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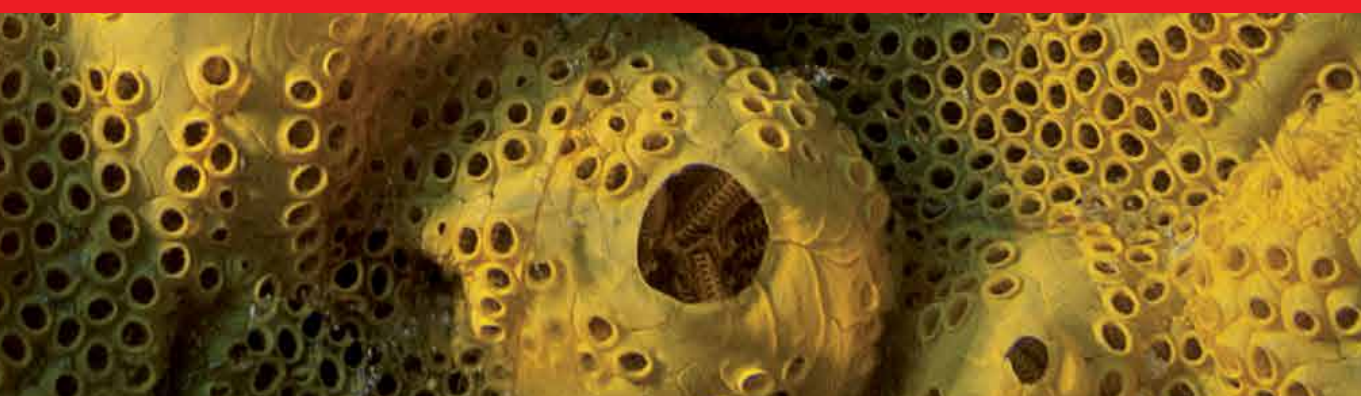
Medicinal Plants

Use in Prevention and Treatment of Diseases

Edited by Bassam Abdul Rasool Hassan



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Meet the editor

Bassam Abdul Rasool Hassan, PhD, worked as a senior lecturer at the Department of Pharmacy, Faculty of Medicine, University of Malaysia (2014–2017), and at the Department of Pharmacy Practice, Faculty of Pharmacy, Universiti Teknologi MARA (2017–2019). He is currently working as a senior lecturer at the Department of Pharmacy, Al-Rafidain University College, Baghdad, Iraq.

Contents

Preface

Cancer has become one of the major causes of death in both developed and developing countries. This disease is a result of gene mutation, which causes deformation of normal cells. It is characterized by its ability to metastasize to other parts of the body. Although a large collection of effective chemotherapeutic agents has been discovered and developed over the last 50 years, advanced metastasized cancer is still untreatable. Besides, the usage of these treatments is found to cause many critical side effects. Therefore, the demand for new, safe, and effective treatments that can significantly prevent, delay, and/or cure cancer is growing. Many types of new therapies have emerged and some of them use extracts from plants that have shown very promising anticancer properties. Recently, some plants have been considered as good sources for new anticancer treatments due to their multiple chemical compounds. Many researchers have recommended that future studies should focus more on plants as a source of safe and effective anticancer treatments and to detect their active ingredients and mechanisms of action. Therefore, this book focuses on clarifying the anticancer effects (i.e., apoptotic, antiproliferative, antimetastatic, antiangiogenic) and mechanisms of most of the medicinal plants found in the world against solid and/or hematological cancers.

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Section 1

Pharmacological Properties of Some Medicinal Plants

Chapter 1

Analgesic and Anti-Inflammatory Effect of Ghanaian Medicinal Plants

Evelyn Asante-Kwatia, Abraham Yeboah Mensah and Michael Frimpong Baidoo

Abstract

Medicinal plants continue to be used in various cultures of the world as safe therapeutic agents against various issues including pain and inflammation which underlie almost every disease process. In Ghanaian traditional medicine, various parts of several plants have been used alone or in combination of therapies for the treatment of various painful inflammatory conditions. In this chapter, the anti-inflammatory and analgesic (antinociceptive) properties of selected medicinal plants from Ghana are reviewed. Evidence of pharmacological activities of crude extracts and fractions in *in-vitro* and *in-vivo* models, bioactive anti-inflammatory and antinociceptive compounds isolated as well as possible mechanisms of anti-inflammatory and antinociceptive action are discussed.

Keywords: inflammation, nociception, analgesia, herbal medicine, Ghana

1. Introduction

Inflammation is a complex defensive and protective response of living tissues to injury, irritation or infection which is accompanied by typical symptoms of pain, swelling, redness and fever. It is a mechanism by which the body identifies and neutralises noxious stimuli by increasing the blood flow to the site of tissue injured. Inflammation is a defensive mechanism but the complexity of events as well as the mediators released often result in the induction or aggravation of several disease conditions [1, 2]. Painful conditions such as rheumatoid arthritis, osteoarthritis, asthma, inflammatory bowel disease, colitis and hepatitis as well as other chronic diseases including cardiovascular and neurodegenerative diseases are all conditions whose pathophysiology involves inflammation [3]. These diseases impose a huge social and economic burden on individual victims, their families, and societies as a whole. Moreover, they can cause disability, impairing the social function of people, reducing their quality of life and sometimes resulting in death [2]. Millions of people suffering from different types of painful inflammatory conditions wish to find effective interventions with fewer or no side effects [4].

1.1 Current drugs for the treatment of inflammation and pain and their major side effects

The range of anti-inflammatory and analgesic agents currently available all work to relieve pain, reduce inflammation, and slow down or stop tissue damage. These include non-steroidal anti-inflammatory drugs (NSAIDs), disease modifying anti-rheumatic drugs (DMARDs), opioids and corticosteroids. Some antidepressants and anti-convulsants have also been shown to increase patients' threshold to pain [5].

NSAIDS such as diclofenac, ibuprofen and aspirin act by blocking certain stages of the arachidonic acid pathway, specifically by inhibiting lipoxygenase (LOX) and cyclooxygenase enzymes (COX-1 and COX-2) responsible for converting arachidonic acid to prostaglandins (PGs). Though effective, NSAIDS are associated with major adverse effects such as gastrointestinal ulceration, intestinal perforation, cardiovascular risks, hepatotoxicity and renal failure after long term use [6].

DMARDs such as methotrexate, sulfasalazine, gold compounds and penicillamine slow the progression of joint destruction in chronic inflammatory conditions like arthritis but are reported to cause kidney failure, skin reactions, liver problems and gastrointestinal side effects [7].

Corticosteroids such as prednisone, cortisone and methylprednisolone act by inhibiting the action of phospholipase A_2 which subsequently blocks the biosynthesis of inflammatory mediators such as prostaglandins and leukotrienes. Adverse effects such as delayed wound healing, hypertension, fluid retention, weight gain and osteoporosis are reported [8].

Opioids such as morphine, codeine and pethidine are very effective centrally working analgesics which increase the threshold of pain at the spinal level. These are associated with unwanted behavioural tendencies such as physical dependence, development of tolerance and respiratory depression [9].

1.2 Anti-inflammatory and analgesic agents extracted from medicinal plants

The adverse effects of most currently used orthodox drugs for the management of painful inflammatory conditions give a strong motivation for researchers to search for other appropriate and effective treatment [10]. Through this search, drugs of plant origin have attracted much attention due to their wide acceptance, availability, reported effectiveness and safety. The discovery of the anti-inflammatory agent salicin and subsequently, aspirin from *Salix fragilis* was a significant evidence to affirm the ability of plants to produce anti-inflammatory compounds [11]. Other plant products such as capsaicin (*Capsicum annum*), curcumin (*Curcuma longa*) and frankincense (*Boswellia serrata*) have been effectively utilised as adjuncts in the treatment of inflammatory conditions and pain [12–14]. Apart from being potent, these products have an added advantage of causing no significant adverse effect or toxicity to liver and kidney cells like other synthetic agents. Medicinal plants are therefore considered as sources of anti-inflammatory and analgesic agents and as practicable alternatives to conventional medicines [15].

1.3 Experimental methods used for screening anti-inflammatory and antinociceptive activities of herbal extracts

Based on the symptoms of inflammation, several *in-vivo* and *in-vitro* screening methods have been employed to evaluate the anti-inflammatory activity of plant extracts and natural compounds.

To investigate the anti-inflammatory activity of plant extracts against acute and chronic inflammation *in-vivo*, oedema, granuloma and arthritis models

have been used. To induce inflammation, phlogistic substances or irritants such as carrageenan, mustard, dextran, egg-white, yeast, zymosan-LOX, serotonin, histamine, kaolin, etc. are employed. Some applicable methods described in literature are the carrageenan-induced paw oedema in rats or chicks, croton-oil or oxazolone-induced ear oedema in mice, UV erythema in guinea pigs, granuloma pouch technique and pleurisy in mice [16]. Adjuvant-induced and collagen-induced arthritis models are also efficient in chronic inflammation studies. *In-vitro* methods have mainly focused on the inhibition of the activation of local inflammatory mediators such as leukotrienes [tumour necrosis factor alpha (TNF-α), interleukins (IL-6, IL-1β)], prostaglandins (PGE_2), prostacyclin, thromboxane A2, interferon-λ (IFN-λ), inducible nitric oxide synthase (iNOS) and reactive oxygen species. The level of these mediators at the inflamed site is measured and compared to control groups [17, 18]. Other *in-vitro* methods include human red blood cell stabilisation and protein denaturation assays.

To determine the antinociceptive effects of herbal extracts, chemically-induced (formalin and acetic acid-induced writhing test) and thermal-induced pain models (hot plate, tail immersion, tail flick, Hargreaves paw withdrawal methods) in experimental animals are commonly used [19].

1.4 Use of herbal medicine in Ghana

Like other developing countries, Ghana continues to search for more effective and appropriate ways of providing the health needs of its developing populace. Generally, the high cost of Western therapeutic medications and additionally their unavailability to the rural communities has prompted a high interest for herbal medicines [20]. In this regard, intensive efforts are being made to explore plants that might be of therapeutic significance to the Ghanaian community. Several reports cutting across the boundaries of botany, medicine and pharmacy have highlighted the use of different plants alone or in combination therapies for the treatment diseases [21–23].

Considering the evolving interest in studying traditional systems of healthcare and exploiting the potential of natural products for future drug development, this communication presents a compilation of data on plants with promising anti-inflammatory and analgesic activity with special emphasis on plants found in Ghana. Their pharmacological action, anti-inflammatory or analgesic constituents and possible mechanisms of actions are hereby discussed. It is envisioned that this information will be helpful to the indigenes for their primary healthcare and for researchers, to further identify the active chemical constituents and mechanisms responsible for the analgesic and anti-inflammatory potential of these plants [24].

1.5 Methods used for identifying herbal materials with anti-inflammatory and analgesic activities

Electronic databases including PubMed, SciFinder and Google Scholar were employed in the search for medicinal plants with reported anti-inflammatory and analgesic activities collected from various parts of Ghana. The inclusion criteria were that (i) plant should be used in Ghanaian traditional medicine for treatment of inflammatory condition or pain; (ii) validated *in-vitro* and *in-vivo* models for screening anti-inflammatory and antinociceptive activity were employed; (iii) the right botanical names, plant parts used, types of extracts prepared, active constituents and mechanisms of action if identified were mentioned. Consideration was also given to plants with significant activity differences with reference to control groups.

2. Plants with anti-inflammatory and analgesic activities from Ghana

2.1 *Albizia zygia* (DC.) J.F. Macbr. (*Leguminosae-Mimosoideae*)

Albizia zygia is a medium-sized ornamental shade tree widely distributed in secondary forest and semi-deciduous forest zones of West and East Africa. It grows up to about 30 m tall, has a branchless cylindrical bole with a greenish-grey smooth outer bark and an orange-brown fibrous inner bark. It has alternate bipinnately compound leaves and bears oblong flat pods. It is commonly known as the West African walnut and locally called '*okuro*' in Ghana (Akan). The leaf infusion is used for the treatment of lumbago, fever, waist pain and sexually transmitted infection. The bark decoction is administered to treat respiratory tract disease, malaria fever, constipation and worm infestation. The crushed bark is applied topically to treat yaws, heal wounds and toothache [25].

In previous studies, the leaves and roots were evaluated for their analgesic properties in animal models. Oral administration of the 70% ethanolic leaf extract in rats caused a significant reduction in both neurogenic and inflammatory phases of formalin-induced paw licking with maximal inhibition of 67.81 ± 8.73% and 72.85 ± 12.74% respectively [26]. The hydro-alcoholic root extract also caused a significant diminishing of acetic acid-induced visceral pain, formalin-induced paw pain, thermal and carrageenan-induced mechanical hyperalgesia in animals *via* opioidergic, adenosinergic and muscarinic cholinergic mechanisms [27].

To validate its anti-inflammatory effects, the hydro-alcoholic root extract was evaluated in carrageenan-induced paw oedema and caused a significant reduction of paw oedema in cockerels. The extract was found to increase the expression of endogenous antioxidants such as superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) as well as reduced the action of myeloperoxidase (MPO) and malondialdehyde (MDA) levels at the inflamed site [26].

2.2 *Anopyxis klaineana* (Pierre) Engl. (*Rhizophoraceae*)

A. klaineana is a medium sized to large tree found in the evergreen and semi-deciduous forest of tropical Africa. It grows up to about 50 m tall, has a branchless, cylindrical bole with longitudinally fissured greyish-brown outer bark and a thick pale orange inner bark. It has simple leathery, glabrous leaves which occur in whorls of 3–4 and bears greenish-white hairy flowers. *A. klaineana* is locally traded as '*kokoti(e)*' in Ghana and '*bodioa*' in Cote d'Ivoire. The stem bark decoction is used to treat joint pain, gonorrhoea, skin and respiratory tract infection, pneumonia, bronchitis and malaria. Its leaves are also applied as a poultice to heal wounds [28].

The anti-inflammatory activity of the stem bark of *A. klaineana* was evaluated in previous studies. Various solvent extracts including the petroleum ether, ethyl acetate and methanol extracts showed anti-inflammatory activity in a time and dose-dependent manner, by suppressing carrageenan-induced foot pad swelling in chicks. A tetranortriterpenoid called methyl angolensate was isolated as the major constituent of the stem bark and showed anti-inflammatory activity by significantly suppressing foot pad oedema in chicks with an ED_{50} of 4.05 ± 0.0034 [29]. In another study, a tirucallane triterpenoid isolated from the stem bark namely 3,23-dioxotirucalla-7,24-dien-21-oic acid, exhibited remarkable anti-inflammatory activity in a PGE_2 competitive inhibition immunoassay with an IC_{50} value of 3.63 μM which was comparable to the positive control, cortisone (IC_{50} = 2.59 μM). Methyl angolensate further demonstrated remarkable competitive inhibition of PGE_2 with an IC_{50} of 10.23 μM confirming its *in-vivo* anti-inflammatory effect [30].

2.3 *Calotropis procera* (Ait) f. (*Apocynaceae*)

C. procera is a medium-large sized bushy shrub which grows on coarse, sandy or alkaline soils of West and East Africa also found in the Indian Ocean islands and the north of South Africa. The plant can be identified by decussate, broadly ovate, leathery leaves and bears purple flowers with erect lobes [31]. It is locally called '*mpatu-asa*' (Akan) in Ghana. In traditional medicine, the bark decoction is used for the treatment of rheumatism, arthritis, headache and general body pain. The latex from leafy twigs and flowers is used for treating conjunctivitis, nasopharyngeal infection, tooth ache, wound healing, and vermifuge. The root bark decoction is used for treating cutaneous and subcutaneous skin infection and yaws [32, 33].

A study was conducted to determine the anti-inflammatory effect of the alcoholic extract of *C. procera* leaf in *in-vitro* models including the heat-induced haemolysis, hypotonic-induced haemolysis, albumin denaturation and the bovine serum albumin assay. The 70% alcoholic leaf extract at 1000 μg/mL significantly demonstrated anti-inflammatory effect by stabilising human red blood cells exposed to heat (69.24% inhibition) and hypotonic solution (85.09% inhibition). The extract prevented denaturation of protein (albumin) as well as bovine serum by 87.8% and 96.86% respectively. In *in-vivo* studies, the extract caused significant reduction of carrageenan-induced paw oedema in both acute and chronic inflammation [34].

2.4 *Capparis erythrocarpos* Isert (*Capparaceae*)

C. erythrocarpos is a climbing shrub distributed in the coastal scrubs and inlands of many African countries and commonly referred to as 'salt bush'. The plant is densely thorny and branched with re-curved hooks, growing up to about 6m in height. Its bears green elliptical leaves, which are alternately arranged [35]. The roots are used in traditional medicine for the management of rheumatism and arthritis. Other plant parts also find use in the treatment of eye and ear infection, fever, epilepsy and as aphrodisiac. The powdered root is used at the Center for Scientific Research in to Plant Medicine (CSRPM), Mampong, Ghana for the management of arthritis [21].

To validate the analgesic effect of *C. erythrocarpos*, the 70% ethanol extract of the root, stem bark and leaf were investigated in the formalin-induced nociception, hot plate and acetic acid-induced writhing assays in mice and rats. The root extract (100 mg/kg *p.o.*) was found to significantly and dose-dependently reduce pain in the early and late phases of formalin-induced pain by 47.54 ± 5.65% and 80.01 ± 3.77% respectively via interaction with adenosinergic receptors [36]. In other studies, the leaf extract at 200 mg/kg *p.o.* showed significant analgesic effects by reducing acetic acid writhing by 27.43% and increasing the pain threshold in the hot plate assay by 184.5% [37].

To validate its anti-inflammatory effect in both acute and chronic inflammation, the 70% alcoholic root extract was investigated in the carrageenan-induced paw oedema and Freund's adjuvant-induced arthritis models respectively. The extract at 30 mg/kg *p.o.* caused marked reduction in foot oedema by 48.86 ± 20.41% and significantly reduced knee joint swelling in arthritis by 34.19 ± 15.73%. The extract prevented systemic spread of inflammation from ipsilateral to contralateral limbs [38]. In another study, the leaves, stem bark and roots also demonstrated marked anti-arthritic activity by reducing rat paw volumes in the Complete Freund's adjuvant model with ED50 values (mg/kg) of 182.5, 181.5 and 36.4 respectively [39].

2.5 *Cassia sieberiana* D.C. (*Caesalpinaceae*)

C. sieberiana is a tropical woody shrub found growing in the bushy savannahs and coastal shrubs of many African countries. The plant grows up to about 20 m tall, has a short twisted bole, with a greyish-brown fissured bark. It has spirally arranged paripinnately compound leaves which bear bright yellow flowers and dehiscent pods as fruits. The entire plant is purgative and diuretic. The root decoction and leaf infusions are used as pain reliever in rheumatism and arthritis, for treatment of ear infection, skin disease, malaria fever, gastrointestinal infection, oedema, sexually transmitted infection, as laxative and vermifuge. The boiled and squeezed fresh leaves are applied topically to heal wounds, pleurisy and boils [25, 40].

A study conducted to investigate the analgesic effects of the aqueous and ethyl acetate root extracts indicated that in the hot plate assay, the aqueous extract attenuated hyperalgesia in a dose-dependent manner with an ED_{50} of 9.7 ± 3.9 mg/kg. The ethyl acetate fraction also showed antinociceptive activity in the formalin-induced nociception, yeast induced hyperalgesia, hot plate and acetic acid writhing tests. The analgesic effect was significantly blocked by Naloxone, atropine and theophylline indicating interactions with the opioidergic, muscarinic cholinergic or adenosinergic pathways [41, 42].

In-vivo study detected that the ethyl acetate extract of *C. sieberiana* root exhibits anti-inflammatory activity by reducing carrageenan-induced foot oedema in chicks [42]. Furthermore, the 70% ethanolic root extracts dose-dependently attenuated *Mycobacterium tuberculosis*-carrageenan-induced inflammation in the rats. Serum levels of IL-1α, IL-6 and TNF-α were reduced with increasing levels of IL-10 suggesting that the anti-inflammatory activity of the root bark extract may be as a result of its immune-modulatory effects *via* interactions with these pro-inflammatory mediators [43].

2.6 *Commelina diffusa* Burm. f. (*Commelinaceae*)

C. diffusa is a perennial herb distributed in tropical African countries including Ghana, Nigeria, Ivory Coast, Gabon and Congo. The plant is a smooth and sparsely hairy herb with mucilaginous leaves and creeping stems which ascends above and roots at the nodes. It is commonly called 'climbing day flower'. The Akans in Ghana fancifully refer to it as '*Nyame bewu ansa na mawu*' meaning '*God will die before I die*' alluding to its tenacity to life. In Ghana and Nigeria, the pounded leaves are applied topically to boils and swollen glands and as a rubefacient to relief pain in rheumatism and arthritis. Other reported uses include for the treatment of skin abscess, wound, gonorrhoea, ear infection and for the relief of severe menstrual pain [44].

To evaluate its anti-inflammatory effect, the 70% ethanolic leaf extract was investigated in the carrageenan-induced foot pad oedema in chicks. The extract (30, 100 and 300 mg/kg *p.o.*) showed a dose-dependent inhibition of foot pad oedema with the maximum inhibition of 43.55% at 300 mg/kg confirming it anti-inflammatory effects [44].

2.7 *Erythrophleum ivoren*se (A Chev.) (*Fabaceae*)

E. ivorense is a large tree widely distributed in the evergreen primary and secondary forests of tropical Africa. It grows to about 40 m tall, with a cylindrical bole, sometimes fluted at the base. It is called by names like 'forest ordeal tree', 'red water tree' and 'sasswood tree' in West African countries. Among the Akan tribe in Ghana, *E. ivorense* is known as '*potrodum*'. The stem-bark and roots are usually

employed in the treatment of epilepsy, emesis, pain, oedema, constipation and worm infestation [45].

The carrageenan-induced foot pad oedema in chick was used to evaluate the anti-inflammatory activity of the roots of *E. ivorense*. The 70% alcoholic root extract suppressed foot pad oedema in a time and dose-dependent manner. Three constituents, a casein type diterpene namely erythroivorensin A, betulininc acid and the flavonoid, eriodictyol isolated from the roots exhibited significant reduction of carrageenan-induced foot pad oedema better than the standard drug diclofenac [46].

2.8 *Ficus exasperata* Vahl (*Moraceae*)

F. exasperata, commonly known as 'sand paper tree', is a deciduous, shrub growing up to about 30 m tall. It has a buttressed bole with a pale grey-green outer bark and creamy-white inner bark which exudes a clear, viscid sap when damaged. It has alternate simple pubescent leaves which are elliptical in shape. In Ghana, it is locally called '*onyankyerɛn*' (Akan), '*nyadɛlɛ*' (Nzema) or *nyadkese* (Ga). The plant is used in folk medicine for the treatment of sprain, arthritis, rheumatism, intestinal and stomach infection, high blood pressure, abscesses and respiratory tract disease [47].

The analgesic activity of the 70% alcoholic leaf extract was investigated in murine models. The extract elicited a dose-dependent significant antinociceptive effect in the formalin-induced nociception assay through interactions with adenosinergic and opioidergic pathways [48]. The leaf extract also caused significant reduction in acute carrageenan-kaolin-induced muscle hyperalgesia (ED_{50} = 31.23 ± 11.91). Significant attenuation of chronic muscle hyperalgesia in both ipsilateral and contralateral paws and total reversal of the chronic muscle hyperalgesia in rats was produced by the leaf extract [49].

The hydro-alcoholic stem bark and leaf extracts at 30–300 mg/kg, *p.o.* dose-dependently inhibited carrageenan-induced foot oedema with ED_{50}s of 50.65 ± 0.012 and 46.05 ± 12.3 respectively [50]. Moreover, the leaf extract significantly reduced the arthritic oedema in ipsilateral paws of rats with a maximal inhibition of 34.46 ± 11.42% and significantly prevented the systemic spread to the contralateral paws [51]. Furanocoumarins namely bergapten, oxypeucedanin hydrate and the sterolin, sitosterol-3-O-β-D-glucopyranoside isolated from the stem bark also exhibited anti-inflammatory activities [52].

2.9 *Glyphaea brevis* (Spreng) Monachino (*Tiliaceae*)

G. brevis is a medium sized spreading climber usually found growing in forest re-growths, rocky savannahs and swampy areas of tropical Africa. It possesses straggling sparsely stellate branchlets, which bear ovate-oblong leaves and lemon-yellow flowers. Its fruits are spindle-shaped and brown in colour with irregularly ellipsoid seeds. The leaves are used to treat dyspepsia, gastric ulcer, oedema, pain and worm infestation. The root decoction is used to treat male sexual impotence, constipation, chest pain and gastrointestinal infection [53].

The anti-inflammatory effects of the 70% ethanol extracts of the leaves and stem bark were investigated by the carrageenan induced foot pad oedema method. The extracts exhibited potent anti-inflammatory activity in doses of 30, 100 and 300 mg/kg *p.o.*, by reducing foot oedema with similar potencies at ED_{50}s ~ 21.00 mg/kg [54]. In another study, oral administration of the 70% ethanol extract of the stem bark exerted inhibitory effects on carrageenan-induced paw oedema, systemic anaphylaxis and chronic inflammation in the Freund's adjuvant-induced arthritis models. The effect was significant when the extract was given both prophylactically and therapeutically [55].

2.10 *Haematostaphis barteri* Hook. f. (*Anarcadiaceae*)

H. barteri is a woody plant typical of tropical Africa widely distributed in rocky savanna areas of Ghana, Upper Volta, Nigeria, Cameroon and Sudan. It reaches up to about 8 m high, about 65 cm in girth with a bark that contains a clear gum. It bears characteristic reddish-purple drupes which are edible with an acrid taste [33]. It is commonly called 'blood plum' and in the Upper West region of Ghana where it is locally referred to as '*zimbringa*' (Dagaari). In traditional medicine, the boiled leaves are used to treat malaria. The stem bark decoction is used for the treatment of hepatitis and sleeping sickness, while the roots are used in the treatment of oedema, pain and swelling [56].

The antinociceptive and anti-inflammatory effects of the plant were investigated in previous studies. The aqueous leaf extract significantly blocked the progression of the neurogenic and inflammatory phases of formalin-induced nociception in a dose-dependent manner. The study further revealed that *H. barteri* inhibits nociception in mice by modulating the opioidergic, adrenergic, muscarinic, ATP-sensitive K^+ channels and adenosinergic nociceptive pathways [57]. Moreover, the aqueous leaf extract inhibited carrageenan, histamine and serotonin-induced rat paw oedema significantly [58].

2.11 *Hilleria latifolia* (Lam.) H. Walt. (*Phytolaccaceae*)

H. latifolia is a woody perennial herb about 2 m tall, with weak spiky hairs on young branches. It has alternate, simple elliptical leaves, bears several whitish-green sepals and a lens-shaped fruit with a thin wrinkled pericarp. In Ghana it is locally called '*Avegboma*' (Ewe) and '*Anafranaku*' (Akan-Twi) and used for the treatment of arthritis, rheumatism, oedema, gout, worm infestation, parasitic and viral infection of the skin, respiratory and pulmonary disease including asthma [25, 59].

The ethanolic extract of the aerial plant parts of the plant was investigated for analgesic and anti-inflammatory effect *in-vivo*. The extract in doses of 30–300 mg/kg *p.o.* demonstrated remarkable antinociceptive activity in the chemical and thermal-induced pain models. It produced a dose-related analgesic effect and significantly suppressed the development of morphine tolerance after repeated co-administration with morphine [60]. Its analgesic effect is *via* alteration of adenosinergic, muscarinic cholinergic and opioid pathways [61].

The 70% ethanolic extract of the aerial parts also significantly inhibited acute inflammation in the carrageenan-induced foot oedema [61] and significantly reduced poly-arthritic oedema in the ipsilateral paw of rats but was unable to prevent systemic spread to contralateral limbs in the Freund's adjuvant-induced arthritis model [62].

2.12 *Jatropha curcas* L. (*Euphorbiaceae*)

J. curcas is a shrub or small tree about, 2–5 m tall, with a smooth bark and sparsely lenticellate branches. The leaves are broadly palmate and inflorescences greenish-yellow. At maturity it produces ellipsoidal capsules containing black seeds. The seed oil is used to treat eczema, skin disease and to soothe rheumatic pain. The root powder is topically applied as a paste to treat swelling and inflammatory condition such as gout [63].

In studies of its analgesic activity, the 70% ethanolic root extract (30–300 mg/kg, *p.o.*) significantly inhibited acute and chronic skeletal hyperalgesia induced by 3% kaolin-carrageenan mixture in both ipsilateral and contralateral limbs of rats [64].

2.13 *Lannea acida* A. Rich (*Anacadiaceae*)

The name of the genus, *Lannea*, originates from the Latin word '*lana*' which translates to 'wool' alluding to the densely hairy young plant parts or possibly to the wool on the roots of some *Lannea* species. The plant occurs in different habitats in Sub-Saharan Africa including Benin, Burkina Faso, Cameroon, Central African Republic, Côte d'Ivoire, Gambia, Ghana, Guinea, Mali, Niger, and Nigeria. It usually grows in wooded savannah, forest edges, bushed grassland, rocky outcrops, and near rivers on sandy soils. It bears berry-like fruits which occur in large clusters and are consumed either fresh or dried. The fruits have a slightly acidic but pleasant taste. In traditional medicine, *L. acida* is used for the treatment of inflammatory condition, pain, schistosomiasis, haemorrhoid and toothache [65].

The aqueous stem bark extract was evaluated for anti-inflammatory effect and caused a significant dose-dependent reduction of PGE_2-induced rat paw oedema with maximal oedema inhibition of 67.1%. The stem bark extract also inhibited writhing movement in the acetic acid-induced writhing test in mice models [66].

2.14 *Newbouldia laevis* Seem. (*Bignoniaceae*)

N. laevis is a shrubby small to medium sized ornamental tree with several vertically ascending stems usually found growing in the wooded savanna and deciduous forests across tropical Africa. The plant has shiny dark green leaves and bears large terminal purple flowers. *N. laevis* finds use in folk medicine for the treatment of epilepsy, elephantiasis, haemorrhoid, pelvic pain, peptic and skin ulcer, rheumatism and as antidote to snake bite [40].

The analgesic and anti-inflammatory activity of the leaves have been investigated in several models. At 300 mg/kg *p.o.*, the 70% ethanol leaf extract significantly increased the paw withdrawal latency of mice in a tail immersion (withdrawal) test by 88.45 ± 19.81% indicating decreased sensitivity to pain. The leaf extract further inhibited the neurogenic (54.47 ± 8.60%) and inflammatory phases (83.62 ± 6.03%) of formalin-induced nociception and blocked the effect of carrageenan-induced thermal hyperalgesia by 37.60 ± 7.26% [67]. In another study, the hydro-alcoholic stem bark extract significantly and dose-dependently decreased formalin-induced nociceptive behaviour in rats [68].

The ethanolic leaf extract significantly and dose-dependently, inhibited carrageenan-induced foot oedema with maximal inhibition of 64.41 ± 11.47% [67]. In another study, the ethanol stem bark extract inhibited the poly-arthritic phase limb swelling in rat adjuvant-induced arthritis by 28.11 ± 2.02% justifying the use of the stem bark in the management of arthritis [69].

2.15 *Palisota hirsuta* K. Schum (*Commelinaceae*)

P. hirsuta is one of the most commonly used species of Commelinaceae. It is a robust perennial herb with lax inflorescences, lateral branches, purplish flowers and black glossy fruits. It is usually found in lowland rain-forest of West Africa. In Ghana it is commonly called '*somenini*' or '*mpentemi*' in Akan, '*sumbe*' in Ewe and '*sombenyin*' in Fante languages. Various parts of the plant are used in traditional medicine for the treatment of general body pain, earache, pelvic pain, piles, tooth ache, swelling and wound [70].

The ethanolic leaf extract of *P. hirsuta* was investigated for its analgesic effect. The extract (30–300 mg/kg *p.o.*) caused a significant increase in tail withdrawal latency by 73.75 ± 14.99%; reversed carrageenan-induced hyperalgesia with a percentage maximum effect of 154.79 ± 15.84%; reduced the number of acetic acid

writhing with an ED_{50} of 80.20 ± 0.58 mg/kg and decreased formalin-induced nociception by 83.46 ± 6.67% and 94.56 ± 4.12% in the early and late phases respectively [71].

In other studies, oral administration of the leaf extract (30–300 mg/kg *p.o.*) resulted in a dose-dependent complete reversal of vincristine-induced neuropathic pain in rats [72]. An ecdysteroid called 20-hydroxyecdysone was isolated from the root and was found to inhibit formalin-induced nociception in rats by 71.39 ± 9.19% and 89.19 ± 3.81% respectively in the early and late phases [73].

The ethanolic root extract (50–400 mg/kg *p.o.*) demonstrated remarkable reduction of carrageenan-induced foot oedema in chicks in both curative (62.52 ± 4.73%) and prophylactic (58.90 ± 11.38%) treatment regimens [74]. Further, the ethanolic leaf extract caused significant reduction in arthritic oedema induced by Freund's adjuvant and prevented the systemic spread of arthritis from the ipsilateral to the contralateral limb [75].

2.16 *Picralima nitida* (Stapf) T. Durand & H. Durand (*Apocynaceae*)

P. nitida is a medium sized to large tree which reaches up to 35 m in height with a dense crown, a pale yellow, fine grained inner wood and a cylindrical trunk. The leaves are broadly oblong with hard tiny lateral nerves and bear white flowers with ovoid fruits which turn yellow at maturity. *P. nitida* is widely distributed in the deciduous forests of West and Central Africa. In Ghana, the seeds are locally known as '*akuama*' (Asante-Akan) or '*onwema*' (Fante) and are used for the treatment of pain of various aetiologies as well as fever. Other plant parts find use in folk medicine for the treatment of malaria, fever, worm infestation, venereal disease, respiratory tract infection, constipation and jaundice [76].

Investigation of the analgesic effects of seeds collected from Ghana established that the aqueous seed extract possessed significant antinociceptive effect in murine models tested by the hot plate assay. Indole alkaloids isolated from the seeds, namely akuammidine, akuammine, akuammicine, akuammigine and pseudoakuammigine also exhibited potent analgesic effects in an isolated tissue and radio-ligand binding assay, demonstrating varying degrees of agonist and antagonist activity at μ-, δ-, and κ-opioid receptors [77, 78].

In anti-inflammatory studies, the hydro-ethanolic extract of the seeds demonstrated a dose-dependent suppression of paw oedema in the carrageenan-induced paw oedema assay. The extract further showed inhibition of chronic inflammation in rat adjuvant-induced arthritis. The total alkaloidal extract at 75–300 mg/kg *p.o.* caused a significant dose-dependent inhibition of total oedema formation in carrageenan-induced paw oedema assay and reduced adjuvant-induced knee joint swelling in rats [79]. Pseudoakuammigine displayed significant dose-dependent suppression of total paw oedema by 82.8 ± 94.6% [78].

2.17 *Phyllanthus muellerianus* (Kuntze) Exell. (*Euphorbiaceae*)

P. muellerianus is a straggling shrub about 12 m tall with spreading branches and several short axillary shoots dispersed in the deciduous and secondary forests of tropical Africa. It has simple alternate glabrous leaves and clustered whitish-green flowers. The plant bears fleshy six-seeded smooth capsules which are green when young and black at maturity. The fresh twigs are chewed to prevent toothache and also used to treat dysmenorrhea, dropsy, wound, swelling, oedema, tumour, paralysis and epilepsy [80].

The aerial part of the plant was investigated for analgesic and anti-inflammatory effects in various models. Oral administration of the aqueous extract in doses

of 30, 100, 300 mg/kg, produced significant antinociceptive effect in the acetic acid-induced abdominal writhing and formalin-induced nociception models in rats [81]. The antinociceptive effect of its major constituent geraniin was demonstrated *via* interaction with opioidergic receptors. Geraniin was found to potentiate the antinociceptive effects of diclofenac and morphine when co-administered [82].

In the carrageenan-induced acute inflammation model, the 70% ethanolic extract of the whole plant and 10 mg/kg of its major constituent geraniin significantly reduced paw oedema by 46.75 ± 4.97% and 61.65 ± 6.70% respectively. The extract and geraniin further attenuated arthritis by reducing total limb swelling in the Freund's adjuvant-induced arthritis model. Histomorphological analysis revealed reduced bone damage in both extract and geraniin treated groups [83].

2.18 *Secamone afzelii* (Schult.) K. Schum (*Asclepiadaceae*)

S. afzelii is a slender creeping woody climber about 12 m long, with dark brown branches which contain whitish latex. Its leaves are pinnately compound with entire margins and exude an odourless white gummy substance with slightly acrid taste when cut. It bears numerous flowers and achene (cypsela) fruits. In West Africa, the leaves are used to treat constipation, pain in rheumatism and arthritis, gastrointestinal discomfort, urinary tract and sexually transmitted infection [84].

To evaluate the anti-inflammatory effect of the plant, the ethanolic leaf extract (30–300 mg/kg *p.o.*) was examined in the carrageenan-induced foot oedema in chicks and caused a dose-dependent inhibition foot oedema. The highest dose of the extract gave a 44.26% inhibition of oedema [85].

2.19 *Synedrella nodiflora* (Linn.) Gaertn. (*Asteraceae*)

S. nodiflora is a common weed usually found growing along the banks of rivers, streams and roadsides of tropical African countries. It is an erect branched annual herb with ascending woody stems branching dichotomously from the base. It leaves occur in opposite pairs, elliptic in shape with finely toothed margins and bear small crowded yellow flowers at nodes. The whole plant is boiled in water and drank for the treatment of convulsion, threatened miscarriage, constipation, arthritis and as haemostatic [86].

The analgesic effect of the whole plant was investigated in several animal models. The ethanolic extract of the whole plant (100–1000 mg/kg *p.o.*) significantly reduced the number of writhes in mice during an acetic acid-induced writhing assay (ED_{50} = 141.9 ± 37.16) and inhibited both neurogenic (ED_{50} = 25.98 ± 14.59) and inflammatory (ED_{50} = 30.24 ± 18.08) phases of the nociceptive pain produced by formalin *via* adenosinergic mechanisms [87]. In other studies, the hydroethanolic extract of the whole plant (100–1000 mg/kg *p.o.*) caused a significant decrease to pain perception in mechanical, tactile, cold water and thermal hyperalgesia in paclitaxel and vincristine-induced neuropathic pain [88, 89].

2.20 *Trichilia monadelpha* (Thonn) JJ De Wilde (*Meliaceae*)

T. monadelpha is an evergreen, small to medium-sized tree with a straight cylindrical bole, smooth greyish outer bark and a pale pink inner wood. Its leaves are alternate, imparipinnately compound. It bears greenish yellow flowers and an obovoid 6-seeded dehiscent capsule. It is commonly known as '*otanduru*' (Akan-Twi) in Ghana and found growing at the river banks near evergreen semi deciduous forests. Various parts of the plant find use in traditional medicine for the treatment of inflammatory condition and neurological disorder such as epilepsy and psychosis [90].

Various solvent extracts (pet-ether, ethyl acetate and methanol) of the stem bark were evaluated for analgesic and anti-inflammatory effect. A significant dose-dependent antinociceptive activity in the chemical, thermal and mechanical models of pain was elicited by interaction with opioidergic, muscarinic cholinergic and adenosinergic pathways [91].

The aqueous and pet-ether stem bark extracts suppressed carrageenan-induced foot oedema in chicks by 57.79 ± 3.92% and 63.83 ± 12.0% respectively. In a Complete Freund's Adjuvant-induced arthritis assay, the aqueous extract (100 mg/kg *p.o.*) caused a significant attenuation of chronic inflammation by reducing joint thickness by 64.41 ± 5.56% [92]. Moreover, the stem bark extract caused significant reduction in the high levels of TNF-α, IL-6, malonaldehyde and myeloperoxidase and increased the levels of superoxide dismutase [93] and improved arthritic score by reducing redness, swelling and joint stiffness in rats. Hyperplasia, formation of pannus and exudation of inflammatory cells into synovial spaces were also reduced [94].

2.21 *Vernonia amygdalina* Delile. (*Compositae*)

V. amygdalina is a widely grown shrub in many African countries including Ghana, Nigeria, Cameroon, Togo, Benin, Guinea and Sierra Leone. It reaches up to about 10 m tall and is severally branched with a greyish-brown smooth bark. Its leaves are ovate-elliptical in shape, simple and alternately arranged with minutely toothed margin. It bears a 10-ribbed achene pubescent dark brown to black fruit. Due to the bitterness of its leaves, the plant is called 'bitter leaf' in many countries. In Ghana, the Akans refer to it as '*awonwene*' (Twi) literally meaning 'bitterness'. The leaves, stem bark and roots are used to treat malaria, fever, worm infestation, skin and nasopharyngeal infection, diarrhoea, dysentery, diabetes and as pain reliever in arthritis and rheumatism [95].

In previous studies, the anti-inflammatory properties of the leaves were evaluated in various models. The ethanol extracts of the young and old leaves (200 mg/kg *p.o.*) caused a significant dose-dependent inhibition of carrageenan-induced cold allodynia, increased the tail withdrawal latency in the tail immersion test and reduced the paw licking time in formalin-induced nociception test in rats *via* opioidergic, nitric oxide cyclic GMP and the muscarinic cholinergic pathways [96].

The young leaf extract at 50, 100 and 200 mg/kg *p.o.* significantly and dose-dependently reduced carrageenan-induced foot pad oedema by 59.61%, 67.52% and 86.31% respectively. Similarly, the old leaf extract at same doses exhibited remarkable suppression of oedema formation by 56.11%, 63.37% and 67.41% respectively [96].

2.22 *Wissadula amplissima* var. rostrata (Schum. & Thonn.) (*Malvaceae*)

W. amplissima is an erect, shrubby herb which grows up to 2.5 m tall on rocky and loamy soils of grassland, bushes and forests in tropical Africa. The leaves have entire or slightly toothed margins, densely pubescent on the lower surface but with sparsely stellate hairs on the dark green upper surface. The leaves are used as a poultice to relief spider bite and sting by venomous insects [25, 97].

The anti-inflammatory activity of the pet ether, chloroform and methanol fractions was investigated in 7-day old chicks and showed significant dose-dependent reduction of carrageenan-induced foot oedema. Maximal oedema inhibition was recorded as 68.25 ± 2.03%, 77.83 ± 0.81% and 62.21 ± 2.61% for the three extracts respectively [98].

2.23 *Xylopia aethiopica* (Dunal) A. Rich. (*Annonaceae*)

X. aethiopica is a tall evergreen aromatic tree with a smooth greyish-brown bark, severally branched crown and a buttressed bole. Its leaves are coriaceous, green on the upper surface and greenish-brown to orange on the lower surface. It bears small dark brown, cylindrical twisted bean-like aromatic pods, with about 5–8 black seeds per pod. The tree is usually found in lowland rainforests, coastal brackish swamps and deciduous forests of tropical Africa. The fruit is the most important part of the plant and is commonly known as the 'African pepper'. In Ghana it is locally referred to as '*hwentia*' (Twi), '*tso*' (Ewe) and '*soo*' (Ga). It is used as a flavouring in the preparation of soups and for the treatment of inflammatory conditions such as arthritis, bronchitis, rheumatism, lumbago, headache, neuralgia and colic pain [25].

The ethanolic fruits extract and its major diterpene constituent, xylopic acid were investigated for analgesic effects in several pain models. The fruit extract (XAE, 30–300 mg/kg *p.o.*) and xylopic acid (XA, 10–100 mg/kg *p.o.*) inhibited acetic acid-induced visceral nociception, formalin-induced paw pain, thermally-induced as well as carrageenan-induced mechanical and thermal hyperalgesia [99]. XAE and XA also exhibited anti-hyperalgesic and anti-allodynic properties in vincristine and paclitaxel-induced neuropathic pain [100, 101]. Co-administration of XA and pregabalin synergistically reduced paclitaxel induced neuropathic pain without causing any toxicity [102]. XAE and XA dose-dependently reduced both acute and chronic carrageenan-induced musculoskeletal pain [103] *via* opioidergic, adenosinergic, adrenergic, bradykinin and prostaglandin nociceptive pathways [104].

In anti-inflammatory studies, the aqueous fruit extract (300 mg/kg *p.o.*) caused a significant reduction of carrageenan-induced paw oedema in mice through inhibition of histamine release from mast cells [105]. Histopathology revealed substantial reduction in mononuclear infiltration, formation of pannus and bone erosion [106]. XA also caused inhibition of inhibition of histamine, serotonin, bradykinin and prostaglandin E_2-induced inflammation [107].

2.24 *Ziziphus abyssinica* Hochst Ex A. Rich (*Rhamnaceae*)

Z. abyssinica is a thorny, semi-deciduous plant, varying in habit from an erect shrub, a climbing plant or a tree with sagging branches that form a heavy, rounded crown. It usually reaches up to about 12 m tall and has a straight bole. It is commonly known as 'Catch thorn' in English and '*larukluror*' among the Sissala people of Ghana. The root and leaves are useful in folk medicine for treatment of pneumonia, tonsillitis, burn wound, chest pain, migraine and as a general pain-killer [108].

The analgesic and anti-inflammatory effects of the roots were investigated. The hydro-ethanolic root bark extract (30–300 mg/kg, *p.o.*) dose-dependently inhibited acetic acid-, formalin- and glutamate-induced nociception with maximal inhibition of 86.29 ± 2.27%, 84.97 ± 5.35%, and 82.81 ± 5.97% respectively. The paw withdrawal latencies in both tail-immersion and carrageenan-induced hyperalgesia were also prolonged [109]. Moreover, the root extract reversed hyper-nociception induced by intra-plantar injection of TNF-α, IL-1β, bradykinin and prostaglandin E_2 *via* interactions with opioidergic, adenosinergic, ATP-sensitive potassium channels and nitric oxide cyclic GMP pathways [110].

In an *in-vitro* assay, the hydro-alcoholic root extract at 100 μg/mL inhibited heat and hypotonic-induced haemolysis of human red blood cells by 61.8% and 42.98% respectively. The extracts also inhibited protein (albumin) and bovine serum albumin denaturation. Significant reduction of carrageenan-induced paw oedema

and a decreased the level of neutrophils in the peritoneal cavity were observed after oral administration of the root extract [111].

3. Conclusion

The Ghanaian flora provides a potent promising source for new therapeutic interventions for local population. The anti-inflammatory and analgesic activities of the crude extracts and fractions of several medicinal plants employed in Ghanaian traditional medicine have been validated in several models. However, the specific bioactive constituents are not yet identified. Therefore further studies to isolate and verify these anti-inflammatory and analgesic compounds are highly recommended. Further evaluation of safety profiles and standardisation of most active plants will add substantial value to the reported bioactivities and make these plants attractive for adaptation to pharmaceutical companies for further development.

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Conflict of interest

Authors have no conflict of interest to declare.

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References

[1] Kunnumakkara AB, Sailo BL, Banik K, Harsha C, Prasad S, Gupta SC, et al. Chronic diseases, inflammation, and spices: How are they linked? Journal of Translational Medicine. 2018;**16**(1):1-14

[2] Fürst R, Zündorf I. Plant-derived anti-inflammatory compounds: Hopes and disappointments regarding the translation of preclinical knowledge into clinical progress. Mediators of Inflammation. 2014;**2014**:1-9

[3] Allison D, Ditor D. Immune dysfunction and chronic inflammation following spinal cord injury. Spinal Cord. 2015;**53**(1):14-18

[4] Bahmani M, Shirzad H, Majlesi M, Shahinfard N, Rafieian-Kopaei M. A review study on analgesic applications of Iranian medicinal plants. Asian Pacific Journal of Tropical Medicine. 2014;**7**:S43-S53

[5] Modi C, Mody S, Patel H, Dudhatra G, Kumar A, Avale M. Toxicopathological overview of analgesic and anti-inflammatory drugs in animals. Journal of Applied Pharmaceutical Science. 2012;**2**(1):149-157

[6] Pelletier J-P, Martel-Pelletier J, Rannou F, Cooper C. Efficacy and safety of oral NSAIDs and analgesics in the management of osteoarthritis: Evidence from real-life setting trials and surveys. Seminars in Arthritis and Rheumatism. 2016;**45**(4):S23-S27

[7] Ramiro S, Sepriano A, Chatzidionysiou K, Nam JL, Smolen JS, Van Der Heijde D, et al. Safety of synthetic and biological DMARDs: A systematic literature review informing the 2016 update of the EULAR recommendations for management of rheumatoid arthritis. Annals of the Rheumatic Diseases. 2017;**76**(6):1101-1136

[8] Oray M, Abu Samra K, Ebrahimiadib N, Meese H, Foster CS. Long-term side effects of glucocorticoids. Expert Opinion on Drug Safety. 2016;**15**(4):457-465

[9] Manglik A, Lin H, Aryal DK, McCorvy JD, Dengler D, Corder G, et al. Structure-based discovery of opioid analgesics with reduced side effects. Nature. 2016;**537**(7619):185-190

[10] Stark TD, Mtui DJ, Balemba OB. Ethnopharmacological survey of plants used in the traditional treatment of gastrointestinal pain, inflammation and diarrhea in Africa: Future perspectives for integration into modern medicine. Animals. 2013;**3**(1):158-227

[11] Chang T-N, Deng J-S, Chang Y-C, Lee C-Y, Jung-Chun L, Lee M-M, et al. Ameliorative effects of scopoletin from *Crossostephium chinensis* against inflammation pain and its mechanisms in mice. Evidence-Based Complementary and Alternative Medicine. 2012;**2012**:1-10

[12] Derry S, Rice AS, Cole P, Tan T, Moore RA. Topical capsaicin (high concentration) for chronic neuropathic pain in adults. Cochrane Database of Systematic Reviews. 2017;**1**:1-69

[13] He Y, Yue Y, Zheng X, Zhang K, Chen S, Du Z. Curcumin, inflammation, and chronic diseases: How are they linked? Molecules. 2015;**20**(5):9183-9213

[14] Al-Yasiry ARM, Kiczorowska B. Frankincense-therapeutic properties. Advances in Hygiene & Experimental Medicine. 2016;**70**:380-391

[15] Jamshidi-Kia F, Lorigooini Z, Amini-Khoei H. Medicinal plants: Past history and future perspective. Journal of Herbmed Pharmacology. 2018;**7**(1):1-7

[16] Patel M, Murugananthan G, Gowda K. *In-vivo* animal models in preclinical evaluation of anti-inflammatory activity—A review. International Journalof Pharmaceutical Research and Allied Sciences. 2012;**1**(2):1-5

[17] Hariram Nile S, Won PS. Optimized methods for *in-vitro* and *in-vivo* anti-inflammatory assays and its applications in herbal and synthetic drug analysis. Mini Reviews in Medicinal Chemistry. 2013;**13**(1):95-100

[18] Morris CJ. Carrageenan-induced paw edema in the rat and mouse. In: Winyard PG, Willoughby DA, editors. Inflammation Protocols. Totowa, NJ: Humana Press; 2003. pp. 115-121

[19] Patel PK, Sahu J, Chandel SS. A detailed review on nociceptive models for the screening of analgesic activity in experimental animals. International Journal of Neurologic Physical Therapy. 2016;**2**:44-50

[20] Agyei-Baffour P, Kudolo A, Quansah DY, Boateng D. Integrating herbal medicine into mainstream healthcare in Ghana: Clients' acceptability, perceptions and disclosure of use. BMC Complementary and Alternative Medicine. 2017;**17**(513):1-9

[21] Adeniyi A, Asase A, Ekpe PK, Asitoakor BK, Adu-Gyamfi A, Avekor PY. Ethnobotanical study of medicinal plants from Ghana; confirmation of ethnobotanical uses, and review of biological and toxicological studies on medicinal plants used in Apra Hills Sacred Grove. Journal of Herbal Medicine. 2018;**14**:76-87

[22] Appiah K, Oppong C, Mardani H, Omari R, Kpabitey S, Amoatey C, et al. Medicinal plants used in the Ejisu-Juaben municipality, Southern Ghana: An Ethnobotanical Study. Medicines. 2019;**6**(1):1-27

[23] Asase A, Yohonu DT. Ethnobotanical study of herbal medicines for management of diabetes mellitus in Dangme West District of southern Ghana. Journal of Herbal Medicine. 2016;**6**(4):204-209

[24] Sengupta R, Sheorey SD, Hinge MA. Analgesic and anti-inflammatory plants: An updated review. International Journal of Pharmaceutical Sciences Review and Research. 2012;**12**(2):114-119

[25] Neuwinger HD. African Traditional Medicine: A Dictionary of Plant Use and Applications. With Supplement: Search System for Diseases. Stuttgart, Germany: Medpharm Scientific; 2000

[26] Abotsi WKM, Lamptey SB, Afrane S, Boakye-Gyasi E, Umoh RU, Woode E. An evaluation of the anti-inflammatory, antipyretic and analgesic effects of hydroethanol leaf extract of *Albizia zygia* in animal models. Pharmaceutical Biology. 2017;**55**(1):338-348

[27] Abotsi WKM, Lamptey SB, Boakye-Gyasi E, Woode E. *Albizia zygia* (DC.) JF Macbr. (Leguminosae-Mimosoideae) root extract exhibits anti-nociceptive and antipyretic activities in murine models. Journal of Ethnopharmacology. 2017;**199**:183-193

[28] Oteng-Amoako A, Essien C. *Anopyxis klaineana* (Pierre) Engl. Wageningen, Netherlands: Plant Resources of Tropical Africa/Ressources Végétales de l'Afrique Tropicale; 2011

[29] Mireku EA, Mensah AY, Mensah ML, Tocher DA, Habtemariam S. Anti-inflammatory properties of the stem-bark of *Anopyxis klaineana* and its major constituent. Methyl Angolensate. Phytotherapy Research. 2014;**28**(12):1855-1860

[30] Mireku EA, Kusari S, Eckelmann D, Mensah AY, Talontsi FM, Spiteller M.

Anti-inflammatory tirucallane triterpenoids from *Anopyxis klaineana* Pierre (Engl.),(Rhizophoraceae). Fitoterapia. 2015;**106**:84-91

[31] Murti Y, Yogi B, Pathak D. Pharmacognostic standardization of leaves of *Calotropis procera* (Ait.) R. Br. (Asclepiadaceae). International Journal of Ayurveda Research. 2010;**1**(1):14-17

[32] Yogi B, Gupta SK, Mishra A. *Calotropis procera* (Madar): A medicinal plant of various therapeutic uses—A review. Bulletin of Environment, Pharmacology and Life Sciences. 2016;**5**:74-81

[33] Arbonnier M. Arbres, arbustes et lianes des zones sèches d'Afrique de l'Ouest. Paris, France: CIRAD–MNHN, Margraf publishers, Gmbh; 2002

[34] Obese E, Ameyaw EO, Biney RP, Henneh IT, Edzeamey FJ, Woode E. Phytochemical screening and anti-inflammatory properties of the hydroethanolic leaf extract of *Calotropis procera* (Ait). R. Br.(Apocynaceae). Journal of Pharmaceutical Research International. 2018;**23**(1):1-11

[35] Twumasi MA, Ekuadzi E, Mante PK, Boakye-Gyasi ME, Mensah MLK, Woode E. Pharmacognostic studies of the leaves, stem and root of *Capparis erythrocarpos* Isert (Capparaceae). Pharmacognosy Journal. 2019;**11**(1):112-118

[36] Woode E, Ansah C. Antinociceptive effect of an Ethanolic extract of *Capparis erythrocarpos* Isert root in the mice formalin test. International Journal of Pharmacology. 2009;**5**(6):354-361

[37] Kumatia EK, Antwi S, Brew-Daniels H, Appiah AA, Ocloo A. *In-vivo* comparative anti-inflammatory and analgesic activities of root bark, stem and leaf extracts of *Capparis erythrocarpus* (Capparaceae). Pharmacognosy Journal. 2019;**11**(3):515-520

[38] Danquah CA, Woode E, Gyasi EB, Duwiejua M, Ansah C. Anti-inflammatory and antipyretic effects of an ethanolic extract of *Capparis erythrocarpos* Isert roots. Research Journal of Medicinal Plant. 2011;**5**(2):158-168

[39] Twumasi M, Tandoh A, Mante P, Ekuadzi E, Boakye-Gyasi M, Benneh C, et al. Leaves and stems of *Capparis erythrocarpos*, more sustainable than roots, show antiarthritic effects. Journal of Ethnopharmacology. 2019;**238**. DOI: 10.1016/j.jep.2019.111890

[40] Arbonnier M. Trees, Shrubs and Lianas of West African Dry Zones. Paris, France: CIRAD-MNHN, Margraf Publishers, Gmbh; 2004

[41] Duwiejua M, Panyin AB, Weremfo A, Woode E, Ansah C. Antinociceptive activity of the ethanolic extract of the root bark of *Cassia sieberiana* (Fam. Caesalpinaceae). Journal of Pharmacy & Bioresources. 2008;**4**:49-58

[42] Donkor K, Okine LN, Abotsi WK, Woode E. Antiinflammatory and anti-nociceptive effects of ethyl acetate fraction of root bark of *Cassia sieberiana* DC in murine models. Pharmacologia. 2013;**4**(4):301-310

[43] Donkor K, Woode E, Okine LK. Immunoregulatory activity of root bark of *Cassia sieberiana* DC in a modified adjuvant-induced arthritis in rat. Journal of Medicinal Plants Research. 2017;**11**(39):613-620

[44] Mensah AY, Mireku EA, Oppong-Damoah A. Amponsah IK. Anti-inflammatory and antioxidant activities of *Commelina diffusa* (Commelinaceae). 2014;**2**(10):1159-1165

[45] Adu-Amoah L, Agyare C, Kisseih E, Ayande PG, Mensah KB. Toxicity assessment of *Erythrophleum ivorense* and *Parquetina nigrescens*. Toxicology Reports. 2014;**1**:411-440

[46] Armah FA, Annan K, Mensah AY, Amponsah IK, Tocher DA, Habtemariam S. Erythroivorensin: A novel anti-inflammatory diterpene from the root-bark of *Erythrophleum ivorense* (A Chev.). Fitoterapia. 2015;**105**:37-42

[47] Niangadouma R. *Ficus exasperata Vahl*. Plant Resources of Tropical Africa/ Ressources végétales de l'Afrique tropicale. 2010. Available from: http://www.prota4u.org/search.asp. [Accessed: 31 July 2019]

[48] Woode E, Poku RA, Ainooson GK, Boakye-Gyasi E, Abotsi WKM, Mensah TL, et al. An evaluation of the anti—Inflammatory, antipyretic and antinociceptive effects of *Ficus exasperata* (Vahl) leaf extract. Journal of Pharmacology and Toxicology. 2009;**4**(4):138-151

[49] Boakye-Gyasi E, Abotsl WKM, Biney RP, Amevaw EO, Woode E. Effects of hydroalcoholic leaf extract of *Ficus Exasperata* Vahl (Moraceae) in carrageenan/kaolin-induced acute and chronic musculoskeletal pain in rats. West African Journal of Pharmacy. 2014;**25**(1):27-37

[50] Fleischer T, Annan K, Dickson R, Mensah A, Sarpong F. Anti-inflammatory, antioxidant and antimicrobial activity of the stem bark extract and fractions of *Ficus exasperata* Vahl.(Moraceae). Journal of Pharmacognosy and Phytochemistry. 2013;**2**(3):38-44

[51] Abotsi WM, Woode E, Ainooson GK, Amo-Barimah AK, Boakye-Gyasi E. Antiarthritic and antioxidant effects of the leaf extract of *Ficus exasperata* P. Beauv.(Moraceae). Pharmacognosy Research. 2010;**2**(2):89-97

[52] Amponsah IK, Fleischer TC, Dickson RA, Annan K, Thoss V. Evaluation of anti-inflammatory and antioxidant activity of Furanocoumarins and Sterolin from the stem bark of *Ficus exasperata* Vahl (Moraceae). Journal of Scientific and Innovative Research. 2013;**2**(5):880-887

[53] Osafo N, Boakye YD. *Glyphaea brevis* (Spreng.) Monach.: A review of the ethno-medical, phytochemical and pharmacological investigations. Journal of Pharmaceutical Research International. 2016;**12**(2):1-18

[54] Dickson R, Ekuadzi E, Annan K, Komlaga G. Antibacterial, anti-inflammatory, and antioxidant effects of the leaves and stem bark of *Glyphaea brevis* (Spreng) Monachino (Tiliaceae): A comparative study. Pharmacognosy Research. 2011;**3**(3):166

[55] Obiri DD, Osafo N, Abotsi RE. Antiallergic and antiarthritic effects of stem bark extract of *Glyphaea brevis* (Spreng) Monachino (Tiliaceae) in murine models. ISRN Pharmacology. 2013;**2013**:1-13

[56] Asase A, Oteng-Yeboah AA, Odamtten GT, Simmonds MS. Ethno-botanical study of some Ghanaian anti-malarial plants. Journal of Ethnopharmacology. 2005;**99**(2):273-279

[57] Ameyaw EO, Kukuia KKE, Thomford AK, Kyei S, Mante PK, Boampong JN. Analgesic properties of aqueous leaf extract of *Haematostaphis barteri*: Involvement of ATP-sensitive potassium channels, adrenergic, opioidergic, muscarinic, adenosinergic and serotoninergic pathways. Journal of Basic and Clinical Physiology and Pharmacology. 2016;**27**(6):557-561

[58] Boampong JN. *In-vivo* antiplasmodial, anti-inflammatory, and analgesic properties, and safety profile of root extracts of *Haematostaphis barteri* hook F.(Anacardiaceae). Journal of Parasitology Research. 2015;**6**(3):182-188

[59] Amponsah IK, Mensah AY, Otoo A, Mensah MLK, Jonathan J.

