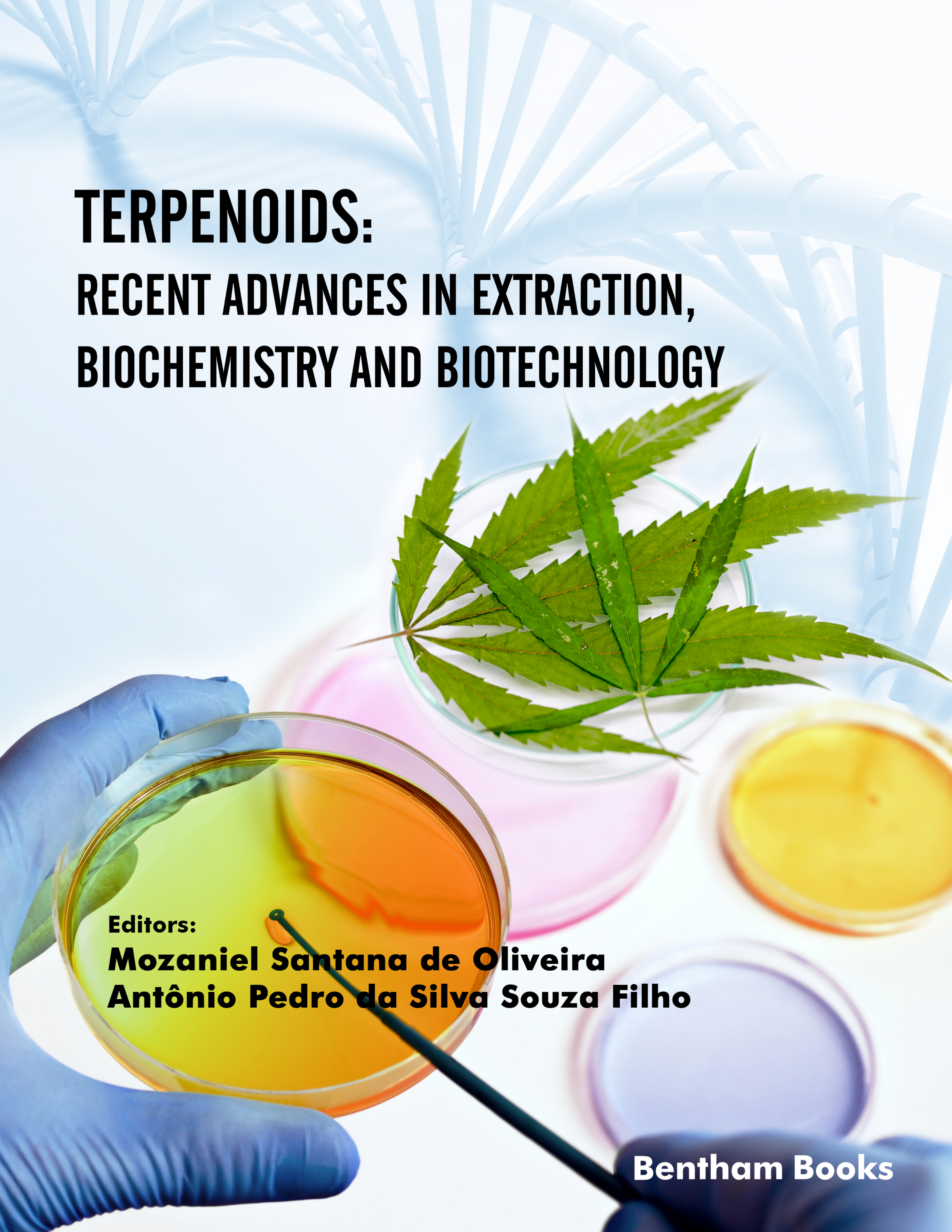

TERPENOIDS:
RECENT ADVANCES IN EXTRACTION, BIOCHEMISTRY AND BIOTECHNOLOGY

Editors:
Mozaniel Santana de Oliveira
Antônio Pedro da Silva Souza Filho

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Terpenoids: Recent Advances in Extraction, Biochemistry and Biotechnology

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Terpenoids: Recent Advances in Extraction, Biochemistry and Biotechnology

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CONTENTS

PREFACE

In natural systems, such as forest areas, and in artificial systems, such as agroecosystems, different types of interactions can occur, promoting changes in the dynamics and density of components, favoring certain components, or harming others. Many of these interactions are due to competition for factors that are essential for the survival of each component, and in others, the interactions are mediated by chemical compounds released by different components in the environment. Plant-plant, plant-fungal, and plant-insect interactions, among others, are good examples of chemical interactions.

Although only in the recent past have significant advances in this area made it possible to understand the real possibilities that this knowledge represented in practical terms for the consolidation of agriculture aimed at meeting the demands of society, the perception of the occurrence of these interactions dates back to a very remote time. Over the years, teams of researchers focused on the subject, and the implementation of properly equipped laboratories enabled the development of research projects that resulted in a substantial accumulation of information on the chemical classes involved in the process and the components of each class.

As new equipment was made available and incorporated into existing laboratories, other laboratories were set up around the world, especially in countries with little tradition in science. In the wake of this process, other researchers were joining the groups already formed, boosting the research even more. As a result of these efforts, several chemicals were isolated and their biological activities identified. Among these studies, the class of terpenoids deserves to be highlighted, representing the group with the largest number of components and the greatest range of activity for the control of weeds, insects, and fungi, among others.

Terpenoids are composed of various chemicals with different polarities, which include both essential oils, formed by monoterpenes, diterpenes, sesquiterpenes, hydrocarbons, and triterpenes, and terpenes and tetranorterpenoids. Transforming available information about this class into a finished product to fight pests and diseases is a challenge that has been overcome.

ACKNOWLEDGEMENTS

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We are grateful to the author, Dr. Mozaniel Santana de Oliveira and would like to give special thanks to PCI-MCTIC/MPEG, as well as CNPq for the scholarship (process number 302050/2021-3).

DEDICATION

To the dream that inhabits in everyone's heart, those who propose to offer alternatives to the cravings of society.

The author Dr. Mozaniel de Oliveira dedicates this work to his parents and Maria and Manoel de Oliveira and wife Joyce Fontes.

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CHAPTER 1

Biosynthesis of Terpenoids By Plants

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Abstract: Terpenoids are a class of chemicals with over 50,000 individual compounds, highly diverse in chemical structure, founded in all kingdoms of life, and are the largest group of secondary plant metabolites. Also known as isoprenoids, their structure began to be elucidated between the 1940s and 1960s, when their basic isoprenoid building blocks were characterized. They play several basic and specialized physiological functions in plants through direct and indirect interactions. Terpenoids are essential to metabolic processes, including post-translational protein modifications, photosynthesis, and intracellular signaling. All terpenoids are built through C_5 units condensed to prenyl diphosphate intermediates. The fusion of these C_5 units generates short C_{15}-C_{25}, medium C_{30}-C_{35}, and long-chain C_{40}-Cn terpenoids. Along with the extension of the chain, the introduction of functional groups, such as ketones, alcohol, esters and, ethers, forms the precursors to hormones, sterols, carotenoids, and ubiquinone synthesis. The biosynthesis of terpenoids is regulated by spatial, temporal, transcriptional, and post-transcriptional factors. This chapter gives an overview of terpenoid biosynthesis, focusing on both cytoplasmic and plastid pathways, and highlights recent advances in the regulation of its metabolic pathways.

Keywords: Abscisic Acid, Brassinosteroids, Carotenoids, Dimethylallyl Diphosphate, Gene Regulation, Gene Expression, Glycosylation, Isopentenyl Diphosphate, Isoprene, Isoprenoids, MEP Pathway, MVA Pathway, Plant Hormones, Prenyl Diphosphate, Secondary Metabolites, Sterols, Terpene Synthesis, Terpenoid, Ubiquinone, Volatile Terpenes.

INTRODUCTION

Terpenoids, also called isoprenoids, are the most diverse class of chemical groups produced by plants. They are the largest category of secondary metabolites derived from the universal 5-carbon compound, isopentenyl diphosphate (IPP),

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and its allylic isomer dimethylallyl diphosphate (DMAPP) [1] (Fig. **1**). The condensation of IPP and/or DMAPP units to prenyl diphosphate intermediates are used as precursors for the biosynthesis of terpenoids.

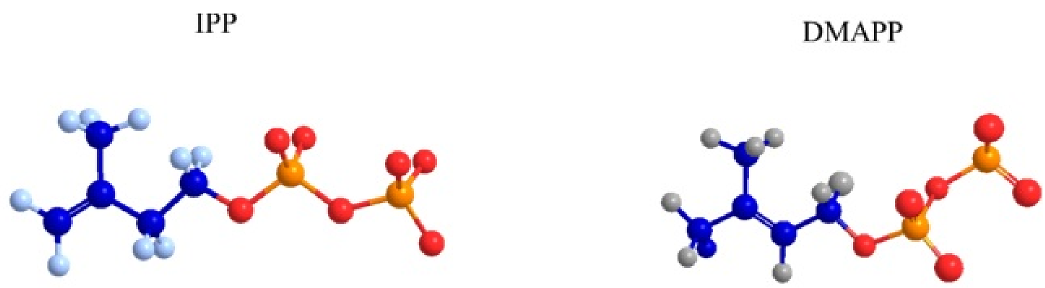

Fig. (1). Isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) molecules structure.

