IntechOpen

IntechOpen Book Series
Biochemistry, Volume 21

Terpenes and Terpenoids

Recent Advances

Edited by Shagufta Perveen
and Areej Mohammad Al-Taweel

Terpenes and Terpenoids-Recent Advances

Edited by Shagufta Perveen and Areej Mohammad Al-Taweel

Published in London, United Kingdom



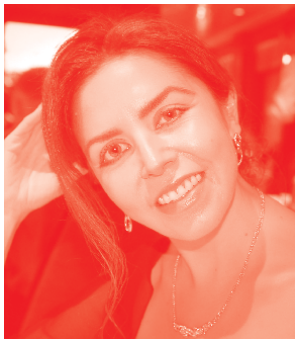




IntechOpen





Supporting open minds since 2005

Terpenes and Terpenoids-Recent Advances
http://dx.doi.org/10.5772/intechopen.87558
Edited by Shagufta Perveen and Areej Mohammad Al-Taweel

Part of IntechOpen Book Series: Biochemistry, Volume 21
Book Series Editor: Miroslav Blumenberg

Contributors
Zetty-Norhana Balia Yusof, Umme Tamanna Ferdous, Nanik Siti Aminah Aminah, Paco Noriega, Fernando Ramos, Alexandra Valencia, Frank Romero-Orejon, Adriana Viñas-Ospino, Dayana Barriga-Rodriguez, Ana Maria Muñoz, Jose Henrique Leal Cardoso, Ana Carolina Cardoso-Teixeira, Klausen Oliveira-Abreu, Andrelina Noronha Coelho-De-Souza, Levy Gabriel De Freitas Brito, Bechir Baccouri, Rajhi Imen, Dwi Setyorini Setyorini, Shagufta Perveen, Khun Nay Win Tun, Alfinda Novi Kristanti, Hnin Thanda Aung, Yoshiaki Takaya

First published in London, United Kingdom, 2021 by IntechOpen
IntechOpen is the global imprint of INTECHOPEN LIMITED, registered in England and Wales, registration number: 11086078, 5 Princes Gate Court, London, SW7 2QJ, United Kingdom
Printed in Croatia

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

Additional hard and PDF copies can be obtained from orders@intechopen.com

Terpenes and Terpenoids-Recent Advances
Edited by Shagufta Perveen and Areej Mohammad Al-Taweel
p. cm.
Print ISBN 978-1-83881-916-3
Online ISBN 978-1-83881-917-0
eBook (PDF) ISBN 978-1-83881-918-7
ISSN 2632-0983

IntechOpen Book Series

Biochemistry

Volume 21

Prof. Shagufta Perveen is a Distinguish Professor in the Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. Dr. Perveen has acted as the principal investigator of major research projects funded by the research unit of King Saud University. She has more than ninety original research papers in peer-reviewed journals of international repute to her credit. She is a fellow member of the Royal Society of Chemistry UK and the American Chemical Society of the United States.

Prof. Areej Al-Taweel obtained her Ph.D. in Pharmacognosy from King Saud University, Riyadh, Saudi Arabia. Her research focuses on different Saudi Arabian medicinal plants and she has isolated many bioactive natural products. Dr. Al-Taweel has published more than fifty-five papers in ISI-ranking journals.

Editors of Volume 21:
Shagufta Perveen and Areej Al-Taweel
Department of Pharmacognosy, College of Pharmacy
King Saud University, Riyadh, Saudi Arabia

Book Series Editor: Miroslav Blumenberg
NYU Langone Medical Center, New York, USA

Scope of the Series

Biochemistry, the study of chemical transformations occurring within living organisms, impacts all of the life sciences, from molecular crystallography and genetics, to ecology, medicine and population biology. Biochemistry studies macromolecules - proteins, nucleic acids, carbohydrates and lipids –their building blocks, structures, functions and interactions. Much of biochemistry is devoted to enzymes, proteins that catalyze chemical reactions, enzyme structures, mechanisms of action and their roles within cells. Biochemistry also studies small signaling molecules, co-enzymes, inhibitors, vitamins and hormones, which play roles in the life process. Biochemical experimentation, besides coopting the methods of classical chemistry, e.g., chromatography, adopted new techniques, e.g., X-ray diffraction, electron microscopy, NMR, radioisotopes, and developed sophisticated microbial genetic tools, e.g., auxotroph mutants and their revertants, fermentation, etc. More recently, biochemistry embraced the 'big data' omics systems.

Initial biochemical studies have been exclusively analytic: dissecting, purifying and examining individual components of a biological system; in exemplary words of Efraim Racker, (1913 –1991) "Don't waste clean thinking on dirty enzymes." Today, however, biochemistry is becoming more agglomerative and comprehensive, setting out to integrate and describe fully a particular biological system. The 'big data' metabolomics can define the complement of small molecules, e.g., in a soil or biofilm sample; proteomics can distinguish all the proteins comprising e.g., serum; metagenomics can identify all the genes in a complex environment e.g., the bovine rumen. This Biochemistry Series will address both the current research on biomolecules, and the emerging trends with great promise.

Contents

Preface

Terpenes belong to the diverse class of chemical constituents isolated from materials found in nature (plants, fungi, insects, marine organisms, plant pathogens, animals and endophytes). These metabolites have simple-to-complex structures derived from Isopentyl diphosphate (IPP), dimethylallyl diphosphate (DMAPP), mevalonate and deoxyxylulose biosynthetic pathways. Terpenes play a very important role in human health and have significant biological activities (anticancer, antimicrobial, anti-inflammatory, antioxidant, anti-allergic, skin permeation enhancer, anti-diabetic, immunomodulatory, anti-insecticidal). According to new research, cineole (a spicy eucalyptus-derived flavoring oil) terpenes are ready to be directly converted to biofuel as soon as they are produced. This book provides an overview and highlights recent research in the phytochemical and biological understanding of terpenes and terpenoids and explains the most essential functions of these kinds of secondary metabolites isolated from natural sources.

Dr. Shagufta Perveen
Professor,
Distinguish Professor, FRSC-UK,
Department of Pharmacognosy,
College of Pharmacy,
King Saud University,
Riyadh, Saudi Arabia

Dr. Areej Al-Taweel
Professor,
Department of Pharmacognosy,
College of Pharmacy,
King Saud University,
Riyadh, Saudi Arabia

Chapter 1

Introductory Chapter: Terpenes and Terpenoids

Shagufta Perveen

1. Terpenes and terpenoids

Terpenes are the largest class of secondary metabolites found in nature (plants, fungus, marine organisms, animals). Terpenes are mainly present as a main constituent of essential oils. It consists of isoprene units (CH_2=C(CH_3)CH=CH_2, C_5H_8) which are known as the building block of all types of terpenes, containing five carbon and eight hydrogen atoms. Monoterpenes have two isoprene units (C_{10}), sesquiterpenes have three (C_{15}), diterpenes have four (C_{20}), sesterpene have five (C_{25}), triterpenes have six (C_{30}) and tetraterpenes have eight isoprene units (C_{40}). Terpenes and terpenoids based chemical constituents are characterized by different chemical diversity with a wide range of therapeutic effects. This class of metabolites has been an enormous source of novel medicinal agents. Many terpenoids or terpenoid derivatives are used as traditional drugs with different medicinal values identified from different natural sources. *Artemisia annua* (sweet wormwood) a medicinal plant belongs to the family Asteraceae provided a drug artemisinin and its related derivatives which used as an antimalarial drug all over the world. Scientists Professor Tu Youyou was awarded Nobel Prize 2015 in Physiology or Medicine for her efforts toward the discovery of this important drug. Artemisinin and its derivatives are mainly sesquiterpenes (fifteen Carbons containing terpenes) which is known as a magical drug which served as the foundation for antimalarial treatment. Currently, many research groups have been reported the therapeutic potential of terpenes and its extract (terpene rich plant extracts) against anticancer, anti-inflammatory and SARS-CoV-2 and performed many tests and screenings. Many studies have been done for testing the efficacy of cannabis terpene for the treatment of this new viral infections [1, 2]. This chapter provides information about recently published terpenes which showed significant biological activities have unique skeletons.

2. Hemiterpene

Hemiterpenes are the basic unit of terpenes and its consists of five carbon atoms (CH_2=C(CH_3)CH=CH_2) or one isoprene unit. It is usually found in different types of plants especially Coniferous, Willow and Oaks. Many types of hemiterpenes have isolated from different marine derived fungi (*Acremonium persicinum*, *Penicillium bialowiezense*) which are known as merohemiterpenoid]. Herein, we are discussing some of the recently published chemical diverse emiterpenes (**Table 1**, **Figure 1**).

Name	Source	Activity	Ref
Securiterpenoside G	*Securidaca inappendiculata* found in China	The potential anti-inflammatory activities of compounds were evaluated through inhibiting nitric oxide (NO) overproduction in LPS-stimulated mouse macrophage RAW264.7 model. Cell viability was measured by the MTT assay. None of them showed the obvious cytotoxicity at the dosage of 50 μM and significant anti-inflammatory activities (IC50 145.3, 57.5 μM, respectively). Dexamethasone was used as positive control (IC50 2.5 μM).	[3]
(±)-Cytorhizophin A, Cytorhizophin B	Endophytic fungus *Cytospora rhizophorae* from the plant *Morinda officinalis*	These compounds were evaluated for antimicrobial activities against the bacteria *Escherichia coli* and *Staphylococcus aureus*. However, all of them were found to be devoid of significant activity even at a concentration of 100 μg mL^{-1}.	[4]

Table 1.
Source and biological activities of some hemiterpenes.

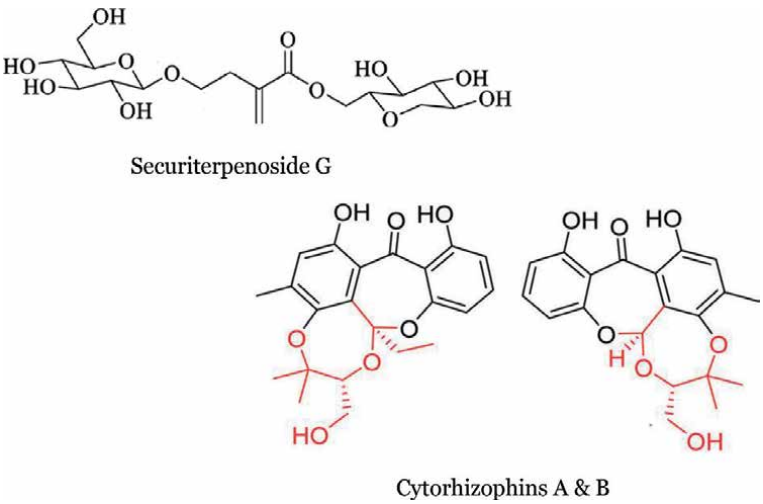


Figure 1.
Structure of hemiterpene.

3. Monoterpenes

These types of terpenes consist on ten carbon atom or two isoprene units. Each type of monoterpenes has a particular aroma for the related plant such as: Citrus, grapes, rose etc. Many monoterpenes and their isomers have been isolated from different marine sources. Herein, we are discussing some of the recently published monoterpenes (**Table 2**, **Figure 2**).

Name	Source	Activity	Ref
N-glucopyranosyl vincosamide, vincosamide	*Psychotria leiocarpa* Leaves found in Brazil	Vincosamide with a preliminary dose-dependent activity inhibiting at 50 μg mL^{-1} 99% of DENV infectious particles in the conditioned medium of infected HepG2 culture can be highlighted among the other isolated alkaloids as a potential anti-dengue agent.	[5]

Name	Source	Activity	Ref
Callistrilones H & I	*Callistemon rigidus* found in China	Compounds exhibited moderate inhibitory activities against HSV- 1 with IC_{50} values of 10.00 ± 2.50 and 12.50 ± 1.30 μM, respectively.	[6]
Plaxenones A & B	South African red seaweed *Plocamium maxillosum*	Plaxenones A and B were evaluated for activity against the metastatic breast carcinoma (MDA-MB-231) cell line and showed moderate antiproliferative effects with IC_{50} values of 10.78 ± 1.01 and 22.30 ± 1.13 μM, respectively.	[7]
Melodinines Y1		It showed cytotoxicity toward six cancer cell lines. The new modification of the isolated compounds expands the chemical diversity of this family. Cytotoxicity assays have demonstrated that compound significantly inhibited HL-60 cancer cell line, presenting with a great opportunity to discover promising natural agents for new antitumor leadings.	[8]

Table 2.
Source and biological activities of some monoterpenes.

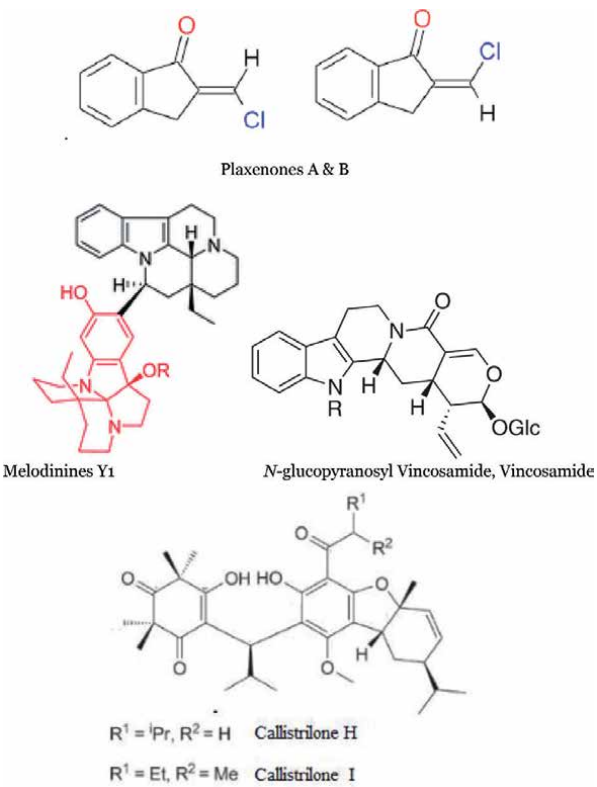


Figure 2.
Structure of monoterpene.

4. Sesquiterpenes

Sesquiterpenes are the class of terpenes with C15 carbon atoms having many uses like medicine, sanitary, agriculture, cosmetics and foods. These types of terpenes have many biological activities like, antibacterial, antifungal, antiviral and

ant insecticidal which provokes the researcher to work on the sesquiterpene rich natural sources. It is usually found in Asteraceae family plants. Herein we are tabulating few important sesquiterpene with their structure and biological information (**Table 3**, **Figure 3**).

Name	Source	Activity	Ref
Minusolide G	*Carpesium minus*	It exhibited cytotoxic activities against MDA-MB-231, A549, and HCT-116 cells with IC_{50} values of 6.1 ± 0.2, 8.4 ± 0.6, and 3.7 ± 0.6 μM, respectively. It induced the apoptosis of HCT-116 cells via suppression of PARP and promoting cleavage of PARP.	[9]
Penisarins A & B	Endophytic *Penicillium* sp. found in China	Penisarin B showed significant cytotoxicities against two human cancer cell lines, HL-60 and SMMC-7721, with IC_{50} values of 3.6 ± 0.2 and 3.7 ± 0.2 μM, respectively.	[10]
Cedrol	*Cedrus atlantica* Cedarwood oil	Cedrol-treated mice exhibited no significant differences in body weight and improved TMZ-induced liver damage. These results imply that cedrol may be a potential novel agent for combination treatment with TMZ for GBM therapy that deserves further investigation.	[11]

Table 3.
Source and biological activities of some sesquiterpenes.

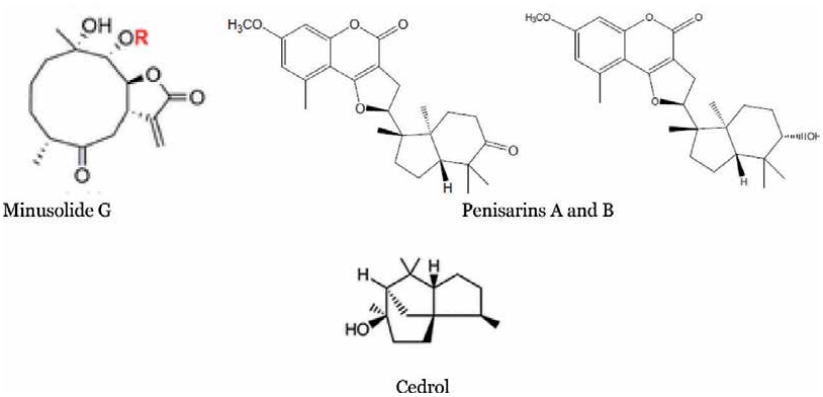


Figure 3.
Structure of sesquiterpene.

5. Diterpenes

It consists on C20 carbon atom having four isoprene units. These are very famous class of compounds as many are using in market for curing cancer

Name	Source	Activity	Ref
Kaemgalangols B-D	Edible rhizomes of *Kaempferia galanga* found in India	The antiproliferative activity of compounds was screened against CCRF-CEM leukemia cells using a fixed concentration of 30 μM. Dose response curve of (2*R*)-ent-2-hydroxyisopimara-8(14),15-dien showed IC_{50} values of ≤ 50 μM against CCRF-CEM, MDAMB-231-pcDNA and HCT116 (p53+/+).	[12]

Name	Source	Activity	Ref
Wickerols A & B	Fungus *Trichoderma atroviride* FKI-3849 from a soil sample	Wickerol A was highly active against two A/H1N1 viruses, but not active against two A/H_3N_2 viruses or a B-type virus.	[13, 14]
Nukiangendines A & B	*Abies Nukiangensis* found in China	Compounds were subjected to an in vitro bioassay for anti-hepatitis C virus (HCV) infection activity. Nukiangendine A exhibits a significant effect at 10 μM with an inhibition rate of 70.0%, compared to 99.0% for sofosbuvir (the positive control) at 0.01 μM.	[15]
Stachyonic acid A & B	*Basilicum polystachyon*	Stachyonic acids A & B was tested for cytotoxicity against human cells, breast and melanoma along with primary neonatal foreskin fibroblast cells. Mixture of both showed limited cytotoxicity toward all cell lines investigated. Stachyonic acid A, was found to display potent inhibitory activity against dengue virus.	[16, 17]
Andrographolide	*Andrographis paniculata* from Thailand	The study demonstrated anti-SARS-CoV-2 activity of *A. paniculata* and andrographolide using a Calu-3-based anti-SARS-CoV-2 assay. Potent anti-SAR-CoV-2 activities, together with the favorable cytotoxicity profiles, support further development of *A. paniculata* extract and especially andrographolide as a monotherapy or in combination with other effective drugs against SARS-CoV-2 infection.	[18]

Table 4.
Source and biological activities of some diterpenes.

disease such as; Taxol and etc. Herein, we are summarizing few recently published diterpenes structures, sources, origin and biological activities (**Table 4**, **Figure 4**).

6. Sesterpenes

Sesterpenes are the small class of terpenoids family which consists on twenty-five carbon atoms (tricyclic 5-8-5 carbotricyclic core, five isoprene units). These types of constituents usually found in plants, fungus culture, insects and marine organism. Sesterterpenes type compounds has complex structures due to the presence of many ring systems which makes its unique skeletons. These compounds have significant biological activities such as cytotoxic, nematocidal, anti-influenza, enzyme inhibition, anti-inflammatory and antimicrobial activities. In this chapter we are discussing, some recently published sesterterpene, including their structures, source, origin and pharmacology (**Table 5**, **Figure 5**).

7. Meroterpenes

Meroterpenes are mainly found in marine organisms and abundant in brown algae and other natural sources like microorganisms and invertebrates (sponges and

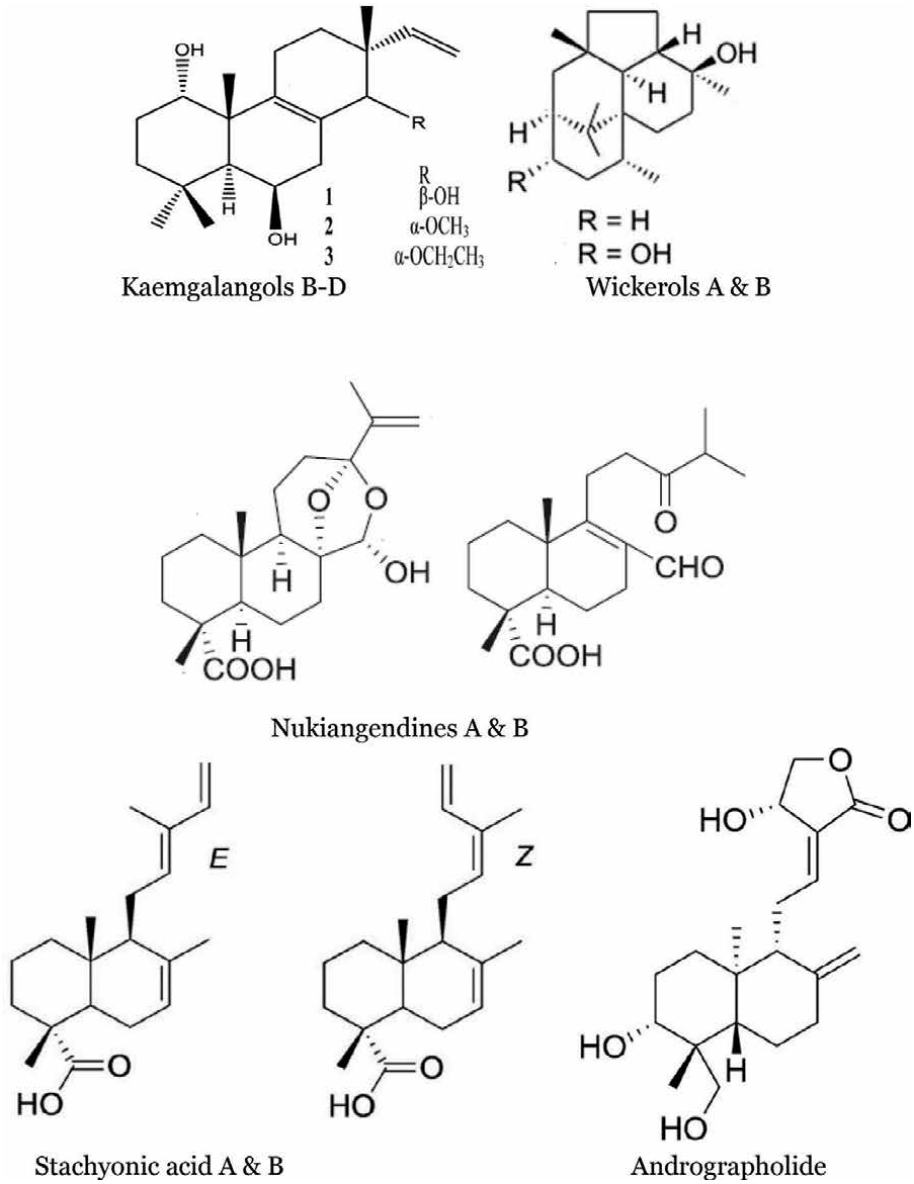


Figure 4.
Structure of diterpene.

Name	Source	Activity	Ref
Manoalide derivatives	Sponge *Luffariella variabilis* from the South China Sea	manoalide derivatives demonstrated cytotoxic activities against several human cancer cell lines with IC_{50} values ranging from 2 to 10 μM.	[19]
Drophiobiolins A & B	*Dreschslera gigantea s* found in China	Both of the newly identified ophiobolins showed significant phytotoxicity. Drophiobolins A & B exhibited cytotoxicity against Hela B cells with an IC_{50} value of 10 μM.	[20]

Table 5.
Source and biological activities of some sesterpenes.

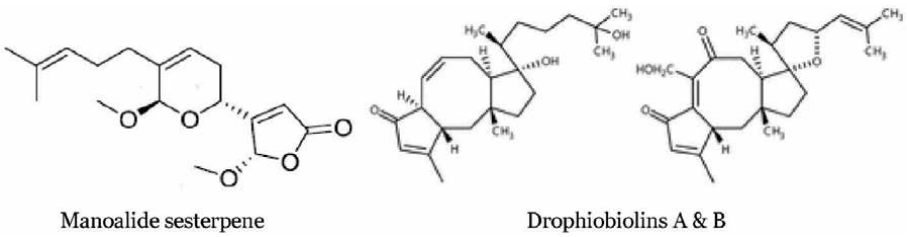


Figure 5.
Structure of sesterterpene.

tunicates). These types of compounds have many chemical diversities. Herein, we are discussing some recently published biological active meroterpenes (**Table 6**, **Figure 6**).

Name	Source	Activity	Ref
Peniclactone C	Endophytic fungus *Penicillium* sp. GDGJ-285	Bioassays showed that peniclactone C inhibited nitric oxide production in lipopolysaccharide-induced RAW 264.7 macrophage cells with an IC_{50} value of 39.03 μM.	[21]
Gancochlearols E – I	Ganoderma cochlear	Biological results revealed the significantly inhibitory effects of the Gancochlearols E – I on COX-2 activity and the migration of TNBC cells. In results not only enrich the structure type of meroterpenoids in Ganoderma, but also present novel structural template for developing nonsteroidal anti-inflammatory drug (NSAID) and anti-cancer drug against metastatic TNBC.	[22]

Table 6.
Source and biological activities of some meroterpenes.

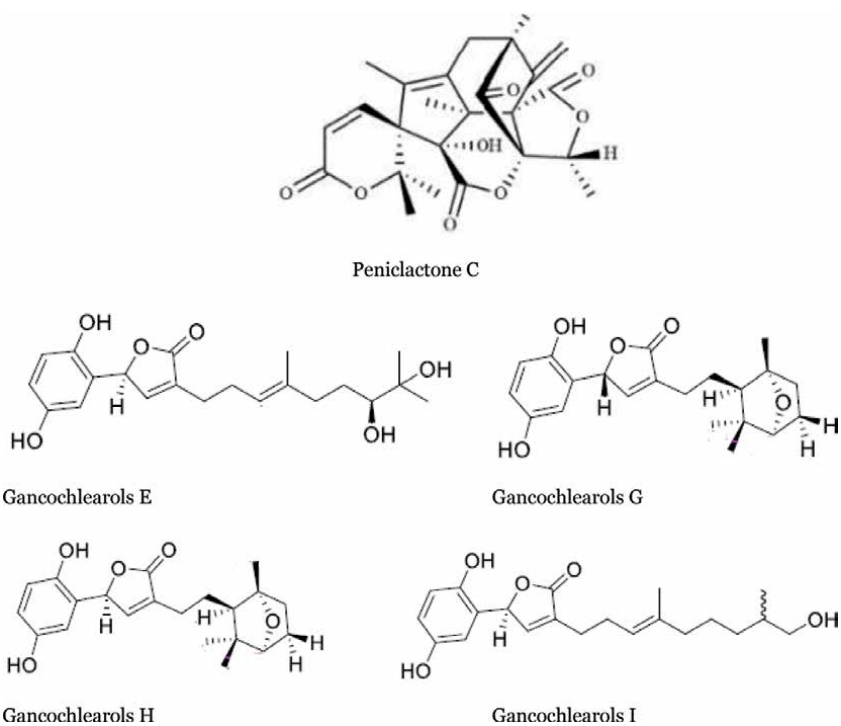


Figure 6.
Structure of meroterpene.

Name	Source	Activity	Ref
Arenarosides A	*Polycarpaea arenaria* found in Brazil	Compound displayed promising antiangiogenesis effects with IC_{50} values <5 μM in the test system used. It exhibited the most potent inhibitory effects, not only in cancer cell proliferation but also in angiogenic activities.	[23]
Ganoweberianones A & B	Fruiting bodies of Basidiomycete *Ganoderma weberianum*	These compounds were evaluated for Ganoweberianone A exhibited significant antimalarial activity against *Plasmodium falciparum* K1 (multidrug-resistant strain) with an IC_{50} value of 0.050 μM.	[24]
Longipetalol A	Dichapetalum longipetalum	Compound exhibited inhibitory effects on nitric oxide production in lipopolysaccharide-induced RAW264.7 macrophages.	[25]
Periploside A5	Root barks of *Periploca sepium*	Periploside showed significant suppressive effects on T lymphocyte proliferation with IC_{50} values ranging from 0.16 to 3.9 μM and displayed potent inhibitory activity on B lymphocyte proliferation with IC_{50} data at between 0.17 and 5.9 μM. IC_{50} data of Periploside A5 were 0.30 μM and 0.55 μM for T and B lymphocytes, and with the most favorite selective index values 176 and 96.9, respectively.	[26]

Table 7.
Source and biological activities of some triterpenes.

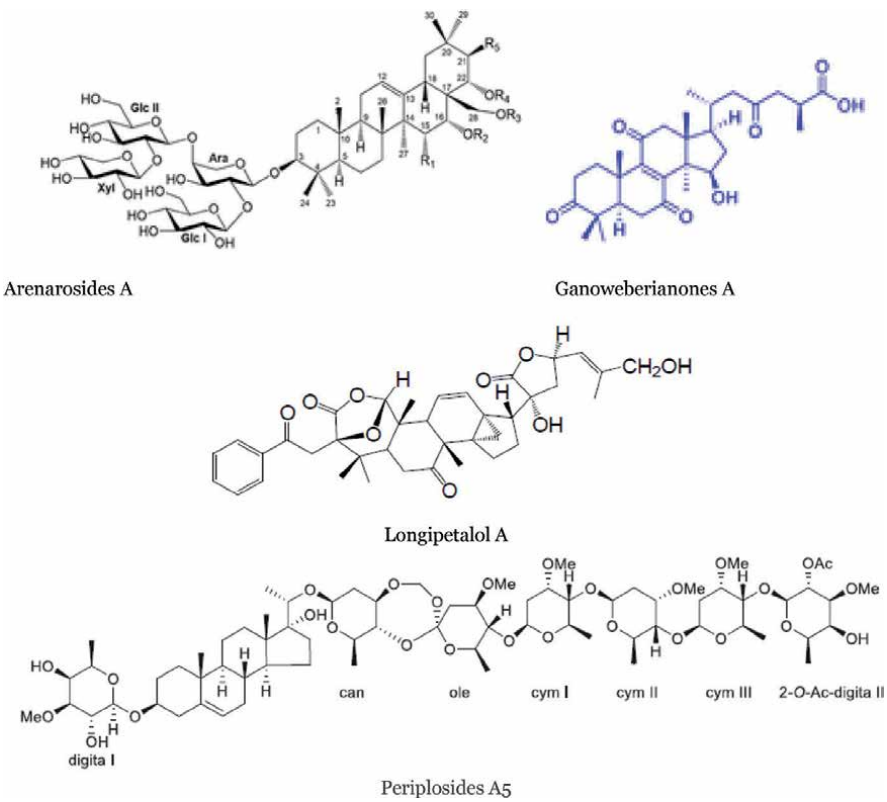


Figure 7.
Structure of triterpene.

8. Tripterpenes

A major class of secondary metabolites are known as triterpenes and it usually contains thirty carbons consisting of six isoprene units. Different class of triterpenes are known as lanostanes, euphanes,holostanes, tetranortriterpenoids, cycloartanes, cucurbitanes, dammaranes, tirucallanes, quassinoids, oleananes, lupanes, friedelanes, ursanes, hopanes, serratanes, isomalabaricanes which derived from the squalene biosynthesis (**Table 7**, **Figure 7**).

Author details

Shagufta Perveen
Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Kingdom of Saudi Arabia

*Address all correspondence to: shagufta792000@yahoo.com; shakhan@ksu.edu.sa

References

[1] Diniz LRL, Castillo-P Y, Hatem A. Elshabrawy, Filho CSMV, de Sousa DP. Bioactive Terpenes and Their Derivatives as Potential SARS-CoV-2 Proteases Inhibitors from Molecular Modeling Studies. Biomolecules 2021;11(74). DOI: 10.3390/biom 11010074.

[2] Anil MS, Shalev N, Vinayaka AC, Nadarajan S, D Namdar, Belausov E, Shoval I, Mani KA, Mechrez G, Kolta H. Cannabis compounds exhibit anti-infammatory activity in vitro in COVID-19-related infammation in lung epithelial cells and pro-infammatory activity in macrophages. Scientifc Reports. 2021;11;1462. DOI: 10.1038/s41598-021-81049-2

[3] Yang C, Wang Z, Qiu Y, Zha H,Yang X. New hemiterpene and furolactone-type lignan glycosides from *Securidaca inappendiculata* Hassk. Phytochemistry Letters. 2020; 37:42-46. DOI: 10.1016/j.phytol.2020.04.001

[4] Liu H, Tan H, Wang W, Zhang W, Chen Y, Li S, Liu Z, Lia H, Zhang W. Cytorhizophins A and B, benzophenonehemiterpene adducts from the endophytic fungus Cytospora rhizophorae. Organic Chemistry Frontries. 2019; 6:591-596. DOI: 10.1039/C8QO01306C

[5] Costa JO, Barboza RS, Valente LMM, Gomes TWM, Gallo B, Berrueta LA, Guimarães-Andrade IP, Gavino-Leopoldinod D, Assunção-Miranda I. One-Step Isolation of Monoterpene Indole Alkaloids from Psychotria leiocarpa. Leaves and Their Antiviral Activity on Dengue Virus Type-2. Brazilian Chemical Society. 2020:10(31): 2104-2113. DOI: 10.21577/0103-5053.20200111

[6] Cao J-Q, Wu Y, Zhong Y-L, Li N-P, Chen M, Li M-M, Ye W-C, Wang L. Antiviral Triketone-Phloroglucinol-Monoterpene Adducts from Callistemon rigidus. Chemistry & Biodiversity 2018;15: e1800172. DOI: 10.1002/cbdv.201800172

[7] Knotta MG, de la Marec J.A, Edkinsc AL, Zhangd A, Stillmand MJ, Boltone JJ, Antunesf EM, Beukes DR. Plaxenone A and B: Cytotoxic halogenated monoterpenes from the South African red seaweed Plocamium maxillosum. Phytochemistry Letters. 2019;29:182-185. DOI: 10.1016/j.phytol.2018.12.009

[8] Fa-Lei Zhang, Juan He, Tao Feng, Ji-Kai Liu. Melodinines Y1–Y4, four monoterpene indole alkaloids from Melodinus henryi. RSC Advances. 2021, 11, 23-29. DOI:10.1039/D0RA09819A

[9] Zhu L, Liu X-Q, Lin Y-L, Wang W-L, Luo J-G, Kong L-Y. Cytotoxic Germacranolides from the Whole Plant of *Carpesium minus.* Journal of Natural Products. 2020 25;83(11):3230-3238. DOI: 10.1021/acs.jnatprod.0c00428

[10] Li W, Shao YT, Yin TP, Yan H, Shen BC, Li YY, Xie HD, Sun ZW, Ma YL. Penisarins A and B, Sesquiterpene Coumarins Isolated from an Endophytic *Penicillium* sp. Journal of Natural Products. 2020, 83(11):3471-3475. DOI: 10.1021/acs.jnatprod.0c00393

[11] Chang K-F, Huang X-F, Chang J-T, Huang Y-C, Lo W-S, Hsiao C-Y, Tsai N-M. Cedrol, a Sesquiterpene Alcohol, Enhances the Anticancer Efficacy of Temozolomide in Attenuating Drug Resistance via Regulation of the DNA Damage Response and MGMT Expression. Journal of Natural Products. 2020;23;83(10):3021-3029. DOI: 10.1021/acs.jnatprod.0c00580

[12] Elshamy AI, Mohamed TA, Swapana N, Yoneyama T, Noji M, Efferth T, Hegazy M-EF, Akemi

Umeyama. Cytotoxic polyoxygenated isopimarane diterpenoids from the edible rhizomes of *Kaempferia galanga* (kencur). Industrial Crops & Products 2020; 158:112965. DOI:10.1016/j.indcrop.2020.112965

[13] Yamamoto T, Izumi N, Ui H, Sueki A, Masuma R, Nonaka K, Hirose T, Sunazuka T, Nagai T, Yamada H, Ōmura S, Shiomi K. Wickerols A and B: novel anti-influenza virus diterpenes produced by *Trichoderma atroviride* FKI-3849. Tetrahedron. 2012;45(68):9267-9271. DOI:10.1016/j.tet.2012.08.066

[14] Deng J, Ning Y, Tian H, Gui J. Divergent Synthesis of Antiviral Diterpenes Wickerols A and B. Journal of American Chemical Society. 2020;142(10), 4690-4695. DOI: 10.1021/jacs.9b11838

[15] LiLi-Y, Zhang O, Wu J-J, Xue L-J, Chen L-M, Tian J-M, Xu Z-N, Chen Y, Yang X-W, Hao X-J, Li J. Nukiangendines A and B, two novel 13,14-*seco*-abietanes from *Abies nukiangensis.* Tetrahedron Letters. 2019;10(60):751-753. DOI: 10.1016/j.tetlet.2019.02.008

[16] Tan YP, Houston SD, Modhiran N, Savchenko AI, Boyle GM, Young PR, Watterson D, Williams C.M. Stachyonic Acid: A Dengue Virus Inhibitor from *Basilicum Polystachyon*. Chemistry A Eurpion Journal 2019; 25:5664-5667. DOI:10.1002/chem.201900591

[17] Yuen P. Tan, Sevan D. Houston, Naphak Modhiran, Andrei I. Savchenko, Glen M. Boyle, Paul R. Young, Daniel Watterson, Craig M. Williams. Stachyonic Acid: A Dengue Virus Inhibitor from *Basilicum Polystachyon*. Chemistry A Eurpion Journal 2019;25,5664-5667. DOI:10.1002/chem.201900591

[18] Sa-ngiamsuntorn K, Suksatu A, Pewkliang Y, Thongsri P, Kanjanasirirat P, Manopwisedjaroen S, Charoensutthivarakul S, Wongtra koongate P, Pitiporn S, Chaopreecha J, Kongsomros S, Jearawuttanakul K, Wannalo W, Phisit K, Chuti pongtanate S, Borwornpinyo S, Thitithanyanont A, Hongeng S. Anti-SARS-CoV-2 Activity of *Andrographis paniculata* Extract and Its Major Component Andrographolide in Human Lung Epithelial Cells and Cytotoxicity Evaluation in Major Organ Cell Representatives. Journal of Natural Products. 2021;84(4):1261-1270. DOI: 10.1021/acs.jnatprod.0c01324

[19] Luo X, Wang Q, Tang X, Xu J, Wang M, Li P, Li G. Cytotoxic Manoalide-Type Sesterterpenes from the Sponge *Luffariella variabilis* Collected in the South China Sea. Journal of Natural Products. 2021;84(1):61-70. DOI: 10.1021/acs.jnatprod.0c01026

[20] Zatout R, Masi M , Sangermano F, Vurro M, Zonno MC, Santoro E, Viola Calabrò V, Superchi S, Evidente A. Drophiobiolins A and B, Bioactive Ophiobolan Sestertepenoids Produced by *Dreschslera gigantea.* Journal of Natural Products. 2020;83(11):3387-3396. DOI: 10.1021/acs.jnatprod.0c00836

[21] Mo T-X, Huang X-S, Zhang W-X, Schäberle TF, Qin J-K, Zhou D-X, Qin X-Y, Xu Z-L, Li J, Yang R-Y. A series of meroterpenoids with rearranged skeletons from an endophytic fungus *Penicillium* sp. GDGJ-285. Organic Chemistry Frontiers. 2021. DOI: 10.1039/d1qo00173f

[22] Li Y-P, Jiang X-T, Qin F-Y, Zhang H-X, Cheng Y-X. Gancochlearols E-I, meroterpenoids from *Ganoderma cochlear* against COX-2 and triple negative breast cancer cells and the absolute configuration assignment of ganomycin K. Bioorganic Chemistry 2021;109: 104706. DOI: 10.1016/j.bioorg.2021.104706

[23] Nguyen N-L, Vo T-H, Lin Y-C, Liaw C-C , Lu M-K , Cheng J-J, Chen M-C, Kuo Y-H. Arenarosides A-G, Polyhydroxylated Oleanane-Type Saponins from *Polycarpaea arenaria* and their Cytotoxic and Antiangiogenic Activities. Journal of Natural Products. 2021;84(2):259-267. DOI: 10.1021/acs.jnatprod.0c00919

[24] Isaka M, Chinthanom P, Vichai V, Sommai S, Choeyklin R. Ganowe berianones A and B, Antimalarial Lanostane Dimers from Cultivated Fruiting Bodies of the Basidiomycete Ganoderma weberianum. *Journal of Natural Products* 2020;83(11):3404-3412. DOI: 10.1021/acs.jnatprod.0c00879

[25] Zhang D-L, Li M, Han G-F, Li S-Y, Jin D-J, Tang S-A. Longipetalol A: A Highly Modified Triterpenoid from *Dichapetalum longipetalum*. Journal of Natural Products. 2021. DOI: 10.1021/acs.jnatprod.1c00068.

[26] Shao X-C, Chen Z-H, Liu S-S, Wu F, Mu H-Y, Wei W-H, Feng Y, Zuo J-P, Zhang J-Q, He S-J, Zhao W-*M. minor* immunosuppressive spiroorthoester group-containing pregnane glycosides from the root barks of *Periploca sepium*. Bioorganic Chemistry 2021; 108:104641. DOI: 10.1016/j.bioorg.2021.104641

Chapter 2

Terpenes in Essential Oils: Bioactivity and Applications

Paco Noriega

Abstract

Secondary metabolites from plant organisms have always been excellent options for the pharmaceutical, cosmetic, and food industries. Essential oils are a type of metabolites found in vegetables, and their chemical composition is diverse; however, monoterpenes and sesquiterpenes are inside the most abundant molecules. These terpenes have a diverse chemical composition that range from a simple molecule with carbon and hydrogen to more complex molecules with oxygenated organic groups, such as alcohols, aldehydes, ketones, and ethers. Many of these molecules with 10 and 15 carbon atoms have an especially important biological activity, being important the antimicrobial, antifungal, antioxidant, anti-inflammatory, insecticide, analgesic, anticancer, cytotoxic, among others. Some of these substances are potentially toxic, and hence, they should be handled with caution, especially when they are pure. They are easily obtained by different methods, and their industrial value grows every year, with a market of several million dollars. This chapter seeks to provide a better understanding of this type of bioactive molecules, with an emphasis in those whose information is remarkable in the scientific literature and whose value for health and human well-being makes them extremely important.

Keywords: terpenes, essential oils, bioactivity, chemical analysis

1. Introduction

Terpenes are chemical molecules synthesized from isoprene, 2-methyl-1,3 butadiene which are polymerized, thus obtaining one of nature's most diversified families of secondary metabolites.

The chemical diversity of terpenes is determined by the polymerization capacity of isoprene; because of this their classification is linked to the addition of five carbons to the basic molecular unit. The biosynthesis of the chemical precursors of isoprene, dimethylallyl pyrophosphate (DMAPP) and isopentenyl pyrophosphate (IPP) is produced by two diversified metabolic routes, the mevalonate route (MEV) and the 2C-Methyl-D-erythirol-4-phosphate (MEP) route [1].

DMAPP and IPP are hemiterpenes and are responsible of forming the various subclasses of compounds that make up the terpenes. Additionally, these isoprene polymers can be linear or can form rings and adhere to their structure oxygen and nitrogen atoms. The approximate number of known terpenes is close to 55,000 compounds [2].

Traditionally they are classified as [3]:

Hemiterpenes. These are constituted by five carbon atoms and are the basic units of the terpenes, the best-known example is 2-methyl-1,3 butadiene or isoprene.

Monoterpenes. These are constituted by 10 carbon atoms, resulting from the union of two units of isoprene, which are abundant in essential oils. Some important substances are: pinene, myrcene, limonene, thujene, etc.

Sesquiterpenes. These are formed by 15 carbon atoms, which are the result of the junction of three units of isoprene, some examples are: bisabolene, zingiberene, germacrene, caryophyllene, etc.

Diterpenes. These are formed by 20 carbon atoms or four units of isoprene; some important compounds are retinol, taxol and phytol.

Triterpenes. Squalene and several phytosterols such as sitosterol stand out among the terpenes containing 30 carbon atoms or six units of isoprene.

Tetraterpenes. These are constituted by 40 carbon atoms and eight units of isoprene, many of them are dyes like carotenes, among these the most important are carotene, lycopene and bixin.

Polyterpenes. These are composed of more than 40 carbon atoms; they are often found in gums and latex of various plant species.

2. Essential oils

Essential oils are common secondary metabolites in vegetables. From 10 to 200 compounds can be found in an essential oil, and their main characteristic is their ability to evaporate at room temperature. The chemical variability in an oil is significant; however, its components can be classified into three large groups (**Figure 1**).

Terpenes are the majority group, being monoterpenes and sesquiterpenes the most abundant. These can be present as hydrocarbons, consisting of carbon and

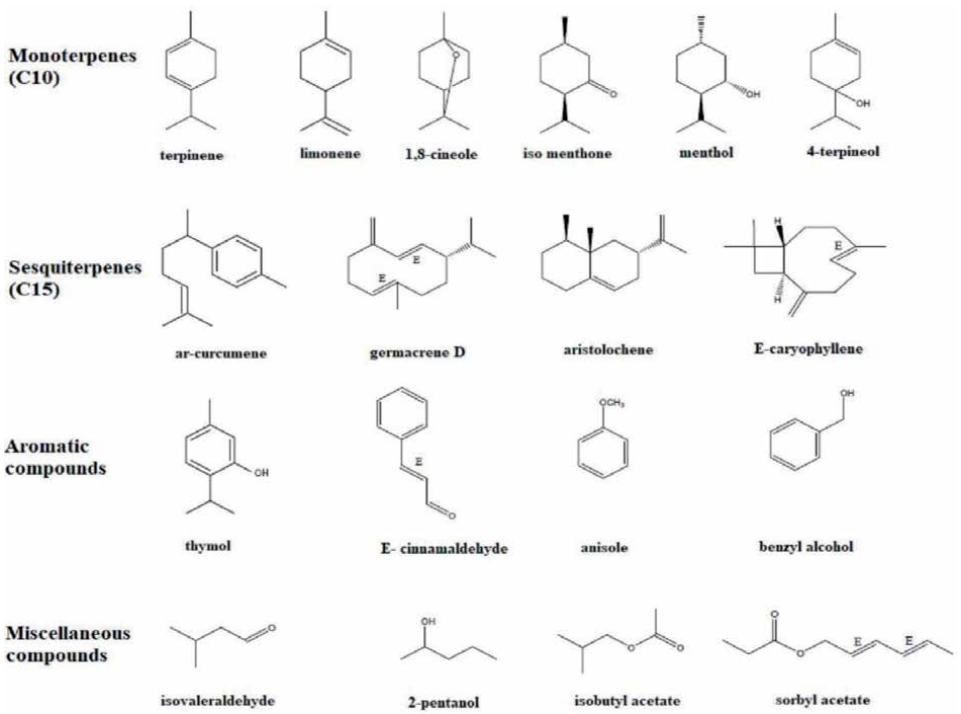


Figure 1.
Main molecules of essential oils.

hydrogen, or can have various functional groups such as alcohols, thiols, aldehydes, ketones, and ethers.

The second group of importance is aromatic compounds, many of them with an important biological activity such as derivatives of cinnamaldehyde, thymol, anethole or carvacrol.

There is a third miscellaneous group in a lower proportion that groups various molecules such as hydrocarbons, aldehydes, ketones, esters, etc. Examples of these substances are isovaleraldehyde or dodecanal.

Essential oils are usually found in low concentrations in plant organisms, ranging from 0.1 to 1%. They can exceed this value as is the case of clove oil with up to 10%, and are present in all plant organs and leaves: *Mentha piperita*, *Origanum majorana*, *Thymus vulgaris*; flowers: *Rosa damascena*, *Matricaria chamomilla*, *Lavandula officinale*; stems: *Cinnamomum verum*, *Ocotea quixos*, *Santalum álbum*; roots: *Valeriana officinale*; fruits: *Citrus bergamia*; rhizomes: *Zingiber officinale, Curcuma longa*; and seeds: *Pimpinella anisum*, *Syzygium aromaticum* and *Cuminum cyminum*.

The extraction processes are diverse, depending on the part of the plant used; the simplest and most widespread is the extraction by distillation with steam current, which does not require expensive equipment. Other methods are mechanical extraction used mainly to obtain oil from citrus pericarps, extraction using solvents which is useful when components can be affected by high temperatures and extraction using a supercritical CO_2 current, which does not need high temperatures while maintaining the chemistry of molecules, but it is very expensive to implement.

About 4000 species have been investigated by their ability to produce essential oils, but only about 30 are marketed massively globally; their main use is intended for the cosmetic industry and aromatherapy, although several of the compounds from essences could be valuable to the pharmaceutical industry. There are certainly still species whose essential oils have not been analyzed in their chemical composition or in their bioactivity, which could be interesting as a source of new secondary metabolites.

3. Chemical analysis

Since they are volatile metabolites, their low boiling points make it possible to have them as steam in a remarkably simple way; for this reason the ideal analysis is gas chromatography with GC/MS mass spectrometry.

The use of capillary columns has made it possible to have defined separations in essential oils that exceed 100 compounds, usually chromatographic separation is made in nonpolar columns with 95% dimethylpolysiloxane, due to the fact that several components of an essential oil contain polar groups such as hydroxyl (OH); the realization of these components using columns of intermediate polarity has been made. Both assays result in a complete chemical inquiry of molecules and are complementary. The correct structural elucidation is performed by combining several analyses such as comparison with spectrum databases and the theoretical and experimental determination of the retention rates of the compounds. For this purpose, there are databases, being the most used the "Identification of essential oil components by gas chomatography/mass spectrometry," with approximately 4000 compounds from essential oils [4].

The GC/MS technique is limited in the fact that it is ineffective in evaluating stereoisomers, in such cases it is necessary to use chiral columns or techniques such as nuclear magnetic resonance imaging.

A more thorough investigation of the chemical identity of the molecules of an essential oil can be done with an equipment that couples gas chromatography with spectrophotometric techniques, such as nuclear magnetic resonance imaging and infrared spectroscopy. It is also possible to analyze NMR or IR spectra in previously isolated molecules by column or thin layer chromatography [5].

4. Monoterpenes with therapeutic importance

Several monoterpenes have a diverse and useful biological activity for treating diseases and ailments; some have valuable aromatic characteristics in cosmetics and perfumery. Those molecules that have relevant information and studies are analyzed to verify their use as phytotherapeutic elements (**Figure 2**).

Pinenes. These have alpha and beta isomers; their formula is $C_{10}H_{10}$ and they are common in essential oils from conifers, although they can be found in many other species such as rosemary and lavender [6–8]; oils with high concentrations of pinenes generally have antimicrobial activity [8, 9]. Traditionally many plants containing pinene-rich essential oils are used in respiratory system disease [9].

1–8 cineol (Eucalyptol). Oxygenated monoterpene has a $C_{10}H_{18}O$ formula whose functional group is an ether that is present in many varieties of eucalyptus. Among its most noteworthy properties are analgesic, anti-inflammatory and antimicrobial [10]. Plants with eucalyptol-rich essential oils are used for expectorant and decongestant properties of the respiratory system [11].

Limonene. It is a monoterpene whose formula is $C_{10}H_{16}$; it has two optical isomers R-limonene or D- limonene and S-limonene or L-limonene, which stand out by the insecticide [12, 13] and antimicrobial properties [14].