Pharmacognostic standardisation of *Hilleria latifolia* (Lam.) H. Walt. (Phytolaccaceae). Asian Pacific Journal of Tropical Biomedicine. 2014;**4**(12):941-946

[60] Woode E, Abotsi WK. Antinociceptive effect of an ethanolic extract of the aerial parts of *Hilleria latifolia* (Lam.) H. Walt. (Phytolaccaceae). Journal of Pharmacy and Bioallied Sciences. 2011;**3**(3):384-396

[61] Abotsi WKM, Boakye-Gyasi E, Tandoh A, Lamptey BS, Woode E. Further studies on the anti-nociceptive and anti-inflammatory effects of the aerial parts of *Hilleria latifolia* (Lam.) H. Walt. 2014;**3**(5):367-373

[62] Abotsi WKM, Ainooson GK, Woode E. Anti-inflammatory and antioxidant effects of an ethanolic extract of the aerial parts of *Hilleria latifolia* (Lam.) H. Walt. (Phytolaccaceae). African Journal of Traditional, Complementary and Alternative Medicines. 2012;**9**(1):138-152

[63] Ameyaw E, Agyei P, Boampong J, Kyei S, Koranteng K. Ethanolic root extract of *Jatropha curcas* L. ameliorates paclitaxel-induced neuropathic pain in rats. Research and Reviews Journal of Pharmacology. 2013;**3**:10-15

[64] Ofori AE. Ethanolic root extract of *Jatropha curcas* L. relieves hyperalgesia in arthritis model in rats. International Journal of Pharmaceutical Reseaarch. 2014;**4**(1):1-18

[65] Ziblim IA, Timothy KA, Deo-Anyi EJ. Exploitation and use of medicinal plants, Northern Region, Ghana. Journal of Medicinal Plants Research. 2013;**7**(27):1984-1993

[66] Owusu G, Ofori-Amoah J. Anti-inflammatory and analgesic effects of an aqueous extract of *Lannea acida* stem bark. British Journal of Pharmaceutical Research International. 2017;**16**(6):1-8

[67] Boakye-Gyasi E, Abotsi W, Woode E. Anti-inflammatory and analgesic effects of ethanolic leaf extract of *Newbouldia laevis* (P. Beauv.) Seemann Ex Bureau (Bignoniaceae). Der Pharmacia Sinica. 2013;**4**(3):30-43

[68] Ainooson GK, Woode E, Obiri DD, Koffour GA. Antinociceptive effects of *Newbouldia laevis* (P. Beauv.) stem bark extract in a rat model. Pharmacognosy Magazine. 2009;**5**(17):49-54

[69] Woode E, Ainooson GK, Boakye-Gyasi E, Ansah C, Obiri DD, Koffour GA, et al. Anti-arthritic and antioxidant properties of the ethanolic stem bark extract of *Newbouldia laevis* (P. Beauv.) Seaman ex Bureau (Bignoniaceae). Journal of Medicinal Plants Research. 2008;**2**(8):180-188

[70] Sarpong FM, Armah FA, Kingsley I, Amponsah PKA, Ampofo EK, Nortey NND. Pharmacognostic and physicochemical investigation of *Palisota hirsuta* (K. Schum) (Commelinaceae). Journal of Pharmacognosy and Phytochemistry. 2017;**6**(1):187-191

[71] Woode E, Boakye-Gyasi E, Ainooson G, Ansah C, Duwiejua M. Anti-nociceptive effects and the mechanism of *Palisota hirsuta*. International Journal of Pharmacology. 2009;**5**(2):101-113

[72] Boakye-Gyasi E, Kasanga EA, Biney RP, Abotsi WKM, Mensah KB, Woode E. Ameliorative effects of ethanolic leaf extract of *Palisota hirsuta* K. Schum (Commelinaceae) on Vincristine-Induced Neuropathic Pain in Rats. 2014;**4**(11):35-41

[73] Sarpong FM, Armah FA, Amponsah IK, Atchoglo PK. Antinociceptive ecdysteroids and other constituents of *Palisota hirsuta*

K. Schum (Commelinaceae). Journal of Applied Pharmaceutical Science. 2016;**6**(10):147-153

[74] Boakye-Gyasi E, Woode E, Ainooson G, Obiri D, Ansah C, Duwejua M, et al. Anti-inflammatory and antipyretic effects of an ethanolic extract of *Palisota hirsuta* K. Schum roots. African Journal of Pharmacy and Pharmacology. 2008;**2**(9):191-199

[75] Woode E, Boakye-Gyasi E, Danquah C, Ansah C, Duwiejua M. Anti-arthritic effects of *Palisota hirsuta* K. Schum. leaf extract in Freund's adjuvant-induced arthritis in rats. International Journal of Pharmacology. 2009;**5**:181-190

[76] Erharuyi O, Falodun A, Langer P. Medicinal uses, phytochemistry and pharmacology of *Picralima nitida* (Apocynaceae) in tropical diseases: A review. Asian Pacific Journal of Tropical Medicine. 2014;**7**(1):1-8

[77] Menzies JR, Paterson SJ, Duwiejua M, Corbett AD. Opioid activity of alkaloids extracted from *Picralima nitida* (fam. Apocynaceae). European Journal of Pharmacology. 1998;**350**(1):101-108

[78] Duwiejua M, Woode E, Obiri D. Pseudo-akuammigine, an alkaloid from *Picralima nitida* seeds, has anti-inflammatory and analgesic actions in rats. Journal of Ethnopharmacology. 2002;**81**(1):73-79

[79] Woode E, Obiri D, Ansah C, Duwiejua M, Kuffuor G. Total alkaloidal extract of *Picralima nitida* (Fam. Apocynaceae) seeds has anti-inflammatory actions. Journal of the Ghana Science Association. 2006;**8**(1):70-78

[80] Ben-Bala K. *Phyllanthus muellerianus (Kuntze) Exell*. Plant Resources of Tropical Africa/Ressources Végétales de L'Afrique Tropicale. 2008. Available from: http://www.prota4u.org/search.asp. [Accessed: 01 August 2019]

[81] Boakye-Gyasi E, Kasange EA, Biney RP, Boadu-Mensah K, Agyare C, Woode E. Anti-nociceptive effects of geraniin and an aqueous extract of the aerial parts of *Phyllanthus muellerianus* (Kuntze) Exell. in murine models of chemical nociception. Iranian Journal of Pharmaceutical Sciences. 2016;**12**(3):17-30

[82] Boakye-Gyasi E, Kasanga EA, Ameyaw EO, Abotsi WKM, Biney RP, Agyare C, et al. An isobolographic analysis of the anti-nociceptive effect of geraniin in combination with morphine or diclofenac. Journal of Basic and Clinical Physiology and Pharmacology. 2018;**29**(2):201-209

[83] Boakye YD, Agyare C, Abotsi WKM, Ayande PG, Ossei PPS. Anti-inflammatory activity of aqueous leaf extract of *Phyllanthus muellerianus* (Kuntze) Exell. and its major constituent, geraniin. Journal of Ethnopharmacology. 2016;**187**:17-27

[84] Abere TA, Onwukaeme DN. Pharmacognostic evaluation of the leaves of *Secamone afzelii* (Schult) K Schum (Asclepiadaceae). Tropical Journal of Pharmaceutical Research. 2012;**11**(1):125-131

[85] Mensah A, Mireku E, Okwuonu V. Anti-inflammatory and anti-oxidant activities of *Secamone afzelii* (Rhoem) Ascleipiadaceae. Journal of Medical and Biomedical Sciences. 2014;**3**(1):23-30

[86] Bhogaonkar P, Dagawal M, Ghorpade D. Pharmacognostic studies and antimicrobial activity of *Synedrella nodiflora* (L.) Gaertn. Bioscience Discovery. 2011;**2**(3):317-321

[87] Woode E, Amoateng P, Ansah C, Duwiejua M. Anti-nociceptive effects of an ethanolic extract of the whole plant of *Synedrella nodiflora* (L.)

Gaertn in mice: Involvement of adenosinergic mechanisms. Journal of Pharmacology and Toxicology. 2009;**4**(1):17-29

[88] Amoateng P, Adjei S, Osei-Safo D, Ameyaw EO, Ahedor B, N'guessan BB, et al. A hydro-ethanolic extract of *Synedrella nodiflora* (L.) Gaertn ameliorates hyperalgesia and allodynia in vincristine-induced neuropathic pain in rats. Journal of Basic and Clinical Physiology and Pharmacology. 2015;**26**(4):383-394

[89] Amoateng P, Adjei S, Osei-Safo D, Kukuia KKE, Kretchy IA, Sarkodie JA, et al. Analgesic effects of a hydro-ethanolic whole plant extract of *Synedrella nodiflora* (L.) Gaertn in paclitaxel-induced neuropathic pain in rats. BMC Research Notes. 2017;**10**(226):1-7

[90] Lemmens R. *Trichilia monadelpha* (Thonn.) JJ de Wilde. Plant Resources of Tropical Africa. 2008;**7**(1):561-563

[91] Woode E, Amoh-Barimah AK, Abotsi WKM, Ainooson GK, Owusu G. Analgesic effects of stem bark extracts of *Trichilia monadelpha* (Thonn.) JJ De Wilde. Indian Journal of Pharmacology. 2012;**44**(6):765-773

[92] Ainooson G, Owusu G, Woode E, Ansah C, Annan K. *Trichilia monadelpha* bark extracts inhibit carrageenan-induced foot-oedema in the 7-day old chick and the oedema associated with adjuvant-induced arthritis in rats. African Journal of Traditional, Complementary and Alternative Medicines. 2012;**9**(1):8-16

[93] Ben IO, Woode E, Koffuor GA, Boakye-Gyasi E, Ehigiator BE. Effect of *Trichilia monadelpha* (Thonn.) JJ De Wilde (Meliaceae) extracts on haematology, cytokines and oxidative stress biomarkers in rats adjuvant-induced arthritis. Pharmacologia. 2016;**7**(1):32-43

[94] Ben IO, Woode E, Koffuor GA, Boakye-Gyasi E, Titiloye NA. Effect of *Trichilia monadelpha* (Meliaceae) extracts on bone histomorphology in complete Freund's adjuvant-induced arthritis. Journal of Intercultural Ethnopharmacology. 2017;**6**(2):177

[95] Ucheck FF. *Vernonia Amygdalina Delile*. Plant Resources of Tropical Africa/Ressources végétales de l'Afrique Tropicale. 2004. Available from: http://www.prota4u.org/search.asp. [Accessed: 01 August 2019]

[96] Asante D-B, Henneh IT, Acheampong DO, Kyei F, Adokoh CK, Ofori EG, et al. Anti-inflammatory, anti-nociceptive and antipyretic activity of young and old leaves of *Vernonia amygdalina*. Biomedicine & Pharmacotherapy. 2019;**111**:1187-1203

[97] Oyen LPA. Wissadula rostrata (Schumach. & Thonn.) Planchon ex Hook. f. Plant Resources of Tropical Africa/Ressources Végétales de l'Afrique Tropicale. 2011. Available from: http://www.prota4u.org/search.asp. [Accessed: 01 August 2019]

[98] Mensah A, Donkor P, Fleischer T. Anti-inflammatory and antioxidant activities of the leaves of *Wissadula amplissima* var Rostrata. African Journal of Traditional, Complementary and Alternative Medicines. 2011;**8**(2):185-195

[99] Woode E, Ameyaw EO, Boakye-Gyasi E, Abotsi WK. Analgesic effects of an ethanol extract of the fruits of *Xylopia aethiopica* (Dunal) A. Rich (Annonaceae) and the major constituent, xylopic acid in murine models. Journal of Pharmacy & Bioallied Sciences. 2012;**4**(4):291

[100] Ameyaw EO, Woode E, Boakye-Gyasi E, Abotsi WK, Kyekyeku JO, Adosraku RK. Anti-allodynic and anti-hyperalgesic effects

of an ethanolic extract and xylopic acid from the fruits of *Xylopia aethiopica* in murine models of neuropathic pain. Pharmacognosy Research. 2014;**6**(2):172-179

[101] Ameyaw E, Boampong J, Kukuia K, Amoateng P, Obese E, Osei-Sarpong C, et al. Effect of xylopic acid on paclitaxel-induced neuropathic pain in rats. Journal of Medical and Biomedical Sciences. 2013;**2**(4):6-12

[102] Ofori AE, Eric W, Samuel K, Robert BP, Nyarko BJ. Anti-nociceptive synergism of pregabalin and xylopic acid co-administration in paclitaxel induced neuropathy: Isobolographic analysis. Pharmacognosy Journal. 2015;**7**(6):363-368

[103] Woode E, Ameyaw EO, Boakye-Gyasi E, Abotsi WKM, Oppong Kyekyeku J, Adosraku R, et al. Effects of an ethanol extract and the diterpene, xylopic acid, of *Xylopia aethiopica* fruits in murine models of musculoskeletal pain. Pharmaceutical Biology. 2016;**54**(12):2978-2986

[104] Woode E, Ameyaw EO, Ainooson G, Abotsi W, Gyasi E, Kyekyeku J. Analgesic effects of an ethanol extract of the fruits of *Xylopia aethiopica* and xylopic acid in murine models of pain: Possible mechanism(s). Pharmacologia. 2013;**4**(4):285-300

[105] Obiri DD, Osafo N. Aqueous ethanol extract of the fruit of *Xylopia aethiopica* (Annonaceae) exhibits anti-anaphylactic and anti-inflammatory actions in mice. Journal of Ethnopharmacology. 2013;**148**(3):940-945

[106] Obiri DD, Osafo N, Ayande PG, Antwi AO. *Xylopia aethiopica* (Annonaceae) fruit extract suppresses Freund's adjuvant-induced arthritis in Sprague-Dawley rats. Journal of Ethnopharmacology. 2014;**152**(3):522-531

[107] Osafo N, Obiri DD, Antwi AO, Yeboah OK. The acute anti-inflammatory action of xylopic acid isolated from *Xylopia aethiopica*. Journal of Basic and Clinical Physiology and Pharmacology. 2018;**29**(6):659-669

[108] Ruffo CK, Birnie A, Tengnäs B. Edible Wild Plants of Tanzania. Vol. 27. Nairobi, Kenya: Regional Land Management Unit, RELMA/Sida; 2002

[109] Boakye-Gyasi E, Henneh IT, Abotsi WKM, Ameyaw EO, Woode E. Hydro-ethanolic leaf extract of *Ziziphus abyssinica* Hochst Ex A. Rich (Rhamnaceae) exhibits anti-nociceptive effects in murine models. BMC Complementary and Alternative Medicine. 2017;**17**(231):1-12

[110] Boakye-Gyasi E, Henneh IT, Abotsi WKM, Ameyaw EO, Woode E. Possible mechanisms involved in the anti-nociceptive effects of hydro-ethanolic leaf extract of *Ziziphus abyssinica*. Pharmaceutical Biology. 2017;**55**(1):1962-1971

[111] Henneh IT, Ameyaw EO, Biney RP, Armah FA, Obese E, Konjah D, et al. *Ziziphus abyssinica* hydro-ethanolic root bark extract attenuates acute inflammation possibly through membrane stabilization and inhibition of protein denaturation and neutrophil degranulation. West African Journal of Pharmacy. 2018;**29**(2):81-94

Chapter 2

Antimicrobial Potential of Genes from Garlic (*Allium sativum* L.)

Hafiz Muhammad Khalid Abbas, Xi Kong, Jia Wu, Mohsin Ali and Wubei Dong

Abstract

With the advancements in agriculture, farming community less or more started to rely on synthetic chemicals to increase the crop production and protection. But the negative impact of these chemicals on environment and cropping system urges the scientists to discover some new ways to combat with crop disease. By keeping in view, garlic is a well-known economically important vegetable throughout the world and recognized as reservoir for a number of bioactive compounds to treat various diseases; scientists have developed a strategy to identify and isolate antimicrobial genes from garlic. By using *B. subtilis* expression systems, a total of 48 antimicrobial genes, including *AsR 416*, were identified with the potential to significantly retard the growth of economically important fungal and bacterial pathogens. Furthermore, these antimicrobial genes exhibited the thermal stability along with nontoxic effects on mammalian blood cells, which indicate its potential use in the development of human medicines. These genes can revolutionize the way to treat with pathogens and also give a new wave of knowledge to explore the other organisms for the search of antimicrobial genes. This will also help to search the other cost-effective ways for the treatment of plant and human diseases.

Keywords: *Allium sativum* L., antimicrobial peptide, *Bacillus subtilis*, resistance gene

1. Introduction

Garlic (*Allium sativum* L.) is one of the most important species of the genus *Allium* and recognized as economically important vegetable throughout the world, especially around the Mediterranean basin where it is considered as main agricultural product [1, 2]. It is also of great importance because of its therapeutic properties and health-related benefits against various kinds of diseases such as aches, deafness, diarrhea, constipation, tumors, and respiratory problems. Health benefits from *Allium* species, especially garlic, have been used for centuries to treat various kinds of disorders, and still, there is need of research to explore its health-related potential [3–5]. It is a historic medicinal plant, originated from central Asia about 6000 years ago, and had been started to use as medicine in India since 5000 years ago and 3000 years ago in China [6, 7]. Volatile sulfur compounds, especially thiosulfates, responsible for pungent aroma, are the main compounds

responsible for its physiological effects [8]. Because of its health benefits, garlic is usually recommended as dietary supplement.

During the past few decades, antimicrobial resistance has become one of the most serious and challenging threat for the prevention and treatment of the infectious diseases [9, 10]. Nowadays, much of the attention has been paid to search some new and natural therapeutic agents, which can be used to treat human diseases with high efficacy and minimum adverse effects [11, 12]. Recent advances in research have revealed that there are several natural products with the potential to eliminate or alleviate several serious human diseases, especially cardiovascular, neurodegeneration, cancer, and several other important diseases [13–15]. A large number of researches have elaborated several herbs with the ability to produce antimicrobial compounds as their defense response against the number of different stresses including microbes [16, 17].

With the advancements in agriculture, farming community started to rely more on synthetic chemicals, which have been considered as an important source for crop production and protection. But, hazardous effects of these synthetic chemicals to environment and cropping system make their use questionable [18, 19]. Besides, pathogens also tended to increase their resistance against these synthetic chemicals and threaten the agriculture sustainability [20, 21]. By keeping these challenges in view, the need of identifying new strategies as an alternative source is increasing interestingly. Recently, scientists are trying to understand the chemistry of secondary metabolites from plants, as studies have revealed these secondary metabolites important in several ways, especially allelopathy, biological control, and biofertilizers, and also some compounds have been identified as biostimulants [22–24]. Consequently, understanding the mechanism of these secondary metabolites/bioactive compounds from plants can be useful for agricultural community.

2. Antimicrobial potential of *Allium* species

A number of *Allium* species have antimicrobial potential against variety of microbes including fungi, bacteria, viruses, and other parasites. Among all the *Allium* species, garlic is considered most for antimicrobial research after onion [25].

2.1 Antibacterial potential

Allium extracts containing thiosulfinates have the potential to retard the growth of Gram-positive and Gram-negative bacteria. It is, however, reported that garlic can inhibit the Gram-negative bacteria more than Gram-positive bacteria [26]. The permeability of inhibitory compounds from *Allium* might be affected by the cell wall and cell membrane structure. However, the results were quite opposite with diallyl trisulfide and dimethyl trisulfide and with garlic extracts to conclude that the Gram-positive bacteria were more sensitive than Gram-negative bacteria [27, 28].

Extracts from the garlic are reported to exhibit the effective results against saprobic and pathogenic bacteria, which are resistant to various drugs [29]. Garlic along with ciprofloxacin exhibits the pronounced inhibition of *E. coli* Z17, O2:K1:H- and *Helicobacter pylori*, but no significant evidence was found in the case of *H. pylori* infection in human [30, 31]. It has previously been proved that allicin is the main compound in garlic responsible for the antimicrobial activity, as garlic oil and extracts deficient in allicin do not exhibit any kind of antimicrobial activity [32]. It was later found that garlic oil and its constituting sulfides exhibit the more and significant inhibition of microbes and work as strong antifungal than the antibacterial agent [28].

Studies have reported that oils and sulfides from elephant (*A. ampeloprasum*) and shallot (*A. ascalonicum* L.) garlic have the potential to inhibit the food-borne pathogenic bacteria [33, 34]. Ajoene, an unsaturated disulfide, has been reported for its broad-spectrum antibacterial activities, which can be reduced by cysteine, a sulfhydryl compound [35]. Later, it was proved that disulfide in ajoene is a necessary component for the inhibition of bacteria as reduction by sulfhydryl compounds reduces the antibacterial activity. Gram-positive bacteria and yeast are more sensitive to ajoene than Gram-negative bacteria.

2.2 Antifungal potential

It is reported in different studies that oils and sulfides from the *Allium* have the more potential to inhibit the fungi than bacteria [28, 36]. Antifungal activity of sulfide molecules is directly proportional to increase in the number of sulfur atoms up to sulfur number three or four in sulfide molecules [28, 37].

Another study has also reported that sterilized/autoclaved garlic and its active compounds exhibit significant antifungal activities than that of antibacterial. Further analysis of garlic antimicrobial products revealed that these products are the heterocyclic sulfides [38], allyl alcohol [39], and 3-(allyltrisulfanyl)-2-aminopropanoic acid [40]. For bacteria and yeasts, minimum inhibitory concentrations (MICs) of heterocyclic sulfides are more than 100 and 1–6 ppm [38], respectively, while for the allyl alcohol, 4% and 55–140 ppm MICs are recorded for bacteria and yeasts [39], respectively. In the case of 3-(allyltrisulfanyl)-2-aminopropanoic acid, 100 ppm and less than 50 ppm MICs are observed for bacteria and yeasts [40], respectively. In previous studies, it was mistakenly stated that autoclaved garlic exhibit less antimicrobial activities than fresh garlic. For this statement, the only reason was that they tested autoclaved garlic against bacteria, which was already very less sensitive than yeasts against garlic [41]. Recent studies have explored the germicidal potential of sterilized/autoclaved garlic.

2.3 Antiviral activity

Diallyl polysulfides, as transformation product of allicin, and ajoene exhibit the antiviral activities. From all the reported *Allium* products, it is observed that ajoene exhibits more inhibition than other compounds like allicin and thiosulfinates, but on the other hand, allicin is considered as strong antimicrobial agent [42, 43]. It is thought that antimicrobial compounds from garlic react with viral envelope and inhibit the penetration and exponentiation of influenza virus in animal kidney cells [44]. Garlic aqueous extracts have also been studied to observe the inhibition against potato virus Y under in vivo and in vitro conditions [45].

2.4 Antiparasitic potential

A number of parasites, including *Leishmania donovani* [46], *Spironucleus vortens* [47], and *Eimeria papillata* [48], are sensitive to garlic extracts. The MIC values of allicin, dithiins, and ajoene for the inhibition of *S. vortens* growth are higher than the MICs reported for the inhibition of bacteria and fungi, indicating the high tolerance of *S. vortens* for *Allium* extracts [46].

From the above discussed literature, it is clear that garlic has a certain pool of antimicrobial genes which can be isolated and studied further to explore their mechanisms. It will provide some new directions for antimicrobial research. Now we will discuss some techniques to isolate and study the antimicrobial genes from garlic.

3. Systems for the isolation of antimicrobial genes from garlic

3.1 *Bacillus subtilis* and *Escherichia coli* expression systems

An experiment was designed to study the antimicrobial genes from the garlic. For this purpose, cDNA libraries from garlic were constructed by using two different vectors, pBE-s and pET22 (b), and then transformed into expression systems, *B. subtilis* and *E. coli*, respectively. For the library quality analysis, two parameters were considered, recombination rate and library titer [49]. For the *E. coli* expression system, 96.7% and 4.6×10^6 pfu/ml, recombination rate and library titer were observed, respectively. On the other hand, recombination rate and library titer for *B. subtilis* expression system were 91.7% and 7.8×10^6 pfu/ml, respectively. Quality analysis revealed gene library in *E. coli* expression system was marginally better than that of the *B. subtilis* expression system.

For the screening of libraries, it was considered that because of the toxicity of protein products of cDNA libraries, *B. subtilis* and *E. coli* cells would be showing autolysis to indicate the antimicrobial potential of these libraries' inserts. For more confirmation, trypan blue dye was also used to indicate the viability of *E. coli* cells [50]. By using this strategy, a number of antimicrobial genes were screened from garlic to reveal its further potentials. For example, in case of *B. subtilis* expression system, a total of 48 antimicrobial genes were screened, including *AsR 416*, while *AsRE 67* was identified by using *E. coli* expression system [51].

3.2 Antimicrobial potential of genes from *A. sativum*

Antimicrobial potential of *A. sativum* genes was studied against fungi and Gram-positive and Gram-negative bacteria [50], and the results were observed as follows (**Tables 1–3**).

3.3 Action mechanism of antimicrobial proteins

A study was designed to explore the action mechanism of antimicrobial peptides. In this study, *B. subtilis* cells were treated with antimicrobial peptide, AsR 416,

Genes	*Fusarium* spp.	*Botrytis cinerea*	*Phytophthora capsici*
WB800	—	—	+
AsR 379	—	—	—
AsR 117	—	—	+
AsR 412	—	—	—
AsR 416	—	—	—
AsR 453	—	—	+
AsR 36	—	—	—
AsR 174	—	—	—
AsR 864	—	—	—
AsR 498	—	—	—
AsR 845	—	—	+
AsR 853	—	—	—

—, indicate no inhibition, +, indicate inhibition.

Table 1.
Antimicrobial potential of A. sativum genes against fungi.

	Gram-negative bacteria			
Genes	***Xanthomonas campestris* pv. *oryzicola***	***Agrobacterium tumefaciens***	***E. coli* DE3**	***Ralstonia solanacearum***
WB800	—	—	—	+
AsR 379	—	—	—	+
AsR 117	—	—	—	+
AsR 412	—	—	—	+
AsR 416	—	—	—	+
AsR 453	—	—	—	+
AsR 36	—	—	—	+
AsR 174	—	—	—	+
AsR 864	—	—	—	+
AsR 498	—	—	—	+
AsR 845	—	—	—	+
AsR 853	—	—	—	+

—, indicate no inhibition, +, indicate inhibition.

Table 2.
Antimicrobial potential of A. sativum genes against Gram-negative bacteria.

	Gram-positive bacteria						
Genes	***Clavibacter michiganensis* subsp.**	***C. fangii***	***B. anthracis***	***B. subtilis* 330–2**	***B. cereus***	***B. subtilis* 168**	***B. subtilis* WB800**
WB800	—	+	—	+	+	—	—
AsR 379	+	+	+	+	+	+	+
AsR 117	+	+	+	+	+	+	+
AsR 412	+	+	+	+	+	+	+
AsR 416	+	+	+	+	+	+	+
AsR 453	+	+	+	+	+	+	+
AsR 36	+	+	+	+	+	+	—
AsR 174	+	+	+	+	+	+	—
AsR 864	+	+	+	+	+	+	+
AsR 498	+	+	+	+	+	+	+
AsR 845	+	+	+	+	+	+	—
AsR 853	+	+	+	+	+	+	+

—, indicate no inhibition, +, indicate inhibition.

Table 3.
Antimicrobial potential of A. sativum genes against Gram-positive bacteria.

and then PI (propidium iodide) staining was performed [51]. PI is fluorescent agent that has the ability to bind with DNA through broken cell membrane. Red fluorescence in all bacterial cells treated with antimicrobial peptide was observed under confocal laser microscope [52], while the flow cytometry analysis revealed that cell membrane damages increase with increase in the protein concentration [53].

All findings collectively support that the target of antimicrobial peptide is to destroy the cell membrane of target bacteria.

3.4 Thermal stability and safety analysis of antimicrobial proteins

Proteins from *AsR 117*, *AsR 416*, and *AsR 498* were heated at different temperatures for 15 min, and it was found that *AsR 117* and *AsR 416* proteins were thermally stable at all temperature ranges, while *AsR 498* became thermally unstable after 50°C, as it exhibited the reduced antimicrobial activity. For the safety analysis, these proteins were analyzed against sheep red blood cells [54, 55]. This analysis revealed these antimicrobial proteins as nontoxic to mammalian cells with maximum 1000 μg/ml concentration [51]. From the thermal and safety analyses, it is also obvious that antimicrobial genes from garlic can also be used in human medicines in the future, which needs further investigations.

4. Conclusion

It is an adverse need of modern agriculture to search cost-effective ways to treat the crop diseases, as the potential use of synthetic chemicals also increases the resistance in pathogens. Garlic is a famous vegetable for its potential to treat various kinds of diseases. So, it is obvious that antimicrobial genes from garlic are the best source to incorporate resistance in plants without affecting the other environmental factors. This way of introducing resistance can also help to understand the mechanisms of plant biology to further explore the new strategies.

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Conflict of interest

The authors declare that they have no conflict of interest.

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References

[1] Casella S, Leonardi M, Melai B, Fratini F, Pistelli L. The role of diallyl sulfides and dipropyl sulfides in the in vitro antimicrobial activity of the essential oil of garlic, *Allium sativum* L., and leek, *Allium porrum* L. Phytherapy Research. 2013;**27**(3):380-383

[2] El-Hamidi M, El-Shami SM. Scavenging activity of different garlic extracts and garlic powder and their antioxidant effect on heated sunflower oil. American Journal of Food Technology. 2015;**10**(4):135-146

[3] Martins N, Petropoulos S, Ferreira ICFR. Chemical composition and bioactive compounds of garlic (*Allium sativum* L.) as affected by pre- and post-harvest conditions: A review. Food Chemistry. 2016;**211**:41-50

[4] Zeng Y, Li Y, Yang J, Pu X, Du J, Yang X, et al. Therapeutic role of functional components in alliums for preventive chronic disease in human being. Evidence-based Complementary and Alternative Medicine. 2017;**2017**:1-13

[5] Hannan A, Ullah MI, Usman M, Hussain S, Absar M, Javed K. Anti-mycobacterial activity of garlic (*Allium sativum*) against multi-drug resistant and non-multi-drug resistant Mycobacterium tuberculosis. Pakistan Journal of Pharmaceutical Sciences. 2011;**24**(1):81-85. Available from: https://www.mendeley.com/catalogue/antimycobacterial-activity-garlic-allium-sativum-against-multidrug-resistant-nonmultidrug-resistant/

[6] Galeone C, Pelucchi C, Levi F, Negri E, Franceschi S, Talamini R, et al. Onion and garlic use and human cancer. The American Journal of Clinical Nutrition. 2006;**84**(5):1027-1032

[7] Rivlin RS. Historical perspective on the use of garlic. Journal of Nutrition. 2001;**131**(3s):951S-954S. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11238795

[8] Agarwal KC. Therapeutic actions of garlic constituents. Medicinal Research Reviews. 1996;**16**(1):111-124. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8788216

[9] Nathan C, Cars O. Antibiotic resistance—Problems, progress, and prospects. The New England Journal of Medicine. 2014;**371**(19):1761-1763. Available from: http://www.nejm.org/doi/10.1056/NEJMp1408040

[10] Paphitou NI. Antimicrobial resistance: Action to combat the rising microbial challenges. International Journal of Antimicrobial Agents. 2013;**42**(Suppl. 1):S25-S28

[11] Alinezhad H, Azimi R, Zare M, Ebrahimzadeh MA, Eslami S, Nabavi SF, et al. Antioxidant and antihemolytic activities of ethanolic extract of flowers, leaves, and stems of *Hyssopus officinalis* L. Var. angustifolius. International Journal of Food Properties. 2013;**16**(5):1169-1178

[12] Cooper AI, Poliakoff M. High-pressure reactions in polyethylene films, a new development in matrix isolation. The photochemical reaction of $Fe(CO)_5$ with N_2 and the thermal reaction of $Fe(CO)_4$ (N_2) with H_2. Chemical Physics Letters. 1993;**212**(6):611-616

[13] Vasanthi HR, ShriShriMal N, Das DK. Phytochemicals from plants to combat cardiovascular disease. Current Medicinal Chemistry. 2012;**19**(14): 2242-2251. Available from: http://www.eurekaselect.com/openurl/content.php?genre=article&issn=0929-8673&volume=19&issue=14&spage=2242

[14] Cragg GM, Newman DJ. Natural products: A continuing source of novel drug leads. Biochimica et Biophysica Acta - General Subjects. 2013;**1830**:3670-3695

[15] Nabavi SF, Russo GL, Daglia M, Nabavi SM. Role of quercetin as an alternative for obesity treatment: You are what you eat! Food Chemistry. 2015;**179**:305-310

[16] Bednarek P. Chemical warfare or modulators of defence responses—The function of secondary metabolites in plant immunity. Current Opinion in Plant Biology. 2012;**15**:407-414

[17] Daglia M. Polyphenols as antimicrobial agents. Current Opinion in Biotechnology. 2012;**23**:174-181

[18] Sugeng AJ, Beamer PI, Lutz EA, Rosales CB. Hazard-ranking of agricultural pesticides for chronic health effects in Yuma County, Arizona. Science of the Total Environment. 2013;**463-464**:35-41

[19] Hadi F, Hussain F, Hussain M, Ahmad A, Ur Rahman S, Ali N. Phytoextraction of Pb and Cd; the effect of urea and EDTA on *Cannabis sativa* growth under metals stress. International Journal of Agricultural Research. 2014

[20] Talukdar PK, Rahman M, Rahman M, Nabi A, Islam Z, Hoque MM, et al. Antimicrobial resistance, virulence factors and genetic diversity of *Escherichia coli* isolates from household water supply in Dhaka, Bangladesh. PLoS One. 2013;**8**(4)

[21] Kahmann R, Basse C. Fungal gene expression during pathogenesis-related development and host plant colonization. Current Opinion in Microbiology. 2001;**4**:374-380

[22] Gniazdowska A, Bogatek R. Allelopathic interactions between plants. Multi site action of allelochemicals. Acta Physiologiae Plantarum. 2005;**27**:395-407

[23] Cantor A, Hale A, Aaron J, Traw MB, Kalisz S. Low allelochemical concentrations detected in garlic mustard-invaded forest soils inhibit fungal growth and AMF spore germination. Biological Invasions. 2011;**13**(12):3015-3025

[24] Gurjar MS, Ali S, Akhtar M, Singh KS. Efficacy of plant extracts in plant disease management. Agricultural Sciences. 2012;**03**(03):425-433. Available from: http://www.scirp.org/journal/doi.aspx?DOI=10.4236/as.2012.33050

[25] Kyung KH. Antimicrobial properties of allium species. Current Opinion in Biotechnology. 2012;**23**(2):142-147. Available from: https://www.sciencedirect.com/science/article/pii/S0958166911006720

[26] Perry CC, Weatherly M, Beale T, Randriamahefa A. Atomic force microscopy study of the antimicrobial activity of aqueous garlic versus ampicillin against *Escherichia coli* and *Staphylococcus aureus*. Journal of the Science of Food and Agriculture. 2009;**89**(6):958-964

[27] Fujisawa H, Watanabe K, Suma K, Origuchi K, Matsufuji H, Seki T, et al. Antibacterial potential of garlic-derived allicin and its cancellation by sulfhydryl compounds. Bioscience, Biotechnology, and Biochemistry. 2009;**73**(9):1948-1955. Available from: http://www.tandfonline.com/doi/full/10.1271/bbb.90096

[28] Kim JW, Kim YS, Kyung KH. Inhibitory activity of essential oils of garlic and onion against bacteria and yeasts. Journal of Food Protection. 2004;**67**(3):499-504. DOI: 10.4315/0362-028X-67.3.499

[29] Dubey D, Rath S, Sahu MC, Debata NK, Padhy RN. Antimicrobials of plant origin against TB and other infections and economics of plant drugs—Introspection. Indian Journal of Traditional Knowledge. 2012;**11**(2):225-233

[30] Graham DY, Anderson SY, Lang T. Garlic or jalapeno peppers for treatment of *Helicobacter pylori* infection. The American Journal of Gastroenterology. 1999;**94**(5):1200-1202

[31] Sohn DW, Han CH, Jung YS, Kim SI, Kim SW, Cho YH. Anti-inflammatory and antimicrobial effects of garlic and synergistic effect between garlic and ciprofloxacin in a chronic bacterial prostatitis rat model. International Journal of Antimicrobial Agents. 2009;**34**(3):215-219. Available from: https://www.sciencedirect.com/science/article/pii/S0924857909000971

[32] Booyens J, Thantsha MS. Fourier transform infra-red spectroscopy and flow cytometric assessment of the antibacterial mechanism of action of aqueous extract of garlic (*Allium sativum*) against selected probiotic Bifidobacterium strains. BMC Complementary and Alternative Medicine. 2014;**14**(1)

[33] Rattanachaikunsopon P, Phumkhachorn P. Antimicrobial activity of elephant garlic oil against *Vibrio cholerae* in vitro and in a food model. Bioscience, Biotechnology, and Biochemistry. 2009;**73**(7):1623-1627. DOI: 10.1271/bbb.90128

[34] Rattanachaikunsopon P, Phumkhachorn P. Shallot (*Allium ascalonicum* L.) oil: Diallyl sulfide content and antimicrobial activity against food-borne pathogenic bacteria. African Journal of Microbiological Research. 2009;**3**(11):747-750. Available from: http://www.academicjournals.org/ajmr

[35] Terzioğlu S, Başkent EZ, Sivrikaya F, Çakir G, Kadioğullari AI, Başkaya Ş, et al. Monitoring forest plant biodiversity changes and developing conservation strategies: A study from Camili Biosphere Reserve area in NE Turkey. Biologia (Bratislava). 2010;**65**(5):843-852

[36] Mahmoudabadi AZ, Nasery MKG. Antifungal activity of shallot, *Allium ascalonicum* Linn. (Liliaceae), in vitro. Journal of Medicinal Plants Research. 2009;**35**(12):2531-2541

[37] O'Gara EA, Hill DJ, Maslin DJ. Activities of garlic oil, garlic powder, and their diallyl constituents against *Helicobacter pylori*. Applied and Environmental Microbiology. 2000;**66**(5):2269-2273

[38] Choi MK, Chae K-Y, Lee J-Y, Kyung KH. Antimicrobial activity of chemical substances derived from S-alk(en) yl-L-cysteine sulfoxide (alllin) in garlic, *Allium sativum* L. Food Science and Biotechnology. 2007;**16**(1):1-7

[39] Kyung KH, Choi JH. Allyl alcohol is the sole antiyeast compound in heated garlic extract. Journal of Food Science. 2005;**70**(6):305-309

[40] Kang S-S, Lim DR, Kyung KH. 3-(Allyltrisulfanyl)-2-aminopropanoic acid, a novel nonvolatile water-soluble antimicrobial sulfur compound in heated garlic. Journal of Medicinal Food. 2010;**13**. DOI: 10.1089/jmf.2010.1059

[41] Walton L, Herbold M, Lindegren CC. Bactericidal effects of vapors from crushed garlic. Journal of Food Science. 1936;1(2):163-169. Available from: 10.1111/j.1365-2621.1936.tb17778.x

[42] Weber ND, Andersen DO, North JA, Murray BK, Lawson LD, Hughes BG. In vitro virucidal effects of *Allium sativum* (garlic) extract and compounds. Planta Medica. 1992;**58**(5):417-423. Available

from: http://www.ncbi.nlm.nih.gov/pubmed/1470664

[43] Schäfer G, Kaschula C. The immunomodulation and anti-inflammatory effects of garlic organosulfur compounds in cancer chemoprevention. Anti-Cancer Agents in Medicinal Chemistry. 2014;**14**(2):233-240. Available from: http://www.eurekaselect.com/openurl/content.php?genre=article&issn=1871-5206&volume=14&issue=2&spage=233

[44] Mehrbod P, Aini I, Amini E, et al. Assessment of direct immunofluorescence assay in detection of antiviral effect of garlic extract on influenza virus. African Journal of Microbiology Research. 2013;**7**(21):2608-2612. Available from: http://academicjournals.org/journal/AJMR/article-abstract/C7BAEBF12939

[45] Mohamed EF. Antiviral properties of garlic cloves juice compared with onion bulbs juice against potato virus Y (PVY). Journal of American Science. 2010;**6**. Available from: http://www.americanscience.orgeditor@americanscience.org302

[46] Sharma U, Velpandian T, Sharma P, Singh S. Evaluation of anti-leishmanial activity of selected Indian plants known to have antimicrobial properties. Parasitology Research. 2009;**105**(5):1287-1293

[47] Millet COM, Lloyd D, Williams C, Williams D, Evans G, Saunders RA, et al. Effect of garlic and allium-derived products on the growth and metabolism of *Spironucleus vortens*. Experimental Parasitology. 2011;**127**(2):490-499

[48] Dkhil MA, Abdel-Baki AS, Wunderlich F, Sies H, Al-Quraishy S. Anticoccidial and antiinflammatory activity of garlic in murine *Eimeria papillata* infections. Veterinary Parasitology. 2011;**175**(1-2):66-72

[49] Park NJ, Zhou X, Yu T, Brinkman BMN, Zimmermann BG, Palanisamy V, et al. Characterization of salivary RNA by cDNA library analysis. Archives of Oral Biology. 2007;**52**(1):30-35. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17052683

[50] Cheng X, Liu G, Ye G, Wang H, Shen X, Wu K, et al. Screening and cloning of antimicrobial DNA sequences using a vital staining method. Gene. 2009;**430**((1-2)): 132-139. Available from: https://www.sciencedirect.com/science/article/pii/S0378111908005453

[51] Kong X, Yang M, Abbas HMK, Wu J, Li M, Dong W. Antimicrobial genes from *Allium sativum* and *Pinellia ternata* revealed by a *Bacillus subtilis* expression system. Scientific Reports. 2018;**8**(1):14514. Available from: http://www.nature.com/articles/s41598-018-32852-x

[52] Xie J, Gou Y, Zhao Q , Wang K, Yang X, Yan J, et al. Antimicrobial activities and membrane-active mechanism of CPF-C1 against multidrug-resistant bacteria, a novel antimicrobial peptide derived from skin secretions of the tetraploid frog *Xenopus clivii*. 2014. DOI: 10.1002/psc.2679

[53] Lee H, Hwang J-S, Lee J, Kim JI, Lee DG. Scolopendin 2, a cationic antimicrobial peptide from centipede, and its membrane-active mechanism. Biochimica et Biophysica Acta – Biomembranes. 2015;**1848**(2): 634-642. Available from: https://www.sciencedirect.com/science/article/pii/S0005273614004143

[54] Joshi S, Bisht GS, Rawat DS, Kumar A, Kumar R, Maiti S, et al. Interaction studies of novel cell selective antimicrobial peptides with model membranes and *E. coli* ATCC 11775. Biochimica et Biophysica Acta—Biomembranes. 2010;**1798**(10):

1864-1875. Available from: https://www.sciencedirect.com/science/article/pii/S0005273610002142

[55] Wingfield P. Protein precipitation using ammonium sulfate. Current Protocols in Protein Science. 2001;**3**(3):Appendix 3F. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18429073

Chapter 3

Medicinal Plants Used for Treatment of Prevalent Diseases in Northern Pakistan of Western Himalayas

Khafsa Malik, Mushtaq Ahmad, Muhammad Zafar, Shazia Sultana, Athar Tariq and Neelam Rashid

Abstract

In this research study, we have scientifically assessed medicinal species and herbal preparations used by inhabitants of Northern Pakistan to treat joint pain, hypertension, skin diseases and glottis infections. The aim of the study is to document and highlight the ethnopharmacological significance and compare the uses of medicinal herbs for curing prevalent ailments in Northern Pakistan. Ethnomedicinal data were collected from 180 informants using semi-structured interviews and group meetings. A total of 80 plant species in 54 families were reported for the treatment of various health conditions. *Heliotropium lasiocarpum, Geranium wallichianum, Parkinsonia aculeata, Rubia cordifolia* and *Salvadora persica* were the favored plants for curing these diseases. Highest RFC was recorded for *Neolitsea chinensis* (0.956), *Rubia cordifolia* (0.928). The similarity of the informer's knowledge about used medicines was found in *Aesculus indica* and *Abies pindrow* with high UV. *Cuscuta reflexa* and *Lawsonia inermis* had 98–99% fidelity level for management of joint pain, skin diseases, glottis infection and hypertension respectively. In Northern Pakistan, a rich diversity of medicinal plants was used in curing various diseases. The results of this study help us in screening of herbal plants for further phytochemical and pharmacological study which leads to discovery of natural drug and development with global interest for cure of various ailments.

Keywords: herbaceous diversity, ethnomedicinal, diseases, Northern Pakistan, herbal preparation, frequency of citation

1. Introduction

1.1 Ethnobotany: concept and significance

Ethnomedicinal literature put emphasis on the relation between the indigenous communities and the usage of plants [1]. Plants are important for all biomes and the working of all social societies [2]. Traditional herbal drugs have been effective as a remedy for wide variety of diseases [3]. Traditional medicinal species and plant derivative treatments are extensively utilized in old medicinal systems worldwide,

and the therapeutic use of plant species is becoming gradually popular in modern society as natural alternatives to synthetic medications [4]. Ethnomedicinal assessment of medicinal species is essential for preserving security and valuable for incipient plant medicines [5]. Many people of the rural areas retain indigenous knowledge of therapeutic plant species [6] and such plant material still exists because it is transferred from generation to generation [7]. Thus, the race of human generally relies on plant species and their needs are increasing with passage of time [8].

1.2 Medicinal plants used at global level

The usage of medicinal plant species is common, as they have little side effects, less price, easily accessible, consistent by numerous beliefs and traditional performs [9]. Native utilization of medicinal species becomes unavoidable in giving as a source of food and drugs for health care for the rural communities and low income class. The ethnic system of old herbal drugs rely on the utilization of medicinal flora by the people of native populations and has been experienced for spans [10]. These medicinal plants were commonly used by local inhabitants and were of great value so that lot of people was engaged in the trade of essential medicinal species throughout the world [11]. Medicinal herbs gained attention due to elevation in prices of allopathic drugs for the wellbeing, biomedical benefits and accessibility and maintenance of personal health, [12]. So, conservation and sustainability of traditional medicinal system is needed [13].

World Health Organization (WHO) stated that, in developing nations around 80% of the population of the world dependent on indigenous herbal drugs (THD) for treating various diseases. Internationally, 422,000 flowering species are stated [10]. Out of these, around 50,000 plant species are used as medicinal plants and only 5000 species have separated phytochemically to examine their active chemical compounds [14]. In developed nations, 25% of medications are based on plant species and their derivatives [14]. Consequently pharmaceutical companies have made a huge amount of clinical agents, still traditional knowledge of herbal medications and phytotherapies are running in different areas of the globe. The importance of the indigenous traditional medicinal system was highlighted by the WHO that the most of population the rural communities of the emerging states is still relies on the medications for healthcare [15].

Pakistan has about 6000 medicinal plant species out of which 600 are considered to be significant from medicinal point of view [16]. These medicinal herbs are recommended by the local healers, akhuns and hakims who give health care tips within the rural areas. Around 80% of the rural people of Pakistan depend on Unani medicinal system, derived from medicinal species directly or their products [17]. The rich biodiversity of Pakistan has nine major ecological amplitudes in which the areas of Northern Pakistan are blessed with a unique biodiversity [4]. Variety of economically essential medicinal plant species for indigenous communities is fairly rich in Northern Pakistan [18]. Therapeutic species have remained utilized as a base of herbal medicinal treatment since human civilization in these areas [19]. Because of diverse climatic conditions and unique phytogeography, the area has a high variety of aromatic and medicinal plant species [20]. People living in hilly areas of Pakistan utilized medicinal species for numerous diseases and they also reliant on herbal products for their shelter, fuel, food, health, and further needs [21].Field of ethnobotany has been presented currently in Pakistan in comparison to other nations however in the recent era much effort was performed in this research study by several scientists in various regions of country [17, 19, 22–33]. Although, a lot of work has been done on medicinal species in several areas in Pakistan, yet, no study

has been carried out on areas of Northern Pakistan in relation to special emphasis on hypertension diseases, skin infections, glottis problems and musculoskeletal disorders. Further, this is the first ever report on these prevalent disorders from Northern Pakistan.

1.3 Objectives

This study has been planned with the objective to document the folkloric knowledge of commonly used therapeutic species from different regions of Northern Pakistan, to save the medicinal knowledge. The current work focused to quantitatively calculate consensus of plants usage for treatment of diseases. This study also aims to form a baseline data for future comprehensive research on bioactive constituents.

2. Methodology

2.1 Study sites

The Northern part of Pakistan in Western Himalayas is situated among world's largest peaks and high heaps i.e., Himalaya ranges, Alai Ranges, Karakorum, Kunlun, Tien Shan and Hindu Kush [34]. The Himalaya ranges have of world largest peak i.e. Mount Everest and K2 present in this range and the lesser Himalayas Mountains are located on 2000–3000 m elevation. Its topography diverges from desiccated rocky areas in north to forest and green plains in the south. Northern part of Pakistan has rich floral variety particularly of therapeutic plant species [35]. The areas included in the research work were Bannu, Swat, Mahnshera, Dir, Abbottabad, Naran, Khaghan, Hazara division, and other tribal areas of northern parts (**Figure 1**). It is located at 72°35- to 73°31- east latitude and 33°50- to 34°23- north latitudes. It shares border with FATA (Federal Administered Tribal Areas) in the Western South part, in Northern side Azad Jammu and Kashmir, Gilgit Baltistan in north east while the Punjab in south east. Northern areas of Pakistan are home of the largest peaks these covers 72,496 km^2. Mean lowest temperature in January was documented to be 1.7°C, while average highest temperature to be 32.41°C in June. These Northern areas have also very severe winter with heavy rainfall [33]. The chief tribes of the area are Marwat, Shinwari, Afridi, Mohmand, Abbassies, Tareen, Khattak, Mashwani, Jadoon, Tanolis, Awans, Yusufzai, Sardars, Qureshis and

Figure 1.
Dominant families of medicinal plants.

Orakzai [30]. Majority of people speaks Pushto other local languages are Potohari, Gujrati and Hindko.

2.2 Ethnobotanical data collection

This work was mainly focused on communities exploiting conventional plant resources for treatment of hypertension, glottis disorders, skin infection, joint pain and throat diseases. The people living in Northern Pakistan have information on the usage of natural resources. The field work was performed for 6 months (from March to September, 2016). Semi-structured interviews were taken from 180 informants having traditional curing methods against variety of ailments after receiving their prior consent. The data about medicinal uses of these plants was collected from local informers and healers and medicinal practionists. Questionnaire forms was comprised of two sections; first section involves the demographic information of participants and the other section contains data about plants vernacular name, part used and mode of administration used against these diseases. Further evaluation of data obtained during field study was done by using quantitative indices.

2.3 Plant collection and preservation

The medicinal plants exploited for different ailments in the Northern Pakistan were first collected and vouchers were constituted for identification at Herbarium of Quaid -i- Azam University Islamabad Pakistan (ISL). Correct scientific families and names were confirmed by database of KEW medicinal plant name services (mpns: http://www.kew.org/mpns) and flora of Pakistan [36]. Each plant sample contains vital parts such as stems, seeds, roots, bark, fruits, flowers and leaves, whole plants was generally collected for small herbaceous plant specimen.

2.4 Quantitative analysis of ethnobotanical data

2.4.1 Use value citations (UV)

UV was assessed by means of standard procedure of [24].

$$UV = u/n \tag{1}$$

"u" denoted the total respondent citing different usages of a medicinal species. Use value is usually larger at close to (1) incase numeral of usage is higher and UV of plant noticeably lowers if it is close to (0). Use values do not deliver data for only one or numerous uses of plants.

2.4.2 Relative frequency of citation (RFC)

The computation of RFC was done by using formula:

$$RFC = Fc/N \tag{2}$$

The number of respondents stated by "Fc" that specified about therapeutic use related to herbal medicinal plants whereas "N" stands for numeral total value related to the respondents [37–39].

2.4.3 Family importance value (FIV)

FIV of the plant species being evaluated by using formula as under [40].

$$\text{FIV} = \text{FC}/\text{N} \times 100 \tag{3}$$

where "Fc" is the numeral value of respondents stating the use of the family and N denotes to the total numeral value of respondents contributing in the research work.

2.4.4 Fidelity level (FL)

FL is measured by following formula:

$$\text{FL}\ (\%) = \text{Np}/\text{N} \times 100 \tag{4}$$

where "Np" is the numeral value of participants who defined medicinal plants as a remedy for particular ailments while "N" is the total number of informants [41].

3. Results and discussion

3.1 Socio-demographic profile of informants

Demographic information of the participants was taken from semi structured questionnaires. A total of 180 respondents were questioned in this field survey. Of the 180 informants, 113 were Indigenous people and the rest (67) were local health practitioners, rest of all information is stated in (**Table 1**).

3.2 Medicinal plant diversity

Present research stated 80 medicinal plants used to treat some prevalent diseases in Northern Pakistan (**Table 2**). These medicinal plants were distributed in 54 families. They show diversity in growth and presented by all growth forms with high proportion of herbs (54%), shrubs (30%) and trees (16%) (**Table 3**). The main cause for herbs dominancy in the research area may be the easily accessibility resultant from bulk growing in wild area. The native respondent described that most of the hakims and healers commonly use herbs for treatment of ailments because of their easy attainability and availability.

The recorded medicinal species and medicinal uses along with local name, part used, preparations and mode of utilization had been documented in **Table 2**. The plant family that have higher number of medicinal specie was *Lamiaceae* (7 species) followed by (4 species) of Papaveraceae, (3 species) of Malvaceae, Apiaceae, Asteraceae and Brassicaceae, (2 species) Acanthaceae, Pinaceae, Myrtaceae, Rubiaceae, Lythraceae, Plantaginaceae, Cactaceae and Capparaceae, (1 species) Ranunculaceae, Berberidaceae, Saxifragaceae, Umbelliferae, Moraceae, Papilionaceae, Poaceae, Oleaceae, Fabaceae, Salvadoraceae, Solanaceae, Rutaceae, Meliaceae and rest of the families presented one medicinal plant (**Figure 1**). *Lamiaceae* documented higher diversity of medicinal species followed by Asteraceae and Solanaceae; *Lamiaceae* also indicated greater diversity of medicinal flora plants [42]. *Lamiaceae* a diverse family with mostly herbaceous plants producing volatile aroma over all aerial parts, has been described as dominant plant family by [43]. In ethnobotanical studies of lesser Himalayas the high percentage of medicinal

Serial number	Variables	Categories	No of person	Percentages
1.	Gender	Female	84	47
		Male	96	53
2.	Age group	37–47	12	7
		47–57	36	20
		57–67	49	27
		67–77	53	29
		77>	30	17
3.	Occupation	Retired	48	27
		Unemployed	24	13
		Employed	38	21
		Housewife's	50	28
		Others	20	11
4.	Education	Primary level	52	29
		Secondary level	31	17
		Tertiary level	20	11
		Others	19	10
		Uneducated	58	33
5.	Informant category	Indigenous people	113	63
		Local health practitioners	67	37
6.	Residence	Rural	41	23
		Urban	139	77
7.	Marital status	Married	83	46
		Un-married	97	54

Table 1.
Demographic data of informants of Western Himalayas.

plants in the families' Papaveraceae, Moraceae and Fabaceae has previously been stated by [44].

3.3 Plant parts used as a medicine

In this ethnobotanical study, the part of plant most frequently utilized is was leaves (41%), and seeds and roots (10%) (**Table 4**). Leaves were used as main part of plant, it has been stated within different ethnomedicinal research of Thailand, India, Bangladesh, Colombia, Pakistan, and China [45–51]. Leaves are the dominantly used plant part because it is easily attainable plant part and requires small effort to collect as compared to other plant parts [43]. Moreover, other important fact of leave utilization is important for conservation and maintenances of plant because collection of other plant parts and roots may kill the plant or endangered the specie [52]. Bulk use of whole plant, fruits, seeds, roots and bark in herbal medicinal preparations may results in decreasing population of plants in nature [53].

