit Hyperactivity Disorder and Anxiety of Depression take herbal products a part of their treatments. The use of CAM in children with medical comorbidities, excessive sleep problems or insomnia is 1.8 times higher than children without such difficulties [39]. It is reported that herbs significantly decreasing body temperature, cough and breathing difficulties, and improving absorption of pulmonary infiltration and quality of life on the severe acute respiratory syndrome (SARS) [40]. The herbal formula (Ma Xin Shi Gan Tang) was claimed to antiviral effect on which inhibits the entry of influenza virus and have potential in managing seasonal pandemics of influenza infection [41].

4. Toxicity of herbs

Herbal products were used mainly because of hearsay recommendation, dissatisfaction with conventional medicine, and fear of adverse-effects of drugs [30]. It is important to understand that mothers consider herbs to be 'natural and safe' and are therefore more willing to try herbal remedies such as herbs, olive oil, and food for their babies' health. This is no different to any other country, where the local 'health food shop' and vitamin bars in pharmacies are well frequented by concerned parents. For some participants, decisions regarding use of traditional practices are usually made by an extended family member or through advice from virtual support groups and social media [42].

The herbal medicines have been obtainable without prescription and professional advices. This practice could lead to harm in children. Because of the variability in herbal product ingredients, the actual dose of active ingredients being consumed is often variable and unknown. When compared with adults, since children have smaller sizes and their immature systems they may be particularly susceptible to the effects of such dosage variations [29]. For this reason, before the administration of any therapeutic agents into children's body, the mothers must be taken into attention to consider the anatomy and physiology of them. The development level of organs such as brain, liver, and kidney affect rate of the absorption, distribution, metabolism and excretion of drugs. The inappropriate doses could lead to the accumulation of drugs in the body and finally cause the toxic effects. The unstandardized preparation of herbal medicines by manufacturer and contaminants (metals, chemical drugs, etc.) create a risk for children's health [29, 43]. Herbal products are widespread usages in children; professionals should be aware of this and be alert for possible side-effects/interactions [30].

Herbal medicines have some drawbacks such as contamination with chemicals. One of the most contaminants is pesticide on the herbal products [44]. The wide spread use of pesticides in agriculture has caused severe environmental pollution and possible health hazards including severe acute and chronic poisonings. WHO estimates that the incidence of pesticide poisonings in developing countries has doubled during the past decade [45, 46].

Herbal products can also produce adverse side effects that range from mild one to fatal ones. For example; herbs believed to have an effect on blood-clotting abilities may cause serious side effects for patients with certain blood-related conditions such as hemophilia. Some herbs may increase the effects of anticoagulant medications, and then it may be creating the risk of bleeding [1].

5. Advice to parents

Health care practitioners might be considered some practical points, when parents come to counsel about herbal medicine. Parents should realize that all herbal medicine is not safe [30]. Parents have to be informed about the potential risk or adverse effects of the long-term use of herbal products [47]. When parents are informed about herbal products it maybe prevents the negative interactions [30, 48]. Parents have to be understood that natural is not equal to safe [29].

Unless parents must have the essential knowledge on herbal products, they do not give the herbal remedies to their children [18, 43]. It is important for clinicians to ask the question to find out the beliefs and alternative therapies of the parents, and it is necessity to understand whether they give the herbal remedies in their children [29]. Lack of information about taken herbal remedies by child can prolong a hospital stay or hamper the clinician's approach to diagnosis and management [29].

6. Herbal medicine for the treatment COVID-19

On these days, herbal medicine plays a major role in the prevention and treatment of many diseases also as the novel coronavirus. Chinese medicine is the pioneer of herbal medicine among all of the countries [49]. There was wide usage of traditional Chinese medicine through the last SARS-COV outbreak. The five most famous applied herbs were Astragali Radix (Huangqi), Saposhnikoviae Radix (Fangfeng), Glycyrrhizae Radix Et Rhizoma (Gancio), Atractylodis Macrocephalae Rhizoma (Baizhu), and Lonicerae Japonicae Flo [50].