Myrcene. It is a monoterpene whose formula is $C_{10}H_{16}$; it is the main component of *Cannabis sativa* essential oil [15]. Several studies highlight its analgesic-sedative [16, 17] and anti-inflammatory activity [18].

Linalool. It is a hydroxylated monoterpene with $C_{10}H_{18}O$ formula, its pleasant aroma makes it widely used in perfumery. Its action on the central nervous system is evidenced by its sedative, anxiolytic, analgesic and anti-inflammatory

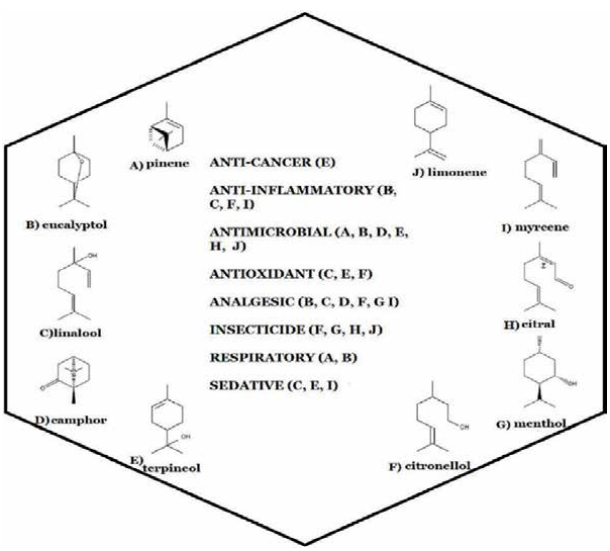


Figure 2.
Monoterpene molecules with therapeutic importance.

properties [19, 20]. Its antimicrobial and antioxidant properties have been evaluated with good results [21, 22].

Citral. It is an oxygenated monoterpene containing a group of aldehyde; its formula is $C_{10}H_{16}O$. There are two isomers known as neral (cis isomer) and geranial (trans isomer) [23], which are abundant in species such as *Backhousia citriodora* [24] and *Cymbopogon citratus* [25]. Its antimicrobial [25] and insect repellent action is noteworthy [26].

Camphor. It is an oxygenated monoterpene whose functional group is a ketone; its formula is $C_{10}H_{16}O$ and it is present in two optical isomers R and S, which are abundant in the species *Cinnamomum camphora* [27]. Traditionally camphor has been used in traditional Asian medicine, and it is known to have digestive effects, but its most important use is related to its analgesic and antiseptic effect, being very popular its inclusion in topical formulations such as liniments and creams [28, 29].

Menthol. It is a hydroxylated monoterpene, with a $C_{10}H_{20}O$ formula, which has seven isomers that are very common in mint varieties such as Peppermint. It is one of the most used compounds in the food, cosmetic, pharmaceutical industries, and pesticides, among others. Its aromatic properties are very well known [30]; however, its most noticeable and known effect is that of analgesia at the topical level [31, 32].

Terpineol. It is a hydroxylated monoterpene with a $C_{10}H_{18}O$ formula. It is known by having five isomers (α, β, γ, δ and 4-terpineol) [33], which are abundant in the essential oil of tea tree (*Malaleuca alternifolia*) [34]. The outstanding properties are antihypertensive, anticancer, antioxidant, antimicrobial, antifungal and sedative [33, 35].

Citronellol. It is a hydroxylated monoterpene with a $C_{10}H_{20}O$ formula. There are two enantiomers (+)-citronellol and (−)-citronellol [36]. The first is quite common in citronella oil, and the second is abundant in rose oil [37], which is used in perfumery. Its properties are insecticide [38], analgesic and anti-inflammatory [39], and antioxidant [40].

5. Sesquiterpenes with therapeutic importance

Several molecules with interesting properties can be found in C_{15} sesquiterpene (**Figure 3**). From a therapeutic view, there is evidence that validates its biological activity, highlighting anti-inflammatory, analgesic and anticancer trials.

Bisabolol. These are isomers, out of which stand (−)-α-Bisabolol, (−)-epi-α-Bisabolol, (+)-α-Bisabolol and (+)-epi-α-Bisabolol which are abundant in the species *Matricaria camomilla* [41], and in other species such as *Salvia runcinata* [42]. The most well-known effects in the molecule are analgesic and anti-inflammatory [43], antimicrobial and antioxidant properties; for this reason, the molecule is widely used in the cosmetic industry [44].

β-Caryophyllene. It has a $C_{15}H_{24}$ formula. It is one of the most abundant sesquiterpenes in essential oils. Various bioactivity studies have been carried out in this molecule with good results, such as analgesic [45, 46], anti-inflammatory [47] and anticancer [48].

Chamazulene. With a $C_{14}H_{16}$ formula, it is a molecule derived from the sesquiterpene matricina, which is one of the few aromatic molecules that have a blue coloration. It is found in *Matricaria camomilla*, being its most known property the anti-inflammatory [49, 50]. Several studies highlight its antioxidant effects [51, 52].

Caryophyllene oxide. It is an oxygenated sesquiterpene with a $C_{15}H_{24}O$ formula; it has properties similar to those of caryophyllene, such as analgesic and anti-inflammatory [53].

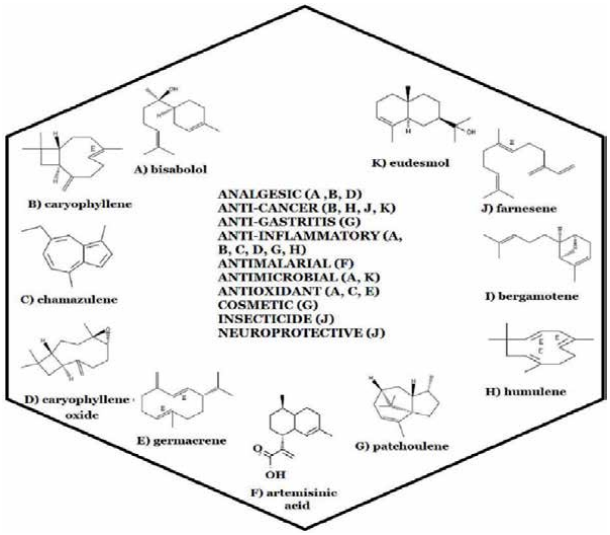


Figure 3.
Sesquiterpene molecules with therapeutic purposes.

Germacrene. It belongs to the sesquiterpenes family, and it has three double links in its structure. There are five types of germacrenes: A, B, C, D, E. Recent studies mention its antioxidant potential [5, 54].

Artemisinic acid. It has a $C_{15}H_{22}O_2$ formula, and it is one of the most interesting sesquiterpenes for health due to its antimalarial properties [55]. It is abundant in the species *Artemisia annua*, and it is generally found as a sesquiterpenic lactone [56].

Patchoulene. It is a sesquiterpene with a $C_{15}H_{24}$ formula. It is common to find its isomers α, β, α, and δ in essential oils. It is attributed to various types of bioactivity, the most relevant being those found in β-patchoulene as anti-inflammatory [57], antigastritis [58, 59], and cosmetic [60].

Humulene. Also known as α-caryophyllene, its formula is $C_{15}H_{24}$. It is named after the essential oil of the species *Humulus lupulus* [61]. It has anti-inflammatory [62, 63] and anticancer properties [64].

Bergamotene. It is a sesquiterpene with a $C_{15}H_{24}$ formula. It has four isomers α-cis, β-cis, α-trans and β-trans. It is found in several citric species such as *Citrus bergamia* [65]. One of the properties of this molecule is to act as a pheromone [66, 67].

Farnesene. It has a $C_{15}H_{24}$ formula. It is a molecule found in several essential oils, and it is a precursor to many other sesquiterpenes since its open-chain structure and its 4-double bonds contribute to this action, as well as in the possibility of having a wide variety of isomers between geometrics and stereoisomers. Its cytotoxic and genotoxic [68], insecticide [69] and neuroprotective effects [70, 71] have been evaluated.

Eudesmol. Hydroxylated sesquiterpene with a $C_{15}H_{26}O$ formula is a very interesting molecule by the multiple positive bioactivity assays, highlighting antimicrobial and antifungal [72], anticancer [73, 74] and antiangiogenic [75].

6. Toxicity

Most of the terpenes present in essential oils have some degree of toxicity, which is not detected when consuming aromatic species directly because in most cases

the oil yield is low. Many commonly used essential oil components are potentially dermal irritating with restrictions on application concentrations [76, 77]. There are also some terpenes whose toxicity is much more dangerous, such as pulegone which causes liver damage and seizures [78], and thujone that can cause dementia by being neurotoxic [79].

7. Conclusion

This brief review has shown the chemical and biological importance of low molecular weight and volatile terpenes. For this reason, components of secondary metabolites are known as essential oils. The abundance of these molecules is much higher than the one presented in this chapter, since the information presented covers those whose scientific evidence and industrial importance are references in this family of metabolites. There is still much research to be carried out on the hundreds of molecules from which there is still little or no information. There are still aromatic species whose essential oils have not yet been described and that could be a source of new monoterpenes and sesquiterpenes that are beneficial to humans.

Author details

Paco Noriega
Salesian Polytechnic University, Quito, Ecuador

*Address all correspondence to: pnoriega@ups.edu.ec

References

[1] Natanya C. Natural Products in Chemical Biology. Hoboken, New Jersey: John Wiley & Sons; 2012. pp. 127-129

[2] Guimarães AG, Serafini MR, Quintans-Júnior LJ. Terpenes and derivatives as a new perspective for pain treatment: A patent review. Expert Opinion on Therapeutic Patents. 2014;**24**(3):243-265

[3] Perveen S, Al-Taweel A. Introductory chapter: Terpenes and terpenoids. In: Terpenes and Terpenoids. London: IntechOpen; 2018. pp. 1-7

[4] Adams RP. Identification of Essential Oils by Ion Trap Mass Spectroscopy. San Diego, California: Academic Press; 2012

[5] Noriega P, Guerrini A, Sacchetti G, Grandini A, Ankuash E, Manfredini S. Chemical composition and biological activity of five essential oils from the Ecuadorian Amazon rain forest. Molecules. 2019;**24**(8):1637

[6] Jiang Y, Wu N, Fu YJ, Wang W, Luo M, Zhao CJ, et al. Chemical composition and antimicrobial activity of the essential oil of rosemary. Environmental Toxicology and Pharmacology. 2011;**32**(1):63-68

[7] Zheljazkov VD, Astatkie T, Hristov AN. Lavender and hyssop productivity, oil content, and bioactivity as a function of harvest time and drying. Industrial Crops and Products. 2012;**36**(1):222-228

[8] Silva ACRD, Lopes PM, Azevedo MMBD, Costa DCM, Alviano CS, Alviano DS. Biological activities of a-pinene and β-pinene enantiomers. Molecules. 2012;**17**(6):6305-6316

[9] Rivera PFN, Paredes EA, Gómez ED, Lueckhoff A, Almeida GA, Suarez SE. Composición química y actividad antimicrobiana del aceite esencial de los rizomas de *Renealmia thyrsoidea* (Ruiz & Pav) Poepp. & Eddl (shiwanku muyu). Revista Cubana de Plantas Medicinales. 2017;**22**(2)

[10] Bhowal M, Gopal M. Eucalyptol: Safety and pharmacological profile. Journal of Pharmaceutical Sciences. 2015;**5**:125-131

[11] Adnan M. Bioactive potential of essential oil extracted from the leaves of *Eucalyptus globulus* (Myrtaceae). Journal of Pharmacognosy and Phytochemistry. 2019;**8**(1):213-216

[12] Karr LL, Costas JR. Insecticidal properties of d-limonene. Journal of Pesticide Science. 1988;**13**(2):287-290

[13] Malacrinò A, Campolo O, Laudani F, Palmeri V. Fumigant and repellent activity of limonene enantiomers against *Tribolium confusum* du Val. Neotropical Entomology. 2016;**45**(5):597-603

[14] Espina L, Gelaw TK, de Lamo-Castellví S, Pagán R, García-Gonzalo D. Mechanism of bacterial inactivation by (+)-limonene and its potential use in food preservation combined processes. PLoS One. 2013;**8**(2):e56769

[15] Gulluni N, Re T, Loiacono I, Lanzo G, Gori L, Macchi C, et al. Cannabis essential oil: A preliminary study for the evaluation of the brain effects. Evidence-Based Complementary and Alternative Medicine. 2018;(1):1-11

[16] Mirghaed AT, Ghelichpour M, Hoseini SM. Myrcene and linalool as new anesthetic and sedative agents in common carp, *Cyprinus carpio* - Comparison with eugenol. Aquaculture. 2016;**464**:165-170

[17] Rao VSN, Menezes AMS, Viana GSB. Effect of myrcene on nociception in mice. The Journal of Pharmacy and Pharmacology. 1990;**42**(12):877-878

[18] Rufino AT, Ribeiro M, Sousa C, Judas F, Salgueiro L, Cavaleiro C, et al. Evaluation of the anti-inflammatory, anti-catabolic and pro-anabolic effects of E-caryophyllene, myrcene and limonene in a cell model of osteoarthritis. European Journal of Pharmacology. 2015;**750**:141-150

[19] Aprotosoaie AC, Hăncianu M, Costache II, Miron A. Linalool: A review on a key odorant molecule with valuable biological properties. Flavour and Fragrance Journal. 2014;**29**(4):193-219

[20] Pereira I, Severino P, Santos AC, Silva AM, Souto EB. Linalool bioactive properties and potential applicability in drug delivery systems. Colloids and Surfaces, B: Biointerfaces. 2018;**171**:566-578

[21] Herman A, Tambor K, Herman A. Linalool affects the antimicrobial efficacy of essential oils. Current Microbiology. 2016;**72**(2):165-172

[22] Hu J, Liu S, Deng W. Dual responsive linalool capsules with high loading ratio for excellent antioxidant and antibacterial efficiency. Colloids and Surfaces, B: Biointerfaces. 2020;**190**:110978

[23] Sacks J, Greenley E, Leo G, Willey P, Gallis D, Mangravite JA. Separation and analysis of citral isomers: An undergraduate organic laboratory experiment. Journal of Chemical Education. 1983;**60**(5):434

[24] Southwell IA, Russell M, Smith RL, Archer DW. *Backhousia citriodora* F. Muell. (Myrtaceae), a superior source of citral. Journal of Essential Oil Research. 2000;**12**(6):735-741

[25] Onawunmi GO, Yisak WA, Ogunlana EO. Antibacterial constituents in the essential oil of *Cymbopogon citratus* (DC.) Stapf. Journal of Ethnopharmacology. 1984;**12**(3):279-286

[26] Hao H, Wei J, Dai J, Du J. Host-seeking and blood-feeding behavior of *Aedes albopictus* (Diptera: Culicidae) exposed to vapors of geraniol, citral, citronellal, eugenol, or anisaldehyde. Journal of Medical Entomology. 2014;**45**(3):533-539

[27] Pragadheesh VS, Saroj A, Yadav A, Chanotiya CS, Alam M, Samad A. Chemical characterization and antifungal activity of *Cinnamomum camphora* essential oil. Industrial Crops and Products. 2013;**49**:628-633

[28] Green BG. Sensory characteristics of camphor. The Journal of Investigative Dermatology. 1990;*94*(5):662-666

[29] Craven R. The comfort of camphor. Nature Reviews. Neuroscience. 2005;**6**(11):826-826

[30] Kamatou GP, Vermaak I, Viljoen AM, Lawrence BM. Menthol: A simple monoterpene with remarkable biological properties. Phytochemistry. 2013;**96**:15-25

[31] Galeotti N, Mannelli LDC, Mazzanti G, Bartolini A, Ghelardini C. Menthol: A natural analgesic compound. Neuroscience Letters. 2002;**322**(3):145-148

[32] Pan R, Tian Y, Gao R, Li H, Zhao X, Barrett JE, et al. Central mechanisms of menthol-induced analgesia. The Journal of Pharmacology and Experimental Therapeutics. 2012;**343**(3):661-672

[33] Khaleel C, Tabanca N, Buchbauer G. α-Terpineol, a natural monoterpene: A review of its biological properties. Open Chemistry. 2018;**16**(1):349-361

[34] Papadopoulos CJ, Carson CF, Chang BJ, Riley TV. Role of the

MexAB-OprM efflux pump of Pseudomonas aeruginosa in tolerance to tea tree (*Melaleuca alternifolia*) oil and its monoterpene components terpinen-4-ol, 1, 8-cineole, and α-terpineol. Applied and Environmental Microbiology. 2008;**74**(6):1932-1935

[35] Kong Q, Zhang L, An P, Qi J, Yu X, Lu J, et al. Antifungal mechanisms of α-terpineol and terpene-4-alcohol as the critical components of *Melaleuca alternifolia* oil in the inhibition of rot disease caused by *Aspergillus ochraceus* in postharvest grapes. Journal of Applied Microbiology. 2019;**126**(4):1161-1174

[36] Ravid U, Putievsky E, Katzir I, Ikan R, Weinstein V. Determination of the enantiomeric composition of citronellol in essential oils by chiral GC analysis on a modified γ-cyclodextrin phase. Flavour and Fragrance Journal. 1992;**7**(4):235-238

[37] Katsukawa M, Nakata R, Koeji S, Hori K, Takahashi S, Inoue H. Citronellol and geraniol, components of rose oil, activate peroxisome proliferator-activated receptor α and γ and suppress cyclooxygenase-2 expression. Bioscience, Biotechnology, and Biochemistry. 2011;**75**(5):1010-1012

[38] Brari J, Thakur DR. Insecticidal potential properties of citronellol derived ionic liquid against two major stored grain insect pests. Journal of Entomology and Zoology Studies. 2016;**4**(3):365-370

[39] Brito RG, Guimarães AG, Quintans JS, Santos MR, De Sousa DP, Badaue-Passos D, et al. Citronellol, a monoterpene alcohol, reduces nociceptive and inflammatory activities in rodents. Journal of Natural Medicines. 2012;**66**(4):637-644

[40] Jagdale AD, Kamble SP, Nalawade ML, Arvindekar AU. Citronellol: A potential antioxidant and aldose reductase inhibitor from *Cymbopogon citratus*. International Journal of Pharmacy and Pharmaceutical Sciences. 2015;**7**(3):203-209

[41] Isaac O, Thiemer K. Biochemical studies on camomile components/III. In vitro studies about the antipeptic activity of (−)-alpha-bisabolol (author's transl). Arzneimittel-Forschung. 1975;**25**:1352-1354

[42] Viljoen AM, Gono-Bwalya AB, Kamatou GPP, Baser KHC, Demirci B. The essential oil composition and chemotaxonomy of *Salvia stenophylla* and its allies *S. repens* and *S.runcinata*. Journal of Essential Oil Research. 2006;**18**:37-45

[43] Rocha NFM, Rios ERV, Carvalho AMR, Cerqueira GS, de Araújo LA, Leal LKAM, et al. Anti-nociceptive and anti-inflammatory activities of (−)-α-bisabolol in rodents. Naunyn-Schmiedeberg's Archives of Pharmacology. 2011;**384**(6):525-533

[44] Kamatou GP, Viljoen AM. A review of the application and pharmacological properties of α-bisabolol and α-bisabolol-rich oils. Journal of the American Oil Chemists' Society. 2010;**87**(1):1-7

[45] Ghelardini C, Galeotti N, Mannelli LDC, Mazzanti G, Bartolini A. Local anaesthetic activity of β-caryophyllene. Il Fármaco. 2001;**56**(5-7):387-389

[46] Fidyt K, Fiedorowicz A, Strządała L, Szumny A. β-Caryophyllene and β-caryophyllene oxide—Natural compounds of anticancer and analgesic properties. Cancer Medicine. 2016;**5**(10):3007-3017

[47] Tambe Y, Tsujiuchi H, Honda G, Ikeshiro Y, Tanaka S. Gastric cytoprotection of the non-steroidal anti-inflammatory sesquiterpene, β-caryophyllene. Planta Medica. 1996;**62**(05):469-470

[48] Legault J, Pichette A. Potentiating effect of β-caryophyllene on anticancer activity of α-humulene, isocaryophyllene and paclitaxel. The Journal of Pharmacy and Pharmacology. 2007;**59**(12):1643-1647

[49] Ma D, He J, He D. Chamazulene reverses osteoarthritic inflammation through regulation of matrix metalloproteinases (MMPs) and NF-kβ pathway in in-vitro and in-vivo models. Bioscience, Biotechnology, and Biochemistry. 2020;**84**(2):402-410

[50] Flemming M, Kraus B, Rascle A, Jürgenliemk G, Fuchs S, Fürst R, et al. Revisited anti-inflammatory activity of matricine in vitro: Comparison with chamazulene. Fitoterapia. 2015;**106**:122-128

[51] Rekka EA, Kourounakis AP, Kourounakis PN. Investigation of the effect of chamazulene on lipid peroxidation and free radical processes. Research Communications in Molecular Pathology and Pharmacology. 1996;**92**(3):361-364

[52] Capuzzo A, Occhipinti A, Maffei ME. Antioxidant and radical scavenging activities of chamazulene. Natural Product Research. 2014;**28**(24):2321-2323

[53] Chavan MJ, Wakte PS, Shinde DB. Analgesic and anti-inflammatory activity of caryophyllene oxide from *Annona squamosa* L. bark. Phytomedicine. 2010;**17**(2):149-151

[54] Casiglia S, Bruno M, Bramucci M, Quassinti L, Lupidi G, Fiorini D, et al. *Kundmannia sicula* (L.) DC: A rich source of germacrene D. Journal of Essential Oil Research. 2017;**29**(6):437-442

[55] Ro DK, Ouellet M, Paradise EM, Burd H, Eng D, Paddon CJ, et al. Induction of multiple pleiotropic drug resistance genes in yeast engineered to produce an increased level of anti-malarial drug precursor, artemisinic acid. BMC Biotechnology. 2008;**8**(1):83

[56] Ram M, Gupta MM, Naqvi AA, Kumar S. Effect of planting time on the yield of essential oil and artemisinin in *Artemisia annua* under subtropical conditions. Journal of Essential Oil Research. 1997;**9**(2):193-197

[57] Zhang Z, Chen X, Chen H, Wang L, Liang J, Luo D, et al. Anti-inflammatory activity of β-patchoulene isolated from patchouli oil in mice. European Journal of Pharmacology. 2016;**781**:229-238

[58] Liu Y, Liang J, Wu J, Chen H, Zhang Z, Yang H, et al. Transformation of patchouli alcohol to β-patchoulene by gastric juice: β-patchoulene is more effective in preventing ethanol-induced gastric injury. Scientific Reports. 2017;**7**(1):1-13

[59] Liang J, Dou Y, Wu X, Li H, Wu J, Huang Q, et al. Prophylactic efficacy of patchoulene epoxide against ethanol-induced gastric ulcer in rats: Influence on oxidative stress, inflammation and apoptosis. Chemico-Biological Interactions. 2018;**283**:30-37

[60] Api AM, Belsito D, Botelho D, Browne D, Bruze M, Burton GA, et al. RIFM fragrance ingredient safety assessment β-Patchoulene, CAS Registry Number 514-51-2. Food and Chemical Toxicology. 2018;**115**:S256-S263

[61] Nance MR, Setzer WN. Volatile components of aroma hops (*Humulus lupulus* L.) commonly used in beer brewing. Journal of Brewing and Distilling. 2011;**2**(2):16-22

[62] Rogerio AP, Andrade EL, Leite DF, Figueiredo CP, Calixto JB. Preventive and therapeutic anti-inflammatory properties of the sesquiterpene α-humulene in experimental airways allergic inflammation. British Journal of Pharmacology. 2009;**158**(4):1074-1087

[63] Fernandes ES, Passos GF, Medeiros R, da Cunha FM, Ferreira J, Campos MM, et al. Anti-inflammatory effects of compounds alpha-humulene and (−)-trans-caryophyllene isolated from the essential oil of *Cordia verbenacea*. European Journal of Pharmacology. 2007;**569**(3):228-236

[64] El Hadri A, del Rio MG, Sanz J, Coloma AG, Idaomar M, Ozonas BR, et al. Cytotoxic activity of α-humulene and transcaryophyllene from *Salvia officinalis* in animal and human tumor cells. Anales de la Real Academia Nacional de Farmacia. 2010;**76**(3):343-356

[65] Navarra M, Mannucci C, Delbò M, Calapai G. *Citrus bergamia* essential oil: From basic research to clinical application. Frontiers in Pharmacology. 2015;**6**:36

[66] Cônsoli FL, Williams HJ, Vinson SB, Matthews RW, Cooperband MF. Trans-bergamotenes—Male pheromone of the ectoparasitoid *Melittobia digitata*. Journal of Chemical Ecology. 2002;**28**(8):1675-1689

[67] Haber AI, Sims JW, Mescher MC, De Moraes CM, Carr DE. A key floral scent component (β-trans-bergamotene) drives pollinator preferences independently of pollen rewards in seep monkeyflower. Functional Ecology. 2019;**33**(2):218-228

[68] Çelik K, Toğar B, Türkez H, Taşpinar N. In vitro cytotoxic, genotoxic, and oxidative effects of acyclic sesquiterpene farnesene. Turkish Journal of Biology. 2014;**38**(2):253-259

[69] Sun Y, Qiao H, Ling Y, Yang S, Rui C, Pelosi P, et al. New analogues of (E)-β-farnesene with insecticidal activity and binding affinity to aphid odorant-binding proteins. Journal of Agricultural and Food Chemistry. 2011;**59**(6):2456-2461

[70] Arslan ME, Türkez H, Mardinoğlu A. In vitro neuroprotective effects of farnesene sesquiterpene on alzheimer's disease model of differentiated neuroblastoma cell line. The International Journal of Neuroscience. 2020;**130**:1-10

[71] Turkez H, Sozio P, Geyikoglu F, Tatar A, Hacimuftuoglu A, Di Stefano A. Neuroprotective effects of farnesene against hydrogen peroxide-induced neurotoxicity in vitro. Cellular and Molecular Neurobiology. 2014;**34**(1):101-111

[72] Noriega P, Ballesteros J, De la Cruz A, Veloz T. Chemical composition and preliminary antimicrobial activity of the hydroxylated sesquiterpenes in the essential oil from Piper barbatum Kunth leaves. Plants. 2020;**9**(2):211

[73] Plengsuriyakarn T, Karbwang J, Na-Bangchang K. Anticancer activity using positron emission tomography-computed tomography and pharmacokinetics of β-eudesmol in human cholangiocarcinoma xenografted nude mouse model. Clinical and Experimental Pharmacology and Physiology. 2015;**42**(3):293-304

[74] Miyazawa M, Shimamura H, Nakamura SI, Kameoka H. Antimutagenic activity of (+)-β-eudesmol and paeonol from *Dioscorea japonica*. Journal of Agricultural and Food Chemistry. 1996;**44**(7):1647-1650

[75] Tsuneki H, Ma EL, Kobayashi S, Sekizaki N, Maekawa K, Sasaoka T, et al. Antiangiogenic activity of β-eudesmol in vitro and in vivo. European Journal of Pharmacology. 2005;**512**(2-3):105-115

[76] Mekonnen A, Tesfaye S, Christos SG, Dires K, Zenebe T, Zegeye N, et al. Evaluation of skin irritation and acute and subacute oral toxicity of *Lavandula angustifolia* essential oils in rabbit and mice. Journal of Toxicology. 2019;(1):1-8

[77] Lee CJ, Chen LW, Chen LG, Chang TL, Huang CW, Huang MC, et al. Correlations of the components of tea tree oil with its antibacterial effects and skin irritation. Journal of Food and Drug Analysis. 2013;**21**(2):169-176

[78] Gordon WP, Forte AJ, McMurtry RJ, Gal J, Nelson SD. Hepatotoxicity and pulmonary toxicity of pennyroyal oil and its constituent terpenes in the mouse. Toxicology and Applied Pharmacology. 1982;**65**(3):413-424

[79] Pelkonen O, Abass K, Wiesner J. Thujone and thujone-containing herbal medicinal and botanical products: Toxicological assessment. Regulatory Toxicology and Pharmacology. 2013;**65**(1):100-107

Chapter 3

Terpene Compounds of New Tunisian Extra-Virgin Olive Oil: Effect of Ripening Stage

Bechir Baccouri and Imene Rajhi

Abstract

The volatile profiles of Tunisian virgin olive oils were established by solid phase micro-extraction (SPME) and gas chromatography (GC), using flame ionisation and mass spectrometer detectors. Terpenes compounds were identified and characterized. Limonene, the main terpene compound extracted by SPME, characterized the studied olive oil. Significant differences in the proportions of terpenes constituents from oils of different maturity index were detected. The results demonstrated that the accumulation of the terpenes compounds in the studied oils obtained from different ripeness stage was strictly connected with the ripeness stage.

Keywords: virgin olive oil, headspace-solid-phase microextraction (HS-SPME), volatile compounds, terpenes

1. Introduction

Virgin olive oil is characterized through its distinctive perfume, which is synthesized after olive fruits are crushed during industrial oil production. Extra virgin olive oil (EVOO) is unique for its high monounsaturated fatty acids levels and the existence of a wide range of minor components responsible for their organoleptic characteristics and health properties [1]. EVOO attract the interest of the scientific community for its health properties; is an indispensable element of the Mediterranean diet [2]. Its consumption is correlated with a lower incidence of a number of diseases correlated to inflammatory processes such as cardiovascular diseases, diabetes, arthritis, Alzheimer and certain types of cancer [3].

A potential interacting impact of phenolic compounds on EVOO aroma release and perception has been recently described. Volatile minor components are consequently responsible for the aroma of EVOO whereas phenolics are closely related to the bitter and pungent sensory notes [4].

Aldehydes and alcohols of six straight-chain carbons (C6), as well as their corresponding esters, are the most important compounds in EVOO volatile compounds, also quantitatively or qualitatively. Linolenic (LnA) and Linoleic (LA) acids are the main substrates for this synthesis. Lastly, terpenes found in the volatile fraction of EVOO seem not to be important contributors to EVOO aroma due to their low concentration and high odor threshold [1, 5].

Baccouri et al. [6] revealed that Solid-phase microextraction (SPME) of the head space (HS) in combination with mass spectrometry (GC–MS) and gas

chromatography is a very powerful technique that is used quite regularly for the analysis of aroma compounds in foods. HS-SPME–GG–MS has been applied to study the volatile of products derived from the olive fruit, such as oil or table olives [7]. Actually, SPME-GC–MS is the technique of reference to validate the discriminating power of new e-sensing technologies such as the electronic nose for olive oil [8–10].

The main triterpenes present in EVOO are two hydroxyl pentacyclic triterpene acids (oleanolic and maslinic acid) and two dialcohols (uvaol and erythrodiol) [2]. These compounds are mostly found in the epicarp of drupes, therefore, pomace olive oil extracted from olive pomace after the first press with the use of solvents or other chemical processes generally contains 10-fold higher concentrations than EVOO [11].

In in vitro studies, EVOO triterpenes have been described as potent inhibitors of LDL oxidation [12] and to possess antiatherogenic properties via preventing LDL-supporting thrombin generation [3]. A role of these compounds in atherosclerosis protection has been further suggested in a feed work with apolipoprotein (apo) E knockout (KO) mice developing a spontaneous atherosclerosis that mimics most of the features of human atherogenesis [11].

EVOO triterpenes together with hydrocarbons and lignans inhibited cell proliferation and DNA synthesis in Caco-2 colon cancer cell cultures induced through oleic acid, as oleic acid in deficiency of growth factors was capable to induce Caco-2 propagation [13]. In addition, pentacyclic triterpenes from olives established an antiproliferative, and proapoptotic action on on HT-29 colon cancer cells and MCF-7 human breast cancer cells [14].

2. Material and methods

2.1 Sampling

This work was carried out on the study of Effect of ripening period on Terpene compounds of new Tunisian extra-virgin olive oil obtained through controlled crossings on Meski variety. Preliminary work evaluating the oil fatty acid composition of the oil of 50 hybrids showed the performance of these cultivars (9d) among the studied descendants. This new cultivars have an improved oil composition compared to that of Chemlali, the most abundant variety in Tunisia. Samples, obtained from homogeneous olive have picked by hand at a known ripening degree during the crop season 2018/2019. Healthy fruits, without any infection or physical damage, were processed. The olives were washed, deleafed and crushed with a hammer crusher, and the paste mixed at 25 °C for 30 min, centrifuged without addition of warm water and then transferred into dark glass bottles.

2.2 Analysis of volatile compounds: HS–SPME analysis

Before use, the fibre was conditioned; the fibre used for the extraction of the volatile components was divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 50/30 mm.

5 g olive oil was placed in a 20 ml vial closed by PTFE/silicone septum. Before extraction, the stabilization of the headspace in the vial was accomplished by equilibration for 60 min at 25 °C. The extraction was carried out at room temperature, with magnetic stirring (900 trs/min). To determine the optimal adsorption time of the fibre with the sample headspace, the fibre DVB/CAR/PDMS was exposed for time periods of 10, 30, 60, 90 and 120 min [5].

The injections were performed using a SPME autosampler. The fibre was thermally desorbed into a GC and left in the injection port for 4 min. The injector was set at 250 °C and operated in the splitless mode for 2 min unless otherwise stated. The fibre was reconditioned for 5 min in a washing port at 250 °C and blank runs were done periodically during the study [5].

2.3 GC–MS analyses

Each oil was analysed by GC–MS using an Agilent 6890N/5973N system, with fused-silica capillary columns HP-1 (50 m X 0.20 mm; film thickness: 0.5 mm). The identification of the constituents was based on comparison of the retention times with those of authentic samples. Several structures were also confirmed by standard compounds injection. All chemicals were purchased from Fluka or Sigma–Aldrich (Saint Quentin Fallavier, France).

3. Result and discussion

Sesquiterpene hydrocarbons were a major class of compounds identified in the EVOOs samples. Monoterpenes and sesquiterpenes are the lower molecular weight representatives of the terpenoid compounds; they are produced by two and three isoprene units, respectively.

Studied EVOOs made during ripeness process were exposed. Ten sesquiterpenes (**Table 1**), acyclic, monocyclic, bicyclic and tricyclic, were studieded. The variables which were more decisive to discriminate among ripeness stages were sesquiterpenes and aldehydes, such as limonene, α-agarofuran, α-muurolene, *trans*-α-bergamotene, α-farnesene, α-copaene, β-selinene, β-elemene, β-dihydroagarofuran, β-caryophyllene, (Z)-β-farnesene, δ-cadinene, (*E,E*)- (*E*)-β-ocimene, (*Z*)-3-hexenal and nonanal. This finding is in very good agreement with previous studies on other varieties [2, 8].

Several terpene hydrocarbons (mono- and sesquiterpenes) were often detected, and they totally accounted for 2.9–13.5% of the whole volatiles (**Table 1**). (E,E)-α-farnesene (0.1–0.7%), a mono-unsaturated sesquiterpene, was the main one. Besides (E,E)- α-farnesene, other important sesquiterpenes were cyclosativene and α-muurolene, a tetra-unsaturated sesquiterpene that has already been detected in Spanish oils, mainly in those obtained from olives of the Hojiblanca variety [15].

Sesquiterpene hydrocarbons, tended to increase during the maturation process. The highest value (1.3%) was registered in EVOOs obtained from fruits at MI = 6, and the lowest (0.1%) in EVOOs from fruits at MI = 2.

During maturation process, α-copaene remained almost constant during the two maturation stages followed the general trend described above, varying from 0.2% (MI = 2) to 0.4% (MI = 6). further sesquiterpene, such as β-slinene, γ-muurolene, cyclosativene, α-ylangene and α-cubebene, appeared in small amounts only at the highest MI value (**Table 1**).

Monoterpene hydrocarbons, represented by limonene, p-cymene and (E)-β-ocimene, showed a constant increment of its levels during the three maturation stages. Limonene showed a constant increment of its levels passing from 2.2 to 9.4%.

This strong dependence on maturity process, makes terpenes good candidates suitable for the discrimination of oils with different ripeness index. Vichi et al. [15] demonstrated that the amounts of α-muurolene, α-copaeneand and α-farnesene may be used to construct a decisional tree that successfully classifies Western-Liguria extra-virgin olive oils from further Mediterranean oils. Vichi et al. [15] confirmed also for the first time that the enhance of sesquiterpene is Maturation-dependent in olive, and consequently that ripening must be taken carefully into

Constituents	RI	9d Im2	9d Im4	9d Im6
p-cymene	028	0,2	1,7	2,6
limonene	032	2,2	3,9	9,4
(E)-β-ocimene	052	0,2	0,2	0,2
α-cubebene	352			0,1
cyclosativene	370			0.2
α-ylangene	371			0,1
α-copaene	377	0,2	0,2	0,4
β-selinene	487			0,2
α-muurolene	499			0,2
(E,E)-α-farnesene	507	0,1	0,2	0,5
Monoterpene hydrocarbons		2,6	4,3	12,2
Sesquiterpene hydrocarbons		0,3	0,4	1,5
Total terpene hydrocarbons		2,9	4,7	13,7
erythrodiol		0,5	0,8	2,2
uvaol		0,1	0,4	0,9
erythrodiol +uvaol		0,57	1,2	3,14

Table 1.
Terpene composition of the studied olive oil at different stage of maturity.

account when analyzing terpenes [16]. Our results are in agreement with the study of Vichi et al. [15]. These hydrocarbons may be used as markers to distinguish EVOO of different geographical origins [10].

The volatile fraction of the oil from Sfax (South of Tunisia) was characterized by the pre-eminence of α-copaene (24.5%) that may be used as markers to differentiate EVOO of different sites. The other main compounds detected were (E,E)- α -farnesene (6.8%), α -muurolene (4.8%), cyclosativene (3.0%), aromadendrene (1.8%) and longicyclene (1.7%).

A comparison with literature data on the chemical composition of olive oils is complicated because of the big variability of the volatile profiles. In fact, it has been reported that the concentrations of compounds depend on the enzymatic activity though external parameters (soil, climate, harvesting and extraction conditions) may alter the inherent olive oil sensory profile. The variation in levels of C6 aldehydes and alcohols for oil samples from different soils implies that pedologic conditions may influence the activity of alcohol dehydrogenase (ADH).

The triterpenic dialcohols (erythrodiol and uvaol), which are also part of the unsaponifiable fraction of the olive oil, are usually analysed together with the sterol fraction [1]. The erythrodiol and uvaol content of the studied olive oils varied according to maturity, ranging from 0.5 to 2.22% and from 0.1 to 0.92%, respectively (**Table 1**). The sum of erythrodiol and uvaol in all ripeness index was below the established limit of 4.5% for the "extra virgin" olive oil category. These results are consistent with the findings of other authors [15].

4. Conclusions

In conclusion, results demonstrated that the studied olive oils presents a elevated level of variability in terms of the volatile fraction. This aroma variability and the

high genetic diversity of the cultivar germplasm collection suggest that it is possible both to identify old olive cultivars that give rise to oils with a high organoleptic quality and to select optimal parents for olive breeding programs with the aim of finding new cultivars with improved oil aroma. The application of SPME to the analysis of virgin olive oil headspace allowed the detection of significant differences in the proportion of volatile constituents from oils of different maturity index. The results indicate that the ripeness time influence the quali-quantitative production of volatiles. These results permit to use the volatile composition as an indicator of each maturity stage.

Conflict of interest

The authors declare no conflicts of interest.

Author details

Bechir Baccouri[1*] and Imene Rajhi[2]

1 Laboratory of Olive Biotechnology, Centre of Biotechnology of Borj-Cédria, Tunisia

2 Laboratory of Legumes Centre of Biotechnology of Borj-Cédria, Tunisia

*Address all correspondence to: bechirbaccouri@yahoo.fr

References

[1] Baccouri B., Ben Temime S., Daoud D., M'sallem M. and Zarrouk M. (2007). Analytical characteristics of virgin olive oils from two new varieties obtained by controlled crossing on Meski variety. Journal of Food Lipids *14,* 19-34

[2] Ben Temime S, Manai H, Methenni K, Baccouri B, Abaza L, Daoud D, (2008), Sterolic composition of Chetoui virgin olive oil: Influence of geographical origin. Food Chem 110:368-374.

[3] R. K. Manoharan, J. Lee, and J. Lee, (2017). "Antibiofilm and antihyphal activities of cedar leaf essential oil, camphor, and fenchone derivatives against Candida albicans," Front. Microbiol. 8, 1476.

[4] Mills, J.J.; Chari, R.S.; Boyer, I.J.; Gould, M.N.; Jirtle, R.L. (1995). Induction of apoptosis in liver tumors by the monoterpene perillyl alcohol. Cancer Res., *53*, 955-979.

[5] Baccouri B, Zarrouk W, Khichene D, Nouari I, Ben Youssef N, Daoud D, (2007). Influence of fruit ripening and crop yield on chemical properties of virgin olive oils from seven selected oleasters (*Olea europea* L.). J Agron 6:388-396.

[6] Baccouri, B., Zarrouk, W., Krichene, D., Nouairi, I., Ben youssef, N., Daoud, D., Zarrouk, M., (2007b). Influence of fruit ripening and crop yield on chemical properties of virgin olive oils from seven selected oleasters (Olea Europea L.). J. Agron. 6 (3), 388-396.

[7] Baccouri, B., Guerfel, M., Zarrouk, W., Taamalli, W., Daoud, D., Zarrouk, M., (2011). Wild olive (olea europea L.) selection for quality oil production. J. Food Biochem. 35, 161-176.

[8] Baccouri, B., Ben Temime, S., Campeol, E., Cioni, P.L., Daoud, D., Zarrouk, M., (2007a). Application of solid-phase microextraction to the analysis of volatile compounds in virgin olive oils from five new cultivars. Food Chem. 102, 850-856.

[9] Baccouri, B., Zarrouk, W., Guerfel, M., Baccouri, O., Nouairi, I., Krichene, D., Daoud, D., Zarrouk, M., (2008). Composition, quality and oxidative stability of virgin olive oils from some selected wild olives (Olea europaea L. subsp. Oleaster). Grasas Aceites 59 (4), 346-351.

[10] Ben Temime, S., Baccouri, B., Taamalli, W., Abaza, L., Daoud, D., Zarrouk, M., (2006). Location effects on Chetoui virgin olive oil stability. J. Food Biochem. 30, 659-670.

[11] Beltran G, Aguilera MP, Del Rio C(2005), Sanchez S and Martinez L, Influence of fruit ripening process on the natural antioxidant content of Hojiblanca virgin olive oils. Food Chem 89:207-215.

[12] H. Sebai, S. Selmi, K. Rtibi, A. Souli, N. Gharbi, and M. Sakly, (2013). "Lavender (Lavandula stoechas L.) essential oils attenuate hyperglycemia and protect against oxidative stress in alloxan-induced diabetic rats," Lipids Health Dis. 12, 189.

[13] M. Božović and R. Ragno, (2017). "Calamintha nepeta (L.) Savi and its main essential oil constituent pulegone: Biological activities and chemistry," Molecules 22, 290.

[14] Urbina A., Martin M., Montero M., Carron R., Sevilla M., and L. Roman, (1990). "Antihistaminic activity of pulegone on the guinea-pig ileum," J. Pharm. Pharmacol. 42, 295-296.

[15] Vichi, S., Castellote, A.I., Pizzale, L., Conte, L.S., Buxaderas, S. & Lopez-Tamames, E. (2003). Analysis of

virgin olive oil volatile compounds by headspace solid-phase microextraction coupled to gas chromatography with mass spectrometric and flame ionization detection. Journal of Chromatography A, 983, 19-33.

[16] Heuberger, E.; Ilmberger, J.; Hartter, E.; Buchbauer, G. (2008) Physiological and behavioral effects of 1,8 cineol and (±)-linalool: A comparison of inhalation and massage aromatherapy. Nat. Prod. Commun., , *3*, 1103-1110.

Chapter 4

Sacha Inchi Seed (*Plukenetia volubilis* L.) Oil: Terpenoids

Alexandra Valencia, Frank L. Romero-Orejon, Adriana Viñas-Ospino, Dayana Barriga-Rodriguez, Ana María Muñoz and Fernando Ramos-Escudero

Abstract

Sacha inchi oil is a product obtained from oilseed (*Plukenetia volubilis* L.) and is an excellent source of bioactive compounds, especially in polyunsaturated fatty acids, tocopherols, and sterols. These compounds are causally related to their positive impact on human health. In this study summarizes some monoterpenes, sesquiterpenes, and triterpenes reported in Sacha inchi oil seeds and reviews their sensory properties. The terpenoids that characterize Sacha inchi seed oil are: α-pinene, sabinene, limonene, aristolene, cycloartenol, 24-methylene cycloartenol, lanosterol, β-sitosterol, stigmasterol, campesterol and phytol. The sensory properties of this oil are due to a set of volatile compounds including terpenoids, the odor descriptors of monoterpenes, sesquiterpenes and diterpenes are: flower, pine, turpentine, pepper, wood, lemon, orange, and sweet. These compounds were characterized by gas chromatography with different detectors.

Keywords: sacha inchi seed oil, terpenoids, sensory properties, chromatographic analysis

1. Introduction

The Sacha inchi (*Plukenetia volubilis* L.) plant is a crop that has expanded rapidly in recent decades. This endemic crop of the South American Amazon is found mainly in Peru, Colombia, Ecuador, and Brazil. Other geographical regions of the world where Sacha inchi cultivation has flourished include China, Thailand, Vietnam, and Malaysia [1–4]. Its oleaginous plant has become a crop of economic importance for the food, pharmaceutical and cosmetic industries. Exports in Peru have grown notably for the year 2017, especially for its main products such as oil, roasted seed, and powder, having as main destinations, South Korea, United States, Japan, Canada and France [5].

Kodahl [6] mentioned that Sacha inchi seed has an unusual chemical composition as it contains remarkably high amounts of polyunsaturated fatty acids. According to the NTP [7] indicates that the requirements for the polyunsaturated fatty acids (PUFAs) profile is as follows: α-linolenic acid (ω-3, greater than 42%), linoleic acid (ω-6, greater than 32%) and polyunsaturated fatty acids (greater than 80%) of the total lipid fraction. Other main representatives of the unsaponifiable fraction are tocopherols, which are distributed in the oil as follows: α-tocopherol

(60–70 mg/kg), β-tocopherol (18–29 mg/kg), γ-tocopherol (1108–1367 mg/kg), δ-tocopherol (641–856 mg/kg), and sterols fraction of commercial oils was 1130–3635 mg/kg, and the main sterols were β-sitosterol, stigmasterol, campesterol and Δ5-avenasterol [8, 9]. Other compounds of interest are phenolic compounds (the main classes of phenols found in sacha inchi seed oil (SISO) are phenyl alcohol, isocoumarin, flavonoid, secoiridoid, and lignan) [10], volatile organic compounds (while the classes of VOCs identified in commercial oil were aldehydes, hydrocarbons, alcohols, ketone, furan, and carboxylic acid), and terpenoids [11].

Terpenoids are a large family of chemical compounds which can be found in a large number of plants, many of which have characteristic odors, flavors, and colors, and are main components of essential oils (especially monoterpenes and sesquiterpenes) [12]. Terpenoids can be structurally decomposed into two or more isoprene units or 2-methyl-1,3-butadiene and classified as monoterpenes (C10H16), sesquiterpenes (C15H24), diterpenes (C20H32), triterpenes (C30H48), and tetraterpenes or carotenes (C40H64) [13]. In vegetable oils, several terpenoids have been identified, these compounds provide aromatic properties (monoterpenoids: myrcene, citral, linalool, thymol, menthol, carvone, eucalyptol, α- and β-pinene, etc.), and are natural fat-soluble pigments (tetraterpenoids: lycopene, γ-carotene, β-carotene, lutein, zeaxanthin, etc.) [14], this last group of chemical species are responsible for transmitting the chromatic characteristics in vegetable oils. A list of oils from conventional and non-conventional plant sources where terpenoids have

Terpenoids	Class	Effects	Reference
α-Pinene	Monoterpene	Cytogenetic, gastroprotective, anxiolytic, cytoprotective, anticonvulsant, and neuroprotective	[27]
Sabinene	Monoterpene	Antioxidant, antibacterial and antifungal	[29, 30]
Limonene	Monoterpene	Gastroprotective, anti-inflammatory, bradycardic, antiarrhythmic, antitumor, antiviral, and antibacterial	[31–33]
Aristolene	Sesquiterpene	Antifungal, antioxidant, and anticancer	[34, 35]
Cycloartenol	Triterpene	Anticancer, and antidiabetic	[36, 37]
24-Methylene cycloartenol	Triterpene	Antidiabetic	[37]
Lanosterol	Triterpene	Cytotoxic and immunomodulatory	[38, 39]
β-Sitosterol	Sterol	Anticancer, lipid-lowering, anti-inflammatory, and antioxidant	[40–43]
Stigmasterol	Sterol	Lipid-lowering, antiasthmatic, immunomodulatory, antioxidant, and anti-inflammatory	[41, 44]
Campesterol	Sterol	Anti-inflammatory, and cytotoxic	[45]
Phytol	Diterpene	Antitumoral, antimutagenic, antimicrobial, anxiolytic, metabolism-modulating, cytotoxic, antioxidant, autophagy- and apoptosis-inducing, antinociceptive, anti-inflammatory, immune-modulating, antidiabetic, anti-atherogenic, lipid-lowering, antispasmodic, antiepileptic, antidepressant and immunoadjuvant	[46, 47]

Table 1.
Summary of terpenoids of Sacha inchi seed oil and biological effects.

been identified: soybean, olive, rapeseed, sunflowerseed, flaxseed, sesame, pumpkin, pistachio, almond, hazelnut, safflower, hempseed, sacha inchi oils [15–20].

Traditionally, plant-based terpenoids have been used by humans in the food (terpenoids as natural flavorings compounds, preservatives for dairy products, stability of edibles oils flavored with essential oils) [21–23], pharmaceutical (production of pharmaceutical terpenoids for the treatment of human diseases) [24, 25], and chemical industries (natural additives for food or fragrances in perfumery) [26]. Various studies have shown the efficacy of terpenoids due to their biological and medical properties [25, 27, 28]. **Table 1** summarizes most of the effects, however some of heightened interest are mentioned in this section.

This document summarizes some monoterpenes, sesquiterpenes, and triterpenes reported in Sacha inchi oil seeds and reviews their sensory properties.

2. Overview of terpenoids biosynthesis in Sacha inchi seed oil

The biosynthesis of these compounds occurs via the methylerythritol phosphate pathway (MEP) or mevalonate (MVA) pathway involves several reactions to isopentenyl diphosphate production from acetyl CoA. The isopentenyl diphosphate (IPP) combines with dimethyl-allyl diphosphate (DMAPP) to that subsequently converted to geranyl pyrophosphate (GPP) by enzymatic catalysis of isopentenyl diphosphate isomerase. Geranyl pyrophosphate is the substrate to produce monoterpenoids. The enzymatic reaction is mediated by monoterpene synthases [48]. The monoterpenes found in SISO were α-pinene, sabinene and limonene (**Figure 1**). α-Pinene (C10H16) is the main bicyclic monoterpene found in this oil, it is also widely distributed in nature. The sesquiterpenes are formed by the condensation of IPP with GPP to yield farnesyl pyrophosphate (FPP) [50]. The GPP to FPP reaction is mediated by farnesyl pyrophosphate synthase. The only sesquiterpene found in SISO is the aristolene (C15H24) [20]. On the other hand, this biochemical pathway may be used for triterpene (some triterpenes were found in SISO, namely cycloartenol, 24-methylene cycloartenol and lanosterol isomers) and probably sterols (individual sterols found in SISO, namely β-sitosterol, stigmasterol, campesterol, Δ5-avenasterol, Δ5,24- stigmastadienol, Δ7-stigmastenol, Δ7-avenasterol, etc.) [8, 9, 51], and brassinosteroids biosynthesis, whereas geranylgeranyl pyrophosphate (GGPP) is utilized for the biosynthesis of photosynthetic pigments such as carotenoids, chlorophylls and diterpenes (phytol) (**Figure 1**) [9, 52, 53].