While fruits (9%), whole plant (7%), stem and aerial part (6%), bark (4%), flower (3%), shoots and rhizomes (2%) were also frequently used (**Table 4**). Fruits,

Sr. no	Taxonomic names/voucher no/families	Local name	Life form	Part used	Mode of utilization	Diseases category	Recipes	FC	RFC	UR	UV	FL
1.	*Abelmoschus esculentus* (L.) Moench KN 1011/Malvaceae	Bhindi	Herbs	Seed	Teas	pimple	Plants seeds are boil in water and make tea which is usage in treatment of pimples for 2 weeks	25	0.139	1	0.040	80.00
2.	*Abies pindrow* (Royle ex D. Don) Royle KN 1015/Pinaceae	Kachal/Achal	Trees	Leave and shoot	Decoctions	Throat and cough infection	Leave decoction of are given for 3–4 weeks for treatment of throat diseases	28	0.156	2	0.071	78.57
3.	*Achyranthes aspera* L. KN 1017/ Amaranthaceae	Put kandha	Herbs	Leave	Juices	Rheumatism	The 20 g fresh leave juice is used for treatment of rheumatism after every meal for month	18	0.100	1	0.056	55.56
4.	*Aconitum chasmanthum* Stapf ex Holmes KN 1019/ Ranunculaceae	Mori, Bishmoulo	Herbs	Rhizome	Decoctions	Measles and mumps	The rhizomes of the plant is boiled in water and decoction is made, are given for weeks to cure diseases	42	0.233	2	0.048	76.19
5.	*Acorus calamus* Linn KN 1010/ Acoraceae	Wajh	Herbs	Root	Infusions	Hypertension	20-30 g of root infusion are given to relief hypertension until it is controlled	110	0.611	1	0.009	92.73
6.	*Adiantum venustum* D. Don KN 1013/Pteridaceae	Pata, kakwa	Herbs	Leave	Paste	Healing of wound	The 50 g of leaves are dried and make paste for healing wounds	144	0.800	1	0.007	84.72
7.	*Aesculus indica* (Wall. ex Cambess.) Hook. OP KN 1014/ Hippocastanaceae	Bankh khore	Trees	Leave	Extracts	Whooping and cough	Leaves extract are used to treat whooping and cough	22	0.122	2	0.091	81.82
8.	*Ajuga bracteosa* Benth. AK KN 1017/*Lamiaceae*	Kahri bhooti	Herbs	Whole plants	Decoction and infusion	Rheumatism and body pain	20 g of whole plant is boiled in water and decoction is made this is given to treat body pain	10	0.056	2	0.200	80.00
9.	*Argemone mexicana* Linn KN 1019/Papaveraceae	Kandiarhi	Herbs	Aerial part	Decoction and infusion	Dislocate pain and joint pain	Infusion and decoction of aerial part of plant is suggested for 5 days	48	0.267	2	0.042	70.83
10.	*Barleria cristata* L. KN 1020/ Acanthaceae	Janglhi pool	Shrubs	Roots and stem	Poultices	Rheumatic pain	25 g of stem and roots are powdered and mix with water and make paste that is used as poultice to treat rheumatism	62	0.344	1	0.016	85.48

Sr. no	Taxonomic names/voucher no/families	Local name	Life form	Part used	Mode of utilization	Diseases category	Recipes	FC	RFC	UR	UV	FL
11.	*Begonia reniformis* Bedd. KN 1021/Begoniaceae		Shrubs	Flower	Infusions	Hypertension	Infusion of dry flower is taken to relieve hypertension	20	0.111	1	0.050	65.00
12.	*Berberis lycium* Royle KN 1024/ Berberidaceae	ZiarLargay	Shrubs	Leave	Decoction s	Sore throat and throat infection &	2 cups of decoction is taken twice a day for week for curing throat infections	36	0.200	2	0.056	69.44
13.	*Bergenia ciliata* (Haw.) Sternb KN 1026/Saxifragaceae	Batweyaha	Herbs	Bark	Pastes	Wound healing	Paste of Bark is used to heal up wounds	16	0.089	1	0.063	75.00
14.	*Bryophyllum pinnatum* (Lam.) Oken KN 1027/Crassulaceae	Zakam e Hayhat	Herbs	Leave	Decoction s	Hypertension	15 g of leaves are boiled in water and decoction is made, 1 cup of decoction is used once a day for hypertension	33	0.183	1	0.030	90.91
15.	*Buxus papillosa* KN 1028/ Buxaceae	Angaroo	Shrubs	Leave	Oils	Skin problem	Oil of leaves are applied on skin to treat skin problem	22	0.122	1	0.045	86.36
16.	*Camellia sinensis* (L.) Kuntze KN 1029/Camelliaceae	Chaieh	Shrubs	Leave	Teas	Throat infection and Cough	50 g of leaves are boiled in water to make tea which is used for 2 weeks for cough and throat infections	32	0.178	2	0.063	90.63
17.	*Capparis decidua* (Forssk.) Edgew. KN 1030/Capparaceae	Keehra	Trees	Seed	Decoctions	Healing of wound	Seeds decoction used 3 cups daily for treatment of disease	21	0.117	1	0.048	71.43
18.	*Capparis spinosa* L. KN 1032/ Capparaceae	Kabhar	Shrubs	Seed	Powders	Hypertension	Powder of the seeds is taken with water 3 times a day to cure hypertension	56	0.311	1	0.018	60.71
19.	*Commelina diffusa* Burm. f KN 1034/Commelinaceae		Herbs	Whole plants	Juices	Hypertension	30–35 g of whole plants are crushed to attain juice which is better for hypertension for 3 weeks	62	0.344	1	0.016	79.03
20.	*Commiphora stocksiana* (Engl.). KN 1036/Burseraceae	Chandrhu	Shrubs	Leave and root	Paste and Poultice	Backache Joint pain and bone fracture	Leaves paste and poultice are applied on joints for treatment of rheumatic disorders until its cure	76	0.422	3	0.039	88.16
21.	*Cuminum cyminum* L. KN 1038/ Apiaceae	Zhira	Herbs	Fruit	Infusions	Cough	80 g of fruits of the plants are dipped whole night in water and make infusion given twice a day for 2 weeks	40	0.222	1	0.025	55.00

Sr. no	Taxonomic names/voucher no/families	Local name	Life form	Part used	Mode of utilization	Diseases category	Recipes	FC	RFC	UR	UV	FL
22.	*Cuscuta reflexa* Roxb KN 1040/ Convolvulaceae	Aftimhoon	Herbs	Leave	Decoctions	Hypertension	10-20 g of leave decoction is used for hypertension for 3 weeks	111	0.617	1	0.009	99.10
23.	*Daphne mucronata* Royle. KN 1042/Thymelaeaceae	Daphnee plants	Shrubs	Leave bark & aerial part	Decoctions cooked and Powders	Rheumatisms	Powder of aerial parts and bark is taken after each meal for treatment of rheumatic disorders. Leaves are dried mix with water and taken for 4–5 days. Aerial parts are cooked and used for cure of diseases	83	0.461	1	0.012	96.39
24.	*Descurainia sophia* (L.) Webb ex Prantl KN 1043/Brassicaceae	Burriborhy	Herbs	Whole plants	Decoctions	Cough and throat infection	30 g of leave decoctions is used for a 2 week to cure throat infection	12	0.067	2	0.167	83.33
25.	*Equisetum arvense* L. KN 1045/ Equisetaceae		Herbs	Aerial parts	Extracts	Hypertension	70 g of aerial parts are required for extraction that is used for hypertension for 20 days	102	0.567	1	0.010	89.22
26.	*Eruca sativa* (L.) Cav. KN 1048/ Brassicaceae	Jambhoo	Herbs	Shoot & seed	Pastes and decoctions	Rheumatic disorders	2 cups of hot water is taken and add 2 teaspoons of grinded plant mixed well and used thrice a day for treatment. Seeds paste is used for Rheumatism	109	0.606	1	0.009	90.83
27.	*Eucalyptus globulus* Labill. KN 1049/Myrtaceae	Lachi, Sufaida	Trees	Leave & Stem	Decoctions	Sore throat	25 g of eaves decoction is used twice a day after every meal	28	0.156	1	0.036	71.43
28.	*Euphorbia helioscopia* L. KN 1051/Euphorbiaceae	Catt milk	Herbs	Leave	powder	Healing of wounds	Dried 40 g of leaves are powdered and mix with water and taken orally for 4–5 days	21	0.117	1	0.048	66.67
29.	*Ferula asafoetida* L. KN 1053/ Umbelliferae	Hinhg	Herbs	Rhizomes	Decoction s	Coughs	A rhizomes of the plant is boiled in water and decoction is made taken 2 cups daily for cough	18	0.100	1	0.056	66.67
30.	*Ficus virgata* Reinw. ex Blume KN 1055/Moraceae	Anjheer Zardh	Trees	Fruit	Raw	Hypertension	Fruits are eaten orally for treatment of diseases	100	0.556	1	0.010	89.00
31.	*Fumaria officinalis* L.KN 1057/ Papaveraceae	Shahtaraha	Herbs	Aerial part	Juices and Extracts	Hypertension	45 g of dried leaves are used for making decoction and juice. 1 cup is used for hypertension	48	0.267	1	0.021	60.42

Sr. no	Taxonomic names/voucher no/families	Local name	Life form	Part used	Mode of utilization	Diseases category	Recipes	FC	RFC	UR	UV	FL
32.	*Galium abaujense* Borbás KN 1058/Rubiaceae	Khrrhatanii	Herbs	Leave	Poultices	Wound healing and skin problem	Poultice of leaves is applied on wounds	17	0.094	4	0.235	52.94
33.	*Geranium wallichianum* D. Don ex sweet KN 1059/Geraniaceae		Herbs	Root	Extracts	Hypertension	50 ml of extract of 80 g of dried roots is use for hypertension	150	0.833	1	0.007	92.00
34.	*Glycyrrhiza glabra* L. KN 1060/ Papilionaceae	Malhathii	Herbs	Root	Decoction s	Sore throats	Roots of plants are boil in water then used as decoction for treating sore throat infections	23	0.128	1	0.043	78.26
35.	*Grewia optiva* J. R. Drumm. ex Burret KN 1062/Malvaceae	Tamhar	Shrubs	Leave and bark	Decoctions and powders	Rheumatism and bone dislocation	Paste of leaves are apply on bones & plant decoction are used for treatment of diseases	99	0.550	2	0.020	76.77
36.	*Gymnosporia senegalensis* (Lam.) Loes.KN 1064/Celastraceae		Shrubs	Leave and root	Decoctions	Hypertension	Decoctions of roots and leaves are used for cure of hypertension	73	0.406	1	0.014	93.15
37.	*Heliotropium lasiocarpum* Fisch KN 1067/Boraginaceae	Chulai	Herbs	Whole plants	Decoction s	Hypertension	35 g of whole plant is boiled in water and decoction is made that is used for hypertension	135	0.750	1	0.007	89.63
38.	*Hippophae rhamnoides* L. KN 1069/Elaeagnacea*e*		Trees	Fruit and seed	Decoctions	Skin problem	Fruits decoction are used for skin problems	132	0.733	1	0.008	95.45
39.	*Hordeum vulgare* L. KN 1070/ Poaceae	Jahoo	Herbs	Seed	Decoctions	Whooping cough	Seeds decoction are used to cure cough, 2 cups of decoctions taken daily twice a day	28	0.156	1	0.036	67.86
40.	*Heracleum candicans* Wall. ex DC. KN 1072/Apiaceae	Kadupanhra	Herbs	Whole plants	Juices	Rheumatic pain	Whole plant is grinded to make juice and this juice is taken 2 glasses a day	76	0.422	1	0.013	85.53
41.	*Impatiens edgeworthii* Hook. f KN 1074/Balsaminaceae	Buntilh	Herbs	Whole plants	Pastes	Skin burns	Paste of the plant is applied externally for burns	30	0.167	1	0.033	93.33
42.	*Isodon rugosus* (Wall. ex Benth.) KN 1075/*Lamiaceae*	Sperkaye	Shrubs	Leave	Powders	Wound healings and skin problem	15 g of leaves powder is used 2 times a day after each meal	122	0.678	2	0.016	93.44

Sr. no	Taxonomic names/voucher no/families	Local name	Life form	Part used	Mode of utilization	Diseases category	Recipes	FC	RFC	UR	UV	FL
43.	*Juglans regia* L. KN 1076/ Juglandaceae	Akhroot	Trees	Aerial part, leave and stem	Decoction s	Hypertension	40 g of dried leaves and roots extract and decoction are used in hypertension for 1 month thrice a day	52	0.289	1	0.019	92.31
44.	*Justicia adhatoda* L.KN 1079/ Acanthaceae	Behkare	Shrubs	Leave	Raw	Wound healing	Leaves are directly applied on wounds for healing	25	0.139	1	0.040	72.00
45.	*Lagenaria siceraria* (Molin) KN 1082/Cucurbitaceae		Herb	Fruit,	Raw	Muscle and rheumatic pain	Fruit is eaten directly for curing a diseases	19	0.106	2	0.105	68.42
46.	*Lavandula angustifolia* Mill KN 1084/*Lamiaceae*	Khushbudhar	Shrub	Leave	Infusions & decoction	Hypertension	20 g of leaves are taken and dipped in water for 2 days, its infusion is antihypertensive	62	0.344	1	0.016	69.35
47.	*Lawsonia inermis* KN 1085/ Lythraceae	Mehndi	Shrubs	Leaves	Infusions	Boils and skin burn	Dried and crushed leaves are dissolved in water and are applied for 5–6 days	140	0.778	2	0.014	98.57
48.	*Laphangium affine* (D.Don) Tzvelev KN 1086/Asteraceae	Janglii dodhal	Herbs	Leaves	Decoctions	Throat and cough infection	10-20 g of leaves decoction is use for throat infection twice a day for 2 weeks	49	0.272	2	0.041	59.18
49.	*Malvastrum coromandelianum* (L.) Garcke KN 1087/ Malvaceae	Dhamnii bhooti	Herbs	Leave and rhizomes	Powders	Muscular pain	3–5 rhizomes of the plants are taken, powder them tea can be made by addition of 2–6 g of powder in 3 cups of water. This is used for cure of muscular diseases	52	0.289	1	0.019	88.46
50.	*Mentha longifolia* (L.) Huds. KN 1089/*Lamiaceae*	Jangli Podhina	Herbs	Flower and leave	Extracts	Hypertension	50 g of leaves extract o is used to cure hypertension	75	0.417	1	0.013	81.33
51.	*Musa acuminata* Colla KN 1090/ Musaceae	Kelha	Trees	Fruits and stem	Decoctions	Hypertension	Stems, fruits and flowers decoctions are effective in relieving from hypertension	51	0.283	1	0.020	90.20
52.	*Myrsine africana* L. KN 1092/ Myrsinaceae	Gughal	Shrubs	Leave	Decoctions	Skin problems	Leaves were especially used against cough, cold, flue and skin diseases	39	0.217	1	0.026	76.92
53.	*Myrtus communis* L. KN 1094/ Myrtaceae	Manrhoo	Trees	Fruit	Decoctions and boils	Muscle pain	25 g of fruit decoction is taken for muscle disorder for 2 weeks, twice a day	67	0.372	1	0.015	70.15

Sr. no	Taxonomic names/voucher no/families	Local name	Life form	Part used	Mode of utilization	Diseases category	Recipes	FC	RFC	UR	UV	FL
54.	*Neolitsea chinensis* Chun KN 1096/Lauraceae	Maiddasak	Trees	Shoot & bark	Powders and pastes	Muscular problem	Paste of the 39 g of bark and shoot are apply over muscles for several days until pain is cure	172	0.956	1	0.006	97.09
55.	*Nepeta praetervisa* Rech. f. KN 1098/*Lamiaceae*	Simsok	Herbs	Leave	Teas	Cough and throat infection	Leaves are boil in water, make tea which is used for throat infection	32	0.178	2	0.063	53.13
56.	*Nerium oleander* L. KN 1099/ Apocynaceae	Kaneer	Shrubs	Leave	Extracts	Hypertension	30 g of leave extracts are mixed with oil are used for hypertension	54	0.300	1	0.019	75.93
57.	*Ocimum basilicum* L. KN 1100/ *Lamiaceae*	JangliTulsi	Herb	Leave	Infusions	Hypertension	Leaves infusion in 1 l water is prepared and taken two times/day as needed until improvement occurs	81	0.450	1	0.012	81.48
58.	*Oenothera rosea* L'Hér. ex Aiton KN 1101/Onagraceae	Jungligulahb	Herbs	Whole plants	Infusions	Whooping Cough	Leaves are dipped in water for 2 days than infusion is used for cough for a week	20	0.111	1	0.050	50.00
59.	*Olea europaea subsp. cuspidata* (Wall. & G. Don) Cif KN 1104/ Oleaceae	Ghawarejha	Shrubs	Seed and leave	Teas	Skin problem	70 g of dried leaves of plants are boiled and tea is used orally for mouth ulcer and skin diseases for 1 month	32	0.178	1	0.031	84.38
60.	*Opuntia dillenii* (Ker Gawl.) Haw. KN 1106/Cactaceae	Zuqham	Shrubs	Fruit	Juices	Cough and throat infection	Juice of 90 g of fruit is good in throat infection	32	0.178	2	0.063	56.25
61.	*Opuntia ficus-indica* (L.) Mill KN 11071/Cactaceae	Zahqam	Shrubs	Fruit	Raw	Muscular pain	Raw fruits are taken daily to treat muscle pain	117	0.650	1	0.009	96.58
62.	*Pinus sylvestris* L. KN 1108/ Pinaceae	Snobher	Trees	Leave	Extracts	Hypertension	Few grams of leaves extract is good for curing disease for 3 weeks	56	0.311	1	0.018	76.79
63.	*Papaver somniferum* L.KN 1110/ Papaveraceae	Post	Herbs	Fruit	Teas	Whooping, cough	Fruits are boil in water to make tea and 2 cups of tea are taken thrice a day for cough	43	0.239	1	0.023	65.12
64.	*Parkinsonia aculeata* L. KN 1112/Fabaceae	Janglii baabar	Shrubs	Leave, seed and root	Oils	Joint pain and body swelling	Seeds oil of plants are used for joints pain	163	0.906	2	0.012	85.28
65.	*Picrorhiza kurrooa* Royle. ex Benth KN 1114/Plantaginaceae	Kutakhi safed	Herbs	Root	Raw	Skin burns	It is beneficial in the treatment of burning sensation	40	0.222	1	0.025	50.00

Sr. no	Taxonomic names/voucher no/families	Local name	Life form	Part used	Mode of utilization	Diseases category	Recipes	FC	RFC	UR	UV	FL
66.	*Plantago major* L. KN 1117/ Plantaginaceae	Barthange	Herbs	Leave	Decoctions	Hypertension	Leaves decoction is taken for 3 week to cure hypertension	97	0.539	1	0.010	81.44
67.	*Prunus persica* (L.) Batch KN 1118/Rosaceae	Aruu	Trees	Leave and fruit	Decoctions	Skin problem	20 g of dry leaves are taken boiled in water, decoction is made that is given for week for skin problems	18	0.100	1	0.056	50.00
68.	*Rhazya stricta* Decne. KN 1120/ Apocynaceae	Harmaal, venna	Shrubs	Leave	Infusions	Sore throats	65 g of leaves are dipped in water whole night and infusion is made that is used to cure sore throat	28	0.156	1	0.036	64.29
69.	*Rhododendron arboreum* Sm. KN 1122/Ericaceae	Rantool	Trees	Leave	Powders	Rheumatic disorder	13 g of leaves are dried and powder are taken against chronic rheumatic disorders for 2 weeks	124	0.689	1	0.008	95.16
70.	*Rubia cordifolia* L. KN 1124/ Rubiaceae	Majithe	Herbs	Whole plants and root	Decoction s and Pastes	Joint pains	40 g of roots are powdered and make paste that are applied at joints for 2 weeks	167	0.928	1	0.006	79.64
71.	*Rydingia limbata* (Benth.) Scheen & V. A. Albert KN 1127/ *Lamiaceae*	Ghawarejha	Shrubs	Leave	Extracts	Skin problems	30 ml of extracts of leaves are taken orally against mouth ulcers and skin diseases	117	0.650	1	0.009	86.32
72.	*Salvadora persica* L. KN 1128// Salvadoraceae	Piilu	Shrubs	Stems and leave	Poultices	Rheumatic diseases	Leaves of plants are heated and tied in cloth and applied over pain areas	138	0.767	1	0.007	72.46
73.	*Senecio chrysanthemoides* DC KN 1129/Asteraceae		Herbs	Leave	Oils	Skin problems	Oil of 60 g of leaves are used for treatment	102	0.567	1	0.010	87.25
74.	*Sisymbrium irio* L.KN 1131/ Brassicaceae	Janglii sarso	Herbs	Leave	Infusions	Throat infection & cough	50 ml of infusion of leaves is effective against throat and cough ailment	30	0.167	2	0.067	76.67
75.	*Tagete serecta* L. KN 1134/ Asteraceae	Satveerga	Herbs	Leave	Poultices	Muscular pain & swelling of body	Hot oil is mixed in leaves and applied on used on swelling body parts	153	0.850	2	0.013	95.42
76.	*Trachyspermum ammi* (L.) Sprague KN 1136/Apiaceae	Ajwaain	Herbs	Seed	Decoctions	Throat infection and cough	50 g of seed decoction is used for 3 weeks for throat infections	139	0.772	2	0.014	88.49

Sr. no	Taxonomic names/voucher no/families	Local name	Life form	Part used	Mode of utilization	Diseases category	Recipes	FC	RFC	UR	UV	FL
77.	*Urtica dioica* L. KN 1137/ Urticaceae	Bichoo bhooti	Herbs	Root	Decoctions	Cough and throat infection	30-40 g of root are boiled in water and make decoction that is used for throat infection	129	0.717	2	0.016	86.05
78.	*Verbascum thapsus* L. KN 1138/ Scrophulariaceae	Gadikhand	Herbs	Aerial part	Infusions	Pimples and skin problems	Aerial plants are grinded and dissolved in water and make infusion that is taken for 3–4 days	109	0.606	2	0.018	96.33
79.	*Withania somnifera* (L.) Dunal KN 1139/Solanaceae	Aksaan	Herbs	Leave & root	Pastes and powders	Joint pains	Root of plant are taken, rinse with water, dried and crushed to make powder then it is given in lessen amount for joint disorders. Paste of leaves is used to cure pain	112	0.622	1	0.009	87.50
80.	*Zanthoxylum armatum* DC. KN 1142/Rutaceae		Trees	Seed	Decoctions	Sore throat	18 g of seeds decoction are given for 3 week to treat sore throat	132	0.733	1	0.008	75.00

Table 2.
Medicinal plants use for some prevalent diseases in Northern Pakistan.

S/No	Life form	Percentage (%)
1.	Herbs	54
2.	Shrubs	30
3.	Trees	16

Table 3.
Life form of medicinal plant.

S/No	Part used	Percentage (%)
1.	Leaves	41
2.	Seeds	10
3.	Roots	10
4.	Fruits	9
5.	Whole plant	7
6.	Stem	6
7.	Aerial parts	6
8.	Bark	4
9.	Flowers	3
10.	Rhizomes	2
11.	Shoots	2

Table 4.
Plant part used as medicines.

leaves and stem were the key source of herbal medicines in the research study area. In earlier described studies from various parts of the Pakistan whole plant, flower and fruit therapy is very common and it is present among the top of the plant parts usage [54]. Rhizomes, roots and fleshy parts of the plant species have a high amount of bio-active compounds [55].

3.4 Types of herbal preparations

Medicinal species utilized and administrated in herbal medicines in numerous forms in the area. The common preparation methods were categorized into decoction (33%), powder (14%), paste and extracts (11%), infusion (1o%). Juice (5%), tea (7%), poultice (3%), raw (2%) and Oil and cooked (2%) (**Table 5**). There are several routes of administration, such as, topical use and oral ingestion for the treatment of different diseases (**Table 2**). Local traditional healers use ingestion to cure most diseases, but topical use is an important route of intake to cure diseases such as skin disorders, glottis diseases, joint pain, hypertension, wounds, and body pain, weakness and poisonous bites [54]. The particular parts of plants and definite quantity of dosages taken for ailments control mainly depends on patients physical health [35].Some individuals use, orange peel, sugar, lemon, banana pulp, tobacco leaf, black pepper and camphor, as adjuvant with various diluents.

Large number of the plant drugs (74%) was made from fresh part of plants neither the dry parts of plants. In this study it was observed that there are ambiguities in taking exact quantities of medicines between the respondents due to

S/No	Mode of preparation	Percentage (%)
1.	Poultice	3
2.	Decoction	33
3.	Infusion	10
4.	Powder	14
5.	Paste	11
6.	Extract	11
7.	Juice	5
8.	Tea	7
9.	Oil	2
10.	Raw	2
11.	Cooked	2

Table 5.
Mode of utilization of medicinal plant.

variation of person's experiences and difference in ethnical information of the respondents.

3.5 Use of phototherapies

This ethnobotanical survey showed indigenous people utilized therapeutic plant species most often for the cure of hypertension (20 reports) followed by cough (14 reports), skin problem (11 reports), rheumatism (10 reports). This survey specified fact that indigenous communities used medicinal plants frequently exploited in skin diseases, respiratory disorders, cough, throat infections, joint pain and hypertension specified that the plant of this zone have versatile medicinal usages against disease [56].The other noticeable diseases were throat infections (10 reports), wound healing (7 reports), Sore throat (5 reports), joint pain (4 reports), skin burn (3 reports). Though, skin problems were followed by pimples and swelling of body (2 reports) and boils and body pain (1 report) (**Tables 2** and **6**). Recent studies shown that maximum of local populations were dependent on a diversity of native plant species to treat several diseases as the modern health services were out of reached. It was noticed in throughout the field study that old information of indigenous therapeutic species is about to vanishing upcoming age groups belonging to research region. This is because of absence of attention by modern cultures, as they thought herbal medicines are less useful in comparison to allopathic medicines. Despite the fact when these elder persons die than these conventional medicinal practices might be quickly disappeared [57].

3.6 Quantitative analysis

3.6.1 Relative frequency of citation

For examining the ethnobotanical knowledge quantitative analysis was recorded in this study. Most stated plant species identified by a large number of respondents for medicinal purposes. Maximum RFC was documented for *Neolitsea chinensis* (0.956), *Rubia cordifolia* (0.928), *Parkinsonia aculeate* (0.906) and *Tagetes erecta* (0.850) (**Table 2**). These findings are related to the fact that a large number of respondents cited the plant species and RFC directly related to the number of

S/No	Mode of preparation	Percentage (%)
1.	Hypertension	22
2.	Cough	15
3.	Throat infection	11
4.	Sore throat	6
5.	Wound healing	8
6.	Rheumatism	11
7.	Joint pain	4
8.	Swelling of body	2
9.	Muscular pain	2
10.	Body pain	1
11.	Skin problem	12
12.	Skin burn	3
13.	Boils	1
14.	Pimples	2

Table 6.
Categories of disease.

respondents describing the usage of this medicinal species [19]. It was followed by *Lawsonia inermis* (0.778), *Zanthoxylum armatum* (0.733), *Utica dioica* (0.717), *Opuntia ficus-indica (0.65), Rhododendron arboretum* (0.689), *Geranium wallichianum* (0.833), *Hippophae rhamnoides* (0.73), *Cuscuta reflexa* (0.617) and *Ficus virgate* (0.556). Another cause of why medicinal plant stated repeatedly because of; (1) the trust of people on medicinal plant and old age relationship of the easily accessible medicinal species with people and (2) the comparatively high price of synthetic drugs and non-approach to the systems of medicine [41].

3.6.2 Use value of medicinal plants

Mostly local health practitioners in study area used these species to cure diseases from other communities. The current research showed that the use value varies from 0.094 to 0.006 (**Table 2**). Plant species recorded with high use values were *Aesculus indica*, *Abies pindrow, Opuntia dillenii, Nepeta praetervisa, Begonia reniformis* and *Berberis lyceum.* These plants were commonly found in people's homes, the decoction, tea, extract made from leaves was found very effective in hypertension, joint pain and glottis infection. Thus it should be recommended that medicinal plants have maximum UV values, would be further studied for phyto-chemical and pharmacological evaluation for developing medicinal system of herbal drugs [58]. *Neolitsea chinensis* (Use value 0.006) revealed least UVs because they were not abundant in the research area. Used value was less in some conditions due to the lower information of the informants about the medicinal plants, that may be of exotic source [59].

3.6.3 Fidelity level (FL)

To find the plant that is most chosen by the respondents for the cure of specific disease is fidelity level. FL in the present study varied from 50 to 99%. *Cuscutareflexa* (99%), *Lawsonia inermis* (98%), *Daphne mucronata* (96%),

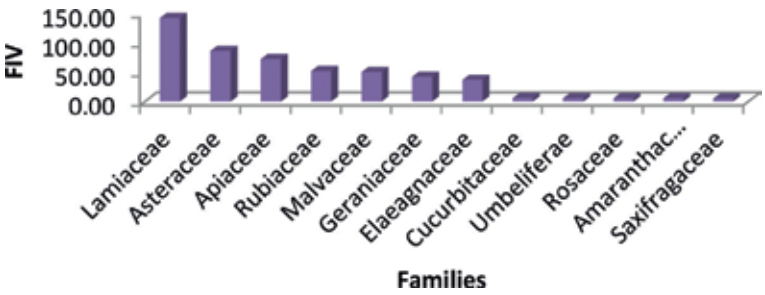

Figure 2.
Family importance value of medicinal plants.

Hippophae rhamnoides (95%), *Impatiens edgeworthii*, *Isodon rugosus* and *Gymnosporia senegalensis* (93%), *Geranium wallichianum* and *Acorus calamus* (92%) *Bryophyllum pinnatum, Camellia sinensis* and *Eruca sativa* (90%), *Heliotropium lasiocarpum*, *Equisetum arvense* and *Ficus virgata* (89%), *Commiphora stocksiana* and *Malvastrum coromandelianum (88%), Withania somnifera* (87%), *Buxus papillosa* (86%), *Heracleum candicans* and *Barleria cristata* (85%), *Adiantum venustum* (84%), *Descurainia sophia* (83%), *Mentha longifoli*a and *Aesculus indica* (81%), *Abelmoschus esculentus* and *Ajuga bracteosa* (80%) had high fidelity levels for the treatment of muscular pain, skin infections, dermatological diseases and hypertension (**Table 2**). High FL values of medicinal plant shows the selection of plant by respondents to cure particular disease [60, 61]. These plants might be confirmed as significant medicinal species by further evaluation and assessment by pharmaceutical, phytochemical and biological actions [62]. The species with least FL cannot be ignored as it causes the next generation to control the risk of gradually decreasing medicinal knowledge [63].

3.6.4 Family importance value (FIV) of medicinal flora

The evaluation of family importance value of plant species revealed that *Lamiaceae* was most prevailing group of plants having FIV of (142.50) then Asteraceae (86.1), Apiaceae (72.50), Rubiaceae (52.22), Malvaceae (50.56), Geraniaceae (42.22) and Elaeagnaceae (37.22) etc. Lower values of FIV were calculated for Saxifragaceae (5.00), Amaranthaceae, Rosaceae and Umbelliferae (5.56), Cucurbitaceae (5.83), Euphorbiaceae (6.39), Hippocastanaceae and Buxaceae (6.67), Fabaceae (6.94) and Poaceae (8.33) (**Figure 2**). The ethnobotanical study revealed by [35] showed that maximum FIV was observed by family Asclepiadaceae (FIV 18.5) then Punicaceae (FIV 17.9) whereas minimum value was observes by Myrtaceae (FIV 2.3). All these findings are dissimilar from current research as the numerical ethnomedicinal facts varies because of change in geo-climate and vegetation of the region [19].

4. Conclusion

The ethnobotanical data revealed that the conventional knowledge of therapeutic plants in the Northern Pakistan is mostly sustained by elders, and this knowledge was transferred from their forefathers. This study revealed that the most frequently exploited plants were present in *Lamiaceae* and Papaveraceae, The common method of utilization was decoction. Numerical indices of FC, UV, RFC, FL, and FIV reveal that a greater variety of medicinal species is still utilized between the native inhabitants as treatment of various ailments in the study site. Particularly,

this ethnobotanical study suggested that the studied species of far-off valley should be further assessed for appropriate research and pharmacological activities to validate their present traditional usage that may help as the primary means to produce plant-derived prescriptions. Future study on the security and usefulness of medicinal herbs, along with ecological and traditional management works, which are required intended for the maintainable development of herbal drugs in the Northern Pakistan.

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Abbreviations

WHO	World Health Organization
THD	traditional herbal drugs
FATA	federal administered tribal areas
ISL	Islamabad
UV	used value
FC	frequency of citation
RFC	relative frequency of citation
FIV	family importance value
FL	fidelity level

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References

[1] Pereira JA, Oliveira I, Sousa A, Ferreira IC, Bento A, Estevinho L. Bioactive properties and chemical composition of six walnut (Juglans regia L.) cultivars. Food and Chemical Toxicology. 2008;**46**(6):2103-2111

[2] Smita R, Sangeeta R, Kumar SS, Soumya S, Deepak P. An ethnobotanical survey of medicinal plants in Semiliguda of Koraput District, Odisha, India. Research Journal of Recent Sciences. 2012;**2277**:2502

[3] Malik K, Ahmad M, Bussmann R, Tariq A, Alqahtani A, Shahat A, et al. Ethnobotany of anti-hypertensive plants used in northern Pakistan. Frontiers in Pharmacology. 2018;**9**:789

[4] Malik K, Ahmad M, Zhang G, Rashid N, Zafar M, Sultana S, et al. Traditional plant based medicines used to treat musculoskeletal disorders in northern Pakistan. European Journal of Integrative Medicine. 2018; **19**:17-64

[5] Rashid N, Gbedomon RC, Ahmad M, Salako VK, Zafar M, Malik K. Traditional knowledge on herbal drinks among indigenous communities in Azad Jammu and Kashmir, Pakistan. Journal of Ethnobiology and Ethnomedicine. 2018;**14**(1):16

[6] Ahmad M, Malik K, Tariq A, Zhang G, Yaseen G, Rashid N, et al. Botany, ethnomedicines, phytochemistry and pharmacology of Himalayan paeony (Paeonia emodi Royle.). Journal of Ethnopharmacology. 2018;**220**:197-219

[7] Sadia S, Tariq A, Shaheen S, Malik K, Ahmad M, Qureshi H, et al. Ethnopharmacological profile of anti-arthritic plants of Asia-a systematic review. Journal of Herbal Medicine. 2018;**13**:8-25

[8] Sher H, Hussain F. Ethnobotanical evaluation of some plant resources in northern part of Pakistan. African Journal of Biotechnology. 2009;**8**(17): 4066-4076

[9] Gradé JT, Tabuti JR, Van Damme P. Ethnoveterinary knowledge in pastoral Karamoja, Uganda. Journal of Ethnopharmacology. 2009;**122**(2):273-293

[10] Ahmad M, Sultana S, Fazl-i-Hadi S, Ben Hadda T, Rashid S, Zafar M, et al. An Ethnobotanical study of medicinal plants in high mountainous region of Chail valley (district swat-Pakistan). Journal of Ethnobiology and Ethnomedicine. 2014;**10**(1):36

[11] Sher H, Elyemeni M, Sher H, Hussain K. Ethnobotanical and economic observations of some plant resources from the northern parts of Pakistan. Ethnobotany Research and Applications. 2011;**9**:027-041

[12] Yabesh JM, Prabhu S, Vijayakumar S. An ethnobotanical study of medicinal plants used by traditional healers in silent valley of Kerala, India. Journal of Ethnopharmacology. 2014; **154**(3):774-789

[13] Shah NA, Khan MR, Nadhman A. Antileishmanial, toxicity, and phytochemical evaluation of medicinal plants collected from Pakistan. BioMed Research International. 2014:1-7

[14] Ali H, Sannai J, Sher H, Rashid A. Ethnobotanical profile of some plant resources in Malam Jabba valley of swat, Pakistan. Journal of Medicinal Plants Research. 2011;**5**(18):4676-4687

[15] Murad W, Azizullah A, Adnan M, Tariq A, Khan KU, Waheed S, et al. Ethnobotanical assessment of plant resources of Banda Daud Shah, district Karak, Pakistan. Journal of

Ethnobiology and Ethnomedicine. 2013; **9**(1):77

[16] Jan G, Khan MA, Farhatullah JF, Ahmad M, Jan M, Zafar M. Ethnobotanical studies on some useful plants of Dir Kohistan valleys, KPK. Pakistan. Pak J Bot. 2011;**43**(4): 1849-1852

[17] Ali H, Qaiser M. The ethnobotany of Chitral valley, Pakistan with particular reference to medicinal plants. Pakistan Journal of Botany. 2009;**41**(4): 2009-2041

[18] Alam N, Shinwari Z, Ilyas M, Ullah Z. Indigenous knowledge of medicinal plants of Chagharzai valley, district Buner. Pakistan. Pak J Bot. 2011; **43**(2):773-780

[19] Bibi T, Ahmad M, Tareen RB, Tareen NM, Jabeen R, Rehman S-U, et al. Ethnobotany of medicinal plants in district Mastung of Balochistan province-Pakistan. Journal of Ethnopharmacology. 2014;**157**:79-89

[20] Bano A, Ahmad M, Zafar M, Sultana S, Rashid S, Khan MA. Ethnomedicinal knowledge of the most commonly used plants from Deosai plateau, Western Himalayas, Gilgit Baltistan, Pakistan. Journal of Ethnopharmacology. 2014;**155**(2): 1046-1052

[21] Ahmad S, Islam M, Bano G, Aslam S, Koukab S. Seasonal variation in current season and dead biomass of chrysopogon aucheri (boiss) stapf. And cymbopogon jwarancusa (jones) schult. in highland Balochistan, Pakistan. Pakistan Journal of Botany. 2009;**41**(2): 519-527

[22] Ahmad F, Khan MA, Ahmad M, Zafar M, Mahmood T, Jabeen A, et al. Ethnomedicinal uses of grasses in the salt range region of northern Pakistan. Journal of Medicinal Plant Research. 2010;**4**(5):362-369

[23] Hazrat A, Nisar M, Shah J, Ahmad S. Ethnobotanical study of some elite plants belonging to Dir, Kohistan valley, Khyber Pukhtunkhwa, Pakistan. Pakistan Journal of Botany. 2011;**43**(2): 787-795

[24] Rashid S, Ahmad M, Zafar M, Sultana S, Ayub M, Khan MA, et al. Ethnobotanical survey of medicinally important shrubs and trees of Himalayan region of Azad Jammu and Kashmir, Pakistan. Journal of Ethnopharmacology. 2015;**166**:340-351

[25] Gilani SA, Qureshi RA, Gilani SJ. Indigenous uses of some important ethnomedicinal herbs of Ayubia National Park, Abbottabad, Pakistan. Ethnobotanical Leaflets. 2006; **2006**(1):32

[26] Hussain K, Shahazad A, Zia-ul-Hussnain S. An ethnobotanical survey of important wild medicinal plants of Hattar district Haripur, Pakistan. Ethnobotanical Leaflets. 2008;**2008**(1):5

[27] Afzal S, Afzal N, Awan MR, Khan TS, Gilani A, Khanum R, et al. Ethno-botanical studies from northern Pakistan. Journal of Ayub Medical College, Abbottabad. 2009;**21**(1):52-57

[28] Ahmad M, Qureshi R, Arshad M, Khan MA, Zafar M. Traditional herbal remedies used for the treatment of diabetes from district Attock (Pakistan). Pakistan Journal of Botany. 2009;**41**(6): 2777-2782

[29] Qureshi RA, Ghufran MA, Gilani SA, Yousaf Z, Abbas G, Batool A. Indigenous medicinal plants used by local women in southern Himalayan regions of Pakistan. Pakistan Journal of Botany. 2009;**41**(1):19-25

[30] Abbasi AM, Khan M, Ahmad M, Zafar M, Jahan S, Sultana S. Ethnopharmacological application of medicinal plants to cure skin diseases and in folk cosmetics among the tribal

communities of North-West Frontier Province, Pakistan. Journal of Ethnopharmacology. 2010;**128**(2): 322-335

[31] Ishtiaq M, Mumtaz AS, Hussain T, Ghani A. Medicinal plant diversity in the flora of Leepa Valley, Muzaffarabad (AJK), Pakistan. African Journal of Biotechnology. 2012;**11**(13): 3087-3098

[32] Butt MA, Ahmad M, Fatima A, Sultana S, Zafar M, Yaseen G, et al. Ethnomedicinal uses of plants for the treatment of snake and scorpion bite in northern Pakistan. Journal of Ethnopharmacology. 2015;**168**:164-181

[33] Khan MPZ, Ahmad M. Traditional preference of wild edible fruits (WEFs) for digestive disorders (DDs) among the indigenous communities of Swat Valley-Pakistan. Journal of Ethnopharmacology. 2015;**174**:339-354

[34] Abbas Z, Khan SM, Abbasi AM, Pieroni A, Ullah Z, Iqbal M, et al. Ethnobotany of the Balti community, Tormik valley, Karakorum range, Baltistan, Pakistan. Journal of Ethnobiology and Ethnomedicine. 2016; **12**(1):38

[35] Kayani S, Ahmad M, Zafar M, Sultana S, Khan MPZ, Ashraf MA, et al. Ethnobotanical uses of medicinal plants for respiratory disorders among the inhabitants of Gallies–Abbottabad, northern Pakistan. Journal of Ethnopharmacology. 2014;**156**:47-60

[36] Ahmad M, Khan MPZ, Mukhtar A, Zafar M, Sultana S, Jahan S. Ethnopharmacological survey on medicinal plants used in herbal drinks among the traditional communities of Pakistan. Journal of Ethnopharmacology. 2016;**184**:154-186

[37] Shah G, Ahmad M, Arshad M, Khan M, Zafar M, Sultana S. Ethno-phyto-veterinary medicines in northern Pakistan. Journal of Animal and Plant Sciences. 2012;**22**:791-797

[38] Kayani S, Ahmad M, Sultana S, Shinwari ZK, Zafar M, Yaseen G, et al. Ethnobotany of medicinal plants among the communities of alpine and sub-alpine regions of Pakistan. Journal of Ethnopharmacology. 2015;**164**:186-202

[39] Fatima A, Ahmad M, Zafar M, Yaseen G, Khan MPZ, Butt MA, et al. Ethnopharmacological relevance of medicinal plants used for the treatment of oral diseases in Central Punjab-Pakistan. Journal of Herbal Medicine. 2017;**166**:340-351

[40] Bibi T, Ahmad M, Tareen NM, Jabeen R, Sultana S, Zafar M, et al. The endemic medicinal plants of northern Balochistan, Pakistan and their uses in traditional medicine. Journal of Ethnopharmacology. 2015;**173**:1-10

[41] Yaseen G, Ahmad M, Sultana S, Alharrasi AS, Hussain J, Zafar M. Ethnobotany of medicinal plants in the Thar Desert (Sindh) of Pakistan. Journal of Ethnopharmacology. 2015;**163**:43-59

[42] Saqib Z, Mahmood A, Malik RN, Mahmood A, Syed JH, Ahmad T. Indigenous knowledge of medicinal plants in Kotli Sattian, Rawalpindi district, Pakistan. Journal of Ethnopharmacology. 2014;**151**(2): 820-828

[43] Mardani-Nejhad S, Vazirpour M. Ethno-botany of medicinal plants by Mobarakeh's people (Isfahan). Journal of Herbal Drugs (An International Journal on Medicinal Herbs). 2012;**3**(2): 111-126

[44] Ai AM, Khan MA, Shah MH, Shah MM, Pervez A, Ahmad M. Ethnobotanical appraisal and cultural values of medicinally important wild edible vegetables of lesser Himalayas-Pakistan. Journal of Ethnobiology and Ethnomedicine. 2013;**9**(1):66

[45] Singh H, Husain T, Agnihotri P, Pande P, Khatoon S. An ethnobotanical study of medicinal plants used in sacred groves of Kumaon Himalaya, Uttarakhand, India. Journal of Ethnopharmacology. 2014;**154**(1): 98-108

[46] Kadir MF, Sayeed MSB, Setu NI, Mostafa A, Mia M. Ethnopharmacological survey of medicinal plants used by traditional health practitioners in Thanchi, Bandarban Hill tracts, Bangladesh. Journal of Ethnopharmacology. 2014; **155**(1):495-508

[47] Ismail M, Ibrar M, Iqbal Z, Hussain J, Hussain H, Ahmed M, et al. Chemical constituents and antioxidant activity of Geranium wallichianum. Records of Natural Products. 2009; **3**(4):193

[48] Ibrar M, Hussain F. Ethnobotanical studies of plants of Charkotli hills, Batkhela district, Malakand, Pakistan. Frontiers of Biology in China. 2009; **4**(4):539

[49] Teklehaymanot T. Ethnobotanical study of knowledge and medicinal plants use by the people in Dek Island in Ethiopia. Journal of Ethnopharmacology. 2009;**124**(1):69-78

[50] Tabuti JR, Kukunda CB, Waako PJ. Medicinal plants used by traditional medicine practitioners in the treatment of tuberculosis and related ailments in Uganda. Journal of Ethnopharmacology. 2010;**127**(1):130-136

[51] Upadhyay B, Dhaker AK, Kumar A. Ethnomedicinal and ethnopharmaco-statistical studies of eastern Rajasthan, India. Journal of Ethnopharmacology. 2010;**129**(1):64-86

[52] Kadir MF, Sayeed MSB, Mia M. Ethnopharmacological survey of medicinal plants used by indigenous and tribal people in Rangamati, Bangladesh. Journal of Ethnopharmacology. 2012; **144**(3):627-637

[53] Ghimire SK, Gimenez O, Pradel R, McKey D, Aumeeruddy-Thomas Y. Demographic variation and population viability in a threatened Himalayan medicinal and aromatic herb Nardostachys grandiflora: Matrix modelling of harvesting effects in two contrasting habitats. Journal of Applied Ecology. 2008;**45**(1): 41-51

[54] Mahmood A, Mahmood A, Tabassum A. Ethnomedicinal survey of plants from district Sialkot, Pakistan. Journal of Applied Pharmacology. 2011; **3**:212-220

[55] Munir M, Khan MA, Ahmed M, Seema N, Ahmed SN, Tariq K, et al. Foliar epidermal anatomy of some ethnobotanically important species of wild edible fruits of northern Pakistan. Journal of Medicinal Plant Research. 2011;**5**(24):5873-5880

[56] Akhtar N, Rashid A, Murad W, Bergmeier E. Diversity and use of ethno-medicinal plants in the region of swat, North Pakistan. Journal of Ethnobiology and Ethnomedicine. 2013; **9**(1):25

[57] Khan SW, Khatoon S. Ethnobotanical studies on some useful herbs of Haramosh and Bugrote valleys in Gilgit, northern areas of Pakistan. Pakistan. Journal of Botany. 2008; **40**(1):43

[58] Qureshi R, Bhatti GR, Memon RA. Ethnomedicinal uses of herbs from northern part of Nara desert. Pakistan. Pak J Bot. 2010;**42**(2):839-851

[59] Rokaya MB, Münzbergová Z, Timsina B. Ethnobotanical study of medicinal plants from the Humla district of western Nepal. Journal of

Ethnopharmacology. 2010;**130**(3): 485-504

[60] Rajakumar N, Shivanna M. Ethnomedicinal application of plants in the eastern region of Shimoga district, Karnataka, India. Journal of Ethnopharmacology. 2009;**126**(1):64-73

[61] Islam MK, Saha S, Mahmud I, Mohamad K, Awang K, Uddin SJ, et al. An ethnobotanical study of medicinal plants used by tribal and native people of Madhupur forest area, Bangladesh. Journal of Ethnopharmacology. 2014; **151**(2):921-930

[62] Ugulu I, Baslar S, Yorek N, Dogan Y. The investigation and quantitative ethnobotanical evaluation of medicinal plants used around Izmir province, Turkey. Journal of Medicinal Plants Research. 2009;**3**(5):345-367

[63] Shinwari S, Qureshi R, Baydoun E. Ethnobotanical study of Kohat pass (Pakistan). Pakistan Journal of Botany. 2011;**43**(SI):135-139

Chapter 4

Bioinorganic Chemistry and Computational Study of Herbal Medicine to Treatment of Tuberculosis

Sri Widyarti, Mudyawati Kamaruddin, Sherry Aristyani, Meity Elvina, Saraswati Subagjo, Tintrim Rahayu and Sutiman Bambang Sumitro

Abstract

Tuberculosis (TB) is one of the leading infectious diseases in the world. The disease is commonly caused by *Mycobacterium tuberculosis* (Mtb) bacteria which are capable of rapidly spreading through droplet transmission. In developing countries, poverty and malnutrition cause immunodeficiency which is considered as the main risk factor for the incidence of TB. Treatment of TB has been proven to be difficult because treatment options are very limited and found to be expensive specifically in developing countries. Moreover, the existence of extensively drug-resistant TB phenomena is frequently happening in these countries because of mishandling treatments used for this disease. In Indonesia, the traditional herbal medicine, namely, jamu, has been utilized since a long time ago to treat diseases including TB. The present study by using computational methods found that there are many active compounds that can bound and influence proteins responsible for TB pathogenesis. Besides, these compounds have the potency to modulate the host immune system. The current chapter discussed the possible interaction of the antioxidant compounds with the chelating potential to form a complex with transitional metal as the central atom. In the perspective of bioinorganic chemistry, this complex has a scavenging activity which is expected to have a role in overcoming energy management of the host cell during infection pathogenesis. It is important to involve bioinorganic chemistry in energy management during infection, correlated with impairing of niacin metabolism of the host cell in which the host cell mitochondria cannot competitively gain free radicals during infection. This phenomenon is the main reason to propose herbal medicine as a source of niacin and provide a proper environment for gastrointestinal commensal microbiota to treat and govern protection from TB infection.

Keywords: bioinorganic chemistry, gastrointestinal microbiota, herbal medicine, LMWA, TB

1. Introduction

Tuberculosis is a major cause of morbidity and mortality, especially in low-income and middle-income countries [1]. The World Health Organization (WHO) mentioned in the Global Tuberculosis Report 2016 that there were approximately 10.4 million people infected with TB. Among those, 1.4 million patients lost their life leaving poor families in helpless situations. Although the mortality rate of TB patients had been decreased by 22% between 2000 and 2015, resistant and persistent types of disease remain as a major problem till present [2].

Over the past few years, TB treatment depended on the use of antibacterial compounds such as isoniazid, rifampicin, pyrazinamide, and ethambutol as first-line drugs. But recently it had proven that the anti-TB drug(s) had caused mutations in *Mycobacterium tuberculosis* (Mtb) bacteria giving rise to a new kind of multidrug-resistant TB (MDR-TB) [3]. Therefore, the treatment of the disease becomes extremely hard and forces the physicians to start using the second-line of drugs such as aminoglycosides, polypeptides, fluoroquinolones, thioamides, cycloserine, and para-aminosalicylic. These drugs were also causing mutations in Mtb which gives rise to new cases such as extensively drug-resistant TB (XDR-TB), in which Mtb become resistant to both the first- and the second-line of treatments [4, 5].

TB treatment based on eradicating bacteria. In this way, TB therapy needs to be carried out comprehensively through improving nutritional balance and immune system modulation [6]. The mechanism of pathogenesis of Mtb contains competition between host and bacteria, for iron (Fe) involves the secretion of superoxide dismutase (SOD) enzymes which is related with the bioinorganic chemistry of complex compounds that synergistically work as energy delivering system underlies [7]. The SOD enzyme indirectly becomes part of the energy and electron transfer system because of its catalytic activity which converts superoxide radicals (O_2^-) to hydrogen peroxide (H_2O_2) and oxygen (O_2) to minimize the toxic effects of O_2^- [8]. This is what causes Mtb to avoid the reactive oxygen species (ROS) produced by host macrophages as an immune system defense effort. But because Mtb secrete SOD, Mtb can develop well in macrophages, and to synthesize SOD by TB bacteria, it needs Fe, and the main source will be the infected host [9]. On the other hand, the host also needs Fe for hemoglobin of red blood cells [10]. This competition will significantly disturb energy transfer system of the host and will cause lack of hemoglobin, i.e., anemia [11]. As a result of that, in Mtb infection, there is an energy competition, meaning that even though Mtb and hosts do not intersect in their energy management system, the electron flow moves toward Mtb cells rather than hosts. This is due to lower electrical potential state of Mtb. When the immune system is in proper condition, Mtb get more energy to divide and migrate. The balance between cellular and metal ion components is indirect but mediated by a special set of proteins, namely, SOD, which are controlled and organized interactions of energy transfer within the mitochondria [12].

The active compound in herbal medicine that functions as a radical scavenger in bioinorganic chemistry is known as low molecular weight antioxidant (LMWA) complex. This LMWA contains metal ions such as Fe, Mn, Cu, and Zn [13, 14]. The complex can overcome the impairing energy transfer system of the host because they can transfer electron when electron transfer system is impaired due to anemia in the case of TB infection. The potential of LMWA complex as a free radical scavenger is also important as an anti-inflammation agent.

2. Computational studies of active compound of Indonesian herbal medicine used in treating TB

Computational drug discovery has been widely described as a tool and database for saving, managing, analyzing, and modeling compounds. This is proven as an effective strategy to guide and improve the discovery and development of new therapy. The real aim of drug discovery is to find new "lead" compounds and new chemical entities that could potentially be developed into new medicines, gaining the target specificity related to disease development or changing the target function. In the steps of drug discovery, computational drug discovery is involved in designing the main compounds with computer programs, identifying target and validity, and observing the effectiveness, the bioavailability, and the side effect possibility of lead compounds and lead candidates. Recently, a lot of researches have shown that the application of computational drug discovery can significantly help in identification and/or development of an effective treatment [15, 16].

In fact, computational drug discovery can also be utilized to approve traditional treatments that have been used for a long time to treat several diseases. In this case, computational drug discovery tools have been used for observing and analyzing the active compounds of Indonesian medicinal plants used for treating TB. Some studies showed that natural compounds from indigenous Indonesian medicinal plants could inhibit the incidence of TB infection by balancing immunity system and eradicating mycobacteria [17–20].

Based on computational drug discovery tools, it has been detected that Indonesian native plants such as *Euphorbia hirta*, *Foeniculum vulgare*, *Ocimum basilicum*, *Zingiber officinalle* Rosc., *Curcuma domestica*, *Plantago major*, *Curcuma*

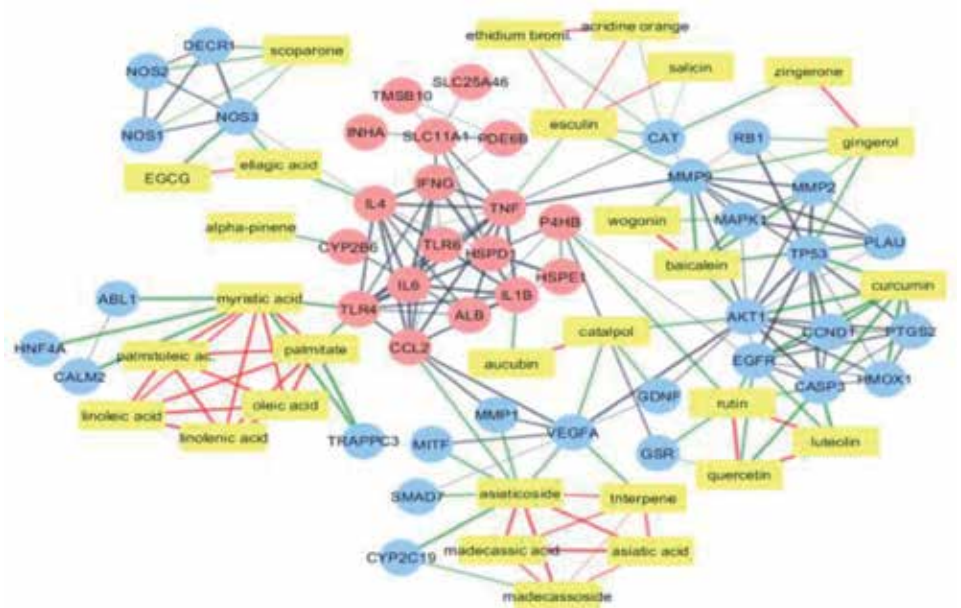

Figure 1.
Interaction between active compounds of Indonesian medicinal plants with protein-related tuberculosis. The red circle indicates protein involved in tuberculosis mechanism, while the blue one indicates protein unrelated to tuberculosis mechanism. The yellow rectangle indicates active compound. The green line indicates active compound-protein interaction. The red line indicates active compound-active compound interaction [21].

zedoaria, *Centella asiatica*, *Coffea arabica*, *Ageratum conyzoides* L., *Tamarindus indica*, *Citrus aurantifolia*, *Petiveria alliacea*, and *Lantana camara* L. contain several active compounds that interacted with protein-related tuberculosis like IL-4, TNF-α, IL-1B, CCL-2, TLR4, and P4HB (**Figure 1**); by immunity balancing they can improve the therapy outcomes and avoid Mtb reaction [21, 22]. Moreover, some studies showed that cytokines like IL-4, IL-1B, and CCL-2 could eradicate mycobacteria [23]. TNF-α, a proinflammatory cytokine, had high expression during the latent phase of infection, suggesting that it might be correlated with IFN-γ for controlling mycobacterial replication [24]. TLR4, a transmembrane protein and a member of the toll-like receptor family, has been reported to be expressed in macrophage and dendritic cell to recognize mycobacteria. P4HB, a disulfate enzyme catalyzation, increased the Th-2 cell migration. In addition, the active compounds not only interacted with protein-related immunity but also another protein like CYP2B6, a cytochrome 450 enzyme, which could be an indicator for tuberculosis therapy [25].

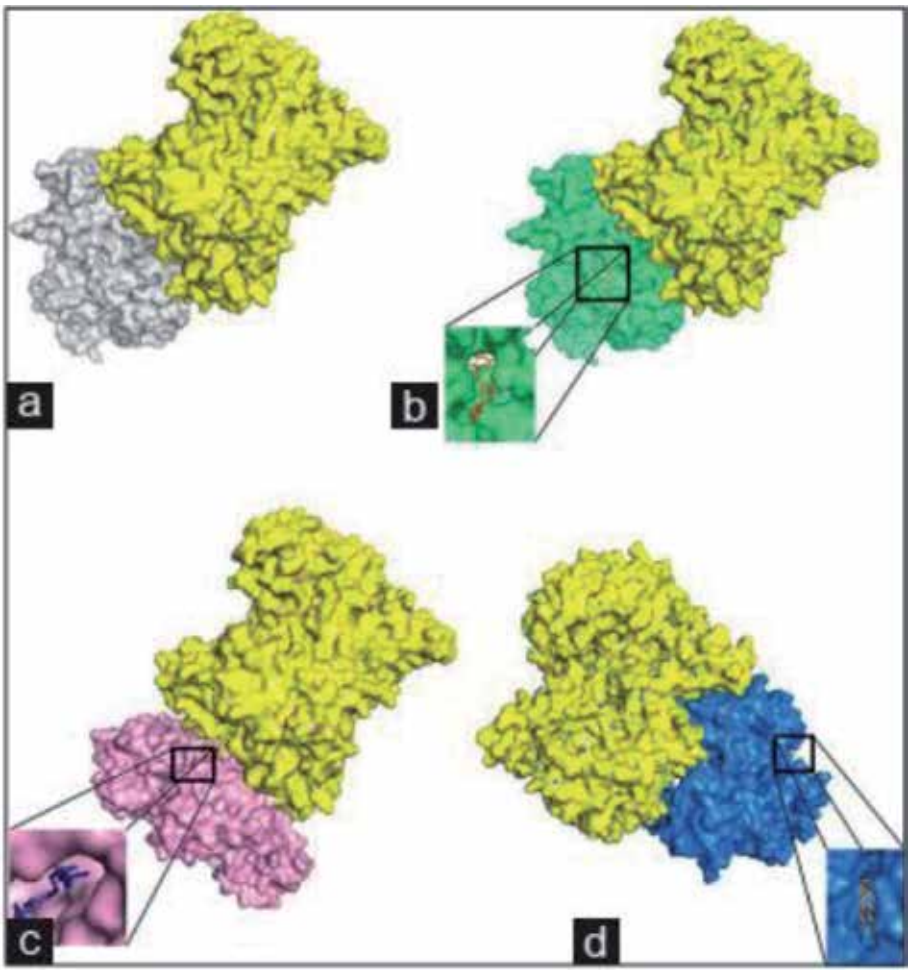

Figure 2.
Src complex. (a) Src-PI3K (wild type), (b) Src-tuberculosis drug-PI3K, (c) Src-phytol-PI3K, (d) Src-oleic acid-PI3K. Phytol and oleic acid could change the binding position of Src-PI3K, whereas the tuberculosis drug did not change the position of Src-PI3K. Gray structure, Src wild-type protein; yellow structure, PI3K; green structure, Src-drug complex; pink structure, Src-phytol; blue structure, Src-oleic complex [21].

A study conducted by Aristyani detected the incidence of interaction between active compounds extracted from Indonesian medicinal plants, *Tamarindus indica* and *Curcuma xanthorrhiza* Roxb., with Mtb's proteins by using CADD tools. Results showed that bisdemethoxycurcumin, alpha-pinene, isoamyl alcohol, furfural, lauric acid, and salicylate correlated with proteins of Mtb. Bisdemethoxycurcumin interacted with Pks 11 and Pks 18 which are involved in the α-pyrones synthesis, an essential compound in the mycobacterium cell wall. α-Pinene correlated with cyp144 which has roles for mycobacterial survival and pathogen in human cells. Both isoamyl alcohol and furfural associated with alcohol dehydrogenase proteins, while lauric acid interacted with fatty acid synthase. Salicylate bonds with various proteins such as caeA for modifying envelope structure, lpqp for encoding a membrane-bound lipoprotein, lipT for hydrolyzing from liposome suspensions,

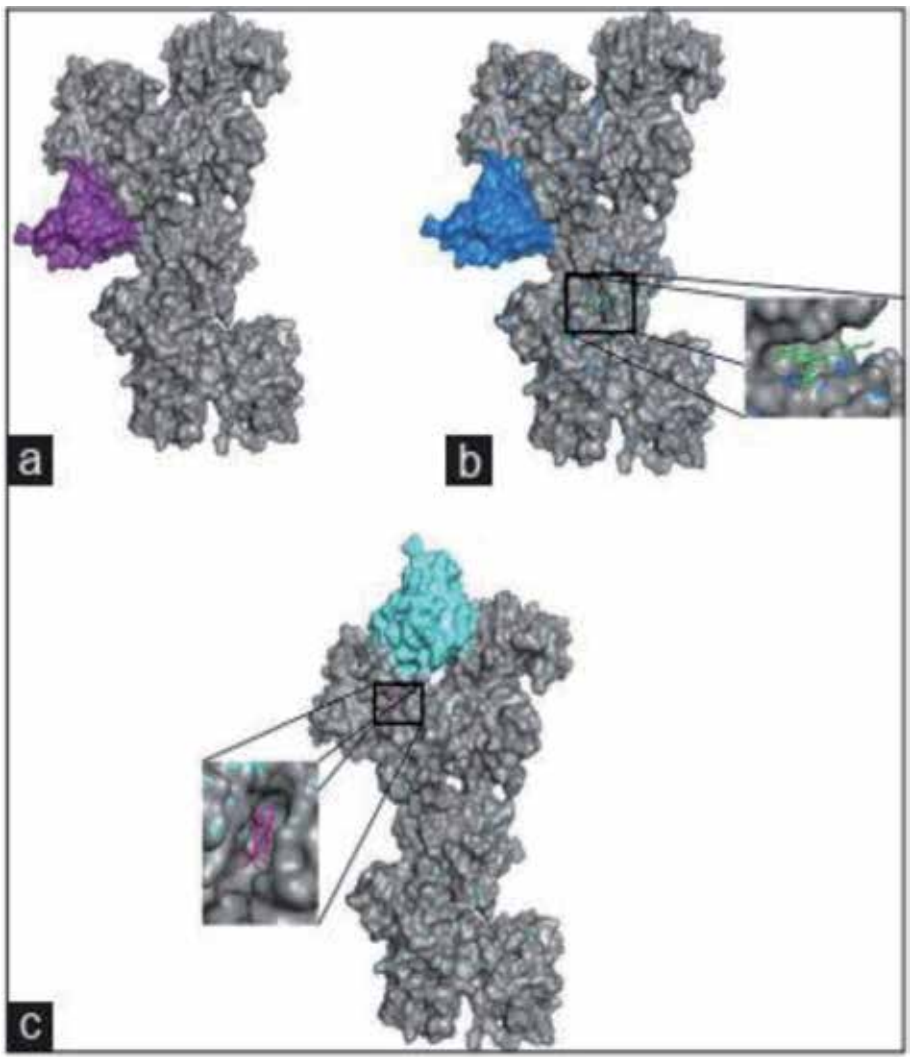

Figure 3.
PknB complex. (a) PknB-FhaA, (b) PknB-drug-FhaA, (c) PknB-phytol-FhaA. The position of FhaA was altered when PknB complexed with phytol; meanwhile, when the complex form of tuberculosis drug and PknB bound with FhaA, it had a similar position with the wild-type form. Gray structure, PknB; purple, FhaA in PknB-FhaA complex; dark blue, FhaA in PknB-drug complex; light blue, FhaA in PknB-phytol complex [21].

and estB for hydrolyzing and has peroxidase activity. Depending on that it can be concluded that natural compounds might treat tuberculosis by directly interfering the cellular system of mycobacteria [26].