In plants, IPP and DMAPP are produced by two independent pathways: the mevalonate-dependent pathway, also known as mevalonic acid pathway (MVA), and the methylerythritol phosphate pathway (MEP). Both pathways are regulated at the transcript and protein level and by feedback. The enzyme IPP isomerase is the one responsible to convert IPP to DMAPP, the reaction occurring in both directions [2]. Subsequently, IPP and DMAPP fusion generate short, medium and long-chains of prenyl diphosphates, which then can be modified for many different enzymes downstream in the terpenoid biosynthetic pathways [3]. From the MVA pathway in the cytosol, many compounds are generated, such as brassinosteroid, cytokinin, and protein prenylation. From the MEP pathway in the plastids, we have the generation of carotenoids (and subsequently strigolactones and abscisic acid), gibberellins, cytokinin, ubiquinone, and chlorophyll.

Mevalonic Acid Pathway

The MVA pathway is primarily cytosolic and is present in most organisms, including animals, plants, archaebacteria and gram-positive bacteria, and yeasts [4]. It consists of six steps initiated with a condensation reaction of two molecules of acetyl-CoA to acetoacetyl-CoA. This condensation is catalyzed by acetoacetyl-CoA thiolase (AACT) (Fig. **2**). The second step is catalyzed by hydroxymethy-glutaryl-CoA synthase (HMGS), where acetoacetyl-CoA is condensed with another acetyl-CoA molecule to form the C6-compound S-3-hydroxy-3-methylglutaryl-CoA (S-HMG-CoA). In the third step, hydroxymethyglutaryl-CoA reductase (HMGR) catalyzes the conversation of S-HMG-CoA to mevalonate using two NADPH. Mevalonate is phosphorylated to mevalonate-5-phosphate in the 5-OH position in a reaction catalyzed by mevalonate kinase (MK). Mevalonate-5-phosphate produces mevalonate-diphosphate in a reaction catalyzed by phosphomevalonate kinase (PMK). In the last step of the mevalonic acid pathway, mevalonate diphosphate decarboxylase (MPDC) catalyzes the decarboxylative elimination reaction of mevalonate-diphosphate to IPP. The three last steps use one ATP in each reaction.

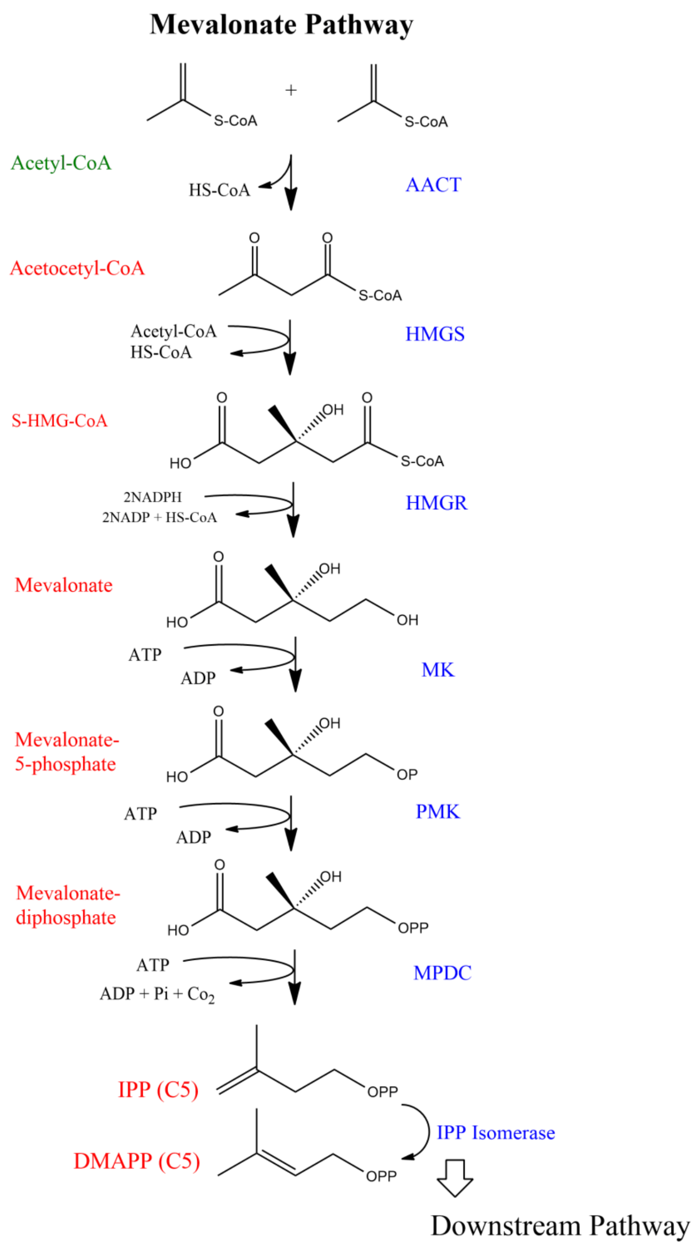

Fig. (2). Enzymatic steps of MVA pathway in terpenoid precursor biosynthesis.

CHAPTER 2

Green Extraction Techniques to Obtain Bioactive Concentrates Rich in Terpenoids

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Abstract: Terpenoids, also called isoprenoids or terpenes, are a large class of natural products which display a wide range of biological activities. They are major constituents of essential oils produced by aromatic plants and tree resins. Due to their notable biological activities, these compounds have enormous economic importance, being widely used as bioactive ingredients in the food, cosmetic, and pharmaceutical industries. The growing demand from consumers and regulatory agencies to develop green sustainable industrial processes has resulted in the emergence of new technologies for obtaining bioactive compounds from natural sources. Thus, many works have been reported in the literature regarding the development and application of new methods for obtaining terpenoids from natural sources that meet the demands of green processes, with reduced consumption of solvent and energy, less waste generation, and use of non-toxic solvents. This chapter proposes to present the main methods of green extraction to obtain terpenoids-rich extracts, with an emphasis on low-pressure methods, such as microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE); and high-pressure methods (here considered as pressures greater than 5 bar), including extraction with supercritical fluids (SFE), subcritical water (SWE) and liquefied petroleum gas extraction (LPG). In addition, the future perspectives and the main challenges regarding the development of alternative methods for the recovery of terpenoids are presented and discussed.

Keywords: Bioactive Compounds, Carbon Dioxide, Conventional Extraction, Green, High-Pressure Extraction, Innovative, Isoprenoids, Low-Pressure Extraction, Microwave-Assisted Extraction, Pressure, Pressurized Liquid Extraction, Supercritical Fluid Extraction, Subcritical Water Extraction, Solvent, Sustainable, Terpenoids, Terpenes, Thermolabile, Temperature, Technologies.

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INTRODUCTION

Terpenoids, also called isoprenoids or terpenes, are a large class of natural compounds since over 60,000 structures have already been identified from natural sources [1, 2]. Terpenoids present an extensive range of biological activities, which is often assumed, for certain terpenoids, due to their lipophilicity and ability to partition into cellular membranes, interact with membrane-bounded proteins and disrupt membrane integrity [3]. Terpenoids are major constituents of essential oils produced by aromatic plants and tree resins. Monoterpenes and sesquiterpenes and their oxygenated derivatives are the most abundant groups of chemical substances in essential oils. Although their biological activities have been scientifically proven, many plants and terpenoid-rich extracts were already widely used in traditional medicine for their anti-inflammatory and pain-relieving properties [4 - 6]. Due to their notable sensory aspects and biological activities, these compounds have enormous economic importance, being widely used as bioactive ingredients in the food, cosmetic, and pharmaceutical industries.

Bioactive compounds, including essential oils, carotenoids, fatty acids, phenolic acids, and flavonoids, were conventionally extracted by steam distillation, solvent extraction, Soxhlet extraction, pressing method, and hydro-distillation, mainly due to their equipment and operation simplicity. However, many drawbacks of conventional extraction methods have been recently recognized. For instance, for Soxhlet extraction, the main disadvantages comprise the long extraction time, the use of toxic solvents, usually in large amounts, the necessity of further evaporation or concentration operation to remove the excess of solvent, besides the possibility of thermal degradation of the targeted compounds due to the harsh extraction conditions (high temperature, long time, presence of oxygen and light, *etc*.) [7]. Most of these limitations also apply to other conventional extraction methods, especially a large amount of solvent required.

Regarding the extraction of terpenoids, thermal degradation is notably a major issue. Many terpenoids, such as α-pinene, limonene, camphor, citronellol, carvacrol, camphene, Δ^3-carene, and γ-terpinene are thermolabile at temperatures above 100 ºC, under subcritical water conditions [8] and hot air [9]. Large-scale extraction of terpenoids commonly uses organic solvents such as methanol or 2-propanol, ethyl acetate, and light petroleum (1:1:1) at temperatures ranging from 40 ºC to 190 ºC [10].

The fact that many bioactive compounds are thermolabile, combined with the growing demand from consumers and regulatory agencies to develop green sustainable industrial processes, has resulted in the emergence of new technologies for obtaining bioactive compounds from natural sources [11]. Thus, innovative strategies to extract and isolate bioactive compounds from plant-based materials are gaining attention in the research and development domains.