3. Terpenoids in Sacha inchi seed oil

In the scientific literature there are few reports on the volatile composition of sacha inchi oil [20, 49]. The terpenoid fractions in the Sacha inchi oil is observed in **Table 2**. The identification of the classes of terpenoids found in Sacha inchi seed oil and commercial Sacha inchi oil were monoterpenes, sesquiterpenes, diterpenes, triterpenes and sterols. The first terpenoids identified in this oil were sterols: β-sitosterol > stigmasterol > campesterol > Δ5-avenasterol [51]. The sterol composition of these main compounds is around ~96%. The sterol content in the Sacha inchi seed oil was reported as 2472 mg/kg. While the sterol contents in commercial oils ranging from 1130 to 3635 mg/kg [8, 9].

The sterol content in Sacha inchi seed oil is represented by the content of β-sitosterol, stigmasterol and campesterol (**Table 2**). The β-sitosterol, followed by stigmasterol or campesterol and other minor sterols (triterpenes) such as

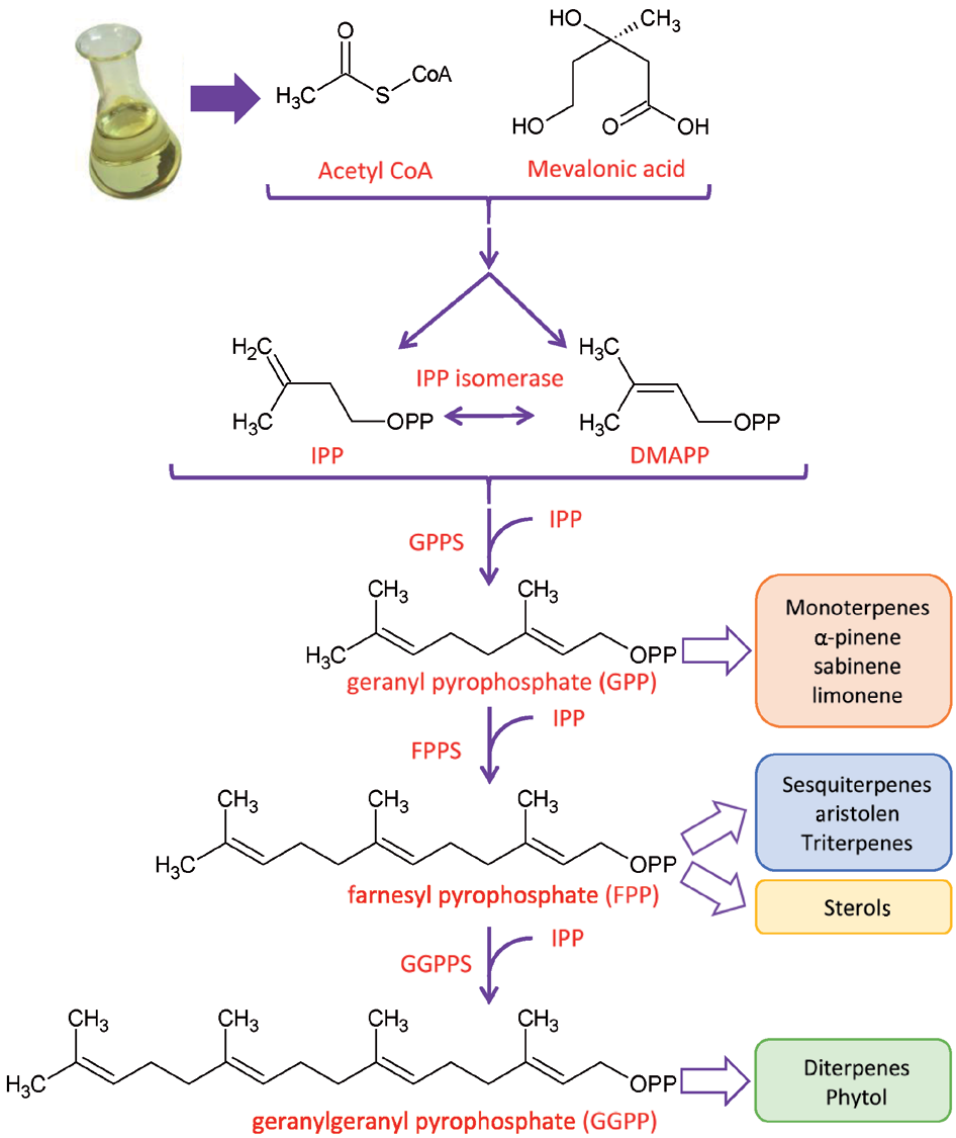


Figure 1.
Biosynthetic pathway of terpenoids and chemical compounds found in Sacha inchi seed oil. The diagram was modified according to Feng et al. [49]. Isopentenyl diphosphate (IPP), dimethyl-allyl diphosphate (DMAPP), geranyl pyrophosphate synthase (GPPS), farnesyl pyrophosphate synthase (FPPS), geranylgeranyl pyrophosphate synthase (GGPPS).

fucosterol, and Δ5-avenasterol are the most representative in vegetable oils. In addition, 50% to 80% of the plant sterols intake comes from oils, spreads, butters, breads, cereals, grains, pastes, and vegetables [55]. On the other hand, other triterpenoids such as cycloartenol, 24-Methylene cycloartenol, and lanosterol were detected in commercial Sacha inchi oil, the contents ranged from 0.10 to 47.44%, 2.59 to 24.15%, 0.80 to 11.79%, respectively. A sole example of diterpene such as phytol were found in the range of 0.10 to 43.51% [9]. The monoterpenoids and sesquiterpene in the sacha inchi oil were α-pinene, sabinene, limonene and aristolene these compounds were also identified by Monroy-Soto et al. [11]. In addition, it has been reported that this class of terpenoids are considered potentiators. In this context, the minimum inhibitory concentration of some monoterpenoids

Terpenoids	Sacha inchi seed oil	Commercial Sacha inchi oil
α-Pinene (μg/kg)		(3.35–1179.24) μg/kg
Sabinene (μg/kg)		(0.87–416.51) μg/kg
Limonene (μg/kg)		(0.93–187.83) μg/kg
Aristolene (μg/kg)		(3.99–34.82) μg/kg
Cycloartenol (%)		(2.59–34.54) %
24-Methylene cycloartenol (%)		(0.80–11.79) %
Lanosterol (%)		(0.10–47.44) %
β-Sitosterol (%)	127.4 mg/100 g	(21.45–68.91) %
Stigmasterol (%)	58.7 mg/100 g	(10.4–27.4) %
Campesterol (%)	15.3 mg/100 g	(5.1–18.9) %
Δ5-Avenasterol (%)		(0.10–7.78) %
Phytol (%)		(0.10–43.51) %

References, for Sacha inchi seed oil: Chirinos et al. [54]. For commercial Sacha inchi oil: Chasquibol et al. [8]; Ramos-Escudero et al. [9].

Table 2.
Summary of terpenoids identified in Sacha inchi oil.

(α-pinene and limonene) on bacteria such as *Escherichia coli*, *Salmonella enterica* and *Staphylococcus aureus* have been reported previously [56]. Furthermore, these monoterpenoids have shown a potent antioxidant activity, especially α-pinene followed by limonene, both presented a 50% inhibitory concentration values (IC_{50}) equal to 12.57 and 13.35 mg/mL, respectively. In this regard, terpenoids have huge potential as natural food preservatives for use in the food industry [57].

The storage food products are subject to changes in the chemical composition and as a result the formation of undesirable volatile compounds. Therefore, terpenoids as natural preservatives can be used to slow down food spoilage. Some monoterpenoids such as limonene can be used as substitutes for synthetic antioxidants (TBHQ, BHA, BHT) and improves oxidative stability in edible oils [58]. Wang et al. [58] have mentioned that monoterpenoids can be used as a reference for the food manufacturing, lifestyle, and nutrition in the future.

4. Terpenoids and sensory properties in Sacha inchi seed oil

Terpenoids are compounds responsible for the smell of most plants. Phytol, α-pinene, sabinene, limonene, and aristolene have been found in Sacha inchi oil (**Table 3**). These compounds provide some odor notes such as flower, pine, turpentine, pepper, wood, lemon, orange, and sweet. The content of monoterpenoids and sesquiterpenoids in Sacha inchi oil, fraction constituted about 9.0% of total volatile fraction. Ramos-Escudero et al. [20] have mentioned that these compounds are responsible for the floral aroma in this oil. However, the sensory characteristics of Sacha inchi oil not only correspond to the sensory notes of the terpenoids, but to a combination of sensory attributes such as herbal, green, nutty, seeds, butter, rancid, fruity, floral, and woody [20, 59]. Different volatile compounds including terpenoids have been identified in vegetable oils and each compound has different characteristics of key odorants. For example, in virgin sunflower oil the most preferred attributes were sweet and wood/vegetable resin, the latter possibly due

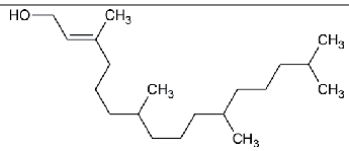
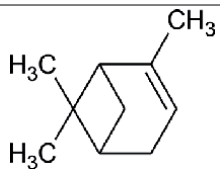
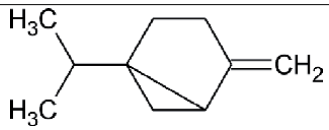
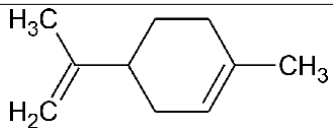
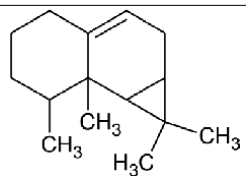
Terpenoids	MF/MW	Structure	Percepts
Phytol	$C_{20}H_{40}O$ 296.54 g/mol		flower
α-Pinene	$C_{10}H_{16}$ 136.23 g/mol		pine, turpentine
Sabinene	$C_{10}H_{16}$ 136.23 g/mol		pepper, turpentine, wood
Limonene	$C_{10}H_{16}$ 136.23 g/mol		lemon, orange
Aristolene	$C_{15}H_{24}$ 204.35 g/mol		flower, sweet

Table 3.
Terpenoids, structures, and percepts of in Sacha inchi seed oil.

to the presence of terpenes such as linalool and α- and β-pinene. Furthermore, the sensory profile of Niger seed oil showed positive attributes such as dried fruit, spicy and bitter, which could be related to the presence of some terpenes, specifically limonene and phellandrene. On the other hand, the sensory notes of pine perceived in the pine nut (*Pinus pinea*) oil were described under the wood/plant resin attribute, which could be attributable to the high contents of α-pinene as well as other terpenes such as β-pinene, β-myrcene and α- and γ-terpinene present in its volatile composition [15].

5. Comparison of terpenoid contents in other vegetable oils

Information about the volatile composition, including some terpenoids in vegetable oils can be found in published reports. Aguilar-Hernández et al. [60] reported the profile of terpenoids including monoterpenes and sesquiterpenes in lemon peel oil. In this oil around 23 terpenoids have been found, the most relevant being limonene, γ-terpinene, sabinene, α-pinene, β-pinene, α-thujene, terpinolene, α-terpineol, neral, geranial, and trans- α- bergamotene. Ivanova-Petropulos et al. [17] reported a higher content of terpenoids in sunflower seed oil and pumpkin seed oil. The most common monoterpenoids and sesquiterpenoids in both oils were: α-thujene, α-pinene, α-fenchene, camphene, verbenene, sabinene, 2-β-pinene, α-phellandrene, α-terpinene, DL-limonene, β-phellandrene, 1,8-cineole, *o*-cymene, *p*-cymene, γ-terpinene, α-terpinolene, α-campholenal,

	Compounds	Flaxseed oil	Rapeseed oil	Sesame seed oil	Sunflower seed oil	Pumpkin oil	Sacha inchi oil	Pistachio oils	Almond oil	Hazelnut oil
1	α-thujene	X			X	X		X		
2	α-pinene	X	X	X	X	X	X	X		
3	β-pinene							X		
4	α-fenchene	X			X	X				
5	camphene	X	X		X	X		X		
6	verbenene	X	X		X	X				
7	sabinene				X	X	X			
8	2-β-pinene	X	X		X	X				
9	α-phellandrene	X		X	X	X				
10	3-carene	X						X		
11	4-carene							X		
12	α-terpinene	X	X	X	X	X		X		
13	β-ocimene			X						
14	limonene						X	X		
15	β-phellandrene	X			X	X				
16	1,8-cineole	X	X		X	X				
17	o-cymene				X	X		X		
18	p-cymene	X	X	X	X	X				
19	γ-terpinene	X	X		X	X				
20	α-terpinolene	X			X	X		X		
21	α-campholenal	X			X	X				

	Compounds	Flaxseed oil	Rapeseed oil	Sesame seed oil	Sunflower seed oil	Pumpkin oil	Sacha inchi oil	Pistachio oils	Almond oil	Hazelnut oil
22	trans-pinocarveol				X	X				
23	α-phellandren-8-ol				X					
24	borneol				X	X				
25	4-terpineol				X	X				
26	3-pinanone				X	X				
27	2-pinen-10-ol				X	X				
28	myrtenal				X	X				
29	verbenone				X	X				
30	α-cubebene				X	X				
31	camphor	X			X	X				
32	α-copaene				X	X				
33	β-elemene				X	X				
34	β-bourbonene				X	X				
35	β-selinene				X	X				
36	β-myrcene							X		
37	2-norpinene			X	X	X				
38	aristolen				X	X	X			
39	γ-cadinene				X	X				
40	calarene				X	X				
41	α-amorphene				X	X				

	Compounds	Flaxseed oil	Rapeseed oil	Sesame seed oil	Sunflower seed oil	Pumpkin oil	Sacha inchi oil	Pistachio oils	Almond oil	Hazelnut oil
42	β-bisabolene				X	X				
43	δ-cadinene				X	X				
44	longifolene							X	X	X
45	*α*-terpineol							X	X	X

Table 4.
Chemical characterization of terpenoids detected in vegetable oils.

trans-pinocarveol, borneol, 4-terpineol, 3-pinanone, 2-pinen-10-ol, myrtenal, verbenone, α-cubebene, camphor, α-copaene, β-elemene, β-bourbonene, β-selinene, 2-norpinene, aristolen, γ-cadinene, calarene, α-amorphene, β-bisabolene, δ-cadinene. While the main terpenoids in the following oils were: flaxseed (α-pinene, camphene, verbenene, 2-β-pinene, 3-carene, α-terpinene, DL-limonene, 1,8-cineole, γ-terpinene, and α-terpinolene), rapeseed (*p*-cymene), and sesame seed (2-norpinene). Other vegetable oils have different terpene profiles [18, 19] (**Table 4**).

6. Application of chromatographic techniques in Sacha inchi seed oil

There are few reports about the chemical characterization of the terpenoids in the Sacha inchi oil (**Table 5**). The separation of the different analytes from the sterol fraction was conducted using the following columns: SAC™-5/Merck (Phase: 5% diphenyl/95% dimethyl polysiloxane), HP-5/Agilent J&W (Phase: 5% phenyl-methylpolysiloxane), and SPB-5/Merck (5% diphenyl/95% dimethyl polysiloxane). While the separation of volatile compounds was carried out using columns with high polar (DB-WAX/Agilent J&W, and TRB-WAX/Teknokroma/ 100% polyethylene glycol) and nonpolar (DB-5/Agilent J&W/5% phenyl-methylpolysiloxane) stationary phases.

Monroy-Soto et al. [11] evaluated the volatile composition of Colombian commercial Sacha inchi oil using headspace-solid phase microextraction coupled GC–MS-O. Ramos-Escudero et al. [20] analyzed the Peruvian commercial Sacha inchi by HS-SPME/GC–MS, through which 16 volatile compounds (among them limonene, α-pinene, and sabinene) may have a significant influence upon perceived flavor and odor.

Analytes	Column	Technique	Methods	Extraction	Reference
Sterol	SAC™-5 (30 m x 0.25 mm ID)	GC-FID	C	S	[61, 62]
Sterol	HP-5 (30 m x 0.32 mm ID)	GC-FID/MS	C, A	P	[9]
Sterol	SPB-5 (30 m x 0.32 mm ID)	GC-FID	C	P	[8, 63]
Terpenes	DB-WAX (30 m x 0.25 mm ID) DB5 (30 m x 0.25 mm ID)	HS-SPME-GC–MS-O	C	P	[11]
Terpenes	ATR-WAX (60 m x 0.25 mm ID)	HS-SPME/ GC–MS	C, A	P	[20]

List of abbreviations: GC-FID, Gas chromatography-flame ionization detector; GC–MS, Gas chromatography–mass spectrometry; HS-SPME, Headspace-solid phase microextraction; GC–MS-O, Gas Chromatography–Mass Spectrometry-Olfactometry. A, authentication; C, characterization; P, cold pressed; S, solvent.

Table 5.
Characterization and authentication of Sacha inchi oil.

7. Conclusions

Sacha inchi oil is a product of economic importance that has been characterized according to its chemical composition. At present several classes of chemical compounds have been identified and quantified, and more recently the volatile

composition. The volatile organic compounds correspond to notes generated by alcohols, aldehydes, ketones, and terpenoids. The classes of terpenoids found in Sacha inchi oil were monoterpenes, sesquiterpenes, diterpenes, and triterpenes. These compounds provide different sensory properties in the oil. Furthermore, the characterization is conducted mainly by gas chromatography (GC) coupled to flame ionization detector (FID) and mass spectrometry (MS) detection.

Conflict of interest

The authors declare no conflict of interest.

Author details

Alexandra Valencia[1], Frank L. Romero-Orejon[1], Adriana Viñas-Ospino[1,2], Dayana Barriga-Rodriguez[3], Ana María Muñoz[1,4] and Fernando Ramos-Escudero[1,4]*

1 Unidad de Investigación en Nutrición, Salud, Alimentos Funcionales y Nutraceúticos, Universidad San Ignacio de Loyola (UNUSAN-USIL), Lima, Perú

2 Nutrition and Food Chemistry, University of Valencia, Burjassot, Spain

3 Facultad de Ciencias de la Salud, Universidad San Ignacio de Loyola, Lima, Perú

4 Instituto de Ciencias de los Alimentos y Nutrición, Universidad San Ignacio de Loyola (ICAN-USIL), Campus Pachacamac, Sección B, Lima, Perú

*Address all correspondence to: diomedes.fernando@gmail.com

References

[1] Srichamnong W, Ting P, Pitchakarn P, Nuchuchua O, Temviriyanukul P. Safety assessment of *Plukenetia volubilis* (Inca peanut) seeds, leaves, and their products. Food Science and Nutrition. 2018;**6**:962-969. DOI: 10.1002/fsn3.633

[2] Minh NP, Trang THP, Trang NTT, Bach LG. Effect of different drying methods on antioxidant of sacha inchi (*Plukenetia volubilis* L.) nut. Research on Crops. 2019;**20**:180-186. DOI : 10.31830/2348-7542.2019.025

[3] Sethuraman G, Nizar NMM, Muhamad FN, Gregory PJ, Azam-Ali S. Nutritional composition of Sacha inchi (*Plukenetia volubikis* L.). International Journal for Innovative Research in Science Technology. 2020;**7**:271-277.

[4] Mai HC, Nguyen DC, Nhan NPT, Bach LG. Physico-chemical properties of sacha inchi (*Plukenetia volubilis* L.) seed oil from Vietnam. Asian Journal of Chemistry. 2020;**32**:335-338. DOI: 10.14233/ajchem.2020.22233

[5] SIICEX. Exportación del producto Sacha inchi según sus principales presentaciones en kg 2016-2021. Sistema Integrado de información de Comercio Exterior. [Internet]. 2020. Available from: https://www.siicex.gob.pe/siicex/apb/ReporteProducto.aspx?psector=1025&preporte=prodpresvolu&pvalor=1945 [Accessed: 2021-02-05]

[6] Kodahl N. Sacha inchi (*Plukenetia volubilis* L.)-from lost crop of the Incas to part of the solution to global challenges?. Planta. 2020;**251**:80. DOI: 10.1007/s00425-020-03377-3

[7] NTP. Norma Tecnica Peruana 151.400, amendment to NTP 151.400, 2014. Sacha inchi oil. Requirements, Lima, Peru: R.D. N° 047-2018-INACAL/DN; 2018. p. 1-24.

[8] Chasquibol NA, Gómez-Coca RB, Yácono JC, Guinda Á., Moreda W, del Aguila C, Pérez-Camino MC. Markers of quality and genuineness of commercial extra virgin sacha inchi oils. Grasas y Aceites. 2016;**67**:e169. DOI: 10.3989/gya.0457161

[9] Ramos-Escudero F, Muñoz AM, Ramos Escudero M, Viñas-Ospino A, Morales MT, Asuero AG. Characterization of commercial Sacha inchi oil according to its composition: tocopherols, fatty acids, sterols, triterpene and aliphatic alcohols. Journal of Food Science and Technology. 2019;**56**:4503-4515. DOI: 10.1007/s13197-019-03938-9

[10] Fanali C, Dugo L, Cacciola F, Beccaria M, Grasso S, Dachà M, Dugo P, Mondello L. Chemical characterization of Sacha Inchi (*Plukenetia volubilis* L.) oil. Journal of Agricultural and Food Chemistry. 2011;**59**:13043-13049. DOI: 10.1021/jf203184y

[11] Monroy-Soto LT, López-Cordoba CA, Araque-Marín P, Torijano-Gutierrez SA, Zapata-Ochoa JA. Caracterización de los compuestos de aroma del aceite de sacha inchi (*Plukenetia volubilis* L.) por HS-SPME-GC-MS-O. Revista Colombiana de Química. 2019;**48**: 45-50. DOI: 10.15446/rev.colomb.quim.v48n3.78979

[12] Stephane FFY, Jules BKJ. Terpenoids as important bioactive constituents of essential oils. In: de Oliveira MS, Silva S, Da Costa WA, editors. Essential Oils: Bioactive Compounds, New Perspectives and Applications. London, UK: IntechOpen Limited; 2020. p. 1-32. DOI: 10.5772/intechopen.91426

[13] Yazaki K, Arimura G-I, Ohnishi T. 'Hidden' terpenoids in plants: Their biosynthesis, localization and ecological roles. Plant and Cell Physiology.

2017;**58**:1615-1621. DOI: 10.1093/pcp/pcx123

[14] Ludwiczuk A, Skalicka-Woźniak A, Georgiev MI. Terpenoids. In: Badal S, Delgoda R, editors. Pharmacognosy: Fundamentals, Applications and Strategies. London, UK: Elsevier Inc; 2017. p. 233-266. DOI: 10.1016/B978-0-12-802104-0.00011-1

[15] Navas Hernández PB. Componentes minoritarios y propiedades antioxidantes de aceites vírgenes y tortas residuales obtenidos por presión en frío a partir de fuentes vegetales convencionales y no convencionales [thesis]. Ciudad Real: Universidad de Castilla La Mancha; 2010.

[16] Sanz C, Belaj A, Sánchez-Ortiz A, Pérez AG. Natural variation of volatile compounds in virgin olive oil analyzed by HS-SPME/GC-MS-FID. Separations. 2018;**5**:24. DOI: 10.3390/separations5020024

[17] Ivanova-Petropulos V, Mitrev S, Stafilov T, Markova N, Leitner E, Lankmayr E, Siegmund B. Characterization of traditional Macedonian edible oils by their fatty acid composition and their volatile. Food Research International. 2015;**77**:506-514. DOI: 10.1016/j.foodres.2015.08.014

[18] Ojeda-Amador RM, Fregapane G, Salvador MD. Chemical characterization of virgin almond and hazelnut oils and their by-products. European Journal of Lipid Science and Technology. 2019;121:1900114. DOI: 10.1002/ejlt.201900114

[19] Ojeda-Amador RM, Fregapane G, Salvador MD. Influence of cultivar and technological conditions on the volatile profile of virgin pistachio oils. Food Chemistry. 2020;**311**:125957. DOI: 10.1016/j.foodchem.2019.125957

[20] Ramos-Escudero F, Morales MT, Ramos Escudero M, Muñoz AM, Cancino Chavez K, Asuero AG. Assessment of phenolic and volatile compounds of commercial Sacha inchi oils and sensory evaluation. Food Research International. 2021;140:110022. DOI: 10.1016/j.foodres.2020.110022

[21] Caputi L, Aprea E. Use of terpenoids as natural flavouring compounds in food industry. Recent Patents on Food, Nutrition and Agriculture. 2011;3:9-16. DOI: 10.2174/2212798411103010009

[22] Chandran J, Nayana N, Roshini N, Nisha P. Oxidative stability, thermal stability and acceptability of coconut oil flavored with essential oils from black pepper and ginger. Journal of Food Science and Technology. 2017;**54**:44-152. DOI: 10.1007/s13197-016-2446-y

[23] Mishra AP, Devkota HP, Nigam M, Adetunji CO, Srivastava N, Sarla Saklani, Ila Shukla, Lubna Azmi, Mohammad Ali Shariati, Henrique Douglas Melo Coutinho, Amin Mousavi Khaneghah. Combination of essential oils in dairy products: A review of their functions and potential benefits. LWT-Food Science and Technology. 2020;**133**,110116. DOI: 10.1016/j.lwt.2020.110116

[24] Jaeger R, Cuny E. Terpenoids with special pharmacological significance: A review. Natural Product Communications. 2016;**9**:1373-1390. DOI: 10.1177/1934578X1601100946

[25] Lu X, Tang K, Li P. Plant metabolic engineering strategies for the production of pharmaceutical terpenoids. Frontiers in Plant Science. 2016;7:1647. DOI: 10.3389/fpls.2016.01647

[26] Stepanyuk A, Kirschning A. Synthetic terpenoids in the world of fragrances: Iso E Super® is the showcase. Beilstein Journal of Organic Chemistry. 2019;**15**:2590-2602. DOI: 10.3762/bjoc.15.252

[27] Salehi B, Upadhyay S, Orhan IE, Jugran AK, Jayaweera SLD, Dias DA, Sharopov F, Taheri Y, Martins N, Baghalpour N, Cho WC, Sharifi-Rad J. Therapeutic potential of α- and β-pinene: A miracle gift of nature. Biomolecules. 2019;**9**:738. DOI: 10.3390/biom9110738

[28] Bergman ME, Davis B, Phillips MA. Medically useful plant terpenoids: Biosynthesis, occurrence, and mechanism of action. Molecules. 2019;**24**:3961. DOI: 10.3390/molecules24213961

[29] Quiroga PR, Asensio CM, Nepote V. Antioxidant effects of the monoterpenes carvacrol, thymol and sabinene hydrate on chemical and sensory stability of roasted sunflower seeds. Journal of the Science of Food and Agriculture. 2015;**95**:471-479. DOI: 10.1002/jsfa.6744

[30] Fidan H, Stefanova G, Kostova I, Stankov S, Damyanova S, Stoyanova A, Zheljazkov VD. Chemical Composition and Antimicrobial Activity ofLaurus nobilisL. Essential Oils from Bulgaria. Molecules. 2019;**24**:804. DOI: 10.3390/molecules24040804

[31] de Souza MC, Vieira AJ, Beserra FP, Pellizzon CH, Nóbrega RH, Rozza AL. Gastroprotective effect of limonene in rats: Influence on oxidative stress, inflammation and gene expression. Phytomedicine. 2019;**53**:37-42. DOI: 10.1016/j.phymed.2018.09.027

[32] Alves QL, Silva DF. D-Limonene: A promising molecule with bradycardic and antiarrhythmic potential. Arquivos Brasileiros de Cardiologia. 2019; **113**:933-934. DOI: 10.5935/abc.20190233

[33] Mukhtar YM, Adu-Frimpong M, Xu X, Yu J. Biochemical significance of limonene and its metabolites: future prospects for designing and developing highly potent anticancer drugs. Bioscience Reports. 2018;**38**:BSR20181253. DOI: 10.1042/BSR20181253

[34] Juárez ZN, Bach H, Sánchez-Arreola E, Bach H, Hernández LR. Protective antifungal activity of essential oils extracted from *Buddleja perfoliata* and *Pelargonium graveolens* against fungi isolated from stored grains. Journal of Applied Microbiology. 2016;120:1264-1270. DOI: 10.1111/jam.13092

[35] da Anunciação TA, Costa RGA, de Lima EJSP, Silva VR, Santos LS, Soares MBP, Días RB, Rocha CAG, Costa EV, da Silva FMA, Koolen HHF, Bezerra DP. *In vitro* and *in vivo* inhibition of HCT116 cells by essential oils from bark and leaves of *Virola surinamensis* (Rol. ex Rottb.) Warb. (Myristicaceae). Journal of Ethnopharmacology, 2020;262:113166. DOI: 10.1016/j.jep.2020.113166

[36] Niu H, Li X, Yang A, Jin Z, Wang X, Wang Q, Yu C, Wei Z, Dou C. Cycloartenol exerts anti-proliferative effects on Glioma U87 cells via induction of cell cycle arrest and p38 MAPK-mediated apoptosis. Journal of the Balkan Union of Oncology. 2018;23:1840-1845.

[37] Nair ANS, Nair RVR, Nair APR, Nair AS, Thyagarajan S, Johnson AJ, Baby S. Antidiabetes constituents, cycloartenol and 24-methylene cycloartanol, from *Ficus krishnae*. PLoSONE. 2018;15:e0235221. DOI: 10.1371/journal.pone.0235221

[38] Upadhyay A, Amanullah A, Mishra R, Kumar A, Mishra A. Lanosterol suppresses the aggregation and cytotoxicity of misfolded proteins linked with neurodegenerative diseases. Molecular Neurobiology. 2018;55:1169-1182. DOI: 10.1007/s12035-016-0377-2

[39] Araldi E, Fernández-Fuertes M, Canfrán-Duque A, Tang W, Cline GW, Madrigal-Matute J, Pober JS, Lasunción MA, Wu D, Fernández-Hernando C, Suárez Y. Lanosterol modulates TLR4-mediated innate immune responses in macrophages. Cell Reports. 2017;19: 2743-2755. DOI: 10.1016/j.celrep.2017.05.093

[40] Bin Sayeed MS, Ameen SS. Beta-sitosterol: A promising but orphan nutraceutical to fight against cancer. Nutrition and Cancer. 2015;67:1214-1220. DOI: 10.1080/01635581.2015.1087042

[41] Feng S, Dai Z, Liu AB, Huang J, Narsipur N, Guo G, Kong B, Reuhl K, Lu W, Luo Z, Yang CS. Intake of stigmasterol and β-sitosterol alters lipid metabolism and alleviates NAFLD in mice fed a high-fat western-style diet. Biochimica et Biophysica Acta-Molecular and Cell Biology of Lipids. 2018;1863:1274-1284. DOI: 10.1016/j.bbalip.2018.08.004

[42] Yang Q, Yu D, Zhang Y. β-sitosterol attenuates the intracranial aneurysm growth by suppressing TNF-α-mediated mechanism. Pharmacology. 2019;104:303-311. DOI: 10.1159/000502221

[43] Wang S, Wu S, Liu S. Integration of (+)-catechin and β-sitosterol to achieve excellent radical-scavenging activity in emulsions. Food Chemistry. 2019;272:596-603. DOI: 10.1016/j.foodchem.2018.08.098

[44] Antwi AO, Obiri DD, Osafo N. Stigmasterol modulates allergic airway inflammation in guinea pig model of ovalbumin-induced asthma. Mediators of Inflammation. 2017;2017:2953930. DOI: 10.1155/2017/2953930

[45] Moreno-Anzúrez NE, Marquina S, Alvarez L, Zamilpa A, Castillo-España P, Perea-Arango I, Torres PN, Herrera-Ruiz M, Díaz García ER, García JT, Arellano-García J. A cytotoxic and anti-inflammatory campesterol derivative from genetically transformed hairy roots of *Lopezia racemosa* Cav. (Onagraceae). Molecules. 2017;22:118. DOI: 10.3390/molecules22010118

[46] Islam MT, Ali ES, Uddin SJ, Shaw S, Islam MA, Ahmed MI, Chandra Shill M, KarmakarUK,YarlaNS,KhanIN,BillahMM, Pieczynska MD, Zengin G, Malainer C, Nicoletti F, Gulei D, Berindan-Neagoe I, Apostolov A, Banach M, Yeung AWK, El-Demerdash A, Xiao J, Dey P, Yele S, Jóźwik A, Strzałkowska N, Marchewka J, RengasamyKRR,HorbańczukJ,KamalMA, Mubarak MS, Mishra SK, Shilpi JA, Atanasov AG. Phytol: A review of biomedical activities. Food and Chemical Toxicology. 2018;121:82-94. DOI: 10.1016/j.fct.2018.08.032

[47] Alencar MVOB, Islam MT, Ali ES, Santos JVO, Paz MFCJ, Sousa JMC, Dantas SMMM, Mishra SK, Cavalcante AACM. Association of phytol with toxic and cytotoxic activities in an antitumoral perspective: A meta-analysis and systemic review. Anti-Cancer Agents in Medicinal Chemistry. 2018;18:1828-1837. DOI: 10.2174/1871520618666180821113830

[48] Gutensohn M, Orlova I, Nguyen TTH, Davidovich-Rikanati R, Ferruzzi MG, Sitrit Y, Lewinsohn E, Pichersky E, Dudareva N. Cytosolic monoterpene biosynthesis is supported by plastid-generated geranyl diphosphate substrate in transgenic tomato fruits. The Plant Journal. 2013;75:351-363. DOI: 10.1111/tpj.12212

[49] Feng Y, Morgan RML, Fraser PD, Hellgardt K and Nixon PJ. Crystal structure of geranylgeranyl pyrophosphate synthase (CrtE) involved in Cyanobacterial terpenoid biosynthesis. Frontier in Plant Science. 2020;11:589. DOI: 10.3389/fpls.2020.00589

[50] Dhar MK, Koul A, Kaul S. Farnesyl pyrophosphate synthase: a key enzyme in isoprenoid biosynthetic pathway and potential molecular target for drug development. New Biotechnology. 2013;30:114-123. DOI: 10.1016/j.nbt.2012.07.001

[51] Bondioli P, Della Bella L, Rettke *P.* Alpha linolenic acid rich oils. Composition of *Plukenetia volubilis* (Sacha Inchi) oil from Perú. Rivista Italiana delle Sostanze Grasse. 2006;83:120-123.

[52] Toyomasu T, Sassa T. Diterpenes. In: Liu HW, Mander L, editors. Comprehensive Natural Products II: Chemistry and Biology. London, UK: Elsevier Ltd; 2010. p. 643-672. DOI: 10.1016/B978-008045382-8.00006-X

[53] Manzano D, Andrade P, Caudepón D, Altabella T, Arró M, Ferrer A. Suppressing farnesyl diphosphate synthase alters chloroplast development and triggers sterol-dependent induction of jasmonate- and Fe-related responses. Plant Physiology. 2016;172:93-117. DOI: 10.1104/pp.16.00431

[54] Chirinos R, Pedreschi R, Domínguez G, Campos D. Comparison of the physico-chemical and phytochemical characteristics of the oil of two *Plukenetia* species. Food Chemistry. 2015;173:1203-1206. DOI: 10.1016/j.foodchem.2014.10.120

[55] Scolaro B, de Andrade LFS, Castro IA. Cardiovascular disease prevention: The earlier the better? A review of plant sterol metabolism and implications of childhood supplementation. International Journal of Molecular Science. 2019;21:128. DOI: 10.3390/ijms21010128

[56] Wang C-Y, Chen Y-W, Hou C-Y. Antioxidant and antibacterial activity of seven predominant terpenoids. International Journal of Food Properties. 2019;22:230-238. DOI: 10.1080/10942912.2019.1582541

[57] Lyu X, Lee J, Chen WN. Potential natural food preservatives and their sustainable production in yeast: Terpenoids and polyphenols. Journal of Agricultural and Food Chemistry. 2019;67:4397-4417. DOI: 10.1021/acs.jafc.8b07141

[58] Ibáñez M.D, Sánchez-Ballester NM, Blázquez MA. Encapsulated limonene: A pleasant lemon-like aroma with promising application in the agri-food industry. A review. Molecules. 2020;25:2598. DOI: 10.3390/molecules25112598

[59] Gutiérrez L-F, Sanchez-Reinoso Z, Quiñones-Segura Y. Effects of dehulling Sacha inchi (*Plukenetia volubilis* L.) seeds on the physicochemical and sensory properties of oils extracted by means of cold pressing. Journal of the American Oil Chemists' Society. 2019;96:1187-1195. DOI: 10.1002/aocs.12270

[60] Aguilar-Hernández MG, Sánchez-Bravo P, Hernández F, Carbonell-BarrachinaAA, Pastor-PérezJJ, Legua P. Determination of the volatile profile of Lemon peel oils as affected by rootstock. Foods. 2020;9,241. DOI: 10.3390/foods9020241

[61] Chirinos R, Zuloeta G, Pedreschi R, Mignolet E, Larondelle Y, Campos D. Sacha inchi (*Plukenetia volubilis*): a seed source of polyunsaturated fatty acids, tocopherols, phytosterols, phenolic compounds and antioxidant capacity. Food Chemistry. 2013;141:1732-1739. DOI: 10.1016/j.foodchem.2013.04.078

[62] Chirinos R, Zorrilla D, Aguilar-Galvez A, Pedreschi R, Campos D. Impact of roasting on fatty acids, tocopherols, phytosterols, and phenolic compounds present in *Plukenetia*

huayllabambana seed. Journal of Chemistry, 2016;2016:6570935.. DOI: 10.1155/2016/6570935

[63] Chasquibol N, Gallardo G, Gómez-Coca RB, Trujillo D, Moreda W, Pérez-Camino MC. 2019. Glyceridic and unsaponifiable components of microencapsulated Sacha inchi (*Plukenetia huayllabambana* L. and *Plukenetia volubilis* L.) edible oils. Foods. 2019;8:671. DOI: 10.3390/foods8120671

Chapter 5

Potential Antioxidant Activity of Terpenes

Bechir Baccouri and Imen Rajhi

Abstract

Terpenes play a key part in the metabolic processes of a wide variety of animals, plants and microorganisms in which they are produced. In nature, terpenoids serve a variety of purposes including defense, signaling and as key agents in metabolic processes. Terpenes have been used in perfumery, cosmetics and medicine for thousands of years and are still extracted from natural sources for these uses. Terpenes antioxidant activities may sometimes explain their capacity to adjust inflammation, immunological effects and neural signal transmission. They offer pertinent protection under oxidative stress situations including renal, liver, cancer, cardiovascular diseases, neurodegenerative and diabetes as well as in ageing mechanisms.

Keywords: terpenes, terpenoids, antioxydant, ROS, health

1. Introduction

Terpenes occur widely in nature. They are a large and varied class of hydrocarbons that are produced by varied plants and some animals. Thus, terpenes defend plants against pathogens like bacteria, fungus and can attract pollinating insects or repel herbivores [1]. Numerous plants produce volatile terpenes in order to attract specific insects for pollination or otherwise to expel certain animals using these plants as food [1].

They are also abundantly found in fruits and flowers. In plants, they function as infochemicals, attractants or repellents, as they are responsible for the typical perfume of many plants [2]. Last, but not least, terpenes play an important role as signal compounds and growth regulators (phytohormones) of plants, as shown by some studies [1]. Thousands of terpenes have been found across the *plantae*, but only a small percentage of all terpenes have been known. Terpenes are biosynthetically derived from isoprene units with the molecular formula C5H8 [1]. The basic formula of all terpenes is (C5H8)n, where n is the number of linked isoprene units [1].

Terpenes presented over 25,000 well defined compounds isolated from all biological kingdoms [3]. The numerous terpene synthases in plants are primarily responsible for terpene diversity; some of them produce different products from a single substrate [4].

The nomenclature of terpenes is based on the number of isoprene structures that they contain. Accordingly, these compounds are classified as sesquiterpenes, monoterpenes, diterpenes, triterpenes, tetraterpenes, and polyterpenes [5]. Monoterpenes, sesquiterpenes, and diterpenes are considered secondary metabolites as they are not essential for viability [5].

Including neurodegenerative diseases (Alzheimer's and Parkinson's diseases), cancer, cardiovascular diseases, liver diseases, diabetes, and other diseases; oxidative stress is involved in the pathological development of many diseases. Antioxidant therapy, via direct and indirect mechanisms, has become one of the main and promising strategies to face oxidative stress-induced cellular damage [1, 6]. Studies have shown that both natural terpenes and their synthetic derivatives enjoy diverse pharmacological properties, including antioxidant, antifungal, anti-inflammatory, antiviral, anticancer, antibacterial, antinociceptive, antiarrhythmic, antispasmodic, antiaggregating, local anesthetic and antihistaminic activities [6, 7]. These interesting characteristics were used in pharmaceuticals and cosmetic industries. In this context, the search for antioxidant compounds among natural terpene products has significantly increased in the last recent years. As shown throughout this chapter, terpenes can function as antioxidant compounds through modulating the endogenous antioxidant system and direct ROS scavenging pathway.

2. Oxidative stress

Reactive oxygen species (ROS) comprise a series of chemical molecules derived from molecular oxygen whose reactivity is much greater than that of this element in its basal state [8]. Intracellular ROS can oxydize lipids, proteins and DNA thus damaging many cellular components and even causing genetic damage and cell death, mainly by apoptosis [1, 8, 9]. These species include oxygen ions as atomic oxygen (O), ozone (O_3) and singlet oxygen ($1O_2$) free radicals such as superoxide radical (O_2• -), hydroxyl radical (OH •), the alkoxy radical (RO •) and peroxyl radical (ROO •), and peroxides such as hydrogen peroxide (H_2O_2) and peroxynitrite (ONOO-) [1, 8].

Molecules such as, ascorbic acid (vitamin C), α tocoferol (vitamin E), bilirubin, selenium and glutathione, between many others, proceed as ROS scavengers, preventing oxidative cellular damage [10, 11]. Among these, glutathione, the antioxidant compound, plays an important role in protecting vital functions [8, 11].

In addition to nonenzymatic compounds, during the antioxidant enzymes action including superoxyde dismutase (SOD), catalase (CAT), glutathione peroxydase (GPx) and heme oxygenase-1 (HO-1), ROS can be detoxyfied or converted into nontoxic forms [8]. Catalase, located principally in peroxisomes, efficiently converts hydrogen peroxide to water and oxygen [12]. The efficiency of this enzyme is such that one CAT molecule is able to turn 6 million of hydrogen peroxide molecules into water and oxygen per minute. In addition, this enzyme cannot be saturated at any hydrogen peroxide concentration [13]. Superoxyde dismutase produces molecular oxygen and hydrogen peroxide through dismutation of superoxide anion and this reaction is over 4 times faster than the non-enzymatic reaction [12]. GPx reduces hydroperoxides using glutathione (GSH) as substrate. The resulting GSSG is reduced back to GSH by the action of GR. The gene expression of all of these antioxidant proteins is regulated by nuclear factor erythroid-2 (Nrf2), through its binding to a specific DNA sequence called antioxidant response element (ARE) [14].

Nevertheless, under various pathological conditions, this endogenous cellular antioxyant defense system cannot remove excessive amounts of ROS, resulting in an oxidant-antioxidant imbalance called oxidative stress [1].

3. Terpenes antioxidants potential

The main triterpenes present in EVOO are two hydroxyl pentacyclic triterpene acids (oleanolic and maslinic acid) and two dialcohols (uvaol and erythrodiol)

(**Figure 1**), whose concentrations oscillate between 8.90 and 112.36 mg kg^{-1} [15]. Terpenes compounds are mostly found in the epicarp, then, pomace olive oil generally contains 10-fold elevated concentrations than EVOO [15].

In the incessant search for new bioactive natural products against oxidation and inflammation, terpenes are emerging as a rich source of these compounds. Some monoterpenes possess both anti-inflammatory and antioxidant properties [16, 17]. (+)-limonene, and 1,8-cineole demonstrated strong antioxidant, anti-inflammatory and anticancer properties in assays using DPPH method, pleural cell migration, and U251, UACC-62, MCF-7, NCI-ADR/RES, OVCAR-3 human cancer cell lines, respectively [16, 17].

Menthol is present in the aroma oil of numerous species of mint plants, such as cornmint oil from *M. arvensis* (wild mint) and peppermint oil derived from *Mentha piperita* (peppermint). Menthol and 1,8-Cineole ([11] had antioxidant characteristics in the ABTS-radical caption scavenging assay [18]. Cornmint and peppermint oils contain 70 and 50%, respectively, of menthol. Menthol can be extracted from other essential oils, such as citronella, eucalyptus and Indian turpentine oils.

Previous works have demonstrated that the antioxidant and prooxidant behaviour of a particular terpene depend most of all on it amount: at high concentrations, terpenes can act as a prooxidant compounds whereas at low concentrations, they can act as antioxidant compounds [19].

Ruberto and Baratta [20] studied the antioxidant activity of monoterpene and sesquiterpene compounds found abundantly in essential oils Two lipid model systems were used: one for evaluating the formation of thiobarbituric acid reactive species (TBARS), utilizing egg yolk as lipid oxydable substrate and the other one for evaluating the peroxides that are formed during linoleic acid oxydation in a micellar system. Among monoterpene hydrocarbons, such as terpinolene, α-terpinene, γ-terpinene and sabinene were the most active [19]. Among oxygenated monoterpenes the order of antioxidant activity effectiveness was monoterpene phenols (thymol and carvacrol > allylic alcoholes (nerol), perillyl alcohol, geraniol and cisverbenol > monoterpenes aldehydes and ketones. Concerning the sesquiterpene group [1], the radical

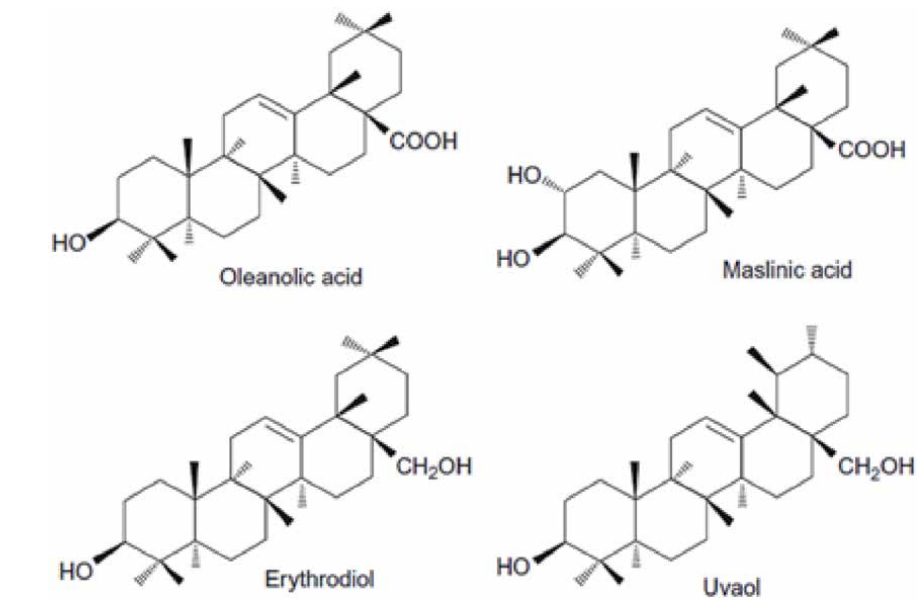


Figure 1.
Chemical structure of EVOO triterpenes.

scavenging properties of the hydrocarbons-type were quite low and lower than that of the monoterpene hydrocarbons cluster, but between the oxygenated type, mainly allylic alcohols (i.e. farnesol, guaiol, (+)-8- (15)-cedren-9-ol showed good scavenging properties, similar to those of oxygenated monoterpenes [1, 19].

Several terpenes display also a protective effect against the oxidative stress induced by heavy metals. Pretreatments with the triterpene arjunolic acid recovered almost completely from reduced antioxidant protection (SOD, CAT, GR, GPx GST, GSH) and increased oxidative damage (lipid peroxydation and protein carbonyl content), mainly via radical scavenging, in murine brain treated with arsenic. El-Missiry and Shalaby [21] indicated that treatments with the ß-carotene (tetra-terpene) protected against cadmium oxidative stress in brain with an associated increase in SOD, GST and non-enzymatic (GSH) antioxidant status [1], a decline in LDH activity and lipid peroxydation and an rise of ATPase activity [1, 22].

The oxidative pathway is also one of the described mechanisms to clarify glutamate toxicity. This excitatory neurotransmitter depletes intracellular GSH, produces ROS and augments lipid peroxydation levels. Koo et al. [23] identified the diterpene 15- methoxypinusolidic acid, obtained from the leaves of *Biota orientalis* L., as protective neuroagent with antioxidant activity in primary cultured cortical rat cells. Moreover, the monoterpenes from *Scrophularia buergeriana* Miq. were capable to ameliorate the antioxidant defense system in primary cultures of rat cerebral cortical cells in glutamate-mediated oxidative stress conditions [1].

Kim et al. [22] focused on the search for antioxidant compounds that delay or prevent oxidant/antioxidant imbalances and its harmful consequences, since oxidative stress is associated with Parkinson's disease pathology. The monoterpene catalpol, isolated from the roots of *Rehmannia glutinosa*, has demonstrated to protect cultured mesencephalic neurons against MPP+-induced toxicity by preventing the inhibition of the mitochondrial complex I, and thus avoiding mitochondrial dysfunction, and by diminishing the level of MDA content and increasing the activity of the antioxidant enzymes (SOD and GPx) [22]. The exogenous neurotoxins 1-methyl-4- phenylpyridinium (MPP+) and 6-hydroxydopamine (6-OHDA) are frequently used in experimental Parkinson models since these chemical compounds induce selectively oxidative stress in nigrostriatal dopaminergic neurons [24].

Its beneficial effects may be partly due to ROS scavenging and enhancement of endogenous antioxidants. Concerning fungi-derived terpenes, the labdane diterpenes, obtained from the fruiting body of the parasitic fungus *Antrodia camphorate* [24]. On the other hand, the carotene astaxanthin resulted to be a potent mitochondria-targeted antioxidant in dopaminergic SH-SY5Y cells treated with 6-OHDA [25].