A computational docking method has been also used to explore the activity of active compounds of Indonesian medicinal plants against TB. In this term, the active compounds of *Curcuma xanthorrhiza* Roxb., *Zingiber officinale* var. Rubrum, *Tamarindus indica* L., and *Citrus aurantifolia* were docked with human proteins related to tuberculosis. Results showed that tyrosine-protein kinase Src (Src), which is a non-receptor protein kinase, could reduce the survival of Mtb in macrophage; matrix metallopeptidase (MMP1), a metalloproteinase protein, can degrade the granuloma, severity of TB directly correlated with MMP1 expression [27, 28], and Mtb's proteins, protein kinase B (PknB), a transmembrane signal kinase which takes a part in mycobacteria growth and division mechanism, and KatG, a multifunctional catalase peroxynitrite; and NADH oxidase has a role in synthesizing mycolic acid of mycobacteria. The results described that active compounds such as curcumin, demethoxycurcumin, 8-gingerol, phytol, oleic acid, and linoleic acid had a higher binding scores to all the target proteins than the binding score of tuberculosis drug with the target proteins. Moreover, when the oleic acid and phytol bound with Src, it changed the binding position of PI3K, a ligand of Src. Phytol also gave a similar effect not only in Src but also in PknB. It altered the binding position of FhaA, a ligand of PknB. It might be concluded that these compounds could inhibit the activity of downstream protein target which could suppress the survival and growth of Mtb (**Figures 2** and **3**) [21].

3. Bioinorganic chemistry as a new perspective of herbal medicine to treat diseases

Recently, bioinorganic chemistry has been used in the broad areas of biological, medical, microbial, and food industry, etc. Bioinorganic chemistry is concerned about complex organic compound containing one or more metals as a center atom. These organic compounds in biological system which are bound with protein may serve multiple activities such as radical scavenging, antibacterial, antioxidant, electron transfer, or enzyme [29–33]. The section below will describe the natural bioinorganic chemistry in a plant that inspires bioinorganic compound in herbal medicine. Cause metals have a pivotal role on energy or electron transfer involves variety of small molecules, nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) as electron carriers, and metalloprotein such as superoxide dismutase (SOD) enzymatic antioxidant, host-pathogen energy interaction can be explained based on bioinorganic chemistry approach.

3.1 Bioinorganic chemistry associated with radical scavenging activity

Bioinorganic chemistry study involves the role of metal complexes in biological systems, including metals which form complexes naturally with proteins (called metalloproteins) or artificially metal complexes [29, 30]. Transition metals such as iron (Fe), copper (Cu), manganese (Mn), and zinc (Zn) are required for proliferation and survival of all living organisms. They have important roles as enzymes and cofactors and environmental sensors. Iron is the most commonly used divalent metal cofactor. Iron containing enzymes or proteins is involved, among other processes, in electron transfer, maintaining redox balance, and detoxification. Hemoglobin in red blood cells is a globin protein molecule that forms a complex

with porphyrin with an iron ion center. Manganese has the strongest affinity for ATP and is the preferred cofactor in cAMP production. Zinc is used as cofactor by numerous enzymes and DNA binding proteins and additionally functions to scaffold additional proteins [34]. Enzymes such as superoxide dismutase (SOD) are enzymatic antioxidants against superoxide radicals that require Fe, Cu, or Mn for their activation. In plants, chlorophyll is a porphyrin complex with the center of magnesium ions. Secondary plant metabolites such as flavonoids possess three possible metal-chelating sites that can bind metal ions Fe, Cu, Mn, Mg, Al, Zn, and Ni [31–33].

According to bioinorganic chemistry in the case of TB pathogenesis, iron plays a pivotal role in host-pathogen interactions. Iron is one of the most important aspects needed for the initiation and establishment of infection. The mechanism of bacterial infection requires iron against host defenses, while the main component of host natural immunity limits the availability of iron for bacteria [35–38]. Mtb require iron for normal growth but faces limited metal ions because of its low solubility at biological pH and limitation of iron availability by mammalian hosts. Because of high affinity for Fe^{3+}, plasma transferrin, ferritin, and lactoferrin in extracellular fluids and polymorphonuclear leukocytes play an important role in reducing iron availability for pathogens [39]. Mtb express Fe^{3+}-specific specifications of siderophore mycobactin and carboxymycobactin to chelate insoluble metals and form host plasma proteins. Iron absorption mediated by siderophore is very important for the survival of Mtb in media with low iron levels and in macrophages. Mtb bacteria have regulation of iron absorption to maintain optimal levels of intracellular iron to prevent iron toxicity [40]. Administration of chelating agents to limit the availability of iron actually stimulates the microbes that express siderophore and carboxymycobactin to improve the acquisition of iron. Subjects who recovered from active TB tended to relapse if they received iron-rich supplements (in tonic form) compared with patients who did not receive supplements and were somewhat anemic. Limiting the availability of iron for microbes using herb medicine that form complexes with metals has a better effect to hold up bacterial proliferation [36].

For this purpose, many plant species have been used for treating tuberculosis. These herbal products have been widely proven to have an antimicrobial activity. In addition, these herbal products are used in combination with synthetic treatment to increase the efficacy of conventional drugs and also to reduce side effects and inhibit incidence of antibiotic resistance. Traditional healing systems like Ayurveda have been applied to cure TB from Africa to Asia or China and so on [41]. Almost all of these traditional herbs have been tested for their activities as antimicrobials especially on Mtb, antioxidant, as well as anti-inflammatory [41–44].

Sukmaningsih et al. [14] proved that the fruit of java plum (*Syzygium cumini*) contains naturally occurring bioinorganic compounds. The X-ray fluorescence (XRF) characterization results indicate that the primary metal contents in java plum were K, Ca, P, Cu, Ni, and Fe. The last three elements are classified as transition metals. The electron configuration of the atomic valence of Fe is $3p^6\ 4s^2\ 3d^6$, Ni is $3p^6\ 4s^2\ 3d^8$, and Cu is $3p^6\ 4s^2\ 3d^9$. The electron configuration shows that the transition metal d orbitals contain unpaired electrons that can accept electrons to occupy the orbital. The electrons interact with the molecule or specific anion through coordination bonds formed ion complex or compound. The metals Fe, Cu, and Ni that have unpaired electrons will act as the central atom and play an important role in complex formation. Analysis of LC/MS shows that the main ingredient in java plum is the flavonoid complex, namely, anthocyanins. In this compound, Fe is the center of the atom, whereas anthocyanin is a ligand. The results of the study using ESR concluded that the java plum had activity as a radical scavenger as evidenced by its ability to reduce the radical intensity of DPPH. When receiving

electrons, the anthocyanin complex does not produce new radicals as well as single compound antioxidants. This is because the metal center in the flavonoid complex has paramagnetic properties (as giving electrons) that will become diamagnetic (as get electrons) when performing its functions as free radical scavengers [14]. The mechanism activity of radical scavenging is related with delocalized electron. In the case of flavonoid and anthocyanin, the electrons came from phenolic hydroxyl group as a source of electron by denoting hydrogen atom or through the transfer of a single electron.

As reported by Aristyani, selected Indonesian medical plants used by Indonesian local people to treat TB were *Curcuma xanthorrhiza* Roxb., *Tamarindus indica* L., *Citrus aurantifolia*, and *Zingiber officinale* var. Rubrum. From the 17 selected compounds, there are 5 compounds predicted to have multitarget that are curcumin, demethoxycurcumin, phytol, oleic acid, and linoleic acid that can bind to the host metallopeptidase 1 and Src matrix proteins and PknB and catalase-peroxidase proteins from Mtb [26]. The antioxidant mechanism of these compounds is the result of interactions between phenols and transition metal ions such as copper or iron [45]. Antioxidant compound may be naturally present in different forms in the plant microstructure. Low molecular weight antioxidants (LMWA) are free from chemical or physical interaction with other plant macromolecules. LMWA are nonenzymatic antioxidant compounds capable of preventing oxidative damage by directly interacting with ROS or indirectly by chelating metals [46]. Because of its small size, LMWA pass through cellular membranes, so it can access near to molecule targets. Different nonenzymatic antioxidants can interact synergic to scavenge free radicals. Endogenous LMWA synthesized by living cells is limited, so exogenous nonenzymatic antioxidants from plant diet and phytochemicals are required. The action of LMWA in cellular metabolism can be bound as a component of endogenic enzymes, or other proteins containing transition metal could be the components of antioxidative enzymes or acting as LMWA alone and non-nutrients such as phenolic compound (phenolic acid, flavonoids, lignans), phytic acid, reduced glutathione, and melatonin to manage the free radicals by preventing free radicals to involve in Haber-Weiss reaction [46, 47].

The complex with transitional metal makes flavonoid an other organic chelating compound becoming water-soluble. This means they can interact with protein and DNA to increase bioavailability as antioxidation, antibacterial, and antitumor and affect various types of enzymatic activity [48]. Metal ions have an impact on the hydrogen atom transferring ability of the complex revealing that complexes deactivate oxidants through hydrogen atom transfer. Fe3+-primuletin (5-hydroxyflavone) is expressed as an antioxidant in in vivo system through direct scavenging of free radicals by decreasing total ROS and at the same time through enhancement of SOD and catalase activities endogenously [49].

Phenolic compounds have strong antioxidant properties in vitro, associated with their ability to trap the chain-carrying peroxyl radicals with the formation of hydroperoxides, to scavenge free radicals in Fenton reaction and chelating metal ion [50, 51]. The deprotonated phenolic group has an oxygen center that possesses a high charge density. The pKa value of most phenols is around 9.0–10.0, but in the presence of cations such as Fe(III) or Cu(II), the proton is donated at physiological pH 5.0–8.0 [52]. The ability of phenol in metal chelation can inhibit lipid peroxidation by binding metal ions to stable complex forms or preventing the interaction of metal ions with lipid hydroperoxides, which are consistently produced in living cells [53, 54]. The scavenger potencies of flavonoid metal complexes were significantly higher than those of the parent flavonoids. In addition, this metal complex also has pharmacological activities such as SOD [55].

Metal complexes have more lipophilic properties so that they easily penetrate cell membranes. The lipophilic properties of metal complexes can be explained according to coordination theory. According to the coordination molecular theory, the overlap of orbitals between metals and ligands reduces the positive charge on metal ions by accepting electrons from ligands. This causes an increase in π-electron delocalization throughout the coordination ring. This results in increased metal complex lipophilicity [56]. Therefore, research on flavonoid metal complexes is very helpful in developing new TB drugs. Accordingly, in the condition of a host of SOD depletion, flavonoid metal complex antioxidants can perform as enzymatic antioxidant SOD.

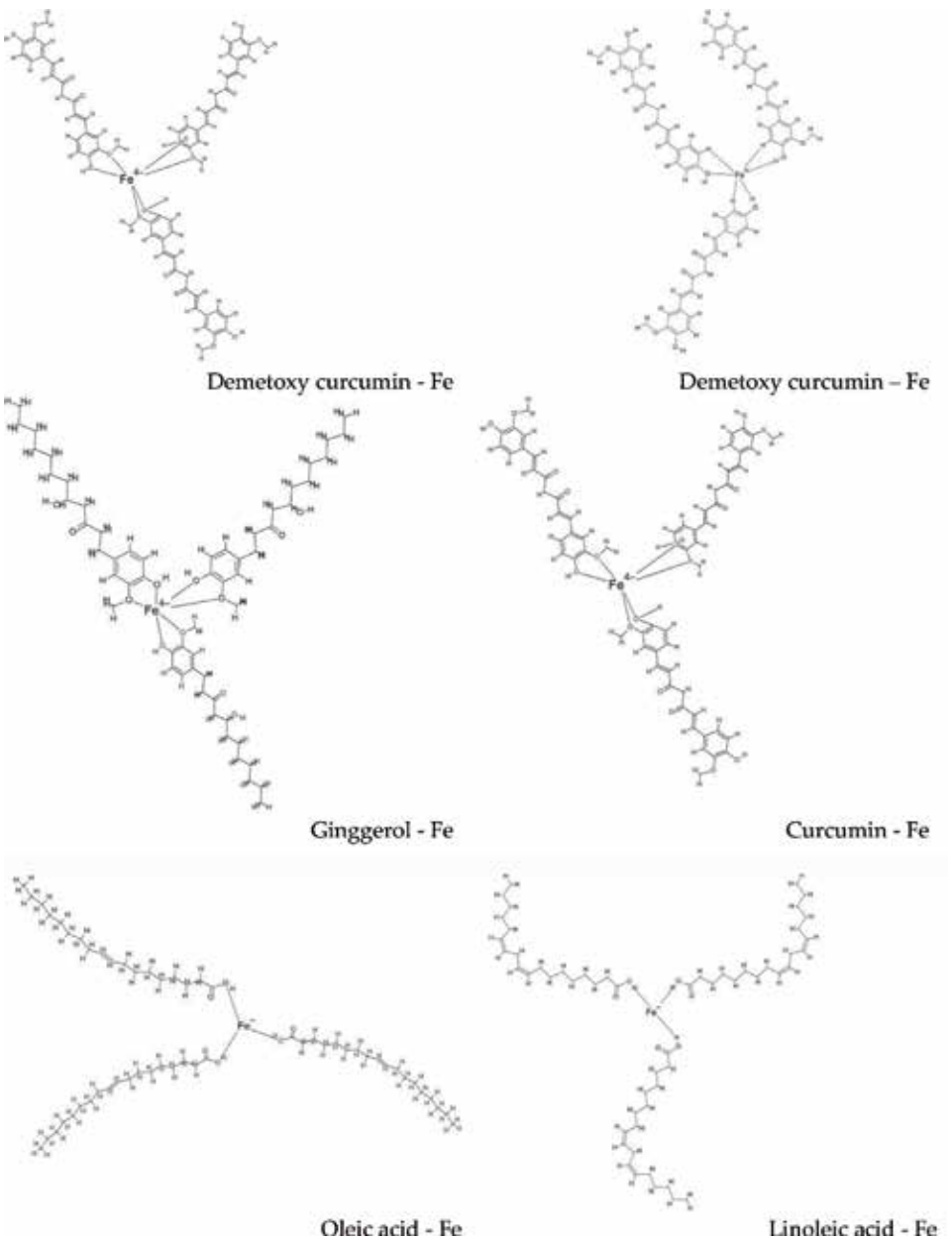

Figure 4.
Structures of the iron-demetoxy curcumin, iron -ginggerol, iron -curcumin, iron-oleic acid, and iron-linoleic acid complexes predicted by ChemSketch open babel software [57].

Given the large variety of active compounds in nature, most of them have the ability to chelate metals. In silico analysis using molecular modeling programs such as ChemSketch can be used to predict this ability. Using ChemSketch open babel software, we predicted that the chelator compounds such as curcumin, demethoxycurcumin, 8-gingerol, phytol, oleic acid, and linoleic acid can interact with transition metal Fe (**Figure 4**) [57]. It shown that the efficacy of this herbal medicine can be studied using some instrumentation such as X-ray diffraction (XRD), X-ray fluorescence (XRF), and scanning electron microscopy (SEM) and be discussed using bioinorganic chemistry perspective.

In practice, it means that herbal application for therapy can be in the form of food or drink as well as scrubbing throughout the skin. In Indonesia the herbal application for scrubbing includes fermented herbs. This scrubbing treatment aimed to leach overwhelming free radicals to sweat out the body through the skin. After several times of scrubbing, patients will gain better quality of life such as sleeping well and good appetite [58].

3.2 Bioinorganic compound associated with energy balance during TB pathogenesis

In the twenty-first century, the concept of oxidative stress has been well acknowledged in relation to understand the underlying mechanism involved in various human diseases including infectious disease such as TB [59]. Tuberculosis has been linked to free radical production either by bacteria or phagocytes. This process is closely related to the gradual loss of mitochondrial efficiency that will contribute to worsening of the disease [60].

The excessive production of reactive oxygen species (ROS) is widely associated with cellular damage. The cellular damage has been associated with depletion of coenzyme that is intricately tied to the pyridine dinucleotide. The important coenzyme in all living cells involved in oxidation–reduction reactions is known as nicotinamide adenine dinucleotide (NAD+); it is a key metabolite derived from niacin or vitamin B3 [61, 62]. NAD+ is an essential electron transporter in mitochondrial respiration and oxidative phosphorylation. NAD+ serves as an electron acceptor for glycolysis, a process that metabolizes glucose or glycogen, and is reduced to NADH. NAD+ instead of eventually transferring electrons from NADH to the mitochondrial respiratory chain. The NAD+/NADH ratio is also vital for cell physiology as it contributes to the synthesis of ATP as the energy currency of the cell. NAD+ must be regenerated from NADH for continued glycolytic flux, a process that happens within the mitochondria. If NAD+ is not regenerated, glycolysis and other metabolic pathways will stop, creating a disruption in mitochondrial metabolism and an imbalance in cellular redox homeostasis that will lead to cell death. Also NAD+ is an important precursor for the phosphorylated dinucleotide pair NADP+/NADPH in several cellular biosynthetic pathways in order to protect cells from free radicals [63]. Moreover, it plays a major role in perturbed immune responses. Thus, NAD+ dictates the host's innate and adaptive immune responses. Furthermore, it can be the strategy treatment of human diseases including TB, suggesting the potential of targeting the cellular NAD+. Four years ago, the Niederweis group described the first toxin ever found in Mtb. They found that tuberculosis necrotizing toxin (TNT) enzymatically hydrolyzes NAD+. The depletion of NAD+ inside the macrophages somehow leads to necrotic cell death of the macrophage that will cause the release of Mtb to infect more cells [64].

Mitochondrial dynamics are supposed to have an important physiological role in maintaining intracellular energy balance and energy transduction; thus, it is generally accepted that the mitochondrial electron transport chain (mETC) is the major

site for the generation of ROS. The mETC is continuously involved in reducing molecular oxygen to water in a four electron reduction processes [65]. Even a small percentage of the oxygen consumed escapes from the mETC as superoxide radical ($O2^{-}$), which can generate other endogenous ROS that will induce a great threat to aerobic organisms [66].

Herbal medicine was found to be a good source of niacin, i.e., a precursor for NAD+ synthesis. Many studies suggest that the conversion of niacin and its derivative, such as nicotinamide, was synthesized from tryptophan. The human studies have shown that 1 mg of nicotinamide is produced from 67 mg of tryptophan intake [67]. The recommended dietary tryptophan daily dose for human adults ranges from 250 to 425 mg/day, corresponding to 3.5–6 mg kg^{-1} (meanly 4 mg kg^{-1}) body weight per day [68, 69]. Thus, the herbal remedy focuses on supplementing tryptophan as precursor for niacin to enhance cofactor NAD+ to prevent the depletion of NAD+ [70]. Mammals, including humans, can synthesize vitamin B3 (nicotinamide) from tryptophan through ingestion process in the gastrointestinal system (gut and liver). Several studies report that the gastrointestinal system plays a critical role in nicotinamide supply. This was involved to the changes in tryptophan-nicotinamide metabolism, particularly on niacin nutritional status [71].

The important role of the healthy gut due to the gastrointestinal tract is prone to the attack of ROS in various diseases including infectious disease such as TB [72]. In the gastrointestinal tract, there are many major endogenous oxidative enzymatic reactions [73]. Managing oxidative stress through the healthy gut will restore the commensal microbiota for helping in tryptophan metabolism. A balanced microbial community is a key regulator of the immune response. In terms of antioxidant or free radical scavenging, tryptophan plays an important regulatory role in restoring the body antioxidant system. Tryptophan as essential amino acid must be ingested from exogenous natural sources (such as polyherbal). Increasing tryptophan concentration will increase NAD+ level in human cellular, particularly encouraging the performance of macrophage in ingestion and killing of pathogen [71, 74].

4. Role of herbal medicine in supporting gastrointestinal microenvironment during TB infection

The presence of commensal microbiota and its metabolites in the body has beneficial roles in human physiology, such as metabolism, formation of the immune system, anti-inflammatory activity, homeostasis maintenance, and vitamin production [75]. Intestinal microbiota was found to play a major role in regulating immune cell homeostasis. The explanation of "gut-lung axis" statement showed that the intestinal microbiota can regulate lung immunity and influence the lung microbiota through microbial products and immunomodulators released upon recognition of commensals and pathogens by intestinal immune cells. This explains why the reduction in commensal flora in the intestine is directly related to the severity of the inflammatory response in the lungs. This was as examined by Tsay et al. that there was a significant effect of commensal microbiota depletion on *Escherichia coli* pneumonia-induced myeloperoxidase (MPO) activity in the lungs and bacterial killing activities of alveolar macrophages by using the commensal depletion model in mutant WT and toll-like receptor 4 (TLR4) mutant mice. The study demonstrated that gut microbiota involved in stimulating lung inflammatory reaction against bacterial challenge through TLR4 which binds to lipopolysaccharide (LPS) [76, 77]. The innate immune system detects invasion of microorganisms via TLR, which can recognize microbial components and trigger an inflammatory response. TLR is a germ-coding pattern recognition receptor, and several have been

identified, such as TLR4 which recognizes LPS and TLR2 as receptor of lipoteichoic acid (LTA) [78]. In addition, the gut microbiota is important in increasing nuclear factor kappa beta (NF-κB) activation, an important early step in innate immune cell activation, in the lung through TLR4 and LPS supplements which serve to increase lung defense through the TLR4 and NF-κB signaling pathways. This confirms that gut commensal microbiota is crucial in maintaining lung defense against bacterial challenge through enhancement of alveolar macrophage activity and neutrophil infiltration [76].

Therefore, antibiotic therapy effect on intestinal microbiota may cause unbalanced inflammatory response in the lung [79]. Components of herbal medicines can improve the composition of the gut microbiota to turn in homeostasis, thus restoring dysfunction and associated pathological conditions. This is because the gut microbiota can metabolize herbal medicine chemicals and the generated metabolites have bioavailability, bioactivity, and toxicity that are different from their precursors [80]. Commonly, polar compounds are found in herbal medicine extracts, and the bioavailability of these chemicals is usually very low due to poor lipophilicity. However, the gut microbiota converts the molecules to be smaller parts that are less polar and more lipophilic [81].

Notable interactions between the active components of herbal medicines and gut microbiota are also being vigorously inspected such as glycosides affluent in many herbal medicines that always hold limited intestinal absorption because of poor intestinal permeability. The gut microbiota which are encoded with glycoside hydrolase genes can cleavage glycosyl or glucuronosyl parts from the backbone. Normally, the secondary glycosides produced by this process hold better bioavailability and thus can be absorbed properly by the intestine [82]. Biotransformation of herbal medicine components carried out by the intestinal microbiota can be done in various ways, for example, hydrolation such as deglycosylation; oxidation such as fission, hydration, hydrogenation, hydroxylation, methylation, and oxygenation; reduction such as dehydration, dehydroxylation, demethylation, decarboxylation, and dehydrogenation; rearrangement; isomerization; condensation; ester hydrolysis; esterification; and intramolecular cyclization.

Furthermore, depending on those various reaction mechanisms, the gut microbiota can trigger a series of metabolic reactions simultaneously and successively against the structural property of the herbal medicine compound. For example, glycosides in herbal medicines are normally metabolized first by gradual hydrolysis, through deglycosylation and esterlysis and then by hydrolysates generating secondary glycoside or aglycone. The secondary glycosides are further converted by skeleton-retained modification (triterpene glycosides), by skeleton fission (flavonoid glycosides), or by skeleton rearrangement (iridoid glycosides). Generally, different bacteria in the gut microbiota can cooperate to promote metabolism of a single compound, while one single bacterial strain is able to transform different compounds [83]. In the human intestinal microbial metabolism of quercitrin, an active compound of Indonesian herbal medicine for tuberculosis treatment [21] showed *Fusobacterium* K-60 deglycosylates the quercitrin, whereas four other strains of bacteria, specifically *Bifidobacterium* B-9, *Pediococcus* Q-5, *Streptococcus* S-3, and *Bacteroides* JY-6, were bounded in further fission of the quercetin (aglycone) [84]. Meanwhile, several compounds such as geniposide, aconitine, and shikonin belonging to different structural types were transformed by *Clostridium butyricum* via various reaction mechanisms: deglycosylation, dehydration, condensation, dehydrogenation, and intramolecular cyclization [82].

Interestingly, herbal medicines can modify the composition of gut microbial community while being metabolized. Some experiments have been tried using fecal transplantation to show that herbal medicine therapy targets the gut microbiota.

For example, the heat shock protein (HSP) expression levels of the murine liver and intestine were altered by a herbal medicine formula that contains *Panax ginseng* root and rhizome, *Ligusticum chuanxiong* rhizome, *Poria cocos* sclerotium, *Atractylodes macrocephala* rhizome, *Glycyrrhiza uralensis* root and rhizome, *A. sinensis* root, *Paeonia lactiflora* root, *Rehmannia glutinosa* tuberous root, *Cinnamomum cassia* cortex, and *Astragalus membranaceus* root. Thus, the alteration only occurred in intestinal microbiota rather than in germ-free mice [85].

5. Conclusion

Herbal medicine used for treating TB produces its pharmacological effect through the following. First, the immune system perspective. The target of most herbal medicine active compounds are proteins involved in the immune system. It can be indicated that these compounds have potential as an immune system modulator to treat tuberculosis diseases. Second, bioinorganic chemistry perspective. Herbal medicine has been endowed with the extraordinary ability as an antioxidant. This antioxidant is grouped as low molecular weight antioxidants (LMWA) that easily permeate to cell membranes. Bioinorganic chemistry perspective discusses LMWA-centered transition metal (such as Fe, Cu, Mn, Zn). Transition metals are directly interacting chelated by LMWA, thereby preventing them from participating in metal-based ROS production. Third, managing free radical. The collaboration between LMWA and metal ion has the ability to manage excessive electron to become moderate-level free radical that is beneficial on a host. Fourth, supporting the gastrointestinal system. The proper herbal medicine formulation will provide nutrition for NAD+ precursor on a host as well as determine gut commensal microbiota diversity that influences the microbiome in the lung. Finally, herbal medicine has a great prospect to treat TB instead of an antibiotic.

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Conflict of interest

The authors declare no conflict of interest.

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References

[1] GBD Tuberculosis Collaborators. The global burden of tuberculosis: Results from the global burden of disease study 2015. The Lancet Infectious Diseases. 2018;**18**:261-284. DOI: 10.1016/S1473-3099(17)30703-X

[2] World Health Organization. Global Tuberculosis Report 2016. Geneva: World Health Organization WHO; 2016

[3] Gandhi NR, Nunn P, Dheda K, Schaaf HS, Zignol M, van Soolingen D, et al. Multidrug-resistant and extensively drug-resistant tuberculosis: A threat to global control of tuberculosis. Lancet. 2010;**375**:1830-1843

[4] Nguyen L. Antibiotic resistance mechanisms in *M. tuberculosis*: An update. Archives of Toxicology. 2016;**90**:1585-1604

[5] Jain A, Mondal R. Extensively drug-resistant tuberculosis: Current challenges and threats. FEMS Immunology and Medical Microbiology. 2008;**53**:145-150

[6] WHO. Nutritional Care And Support For Patients With Tuberculosis. 2013. Available from: https://www.who.int/tb/TBnutrition.pdf?ua=1

[7] Liao D, Fan Q, Bao L. The role of superoxide dismutase in the survival of *Mycobacterium tuberculosis* in macrophages. Japanese Journal of Infectious Diseases. 2013;**66**:480-488

[8] Tian Y, Shioda M, Kasahara S, Okajima T, Mao L, Hisabori T, et al. A facilitated electron transfer of copper–zinc superoxide dismutase (SOD) based on a cysteine-bridged SOD electrode. Biochimica et Biophysica Acta (BBA) - General Subjects. 2002;**1569**(1-3):151-158. DOI: 10.1016/s0304-4165(01)00246-x

[9] Saunders BM, Britton WJ. Life and death in the granuloma: Immunopathology of tuberculosis. Immunology and Cell Biology. 2007;**85**(2):103-111

[10] Rodriguez GM, Neyrolles O. Metallobiology of tuberculosis. Microbiology Spectrum. 2014;**2**(3):MGM2-0012-2013. DOI: 10.1128/microbiolspec.MGM2-0012-2013

[11] Umakanth M. Anemia among tuberculosis patient in teaching hospital Batticaloa, Sri Lanka. International Journal of Medical and Health Research. 2017;**3**(5):95-97

[12] Piddington DL, Fang FC, Andrea M, Cooper AM, Orme IM, Laessig T, et al. Zn superoxide dismutase of *Mycobacterium tuberculosis* contributes to survival in activated macrophages that are generating an oxidative burst. Infection and Immunity. 2001;**69**(8):4980-4987. DOI: 10.1128/Iai.69.8.4980±4987

[13] Sumitro SB, Sukmaningsih AASA. Herbal medicine, radical scavenger and metal detoxification: Bioinorganic, complexity and nano science perspectives. IOP Conference Series: Earth and Environmental Science. 2018;**130**:012003. DOI: 10.1088/1755-1315/130/1/012003

[14] Sukmaningsih AASA, Permana S, Santjojo DJDH, Wardoyo AYP, Sumitro SB. Investigating natural transition metal coordination anthocyanin complex in java plum (*Syzygium cumini*) fruit as free radical scavenging. Rasayan Journal of Chemistry. 2018;**11**(3):1193-1203

[15] Ou-Yang S, Lu J, Kong X, Liang Z, Luo C, Jiang H. Computational drug discovery. Acta Pharmacologica Sinica. 2012;**33**:1131-1140

[16] Leelananda SP, Lindert S. Computational methods in drug discovery. Beilstein Journal of Organic Chemistry. 2016;**12**:2694-2718

[17] Fatimah C, Harahap U. Rosidah, Suryanto D. anti-tuberculosis assay of nanoherbal and Ethanolic extract of *Lantana camara* Linn flos *in vitro* and *in vivo*. IIOABJ. 2017;**8**(Suppl 2):92-97

[18] Fauziyah PN, Sukandar EY, Ayuningtyas DK. Combination effect of Antituberculosis drugs and Ethanolic extract of selected medicinal plants against multi-drug resistant *Mycobacterium tuberculosis* isolates. Scientia Pharmaceutica. 2017;**85**(14):pii:E14. DOI: 10.3390/scipharm85010014

[19] Radji M, Kurniati M, Kiranasari A. Comparative antimycobacterial activity of some Indonesian medicinal plants against multi-drug resistant *Mycobacterium tuberculosis*. Journal of Applied Pharmaceutical Science. 2015;**5**(01):19-022. DOI: 10.7324/JAPS.2015.50104

[20] Putri DE, Rintiswati N, Soesatyo MHNE, Haryana SM. Immune modulation properties of herbal plant leaves: *Phyllanthus niruri* aqueous extract on immune cells of tuberculosis patient–*in vitro* study. Natural Product Research. 2017;**32**(4):463-467. DOI: 10.1080/14786419.2017.1311888

[21] Aristyani S, Widyarti S, Sumitro SB. Network analysis of indigenous Indonesia medical plants for treating tuberculosis. Pharmacognosy Journal. 2018;**10**(6):1159-1164

[22] Vyas SP, Goswami R. Striking the right immunological balance prevents progression of tuberculosis. Inflammation Research. 2017;**66**(12):1031-1056

[23] Van Creve LR, Ottenhoff TH, Van der Meer JW. Innate immunity to *Mycobacterium tuberculosis*. Clinical Microbiology Reviews. 2002;**15**(2):294-309

[24] Singh P, Goyal A. Interleukin-6: A potent biomarker of mycobacterial infection. Springerplus. 2013;**2**(1):686. DOI: 10.1186/2193-1801-2-686

[25] Fernandes DC, Santos NP, Moraes MR, Braga AC, Silva CA, Ribeiro-dos-Santos A, et al. Association of the CYP2B6 gene with anti-tuberculosis drug-induced hepatotoxicity in a Brazilian Amazon population. International Journal of Infectious Diseases. 2015;**33**:e28-e31

[26] Aristyani S, Widyarti S, Sumitro SB. Computational docking study of multitarget bioactive compounds in Indonesia traditional herbal medicines for tuberculosis therapy. International Journal of Green Pharmacy. 2018;**12**(4):258-266

[27] Elkington P, Shiomi T, Breen R, Nuttall RK, Ugarte-Gil CA, NhalliF W, et al. MMP-1 drives immunopathology in human tuberculosis and transgenic mice. The Journal of Clinical Investigation. 2011;**121**:1827-1833

[28] Salgame P. MMPs in tuberculosis: Granuloma creators and tissue destroyers. The Journal of Clinical Investigation. 2011;**121**:1686-1688

[29] Ahmad S, Isab AA, Ali S, Al-Arfaj AR. Perspectives in bioinorganic chemistry of some metal based therapeutic agents. Polyhedron. 2006;**25**:1633-1645. DOI: 10.1016/j.poly. 2005.11.004

[30] Bertini I, Mori M. Inorganic and bioinorganic chemistry. In: Bertini I, editor. Inorganic and Bio-Inorganic Chemistry. Vol. I. Oxford, United Kingdom: EOLSS Publishers Co. Ltd; 2016

[31] Uivarosi V, Munteanu A. Flavonoid Complexes as Promising Anticancer

Metallodrugs. 2017; Available from: http://dx.doi.oskaarrg/10.5772/67879

[32] Symonowicz M, Kolanek M. Flavonoids and their properties to form chelate complexes. Biotechnology and Food Sciences. 2012;**76**(1):35-41

[33] Mahal HS, Kapoor S, Satpati AK, Mukherjee T. Radical scavenging and catalytic activity of metal-phenolic complexes. The Journal of Physical Chemistry. B. 2005;**109**(50):24197-24202

[34] Zondervan NA, van Dam JCJ, Schaap PJ, dos Santos VAPM, Suarez-Diez M. Regulation of three virulence strategies of *Mycobacterium tuberculosis*: A success story. International Journal of Molecular Sciences. 2018;**19**:347. DOI: 10.3390/ijms19020347

[35] Skaar EP, Raffatellu M. Metals in infectious diseases and nutritional immunity. Metallomics. 2015;**7**(6):926-928. DOI: 10.1039/c5mt90021b

[36] McDermid JM, Prentice AM. Iron and infection: Effects of host iron status and the iron-regulatory genes haptoglobin and NRAMP1 (SLC11A1) on host–pathogen interactions in tuberculosis and HIV. Clinical Science. 2006, 2006;**110**:503-524. DOI: 10.1042/CS20050273

[37] Pandey M, Talwar S, Bose S, Pandey AK. Iron homeostasis in *Mycobacterium tuberculosis* is essential for persistence. Scientific Reports. 2018;**8**:17359. DOI: 10.1038/s41598-018-35012-3

[38] Niederweis M, Wolschendorf F, Mitra A, Neyrolles O. Mycobacteria, metals, and the macrophage. Immunological Reviews. 2015;**264**(1):249-263. DOI: 10.1111/imr.12265

[39] Lounis N, Truffot-Pernot C, Grosset J, Gordeuk VR, Boelaert JR. Iron and *Mycobacterium tuberculosis* infection. Journal of Clinical Virology. 2001;**20**:123-126

[40] Sritharan M. Iron homeostasis in *Mycobacterium tuberculosis*: Mechanistic insights into siderophore-mediated iron uptake. Journal of Bacteriology. 2016;**198**(18):2399-2409. DOI: 10.1128/JB.00359-16

[41] Javad Sharifi-Rad J et al. Medicinal plants used in the treatment of tuberculosis Ethnobotanical and ethnopharmacological approaches. Biotechnology Advances. 2017. PII: S0734-9750(17)30077-0. DOI: 10.1016/j.biotechadv.2017.07.001

[42] Moe S, Naing KS, Htay MNH. Herbal medicines used by tuberculosis patients in Myanmar. European Journal of Medicinal Plants. 2018;**2**(1):1-10. DOI: 10.9734/EJMP/2018/ 37341

[43] Nguta JM et al. Medicinal plants used to treat TB in Ghana. International Journal of Mycobacteriology. 2015;**4**(2):116-123. DOI: 10.1016/j.ijmyco.2015.02.003

[44] Semenya S, Maroyi A. Medicinal plants used for the treatment of tuberculosis by Bapedi traditional healers in three districts of the Limpopo province, South Africa. African Journal of Traditional, Complementary, and Alternative Medicines. 2013;**10**(2):316-323. DOI: 10.4314/ajtcam.v10i2.17

[45] Cherrak SA, Mokhtari-Soulimane N, Berroukeche F, Bensenane B, Cherbonnel A, Merzouk H, et al. *In Vitro* antioxidant *versus* metal ion chelating properties of flavonoids: A structure-activity investigation. PLoS One. 2016;**11**(10):e0165575. DOI: 10.1371/journal.pone.0165575

[46] Engwa GA. Free radicals and the role of plant phytochemicals as antioxidants against oxidative

stress-related diseases. In: Phytochemicals - Source of Antioxidants and Role in Disease Prevention. 2018. DOI: 10.5772/intechopen.76719

[47] Zielinska D, Zielinski H. Low molecular weight antioxiants and other biologically active components of buckwheat seeds. The European Journal of Plant Science and Biotechnology. 2009;**3**(1):29-38

[48] Liu Y, Guo M. Studies on transition metal-Quercetin complexes using electrospray ionization tandem mass spectrometry. Molecules. 2015;**20**(5):8583. DOI: 10.3390/molecules 20058583

[49] Jabeen E, Janjua NK, Ahmed S, Murtaza I, Ali T, Hameed S. Radical scavenging propensity of Cu^{2+}, Fe^{3+} complexes of flavonoids and in-vivo radical scavenging by Fe^{3+}-primuletin. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2017;**71**:432-438

[50] Scalbert A, Johnson IT, Saltmarsh M.Polyphenols: Antioxidants and beyond. American Journal of Clinical Nutrition. 2005;**81**:215S-217S

[51] Genaro-Mattos TC, Maurício ÂQ, Rettori D, Alonso A, Hermes-Lima M. Antioxidant activity of Caffeic acid against iron-induced free radical generation—A chemical approach. PLoS One. 2015;**10**(6):e0129963. DOI: 10.1371/journal.pone.0129963

[52] Zhang L, Liu Y, Wang Y. Deprotonation mechanism of methyl Gallate: UV spectroscopic and computational studies. International Journal of Molecular Sciences. 2018;**19**(10):3111. DOI: 10.3390/ijms19103111

[53] Huyut Z, Beydemir Ş, Gülçin İ. Antioxidant and antiradical properties of selected flavonoids and phenolic compounds. Biochemistry Research International. 2017:1-10. DOI: 10.1155/2017/7616791

[54] Chang E, Kim CY. Lipid peroxidation and antioxidant activities of the aqueous rhizome extract of *Rheum officinale* Baillon. Journal of Food Quality. 2018:1-7. DOI: 10.1155/2018/5258276

[55] Kostyuk VA, Potapovich AI, Strigunova EN, Kostyuk TV, Afanas'ev IB. Experimental evidence that flavonoid metal complexes may act as mimics of superoxide dismutase. Archives of Biochemistry and Biophysics. 2004;**428**:204-208. DOI: 10.1016/j.abb.2004.06.008

[56] Mandewale MC, Thorat B, Shelke D, Yamgar R. Synthesis and biological evaluation of new Hydrazone derivatives of Quinoline and their Cu(II) and Zn(II) complexes against *Mycobacterium tuberculosis*. Bioinorganic Chemistry and Applications. 2015;**2015**:153015. DOI: 10.1155/2015/153015

[57] Widyarti S, Sumitro SB, Winarsih S. Bioinorganic chemistry study in utilization Polyherbal to XDR-TB (extensive drug resistant tuberculosis): An approach based-on interaction the energy transfer system. Mid-Year Research Report. Malang, Indonesia: Fakultas MIPA, Universitas Brawijaya; 2019:7-9

[58] Jayanti GE, Balur SS. Improving quality of life. Journal of Tropical Life Science. 2016;**6**(1):35-40

[59] Kavitha V, Srinivas B, Shashikanth P, Muralikrishna B. Assesment of quality of life and oxidative stress In tuberculosis patients visiting directly observed treatment short course Centres of Warangal. Asian Journal of Pharmaceutical and Clinical Research. 2016;**9**(2):200-205

[60] Duan L, Gan H, Golan DE, Remold HG.Critical role of mitochondrial damage in determining outcome of macrophage infection with *Mycobacterium tuberculosis*. The Journal of Immunology. 2002;**169**(9):5181-5518. DOI: 10.4049/jimmunol. 169.9.5181

[61] Sorci L, Kurnasov O, Rodionov DA, Osterman AL. Genomics and Enzymology of NAD Biosynthesis, Burnham Institute for Medical Research, 7th ed. Elsevier Ltd, CA, USA; 2010.(8):213-257

[62] Sauve AA. Perspectives in pharmacology NAD+ and vitamin B3: From metabolism to therapies. The Journal of Pharmacology and Experimental Therapeutics. 2007;**324**(3):11-22

[63] Stein LR, Imai S. The dynamic regulation of NAD metabolism in mitochondria. Trends in Endocrinology and Metabolism. 2012;**23**(9):420-428. DOI: 10.1016/j.tem.2012.06.005

[64] Sun J, Siroy A, Lokareddy RK, Speer A, Niederweis M. The tuberculosis necrotizing toxin kills macrophages by hydrolyzing NAD+. Nature Structural & Molecular Biology. 2015;**22**(9):672-678

[65] De Araujo RF, Martins DB, Borba MA. Book of a Master Regulator of Oxidative Stress, Chapter Oxidative Stress and Disease. Vol. 7. London: Intechopen; 2016. pp. 556-655

[66] Birben E, Sahiner UM, Sackesen C, Erzurum S, Review Article KO. Oxidative stress and antioxidant defense. World Allergy Organization Journal. 2012;**5**:9-19

[67] Fukuwatari T, Shibata K. Nutritional aspect of tryptophan metabolism. International Journal of Tryptophan Research. 2013;**6**(Suppl 1):3-8

[68] World Health Organization. Protein and amino acid requirements in human nutrition. Report of a joint WHO/FAO/ expert consultation. In: WHO Technical Report Series 935. Geneva, Switzerland: World Health Organization (WHO); 2007

[69] Lynch H, Johnston C, Wharton C. Plant-based diets: Considerations for environmental impact, protein quality, and exercise performance. Nutrients. 2018;**10**(12):1841. DOI: 10.3390/ nu10121841

[70] Biefer HRC, Vasudevan A, Elkhal A. Aspects of tryptophan and Nicotinamide adenine dinucleotide in immunity: A new twist in an old tale. International Journal of Tryptophan Research. 2017;**10**:1-8. DOI: 10.1177/1178646917713491

[71] Roager HM, Licht TR. Microbial tryptophan catabolites in health and disease. Nature Communications. 2018;**9**:3294. DOI: 10.1038/ s41467-018-05470-4

[72] Reddy YN, Murthy SV, Krishna DR, Prabhakar MC. Role of free radicals and antioxidants in tuberculosis patients. The Indian Journal of Tuberculosis. 2004;**51**:213-218

[73] Bhattacharyya A, Chattopadhyay R, Mitra S, Crowe SE. Oxidative stress: An essential factor in the pathogenesis of gastrointestinal mucosal diseases. Physiological Reviews. 2014;**94**(2):329-354

[74] Minhas PS et al. Macrophage de novo NAD synthesis specifies immune function in aging and inflammation. Nature Immunology. 2019;**20**:50-63. DOI: 10.1038/s41590-018-0255-3

[75] Backhed F, Fraser CM, Ringel Y, Sanders ME, Sartor RB, Sherman PM, et al. Defining a healthy human gut microbiome: Current concepts, future directions, and clinical applications. Cell Host & Microbe. 2012;**12**:611-622. DOI: 10.1016/j. chom.2012.10.012

[76] Tsay TB, Yang MC, Chen PH, Hsu CM, Chen LW. Gut flora enhance bacterial clearance in lung through toll-like receptors 4. Journal of Biomedical Science. 2011;**18**(1):68. Available from: http://www.jbiomedsci.com/content/18/1/68

[77] Kamaruddin M, Sargowo D, Muliartha IK, Sumarno. *Helicobacter Pylori* lipopolysaccharide increase degradation of type IV collagen in acute myocardial infarction. ASEAN Heart Journal. 2011;**19**:24-32

[78] Takeda K, Kaisho T, Akira S. Toll-like receptors. Annual Review of Immunology. 2003;**21**:335-376

[79] Samuelson DR, Welsh DA, Shellito JE. Regulation of lung immunity and host defense by the intestinal microbiota. Frontiers in Microbiology. 2015;**6**:1085. DOI: 10.3389/fmicb.2015.01085

[80] Jia W, Li HK, Zhao LP, Nicholson JK. Gut microbiota: A potential new territory for drug targeting. Nature Reviews. Drug Discovery. 2008;**7**(2):123-129

[81] Liu JY, Lee KF, Sze CW, Tong Y, Tang SCW, Ng TB, et al. Intestinal absorption and bioavailability of traditional Chinese medicines: A review of recent experimental progress and implication for quality control. The Journal of Pharmacy and Pharmacology. 2013;**65**(5):621-633

[82] El Kaoutari A, Armougom F, Gordon JI, Raoult D, Henrissat B. The abundance and variety of carbohydrate-active enzymes in the human gut microbiota. Nature Reviews. Microbiology. 2013;**11**(7):497-504

[83] Xu J, Chen HB, Li SL. Understanding the molecular mechanisms of the interplay between herbal medicines and gut microbiota. Medicinal Research Reviews. 2017;**37**(5):1140-1185

[84] Kim DH, Kim SY, Park SY, Han MJ. Metabolism of quercitrin by human intestinal bacteria and its relation to some biological activities. Biological & Pharmaceutical Bulletin. 1999;**22**(7):749-751

[85] Kato M, Ishige A, Anjiki N, Yamamoto M, Irie Y, Taniyama M, et al. Effect of herbal medicine Juzentaihoto on hepatic and intestinal heat shock gene expression requires intestinal microflora in mouse. World Journal of Gastroenterology. 2007;**13**(16):2289-2297

Chapter 5

Black Garlic and Its Therapeutic Benefits

Gia-Buu Tran, Tan-Viet Pham and Ngoc-Nam Trinh

Abstract

Black garlic is a functional food produced from fresh garlic (*Allium sativum* L.) via fermentation with the whole bulbs or peeled cloves in a chamber in which temperature (60–90°C) and humidity (70–90%) are regulated for a period of time. Black garlic possesses an abundant amount of antioxidant compounds such as polyphenols, flavonoids, tetrahydro-β-carboline derivatives, and organosulfur compounds, including S-allyl-cysteine and S-allyl-mercaptocysteine, as compared with fresh garlic. Note that fermentation not only alters the nutrition components and sensory attributes but also enhances the bioactivity of black garlic. A growing body of evidences demonstrating therapeutic effects of black garlic, including anticancer, anti-obesity, immunomodulatory, hypolipidemic, antioxidant, hepatoprotective, and neuroprotective effects, have been reported in the last few decades. This book chapter provides a literature review of therapeutic effects of black garlic from fundamental to clinical studies that can be used by food and nutrition experts, researchers, and scientists to improve people's health and wellness.

Keywords: functional food, therapeutic benefits, black garlic, nutrition, bioactivity

1. Introduction

Garlic (*Allium sativum* L.), a member of Alliaceae family, is a popular ingredient used for culinary art and traditional and modern medicine. It possesses not only a strong flavor and distinctive taste but also a variety of bioactive organosulfur compounds, comprising of allicin, allixin, diallyl disulfide, diallyl trisulfide, S-allyl-cysteine, S-allyl-mercaptocysteine, allixin, 1-propenyl allyl thiosulfonate, (E,Z)-4,5,9-trithiadodeca-1,6,11-triene 9-oxide, allyl methyl thiosulfonate, etc. Therefore, garlic has been documented in several literatures as a prominent remedy by reason of its several health benefits such as anticancer, antioxidant, antibiotic, antihyperlipidemic, antidiabetic, anticoagulant, antimicrobial, anti-constipation, antiparasitic, diuretic, and hepatoprotective effects [1–3]. In Vietnamese traditional medicinal system, garlic is prescribed to treat amebic and bacillary dysenteries, wound infection, chronic bronchitis, hypertension, whooping cough, and threadworm infection [4]. Furthermore, bioactivity and garlic components are also quite disparate among different cultivars [5, 6]. Garlic could be used directly or as food processing or brewery products including black garlic, smoked garlic, garlic oil, garlic oil macerate, extract, powder, supplement pill, garlic juice, alcoholic tincture, etc. [7].

Among the garlic processing products, black garlic is emerging as one of the most well-known functional foods in the market. As compared with the raw garlic, black garlic has a typical black color, sweet taste, and chewy texture without the

offensive odor. Moreover, several bioactivities of black garlic including anticancer, anti-obesity, immunomodulatory, hypolipidemic, antioxidant, hepatoprotective, and neuroprotective effects have been documented in literature [8]. However, a systematic review of black garlic and its therapeutic effects from fundamental to clinical studies is still lacking. This chapter of the book provides food and nutrition experts, researchers, and scientists an overview of application of black garlic in functional food for a variety of specific diseases with clinical evidences to improve people's health and wellness.

2. Black garlic and its production

The people from Asian countries such as Thailand, South Korea, and Japan have produced and used black garlic as a traditional food for centuries, but it has been introduced into global market in recent decades. In brief, black garlic is produced by fermentation of whole bulb of fresh garlic at high humidity and temperature which in turn results in garlic to turn black via a set of nonenzymatic browning reactions, including Maillard reaction, oxidation of phenols, and caramelizing. When garlic undergoes fermentation, not only physiochemical characteristics of garlic are altered, but also the concentration of bioactive compounds is also improved [8]. Choi and collaborators [9] showed that the moisture of garlic and pH decreased along with the fermentation process, whereas the reducing sugar and total acidity were accumulated. On the other hand, color spectra and composition of amino acids of black garlic also were altered as compared with fresh garlic [9]. As the consequence, black garlic has elastic and chewy texture, as well as sweet taste without offensive flavor of garlic (**Figure 1**). Furthermore, black garlic possesses an abundant amount of antioxidant compounds such as polyphenols, flavonoids, tetrahydro-β-carboline derivatives, and organosulfur compounds, including S-allyl-cysteine and S-allyl-mercaptocysteine, as compared with fresh garlic. Kim and collaborators suggested that the total polyphenol and flavonoid of black garlic increase 9.3- and 1.5-folds, respectively, after a program heat schedule as compared with fresh garlic [10]. The concentration of S-allyl-cysteine, one of the most important organosulfur bioactive compounds of garlic, also increases in black garlic from 4.3- to 6.3-folds depending of heating treatment [11].

Due to its palatability and abundant amount of bioactive compounds, black garlic has become one of the most well-known and prominent products in nutraceuticals and functional food market with a remarkable growth of consumption demand and profitability during recent years. Furthermore, black garlic has attracted not only

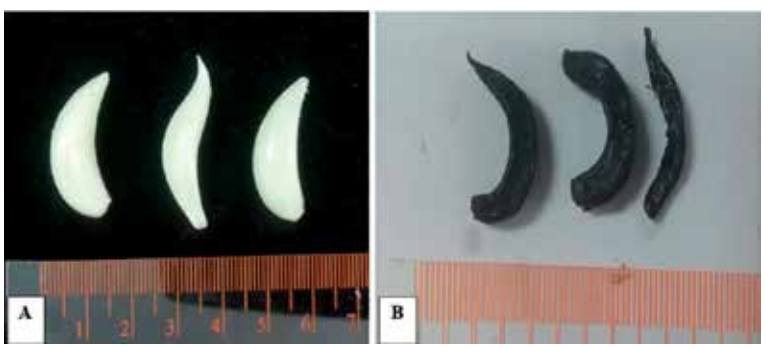

Figure 1.
Fresh peeled garlic (A) and black garlic produced from peeled multi-clove garlic by fermentation in high humidity (90%) and temperature (75°C) after 15 days (B).

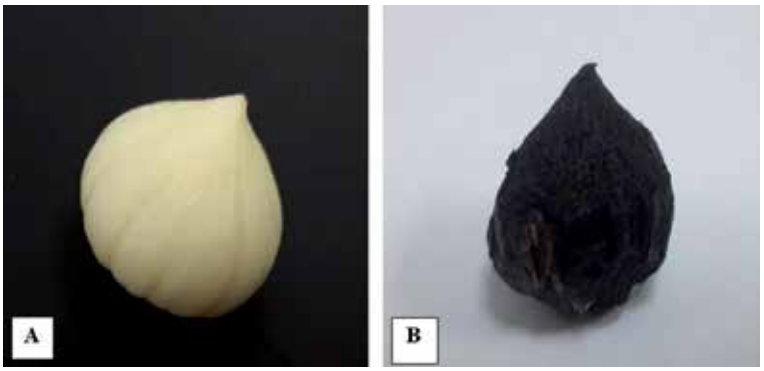

Figure 2.
Single-clove garlic, atypical product of garlic bulb-forming process (A) and black garlic produced from single-clove garlic (B).

consumer attention but also the researcher and manufacturers in the improvement of its production procedure as well as the innovation of new processing products of black garlic. Manufacturing processes of black garlic are diversely programmed depending on temperature, relative humidity, time, and materials. In previous study, Zhang and collaborators produced black garlic from a variety of thermal treatment from 60 to 90°C, and they concluded that higher temperature could shorten the maturity time and the sensory score of black garlic fermented at 70°C was the highest score as compared to other temperatures [12]. Moreover, Kang also investigated the production and physiochemical characteristics of black garlic fermented in programmed stepwise heating process at 60–90°C with 50–100% relative humidity [13]. The effect of time period on the production and antioxidant capacity of black garlic also has been proved in Choi's report [8]. On the other hand, the researchers have investigated the production of black garlic from a variety of materials from multi-clove garlic to single clove of garlic as well as from the unpeeled cloves of garlic to peeled cloves of garlic (**Figures 1** and **2**) [14, 15]. Recently, some black garlic processing products such as black garlic molasses, purée, paste, extract, and supplement pills have been introduced in Vietnamese and global market.

3. Therapeutic effects of black garlic

3.1 Anticancer effect of black garlic

Cancer, which has been regarded as one of leading cause of death in worldwide, is a type of disease related with uncontrolled or abnormal growth of cells or tissues in our bodies. With the growing number of the evidences reported for anticancer effect of black garlic in recent decades, some researchers suggested that black garlic could be used as a dietary product for preventing and treating cancers from gastric cancer to leukemia. The mechanism of anticancer effects of black garlic in different types of cancer diseases is various comprising of the induction of apoptosis, stopping the cell cycle, and inhibition of tumor growth and invasion. In 2011, Wang and collaborators suggested that aqueous extract of black garlic could inhibit proliferation and triggered the apoptosis of SGC-7901 cells, a human gastric cancer cell line, with dose-dependent manner. The authors also observed the inhibitory effect of black garlic on the growth of tumor in tumor-bearing mice [16]. Moreover, black garlic has the anti-invasive effect and prevents tumor metastasis in human gastric carcinoma AGS cells through the increase of tightness of tight junction as well as the

downregulation of matrix metalloproteinases (MMP)-2 and metalloproteinases-9, which play a role as mediators of metastasis and invasiveness [17]. The anticancer effect of black garlic on colon cancer cell line also has been demonstrated. Moreover, treatment with alcoholic extract of black garlic could upregulate PTEN and downregulate Akt/pAkt expression, the members of phosphatidylinositol 3-kinase protein kinase B (PI3K/Akt) signal transduction pathway, leading to the modulation of p70S6K1 protein, induction of apoptosis, and arresting of the cell cycle of HT29 human colon cancer cell line [18]. Park and collaborators have reported that hexane extract of black garlic could reduce the cell viability of U937 cells, human histiocytic lymphoma. It has been found that hexane extract of black garlic exhibited induction of both intrinsic and extrinsic pathways through the alteration of the expression of apoptosis-relating proteins. They found that black garlic upregulates death receptor (DR)-4 and Fas ligand, increases Bax/Bcl-2 ratio, as well as induces the truncation of Bid protein, which involves not only endogenous mitochondrial pathway but also death receptor-mediated apoptotic pathway [19]. Some authors also proved that black garlic could inhibit cell growth and enhance sensitization of Lewis cells, lung cancer cell line, against ionizing radiation [20].

3.2 Benefit effect on dyslipidemia and anti-obesity

Obesity is a threatening problem to public health in Western and developed countries which causes several metabolic syndromes and chronic diseases. Obesity can be prevented by a combination of physical activity and healthy diet balance between energy intake and expenditure. Note that several functional foods such as γ-oryzanol, butyric acid, legumes, bromelain, peas, lentil, fabas beans, conjugated linoleic acid, diacylglycerols, purified black raspberries, apples, bilberries, sea buckthorn, etc. have been indicated as preventing and/or treating obesity agents via several mechanisms including the induction of satiation, decreasing of appetite, regulation of lipid metabolism, and thermogenesis [21]. Among them, black garlic is known as a prominent lipid and weight-lowering ingredient. In 2015, Ha and collaborators showed that the diet supplemented with 1.5% black garlic extract could only reduce weight but also decrease kidney and epididymal fat in high-fat model [22]. Moreover, black garlic extract attenuates dyslipidemia induced by high-fat diet. In Ha's work, the authors observed the lowering effect of black garlic on the plasma level of total lipid, total cholesterol, and triglyceride. On the contrast, high-density lipoprotein cholesterol (HDL) of black garlic group was higher than high-fat diet group. Of note, treatment with black garlic reduces both glucose and insulin in plasma levels in high-fat diet. One of the explanations for the anti-obesity effect of black garlic is that black garlic could ameliorate diet-induced obesity through downregulation of transcription factors and enzymes related with fat and cholesterol syntheses such as sterol regulatory element-binding protein-1c (SREBP-1c), acetyl-coA carboxylase (ACC), fatty acid synthase (FAS), glucose-6-phosphate dehydrogenase (G6PDH), hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, and acyl-CoA cholesterol acyltransferase (ACAT) or fatty acid oxidation rate via increase of the expression of carnitine palmitoyltransferase-1 (CPT-1), a key enzyme related with lipolysis and fatty acid breakdown [22]. Furthermore, Chen and collaborators also recommended that the anti-obesity effect of methanol extract of black garlic could be related with regulation of lipogenesis, adipokine biosynthesis, fatty acid oxidation, fatty acid and glucose transport, and lipolysis in both the adipose tissue and liver [23]. Furthermore, Seo and collaborators found that black garlic extract could augment the anti-obesity and cholesterol-lowering effect of exercise in animal models [24]. Combination of black garlic and exercise showed a greater effect on decreasing visceral fat, epididymal fat, and liver weight

as compared to exercise-alone group and exhibited lowering triglyceride effects in high-fat diet-induced rats. On the other hand, consumption of black garlic (6 g/day) for a long term (12 weeks) also has the cardioprotective effect in patients from a double-blind, randomized placebo-controlled trial by diminishment of atherosclerosis markers and improvement of dyslipidemia. In Jung study, black garlic supplement group exhibited a significant increase of high-density lipoprotein cholesterol levels and low-density lipoprotein cholesterol/apolipoprotein B along with a decline of apolipoprotein B as compared to placebo group [25]. Recently, some researchers have developed fermented products from garlic extract that also manifested an anti-obesity effect similar with black garlic extract. Jung and collaborators demonstrated that fermented black garlic extract, a product created by fermented *Saccharomyces cerevisiae* (KTCT7910) in medium supplemented with 5%, also exhibited the hypolipidemic and anti-obesity effects but not hypoglycemic effect [26]. In 2016, Lee and collaborators fermented black garlic extract with *Lactobacillus plantarum* BL2 to produce functional food that could reduce body weight and epididymal, retroperitoneal, and mesenteric adipose tissue mass as well as plasma level of triglyceride and total cholesterol in diet-induced obese mice. The fermented product inhibited adipose tissue hypertrophy via the downregulation of a set of proteins related with adipogenesis and lipogenesis including peroxisome proliferator-activated receptor γ (PPARγ), CCAAT-enhancer-binding protein α (C/EBPα), stearoyl-CoA desaturase (SCD-1), SREBP-1c, and FAS [27].