According to Chemat, Vian and Cravotto [12], green extraction of natural products is based on the discovery and design of extraction processes that will reduce energy consumption, allow the use of alternative solvents and renewable natural products, and ensure a safe and high-quality extract/product. Therefore, microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), supercritical fluid extraction (SFE), and pressurized liquid extraction (PLE) [13 - 16], which are readily accessible and environmentally sustainable, can be considered green technologies. Many of these green sustainable extraction methods have already been used to recover different terpenoids from plant matrices. The results obtained so far have demonstrated excellent performance of these processes compared to conventional extraction methods.

In this chapter, we will present the main methods of green extraction techniques to obtain terpenoids-rich extracts, with an emphasis on low-pressure methods, such as MAE and UAE; and high-pressure methods, including SFE, subcritical water (SWE) and liquefied petroleum gas extraction (LPG). In addition, the future perspectives and the main challenges regarding the development of alternative methods for the recovery of terpenoids are presented and discussed.

LOW-PRESSURE EXTRACTION METHODS

Microwave-Assisted Extraction (MAE)

Microwaves are radiation of the electromagnetic spectrum ranging in frequency from 300 MHz (radio radiation) to 300 GHz. When applied in chemical processes, the frequencies of 2.45 GHz and 915 MHz are used for laboratory-scale and industrial-scale equipment, respectively [17].

The microwave photon energy corresponding to the frequency used in the microwave heating system ($3.78x10^{-6}$ to $1.01x10^{-5}$ eV) cannot affect the molecular structure since it is lower than the typical ionization energies of chemical bonds (3–8 eV) and hydrogen bonds (0.04-0.44 eV) [18]. As microwave radiation is nonionized, the interaction with materials that absorb the microwave energy occurs by heating. Thus, the efficiency of microwave heating (at a given frequency and temperature) is a function of the capacity of the material to absorb electromagnetic energy and dissipate heat.

Briefly, MAE uses microwave energy to heat solvents containing samples, thereby partitioning analytes from a sample matrix into the solvent. The main advantage of MAE is its capacity to rapidly heat the sample solvent mixture,

CHAPTER 3

Terpenoids Produced by Plant Endophytic Fungi from Brazil and their Biological Activities: A Review from January 2015 To June 2021

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Abstract: Endophytic fungi are fungi that live inside plant tissues at any moment of their life cycle without causing damage or disease symptoms to their hosts. These microorganisms are producers of important substances with several biological activities. Terpenoids are one of the main classes of natural products produced by endophytic fungi, and have a wide range of biological activities, such as anti-inflammatory, anticancer, antioxidant, antifungal, antimicrobial, anticholinesterase, antidepressant, antipyretic, antimalarial, among others. Brazil has one of the largest plant reserves on the planet, consisting of an almost untapped source of endophytic fungi. Thus, in this review chapter, we present the results of the research work of Brazilian researchers, with a focus on the isolation and identification of secondary metabolites of the terpenoid class produced by endophytic fungi and their biological activities. The review period includes January 2015 and June 2021.

Keywords: Bioactive Compounds, Diterpenoids, Endophytic Fungi, Isoprenoids, Meroterpenes, Microorganism, Monoterpenes, Monoterpenoids Diterpenes, Sesquiterpenes, Sesquiterpenoids, Terpenoids, Terpenes, Triterpenes, Triterpenoids.

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Mozaniel Santana de Oliveira & Antônio Pedro da Silva Souza Filho (Eds.)

INTRODUCTION

Natural products are compounds isolated from different natural sources such as plants, animals, microbes, insects, plant pathogens, and endophytes and marines [1]. Microorganisms are very versatile and found everywhere, even in inhospitable habitats, in all ecosystems around the globe. It is preconized that less than 1% of all bacteria species and less than 5% of all fungi species are described, suggesting at least 10 million microbial species are unknown, remaining hidden in nature [2]. Besides, based on genetic research, 90% of biosynthetic skills of microorganisms keep unattainable, which ratifies the significance of microbial natural products research for drug discovery and, even for complete biodiversity knowledge and ecological relationships understanding [3].

Endophytic fungi are fungi that live inside plant tissues at any moment of their life cycle, without causing damage or disease symptoms to their hosts [4 - 8]. These microorganisms are producers of important substances with several biological activities, such as anti-inflammatory, anticancer, antioxidante, antifungal, antimicrobial, anticholinesterase, antidepressant, antipyretic, antimalarial, among others [9 - 12].

Endophytic fungi are one of the most important elements in plant micro-ecosystems and have relevant influences on the growth and development of host plants. Basic knowledge about the relationships between endophytic fungi and their host plants is of significant importance [13, 14]. Any plant-fungal interaction is preceded by a physical encounter between a plant and a fungus, followed by several physical and chemical barriers that must be overcome to successfully establish an association (Fig. **1**) [15 - 17]. To know how an endophyte avoids activating the host defenses, ensures self-resistance before being in capacitated by the toxic metabolites of the host, and manages to grow within its host without causing visible manifestations of infection or disease was initially proposed by the *balanced antagonism* hypothesis [13, 14]. This hypothesis proposed that asymptomatic colonization is a balance of antagonisms between the host and the endophyte. Endophytes and pathogens both possess many virulence factors that are countered by plant defense mechanisms. If fungal virulence and plant defense are balanced, the association remains apparently asymptomatic and avirulent (Fig. **1B**). This phase is only a transitory period where environmental factors play a major role to destabilize the delicate balance of antagonisms. If the plant defense mechanisms completely counteract the fungal virulence factors, the fungus will perish (Fig. **1C**). Conversely, if the plant succumbs to the virulence of the fungus, a plant- pathogen relationship would lead to plant disease (Fig. **1A**). They might be influenced by certain intrinsic or environmental factors to express factors that lead to pathogenicity because many endophytes could possibly be latent pathogens [15]. The plant-endophyte interaction might not be just an equilibrium between virulence and defense, but a much more complex and precisely controlled interaction. Endophytes might protect host plants by creating a heterogeneous chemical composition within and among plant organs that are otherwise genetically uniform [16], according to the *mosaic effect* theory.

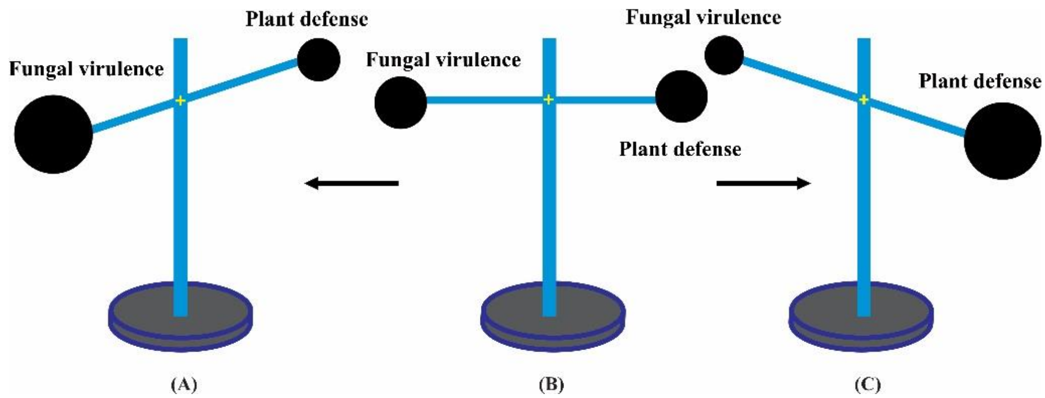

Fig. (1). Balanced antagonism hypothesis: **A)** equilibrium situation; **B)** phytopathogenicity; **C)** healthy plant.

A study based on the report of several authors indicated some benefits promoted by endophytic fungi to their host plants after colonization [18]. Three different beneficial aspects after an interaction are listed: a) First, some endophytic fungi could produce different plant hormones to enhance the growth of their host plants [19]. For example, the growth of wheat (*Triticum aestivum* L.) could be enhanced by *Azospirillum* sp. under drought stresses [20]; b) Second, some endophytic fungi would produce different bioactive compounds, such as alkaloids, diterpenes, flavonoids, and isoflavonoids, to increase the resistance to biotic and abiotic stresses of their host plants [21, 22], and c) Third, some endophytic fungi could promote the accumulation of secondary metabolites (including important medicinal components or drugs) originally produced by plants. These metabolites may be produced by both the host plants or/and endophytic fungi, according to the references surveyed [23].

De Bary described the first endophytic fungi in 1866 [24], but the greatest attention given to these microorganisms as producers of biologically active substances occurred with the isolation of the diterpenoid Taxol (**1**) in 1993, from the endophytic fungus named *Taxomyces andreanae* associated to the phloem of *Taxus brevifalia* Nutt [25]. From then on, endophytes have been recognized as important sources of secondary metabolites such as terpenoids, polyketides, alkaloids, benzopyranones, benzoquinones, naphthoquinones, phenols, steroids, tetralones and xanthones [7, 26 - 30]. The selection of the host plant is an

CHAPTER 4

Volatile Terpenoids in Myrtaceae Species: Chemical Structures and Applications

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Abstract: Terpenes are compounds derived from the secondary metabolism of plants, which act biologically in several functionalities, fighting several predators such as fungi and bacteria. Monoterpenes and sesquiterpenes are some of the main compounds that characterize the chemical composition of essential oils. However, this concentration depends on several factors, such as the type of ecosystem, climate, temperature, and other circumstances that can directly impact the chemical composition of essential oil. The Myrtaceae family is considered one of the main families of Brazilian flora and presents a wide diversity of species. Within this family, some species produce essential oils rich in terpenoids, which, besides being responsible for some biological activities, have contributed to the expansion and search for new natural bioactive substances present in such volatile substances. Given the above, this chapter presents a literature search with current studies that prove the biological and antioxidant activities of terpenoids present in essential oils of species of the Myrtaceae family.