Naval and Gómez-Serranillos [26] reviewed the neuroprotective activity of ginseng constituents, based on their antioxidant activities. Herein, we highlight some examples. *In vitro* studies concerning the neuroprotective activity of the isolated ginsenosides Rb1, Rb2, Rc, Rd., Re and Rg1 under hydrogen peroxide-induced oxidative stress in astrocytes revealed that the triterpene compound Re was the most effective among all tested ones since this compound could decrease cell death, improve SOD, GR and GPx activities and inhibit ROS production [27]. In addition, oxidative stress markers such as high ROS and MDA levels, low amounts of GHS and decreased antioxidant enzyme (SOD, CATand GPx) activity have been detected during oxygen–glucose privation and reoxygenation processes on hippocampal neurons. The ginsenoside Rd. lets return all these oxidant parameters to basal levels [27, 28].

Pretreatments with arjunolic acid isolated from the bark of *Terminalia arjuna* (Roxb.). Wight and Arn. prevented cardiac tissues from arsenic-induced oxidative

stress by restoring antioxidant status and inhibiting lipid peroxydation and protein carbonyl accumulation. There is evidence supporting a link between oxidative stress and cardiovascular tissue injury. Some studies have been conducted on the cardioprotective impacts of terpenes in response to cardiovascular pathological situations oxidative stress-related including hypertension and atherosclerosis, among others [29].

Moreover, antihypertensive beneficial effects through antioxidant actions have been also observed for astaxanthin. In another study, the endothelial function of resistance of arteries was improved in those experimental animals that had been during eight weeks on an astaxanthinenriched diet. Astaxanthin decreased NADPH enhanced O2·- production by direct ROS scavenging and improved NO bioavailability [30].

As it has been previously demonstrated, excessive cigarette smoking and alcohol drinking are both risk factors for triggering atherosclerosis. In a randomized double-bind placebo-controlled study undertaken in over 100 habitual cigarette smokers and alcohol consumers, men 22–57-aged, the possible protective effect of lycopene against heart disease was evaluated in these oxidative stress conditions (smoke and alcohol) [30].

Additionally, Bansal et al. [31] confirmed that the carotenoid lycopene acts as a myocardial protective agent for the prevention of oxidative stress caused after ischemia reperfusion in the heart of rats through lipid peroxydation reduction and antioxiydant capacity enhancement [31]. Through antioxidant mechanisms, particularly scavenging of oxygen free radicals, prevention of lipid peroxydation and upregulation of the Bcl-2/Bax ratio, the diterpene tanshinone IIA also exhibited a protective role on cardiomyocytes against ischemic injury [32].

ROS formation and subsequent oxidative stress events are one of the mechanisms of liver injury with hepatotoxic chemicals injury [32]. Several terpenes have shown hepatoprotective activity against this toxic chemical compound [27]. The kaurane diterpenes kahweol and cafestol, found in coffee beans, inhibited the production of superoxide anion radicals, reduced the level of the lipid peroxydation product malondialdehyde (MDA) and prevented the depletion of intracellular glutathione (GSH) injury [27, 32]. The labdane diterpenes neoandrographolide and andrographiside isolated from the plant species *Andrographis paniculata* (Burm.f.).

Moroever, *in vivo* studies demonstrated an increase in the concentration of reduced glutathione (GSH) in the liver of those rats after chronic alcohol consumption but fed with a diet containing the carotenoid ß-carotene [33].

Several environmental pollutants are able to induce oxidative stress, liver being the organ mostly affected [30]. Also, the ß-carotene (tetraterpene), behaving as an antioxidant, protected from liver damage associated with oxidative stress caused by bile acid or as a side effect of chemotherapy with methotrexate [34].

Among the monoterpene class, catalpol may hold promising protective actions against encephalopathy under hyperglycemic conditions [22].

Moreover, the protective effect of terpenes as antioxidants against excessive ROS production, it is worth to indicate the role of these compounds as chemoprotective agents against tumor cells. Many papers have demonstrated that terpenes could have a very efficient activity in different cancer types [1]. Anticancer therapy of terpenes targeting the apoptotic pathway rather than the antioxidant pathway [1, 35].

The protective effect of carotenoids was attributed to its capacity to inhibit lipid peroxydation, restore GSH levels and improve the activities of the enzymes superoxide dismutase, catalase and glutathione S-transferase (**Figure 2**) [36].

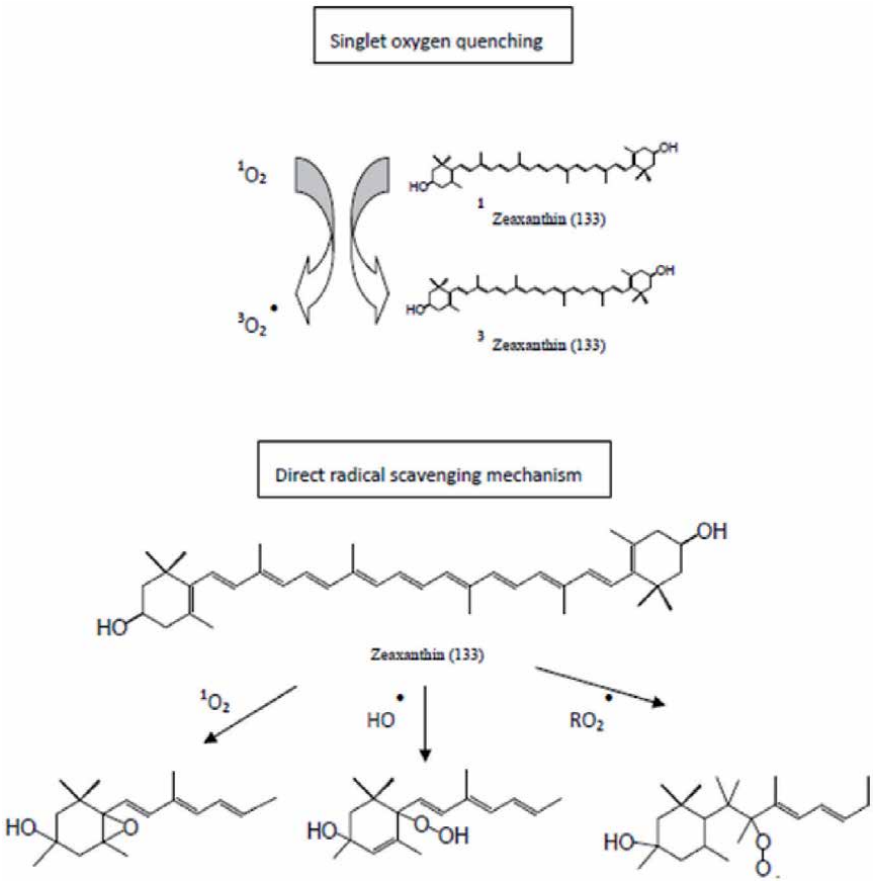


Figure 2.
Interaction of zeaxanthin With ROS.

4. Conclusions

Concerning the number of *in vivo* and *in vitro* studies that have evaluated the terpenes antioxidant activities it is relatively little when compared to the enormous number of identified Terpenes in nature. They have a ample biological activities including anti-inflammatory, anticancer, antimicrobial, antioxidant etc.. Several other as yet undiscovered compounds can exist with immense antioxidant potentials.

Conflict of interest

The authors declare that they have no conflict of interests.

"Reviewed by Pr. Mokhtar Zarrouk, Centre of Biotechnology of Borj Cédria, Tunisia".

Author details

Bechir Baccouri[1*] and Imen Rajhi[2]

1 Laboratory of Olive Biotechnology, Center of Biotechnology of Borj-Cédria, Tunisia

2 Laboratory of Legumes Center of Biotechnology of Borj-Cédria, Tunisia

*Address all correspondence to: bechirbaccouri@yahoo.fr

References

[1] Wang, S.Y.; Wu, J.H.; Shyur, L.F.; Kuo, Y.H.; Chang, S. Antioxidant activity of abietane-type diterpenes from heartwood of *Taiwania cryptomerioides* Hayata. Holzforschung, 2002, *56*, 487-492.

[2] Schulz, G.E.; Schirmer, R.H.; Sachsenheimer, W.; Pai, E.F. The structure of the flavoenzyme glutathione reductase. Nature. 1978, *273*, 120-124.

[3] Buckingham, J. *Dictionary of natural products on CD-ROM*, version 6.1; Chapman & Hall: London, 1998. antioxidant polyoxygenated triterpenes from *Salsola baryosma*, by 1D and 2D NMR spectroscopy.

[4] Ruzlcka, L. The isoprene rule and the biogenesis of terpenic compounds. Experientia, 1953, *9*, 357-367.

[5] Flesh, G.; Rohmer, M. Prokaryotic hopanoids: the biosynthesis of the bacteriohopane skeleton. Formation of isoprenic units from two distinct acetate pools and a novel type of carbon/ carbon linkage between a triterpene and Dribose. Eur. J. Biochem., 1988, *175*, 405-411.

[6] Avery, S.V. Molecular targets of oxidative stress. Biochem. J., 2011, *434*, 201-210.

[7] Valko, M.; Morris, H.; Cronin, M.T. Metals, toxicity and oxidative stress. Curr. Med. Chem., 2005, *12*, 1161-1208.

[8] D'Autreaux, B.; Toledano, M.B. ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. Nat. Rev. Mol. Cell Biol., 2007, *8*, 813-824.

[9] Stadtman, E.R.; Moskovitz, J.; Levine, R.L. Forum Mini Review, Oxidation of methionine residues of proteins: Biological consequences. Antioxidatns & Redox Signaling, 2003, *5*, 577-582.

[10] Li, Y.; Cao, Z.; Zhu, H. Upregulation of endogenous antioxidants and phase 2 enzymes by the red wine polyphenol, resveratrol in cultured and aortic smooth muscle cells leads to cytoprotection against oxidative and electrophilic stress. Pharmacol. Res., 2006, *53*, 6-15.

[11] Townsend, D.M.; Tew, K.D.; Tapiero, H. The importance of glutathione in human disease. Biomed. Pharmacother., 2003, *57*, 145-155.

[12] Valko, M.; Rhodes, C. J.; Moncol, J., Izakovic, M.; Mazur, M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem. Biol. Interact., 2006, *160*, 1-40.

[13] Mates, M. Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. Toxicol., 2000, *153*, 83-104.

[14] Lee, J.M.; Johnson, J.A. An important role of Nrf2-ARE pathway in the cellular defense mechanism. J. Biochem. Mol. Biol., 2004, *37*, 139-143.

[15] Baccouri, B., Manaia, H., Casas, J.S., Osorio, E. & Zarrouk, M., (2018). Tunisian wild olive (Olea europaea L. subsp. oleaster) oils: Sterolic and triterpenic dialcohol compounds. Ind. Crops Prod. 120, 11-15.

[16] Luis, J.C.; Johnson, C.B. Seasonal variations of rosmarinic and carnosic acids in rosemary extracts. Analysis of their *in vitro* antiradical activity. *Spanish J. Agr. Res*., 2005a, *3*, 106.

[17] Sinha, M.; Manna, P.; Sil, P.C. Protective effect of arjunolic acid against arsenic-induced oxidative stress in mouse brain. J Biochem. Mol. Toxicol., 2008, *22*, 15-26.

[18] Qiu, L.H.; Xie, X.J.; Zhang, B.Q. Astragaloside IV improves homocysteineinduced acute phase endothelial dysfunction via antioxidation. Biol. Pharm. Bull., 2010, *33*, 641-646.

[19] Maleknia, S.D.; Adams, M.A. Reactions of oxygen-containing terpenes with peptides and proteins. *Proc. 4th Intl. Peptide Symp*., 2007, 334-335.

[20] Ruberto, G.; Baratta, M.T. Antioxidant activity of selected essential oil components in two lipid model systems. Food Chem., 2000, *69*, 167-174.

[21] El-Missiry, M.A.; Shalaby, F. Role of beta-carotene in ameliorating the cadmium-induced oxidative stress in rat brain and testis. J. Biochem. Mol. Toxicol., 2000, *14*, 238-243.

[22] Kim, S.R.; Koo, K.A.; Sung, S.H.; Ma, C.J.; Yoon, J.S.; Kim, Y.C. Iridoids from *Scrophularia buergeriana* attenuate glutamate-induced neurotoxicity in rat cortical cultures. *J. Neurosci. Res*., 2003, *74*, 948-955.

[23] Koo, K.A.; Kim, S.H.; Lee, M.K.; Kim, Y.C. 15-Methoxypinusolidic acid from *Biota orientalis* attenuates glutamate-induced neurotoxicity in primary cultured rat cortical cells. Toxicol. In Vitro. 2006, *20*, 936-941.

[24] Tian, Y.Y.; Jiang, B.; An, L.J.; Bao, Y.M. Neuroprotective effect of catalpol against MPP(+)-induced oxidative stress in mesencephalic neurons. Eur. J. Pharmacol., 2007, *568*, 142-148.

[25] Liu, X.; Osawa, T. Astaxanthin protects neuronal cells against oxidative damage and is a potent candidate for brain food. Forum Nutr., 2009, *61*, 129-135.

[26] Naval-López, M.V.; Gómez-Serranillos, M.P. In: Ginseng research in the era of systems biology; Yan J, Ed; Int. J. Curr. Biomed. Pharmaceut. Res., 2012, pp. 1-10.

[27] López, M.V.; Cuadrado, M.P.; Ruiz-Poveda, O.M.; Del Fresno, A.M.; Accame, M.E. Neuroprotective effect of individual ginsenosides on astrocytes primary culture. Biochim. Biophys. Acta. 2007, *1770*, 1308-1316.

[28] Ye, R.; Li, N.; Han, J.; Kong, X.; Cao, R.; Rao, Z.; Zhao, G. Neuroprotective effects of ginsenoside Rd against oxygen-glucose deprivation in cultured hippocampal neurons. Neurosci. Res., 2009, *64*, 306-310.

[29] Lakshmi, S.V.; Padmaja, G.; Kuppusamy, P.; Kutala, V.K. Oxidative stress in cardiovascular disease. Indian J. Biochem. Biophys., 2009, *46*, 421-440.

[30] Sudhahar, V.; Kumar, S.A.; Varalakshmi, P. Role of lupeol and lupeol linoleate on lipemic-oxidative stress in experimental hypercholesterolemia. Life Sci., 2006, *78*, 1329-1335.

[31] Bansal, P.; Gupta, S.K.; Ojha, S.K.; Nandave, M.; Mittal, R.; Kumari, S.; Arya, D.S. Cardioprotective effect of lycopene in the experimental model of myocardial ischemia-reperfusion injury. Mol. Cell. Biochem., 2006, *289*, 1-9.

[32] Fu, J.; Huang, H.; Liu, J.; Pi, R.; Chen, J.; Liu, P. Tanshinone IIA protects cardiac myocytes against oxidative stress-triggered damage and apoptosis. Eur. J. Pharmacol., 2007, *568*, 213-221.

[33] Lin, W.T.; Huang, C.C.; Lin, T.J.; Chen, J.R.; Shieh, M.J.; Peng, H.C.; Yang, S.C., Huang, C.Y. Effects of beta-carotene on antioxidant status in rats with chronic alcohol consumption. Cell Biochem. Funct., 2009, 27, 344-350.

[34] Vardi, N.; Parlakpinar, H.; Cetin, A.; Erdogan, A.; Cetin Ozturk, I. Protective effect of beta-carotene on

methotrexate-induced oxidative liver damage. *Toxicol. Pathol*., 2010, *38*, 592-597.Vqrdi et al., 2010.

[35] Aune, D., Chan, D.S., Vieira, A.R., Navarro Rosenblatt, D.A., Vieira, R., Greenwood, D.C., Norat, T. Dietary compared with blood concentrations of carotenoids and breast cancer risk: a systematic review and meta-analysis of prospective studies. Am. J. Clin. Nutr. 2012, *96*, 356-373.

[36] Gupta, S.K.; Trivedi, D.; Srivastava, S.; Joshi, S.; Halder, N.; Verma, S.D. Lycopene attenuates oxidative stress induced experimental cataract development: an *in vitro* and *in vivo* study. Nutrition. 2003, *19*, 794-799.

Chapter 6

Algal Terpenoids: A Potential Source of Antioxidants for Cancer Therapy

Umme Tamanna Ferdous and Zetty Norhana Balia Yusof

Abstract

In cancer treatment, increase in drug resistance and decrease in new chemotherapeutic drugs have become a pressing problem. Hence, searching for novel anticancer agents with less toxicity and high sensitivity is expanding gradually. Many preclinical and clinical studies indicate that natural antioxidants can help combating carcinogenicity and reduce the adverse effects on cancer therapy, when used alone or as adjuvant in chemotherapy. Consequently, marine algae pave the way for exploring more potential antioxidant compounds which have pharmaceutical importance. Algal terpenoids comprise a large group of bioactive compounds that have excellent antioxidative property and can be used as source of antioxidant in cancer therapy. This chapter summarizes the potential role of terpenoids from algal sources in inhibiting cancer cells, blocking cell cycle, hindering angiogenesis and metastasis as well as in inducing apoptosis.

Keywords: algal terpenoids, antioxidant, cancer, chemotherapy, marine algae

1. Introduction

Though cancer is the prime reason for the premature death and responsible for more than nine million death globally in 2018, cancer treatments are still facing challenges in terms of their potency and safety [1]. Over fifty percent of the existing cancer drugs are from natural origin, therefore, exploration of cancer therapeutics from natural reservoir has been escalated currently [2]. In accordance with this natural anti-cancer drug discovery, natural antioxidants can be considered as an alternative source of cancer therapeutics. Many antioxidants, for instance, vitamins, carotenoids, genistein, curcumin, resveratrol, gingerol etc. exhibited promising outcomes in preclinical and clinical studies [3]. Currently, researchers are looking for more novel phytochemicals that can be further used as cancer drug discovery.

Terpenoids are the broadest class of diverse phytochemicals which are widely available in marine algae. These secondary metabolites have excellent antioxidative property and exerted *in vitro* as well as *in vivo* anticancer activity [4]. Algal terpenoids mainly comprised of mono-, di-, tri-, tetra-, mero- and sesquiterpenoids. Tetraterpenoid which is mostly carotenoid, is widely studied algal terpenoid. Carotenoids isolated from macro- and microalgae have been used widely in health-related industries and they have been reported to display strong anticancer activity

against different cancer cells [5]. Besides these tetraterpenoids, other terpenes and terpenoids have also significant anticancer property. This chapter focus on the usage of antioxidants in cancer therapy, presenting the anticancer property of algal terpenoids with their mechanism of action in cancer cells.

2. Role of antioxidant in cancer therapy

Antioxidants are molecules which can detoxify the reactive species (reactive oxygen species (ROS), reactive nitrogen species (RNS), reactive sulfur species (RSS), reactive carbonyl species (RCS) and reactive selenium species (RSeS)), that are generated through body's normal metabolism or can be obtained from environment [6]. These reactive species give rise to oxidative stress which is of two types, oxidative eustress and oxidative distress. Oxidative eustress is considered as good stress, which under basal intensity, maintains redox homeostasis, responsible for controlled cell growth and reversible oxidative modification which ensure normal physiology. On the other hand, oxidative distress is known as bad stress, that in higher intensity, damage biomolecules and consequently disrupt redox signaling and give rise to different diseases, e.g. cancer [7].

Antioxidants protect cellular damage from free radicles through their organized defense mechanism (**Figure 1**), where they either inhibit new free radicle formation or scavenge the formed free radicles. They can also repair the damaged DNA and biomolecules [8]. In cancer cells, ROS level is excessively high which helps in protumorigenic cell signaling while prolonging the cell death. Some chemotherapeutic agents also can induce production of high amount of ROS, which is often considered as one of the main reasons for chemotherapeutic treatment side effects. However, antioxidants, when used in therapeutic dose in adjuvant chemotherapy, can hinder this high production of ROS and thus, potentiate the efficacy of cancer treatments, reduce the adverse effects of the therapy and improves the overall health status of the cancer patients. Antioxidants can inhibit cancer proliferation, angiogenesis and metastasis [9]. Dietary antioxidants supplements are frequently in cancer treatment. About 20–80% of the cancer patients use antioxidant supplements after cancer diagnosis [10]. The efficacy of using antioxidants in adjuvant chemotherapy has been assessed in many clinical trials. The clinical studies of antioxidant administration, especially vitamin, glutathione, melatonin, Coenzyme Q10, during chemotherapy have been revealed the reduction of chemotherapy induced toxicity and improvement of patient health [11].

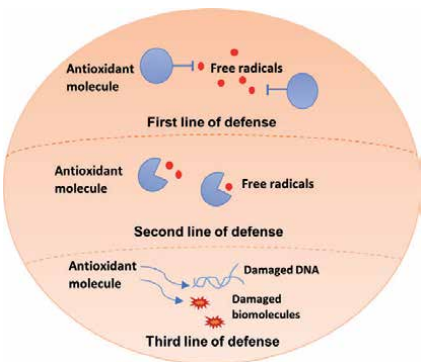


Figure 1.
Three lines of defense system of antioxidant in cell.

3. Algal terpenoids as prospective candidate in cancer therapy

Seaweed are more studied, in terms of their terpenoid profile, compared to marine microalgae. Though the anticancer activity of tetraterpenoids from marine microalgae has been reported broadly, brown macroalgae are good source of carotenoids. Anticancer activity of carotenoids (zeaxanthin, lutein, β-carotene, violaxanthin) was reported in Malaysian green and brown macroalgae [12].

3.1 Monoterpenoids

Monoterpenoids, found in different plant parts, like in bark, root, seeds or leaves, have antioxidant and anticancer activity. For instance, carvacrol, thymol, linalool as well as eugenol are good antioxidant and at the same time, exert antitumor activity against liver, prostate and breast cancer cells [13, 14]. Limonene and perillyl alcohol were subjected to phase I clinical trials in cancer patients [15].

Plocamium cartilagineum, a red alga, produces halogenated monoterpenes like furoplocamioid C, prefuroplocamioid, pirene and cyclohexane which have selective cytotoxicity against human melanoma, human and murine colon cancer cells as well as HeLa cells [16]. Similarly, *Plocamium sp.* from Namibia possesses halogenated monoterpene that have better antioxidant property than that of standard antioxidant [17]. *Sargassum ringgoldianum,* Korean brown seaweed, showed antioxidative activity through monoterpene lactone, that also gave protection against H_2O_2-induced damage in Vero cells [18].

3.2 Diterpenoids

A new diterpenoid has been isolated from green alga *Gracilaria Salicornia*, which displays antioxidant activity equivalent to α-tocopherol [19]. Brown alga *Bifurcaria bifurcate* has been reported to produce diterpenes, namely eleganolone and eleganonal which have better antioxidant activity in comparison to standard antioxidant, as well as exert neuroprotective effect on neuroblastoma [20]. Likewise, diterpenes from brown seaweed *Dictyota dichotoma* has good antioxidant capacity and shows cytotoxicity to liver and breast cancer cell lines [21]. Rodrigues et al., isolated a new diterpene sphaerodactylomelol from *Sphaerococcus coronopifolius* which blocked proliferation of human liver cancer cells at an IC_{50} of 280 μM, while killed the cancer cells at IC_{50} of 720 μM [22].

However, diterpenoids can induce apoptosis in cancer cells through downregulating Bcl2 and regulatory pathways like, JAK2/STAT3, PI3K/Akt and NF-κB. They can arrest cell cycle at G1 and G2-M checkpoint. Besides, diterpenoids can also inhibit metastasis and angiogenesis by hindering PI3K/Akt/mTOR and VEGFR-2 signaling pathways [23].

3.3 Triterpenoids

Triterpenoid (benzene dicarboxylic acid, diisooctyl ester) from the dichloromethane extract of *Sargassum wightii* displayed excellent radical scavenging and reducing activity [24]. Similarly, triterpenoids from the methanolic extracts of *Sargassum sp.* and *Eucheuma cottonii* could be responsible for their strong antioxidant activity [25]. Methanolic extract of *Gracilaria salicornia*, isolated from Persian Gulf, has inhibited human colon cancer cells at an IC_{50} of 58.6 μg/mL and also has good antioxidant property. Phytochemical analysis has been revealed that

triterpenes are present in ample amount in that extract which could be attributed for these activities [26]. On the other hand, Indonesian seaweed *Eucheuma cottonii* contains triterpenoid which exhibited cytotoxicity against lung cancer cells at an IC_{50} of 251.73 μg/mL [27]. *Padina boergesenii* has been reported to produce triterpenes that have antiangiogenic activity against renal carcinoma [28]. Ethanolic extract of edible seaweed *Kjellmaniella crassifolia* has been reported to contain three terpenoids, namely dihydrocimicifugenol, 3-epicyclomusalenol and cyclosadol with chemo-preventive property [29]. Anti-cancerous triterpenoids can also be found in *Laurencia mariannensis*, *L. viridis* and *L. obtuse* [30].

3.4 Tetraterpenoids

Algal tetraterpenoids mainly consist of carotenoids, namely, β-carotene, lutein, fucoxanthin, astaxanthin, canthaxanthin, zeaxanthin, cryptoxanthin, violaxanthin, neoxanthin and siphonaxanthin (**Figure 2**). Theses carotenoids have both antioxidative and anticancer activity with other pharmaceutical importance.

3.4.1 Lutein

Lutein from *Botryococcus braunii* has been reported to exhibit both *in vitro* and *in vivo* antioxidant activity [31].

3.4.2 β-carotene

β-Carotene from *Dunaliella salina* is responsible for apoptotic cell death in human prostate carcinoma [32].

3.4.3 Fucoxanthin

Phaeodactylum tricornutum, *Odontella aurita*, *I. galbana, C. calcitrans, D. salina, C. gracilis, Navicula sp., Thalassiosira sp., Pavlova lutheri, Cylindrotheca closterium* can produce ample amount of fucoxanthin with antioxidative property [33–36]. *P. tricornutum* and *C. calcitrans* possess fucoxanthin which exhibits strong anticancer activity [33, 37]. Fucoxanthin, obtained from brown macroalgae *Padina tetrastromatica,* exhibited cytoprotective effect against oxidative damage [38].

3.4.4 Zeaxanthin

Zeaxanthin separated from *Nannochloropsis oculata, Scenedesmus obliquus, Porphyridium aerugineum* has showed antioxidative property [39, 40]. Zeaxanthin from *Porphyridium purpureum* induced apoptosis human melanoma. Moreover, ZX from this *P. purpureum* potentiates the efficacy of chemotherapeutic drug, vemurafenib towards human melanoma [41].

3.4.5 Violaxanthin

Violaxanthin with antioxidative and anti-inflammatory activities has been isolated from *Chlorella vulgaris*, *N. oceanica*, *Dunaniella salina*, *Tetraselmis spp*., *Isochrysis galbana*, *Pavlova lutheri*, *P. salina* and *Chaetoceros spp*. *Eustigmatos cf. polyphem* [42–46]. Violaxanthin from *Dunaliella tertiolecta* and *Chlorella ellipsoidea* inhibited breast and colon carcinoma, respectively [47].

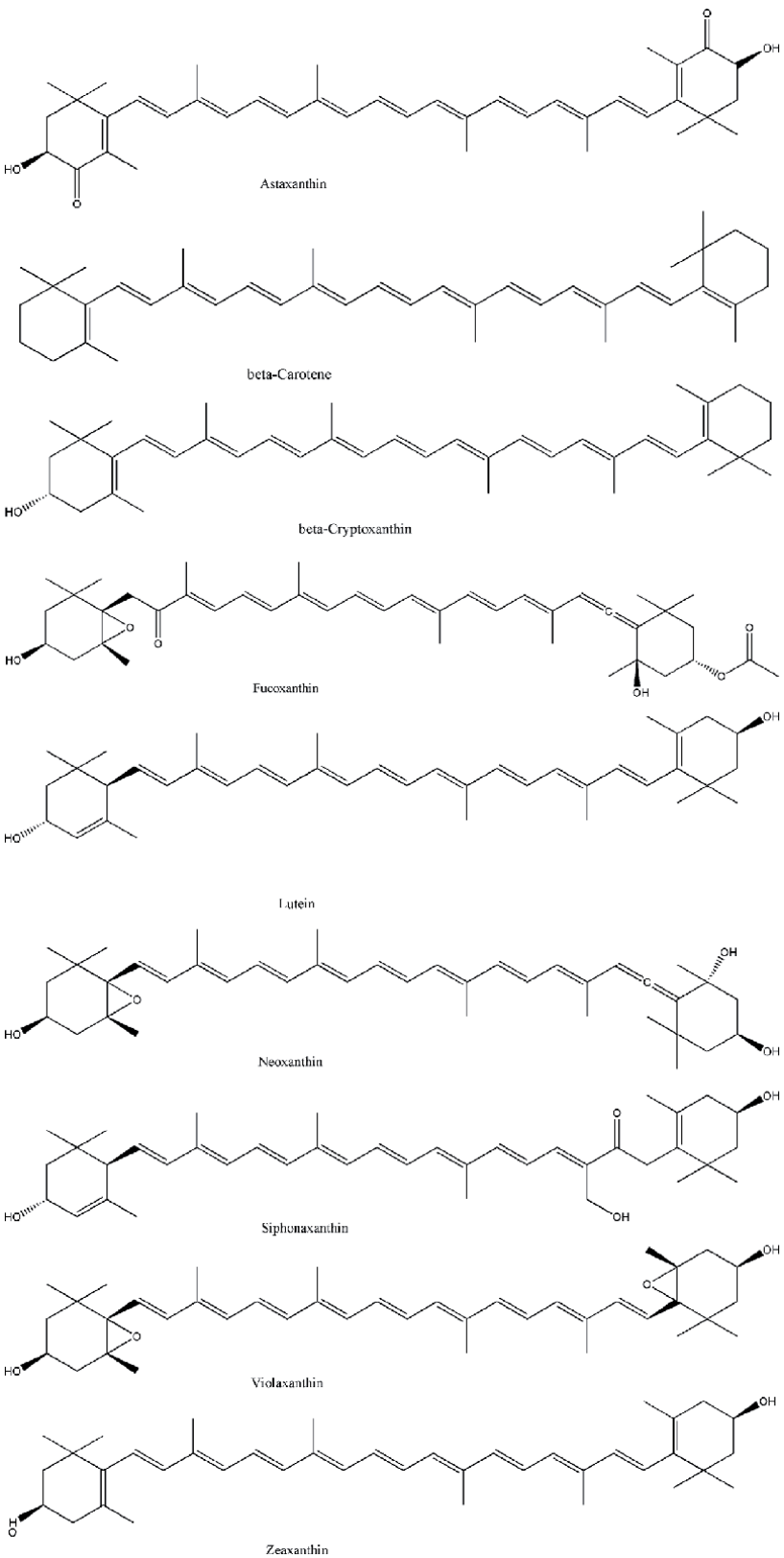


Figure 2.
Chemical structure of some algal tetraterpenoids.

3.4.6 Neoxanthin

The antioxidative property of neoxanthin was found in *Scenedesmus sp.*, *Chlorella sp.* and *Tetraselmis suecica* [48, 49].

3.4.7 Astaxanthin

Astaxanthin from *H. pluvialis* inhibits the oxidative stress inside the cells [50].

3.4.8 β-Cryptoxanthin

β-Cryptoxanthin obtained from *Cyanophora paradoxa* exerted cytotoxicity against human skin, breast and lung cancer cells [51].

3.4.9 Siphonaxanthin

Siphonaxanthin from green microalgae *Codium fragile* exhibited apoptosis in human leukemia cells through TRAIL induction with the augmentation of GADD45a and DR5 expression and reduced Bcl-2 and thus, showed more effective anticancer property compared to FX [52].

3.5 Sesquiterpenoids

Sesquiterpenoids have also high antioxidative and anticancer properties. Green seaweed *Ulva fasciata*, isolated from south Indian rocky shore, produced five sesquiterpenoids with radical scavenging activity and among them, 3,4,5,5-tetramethyl-4-(30-oxopentyl)-2-cyclohexen-1-one was revealed as one of the most potent radical scavengers [53]. Isozonarol, a sesquiterpenoid, has been identified from *Dictyopteris undulata* that can scavenge DPPH with an EC_{50} of 71 μM which is similar to α-tocopherol [54].

Sesquiterpenoids from *Laurencia composita* Yamada, namely compositacin D and G, as well as cycloelatanene A and B inhibited the growth of lung cancer cells at IC_{50} values ranging from 48.6 to 85.2 μM [55]. *Laurencia spp.* are good sources of anti-cancer sesquiterpenoids [56]. For instance, *Laurencia okamurai* produced laurinterol which inhibited melanoma cells by causing apoptosis via p53-dependent pathway and caspase activation [57]. Likewise, teuhetenone from *Laurencia obtuse* has been reported to display anticancer property against breast cancer cell line with an IC_{50} of 22 μM which is more effective in inhibiting breast cancer cells compared to chemotherapeutic drug, cisplatin (59 μM) [58]. Another major sesquiterpene, caulerpenyne was separated from *Caulerpa taxifolia,* that hindered human neuroblastoma cells (IC_{50} = 10 μM), while blocked cell cycle at G2/M phase [59].

3.6 Meroterpenoids

Cystoseira usneoides, brown macroalgae, is a rich source of meroterpenoids. Eight meroterpenoids have been isolated from this seaweed which have anti-colon and anti-lung cancer activity. These meroterpenoids can hinder growth and migration of colon cancer cells by suppressing ERK/JNK/AKT pathways, as well as can arrest cells at G2/M phase [60]. Similarly, these meroterpenoids displayed anticancer effect against lung carcinoma, while blocks lung cancer cells at G2/M and S phases [61]. Another brown seaweed *Stypopodium flabelliforme* produced meroterpenoids, namely epitaondiol, epitaondiol monoacetate and stypotriol triacetate which exhibited anticancer property against human colon and brain carcinoma [62].

Sargassum muticum can produce tetraprenyltoluquinol meroterpenoid that has antioxidant activity and can give protection against oxidative damage [63]. Likewise, highly oxygenated meroterpenoids with antioxidant property have been found from *Kappaphycus alvarezii*, a red macroalgae [64]. *Hypnea musciformis* has meroterpenoid like 2-(tetrahydro-5-(4-hydroxyphenyl)-4-pentylfuran-3-yl)-ethyl-4-hydroxy benzoate which shows antioxidative properties comparable to gallic acid [65]. Meroterpenoids from ethanolic extract of *Sargassum serratifolium* have the capability to protect liver from the oxidative damage generated from pro-oxidant tert-butyl hydroperoxide [66].

4. Conclusion

The investigation on the anticancer properties of algal terpenoids is still in its infancy, albeit the anticancer efficacy of these phytochemicals is quite persuasive. Marine algae contain a wide array of promising terpenes and terpenoids that can strongly inhibit the proliferation of cancer cells. Extensive research on these algal terpenoids regarding their mechanism of action in the cancer cells and more clinical studies will open the door to develop novel drugs for treating cancer.

Author details

Umme Tamanna Ferdous[2] and Zetty Norhana Balia Yusof[1,2,3*]

1 Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

2 Aquatic Animal Health and Therapeutics Laboratory (AquaHealth), Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

3 Bioprocessing and Biomanufacturing Research Center, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

*Address all correspondence to: zettynorhana@upm.edu.my

References

[1] 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. DOI: 10.3322/caac.21492

[2] Ovadje P, Roma A, Steckle M, Nicoletti L, Arnason JT, Pandey S. Advances in the research and development of natural health products as main stream cancer therapeutics. *Evidence-Based Complementary and Alternative Medicine*. 2015;*2015*. DOI: 10.1155/2015/751348

[3] Calvani M, Pasha A, Favre C. Nutraceutical boom in cancer: Inside the labyrinth of reactive oxygen species. *International Journal of Molecular Sciences*. 2020;*21*(6). DOI: 10.3390/ijms21061936

[4] Huang M, Lu JJ, Huang MQ, Bao JL, Chen XP, Wang YT. Terpenoids: Natural products for cancer therapy. *Expert Opinion on Investigational Drugs*. 2012;*21*(12):1801-1818. DOI: 10.1517/13543784.2012.727395

[5] Sathasivam R, Ki JS. A review of the biological activities of microalgal carotenoids and their potential use in healthcare and cosmetic industries. *Marine Drugs*. 2018;*16*(1). DOI: 10.3390/md16010026

[6] Borek C. Dietary Antioxidants and Human Cancer. *Journal of Restorative Medicine*. 2017;*6*(1):53-61 https://doi.org/10.14200/jrm.2017.6.0105

[7] Sies, H. (2019). Oxidative Stress: Eustress and Distress in Redox Homeostasis. In *Stress: Physiology, Biochemistry, and Pathology*. doi:10.1016/B978-0-12-813146-6.00013-8

[8] Mut-Salud N, Álvarez PJ, Garrido JM, Carrasco E, Aránega A, Rodríguez-Serrano F. Antioxidant Intake and Antitumor Therapy: Toward Nutritional Recommendations for Optimal Results. *Oxidative Medicine and Cellular Longevity*. 2016;*2016*. DOI: 10.1155/2016/6719534

[9] Ilghami R, Barzegari A, MashayekhiMR, LetourneurD, CrepinM, Pavon-Djavid G. The conundrum of dietary antioxidants in cancer chemotherapy. *Nutrition Reviews*. 2020;*78*(1):65-76. DOI: 10.1093/nutrit/nuz027

[10] Marian MJ. Dietary Supplements Commonly Used by Cancer Survivors: Are There Any Benefits? *Nutrition in Clinical Practice*. 2017;**32**:607-627. DOI: 10.1177/0884533617721687

[11] Singh K, Bhori M, Kasu YA, Bhat G, Marar T. Antioxidants as precision weapons in war against cancer chemotherapy induced toxicity – Exploring the armoury of obscurity. *Saudi Pharmaceutical Journal*. 2018;*26*(2):177-190. DOI: 10.1016/j.jsps.2017.12.013

[12] Othman, R., Amin, N. A., Sani, M. S. A., Fadzillah, N. A., & Jamaludin, M. A. (2018). Carotenoid and chlorophyll profiles in five species of Malaysian seaweed as potential Halal Active Pharmaceutical Ingredient (API). *International Journal on Advanced Science, Engineering and Information Technology*, *8*(4-2), 1610-1616. https://doi.org/10.18517/ijaseit.8.4-2.7041

[13] Rajput JD, Bagul SD, Pete UD, Zade CM, Padhye SB, Bendre RS. Perspectives on medicinal properties of natural phenolic monoterpenoids and their hybrids. *Molecular Diversity*. 2018;*22*(1):225-245. DOI: 10.1007/s11030-017-9787-y

[14] Sun XB, Wang SM, Li T, Yang YQ. Anticancer activity of linalool

terpenoid: Apoptosis induction and cell cycle arrest in prostate cancer cells. *Tropical Journal of Pharmaceutical Research*. 2015;*14*(4):619-625. DOI: 10.4314/tjpr.v14i4.9

[15] Gould MN. Cancer chemoprevention and therapy by monoterpenes. *Environmental Health Perspectives*. 1997;*105*:977-979. DOI: 10.2307/3433313

[16] De Inés C, Argandoña VH, Rovirosa J, San-Martín A, Díaz-Marrero AR, Cueto M, et al. Cytotoxic activity of halogenated monoterpenes from Plocamium cartilagineum. *Zeitschrift Fur Naturforschung - Section C Journal of Biosciences*. 2004;*59*(5-6):339-344. DOI: 10.1515/znc-2004-5-609

[17] Shapumba CW, Knott M, Kapewangolo P. Antioxidant activity of a halogenated monoterpene isolated from a Namibian marine algal Plocamium species. *Journal of Food Science and Technology*. 2017;*54*(10):3370-3373. DOI: 10.1007/s13197-017-2784-4

[18] Yang X, Kang M-C, Lee K-W, Kang S-M, Lee W-W, Jeon Y-J. Antioxidant activity and cell protective effect of loliolide isolated from Sargassum ringgoldianum subsp. coreanum. *Algae*. 2011;*26*(2):201-208. DOI: 10.4490/algae.2011.26.2.201

[19] Antony T, Chakraborty K. First report of antioxidant abeo-labdane type diterpenoid from intertidal red seaweed Gracilaria salicornia with 5-lipoxygenase inhibitory potential. *Natural Product Research*. 2020;*34*(10):1409-1416. DOI: 10.1080/14786419.2018.1508150

[20] Silva J, Alves C, Freitas R, Martins A, Pinteus S, Ribeiro J, et al. Antioxidant and neuroprotective potential of the brown seaweed bifurcaria bifurcata in an in vitro Parkinson's disease model. *Marine Drugs*. 2019;*17*(2):1-16. DOI: 10.3390/md17020085

[21] Ayyad SEN, Makki MS, Al-Kayal NS, Basaif SA, El-Foty KO, Asiri AM, et al. Cytotoxic and protective DNA damage of three new diterpenoids from the brown alga Dictyota dichotoma. *European Journal of Medicinal Chemistry*. 2011;*46*(1):175-182. DOI: 10.1016/j.ejmech.2010.10.033

[22] Rodrigues D, Alves C, Horta A, Pinteus S, Silva J, Culioli G, et al. Antitumor and antimicrobial potential of bromoditerpenes isolated from the Red Alga, Sphaerococcus coronopifolius. *Marine Drugs*. 2015;*13*(2):713-726. DOI: 10.3390/md13020713

[23] Jian B, Zhang H, Han C, Liu J. Anti-cancer activities of diterpenoids derived from euphorbia fischeriana steud. *Molecules*. 2018;*23*(2):1-11. DOI: 10.3390/molecules23020387

[24] Syad AN, Shunmugiah KP, Kasi PD. Antioxidant and anti-cholinesterase activity of Sargassum wightii. *Pharmaceutical Biology*. 2013;*51*(11):1401-1410. DOI: 10.3109/13880209.2013.793721

[25] Nurjanah, Nurilmala M, Anwar E, Luthfiyana N, Hidayat T. Identification of bioactive compounds of seaweed sargassum sp. and eucheuma cottonii doty as a raw sunscreen cream. *Proceedings of the Pakistan Academy of Sciences: Part B*. 2017;*54*(4):311-318

[26] Ghannadi A, Shabani L, Yegdaneh A. Cytotoxic, antioxidant and phytochemical analysis of Gracilaria species from Persian Gulf. *Advanced Biomedical Research*. 2016;*5*(1):139. DOI: 10.4103/2277-9175.187373

[27] Arsianti A, Kurniawan G, Tejaputri NA, Qorina F, Fithrotunnisa Q, Azizah NN, et al. Phytochemical Profile, Antioxidant

Activity and Cell Line Study of Marine Red Macroalgae Eucheuma cottonii on Lung A-549 Cancer Cells. *Pharmacognosy Journal*. 2020;*12*(2):276-281. DOI: 10.5530/pj.2020.12.43

[28] Rajamani K, Balasubramanian T, Thirugnanasambandan SS. Bioassay-guided isolation of triterpene from brown alga Padina boergesenii possess anti-inflammatory and anti-angiogenic potential with kinetic inhibition of β-carotene linoleate system. *LWT - Food Science and Technology*. 2018. DOI: 10.1016/j.lwt.2018.04.010

[29] Wu ZH, Liu T, Gu CX, Shao CL, Zhou J, Wang CY. Steroids and triterpenoids from the brown alga Kjellmaniella crassifolia. *Chemistry of Natural Compounds*. 2012;*48*(1):158-160. DOI: 10.1007/s10600-012-0190-8

[30] Li YX, Himaya SWA, Kim SK. Triterpenoids of marine origin as anti-cancer agents. *Molecules*. 2013;*18*(7):7886-7909. DOI: 10.3390/molecules18077886

[31] Rao AR, Sarada R, Baskaran V, Ravishankar GA. Antioxidant activity of Botryococcus braunii extract elucidated in vitro models. *Journal of Agricultural and Food Chemistry*. 2006;*54*(13):4593-4599. DOI: 10.1021/jf060799j

[32] Jayappriyan KR, Rajkumar R, Venkatakrishnan V, Nagaraj S, Rengasamy R. In vitro anticancer activity of natural β-carotene from Dunaliella salina EU5891199 in PC-3 cells. *Biomedicine and Preventive Nutrition*. 2013;*3*(2):99-105. DOI: 10.1016/j.bionut.2012.08.003

[33] Neumann U, Derwenskus F, Flister VF, Schmid-Staiger U, Hirth T, Bischoff SC. Fucoxanthin, a carotenoid derived from Phaeodactylum tricornutum exerts antiproliferative and antioxidant activities in vitro. *Antioxidants*. 2019;*8*(6):1-11. DOI: 10.3390/antiox8060183

[34] Peraman M, Nachimuthu S. Bioautography-based Identification of Antioxidant Metabolites of Solanum nigrum L. and Exploration Its Hepatoprotective Potential agChester, K. et al. (2017) 'Bioautography-based Identification of Antioxidant Metabolites of Solanum nigrum L. and Explorati. *Pharmacognosy Magazine*. 2019;*15*:S243-S249. DOI: 10.4103/pm.pm

[35] Rijstenbil JW. Effects of UVB radiation and salt stress on growth, pigments and antioxidative defence of the marine diatom Cylindrotheca closterium. *Marine Ecology Progress Series*. 2003;*254*(June 2003):37-48. DOI: 10.3354/meps254037

[36] Xia S, Wang K, Wan L, Li A, Hu Q, Zhang C. Production, characterization, and antioxidant activity of fucoxanthin from the marine diatom odontella aurita. *Marine Drugs*. 2013;*11*(7):2667-2681. DOI: 10.3390/md11072667

[37] Foo SC, Yusoff FM, Imam MU, Foo JB, Ismail N, Azmi NH, et al. Increased fucoxanthin in Chaetoceros calcitrans extract exacerbates apoptosis in liver cancer cells via multiple targeted cellular pathways. *Biotechnology Reports*. 2018;*20*(e00296). DOI: 10.1016/j.btre.2018.e00296

[38] Raguraman VLSA, MubarakAli D, Narendrakumar G, ThirugnanasambandamR,KirubagaranR, Thajuddin N. Unraveling rapid extraction of fucoxanthin from Padina tetrastromatica: Purification, characterization and biomedical application. *Process Biochemistry*. 2018;*73*:211-219. DOI: 10.1016/j.procbio.2018.08.006

[39] Banskota AH, Sperker S, Stefanova R, McGinn PJ, O'Leary SJB. Antioxidant properties and lipid composition of selected microalgae. *Journal of Applied Phycology*. 2019;*31*(1):309-318. DOI: 10.1007/s10811-018-1523-1

[40] Cho YC, Cheng JH, Hsu SL, Hong SE, Lee TM, Chang CMJ. Supercritical carbon dioxide anti-solvent precipitation of anti-oxidative zeaxanthin highly recovered by elution chromatography from Nannochloropsis oculata. *Separation and Purification Technology*. 2011;*78*(3):274-280. DOI: 10.1016/j.seppur.2011.02.017

[41] Juin, C., Oliveira Junior, R. G. de, Fleury, A., Oudinet, C., Pytowski, L., Bérard, J. B., ... Picot, L. (2018). Zeaxanthin from Porphyridium purpureum induces apoptosis in human melanoma cells expressing the oncogenic BRAF V600E mutation and sensitizes them to the BRAF inhibitor vemurafenib. *Brazilian Journal of Pharmacognosy*, *28*(4), 457-467. doi:10.1016/j.bjp.2018.05.009

[42] Ahmed F, Fanning K, Netzel M, Turner W, Li Y, Schenk PM. Profiling of carotenoids and antioxidant capacity of microalgae from subtropical coastal and brackish waters. *Food Chemistry*. 2014;*165*:300-306. DOI: 10.1016/j.foodchem.2014.05.107

[43] Kim HM, Jung JH, Kim JY, Heo J, Cho DH, Kim HS, et al. The Protective Effect of Violaxanthin from Nannochloropsis oceanica against Ultraviolet B-Induced Damage in Normal Human Dermal Fibroblasts. *Photochemistry and Photobiology*. 2019;*95*(2):595-604. DOI: 10.1111/php.13030

[44] Kim J, Kim M, Lee S, Jin ES. Development of a Chlorella vulgaris mutant by chemical mutagenesis as a producer for natural violaxanthin. *Algal Research*. 2020;*46*(September 2019):101790. DOI: 10.1016/j.algal.2020.101790

[45] Soontornchaiboon W, Joo SS, Kim SM. Anti-inflammatory effects of violaxanthin isolated from microalga Chlorella ellipsoidea in RAW 264.7 macrophages. *Biological and Pharmaceutical Bulletin*. 2012;*35*(7):1137-1144. DOI: 10.1248/bpb.b12-00187

[46] Wang F, Huang L, Gao B, Zhang C. Optimum production conditions, purification, identification, and antioxidant activity of violaxanthin from microalga eustigmatos cf. Polyphem (eustigmatophyceae). *Marine Drugs*. 2018;*16*(6). DOI: 10.3390/md16060190

[47] Pasquet V, Morisset P, Ihammouine S, Chepied A, Aumailley L, Berard JB, et al. Antiproliferative activity of violaxanthin isolated from bioguided fractionation of Dunaliella tertiolecta extracts. *Marine Drugs*. 2011;*9*(5):819-831. DOI: 10.3390/md9050819

[48] Patias LD, Fernandes AS, Petry FC, Mercadante AZ, Jacob-Lopes E, Zepka LQ. Carotenoid profile of three microalgae/cyanobacteria species with peroxyl radical scavenger capacity. *Food Research International*. 2017;*100*:260-266. DOI: 10.1016/j.foodres.2017.06.069

[49] Sansone C, Galasso C, Orefice I, Nuzzo G, Luongo E, Cutignano A, et al. The green microalga Tetraselmis suecica reduces oxidative stress and induces repairing mechanisms in human cells. *Scientific Reports*. 2017;*7*(December 2015):1-12. DOI: 10.1038/srep41215

[50] Régnier P, Bastias J, Rodriguez-Ruiz V, Caballero-Casero N, Caballo C, Sicilia D, et al. Astaxanthin from Haematococcus pluvialis prevents oxidative stress on human endothelial cells without toxicity. *Marine Drugs*. 2015;*13*(5):2857-2874. DOI: 10.3390/md13052857

[51] Baudelet PH, Gagez AL, Bérard JB, Juin C, Bridiau N, Kaas R, et al. Antiproliferative activity of Cyanophora paradoxa pigments in melanoma, breast and lung cancer cells.