3.3 Black garlic and its antioxidant properties

Black garlic contains abundant antioxidant compounds including polyphenols, alkaloids, flavonoids, S-allyl-cysteine, and antioxidant intermediate products derived from Maillard reaction [8, 9]. Several studies suggest that black garlic not only scavenges the free radicals in vitro but also activates the antioxidant enzymes in vivo. Wang and Sun reported that black garlic ethanol extract has an identical DPPH radical inhibitory effect with vitamin C in concentrations 200 and 250 μg/ml and comparable OH radical scavenging effect with vitamin C in concentrations 400 and 500 μg/ml. Black garlic extract could reduce malondialdehyde (MDA) concentration in serum, an end product of lipid peroxidation, and enhance serum superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities in oxidative damage murine model induced by benzene bromide [28]. These results were similar with Lee and collaborators work. A diet supplemented with 5% black garlic aqueous extract could decrease oxidative stress and diabetes complications. Black garlic exerts a strong antioxidant capacity through the strong scavenging 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) or ABTS radical activity, which is four times higher than those of raw garlic, and suppression of oxidative stress marker, such as thiobarbituric acid reactive substances (TBARS) content in the liver, and activation of antioxidant defense system, including SOD, GSH-Px, and catalase (CAT) in db/db mice, a genetically modified model for diabetes mellitus [29]. Additionally, Ha and collaborators suggested that black garlic could specifically upregulate the mRNA expression of nuclear factor erythroid 2-like factor (Nrf2)-related antioxidant proteins, such as heme oxygenase-1 (HO-1), glutathione S-transferase alpha 2 (GSTA2), and NAD(P)H quinone oxidoreductase-1 (NQO1) to prevent accumulation of reactive oxidative species in the liver [30]. According to a Hungarian research, black garlic also improves superior postischemic cardiac function, infarct size reduction, and HO1 and inducible NOS (iNOS) levels after ischemia/reperfusion, which in turn exerts a cardioprotective effect [31]. Moreover, antioxidant effect of black garlic has also been proved in clinical study. In previous study, Wang and collaborators performed a double-blind,

parallel design study with a population of untrained males with similar age and body mass index during 14 days to compare the effect of black garlic and placebo on exercise-induced oxidative stress and recovery of muscle function [32]. They found that black garlic improved the recovery rate of circumference of biceps brachii after eccentric exercise and diminished reactive oxygen metabolites (dROMs), lipid peroxide, and 8-iso prostaglandin F2α concentrations, a new indicator of oxidative stress and related with atherosclerosis [32, 33]. Recently, Liu and collaborators have demonstrated the beneficial effect of black garlic on coronary heart disease patient. Black garlic exhibits an augmentation on chronic heart failure by increasing the left ventricular ejection fraction value and the scores of the quality of life and circulating antioxidant levels along with a decline of brain natriuretic peptide (BNP) precursor N-terminal, a biomarker for severity of heart failure [34]. Note that some research implies that the processing method has a remarkable effect on antioxidant of black garlic [8, 15, 35]. The temperature and moisture are the most important parameters that determine the quality and antioxidant capacity of black garlic. In Sun and Wang's work, scavenging DPPH free radical activity of black garlic produced at 85°C in 85% humidity was higher than ones generated at 75 and 60°C in the same humidity after fermentation. The authors observed that at the same temperature, the indicated humidity (75%) would produce black garlic with highest Trolox equivalent antioxidant capacity followed by 85, 80, and 70% humidity [35]. The separation of garlic cloves also has positive correlation with antioxidant capacity. Angeles and collaborators proposed that peeled black garlic cloves exhibited a higher antioxidant capacity than whole black garlic bulbs fermented at same condition in the end of production [15].

3.4 Black garlic and its anti-inflammatory effect

Inflammation is a process by which our immune system responds to injury, infection, and toxin. Inflammation plays a vital role not only in wound healing and repairing process but also in protecting our body from foreign invaders, including viruses and bacteria. However, chronic inflammation may have a negative impact on our health which has been manifested in a variety of chronic diseases from heart disease to rheumatoid arthritis and lupus. Consumption of anti-inflammatory ingredients or foods, such as ώ-3 polyunsaturated fatty acid, monounsaturated fatty acids, β-cryptoxanthin, quercetin, kaempferol, malvidin, peonidin, daidzein, genistein, extra virgin olive oil, tomato juice, walnut, red wine, flaxseed flour, and cherry, may help combat certain diseases related to chronic inflammation [36]. Recently, some reports have suggested black garlic as a prominent agent for treatment of inflammation and septicemia-related diseases. Aqueous extract of black garlic hinders the production of nitric oxide (NO) and proinflammatory cytokines, including tumor necrosis factor α (TNFα) and prostaglandin (PG)-E2, and suppresses NO synthase and TNFα and cyclooxygenase-2 expression through a mechanism-related mitogen-activated protein kinase and nuclear factor-KB in liposaccharide (LPS)-stimulated murine macrophages. Furthermore, black garlic extract supplement impedes serum TNFα, interleukin-6 (IL6), and interleukin-1 β (IL1β) production and prevents mice from LPS-induced death [37, 38]. These findings are identical with the results from Zhang and Jilg experiments, in which six different black garlic extracts, including hot aqueous extract, ethanol supernatant extract, ethanol precipitate extract, deproteinized ethanol supernatant extract, and deproteinized ethanol precipitate extract, not only ameliorate regulatory effect of LPS on macrophages growth inhibition but also abate TNF α, IL-6, and IL1β generation in LPS-treated macrophages [39]. Moreover, the chloroform extract of black

garlic inhibits TNFα-induced reactive oxygen species (ROS) formation, mRNA and protein expression of vascular cell adhesion molecule-1 (VCAM1), and activation of NFKB pathway and reduces adhesiveness of THP-1 monocytes to human umbilical vein endothelial cells (HUVECs) [40]. In previous research, hexane extract of black garlic also regulates human endometrial stromal cell proliferation and cell progression via suppression of c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK). Moreover, Kim and collaborators demonstrate that hexane extract of black garlic has potential to render the NFKB and activator protein 1 (AP1) activation, which in turn decreases VCAM1 and ICAM1 expression [41]. Fermented black garlic, a product of fermentation of *Lactobacillus rhamnosus,* also hinders production of inflammatory mediators, such as TNF α, IL-6, IL1β, iNOS, and COX2, and retards an inflammatory signal transduction pathway, the NFKB pathway [42].

3.5 Neuroprotective effect of black garlic

A growing body of literature indicates that black garlic has beneficial effects to memory and nerve system through antiamnesic effect, improvement of cognitive impairment, and prevention from neuroinflammation and neurotoxicity. In previous study, Nurmasitoh and collaborators demonstrated that ethanol extract of black garlic has manifested a strong protection of murine medial prefrontal cortex from monosodium glutamate-induced oxidative stress via improvement of the working memory performance and prevention of the pyramidal neurons from modification of neuronal architecture [43]. Furthermore, a variety of doses of black garlic extracts (from 2.5 to 10 mg/200 g body weight) also inhibit deleterious effect of monosodium glutamate on spatial memory and total number of pyramidal neurons in CA1 region of the hippocampus [44]. These findings are identical with the results from Indonesian report, in which black garlic improves motor coordination function and the number of Purkinje cells in the cerebellar cortex of the rat brain [45]. The protection effect of black garlic on nerve system from neuroinflammation, a pathological evidence of Alzheimer's disease, and cognitive impairment has been well documented [46]. B amyloid (Aβ) deposition leads to inflammation of the neuron, which triggers a host defense response to neuronal damage and eventually neuronal degeneration. Nillert and collaborators had established a neuronal degenerative model by treatment with 1 μl of aggregated Aβ(1–42) in the lateral ventricles; eventually they observed that ethanol extract-aged black garlic, a variant form of black garlic produced by aging the fresh garlic in room temperature, could ameliorate short-term recognition memory and inhibit activation of microglia as well as production of IL1β, a proinflammatory cytokine [46]. Additionally, ethyl acetate fraction of aged garlic extract protects PC12 neuron-like cells and ICR mice from neurotoxicity and amnesia induced by Aβ(25–35) [47]. Note that both aged and black garlics are rich of S-allyl-cysteine content, a stable bioactive organosulfur compound which also exerts anti-inflammatory, neuroprotective, and antioxidant effects [48].

3.6 Hepatoprotective effect of black garlic

The liver is a vital organ which exerts detoxicity, protein biosynthesis, and digestive biochemical production. However, the liver is vulnerable with medications, chemicals, alcohol, solvents, infection, and nutritional supplement. Black garlic has proved to protect the liver from side effects including hepatoxicity and apoptosis of cyclophosphamide, an anticancer medicine [49]. Ahmed indicated that black garlic supplement at the dose 200 mg/kg body weight recovers the histological

change, DNA damage, and blood biochemical parameter alteration (bilirubin, alanine transaminase (ALT), aspartate transaminase (AST)) as well as increases the hepatic antioxidant enzyme levels (CAD, SOD, GSH-Px) as compared with cyclophosphamide-treated group. According to Lee's research, black garlic could prevent rat clone-9 hepatocytes from hepatic damage induced by tert-butyl hydroperoxide in vitro [50]. One of explanations for hepatoprotective effect of black garlic is that black garlic could improve cell death and reduce lipid peroxidation, oxidative stress, and inflammation through regulation of JNK signaling cascade. Note that black garlic exerts the hepatoprotective effect not only in acute toxicity but also in chronic conditions. One study from Korea reports that black garlic decreases the fat accumulation, epididymal, total fat pad, and liver weight alteration and reduces elevation of hepatic enzymes (ALT, AST, alkaline phosphatase (ALP), and lactate dehydrogenase (LDH)) and blood lymphocyte DNA damage in chronic alcohol-induced hepatic damage model [51]. They also observed a decrease of TBARS content in the liver, heart, and plasma and reduction of cytochrome P450 2E1 activity companion with a rise of hepatic GSH level and antioxidant enzyme activities such as GSH-Px, CAT, and glutathione reductase in black garlic-treated group. From these evidences, the authors imply that the strong antioxidant effect of black garlic is related to the mechanism of protection against liver damage induced by chronic alcohol exposure. Moreover, Shin and collaborators suggested that black garlic supplement not only protects the liver from acute toxicity induced by carbon tetrachloride or D-galactosamine but also improves lipid profile and liver injury in hepatic steatosis model [52]. A Vietnamese report also proved the hepatoprotective effect of single-clove black garlic on liver injury in sub-chronic toxicity model (**Figure 3**) [14].

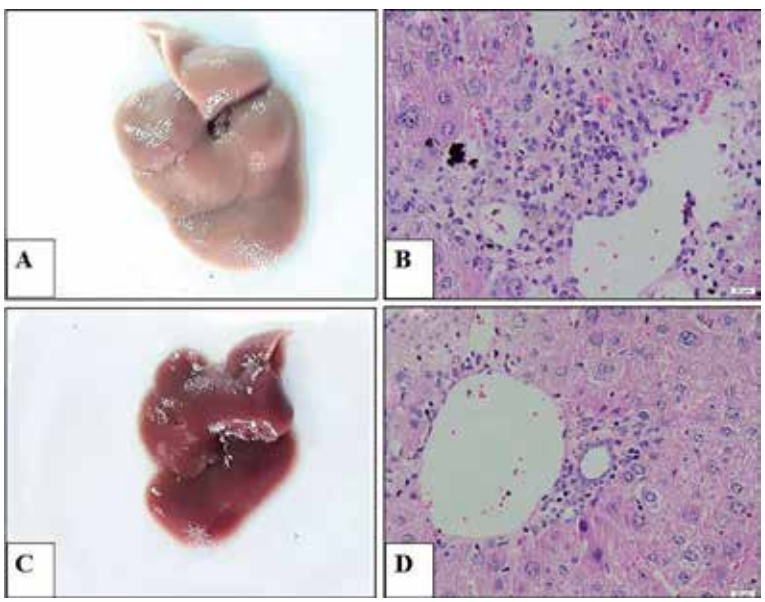

Figure 3.
Liver sections from carbon tetrachloride (CCl_4)-intoxicated mice and CCl_4 treated with single-clove black garlic extract mice. Carbon tetrachloride treatment (1 ml of mixture of CCl4 in 50% olive oil/kg body weight, twice per week, for 28 days) not only alters the gross appearance of the liver (swelling liver, hard texture, pale brown with coarse surface) but also results in a severe hepatic inflammation and necrosis in microscopic level (panels A and B, respectively). Supplement with single-clove black garlic extract (200 mg/kg body weight) could improve liver morphology (semihard texture, redness, slight coarse surface) and histological structure of the liver along with reduction of inflammation (panels C and D, respectively).

4. Conclusions

Black garlic is a well-known garlic preparation which is fermented in regulated high humidity and temperature not only to remove strong unpleasant flavor of fresh garlic but also to improve its nutrient composition, bioactivites, and taste values. After being introduced in the market in last few decades, black garlic has become an emerging functional food on account of its wide-range biological functions, including antioxidant, anti-inflammatory, anticancer, lowering hyperlipidemia, anti-obesity, hepatoprotective, and neuroprotective effects. Its bioactivities and therapeutic benefits have been the subjects to a numerous extensive researches in both in vitro and in vivo levels. In recently, there are only a few clinical studies which prove the health benefits of black garlic on cardiovascular diseases. Therefore, further researches focused on other medical application and safety aspect of black garlic are required to provide a comprehensive overview about therapeutic effects of black garlic.

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Conflict of interest

The authors declare there are no conflicts of interest regarding the publication of this work.

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References

[1] Bayan L, Koulivand PH, Gorji A. Garlic: A review of potential therapeutic effects. Avicenna Journal of Phytomedicine. 2014;**4**(1):1-14. DOI: 10.22038/ajp.2014.1741

[2] Ried K, Toben C, Fakler P. Effect of garlic on serum lipids: An updated meta-analysis. Nutrition Reviews. 2013;**71**(5):282-299. DOI: 10.1111/nure.12012

[3] Adaki S, Adaki R, Shah K, Karagir A. Garlic: Review of literature. Indian Journal of Cancer. 2014;**51**(4):577-581. DOI: 10.4103/0019-509X.175383

[4] Do TL, editor. Vietnamese Medicinal Plants and Remedies. 12th ed. Hanoi: Hanoi Medical Publishing House; 2004. 1274 p. (In Vietnamese)

[5] Chen S, Shen X, Cheng S, Li P, Du J, Chang Y, et al. Evaluation of garlic cultivars for polyphenolic content and antioxidant properties. PLoS One. 2013;**8**(11):e79730. DOI: 10.1371/journal.pone.0079730

[6] Naji KM, Al-Shaibani ES, Alhadi FA, Al-Soudi SA, D'souza MR. Hepatoprotective and antioxidant effects of single clove garlic against CCl4-induced hepatic damage in rabbits. BMC Complementary and Alternative Medicine. 2017;**17**(1):411. DOI: 10.1186/s12906-017-1916-8

[7] Santos FCC, Carvalho NUM. Alcoholic tincture of garlic (*Allium sativum*) on gastrointestinal endoparasites of sheep-short communication. Ciência Animal Brasileira. 2014;**15**(1):115-118. DOI: 10.5216/cab.v15i1.23284

[8] Kimura S, Tung YC, Pan MH, Su NW, Lai YJ, Cheng KC. Black garlic: A critical review of its production, bioactivity, and application. Journal of Food and Drug Analysis. 2017;**25**(1): 62-70. DOI: 10.1016/j.jfda.2016.11.003

[9] Choi IS, Cha HS, Lee YS. Physicochemical and antioxidant properties of black garlic. Molecules. 2014;**19**:16811-16823. DOI: 10.3390/molecules191016811

[10] Kim JS, Kang OJ, Gweon OC. Comparison of phenolic acids and flavonoids in black garlic at different thermal processing steps. Journal of Functional Foods. 2013;**5**(1):80-86. DOI: 10.1016/j.jff.2012.08.006

[11] Bae SE, Cho SY, Won YD, Lee SH, Park HJ. Changes in S-allyl cysteine contents and physicochemical properties of black garlic during heat treatment. LWT-Food Science and Technology. 2014;**55**(1):397-402. DOI: 10.1016/j.lwt.2013.05.006

[12] Zhang X, Li N, Lu X, Liu P, Qiao X. Effects of temperature on the quality of black garlic. Journal of the Science of Food and Agriculture. 2016;**96**(7): 2366-2372. DOI: 10.1002/jsfa.7351

[13] Kang OJ. Physicochemical characteristics of black garlic after different thermal processing steps. Preventive Nutrition and Food Science. 2016;**21**(4):348-354. DOI: 10.3746/pnf.2016.21.4.348

[14] Tran GB, Dam SM, Le TNT. Amelioration of single clove black garlic aqueous extract on dyslipidemia and hepatitis in chronic carbon tetrachloride intoxicated Swiss albino mice. International Journal of Hepatology. 2018;**2018**:9383950. DOI: 10.1155/2018/9383950

[15] Toledano-Medina MA, Pérez-Aparicio J, Moreno-Rojas R, Merinas-Amo T. Evolution of some physicochemical and antioxidant properties of black garlic whole bulbs

and peeled cloves. Food Chemistry. 2016;**199**:135-139. DOI: 10.1016/j.foodchem.2015.11.128

[16] Wang X, Jiao F, Wang QW, Wang J, Yang K, Hu RR, et al. Aged black garlic extract induces inhibition of gastric cancer cell growth in vitro and in vivo. Molecular Medicine Reports. 2012;**5**(1):66-72. DOI: 10.3892/mmr.2011.588

[17] Shin DY, Yoon MK, Choi YW, Gweon OC, Kim JI, Choi TH, et al. Effects of aged black garlic extracts on the tight junction permeability and cell invasion in human gastric cancer cells. Journal of Life Sciences. 2010;**20**(4):528-534. DOI: 10.3892/mmr.2011.588

[18] Dong M, Yang G, Liu H, Liu X, Lin S, Sun D, et al. Aged black garlic extract inhibits Ht29 colon cancer cell growth via the PI3K/Akt signaling pathway. Biomedical Reports. 2014;**2**:250-254. DOI: 10.3892/br.2014.226

[19] Park C, Park S, Chung YH, Kim GY, Choi YW, Kim BW, et al. Induction of apoptosis by a hexane extract of aged black garlic in the human leukemic U937 cells. Nutrition Research and Practice. 2014;**8**(2):132-137. DOI: 10.4162/nrp.2014.8.2.132

[20] Yang GQ, Wang D, Wang YS, Wang YY, Yang K. Radiosensitization effect of black garlic extract on lung cancer cell line Lewis cells. Zhongguo Zhong Xi Yi Jie He Za Zhi. 2013;**33**(8):1093-1097

[21] Sunkara R, Verghese M. Functional foods for obesity management. Food and Nutrition Sciences. 2014;**5**:1359-1369. DOI: 10.4236/fns.2014.514148

[22] Ha AW, Ying T, Kim WK. The effects of black garlic (*Allium sativum*) extracts on lipid metabolism in rats fed a high fat diet. Nutrition Research and Practice. 2015;**9**(1):30-36. DOI: 10.4162/nrp.2015.9.1.30

[23] Chen YC, Kao TH, Tseng CY, Chang WT, Hsu CL. Methanolic extract of black garlic ameliorates diet-induced obesity via regulating adipogenesis, adipokine biosynthesis, and lipolysis. Journal of Functional Foods. 2014;**9**:98-108. DOI: 10.1016/j.jff.2014.02.019

[24] Seo DY, Lee S, Figueroa A, Kwak YS, Kim N, Rhee BD, et al. Aged garlic extract enhances exercise-mediated improvement of metabolic parameters in high fat diet-induced obese rats. Nutrition Research and Practice. 2012;**6**(6):513-519. DOI: 10.4162/nrp.2012.6.6.513

[25] Jung ES, Park SH, Choi EK, Ryu BH, Park BH, Kim DS, et al. Reduction of blood lipid parameters by a 12-wk supplementation of aged black garlic: A randomized controlled trial. Nutrition. 2014;**30**(9):1034-1039. DOI: 10.1016/j.nut.2014.02.014

[26] Jung YM, Lee SH, Lee DS, You MJ, Chung IK, Cheon WH, et al. Fermented garlic protects diabetic, obese mice when fed a high-fat diet by antioxidant effects. Nutrition Research. 2011;**31**(5):387-396. DOI: 10.1016/j.nutres.2011.04.005

[27] Lee HS, Lim WC, Lee SJ, Lee SH, Lee JH, Cho HY. Antiobesity effect of garlic extract fermented by *Lactobacillus plantarum* BL2 in diet-induced obese mice. Journal of Medicinal Food. 2016;**19**(9):823-829. DOI: 10.1089/jmf.2016.3674

[28] Wang W, Sun Y. In vitro and in vivo antioxidant activities of polyphenol extracted from black garlic. Food Science and Technology. 2017;**37**(4):681-685. DOI: 10.1590/1678-457x.30816

[29] Lee YM, Gweon OC, Seo YJ, Im J, Kang MJ, Kim MJ, et al. Antioxidant effect of garlic and aged black garlic in animal model of type 2 diabetes mellitus. Nutrition Research and Practice. 2009;**3**(2):156-161. DOI: 10.4162/nrp.2009.3.2.156

[30] Ha AW, Kim WK. Antioxidant mechanism of black garlic extract involving nuclear factor erythroid 2-like factor 2 pathway. Nutrition Research and Practice. 2017;**11**(3):206-213. DOI: 10.4162/nrp.2017.11.3.206

[31] Czompa A, Szoke K, Prokisch J, Gyongyosi A, Bak I, Balla G, et al. Aged (black) versus raw garlic against ischemia/reperfusion-induced cardiac complications. International Journal of Molecular Sciences. 2018;**19**(4):1017. DOI: 10.3390/ijms19041017

[32] Wang L, Mimura K, Fujimoto S. Effects of black garlic supplementation on exercise-induced physiological responses. The Journal of Physical Fitness and Sports Medicine. 2012;**1**(4):685-694. DOI: 10.7600/jpfsm.1.685

[33] Mueller T, Dieplinger B, Gegenhuber A, Haidinger D, Schmid N, Roth N, et al. Serum total 8-iso-prostaglandin F2α: A new and independent predictor of peripheral arterial disease. Journal of Vascular Surgery. 2004;**40**(4):768-773. DOI: 10.1016/j.jvs.2004.07.044

[34] Liu J, Zhang G, Cong X, Wen C. Black garlic improves heart function in patients with coronary heart disease by improving circulating antioxidant levels. Frontiers in Physiology. 2018;**9**:1435. DOI: 10.3389/fphys.2018.01435

[35] Sun YE, Wang W. Changes in nutritional and bio-functional compounds and antioxidant capacity during black garlic processing. Journal of Food Science and Technology. 2018;**55**(2):479-488. DOI: 10.1007/s13197-017-2956-2

[36] Galland L. Diet and inflammation. Nutrition in Clinical Practice. 2010;**25**(6):634-640. DOI: 10.1177/0884533610385703

[37] Kim MJ, Yoo YC, Kim HJ, Shin SK, Sohn EJ, Min AY, et al. Aged black garlic exerts anti-inflammatory effects by decreasing no and proinflammatory cytokine production with less cytotoxicity in LPS-stimulated raw 264.7 macrophages and LPS-induced septicemia mice. Journal of Medicinal Food. 2014;**17**(10):1057-1106. DOI: 10.1089/jmf.2013.3043

[38] Oh HL, Kim MJ, You BR, Kim MR. Anti-inflammatory action of black garlic through downregulation of NF-κB activation and MAP kinase phosphorylation. The FASEB Journal. 2012;**26**(1_supplement):823-833

[39] Zhang J, Hao J. Antioxidant and anti-inflammatory activities of black garlic extracts. Journal of Food Safety and Quality. 2017;**8**(7):2635-2643

[40] Lee EN, Choi YW, Kim HK, Park JK, Kim HJ, Kim MJ, et al. Chloroform extract of aged black garlic attenuates TNF-α-induced ROS generation, VCAM-1 expression, NF-κB activation and adhesiveness for monocytes in human umbilical vein endothelial cells. Phytotherapy Research. 2011;**25**(1):92-100. DOI: 10.1002/ptr.3230

[41] Kim KH, Park JK, Choi YW, Kim YH, Lee EN, Lee JR, et al. Hexane extract of aged black garlic reduces cell proliferation and attenuates the expression of ICAM-1 and VCAM-1 in TNF-α-activated human endometrial stromal cells. International Journal of Molecular Medicine. 2013;**32**(1):67-78. DOI: 10.3892/ijmm.2013.1362

[42] Tak HM, Kang MJ, Kyoung MK, Kang D, Han S, Shin JH. Anti-inflammatory activities of fermented black garlic. Journal of the Korean Society of Food Science and Nutrition. 2014;**43**:1527-1534. DOI: 10.3746/jkfn.2014.43.10.1527

[43] Nurmasitoh T, Sari DCR, Partadiredja G. The effects of black garlic on the working memory and pyramidal cell number of

medial prefrontal cortex of rats exposed to monosodium glutamate. Drug and Chemical Toxicology. 2018;**41**(3):324-329. DOI: 10.1080/01480545.2017.1414833

[44] Hermawati E, Sari DCR, Partadiredja G. The effects of black garlic ethanol extract on the spatial memory and estimated total number of pyramidal cells of the hippocampus of monosodium glutamate-exposed adolescent male Wistar rats. Anatomical Science International. 2015;**90**:275. DOI: 10.1007/s12565-014-0262-x

[45] Aminuddin M, Partadiredja G, Sari DCR. The effects of black garlic (*Allium sativum* L.) ethanol extract on the estimated total number of Purkinje cells and motor coordination of male adolescent Wistar rats treated with monosodium glutamate. Anatomical Science International. 2015;**90**:75. DOI: 10.1007/s12565-014-0233-2

[46] Nillert N, Pannangrong W, Welbat JU, Chaijaroonkhanarak W, Sripanidkulchai K, Sripanidkulchai B. Neuroprotective effects of aged garlic extract on cognitive dysfunction and neuroinflammation induced by β-amyloid in rats. Nutrients. 2017;**9**(1):24. DOI: 10.3390/nu9010024

[47] Jeong JH, Jeong HR, Jo YN, Kim HJ, Shin JH, Heo HJ. Ameliorating effects of aged garlic extracts against Aβ-induced neurotoxicity and cognitive impairment. BMC Complementary and Alternative Medicine. 2013;**13**:268. DOI: 10.1186/1472-6882-13-268

[48] Colín-González AL, Ali SF, Túnez I, Santamaría A. On the antioxidant, neuroprotective and anti-inflammatory properties of S-allyl cysteine: An update. Neurochemistry International. 2015;**89**:83-91. DOI: 10.1016/j.neuint.2015.06.011

[49] Ahmed RA. Hepatoprotective and antiapoptotic role of aged black garlic against hepatotoxicity induced by cyclophosphamide. The Journal of Basic and Applied Zoology. 2018;**79**:8. DOI: 10.1186/s41936-018-0017-7

[50] Lee K, Teng C, Shen C, Huang W, Lu C, Kuo H, et al. Protective effect of black garlic extracts on tert-butyl hydroperoxide-induced injury in hepatocytes via a c-Jun N-terminal kinase-dependent mechanism. Experimental and Therapeutic Medicine. 2018;**15**:2468-2474. DOI: 10.3892/etm.2018.5719

[51] Kim MH, Kim MJ, Lee JH, Han JI, Kim JH, Sok DE, et al. Hepatoprotective effect of aged black garlic on chronic alcohol-induced liver injury in rats. Journal of Medicinal Food. 2011;**14**(7-8):732-738. DOI: 10.1089/jmf.2010.1454

[52] Shin JH, Lee CW, Oh SJ, Yun J, Kang MR, Han SB, et al. Hepatoprotective effect of aged black garlic extract in rodents. Toxicological Research. 2014;**30**(1):49-54. DOI: 10.5487/TR.2014.30.1.049

Chapter 6

Medicinal Plants Having Antifungal Properties

Koushlesh Kumar Mishra, Chanchal Deep Kaur, Anil Kumar Sahu, Rajnikant Panik, Pankaj Kashyap, Saraswati Prasad Mishra and Shweta Dutta

Abstract

In the past few decades, a worldwide increase in the incidence of fungal infections has been observed as well as rise in the resistance of some species of fungi to different fungicidal used in medicinal practice. Besides, fungi are the one of the most neglected pathogens as demonstrated by the fact that the amphotericin B and other sold treatments are still used as gold standard as antifungal therapy. The majority of used antifungal treatments have various drawbacks in terms of toxicity, efficacy as well as cost and their frequent use has also led to the emergence of resistant strains. Hence, there is a great demand for developing an antifungal belonging to a wide range of structural classes, selectively acting on new targets with least side effects. Natural products, either as pure phytocompounds or as standardized plant extracts, provide unlimited opportunities for new drug lads because of their having normally matchless chemical diversity. Present chapter focused on the work done in the field of antifungal activities of various plant components and novel approaches which will be the future prospective for the new drug discoveries and providing better antifungal therapy.

Keywords: antifungal, phytocompounds, fungicidal, antifungal therapy, fungal infections

1. Introduction to fungal disease

Fungal infections are one of the most deadly infections accounting in excess of 1.5 million deaths annually worldwide. The major reason that makes fungal infections more life threatening because they are been neglected by the society. Though in last 20 years there are many developments in the diagnosis and treatment of fungal disease but still majority of population are devoid of the benefits of these developments [1]. Among all the fungal diseases, infection of skin hold the 4th position and it accounts for the majority of death also [2].

Plant kingdom has always been a hub for many natural compounds with novel structure and this keep the investigators interested in doing research about many plants species till today. Results of new researchers showed that plants are enrich of many bioactive secondary metabolites such as saponins, alkaloids and terpenoids which characterized by antifungal property. Depending on that, these plants can be considered as a potent future source for anti-fungal drugs [3]. When recent scenario regarding fungal diseases and antifungal drugs are taken into consideration it

has been seen that the development of resistance of fungus towards the presently used antifungal drugs has increased [4–11]. With the challenges like morbidity and mortality there always lies difficulty in antifungal treatment for patients receiving therapy for AIDS, diabetes, chemotherapy or organ transplant as some of the molecular processes of fungus are similar to humans, so toxicity to fungal cells could affect human cells too [12]. In the last 30 years few drugs have made an impact in the treatment of fungal infection as shown in (**Table 1**), one of them is amphotericin B which is among the few fungicidal drugs present antifungal therapy has but it also showed several critical side effects (**Table 2**) [13]. In addition to this, during the period between late of 1980s and the beginning of 1990s emergence of Imidazoles and Triazoles was seen. These classes of drugs were efficient in inhibiting processes associated with fungal cells. The major drawback associated with them was relapse of infection and resistance developed by the fungus towards them [14]. Therefore, it become an oblige for the research to discover and produce a new, efficient, and safe anti-fungal treatments from new sources like plants. Therefore current chapter attempts to elaborate the current scenario about the important plants and their antifungal derivatives that can be future prospective to work on for development of more potent antifungal drugs.

There are around 2 million of fungal species found in the whole world but only 600 of them cause infection. The major species that are mostly involved in causing infection are *Cryptococcus*, *Candida, Trichophyton and Aspergillus. All the fungal infections which affects human, that are prevailing in the world can be grouped into five types.* The types are as follows:

1. *Invasive fungal infections*: examples are cryptococcal meningitis, Candida bloodstream infection, invasive aspergillosis, Pneumocystis pneumonia

2. *Chronic lung or deep tissue infection*: under this type example is chronic pulmonary aspergillosis

3. *Allergic fungal disease*: examples are allergic bronchopulmonary aspergillosis also known as ABPA and severe asthma with fungal sensitization (SAFS)

S. No	Class	Drugs	Uses
1.	Azole antifungals	Clotrimazole, Econazole, Isoconazole, Miconazole, Ketoconazole, Itraconazole	Topical fungal infections, Candidiasis, aspergillus and candida infections, vaginal yeast infections
2.	Echinocandins	Caspofungin, Micafungin	Esophageal Candidiasis, Salvage therapy
4.	Polyenes	Amphotericin B, Nystatin	Systemic mycosis, superficial mycosis
5.	Phenolic cyclohexane	Griseofulvin	Dermatophytic infections
6.	Synthetic pyrimidines	Flucytosine	Cryptococcosis, severe invasive aspergillosis, cryptococcal meningitis treated along with other antifungals
7.	Morpholines	Amorolfine	Topical fungal infections
8.	Pyridines	Buthiobate, Pyrifenox	Dermatophytic infections, Tinea conditions
9.	Phthalimides	Captan	Invasive dermatophytic conditions and candida infections

Table 1.
The synthetic drugs available in market for treatment of fungal diseases are – [15].

S. No	Side effects	Drugs
1.	Non-melanoma skin cancer prolonged therapy	Voriconazole
2.	Fever, Chills	Isavuconazole, Ketoconazole, Voriconazole, Flucytosine, Anidulafungin, Caspofungin
3.	Rash	Flucytosine, Fluconazole, Ketoconazole, Clotrimazole, Voriconazole
4.	Nausea, vomiting	Isavuconazole, Itraconazole, Flucytosine, Fluconazole, Ketoconazole, Clotrimazole, Voriconazole
5.	Abdominal pain	Flucytosine, Ketoconazole, Isavuconazole, Voriconazole
6.	Anemia	Amphotericin B, Caspofungin, Flucytosine
7.	Leukopenia, Thrombocytopenia	Flucytosine, Fluconazole
8.	Decreased renal function	Amphotericin B, Caspofungin, Voriconazole
9.	Headache	Flucytosine, Fluconazole, Ketoconazole, Isavuconazole, Voriconazole, Caspofungin
10.	Dark urine, clay-colored stools, jaundice	Anidulafungin C, Micafungin

Table 2.
Adverse side effects of different antifungals.

4. *Mucosal infection*: oral and esophageal candidiasis, Candida vaginitis are examples of this group.

5. *Skin, hair and nail infection*: examples of this kind of infections are athlete's foot tinea capitis and onychomycosis [16].

2. Plants having antifungal activity

The epidemiological data suggest that the incidence and prevalence of serious mycoses continues to be a public health problem. The increased use of antifungal agents has resulted in the development of resistance to these drugs. The spread of multidrug-resistant strains of fungus and the reduced number of drugs available make it necessary to discover new classes of antifungals from natural products

S. No.	Botanical name	Family	Parts used	Chemical classes	Microorganism tested
1.	*Eugenia uniflora*	Myrtaceae	Leaves	Sesquiterpenes, Monoterpene, hydrocarbons	*C. albicans, C. dubliniensis, C. glabrata, C. krusei* [17]
2.	*Psidium guajava*	Myrtaceae	Leaves	Methanolic extract	*C. albicans, C. dubliniensis, C. glabrata, C. krusei* [17]
3.	*Curcuma longa*	Zingiberaceae	Rhizome	Turmeric oil	*C. albicans, C. dubliniensis, C. glabrata, C. krusei* [17]
4.	*Piptadenia colubrina*	Mimosaceae	Stem bark	—	*C. albicans, C. dubliniensis, C. glabrata* [17]
5.	*Schinus terebinthifolius*	Anacardiaceae	Stem bark	Extract	*C. albicans, C. dubliniensis* [17]

S. No.	Botanical name	Family	Parts used	Chemical classes	Microorganism tested
6.	*Persea americana*	Lauraceae	Leaves	Chromene	*C. albicans C. dubliniensis C. glabrata, C. krusei* [17]
7.	*Parapiptadenia rigida*	Fabaceae	Stem bark	Pyrrolidine amide	*C. albicans* [17]
8.	*Ajania fruticulosa*	Asteraceae	Fruits	Guaianolides	*Candida albicans, C. glabrata A. fumigatus* [17]
9.	*Alibertia macrophylla*	Rubiaceae	Leaves	Extract	*Cladosporium sphaerospermum; C. cladosporioides; A. niger; Colletotrichum gloeosporioides* [17]
10.	*Aniba panurensis*	Lauraceae	Whole plant	—	*C. albicans* [17]
11.	*Aquilegia vulgaris*	Ranunculaceae	Leaves and stems	Bis (benzyl)	*A. niger* [17]
12.	*Mimosa tenuiflora*	Mimosaceae	Stem bark	Sesquiterpene lactone	*C. albicans, C. dubliniensis, C. glabrata, C. krusei* [17]
13.	*P. regnellii*	Piperaceae	Leaves	Extract	*Trichophyton rubrum, Trichophyton mentagrophytes, Microsporum canis* [18]
14.	*Rubia tinctorum*	Rubiaceae	Root	Triterpene	*A. niger, Alternaria alternaria, P. verrucosum, Mucor mucedo* [19]
15.	*Tithonia diversifolia*	Asteraceae	Whole plant	Contained saponins, Polyphenols	*Microbotryum violaceum, Chlorella fusca* [20]
16.	*Vernonanthura tweedieana*	Asteraceae	Root	Extracts	*T. mentagrophytes* [21]
17.	*Zingiber officinale*	Zingiberaceae	Rhizomes	Steroidal saponin	*P. oryzae* [22]
18.	*Datura metel*	Solanaceae	Whole plant	Diterpenoid, Alkaloids	*C. albicans, C. tropicalis* [23]
19.	*Lupinus albus*	Leguminosae	Leaf surface	—	*T. mentagrophytes* [24]
20.	*Ecballium elaterium*	Cucurbitaceae	Fruit	Extract	*Boitylis cinerea* [25]
21.	*Cassia tora*	Leguminosae	Seeds	Anthraquinone	*Botrytis cinerea, Erysiphe graminis, Phytophthora infestans, Puccinia recondita, Pyricularia grisea* [26]
22.	*Chamaecyparis pisifera*	Cupressaceae	Leaves and Twigs	Isoflavone	*P. oryzae* [27]
23.	*Prunus yedoensis*	Rosaceae	Leaves	Diterpenes	*C. herbarum* [28]

Table 3.
List of plants having antifungal activity against pathogenic fungi.

including medicinal plants. Medicinal plants have also been reported in traditional systems of medicine for the treatment of both human and animal mycoses, and are considered to be a valuable source for the discovery of new antifungal drugs. Many books have also reported and recorded the use of medicinal plants in the traditional system of medicine. Therefore, we have focused here mainly on the antifungal plants and their use against pathogenic fungi. The antifungal activity associated plants are illustrated in (**Table 3**).

3. Phytochemicals and their antifungal activity

Plants and their biologically active chemical constituents, sometimes called secondary metabolites or bioactives, present numerous opportunities for the improvement of livestock production by inclusion in the diet. Several papers and reviews have been published on the occurrence of antifungal compounds in plant. However, literature and systematic reviews on the natural products as an alternative to antifungal drugs are still scanty. The distribution of antifungal compounds can be defined either on the basis of their taxonomic distribution or on the basis of their chemical classes. **Table 4** shows the antifungal natural products belonging to

S. No	Plants	Plant part	Phytochemicals
1	*Aegle marmelos*	Leaves	Essential oils
2	*Alpinia galangal*	Seeds	Diterpenes
3	*Ananas comosus*	Leaves	Protein
4	*Blumea balsamifera*	Leaves	Flavonoid luteolin
5	*Camptotheca acuminate*	Leaves	Flavonoid
6	*Capsicum frutescens*	Whole plant	Triterpene saponin
7	*Cassia tora*	Whole plant	Emodin, physcion and rhein
8	*Datura metel*	Whole plant	Alkaloid
9	*Euonymus europaeus*	Leaves	Protein
10	*Haloxylon salicornium*	Aerial part	Alkaloid
11	*Juniperus communis*	Leaves	Essential oil
12	*Khaya ivorensis*	Stem bark	Triterpenes
13	*Lycium chinense*	Root bark	Phenolic compounds
14	*Musa acuminate*	Banana	Protein
15	*Ocimum gratissimum*	Bark	Essential oil
16	*Pinus pinaster*	Leaves	Pinosylvin
17	*Polygonum punctatum*	Whole plant	Sesquiterpene
18	*Smilax medica*	Root	Saponins
19	*Solanum tuberosum*	Tubers	Protein
20	*Thymus vulgaris*	Whole plant	Essential oil
21	*Trachyspermum ammi*	Leaves, flowers	Essential oil
22	*Trigonella graecum*	Whole plants	Peptides
23	*Zingiber officinalis*	Rhizome	Protein

Table 4.
List of plant components having antifungal property [29].

all major classes of secondary metabolites such as phenolics, alkaloids, terpenoids, saponins, flavonoids, proteins, and peptides, etc.

4. Novel approaches for antifungal plant components and their extracts

Novel drug delivery system has shown tremendous potential to deliver herbal drugs in the form of microcapsules, implants, nanoparticles micro particles sustained release tablets or extended release forms. Many herbal drugs using novel drug delivery system has made a mark in the market and few of them are in the developmental stage in the laboratory [30].

Active herbal components such as curcumin, digoxin, atropine, bromelain can be promising prospects for treatment of conditions like cancer or inflammation [31].

The popularity experienced by novel drug delivery system is due to its ability to deliver the herbal drugs in a better way providing enhanced therapeutic efficacy with lower toxicity [32]. In addition to this it also counteract the limitations of lower absorption and lack of specificity experienced by the available formulation of drugs. Advantages of novel drug delivery system over the presently available drug therapy is that it is specific, has rapid onset of action with faster absorption due to enhanced surface area and lastly nanoparticles provides better penetration in Blood Brain Barrier (BBB) [33].

For any herbal drugs to show expected therapeutic efficacy optimum amount of active constituent must reach the target tissues. Herbal drugs are prone to be degraded by first pass metabolism of by the pH difference of GIT. Various novel drug delivery systems such as nanoparticles, nanoemulsions, phytosomes, transferosomes and liposomes by passes all the hurdles of acidic pH as well as first pass metabolism to carry optimum amount of drugs to target tissues. Being smaller in size nano carriers also provides rapid onset of actions [34].

For delivery of drug by using novel drug delivery system, herbal drugs present themselves as potential candidate because of following reasons:

- The side effects that are seen with other drugs are absent with natural compounds.
- Natural compounds shows synergistic effect when they contain multifunctional molecules.
- Natural compounds have traditional backing for their action and safety potential whereas modern medicines are more toxic even if they are experimentally proven for their action [35].

4.1 Novel carrier systems used to treat different fungal infections

The major benefit provided by novel drug delivery system is to elicit better therapeutic response with minimum doses. Types of carriers used for herbal drug delivery and synthetic drugs are as follows:

4.1.1 Phytosomes

The name came from two words "Phyto" and "some" which means plant and cell-like respectively. Phytosomes contains lipid soluble complex of phospholipids and phyto-constituents. Some literatures also referred Phytosomes as ribosome [36]. Green tea phytosomes, *Ginkgo biloba* phytosomes, Centella phytosomes,

Meriva phytosome, Zanthalene phytosomes, Sericoside phytosomes are some examples of phytosomes which are recently developed and characterized for different ailments. Among all of them Zanthalene phytosomes are prepared especially for the treatment of fungal disease.

Advantages of Phytosomes

- Phytosomes are not degraded by bacteria or digestive secretion of guts.
- It has better stability because of the formation of bonds chemically connecting phytoconstituents and phosphatidylcholine molecules.
- Phytosomes delivers herbal drug to the respective target tissues [37].
- It shows greater therapeutic benefit due to better absorption of lipid insoluble polar phytoconstituents in turn shows better bioavailability [38].

4.1.2 Liposomes

Liposomes contain microscopic vesicles made up of lipid bilayer arranged in concentric fashion and the separation is filled with aqueous medium. Lipophilic substances are inserted into the lipid bilayer whereas aqueous compartment traps hydrophilic substance. Liposomes show better bioavailability, stability and enhanced pharmacokinetic property [39]. There are various herbal and synthetic liposomes are prepared for the effective treatment of different skin diseases. In 2017, a herbal liposomal gel containing ketoconazole and neem extract was developed for the effective treatment of seborrheic dermatitis against *Aspergillus niger* and *Candida albicans*. The results indicated that developed liposomal gel have great potential and showed synergetic effect for the treatment.

Advantages of liposomes

- Liposome formulation is better options for producing sustained release formulations as it enhances drug solubility.
- It is easy to load phytoconstituents of any chemical nature whether it is hydrophilic, amphiphilic or lipophilic [40].

4.1.3 Nanoparticles

This carrier system has particle size within the range between 1 and 100 nm. The particles which are of nano size are made up of polymer of synthetic or semisynthetic origin. Nanoparticles are microencapsulated to protect them from any kind of losses. Nanoparticles were made to encounter the problem of solubility and toxicity associated with triptolide [41].

Advantages of herbal nanoparticle delivery system:

- Nanoparticles having smaller size shows better dissolution in turn enhances solubility of dosage form and it also delivers drug with specificity thereby enhancing the efficacy [42].

4.1.4 Microemulsions and nanoemulsion

These are emulsions of O/W type and the particle size of the particulate is of micron. In this drug delivery system inner phase stores the drugs and because of its

contact with tissue directly drug release is slow. As per few reports oil of Pterodon emarginatus are considered to have property to enhance anti-inflammatory activity [43]. Formulation, development and evaluation of microemulsion gel of hydroalcoholic extract of *Quercus infectoria* in the treatment of different skin ailments was successfully prepared. Tannins which are prime constituent of galls can be effectively treat different skin conditions.

4.1.5 Microsphere

This drug delivery system have matrix and the drug is dispersed in a polymer which are present inside this matrix. Particle size that can be used is in between 1 and 300 μm. The release of drug is dependent on the dissolution and degradation rate of the said matrix. Release of drug occurs according to first order kinetic. For example, development and evaluation of floating microspheres of curcumin prepared by emulsion solvent diffusion method for treatment of onychomycosis. The result shows improved absorption kinetics of curcumin.

Advantages of microsphere formulations

- The major advantage of this kind of formulation is that it taken orally or parentally and their site of release can also be targeted [44].

4.1.6 Niosomes

Niosomes are similar as liposomes bjt are far more stable than liposomes. Niosomes are made up of surfactant like dialkyl polyglycerol which is noninonic in nature and are able encapsulate variety of drugs. Niosomes are more economical than liposomes [32]. Chitosan niosomal gel, miconazole niosomes are prepared as an effective nanocarrier against both dermatophytes and yeasts.

4.2 Transdermal drug delivery system

In this system of drug delivery, patches encapsulating drugs are prepared and are placed on the skin. Through the skin drug enters into the blood vessels. This system is beneficial when the required effect of oral therapy was not found to be up to the mark. Patches of antismoking and anti-motion sickness are available in market [45].

Advantages of transdermal drug delivery

- The transdermal delivery system has advantages such as it provides enhanced bioavailability and provides a better alternative of dosage form for unconscious or vomiting patients [46].

4.2.1 Ethosomes

Ethosomes are composed of phospholipids and ethanol and are in the form of sac. Ethanol present in ethosomes acts as permeability enhancer. Ethosomes are found in the form of cream and gel for better patient compliance [47]. Now a days, Transethosomes and Nanoethosomes used most widely which are the advanced type of ethosomes having edge activator in it. These advanced novel carrier system is much better than conventional novel carriers like transferosomes and liposomes [48]. Clotrimazole, Itraconazole, Miconazole are synthetic drugs which are prepared and evaluated successfully for the treatment of

dermatophytosis or ringworm. *Tridax procumbens* and *Galinsoga parvifolia* are two herbs used into ethosomal gel against Trichophyton species.

Advantages of Ethosome

- Ethosomes can entrap all type of drugs and have better skin permeability [46].

4.2.2 Transferosomes

Transferosomes contains phospholipids sac which behaves as carrier for delivery of drug through the skin. As Transferosomes are flexible in nature they cross the skin through the intracellular space found within the skin. Transferosomes of Colchicines shows lesser side effect than its oral form [49].

Advantages of Transferosomes:

- Transferosomes being flexible can pass through narrow openings of skin.
- It shows high efficiency of entrapment which may increase up to 90% in case of lipophilic drug [50].

4.2.2.1 Complexation

The problem associated with herbal drug formulation is their solubility. To counter this solubility problem, complex formation is done which gives particulates with well-defined stoichiometry. Few commonly used complexing agents are EDTA and cyclodextrin [51].

Drugs/Plant components	**Novel carriers**	**Indication**	**Microorganism tested**
Essential oil (*Bidens tripartite*)	Microemulsion gel	Candidiasis	*Candida albicans*
Curcumin	Phytosome	Onychomycosis	Yeast sp.
Clotrimazole, Econazole nitrate, Fluconazole	Micelles	Superficial fungal infection	*Trichophyton* sp.
Miconazole	Solid lipid nanoparticles and nanostructured lipid carriers	Candidiasis	*Candida albicans*
Fluconazole, Ketoconazole, Itraconazole, Voriconazole, Econazole	Microemulsion	*Tinea corporis, Tinea circinata, Tinea pedis*	*Candida albicans*
Amphotericin B	Microemulsion	Invasive fungal infection	*Trichophyton rubrum*
Griseofulvin	Microemulsion gel	Dermatophytosis	*Trichophyton* sp.
Terbinafine Hcl	Niosomes	Fungal infection	*Aspergillus niger*
Griseofulvin, Amphotericin B	Transferosomes	Dermatophytosis	*Trichophyton rubrum*
Clotrimazole, Econazole	Ethosomes	Localized skin fungal infection	*Candida* sp.

Table 5.
List of some novel carriers for antifungal plant components and synthetic drugs [55].

4.2.2.2 Hydrogels

The hydrogel are three dimensional structures with cross linking of polymers. As name suggest hydrogels are hydrophilic in nature. Hydrogels can be designed into different forms according to the needs. The form can be of slabs, films and nanoparticle coating [52]. Hydrogels have the potential to bind both herbal as well as synthetic drug, this ability can be treated as avenue for further research [53]. There are many marketed formulation of novel drug delivery available in the market [46, 54] Here are list of some novel carriers used with their plant components or synthetic drugs combinations for different fungal infections (**Table 5**).

5. Conclusion

The last 20 years has shown an increase in number of fungal infection. Currently used drugs in treatment of fungal infection are having many side effects, and development of resistance is very common against these drugs. Plants have been considered as traditional source of antifungal medicines for past many years. Plant bioactive with antifungal activity can be considered as an option for development of new and improved alternative formulations in antifungal therapy. Development of improved formulations with plant phytcompounds is the need of the hour for efficient treatment of fungal diseases. Further research on this field can provide us with increased number of options in treatment of fungal diseases that will give the patients with a better quality of life.

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References

[1] Sanglard D. Clinical relevance of mechanisms of antifungal drug resistance in yeasts Importancia clínica de los mecanismos de resistencia a los antifúngicos en levaduras. Enfermedades Infecciosasy Microbiología Clínica. 2002;**20**(9):462-470

[2] Hay RJ, Johns NE, Williams HC, Bolliger IW, Dellavalle RP, Margolis DJ, et al. The global burden of skin disease in 2010: An analysis of the prevalence and impact of skin conditions. The Journal of Investigative Dermatology. 2013;**134**(6):1527-1534. DOI: 10.1038/jid.2013.446

[3] Arif T, Bhosale JD, Kumar N, Mandal TK, Bendre RS, Lavekar GS, et al. Natural products–antifungal agents derived from plants. Journal of Asian Natural Products Research. 2009;**11**(7):621

[4] Fischer MC, Henk DA, Briggs CJ, Brownstein JS, Madoff LC, McCrwa SL, et al. Emerging fungal threats to animal, plant and ecosystem health. Nature. 2012;**484**:186

[5] Sanglard D. Resistance and tolerance mechanisms to antifungal drugs in fungal pathogens. Mycologist. 2003;**17**:74

[6] Rodriguez-Tudela JL, Alcazar-Fuoli L, Cuesta I, Alastruey-Izquierdo A, Monzon A, Mellado E, et al. Clinical relevance of resistance to antifungals. International Journal of Antimicrobial Agents. 2008;**32**:S111

[7] Manavathu EK, Vazquez JA, Chandrasekhar PH.
Reduced susceptibility in laboratory-selected mutants of Aspergillus fumigatus to itraconazole due to decreased intracellular accumulation of the antifungal agent. International Journal of Antimicrobial Agents. 1999;**12**:213

[8] Sanglard D. Resistance of human fungal pathogens to antifungal drugs. Current Opinion in Microbiology. 2002;**5**:379

[9] Pfaller MA, Casatanheira M, Messer SA, Moet GJ, Jones RN. Diagnostic Microbiology and Infectious Disease. 2010;**68**:278

[10] Odds FC. Resistance of clinically important yeasts to antifungal agents. International Journal of Antimicrobial Agents. 1996;**6**:145

[11] Sanglard D. Clinical relevance of mechanisms of antifungal drug resistance in yeasts. Odda FC. 2002;**2**:73

[12] Beck-Sague C, Banerjee S, Jarvis WR. Analyzing socioeconomic and racial/ethnic patterns in health and health care. American Journal of Public Health. 1993;**83**:1739

[13] Tripathi KD. Essentials of Medical Pharmacology. New Delhi, India: Jaypee Brothers Medical Publishers (P) Ltd.; 2009. p. 757

[14] Rex JH, Rinaldi MG, Pfaller MA. Resistance of Candida species to fluconazole. Antimicrobial Agents and Chemotherapy. 1995;**39**:1

[15] Revankar SJ. Wayne State University School of Medicine, Merck Manual professional version, Antifungal drugs; 2018

[16] Kobayashi GS. Chapter 74, disease mechanism of fungi. In: Baron S, editor. Medical Microbiology. 4th ed. The University of Texas Medical Branch at Galveston. 1996

[17] Ferreira MRA, Santiago RR, Langassner SMZ, de Mello JCP, Svidzinski TIE, Soares LAL. Antifungal activity of medicinal plants from northeastern Brazil. Journal

of Medicinal Plant Research. 2013;**7**(40):3008-3013

[18] Koroishi AM, Foss SR, Cortez DAG, Nakamura TU, Nakamura CV, Filho BPD. In vitro antifungal activity of extracts and neolignans from Piper regnellii against dermatophytes. Journal of Ethnopharmacology. 2008;**117**:270-277

[19] Manojlovic NT, Solujic S, Sukdolak S, Milosev M. Antifungal activity of Rubia tinctorum, Rhamnus frangula and Caloplaca cerina. Fitoterapia. 2005;**76**:244-246

[20] Yemele-Bouberte M, Krohn K, Hussain H, Dongo E, Schulz B, Hu Q. Tithoniamarin and tithoniamide: A structurally unique isocoumarin dimer and a new ceramide from Tithonia diversifolia. Natural Product Research. 2006;**20**:842-849

[21] Portillo A, Vila R, Freixa B, Adzet T, Canigueral S. Antifungal activity of Paraguayan plants used in traditional medicine. Journal of Ethnopharmacology. 2001;**76**:93-98

[22] Endo K, Kanno E, Oshima Y. Structures of antifungal diarylheptenones, gingerenones a, B, C and isogingerenone B, isolated from the rhizomes of Zingiber officinale. Phytochemistry. 1990;**29**:797

[23] Dabur R, Chhillar AK, Yadav V, Kamal PK, Gupta J, Sharma GL. In vitro antifungal activity of 2-(3,4-dimethyl-2,5-dihydro-1H-pyrrol-2-yl)-1-methylethyl pentanoate, a dihydro – Pyrrole derivative. Journal of Medical Microbiology. 2005;**54**:549-552

[24] Ingham JL, Tahara S, Harborne JB. Fungitoxic isoflavones from Lupinus albus and other Lupinus species. Zeitschrift für Naturforschung. 1983;**38c**:194-200

[25] Har-Nun N, Meyer AM. Cucurbitacins protect cucumber tissue against infection by Botrytis cinerea. Phytochemistry. 1990;**29**:787-791

[26] Kim KY, Davidson PM, Chung HJ. Antibacterial activity in extracts of Camellia japonica L. petals and its application to a model food system. Journal of Food Protection. 2001;**64**:1255-1260

[27] Kobayashi K, Nishino C, Tomita H, Fukushima M. Antifungal activity of pisiferic acid derivatives against the rice blast fungus. Phytochemistry. 1987;**26**:3175-3179

[28] Ito T, Kumazawa K. Antifungal substances from mechanically damaged cherry leaves (Prumus yedoensis matsumura). Bioscience, Biotechnology, and Biochemistry. 1992;**56**:1655

[29] Meena MR, Sethi V. Antimicrobial activity of essential oils from species. Journal of Food Science and Technology. 1994;**31**:68-70

[30] Devi VK, Jain N, Valli SK. Importance of novel drug delivery systems in herbal medicines. Pharmacognosy Reviews. 2010;**4**(7):27-31

[31] Yadav D, Suri S, Chaudhary AA, Asif M. A novel approach: Herbal remedies and natural products in pharmaceutical science as nano drug delivery systems. International Journal of Pharmacy and Technology. 2011;**3**(3):3092-3116

[32] Beyatricks KA, Kumar KS, Suchitra D, Jainab HN, Anita A. Recent microsphere formulation and its applications in herbal drugs. International Journal of Pharmaceutical Development and Technology. 2014;**4**(1):58-62

[33] Chakraborty K, Shivakumar A, Ramachandran S. Nanotechnology in

herbal medicine. International Journal of Herbal Medicine. 2016;**4**(3):21-27

[34] Indalkar YR, Pimpodkar VP, Godase AS, Gaikwad PS. A compressive review on the study of nanotechnology for herbal drugs. Asian Pharma Press. 2015;**5**(4):203-207

[35] Sharma AT, Mitkare SS, Moon RS. Multicomponent herbal therapy: A review. International Journal of Pharmaceutical Sciences Review and Research. 2011;**6**(2):185-187

[36] Ravi GS, Chandur V, Shabaraya AR, Sanjay K. Phytosomes: An advanced herbal drug delivery system. International Journal of Pharmaceutical Research and Bioscience. 2015;**4**(3):415-432

[37] Kareparamban JA, Nikam PH, Jadhav PA, Kadam VJ. Phytosome: A novel revolution in herbal drugs. International Journal of Research in Pharmacy and Chemistry. 2012;**2**(2):299-310

[38] Deshpande PK, Pathak AP, Gothalwal R. Phytosomes: A novel drug delivery system for phytoconstituents. Journal on New Biological Reports. 2014;**3**(3):212-220

[39] Sharma M. Applications of nanotechnology based dosage forms for delivery of herbal drugs. Research & Reviews: Journal of Pharmaceutics and Nanotechnology. 2014;**2**(1):23-30

[40] Abhinav M, Neha J, Anne G, Bharti V. Role of novel drug delivery systems in bioavailability enhancement: At a glance. International Journal of Drug Delivery Technology. 2016;**6, 26**(1):7

[41] Abirami A, Halith SM, Pillai KK, Anbalagan C. Herbal nanoparticle for anticancer potential - a review. World Journal of Pharmacy and Pharmaceutical Sciences. 2014;**3**(8):2123-2132

[42] Sachan AK, Gupta A. A review on nanotized herbal drugs. International Journal of Pharmaceutical Sciences and Research. 2015;**6**(3):961-970

[43] Jadhav V, Bhogale V. Novel drug delivery system in herbal. International Journal of Pharma Wave. 2015;**1**(2):85-103

[44] Pascoa H, Diniz DA, Florentino IF, Costa EA, Bara MF. Microemulsion based on Pterodon emarginatus oil and its anti inflammatory potential. Brazilian Journal of Pharmaceutical Sciences. 2015;**51**(1):117-126

[45] Amol K, Pratibha P. Novel drug delivery system in Herbal's. International Journal of Pharmaceutical, Chemical and Biological Sciences. 2014;**4**(4):910-930

[46] Yadav M, Bhatia VJ, Doshi G, Shastri K. Novel techniques in herbal drug delivery systems. International Journal of Pharmaceutical Sciences Review and Research. 2014;**28**(2):83-89

[47] Ghulaxe C, Verma R. A review on transdermal drug delivery system. The Pharma Innovation Journal. 2015;**4**(1):37-43

[48] Mishra KK, Kaur CD, Verma S, Sahu AK, Dash DK, Kashyap P, et al. Transethosomes and nanoethosomes: Recent approach on transdermal drug delivery system. Nanomedicine. 2019;**2**:33-54

[49] Fatima GX, Rahul RS, Reshma I, Sandeep T, Shanmuganathan S, Chamundeeswari D. Herbal ethosomes - a novel approach in herbal drug technology. American Journal of Ethnomedicine. 2014;**1**(4):226-230

[50] Ajazuddin S. Applications of novel drug delivery system for herbal formulations. Fitoterapia. 2010;**81**:680-689

[51] Sachan R, Parashar T, Soniya SV, Singh G, Tyagi S, Patel C, et al. Drug carrier transferosomes: A novel tool for transdermal drug delivery system. International Journal of Research and Development in Pharmacy & Life Sciences. 2013;**2**(2):309-316

[52] Butler MS. The role of natural product chemistry in drug discovery. Journal of Natural Products. 2004;**67**(12):2141-2153

[53] Kushwaha SKS, Rastogi A, Rai AK, Singh S. Novel drug delivery system for anticancer drug: A review. International Journal of PharmTech Research. 2012;**4**(2):542-553

[54] Lai WF, Al R. Hydrogel based materials for delivering herbal drugs. ACS Applied Materials & Interfaces. 2017;**9**(13):11309-11320

[55] Bseiso EA, Nasr M, Sammour O, Gawad NA. Recent advances in topical formulation carriers of antifungal agents. Indian Journal of Dermatology, Venereology and Leprology. 2015;**8**(5):457-463

Chapter 7

The Utilization of Traditional Herbal Medicine for Treatment in Traditional Korean Medicine Clinics

Ji Yeon Ryu, Jung Youn Park, Angela Dongmin Sung and Soo Hyun Sung

Abstract

A cross-sectional study has been conducted to detect the facts about the use of traditional herbal medicines (THMs) in South Korea. The questionnaire has been adopted from the 2017 National Survey for the usage of traditional Korean medicine (TKM) and consumption of THMs. A total number of 1346 participants have been involved in this study. Results showed that the non-decoction types of herbal medicines, which are mostly used for therapeutic purposes (89.0%), and the decoction types of herbal medicines were not only used for the purpose of treatment of diseases (62.5%) but also health improvement purposes (21.9%). Results presented that decoction types of THMs are used for musculoskeletal diseases (56.0%), digestive diseases (21.3%), and respiratory diseases (6.3%), whereas the non-decoction types of THMs are commonly used in musculoskeletal diseases (55.6%), respiratory diseases (20.5%), and digestive diseases (18.1%). Future studies are highly recommended to detect more details about the medical use of THMs in South Korea.