Keywords: Biological Activities, Bioactive Compounds, Volatile Compounds.

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INTRODUCTION

Essential oils are complex, highly volatile mixtures of low molecular weight [1], originating from the secondary metabolism of plants, which present in their chemical composition several organic compounds such as terpenes (monoterpenes and sesquiterpenes), alcohols, ethers, esters, ketones, aldehydes, phenols, lactones, and phenolic ethers (oxygenated groups) [2].

However, it is important to mention that terpenes are also responsible for the application of essential oils in several sectors. For instance, menthol is used in the preparation of perfumes and fragrances; limonene and citronella are used in the manufacture of repellents, while pinene and limonene are used as air purifiers. Others are applied as expectorants, diuretics, and in the production of ointments for itching and pain relief [3].

Terpenoids are biologically active compounds produced by plants, which can be classified according to the number of carbon atoms: hemiterpenes ($_{5}$C), monoterpenes ($_{10}$C), sesquiterpenes ($_{15}$C), diterpenes ($_{20}$C), sesterterpenes ($_{25}$C), triterpenes ($_{30}$C), tetraterpenes ($_{40}$C), and polyterpenes ($_{n}$C) [4]. However, it is important to note that in the chemical composition of essential oils, the strong presence of monoterpenes and sesquiterpenes is peculiar, and monoterpenes can represent about 90% of the essential oil, depending on the type of species studied [5].

In the biosynthesis of terpenic compounds, there are two universal precursors: isopentenyl pyrophosphate (IPP) and dimethylallyl diphosphate (DMAPP). In plants, IPP is biosynthesized through two pathways: *via* mevalonate (MVA) and non-mevalonate (mevalonate-independent), or the deoxyxylulose phosphate pathway, as shown in Fig. (**1**). Through the mevalonate pathway, the IPP intermediate is formed through melavonic acid, which condenses three parts of acetyl coenzyme-A. The non-mevalonate pathway involves 2-C-methyl-*D*-erythritol-4-phosphate (MEP) and 1-deoxy-*D*-xylulose-5-phosphate (DOXP), and results in the condensation of glyceraldehyde phosphate and pyruvate [6]. The first pathway occurs in the cytoplasm, in which most sesquiterpenes are formed, while the second one occurs in chloroplasts, in which there is the formation mainly of monoterpenes and diterpenes [6 - 7].

Monoterpenes are widely distributed in the plant kingdom, especially in some plant species of the Myrtaceae family [5]. They are defined as natural constituents present in the essential oils of plants, which are mostly presented as unsaturated hydrocarbons (C_{10}). Moreover, these compounds have some functions, such as antibacterial, analgesic, stimulant, and expectorant properties [2].

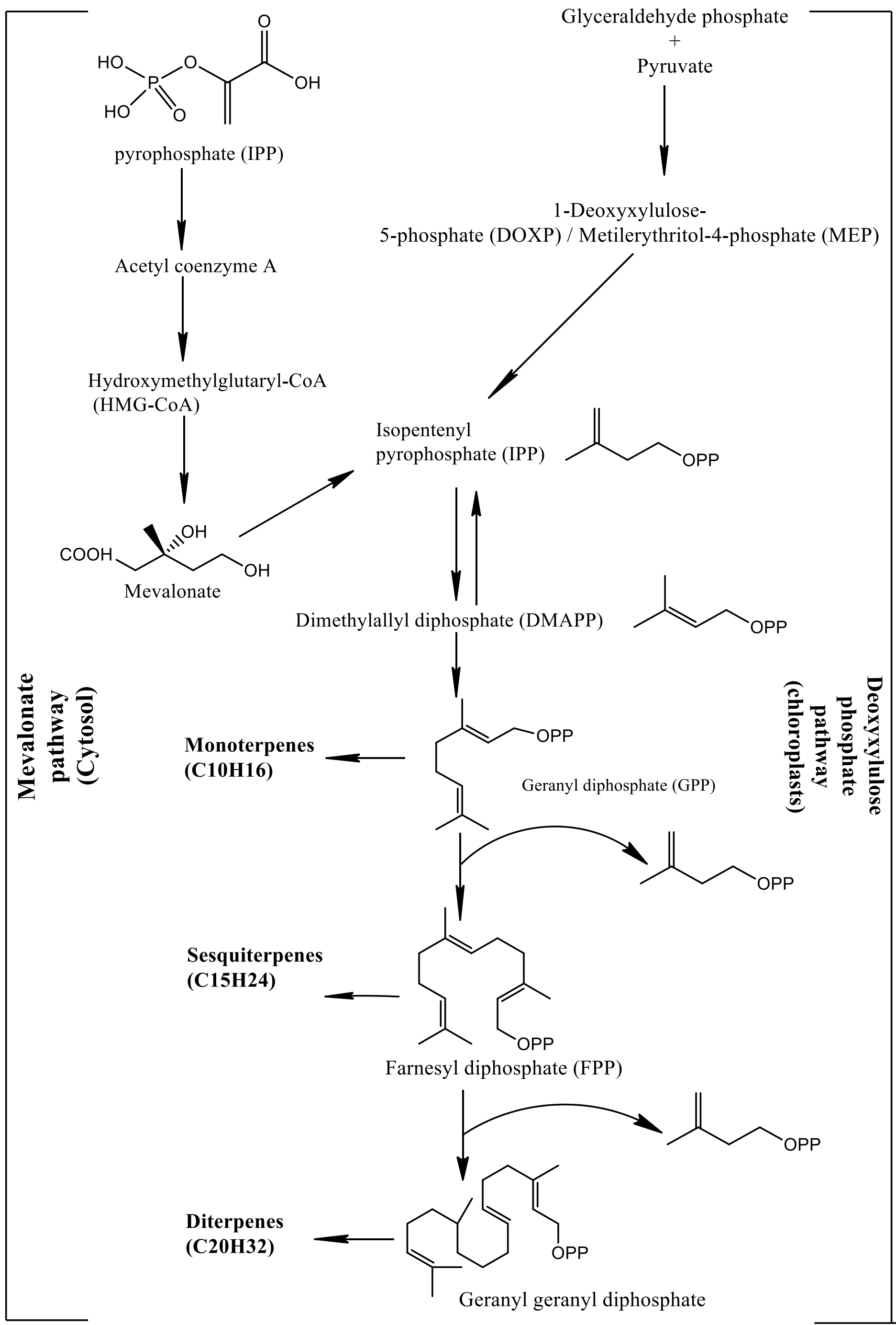

Fig. (1). Biosynthesis of terpenes [6].

CHAPTER 5

Volatile Terpenoids of Annonaceae: Occurrence and Reported Activities

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Abstract: Annonaceae includes 2,106 species. Some species of this family have an economic interest in the international fresh fruit market and are often used as raw materials for cosmetics, perfumes and folk medicine. The most cited species are mainly those belonging to the genera *Annona*, *Guatteria* and *Xylopia*. Chemical investigations indicate that the characteristic constituents of the Annonaceae are terpenoids, including mono and sesquiterpenoids, such as α-pinene, β-pinene, limonene, (*E*)-caryophyllene, bicyclogermacrene, caryophyllene oxide, germacrene D, spathulenol and β-elemene. Antimicrobial, antioxidant, larvicidal, antiproliferative, trypanocidal, antimalarial and anti-inflammatory effects have been described in these terpenes. This work is an overview of the chemical properties and biological effects of the volatile terpenoids from Annonaceae species.

Keywords: Antioxidant Potential, Biological Effects, Essential Oils, Monoterpenes, Sesquiterpenes.

INTRODUCTION

Annonaceae are flowering plants that consist of trees, shrubs and lianas, which have a combination of striking characters, being one of the most uniform botanical families from both an anatomical and structural point of view. It is one of the most primitive of Angiosperms, belongs to the Magnoliopsida class, subclass Magnoliidae and order Magnoliales [1].

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Annonaceae consists of 2,106 species, and more than 130 genera, concentrated in the Tropics, about 900 species are Neotropical, 450 are Afrotropical, and the remaining species are Indomalayan [2]. Annonaceae plays an important ecological role in terms of species diversity, especially in tropical forest ecosystems [3].

Some Annonaceae species are important in the international fresh fruit market, such as *Annona cherimola* Mill. ("cherimólia") and *Annona squamosa* L. ("pinha") [4]. In Brazil, some *Annona* fruits are very popular, such as those of *Annona crassiflora* Mart. ("araticum"), *Annona squamosa* L. ("fruta do conde") and *Annona muricata* L. ("graviola") [5]. In addition, some Annonaceae are often used as raw materials for cosmetics, perfumes and folk medicinal plants [6]. The most cited species in folk medicine are mainly those belonging to the genera *Annona*, *Guatteria* and *Xylopia* [7].

Numerous species of Annonaceae are odoriferous, and these fragrances are due to the presence of essential oils (EOs) [8]. In nature, EOs have many important functions, such as attracting insects or allelopathic communication between plants [9], in addition, they can act as antibacterials, antivirals, anti-inflammatories, and antifungals [10]. About 1% of these volatile constituents are known to date and are mainly represented by terpenoids, phenylpropanoids/benzenoids, fatty acids and amino acid derivatives [11].