Marine Drugs. 2013;*11*(11):4390-4406. DOI: 10.3390/md11114390

[52] Ganesan P, Noda K, Manabe Y, Ohkubo T, Tanaka Y, Maoka T, et al. Siphonaxanthin, a marine carotenoid from green algae, effectively induces apoptosis in human leukemia (HL-60) cells. *Biochimica et Biophysica Acta - General Subjects*. 2011;*1810*(5):497-503. DOI: 10.1016/j.bbagen.2011.02.008

[53] Chakraborty K, Paulraj R. Sesquiterpenoids with free-radical-scavenging properties from marine macroalga Ulva fasciata Delile. *Food Chemistry*. 2010;*122*(1):31-41. DOI: 10.1016/j.foodchem.2010.02.012

[54] Kumagai M, Nishikawa K, Matsuura H, Umezawa T, Matsuda F, Okino T. Antioxidants from the brown alga dictyopteris undulata. *Molecules*. 2018;*23*(5):1-8. DOI: 10.3390/molecules23051214

[55] Yu XQ, Jiang CS, Zhang Y, Sun P, Kurtán T, Mándi A, et al. Compositacins A–K: Bioactive chamigrane-type halosesquiterpenoids from the red alga Laurencia composita Yamada. *Phytochemistry*. 2017;*136*:81-93. DOI: 10.1016/j.phytochem.2017.01.007

[56] Rocha DHA, Seca AML, Pinto DCGA. Seaweed secondary metabolites in vitro and in vivo anticancer activity. *Marine Drugs*. 2018;*16*(11):1-27. DOI: 10.3390/md16110410

[57] Kim MM, Mendis E, Kim SK. Laurencia okamurai extract containing laurinterol induces apoptosis in melanoma cells. *Journal of Medicinal Food*. 2008;*11*(2):260-266. DOI: 10.1089/jmf.2007.575

[58] Alarif WM, Al-Footy KO, Zubair MS, Halid Ph M, Ghandourah MA, Basaif SA, et al. The role of new eudesmane-type sesquiterpenoid and known eudesmane derivatives from the red alga Laurencia obtusa as potential antifungal-antitumour agents. *Natural Product Research*. 2016;*30*(10):1150-1155. DOI: 10.1080/14786419.2015.1046378

[59] Barbier P, Guise S, Huitorel P, Amade P, Pesando D, Briand C, et al. Caulerpenyne from Caulerpa taxifolia has an antiproliferative activity on tumor cell line SK-N-SH and modifies the microtubule network. *Life Sciences*. 2001;*70*(4):415-429. DOI: 10.1016/S0024-3205(01)01396-0

[60] Zbakh H, Zubía E, de Los Reyes C, Calderón-Montaño JM, Motilva V. Anticancer Activities of Meroterpenoids Isolated from the Brown Alga Cystoseira usneoides against the Human Colon Cancer Cells HT-29. *Foods*. 2020b;*9*:300. DOI: 10.3390/foods9030300

[61] Zbakh H, Zubía E, de los Reyes C, Calderón-Montaño JM, López-Lázaro M, Motilva V. Meroterpenoids from the brown alga cystoseira usneoides as potential anti-inflammatory and lung anticancer agents. *Marine Drugs*. 2020a;*18*(4). DOI: 10.3390/md18040207

[62] Pereira DM, Cheel J, Areche C, San-Martin A, Rovirosa J, Silva LR, et al. Anti-proliferative activity of meroditerpenoids isolated from the brown alga Stypopodium flabelliforme against several cancer cell lines. *Marine Drugs*. 2011;*9*(5):852-862. DOI: 10.3390/md9050852

[63] Balboa EM, Li YX, Ahn BN, Eom SH, Domínguez H, Jiménez C, et al. Photodamage attenuation effect by a tetraprenyltoluquinol chromane meroterpenoid isolated from Sargassum muticum. *Journal of Photochemistry and Photobiology B: Biology*. 2015;*148*:51-58. DOI: 10.1016/j.jphotobiol.2015.03.026

[64] Makkar F, Chakraborty K. Antioxidant and anti-inflammatory oxygenated meroterpenoids from the

thalli of red seaweed Kappaphycus alvarezii. *Medicinal Chemistry Research*. 2018;*27*(8):2016-2026. DOI: 10.1007/s00044-018-2210-0

[65] Chakraborty K, Joseph D, Joy M, Raola VK. Characterization of substituted aryl meroterpenoids from red seaweed Hypnea musciformis as potential antioxidants. *Food Chemistry*. 2016;*212*:778-788. DOI: 10.1016/j.foodchem.2016.06.039

[66] Lim S, Kwon M, Joung EJ, Shin T, Oh CW, Choi JS, et al. Meroterpenoid-Rich fraction of the ethanolic extract from sargassum serratifolium suppressed oxidative stress induced by tert-butyl hydroperoxide in HepG2 cells. *Marine Drugs*. 2018;*16*(10). DOI: 10.3390/md16100374

Chapter 7

Sesquiterpene from Myanmar Medicinal Plant (*Curcuma comosa*)

Khun Nay Win Tun, Nanik Siti Aminah, Alfinda Novi Kristanti, Hnin Thanda Aung and Yoshiaki Takaya

Abstract

Curcuma comosa (Zingiberaceae) is widely grown in tropical and subtropical areas of Asia, like Thailand, Indonesia, Malaysia, and Myanmar. In Myanmar, the rhizome of *Curcuma comosa* is called Sa-nwin-ga, and local people had used it as a traditional medicine for stomach ache, diabetes mellitus, and hypertension. This species produces secondary metabolites of phenolic and nonphenolic groups. Phenolic groups like diarylheptanoids and flavonoids. While nonphenolics are terpenoids, especially sesqui- and monoterpenes. In this chapter, the group of sesquiterpene compounds from *Curcuma comosa* starts from the isolation technique, followed by the elucidation of the molecular structure, and their activity tests have been discussed.

Keywords: *Curcuma comosa*, Myanmar, sesquiterpenes, Zingiberaceae, Sa-nwin-ga

1. Introduction

Terpenes are formally derived from the carbon backbone of isoprene and based on the polymers of the active building blocks head-to-tail and tail-to-tail. Virtually all parts of the plant, especially flowers, leaves, fruits, and roots, contain different quantities of terpenes and terpenoids which are separated by means of methods such as distillation, extraction and other techniques. More than 30,000 terpenes and terpenoids are known to date. Their role in nature is still unknown and undergoes further research. Essential oils play an important role in defense and signaling as a product of plant secondary metabolism. Today, herbs and spices have an important role to play in disease prevention. In vitro trials have shown that terpenes can inhibit or sometimes induce pathways that regulating cell division, cell proliferation and detoxification [1]. *Curcuma comosa* (Zingiberaceae), widely grown in tropical and subtropical area of Asia, like Thailand, Indonesia, Malaysia, and Taunggyi (Shan State of Myanmar). It is popularly known for its beneficial effect in human health, being traditionally used in folk medicine in Asian countries, including Myanmar, Malaysia, Indonesia, and Thailand. In Taunggyi, the rhizome of *Curcuma comosa* is called **Sa-nwin-ga** and local people had used as a traditional medicine for stomach ache, diabetes mellitus and hypertension. In Thailand, the

rhizome of *Curcuma comosa* is called **Waamchak mod luuk** and had been used for the treatment of reproductive disorders in women, and for relief of unpleasant menopausal symptoms among postmenopausal women. Phytochemical investigations of this plant led to the isolation of several compounds. Two major groups of structures reported constituents include sesquiterpenes and diarylheptanoids [2, 3].

2. Classification of terpenes

Terpenes are typically classified according to the number of biogenetically derived isoprene units (**Figure 1**). (i) Hemiterpenes: They are made up of C_5 unit or 1 residues of isoprene. (ii) Monoterpenes: They are made up of C_{10} unit or 2 residues of isoprene. (iii) Sesquiterpenes: They are made up of C_{15} unit or 3 residues of isoprene. (iv) Diterpenes: They are made up of C_{20}unit or 4 residues of isoprene. (v) Sesterterpenes: They are made up of C_{25} unit or 5 residues of isoprene. (vi) Triterpenes: They are made up of C_{30} unit or 6 residues of isoprene. (vii) Tetraterpenes: They are made up of C_{40} unit or 8 residues of isoprene [4, 5].

2.1 Sesquiterpenes

Sesquiterpenes can be classified into five sub-groups (**Figure 2**): (i) germacrane-type sesquiterpenes, (ii) guaiane-type sesquiterpenes, (iii) bisaborane-type sesquiterpenes, (iv) carabrane-type sesquiterpenes, and (v) eudesmane-type sesquiterpenes [6].

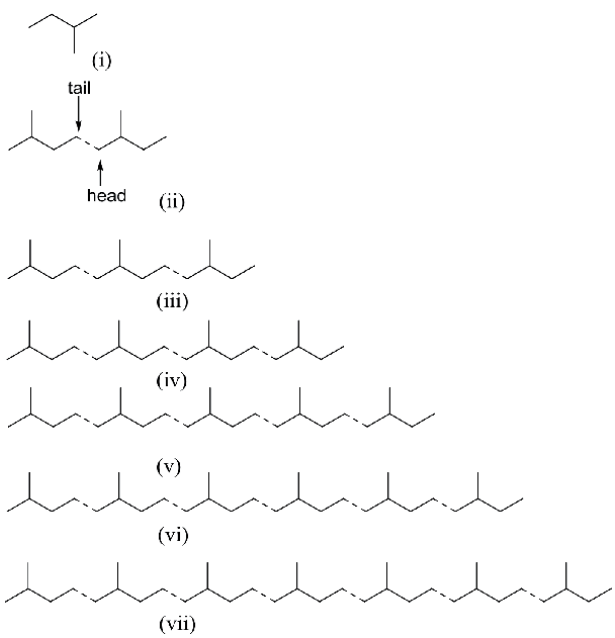


Figure 1.
Classification of terpenes [4].

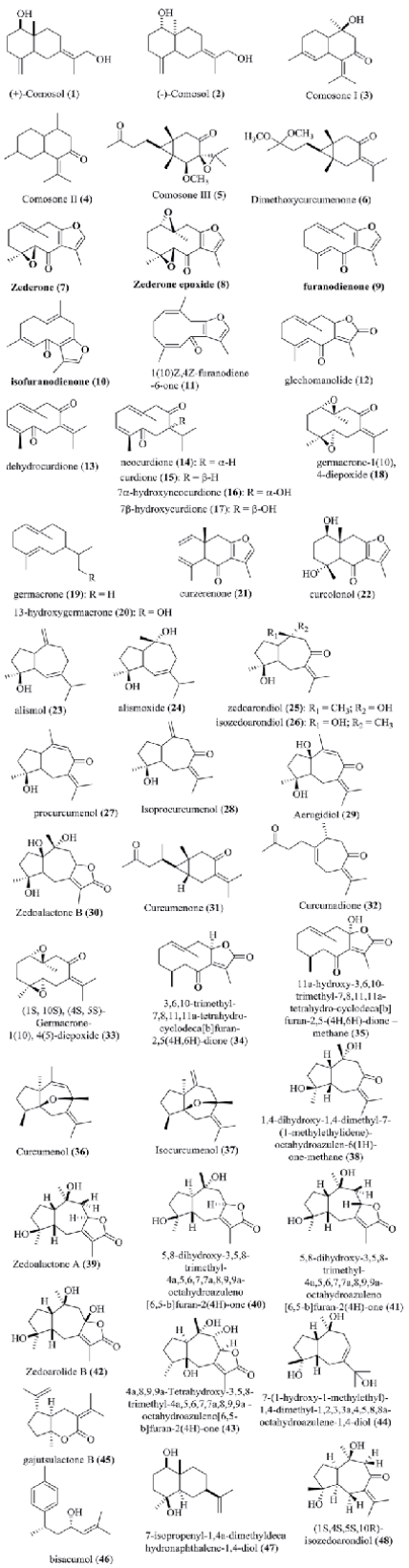


Figure 2.
Sesquiterpenes (1-48) from Curcuma comosa *[6, 18–20].*

3. Sample collection and preparation

Plant material may be obtained from fresh or dried plant parts such as leaves, barks, stem barks, roots, rhizomes, fruits, and flowers. The plant materials were dried at room temperature. These were cut into small pieces. The air-dried samples were kept in a covered glass container to protect them from humidity and light prior to extraction.

3.1 Extraction

Plant materials are an immensely complicated system containing a broad range of natural compounds. The most relevant techniques can effortlessly be used for especially selective and reliable extraction of specific components found in complex matrices. These techniques comprise maceration, percolation, decoction, reflux extraction, soxhlet extraction, pressurized liquid extraction, ultrasonic extraction, (sonication), microwave-assisted extraction (MAE), accelerated solvent extraction (ASE), supercritical fluid extraction (SFE), pulsed electric field extraction, enzyme assisted extraction, hydro distillation, and steam distillation. The point of the method for extraction is to optimize the number of goal compounds and to realize most biological activity [7, 8].

3.2 Examination of the crude extract

Analytical TLC was used to examine the composition of the unrefined extracts. The visualizations were assisted either by the UV detection of the TLC or by anisaldehyde dipping, accompanied by warming at 100°C. The TLC has been changed more than once by altering solvent processes to achieve the best separation [9].

3.3 Fractionation

Fractionation is the method of classification by physical or chemical characteristics of a specific sample of an analyte or group of analytes. Raw extracts can contain thousands of compounds in a complicated mix. It would not be possible to produce a single compound from crude extract with a single separation procedure. It is therefore also important to divide the crude extract into different fractions that contain a similar group of polarities or molecular compounds [10].

3.4 Isolation and purification

Solvent extraction and partition accompanied by column chromatography (CC), vacuum-liquid chromatography (VLC), thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), and gas chromatography–mass spectrometry (GCMS) are the prevalent separation techniques for sesquiterpenes. Resembling extraction, the most significant factor to be considered before choosing an isolation protocol is the nature of the goal compound(s) present in the crude extracts or fractions. Chromatography is a technique that allows qualitative and quantitative analysis to separate, identify and purify the mixture of a compound. Chromatography is based on the concept under which the mixed molecules deposited on or in the solid and fluid stationary phases are separated with the aid of a mobile phase. The stationary phase normally employed is silica gel with the mobile the solvent(s) of choice to fractionate or extract bioactive compounds [11–13].

3.5 Structure elucidation

A mixture of physical (melting point, CD and alpha-D) and spectroscopic (UV, IR, 1D-, 2D- NMR, and HR-MS) techniques have typically used to characterize the structures of the isolated pure sesquiterpenes. UV–Vis spectroscopy is widely used in analytical chemistry for the measurement of various analyze, such as strongly multiple bonds or aromatic conjugation within molecules, bioprocess, and fermentation of food production. Fourier-transform infrared (FTIR) spectroscopy is an effective method to classify the functional groups found in the sesquiterpenes compound. Nuclear magnetic resonance (NMR) may be the capable spectroscopy that gives complete data on atomic structure and is well appropriate for the identification of simple molecules. NMR spectroscopy is primarily partitioned into one dimensional (1D-NMR) and two-dimensional techniques (2D-NMR). The ^{1}H-NMR and ^{13}C-NMR one-dimension techniques provide information about the numbers and types of protons and carbon atoms in the sesquiterpenes compound. There are five 2D-NMR techniques commonly used to determine the sesquiterpenes structure, double quantum filtered correlated spectroscopy (DQF-COSY), nuclear Overhauser enhancement spectroscopy (NOESY), heteronuclear multiple-bond correlation (HMBC), heteronuclear single-quantum correlation spectroscopy (HSQC)/heteronuclear multiple-quantum coherence (HMQC), rotating frame Overhauser enhancement spectroscopy (ROESY), and total correlation spectroscopy (TOCSY) [13–16].

4. Sesquiterpenes from *C. comosa*

Xu et al., isolated six new sesquiterpenes (**1-6**) from the EtOAc soluble portion of the methanol rhizomes extract of *C. comosa* by using silica gel column chromatography, octa decyl silica (ODS) column chromatography, and high-performance-liquid-chromatography (HPLC) [17]. Qu et al., also isolated 26 known compounds (**7-32**) from the EtOAc soluble layer of the methanol rhizomes extract of *C. comosa* by using silica gel column chromatography, octa decyl silica (ODS) column chromatography, and high-performance-liquid-chromatography (HPLC) [18]. Khine isolated 25 sesquiterpenes (**7**, **15**, **24-28**, and **30-47**) from the hexane extract and *n*-butanol fraction of *C. comosa* by using different chromatographic techniques [6]. In our previous work, 3 known sesquiterpenes (**25**, **36**, and **48**) were isolated from the MeOH soluble fraction of *C. comosa* by using vacuum-liquid chromatography and successive repeated column chromatography [19]. The physical and spectroscopic data of the isolated compounds are depicted in **Table 1**.

5. Biological activities

Several studies have reported that *Curcuma comosa* have been successfully used for various diseases **Table 2**.

6. Conclusion

Work on natural products has recently experienced rapid expansion due to improvement in isolation techniques and the design of synthesis methods and also for the identification of a wide range of biological properties of these compounds. In

Compound	Physical and spectral data
(+)-Comosol (**1**) [17]	A colorless oil; $[\alpha]_D^{23}$ +34.7° (*c* = 0.2, $CHCl_3$); IR (cm^{-1}): 3420, 2936, 1655, 1541, 754. ^1H NMR (600 MHz, $CDCl_3$) δ_H: 0.80, 1.79 (each 3H, s H_3-14, 13), 1.16, 1.99 (1H each, both m, H_2-10), 1.59, 1.82 (1H each, both m, H_2-3), 1.73 (1H, br d, J = *ca*. 12 Hz, H-6), [1.88 (1H, dd, J = 13.0, 11.7 Hz), 2.53 (1H, br d, J = *ca*. 13 Hz), H_2-7], [1.94 (1H, dd like, J = 13.7 Hz), 2.67 (1H, br d, J = ca. 14 Hz), H_2-9], 2.10, 2.32 (1H each, both m, H_2-4), 3.39 (1H, dd, J = 11.6, 4.1 Hz, H-2), 4.16 (2H, s, H_2-12), 4.57, 4.82 (1H each, both br s, H_2-15). ^{13}C NMR (150 MHz, $CDCl_3$) δ_C: 40.5 (C-1), 79.1 (C-2), 31.4 (C-3), 34.1 (C-4), 148.8 (C-5), 48.1 (C-6), 28.1 (C-7), 136.5 (C-8), 25.0 (C-9), 38.3 (C-10), 125.4 (C-11), 63.4 (C-12), 16.4 (C-13), 9.8 (C-14), 107.0 (C15). EI-MS m/z: 236 [M^+] (6), 218 [M -H_2O]$^+$ (100). HR-EI-MS m/z: 236.1771 (Calcd for $C_{15}H_{24}O_2$: 236.1776).
(−)-Comosol (**2**) [17]	A colorless oil; $[\alpha]_D^{22}$ −34.7° (*c* = 0.2, $CHCl_3$); IR (cm^{-1}): 3420, 2936, 1655, 1541, 754. ^1H NMR (600 MHz, $CDCl_3$) δ_H: 0.80, 1.79 (3H each, both s, H_3-14, 13), 1.16, 1.99 (1H each, both m, H_2-10), 1.59, 1.82 (1H each, both m, H_2-3), 1.73 (1H, br d, *J* = *ca.* 12 Hz, H-6), [1.88 (1H, dd, J = 13.0, 11.7 Hz), 2.53 (1H, br d, *J* = *ca.* 13 Hz), H2-7], [1.94 (1H, dd like, *J* = 13.7 Hz), 2.67 (1H, br d, *J* = *ca.* 14 Hz), H_2-9], 2.10, 2.32 (1H each, both m, H_2-4), 3.39 (1H, dd, *J* = 11.6, 4.1 Hz, H-2), 4.16 (2H, s, H_2-12), 4.57, 4.82 (1H each, both br s, H_2-15). ^{13}C NMR (150 MHz, $CDCl_3$) δ_C: 40.5 (C-1), 79.1 (C-2), 31.4 (C-3), 34.1 (C-4), 148.8 (C-5), 48.1 (C-6), 28.1 (C-7), 136.5 (C-8), 25.0 (C-9), 38.3 (C-10), 125.4 (C-11), 63.4 (C-12), 16.4 (C-13), 9.8 (C-14), 107.0 (C-15). EI-MS *m/z*: 236 [M^+] (6), 218 [M - H2O]$^+$ (100). HR-EI-MS *m/z*: 236.1771 (Calcd for $C_{15}H_{24}O_2$: 236.1776).
Comosone I (**3**) [17]	A colorless oil; $[\alpha]_D^{25}$ +15.4° (*c* = 0.80, MeOH). UV λ_{max} (MeOH) nm (log ε): 221 (3.78). IR (cm^{-1}): 3420, 2936, 1655, 1541, 754. ^1H NMR (600 MHz, $CDCl_3$) δ_H: 1.22, 1.74 (1H each, both m, H_2-2), 1.34, 1.65, 1.92, 1.92 (3H each, all s, H_3-14, 15, 12, 13), 1.92 (2H, m, H_2-3), 1.96 (1H, m, H-1), 2.35 (1H, dd, *J* = 18.3, 1.6 Hz, H-9b), 2.50 (1H, d, J = 18.3 Hz, H-9a), 3.74, 5.33 (1H each, both br s, H-6, 5). ^{13}C NMR (150 MHz, $CDCl_3$) δ_C: 43.9 (C-1), 23.5 (C-2), 29.6 (C-3), 134.8 (C-4), 121.6 (C-5), 36.0 (C-6), 134.3 (C-7), 203.4 (C-8), 51.5 (C-9), 72.9 (C-10), 141.6 (C-11), 22.4 (C-12) 22.5 (C-13), 27.6 (C-14), 23.7 (C-15). EI-MS m/z: 234 (M^+) (28), 216 (M - H_2O)$^+$ (30), 43 (M - 191) (100). HR-EI-MS m/z: 234.1616 (Calcd for $C_{15}H_{22}O_2$: 234.1620).
Comosone II (**4**) [17]	A colorless oil; $[\alpha]_D^{27}$ +10.1° (*c* = 0.70, MeOH). UV λ_{max} (MeOH) nm (log ε): 237 (3.77). IR (cm^{-1}): 1665, 1651, 1515, 1439, 1379, 754. ^1H NMR (600 MHz, $CDCl_3$) δ_H: 1.58, 1.87, 1.93, 2.06 (3H each, all s, H_3-15, 13, 14, 12), 1.82 (2H, m, H_2-3), 1.83, 2.20 (1H each, both m, H_2-2), 2.75 (1H, m, H-1), 3.76 (1H, br s, H-6), 4.92 (1H, br s, H-5), 5.90 (1H, s, H-9). ^{13}C NMR (150 MHz, $CDCl_3$) δ_C: 38.3 (C-1), 25.3 (C-2), 26.0 (C-3), 135.1 (C-4), 122.0 (C-5), 39.8 (C-6), 1333.5 (C-7), 191.8 (C-8), 130.8 (C-9), 158.6 (C-10), 141.8 (C-11), 23.0 (C-12), 21.9 (C-13), 20.8 (C-14), 23.5 (C-15). EI-MS m/z: 216 (M^+) (100). HR-EI-MS m/z: 216.1509 (Calcd for $C_{15}H_{20}O$: 216.1514).
Comosone III (**5**) [17]	A colorless oil; $[\alpha]_D^{24}$ +23.9° (*c* = 0.5, MeOH). IR (cm^{-1}): 1713, 1092. ^1H NMR (600 MHz, $CDCl_3$) δ_H: 0.79 (1H, ddd, *J* = 8.1, 5.4, 5.4 Hz, H-1), 1.13 (1H, t like, *J* = *ca*. 5 Hz, H-5), 1.18, 1.21, 1.39, 2.17 (3H each, all s, H_3-12, 14, 13, 15), 1.64, 1.76 (1H each, both m, H_2-2), 2.56 (2H, t, *J* = 7.6 Hz, H_2-3), 2.68, 2.77 (1H each, both d, *J* = 19.9 Hz, H_2-9), 3.43 (3H, s, OCH_3-6), 3.88 (1H, d, *J* = 4.1 Hz, H-6). ^{13}C NMR (150 MHz, $CDCl_3$) δ_C: 25.9 (C-1), 23.09 (C-2), 43.3 (C-3), 208.0 (C-4), 30.4 (C-5), 79.3 (C-6), 69.9 (C-7), 204.9 (C-8), 47.2 (C-9), 18.8 (C-10), 62.6 (C-11), 19.4 (C-12), 20.8 (C-13), 19.3 (C-14), 30.0

Compound	Physical and spectral data
	(C-15), 57.7 (C-16). EI-MS m/z: 280 [M^+] (2), 265[M - Me]$^+$ (3), 139 [M - 141]$^+$ (100). HR-EI-MS m/z: 280.1676 (Calcd for $C_{16}H_{24}O_4$: 280.1674).
Dimethoxycurcumenone (**6**) [17]	A colorless oil; $[\alpha]_D^{25}$ −10.1° (*c* = 1.4, MeOH). UV λ_{max} (MeOH) nm (log ε): 255 (3.59). IR (cm^{-1}): 1682, 1601, 1458, 1375, 1055, 853. ^{1}H NMR (600 MHz, $CDCl_3$) δ_H: 0.47, 0.66 (1H each, both m, H-1, 5), 1.13, 1.23, 1.79, 2.10 (3H each, all s, H_3-14, 15, 13, 12), 1.34, 1.65 (2H each, both m, H_2-2, 3), 2.51, 2.56 (1H each, both d, *J* = 15.6 Hz, H2-9), 2.83 (2H, br s, H_2-6), 3.15, 3.15 (3H each, both s, OCH_3-4). ^{13}C NMR (150 MHz, $CDCl_3$) δ_C: 24.7 (C-1), 23.9 (C-2), 36.7(C-3), 101.3 (C-4), 24.1 (C-5), 28.0 (C-6), 128.2 (C-7), 201.7 (C-8), 49.0 (C-9), 20.0 (C-10), 147.0 (C-11), 23.4 (C-12), 23.4 (C-13), 19.1 (C-14), 20.9 (C-15), 48.0 (C-16), 48.0 (C-17). EI-MS m/z: 280 [M^+] (3), 85 [M – 195]$^+$ (100). HR-EI-MS m/z: 280.2046 (Calcd for $C_{17}H_{28}O_3$: 280.2038).
Zederone (**7**) [20, 21]	Colorless plates; melting point: 153 ∼ 154°C; $[\alpha]_D^{20}$ +220° (*c* = 0.10, $CHCl_3$). UV λ_{max} (MeOH) nm (log ε): 232 (5010), 285 (2450). IR (cm^{-1}) 1662. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 1.56 (3H, br.s, H_3-15), 1.30 (3H, s, H_3-14), 2.07 (3H, s, H_3-13), 7.04 (1H, br.s, H-12), 3.66, 3.70 (2H, m, H_2-9), 3.77 (1H, s, H-5), 1.24, 2.27 (2H, m, H_2-3), 2.24, 2.46 (2H, m, H_2-2), 5.46 (1H, d, J = 11.8 Hz, H-1). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 131.2 (C-1), 24.7 (C-2), 38.0 (C-3), 64.0 (C-4), 66.6 (C-5), 192.2 (C-6), 123.2 (C-7), 157.2 (C-8), 41.9 (C-9), 131.1 (C-10), 122.2 (C-11), 138.1 (C-12), 10.3 (C-13), 15.2 (C-14), 15.8 (C-15). MS m/z: 246 (M^+, 18%), 188 (15), 175 (100), 161, 119 (50), 43 (27). HR-TOF-MS m/z: 247.0889 ($C_{15}H_{18}O_3$).
Zederone epoxide (**8**) [22]	White amorphous powder;$[\alpha]_D^{25}$ +38.3° (*c* = 0.3, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ_H: 7.08 (1H, br. s, H-12), 3.78 (1H, s, H-5), 3.68 (1H, d, *J* = 17.0 Hz, H-9a), 2.93 (1H, br. d, *J* = 10.0 Hz, H-1), 2.82 (d, J = 17.0 Hz, H-9b); 2.41 (1H, br. d, J = 11.0 Hz, H-3a), 2.21 (1H, br d, J = 14.0 Hz, H-2a), 2.16 (3H, s, H_3-13), 1.52 (1H, m, H-2b), 1.47 (1H, m, H-3b), 1.32 (3H, s, H_3-14), 1.15 (3H, s, H_3-15). ^{13}C NMR (125 MHz, $CDCl_3$): 189.8 (C-6), 156.1 (C-8), 138.4 (C-12), 123.4 (C-11), 122.6 (C-7), 69.0 (C-1), 63.6 (C-4), 63.2 (C-5), 57.9 (C-10), 39.5 (C-9), 36.1 (C-3), 23.8 (C-2), 16.8 (C-14), 15.3 (C-15), 10.5 (C-13). EI-MS: 262 (18.2, Mþ), 43 (100,$C_3H_7^+$).
Furanodienone (**9**) [23, 24]	Colorless prisms; melting point 87 ∼ 88°C. UV λ_{max} (EtOH) nm (ε): 241 (9150), 269 (6800). IR (cm^{-1}) 1664, 1608, 1231, 1013, 755. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 5.15 (1H, dd, *J* = 11.4, 4.1 Hz, H-1), 2.16 (1H, td, *J* = 12.4, 3.5 Hz, H-2α), 2.30 (1H, td, *J* = 12.4, 4.1 Hz, H-2β), 1.85 (1H, td, *J* = 11.4, 4.1 Hz, H-3α), 2.44 (1H, ddd, *J* = 11.4, 6.9, 3.4 Hz, H-3β), 5.78 (1H, br s, H-5), 3.66 (1H, br d, *J* = 14.5 Hz, H-9α), 3.70 (1H, br d, *J* = 14.5 Hz, H-9β), 7.05 (1H, br s, H-12), 2.11 (3H, s, H_3-13), 1.97 (3H, s, H_3-14), 1.28 (3H, s, H_3-15). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 130.5 (C-1), 26.4 (C-2), 40.6 (C-3), 145.8 (C-4), 132.4 (C-5), 190.0 (C-6), 123.9 (C-7), 156.5 (C-8), 41.7 (C-9), 135.4 (C-10), 122.0 (C-11), 138.0 (C-12), 9.5 (C-13), 18.9 (C-14), 15.7 (C-15). MS m/z: 230 (M^+, 47%), 150 (50), 122 (100), 94 (26), 81 (48).
Isofuranodienone (**10**) [24, 25]	Needles; melting point: 70-71°C; .$[\alpha]_D$ ±0°145° (*c* = 10.0). UV λ_{max} (EtOH) nm (ε): 223 (4.17), 248 (3.95). IR (KBr) cm^{-1}: 1667. ^{1}H NMR ($CDCl_3$, 400 MHz), δ_H: 5.25 (1H, br t, *J* = 8.6 Hz, H-1), 1.78 (1H, m, H-2α), 2.09 (1H, m, H-2β), 2.20 (1H, m, H-3α), 2.25 (1H, m, H-3β), 5.84 (1H, br s, H-5), 3.03 (1H, d, *J* = 14.5 Hz, H-9α), 3.57 (1H, d, *J* = 14.5 Hz, H-9β), 7.05 (3H, br s, H-12), 2.16 (3H, br s, H_3-13), 1.73 (3H, s, H_3-14), 1.63 (3H, s, H_3-15). ^{13}C NMR ($CDCl_3$, 100 MHz), δ_C: 123.9 (C-1), 26.1 (C-2), 36.3 (C-3), 141.1 (C-4), 129.0 (C-5), 193.8 (C-6), 123.9 (C-7), 161.5 (C-8), 32.8 (C-9), 134.0

Compound	Physical and spectral data
	(C-10), 122.1 (C-11), 138.4 (C-12), 9.5 (C-13), 22.6 (C-14), 19.1 (C-15). MS m/z: 230 [M^+], 122 (100%).
1(10)Z,4Z-furanodiene-6-one (**11**)	No data
Glechomanolide (**12**)	No data
Dehydrocurdione (**13**) [26, 27]	Colorless needles; melting point 40-42°C.$[\alpha]_D^{23}$ +145° (*c* = 1.1, MeOH): $[\theta]_{303}$ +13,671. UV λ_{max} (EtOH) nm (ε): 207 (1.16). IR ($CHCl_3$) cm^{-1}: 1742, 2934, 1680, 1453, 1375. ^1H NMR (400 MHz, $CDCl_3$) δ_H: 5.13 (1H, t, *J* = 8.24 Hz, H-1), 2.10 (2H, m, H_2-2), 2.0 (2H, m, H_2-3), 2.38 (1H, m, H-4), 3.21/3.29 (each 1H, dd, *J* = 16.48 Hz, H_2-6), 3.06/3.23 (each 1H, dd, *J* = 11.44 Hz, H_2-9), 1.76 (3H, s, H_3-12), 1.73 (3H, s, H_3-13), 1.01 (3H, d, *J* = 6.88 Hz, H_3-14), 1.3 (3H, s, H_3-15). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 133.0 (C-1), 26.4 (C-2), 34.2(C-3), 46.4(C-4), 211.1(C-5), 43.4(C-6), 129.3(C-7), 207.2(C-8), 57.0(C-9), 129.9(C-10), 137.0(C-11), 21.0(C-12), 22.1 (C-13), 18.4(C-14), 16.3(C-15). MS m/z: 234 (M^+) ($C_{15}H_{22}O_2$).
Neocurdione (14) [27]	Colorless needles; melting point 45-47°C (hexane). .$[\alpha]_D^{23}$ −190° (*c* = 2.1, $CHCl_3$): CD (*c* = 0.022, MeOH): $[\theta]_{301}$ −29,230. UV λ_{max} (EtOH) nm (ε): 203 (3.73). IR (KBr) cm^{-1}: 1696, 1682, 1395, 1282. ^1H NMR ($CDCl_3$): 0.92 (3H, d, *J* = 6.6 Hz, H_3-14), 0.98 (3H, d, *J* = 6.8 Hz, H_3-12 or −13), 1.03 (3H, d, J = 6.8 Hz, H_3-13 or −12), 1.67 (3H, s, H_3-15), 5.18 (1H, br t, *J* = 7.0 Hz, H-1). ^{13}C NMR ($CDCl_3$) δ_C: 131.1 (C-1), 25.5 (C-2), 32.8 (C-3), 45.8 (C-4), 210.2 (C-5), 42.4 (C-6), 52.6 (C-7), 212.5 (C-8), 55.3 (C-9), 129.1 (C-10), 30.9 (C-11), 20.2 (C-12), 21.1 (C-13), 18.2 (C-14), 18.2 (C-15). MS m/z: 236.1763 [M^+] (Calcd for $C_{15}H_{24}O_2$ 236.1777).
Curdione (**15**) [23, 27, 28]	Colorless prisms; melting point 53-54°C (MeOH).$[\alpha]_D^{23}$ +214° (*c* = 1.6, MeOH). CD (*c* = 0.0033, $CHCl_3$) $[\theta]_{309}$ +26,655. IR (KBr) cm^{-1}: 1690, 1460, 1420, 1170, 1060. ^1H NMR ($CDCl_3$, 400 MHz), δ: 5.14 (1H, br s, H-1), 2.08-2.13 (2H, m, H_2-2), 1.56 (1H, m, H-3α), 2.08-2.13 (1H, m, H-3β), 2.30 (1H, br s, H-4), 2.37 (1H, dd, *J* = 16.4, 1.5 Hz, H-6α), 2.65 (1H, m, H-6β), 2.88 (1H, ddd, *J* = 16.4, 8.5, 7.8 Hz, H-7), 2.91 (1H, d, *J* = 10.7 Hz, H-9α), 3.04 (1H, d, J = 10.7 Hz, H-9β), 1.85 (1H, m, H-11), 0.85 (3H, d, J = 6.5 Hz, H_3-12), 0.92 (3H, d, J = 6.5 Hz, H_3-13), 0.95 (3H, d, *J* = 6.9 Hz, H_3-14), 1.62 (3H, s, H_3-15).^{13}C NMR ($CDCl_3$, 100 MHz) δ_C: 131.5 (C-1), 26.3 (C-2), 34.0 (C-3), 46.7 (C-4), 214.6 (C-5), 44.2 (C-6), 53.5 (C-7), 211.1 (C-8), 55.8 (C-9), 129.2 (C-10), 29.9 (C-11), 21.1 (C-12), 19.8 (C-13), 18.5 (C-14), 16.5 (C-15). EI-MS m/z (rel. Int.): 236 (2), 208 (1), 180 (33), 167 (28), 109 (52), 95 (23), 83 (13), 69 (100), 55 (76). MS m/z 236[M^+] $C_{15}H_{24}O_2$.
7α-hydroxyneocurdione (**16**)	No data
7β-hydroxycurdione (**17**)	No data
Germacrone-1(**10**),4-diepoxide (**18**) [6]	White powder; melting point 84-86°C.$[\alpha]_D$ = +71.17° (*c* = 0.14, MeOH). UV (MeOH) λ_{max} nm (log ε): 256 (4.22), 315 (2.30). IR (KBr) cm^{-1}. 1678, 1645. ^1H NMR (400 MHz, $CDCl_3$) δ_H: 1.143 (3H, s, H_3-14), 1.26-1.32 (1H, m, H-3b), 1.444 (3H, s, H_3-15), 1.45-1.50 (1H, m, H-2b), 1.794 (3H, s, H_3-12), 1.862 (3H, s,H_3-13), 2.02-2.08 (1H, m, H-2a), 2.19-2.24 (1H, m, H-3a), 2.260 (1H, dd, *J* = 14.2/ 10.8 Hz, H-6b), 2.644 (1H, d, *J* = 10.8 Hz, H-9b), 2.652 (1H, dd, *J* = 10.9/2.2 Hz H-5), 2.855 (1H, dd, *J* = 14.2/2.2 Hz, H-6a), 2.918 (1H, d, *J* = 10.8 Hz, H-1), 3.007 (1H, *J* = 10.8 Hz, H-9a). EI-MS m/z: 124.9 (100), 122 (80). ^{13}C NMR (500 MHz, $CDCl_3$) δ_C: 61.3 (C-1), 22.8 (C-2), 35.7 (C-3), 60.1 (C-4), 64.0 (C-5), 29.2 (C-6), 134.3 (C-7), 207.2 (C-8), 54.5 (C-9), 58.4 (C-10), 137.8 (C-11), 22.9 (C-12), 20.8 (C-13), 15.5 (C-14), 17.3 (C-15). HR-ESI-MS: $C_{15}H_{22}O_3Na[M + Na]^+$ calcd. 273.14611 found 273.14575.

Compound	Physical and spectral data
Germacrone (**19**) [20, 27]	Colorless prisms; melting point: 53-54°C (MeOH). IR (cm^{-1}): 1679, 1665, 1445, 1294, 1135. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 1.62 (3H, s, H_3-15), 1.43 (3H, s, H_3-14), 1.76 (3H, s, H_3-13), 1.73 (1H, s, H-12), 3.42, 2.95 (2H, dd, *J* = 11, 3.68 Hz, H_2-9), 2.86 (2H, m, H_2-6), 4.71 (1H, d, *J* = 11 Hz, H-5), 2.15 (2H, m, H_2-3), 2.08, 2.35 (2H, m, H_2-2), 4.94 (1H, d, J = 11.8 Hz, H-1). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 132.8 (C-1), 24.0 (C-2), 38.1 (C-3), 126.0 (C-4), 125.4 (C-5), 29.3 (C-6), 129.0 (C-7), 208.0 (C-8), 56.0 (C-9), 135.1 (C-10), 137.0 (C-11), 20.0 (C-12), 22.4 (C-13), 15.6 (C-14), 16.8 (C-15). MS m/z: 218 (M^+) ($C_{15}H_{22}O$).
13-hydroxygermacrone (**20**) [29]	Colorless oil ($CHCl_3$); IR (KBr) cm^{-1}: 3452, 1679. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 4.95 (1H, br. d, *J* = 10.8 Hz, H-1), 4.63 (1H, dd, *J* = 10.0, 3.2 Hz, H-5), 4.25 (1H, d, J = 12.4 Hz, H-13a), 4.13 (1H, d, *J* = 12.4 Hz, H-13b), 3.40 (1H, d, *J* = 10.4 Hz, H-9a), 2.95 (1H, d, *J* = 10.4 Hz, H-9b), 2.92 (2H, overapped, H_2-6), 2.33 (1H, m, H-2a), 2.14 (1H, m, H-3a), 2.04 (1H, m, H-3b), 1.89 (1H, m, H-2b), 1.78 (3H, s, H_3-12), 1.59 (3H, s, H_3-15), 1.40 (3H, s, H_3- 14). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 133.08 (C-1), 24.03 (C-2), 38.02 (C-3), 135.70 (C-4), 124.94 (C-5), 28.55 (C-6), 131.43 (C-7), 207.32 (C-8), 55.48 (C-9), 126.20 (C-10), 139.80 (C-11), 17.73 (C-12), 62.65 (C13), 15.56 (C-14), 16.60 (C-15). EI-MS m/z: 234.
Curzerenone (**21**) [30, 31]	Yellowish oil. 1H-NMR (400 MHz, $CDCl_3$) δ: 7.07 (1H, brs, H-11), 5.81 (1H, brs, H-5), 5.18 (1H, t, *J* = 7.5 Hz, H-1), 3.72 (2H, AB-system, *J* = 15 Hz, H-9a, 9b), 2.20 (3H, d, *J* = 1.5 Hz, H_3-13), 1.76 (3H, d, *J* = 1.5 Hz, H_3-14), 1.31 (3H, s, H_3-15), 1.60–2.48 (4H, m, H_2-2 and H_2-3). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 130.5 (C-1), 26.4 (C-2), 41.6 (C-3), 145.7 (C-4), 132.4 (C-5), 189.7 (C-6), 122.2 (C-7), 156.5 (C-8), 40.6 (C-9), 135.4 (C-10), 138.1 (C-11), 123.7 (C-12), 9.5 (C-13), 18.9 (C-14), 15.7 (C-15). ESI-MS m/z: 231 $[M + H]^+$ ($C_{15}H_{18}O_2$, M = 230).
Curcolonol (**22**) [32]	Colorless prisms (acetone); melting point 183-184°C;$[\alpha]_D^{25}$ = 0° (*c* = 2.0, EtOH). IR (cm^{-1}): 3420, 2934, 2872, 1723, 1653, 1562, 1426, 1381, 1275, 1126, 1067, 1040, 922, 742. ^{1}H NMR (500 MHz, Acetone-d_6) δ_H: 3.69 (1H, m, H-1), 1.73 (1H, m, H-2 eq), 1.63 (1H, m, H-2ax), 1.58 (2H, m, H_2-3), 2.61 (1H, s, H-5), 3.03 (d, *J* = 17 Hz, H-9 eq), 2.84 (d, *J* = 17 Hz, H-9ax), 7.29 (1H, br s, H-11), 2.14 (3H, d, *J* = 1.3 Hz, H_3-13), 1.40 (3H, s, H_3-14), 0.97 (3H, s, H_3-15). ^{13}C NMR (50 MHz, Acetone-d_6) δ_C: 77.9 (C-1), 28.8 (C-2), 39.3 (C-3), 71.5 (C-4), 62.8 (C-5), 198.4 (C-6), 119.8 (C-7), 167.6 (C-8), 40.3 (C-9), 45.4 (C-10), 140.6 (C-11), 119.6 (C-12), 9.1 (C-13), 25.0 (C-14), 15.0 (C-15). EIMS m/z (rel int) 264 [m] + (13, 249 (29), 246 (15), 231 (5), 228 (5), 213 (12), 163 (100), 135 (35), 122 (37), 107 (31), 94 (14); HR-EI-MS m/z: 264.1354 (calcd for $C_{15}H_{20}O_4$, 264.1362).
Alismol (**23**) [33, 34]	Colorless oil; (+)-alismol:$[\alpha]_D^{25}$ = +38.8° (*c* = 0.80, $CHCl_3$,), (−)-alismol:$[\alpha]_D^{25}$ = −38.6° (*c* = 0.80, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ_H: 0.99 (3H, d, J = 6.9 Hz), 1.00 (3H, d, *J* = 6.9 Hz), 1.25 (3H, s), 1.72 -1.80 (2H, m), 1.92 (1H, m), 1.99-2.08 (1H, m), 2.02-2.09 (1H, m), 2.19-2.25 (1H, m), 2.25-2.28 (1H, m), 2.03 (2H, m), 2.51 (1H, m), 4.71 (1H, s), 4.77 (1H, s), 5.55 (1H, s). ^{13}C NMR (400 MHz, $CDC1_3$) δ_C: 47.3 (C-1), 24.7 (C-2), 40.2 (C-3), 80.7 (C-4), 55.0 (C-5), 121.3 (C-6), 149.8 (C-7), 30.0 (C-8), 37.1 (C-9), 153.9 (C-10), 37.4 (C-11), 21.5, 21.3 (C-12 and C-13), 24.1 (C-14), 106.5 (C-15). MS: m/z %: 220 [M^+](6), 205 (12), 202 (10), 187 (16), 177 (18), 162 (53), 159 (52), 149 (18), 147 (37), 145 (16), 134 (25), 131 (23), 119 (100), 117 (30), 107 (39), 105 (47), 93 (48), 91 (76), 85 (9), 81 (15), 79 (36), 77 (28), 71 (15), 69 (14), 67 (16), 55 (25),53 (16), 43 (87), 41 (38).