Keywords: medicinal herb, herbal medicine, traditional Korean medicine, traditional herbal medicine, medical use of herbal medicine

1. Introduction

1.1 Herbal medicine

Traditional herbal medicines (THMs) are the most popular and preferred forms of traditional medicines (TMs) [1–3]. The World Health Organization (WHO) estimated that 80% of the population in developing countries rely on traditional herbs for their primary health care [4]. Compared with the developed countries, in the United States of America (USA), 19% of the population uses THMs for their treatment [5].

THMs have gained an increasing popularity in the last two decades in the industrialized countries [6–8]. The Europe market for THMs is estimated to be valued at $5.18 billion in 2016 [9]. The American botanical council reported that the sales of THMs in the USA reached a total of $7.45 billion in 2016 [10]. Thus, the global THM market is expected to grow and reach $129.68 billion by 2023 [11].

TMs, such as traditional Chinese medicines (TCMs), Ayurveda, Kampo, traditional Korean medicines (TKMs), and Unani, have used herbal medicines for hundreds or even thousands of years worldwide [6, 12–15]. Before Western medicine was introduced, TMs, especially herbal medicines, were used for preventing and treating diseases in many nations [16]. The use of THMs is well established, and it is widely known to be safe and effective [17].

TM practitioners in Asian countries, such as China, South Korea, and Taiwan, practice TM treatment in medical clinics as doctors defined by law [18, 19]. South Korea has the highest percentage (15.26%) of TM doctors in medical clinics in East Asia, followed by China (12.63%) and the Taiwan region (9.69%) [18].

1.2 Traditional herbal medicines in Korea

Koreans have traditionally used herbs for treatment and prevention of diseases. In 1894, Je Ma Lee, a TKM doctor, established the theory of Sasang constitutional medicine (SCM), which is a unique TKM form [14, 20]. Based on the SCM theory, humans are categorized into four constitution types: Taeyangyin (Greater Yang type), Tae-eumin (Greater Yin type), Soyangyin (Lesser Yang type), and Soeumin (Lesser Yin type) [14, 20]. SCM has classified therapeutic THMs according to the four constitutions because THMs that respond to the characteristics of four constitutions are different [14, 20].

In South Korea, TKM is legally institutionalized and covered by national insurance [18]. Since 1987 herbal extracts based on good manufacturing practice (GMP) are covered by health insurance, but the decoction type of traditional herbal medicines (THMs) which is combined with two or more medicinal herbs is still not insured yet [18]. In addition, as a member of the WHO Pharmacovigilance, Korea is monitoring adverse drug reactions, including THMs [21].

1.2.1 Medicinal use of herbs in traditional Korean medicine clinics

The Korean Ministry of Food and Drug Safety (MFDS) introduced the Good Manufacturing Practice for herbs (hGMP) in 2012 and made it mandatory in 2015 [22]. Thus, medicinal herbs must be manufactured by the hGMP facilities that are licensed by the Korean MFDS [22].

Standards for commonly used herbal materials and preparations are included in the "Korean Pharmacopoeia (KP)" and "Korean Herbal Pharmacopoeia (KHP)" [23]. As of 2019, there are 601 kinds of herbal materials called medicinal herbs listed in the "Korean Pharmacopeia" and "Korean Herbal Pharmacopoeia" [24, 25]. The amount of hazardous substances (e.g., heavy metals, pesticides, aflatoxins, sulfur dioxide, and benzopyrene) in herbal materials is restricted by the "Regulations on Limits and Test Methods for Residues and Contaminants in Herbal Medicines" [26].

According to the Korea medical law, TKM clinics should utilize medicinal herbs certified by the Korean MFDS [22]. Since 2015, TKM clinics have been using THMs composed of medicinal herbs for treating diseases and promoting health.

THMs are the second most commonly used treatment in South Korea, mainly utilized as a combination of two or more medicinal herbs [27, 28]. In addition, they are used in various forms, such as a decoction, powder, tablet, soft extract, paste, and pill [27].

In previous studies related to Korean THMs, Fan [23] reviewed the role of MFDS in THM management: pre-market approval, post-market inspection, and management of the product quality system. Choi [29] reported that THMs are

standardized, regulated, and quality controlled by MFDS guidelines such as KP and KHP. Park [18] investigated the TKM system and summarized development of TM system, policy, education system, medical insurance coverage, and herbal drug monitoring system. Yarnell [30] introduced herbal medicine market and modernized decoction device of TKM hospitals.

To the best of our knowledge, this is the first study aims to *shed* some *light on* the overall status of THM usage in South Korea (TKM clinics). The objective of this research was to examine the medical use of THMs, particularly mixtures of THMs, in South Korea, a country with an extensive knowledge and experience in THM use for preventing and treating diseases.

2. Methods

2.1 Study design

The cross-sectional study design has been used to address the research goals.

2.2 Study population

The population of the study included TKM doctors who worked at the TKM clinics. Inclusion and exclusion criteria are as follows:

2.2.1 Inclusion criteria

All TKM doctors who used to prescribe THMs and provide their consent form have been included.

1. How many prescription cases are being used in the practice? (1) Decoction types of THMs: _____ cases (2) Non-decoction types of THMs: _____ cases
2. What is the prescription purpose rate when prescribing herbal medicines? (total 100%) (1) Diseases treatment _____% (2) Health improvement _____% (3) Beauty _____% (4) Traffic accident _____% (5) Others _____%
3. Which disease prescribed herbal medicine the most when treating the disease? (1) Which disease prescribed decoction types of THMs the most when treating the disease? ① Musculoskeletal disease ② Neurological disease ③ Cerebrovascular disease ④ Cardiovascular disease ⑤ Cancer ⑥ Endocrine disease ⑦ Digestive disease ⑧ Respiratory disease ⑨ Mental disease ⑩ Obstetrics and gynecology disease ⑪ Urogenital disease ⑫ Skin disease ⑬ Disease in eyes and ears ⑭ Others (2) Which disease prescribed non-decoction types of THMs the most when treating the disease? ① Musculoskeletal disease ② Neurological disease ③ Cerebrovascular disease ④ Cardiovascular disease ⑤ Cancer ⑥ Endocrine disease ⑦ Digestive disease ⑧ Respiratory disease ⑨ Mental disease ⑩ Obstetrics and gynecology disease ⑪ Urogenital disease ⑫ Skin disease ⑬ Disease in eyes and ears ⑭ Others
4. Which disease prescribed herbal medicine the most when treating the disease? 1. Which disease prescribed decoction types of THMs the most when treating the disease? 2. Which disease prescribed non-decoction types of THMs the most when treating the disease?
TKM: Traditional Korean medicine *THM: Traditional herbal medicine*

Table 1.
Questionnaire on THM prescriptions of TKM doctors.

2.3 Sample selection

The survey group of THM consumption included individuals who worked at TKM clinics, hospitals (TKM, long-term care, and general hospitals), traditional herbal medicine pharmacies, and community pharmacies. In the study, 1354 TKM doctors working in TKM clinics were included. Among them, 1346 respondents were selected for the final analysis, excluding 8 who said they do not prescribe THMs.

2.4 Questionnaire

The questionnaire adopted and used in this study has been developed by the Korea Ministry of Health and Welfare (MHW), and the National Statistics of Korea reviewed and approved the questionnaire [27]. The 10-page structured questionnaire contained questions on the use, experience, treatment, management status, preference form, number of prescriptions, prescription purpose, frequently prescribed THMs for treatment, ways to purchase herbs, reasons for difficulty in purchase herbs, and plans to expand the use of THMs [27].

There are four sections used in the analysis: the number of THM prescriptions per year, prescription purpose when prescribing THMs, diseases treated with THMs, and THMs used for the disease [27]. The used questionnaire is shown in **Table 1**.

2.5 Data analysis

Descriptive statistics (mean and proportions) were used to describe the characteristics of the representatives of TKM clinics in the sample. Cross-analysis was used to determine the relationships between the high frequency of disease and prescription. All data analyses were performed using SPSS software (version 21.0 for Windows; IBM cop., Armonk, United States).

3. Results

3.1 Basic characteristics of participant TKM doctors

A total number of 1346 TKM doctors participated in this study, 1204 were men (89.5%) and 142 were women (10.5%). Most of them (73.5%) aged between 41 and 60. Out of the 1346 clinics, 779 (57.9%) were located in the capital area, while the rest 567 (42.1%) were located in the noncapital area. Majority of the participants 882 (65.5%) were with less than 20 years of clinical experience, 417 (31.0%) were with 21–38 years of treatment experience, and 47 (3.5%) of them were with 39–56 years of experience, as shown in **Table 2**.

3.2 Types of THMs prescribed in TKM clinics

3.2.1 Dispensing of decoction types of THMs

Results showed that 1346 of the TKM doctors prescribed an average of 590.4 ± 1105.5 decoction types of THMs/year, and the prescription distribution was as follows: 378 (28.1%) had ≤200 cases, 428 (31.8%) had 201–400 cases, 256 (19.0%) had 401–600 cases, and 284 (21.1%) had ≥601 cases (**Table 3**).

Factors	*N* (%)
Gender	
Male	1204 (89.5)
Female	142 (10.5)
Age (years)	
30–40	191 (14.2)
41–50	550 (40.8)
51–60	440 (32.7)
≥61	165 (12.3)
Place of work	
Capital area	779 (57.9)
Chungcheong Province	122 (9.1)
Gyeongsang Province	287 (21.3)
Jeolla Province	158 (11.7)
Clinical experience	
≤20	882 (65.5)
21–38	417 (31.0)
39–56	47 (3.5)

TKM: Traditional Korean medicine

Table 2.
Demographics of participating TKM doctors.

Prescription frequency	*N* (%)	Mean ± SD
≤200	378 (28.1)	590.4 ± 1105.5
201–400	428 (31.8)	
401–600	256 (19.0)	
≥601	284 (21.1)	

THM: Traditional herbal medicine
TKM: Traditional Korean medicine

Table 3.
Decoction types of THMs prescribed in TKM clinics.

3.2.2 Dispensing of non-decoction types of THMs

Out of the total 1346 respondents, 1070 TKM doctors prescribed non-decoction types of THMs. The average of prescription cases was 1775.9 ± 2349.1 non-decoction types of THMs/year, and prescription distribution was as follows: 222 (20.7%) had ≤200 cases, 120 (11.2%) had 201–400 cases, 92 (8.6%) had 401–600 cases, and 636 (59.4%) had ≥601 cases (**Table 4**).

3.3 Purpose of prescribed THMs

3.3.1 Purpose of prescribed decoction types of THMs

Decoction types of THMs have been prescribed in the TKM clinics for various reasons: disease treatment (62.5%), health promotion (21.9%), traffic accidents

Prescription frequency	*N* (%)	Mean ± SD
≤200	222 (20.7)	1775.9 ± 2349.1
201–400	120 (11.2)	
401–600	92 (8.6)	
≥601	636 (59.4)	

THM: Traditional herbal medicine
TKM: Traditional Korean medicine

Table 4.
Non-decoction types of THMs prescribed in TKM clinics.

(8.6%), beauty (5.9%), and others (1.1%). It was found that most TKM clinics prescribe decoction of Korean medicine herbs for treatment of diseases, as shown in **Table 5**.

3.3.2 Purpose of prescribed non-decoction types of THMs

Same as above, non-decoction types of THMs have been prescribed for several reasons: disease treatment (89.0%), health promotion (6.0%), car accidents (2.8%), beauty (1.4%), and others (0.9%), as shown in **Table 6**.

3.4 Disease proportions and THMs prescribing pattern

3.4.1 Decoction types of THMs

The decoction types of THMs were prescribed for patients suffering from musculoskeletal diseases (56.0%), digestive diseases (21.3%), respiratory diseases (6.3%), and obstetrics and gynecology disease (4.2%).

Results showed that decoction types of THMs frequently used for musculoskeletal diseases were Ojeoksan (22.5%), Bojungikgitang (10.3%), and Sipjeondaebotang (7.5%); for digestive diseases were Bojungikgitang (12.5%), Sipjeondaebotang (8.8%), and Ojeoksan (8.8%); for respiratory diseases were Bojungikgitang (9.9%), Socheongryongtang (9.9%), Sipjeondaebotang (8.6%), and Ojeoksan (8.6%); for obstetrics and gynecology diseases were Jogyeongjongogtang (11.1%), Ojeoksan (9.3%), and Bojungikgitang (7.4%); for skin diseases (n = 38) were Ojeoksan (10.5%) and Bojungikgitang (7.9%); for neurological diseases were Ojeoksan (16.7%), Galgeuntang (8.3%), and Yukmijihwangtang (8.3%); for cerebrovascular diseases were Ojeoksan (18.8%), Ganghwangyupungtang (12.5%), and Sipjeondaebotang (9.4%); for mental diseases were Gamiondamtang (11.5%), Kwibitang (7.7%), Bunsimgieum (7.7%), and Ojeoksan (7.7%); for cardiovascular diseases was Gamiondamtang (30.0%); for endocrine diseases was Sipjeondaebotang (25.0%); for urogenital diseases were Ssanghwatang (33.3%), Ojeoksan (33.3%), and Yukmijihwangtang (33.3%); for diseases in eyes and ears was Ojeoksan (100.0%); and for cancer was Banhasasimtang (100.0%), as shown in **Table 7**.

3.4.2 Non-decoction types of THMs

The non-decoction types of THMs were prescribed for patients suffering from musculoskeletal diseases (55.6%), respiratory diseases (20.5%), and digestive diseases (18.1%).

Prescription purpose	Mean ± SD
Disease treatment	62.5 ± 24.7
Health improvement	21.9 ± 19.6
Traffic accidents	8.6 ± 12.5
Beauty	5.9 ± 12.5
Others	1.1 ± 4.6

THM: Traditional herbal medicine

Table 5.
Purpose of prescribed decoction types of THMs.

Purpose of prescription	Mean ± SD
Disease treatment	89.0 ± 19.3
Health improvement	6.0 ± 13.3
Beauty	2.8 ± 6.5
Traffic accidents	1.4 ± 9.1
Others	0.9 ± 5.1

THM: Traditional herbal medicine

Table 6.
Purpose of prescribed non-decoction types of THMs.

Types of diseases	Top 3 used THMs	*N* (%)
Musculoskeletal disease (*n* = 716)	Ojeoksan (五積散)	161 (22.5)
	Bojungikgitang (補中益氣湯)	74 (10.3)
	Sipjeondaebotang (十全大補湯)	54 (7.5)
Digestive disease (*n* = 272)	Bojungikgitang (補中益氣湯)	34 (12.5)
	Sipjeondaebotang (十全大補湯) Ojeoksan (五積散)	24 (8.8)
Respiratory disease (*n* = 81)	Bojungikgitang (補中益氣湯) Socheongryongtang (小靑龍湯)	8 (9.9)
	Sipjeondaebotang (十全大補湯) Ojeoksan (五積散)	7 (8.6)
Obstetrics and gynecology disease (*n* = 54)	Jogyeongjongogtang (調經種玉湯)	6 (11.1)
	Ojeoksan (五積散)	5 (9.3)
	Bojungikgitang (補中益氣湯)	4 (7.4)
Skin disease (*n* = 38)	Ojeoksan (五積散)	4 (10.5)
	Bojungikgitang (補中益氣湯)	3 (7.9)
	Dangguisusan (當歸鬚散) Wolbitang (越婢湯) Hwangryunhaedoktang (黃蓮解毒湯)	2 (5.3)
Neurological disease (*n* = 36)	Ojeoksan (五積散)	6 (16.7)
	Galgeuntang (葛根湯) Yukmijihwangtang (六味地黃湯)	3 (8.3)
Cerebrovascular disease (*n* = 32)	Ojeoksan (五積散)	6 (18.8)
	Ganghwangyupungtang (羌活愈風湯)	4 (12.5)
	Sipjeondaebotang (十全大補湯)	3 (9.4)

Types of diseases	Top 3 used THMs	*N* (%)
Mental disease (*n* = 26)	Gamiondamtang (加味溫膽湯)	3 (11.5)
	Kwibitang (歸脾湯) Bunsimgieum (分心氣飮) Ojeoksan (五積散)	2 (7.7)
Cardiovascular disease (*n* = 10)	Gamiondamtang (加味溫膽湯)	3 (30.0)
	Ganghwangyupungtang (羌活愈風湯) Bojungikgitang (補中益氣湯) Sipjeondaebotang (十全大補湯) Ojeoksan (五積散) Yukmijihwangtang (六味地黃湯) Insamnyangyeongtang (人蔘養榮湯) Galgeuntang (葛根湯)	1 (10.0)
Endocrine disease (*n* = 8)	Sipjeondaebotang (十全大補湯)	2 (25.0)
	Gwakhyangjunggisan (藿香正氣散) Banhasasimtang (半夏瀉心湯) Bangpungtongseongsan (防風通聖散) Yukgunjatang (六君子湯) Yukmijihwangtang (六味地黃湯) Palmultang (八物湯)	1 (12.5)
Urogenital disease (*n* = 3)	Ssanghwatang (雙和湯) Ojeoksan (五積散) Yukmijihwangtang (六味地黃湯)	1 (33.3)
Disease in eyes and ears (*n* = 1)	Ojeoksan (五積散)	1 (100.0)
Cancer (*n* = 1)	Banhasasimtang (半夏瀉心湯)	1 (100.0)

THM: Traditional herbal medicine

Table 7.
Disease proportions and pattern of prescribed decoction types of THMs.

Types of diseases	Top 3 used THMs	*N* (%)
Musculoskeletal disease (*n* = 591)	Ojeoksan (五積散)	216 (36.5)
	Gunghatang (芎夏湯)	92 (15.6)
	Ijintang (二陳湯)	45 (7.6)
Respiratory disease (*n* = 218)	Ojeoksan (五積散)	35 (16.1)
	Samsoeum (蔘蘇飮)	30 (13.8)
	Socheongryongtang (小靑龍湯)	
Digestive disease (*n* = 192)	Pyeongwisan (平胃散)	38 (19.8)
	Ojeoksan (五積散)	37 (19.3)
	Hyangsapyungwisan (香砂平胃散)	21 (10.9)
Neurological disease (*n* = 16)	Socheongryongtang (小靑龍湯)	3 (18.8)
	Galgeuntang (葛根湯)	2 (12.5)
	Ojeoksan (五積散)	
	Gumiganghwaltang (九味羌活湯)	
	Samsoeum (蔘蘇飮)	
	Pyeongwisan (平胃散)	

Types of diseases	Top 3 used THMs	*N* (%)
Cerebrovascular disease (*n* = 11)	Ojeoksan (五積散)	3 (27.3)
	Yeongyopaedoksan (蓮翹敗毒散)	2 (18.2)
	Gunghatang (芎夏湯)	1 (9.1)
	Bojungikgi-tang (補中益氣湯)	
	Socheongryongtang (小青龍湯)	
	Ijintang (二陳湯)	
	Jaeumganghwatang (滋陰降火湯)	
	Pyeongwisan (平胃散)	
Cardiovascular disease (*n* = 9)	Banhabackchulchunmatang (半夏白朮天麻湯)	2 (22.2)
	Gumiganghwaltang (九味羌活湯)	1 (11.1)
	Gunghatang (芎夏湯)	
	Bojungikgitang (補中益氣湯)	
	Sosihotang (小柴胡湯)	
	Ijungtang (理中湯)	
	Ijintang (二陳湯)	
	Pyeongwisan (平胃散)	
Obstetrics and gynecology disease (*n* = 8)	Ojeoksan (五積散)	2 (25.0)
	Gamisoyosan (加味逍遙散)	1 (12.5)
	Gunghatang (芎夏湯)	
	Bulhwangeumjeonggisan (不換金正氣散)	
	Samsoeum (蔘蘇飮)	
	Ijintang (二陳湯)	
	Pyeongwisan (平胃散)	
Skin disease (*n* = 5)	Gamisoyosan (加味逍遙散)	1 (20.0)
	Gunghatang (芎夏湯)	
	Ojeoksan (五積散)	
	Insampaedoksan (人蔘敗毒散)	
	Pyeongwisan (平胃散)	
Mental disease (*n* = 5)	Ojeoksan (五積散)	2 (40.0)
	Gamisoyosan (加味逍遙散)	1 (20.0)
	Gumiganghwaltang (九味羌活湯)	
	Daecheongnyongtang (大靑龍湯)	
Urogenital disease (*n* = 3)	Ojeoksan (五積散)	2 (66.7)
	Pyeongwisan (平胃散)	1 (33.3)
Endocrine disease (*n* = 2)	Gumiganghwaltang (九味羌活湯)	1 (50.0)
	Pyeongwisan (平胃散)	
Disease in eyes and ears (*n* = 1)	Ojeoksan (五積散)	1 (100.0)
Cancer (*n* = 1)	Gunghatang (芎夏湯)	1 (100.0)

THM: Traditional herbal medicine

Table 8.
Disease proportions and pattern of prescribed non-decoction types of THMs.

Results showed that decoction types of THMs frequently used for musculoskeletal diseases were Ojeoksan (36.5%), Gunghatang (15.6%), and Ijintang (7.6%); for respiratory diseases were Ojeoksan (16.1%), Samsoeum (13.8%), and Socheongryongtang (13.8%); for digestive diseases were Pyeongwisan (19.8%), Ojeoksan (19.3%), and Hyangsapyungwisan (10.9%); for neurological diseases was Socheongryongtang (18.8%); for cerebrovascular diseases were Ojeoksan (27.3%) and Yeongyopaedoksan (18.2%); for cardiovascular diseases was Banhabackchulchunmatang (22.2%); for obstetrics and gynecology diseases was Ojeoksan (25.0%); for skin diseases were Gamisoyosan (20.0%), Gunghatang (20.0%), Ojeoksan (20.0%), Insampaedoksan (20.0%), and Pyeongwisan (20.0%); for mental diseases was Ojeoksan (40.0%); for urogenital diseases were Ojeoksan (66.7%) and Pyeongwisan (33.3%); for endocrine diseases were Gumiganghwaltang (50.0%) and Pyeongwisan (50.0%); for diseases in eyes and ears was Ojeoksan (100.0%); and for cancer (n = 1) was Gunghatang (100.0%), as shown in **Table 8**.

4. Discussion

This study investigated the use of THMs in TKM clinics in South Korea.

Results showed that average prescription cases of 590.4 ± 1105.5 decoction types of THMs and 1775.9 ± 2349.1 non-decoction types of THMs had been prescribed by doctors per year in the TKM clinics. According to the Korean national survey [27], the use of decoction types of THMs was 54.8%, and non-decoction types of THMs with the exception of insurance herbal extracts was 45.2% [27].

The reason for the preference for decoction types of THMs in South Korea is that the traditional way to boil herbs is familiar to the people, and it is possible to prescribe customized prescription according to the symptoms of the patients [31]. Non-decoction types of THMs such as granule, pill, tablet, soft extract, paste, and powder are convenient to store and carry because it is smaller in size than the decoction types of THMs [27]. In the case of the same prescription, the non-decoction types of THMs found to be cheaper than decoction types of THMs, so that patients may prefer non-decoction types of THMs for economic reason [27].

According to the 2017 national survey, Koreans responded that decoction types of THMs were too expensive (44.3%) and required health insurance coverage with a top priority (55.2%) [27]. This point will be solved soon since the Korean MHW has a plan to conduct a pilot program for insurance coverage expansion to the THMs in the latter half of 2019 [32].

The Korean MHW has a plan to conduct a pilot program for insurance coverage expansion to the THMs in the latter half of 2019 [32]. This point will make decoction types of THMs more affordable; this is mainly because the decoction type is found to be more potent and used in a wide range than the non-decoction type. As mentioned by Yoo and Son [33], unlike the non-decoction types of THMs, which were mostly used for therapeutic purposes (89.0%), the decoction types of THMs were not only used for the purpose of treatment of diseases (62.5%) but also for health improvement purposes (21.9%). THMs, especially decoction types of THMs, have played an important role in the prevention of diseases and health promotion in people in South Korea [33]. It is related to the viewpoint of TKM, which improves the vitality by correcting the imbalance of the human body, and the traditional way of boiling herbs reflects this [33].

In respiratory diseases, non-decoction types of THMs (20.5%) were more frequently prescribed than decoction types of THMs (6.3%). It is important to take the medication early during a cold, because of the need to combat the virus. It relieves

symptoms such as cough, nasal congestion, and rhinorrhea [34]. The decoction types of THMs require about 1 day of preparation time because it takes time to boil the mixed herbs. In the case of non-decoction types of THMs, it is presumed that it is used in respiratory diseases such as cold, because it can be prepared in advance, or ready-made products (pharmaceutical products) can be prescribed immediately.

Koreans suffer from digestive diseases due to the preference of spicy and salty Foods, and frequent use of antibiotics [35, 36]. In general, decoction types of THMs are used for treating digestive diseases [27]. The reason for using decoction types of THMs for treating digestive diseases is related to drug manufacturing method and patient's digestive condition [37]. As a result of that, decoction types of THMs seem to be more preferable and suitable for digestive diseases [27, 37].

There are several limitations in this study. First, some data were limited or deficient due to their limited sources, and the information may be biased despite our efforts to obtain objective data. Since this study was particularly purposed to identify the prescription of THMs for each disease, there is a limitation to the analysis by excluding "other" diseases.

Second, we could not analyze the results of the use of all Korean THMs because the questionnaire was designed specifically for decoction types of THMs. In addition, we could not assess the utilization status of different THM forms (e.g., granule, pill, tablet, soft extract, paste, and powder) and their target diseases, except for the decoction types of THMs. The dosage has an important influence on the treatment [38]. In order to examine the whole use and status of dosage forms of THMs, the questionnaire needs to be revised in a future survey.

Third, we investigated the disease categories, not the specific diseases that THMs were prescribed for. In the 2020 national survey, the questionnaire needs to be corrected so that the respondents can reply in regard to specific diseases.

5. Conclusions

Our study, based on a national survey of TKM doctors, presented the overall status of medical use of THMs in South Korea. However, the 2017 national survey was conducted separately for decoction and non-decoction types of THMs. Therefore, we could not assess the whole status of THM usage and therapeutic effects of each THM formulation. In future survey, we hope to be able to assess the details of medical use of THMs in South Korea.

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Conflict of interest

The authors declare no conflict of interest.

Abbreviations

THM	Traditional herbal medicine
TM	Traditional medicine

TKM	Traditional Korean medicine
MFDS	Ministry of Food and Drug Safety
MHW	Ministry of Health and Welfare

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References

[1] Barnes PM, Bloom B, Nahin RL. Complementary and alternative medicine use among adults and children: United States, 2007. National Health Statistics Reports. 2008;**10**:1-23. DOI: 10.1016/j.sigm.2004.07.003

[2] Thomas K, Coleman P. Use of complementary or alternative medicine in a general population in Great Britain. Results from the national omnibus survey. Journal of Public Health. 2004;**26**:152-167. DOI: 10.1093/pubmed/fdh139

[3] Eardley S, Bishop FL, Prescott P, Cardini F, Brinkhaus B, Santos-Rey K, et al. A systematic literature review of complementary and alternative medicine prevalence in EU. Forschende Komplementärmedizin. 2012;**19**:18-28. DOI: 10.1159/000342708

[4] World Health Organization. Health of Indigenous Peoples. Geneva: World Health Organization; 2007. p. 1132

[5] Gardiner P, Graham R, Legedza ATR, Ahn AC, Eisenberg DM, Phillips RS. Factors associated with herbal therapy use by adults in the United States. Alternative Therapies in Health and Medicine. 2007;**13**:22-29

[6] Msomi NZ, Simelane MBC. Herbal Medicine. London: IntechOpen; 2018. pp. 215-227. DOI: 10.5772/intechopen.72816

[7] Ekor M. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. Frontiers in Pharmacology. 2013;**4**:177. DOI: 10.3389/fphar.2013.00177

[8] Wegener T. Patterns and trends in the use of herbal products, herbal medicine and herbal medicinal products. International Journal of Complementary & Alternative Medicine. 2017;**9**:317. DOI: 10.15406/ijcam.2017.09.00317

[9] Research and Markets. Europe Spice and Herb Extracts Market—Segmented by Type, Application, and Geography—Growth, Trends and Forecasts (2018-2023). Dublin: Research and Markets; 2018

[10] Smith T, Kawa K, Eckl V, Morton C, Stredney R. Herbal supplement sales in US increase 7.7% in 2016. Herbal Gram. 2017;**115**:56-65

[11] Market Research Future. Herbal Medicine Market Research Report—Forecast to 2023. Maharashtra: WantStats Research and Media Pvt. Ltd; 2018

[12] Yuan H, Ma Q, Ye L, Piao G. The traditional medicine and modern medicine from natural products. Molecules. 2016;**21**:E559. DOI: 10.3390/molecules21050559

[13] Pan SY, Litscher G, Chan K, Yu ZL, Chen HQ, Ko KM. Traditional medicines in the world: Where to go next? Evidence-based Complementary and Alternative Medicine. 2014;**2014**:739895. DOI: 10.1155/2014/739895

[14] Kumar H, Song SY, More SV, Kang SM, Kim BW, Choi DK. Traditional Korean East Asian medicine and herbal formulations for cognitive impairment. Molecules. 2013;**18**:14670-14693. DOI: 10.3390/molecules181214670

[15] Vickers A, Zollman C, Lee R. Herbal medicine. The Western Journal of Medicine. 2001;**175**:125-128. DOI: 10.1136/ewjm.175.2.125

[16] Na-Bangchang K, Karbwang J. Traditional herbal medicine for the control of tropical diseases. Tropical

Medicine and Health. 2013;**42**:3-13. DOI: 10.2149/tmh.2014-S01

[17] Li Y, Zhu X, Bensussan A, Li P, Moylan E, Delaney G, et al. Herbal medicine for hot flushed induced by endocrine therapy in women with breast cancer: A systematic review and meta-analysis. Evidence-based Complementary and Alternative Medicine. 2016;**2016**:1327251. DOI: 10.1155/2016/1327251

[18] Park HL, Lee HS, Shin BC, Liu JP, Shang Q, Yamashita H, et al. Traditional medicine in China, Korea, and Japan: A brief introduction and comparison. Evidence-based Complementary and Alternative Medicine. 2012;**2012**:429103. DOI: 10.1155/2012/429103

[19] Cheung F. TCM: Made in China. Nature. 2011;**480**:S82-S83. DOI: 10.1038/480S82a

[20] Kim JY, Pham DD. Sasang constitutional medicine as a holistic tailored medicine. Evidence-based Complementary and Alternative Medicine. 2009;**6**:11-19. DOI: 10.1093/ecam/nep100

[21] Kang DY, Ahn KM, Kang HR, Cho SH. Past, present, and future of pharmacovigilance in Korea. Asia Pacific Allergy. 2017;7:173-178. DOI: 10.5415/apallergy.2017.7.3.173

[22] Korea Ministry of Food and Drug Safety. 2016 Ministry of Food and Drug Safety White Paper. Osang: Ministry of Food and Drug Safety; 2015

[23] Fan TP, Deal G, Koo HL, Rees D, Sun H, Chen S, et al. Future development of global regulations of Chinese herbal products. Journal of Ethnopharmacology. 2012;**140**:568-586. DOI: 10.1016/j.jep.2012.02.029

[24] National Law Information Center. Korean Pharmacopoeia [Internet]. 2019. Available from: http://www.law.go.kr/LSW/admRulInfoP.do?admRulSeq=2100000176764

[25] National Law Information Center. Korean Herbal Pharmacopoeia [Internet]. 2019. Available from: http://www.law.go.kr/LSW/admRulInfoP.do?admRulSeq=2100000176201

[26] Lee YJ, Park CG. A Study on the Collection of Oriental Herbal Medicine and the Production of Standardized Items. Osong: Ministry of Food and Drug Safety; 2015. Available from: http://www.ndsl.kr/ndsl/commons/util/ndslOriginalView.do?dbt=TRKO&cn=TRKO201600010697&rn=&url=&pageCode=PG18

[27] Ministry of Health and Welfare, National Development Institute of Korean Medicine, Gallup Korea. 2017 Years National Survey for Usage and Consumption of Traditional Herbal Medicine. Seoul: National Development Institute of Korean Medicine; 2018. Available from: http://www.koms.or.kr/board/researchReport/view.do?post_no=43&menu_no=21

[28] Kwon SH, Heo SH, Kim DS, Kang SH, Woo JM. Changes in trust and the use of Korean medicine in South Korea: A comparison of surveys in 2011 and 2014. BMC Complementary and Alternative Medicine. 2017;**17**:463. DOI: 10.1186/s12906-017-1969-8

[29] Choi DW, Kim JH, Cho SY, Kim DH, Chang SY. Regulation and quality control of herbal drugs in Korea. Toxicology. 2002;**181-182**:581-586. DOI: 10.1016/S0300-483X(02)00487-0

[30] Yarnell E, Abascal K. Herbal medicine in Korea "alternative" is mainstream. Alternative and Complementary Therapies. 2004;**10**:161-166. DOI: 10.1089/1076280041138298

[31] Pusan National University. A Study on Establishment of

Foundation for Strengthening the Health Insurance Coverage of Herbal Medicine(Decoction). Wonju: National Health Insurance Service; 2018. Available from: http://www.alio.go.kr/popSusiViewB1040.do

[32] Ministry of Health and Welfare. National Health Insurance Comprehensive Plan (2019-2023). Sejong: Ministry of Health and Welfare; 2019. Available from: http://www.mohw.go.kr/react/al/sal0301vw.jsp?PAR_MENU_ID=04&MENU_ID=0403&CONT_SEQ=349272

[33] Yoo SR, Son CG. Survey analysis of 101 subjects using herbal medicine with deer antler. Journal of Korean Medicine. 2008;**29**:41-46. Available from: https://www.jkom.org/journal/view.php?number=3465

[34] Simasek M, Blandino DA. Treatment of the common cold. American Family Physician. 2007;**75**:515-520

[35] Jung HK, Jang BH, Kim YH, Park JY, Park SY, Nam MH, et al. Health care costs of digestive diseases in Korea. The Korean Journal of Gastroenterology. 2011;**58**:323-331. DOI: 10.4166/kjg.2011.58.6.323

[36] Kim BN. Overview of antibiotic use in Korea. Infection & Chemotherapy. 2012;**44**:250-262. DOI: 10.3947/ic.2012.44.4.250

[37] Kim HC. Study on the Standardization of Drug Processing in Traditional Korean Medicine. Sejong: Ministry of Health and Welfare; 2001

[38] Yang XY, Shi GX, Li QQ, Zhang ZH, Wu A, Liu CZ. Characterization of Deqi sensation and acupuncture effect. Evidence-based Complementary and Alternative Medicine. 2013;**2013**:319734. DOI: 10.1155/2013/319734

Chapter 8

Pharmacological Activities and Phytochemicals of *Etlingera pavieana* (Pierre ex Gagnep) R.M.Sm

Klaokwan Srisook and Ekaruth Srisook

Abstract

Etlingera pavieana (Pierre ex Gagnep) R.M.Sm. (Zingiberaceae family) is commonly found in Southeast Asia. The rhizome of the plant is used as a spice and folk medicine in southeastern Thailand and Cambodia. The extracts, essential oil, and compounds from *E. pavieana* were found to possess a variety of pharmacological activities like anti-inflammatory, antioxidant, antiatherogenic, and antimicrobial effects. Furthermore, phytochemical studies have reported the presence of various chemical constituents, the main being phenylpropanoids such as *trans*-4-methoxycinnamaldehyde (MCD) and 4-methoxycinnamyl *p*-coumarate (MCC). Therefore, *E. pavieana* seems to be a potential source of natural products for treatment of various diseases and promotion of good health.

Keywords: *Etlingera pavieana*, pharmacological activity, phytochemical constituents, Zingiberaceae

1. Introduction

Plant-based nutraceuticals or functional foods have gained attention due to their health promotion and safety in comparison to synthetic food ingredients [1, 2]. Presently, worldwide researchers have focus on scientific evaluation of medicinal plants to detect their pharmacological activities. Zingiberaceae family is distributed worldwide and comprises approximately 52 genera and 1587 species [3]. It is about 300 species belonging to 26 genera found in Thailand [4]. The plants in this family are the natural sources for traditional medicine, foods, spice, and other ethnobotanical uses [4]. *Etlingera pavieana* (Pierre ex Gagnep) R.M.Sm. is a member of the Zingiberaceae family and a medicinal plant of southeastern Thailand [5]. Several *in vitro* and *in vivo* pharmacological experiments and phytochemistry studies of *E. pavieana* have been reported. This chapter has presented comprehensive information about morphological characteristics, traditional uses, pharmacology activities, and phytochemical constituents of *E. pavieana*, which provide the data to plan future studies.

2. Botanical description and uses of *Etlingera pavieana* (Pierre ex Gagnep) R.M.Sm.

E. pavieana is endemic to Southeast Asia including Thailand, Cambodia, Laos, and Vietnam. This plant is locally known in Thai as rew-hom/raew hawm [5]. It is a perennial rhizomatous herb. The leafy shoots can grow to a height of 1–2.5 m (**Figure 1A**). The leaves are narrowly obovate, green, and glabrous (**Figure 1B**). The rhizomes are long-creeping, slender (0.7–1.5 cm in diameter), and fragrant (**Figure 1C**). Inflorescence arises from the rhizomes and is partially embedded in the ground (**Figure 1D**). To date *E. pavieana* is found in the natural habitats and cultivated in fruit gardens in southeastern Thailand for commercial purposes as food and herbs [5]. The young shoots are eaten as a culinary vegetable, and the rhizomes are used as a spice, an ingredient in noodle soup "Moo Lieng," and as medicines. The reported medicinal uses of the rhizomes are relieving fever and flatulence and helping the digestive system and diuresis [5, 6]. In Cambodia, the *E. pavieana* rhizome is boiled in water with *Amomum verum* Blackw. and sugar to make a drink as a tonic and as a medicine for relieving stomach disorder and pharyngitis [5].

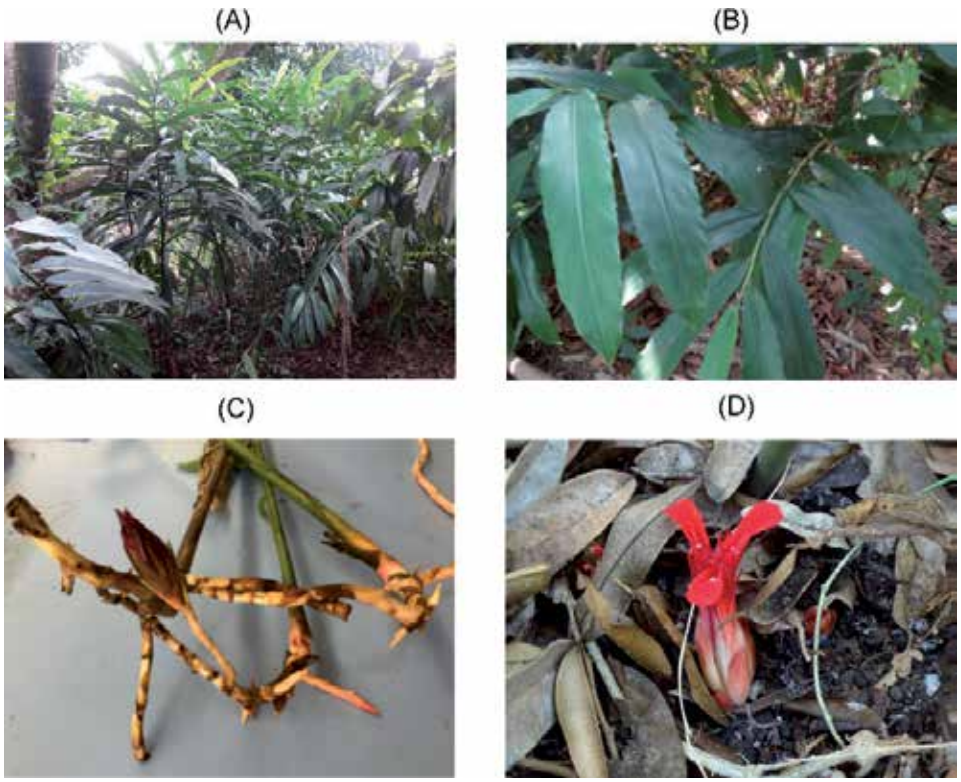

Figure 1.
Etlingera pavieana (Pierre ex Gagnep) R.M.Sm. whole plant (A), leaves (B), rhizomes (C), inflorescence (D) (Photographed by Srisook, K).

3. Phytochemical constituents of *E. pavieana*

It has been recently reported that the investigation of rhizomes of *Etlingera pavieana* from Thailand resulted in isolation of an uncommon compound which is (E)-((E)-3-(4-methoxyphenyl)ally) 3-(4-hydroxyphenyl) acrylate or 4-methoxycinnamyl *p*-coumarate (MCC) (**1**) and a series of phenylpropanoids such as

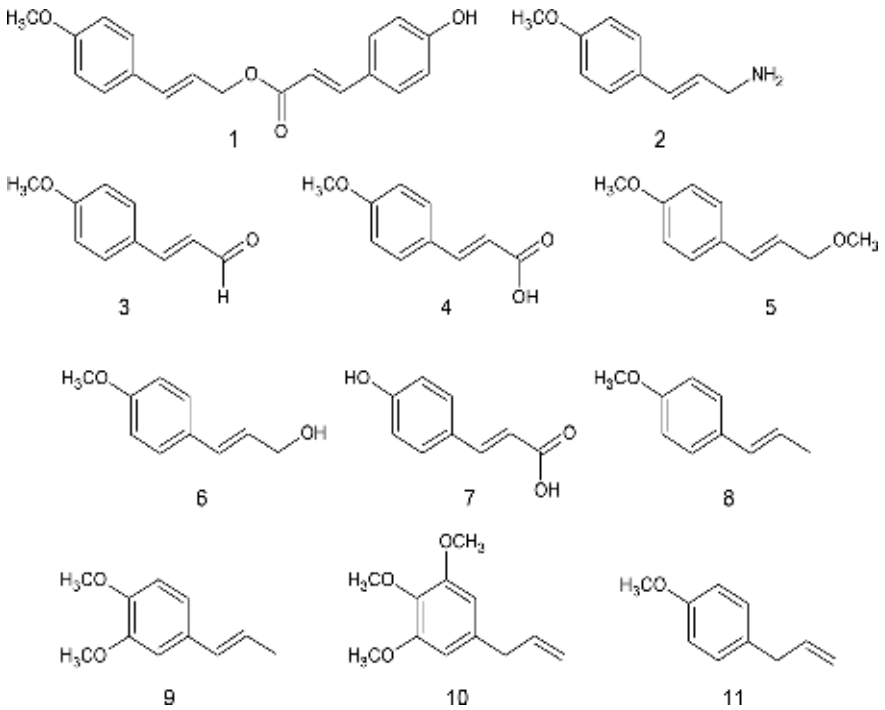

Figure 2.
Chemical structures of some phytochemicals from rhizomes and leaves of E. pavieana.

(E)-3-(4-methoxyphenyl)prop-en-1-amine (**2**), 3-(4-methoxyphenyl) prop-2-enal, or *trans*-4-methoxycinnamaldehyde (MCD) (**3**), (E)-4-methoxycinnamic acid (**4**), and (E)-1-methoxy-4-(3-methoxyprop-1-enyl) benzene (**5**) as shown in **Figure 2** [7]. Moreover, Srisook et al. have reported the isolation of the bioactive compound **1** and its two precursors, (2E)-3-(4-methoxyphenyl)prop-2-en-1-ol or 4-methoxycinnamyl alcohol (MCA) (**6**) and (E)-3-(4-hydroxyphenyl)prop-2-enoic acid or *p*-coumaric acid (CM) (**7**), from the rhizome of *E. pavieana* by activity-guided isolation [8]. The other three common phenylpropanoids, (E)-4-methoxycinnamaldehyde (**3**), *trans*-anethole (**8**), and *trans*-methyl isoeugenol (**9**), were also extracted from the plant by Srisook and colleagues (unpublished data).

The essential oil investigation of rhizome of *E. pavieana* resulted in *trans*-anethole (**8**) (48.5%) and elemicin (**10**, 13.8%) as major compounds [9], while Srisook et al. have investigated that the hydrodistillation of leaves of *E. pavieana* yielded methyl chavicol (**11**) as the most predominant product (93%) (unpublished data).

4. Pharmacological activities of *E. pavieana*

It is reported that *E. pavieana* possess various pharmacological activities such as antioxidant [10, 11]; antimicrobial [7, 12]; anticancer against breast, cervix, liver, colon, and small cell lung cancer [7, 13]; and anti-inflammatory effects[8, 14–16]. The detailed information is shown below.

4.1 Anti-inflammatory effect

Inflammation is a complex biological response to pathogenic agents and chemical as well as physical stimuli to get rid of the stimulants [17]. Macrophages are inflammatory cells that are considered as the first line of defense against various

stimuli [18]. Upon contact with injurious stimuli, macrophages are activated and produce a large amount of inflammatory mediators and cytokines such as reactive oxygen species (ROS), nitric oxide (NO), prostaglandins (PGs), tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6, and IL-12 [19–24]. It is well-known that excess and prolonged secretion of these mediators and cytokines has participated in the pathogenesis of a variety of inflammatory diseases such as inflammatory bowel diseases [25], osteoarthritis [26], cancer [23], multiple sclerosis [27], neurodegenerative diseases like Alzheimer's disease and Parkinson's disease [28–30], atherosclerosis, and cardiovascular diseases [24, 31–33]. Therefore, substances inhibiting the secretions of these mediators and cytokines may be used to treat or prevent a variety of inflammation-related diseases. Since some Zingiberaceae species including *Curcuma comosa* [34], *Amomum tsao-ko* [35], *Kaempferia parviflor*a [36], *Zingiber officinale* [37], *Alpinia officinarum* [38], *Alpinia pricei* Hayata [39], and *Alpinia katsumadai* Hayata [40] exhibit anti-inflammatory effects *in vitro* and *in vivo*, Srisook and colleagues, therefore, designed the experiment to determine anti-inflammatory activity of *E. pavieana* using lipopolysaccharide (LPS)- induced inflammation in RAW 264.7 murine macrophage cell model. A 95% ethanol extract of *E. pavieana* rhizomes was successively fractionated with hexane and ethyl acetate which was evaluated for anti-inflammatory effect on macrophage cells by inhibiting the production of inducible nitric oxide synthase (iNOS)-catalyzed NO. The hexane, ethyl acetate, and water fractions of rhizomal ethanol extracts of *E. pavieana* displayed anti-inflammatory effect on macrophage cells. Among them, ethyl acetate fraction was the most potent fraction. Furthermore, activity-guided isolation of this fraction showed that the bioactive compounds were phenolic compounds including 4-methoxycinnamyl alcohol (MCA), *trans*-4-methoxycinnamaldehyde (MCD), 4-methoxycinnamyl *p*-coumarate (MCC), and p-coumaric acid (CM) [8].

Mankhong et al. reported that 4-methoxycinnamyl *p*-coumarate, as the most potent compound extracted from *E. pavieana* rhizomes, exhibited an inhibitory effect on the production of cyclooxygenase-2 (COX-2)-catalyzed PGE_2, NO, IL-1β, and TNF-α on LPS-stimulated RAW 264.7 murine macrophages with IC_{50} values of 14.1 ± 2.3, 32.7 ± 4.7, 3.0 ± 0.5, and 26.8 ± 5.7 μM, respectively. MCC compound inhibited gene expression of iNOS, COX-2, IL-1β, and TNF-α in a concentration-dependent manner through downregulating NF-κB, PI3K/Akt, and AP-1 signaling pathways [14, 16]. Furthermore, MCC attenuated LPS-reduced COX-1 expression in LPS-stimulated RAW 264.7 macrophages. It suggests that MCC possesses anti-inflammatory activity with selective inhibitory effect on COX expression [14].

Recently, *trans*-4-methoxycinnamaldehyde, another compound isolated from *E. pavieana* rhizomes, suppressed the formation of NO and PGE_2 as well as the expression of their synthesizing enzymes, iNOS and COX-2, on LPS- and Pam3CSK4-induced RAW 264.7 cells. The IC_{50} values of NO and PGE_2 inhibition were 49.9 ± 4.7 and 87.6 ± 5.6 μM, respectively. The mechanism underlying anti-inflammatory activity of MCD could be inactivation of NF-κB and JNK/c-Jun signaling pathways [15]. Moreover, MCD showed a significant anti-inflammatory activity in rat models of acute inflammation. It was evident from this study that MCD (3 mg/ear) reduced ethyl phenylpropiolate (EPP)-induced ear edema by 51.5% inhibition. The second animal model used in this study was carrageenan-induced paw edema. Rats were orally administrated with MCD at doses of 75, 150, and 300 mg/kg for 1 h before injection with carrageenan. MCD can significantly decrease paw edema in a dose-dependent manner at 1, 3, and 5 h after carrageenan stimulation. Since edema formation induced by EPP and carrageenan is attributed to a release of several inflammatory mediators [41, 42], it is implied that the mode of MCD anti-inflammatory action *in vivo* model is mediated in part by suppression of inflammatory mediators.

We also investigated anti-inflammatory effect of essential oil of *E. pavieana* leaves on LPS-induced RAW 264.7 macrophages. The essential oil of *E. pavieana* leaves at 25–100 μg/mL exhibited a concentration-dependent inhibition effect on NO production without significant cytotoxicity (**Figure 3A**). Incubation of cells with essential oil caused reduction of iNOS protein and mRNA expression (**Figure 3B, C**). The compound responsible for anti-inflammatory effect of essential oil of *E. pavieana* leaves might be methyl chavicol, a main phytoconstituent of this essential oil (unpublished data).

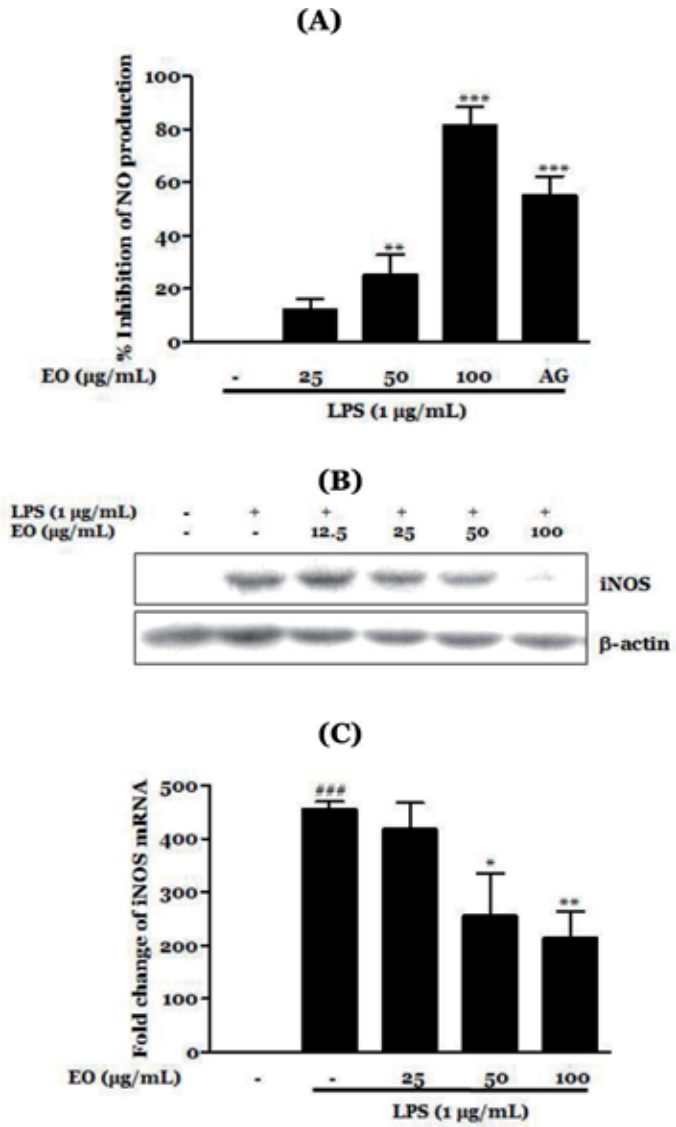

Figure 3.
The inhibitory effect of essential oil of E. pavieana leaves (EO) on LPS-induced NO production (A) and iNOS expression. RAW 264.7 cells were incubated with essential oil of E. pavieana leaves and LPS for 24 h for determination of NO production and iNOS protein as well as 9 h for mRNA expression. Supernatant was collected for the analysis of NO production by Griess reaction. Cells were lysed and determined for protein (B) and mRNA (C) expression by Western blot analysis and real-time RT-PCR, respectively. $^{###}p < 0.001$ *compared to unstimulated control cells,* $^{*}p < 0.05$, $^{**}p < 0.01$, *and* $^{***}p < 0.001$, *was significantly different from LPS-stimulated cells. AG = cells treated with 50 μM aminoguanidine, an iNOS inhibitor.*

4.2 Antiatherogenic effect

Atherosclerosis is a chronic inflammatory disorder which leads to cardiovascular diseases (CVDs) [43, 44]; it is a major cause of death in the world [24, 45]. Endothelial dysfunction is an early step in the development of atherosclerosis. It involves with reduced NO bioavailability, low-grade inflammation, and oxidative stress [44, 46–48]. The initiation of atherosclerotic lesion is influenced by the expression of cell adhesion molecules including vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) on the vascular surface of the endothelial cells resulting in recruitment of leukocytes into the vascular tissue [49]. Inhibiting the expression of ICAM-1 and VCAM-1 leads to reduction of leukocyte emigration and retardation of the development of atherosclerosis. Thus, the substance that inhibits the expression of these cell adhesion molecules may be considered as anti-atherosclerosis agent that prevents vascular inflammatory disorders.

The rhizomal ethanol extract of *E. pavieana* was assessed for its anti-vascular inflammatory effect in human umbilical vein endothelial cells (EA.hy926 cells). Endothelial activation is upregulated by various pro-inflammatory cytokines, including TNF-α secreted under inflammatory conditions. The *E. pavieana* extract inhibited TNF-α-induced expression of ICAM-1 and VCAM-1 protein and mRNA in a concentration-dependent manner. The inhibitory effect of the rhizome extract in endothelial cells is caused from interfering with the activation of NF-κB and JNK/c-Jun signaling pathways. Moreover, Akt activation by *E. pavieana* rhizome is associated with negative regulation of inflammation. This anti-vascular inflammatory activity was attributed in part due to the presence of the two most active phenolic compounds of which were 4-methoxycinnamyl *p*-coumarate and *trans*-4-methoxycinnamaldehyde [50].

It is believed that decrease in endothelium-derived NO, produced by endothelial NO synthase (eNOS), results in reduced NO bioavailability [44, 49]. Another study was carried out to demonstrate antiatherogenic effect of *E. pavieana* rhizome. EA.hy926 endothelial cells incubated with the ethanol extract of *E. pavieana* rhizome (12.5–200 μg/mL) caused increased NO level in a concentration-dependent manner. This induction might be partly mediated by the activation of eNOS enzyme via phosphorylation at Ser1177 [11].

4.3 Antioxidant effect

Accumulating evidences suggest that not only inactivation of eNOS enzyme but also increased superoxide level leads to the reduction in NO bioavailability [24, 49]. Superoxide anion is produced by the mitochondrial electron transport chain and several enzymes in the endothelial cells. Superoxide anion is then oxidized to hydrogen peroxide and other physiological reactive species such as hydroxyl radical and hypochlorous acid [44]. Moreover, it reacts with NO to form highly reactive peroxynitrite leading to eNOS dysfunction [24, 47]. Chronic inflammation is also closely associated with oxidative stress which generated excess ROS [24].

The hexane, ethyl acetate, and water fractions of ethanol extract of *E. pavieana* rhizomes have shown *in vitro* antioxidant effects, which are evaluated by various antioxidant activity assays including 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, reducing power and ferrous ion chelating activity [10]. *E. pavieana* rhizomal extract has also been assayed, its ROS scavenging activity in EA.hy926 endothelial cells, using 2,7-dichlorodihydrofluorescein diacetate (H_2DCF-DA) probe. TNF-α markedly induced ROS formation, while the extract (25–100 μg/mL) concentration dependently inhibited ROS level [11]. Thus, the data indicate antioxidant effect of *E. pavieana* mediated in part by counteract with ROS.

4.4 Anticancer activity

A number of plant-derived compounds can prevent progression of cancer [51, 52] and possess anticancer activity [53]. The ethanol extract from *E. pavieana* rhizomes exhibits cytotoxic effect against several cancer cell lines such as breast adenocarcinoma MDA-MB-231, hepatoma $HepG_2$, cervical carcinoma HeLa, and C33A. The IC_{50} values were ranging from 160 to 192 μg/mL, respectively. Trans-4-methoxycinnamaldehyde, an active compound isolated by cytotoxicity-guided isolation from *E. pavieana* rhizomes, has shown cytotoxic effects against C33A, colorectal carcinoma HCT116, MDA-MB-231, and $HepG_2$ with the IC_{50} values of 34.3, 38.7, 39.3, and 40.7 μM, respectively [13]. Tachai and Nuntawong [7] reported that 4-methoxycinnamyl *p*-coumarate exhibited inhibitory activity on cell growth of human breast cancer (MCF7), human oral cavity cancer (KB), and human small cell lung cancer (NCI-H187) with IC_{50} values of 25.1, 20.2, and 34.8 μM, respectively [7]. Essential oil from the rhizomes was found to have cytotoxic effect against NCI-H187 (IC_{50}: 31.7 μg/mL) [7].