According to a review published by Fournier and coworkers (1999) [12], the main volatile constituents of the EOs of Annonaceae species are monoterpene hydrocarbons in fruit and seed, sesquiterpene hydrocarbons in leaf, and oxygenated sesquiterpenes in bark and roots. After this review (1999), several papers have been published evidencing the presence of terpenoids in EOs from Annonaceae and their biological activities. The present work provides an overview of the chemical composition and the biological effects of the volatile terpenoids from Annonaceae species. Original articles published from 2015 to 2021 were considered for composition.

CHEMICAL DIVERSITY OF VOLATILE TERPENOIDS

Terpenoids are natural products with incredibly diverse structures and activities. So far, more than 40,000 phytoterpenoids have been identified [13]. The terpenoids compose the largest class of plant secondary metabolites with many volatile representatives. Terpenoids originated from the universal five carbon precursors, isopentenyl diphosphate (IPP) and its allylic isomer dimethylallyl diphosphate (DMAPP) [11].

So far, more than 90 volatile terpenoids (>5%) have been obtained from different parts of Annonaceae species. Among these compounds, α-pinene, β-pinene, limonene, (*E*)-caryophyllene, bicyclogermacrene, caryophyllene oxide, germacrene D, spathulenol and β-elemene are the most dominant terpenes reported. The terpenes, the corresponding plant sources and references from which they are derived, are summarized in Table **1**.

Table 1. *Mono and Sesquiterpenoids Identified in Essential Oils of* Annonaceae *Species.*

Annonaceae Species [Refs.]	Part of Plant	Number of Identified Compounds	Total of Identified Compounds (%)	Main Monoterpenoids (>5%)	Main Sesquiterpenoids (>5%)	Monoterpenoids (%)	Sesquiterpenoids (%)
Alphonsea tonkinensis A. DC [14]	Leaf	40	98.7	-	β-Elemene, β-caryophyllene, germacrene D, bicyclogermacrene and caryophyllene oxide	5.6	92.9
A. tonkinensis [14]	Stem	40	99.9	α-Pinene, β-pinene, and limonene	β-Caryophyllene, β-elemene, germacrene D and farnesol	28	71.8
Anaxagorea Brevipes Benth [15]	Leaf	31	75.6	-	Guaiol, γ-eudesmol, β-eudesmol and α-eudesmol	3.3	72.3
Annona exsucca DC. [16]	Leaf	50	99.3	Linalool	β-Elemene, (*E*)-caryophyllene, α-humulene, germacrene D, bicyclogermacrene	13.9	84.5
A. exsucca [16]	Leaf	58	99.1	*p*-Cymene, sylvestrene, terpinolene and linalool	Germacrene D and bicyclogermacrene	62.7	36.4
A. Squamosa L. [17]	Fruit	33	86.0	α-pinene, limonene, and β-cubebene	β-caryophyllene, spathulenol, caryophyllene oxide and α-cadinol	N.I	N.I
A. leptopetala (R.E.Fr.) H. Rainer [18]	Leaf	37	98.1	α-Limonene, linalool and α-terpineol	(*E*)-Caryophylene, bicyclogermacrene, spathulenol and guaiol	44.1	55.9
A. muricata L. [19]	Fruit	31	99.98	-	α-Muurolene, β-caryophyllene, δ-cadinene and α-cadinol	N.I	N.I
A. sylvatica A. St.-Hil Anelise [20]	Leaf	36	98.97	-	β-Selinene, (*Z*)-caryophyllene, γ-gurjunene and hinesol	NI	NI
A. vepretorum Mart. [21]	Leaf	26	97.6	α-Phellandrene, *o*-cymene and (*E*)-β-ocimene	Bicyclogermacrene and spathulenol	30.1	67.4
A. vepretorum [3]	Leaf	19	93.9	α-Pinene and limonene	Spathulenol and caryophyllene oxide	NI	NI
A. vepretorum [22]	Leaf	16	100.0	Limonene and (*E*)-β-ocimene	Germacrene D and bicyclogermacre	NI	NI

CHAPTER 6

Repellent Potential of Terpenoids Against Ticks

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Abstract: Substances used as repellents to avoid contact with ticks and tickborne disease are essential to control. Several compounds have been developed throughout human history to promote repellent activity, and in the last decades, synthetic repellents have been widely used. However, several humans, animal, and environmental health problems have been related to synthetic compounds. The use of natural molecules with low toxicity becomes an alternative to replace these compounds. The natural terpenoids from secondary plant metabolites are an essential group with repellency activity on different arthropods. This chapter addresses the primary terpenes with repellency activity, briefly identifying the effectiveness of tick repellents, test methodology, primary terpenes tested, and activity. The evaluated compound showed good repellent activity on different tick species and stages. However, through this chapter, we show the variations in the techniques used to evaluate the bioprospection of terpenes with possible repellent activity and a lack of *in vivo* repellency studies with terpenes. Finally, we emphasize the repellent activity of terpenes to encourage the use of natural compounds as a strategy to control ticks.

Keywords: Animals, Control, Natural Product, Repellent, Tick.

INTRODUCTION

Repellent compounds are volatile chemicals that cause the arthropod to disorient its movements, removing it and thus preventing infestation or attack on the host (Fig. **1**) [1, 2]. Chemical repellents like DEET, IR3535, DEPA, Icaridin (picaridina) and Permethrin (synthetic pyrethroid) have been the most widely used repellents for repelling arthropods, such as insects and ticks [3, 4], with vehicle formulations in the form of a spray, lotion, and gel and can be applied to clothing or skin [5].

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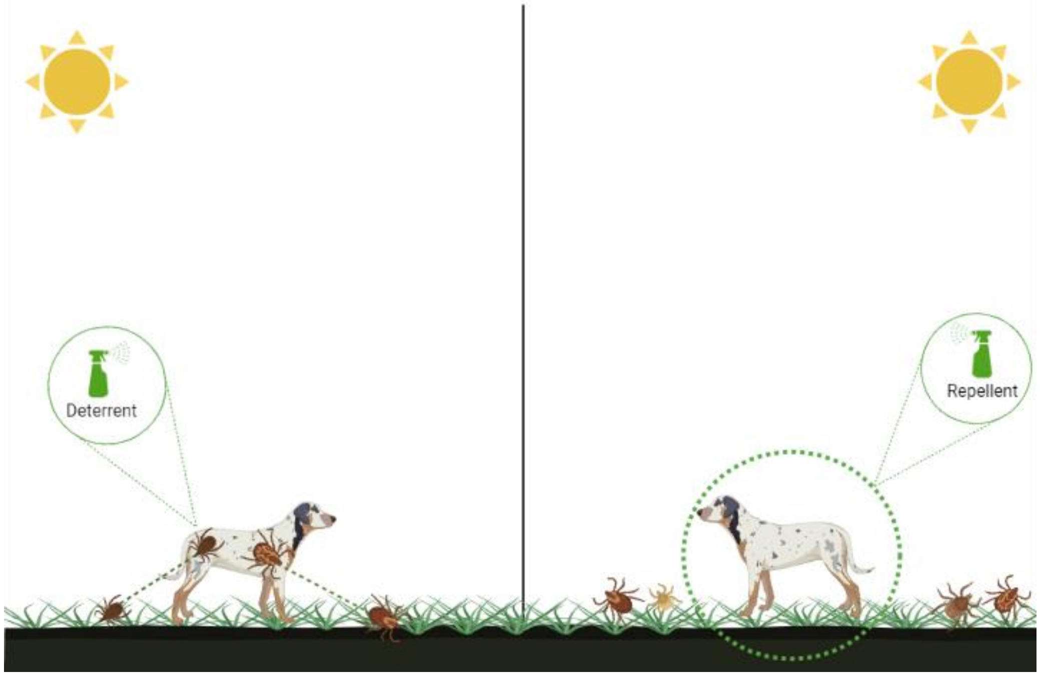

Fig. (1). Deterrent and repellent effect on dogs' ticks.

The repellent products were developed to promote the personal protection of humans against diseases transmitted by insects, such as malaria, dengue, zika, yellow fever, and chikungunya [5]. For many years DEET has been the most used and effective synthetic repellent for this activity, besides being an active compound for many commercial repellents. However, some reported toxicity cases can affect adults and children and may cause environmental and animal health risks [6 - 8]. In this context, growing research for safer, natural, available, and more effective methods for the control and repellency of arthropods parasites [9]. The use of natural products (NP) with repellent effects against arthropods has been promising. Repellent formulations containing citronella, lemon, and eucalyptus essential oil has registered as an insect repellent by US Environmental Protection Agency (US EPA) [10].

Plants have for centuries provided a variety of molecules that have a repellent effect against arthropods, with a large number of descriptions of natural repellents in the literature. NP that has a repellent effect are chemicals produced by the secondary metabolism of plants as a defense mechanism against predatory insects. This repellent action is mainly based on the production of terpenoids (isoprenoids), such as monoterpenes, sesquiterpenes, and phenols (Fig. **2**) [11]. However, classes such as alkaloids, quinones, nitrile, furanes, and lactones have also been described to perform this action [12]. Terpenes represent a diverse chemical group that is part of the secondary metabolism of plants, derivatives of hemiterpene units (C_5) and classified according to the number of carbons found in their chemical skeleton, which can range from monoterpene (C_{10}), sesquiterpenes (C_{15}) to polyterpenes ($>C_{40}$) units [13]. The acyclic and bicyclic isomeric skeletons and functional groups give terpenes the ability to form a wide variety of molecules [14].