Compound	Physical and spectral data
Alismoxide(**24**) [33, 35]	Colorless crystals, mp 138–141°C;$[\alpha]_D^{20}$ = +9.3 (c 0.9 $CHCl_3$,). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 5.44 (1H, br d, J = 3.0 Hz, H-6), 0.98, 1.0 (3H each, d, *J* = 6.9 Hz, H_3-12, −13), 1.25, 128 (3H each, s, H_3-14, −15). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C: 50.5 (C-1), 21.4 (C-2), 40.3 (C-3), 80.0 (C-4), 50.1 (C-5), 121.3 (C-6), 149.4(C-7), 25.0 (C-8), 42.5 (C-9), 75.2 (C-10), 37.2 (C-11), 21.1 (C-12), 21.2 (C-13), 22.4 (C-14), 21.3 (C-15). MS: m/z (%): 220 (M^+ -H_2O)(7), 205 (9), 202 (4), 187 (9), 177 (12), 162 (66), 159 (28), 149 (20), 147 (38), 134 (34), 121 (23), 119 (53), 107 (34), 105 (24), 93 (42), 91 (30), 85 (12), 81 (14), 79 (28), 77 (16), 71 (12), 69 (12), 55 (20), 43 (100), 41 (24).
Zedoarondiol (**25**) [27]	Colorless needles; melting point 134°C ($CHCl_3$);$[\alpha]_D^{23}$ = −44° (*c* = 1.0, MeOH). CD (*c* = 0.03, MeOH): $[\theta]_{321}$− 6468. UV (MeOH) λ_{max} nm (log ε): 258 (3.86). IR (cm^{-1}): 3420, 1662, 1604. ^{1}H NMR ($CDCl_3$) δ_H: 1.18 (3H, s, H_3-14 or −15), 1.20 (3H, s, H_3-15 or −14), 1.84 (3H, s, H_3-12, or −13), 1.94 (3H, s, H_3-13 or −12), 2.60 (1H, d, *J* = 13.0 Hz, H-9β), 2.98 (1H, d, *J* = 13.0 Hz, H-9α). ^{13}C NMR ($CDCl_3$) δ_C: 55.9 (C-1), 22.9 (C-2), 28.5 (C-3), 79.9 (C-4), 52.0 (C-5), 39.7 (C-6), 134.6 (C-7), 202.9 (C-8), 59.8 (C-9), 72.7 (C-10), 142.1 (C-11), 21.9 (C-12), 22.2 (C-13), 22.7 (C-14), 20.6 (C-15). MS m/z: 252 (M^+) ($C_{15}H_{24}O_3$).
isozedoarondiol (**26**) [27]	Colorless needles; melting point 150-156°C.$[\alpha]_D^{23}$ = −147.2° (*c* = 0.8, MeOH). CD (*c* = 0.003, MeOH): $[\theta]_{313}$ −6323. UV (MeOH) λ_{max} nm (log ε): 258 (3.86). IR (cm^{-1}): 3420, 1662, 1604. ^{1}H NMR ($CDCl_3$) δ_H: 1.23 (3H, s, H_3-14), 1.42 (3H, s, H_3-15), 1.86 (3H, s, H_3-12 or −13), 2.03 (3H, s, H_3-13 or −12), 2.42 (H, d, *J* = 16.0 Hz, H-9β), 3.21 (H, d, *J* = 16.0 Hz, H-9α). 53.4 (C-1), 25.2 (C-2), 27.4 (C-3), 82.4 (C-4), 51.7 (C-5), 37.0 (C-6), 134.0 (C-7), 203.0 (C-8), 50.2 (C-9), 73.2 (C-10), 143.7 (C-11), 22.1 (C-12), 22.8 (C-13), 25.0 (C-14), 32.2 (C-15). MS: *Anal*. Calcd for $C_{15}H_{24}O_3$: C, 71.39; H, 9.59. Found: C, 71.65: H, 9.52.
Procurcumenol (**27**) [26, 36]	Viscous oil;$[\alpha]_D^{24}$ = +218.5° (*c* = 0.15, MeOH). UV (MeOH) λ_{max} nm (log ε):248 (3.90), 275 (3.75). IR (cm^{-1}): 3430, 1646. ^{1}H NMR (500 MHz, $CDCl_3$) δ_H: 1.24 (3H, s, H_3-14), 1.75 (3H, s, H_3-13), 1.78 (3H, s, H_3-12), 1.88 (3H, s, H_3-15), 2.18 (1H, dd, *J* = 16.0, 13.0 Hz, H-6α), 2.38 (1H, ddd, J = 10.5, 10.0 Hz, H-1), 2.61 (1H, br d, J = 16.0 Hz, H-6β), 5.88 (1H, br s, H-9). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 50.5 (C-1), 26.9(C-2), 39.9 (C-3), 80.3 (C-4), 53.9 (C-5), 28.6 (C-6), 136.9 (C-7), 199.2(C-8), 129.2 (C-9), 155.1(C-10), 136.3 (C-11), 21.3 (C-12), 22.4(C-13), 23.4 (C-14), 24.3 (C-15). ESI-MS m/z: 235 $[M + H]^+$. $C_{15}H_{20}O_2$. GC MS: RT 29.36 min, 234(M^+, 6.08), 158(35), 121(84), 105(100), 93(60), 43(79).
Isoprocurcumenol (**28**) [26]	Colorless oil; UV (MeOH) λ_{max} nm (log ε): 205 (1.83). IR (CHCl3) cm^{-1}: 3450, 1674, 1610. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 3.22 (1H, q, *J* = 14.68 Hz, H-1), 1.21 (2H, m, H_2-2), 1.39 (2H, m, H_2-3), 1.40 (1H, m, H-5), 2.81 (2H, d, *J* = 14.2 Hz, H_2-6), 2.16 (2H, s, H_2-9), 1.92 (3H, s, H_3-12), 1.82 (3H, s, H_3-13), 1.24 (3H, s, H_3-14), 4.90 (2H, br S, H_2-15). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 51.2 (C-1), 24.7(C-2), 28.2 (C-3), 77.4 (C-4), 58.9 (C-5), 39.8 (C-6), 134.5 (C-7), 203.0 (C-8), 53.8 (C-9), 141.3 (C-10), 143.9 (C-11), 21.9 (C-12), 22.8 (C-13), 24.4 (C-14), 111.6 (C-15). $C_{15}H_{20}O_2$. GC mS: RT 29.36 min, 234 (M^+, 6.08), 158(35), 121(84), 105(100), 93(60), 43(79).
Aerugidiol (**29**)	No data
Zedoalactone B (**30**) [37]	Oil; $[\alpha]_D$ +177.7° (*c* = 0.4, MeOH). UV (MeOH) λ_{max} nm (log ε): 273 (4.33). IR (KBr) cm^{-1}: 3400, 2970, 2940, 2880, 1740, 1660, 1630. ^{1}H NMR (500 MHz, pyridine-d_5) δ_H: 2.06 (1H, ddd, *J* = 8.0, 11.5,

Compound	Physical and spectral data
	13.1 Hz, H-2α), 3.10 (1H, ddd, *J* = 2.0, 9.0, 13.1 Hz, H-2β), 2.15 (1H, ddd, *J* = 2.0, 8.0, 11.5 Hz, H-3α), 2.41 (1H, ddd, *J* = 9.0, 11.5, 11.5 Hz, H-3β), 3.35 (1H, dd, *J* = 3.0, 12.8 Hz, H-5), 3.21 (1H, ddd, *J* = 1.5, 12.8, 17.4 Hz, H-6α), 3.08 (1H, ddd, *J* = 1.5, 3.0, 17.4 Hz, H-6β), 6.09 (1H, s, H-9), 1.71 (3H, d, *J* = 1.5, H_3-13), 1.75 (3H, br s, H_3-14), 1.90 (3H, s, H_3-15), 7.12 (s, 1-OH), 6.22 (s, 4-OH), 6.02 (s, 10-OH). ^{13}C NMR (125 MHz, pyridine-d_5) δ_H: 75.1 (C-1), 35.7(C-2), 41.5(C-3), 79.5(C-4), 50.3 (C-5), 22.0 (C-6), 151.2 (C-7), 148.5 (C-8), 118.8 (C-9), 82.7 (C-10), 125.8 (C-11), 170.2 (C-12), 8.4 (C-13), 23.7(C-14), 26.1 (C-15). EIMS m/z: $[M]^+$ absent, 262 $[M - H_2O]^+$ (13), 244 $[M - 2H_2O]^+$ (33), 226 $[M - 3H_2O]^+$ (100), 211 $[M - 3H_2O - CH_3]^+$ (66). HR-MS, found: $[M\text{-}H_2O]^+$, 262.1195. $C_{15}H_{18}O_4$ requires $[M - H_2O]^+$, 262.1205.
Curcumenone (**31**) [26]	Colorless oil. IR ($CHCl_3$) cm^{-1}: 1679, 1715. UV (MeOH) λmax nm (log ε): 205(1.28). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 0.43 (1H, dt, *J* = 4.56, 7.32 Hz, H-1), 1.64 (2H, q, *J* = 7.32 Hz, H_2-2), 2.47 (2H, t, *J* = 7.36 Hz, H_2-3), 0.67 (1H, q, *J* = 4.56 Hz, H-5), 2.8 (2H, m, H_2-6), 2.52 (2H, d, *J* = 15.6 Hz, H_2-9), 2.07 (3H, s, H_3-12), 1.77 (3H, s, H_3-13), 2.12 (3H, s, H_3-14), 1.10 (3H, s, H_3-15). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 24.1 (C-1), 23.4(C-2), 44.0(C-3), 209.0(C-4), 24.2(C-5), 28.0(C-6), 128.1(C-7), 201.9(C-8), 49.0(C-9), 20.2(C-10), 147.6(C-11), 23.5(C-12), 23.5(C-13), 30.1(C-14), 19.1(C-15). $C_{15}H_{22}O_2$. GC MS: RT 28.9, 234(M^+, 13.5), 176(78), 163(29), 161(48), 149 (43), 133 (37), 107(32), 91(29), 68(91), 67(75), 43(100).
Curcumadione (**32**) [38]	Colorless oil; $[\alpha]_D$ +63.3° (*c* = 0.15, MeOH). UV λ_{max} (EtOH) nm (ε): 207 (1.16). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 1.07 (3H, d, *J* = 6.8 Hz, H_3-15), 1.80, 1.99 (3H each, s, H_3-12, −13), 2.14 (3H, s, H_3-14), 5.52 (1H, t, *J* = 6.6 Hz, H-5). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 140.0 (C-1), 27.8(C-2), 42.6(C-3), 208.1(C-4), 121.1(C-5), 30.2(C-6), 134.7 (C-7), 205.1(C-8), 48.6(C-9), 35.0(C-10), 143.7(C-11), 19.1(C-12), 22.6(C-13), 22.2(C-14), 19.1(C-15). MS m/z: 234.1625 (M^+) (calcd for $C_{15}H_{22}O_2$: 234.1620).
(1S, 10S), (4S, 5S)-Germacrone-1(10), 4(5)-diepoxide (**33**) [6]	^{1}H NMR (500 MHz, $CDCl_3$) δ_H: 2.92 (1H, d, J = 10.8 Hz, H-1), 2.06, 1.46 (m, H_2-2), 2.21, 1.28 (2H, m, H_2-3), 2.65 (1H, dd, J = 10.9, 2.2 Hz, H-5), 2.86 (dd, J = 14.2, 2.2 Hz, H-6a), 2.26 (dd, J = 14.2, 10.8 Hz, H-6b), 3.01 (d, J = 10.8 Hz, H-9a), 2.64 (d, J = 10.8 Hz, H-9b), 1.79 (s, H_3-12), 1.86 (s, H_3-13), 1.14 (s, H_3-14), 1.44 (s, H_3-15). ^{13}C NMR (500 MHz, $CDCl_3$) δ_C: 61.3 (C-1), 22.8 (C-2), 35.7 (C-3), 60.1 (C-4), 64.0 (C-5), 29.2 (C-6), 134.3 (C-7), 207.2 (C-8), 54.5 (C-9), 58.4 (C-10), 137.8 (C-11), 22.9 (C-12), 20.8 (C-13), 15.5 (C-14), 17.3 (C-15).
3,6,10-trimethyl-7,8,11,11-atetrahydrocyclodeca[b]furan-2,5(4H,6H)-dione (**34**) [6]	$[\alpha]_D$ +35.2° (*c* = 0.15, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ_H: 4.92 (1H, br s, H-1), 2.06, 2.20 (2H, m, H_2-2), 1.72, 2.04 (2H, m, H_2-3), 2.44 (1H, m, H-4), 3.36 (2H, m, H_2-6), 4.92 (1H, br s, H-8), 2.04, 2.94 (m, H_2-9), 1.85 (3H, s, H_3-13), 1.09 (3H, d, J = 6.7 Hz, H_3-14), 1.82 (3H, s, H_3-15). ^{13}C NMR (500 MHz, $CDCl_3$) δ_C: 133.4 (C-1), 27.3 (C-2), 35.9 (C-3), 48.0 (C-4), 208.2 (C-5), 41.6 (C-6), 155.2 (C-7), 79.7 (C-8), 46.1 (C-9), 128.9 (C-10), 128.9 (C-11), 173.5 (C-12), 9.2 (C-13), 18.6 (C-14), 16.0 (C-15).
11a-hydroxy-3,6,10-trimethyl-7,8,11,11a-tetrahydrocyclodeca [b]furan-2,5(4H,6H)-dione-methane (**35**) [6]	^{1}H NMR (500 MHz, $CDCl_3$) δ_H: 4.88 (1H, d, J = 10.7 Hz, H-1), 2.00, 2.20 (2H, m, H_2-2), 1.65, 2.10 (2H, m, H_2-3), 2.45 1H, (m, H-4), 3.58 (d, J = 15.4 Hz, H-6a), 3.30 (d, J = 15.7 Hz, H-6b), 2.93 (1H, d, J = 13.4 Hz, H-9a), 2.31 (1H, d, J = 13.4 Hz, H-9b), 1.86 (3H, s, H_3-13), 1.06 (3H, d, J = 6.8 Hz, H_3-14), 1.93 (3H, s, H_3-15). ^{13}C NMR (500 MHz, $CDCl_3$) δ_C: 133.8 (C-1), 27.2 (C-2), 36.0 (C-3), 47.8 (C-4), 209.6 (C-5), 40.2 (C-6), 154.6 (C-7), 106.9 (C-8), 49.7 (C-9), 130.5 (C-10), 129.9 (C-11), 172.3 (C-12), 9.2 (C-13), 18.4 (C-14),

Compound	Physical and spectral data
	16.5 (C-15). HR-ESI-MS: 287.12547[M + Na]$^+$, calcd. For $C_{15}H_{20}O_4Na$ 287.1253802.
Curcumenol (**36**) [19]	Colorless crystals; melting point: 98-100°C. IR (cm^{-1}): 3371, 3321, 1695, 1658, 1274. ^{1}H NMR (600 MHz, $CDCl_3$) δ_H: 1.95 (1H, m, H-1), 1.96 2H, (m, H_2-2), 1.90 (2H, m, H_2-3), 1.93 (1H, m, H-4), 2.11, 2.66 (each, 1H, d, J = 16.9 Hz, H_2-6), 5.77 (1H, s, H-9), 1.60 (3H, s, H_3-12), 1.82 (3H, s, H_3-13), 1.03 (3H, d, J = 6.4 Hz, H-14), 1.67 (3H, s, H-15).^{13}C NMR (151 MHz, $CDCl_3$) δ_C: 51.3 (C-1), 27.6 (C-2), 31.2 (C-3), 40.4 (C-4), 85.7 (C-5), 37.3 (C-6), 137.4 (C-7), 101.5 (C-8), 125.6 (C-9), 139.2 (C-10), 122.2 (C-11), 22.3 (C-12), 18.9 (C-13), 11.8 (C-14), 20.9 (C-15).
Isocurcumenol (**37**) [6]	^{13}C NMR (400 MHz, $CDCl_3$) δ_C: 53.0 (C-1), 28.6 (C-2), 31.0 (C-3), 41.9 (C-4), 87.4 (C-5), 39.2 (C-6), 134.1 (C-7), 104.0 (C-8),36.4 (C-9), 145.4 (C-10), 127.2 (C-11), 22.8 (C-12), 19.2 (C-13), 12.7 (C-14), 112.5 (C-15).
1,4-dihydroxy-1,4-dimethyl-7-(1-methylethylidene) octahydroazulen-6(1H)-one-methane (**38**) [6]	^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 1.16 (s), 1.26 (s), 1.81 (s), 1.89 (s), 1.50-1.80 (m), 2.51 (d, J = 11.7), 2.83 (d, J = 15.6), 2.92 (d, J 11.7). ^{13}C NMR (400 MHz, $CDCl_3$) δ_C: 54.7 (C-1), 21.4 (C-2), 28.0 (C-3), 80.4 (C-4), 50.1 (C-5), 39.9 (C-6), 135.8 (C-7), 205.6 (C-8), 57.3 (C-9), 71.5 (C-10), 140.0 (C-11), 22.0 (C-12), 22.9 (C-13), 22.0 (C-14), 30.0 (C-15).
Zedoalactone A (**39**) [6]	^{1}H NMR (500 MHz, $CDCl_3$) δ_H: 2.71 (1H, m, H-1), 1.85 (1H, m, H-2a), 1.49 (1H, m, H-2b), 1.80 (2H, m, H_2-3), 2.00 (1H, ddd, J = 13.3, 6.6, 3.7 Hz, H-5), 2.71 (1H, m, H-6a), 1.85 (1H, m, H-6b), 4.92 (1H, ddq, J = 6.9, 2.6, 2.0 Hz, H-8), 2.33 (1H, dd, 16.0, 6.9 Hz, H-9a), 2.09 (1H, ddd, J = 16.0, 2.6, 0.7 Hz, H-9b), 1.83 (3H, d, J = 2.0 Hz, H_3-13), 1.34 (3H, s, H_3-14), 1.24 (3H, s, H_3-15). ^{13}C NMR (500 MHz, $CDCl_3$) δ_C: 51.5 (C-1), 24.5 (C-2), 37.1 (C-3), 816 (C-4), 50.8 (C-5), 24.9 (C-6), 161.4 (C-7), 80.8 (C-8), 35.7 (C-9), 73.5 (C-10), 122.5 (C-11), 175.5 (C-12), 8.0 (C-13), 25.0 (C-14), 31.8 (C-15).
5,8-dihydroxy-3,5,8-trimethyl-4a,5,6,7,7a,8,9,9a-octahydroazuleno[6,5-b]furan-2(4H)-one (**40**) [6]	^{1}H NMR (500 MHz, $CDCl_3$) δ_H: 1.97 (1H, m, H-1), 1.82 (m, H-2a), 1.70 (m, H-2b), 1.70 (2H, m, H_2-3), 1.58 (1H, ddd, J = 13.0, 9.0, 2.8 Hz, H-5), 2.30 (1H, dd, J = 15.7, 2.8 Hz, H-6a), 2.06 (1H, dd, J = 14.7 13.3 Hz, H-6b), 5.13 (1H, d, J = 11.2 Hz, H-8), 2.31 (1H, dd, 14.7, 2.7 Hz, H-9a), 1.76 (1H, dd, J = 14.7, 11.3 Hz, H-9b), 1.81 (3H, dd, J = 1.7, 1.7 Hz, H_3-13), 1.28 (3H, s, H_3-14), 1.25 (3H, s, H_3-15). ^{13}C NMR (500 MHz, $CDCl_3$) δ_C: 53.2 (C-1), 23.5 (C-2), 41.2 (C-3), 80.4 (C-4), 48.1 (C-5), 29.8 (C-6), 162.4 (C-7), 79.0 (C-8), 46.3 (C-9), 72.6 (C-10), 122.2 (C-11), 174.2 (C-12), 8.7 (C-13), 23.5 (C-14), 24.0 (C-15).
5,8-dihydroxy-3,5,8-trimethyl-4a,5,6,7,7a,8,9,9a-octahydroazuleno[6,5-b]-furan-2(4H)-one (**41**) [6]	^{1}H NMR (500 MHz, $CDCl_3$) δ_H: 2.86 (1H, dddd, J = 12.3, 7.9, 5.1, 1.4 Hz, H-1), 1.81 (m, H-2a), 1.34 (m, H-2b), 1.72 (2H, m, H_2-3), 2.23 (1H, m, H-5), 2.72 (1H, m, H-6a), 2.23 (1H, m H-6b), 5.28 (1H, dqd, 11.7, 1.8, 1.7 Hz, H-8), 2.28 (1H, ddd, J = 13.7, 3.4, 1.7 Hz, H-9a), 1.68 (1H, dd, 13.7, 11.7 Hz, H-9b), 1.79 (3H, dd, 1.8, 1.4 Hz, H_3-13), 1.40 (3H, s, H_3-14), 1.32 (3H, s, H_3-15). ^{13}C NMR (500 MHz, C_5D_5N) δ_C: 53.1 (C-1), 24.9 (C-2), 37.8 (C-3), 80.7 (C-4), 48.4 (C-5), 24.9 (C-6), 165.4 (C-7), 79.8 (C-8), 41.2 (C-9), 71.2 (C-10), 121.3 (C-11), 174.9 (C-12), 8.8 (C-13), 25.8 (C-14), 32.4 (C-15).
Zedoarolide B (**42**) [6]	^{1}H NMR (400 MHz, C_5D_5N) δ_H: 3.38 (1H, ddd, 3.7, 7.6, 7.6 Hz, H-1), 1.98 (1H, m, H-2a), 1,79 (1H, m, H-2b), 2.08 (1H, m, H-3a), 1.97 (1H, m, H-3b), 2.64 (1H, ddd, J = 3.7, 3.7, 12.8 Hz, H-5), 2.82 (1H, dd, J = 3.7, 12.8 Hz, H-6a), 2.43 (1H, dd, J = 12.8, 12.8 Hz, H-6b), 2.86 (1H, Abq, J = 15.5 Hz, H-9a), 2.80 (1H, Abq, J = 15.5 Hz, H-9b), 1.81 (3H, s, H-13), 1.44 (3H, s, H-14), 1.58 (3H, s, H-15).

Compound	Physical and spectral data
	^{13}C NMR (400 MHz, C_5D_5N) δ_C: 53.1 (C-1), 25.3 (C-2), 38.2 (C-3), 80.7 (C-4), 52.4 (C-5), 24.6 (C-6), 161.5 (C-7), 106.9 (C-8), 44.0 (C-9), 72.1 (C-10), 122.7 (C-11), 173.7 (C-12), 8.0 (C-13), 25.6 (C-14), 32.5 (C-15).
4a,8,9,9a-Tetrahydroxy-3,5,8-trimethyl-4a,5,6,7,7a,8,9,9a-octahydroazuleno[6,5-b]-furan-2(4H)-one (**43**) [6]	^{1}H NMR (400 MHz, C_5D_5N) δ_H: 3.74 (1H, dd, J = 5.0, 3.8 Hz, H-1), 1.77 (2H, m, H_2-2), 2.36 (1H, dddd, J = 11.4, 11.4, 10.7, 6.8 Hz, H-3a), 1.43 (1H, m, H-3a), 2.05 (1H, qd, J = 7.3, 6.8 Hz, H-4), 3.28 (1H, d, J = 15.6 Hz, H-6a), 2.87 (1H, dq, J = 15.6, 1.7, H-6b), 3.99 (1H, s, H-9), 1.87 (3H, d, J = 1.7 Hz, H_3-13), 0.71 (3H, d, J = 7.3 Hz, H_3-14), 1.47 (3H, s, H_3-15). ^{13}C NMR (400 MHz, C_5D_5N) δ_C: 43.3 (C-1), 25.0 (C-2), 33.2 (C-3), 42.9 (C-4), 92.2 (C-5), 32.2 (C-6), 158.8 (C-7), 108.6 (C-8), 81.1 (C-9), 82.0 (C-10), 126.9 (C-11), 172.4 (C-12), 8.7 (C-13), 14.2 (C-14), 19.7 (C-15). MS: m/z 303.12047, $[M - H_2O + Na]^+$. HR-MS 303.12047 $C_{15}H_{22}O_6$.
7-(1-hydroxy-1-methylethyl)-1,4-dimethyl-1,2,3,3a,4,5,8,8a-octahydroazulene-1,4-diol (**44**) [6]	^{1}H NMR (400 MHz, C_5D_5N) δ_H: 3.48 (m, H-1), 1.96 (1H, m, H-2a), 1.78 (1H, m, H-2b), 2.02 (1H, m, H-3a), 1.85 (1H, m, H-3b), 2.41 (dd, J = 12.8, 4.9 Hz, H-5), 2.52 (1H, d, J = 13.9 Hz, H-6a), 2.15 (1H, dd, J = 13.9, 12.8 Hz, H-6b), 6.16 (1H, br dd, J = 8.4, 5.2 Hz, H-8), 2.78 (91H, J = 14.2, 5.2 Hz, H-9a), 2.27 (1H, dd, J = 14.2, 8.4 Hz, H-9b), 1.57 (3H, s, H_3-12), 1.57 (3H, s, H_3-13), 1.62 (3H, s, H_3-14), 1.36 (3H, s, H_3-15). ^{13}C NMR (400 MHz, C_5D_5N) δ_C: 54.2 (C-1), 25.5 (C-2), 37.5 (C-3), 80.9 (C-4), 49.4 (C-5), 26.2 (C-6), 150.9 (C-7), 118.8 (C-8), 35.4 (C-9), 70.6 (C-10), 72.7 (C-11), 29.2 (C-12), 29.4 (C-13), 26.3 (C-14), 31.6 (C-15).
Gajutsulactone B (**45**) [6]	^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 2.88 (ddd, 6.4, 6.4, 9.8 Hz, H-1), 2.06 (1H, m, H-2a), 1.86 (1H, m, H-2b), 1.90 (m, H-3), 2.30 (m, H-5), 2.50 (d, H-6a), 2.24 (d, H-6b), 5.01 (br s, H-9a), 4.84 (br s, H-9b), 2.18 (3H, s, H-12), 1.86 (3H, s, H-13), 1.78 (3H, s, H-14), 1.22 (3H, s, H-15). ^{13}C NMR (300 MHz, $CDCl_3$) δ_C: 42.4 (C-1), 26.2 (C-2), 38.0 (C-3), 85.3 (C-4), 45.7 (C-5), 25.7 (C-6), 120.4 (C-7), 167.5 (C-8), 111.9 (C-9), 145.2 (C-10), 151.8 (C-11), 23.3 (C-12), 23.5 (C-13), 25.2 (C-14), 19.9 (C-15).
Bisacumol (**46**) [6]	^{13}C NMR (300 MHz, $CDCl_3$) δ_C: 144.2 (C-1), 127.2 (C-2), 129.3 (C-3), 135.7 (C-4), 129.3 (C-5), 127.2 (C-6), 36.1 (C-7), 46.1 (C-8), 67.1 (C-9), 128.6 (C-10), 135.0 (C-11), 18.4 (C-12), 26.0 (C-13), 23.3 (C-14), 21.3 (C-15).
7-isopropenyl-1,4a-dimethyldecahydronaphthalene-1,4-diol (**47**) [6]	^{1}H NMR (500 MHz, $CDCl_3$) δ_H: 3.27 (1H, dd, 12.7, 4.2 Hz, H-1), 1.87 (1H, m, H-2a), 1.62 (1H, m, H-2b), 1.72 (1H, m, H-3a), 1.5 (1H, ddd, J = 14.1, 14.1, 4.5 Hz, H-3b), 1.07 (1H, dd, 12.4, 2.6 Hz, H-5), 1.68 (1H, m, H-6a), 1.94 (1H, m, H-6b), 1.62 (1H, m, H-8a), 1.45 (1H, m, H-8b), 1.87 (1H, m, H-9a), 1.11 (1H, dd, J = 13.2, 3.7 Hz, H-9b),1.76 (3H, s, H_3-12), 4.74 1H, (H-13, Z), 4.71 (1H, H-13, E), 1.16 (3H, s, H_3-14), 1.05 (3H, s, H_3-15). ^{13}C NMR (500 MHz, $CDCl_3$) δ_C: 79.7 (C-1), 26.8 (C-2), 39.4 (C-3), 71.4 (C-4), 50.4 (C-5), 25.6 (C-6), 46.1 (C-7), 26.4 (C-8), 39.3 (C-9), 38.9 (C-10), 150.5 (C-11), 20.7 (C-12), 108.6 (C-13), 30.0 (C-14), 12.6 (C-15).
(1S,4S,5S,10R)-isozedoarondiol (**48**) [19]	Yellow oil. IR (cm^{-1}): 3394, 1701, 1665, 1612. ^{1}H NMR (600 MHz, $CDCl_3$) δ_H: 2.79 (1H, m, H-1), 1.63 (2H, m, H_2-2), 1.73 (2H, m, H_2-3), 2.02(1H, d, J = 12.9 Hz, H-5), 2.52, 1.91 (each, 1H, d, J = 13.9 Hz, H_2-6), 2.30 (1H, dd, J = 16.1, 1.2 hz, H-9a), 3.34 (d, J = 16.1 Hz, H-9b), 1.97 (3H, s, H_3-12), 1.88 (3H, s, H_3-13), 1.39 (3H, d, J = 6.4 Hz, H-14), 1.19 (3H, s, H-15).^{13}C NMR (151 MHz, methanol-d_4) δ_C: 51.2 (C-1), 24.6 (C-2), 36.0 (C-3), 81.7 (C-4), 52.6 (C-5), 27.0 (C-6), 134.0 (C-7), 204.7 (C-8), 49.9 (C-9), 72.5 (C-10), 143.0 (C-11), 20.9 (C-12), 21.8 (C-13), 23.4 (C-14), 31.3 (C-15).

Table 1.
Physical and spectral data of sesquiterpenes.

Sample/extract	Biological activity	References
Hexand and DCM	Nematocidal	[39]
EtOAc	Choleretic	[40]
Crude protein	Antioxidant	[41]
EtOH	Antibacterial	[42]
Hexane and EtOH	Anti-inflammatory	[43]
Hexane, EtOAc, and *n*-butanol	Antifungal	[6]
Zedoarondiol (**25**)	Cytotoxic	[19]
(1S, 10S), (4S, 5S)-Germacrone-1(10), 4(5)-diepoxide (**33**)	Cellular viability	[6]
Curcumenol (**36**)	Cytotoxic	[19]
(1S,4S,5S,10R)-isozedoarondiol (**48**)	Cytotoxic	[19]

Table 2.
Biological activities of extracts and compounds from Curcuma comosa.

this chapter, the extraction, isolation, and spectroscopic data of sesquiterpenes from *Curcuma comosa* have been discussed.

Author details

Khun Nay Win Tun[1,2], Nanik Siti Aminah[1*], Alfinda Novi Kristanti[1], Hnin Thanda Aung[3] and Yoshiaki Takaya[4]

1 Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga, Komplek Kampus C UNAIR, JL. Mulyorejo, Surabaya, Indonesia

2 Department of Chemistry, Pathein University, Pathein, Myanmar

3 Department of Chemistry, Mandalay University, Mandalay, Myanmar

4 Faculty of Pharmacy, Meijo University, Tempaku, Nagoya, Japan

*Address all correspondence to: nanik-s-a@fst.unair.ac.id

References

[1] Krings U, Berger RG. Terpene bioconversion—How does its future look? Natural Product Communications. 2010;**5**(9):1507-1522. DOI: 10.1177/1934578X1000500927

[2] Keeratinijakal V, Kongkiatpaiboon S. Distribution of phytoestrogenic diarylheptanoids and sesquiterpenoids components in *Curcuma comosa* rhizomes and its related species. Revista Brasileira de Farmacognosia. 2017;**27**(3):290-296. DOI: 10.1016/j.bjp.2016.12.003

[3] Matsumoto T, Nakamura S, Fujimoto K, Ohta T, Ogawa K, Yoshikawa M, et al. Structure of diarylheptanoids with antiallergic activity from the rhizomes of *Curcuma comosa*. Journal of Natural Medicines. 2014;**69**(1):142-147. DOI: 10.1007/s11418-014-0870-8

[4] Perveen S. Introductory Chapter: Terpenes and Terpenoids. London: IntechOpen; 2018. pp. 1-12. DOI: 10.5772/intechopen.79683

[5] De las Heras B, Rodríguez B, Boscá L, Villar AM. Terpenoids: sources, structure elucidation and therapeutic potential in inflammation. Current Topics in Medicinal Chemistry. 2003;**3**(2):171-185. DOI: 10.2174/1568026033392462

[6] Khine MM. Isolation and characterization of phytoconstituents from Myanmar medicinal plants [Dissertation]. Mathematisch-Naturwissenschaftlich-Technischen Fakultät (mathematisch-naturwissenschaftlicher Bereich) der Martin-Luther-Universität Halle-Wittenberg. Germany; 2006

[7] Zhang QW, Lin LG, Ye WC. Techniques for extraction and isolation of natural products: A comprehensive review. Chinese Medicine. 2018;**13**(20):1-26. DOI: 10.1186/s13020-018-0177-x

[8] Bucar F, Wube A, Schmid M. Natural product isolation-how to get from biological material to pure compounds. Natural Product Reports. 2013;**30**(4):525-545. DOI: 10.1039/c3np20106f

[9] Sawaya ACHF, Souza KS, Marcucci MC, Cunha IBS, Shimizu MT. Analysis of the composition of Brazilian propolis extracts by chromatography and evaluation of their in vitro activity against gram-positive bacteria. Brazilian Journal of Microbiology. 2004;**35**(1-2):104-109. DOI: 10.1590/s1517-83822004000100017

[10] Sarker SD, Nahar L. An introduction to natural products isolation. Methods in Molecular Biology. 2012;**864**:1-25. DOI: 10.1007/978-1-61779-624-1_1

[11] Coskun O. Separation techniques: Chromatography. Northern Clinics of Istanbul. 2016;**3**(2):150-160. DOI: 10.14744/nci.2016.32757

[12] De Castro Vasconcellos P, da Rocha GO, Caramão EB, Machado ME, Krause LC. Chromatographic techniques for organic analytes. Comprehensive Analytical Chemistry. Netherlands: Elsevier; 2015. pp. 267-309. DOI: 10.1016/bs.coac.2015.09.009

[13] Majik MS, Gawas UB, Mandrekar VK. Analytical methods for natural products isolation. In: Advances in Biological Science Research. Elsevier; 2019. pp. 395-409. DOI: 10.1016/b978-0-12-817497-5.00024-0

[14] Silverstein RM, Webster FX, Kiemle D. Spectrometric Identification of Organic Compounds. 7th ed. Hoboken, New Jersey: John Wiley and Sons, Inc; 2005. p. 512

[15] Socrates G. Infrared and Raman Characteristic Group Frequencies: Tables and Charts. 3rd ed. Hoboken,

New Jersey: John Wiley and Sons, Ltd; 2004. p. 366

[16] Roberts J, Power A, Chapman J, Chandra S, Cozzolino D. The use of UV-Vis spectroscopy in bioprocess and fermentation monitoring. Fermentation. 2018;**4**(1):18. DOI: 10.3390/fermentation4010018

[17] Xu F, Nakamura S, Qu Y, Matsuda H, Pongpiriyadacha Y, Wu L, et al. Structures of new sesquiterpenes from *Curcuma comosa*. Chemical & Pharmaceutical Bulletin (Tokyo). 2008; **56**(12):1710-1716. DOI: 10.1248/cpb.56.1710

[18] Qu Y, Xu F, Nakamura S, matsuda H, Pongpiriyadacha Y, Wu L, et al. Sesquiterpenes from *Curcuma comosa*. Journal of Natural Medicines. 2009; **63**(1):102-104. DOI: 10.1007/s11418-008-0282-8

[19] Tun KNW, Aminah NS, Kristanti AN, Ramadhan R, Takaya Y, Aung HT. Isolation of cytotoxic sesquiterpenes from *Curcuma comosa* and characterization of their structures. Journal of the Indian Chemical Society. 2019;**96**:1513-1517

[20] Hamdi OA, Anouar el H, Shilpi JA, Trabolsy ZBKA, Zain SBM, Zakaria NSSZ, et al. A quantum chemical and statistical study of cytotoxic activity of compounds isolated from *Curcuma zedoaria*. International Journal of Molecular Sciences. 2015;**16**(5): 9450-9468. DOI: 10.3390/ijms16059450

[21] Kawabata J, Fukushi Y, Tahara S, Mizutani J. Structures of novel sesquiterpene ketones from *Chloranthus serratus* (Chloranthaceae). Agricultural and Biological Chemistry. 1985;**49**(5): 1479-1485. DOI: 10.1080/00021369.1985.10866891

[22] Wang LJ, Xiong J, Liu ST, Liu XH, Hu JF. Sesquiterpenoids from *Chloranthus henryi* and their anti-neuroinflammatory activities. Chemistry and Biodiversity. 2014;**11**(6): 919-928. DOI: 10.1002/cbdv.201300283

[23] Chokchaisiri R, Pimkaew P, Piyachaturawat P, Chalermglin R, Suksamrarn A. Cytotoxic sesquiterpenoids and diarylheptanoids from the rhizomes of *Curcuma elata* Roxb. Record of Natural Products. 2014; **8**(1):46-50

[24] Kawabata J, Fukushi Y, Tahara S, Mizutani J. Structure of novel sesquiterpenes alcohols from *Chloranthus japonicas* (Chloranthaceae). Agricultural and Biological Chemistry. 1984;**48**(3):713-717. DOI: 10.1080/00021369.1984.10866207

[25] Hikino H, Konno C, Agatsuma K, Takemoto T, Horibe I, Tori K, et al. Sesquiterpenoids. Part XLVII. Structure, configuration, conformation, and thermal rearrangement of furanodienone, isofuranodienone, curzerenone, epicurzerenone, and pyrocurzerenone, sesquiterpenoids of *Curcuma zedoaria*. Journal of the Chemical Society, Perkin Transactions 1. 1975;**5**:478-484. DOI: 10.1039/p19750000478

[26] Hamdi OAA, Ye LJ, Kamarudin MNA, Hazni H, Paydar M, Looi CY, et al. Neuroprotective and antioxidant constituents from *Curcuma zedoaria* rhizomes. Record of Natural Products. 2015;**9**(3):349-355

[27] Kuroyanagi M, Ueno A, Ujiie K, Sato S. Structures of sesquiterpenes from *Curcuma aromatica* Salisb. Chemical and Pharmaceutical Bulletin. 1987;**35**(1):53-59. DOI: 10.1248/cpb.35.53

[28] Yan J, Chena G, Tong S, Feng Y, Sheng L, Lou J. Preparative isolation and purification of germacrone and curdione from the essential oil of the rhizomes of *Curcuma wenyujin* by high-speed counter-current chromatography.

Journal of Chromatography A. 2005; **1070**(1-2):207-210. DOI: 10.1016/j.chroma.2005.02.064

[29] Park JH, Mohamed MA, Jung YJ, Shrestha S, Lee TH, Lee CH, et al. Germacrane sesquiterpenes isolated from the rhizome of *Curcuma xanthorrhiza* Roxb. inhibit UVB-induced upregulation of MMP-1, -2, and -3 expression in human keratinocytes. Archives of Pharmcal Research. 2015; **38**(10):1752-1760. DOI: 10.1007/s12272-014-0525-z

[30] Yang FQ, Wang HK, Chen H, Chen JD, Xia ZN. Fractionation of volatile constituents from Curcuma rhizome by preparative gas chromatography. Journal of Automated Methods & Management in Chemistry. 2011;**2011**:942467. DOI: 10.1155/2011/942467

[31] Boutsada P, Giang VH, Linh TM, Mai NC, Cham PT, Hanh TTH, et al. Sesquiterpenoids from the rhizomes of *Curcuma aeruginosa*. Vietnam Journal of Chemistry. 2018;**56**(6):721-725. DOI: 10.1002/vjch.201800077

[32] Syu WJ, Shen CC, Don MJ, Ou JC, Lee GH, Sun CM. Cytotoxicity of curcuminoids and some novel compounds from *Curcuma zedoaria*. Journal of Natural Products. 1998; **61**(12):1531-1534. DOI: 10.1021/np980269k

[33] Shimizu Y, Imayoshi Y, Kato M, Maed K, Iwabuchi H, Shimomura K. New eudesmane-type sesquiterpenoids and other volatile constituents from the roots of Gynura bicolor DC. Flavour and Fragrance Journal. 2011;**26**(1):55-64. DOI: 10.1002/ffj.2016

[34] Konig WA, Bolow N, Fricke C, Melching S, Rieck A, Mijhle H. The sesquiterpene constituents of the liverwort *Preissia quadrata*. Phytochemistry. 1996;**43**(3):629-633. DOI: 10.1016/0031-9422(96)00354-8

[35] Ellithey MS, Lall N, Hussein AA, Meyer D. Cytotoxic, cytostatic and HIV-1 PR inhibitory activities of the soft coral *Litophyton arboreum*. Marine Drugs. 2013;**11**(12):4917-4936. DOI: 10.3390/md11124917

[36] Chen JJ, Tsai TH, Liao HR, Chen LC, Kuo YH, Sung PJ, et al. New sesquiterpenoids and anti-platelet aggregation constituents from the rhizomes of *Curcuma zedoaria*. Molecules. 2016;**21**(10):1385. DOI: 10.3390/molecules21101385

[37] Takano I, Yasuda I, Takeya K, Itokawa H. Guaiane sesquiterpene lactones from *Curcuma aeruginosa*. Phytochemistry. 1995;**40**(4):1197-1200. DOI: 10.1016/0031-9422(95)00425-7

[38] Kuroyanagi M, Ueno A, Koyama K, Natori S. Structures of sesquiterpenes of *Curcuma* aromatic Salisb. II. Studies on Minor Sesquiterpenes. 1990;**38**(1):55-58. DOI: 10.1248/cpb.38.55

[39] Jurgens TM, Frazier EG, Schaeffer JM, Jones TE, Zink DL, Borris RP. Novel nematocidal Agents from *Curcuma comosa*. Journal of Natural Products. 1994;**57**(2):230-235. DOI: 10.1021/np50104a006

[40] Piyachaturawat P, Gansar R, Suksamrarn A. Choleretic effect of *Curcuma comosa* rhizome extracts in rats. International Journal of Pharmacognosy. 1996;**34**(3):174-178. DOI: 10.1076/phbi.34.3.174.13204

[41] Boonmee A, Srisomsap C, Karnchanatat A, Sangvanich P. An antioxidant protein in *Curcuma comosa* Roxb. rhizomes. Food Chemistry. 2011; **124**(2):476-480. DOI: 10.1016/j.foodchem.2010.06.057

[42] Li T, Zhang D, Oo TN, San MM, Mon AM, Hein PP, et al. Investigation on the antibacterial and anti-T3SS activity of traditional Myanmar medicinal plants. Evidence-based

Complementary and Alternative Medicine. 2018;**2018**:2812908. DOI: 10.1155/2018/2812908

[43] Sodsai A, Piyachaturawat P, Sophasan S, Suksamrarn A, Vongsakul M. Suppression by *Curcuma comosa* Roxb. of pro-inflammatory cytokine secretion in phorbol-12-myristate-13-acetate stimulated human mononuclear cells. International Immunopharmacology. 2007;7(4): 524-531. DOI: 10.1016/j.intimp.2006.12.013

Chapter 8

Effects of Terpenes and Terpenoids of Natural Occurrence in Essential Oils on Vascular Smooth Muscle and on Systemic Blood Pressure: Pharmacological Studies and Perspective of Therapeutic Use

Ana Carolina Cardoso-Teixeira, Klausen Oliveira-Abreu, Levy Gabriel de Freitas Brito, Andrelina Noronha Coelho-de-Souza and José Henrique Leal-Cardoso

Abstract

Terpenes are a class of chemical compounds with carbon and hydrogen atoms in their structure. They can be classified into several classes according to the quantity of isoprene units present in its structure. Terpenes can have their structure modified by the addition of various chemical radicals. When these molecules are modified by the addition of atoms other than carbon and hydrogen, they become terpenoids. Terpenes and terpenoids come from the secondary metabolism of several plants. They can be found in the leaves, fruits, stem, flowers, and roots. The concentration of terpenes and terpenoids in these organs can vary according to several factors such as the season, collection method, and time of the day. Several biological activities and physiological actions are attributed to terpenes and terpenoids. Studies in the literature demonstrate that these molecules have antioxidant, anticarcinogenic, anti-inflammatory, antinociceptive, antispasmodic, and antidiabetogenic activities. Additionally, repellent and gastroprotective activity is reported. Among the most prominent activities of monoterpenes and monoterpenoids are those on the cardiovascular system. Reports on literature reveal the potential effect of monoterpenes and monoterpenoids on systemic blood pressure. Studies show that these substances have a hypotensive and bradycardic effect. In addition, the inotropic activity, both positive and negative, of these compounds has been reported. Studies also have shown that some monoterpenes and monoterpenoids also have a vasorelaxing activity on several vascular beds. These effects are attributed, in many cases to the blocking of ion channels, such as voltage-gated calcium channels. It can also be observed that monoterpenes and

monoterpenoids can have their effects modulated by the action of the vascular endothelium. In addition, it has been shown that the molecular structure and the presence of chemical groups influence the potency and efficacy of these compounds on vascular beds. Here, the effect of several monoterpenes and monoterpenoids on systemic blood pressure and vascular smooth muscle will be reported.

Keywords: terpenes, terpenoids, arterial pressure, pharmacological effect, toxicity, perspective of therapeutic use, anti-hypertensive

1. Introduction

1.1 Terpenes and terpenoids

Terpenes and terpenoids are names frequently interchangeably used. Most frequently terpene is defined as a hydrocarbon with one or several isoprene units. When terpene molecules are modified by the addition of atoms other than carbon and hydrogen, they are more appropriately named terpenoids [1].

Terpenes and terpenoids come from the secondary metabolism of several plants. They can be found in the leaves, fruits, stem, flowers, and roots [2]. They can be classified into several classes according to several criteria: 1—the quantity of isoprene units present in its structure, among which we can mention the monoterpenes, diterpenes, sesquiterpenes, and others; 2—the number of cyclic components in their molecular structure, according to which we have the acyclic, monocyclic, and bicyclic monoterpenes [1, 3, 4].

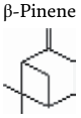
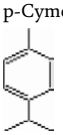
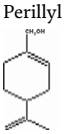
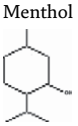
Studies in the literature demonstrate that the natural (present in essential oils—EO) monoterpenes and monoterpenoids have one or several biological/pharmacological activities, among which the most frequently reported are antioxidant, anticarcinogenic, anti-inflammatory, repellent, gastroprotective, antinociceptive, antispasmodic, and antidiabetogenic activities [4, 5].

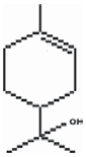
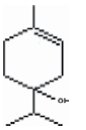
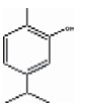
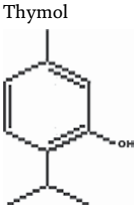
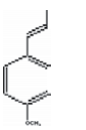
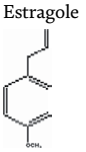
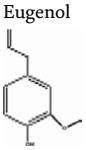
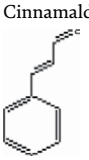
Among the most prominent and therapeutically potentially promising activities of natural monoterpenes and monoterpenoids are those on the cardiovascular system [6]. Reports on literature, here reviewed and discussed, reveal their effect on heart (rate and inotropism), systemic blood pressure (SBP), and blood vessels (direct (myogenic) and indirect (endothelium mediated)) [7–10].

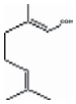
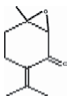
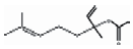
These effects are frequently attributed to activity on ion channels, such as voltage-dependent Ca^{2+} channels (VDCC) [11]. These substances can affect the contractions mediated by electromechanical excitation-contraction coupling (EMC; ex.: the KCl-induced contraction) or pharmacomechanical excitation-contraction coupling (PMC; ex.: the Phenylephrine-induced contraction). In addition, it has been shown that the molecular structure and the presence of chemical groups influence the potency (pharmacodynamic potency) and efficacy (pharmacodynamic efficacy) of these compounds on vascular beds [10]. Here we will call simply maximum efficacy when, at appropriate concentration, total blockade of a response is induced (it is: complete blockade of contraction or Emax = 100%).

Here, we described the effects of monoterpenes and monoterpenoids on SBP and vascular smooth muscle (VSM). This research was carried out predominantly using articles present in the Pubmed and Pubchem databases. The search included articles published between 2000 and 2020. The search included 42 monoterpenes and monoterpenoids. The words used in the research include “monoterpenes”, “monoterpenoids” and the name of the compounds associated with “cardiovascular”, “vasorelaxant”, “hypotension”, “hypotensive”, “antihypertensive”, “vascular

smooth muscle", or "toxicity". For easiness of posterior consultation, we presented the information related to a substance under a heading, which was the common name of the substance and organized the most important information on a table

Substance	Pharmacological cardiovascular activities	Dose (mg/kg)/ concentration (μM)	Ref.	LD_{50}	Plant source
Limonene 	Hypotensive	20–40 mg/kg[A]	[12]	4.4–5.1 g/kg (v.o—rats); 5.6–6.6 g/kg (v.o—mice) [13]	*Citrus limon*, *Lippia alba*
	Vasorelaxant	941.6 ± 28.02 μM[B] 2159.1 ± 203.62 μM[C]	[10]		
α-Pinene 	Hypotensive	1–20 mg/kg[A]	[14]	>2.0 g/kg (v.o.—mice) [15]	*Eucalyptus tereticornis*, *Citrus lemon*
β-Pinene 	Hypotensive	1–20 mg/kg[A]	[14]	>2.0 g/kg (v.o.—mice) [15]	*Eucalyptus tereticornis*, *Citrus lemon*
p-Cymene 	Vasorelaxant	$5.8 \pm 1.6 \times 10^{-5}$ M[C]	[16]	4.7 g/kg (v.o—rat); >5 g/kg (v.o —rabbit) [17]	*Eucalyptus camaldulensis*, *Origanum acutidens*
Citronellol 	Hypotensive	1–20 mg/kg	[14, 18, 19]	3.45 g/kg (v.o —rats) [20]	*Cymbopogon winterianus*, *Lippia alba*
	Vasorelaxant [18]	6.4×10^{-4} to 1.9 M	[18]		
Geraniol 	Vasorelaxant [21]	10–300 μM [A]	[21]	3.6 g/kg (v.o. —rats)[22]	*Cymbopogon martinii*, *C. nardus*, *C. winterianus*.
Linalool 	Hypotensive (normotensive animals)	1–20 mg/kg	[14, 23]	2.7 g/kg (v.o —rats) [24]	*Lavandula angustifólia*, *Ocimum basilicum*, *Citrus bergamia*
	Hypotensive (hypertensive animals)	100–200 mg/kg	[23, 25]		
	Vasorelaxant [23]	6.4×10^{-6}–6.4×10^{-3} M	[23]		
Perillyl alcohol 	Vasorelaxant[10]	277.7 ± 5.46 μM[B], 443.3 ± 66.83 μM[C]	[10]	2.1 g/kg (v.o —rats) [26]	*Dracocephalum kotschyi*
	Vasorelaxant	1–2 mM[A]	[27]		
Carveol 	Vasorelaxant	662.1 ± 32.85 μM[B]; 1333.3 ± 225.20 μM[C]	[10]	3.0 g/kg (v.o. —rats) [28]	*Citrus reticulata*, *Anethum graveolens*
Menthol 	Antihypertensive	Diet plus 0.5% menthol	[29, 30]	2.9–6 g/kg (v. o—mice and rats) [31, 32]	*Mentha* genus
	Vasorelaxant	100–500 mM[A]	[33]		

Substance	Pharmacological cardiovascular activities	Dose (mg/kg)/ concentration (μM)	Ref.	LD_{50}	Plant source
α-Terpineol 	Hypotensive	1–30 mg/kg[A]	[34]	3.0 g/kg (v.o.—mice) [35]	*Eucalyptus camaldulensis*, *Croton nepetaefolius*.
	Vasorelaxant	$10^{-12} - 10^{-5}$ M	[34]		
	Vasorelaxant	300 μg/ml (1.94 mM)	[36]		
Terpinen-4-ol 	Hypotensive	1–10 mg/kg[A], i.v.	[7, 37]	4.3 g/kg (v.o—rats) [32]	*Alpinia zerumbet*, *Croton sonderianus*
	Vasorelaxant	421.43 ± 23.48 μM[B]; 802.50 ± 13.8 μM[C]	[9]		
Borneol 	Vasorelaxant	3×10^{-9}–3×10^{-4} M[B]	[38, 39]	6.5 g/kg (v.o—rats) [40]	*Salvia officinalis*, *Cinnamomum camphora*
Carvacrol 	Hypotensive	100 μg/kg, i.p	[41]	0.8 g/kg (v.o—rats) [42]	*Thymus vulgaris*, *Origanium compactum*, *Lippia sidoides*
	Hypotensive	1–20 mg/kg i.v.	[43]		
	Vasorelaxant	78.80 ± 11.91 μM[B]; 145.40 ± 6.07 μM[C]	[44]		
	Vasorelaxant	10^{-8}–3×10^{-4} M	[43]		
Thymol 	Vasorelaxant	64.40 ± 4.41 μM[B]; 106.40 ± 11.37 μM[C]	[44]	0.9 g/kg (v.o—rats) [44]	*Acalypha phleoides*, *Lippia sidoides*, *L. origanoides*
Anethole 	Antihypertensive	125–250 mg/kg[A]	[45]	<3.0 g/kg (v.o—rats) [46, 17]	*Pimpinella anisum*, *Croton zehntneri*, *Foeniculum vulgare*
	Hypotensive	5–10 mg/kg, i.v.[A]	[47]		
	Vasorelaxant	$9.01 \pm 2.44 \times 10^{-4}$ M[C]	[48]		
Estragole 	Hypotensive	5–10 mg/kg, i.v.[A]	[47]	1.8 g/kg (v.o—rats) [32]	*Croton Zehntneri*, *Ocimum basilicum*, *Artemisia dracunculus*
	Vasorelaxant	$4.34 \pm 0.3 \times 10^{-4}$ M[C]	[48]		
Eugenol 	Hypotensive	1–10 mg/kg, i.v.[A]	[49–51]	2.6 g/kg (v.o—rats) [17]	*Croton zehntneri*, *Ocimum gratissimum*
	Vasorelaxant	0.31 ± 0.05 mM	[49]		
	Vasorelaxant	323.3 ± 14.0 μMB	[11]		
Cinnamaldehyde 	Vasorelaxant	334 ± 30 μM	[52]	2.22 g/kg (v.o—rats) [17]	*Cinnamomum osmophloeum*, *C. zeylanicum*

Substance	Pharmacological cardiovascular activities	Dose (mg/kg)/ concentration (μM)	Ref.	LD_{50}	Plant source
Citral 	Vasorelaxant	110.80 μg/ml (727.8 μM)[B], 99.34 μg/ml (652.56 μM)[C]	[53, 54]	4.9 g/kg (v.o.—rats) [17]	*Lippia alba e Pectis brevipedunculata*
Citronellal 	Antihypertensive	200 mg/kg, v.o.	[55]	2.42 g/kg (v.o.—rats) [56]	*Cymbopogon winterianus*; *Cymbopogon citrates*
	Hypotensive	10–40 mg/kg, i.v.	[55]		
	Vasorelaxant	10^{-6} - 10^{-1} M	[55]		
Carvone 	Vasorelaxant	$6.2 \pm 2.6 \times 10^{-4}$ M[C]	[57]	1.6 g/kg (v.o.—rats) [17]	*Mentha spicata*, *Carum carvi*
Rotundifolone 	Hypotensive	1–30 mg/kg, i.v.	[58]	Not available (for mammals)	*Mentha rotundifolia*, *M. spicata L.*, *and M. x villosa*
	Vasorelaxant	184 (1.1 mM)[B] and 185 (1.11 mM)[C] μg/ml	[58, 59]		
	Vasorelaxant	pD2 = 4.0	[60]		
1,8-cineole 	Antihypertensive	0.1 mg/kg, i.p.	[61]	2.48 g/kg (rat, v.o); >5 g/kg (v.o, rabbit) [17]	*Croton nepetaefolius*; *Alpinia zerumbet*
	Hypotensive	0.3–10 mg/kg, i.v.	[62]		
	Vasorelaxant	1.09 mM[B], 663.2 μg/ml (4.22 mM)[C]	[62, 63]		
Linalyl acetate 	Antihypertensive	10–100 mg/kg, i.p.	[64]	10.0 g/kg (v.o—rats); 13.3 g/kg (v.o—mice) [24]	*Lavandula angustifolia* and *Salvia sclarea*
	Hypotensive	10–100 mg/kg	[64–66]		
	Vasorelaxant	3.6×10^{-4} M[C]	[67]		

Ref., Reference. ip, Intraperitoneally. v.o, Orally. i.v., Intravenous.
[A]Range of doses or concentration employed.
[B]IC_{50} for KCl-induced contraction (electromechanical coupling) in presence of endothelium.
[C]IC_{50} for phenilephrine-induced contraction (pharmacomechanical coupling) in presence of endothelium.

Table 1.
Monoterpenes and monoterpenoids with hypotensive and vasorelaxant effects.

(**Table 1**). In order to allow some basis for evaluation of the therapeutic potential of these compounds, we include information on toxicity (LD_{50} values in mammals; **Table 1**).

2. Monoterpenes

Monoterpenes are compounds with two isoprene units in their structure. They can be subdivided according to the number of cycle components in its structure into acyclic, monocyclic, and bicyclic [68, 69]. Of the natural monoterpenes studied, we have not found, in any publications, report of cardiovascular effects for myrcene, ocimene (acyclic), terpinenes, phellandrenes, terpinolene, thujene (monocyclic) and, −3-carene, camphene, sabinene (bicyclic), and tricyclene on SBP and VSM. However, several studies in the literature demonstrate that EO containing these compounds have interesting cardiovascular effects.

2.1 Limonene

Limonene (LM) is one of the most common monoterpenes on nature. Studies have demonstrated that it has low toxicity and have suggested its promising effect [13]. The LM had a dose-dependent hypotensive effect, associated with bradycardia in rats (**Table 1**). LM is also reported to cause delayed ventricular relaxation and negative inotropism. It has been suggested that these effects are due to an action of LM on VDCC [12]. In spontaneously hypertensive rats (SHR) with cerebral ischemia, LM attenuated the elevation of the blood pressure of the animals [70].