4.5 Antimicrobial activity

Various solvent extracts of *E. pavieana* (ethanol, acetone, dichloromethane, ethyl acetate, petroleum ether, and hexane) were found to be active against Gram-positive bacteria *(Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus*, *Listeria monocytogenes*) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Vibrio parahaemolyticus*, and *Salmonella typhimurium*). Antimicrobial activity of leaves was higher than that of stem and rhizome [12]. Tachai and Nuntawong demonstrated that hexane, dichloromethane, and methanol extracts of *E. pavieana* rhizomes were inactive against *Mycobacterium tuberculosis*, while MCC exhibited its antimicrobial activity against *M. tuberculosis* with minimum inhibitory concentration at 50 μg/mL [7].

5. Conclusion

In this chapter, we have provided the information on botanical aspects, dietary and traditional uses, phytochemicals, and pharmacological activities of *Etlingera pavieana* which is distributed in Southeast Asia. The major active compounds are phenylpropanoids such as *trans*-4-methoxycinnamaldehyde, and 4-methoxycinnamyl *p*-coumarate. Its rhizomes exhibit anti-inflammatory, antioxidant, antiatherogenic, antimicrobial, and anticancer activities. The pharmacological activities reported here confirm therapeutic efficacy of *E. pavieana* rhizomes which might be developed into medicines or nutraceuticals for the treatment and prevention of various diseases, especially inflammation-related diseases. However, in vivo toxicology studies of *E. pavieana* rhizomes should be performed along with the clinical trials.

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Conflict of interest

The authors have no conflict of interest to declare.

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References

[1] Pandey N, Meena RP, Rai SK, Pansey-Rai S. Medicinal plants derived nutraceuticals: A Re-emerging health aid. International Journal of Pharma and Bio Sciences. 2011;**2**(4):420-441

[2] Srivastava RK. Need of nutraceuticals/functional food products for health benefits to world-wide people. Journal of Biotechnology and Biomedical Science. 2018;**1**(4):1-13. DOI: 10.14302/issn.2576-6694.jbbs-18-2408

[3] The Plant List. Version 1.1 [Internet]. 2013. Available from: http://www.theplantlist.org/ (accessed 1st January). [Accessed: 24-07-2019]

[4] Larsen K, Larsen SS. Gingers of Thailand. Chiang Mai, Thailand: Queen Sirikit Botanic Garden; 2006. 184p

[5] Poulsen DA, Phonsena P. Morphological variation and distribution of the useful ginger *Etlingera pavieana* (Zingiberaceae). Nordic Journal of Botany. 2017;**35**:467-475

[6] Phonsena P. Medicinal Plants in Khao Hin Son Herb Garden. Prachinburi: Jettanaromphun Printing; 2007. 301 p. (In Thai)

[7] Tachai S, Nuntawong N. Uncommon secondary metabolites from *Etlingera pavieana* rhizomes. Natural Product Research. 2016;**30**:2215-2219

[8] Srisook E, Palachot M, Mankhong S, Srisook K. Anti-inflammatory effect of *Etlingera pavieana* (Pierre ex Gagnep.) R.M.Sm. rhizomal extract and its phenolic compounds in lipopolysaccharide-stimulated macrophages. Pharmacognosy Magazine. 2017;**13**:s230-s235

[9] Tachai S, Wangkarn S, Nuntawong N. Chemical constituents of the rhizome oils of *Etlingera pavieana* (Pierre ex Gagnep.) R.M.Sm. Biochemical Systematics and Ecology. 2014;**57**:410-415

[10] Srisook K, Srisook E. Evaluation of antioxidant capacity of fractions from *Etlingera pavieana* (Pierre ex Gagnep.) R.M.Sm. rhizome. In: Proceedings of the 3rd International Natural Products for Health and Beauty; 16-18 March 2011; Thailand: Chiang Mai University

[11] Srisook K, Udompong S, Sawai P, Thongyen T. *Etlingera pavieana* rhizome extract decreases oxidative stress and activates eNOS activity via stimulation of Akt phosphorylation in human endothelial cells. Naresuan Phayao Journal. 2018;**11**:23-28

[12] Naksang P, Rachtanapun C. Effect of sample preparation on antibacterial activity of *Etlingera pavieana* (Pierre ex Gagnep.) R.M.Sm. extracts against foodborne bacteria. In: Proceedings of the 53rd Kasetsart University Annual Conference: Innovation for Bio-Health Supplements; February 2015; Thailand: Kasetsart University

[13] Iawsipo P, Srisook E, Ponglikitmongkol M, Tatiyar S, Singaed O. Cytotoxic effects of *Etlingera pavieana* rhizome on various cancer cells and identification of a potential anti-tumor component. Journal of Food Biochemistry. 2018;**42**:e12540:1-9

[14] Mankhong S, Srisook E, Srisook K. Anti-inflammatory activity of 4-methoxycinnamyl *p*-coumarate isolated from *Etlingera pavieana* rhizomes in lipopolysaccharide-induced macrophages. NU International Journal of Science. 2017;**14**:58-66

[15] Srisook K, Mankhong S, Chiranthanut N, Kongsamak K, Kitwiwat N, Tongjurai P, et al.

Anti-inflammatory effect of *trans*-4-methoxycinnamaldehyde from *Etlingera pavieana* in LPS-stimulated macrophages mediated through inactivation of NF-κB and JNK/c-Jun signaling pathways and in rat models of acute inflammation. Toxicology and Applied Pharmacology. 2019a;**371**:3-11

[16] Mankhong S, Iawsipo P, Srisook E, Srisook K. 4-methoxycinnamyl *p*-coumarate isolated from *Etlingera pavieana* rhizomes inhibits inflammatory response via suppression of NF-kB, Akt and AP-1 signaling in LPS-stimulated RAW 264.7 macrophages. Phytomedicine. 2019;**54**:89-97

[17] Kumar V, Abbas AK, Aster JC. Robbins Basic Pathology. 9th ed. Philadelphia, PA: Elsevier/Saunders; 2013. 928 p

[18] Kumar V. Macrophages: The potent immunoregulatory innate immune cells. In: Bhat KH, editor. Macrophage at the Crossroads of Innate and Adaptive Immunity. London: IntechOpen; 2019

[19] MacMicking J, Xie QW, Nathan C. Nitric oxide and macrophage function. Annual Review of Immunology. 1997;**15**:323-350

[20] Sherwood ER, Toliver-Kinsky T. Mechanisms of the inflammatory response. Best Practice and Research. Clinical Anaesthesiology. 2004;**18**:385-405

[21] FujiwaraN, KobayashiK. Macrophages in inflammation. Current Drug Targets. Inflammation and Allergy. 2005;**4**:281-286

[22] Barrientos S, Stojadinovic O, Golinko MS, Brem H, Tomic-Canic M. Growth factors and cytokines in wound healing. Wound Repair and Regeneration. 2008;**16**(5):585-601

[23] Fernandes JV, Cobucci RNO, Jatobá CAN, Fernandes TA, de Azevedo JWV, de Araújo JMG. The role of the mediators of inflammation in cancer development. Pathology and Oncology Research. 2015;**21**(3):527-534. DOI: 10.1007/s12253-015-9913-z

[24] Incalza MA, D'Oria R, Natalicchio A, Perrini S, Laviola L, Giorgino F. Oxidative stress and reactive oxygen species in endothelial dysfunction associated with cardiovascular and metabolic diseases. Vascular Pharmacology. 2018;**100**:1-19

[25] Soufli I, Toumi R, Rafa H, Touil-Boukoffa C. Overview of cytokines and nitric oxide involvement in immuno-pathogenesis of inflammatory bowel diseases. World Journal of Gastrointestinal Pharmacology and Therapeutics. 2016;**7**(3):353-360. DOI: 10.4292/wjgpt.v7.i3.353

[26] Philp AM, Davis ET, Jones SW. Developing anti-inflammatory therapeutics for patients with osteoarthritis. Rheumatology. 2017;**56**(6):869-881. DOI: 10.1093/rheumatology/kew278

[27] Grigoriadis N, van Pesch V. A basic overview of multiple sclerosis immunopathology. European Journal of Neurology. 2015;**22**(Suppl. 2):3-13. DOI: 10.1111/ene.12798

[28] Lull ME, Block ML. Microglial activation and chronic neurodegeneration. Neurotherapeutics. 2010;**7**(4):354-365

[29] Koppula S, Kumar H, Kim IS. Reactive oxygen species and inhibitors of inflammatory enzymes, NADPH oxidase, and iNOS in experimental models of Parkinson's disease. Mediators of Inflammation. 2012;**2012**:16-16

[30] Asiimwe N, Yeo SG, Kim MS, Jung J, Jeong NY. Nitric oxide: Exploring the contextual link with Alzheimer's disease. Oxidative and Cellular

Longevity. 2016. Article ID 7205747, 10 pages. DOI: 10.1155/2016/7205747

[31] Khan R, Spagnoli V, Tardif JC, Allier P. Novel anti-inflammatory therapies for the treatment of atherosclerosis. Atherosclerosis. 2015;**240**:497-509

[32] Shayganni E, Bahmani M, Asgary S, Rafieian-Kopaei M. Inflammaging and cardiovascular disease: Management by medicinal plants. Phytomedicine. 2016;**23**:1119-1126

[33] Welsh P, Grassia G, Botha S, Sattar N, Maffia P. Targeting inflammation to reduce cardiovascular disease risk: A realistic clinical prospect? British Journal of Pharmacology. 2017;**174**:3898-3913

[34] Jantaratnotai N, Utaisincharoen P, Piyachaturawat P, Chongthammakun S, Sanvarinda Y. Inhibitory effect of *Curcuma comosa* on NO production and cytokine expression in LPS-activated microglia. Life Sciences. 2006;**78**:571-577

[35] Lee KY, Kim SH, Sung SH, Kim YC. Inhibitory constituents of lipopolysaccharide-induced nitric oxide production in BV2 microglia isolated from *Amomum tsao-ko*. Planta Medica. 2008;**74**:867-886

[36] Sae-Wong C, Tansakul P, Tewtrakul S. Anti-inflammatory mechanism of *Kaempferia parviflora* in murine macrophage cells (RAW264.7) and in experimental animals. Journal of Ethnopharmacology. 2009;**124**:576-580

[37] Jung HW, Yoon CH, Park KM, Han HS, Park YK. Hexane fraction of *Zingiberis rhizoma* crude extract inhibits the production of nitric oxide and pro-inflammatory cytokines in LPS-stimulated BV2 microglial cells via the NF-kappa B pathway. Food and Chemical Toxicology. 2009;**47**:1190-1197

[38] Lee JS, Kim KA, Jeong SH, Lee SG, Park HJ, Kim NJ, et al. Anti-inflammatory, anti-nociceptive, and anti-psychiatric effects by the rhizomes of *Alpinia officinarum* on complete Freund's adjuvant-induced arthritis in rats. Journal of Ethnopharmacology. 2009;**126**:258-264

[39] Yu YS, Hsu CL, Yen GC. Anti-inflammatory effects of the roots of *Alpinia pricei* Hayata and its phenolic compounds. Journal of Agriculture and Food Chemistry. 2009;**57**:7673-7680

[40] Lee MY, Seo CS, Lee JA, Shin IS, Kim SJ, Ha HK, et al. *Alpinia katsumadai* HAYATA seed extract inhibit LPS-induced inflammation by induction of heme oxygenase-1 in RAW264.7 cells. Journal of Inflammation. 2011;**10**:1007-1019

[41] Carlson R, O'Neill-Davis L, Chang J, Lewis A. Modulation of mouse ear edema by cyclooxygenase and lipoxygenase inhibitors and other pharmacologic agents. Agents and Actions. 1985;**17**:197-204

[42] Mansouri MT, Hemmati AA, Naghizadeh B, Mard SA, Rezaie A, Ghorbanzadeh B. A study of the mechanisms underlying the anti-inflammatory effect of ellagic acid in carrageenan-induced paw edema in rats. Indian Journal of Pharmacology. 2015;**47**:292-298

[43] Stoner L, Lucero A, Palmer BR, Jones LM, Young JM, Faulkner J. Inflammatory biomarkers for predicting cardiovascular disease. Clinical Biochemistry. 2013;**46**:1353-1371

[44] Haybar H, Shahrabi S, Rezaeeyan H, Shirzad R, Saki N. Endothelial cells: From dysfunction mechanism to pharmacological effect in cardiovascular disease. Cardiovascular Toxicology 2019;**19**:13-22

[45] World Health Organization. Global Status Report on Noncommunicable Diseases 2014 [Internet]. 2014. Available from: https://www.who.int/nmh/publications/ncd-status-report-2014/en/ [Accessed: 2 July, 2019]

[46] Clapp BR, Hingorani AD, Kharbanda RK, Mohamed-Ali V, Stephens JW, Vallance P, et al. Inflammation-induced endothelial dysfunction involves reduced nitric oxide bioavailability and increased oxidant stress. Cardiovascular Research. 2004;**64**(1):172-178

[47] Vanhoutte PM, Shimokawa H, Tang EH, Feletou M. Endothelial dysfunction and vascular disease. Acta Physiologica (Oxford). 2009;**196**:193-222

[48] Park KH, Park WJ. Endothelial dysfunction: Clinical implications in cardiovascular disease and therapeutic approaches. Journal of Korean Medical Science. 2015;**30**(9):1213-1225

[49] Čejková S, Králová-Lesná I, Poledne R. Monocyte adhesion to the endothelium is an initial stage of atherosclerosis development. Cor et Vasa. 2016;**58**(4):e419-e425

[50] Srisook K, Potiprasart K, Sarapusit S, Park CS, Srisook E. *Etlingera pavieana* extract attenuates TNF-α induced vascular adhesion molecule expression in human endothelial cells through NF-κB and Akt/JNK pathways. Inflammopharmacology. 2019b submitted

[51] Butt MS, Naz A, Sultan MT, Qayyum MMN. Anti-oncogenic perspectives of spices/herbs: A comprehensive review. EXCLI Journal. 2013;**12**:1043-1065

[52] Madigan M, Karhu E. The role of plant-based nutrition in cancer prevention. Journal of Unexplored Medical Data. 2018;**3**:9. DOI: 10.20517/2572-8180.2018.05

[53] Roy A, Ahuja S, Bharadvaja N. A review on medicinal plants against cancer. Journal of Plant Sciences and Agricultural Research. 2017;**2**(1:008):1-5

Section 2

Medicinal Plants and Cancer Treatment

Chapter 9

Plants and Cancer Treatment

Bassam Hassan

Abstract

In this century, cancer has become one of the major problems and diseases that have caused predominant death, and it will even surpass heart diseases. Since World War I, chemotherapy has become as one of the most important and significant treatments of cancer. Even if it can cure cancer, it is found to cause several critical side effects. Over the past 20 years, many types of new therapies have emerged; some of them were extracted from plants that are found to be effective and safe. Moreover, it has been approved that several plants, herbs, and vegetables can prevent or reduce incidence of cancer in several sites of the human body. Besides, researchers found that they are a great source for developing and producing new, effective, tolerable, and safe anticancer drugs compared with the synthetic ones. As a result of that, researchers recommended future studies to focus more on plant as a source for safe and effective anticancer treatment.

Keywords: cancer, plants, anti-growth, apoptosis, treatment

1. Cancer background

During this century, cancer has become one of the major problems and diseases which has caused predominant death, and it will even surpass heart diseases. Many of the researchers begin to use the term lifetime risk for cancer patients which refer to the time that cancer will progress and developed or the time that the patient will die because of cancer. Cancer does not represent only one disease but it is a group involving about 100 diseases. It is characterized by two things: Firstly there is no control for the growth of cancer cells, and secondly it is the ability of the cancer cells to metastasize and migrate from the original site to different parts of the body. There are two types of tumors which are malignant and benign. Cancer can attack any person, and its occurrence increases as the age of the individual increases too [1, 2]. There are many problems (i.e., side effects) associated with cancer diseases either solid or hematological cancer such as nausea, vomiting, diarrhea, constipation, hypercalcemia, pain, loss of appetite, anemia, fatigue, cachexia, leucopenia, neutropenia, and thrombocytopenia. However the major problems are nausea and vomiting, neutropenia, anemia, thrombocytopenia, and hypercalcemia. Hence due to these reasons, cancer is considered as one of the major diseases that will affect the quality of life [3–6].

2. Chemotherapy background

Chemotherapy was developed and used since the Word War I from the chemical weapon program of the United States of America (USA). Since then chemotherapy

has become as one of the most important and significant treatments of cancer. Its main mechanism of action is by killing the cancer cells which are characterized by their high multiplication and growth rate. It will also kill all the cancer cells that had broken off from the main tumor and spread to the blood or lymphatic system or any part of the body. This killing process of cells is either by a direct effect on deoxyribonucleic acid (DNA) or an effect on the factors involved in mitosis by inhibition of its synthesis or production or uses [7–9]. Chemotherapy drug may lead to complete cure for some types of cancers or may suppress the growth of others or may prevent their spread to other parts of the body. So many types of new therapies have emerged over the past 20 years. Some of them were straight forward, effective, and safe and some have many side effects. However when comparing chemotherapy with other types of treatments, it still remains potentially high risk with many side effects which are difficult to manage. The chemotherapy used required the involvement of various clinical professionals during its various stages of administration, and enormous patient health care is needed to overcome its side effects [7, 10].

3. Chemotherapy side effects

The goal of chemotherapy is to be as effective as possible with tolerable side effects, since the dose of chemotherapy will be toxic to the cancer cells as well as to the normal cells. A proportion of the cancer patients suffer from only mild side effects, whereas others may suffer from serious side effects [10–12].

These side effects are classified as:

1. Acute, which develop within 24 hours after chemotherapy administration
2. Delayed, which developed after 24 hours and up to 6–8 weeks after chemotherapy treatments
3. Short term, combination of both acute and delayed effect
4. Late/long term, which developed after months or years of chemotherapy treatment
5. Expected, which developed among 75% of the patients
6. Common, occurred in 25–75% of the patients
7. Uncommon, happened less than 15% of the patients
8. Rare, occur in only 5% of the patients
9. Very rare, occur on less than 1% of the patients [10–12]

Occurrence of specific side effects will vary according to the chemotherapy used. The most common side effects experienced are nausea and vomiting, anemia, hair lost, bleeding, thrombocytopenia, hyperuricemia, bone marrow depression, alopecia, and mucositis. So different parameters must be taken into consideration to prevent, reduce, and overcome these side effects [10–12].

4. Plants and cancer

Herbal medicine has been used as a major treatment for cancer in various countries in the Middle East and Europe long time ago. Recent reports released by the World Health Organization (WHO) showed that although many advanced countries have considered traditional herbal treatment as an official treatment for cancer, only 5–15% of these herbs have been investigated to detect their bioactive compounds, i.e., anticancer compounds [13–15].

5. Plants and cancer treatment and prevention

According to the two famous Islamic physicians (Rhazes and Avicenna), diseases need to be treated by using a scheme which consists of three options; the first option will be by using physiotherapy and diet, the second one will be by using drugs, and the last option will be surgery. Drugs used on that time have been classified as simple and compound drugs. Treatment of any disease will start with the simple one to avoid drug–drug interaction; unless it did not work, then physician will use the compound drugs, and when second option failed too, then surgery will be used [15].

Regarding cancer treatment, the Islamic scholar "Avicenna" mentioned that "if it is the start of a cancer, it is possible to make it static and prevent it from growth and hence ulceration" [14].

Researchers mentioned that herbal-based medicines are found to be one of the best choices for treating and/or preventing incidence of cancer. This is mainly because of the varieties of active substances that plants contained which work against many types of cancers in several mechanisms. These compounds can be extracted and can be used alone or in combination with other anticancer treatments. In comparison with synthetic drugs, these natural compounds are found to be naturally available, cheaper, and easy to administered orally and have low or minimal side effects, and they are found to be rich of various biologically active chemotypes [14–16, 19].

Avni and colleagues mentioned there are several plants work as "Chemopreventive agents against many types of cancers like; *Abrus precatorius* on Yoshida sarcoma, *Albizia lebbeck* on sarcoma, and *Alstonia scholaries* on forestomach carcinoma". Other plants characterized by anticancer activity like "*Anacardium occidentale* in hepatoma, *Asparagus racemosus* in human epidermoid carcinoma, *Boswellia serrata* in human epidermal carcinoma of the nasopharynx, *Erythrina suberosa* in sarcoma, *Euphorbia hirta* in Freund virus leukemia, *Gynandropsis pentaphylla* in hepatoma, *Nigella sativa* in Lewis lung carcinoma, *paederia foetida* in human epidermoid carcinoma of the nasopharynx, *picrorhiza kurroa* in hepatic cancers, and *Withania somnifera* in various tumors" [20].

One of the most critical problems associated with cancer treatment is chemotherapy resistance, that's why researchers trying their best to prevent or reduce incidence of resistance by detecting new anticancer agents as an alternate [14].

Thazin and colleagues mentioned that natural compounds extracted from plants have not only the ability to work as anticancer agents but also to restore chemotherapy sensitivity, for example, tetrandrine which is an active alkaloid compound extracted from plant enhances doxorubicin anticancer activity against resistant MCF-1/DOX cells in vivo via modulating P-gp-mediated drug efflux. Another natural compound is quercetin (flavonoid) which restores daunorubicin chemosensitivity in resistant HL-60/DOX and K562/DOX cell lines via suppression of P-gp

expression. Curcumin also increases vincristine chemotherapy activity in SGC7901/VCR cell lines by suppressing ABC transporters such as P-gp, MRP1, and ABCG2 proteins [21].

Jana and colleagues conducted in vitro study to determine the anticancer, antiproliferative, and cytotoxic effect of brassinosteroids (BRs) which are steroids extracted from plants against (MCF-7/MDA-MB-468) breast and (LNCaP/DU-145) prostate cancer cell lines and normal cell line. Results showed that RBs significantly arrested MCF-7, MDA-MB-468, and LNCaP cells in G1 phase of the cell cycle and induced apoptosis in MDA-MB-468, LNCaP, and slightly in the DU-145 cells, without any toxic effect against normal cell lines. These results support the point that RB compounds are a promising source for anticancer drugs [22]. Another in vitro study is conducted to detect the antiproliferative and cytotoxic effect of the aqueous extract of *A. ascalonicum* against Wehi164 (mouse fibrosarcoma cells), Jurkat (human acute T-cell leukemia) and K562 (human erythroleukemia), and human umbilical vein endothelial cells (HUVEC) as a normal cell line. Results showed that the extract showed a significant antiproliferative effect against all cancer cell lines and a dose and time cytotoxic effect against them with a very low cytotoxic effect against a normal cell line. These results showed that the *Allium ascalonicum* plant is a promising source for a potent anticancer treatment for several types of cancers [23].

About cancer prevention, it has been approved that several plants, herbs, and vegetables can prevent or reduce the incidence of cancer in several sites of the human body [14].

An in vitro study is conducted by a group of researchers trying to detect the ability of ethyl acetate extract of onion (EEO) to cause inhibition for cancer growth and cause apoptosis in human breast cancer MDA-MB-231. Results showed that EEO cause apoptosis for MDA-MB-231 breast cancer cell line and prevent incidence (i.e., growth) of breast cancer by inhibiting fatty acid synthase (FAS) production and accumulation in adipose tissues [24].

Another in vitro study is conducted by Arif and colleagues to detect the antitumor effect of *Aloe vera* crude extract (ACE) alone and in combination with cisplatin on human breast carcinoma cell line (MCF-7) and human cervical carcinoma cell line (HeLa) [25]. **T**he cytotoxic potential of ***Aloe vera*** crude extract alone or in combination with cisplatin in human breast (MCF-7) and cervical (HeLa) cancer cells was studied by cell viability assay, nuclear morphological examination, and cell cycle analysis. Effects were correlated with the modulation of expression of genes involved in cell cycle regulation, apoptosis, and drug metabolism by RT-PCR. "Results showed that exposure of cells to ACE resulted in considerable loss of cell viability in a dose- and time-dependent fashion, which was found to be mediated by through the apoptotic pathway as evidenced by changes in the nuclear morphology and the distribution of cells in the different phases of the cell cycle. Interestingly, ACE did not have any significant cytotoxicity towards normal cells, thus placing it in the category of safe chemopreventive agent. Further, the effects were correlated with the downregulation of cyclin D1, CYP 1A1, and CYP 1A2 and increased expression of bax and p21 in MCF-7 and HeLa cells. In addition, a low-dose combination of ACE and cisplatin showed a combination index less than 1, indicating synergistic growth inhibition compared to the agents applied individually. In conclusion, these results signify that *Aloe vera* may be an effective antineoplastic agent to inhibit cancer cell growth and increase the therapeutic efficacy of conventional drugs like cisplatin. Thus promoting the development of plant-derived therapeutic agents appears warranted for novel cancer treatment strategies" [25].

6. Anticancer plant-derived drugs

About two-thirds of the anticancer treatments are extracted from plants, and these drugs are divided into several classes depending on their pharmacological effect: antimitotics [vinca alkaloids (e.g., vincristine and vinblastine), podophyllotoxins (e.g., etoposide and teniposide), and taxanes (e.g., paclitaxel, docetaxel)], topoisomerase inhibiters [Topo I (e.g., topotecan and irinotecan), Topo II (e.g., ellipticine and podophyllotoxins)], ROS inducers (e.g., EGCG2 and thymoquinone), angiogenesis inhibitors (e.g., flavopiridol), histone deacetylases (HDAC) inhibitors (e.g., sulforaphane and pomiferin), and mitotic disruptors (e.g., roscovitine) [3, 4, 15].

An *in vitro* study is conducted by Maram and colleagues to detect the antitumor effect of *Aloe vera* (*A. vera*) and *Calligonum* extracts on hepatocellular carcinoma (HepG2) cells. Viability, apoptosis, and DNA damage of these cells have been tested after exposure to different concentrations of the two extracts. Results showed that the extracts of these two plants could have an antitumor effect against HepG2 cells. Thus, these two plants can be promising sources for future anticancer treatment [26].

Nadia and colleagues conducted an *in vitro* study in which the main aim was to detect the anticancer effect of ethyl acetate extract of *Crataegus azarolus* against HCT-116 and HT-29 human colorectal cancer cell lines. Results showed that the extract demonstrated strong cytotoxic and anti-growth activities via several mechanisms. Moreover, its apoptotic effect is associated with the elevation of p21 expression but not through p53 activation. As a result of that, authors concluded that this compound can be used as anticancer for treating colorectal cancer [27].

7. Secondary metabolites extracted from plants used as anticancer agents

Overtime researchers detected that plants found to be enriched with natural compounds called secondary metabolites these metabolites characterized by several points that make them effective antitumor agents. These compounds can be classified into "three main groups which are: terpenoids (polymeric isoprene derivatives and biosynthesized from acetate via the mevalonic acid pathway), phenolics (biosynthesized from shikimate pathways, containing one or more hydroxylated aromatic rings), and the extremely diverse alkaloids (nonprotein nitrogen-containing compounds, biosynthesized from amino acids such as tyrosine, with a long history in medication)" [16]. Yearly several new metabolites are extracted from plants, but limited numbers of them have been used to synthesize new potent anticancer agents.

8. Medicinal plant enhanced chemotherapy and radiotherapy function

As mentioned above one of the crucial problems associated with chemotherapy drug is multidrug resistance (MDR), which happened when cancer cells become insensitive to chemotherapy treatment used for treating it [17]. The main factor that plays a role in the incidence of this phenomenon is the overexpression of ATP-binding cassette (ABC) transporters which their main function is to prevent transportation of chemotherapy drug through the biological membrane of the cell to reach its target [17]. As a result of that, many researchers are working so hard to produce and/or extract efficient and low-toxic inhibitors for ABC drug transporters from natural

sources, i.e., natural products. Their main target is to restore drug sensitivity in MDR cancer cells by improving chemotherapy drug penetration, distribution, and accumulation of the drug inside the tumor cells [17]. Besides that, natural compounds extracted from plants can be used to enhance chemotherapy function in several ways, as clarified in **Table 1**.

Compound	**Dietary source**	**Chemotherapy drug**	**Effect**
			Influence on treatment efficacy
Ginsenosides	Ginseng	Cisplatin 5-Fluorouracil	Enhancement of drug-induced antiproliferative effect Increase in antiproliferative effect
Curcumin	Turmeric	Vinorelbine	Enhancement of chemotherapeutic efficacy
Catechins/theanine	Green tea	Doxorubicin Cisplatin	Enhancement of antitumor activity Increase in reduction of tumor growth
Quercetin	Many foods such as onions, apples, berries, and tea	Doxorubicin Busulfan Cisplatin	Potentiation of growth-inhibitory activity Synergistic antiproliferative activity Increased cytotoxic effect
Genistein	Soy foods	Tamoxifen	Attenuation of inhibitory effect of tamoxifen on tumor cell growth Attenuation of tamoxifen effect on reducing of tumor burden Synergistic growth inhibition
Daidzein	Soy foods	Tamoxifen	Improvement of drug activity to reduce tumor burden
Tangeretin	Tangerine and other citrus peels	Tamoxifen	Complete blocking of growth-inhibitory effect of tamoxifen
			Influence on side effects of chemotherapy
Ginsenosides	Ginseng	Cyclophosphamide	Protection against drug-induced genotoxicity and apoptosis in bone marrow cells and peripheral lymphocytes
Quercetin	Many foods such as onions, apples, berries, and tea	Cisplatin	Protection of normal renal tubular cells from drug toxicity
			Influence on drug resistance
Ginsenosides	Ginseng	Paclitaxel	Chemosensitization
		Doxorubicin	Inhibition of drug efflux from tumor cells

Table 1.
Various natural compounds and their effects on chemotherapy [17, 18].

Plants (family)	Radioprotective/radiosensitizing efficacy
Aegle marmelos (L.) Corr. (Rutaceae)	Reduced decline in hemoglobin level, and leukocytes and lymphocytes counts because of radiotherapy.
Aloe arborescens Mill. (Liliaceae)	Radioprotective efficacy
Alstonia scholaris L. (Apocynaceae)	Radioprotective efficacy
Biophytum sensitivum (L.) DC. (Oxalidaceae)	Prevented γ-radiation-induced DNA damage
Citrus sinensis (L.) Osbeck (Rutaceae)	Significantly counteracted UV-B-induced damage in human keratinocytes
Emblica officinalis L. (Phyllanthaceae)	Significantly caused depletion in lipid peroxidation and elevation in glutathione and catalase levels
Grewia asiatica L. (Malvaceae)	Inhibited γ-radiation-induced glutathione depletion and ameliorating lipid peroxidation
Isatis indigotica Fort. (Brassicaceae)	Reduce the mucosal damage caused by radiation
Mentha piperita and *Mentha arvensis* (Lamiaceae)	Prevented γ-radiation-induced DNA damage
Olea europaea L. (Oleaceae)	Inhibited incidence of skin damage
Panax ginseng L. (Araliaceae)	Prevented γ-radiation-induced DNA damage
Rosmarinus officinalis L. (Lamiaceae)	Prevented γ-radiation
Rubus spp. (Rosaceae)	Prevented UV radiation
Syzygium cumini L. Skeels (Myrtaceae)	Prevented γ-radiation
Tinospora cordifolia (Thunb.)	Prevented γ-radiation
Viscum album L. (Santalaceae)	Palliate radiotherapy side effects
Xylopia aethiopica (Dunal) A. Rich (Annonaceae)	Prevented γ-radiation

Table 2.
Radioprotective/radiosensitizing efficacy of plants against radiotherapy [28].

Regarding radiation therapy, studies found that a substantial fraction of cancers fails to response properly to radiation therapy, and for this case the use of a high dose is recommended, and this will cause incidence of major side effects (like tissue fibrosis, hair loss, myelosuppression, etc.) [28]. This will happen as a result of generation of intercellular reactive species which will cause breakdown for DNA strand and alteration in biomolecules. Moreover, the combination of radiotherapy and chemotherapy can aggravate the situation, for example, renal problems and alopecia will be detected on cancer patients treated with radiotherapy and platinum chemotherapy, while the combination with alkylating agents is found to cause infertility [28]. Therefore, researchers worked so hard to detect compounds from natural and/or synthetic source (s) to overcome radiotherapy damage and side effects. Studies showed that some plants are radioprotectors which are capable of preventing and/or palliating radiotherapy damage and side effects. Other plants are found to be radiosensitizers which are capable of enhancing radiotherapy pharmacological effects i.e., get the desired pharmacological effect with the minimum dose, as shown in **Table 2**.

9. Nutritional approach in chemotherapy and radiotherapy

During this century chemotherapy and radiotherapy remain as the dominant and the most effective treatments against many types of cancers. Extraordinary effort is made by the researchers to improve the efficacy of both of them. The new

vision for doing that is by using natural products (supplements) in concurrent with them [18, 29].

Besides, these supplements will significantly reduce the incidence of many side effects like "oral mucositis, gastrointestinal toxicity, hepatotoxicity, nephrotoxicity, hematopoietic system injury, cardiotoxicity, and neurotoxicity" that can be caused by the use of the pharmacological treatments, and example for these natural supplements are ginseng extract, grape seed extract, and curcumin [18, 30].

Moreover, nutritional supplement can also help in increasing cancer cell apoptosis, reducing multidrug resistance, increasing drug penetration and its concentration inside cancer cells, and reducing incidence of weight loss, malnutrition, and severity of comorbidities. At the same time, they can significantly improve cancer patients' quality of life [18]. As a result of that, clinicians encouraged to administer nutritional supplement in combination with chemotherapy, rather than giving them separately [18].

One of the most important benefits that can be obtained by combining nutritional supplement with chemotherapy is reducing the incidence of drug resistance by inhibiting ABC transporters [example P-glycoprotein (P-gp) and multidrug resistance proteins (MRP-s)] [18]. Currently no synthetic material is found to be efficient and safe for multidrug resistance, but some novel compounds extracted from natural products can help in some ways to solve this crucial problem [18].

An in vitro study is conducted to determine the antiproliferative and apoptotic effect of *B. serrata* plant methanolic extract as a monotherapy and in combination with doxorubicin (DOX) chemotherapy against hepatocellular carcinoma cell lines (HepG2) and (Hep3B). The results showed that the extract of the plant inhibited the proliferation of both cancer cell lines (HepG2 and Hep3B) with IC_{50} values 21.21 ± 0.92 and 18.65 ± 0.71 μg/mL, respectively. About DOX it caused an inhibition for proliferation of both cancer cell lines at IC_{50} values 1.06 ± 0.04 and 1.92 ± 0.09 μg/ML, respectively, while when the extract is used in combination with DOX, results showed that there was a synergistic effect against both cancer cell lines with combination index (CI) of DOX and *B. serrata* extract of 0.53 ± 0.03 to 0.79 ± 0.02. Besides, results showed that the use of the extract alone and in combination with Dox significantly stimulated the activity of caspase-3 and the activation in combination with Dox was higher. A similar result also gotten about the use of the extract with Dox significantly increased the expression of *TNF-α* and IL-6 and reduced the anti-apoptotic protein level which is *NF-κB*. Moreover, the use of the extract showed a significant reduction in liver enzymes SGOT, SGPT, and ALP which have been elevated as a result of the use of Dox alone, i.e., the extract significantly helped in reducing liver toxicity. Also the extract leaded to restored albumin protein level which has been decreased as a result of Dox use. Moreover, the use of the extract with Dox chemotherapy significantly helps in preserving the histological architecture of the liver, which showed a significant change among group of rats receiving Dox chemotherapy alone. All these points confirmed that the use of the extract in combination with Dox will significantly help in improving treatment of hepatocellular cancer and reducing side effects of Dox [31].

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References

[1] Carson-Dewitt R. Cancer. In: Longe JL, editor. The Gale Encyclopedia of Medicine. Farmington Hills: Gale Group; 2002

[2] Markman M. Principles of cancer screening. In: Aziz K, Wu GY, editors. Cancer Screening: A Practical Guide for Physicians. New Jersey: Humana Press; 2002

[3] Dolan S. Anaemia. In: Brighton D, Wood M, editors. The Royal Marsden Hospital Handbook of Cancer Chemotherapy. London, England: Churchill Livingstone, Elsevier; 2005

[4] Henry L. Malnutrition. In: Brighton D, Wood M, editors. The Royal Marsden Hospital Handbook of Cancer Chemotherapy. London, England: Churchill Livingstone, Elsevier; 2005

[5] Sitamvaram R. Gastrointestinal effects. In: Brighton D, Wood M, editors. The Royal Marsden Hospital Handbook of Cancer Chemotherapy. London, England: Churchill Livingstone, Elsevier; 2005

[6] Stephens M. Nausea and vomiting. In: Brighton D, Wood M, editors. The Royal Marsden Hospital Handbook of Cancer Chemotherapy. London, England: Churchill Livingstone, Elsevier; 2005

[7] Weir-Hughes D. Foreword. In: Brighton D, Wood M, editors. The Royal Marsden Hospital Handbook of Cancer Chemotherapy. London: Elsevier/ Churchill Livingstone; 2005

[8] Scurr M, Judson I, Root T. Combination chemotherapy and chemotherapy principles. In: Brighton D, Wood M, editors. The Royal Marsden Hospital Handbook of Cancer Chemotherapy. London: Elsevier/ Churchill Livingstone; 2005

[9] Kelland LR. Cancer cell biology, drug action and resistance. In: Brighton D, Wood M, editors. The Royal Marsden Hospital Handbook of Cancer Chemotherapy. London: Elsevier/ Churchill Livingstone; 2005

[10] Rizzo T, Cloos R. Chemotherapy. In: Thackery E, editor. The Gale Encyclopedia of Cancer. Detroit: Gale Group; 2002

[11] Abrams AC. Drugs used in oncologic disorders. In: Repchinsky C, editor. Clinical Drug Therapy. 36th ed. Ontario: Canadian Pharmacists Association; 2001

[12] Koda-Kimble LYY, Wayne A, Kradjan BJG, Brain KA, Robin LC. Applied therapeutics the clinical use of drugs. In: Troy D, editor. Handbook of Applied Therapeutics. Philadelphia: Lippincott Williams & Wilkins; 2002

[13] Shabani A. A review of anticancer properties of herbal medicines. Journal of Pharmaceutical Care and Health Systems. 2016;**3**:2

[14] Ahmad R, Ahmad N, Naqvi AA, Shehzad A, Al-Ghamdi MS. Role of traditional Islamic and Arabic plants in cancer therapy. Journal of Traditional and Complementary Medicine. 2016;**7**(2):195-204

[15] Zaid H, Silbermann M, Ben-Arye E, Saad B. Greco-Arab and Islamic herbal-derived anticancer modalities: From tradition to molecular mechanisms. Evidence-based Complementary and Alternative Medicine. 2012;**2012**:1-14

[16] Seca AM, Pinto DC. Plant secondary metabolites as anticancer agents: Successes in clinical trials and therapeutic application. International Journal of Molecular Sciences. 2018;**19**(263):1-22

[17] Wu CP, Ohnuma S, Ambudkar SV. Discovering natural product modulators to overcome multidrug resistance in cancer chemotherapy. Current Pharmaceutical Biotechnology. 2011;**12**(4):609-620

[18] Sak K. Chemotherapy and dietary phytochemical agents. Chemotherapy Research and Practice. 2012;**2012**:1-11

[19] Lichota A, Gwozdzinski K. Anticancer activity of natural compounds from plant and marine environment. International Journal of Molecular Sciences. 2018;**19**:1-38

[20] Desai AG, Qazi GN, Ganju RK, El-Tamer M, Singh J, Saxena AK, et al. Medicinal plants and cancer chemoprevention. Current Drug Metabolism. 2008;**9**(7):581-591

[21] Aung TN, Zhipeng Q, Daniel Kortschak R, Adelson DL. Understanding the effectiveness of natural compound mixtures in cancer through their molecular mode of action. International Journal of Molecular Sciences. 2017;**18**(656):1-20

[22] Malıkova J, Swaczynova J, Kolar Z, Strnad M. Anticancer and antiprolifer-ative activity of natural brassinosteroids. Phytochemistry. 2008;**69**:418-426

[23] Mohammadi-Motlagh H-R, Mostafaie A, Mansouri K. Anticancer and anti-inflammatory activities of shallot (*Allium ascalonicum*) extract. Archives of Medical Science. 2011;**7**(1):38-44

[24] Wang Y, Tian W-X, M X-F. Inhibitory effects of onion (*Allium cepa* L.) extract on proliferation of cancer cells and adipocytes via inhibiting fatty acid synthase. The Asian Pacific Journal of Cancer Prevention. 2012;**13**(11):5573-5579

[25] Hussain A, Sharma C, Khan S, Shah K, Haque S. Aloe vera inhibits proliferation of human breast and cervical cancer cells and acts synergistically with cisplatin. Asian Pacific Journal of Cancer Prevention. 2015;**16**(7):2939-2946

[26] Shalabi M, Khilo K, Zakaria MM, Elsebaei MG, Abdo W, Awadin W. Anticancer activity of *Aloe vera* and *Calligonum comosum* extracts separately on hepatocellular carcinoma cells. Asian Pacific Journal of Tropical Biomedicine. 2015;**5**(5):375-381

[27] Mustapha N, Pinon A, Limami Y, Simon A, Ghedira K, Hennebelle T, et al. Crataegus azarolus leaves induce antiproliferative activity, cell cycle arrest, and apoptosis in human HT-29 and HCT-116 colorectal cancer cells. Journal of Cellular Biochemistry. 2016;**117**(5):1262-1272

[28] Hazra B, Ghosh S, Kumar A, Pandey BN. The prospective role of plant products in radiotherapy of cancer: A current overview. Frontiers in Pharmacology. 2012;**2**(94):1-13

[29] Oneschuk D, Younus J. Natural health products and cancer chemotherapy and radiation therapy. Oncology Reviews. 2008;**1**:233-242

[30] Zhang Q-Y, Wang F-X, Jia K-K, Kong L-D. Natural product interventions for chemotherapy and radiotherapy-induced side effects. Frontiers in Pharmacology. 2018;**9**:1-25

[31] Mazzio EA, Lewis CA, Soliman KFA. Transcriptomic profiling of MDA-MB-231 cells exposed to *Boswellia Serrata* and 3-O-acetyl-B-Boswellic acid; ER/UPR mediated programmed cell death. Cancer Genomics and Proteomics. 2017;**14**:409-425

Chapter 10

Herbal Remedies for Breast Cancer Prevention and Treatment

Yahyea Baktiar Laskar, Romen Meitei Lourembam and Pranab Behari Mazumder

Abstract

Breast cancer is among the most common type of cancer in women around the globe. Prevention of breast cancer is better than its treatment. Because of the molecular variation and complexity underlying breast cancer occurrence, its treatment by using chemotherapy and/or radiotherapy is very complicated and often leads to undesirable side effects. Plants and their extracts have been used for centuries for the treatment of almost every disease and breast cancer is not an exception. Herbal products can be trusted for cancer treatment because of their low toxicity. Besides, herbal remedies are easily accepted by the majority of woman suffering from breast cancer because of their easy availability and affordability. In the last decade, a large number of plants and their compounds were reported to show promising anticancerous effects against breast cancer cells in both *in vivo* and *in vitro* models. However, their beneficial effects on breast cancer treatment are still doubtful due to the lack of randomized clinical trials. This chapter is dedicated to reporting the potential of some herbal products for the prevention and/or treatment of breast cancer. Besides, it focused on the anticarcinogenic mechanism of those phytocompounds to report their potential chemotherapeutic role.

Keywords: herbal remedies, phytochemicals, phytoestrogens, breast cancer

1. Introduction

According to World Health Organization (WHO), cancer is the second leading cause of death after cardiovascular diseases and a growing health issue globally. Breast cancer is the most commonly diagnosed type of cancer among females accounting for approximately one-quarter of cancers in females globally. Great research efforts are in place to understand the cause of breast cancer onset, to identify the critical molecular mechanism of its progression, and to define new ways of treating it with lower and limited toxicity. These efforts are certainly encouraging since overall survival has greatly improved in several breast cancer types during the last decade. Since 1990, mortality rates of breast cancer have reduced significantly by 25%, this is at least in part due to the significant improvement in its treatment [1]. Treatment of cancer mainly relies on chemotherapy that uses cytotoxic agents for killing cancer cells. However, these agents or drugs affect both cancer cells as well as healthy cells, causing an array of side effects during the therapy or after the therapy. To overcome these problems, current research is emphasized to explore herbal remedies that selectively targets cancer cells. Besides this, unlike other

cancer types, breast cancer has diverse genetic mutations that affect several pathways [2]. These complexities aid to distinct pathological types with different clinical outcomes [3]. Therefore, response to a certain chemotherapeutic drug may differ in different patients and lack of proper treatment plan may increase the toxicity furthermore. One of the encouraging approaches to overcome drug toxicity is to look for alternative medicines that have less or selective toxicity toward cancer cells [4]. In recent years, many studies have demonstrated selective cytotoxicity of a variety of herbal compounds that can be used as potential chemotherapeutics [4]. Meanwhile, diverse herbal products were reported to prevent and/or palliate the side effects of treatment, improve quality of life, and reduce stress. However, the usefulness of herbal remedies for breast cancer prevention and/or treatment is still ambiguous due to the lack of randomized clinical trials. These objectives will be achievable only if the herbal compounds that showed promising anticancer activity can be successfully transferred to clinical trials.

2. Current scenario and future burden of breast cancer

Cancer of the breast is among the most frequently diagnosed cancer and the leading cause of cancer-related deaths in females globally. According to International Agency for Research on Cancer (IARC's) Globocan data on 2018, breast cancer caused 0.62 million deaths in 2018 and another 2.08 million new cases were identified, which is 11.6% of all cancer types recorded [5]. At the current rate, the number of incident cases is expected to rise to 3.05 million, and the mortality troll is expected to rise to a nerve-racking 6.99 million by 2040 [6]. Approximately 1 in 10 women is diagnosed with breast cancer at some time in their lives [3].

Epidemiological observation shows that the incidence of breast cancer is continuously raising in both industrialized and developing countries [7]. Breast cancer is a disease largely triggered by environmental and lifestyle factors than genetic, which is believed to be responsible for only 10–15% of all breast cancer cases [8]. Various risk factors like age (>50), family history of breast cancer, woman's reproductive history such as early menarche, nulliparity or late pregnancy, and late menopause mainly aid to breast cancer onset [9]. In addition, prolonged use of oral contraceptive and hormone replacement therapy are also known risk factors of this disease among postmenopausal women [10].

3. Molecular feature of breast cancer occurrence, progression, and treatment

The onset of cancer is a result of several sequential molecular events. Most common of them is a mutation in a DNA molecule that codes for a protein that either triggers cell division, proliferation, and growth or that signals termination of all these molecular events [11]. Therefore, damage to DNA or a protein that regulates cell cycle may lead to uncontrolled division and growth of cells, the condition is cancer. It is a hyperproliferative disease that involves molecular alteration resulting in apoptosis dysregulation, proliferation, angiogenesis, and metastasis [12].

Breast cancer is one of the commonest types of cancer and characterized by distinct pathological types with different clinical outcomes. It has different stages that arise from ductal hyperproliferation, which changes into ductal carcinoma *in situ* (DCIS), invasive carcinoma, and metastatic stage. In addition, based on the molecular mechanism of occurrence, breast cancer can be divided into estrogen receptor (ERα) and progesterone receptor (PR) expression and amplification

of human epidermal growth factor receptor (HER2), also known as epidermal growth factor receptor 2 (ErbB2) [3]. Breast cancer 1 (BRCA1) and breast cancer 2 (BRCA2) are the two most important genes that code the proteins BRCA-1 and BRCA-2, which play a key role in DNA damage repair and in maintaining genomic stability [3]. Mutation in these genes leads to 15–20 fold increases the risk of breast cancer occurrence [13]. Additionally, tumor suppressor TP53 is another important gene that codes for the protein p53 that plays a major role in the regulation of cell cycle and in apoptosis induction. Mutation in the TP53 gene increases the risk of breast cancer as well as other cancer types. Breast cancer cell survival, proliferation, motility, and cell metabolism are controlled by various signaling cascades. In around 70% of breast cancers, the phosphatidylinositol 3-kinase (PI3K)/AKT pathway has shown to be mutated [14]. Other frequently mutated signaling cascades in breast cancer are Janus kinase (JAK)/signal transducer, activators of transcription (STAT), and nuclear factor κβ (NF-κβ) pathways [3]. Classification of breast cancer based on the molecular expression has therapeutic implication as it helps in deciding the treatment plan. Based on the relative expression of the above markers, patients either receives hormonal therapy or chemo/targeted therapies. The most promising way of dealing with cancer is to interfere with modulation stages of carcinogenesis—initiation, promotion, and progression as well as altering the carcinogenesis signaling pathways [15, 16].

Breast cancer therapeutics include drugs that protect genomic stability by preventing DNA damage, inhibit the cell cycle by disrupting cellular integrity or by inducing apoptotic cell death, and block certain pathways that are responsible for abnormal cell growth (**Table 1**). Majority of breast cancer cases express the estrogen hormone receptor, which helps the cancer cells to proliferate rapidly by the growth-promoting effects of circulatory estrogens [17]. Therefore, current therapies are targeted at abrogating estrogen dependence for estrogen receptor (ER)-positive breast cancers [17]. One of the successful and efficient approaches is the employment of a selective estrogen receptor modulator (SERM) like tamoxifen, which binds to the ER that induces a conformational change in the receptor resulting in obstruction of estrogenic expression [18, 19]. However, tamoxifen like SERMs exhibits many notable side effects including—secondary cancer, cardiovascular diseases by their estrogenic activity in other tissues and organs. The efficiency of tamoxifen is challenged by the development of highly potent third-generation aromatase inhibitors (AIs) that represents a promising approach in endocrine therapy of breast cancer [20]. The aromatase inhibitor drugs like anastrozole and letrozole reduce estrogen production by competitive inhibition of the enzyme aromatase, although the long-term health effects of AIs are doubtful [21]. Another effective strategy in breast cancer treatment is the implementation of a growth factor inhibitor. One of the first identified targets of these growth inhibitors was the epidermal growth factor receptor (EGFR) that plays a vital role in the survival of cancer cells and developing multidrug resistance [22]. The effectiveness of the small molecule EGFR tyrosine kinase inhibitor like gefitinib is highly appreciated for the treatment of breast cancer; however, it failed to produce notable improvement in advance stages of breast cancer [23].

Approximately 20% of breast cancer cases show overexpression of the HER2 that results in aggressive disease and reduced survival [17]. In present, trastuzumab and lapatinib are the only marketed drugs used to inhibit the HER2-mediated growth and proliferation signaling [17]. Other than this, enzyme-mediated DNA damage is an effective approach used in cancer chemotherapy. Doxorubicin, an anthracycline drug, binds with DNA by intercalation with base pairs, which results in an elevated level of DNA-topoisomerase II covalent complexes inhibiting topoisomerase II activity [24]. Other anticancer drugs inhibit mitosis by interrupting the

Drugs	Target	Mode of Action
Doxorubin/ Daunorubicin	DNA , Topoisomerase II	Binds with DNA by intercalation between base pairs and inhibits topoisomerase II activity by stabilizing DNA-topoisomerase II complex.
Epirubicin	DNA/RNA, Topoisomerase II-α, Chromodomain-helicase-DNA-binding protein 1	It has antimitotic and cytotoxic activity. Inhibits nucleic acid & protein synthesis in many ways. Inhibits DNA helicase activity thus interferes DNA replication & transcription.
Cisplatin	DNA, DNA-3-methyladenine glycosylase, α-2-macroglobulin, serotransferrin, ATOX1	Its an alkylating agent that adds alkyl group to DNA bases, preventing DNA and protein synthesis. Forms cross-links in DNA that prevents synthesis or transcription of DNA and induce mutation by mispairing of nucleotides.
Paclitaxel	Tubulin β-1 chain, Bcl-2, microtubule-associated proteins	It's a mitotic inhibitor that interferes with microtubule growth by hyper-stabilization of their structure. Induce apoptosis by inhibiting Bcl-2 activity.
Cyclophosphamide	DNA	Cross-linking and alkylation of DNA that prevents DNA synthesis and transcription.
Tomaxifen	Estrogen Receptor (ER)	It's a selective estrogen receptor modulator (SERM) that binds to estrogen receptor (ER), inducing a conformational change in the receptor, that results in blockage or change of expression of estrogen dependent genes in the mammary tissue.
Raloxifene	Estrogen Receptor (ER)	Second generation SERM, mode of action is similar to tamoxifen.
Herceptin/ Trastuzumab	Human epidermal growth factor receptor 2 (HER 2)	It's a recombinant humanized IgG1 monoclonal antibody used in protein based therapies that blocks the extracellular ligand-binding domain of HER-2 receptor, subsequently inhibiting HER-2 mediated signaling cascade.
Gefitinib	Epidermal growth factor receptor	Inhibits the activity of EGFR tyrosine kinase, subsequently inhibiting the proliferation of malignant cells.
Bevacizumab	VEGF/ VEGFR	It's a recombinant humanized monoclonal IgG1 antibody that inhibits the activity of human vascular endothelial growth factor by preventing its interaction with VEGFR.
Capecitabine	Thyamidylate synthase	It's a prodrug that converts to fluorouracil in cancer cells and inhibit DNA synthesis.

Table 1.
Commonly used breast cancer chemotherapeutic drugs, their targets, and mechanism of action [26].

microtubule stability, hence blocking the transition from metaphase to anaphase [25]. Subsequently, the cell undergoes mitotic arrest or programmed cell death (apoptosis). For instance, vincristine and vinorelbine inhibit the polymerization of microtubules by binding to either the vinca domain or taxoid-binding domain that interferes between β- and α-subunit of tubulin [25]. On the other hand, microtubule-stabilizing drugs like paclitaxel hyperstabilizes the microtubule assembly by binding to the inner surface of the microtubule at a taxoid-binding site on β-tubulin resulting in mitotic arrest in the cell [25]. All these strategies helped in reducing mortality due to breast cancer and increased the survival rate; however, they appear with certain side effects that may be either low and short term or high and life threatening.

4. Chemotherapeutic-associated toxicity in breast cancer treatment

The role of chemotherapy in curing cancer is still doubtful [27]. Even it decreases the risk of recurrence and helps the patient to live longer with improved quality of life in case of metastatic breast cancer. But its use associated with certain risk factors or side effects—some of the side effects are short term and minor, whereas others may become more serious and life threatening [27]. **Table 2** describes a few commonly used chemotherapeutic drugs and their side effects.

Among the most common side effects of chemotherapeutic drugs is its nonselective toxicity, where it destroys the normal body cells such as those in the hair follicle,

Drugs	Common Side-Effects
Doxorubicin	Cardiotoxicity, infertility, alopecia, nausea & vomiting, low blood counts.
Daunorubicin	Alopecia, nausea & vomiting, mouth sores and low blood count. May cause infertility and cognitive heart failure in exceptional occasions.
Epirubicin	Increased risk of infectious diseases, hair loss, respiratory problems, decreased blood count.
Cisplatin	Nausea & vomiting, kidney toxicity, ototoxicity and decreased blood count.
Paclitaxel	Alopecia, pain in joints and muscles (arthralgia and myalgia), peripheral neuropathy, nausea & vomiting, diarrhea and hypersensitivity.
Cyclophosphamide	Temporary hair loss, nausea & vomiting, poor appetite, discoloration of skin and nails, low blood count, loss of fertility.
Tomaxifen	Cardiotoxicity, respiratory difficulties, abnormal vaginal bleeding, tenderness and numbness in face, hand and legs.
Raloxifen	Hot flashes, flu, joint and muscle pain, rhinitis and blood clots, including deep vein thrombosis in rare cases.
Herceptin/ Pertuzumab	Flu-like syndrome, respiratory problems, insomnia, hypersensitivity, cardiotoxicity, peripheral neuropathy, alopecia, low blood count, nausea & vomiting.
Gefitinib	Eye irritation, hypersensitivity, poor appetite, nausea & vomiting, pulmonary and respiratory problems, liver toxicity.
Bevacizumab	Upper respiratory infection, alopecia, nausea, vomiting, abdominal pain, constipation, nose bleeding, proteinuria and in rare cases cognitive heart failure and nephrotic syndrome was observed.
Capecitabine	Low blood count, risk of infection, hand-foot syndrome, hepatotoxicity, eye irritation, nausea & vomiting, poor appetite and constipation.

Table 2.
Frequently used chemotherapeutic drugs in breast cancer treatment and their common side effects associated with them [26, 28–30].

bone marrow, and cells of other important organs along with the cancer cells. Quite a few chemotherapeutic drugs affect the nerve endings or synaptic gaps in hands and feet that may result into numbness, pain, burning or tingling, sensitivity to cold or heat, or weakness in your extremities [31]. Besides, chemotherapeutic drugs may severely damage the immune cells as wells as the brain cells, making the patient vulnerable to infectious diseases and impaired cognitive functions [32]. These side effects may be temporary and may disappear after a few months of completion of chemotherapy. Other critical side effects that arise due to certain chemotherapeutic drugs may last longer—infertility is one of them [33]. Chemotherapeutics that damage ovaries may lead to menopause symptoms, like hot flashes and vaginal dryness, where menstrual cycle becomes irregular or permanently ceases making pregnancy impossible [34]. Further, early menopause in premenopausal women due to the use of aromatase inhibitor agents in adjuvant therapy causes a hypoestrogenic condition that negatively impacts bone density resulting in osteopenia or osteoporosis [35].

Besides, long-term chemotherapeutic toxicity results in cardiac diseases and may trigger secondary cancer such as marrow neoplasm or leukemia [36, 37]. Chemotherapy-linked cardiotoxicity is another major setback of cancer therapy that increases the mortality rate because of the high prevalence of cardiovascular diseases in cancer patients [38]. The cardiotoxicity leads to congestive heart failure (CHF), which is more prevalence in young and elderly patients. It has been reported

that the breast cancer patients aged between 65 and 70 years, who received adjuvant anthracycline chemotherapy, had significantly higher rates of CHF [39]. In another investigation, a widely used chemotherapeutic drug, doxorubicin, was reported to cause CHF in worryingly 26% of the patients suffering from breast carcinoma [40, 41]. Additionally, it was observed that 0.5% of breast cancer patients developed different types of marrow neoplasm (MN) or leukemia after a few years of chemotherapy [42]. The risk of developing MN is higher in the first few years after chemotherapy. Furthermore, chemotherapeutic drugs may also disrupt the normal psychological state of patients in certain cases [43, 44].

The side effects that arise due the conventional chemotherapy is mainly due to lack of specificity of the drugs for cancer cells. Majority of the widely used chemotherapeutic drugs causes adverse damage to normal cells and key organs, which limits the dose of a drug that can be used [45]. This explains the reason why cancer drugs have a low therapeutic index. Several approaches are being considered to address this issue in order to improve the effectiveness of anticancer drugs. One of the popular approaches among them is searching for natural compounds that inhibit cancer cell growth without disrupting the functioning of healthy cells.

5. Ethnomedicine and herbal compounds used for cancer treatment

Plants have played a key role in the survival and evolution of human beings as they have provided the basic need of mankind like food, clothing, shelter, and medicine since the beginning of the human race. Plants have formed the basis of traditional medicine systems like Ayurveda, Unani, and Chinese traditional medicines that have served mankind with their health needs. A larger part of the population in developing and underdeveloped countries relies on herbal medicine for solving treating their primary health issues. Traditional herbal medicines become popular because of their cost-effectiveness, abundancy, and less or no side effects. In recent years, global emphasis on plant research has increased to find out drug-like substances from traditionally used medicinal plants. Moreover, several naturally occurring plant-based compounds like curcumin, resveratrol, quercetin, and many more showed promising anticancerous effects and are gaining interest as an adjuvant chemotherapeutic agent. Besides, naturally occurring compounds cause less toxicity to healthy cells and in certain cases show selective toxicity against abnormal or diseased cells [46]. This might be the reason that today a large number of drugs being marketed are structurally similar to the structure of naturally occurring compounds.

Herbal compounds show a variety of anticancer activity mainly antioxidant, anti-inflammatory, antimutagenic, and apoptosis-inducing activity that may help prevent cancer development in the early stage (**Figure 1**). Dietary consumption of adequate quantity of these herbal products may help in prevention and treatment of breast cancer by cell cycle arrest, induction of apoptosis, regulating carcinogen metabolism and oncogenic expression, inhibiting cell adhesion, proliferation and migration, and blocking signaling pathways that are essential for cancer progression [47].