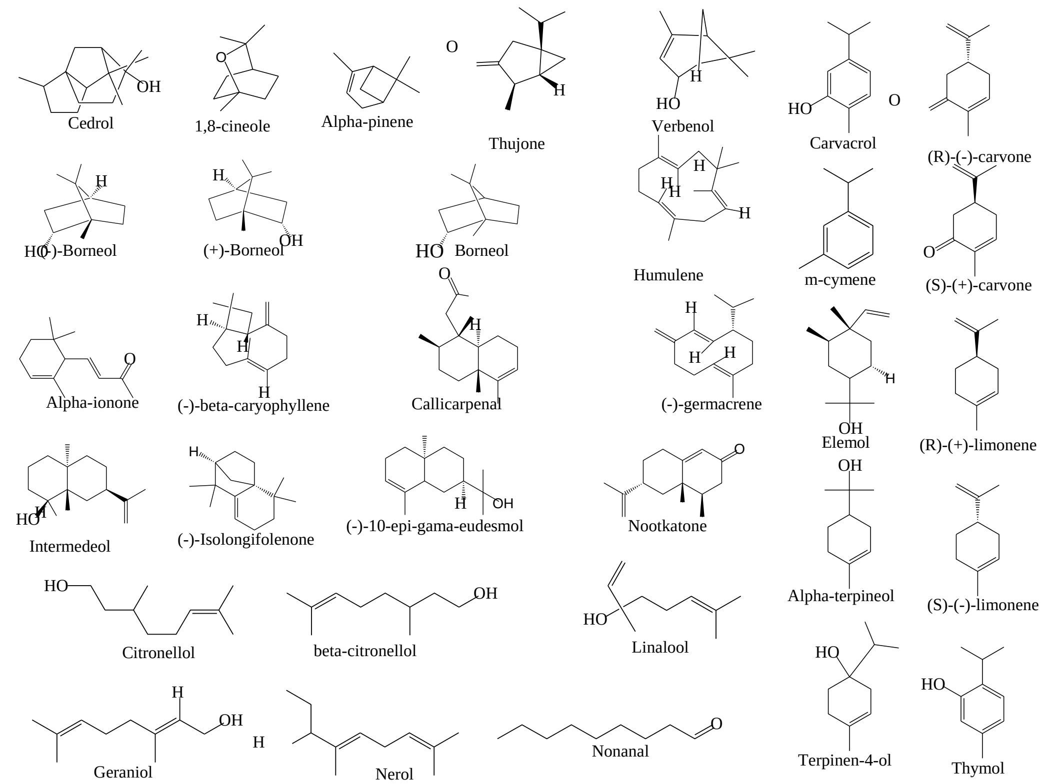

Fig. (2). Terpenoids tested as tick's repellent.

A variety of terpenes have high volatility and lipophilic characteristics, giving them the ability to penetrate the membrane. They are generally colorless and have aromatic odors [15, 16]. Many terpenes are used extensively in the perfumery, cosmetics, and food industries. These compounds show different biological activities; among them, acaricide and repellent against several species of arthropods and have as other functions pollination attractants, herbivore deterrents, antibacterial, anti-inflammatory, allelopathic toxic, antioxidants, thermotolerance, and photoprotection [17 - 20].

CHAPTER 7

Use of Terpenoids to Control Helminths in Small Ruminants

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Abstract: Gastrointestinal nematodes affect the animal's health and cause economic losses in meat, milk, and wool production. Essential oils and their terpenoids have been shown to effectively control gastrointestinal nematodes and may be an alternative to control gastrointestinal nematodes. The great advantage of terpenoids is the possibility of acting on the parasite in a multidirectional way on the neuromuscular system and body structures of nematodes. The current chapter describes the pharmacological basis of the combination of terpenes and synthetic anthelmintics as an alternative for increasing antiparasitic efficacy. It is necessary to evaluate if these combinations show antagonist, additive or synergic effects at the pharmacokinetic and pharmacodynamic levels. The physicochemical properties, pharmacokinetic features and potential drug-drug interactions at the metabolism or transport level of monoterpenes may be relevant for obtaining effective concentrations against different nematodes. In this context, the prediction of absorption, distribution, metabolism and excretion (ADME) is essential to optimize the anthelmintic action of these compounds. The rapid absorption and elimination of monoterpenes after their oral administration may directly influence the drug concentration level attained at the target parasites and the resultant pharmacological effect. Therefore, investigations on the dose schedule, administration route and type of pharmaceutical formulation are necessary. The integration of *in vitro* assays, *in silico* analysis, and *in vivo* pharmaco-parasitological studies are relevant to corroborate the kinetic/metabolic interactions and the efficacy of bioactive natural products combined with synthetic anthelmintics

Keywords: Goat, Natural Product, Nematode, Sheep, Small Ruminant, Synthetic Anthelmintics, Terpenes.

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INTRODUCTION

Gastrointestinal nematodes are especially relevant for small ruminant production. These parasites affect the animal's health and cause economic losses in meat, milk, and wool production [1]. Essential oils and their terpenoids have been shown to effectively control gastrointestinal nematodes [2 - 4]. The terpenoids, compounds from plants, are alternatives to control the gastrointestinal nematodes [5]. However, the mechanism of action of these composts is not quite clear yet.

Since 1950, studies have been performed to better understand anthelmintic compounds' mechanism to control human and animal parasites [6]. The anthelmintic action is associated with the interference of the product in the biochemical process of the parasites. This interference may be related to energy production, muscular coordination, microtubule dynamic, and procedures that can take the parasite's death [7]. Thus, the mechanism of action of the anthelmintic may be invalidated, with alterations that happen in nematode strains, as to the development of parasite defense and are known as resistance [8]. The great advantage of terpenoids is the possibility of acting on the parasite in a multidirectional way [6]. Although the use of control strategies must be well elaborated and planned, it has the most significant effect. For this, a broad knowledge of the mechanism of action is required.

Mechanism of Action of the Anthelmintic Compound

Neuromuscular System and Motility Control

Cys-loop receptors are ligand-gated ion channels activated by several neurotransmitters, like acetylcholine, serotonin, glycine, and GABA [9]. The nervous system of nematodes includes an exclusive and diverse family of cys-loop receptors linked in rapid synaptic transmission, fundamental for worm sensory and locomotor functions [10]. The Cys-loop receptors target widely used anthelmintics, such as levamisole, piperazine, and ivermectin [11 - 13]. The Levamisol-sensitive nicotinic receptors (L-AChR) and GABA (A) (UNC-49) are two target muscular receptors of terpenoids that cause paralyzed effects. Thymol, carvacrol, and eugenol act as inhibitors of L-AChR and UNC-49 receptors from *Caenorhabditis elegans* muscle cells. This result is probably due to the double effects caused by terpenoids on muscle receptors that support antagonistic actions since L-ACHRs are involved in muscle contraction and UNC-49 receptors in muscle relaxation [11].

Terpenoids also act on other different transient receptors. There are 29 nAChR subunits present in *C. elegans*, demonstrating the importance of further studies to explore the selectivity of terpenoids in the nicotinic family [11]. Other terpenoids like carvone, pulegone and eugenol, were also identified as inhibitors of the nAChRs.

Terpenoids with Action in GABA

γ-Aminobutyric acid (GABA) is a family of receptors widely distributed. Nematodes are responsible for regulating motility, feeding, and reproduction [14]. There are distinct forms of GABA receptors: GABAA and GABAB. GABAA is GABA-gated chloride channels located in post-synaptic membranes, while GABAB is G-protein coupled receptors located both in pre-and post-synaptic membranes [15, 16]. Some monoterpenes, such as thymol, thymoquinone, and borneol, are known as positive modulators for GABAA receptors [17]. Recently, with the use of *C. elegans,* it was identified that thymol and carvacrol might be causing paralyzing effects on the worm, linked to the critical receptors in its locomotion. Know well that the activation of neuronal GABA receptors generally results in hyperpolarization and muscle paralysis [11].

The blocking Ca^{2+} channels and positive allosteric activation of the GABAA receptor were attributed to menthol. Menthol, which is well-known for producing a cooling effect, is a TRPM8 agonist. The GABAB receptor activity was found to inhibit TRPV1 sensitization, and TRPV1 activation triggers GABA release [18]. 1.8-cineole, menthol (both (-)- and (+)-), carvone (both (-)- and (+)-), pulegone, linalyl acetate, linalool, carvacrol, estragole, bisabolol, carvone (both (-)- and (+) -), terpinene-4-ol, are known to have analgesic properties targeting Na^{+} and TRP channels [19]. TRPV1-4 are temperature-sensitive channels activated by heat stimuli, whereas TRPM8 and TRPA1 are temperature-sensitive channels activated by cold stimuli [17]. The study suggested the role of glutamatergic neurotransmission and transient receptor potential cation channels (TRP channels) in these actions. Also, monoterpenes with chemical similarity, *e.g*., geraniol, limonene, α-phellandrene, and carvone, may similarly have anti-nociceptive action. These compounds may be ligands of the same receptors and have similar effects [20].