In rat aorta, the LM promoted a marked vasorelaxing effect when administered in presence of the contractions induced by a solution with a high concentration of K^+ or phenylephrine (PHE). The IC_{50} values were dependent on the endothelium and maximum efficacy was documented for both types of contraction. The potency in endothelium-intact arteries was greater in EMC than in PMC. LM was also able to relax the contraction induced by BayK8644, a VDCC activator [71], effect in which the LM presented the greatest potency, suggesting a possible effect of this monoterpene on VDCCs [10].

2.2 Pinene

α- and β-pinene are two isomeric bicyclic monoterpenes [72] which, in awake rats, induced arterial hypotension and tachycardia. (−)-β-pinene was significantly more effective than (+)-α-pinene. The authors suggested that the exocyclic double bond of (−)-β-pinene contributes more to the pharmacological effect than the endocyclic double bond of (+)-α-pinene. They explained tachycardia as a reflex response to the hypotension [14].

2.3 p-cimene

P-cymene is a monocyclic monoterpene that in rat's aorta showed a reversible vasorelaxant effect, with maximum efficacy and in a concentration-dependent manner. This effect, independent of the endothelium, indicated a myogenic effect. Additionally, the participation of K^+ channels in the vasorelaxant effect of p-cymene has been suggested [16].

3. Monoterpenoids

Monoterpenoids are compounds found in several plant species (**Table 1**). Concerning their chemical functions, they can be: alcohols, phenolics, phenylpropanoids, aldehydes, ketones, ethers, or esters. For the following natural monoterpenoids, no studies were found that described effect on SBP and VSM: lavandulol, fenchol, chrysanthenol and nerol (alcohols); apiol, myristicin and safrole (phenylpropanoids).

3.1 Alcohols

3.1.1 Citronellol

Citronellol is a low toxicity acyclic monoterpenoid [20]. Regarding hemodynamic parameters, citronellol (1–20 mg/kg, i.v.) is reported to induce hypotension associated with tachycardia in non-anesthetized rats [18]. This hypotensive effect was interpreted to occur probably due to a direct vasorelaxant action of citronellol

in VSM without the participation of the NO and cyclooxygenase (COX) pathway [18]. In another study, in anesthetized and awake rats, citronellol (1–20 mg/kg, i.v.) also had a hypotensive effect, but associated with bradycardia. As a probable cause of this discrepancy, the chirality of the compound was suggested [19].

In rat mesenteric arteries, citronellol had an endothelium-independent vasorelaxing effect. On the contraction induced by PHE ($IC_{50} \sim 130$ μM) and KCl, the effectiveness reached 100%. This monoterpenoid was able to block the influx of Ca^{2+} and contraction induced by caffeine and this finding led the authors to also suggest that it acts on influx and the mobilization of Ca^{2+} stores [18, 19].

3.1.2 Geraniol

Geraniol (GER) is an acyclic monoterpenoid with low toxicity (**Table 1**) [73]. In diabetic animals, the GER attenuated the cardiac changes caused by diabetes mellitus (DM). The authors suggested that the mechanism for this effect was the attenuation of changes caused by DM in contractility and systolic duration by GER [74].

In the aorta of normoglycemic animals, GER (30–300 μM) had a vasorelaxing effect on contractions induced by PHE and KCl. This effect was more effective on EMC, suggesting inhibition of Ca^{2+} channels in the plasma membrane of smooth cell. The authors demonstrated that the NO, COX, and K^+ channels do not participate in this vasorelaxant effect. In the aorta of diabetic rats, GER (30–300 μM) reduced tissue hyperresponsiveness to PHE [21].

3.1.3 Linalool

Linalool (LN) is an acyclic tertiary alcohol. In normotensive animals, LN (1–20 mg/kg, i.v.) led to hypotension and tachycardia. Hypotension was attenuated by Nω-Nitro-L-arginine methyl ester (L-NAME) and atropine but not by indomethacin, thus suggesting that the NO and muscarinic receptor pathways participate in promoting this effect [14, 23]. In Goldblatt hypertensive animals, LN (200 mg/kg) caused hypotension, of magnitude similar to nifedipine (NIF), without altering heart rate (HR) [23]. LN also had a hypotensive effect at a dose of 100 mg/kg in SHR [25].

In the mesenteric bed of normotensive animals, LN showed an endothelium-independent vasorelaxant effect on PMC and EMC, with maximum efficacy. Additionally, LN inhibited contractions induced by $CaCl_2$ and caffeine, which led the authors to suggest that the mechanism of action involves the mobilization of Ca^{2+} from intracellular stores and the influx of Ca^{2+} through the plasma membrane. The direct relaxing effect of LN on VSM has been suggested to be responsible for the hypotensive effect of this compound. In the aorta of normotensive rats with endothelium, LN (100 μM) had a relaxing effect on PHE-induced contraction [23, 75].

3.1.4 Perillyl alcohol

Perillyl alcohol (POH) is a monocyclic alcohol. It had a reversible vasorelaxing effect in rat aorta, dependent on concentration and maximum efficacy on the KCl (IC_{50} 277.7 ± 5.46 μM) and PHE-induced (IC_{50} 443.3 ± 66.83 μM) contractions (**Table 1**). Among the contractions inhibited by POH, which also inhibited contractions induced by phorbol dibutyrate (PDB) or by BayK8644, the greatest pharmacological potency was over the contractions induced by KCl and BayK8644, which suggested that the mechanism of the relaxing effect of POH was inhibition of VDCCs. However, other mechanisms have not been ruled out [10]. POH (1–2 mM)

when incubated overnight prevented KCl-, 5-HT-, and U46619-induced contractions in coronary arteries [27].

3.1.5 Carveol

Carveol (CV) is a monocyclic alcohol found in the mint EO. In rat aorta, the CV (10–5000 μM) had a vasorelaxing effect, over contractions induced by PHE (IC_{50} 1333.3 ± 225.20 μM) and KCl (IC_{50} 662.1 ± 32.85 μM), which was reversible and independent of the vascular endothelium. The CV also inhibited contractions induced by PDB and BayK8644. Due to the greater potency of CV on contractions induced by KCl and BayK8644, the mechanism of its relaxing effect was attributed to a probable inhibitory effect on VDCCs [10].

In the human umbilical artery, the CV (1–5000 μM) reduced the basal tone by approximately 72% and relaxed contractions induced by 5-HT (IC_{50} of 175.82 μM) and KCl (IC_{50} 344.25 μM) [76].

3.1.6 Menthol

Menthol is a monocyclic alcohol. In hypertensive animals, menthol (0.5% dietary) attenuated the elevation of vasoconstriction (on PHE- and U46619-induced contraction), blood pressure, the production of reactive oxygen species (ROS), and mitochondrial dysfunction. This effect probably occur due to the TRPM8 activation by menthol and involve the calcium signaling–mediated RhoA/Rho kinase pathway [29, 30].

Menthol showed cutaneous vasorelaxing activity in individuals in normotensive or in essential hypertension condition, an activity that has been suggested to involve the endothelium derived hyperpolarizing factor (EDHF) and NO [33, 77].

3.1.7 α-Terpineol

α-Terpineol (1–30 mg/kg, i.v.), a monocyclic alcohol, induced a reduction in SBP and tachycardia. These effects were mitigated by L-NAME and suggested to involve the NO pathway [34].

In mesenteric arteries, α-terpineol induced an endothelium-dependent vasorelaxant effect on contractions induced by PHE. The vasorelaxant effect of α-terpineol was not affected by atropine and indomethacin, but by treatment with L-NAME and 1H-[1,2,4]oxadiazolo[4,3-a]quinoxal in-1-one (ODQ), suggesting the involvement of the NO/cGMP pathway [34]. In a cannulated mesenteric bed contracted by perfusion with KCl, α-terpineol (300 μg/ml (1.94 mM)) increased (by 93%) the mesenteric flow. The vasorelaxant effect of α-terpineol was abolished by L-NAME, suggesting the participation of NO in this effect [36].

3.1.8 Terpinen-4-ol

Terpinen-4-ol (4TERP) is a monocyclic alcohol. In hypertensive DOCA-salt and normotensive animals, uninefrectomized or not, 4TERP (1–10 mg/kg, i.v.) induced a reduction in SBP and bradycardia, with a peak between 20–30 s after administration. This effect lasted 1–10 minutes for all doses [7, 37].

In VSM of rats, 4TERP showed vasorelaxing effect, reversible and with maximum effectiveness, on contractions mediated by EMC (IC_{50} 421.43 ± 23.48 μM) and PMC (IC_{50} 802.50 ± 13.8 μM). It has been suggested that this effect involves the NO and COX pathway. 4TERP relaxed, with similar potency, the contractions

induced by BayK, $BaCl_2$, and K^+, presenting IC_{50} values of 454.2 ± 28.7, 450.5 ± 71.1, and 421.43 ± 23.48 μM, respectively. This suggest the possible inhibitory effect of 4TERP on VDCCs. It has been suggested that 4TERP also acts on other components of myocytes, such as the IP_3 pathway and the sensitivity of contractile proteins to Ca^{2+} [9].

In ventricular myocytes isolated from rats, 4TERP (30 μM) promoted a small increase (10.6 ± 2.6%) of L type Ca^{2+} currents. Above 300 μM the effect was reversed; 4TERP reduced the amplitude of these currents (IC_{50} of 1203 ± 0.224 μM). 4TERP increased the Ca^{2+} spark frequency at low concentrations and decreased the amplitude of Ca^{2+} transients at low and high concentrations [78]. Thus, among the effects of 4TERP on the cardiovascular system, the effect on ion channels was highlighted as a possible mechanism of action.

3.1.9 Borneol

Borneol is a bicyclic alcohol. In rat aorta, borneol had a vasorelaxing effect on contraction induced by KCl and PHE. This effect was reduced by L-NAME and indomethacin, showing the involvement of the NO and COX pathway in its mechanism [38]. In another study, in rat aorta, borneol inhibited contraction induced by $CaCl_2$, BayK8644, and caffeine and the authors suggested probable activities of this monoterpenoid on VDCCs or intracellular Ca^{2+} stocks as components of its mechanism of action [39].

3.2 Phenolics

3.2.1 Carvacrol

In anesthetized rats, carvacrol (100 μg/kg, i.p.) decreased HR, SBP, systolic and diastolic pressure [42]. In normotensive non-anesthetized rats, carvacrol (1–20 mg/kg, i.v.), had a hypotensive and bradycardic effect [43].

On aorta of rats, on contractions induced by KCl and PHE, carvacrol (1–1000 μM) showed a reversible inhibitory effect, with maximum efficacy, concentration-dependent and not dependent on the endothelium. In addition, carvacrol inhibited contractions in a Ca^{2+}-free medium. These data together suggested the hypothesis that the vasorelaxant mechanism of this monoterpenoid involves multiple mechanisms: the IP_3 pathway, the sensitivity of contractile proteins to Ca^{2+}, and the blocking of VDCCs [44]. In mesenteric arteries, carvacrol (10^{-8} – 3×10^{-4} M) had a concentrations-dependent vasorelaxing effect on PHE-, U46619, and KCl-induced contractions. This effect was endothelium independent and probably involves VOCCs, receptor operator channels (ROC), and store operator channels (SOC) channels [43]. Carvacrol also has a vasorelaxing effect on cerebral parenchymal arteries. In this case, this effect was endothelium-dependent and promoted through the activation of TRPV3 channels that consequently activated the low and medium conductance Ca^{2+}-activated K^+ channels [79].

3.2.2 Thymol

Thymol is a carvacrol isomer. In aorta of rats, thymol (1–1000 μM) has a reversible and concentration-dependent vasorelaxant effect (in contractions induced by KCl and PHE). The experiments to elucidate the mechanism of action showed results very similar to those of carvacrol and led to similar conclusions: the involvement of the IP_3 pathway, the sensitivity of contractile proteins to Ca^{2+}, and the blocking of VDCCs [44].

3.3 Phenylpropanoids

3.3.1 Anethole

Anethole (AN) is a phenylpropanoid with very low toxicity, it has been suggested to have great therapeutic potential [46].

AN (5–10 mg/kg, i.v.) induced, in a concentration-dependent manner, in conscious normotensive rats, hypotension and bradycardia (phase 1), followed by pressoric and bradycardic response (phase 2) [47]. In animals with nicotine-induced hypertension associated with immobilization stress, AN (125–250 mg/kg, i.p.) had an anti-hypertensive effect with efficacy similar to physical exercise and NIF [45].

Soares et al. [48] reported, in relation to concentration, a biphasic effect of AN on the aortic artery. Between 10^{-6} to 10^{-4} M, AN induced an increase in basal tone and PHE-induced contraction in preparations with endothelium. Between 10^{-4} and 10^{-3} the Figure 2b [48] shows this contraction vanishes and full relaxation stablishes (maximum efficacy). It was suggested that the activity on VDCC (activation (10^{-6} to 10^{-4} M) and inhibition (10^{-3} to 10^{-2} M)) is the probable mechanism of this effect [48]. Another study with AN in aortic rings reported only vasorelaxing effects, with higher potencies (for EMC and PMC, IC50: 50–75 μg/ml (0.34–0.51 mM))[80]. This discrepancy was not explained.

3.3.2 Estragol

Estragole (ES) is an isomer of AN which, at 5–10 mg/kg, i.v., induced effects on blood pressure very similar to those of AN (see above) [47, 81]. In the aorta artery of rats with intact endothelium, the ES also had a similar effect to the AN (predominantly vasorelaxant), except that the amplification effect of the contraction was smaller and without statistical significance [48].

3.3.3 Eugenol

Eugenol (EUG) is a phenylpropanoid with a long effect half-life and low toxicity [82]. EUG is probably the most investigated monoterpenoid with effects on the cardiovascular system. EUG (1–10 mg/kg, i.v.) caused reduction of SBP and HR, in dose-dependent manner, in normotensive animals (conscious or anesthetized) and in hypertensive animals (DOCA-salt model). It was suggested that the hypotensive effect is due to the direct vasorelaxing activity of the EUG [49, 50].

In blood vessels, EUG has a relaxing, reversible effect, partially dependent on the endothelium [51, 83]. In rat aorta, with endothelium, this phenylpropanoid inhibited PHE-induced contraction in normotensive (EUG at 1–100 μM) and hypertensive animals (EUG at 0.006–6 mM, DOCA-salt model) [48, 75, 84].

The vasorelaxant effect of the EUG was confirmed with flow measurements. In normotensive animals, EUG induced an increase in flow through the vascular mesenteric bed pre-contracted with KCl (IC_{50} 0.31 ± 0.05 mM) or noradrenaline (0.2, 2 or 20 μM) [50, 85].

EUG also has a vasodilatory effect on pressurized cerebral artery of rats (IC_{50} of 234.2 ± 11.3 μM) or pre-contracted with K^+ (IC_{50} of 323.3 ± 14.0 μM)[11].

The hypotensive and vasorelaxing effect of the EUG is due to multiple mechanisms, the effect of which on ion channels stands out. In VSM cells, the EUG blocks VDCCs by the channel pore blocking mechanism and by changing the steady state of channel inactivation [11, 51]. Consistent with this effect, in rat heart muscle, it was suggested that the negative inotropic effect of EUG (0.1–0.5 mM) is due to the

blocking of Ca^{2+} channels without, however, altering the activity of the contractile intracellular machinery [85]. Similar results were also observed in canine myocytes, where EUG reduced the amplitude and changed the kinetics of the Ca^{2+} current of VDCC L-type channel [86].

Studies have also shown that endothelial TRP channels can participate in the vasorelaxant effect of EUG (5 mg/kg, i.v). EUG at low concentrations (100 μM) is able to activate TRPV4 currents in these cells, triggering actions that lead to vasorelaxation [51].

It is known that among the predominant pathological changes in DM are blood vessel alterations. Nangle et al. [87] demonstrated that the EUG (200 mg/kg/day, p.o.) was able to reverse the increase in sensitivity to PHE and the reduction of ACh-induced relaxation in the renal artery of diabetic rats. This mechanism probably occurred through NO and EDHF.

As EUG has low toxicity, affects several vascular beds and has an inhibitory effect on Ca^{2+} channels, it has therapeutic potential in treatment of DM and Hypertension.

3.4 Aldehydes

3.4.1 Cinnamaldehyde

In rat aorta, cinnamaldehyde has an endothelium-dependent relaxing effect on contraction induced by KCl, prostaglandin F2 (PGF2), and NE [88]. Endothelium dependence, however, has been refuted by Xue et al., since the vasorelaxant effect induced by cinnamaldehyde was not mitigated by pretreatment with L-NAME or ODQ [89]. In addition, these authors reported that COX, K^+ channels, and β-adrenergic receptors are not involved in the vasorelaxant effect of Cinnamaldehyde [52, 88, 89].

In the coronary artery, cinnamaldehyde has a concentration-dependent and endothelium-independent relaxing effect of maximum efficacy on contractions induced by U46619 and KCl [90].

The cinnamaldehyde relaxation mechanism is suggested to occur due to alterations of the sensitivity of contractile proteins to Ca^{2+} and, mainly, by inhibiting VDCCs, as this monoterpenoid inhibited the contraction induced by BayK 8644 in the coronary artery [52, 90]. Additionally, cinnamaldehyde has been shown to reduce L-type Ca^{2+} currents in VSM cells (IC_{50} of 0.81 ± 0.02 mM; maximum efficacy) [52].

In the aorta and mesenteric artery of diabetic mice, cinnamaldehyde added to the diet (% 0.02) improved the endothelial response to ACh without changing SBP. Additionally, cinnamaldehyde prevented the production of ROS and depletion of NO, with beneficial effect in DM [91, 92]. In DM, cinnamaldehyde (20 mg/kg/day) also protected against the elevation of diastolic pressure, the increase in responsiveness to contracting agents and the hyporesponsiveness to ACh [93].

3.4.2 Citral

Citral is a monoterpenoid considered to be non-toxic and of therapeutic relevance [54, 96]. Citral reversibly inhibited contractions induced by PHE (IC_{50} 99.34 μg/mL) and KCl (IC_{50} 110.80 μg/mL) in aorta of healthy rats with maximum efficacy. The authors suggested that this effect occurs due to the blockade of VDCCs, since citral inhibited contractions induced by $BaCl_2$ and BayK 8644 [53, 54].

Relaxing effect of citral was observed in the aortic artery of SHR. This monoterpenoid in concentrations of 0.00624 mM–6.24 mM, induced a relaxing effect partially dependent on the NO pathway. Additionally, citral blocked the contraction induced by reposition of Ca^{2+} to nutrient solution, and this suggested the hypothesis that this compound inhibits Ca^{2+} influx through VDCC channel [95].

3.4.3 Citronellal

Citronellal is a monoterpenoid composed of a racemic mixture of two enantiomers present in plants [96]. In normotensive animals, citronellal (10–40 mg/kg) induced hypotension, bradycardia, and sinoatrial node block. The bradycardic effect probably involves muscarinic receptors as it has been inhibited by atropine. In hypertensive animals, citronellal (200 mg/kg) induced a hypotensive effect of greater duration than that of NIF (1 h of NIF × 3 h in citronellal) [55]. On contractions induced by PHE and KCl in the superior mesenteric artery of normotensive rats, citronellal had a endothelium-independent and concentration-dependent vasorelaxing effect, with maximum efficacy [55].

3.5 Ketone

3.5.1 Carvone

Carvone is a monocyclic monoterpenoid. Heuberger and collaborators [97] investigated the effects of (−)-carvone and (+)-carvone inhalation on the autonomic nervous system. Inhalation of (−)-carvone caused an increase in HR and systolic blood pressure; (+)-carvone inhalation increased systolic and diastolic blood pressure [97]. In rat aorta, carvone had a vasorelaxant effect (Emax = 58.9%) for both enantiomers. The IC_{50} values for (+)-carvone in contractions induced by PHE was 0.62 mM [57].

3.5.2 Rotundifolone

Rotundifolone (RT) is a monocyclic monoterpenoid. RT (1–30 mg/kg, i.v.) had a hypotensive (partial efficacy = 51%) and bradycardic (partial efficacy = 87%) effect in non-anesthetized rats. The hypotensive effect of RT was attenuated by atropine and L-NAME, suggesting the participation of muscarinic receptors in this effect [58].

On isolated aorta from rats, RT inhibited contractions induced by KCl (IC_{50} 184 μg/ml (1.1 mM)) and PHE (IC_{50} 185 μg/ml), with maximum efficacy. As a mechanism of this effect, a possible blocking of VDCCs and of the release of Ca^{2+} from sarcoplasmatic reticulum by RT was suggested [58, 59]. Others also observed the vasorelaxing effect of RT in the mesenteric artery of rats contracted with PHE (pD2 = 4.0, maximum efficacy). As a mechanism for this effect, activity on TRPM8 channels, activation of large conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels, and inactivation of VDCCs were suggested [60, 98, 99].

3.5.3 1,8-cineol

1.8 cineole (CIN), also known as eucalyptol, in anesthetized and conscious normotensive animals, CIN (0.3–10 mg/kg, i.v.) induced hypotension, with maximum effect in 20–30 s after administration and duration of 1–5 min. This effect was independent of the autonomic nervous system, and a probable dependence on vascular relaxation was suggested [62]. CIN (0.1 mg/kg, i.p.) has been shown to

attenuate the elevation of systolic blood pressure in hypertensive rats induced by chronic nicotine exposure [61].

In another study, in the aorta of normotensive rats, CIN inhibited PHE-induced contraction (IC_{50} of 663.2 μg/ml (4.29 mM)). This effect was altered by the presence of L-NAME but was not affected by indomethacin or tetrathylamonium [63].

3.5.4 Linalyl acetate

In hypertensive rats, the linalyl acetate (LA, 10–100 mg/kg, i.p.) attenuated the increase in systolic and diastolic blood pressure. LA also modulates the expression of Endothelial NO Synthase (eNOS), preventing its suppression by ROS. This suggested a possible antihypertensive effect of LA [64, 65]. In rats with hypertension induced by chronic exposure to nicotine and stress, LA (10–100 mg/kg), had a hypotensive effect [66].

It was reported that diabetic animals exposed to chronic stress showed a reduction in endothelial function, changes in SBP and HR. LA (100 mg/kg) was able to revert these parameters to close to control values [100].

In a rabbit carotid artery, LA induced a relaxing effect on PHE-induced contraction, with partial efficacy (E_{max} = 88.8%) and IC_{50} 3.6×10^{-4} M. According to the authors, the cGMP-NO pathway and phosphorylation of myosin light chain, are involved in the relaxing effect of this monoterpenoid, since it was attenuated by L-NAME and ODQ [67]. It was also observed that the LA (300 μM) showed a relaxing effect of PHE-induced contraction in the aorta of mice exposed to nicotine [101].

4. Final considerations

Based on these studies, it can be concluded that the vast majority of those monoterpenes and monoterpenoids investigated and here presented have a hypotensive and vasorelaxant effect. Concerning the hypotensive effect, the studies did not include medium or long-term treatments; they were all about acute effects. In terms of results obtained, there was great variation in the repercussion on heart rate: concomitant tachycardia, in most cases, which was generally interpreted as a reflex reaction to a hypotensive effect of primary vascular origin; bradycardia or no change in heart rate in other cases. Concerning the investigation of the hypotensive effect, in terms of the methodology of administration of monoterpene or monoterpenoid, there was great variation in the route of administration employed, intraperitoneal in some cases, intravenous and oral in others, which makes comparisons more difficult. Additionally, concerning the perspective of therapeutic use, this is a relevant issue, since for long lasting treatment, as is the case with essential hypertension, the oral route of administration is largely preferable, if not mandatory.

Regarding the vasorelaxant effect, most studies describe a relaxing effect in rat aortic rings on contractions mediated by EMC and PMC and suggested, as participant in mechanism of action, based on indirect evidence, the inhibitory effect of these pharmacological agents on the activation of L type VDCC. Participation of K^+ and TRP ionic channels, as well as intracellular mechanisms on monoterpene and monoterpenoid-induced relaxation of contraction have been little investigated.

From the point of view of the possible therapeutic use of monoterpenes and monoterpenoids for the treatment of arterial hypertension, it can be concluded that several studies on the pressure and vascular effects have been carried out. These studies point to a potential therapeutic use for several of these agents. However, in general, they were restricted to the initial stages of a preclinical study.

Author details

Ana Carolina Cardoso-Teixeira[1], Klausen Oliveira-Abreu[1], Levy Gabriel de Freitas Brito[1], Andrelina Noronha Coelho-de-Souza[2] and José Henrique Leal-Cardoso[1*]

1 Laboratório de Eletrofisiologia, Instituto Superior de Ciências Biomédicas, Universidade Estadual do Ceará, Ceará, Brazil

2 Laboratório de Fisiologia Experimental, Instituto Superior de Ciências Biomédicas, Universidade Estadual do Ceará, Ceará, Brazil

*Address all correspondence to: lealcard@gmail.com

IntechOpen

References

[1] Cox-Georgian, D.; Ramadoss, N.; Dona, C.; Basu, C. Therapeutic and medicinal uses of terpenes. In *Medicinal Plants: From Farm to Pharmacy*; 2019; pp. 333–359 ISBN 9783030312695.

[2] Lahlou, M. Methods to Study the Phytochemistry and Bioactivity of Essential Oils. *Phyther. Res.* **2004**, *18*, 435–448.

[3] Tetali, S.D. Terpenes and isoprenoids: a wealth of compounds for global use. *Planta* **2019**, *249*, doi: 10.1007/s00425-018-3056-x.

[4] Paduch, R.; Kandefer-Szerszeń, M.; Trytek, M.; Fiedurek, J. Terpenes: substances useful in human healthcare. *Arch. Immunol. Ther. Exp. (Warsz).* **2007**, *55*, 315–327, doi:10.1007/s00005-007-0039-1.

[5] Edris, A. Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: a review. *Phyther. Res.* **2007**, *21*, 308–323, doi:10.1002/ptr.

[6] De Andrade, T.U.; Brasil, G.A.; Endringer, D.C.; Da Nóbrega, F.R.; De Sousa, D.P. Cardiovascular activity of the chemical constituents of essential oils. *Molecules* **2017**, *22*, doi:10.3390/molecules22091539.

[7] Lahlou, S.; Galindo, C.A.B.; Leal-Cardoso, J.H.; Fonteles, M.C.; Duarte, G.P. Cardiovascular effects of the Essential Oil of *Alpinia zerumbet* leaves and its Main Constituent, Terpinen-4-ol, in rats: Role of the Autonomic Nervous System. *Planta Med.* **2002**, *68*, 1097–1102.

[8] Vasconcelos, C.M.L.; Oliveira, I.S.N.; Santos, J.N.A.; Souza, A.A.; Menezes-Filho, J.E.R.; Silva Neto, J.A.; Lima, T.C.; de Sousa, D.P. Negative inotropism of terpenes on Guinea pig left atrium: Structure-activity relationships. *Nat. Prod. Res.* **2018**, *32*, 1428–1431, doi: 10.1080/14786419.2017.1344658.

[9] Maia-Joca, R.P.M.; Joca, H.C.; Ribeiro, F.J.P.; Nascimento, R.V. Do; Silva-Alves, K.S.; Cruz, J.S.; Coelho-de-Souza, A.N.; Leal-Cardoso, J.H. Investigation of terpinen-4-ol effects on vascular smooth muscle relaxation. *Life Sci.* **2014**, *115*, 52–58, doi:10.1016/j.lfs.2014.08.022.

[10] Cardoso-Teixeira, A.C.; Ferreira-da-Silva, F.W.; Peixoto-Neves, D.; Oliveira-Abreu, K.; Pereira-Gonçalves, Á.; Coelho-de-Souza, A.; Leal-Cardoso, J. Hydroxyl Group and Vasorelaxant Effects of Perillyl Alcohol, Carveol, Limonene on Aorta Smooth Muscle of Rats. *Molecules* **2018**, *23*, 1430, doi: 10.3390/molecules23061430.

[11] Peixoto-Neves, D.; Leal-Cardoso, J. H.; Jaggar, J.H. Eugenol dilates rat cerebral arteries by inhibiting smooth muscle cell voltage-dependent calcium channels. *J. Cardiovasc. Pharmacol.* **2014**, *64*, 401–406, doi:10.1038/jid.2014.371.

[12] Nascimento, G.A. do; Souza, D.S. de; Lima, B.S.; Vasconcelos, C.M.L. de; Araújo, A.A. de S.; Durço, A.O.; Quintans-Junior, L.J.; Almeida, J.R.G. da S.; Oliveira, A.P.; Santana-Filho, V.J. de; et al. Bradycardic and antiarrhythmic effects of the D-limonene in rats. *Arq. Bras. Cardiol.* **2019**, *113*, 925–932, doi: 10.5935/abc.20190173.

[13] Sun, J. D-Limonene: safety and clinical applications. *Altern. Med. Rev.* **2007**, *12*, 259–264.

[14] Menezes, I.A.C.; Barreto, C.M.N.; Antoniolli, Â.R.; Santos, M.R.V.; de Sousa, D.P. Hypotensive activity of terpenes found in essential oils. *Zeitschrift fur Naturforsch. - Sect. C J. Biosci.* **2010**, *65 C*, 562–566, doi:10.1515/znc-2010-9-1005.

[15] Felipe, C.F.B.; Albuquerque, A.M.S.; de Pontes, J.L.X.; de Melo, J.Í.V.; Rodrigues, T.C.M.L.; de Sousa, A.M.P.; Monteiro, Á.B.; Ribeiro, A.E. da S.; Lopes, J.P.; de Menezes, I.R.A.; et al. Comparative study of alpha- and beta-pinene effect on PTZ-induced convulsions in mice. *Fundam. Clin. Pharmacol.* **2019**, *33*, 181–190, doi: 10.1111/fcp.12416.

[16] Silva, M.T.M.; Ribeiro, F.P.R.A.; Medeiros, M.A.M.B.; Sampaio, P.A.; Silva, Y.M.S.; Silva, M.T.A.; Quintans, J. S.S.; Quintans-Júnior, L.J.; Ribeiro, L.A. A. The vasorelaxant effect of p -cymene in rat aorta involves potassium channels. *Sci. World J.* **2015**, *2015*, 6–11, doi: 10.1155/2015/458080.

[17] Jenner, P.M.; Hagan, E.C.; Taylor, J. M.; Cook, E.L.; Fitzhugh, O.G. Food flavourings and compounds of related structure I. Acute oral toxicity. *Food Cosmet. Toxicol.* **1964**, *2*, 327–343, doi: 10.1016/S0015-6264(64)80192-9.

[18] Bastos, J.F.A.; Moreira, Í.J.A.; Ribeiro, T.P.; Medeiros, I.A.; Antoniolli, A.R.; De Sousa, D.P.; Santos, M.R.V. Hypotensive and vasorelaxant effects of citronellol, a monoterpene alcohol, in rats. *Basic Clin. Pharmacol. Toxicol.* **2010**, *106*, 331–337, doi:10.1111/ j.1742-7843.2009.00492.x.

[19] Ribeiro-Filho, H.V.; De Souza Silva, C.M.; De Siqueira, R.J.; Lahlou, S.; Dos Santos, A.A.; Magalhães, P.J.C. Biphasic cardiovascular and respiratory effects induced by β-citronellol. *Eur. J. Pharmacol.* **2016**, *775*, 96–105, doi: 10.1016/j.ejphar.2016.02.025.

[20] Lapczynski, A.; Bhatia, S.P.; Letizia, C.S.; Api, A.M. Fragrance material review on dl-citronellol. *Food Chem. Toxicol.* **2008**, *46*, 4–10, doi:10.1016/j. fct.2008.06.043.

[21] El-Bassossy, H.M.; Elberry, A.A.; Ghareib, S.A. Geraniol improves the impaired vascular reactivity in diabetes and metabolic syndrome through calcium channel blocking effect. *J. Diabetes Complications* **2016**, *30*, 1008–1016, doi:10.1016/j. jdiacomp.2016.04.006.

[22] Babukumar, S.; Vinothkumar, V.; Sankaranarayanan, C.; Srinivasan, S. Geraniol, a natural monoterpene, ameliorates hyperglycemia by attenuating the key enzymes of carbohydrate metabolism in streptozotocin-induced diabetic rats. *Pharm. Biol.* **2017**, *55*, 1442–1449, doi: 10.1080/13880209.2017.1301494.

[23] Anjos, P.J.C.; Lima, A.O.; Cunha, P. S.; De Sousa, D.P.; Onofre, A.S.C.; Ribeiro, T.P.; Medeiros, I.A.; Antoniolli, Â.R.; Quintans-Júnior, L.J.; Santos, M.R. V. Cardiovascular effects induced by linalool in normotensive and hypertensive rats. *Zeitschrift fur Naturforsch. - Sect. C J. Biosci.* **2013**, *68 C*, 181–190, doi:10.1515/znc-2013-5-603.

[24] Bickers, D.; Calow, P.; Greim, H.; Hanifin, J.M.; Rogers, A.E.; Saurat, J.H.; Sipes, I.G.; Smith, R.L.; Tagami, H.; Api, A.M. A toxicologic and dermatologic assessment of linalool and related esters when used as fragrance ingredients. *Food Chem. Toxicol.* **2003**, *41*, 919–942, doi:10.1016/S0278-6915(03)00016-4.

[25] Camargo, S.B.; Simões, L.O.; Medeiros, C.F. de A.; de Melo Jesus, A.; Fregoneze, J.B.; Evangelista, A.; Villarreal, C.F.; Araújo, A.A. de S.; Quintans-Júnior, L.J.; Silva, D.F. Antihypertensive potential of linalool and linalool complexed with β-cyclodextrin: Effects of subchronic treatment on blood pressure and vascular reactivity. *Biochem. Pharmacol.* **2018**, *151*, 38–46, doi:10.1016/j. bcp.2018.02.014.

[26] Bhatia, S.P.; McGinty, D.; Letizia, C. S.; Api, a M. Fragrance material review on p-methya-1,8-dien-7-ol. *Food Chem. Toxicol.* **2008**, *46*, S197–S200, doi: 10.1016/j.fct.2008.06.071.

[27] Kennedy, S.; Wadsworth, R.M.; Wainwright, C.L. Effect of antiproliferative agents on vascular function in normal and in vitro balloon-injured porcine coronary arteries. *Eur. J. Pharmacol.* **2003**, *481*, 101–107, doi: 10.1016/j.ejphar.2003.09.010.

[28] Bhatia, S.P.; McGinty, D.; Letizia, C. S.; Api, A.M. Fragrance material review on laevo-carveol. *Food Chem. Toxicol.* **2008**, *46*, 88–90, doi:10.1016/j. fct.2008.06.055.

[29] Sun, J.; Yang, T.; Wang, P.; Ma, S.; Zhu, Z.; Pu, Y.; Li, L.; Zhao, Y.; Xiong, S.; Liu, D.; et al. Activation of cold-sensing transient receptor potential melastatin subtype 8 antagonizes vasoconstriction and hypertension through attenuating RhoA/Rho kinase pathway. *Hypertension* **2014**, *63*, 1354–1363, doi:10.1161/ HYPERTENSIONAHA.113.02573.

[30] Xiong, S.; Wang, B.; Lin, S.; Zhang, H.; Li, Y.; Wei, X.; Cui, Y.; Wei, X.; Lu, Z.; Gao, P.; et al. Activation of transient receptor potential melastatin subtype 8 attenuates cold-induced hypertension through ameliorating vascular mitochondrial dysfunction. *J. Am. Heart Assoc.* **2017**, *6*, 1–17, doi:10.1161/ JAHA.117.005495.

[31] Oz, M.; El Nebrisi, E.G.; Yang, K.H. S.; Howarth, F.C.; Al Kury, L.T. Cellular and molecular targets of menthol actions. *Front. Pharmacol.* **2017**, *8*, 1–17, doi:10.3389/fphar.2017.00472.

[32] Robu, V.; Covaci, G.; Popescu, I.M. The use of essential oils in organic farming. *Res. J. Agric. Sci.* **2015**, *47*, 134–137.

[33] Craighead, D.H.; Alexander, L.M. Menthol-induced cutaneous vasodilation is preserved in essential hypertensive men and women. *Am. J. Hypertens.* **2017**, *30*, 1156–1162, doi: 10.1093/ajh/hpx127.

[34] Ribeiro, T.P.; Porto, D.L.; Menezes, C.P.; Antunes, A.A.; Silva, D.F.; De Sousa, D.P.; Nakao, L.S.; Braga, V.A.; Medeiros, I.A. Unravelling the cardiovascular effects induced by α-terpineol: A role for the nitric oxide-cGMP pathway. *Clin. Exp. Pharmacol. Physiol.* **2010**, *37*, 811–816, doi:10.1111/ j.1440-1681.2010.05383.x.

[35] Bhatia, S.P.; Letizia, C.S.; Api, A.M. Fragrance material review on alpha-terpineol. *Food Chem. Toxicol.* **2008**, *46*, 280–285, doi:10.1016/j.fct.2008.06.027.

[36] Magalhães, P.J.C.; Lahlou, S.; Jucá, D.M.; Coelho-de-Souza, L.N.; da Frota, P.T.T.; da Costa, A.M.G.; Leal-Cardoso, J.H. Vasorelaxation induced by the essential oil of Croton nepetaefolius and its constituents in rat aorta are partially mediated by the endothelium. *Fundam. Clin. Pharmacol.* **2008**, *22*, 169–177, doi: 10.1111/j.1472-8206.2008.00571.x.

[37] Lahlou, S.; Interaminense, L.F.L.; Leal-Cardoso, J.H.; Duarte, G.P. Antihypertensive effects of the essential oil of *Alpinia zerumbet* and its main constituent, terpinen-4-ol, in DOCA-salt hypertensive conscious rats. *Fundam. Clin. Pharmacol.* **2003**, *17*, 323–330.

[38] Santos, S.E.; Ribeiro, F.P.R.A.; Menezes, P.M.N.; Duarte-Filho, L.A.M.; Quintans, J.S.S.; Quintans-Junior, L.J.; Silva, F.S.; Ribeiro, L.A.A. New insights on relaxant effects of (—)-borneol monoterpene in rat aortic rings. *Fundam. Clin. Pharmacol.* **2019**, *33*, 148–158, doi:10.1111/fcp.12417.

[39] Silva-Filho, J.C.; Oliveira, N.N.P.M.; Arcanjo, D.D.R.; Quintans-Júnior, L.J.; Cavalcanti, S.C.H.; Santos, M.R. V.; Oliveira, R. de C.M.; Oliveira, A.P. Investigation of mechanisms involved in (−)-borneol-induced vasorelaxant response on rat thoracic aorta. *Basic Clin. Pharmacol. Toxicol.* **2012**, *110*, 171–177, doi:10.1111/j.1742-7843. 2011.00784.x.

[40] Bhatia, S.P.; McGinty, D.; Letizia, C.S.; Api, A.M. Fragrance material review on l-borneol. *Food Chem. Toxicol.* **2008**, *46*, 81–84, doi:10.1016/j.fct.2008.06.054.

[41] Aydin, Y.; Kutlay, Ö.; Ari, S.; Duman, S.; Uzuner, K.; Aydin, S. Hypotensive effects of carvacrol on the blood pressure of normotensive rats. *Planta Med.* **2007**, *73*, 1365–1371, doi:10.1055/s-2007-990236.

[42] Suntres, Z.E.; Coccimiglio, J.; Alipour, M. The Bioactivity and Toxicological Actions of Carvacrol. *Crit. Rev. Food Sci. Nutr.* **2015**, *55*, 304–318, doi:10.1080/10408398.2011.653458.

[43] Dantas, B.P.V.; Alves, Q.L.; de Assis, K.S.; Ribeiro, T.P.; de Almeida, Mô.M.; de Vasconcelos, A.P.; de Araújo, D.A.M.; de Andrade Braga, V.; de Medeiros, I.A.; Alencar, J.L.; et al. Participation of the TRP channel in the cardiovascular effects induced by carvacrol in normotensive rat. *Vascul. Pharmacol.* **2015**, *67*, 48–58, doi:10.1016/j.vph.2015.02.016.

[44] Peixoto-Neves, D.; Silva-Alves, K. S.; Gomes, M.D.M.; Lima, F.C.; Lahlou, S.; Magalhães, P.J.C.; Ceccatto, V.M.; Coelho-de-Souza, A.N.; Leal-Cardoso, J. H. Vasorelaxant effects of the monoterpenic phenol isomers, carvacrol and thymol, on rat isolated aorta. *Fundam. Clin. Pharmacol.* **2010**, *24*, 341–50, doi:10.1111/j.1472-8206.2009.00768.x.

[45] Seo, E.; Kang, P.; Seol, G.H. Trans - anethole prevents hypertension induced by chronic exposure to both restraint stress and nicotine in rats. *Biomed. Pharmacother.* **2018**, *102*, 249–253, doi:10.1016/j.biopha.2018.03.081.

[46] Coelho-de-Souza, A.N.; Rocha, M. V.A.P.; Oliveira, K.A.; Vasconcelos, Y.A. G.; Santos, E.C.; Silva-Alves, K.S.; Diniz, L.R.L.; Ferreira-da-Silva, F.W.; Oliveira, A.C.; Ponte, E.L.; et al. Volatile oil of Croton zehntneri per oral sub-acute treatment offers small toxicity: perspective of therapeutic use. *Rev. Bras. Farmacogn.* **2019**, *29*, 228–233, doi:10.1016/j.bjp.2018.11.005.

[47] Siqueira, R.J.B. de; Magalhães, P.J. C.; Leal-Cardoso, J.H.; Duarte, G.P.; Lahlou, S. Cardiovascular effects of the essential oil of Croton zehntneri leaves and its main constituents , anethole and estragole , in normotensive conscious rats. *Life Sci.* **2006**, *78*, 2365–2372, doi:10.1016/j.lfs.2005.09.042.

[48] Soares, P.M.G.; Lima, R.F.; de Freitas Pires, A.; Souza, E.P.; Assreuy, A.M.S.; Criddle, D.N. Effects of anethole and structural analogues on the contractility of rat isolated aorta: Involvement of voltage-dependent Ca2 +−channels. *Life Sci.* **2007**, *81*, 1085–93, doi:10.1016/j.lfs.2007.08.027.

[49] Lahlou, S.; Interaminense, L.F.L.; Magalhães, P.J.C.; Leal-Cardoso, J.H.; Duarte, G.P. Cardiovascular Effects of Eugenol , a Phenolic Compound Present in Many Plant Essential Oils , in Normotensive Rats. *J. Cardiovasc. Pharmacol.* **2004**, *43*, 250–257.

[50] Leal Interaminense, L.F.; Leal-Cardoso, J.H.; Caldas Magalhães, P.J.; Pinto Duarte, G.; Lahlou, S. Enhanced hypotensive effects of the essential oil of *Ocimum gratissimum* leaves and its main constituent, eugenol, in DOCA-salt hypertensive conscious rats. *Planta Med.* **2005**, *71*, 376–378, doi:10.1055/s-2005-864109.

[51] Peixoto-Neves, D.; Wang, Q.; Leal-Cardoso, J.H.; Rossoni, L. V.; Jaggar, J.H. Eugenol dilates mesenteric arteries and reduces systemic BP by activating endothelial cell TRPV4 channels. *Br. J. Pharmacol.* **2015**, *172*, 3484–3494, doi:10.1111/bph.13156.

[52] Alvarez-collazo, J.; Alonso-carbajo, L.; López-medina, A.I.; Alpizar, Y.A.; Tajada, S.; Nilius, B.; Voets, T.; López-

López, J.R.; Talavera, K.; Pérez-García, M.T.; et al. Cinnamaldehyde inhibits L-type calcium channels in mouse ventricular cardiomyocytes and vascular smooth muscle cells. *Pflugers Arch - Eur J Physiol* **2014**, *466*, 2089–2099, doi: 10.1007/s00424-014-1472-8.

[53] da Silva, R.E.R.; de Morais, L.P.; Silva, A.A.; Bastos, C.M.S.; Pereira-gonçalves, Á.; Kerntopf, M.R.; Menezes, I.R.A.; Leal-Cardoso, J.H.; Barbosa, R. Vasorelaxant effect of the *Lippia alba* essential oil and its major constituent, citral, on the contractility of isolated rat aorta. *Biomed. Pharmacother.* **2018**, *108*, 792–798, doi:10.1016/j.biopha.2018.09.073.

[54] Pereira, S.L.; Marques, A.M.; Sudo, R.T.; Kaplan, M.C.A.; Zapata-sudo, G. Vasodilator Activity of the Essential Oil from Aerial Parts of Pectis brevipedunculata and Its Main Constituent Citral in Rat Aorta. *Molecules* **2013**, *18*, 3072–3085, doi: 10.3390/molecules18033072.

[55] Andrade, F.C.; Mota, M.M.; Barreto, A.S.; Sousa, D.P.; Quintans-Junior, L.J.; Santos, M.R.V. Antihypertensive therapeutic potential of citronellal. *Lat. Am. J. Pharm.* **2012**, *31*, 767–771.

[56] Citronellal | C10H18O - PubChem Available online: https://pubchem.ncbi.nlm.nih.gov/compound/7794#section=Toxicity (accessed on Sep 15, 2020).

[57] De Sousa, D.P.; Mesquita, R.F.; De Araújo Ribeiro, L.A.; De Lima, J.T. Spasmolytic activity of carvone and limonene enantiomers. *Nat. Prod. Commun.* **2015**, *10*, 1893–1896, doi: 10.1177/1934578x1501001120.

[58] Guedes, D.N.; Silva, D.F.; Barbosa-Filho, J.M.; Medeiros, I.A. Muscarinic agonist properties involved in the hypotensive and vasorelaxant responses of rotundifolone in rats. *Planta Med.* **2002**, *68*, 700–704, doi:10.1055/s-2002-33795.

[59] Guedes, D.N.; Silva, D.F.; Barbosa-Filho, J.M.; Medeiros, I.A. Calcium antagonism and the vasorelaxation of the rat aorta induced by rotundifolone. *Brazilian J. Med. Biol. Res.* **2004**, *37*, 1881–1887, doi:10.1590/S0100-879X2004001200014.

[60] Silva, D.F.; Araújo, I.G.A.; Albuquerque, J.G.F.; Porto, D.L.; Dias, K.L.G.; Cavalcante, K.V.M.; Veras, R.C.; Nunes, X.P.; Barbosa-Filho, J.M.; Araújo, D.A.M.; et al. Rotundifolone-induced relaxation is mediated by BK Ca channel activation and Ca v channel inactivation. *Basic Clin. Pharmacol. Toxicol.* **2011**, *109*, 465–475, doi:10.1111/j.1742-7843.2011.00749.x.

[61] Moon, H.K.; Kang, P.; Lee, H.S.; Min, S.S.; Seol, G.H. Effects of 1,8-cineole on hypertension induced by chronic exposure to nicotine in rats. *J. Pharm. Pharmacol.* **2014**, *66*, 688–693, doi:10.1111/jphp.12195.

[62] Lahlou, S.; Figueiredo, A.F.; Magalhães, P.J.C.; Leal-Cardoso, J.H. Cardiovascular effects of 1,8-cineole, a terpenoid oxide present in many plant essential oils, in normotensive rats. *Can. J. Physiol. Pharmacol.* **2002**, *80*, 1125–1131, doi:10.1139/y02-142.

[63] Pinto, N.; Assreuy, A.; Coelho-de-Souza, A.; Ceccatto, V.; Magalhães, P.; Lahlou, S.; Leal-Cardoso, J. Endothelium-dependent vasorelaxant effects of the essential oil from aerial parts of *Alpinia zerumbet* and its main constituent 1, 8-cineole in rats. *Phytomedicine* **2009**, *16*, 1151–1155, doi: 10.1016/j.phymed.2009.04.007.

[64] Hsieh, Y.S.; Shin, Y.K.; Han, A.Y.; Kwon, S.; Seol, G.H. Linalyl acetate prevents three related factors of vascular damage in COPD-like and hypertensive rats. *Life Sci.* **2019**, *232*, 116608, doi:10.1016/j.lfs.2019.116608.

[65] Hsieh, Y.S.; Kwon, S.; Lee, H.S.; Seol, G.H. Linalyl acetate prevents

hypertension-related ischemic injury. *PLoS One* **2018**, *13*, 1–14, doi:10.1371/journal.pone.0198082.

[66] Kwon, S.; Hsieh, Y.S.; Shin, Y.K.; Kang, P.; Seol, G.H. Linalyl acetate prevents olmesartan-induced intestinal hypermotility mediated by interference of the sympathetic inhibitory pathway in hypertensive rat. *Biomed. Pharmacother.* **2018**, *102*, 362–368, doi:10.1016/j.biopha.2018.03.095.

[67] Koto, R.; Imamura, M.; Watanabe, C.; Obayashi, S.; Shiraishi, M.; Sasaki, Y.; Azuma, H. Linalyl acetate as a major ingredient of lavender essential oil relaxes the rabbit vascular smooth muscle through dephosphorylation of myosin light chain. *J. Cardiovasc. Pharmacol.* **2006**, *48*, 850–856, doi:10.1097/01.fjc.0000238589.00365.42.

[68] Santos, M.R. V.; Moreira, F. V.; Fraga, B.P.; Souza, D.P. De; Bonjardim, L.R.; Quintans-Junior, L.J. Cardiovascular effects of monoterpenes: a review. *Rev. Bras. Farmacogn.* **2011**, *21*, 764–771, doi:10.1590/S0102-695X2011005000119.

[69] Oz, M.; Lozon, Y.; Sultan, A.; Yang, K.H.S.; Galadari, S. Effects of monoterpenes on ion channels of excitable cells. *Pharmacol. Ther.* **2015**, *152*, 83–97, doi:10.1016/j.pharmthera.2015.05.006.

[70] Wang, X.; Li, G.; Shen, W. Protective effects of D-limonene against transient cerebral ischemia in stroke-prone spontaneously hypertensive rats. *Exp. Ther. Med.* **2018**, *15*, 699–706, doi:10.3892/etm.2017.5509.

[71] Marom, M.; Hagalili, Y.; Sebag, A.; Tzvier, L.; Atlas, D. Conformational changes induced in voltage-gated calcium channel Cav1.2 by BayK 8644 or FPL64176 modify the kinetics of secretion independently of Ca 2+ influx. *J. Biol. Chem.* **2010**, *285*, 6996–7005, doi:10.1074/jbc.M109.059865.

[72] Salehi, B.; Upadhyay, S.; Orhan, I.E.; Jugran, A.K.; Jayaweera, S.L.D.; Dias, D. A.; Sharopov, F.; Taheri, Y.; Martins, N.; Baghalpour, N.; et al. Therapeutic potential of α-and β-pinene: A miracle gift of nature. *Biomolecules* **2019**, *9*, 1–34, doi:10.3390/biom9110738.

[73] Crespo, R.; Wei, K.; Rodenak-Kladniew, B.; Mercola, M.; Ruiz-Lozano, P.; Hurtado, C. Effect of geraniol on rat cardiomyocytes and its potential use as a cardioprotective natural compound. *Life Sci.* **2017**, *172*, 8–12, doi:10.1016/j.lfs.2017.01.008.