Between the year 1981 and 2014, 136 anticancer drugs were brought to use around the globe, almost 83% of which were either herbal compounds or their derivatives [48]. A number of anticancer drugs have already in use for the treatment of breast cancer—including vincristine, vinblastine, paclitaxel, and docetaxel [49]. Despite the success of herbal products in curing breast cancer and its associated complexities, not many herbal products are making through preclinical or clinical

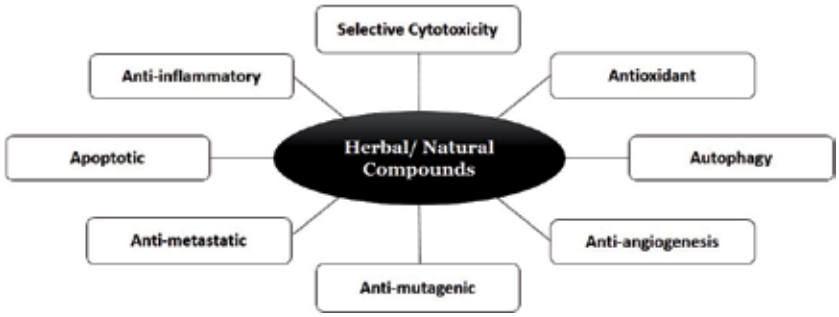


Figure 1.
Features of herbal compounds that attribute to their anticancer activity.

trials. Hence, greater effort is necessary to successfully transfer these agents to an ideal clinical setting to assess their potential for herbal therapies.

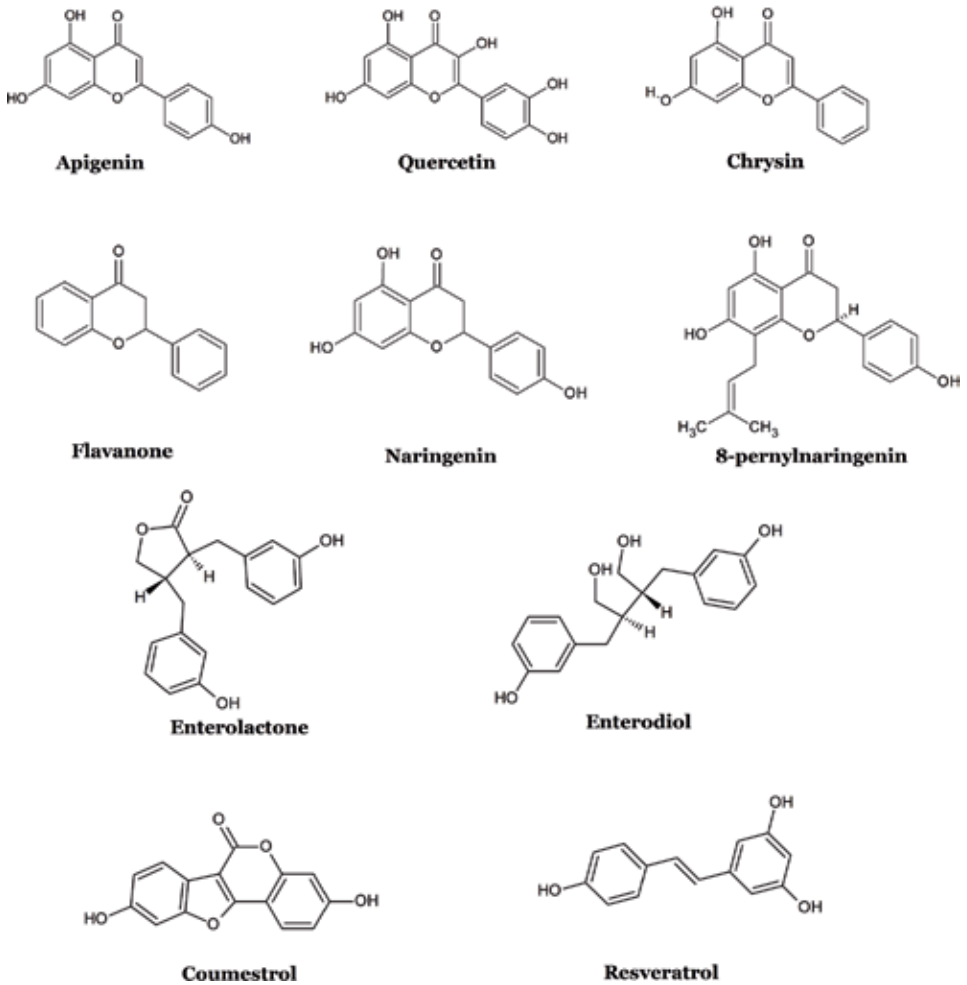


Figure 2.
Some important members of different classes of phytoestrogens [61].

6. Herbal products used for prevention of breast cancer

Breast cancer is a preventable disease [50]. Estrogens play a major role in promoting the proliferation of normal breast cells as well as neoplastic breast epithelium [51]. Almost 40–70% of breast cancers are estrogen receptor positive [52]. Hence, blocking the estrogen receptor for the treatment and chemoprevention of breast cancer is one of the significant approaches. Plant-based estrogen-like compounds or phytoestrogens were originally proposed as cancer-protective agents. This claim was strongly supported by an epidemiological study that revealed a low breast cancer incidence in the soy-consuming population [53, 54]. Phytoestrogens are structural analogues of the mammalian hormone, estrogen, and thus can bind weakly to the hormone receptor [55]. Structurally, phytoestrogen can be grouped into flavones, flavanones, lignans, coumestans, and stilbenes [56]. The structure of important members of different classes of phytoestrogens is given in **Figure 2**. Soybean and soy product is a rich source of isoflavones [57]. Other phytoestrogen classes are legumes and lignans found in seeds, nuts, whole grains, fruit, and vegetables [57]. Historically, the rate of breast cancer occurrence in the United States is 4–7 times higher than that of Asian population where the consumption of dietary isoflavones is comparatively as higher as 20 to 80 mg/d [58]. In addition, epidemiological observations also revealed a modest 30% reduction in breast cancer risk for women with a higher percentage of dietary lignan intake [57]. Therefore, consumption of phytoestrogen-rich diet is one of the many potential protective lifestyles against breast cancer. Recently, there are increasing pieces of evidence that phytoestrogen activity inhibits key steroidogenic enzymes activity involved in the synthesis of estradiol from circulating androgens and estrogen sulfate [7]. Consequently, this activity could play a major role in protection against breast cancer. Besides inhibiting the estrogenic activity, phytoestrogens were also reported to activate the G-protein coupled receptor, GPR30 or GPER-1, described as a novel estrogen receptor and play a significant role in estrogen-dependent diseases like breast cancer [59]. However, the activity of phytoestrogens is unclear and depends on more than one factors that include—its structure, metabolism, its relative availability compared to that of endogenous estrogen [60, 57].

Naturally occurring phenolic compounds namely phenolic acids, flavonoids, tannins, quinones, anthocyanins, and others play an important role in cancer prevention and/or treatment [47]. These phenolic compounds are ubiquitous and rich in medicinal herbs and dietary plants. Several phenolic compounds contribute toward inhibiting carcinogenesis mechanism and show chemopreventive activities by their diverse range of biological activities [62] (**Table 4**).

7. Herbal products used for treatment of breast cancer

A recent population-based survey showed that almost 80% of the women suffering from breast cancer use some form of complementary or alternative medicine for the treatment of cancer [63]. Herbal remedies are the most common and popular form of alternative medicine among them, which is frequently used by women suffering from breast cancer. Here is some evidence that can help to treat breast cancer and its associated toxicity:

7.1 Choosing a selectively cytotoxic herbal cure

One of the interesting features for herbal remedies is their selective toxicity toward cancer cells. There are a number of phytocompounds reported that have

Chemotherapeutic Drugs	Cancer type	Plant Source	Anticancer activity	Clinical status
Paclitaxel	Breast cancer, ovarian cancer	*Taxus brevifolia* L.	Mitotic inhibitor, Microtubule disruptor, Apoptosis inducer	Approved
Docetaxel	Breast cancer, Lung cancer	*Taxus baccata*	Anti-mitotic, Apoptotic	Approved/ Investigational
Sulphoraphane	Breast cancer	Cruciferous vegetables/ Brassica	inhibits tumor growth, anti-proliferative effects	Investigational
Epipodophyllotoxin	Lymphomas, Testicular cancer	*Podophyllum peltatum* L.	Cell cycle disruption, apoptosis	Investigational/ Approved
Vincristine	Breast cancer, Leukemia		Anti-mitotic	Approved, Investigational
Vinblastine	Breast cancer, Lymphoma		Mitotic arrest, cell death	Approved
Vinorelbine	Breast cancer, Hodgkin lymphoma, Lung Cancer	*Catharanthus roseus*	Anti-mitotic, apoptosis	Approved, Investigational
Vindesine	Acute leukaemia, Malignant lymphoma, Hodgkin's disease		Antineoplastic activity, Anti-mitotic, immunomodulatory agent	Approved, Investigational
Vinflunine	Urothelial carcinoma of the bladder		Antineoplastic activity, Anti-mitotic, immunomodulatory agent	Approved, Investigational
Pomiferin	Breast, Lung, prostate and colon cancer	*Maclura pomifera; Derreis malaccensis*	Inhibits histone deacetylases, prevents DNA damage, Apoptotic	Investigational
Epigallacotechin-3-gallate	Prostate cancer, breast cancer	Catechin; green tea; *Hibiscus subdariffa* L.	Anti-mutagenic, DNA protective, anti-proliferative	Experimental
Combretastatin A-4 phosphate	Anaplastic thyroid cancer,	*Combretum caffrum*	Anti-angiogenic, induce necrosis in tumors	Investigational
Roscovitine	Lung cancer, nasopharyngeal carcinoma	*Raphanus sativus* L.	Interfere cell cycle, inhibits cyclin dependent kinases	Experimental
Flavopiridol	Lung cancer, Esophageal cancer, Leukemia, Lymphoma	*Dysoxylum binectariferum*	Inhibits cyclin-dependent kinases, arrests cell cycle, apoptotic, immunomodulatory	Experimental
Noscapine	Lymphoma, Lymphoid leukemia, Multiple myeloma.	*Papaver somniferum*	Anti-proliferative, interfere microtubule stability	Approved/ Investigational

Table 3.
Plant-based cancer therapeutics in different stages of clinical trials and research [68].

selective toxicity toward breast cancer cells. Artemisinin is one among them, isolated from *Artemisia annua* L. proved to be selectively cytotoxic toward breast cancer cells when an adequate amount of iron (i.e., ferrous iron) is present in the cells. Because cancer cells have a higher iron influx, therefore, artemisinin and its analogues can selectively destroy cancer cells under high iron concentration [64]. Besides, polyphenols from *Artemisia annua* L. were reported to inhibit the adhesion and epithelial-mesenchymal transition (EMT) of highly metastatic breast cancer cells, MDA-MB-231 [65]. Other than this, polyphenol-rich extracts of *Hibiscus sabdariffa* and aqueous extract of *Brucea javanica* were also reported to show selective cytotoxicity toward *MCF7* and HTB-126 breast cancer cell lines, respectively [66, 67]. However, further exploration is necessary to isolate the selective cytotoxic ingredients of these plants (**Table 3**).

7.2 Combination therapy by herbal remedies and synthetic drugs

Combination therapy of herbal therapy and synthetic drugs possibly be the last resource for patients in the final stage of breast cancer, where surgery is not possible [69]. The combinatory effect of a herbal drug with conventional cancer drugs might improve the bioavailability of one of them making the treatment more effective [69, 70]. Additionally, the combinatory use of herbal remedies with chemotherapy will reduce the dose of standard medicine resulting in lower toxicity and side effects [71]. Several researchers have suggested that herbal compounds can be used in a therapeutic modality as it enhances the anticancer activity of current drugs. Curcumin, a renowned anticancer herbal compound down-regulated the expression of breast cancer markers *in vivo* and *in vitro* when administered along with

chemotherapeutic drugs cyclophosphamide and paclitaxel that made the cancer cells more viable to the drugs [72, 73]. Similarly, 20S-protopanaxadiol, a metabolite of ginsenosides, inhibited cell proliferation in MCF-7 cells by interfering with estrogenic gene expression when used in combination with tamoxifen [74]. Besides, this combination asynergistically improved the cytotoxicity of tamoxifen in an ER-independent manner [74]. Hence, the benefits of these herbal compounds in synergistic therapy are considerable, and this might help to overcome chemotherapeutic drug resistance and toxicity in breast cancer treatment.

7.3 Herbal supplements and nutraceuticals for breast cancer therapy

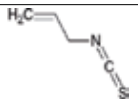
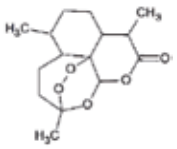
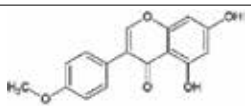
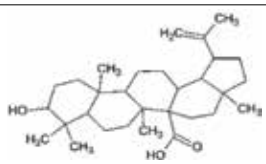
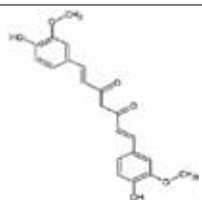
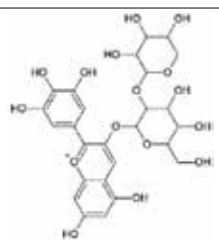
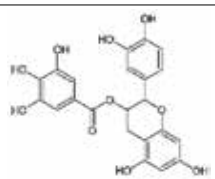
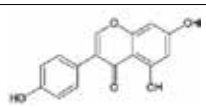
Cancer has been shown to be a preventable disease with changes in nutrition and dietary changes. A previous investigation showed that almost 35% of cancers are related to diet [75]. There are several confirmations from epidemiological and laboratory studies that sufficient intake of fruit, vegetables, and herbal supplements is inversely linked with breast cancer occurrence. A diet composed of adequate quantity of phytoestrogens, polyphenols, and rich sources of other chemopreventive agents helps in reducing breast cancer risk. Dietary supplements of the herbal source are less toxic and easily metabolized. Besides, dietary consumption of these herbal remedies helps in fighting side effects in postchemotherapy patients. One of the primary symptoms of adjuvant chemotherapeutic damage in posttherapy breast cancer patients is hot flushes. Black cohosh or *Actaea racemosa* plant is popularly used by patients of breast cancer to treat hot flushes, which gives conflicting but promising results [76].

8. Molecular mechanism of anticancerous activity of herbal compounds on breast cancer

As discussed in the earlier section, herbal compounds show a verity of anticancer actions—including antioxidant, cytotoxic, antiproliferative, apoptotic activity, etc. Plant-based cancer agents broadly classified into five groups that include—methyltranferase inhibitors, DNA protecting agents, antioxidants, histone deacetylases inhibitors, and mitosis disruptors. Generally, plant-derived compounds contribute toward the anticarcinogenesis mechanism by their antioxidant, cytotoxic, antimitotic, and apoptotic activity (**Table 4**). Others help in chemoprevention by preventing DNA damage, modulating carcinogenesis signaling, and inducing apoptotic cell death (**Table 4**). Several *in vitro* and *in vivo* investigations support the activity of herbal compounds that linked with their anticancer activity. Here's is a few examples of the anticancer mechanism of herbal compounds.

8.1 Antioxidant activity of herbal compounds

Antioxidant activity of herbal compounds of oxidative stress is developed when the balance between the production of reactive oxygen species (free radicals) and antioxidant defense is disturbed [77]. Oxidative stress development and consequent reactive oxygen species (ROS) generation are linked with several disease pathogenesis including cancer. Oxidative stress is dealt with by the body's antioxidant mechanism and several herbal compounds help boosting this machinery. For instance, curcumin enhances the activity of antioxidant enzymes resulting in enhanced cellular resistance to oxidative damage [78]. In addition, curcumin was also found to rise hepatic GSH, SOD, GPx, GR, GST, and CAT activities in paracetamol-treated rats [79]. Other plant-based compounds like epigallocatechin gallate, a component

Compound	Structure	Herbal source	Activity	References
Allyl isothiocyanate		*Brassica nigra, Brassica juncea*	Chemoprevention, detoxification, and reduces cancer risks. Inhibits mitosis and angiogenesis. Shows selective cytotoxicity.	[86, 87]
Artemisinin		*Artemisia annua*	Selective cytotoxicity, mitotic arrest, apoptosis, inhibition of angiogenesis, and ferroptosis.	[88, 89]
Biochanin A		*Trifolium pratense*	Breast cancer preventive agent inhibits tumor growth.	[90, 91]
Bacosine		*Bacopa monnieri*	Anti-metastatic activity.	[92, 93]
Curcumin		*Curcuma longa*	Chemopreventive and antitumoral activities, anti-metastatic, apoptotic, modulate carcinogenesis signaling, help reducing drug toxicity.	[94–96]
Delphinidin 3-sambubioside		*Hibiscus sabdariffa*	Antioxidant, cytotoxic, apoptotic, induces autophagy and necrosis.	[97, 98]
Epicatechin gallate		*Parapiptadenia rigida, Hibiscus sabdariffa,* component of green tea	Induces apoptosis and inhibits tumorigenesis, potential cancer chemopreventive agent.	[99–101]
Genistein		Component of soy products	Chemoprevention, estrogenic activity, inhibition of tumorigenesis, inhibit topoisomerase II activity and angiogenesis	[102–104]

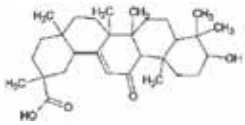
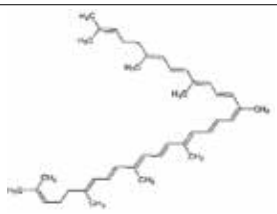
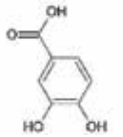
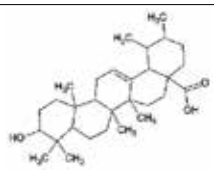
Compound	Structure	Herbal source	Activity	References
Glycyrrhetinic acid		*Glycyrrhiza glabra*	Inhibition of cell proliferation, synergistic effect in combination with an anticancer drug, etoposide. Antineoplastic effect including apoptosis.	[105, 106]
Lycopene		Component of tomatoes, pink grapefruit, apricots, red oranges.	Inhibition of cell cycle progression	[107, 108]
Protocatechuic acid		Dietary polyphenol found in many foods including *Hibiscus sabdariffa, Ginkgo biloba, Hypericum perforatum*	Apoptosis-inducing agent, anti-metastatic.	[109–111]
Ursolic acid		Many plants including *Malus domestica*, *Origanum majorana*	Antitumor, antioxidant, anti-inflammatory, and anti-angiogenesis activity.	[112–114]

Table 4.
Some novel compounds from herbal sources that showed promising anticancerous activity in both in vivo *and* in vitro *studies.*

of in green tea, found to reduce the levels of lipid peroxidation and protein carbonyl content in rats, possibly by enhancing the GSH redox status significantly when administered orally [80]. Likewise, several herbal compounds help to reduce oxidative stress, hence play a preventive role against cancer onset.

8.2 Anti-angiogenesis activity of herbal compounds

Quite a few herbal compounds help to inhibit angiogenesis in breast cancer. Genistein, a flavonoid phytoestrogen, is the most potent angiogenesis inhibitor linked with reduced expression of VEGF, PDGF, uPA, and MMP-2 and MMP-9 [81]. Curcumin was even found to be an effective inhibitor of angiogenesis that reduces the expression of various proangiogenic proteins such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor [82]. Resveratrol and quercetin inhibited the migration and tube formation in bovine aorta endothelial cells consequently inhibiting angiogenesis in those cells [83, 84]. In addition, catechin derivatives, such as epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epigallocatechin-3-gallate (EGCG), present in green tea are potent angiogenesis inhibitors [85]. The anti-angiogenic activity of EGCG was demonstrated by inhibition of vascular endothelial growth factor (VEGF) production and reduction of matrix metalloproteinase-2 (MMP-2) activity in MDA-MB231 breast cancer cells [85].

8.3 Apoptosis-inducing activity of herbal compounds

The apoptosis-inducing activity of herbal compounds is another favorable feature that contributes toward their anticancer effect. Curcumin was found to inhibit the proliferation and inducing apoptosis in several cancer cell lines including breast cancer cells such as T47D, MCF7, MDA-MB-231, and MDA-MB-468 [115]. Curcumin inhibited the phosphorylation of protein kinase B (Akt)/mammalian target of rapamycin (mTOR), decreased BCL2 expression, and elevated BAX expression and cleavage of caspase 3, subsequently inducing apoptosis of breast cancer cells [115]. Protocatechuic acid was also found to be a potent apoptosis inducer in five types of human cancer cell lines including breast, lung, liver, cervix, and prostate cancer cells [111], which was confirmed by DNA fragmentation, changes in mitochondrial membrane potential, and measurement of caspase activity. The flavonoid 8-prenylnaringenin (8PN), a constituent of *Humulus lupulus*, is an effective phytocompound known for its growth-inhibiting and apoptotic activity in various human cancer types including breast cancer [116]. This activity of 8PN in MCF7 breast cancer cells was possibly mediated by interference with an ER-associated PI3K pathway [116]. Other herbal compounds like lycopene inhibit cell cycle progression by reducing cyclin D expression and retention of p27 in cyclin E–cdk2, thus leading to inhibition of G1 CDK activities in human breast cell line MCF-7 and T-47D along with endometrial (ECC-1) cancer cells [108].

Interestingly, artemisinin, which is an ancient Chinese herbal compound for malarial fevers, has been recently found to have potent and selective toxicity against cancer cells. It reacts with iron to form free radicals with alkylating capacity that can kill cells. As cancer cells require a large quantity of iron uptake to proliferate, making them more susceptible to the cytotoxic effect of artemisinin [117]. Besides, oral administration of artemisinin delayed the onset of breast cancer in 7, 12-dimethylbenz [a] anthracene (DMBA)-induced rats [118]. This encouraging results might lead to design novel chemotherapeutics with effective anticancer property and low toxicity.

9. Conclusion

Though, advances in healthcare research lead to the identification and characterization of most breast cancer types and corresponding cure. However, incidence and prevalence of breast cancer is rising in terrifying rate in both developed and developing countries because of various risk factors. Improved synthetic drugs and hormonal therapy emerged in a decline in breast cancer incidences, increased survival, and better life quality. However, prolonged use of synthetic anticancer drugs is linked with several health risks or side effects that consequence from the toxic effect of these drugs in normal cells. Chemoprevention by herbal compounds is of great interest and is considered to be an inexpensive, readily applicable, acceptable, and accessible approach to cancer control and management. Herbal remedies play a significant role in the management of breast cancer and the associated therapeutic toxicity. The adjunct use of herbal products and chemotherapy can be an efficient and cost-effective way to treat breast cancer. Such adjuvant therapy proved to produce a synergistic anticancer effect that reduced the drug toxicity, suppresses drug resistance, and provides quick drug action enhancing the quality of treatment. Besides, combinatory therapy might also increase the therapeutic index of the synthetic partner by improving the efficiency of the drug. Plant-derived anticancer drugs such as vinblastine, vincristine, taxols, etc. showed encouraging chemotherapeutic potential that is currently used in breast cancer treatment and a large number of them are in preclinical or in clinical trials. In the last decade, a vast number of phytochemicals were identified that showed encouraging anticancer

activity *in vivo* and *in vitro* breast cancer models. Interestingly, several compounds like artemisinin and isothiocyanates showed selective toxicity toward cancer cells, which recommend clinical trials of these compounds. Furthermore, phytoestrogens with affinity and capacity to produce functional responses through estrogen receptors revealed unique possibilities of using them in hormone replacement therapy. Overall, this chapter can conclude that understanding the molecular mechanism of interaction between herbal compounds and cancer cells in the tumoral environment can help us to design novel anticancer drugs that are less toxic and affordable. This reflects the fact that these goals will only be attainable if the herbal compounds that showed promising anticancer activity can be successfully transferred to an ideal clinical setting for the use of herbal therapies.

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References

[1] Lukong KE. Understanding breast cancer – The long and winding road. BBA Clinical. 2017;**7**:64-77

[2] Hennessy BT, Gonzalez-Angulo AM, Carey MS, Mills GB. A systems approach to analysis of molecular complexity in breast cancer. Clinical Cancer Research. 2009;**15**(2):417-419

[3] Caffarel MM, Pensa S, Wickenden JA, Watson CJ. Molecular biology of breast cancer. In: eLS. Chichester, UK: John Wiley & Sons, Ltd; 2016. pp. 1-9

[4] Shareef M, Ashraf MA, Sarfraz M. Natural cures for breast cancer treatment. Saudi Pharmaceutical Journal. 2016;**24**(3):233-240

[5] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a Cancer Journal for Clinicians. 2018;**68**(6):394-424

[6] Ferlay J et al. Global Cancer Observatory: Cancer Today. Lyon, France: International Agency for Research on Cancer. Available from :https://gco.iarc.fr/today; 2018

[7] Rice S, Whitehead SA. Phytoestrogens and breast cancer - promoters or protectors? Endocrine-Related Cancer. 2006;**13**(4):995-1015

[8] Anand P et al. Cancer is a preventable disease that requires major lifestyle changes. Pharmaceutical Research. 2008;**25**(9):2097-2116

[9] Lambertini M et al. Reproductive behaviors and risk of developing breast cancer according to tumor subtype: A systematic review and meta-analysis of epidemiological studies. Cancer Treatment Reviews. 2016;**49**:65-76

[10] Mørch LS, Skovlund CW, Hannaford PC, Iversen L, Fielding S, Lidegaard Ø. Contemporary hormonal contraception and the risk of breast cancer. The New England Journal of Medicine. 2017;**377**(23):2228-2239

[11] Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. Cell. 2011;**144**(5):646-674

[12] Lin W, Karin M, Lin W, Karin M. A cytokine-mediated link between innate immunity, inflammation, and cancer find the latest version: Review series a cytokine-mediated link between innate immunity, inflammation, and cancer. The Journal of Clinical Investigation. 2007;**117**(5):1175-1183

[13] Harris TJR, McCormick F. The molecular pathology of cancer. Nature Reviews. Clinical Oncology. 2010;**7**:251-265

[14] Wickenden JA, Watson CJ. Key signalling nodes in mammary gland development and cancer. Signalling downstream of PI3 kinase in mammary epithelium: A play in 3 Akts. Breast Cancer Research. 2010;**12**(2):202

[15] Dayem AA, Choi HY, Yang GM, Kim K, Saha SK, Cho SG. The anti-cancer effect of polyphenols against breast cancer and cancer stem cells: Molecular mechanisms. Nutrients. 2016;**8**(9)

[16] Fresco P, Borges F, Diniz C, Marques MPM. New insights on the anticancer properties of dietary polyphenols. Medicinal Research Reviews. 2006;**26**:747-766

[17] Davies E, Hiscox S. New therapeutic approaches in breast cancer. Maturitas. 2011;**68**(2):121-128

[18] Howell A. The endocrine prevention of breast cancer. Best Practice &

Research. Clinical Endocrinology & Metabolism. 2008;**22**(4):615-623

[19] Bright EE, Petrie KJ, Partridge AH, Stanton AL. Barriers to and facilitative processes of endocrine therapy adherence among women with breast cancer. Breast Cancer Research and Treatment. 2016;**158**(2):243-251

[20] Tjan-Heijnen VCG et al. Extended adjuvant aromatase inhibition after sequential endocrine therapy (DATA): A randomised, phase 3 trial. The Lancet Oncology. 2017;**18**(11):1502-1511

[21] Venturini M, Del Mastro L. Safety of adjuvant aromatase inhibitor therapy. Cancer Treatment Reviews. 2006;**32**(7):548-556

[22] Masuda H, Zhang D, Bartholomeusz C, Doihara H, Hortobagyi GN, Ueno NT. Role of epidermal growth factor receptor in breast cancer. Breast Cancer Research and Treatment. 2012;**136**(2):331-345

[23] Costa R et al. Targeting epidermal growth factor receptor in triple negative breast cancer: New discoveries and practical insights for drug development. Cancer Treatment Reviews. 2017;**53**(2017):111-119

[24] Chen T, Sun Y, Ji P, Kopetz S, Zhang W. Topoisomerase IIα in chromosome instability and personalized cancer therapy. Oncogene. 2015;**34**(31):4019-4031

[25] Van Vuuren RJ, Visagie MH, Theron AE, Joubert AM. Antimitotic drugs in the treatment of cancer. Cancer Chemotherapy and Pharmacology. 2015;**76**(6):1101-1112

[26] Lo EJ et al. DrugBank 5.0: A major update to the drug bank database for 2018. Nucleic Acids Research. 2017. Available from: https://www.drugbank.ca/

[27] Middleton J, Stover D, Hai T. Chemotherapy-exacerbated breast cancer metastasis: A paradox explainable by dysregulated adaptive-response. International Journal of Molecular Sciences. 2018;**19**(11):3333

[28] Tao JJ, Visvanathan K, Wolff AC. Long term side effects of adjuvant chemotherapy in patients with early breast cancer. The Breast. 2015;**24**(3):S149-S153

[29] L SC, Abram R. Side effects of adjuvant treatment of breast cancer. The New England Journal of Medicine. 2001;**344**:1997-2008

[30] Partridge AH, Burstein HJ, Winer EP. Side effects of chemotherapy and combined chemohormonal therapy in women with early-stage breast cancer. Journal of the National Cancer Institute. Monographs. 2001;**2001**:135-142

[31] N. P. Staff, Grisold A, Grisold W, Windebank AJ. Chemotherapy-induced peripheral neuropathy: A current review. Annals of Neurology. 2017;**81**(6):772-781

[32] Galluzzi L, Buqué A, Kepp O, Zitvogel L, Kroemer G. Immunogenic cell death in cancer and infectious disease. Nature Reviews. Immunology. 2017;**17**(2):97-111

[33] Vassilakopoulou M, Boostandoost E, Papaxoinis G, de La Motte Rouge T, Khayat D, Psyrri A. Anticancer treatment and fertility: Effect of therapeutic modalities on reproductive system and functions. Critical Reviews in Oncology/Hematology. 2016;**97**(2015):328-334

[34] Blumenfeld Z. Chemotherapy and fertility. Best Practice & Research. Clinical Obstetrics & Gynaecology. 2012;**26**(3):379-390

[35] U. Department of Health, H. Services, N. Institutes of Health Osteoporosis, and R. Bone Diseases National Resource Center. What People Breast Cancer Survivors Need To Know About Osteoporosis. NIH Publication No: 18-7898. 2018. p. 1-3. Available from: https://www.bones.nih.gov/

[36] Chang H-M, Moudgil R, Scarabelli T, Okwuosa TM, Yeh ETH. Cardiovascular complications of cancer therapy. Journal of the American College of Cardiology. 2017;**70**(20):2536-2551

[37] Keilani M, Hasenoehrl T, Neubauer M, Crevenna R. Resistance exercise and secondary lymphedema in breast cancer survivors—A systematic review. Supportive Care in Cancer. 2016;**24**(4):1907-1916

[38] Fadol AP. Management of chemotherapy-induced left ventricular dysfunction and heart failure in patients with cancer while undergoing cancer treatment: The MD Anderson practice. Frontiers in Cardiovascular Medicine. 2018;**5**(March):1-5

[39] Pinder MC, Duan Z, Goodwin JS, Hortobagyi GN, Giordano SH. Congestive heart failure in older women treated with adjuvant anthracycline chemotherapy for breast cancer. Journal of Clinical Oncology. 2007;**25**(25):3808-3815

[40] Swain SM, Whaley FS, Ewer MS. Congestive heart failure in patients treated with doxorubicin: A retrospective analysis of three trials. Cancer. 2003;**97**(11):2869-2879

[41] Mitry MA, Edwards JG. Doxorubicin induced heart failure: Phenotype and molecular mechanisms. IJC Heart & Vasculature. 2016;**10**:17-24

[42] Wolff AC et al. Risk of marrow neoplasms after adjuvant breast cancer therapy: The national comprehensive cancer network experien. Journal of Clinical Oncology. 2015;**33**:340-348

[43] Hwang KH, Cho OH, Yoo YS. Symptom clusters of ovarian cancer patients undergoing chemotherapy, and their emotional status and quality of life. European Journal of Oncology Nursing. 2016;**21**:215-222

[44] Ahmad SS, Reinius MA, Hatcher HM, Ajithkumar TV. Anticancer chemotherapy in teenagers and young adults: Managing long term side effects. BMJ. 2016;**354**:i4567

[45] Weinberg SE, Chandel NS. Targeting mitochondria metabolism for cancer therapy. Nature Chemical Biology. 2015;**11**(1):9-15

[46] Yuan H, Ma Q, Ye L, Piao G. The traditional medicine and modern medicine from natural products. Molecules. 2016;**21**(5):559

[47] Huang WY, Cai YZ, Zhang Y. Natural phenolic compounds from medicinal herbs and dietary plants: Potential use for cancer prevention. Nutrition and Cancer. 2010;**62**:1-20

[48] Amaral RG. Natural products as treatment against cancer: A historical and current vision. Clinical Oncology. 2019;**4, 2018**(January):1-5

[49] Zyad A, Leouifoudi I, Tilaoui M, Mouse HA, Khouchani M, Jaafari A. Natural products as cytotoxic agents in chemotherapy against cancer. In: Cytotoxicity. Vol. i. Rijeka: InTechOpen; 2018. p. 13

[50] Howell A et al. Risk determination and prevention of breast cancer. Breast Cancer Research. 2014;**16**(5):446

[51] Samavat H, Kurzer MS. Estrogen metabolism and breast cancer. Cancer Letters. 2015;**356**(2):231-243

[52] Orlando L et al. Molecularly targeted endocrine therapies for breast cancer. Cancer Treatment Reviews. 2010;**36**(Suppl 3):S67-S71

[53] Lamartiniere CA. Protection against breast cancer with genistein: A component of soy. The American Journal of Clinical Nutrition. 2000;**71**(6):1705S-1707S

[54] Russo M et al. Understanding genistein in cancer: The 'good' and the 'bad' effects: A review. Food Chemistry. 2016;**196**(March):589-600

[55] Zhao E, Mu Q. Phytoestrogen biological actions on mammalian reproductive system and cancer growth. Scientia Pharmaceutica. 2011;**79**(1):1-20

[56] Alexander S. Phytoestrogens and their effects. European Journal of Pharmacology. 2014;**741**:230-236

[57] Rietjens IMCM, Louisse J, Beekmann K. The potential health effects of dietary phytoestrogens. British Journal of Pharmacology. 2017;**174**(11):1263-1280

[58] de Kleijn MJJ et al. Intake of dietary phytoestrogens is low in postmenopausal women in the United States: The Framingham study. The Journal of Nutrition. 2018;**131**:1826-1832

[59] Molina L, Bustamante FA, Bhoola KD, Figueroa CD, Ehrenfeld P. Possible role of phytoestrogens in breast cancer via GPER-1/GPR30 signaling. Clinical Science. 2018;**132**(24): 2583-2598

[60] Ziegler RG. Phytoestrogens and breast cancer. The American Journal of Clinical Nutrition. 2004;**79**(2):183-184

[61] Kim S et al. PubChem 2019 update: Improved access to chemical data. Nucleic Acids Research. 2019;**47**(D1):D1102-D1109

[62] Ambriz-Perez DL, Leyva-Lopez N, Gutierrez-Grijalva EP, Heredia JB. Phenolic compounds: Natural alternative in inflammation treatment. A review. Cogent Food & Agriculture. 2016;**2**(1):1-14

[63] Johnson SB, Park HS, Gross CP, Yu JB. Use of alternative medicine for cancer and its impact on survival. Journal of the National Cancer Institute (JNCI). 2018;**110**(1):121-124

[64] Singh NP, Lai H. Selective toxicity of dihydroartemisinin and holotransferrin toward human breast cancer cells. Life Sciences. 2001;**70**:49-56

[65] Ko YS et al. Polyphenols from *Artemisia annua* L inhibit adhesion and EMT of highly metastatic breast cancer cells MDA-MB-231. Phytotherapy Research. 2016;**30**:1180-1188

[66] Khaghani S, Razi F, Yajloo MM, Paknejad M, Shariftabrizi A, Pasalar P. Selective cytotoxicity and apoptogenic activity of *Hibiscus sabdariffa* aqueous extract against MCF-7 human breast cancer cell line. Journal of Cancer Therapy. 2011;**02**(03):394-400

[67] Gao H et al. Tumor cell selective cytotoxicity and apoptosis induction by an herbal preparation from *Brucea javanica*. North American Journal of Medical Sciences (Boston). 2011;**4**(2):62-66

[68] Wishart DS et al. DrugBank 5.0: A major update to the DrugBank database for 2018. Nucleic Acids Research. 2018;**46**(D1):D1074-D1082

[69] Efferth T et al. Integration of phytochemicals and phytotherapy into cancer precision medicine. Oncotarget. 2017;**8**(30):50284-50304

[70] Mangla B, Kohli K. Combination of natural agent with synthetic drug for the breast cancer therapy. International

Journal of Drug Development and Research. 2009;**10**(1):22-26

[71] Patwardhan B, Vaidya ADB. Natural products drug discovery: Accelerating the clinical candidate development using reverse pharmacology approaches. Indian Journal of Experimental Biology. 2010;**48**(3):220-227

[72] Royt M, Mukherjee S, Sarkar R, Biswas J. Curcumin sensitizes chemotherapeutic drugs via modulation of PKC, telomerase, NF-kappaB and HDAC in breast cancer. Therapeutic Delivery. 2011;**2**(10):1275-1293

[73] Zhan Y, Chen Y, Liu R, Zhang H, Zhang Y. Potentiation of paclitaxel activity by curcumin in human breast cancer cell by modulating apoptosis and inhibiting EGFR signaling. Archives of Pharmacal Research. 2014;**37**(8):1086-1095

[74] Yu Y, Zhou Q, Hang Y, Bu X, Jia W. Antiestrogenic effect of 20S-protopanaxadiol and its synergy with tamoxifen on breast cancer cells. Cancer. 2007;**109**(11):2374-2382

[75] Doll R, Peto R. The causes of cancer: Quantitative estimates of avoidable risks of cancer in the United States today. Journal of the National Cancer Institute. 1981;**66**(6):1191-1308

[76] Roberts H. Safety of herbal medicinal products in women with breast cancer. Maturitas. 2010;**66**:363-369

[77] Poprac P, Jomova K, Simunkova M, Kollar V, Rhodes CJ, Valko M. Targeting free radicals in oxidative stress-related human diseases. Trends in Pharmacological Sciences. 2017;**38**(7):592-607

[78] Motterlini R, Foresti R, Bassi R, Green CJ. Curcumin, an antioxidant and anti-inflammatory agent, induces heme oxygenase-1 and protects endothelial cells against oxidative stress. Free Radical Biology & Medicine. 2000;**28**(8):1303-1312

[79] Farghaly HS, Hussein MA. Protective effect of curcumin against paracetamol-induced liver damage. Australian Journal of Basic and Applied Sciences. 2010;**4**(9):4266-4274

[80] Kumaran VS, Arulmathi K, Srividhya R. Repletion of antioxidant status by EGCG and retardation of oxidative damage induced macromolecular anomalies in aged rats. Experimental Gerontology. 2008;**43**:176-183

[81] Pratheeshkumar P et al. Cancer prevention with promising natural products: Mechanisms of action and molecular targets. Anti-Cancer Agents in Medicinal Chemistry. 2012;**12**(10):1159-1184

[82] Fu Z, Chen X, Guan S, Yan Y, Lin H, Hua Z-C. Curcumin inhibits angiogenesis and improves defective hematopoiesis induced by tumor-derived VEGF in tumor model through modulating VEGF-VEGFR2 signaling pathway. Oncotarget. 2015;**6**(23):19469-19482

[83] Singh CK, Chhabra G, Ahmad N. Resveratrol and cancer cell biology. In: Resveratrol: State-of-the-Art Science and Health Applications. World Scientific; 2018. pp. 183-207

[84] Balakrishnan S et al. Gold nanoparticle-conjugated quercetin inhibits epithelial-mesenchymal transition, angiogenesis and invasiveness via EGFR/VEGFR-2-mediated pathway in breast cancer. Cell Proliferation. 2016;**49**(6):678-697

[85] Wang Z et al. Broad targeting of angiogenesis for cancer prevention and therapy. Seminars in Cancer Biology. 2015;**35**:S224-S243

[86] Liu P et al. Anti-cancer activities of allyl isothiocyanate and its conjugated silicon quantum dots. Scientific Reports. 2018;**8**(1):1-11

[87] Geng F et al. Allyl isothiocyanate arrests cancer cells in mitosis, and mitotic arrest in turn leads to apoptosis via Bcl-2 protein phosphorylation. The Journal of Biological Chemistry. 2011;**286**:32259-32267

[88] Slezakova S, Ruda-kucerova J. Anticancer activity of artemisinin and its derivatives. Anticancer Research. 2017;**37**(11):5995-6003

[89] Tin AS, Sundar SN, Tran KQ, Park AH, Poindexter KM, Firestone GL. Antiproliferative effects of artemisinin on human breast cancer cells requires the downregulated expression of the E2F1 transcription factor and loss of E2F1-target cell cycle genes. Anti-Cancer Drugs. 2012;**23**(4):370-379

[90] Moon YJ, Shin BS, An G, Morris ME. Biochanin a inhibits breast cancer tumor growth in a murine xenograft model. Pharmaceutical Research. 2008;**25**:2158-2163

[91] Bhushan A, Sehdev V, Lai JCK. Biochanin a modulates cell viability, invasion, and growth promoting signaling pathways in HER-2-positive breast cancer cells. Journal of Oncology. 2009;**2009**

[92] Kishore L, Kaur N, Singh R. Bacosine isolated from aerial parts of Bacopa monnieri improves the neuronal dysfunction in Streptozotocin-induced diabetic neuropathy. Journal of Functional Foods. 2017;**34**;237-247

[93] Mishra SR, Yadav PK, Nandhakumar P, Saini M, Kumar A, Kataria M. In vitro analysis of Bacosine as novel therapeutic agent for murine breast cancer. Proceedings of the National Academy of Sciences, India Section B. 2019;**89**(2):511-515

[94] Liu D, Chen Z. The effect of curcumin on breast cancer cells. Journal of Breast Cancer. 2013;**16**(2):133-137

[95] Aggarwal BB et al. Curcumin suppresses the paclitaxel-induced nuclear factor-κB pathway in breast cancer cells and inhibits lung metastasis of human breast cancer in nude mice. Clinical Cancer Research. 2005;**11**(20):7490-7498

[96] Choudhuri T, Pal S, Agwarwal ML, Das T, Sa G. Curcumin induces apoptosis in human breast cancer cells through p53-dependent Bax induction. FEBS Letters. 2002;**512**(1-3):334-340

[97] Hou DX, Tong X, Terahara N, Luo D, Fujii M. Delphinidin 3-sambubioside, a hibiscus anthocyanin, induces apoptosis in human leukemia cells through reactive oxygen species-mediated mitochondrial pathway. Archives of Biochemistry and Biophysics. 2005;**440**(1):101-109

[98] Wu CH, Huang CC, Hung CH, Yao FY, Wang CJ, Chang YC. Delphinidin-rich extracts of Hibiscus sabdariffa L. trigger mitochondria-derived autophagy and necrosis through reactive oxygen species in human breast cancer cells. Journal of Functional Foods. 2016;**25**:279-290

[99] Du GJ et al. Epigallocatechin gallate (EGCG) is the most effective cancer chemopreventive polyphenol in green tea. Nutrients. 2012;**4**(11):1679-1691

[100] Hong OY et al. Epigallocatechin gallate inhibits the growth of MDA-MB-231 breast cancer cells via inactivation of the β-catenin signaling pathway. Oncology Letters. 2017;**14**(1):441-446

[101] Xiang LP et al. Suppressive effects of tea catechins on breast cancer. Nutrients. 2016;**8**(8):1-15

[102] Liggins J, Bluck LJ, Runswick S, Atkinson C, Coward WA, Bingham SA. Daidzein and genistein content of fruits and nuts. The Journal of Nutritional Biochemistry. 2000;**11**(6):326-331

[103] Spagnuolo C et al. Genistein and cancer: Current status, challenges, and future directions. Advances in Nutrition. 2015;**6**:408-419

[104] Lamartiniere CA. Protection against breast cancer with genistein: A component. The American Journal of Clinical Nutrition. 2018;**71**(March):1705-1707

[105] Cai Y, Zhao B, Liang Q, Zhang Y, Cai J, Li G. The selective effect of glycyrrhizin and glycyrrhetinic acid on topoisomerase IIα and apoptosis in combination with etoposide on triple negative breast cancer MDA-MB-231 cells. European Journal of Pharmacology. 2017;**809**(December):87-97

[106] Wang X-F, Zhou Q-M, Lu Y-Y, Zhang H, Huang S, Su S-B. Glycyrrhetinic acid potently suppresses breast cancer invasion and metastasis by impairing the p38 MAPK-AP1 signaling axis. Expert Opinion on Therapeutic Targets. 2015;**19**(5):577-587

[107] Holzapfel NP, Holzapfel BM, Champ S, Feldthusen J, Clements J, Hutmacher DW. The potential role of lycopene for the prevention and therapy of prostate cancer: From molecular mechanisms to clinical evidence. International Journal of Molecular Sciences. 2013;**14**(7):14620-14646

[108] Nahum A et al. Lycopene inhibition of cell cycle progression in breast and endometrial cancer cells is associated with reduction in cyclin D levels and retention of p27 Kip1 in the cyclin E-cdk2 complexes. Oncogene. 2001;**20**(26):3428-3436

[109] Kakkar S, Bais S. A review on protocatechuic acid and its pharmacological potential. ISRN Pharmacology. 2014;**2014**(4):1-9

[110] Tseng TH et al. Inhibitory effect of hibiscus protocatechuic acid on tumor promotion in mouse skin. Cancer Letters. 1998;**126**(2):199-207

[111] Yin MC, Lin CC, Wu HC, Tsao SM, Hsu CK. Apoptotic effects of protocatechuic acid in human breast, lung, liver, cervix, and prostate cancer cells: Potential mechanisms of action. Journal of Agricultural and Food Chemistry. 2009;**57**(14):6468-6473

[112] Woźniak Ł, Skąpska S, Marszałek K. Ursolic acid - a pentacyclic triterpenoid with a wide spectrum of pharmacological activities. Molecules. 2015;**20**(11):20614-20641

[113] Jäger S, Trojan H, Kopp T, Laszczyk MN, Scheffler A. Pentacyclic triterpene distribution in various plants - rich sources for a new group of multi-potent plant extracts. Molecules. 2009;**14**(6):2016-2031

[114] Mizushina Y, Iida A, Ohta K, Sugawara F, Sakaguchi K. Novel triterpenoids inhibit both DNA polymerase and DNA topoisomerase. The Biochemical Journal. 2000;**350**(3):757-763

[115] Hu S, Xu Y, Meng L, Huang L, Sun H. Curcumin inhibits proliferation and promotes apoptosis of breast cancer cells. Experimental and Therapeutic Medicine. 2018;**16**:1266-1272

[116] Brunelli E, Minassi A, Appendino G, Moro L. 8-Prenylnaringenin, inhibits estrogen receptor-α mediated cell growth and induces

apoptosis in MCF-7 breast cancer cells. The Journal of Steroid Biochemistry and Molecular Biology. 2007;**107**(3-5):140-148

[117] Konstat-Korzenny E, Ascencio-Aragón J, Niezen-Lugo S, Vázquez-López R. Artemisinin and its synthetic derivatives as a possible therapy for cancer. Medical Science. 2018;**6**(1):19

[118] Lai H, Singh NP. Oral artemisinin prevents and delays the development breast cancer in the rat. Cancer Letters. 2006;**231**:43-48

Chapter 11

Chinese Herbal Medicine and Its Application for Female Cancer

Rongyun Wang, Qiuhua Sun, Yifan Lin, Ling Wang, Yuan Liu, Chi Chiu Wang and Lu Li

Abstract

Chinese herbal medicines (CHMs) have been widely used to promote health and treat illnesses in daily medical care throughout Asia while mostly accepted as an alternative medical method in many nations of the western world. CHM has a unique therapeutic effect to reduce adverse effects on cancer patients caused by chemotherapy and surgery; however, we did not find any high-quality review for the claimed effects. In this review, we will summarize the history, basic theories and principles, and clinical applications of CHM for disorders, especially female cancers. Meta-analyses to evaluate the efficacy and safety of CHM in the treatment of ovarian cancer and breast cancer have been conducted. The results showed that combined CHMs and western medicines treatment (CHM-WM) had significantly relieved the symptoms and reduced the side effects after surgery and chemotherapy on both ovarian cancer and breast cancer. However, more high-quality and large-scale RCTs are necessary to confirm the efficacy and safety of CHM-WM intervention.

Keywords: Chinese herbal medicines, female cancer, efficacy and safety, ovarian cancer, breast cancer, clinical application

1. Traditional Chinese medicine and Chinese herbal medicines

1.1 Traditional Chinese medicine

Traditional Chinese medicine (TCM) has a history that spans 5000 years and has been widely used to promote health and treat illnesses [1]. Although TCM has such wide applications as an important part in daily medical care throughout Asia, it is mostly accepted as an alternative medical method in many nations of the western world.

There are nine approaches of TCM, including Chinese medicines (中藥), acupuncture (針灸), food therapy (食療), Qi Gong (氣功), tai chi exercise (太極), tui na (推拿), cupping (拔罐), die da (跌打), and gua sha (刮痧). Most of these therapeutic techniques in TCM have spread aboard since the sixth century BC, and in most western countries, Chinese medicines and acupuncture are the two most popular therapies of TCM nowadays.

With the advantages of Chinese medicines, including less side effects and greater effectiveness in some chronic diseases and cancers than Western medicines, it was gradually accepted by foreigners and now has been spread to over 160 countries [2].

More and more foreign researchers and clinical doctors seriously have interests in it and come to China for further study.

1.2 Chinese herbal medicine books

Most Chinese medicines are derived from nature, including plants, animals, and minerals. Herbal medicines from plants are much more commonly applied than the others. In China, it is not only considered as a primary therapy for treatment but also as a supplementary therapy to promote health in general [2].

Shen Nong's *Herbal Classic* (神農本草經) [3], which is considered the oldest book on oriental medicines, roughly classified 365 species of roots, grass, and woods from plants and furs, animals, and stones into three categories (finest grade, moderate grade, and lowest grade) according to toxicity, effectiveness, and pharmacological effect of them. "Ben Cao" has been developed widely and quickly in China and has been comprehensively studied for about 2000 years. Another world famous book is *Compendium of Materia Medica* (本草綱目), which is also called the *Encyclopedia of China* [4], which considered as the most important and comprehensive review to study herbs by classification, names, property, identification, function, application, formula, and so on. It opened the door to the world for the herbal medicines in Chinese medicine since it was translated into different languages in the seventeenth century [4].

In the late nineteenth century, with the influence of foreign scientific and technical influences, Western medicine spread to China, and it has been in coexistence with Chinese medicines since then [5]. Correspondingly, the community and medical societies defined a new concept of "Chinese herbal medicines (CHMs)" from "Ben Cao" and "Chinese medicines" as different from "Western medicines" [6] to identify the corresponding medicines. After the establishment of the People's Republic China, there comes the faster development of CHM [2].

Chinese Pharmacopeia (中國藥典) and *Chinese Herbal Medicine* (中華本草) were published and considered as the most useful and important reference books as Bibles of the Chinese medicine. In *Chinese Pharmacopeia* over 3700 Chinese medicines were listed, while in *Chinese Herbal Medicine* over 8900 different medicinal substances were recorded. Approximately 600 Chinese herbs are widely used, while about 250 or so are commonly used in clinical practice [7].

These two Bibles gave all the details and information on individual herbs, animals, and mineral products, including formal names, different names, common names, species, source, original plant, cultivation (aquaculture) point, harvest processing, medicine and marketing, medicine identification, chemical composition, pharmacology, processing, properties, effects, application indications, compatibility, usage, dosage, precautious, preparation, clinical research, medicine theory, annals, notes, and references. They also conclude on special topics about history, resources, storage, chemistry, and pharmacology in Chinese medicines [7].

2. Theories and principles of Chinese herbal medicines

2.1 Basic properties of CHM

Four gases (四氣), namely, hot (熱), warm (溫), cold (寒), and cool (涼), refer to four temperature characteristics of the herbs [2, 8]. There is another character called neutral (平), which means the existence of both hot and cold [2].

Five flavors (五味), namely, sour (酸), bitter (苦), sweet (甘), spicy (辛), and salty (咸), refer to five taste properties of the herbs [2].

Lifting-dropping and floating-sinking (升降浮沉) applies the elevation-elimination and outward-inward tendencies of the herbs in the body. For example, good controlling of the Qi and blood is the basic way in stroke treatment in Chinese medicine, and then a doctor may give a Chinese Thorowax root (Chai Hu) and immature bitter orange (Zhi Shi) as basic formula [2, 6, 9]. With the lifting function of Thorowax root, it can clear up the gas accumulated in the liver. Depends on the dropping effect of bitter orange, it can sort out the gas in the intestine and then make the gas flow smoothly within the body.

Take lotus as an example; its flower is used to prevent heat stroke, its nut is applied to treat hypertension, its root can improve gastrointestinal function, and its stem has anti-miscarriage effects [10].

2.2 Application principles of CHM

As in Western medicine, medicines are often applied separately or in combination as cocktail with the same or complementary function. It is important to understand that each medicine has its own effect and function, but in most cases, instead of being prescribed individually, formulae are commonly used by TCM practitioners as therapy to different kinds of health-related problems [11].

Chinese medicines are prescribed in formulae. Based on medical knowledge and personal experience, the TCM practitioners decide the formula according to the clinical presentation [12]. In practice of CHM, the first formula is based on identified health problems according to the basic diagnosis theories, that is, the TCM practitioners will choose one basic formula (consisting of 4–20 herbs) from all traditional formulae related to this disease. Then the doctors will add some other herbal medicines into or subtract other herbal medicines from the basic formula, mostly according to their own experiences. For example, if a patient is suffering from headache, the TCM practitioner will prescribe a formula called "Yin Qiao San (銀翹散)" as basic formula [11], for the patient who is catching a cold. The practitioner will further identify if the patient needs some more medicines; such as if the patient has a heavy headache, mulberry leaf (Sang Ye, *Folium Mori*) will be added into the basic formula [11]. Sometimes, the practitioners will also make changes to the dosages of some herbal medicines or the whole formula to meet the specific needs of individual patients. For example, if the patient is a child, the doctor may consider reducing the total dosage and duration of the formula.

3. Clinical application of Chinese herbal medicines

3.1 Efficacy and safety of CHM for ovarian cancer

Ovarian cancer (OvC) is a malignant tumor which invades the ovarian epithelium and interstitium. Reduction surgery combined with adjuvant chemotherapy is the standard treatment for OvC patients, but the adverse effects due to chemotherapy still remain a major problem. While CHM therapy has a unique therapeutic effect to reduce side effects of chemotherapy by boosting the immune system, the evidence of CHM in the treatment of OvC patients is limited [13].

A systematic review to evaluate the efficacy and safety of CHM in the treatment of OvC after reduction surgery and adjuvant chemotherapy has been conducted.

Chinese National Knowledge Infrastructure (CNKI) and PubMed up to December 31, 2018, were searched to identify relevant studies. Only randomized controlled trials (RCTs) were included, and there was no limitation on language of the publication. Data were extracted from all included studies, and meta-analysis

was performed with ReviewManager 5.3. The study quality was assessed, and pooled risk ratios (RR) or mean difference (MD) with 95% CIs were used to evaluate the efficacy and safety of CHM.

A total of 18 RCTs involving 975 participants were included. There was no placebo, no treatment, and CHM alone. Compared with Western medicine (WM) alone, Chinese herbal medicine combined with WM (CHM-WM) significantly improved TCM syndromes and symptoms, KPS scores, CD4 counts, CA125 levels, and 3-year survival rate ($P < 0.05$). Incidences of gastrointestinal reactions, marrow depression, and urinary system symptoms were significantly lower in CHM-WM group than in WM group ($P < 0.01$). There was no significant difference in CD3 counts, CD8 counts, quality of life, liver function, and peripheral neuropathy between the two groups ($P > 0.05$).

The systematic review indicated that CHM combined with WM is effective and safe as a treatment for OvC patients after reduction surgery and adjuvant chemotherapy. However, more high-quality and large-scale RCTs are needed to confirm the efficacy and safety of CHM intervention.

3.2 Efficacy and safety of CHM for breast cancer

Breast cancer is a disease in which malignant (cancer) cells form in the tissues of the breast. Surgery combined with adjuvant chemotherapy is considered as the standard treatment for breast cancer patients, but the adverse effects after surgery and chemotherapy still cause big problems. While CHM therapy has a unique therapeutic effect to reduce side effects of chemotherapy and surgery by boosting immune system, we did not find any high-quality review for the claimed effects of CHM-WM treatment on breast cancer patients. A systematic review to evaluate the efficacy and safety of CHM-WM treatment in patients with breast cancer has been conducted.

Chinese National Knowledge Infrastructure (CNKI) and PubMed databases (up to August 31, 2019) were searched to identify relevant clinical studies. Only randomized controlled trials (RCTs) were included, and there was no limitation on language of the publication. Data were extracted from all included studies, and meta-analyses were performed with Review Manager 5.3. The study quality was assessed, and pooled risk ratios (RR) or mean difference (MD) with 95% CIs were used to evaluate the efficacy and safety of treatments.

A total of 22 RCTs involving 1256 participants was included. Compared with WM alone, CHM-WM significantly improved skin flap necrosis and upper limb lymphedema after breast cancer surgery ($P < 0.05$). Incidences of nausea and vomiting, constipation, marrow depression, and hot flashes were significantly lower in CHM-WM group than in WM group ($P < 0.05$). There was no significant difference in tumor metastasis and recurrence, survival rate, platelet counts, and CD3 and CD4 levels between the two groups ($P > 0.05$).

Meta-analysis results showed that the combination of CHMs and WMs was an effective and safe therapy for patients with breast cancer after surgery and chemotherapy. However, more high-quality and large-scale RCTs are necessary to confirm the efficacy and safety of CHM-WM intervention.

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References

[1] Loudon I. Western Medicine: An Illustrated History. Oxford, UK: Oxford University Express; 1997. pp. 1-39

[2] Li JB. Zhong Yi Xue. 6th ed. Beijing, China: People's Health Publishing House; 2005. pp. 1-135, 186, 187

[3] Wu P, Sun XY, Sun FJ, et al. ShenNong's Herbal Classic (3 Volumes). 1st ed. Shenyang, China: Liaoning Science and Technology Press; 1995

[4] Li SZ, Chen GT, et al. Compendium of Materia Medica (52 Volumes). Beijing, China: Chinese Medicine Ancient Books Publishing House; 1994

[5] Wang SM, Luo WQ. Image Traditional Chinese Medicine: Research on Historic Images of Traditional Chinese Medicine. 1st ed. Beijing, China: People's Health Publishing House; 2007. pp. 1-100

[6] Zhang JR. Traditional Chinese Pharmacology. Beijing, China: Traditional Chinese Medicine Publishing House; 1997

[7] Chen Z, Shao ML, et al. Chinese Pharmacopoeia. 9th ed. Chemical Industry Press; 2010. pp. 633-983

[8] Wang DR. Concise and Practical Traditional Chinese Medicine. 1st ed. Beijing, China: Traditional Chinese Medicine Publishing House; 1997. pp. 1-233

[9] Yan ZH. Chinese Medicine Pharmacology. 2nd ed. Beijing, China: People's Health Publishing House; 2006

[10] Wang TQ. Lotus medicinal dietary prescription. Oriental Medicinal Diet. 2008;**11**:50-55

[11] Chang MY. Chinese medicines for anti-cancer experiments. China Journal of Chinese Materia Medica. 1997;**22**(11):694-696

[12] Cohen I, Tagliaferri M, Tripathy D. Traditional Chinese medicine in the treatment of breast cancer. Journal of Chinese Medicine. 2002;**68**:40-49

[13] Wang R, Sun Q, Wang F, Liu Y, Li X, Chen T, et al. Efficacy and safety of Chinese herbal medicine on ovarian cancer after reduction surgery and adjuvant chemotherapy: A systematic review and meta-analysis. Frontiers in Oncology. 2019;**9**:730. DOI: 10.3389/fonc.2019.00730

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