The Action of the Terpenoids on Tubulin

Microtubules are involved in the regulation of various cellular functions, such as cell division, cell motility, intracellular trafficking, and maintenance of cell shape [21]. Commercial anthelmintics can interfere with microtubules. Benzimidazoles block the dimerization of the a and b-tubulin, thus inhibiting microtubules formation, mitosis, and resulting in worm mortality [22]. Some terpenoids have microtubules as the target of action. Citral was lethal to *Arabidopsis* seedlings, interfered with cell division, and in microtubules disrupted, without acting on actin filamentous [23].

CHAPTER 8

Terpenes Behavior in Soil

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Abstract: Soil is a complex and dynamic system in constant change due to its natural processes, as well as interaction among physical, chemical and biological characteristics that take part in it. However, the greatest transformation occurred due to the farm business and the adopted management system. Thus, man can manipulate some soil characteristics and make it more suitable for cropping development. Although anthropic action cannot fully control how soil characteristics interact, it is possible to track them. The action of chemical substances should not be disregarded, a product of the secondary metabolism of plants, since they interfere with plant's ability to compete and survive. Such substances can act out as protectors against herbivores and pathogens. They can be attractive or repellent agents in plant-plant competition and plant-microorganism symbiosis. They can also influence the interaction between plant matter and soil organisms. Among these substances, terpenoids are highlighted as the most structurally diverse chemical family in the class of secondary metabolites that are part of natural products. This knowledge allows a better understanding of nutrient decomposition and cycling processes, the influence of environmental factors on production and terpenoid variability in some plants with medicinal and economic importance.

Keywords: Allelochemicals in Soil, Ecological Interactions in Soil, Secondary Metabolism.

INTRODUCTION

Terpenoids

Secondary metabolites of plants can be classified into three chemically distinct groups: phenolic compounds, nitrogen compounds and terpenoids [1]. Terpenoids, also known as isoprenoids or terpenes, constitute the most chemically structurally diverse family of the secondary metabolite class, which are part of natural products. The term terpenoid should rather be more used than terpene, which

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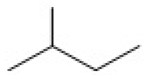
should be used for compounds that are alkenes. The first known terpene structures were α-pinene and camphor, isolated from turpentine [2].

According to Zhou, Picherskym [3], Christianson [4], and Priya *et al.* [5], there are more than 80,000 terpenoid compounds. The terpenome is responsible for almost a third of all compounds described in the Dictionary of Natural Products (http://dnp.chemnetbase.com). These compounds have broad physiological functions, including respiration, photosynthesis, growth, development, reproduction, defense and environmental sensing [3, 4, 6 - 8]. There are also terpenoids derived from animals (cholesterol, dolichol, ubiquinone), which take part in the formation of cell membranes, glycoprotein biosynthesis and intracellular electron transport [9], and others that come from plants (tocopherol, brassinolid and gibberellin). They are responsible for growth regulation and cellular defense [10], and several ecological functions (volatile monoterpenes attract pollinators and sesquiterpenes are present in floral aromas) [3]. These volatile compounds often play an essential role in a plant's defense system, both directly and indirectly, as volatile compounds that repel or attract other insects, respectively [2, 11].

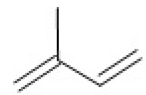
In nature, they also play a significant role in plant-environment interactions, plant-plant communication, and plant-insect and plant-animal interactions [12, 13]. Isoprenoids or terpenoids not only serve as vital allelochemicals in plant defense, but also in several other secondary metabolic processes and plant communication. Some terpenoids are commercially useful, such as pharmaceuticals, flavorings, and biofuels. They are used in food, cosmetic and agricultural industries [14 - 16]. Terpenes can also serve as a source of new drugs or as prototypes for the development of effective pharmaco-therapeutic agents [17, 18].

Despite their structural diversity, all terpenoids are derived from the repetitive bonding of five branching carbons: isopentane (1) - these monomers are referred to as isoprene units (2) - Terpenoids begin with two isoprene-like building blocks, isopentenyl diphosphate (IPP) (3) and dimethylallyl diphosphate (DMAPP) (4) (Fig. **1**) [19, 20].

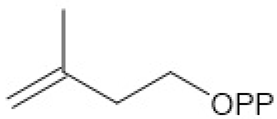
These isoprenic isomers are grouped into categories of natural products, based on their structures, in two pathways for their biosynthesis, and have evolved in different taxonomical organisms [21]. The plants usually use two metabolically separated pathways for IPP and DMAPP biosynthesis in different cell compartments, the mevalonate and non-mevalonate pathways [20, 22].



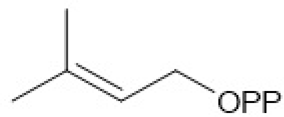
Isopentane **(1)**



Isoprene **(2)**



Isopentenyl diphosphate (IPP) **(3)**



dimethylallyl diphosphate (DMAPP) **(4)**

Fig. (1). 2D isoprene unit formulas.

The non-mevalonate pathway, also known as 2-C-methyl-*D*-erythritol 4-phosphate (MEP) or 1-deoxy-*D*-xylulose 5-phosphate (DXP) pathway, simultaneously produces IPP and DMAPP from the condensation reaction between a pyruvate molecule and 3-phosphate gliceraldehyde, located in plastids, while mevalonic acid (MVA) pathway (Fig. **2**) synthesizes IPP from the reaction of three molecules of Acetyl-CoA to form mevalonic acid. This last acid, after undergoing pyro-phosphorylation, decarboxylation and dehydration reactions, results in IPP and is distributed among cytoplasm, endoplasmic reticulum and peroxisomes in eukaryotes [2, 4, 21, 22], and, despite this compartmentalization, there is some evidence of exchange limited number of common precursors among plastids and cytosol [13, 19, 20].

IPP is a phosphorus-activated compound that becomes its DMAPP isomer, which is biosynthesized by the MEP pathway that occurs in chloroplasts and has an oxygen-pyrophosphate group (OPP). After its oxygen protonation and allelic cation formation, dimerization occurs with geranyl diphosphate formation (GPP) [23 - 25]. Terpenoids are derived from the precursor compounds of IPP and DMAPP and can be classified according to the amount of isoprene residues. They exist as single-unit hemiterpenoid (C_5), monoterpenoid (C_{10}), sesquiterpenoid (C_{15}), diterpenoid (C_{20}), sesteterpenoid (C_{25}), triterpenoid (C_{30}), tetraterpenoid (C_{40}), and polyesterpenoids ($> C_{40}$) and are sub-classified in terms of the degree of cyclization into acyclic, monocyclic or bicyclic [2, 24].

Terpenoids formation occurs by the complete addition of their building blocks (IPP and DMAPP), which are biological equivalents of isoprene, first a head-to-tail condensation of IPP and DMAPP occurs, producing geranyl diphosphate (GPP), a monoterpenoid precursor. The IPP successive addition results in the formation of sesquiterpenoid and diterpenoid precursors, farnesyl diphosphate

CHAPTER 9

Potential Use of Terpenoids in Weed Management

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Abstract: Invasive plants represent a source of economic damage to the agricultural system, and their management has become indispensable from an agronomic point of view, as such plants are known for their competitiveness for resources such as water, light, nutrients, and space. Their control is performed in some cases, such as in Brazil, through the use of pesticides, which can be harmful to human health and other animals. With the change of habits and the search for a better quality of life, the use of these chemicals in management areas is increasingly less encouraged. A possible ecological alternative would be the use of natural products, as secondary metabolites have been shown as potential promoters of phytotoxic activity. Among the allelochemicals produced naturally, terpenoids can be highlighted because their chemical variability can help in the sustainable management of invasive plants.

Keywords: Ecology, Essential oils, Natural Products, Terpenoids, Weed.

INTRODUCTION

In tropical regions, where acidic and low fertility soils predominate, and environmental conditions are highly favorable to the development of biotic agents that are harmful to crops, the success of agricultural activities has always been linked to the use of growth stimulants and agricultural pesticides [1]. While these techniques have ensured satisfactory success, both in terms of productivity and meeting market needs, this scenario has undergone profound changes in recent decades, requiring new paradigms that consider both the values of modern society and the requirements that valorize responsible agriculture in relation to the preservation of natural resources, wildlife, and the humans themselves, who have been seeking food that is increasingly free of chemical residues [2].

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In this context, weeds are one of the most recurrent problems affecting agricultural production and, consequently, the returns on investments applied [3]

[4, 5]. Among the species that infest agricultural areas are those with broad leaves, especially from the families Leguminosae, Malvaceae, Lamiaceae, Convolvulaceae, and Asteraceae [6], and those with narrow leaves, especially from the families Cyperaceae and Poaceae [7 - 9]. The species of these families are characterized by aggressiveness and a high capacity to compete with plants of economic interest, constituting the main component of crop maintenance costs [10, 11]. The control of these species is relevant for crop productivity, and the control methods employed by the producers generate dissatisfaction that promotes insecurity in the sector, especially in relation to chemical products [12, 13].

Many of the current herbicides in use in agriculture have resulted from various weed species being resistant to these products. In recent decades, the number of resistant plant breeds and species has increased significantly in different parts of the world [14 - 16]. In Brazil, the number of herbicide-resistant plants has also increased as a result of the systematic use of herbicides with the same site of action [17, 18]. The use of allelochemicals for the formulation of innovative products can face the challenge of controlling plants resistant to the current products in use, improving the agricultural system, and mitigating the social dissatisfaction arising from the use of herbicides [19, 20].