[74] El-Bassossy, H.M.; Ghaleb, H.; Elberry, A.A.; Balamash, K.S.; Ghareib, S.A.; Azhar, A.; Banjar, Z. Geraniol alleviates diabetic cardiac complications: Effect on cardiac ischemia and oxidative stress. *Biomed. Pharmacother.* **2017**, *88*, 1025–1030, doi:10.1016/j.biopha.2017.01.131.

[75] Kundu, S.; Shabir, H.; Basir, S.F.; Khan, L.A. Inhibition of As(III) and Hg (II) caused aortic hypercontraction by eugenol, linalool and carvone. *J. Smooth Muscle Res.* **2014**, *50*, 93–102, doi:10.1540/jsmr.50.93.

[76] Silva, R.E.R. da; Silva, A. de A.; Pereira-de-Morais, L.; Almeida, N. de S.; Iriti, M.; Kerntopf, M.R.; Menezes, I.R. A. de; Coutinho, H.D.M.; Barbosa, R. Relaxant Effect of Monoterpene (−)-Carveol on Isolated Human Umbilical Cord Arteries and the Involvement of Ion Channels. *Molecules* **2020**, *25*, 1–11.

[77] Craighead, D.H.; Alexander, L.M. Topical menthol increases cutaneous blood flow. *Microvasc. Res.* **2016**, *107*, 39–45, doi:10.1016/j.physbeh.2017.03.040.

[78] Gondim, A.N.S.; Lara, A.; Santos-Miranda, A.; Roman-Campos, D.; Lauton-Santos, S.; Menezes-Filho, J.E. R.; de Vasconcelos, C.M.L.; Conde-Garcia, E.A.; Guatimosim, S.; Cruz, J.S. (−)-Terpinen-4-ol changes intracellular

Ca2+ handling and induces pacing disturbance in rat hearts. *Eur. J. Pharmacol.* **2017**, *807*, 56–63, doi: 10.1016/j.ejphar.2017.04.022.

[79] Pires, P.W.; Sullivan, M.N.; Pritchard, H.A.T.; Robinson, J.J.; Earley, S. Unitary TRPV3 channel ca2+ influx events elicit endothelium-dependent dilation of cerebral parenchymal arterioles. *Am. J. Physiol. - Hear. Circ. Physiol.* **2015**, *309*, H2031–H2041, doi: 10.1152/ajpheart.00140.2015.

[80] Tognolini, M.; Ballabeni, V.; Bertoni, S.; Bruni, R.; Impicciatore, M.; Barocelli, E. Protective effect of *Foeniculum vulgare* essential oil and anethole in an experimental model of thrombosis. *Pharmacol. Res.* **2007**, *56*, 254–260, doi:10.1016/j.phrs.2007.07.002.

[81] Siqueira, R.J.B. De; Leal-Cardoso, J. H.; COUTURE, R.; Lahlou, S. ROLE OF CAPSAICIN-SENSITIVE SENSORY NERVES IN MEDIATION OF THE CARDIOVASCULAR EFFECTS OF THE ESSENTIAL OIL OF CROTON ZEHNTNERI LEAVES IN ANAESTHETIZED RATS. *Clin. Exp. Pharmacol. Physiol.* **2006**, *33*, 238–247.

[82] Guénette, S.; Ross, A.; Marier, J. Pharmacokinetics of eugenol and its effects on thermal hypersensitivity in rats. *Eur. J. ...* **2007**, *562*, 60–67, doi: 10.1016/j.ejphar.2007.01.044.

[83] Criddle, D.N.; Madeira, S.V.F.; de Moura, R.S. Endothelium-dependent and -independent vasodilator effects of eugenol in the rat mesenteric vascular bed. *J. Pharm. Pharmacol.* **2003**, *55*, 359–365, doi:10.1211/002235702694.

[84] Interaminense, L.F.L.; Jucá, D.M.; Magalhães, P.J.C.; Leal-Cardoso, J.H.; Duarte, G.P.; Lahlou, S. Pharmacological evidence of calcium-channel blockade by essential oil of *Ocimum gratissimum* and its main constituent, eugenol, in isolated aortic rings from DOCA-salt hypertensive rats. *Fundam. Clin. Pharmacol.* **2007**, *21*, 497–506, doi:10.1111/j.1472-8206.2007.00514.x.

[85] Damiani, C.E.N.; Moreira, C.M.; Heng, T.Z.; Creazzo, T.L.; Vassallo, D. V. Effects of eugenol, an essential oil, on the mechanical and electrical activities of cardiac muscle. *J. Cardiovasc. Pharmacol.* **2004**, *44*, 688–695, doi: 10.1097/00005344-200412000-00011.

[86] Magyar, J.; Szentandrássy, N.; Bányász, T.; Fülöp, L.; Varró, A.; Nánási, P.P. Effects of terpenoid phenol derivatives on calcium current in canine and human ventricular cardiomyocytes. *Eur. J. Pharmacol.* **2004**, *487*, 29–36, doi: 10.1016/j.ejphar.2004.01.011.

[87] Nangle, M.R.; Gibson, T.M.; Cotter, M.A.; Cameron, N.E. Effects of eugenol on nerve and vascular dysfunction in streptozotocin-diabetic rats. *Planta Med.* **2006**, *72*, 494–500, doi:10.1055/s-2005-916262.

[88] Yanaga, A.; Goto, H.; Nakagawa, T.; Hikiami, H.; Shibahara, N.; Shimada, Y. Cinnamaldehyde Induces Endothelium-Dependent and -Independent Vasorelaxant Action on Isolated Rat Aorta. *Biol. Pharm. Bull.* **2006**, *29*, 2415–2418.

[89] Xue, Y.-L.; Shi, H.-X.; Murad, F.; Bian, K. Vasodilatory effects of cinnamaldehyde and its mechanism of action in the rat aorta. *Vasc. Health Risk Manag.* **2011**, *7*, 273–280, doi:10.2147/VHRM.S15429.

[90] Raffai, G.; Kim, B.; Park, S.; Khang, G.; Lee, D.; Vanhoutte, P.M. Cinnamaldehyde and cinnamaldehyde-containing micelles induce relaxation of isolated porcine coronary arteries : role of nitric oxide and calcium. *Int. J. Nanomedicine* **2014**, *9*, 2557–2566.

[91] Wang, P.; Yang, Y.; Wang, D.; Yang, Q.; Wan, J.; Liu, S.; Zhou, P.;

Yang, Y. Cinnamaldehyde Ameliorates Vascular Dysfunction in Diabetic Mice by Activating Nrf2. *Am. J. Hypertens.* **2020**, *33*, 610–619, doi:10.1093/ajh/hpaa024.

[92] Wang, F.; Pu, C.; Zhou, P.; Wang, P.; Liang, D.; Wang, Q.; Hu, Y.; Li, B.; Hao, X. Cinnamaldehyde Prevents Endothelial Dysfunction Induced by High Glucose by Activating Nrf2. *Cell Physiol Biochem* **2015**, *36*, 315–324, doi:10.1159/000374074.

[93] El-bassossy, H.M.; Fahmy, A.; Badawy, D. Cinnamaldehyde protects from the hypertension associated with diabetes. *Food Chem. Toxicol.* **2011**, *49*, 3007–3012, doi:10.1016/j.fct.2011.07.060.

[94] Gonçalves, E.C.D.; Baldasso, G.M.; Bicca, M.A.; Paes, R.S.; Capasso, R.; Dutra, R.C. Terpenoids, cannabimimetic ligands, beyond the cannabis plant. *Molecules* **2020**, *25*, 1–47, doi:10.3390/molecules25071567.

[95] Devi, R.C.; Sim, S.M.; Ismail, R. Effect of *Cymbopogon citratus* and Citral on Vascular Smooth Muscle of the Isolated Thoracic Rat Aorta. *Evidence-Based Complement. Altern.* **2012**, doi:10.1155/2012/539475.

[96] Lu, J.X.; Guo, C.; Ou, W.-S.; Niu, H. F.; Song, P.; Li, Q.Z.; Liu, Z.; Xu, J.; Li, P.; Zhu, M.-L.; et al. Citronellal prevents endothelial dysfunction and atherosclerosis in rats. *J. Cell Biochem.* **2019**, *120*, 3790–3800, doi:10.1002/jcb.27660.

[97] Heuberger, E.; Hongratanaworakit, T.; Böhm, C.; Weber, R.; Buchbauer, G. Effects of chiral fragrances on human autonomic nervous system parameters and self-evaluation. *Chem. Senses* **2001**, *26*, 281–292, doi:10.1093/chemse/26.3.281.

[98] Silva, D.F.; De Almeida, M.M.; Chaves, C.G.; Braz, A.L.; Gomes, M.A.; Pinho-Da-Silva, L.; Pesquero, J.L.; Andrade, V.A.; De Fátima Leite, M.; De Albuquerque, J.G.F.; et al. TRPM8 channel activation induced by monoterpenoid rotundifolone underlies mesenteric artery relaxation. *PLoS One* **2015**, *10*, 1–17, doi:10.1371/journal.pone.0143171.

[99] Cardoso Lima, T.; Mota, M.M.; Barbosa-Filho, J.M.; Viana Dos Santos, M.R.; De Sousa, D.P. Structural relationships and vasorelaxant activity of monoterpenes. *Daru* **2012**, *20*, 23, doi:10.1186/2008-2231-20-23.

[100] Shin, Y.K.; Hsieh, Y.S.; Kwon, S.; Lee, H.S.; Seol, G.H. Linalyl acetate restores endothelial dysfunction and hemodynamic alterations in diabetic rats exposed to chronic immobilization stress. *J. Appl. Physiol.* **2018**, *124*, 1274–1283, doi:10.1152/japplphysiol.01018.2017.

[101] Kim, J.R.; Kang, P.; Lee, H.S.; Kim, K.Y.; Seol, G.H. Cardiovascular effects of linalyl acetate in acute nicotine exposure. *Environ. Health Prev. Med.* **2017**, *22*, 1–7, doi:10.1186/s12199-017-0651-6.

Chapter 9

Terpenoids: Lycopene in Tomatoes

Dwi Setyorini

Abstract

Terpenoids are compounds that only contain carbon and hydrogen, or carbon, hydrogen and oxygen that are aromatic, some terpenoids contain carbon atoms whose number is a multiple of five called isoprene units. There are many terpenoids in tomatoes, one of which is a tretrapenoid. A type of tetrapenoid, the carotenoids. Lycopene is a terpenoid found in tomatoes. Lycopene is the most carotenoid group in tomatoes. Lycopene plays a very important role in maintaining human health, including its role in the risk of chronic diseases such as cancer, heart disease, and others. The lycopene content in tomatoes depends on genetic factors, in this case the tomato variety, the environment where the tomatoes grow and the fruit storage environment, and the age of the tomatoes. The genetic factor of tomato fruit that greatly affects lycopene content in tomatoes is the color of the fruit. Color is generally an accurate indicator of lycopene content, with yellow cultivars containing less lycopene than red cultivars, and two out of three red cultivars contain more than orange cultivars. Shade tomato plants can increase the lycopene content in tomatoes. Aside from the lack of light in the tomato plant environment, the humidity and air temperature around the tomato plants also greatly affect the lycopene content in the fruit.

Keywords: terpenoids, carotenoids, lycopene, genetics and environment

1. Introduction

Terpene is a group of hydrocarbons that are produced by many plants and animals. In plants, terpenes are contained in the sap and vacuoles of cells. Hydrocarbons are commonly known as terpenes and oxygen-containing compounds called terpenoids are the most important constituents of essential oils. In plants, terpene compounds and their modification, terpenoids, are secondary metabolites. These tarpenes exist in large numbers and in a variety of molecular frameworks, but can be easily recognized by the regularity of the monomers formed from isoprene [1, 2]. Apart from being a secondary metabolite, terpenes are the building blocks of a number of important compounds for living things. Humanity has used terpenes extracted from plants for various purposes, namely as fragrances and flavorings, as pharmaceutical agents and as insecticides. Despite their great commercial value, terpene products have important biological functions in plants. The terpene metabolites are not only important for plant growth and development (eg gibberellin phytochromes) but also an important tool in various plant interactions with the environment [2].

Terpenoids are plant components that have an odor and can be isolated from plant material by distillation, known as essential oils. Essential oils derived from flowers were initially known from a simple structure determination with the ratio

of hydrogen atoms and carbon atoms of a terpenoid compound, which is 8: 5 and with this ratio it can be said that these compounds are in the terpenoid group.. In general, terpenoids consist of elements C and H with the general molecular formula (C5H8) n, the classification usually ranges from the value of n (**Table 1**).

Food carotenoids are generally made of the C40 tetraterpenoid of eight C5 isoprenoid units, joined in reverse order down the middle. A symmetrical linear base framework, which can be cyclically at one or both ends, has a side methyl group separated by six C atoms at the center and five C atoms elsewhere. Cyclization and other modifications, such as hydrogenation, dehydrogenation, double bond migration, shortening or expansion of chains, rearrangement, isomerization, recognition of oxygen function, or a combination of these processes, yield a myriad of structures. Its hallmark is the extensive system of conjugated double bonds, which function as a light-absorbing chromophore responsible for the yellow, orange, or red color this compound imparts to many foods. Hydrocarbon carotenoids (that is, carotenoids consisting only of carbon and hydrogen) are collectively called carotenoids; which contain oxygen are called xanthophiles. In nature, they exist mainly in the more stable all-trans isomer form, but the cis isomer appears. The first two C40 carotenoids formed in the biosynthetic pathway have a 15-cis configuration in plants. The small amount of other cis carotenoid isomers in natural sources is increasingly being reported [4].

A terpenoid compound is a compound that contains only carbon and hydrogen, or carbon, hydrogen and oxygen which are aromatic, some terpenoids contain carbon atoms which are multiples of five containing only carbon and hydrogen, or aromatic carbon, hydrogen, and oxygen, some are terpenoids containing carbon atoms whose number is multiples of five called isoprene units. Terpenoids are grouped based on the number of isoprene units that compose them, namely monoterpenoids, sesquiterpenoids, diterpenoids, triterpenoids, tetraterpenoids, and polterpenoids. Some of these terpenoid compounds are used as anti-tumor drugs because of their cytotoxic effects and some have antiviral activity. Terpenoids are commonly found in plant cells [5]. Terpenoids are chemical compounds made up of several isoprene units. Most terpenoids have a cyclic structure and have one or more functional groups. Terpenoids are generally fat soluble and present in the cytoplasm of plant cells [6].

The terpenoid compounds that contain C40H64 are Pigments and Carotene. For humans, carotenoids or carotenoids play an important role for health, carotenoids with provitamin A activity are important for vision. Other carotenoids that affect human defense function and gap junctional communication (GJC). Moreover, their antioxidant capacity is responsible for the health-promoting properties of fruits and vegetables [7]. The chemical diversity of plant terpenoids can be a reflection of their various biological activities in nature, as natural resources that are widely used

Name	Chemical Formulas	Source
Monoterpen	$C_{10}H_{16}$	Essential oil
Seskuiterpen	$C_{15}H_{24}$	Essential oil
Diterpen	$C_{20}H_{32}$	Pine Resin
Triterpen	$C_{30}H_{48}$	Saponins, Damar
Tetraterpen	$C_{40}H_{64}$	Pigment, Carotene
Politerpen	$(C_5H_8)_n$ n 8	Natural Rubber

Table 1.
Classification of terpenoids [3].

by traditional and modern humans, for example medicines, flavorings, fragrances, food supplements in the form of sweetening vitamins., and pesticides. The terpenoid plant also serves as a volume high feedstock for producing industrial materials. Due to their many different structures, plant terpenoids a group as compounds with many different physical and chemical properties. They can be lipophilic or hydrophilic, volatile or non-volatile, cyclic or acyclic, chiral or achiral. The chemical diversity of terpenoids comes from the biosynthetic pathway of complex terpenoids [8].

In classical biochemistry, it plays an important role in determining features of cellular regulation (eg, possible feedback loops involving allosterism or covalent modification) and in measuring intermediate fluxes and concentrations to provide important metabolic context. With this level of understanding, it should be possible to manipulate transgenic terpenoids directed at biosynthesis to enhance the taste and color of foodstuffs, increase yields of any commercially important compounds, and resistance to pests and pathogens. The possibilities, like the form and function of terpenoid biosynthesis, of orbit are nearly endless [9].

Terpenoids (isoprenoids) encompass more than 40,000 structures and form the largest class of all known metabolic plants. Several terpenoids with typical physiological functions are common to most of the plant species. Historically, terpenoids in particular, along with alkaloids and many phenolics, have been referred to as secondary metabolites. Literature in broad terms, conceptually and empirically, has an essential ecological function in plant biology. Due to their diverse biological activities and their various physical and chemical properties, terpenoid plant chemicals have been exploited by humans as traditional biomaterials in complex compounds or in more or less pure compound form since ancient times [8].

2. Lycopene one of the carotenoids

There are many terpenoids in tomatoes, one of which is tretrapenoid. One type of Tetrapenoid, namely Carotenoids. Carotenoids are important pigments in plant growth. It has been isolated and identified that more than 750 carotenoids have been described as biological substances. These carotenoids are synthesized by plants, algae, fungi, and bacteria, and are also present in animals that eat them [10]. Apart from being an antioxidant, it turns out that carotenoids can now also be used as bio-solar cells. Dye sensitive solar cells (DSSC), also called Graetzel cells, are a new type of solar cell. DSSC becomes more attractive because a variety of dyes including natural dyes can be used as light harvesting elements. The information currently available on the natural dyes that have been used at the DSSC is expected to provide reasonable light harvesting efficiency, sustainability, low cost and easy waste management. Promising natural compounds are carotenoids, polyphenols, and chlorophyll [11].

Carotenoids are found in photosynthetic plants and bacteria, where these compounds have two important functions, namely pigments as accessories in photosynthesis and photoprotection. The first function is as a place for photosynthesis in plant organs. The second function is a consequence of the structure of the carotenoid conjugated polyenes, which allow molecules to absorb light and deactivate single oxygen and free radicals. Humans routinely ingest a variety of different carotenoids, including those that occur naturally in foods (especially fruits and vegetables) and the addition of food coloring to other foods [12].

Several papers reported carotenoid retention of more than 100% in cooked foods calculated on the basis of dry weight. This result cannot be considered an actual improvement; it is unlikely that carotenoids will be biosynthesized during

cooking. The heat treatment activates the enzymes responsible for carotenoid biosynthesis and, in fact, stimulates isomerization and oxidative degradation of carotenoids. This alleged increase could be simply due to carotenoids that are easier to extract from cooked or processed samples compared to carotenoids in fresh foods, which are physically protected or combined with other dietary components. The extraction efficiency of fresh samples must be increased to match those of cooked samples (such as immersing the sample in water or extracting solvents prior to extraction), and the extraction must be thorough. The significant increase may also be due to leaching of sizeable dissolved solids, such as carrots, which concentrate carotenoids per unit weight of food. Calculating the retention of insoluble solid bases has been proposed in this case. In addition, the enzymatic oxidation of carotenoids can substantially decrease their concentration in the raw sample, especially if the sample is left for some time after being cut or shredded [4].

Lycopene (lycopene), often referred to as α-carotene, is a bright red pigment carotenoid, found in tomatoes and other red fruits. Lycopene in nature, is in a thermodynamically stable trans form, dissolves in non-polar solvents and is found in the 446-506 nm wavelength range [13]. Lycopene is a class of carotenoid compounds, and carotenoids including terpenoids, so lycopene is also a terpenoid. Lycopene is found in fruits, giving the fruit their red color. In this study, two variables were observed, namely the ratio of sample versus solvent 1: 1 and 1: 3, and temperature variables of 30° C and 50° C. The results were variable levels of 20% and 75% lycopene. The solvent ratio results in a higher lycopene. One thing that affects the lab results is the cleanliness of the cuvette as it can cause the absorbance and transmittance readings to be wrong [14]. Lycopene is the main pigment of many red meaty fruits and vegetables, such as tomatoes, watermelon, papaya and red guava, and red or pink grapefruit. ζ- Carotene is more ubiquitous but usually present at low levels except in Brazilian passion fruit [15] and in star fruit [16] where it appears as the main pigment.

3. The role of lycopene in life

Lycopene is included in a family of carotenoid compounds found in fruits, vegetables and green plants. In plants, these compounds are part of the plant and are responsible for the yellow, orange, and red colors in fruits and vegetables. They are synthesized by plants and microorganisms [17]. Lycopene is not an essential nutrient for humans, but is found in many foods, especially from foods prepared with tomato sauce. When absorbed from the stomach, lycopene is carried in the blood by various lipoproteins and accumulates in the liver, adrenal glands, and testes. The lycopene content of the different tomato and tomato products was determined. In the following table, we present the lycopene content (mg / 100 g), of the products we studied. The lycopene content in fresh tomato samples ranged from 12 mg / 100 g. In tomato products, lycopene content has the following values: in tomato paste, approximately 16 mg / 100 g, in boiled tomato sauce approximately 4 mg / 100 g, tomato sauce 17 mg / 100 g and spaghetti sauce 16 mg/100 g [18].

Lycopene acts as an inhibiting agent, lycopene eliminates carcinogenesis from the outside (viruses, pollution, radiation, chemicals) with an antioxidant mechanism so that the oxidative stress that occurs does not cause cellular or genetic damage to DNA [19]. Serum concentrations of lycopene, a biomarker of dietary intake rich in tomatoes, may play a role in the early stages of atherogenesis and may have clinical and public health relevance [20]. Lycopene can also decrease H2O2 levels which trigger heart cell damage and decrease the activity of Caspase-3 which

is a key enzyme in cell death in in-vitro testing of H9C2 cardiac cells [21]. Lycopene, a type of biological carotenoid, shows a constant physical cooling rate with the highest singlet oxygen (kq = 31 × 109 M-1 s-1). Constant physical cooling rate with β-carotene singlet oxygen (kq = 14 × 109 M-1 s-1), β-carotene singlet oxygen cooling capacity (0.5 μm in plasma), lycopene singlet oxygen cooling capacity (0.7 μm) in plasma), albumin-bound bilirubin (15 μm in plasma), and α-tocopherol (22 μm in plasma) were comparably large [22]. Lycopene has been shown in several studies to be the most powerful antioxidant, ranking as follows: lycopene> α-tocopherol> α-carotene> β-crypto-xanthin> zeaxanthin = β-carotene> lutein. Carotenoid mixtures are more effective than single compounds. This synergistic effect is most pronounced when lycopene or lutein is present. The superior mixed protection may be related to the specific position of different carotenoids on the membrane [23]. Consumption of tomatoes is usually associated with intake of lycopene and other antioxidants that have health effects. The tomatoes analyzed in this work represent a typology primarily used for fresh consumption in Mediterranean countries. They show remarkable differences in antioxidant abilities and carotenoid and glycocaloid content [24].

Several animal studies have reported a role for lycopene in cancers other than the prostate. Lycopene inhibited the growth and development of C6 glioma cells (malignant brain cells) transplanted into mice [25]. Growth inhibition is more pronounced when given prior to inoculation of glioma cells. Administration of lycopene has been shown to significantly slow down and reduce the growth and development of spontaneous breast tumors in mice [17]. This effect is associated with decreased activity of milk thymidylate synthetase and decreased levels of serum free fatty acids and prolactin, hormones known to be involved in breast cancer development by stimulating cell division. The mice given lycopene developed significantly fewer tumors, and smaller areas of the tumor than mice not given the supplement. β-Carotene has shown no protection against breast cancer development.

Lycopene is an antioxidant that can neutralize free radicals. Free radical damage is one of the main causes of diseases such as heart disease, premature aging, cancer and cataracts. Lycopene has long been used as a preventative measure to prevent prostate cancer, consumption of tomato products is often associated with a lower risk of developing prostate cancer [26]. In vitro studies, showed that lycopene can also inhibit the growth of lung cancer cells [27]. High levels of lycopene and vitamin A in women's blood serum had a 33% lower chance of developing cervical cancer [28] and lycopene was also able to reduce the development of breast cancer [29]. Lycopene can also manage and prevent osteoporosis which is common in women [30].

Lycopene has attracted a lot of attention since 1995, a 6-year study by Harvard University, nearly than 48,000 people found that those who ate at least 10 servings of foods containing tomato or tomato sauce per week, had a 45% less chance of developing cancer prostate [31]. The molecular formula for lycopene is C42H56 with the following formula (**Figure 1**).

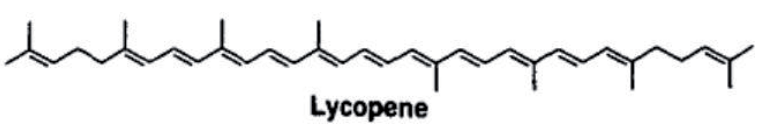


Figure 1.
Lycopene construction formula [31].

4. Effect of genetic factors on lycopene content in tomatoes

The lycopene content in tomatoes is greatly influenced by many factors. Both genetic and environmental factors. The genetic factor of tomatoes that greatly affects the content in tomatoes is the color of the fruit. Color is generally an accurate indicator of lycopene content, with yellow cultivars containing less lycopene than red cultivars, and two out of three red cultivars containing more than orange cultivars. Yellow, orange and red tomatoes can be used as indicators of lycopene and beta-carotene content in tomatoes, but this is not the case for black tomatoes, because black tomatoes "Black Cream" have a higher lycopene content than red. "Celebrity type, however, black tomatoes. "Black Tula" and "Black Plum" have lower lycopene content [24, 32].

The lycopene concentration of fruit growing in the field showed a significant difference based on fruit color. Lycopene concentrations range from 0.14 mg-g bk on Yellow Pear to 1.63 mg-g bk, in Rome, the equivalent of 1.86 mg-100 g - 1 bs in Yellow Pear to 16.30 mg –100 g - 1 bs in Rome [33]. The constructs are introduced into tomato (cv. Moneymarker) through Agrobacterium-mediated transformation and the primary transformants are brought to maturity in the greenhouse. The presence of transgene was tested on leaf DNA via PCR and chromosome complement through leaf nucleus cytometry analysis. Only PCR positive euploid plants were subjected to further research. These plants showed no significant change in growth habits or leaf color phenotypes, and produced normal fruit. Among the transformants, many exhibited a changing color phenotype of the fruit, varying from parent Moneymarker line red (MM) to bright orange. The transform shows a red color, after visual inspection, some shows a slightly darker color [32].

Fruit color is very much determined by the ratio of lycopene and beta-carotene content, where fruit with the same lycopene content but lower beta-carotene content will make the fruit look red, while fruit that has the same lycopene content with higher beta-carotene content results in fruit color appearance more orange [34]. Tomatoes with red color with a higher content of lycopene have better antioxidant activity for the heart than tomatoes with higher levels of beta-carotene and lutein, but this antioxidant activity is better in the form of fruit juice compounds than in the form of lycopene, beta-carotene and lutein pure [35].

The antioxidants in cherry tomatoes have a higher lycopene content than round or cluster tomatoes. Cherry tomatoes contain between 48.9 and 116.7 mg of lycopene per kg of wet weight. Round types ranging from 4.3–47 mg / kg wet weight. The lowest type of lycopene cluster or cluster is 12.6–35 mg / kg wet weight [24, 34]. However, the color of the fruit always gives a different content of lycopene. Red fruit will provide a higher content in yellow fruit, but this is also connected to

Figure 2.
Color Varieties Juliet (N1V1) and Golden Sweet (N1V2) of Cherry Tomatos, Golden Shine (N1V3), and Betavila (N1V4) of Round Tomato.

the ratio A and B in the fruit. So although cherry tomatoes have a higher lycopene content, if the fruit color is more yellow or orange than round tomatoes, the lycopene content in cherry tomatoes is less than that of round tomatoes [36, 37]. One example of a cherry tomato from the Golden Sweet variety which is yellow in color has a lower content of lycopene compared to the red Betavila variety, although it is not a cherry tomato (**Figure 2**).

5. Effect of environmental factors on lycopene content in tomatoes

Apart from genetic factors, there are other factors that affect lycopene levels in tomatoes. The intensity of sunlight greatly affects plant growth, as well as on tomato plants. Tomato plants treated with 25% black color gave higher antioxidant content of lycopene and beta-carotene than plants treated with 40% shade with pearl, red and yellow colors [38]. The lycopene content of tomatoes grown in greenhouses was 40% higher than those grown in open land. Shade by the foliage may be important for maximizing the lycopene content of tomato plants that grow in warm areas with high solar radiation. Partial fruit shade can be achieved by selecting cultivars with closed canopies, by changing pruning techniques to leave the upper lateral shoots, tying this ripening intact and by orienting the crop rows in a north-south direction [39].

In terms of production parameters, plants with a net shade of 40% had a higher tomato production than 50% shade and the highest production when given net shade with pearl and red colors [40]. The sensitivity to shade depends on plant genetics, the production of tomato varieties in Rempai and Bogor varieties will decrease if planted by poly-culture/intercropping, while Palupi varieties have higher production when planted intercropping [41]. The intensity of sunlight greatly affects the temperature around the plant. Research on lycopene content shows that fruit surface temperature is a more accurate predictor of fruit lycopene content than air temperature, especially in situations where the fruit is directly exposed to intense sunlight. The more direct sunlight is exposed to the fruit, the higher the surface temperature of the fruit, which leads to a lower lycopene content of the fruit [39]. This also happened in the study conducted by the author, where 25% shade gave a high enough yield on tomato production and lycopene content. The increase in tomato fruit production can reach 40% in determinate tomatoes (Betavila variety). The increase in lycopene content occurred for Juliet tomatoes, with the highest lycopene content at 50% shade and for Betavila varieties the highest lycopene content with 25% shade [42].

Other environmental factors that can affect the lycopene content of tomatoes are temperature and humidity. Air temperature below 12° C and temperature above 32° C can reduce the antioxidant content [43]. Fruit stored at 15° C and 25° C had a higher lycopene content than when stored at 7° C [44]. Tomato fruit harvested green-ripe and exposed to light for 24 hours of ripening at 25° C in a growth cabinet, had a higher concentration of lycopene than light-ripe green fruit for 8 hours [35]. Tomato plants grown in environmental conditions with a maximum average air temperature of 40.01° C, with an average maximum air humidity of 73%, have a lower lycopene content in tomatoes. Meanwhile plants grown in environmental conditions with an average maximum temperature of 34.52 and 35.85° C with an average humidity of 77.24% and 82.52% had higher lycopene content in Juliet tomatoes [42]. This happens because respiration occurs faster in environments with higher temperatures. This respiration process affects the lycopene content in the fruit. In this respiration process lycopene is degraded into terpenes so that the lycopene content is reduced. On the other hand, the water content in tomatoes will increase with each storage, because one of the results of this process is water [45].

The results of research on greenhouses in anticipation of climate change, with the application of a combined high-pressure fog system and CO_2 enrichment can be applied to reduce the internal temperature of the greenhouse. This can increase the level of CO_2 concentration, humidity, and high ambient temperature, compared to conventional climate strategies. This can increase photosynthesis and other metabolic activities, including increased carbohydrate supply, driven by changing micro-climatic conditions in the greenhouse, thereby accelerating plant growth and increasing dry matter in leaves. The new technology applied to tomato plants in greenhouses has no negative impact on the formation of fruit sets per frame. Climate change with modern greenhouses has decreased in increasing total yield and fruit size, while the occurrence of flower tip rot in tomatoes has decreased. This indicates that the quality of the fruit is better than the fruit grown in conventional climatic conditions. Furthermore, it promotes the biosynthesis of carotenoids and phenolic compounds in tomatoes which are likely to benefit human health [46].

Fruit age also greatly affects the lycopene content in tomatoes. This can be seen from the results of previous studies that at the time of harvest it affects the lycopene content in tomatoes, the fruit harvested on June 11, 2001, has a lower fruit lycopene content than fruit harvested on July 11, 2001. Fruits harvested on 13 September 2001 contain lycopene was lower than the fruit harvested August 14, 2001 and August 29 2001 [39]. Physiologically immature fruit has a lower lycopene content than physiologically ripe fruit, but physiologically overripe fruit also has a lower lycopene content. The carotenoid content, as well as the antioxidant activity of lypo-philic, was more influenced by the maturation stage than the cultivar which was determined to be less although the effect was significant. The glycolipid content depends on the cultivar stage and maturation [23].

The process of respiration affects the content of lycopene, because during storage the process of respiration occurs which makes lycopene degraded into terpenes so that the lycopene content decreases. In fact, the water content in tomatoes will increase with each storage, because one of the results of this process is water [45]. Hydrophilic anti-oxidative activity is typology-dependent, and independent of the maturation stage. Cherry tomatoes have the highest lipophilic and hydrophilic antioxidant abilities; In addition, its high carotenoids combined with low glycocaloid content. Therefore it is necessary to conduct research on the factors related to pre and post harvest conditions that must be taken into account to better understand their effects on the synthesis and accumulation of components such as carotenoids and glycocaloids as well as antioxidant abilities. All of these factors contribute to the determination of tomato quality, particularly in terms of the health-related properties of this fruit [23].

A larger proportion of water results in a decrease in lycopene content. Considerable differences can be observed between greenhouse production and open fields. In the field, climatic factors cannot be controlled and plant stands are exposed to high temperatures and rainfall. Plants grown in the field show lower lycopene content than in greenhouses. Significant differences were also observed between different varieties in terms of lycopene content. An understanding of the relationship between the factors that influence lycopene levels and the content of other compounds with antioxidant properties is necessary if the potential benefits for human health are to taken from tomato consumption [47].

The effect of using various enzyme concentrations on the maximum extraction of lycopene from all dental tumors. The results of the enzyme-assisted extraction showed an increase in lycopene yield of 96.3/g / g (144%), in the case of the cellulase-treated samples. Cellulase acts on cellulose. Lycopene extraction using cellulases and pectinases from various fractions and tomato waste showed that cellulases and pectinases were effective in increasing the yield of lycopene. For whole

tomatoes, pectinase was more effective than cellulose, with an increase in lycopene yield of 108 g / g (224%). For tomato peels used as a source of lycopene, pectinase was also found to be more effective than cellulose, with an increase in lycopene yield of 1104 μg / g (206%). Fruit pulper waste showed an increase in lycopene extraction yield of 119 g / g (23%) for cellulase and 190 g / g (52%) for samples treated with pectinase. Once again, pectinase was proven to be more effective than cellulase for lycopene extraction from pulp waste. However, there was an increase in lycopene yield of 202 g / g (61%) and 156 μg/g (45%) respectively for industrial waste samples treated with cellulase and pectinase. Therefore, cellulase enzymes are more effective than pectinases for lycopene extraction from industrial waste. Of all the fractions and tomato waste studied as a source of lycopene, it turned out that tomato peels showed the highest increase in lycopene yield using the pectinase enzyme. The two enzymes used in this study were found to improve lycopene recovery from tomato waste. In conclusion, the amount of valuable lycopene pigment in tomatoes, which is lost as waste in processing, can be recovered in high yield by extraction using cellulases and pectinases [48].

Processing of tomato pulp in the presence of 5% lipids led to the following results and hypotheses. Adding lipids before processing clearly increases the bio-accessibility of lycopene. However, the type of lipid added is not very important compared to the applied process conditions. Processing can remove cellular barriers to the accessibility of lycopene in tomato-based products. However, it remains unclear which barrier (cell wall or chromoplast) is affected by which unit operation and under what conditions. Therefore, a more detailed study in this context is suggested. As a practical guideline for increasing the bio-accessibility of lycopene, intense thermal processing is recommended for large tomato particles while less intense conditions are sufficient for smaller tomato particles obtained by prior mechanical processing to damage the cellular structure. Although in vitro digestion models have proven to be valuable tools for predicting phytochemical bioavailability in foods, further (in vivo) bioavailability studies are needed to confirm the findings of this study. The results presented can be used as a guide for this in vivo experiment [49]. So the content of lycopene in fruit is not only influenced by plant genetics, environmental factors and fruit processing also greatly affect the content in tomatoes.

Author details

Dwi Setyorini[1,2]

1 Assessment Institute for Agricultural Technology, East Java, Indonesia

2 Indonesian Agency Agricultural for Research and Development, Jakarta, Indonesia

*Address all correspondence to: rinibptpjatim@gmail.com

References

[1] I. Gunawan, I. Gede Bawa, and N. Sutrisnayanti, "Isolasi dan Identifikasi Senyawa Terpenoid yang Aktif Antibakteri pada Herba Meniran(Phyllanthus niruri Linn) (Isolation and Identification of Antibacterial Active Terpenoid Compounds in Meniran Herbs (Phyllanthus niruri Linn))," *J Kim*, vol. 2, no. 1, pp. 31-39, 2008.

[2] D. Tholl, "Terpene synthases and the regulation, diversity and biological roles of terpene metabolism," *Curr Opin Plant Biol*, vol. 9, no. 3, pp. 297-304, 2006, doi: 10.1016/j.pbi.2006.03.014.

[3] S. Lenny, "Senyawa Flavonoida , Fenilpropanoida dan Alkaloida (Flavonoids, Phenylpropanoida and Alkaloida Compounds)," USU Repository, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Sumatera Utara, 2006.

[4] D. B. Rodriguez-Amaya, *A Guide to Carotenoid Analysis in Foods*. Campinas, SP., Brasil: ILSI Press, International Life Sciences Institute, One Thomas Circle, N.W. Washington, D. C. 20005-5802, 2001.

[5] Ramadani, "Senyawa Kimia Bahan Alam Terpenoid (Natural Terpenoid Chemical Compounds)," *Tarbawi J Ilmu Pendidik*, vol. 1, no. 1, pp. 1-9, 2016.

[6] J. Graßmann, "Terpenoids as Plant Antioxidants," *Vitam Horm*, vol. 72, no. 05, pp. 505-535, 2005, doi: 10.1016/S0083-6729(05)72015-X.

[7] C. I. Keeling, "Terpenoid biomaterials," *Plant J*, vol. 54, pp. 656-669, 2008.

[8] D. J. Mcgarvey and R. Croteau, "Terpenoid Metabolism," *Plant Cell*, vol. 7, no. July, pp. 1015-1026, 1995.

[9] G. Britton, S. Liaaen-Jensen, and H. (ed.. Pfander, "Carotenoids. Handbook," in *Photosynthetica*, vol. 42, no. 2, 2004, pp. 186-186.

[10] H. Hug, M. Bader, P. Mair, and T. Glatzel, "Biophotovoltaics : Natural pigments in dye-sensitized solar cells q," *Appl Energy*, vol. 115, pp. 216-225, 2014, doi: 10.1016/j.apenergy.2013.10.055.

[11] S. T. Mayne, "Beta-carotene, carotenoids, and disease prevention in humans.," *FASEB J*, vol. 10, pp. 690-701, 1996.

[12] Hasri, "Kandungan Likopen Buah Tomat (Lycopersicum esculentum l.) terhadap Waktu dan Suhu Pemanasan (Content of Tomato Lycopene (Lycopersicum esculentum l.) On Heating Time and Temperature)," *J Ilm Kim dan Pendidik Kim*, vol. 16, no. 2, pp. 28-35, 2015.

[13] S. Apriliani, "Analisa Kadar Likopen pada Pasta Tomat Dengan Menggunakan Spektrofotometer Genesys 20 Visible (Analysis Content of Lycopen on Tomato Pasta using Genesys 20 Visible Spectrophotometer)." Universitas Diponegoro (Diponegoro University), p. 34, 2015.

[14] A. Z. Mercadante and D. B. Rodriguez-Amaya, "Effects of Ripening, Cultivar Differences, and Processing on the Carotenoid Composition of Mango," *J Agric Food Chem*, vol. 46, no. 1, pp. 128-130, 1998, doi: 10.1021/jf9702860.

[15] J. Gross, "Chlorophyll and carotenoid pigments in Ribes fruits," *Sci Hortic (Amsterdam)*, vol. 18, no. 2, pp. 131-136, 1982.

[16] A. V. Rao, M. R. Ray, and L. G. Rao, "Lycopene," *Adv Food Nutr Res*, vol. 51, no. 06, pp. 99-164, 2006, doi: 10.1016/S1043-4526(06)51002-2.

[17] L. M. Alda *et al.*, "Lycopene content of tomatoes and tomato products,"

J Agroaliment Process Technol, vol. 15, no. 4, pp. 540-542, 2009.

[18] I. Fitricia, D. Winarni, and I. B. R. Pidada, "Pengaruh Pemberian Tomat (*Solanum lycopersicum* L.) Terhadap Histologi Kelenjar Mammae Mencit Yang Diinduksi 7,12-Dimetilbenz (A) Antrasena (DMBA) (Effect of Giving Tomato (*Solanum lycopersicum* L.) on Mice Histology of Mammal Glands, Induced 7,12-Dimethylbe," *J Mat dan Ilmu Pengetah Alam*, vol. 15, no. 2, pp. 52-56, 2012.

[19] Q. P. Arnanda and R. F. Nurwarda, "Penggunaan Radiofarmaka Teknesium-99M dari Senyawa Glutation dan Senyawa Flavonoid Sebagai Deteksi Dini Radikal Bebas Pemicu Kanker (Use of Technetium-99M Radiopharmaceuticals from Glutathione Compounds and Flavonoid Compounds as Early Detection of Cancer," *J Farmaka*, vol. 17, no. 2, pp. 236-243, 2019.

[20] H. Li, Z. Deng, R. Liu, S. Loewen, and R. Tsao, "Carotenoid compositions of coloured tomato cultivars and contribution to antioxidant activities and protection against H(2)O(2)-induced cell death in H9c2," *Food Chem*, vol. 136, no. 2, pp. 878-88, Jan. 2013.

[21] P. Di Mascio, S. Kaiser, and H. Sies, "Lycopene as the most efficient biological carotenoid singlet oxygen quencher," *Arch Biochem Biophys*, vol. 274, no. 2, pp. 532-538, 1989, doi: 10.1016/0003-9861(89)90467-0.

[22] D. Heber and Q. Y. Lu, "Overview of mechanisms of action of lycopene," *Exp Biol Med*, vol. 227, no. 10, pp. 920-923, 2002.

[23] *C. leonardi et al.*, "Antioxidative Activity and Carotenoid and Tomatine Contents in Different Typologies of Fresh Consumption Tomatoes," *J Agric Food Chem*, vol. 48, pp. 4723-4727, 2000.

[24] K. Thanigai Arul, E. Manikandan, and R. Ladchumananandasivam, *Nanoarchitectonics in Biomedicine*, no. March. Bucharest, Romania: Elsevier, 2019.

[25] E. Giovannucci, "Promises and Perils of Lycopene/Tomato Supplementation and Cancer Prevention Tomato: Tomato Products, Lycopene, and Prostate Cancer: A Review of the Epidemiological Literature," *Am Soc Nutr Sci J Nutr*, vol. 135, no. Mei, pp. 2030S–2031S, 2005.

[26] P. Palozza, R. E. Simone, A. Catalano, and M. C. Mele, "Tomato lycopene and lung cancer prevention: from experimental to human studies.," *Cancers (Basel)*, vol. 3, no. 2, pp. 2333-57, Jan. 2011.

[27] Y. M. Peng *et al.*, "Concentrations of carotenoids, tocopherols, and retinol in paired plasma and cervical tissue of patients with cervical cancer, precancer, and noncancerous diseases," *Cancer Epidemiol Biomarkers Prev*, vol. 7, no. 4, pp. 347-350, 1998.

[28] S. Zhang *et al.*, "Measurement of retinoids and carotenoids in breast adipose tissue and a comparison of concentrations in breast cancer cases and control subjects," *Am J Clin Nutr*, vol. 66, no. 3, pp. 626-632, 1997.

[29] A. V. Rao and L. G. Rao, "Carotenoids and human health," *Pharmacol Res*, vol. 55, no. 3, pp. 207-216, 2007.

[30] L. Arab, S. Steck-Scott, and P. Bowen, "Participation of lycopene and beta-carotene in carcinogenesis: defenders, aggressors, or passive bystanders?," *Epidemiol Rev*, vol. 23, no. 2, pp. 211-30, Jan. 2001.

[31] Nurhayat Atasoy, "Biochemistry of lycopene," *J Annu Vet Adv*, vol. 11, no. 15, pp. 2605-2610, 2012.

[32] H. Choi and D. G. Lee, "Lycopene induces apoptosis in Candida albicans

through reactive oxygen species production and mitochondrial dysfunction," *Biochimie*, vol. 115, pp. 108-115, 2015, doi: 10.1016/j.biochi.2015.05.009.

[33] M. Stacewicz-Sapuntzakis and P. E. Bowen, "Role of lycopene and tomato products in prostate health," *Biochim Biophys Acta - Mol Basis Dis*, vol. 1740, no. 2, pp. 202-205, 2005, doi: 10.1016/j.bbadis.2005.02.004.

[34] C. Rosati et al., "Metabolic engineering of beta-carotene and lycopene content in tomato fruit," *Plant J*, vol. 24, no. 3, pp. 413-420, 2000, doi: 10.1046/j.1365-313X.2000.00880.x.

[35] S. E. Cox, C. Stushnoff, and D. A. Sampson, "Relationship of fruit color and light exposure to lycopene content and antioxidant properties of tomato," *Can J Plant Sci*, vol. 83, no. 4, pp. 913-919, 2003.

[36] H. Li, Z. Deng, R. Liu, S. Loewen, and R. Tsao, "Ultra-performance liquid chromatographic separation of geometric isomers of carotenoids and antioxidant activities of 20 tomato cultivars and breeding lines," *Food Chem*, vol. 132, no. 1, pp. 508-517, 2012, doi: 10.1016/j.foodchem.2011.10.017.

[37] J. O. Kuti and H. B. Konuru, "Effects of genotype and cultivation environment on lycopene content in red-ripe tomatoes," *J Sci Food Agric*, vol. 85, no. 12, pp. 2021-2026, 2005, doi: 10.1002/jsfa.2205.

[38] P. P. Tinyane, D. Sivakumar, and P. Soundy, "Influence of photo-selective netting on fruit quality parameters and bioactive compounds in selected tomato cultivars," *Sci Hortic (Amsterdam)*, vol. 161, pp. 340-349, 2013, doi: 10.1016/j.scienta.2013.06.024.

[39] L. Helyes, A. Lugasi, and Z. Pék, "Effect of natural light on surface temperature and lycopene content of vine ripened tomato fruit," *Can J Plant Sci*, vol. 87, no. 4, pp. 927-929, Oct. 2007.

[40] Z. S. Ilić and L. Milenković, "The Influence of Photo-selective Shade Nets on Quality of Tomatoes Grown Under Plastic Tunnels and Field Conditions," 2010.

[41] F. Khumairot, "Pertumbuhan dan Produksi Tomat (Lycopersicon esculantum Mill.) Toleran Naungan pada Pola Tanam Tumpangsari (Shade Tolerance of Tomato (Lycopersicon esculantum Mill.) Growth and Production in Tumpangsari Planting Patterns)," p. 29, 2014.

[42] D. Setyorini, Y. Sugito, N. Aini, and S. Yudho Tyasmoro, "Lycopene, beta-carotene and productivity of tomato varieties at different shade levels under medium land of Indonesia," *J Appl Hortic*, vol. 20, no. 02, pp. 92-96, 2018.

[43] Y. Dumas, M. Dadomo, G. Di Lucca, and P. Grolier, "Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes," *J Sci Food Agric*, vol. 83, no. 5, pp. 369-382, 2003.

[44] R. K. Toor, G. P. Savage, and C. E. Lister, "Seasonal variations in the antioxidant composition of greenhouse grown tomatoes," *J Food Compos Anal*, vol. 19, no. 1, pp. 1-10, 2006.

[45] Bunga Ludya Fitri, "Pengaruh Varietas dan Lama Penyimpanan terhadap Kandungan Lykopen Buah Tomat (Effect of Variety and Storage Time on the Lykopen Content of Tomatoes)," Universitas Islam Negeri Malang, 2007.

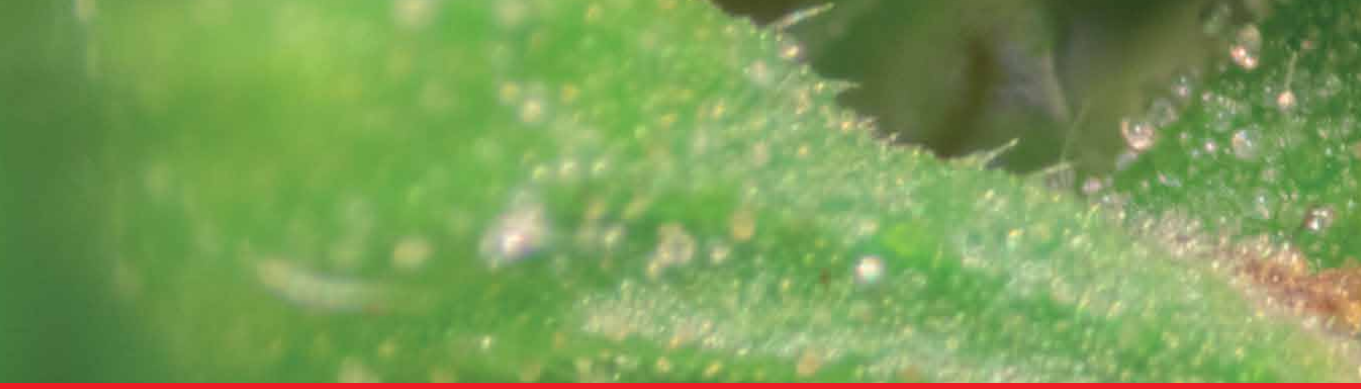
[46] D. Dannehl, C. Huber, T. Rocksch, S. Huyskens-Keil, and U. Schmidt, "Interactions between changing climate conditions in a semi-closed greenhouse and plant development, fruit yield, and health-promoting plant compounds of

tomatoes," *Sci Hortic (Amsterdam)*, vol. 138, pp. 235-243, 2012.

[47] S. Brandt, A. Lugasi, É. Barna, J. Hóvári, Z. Pék, and L. Helyes, "Effects of the growing methods and conditions on the lycopene content of tomato fruits," *Acta Aliment*, vol. 32, no. 3, pp. 269-278, 2003, doi: 10.1556/AAlim.32.2003.3.6.

[48] S. M. Choudhari and L. Ananthanarayan, "Enzyme aided extraction of lycopene from tomato tissues," *Food Chem*, vol. 102, no. 1, pp. 77-81, 2007, doi: 10.1016/j.foodchem.2006.04.031.

[49] I. J. P. Colle, L. Lemmens, S. Van Buggenhout, K. Met, A. M. Van Loey, and M. E. Hendrickx, "Processing tomato pulp in the presence of lipids: The impact on lycopene bioaccessibility," *Food Res Int*, vol. 51, no. 1, pp. 32-38, 2013, doi: 10.1016/j.foodres.2012.11.024.



Edited by Shagufta Perveen and Areej Mohammad Al-Taweel

Terpenes belong to the diverse class of chemical constituents isolated from materials found in nature. They play a very important role in human health and have significant biological activities, including anticancer, antimicrobial, anti-inflammatory, and antioxidant effects. This book provides an overview and highlights recent research in the phytochemical and biological understanding of terpenes and terpenoids, examining the most essential functions of these kinds of secondary metabolites.

ISSN 2632-0983
ISBN 978-1-83881-918-7

Published in London, UK

IntechOpen