Du et al. [51] have summarized the theoretical foundation, potential effect of Chinese herbs on COVID-19 patients, and Yang et al. compared the evidence of current applications of traditional Chinese's herbs in the treatment of COVID-19 patients [52]. Todays, many guidelines related to herbal medicine have been issued for the prevention and treatment of COVID-19 [50, 53]. The herbs were commonly recommended in some symptoms, like fatigue, fever, chills, heavy limbs, and gastrointestinal symptoms in these guidelines [54]. Recent clinical evidence have also showed the therapeutic effectiveness of traditional medicine in treating different stages of COVID-19 [55–59]. Wang et al. [60] investigated effect of traditional Chinese medicine on hospitalized patients with COVID-19. They suggested that patients should receive Kaletra early and should be treated by a combination of Western and Chinese medicines [60]. Li et al. [61] were reported that Lianhua Qingwen had antiviral and anti-inflammatory activity against SARS-CoV-2 in their experimental study. Traditional Chinese Medicine has suggested to prescribe the herbs that are likely to be effective in the diagnosis and treatment plan of COVID-19 [62]. Notably, the usage frequency of Armeniacae Semen was highest among the herbal formulae recommended for the treatment of pediatric COVID-19 [63]. The herbal products' diversity for the recommended treatment of pediatric COVID-19 is lower compared to the adults. This might be due to the difference in the spectrum of diseases between the children and the adults [64].

7. Nurses's role

Overall, the type of herbal products changes all of the worlds. Besides it can be changes in the different societies at one country. Their usage is high prevalence among most traditional society [42]. In Taiwan, about 60% of participants reported that use folk remedies during their child's hospitalization, and the 72% of them would not inform healthcare providers about usage of folk remedies to their children [65]. This illustrates that it is important health practitioners, including nurses, are aware of the use of folk remedies within the community, and investigate about the use of folk remedies or traditional healing practices in a non-judgemental manner [42].

The growing trend of herbal products' uses is a major challenge to health system, children and families. To ensure the quality and safety of nursing interventions to child and mothers, it is important to learn the mothers' knowledge on beliefs and barriers to health care in their living. In addition, nurses need to be open to listening to patients and admitting their practice of traditional remedies while evaluating risks to create a nursing care plan [66]. The nurses have regular contact with parents within the healthcare centers or family health services [67]. Nurses are required to be have sufficient understanding and knowledge about CAM therapies [68]. Therefore, nurses can be credible sources for parents who need the accurate and trust information on herbal medicinal products. Nurses have to ask to parents what methods they used to understand if the parents have any qualms or difficulty obtaining prescription medications for their child [69].

8. Conclusion

The usage of herbal medicines increases day by day. People usually choose the herbal products instead of medical drugs [46]. The use of medical plant species in the treatment of children diseases is a part of traditional knowledge that is handed down by hearsay advices [20].

Herbal medicine can be unconsciously used as though these products are harmless [70, 71]. The use of herbal products in children is a concern, because a few information is available on their benefits and risks at these population [72, 73]. This creates a serious problem in the treatment of children, and it can be occurred a serious hazard in clinical care [74]. Since herbal products are available not only in pharmacies, but also in food stores and supermarkets there is a serious risk to users and remains a major concern about the herbal drug safety issue [18].

As the global use of herbal products continues to increase and many more new products are introduced into the market the risk will be greater for public health day by day [75]. The risk increases because of compromised by lack of suitable quality controls, inadequate labeling, and the absence of appropriate patient information [76].

Most herbal medicines have not been subjected to rigorous clinical trials As a result, it still continues the lack of evidence-based information about the efficacy and safety of herbal products in children [1, 77]. Despite of the high prevalence of herbal remedies' uses, there is a communication problem between CAM users and healthcare professionals. Healthcare professions both have to ask about herbal remedies' uses and inform to their patients or their parents about herbal medicine. Also, parents have to inform to their physicians on herbal remedies' uses to their children during conventional treatment [78]. Health professionals must recommend to parents the correct use of herbal medicine in children, assist in herbal

therapeutic decisions, and monitor for adverse effects and interactions [1, 77]. Finally, herbal products or folk remedies may be inherently unsafe. Owing to the possibility of serious health complications arising from the use of herbal products, it is mandatory to understand their use in the general population in order for appropriate measures to be put into place [32].

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