Allelochemicals can also offer new and innovative molecules with the potential for direct use in the management of weeds, or even make it possible to obtain products as efficient as commercial herbicides [21, 22], without posing any risk to the environment or even to humans, since they have a low permanence rate in the environment, and are quickly degraded by soil microorganisms [23]. Among the various possibilities for this purpose, the terpenoid class deserves to be highlighted due to the wide chemical diversity of its components, which can be classified as monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}), sesterterpenes (C_{25}), triterpenes (C_{30}), tetraterpenes (C_{40}), and polyterpenes ($>C_{40}$) [24].

These compounds have shown phytotoxic activity on invasive plants [25 - 28], which can constitute an advantageous tool to be considered in the strategies of the current agriculture model. Compounds with phytotoxic activity are referred to in the literature as allelochemicals [29 - 34], and in Fig. (**1**), it is possible to observe a form of interaction between plants called allelopathy, in which one of the species produces allelochemicals capable of inhibiting the development of the other one.

Therefore, this work seeks to gather recent information that expresses, in all possibilities, the real potential of using terpenoids in different strategies in weed management.

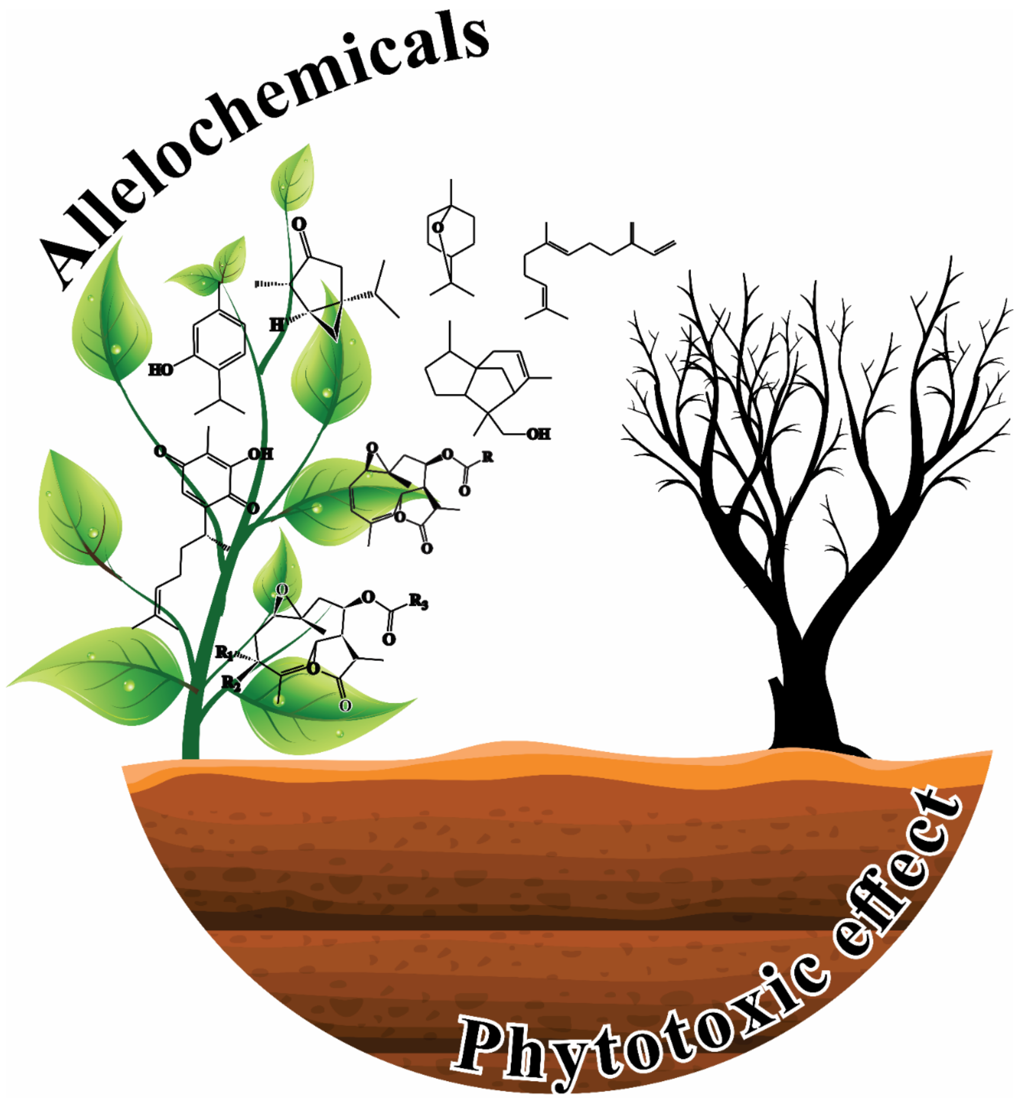

Fig. (1). Illustrative interactions between plants.

Volatile Terpenoids

Terpenoids are an important group of highly diverse chemicals produced by plants that play a leading role in plant defense, and can provide chemical molecules with

CHAPTER 10

Applications of Natural Terpenoids as Food Additives

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Abstract: Food additives are widely used in the food industry in order to ensure the quality of products during processing, storage, packaging and subsequent reaching the consumer's table. The growing concern and doubt of the consumer market regarding artificial additives and their possible harmful effects on public health and safety have caused the demand for the use of natural additives to increase. Consequently, these natural additives have been increasingly sought by the food industry and consumers due to health, safety and sustainability issues. In this framework, terpenoids have great potential to be used with this function because they are a very extensive class of compounds, with wide chemical diversity and several proven applications in foods, mainly as anti-oxidants, anti-microbials, dyes, flavors, sweeteners and nutraceuticals. Therefore, this paper aims to make a literature search on the use of terpenoids as food additives, highlighting the main compounds used and the benefits associated with their use, ranging from the raw material to its extraction and subsequent application in food products.

Keywords: Secondary Metabolites, Additives, Anti-Microbials, Anti-Oxidants, Dyes, Food Industry, Food Chemistry, Flavorings, Food Preservatives, Healthy Life, Natural Additives, Natural Products, Nutraceuticals, Nutritional Fortification, Shelf Life, Sweeteners, Terpenoids, Terpenes.

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INTRODUCTION

The food industry has been using additives for decades in order to give positive attributes to its products, such as longer shelf life and better sensory characteristics. The European Union database represents about 330 authorized compounds, and in the list of Substances Added to Food of FDA (Food and Drug Administration), there are more than 3000. The main additives reported in the composition of ultra-processed foods are nitrates and nitrites, (di/tri/poly) phosphates, sweeteners, monosodium glutamate, sorbate, bixin, caramel, titanium dioxide, tartrazine, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), which can be applied as stabilizers, emulsifiers, dyes, flavorings, preservatives, sweeteners, gelling agents, anti-oxidants, nutrients, among others. Despite the regulations and the benefits generated to the products, researches *in vivo, in vitro* and *in silico* have been showing the harmful effect to the health (allergic, carcinogenic and mutagenic) that the additives, especially the synthetic ones, can bring [1–7].

The interest in products that are sources of bioactive substances of natural origin has increased due to the growing awareness of the consumer market about food safety, healthy eating and the possible damage to health associated with the use of synthetic additives. Thus, natural additives from animals, microorganisms and vegetables have been shown to be an alternative to synthetics and can be used to maintain and prolong food safety. Additives derived from vegetables, such as herbs, spices and their extracts or isolated compounds, contain components that can act in foods as anti-microbial agents, anti-oxidants, flavorings, dyes, and nutraceuticals, among others [8–11].

Terpenoids are the most numerous secondary metabolite group (around 80,000 compounds) and are structurally diverse, being classified according to the number and structural organization of carbons in the linear arrangement. The compounds can be present in natural sources such as plants, animals, microorganisms, insects, plant pathogens and endophytes. They have several functions that can be added to food, cosmetic and pharmaceutical products in the form of food additives, flavorings, fragrances, drug excipients and others. In the food industry, they can be used with different functions, as shown in Table **1**, which presents some terpenes approved by the FDA and the European Union [12–16].

Table 1. Some terpenes approved as food additives on FDA and European Union lists [17, 18].

Function	Compound
Color	Carotenes, bixin, norbixin, capsanthin, capsorubin, lycopene, lutein, canthaxanthin, α-terpineol, caryophyllene.

(Table 1) cont.....

Function	Compound
Antioxidant	α-, β- and δ-tocopherol, β-carotene.
Antimicrobial	Bisabolene.
Masticatory substance	Terpene resin.
Flavor or adjuvant	Lemon terpenes, cedarwood oil terpenes, menthol, α-terpineol, α-terpinene, γ-terpinene, β-terpineol, terpinolene, terpinyl acetate, α-terpinyl anthranilate, terpinyl butyrate, terpinyl cinnamate, terpinyl formate, terpinyl isobutyrate, terpinyl isovalerate, terpinyl propionate, caryophyllene, thymol, carvacrol, eugenol, iso eugenol, phytol, pinene, limonene, tomato lycopene, tocopherols, bisabolene.
Humectant	Natural and synthetic terpene resin.
Solvent or vehicle	Terpene resin.
Nutrient supplement	Carotene, tocopherols.

DIVERSITY AND CHARACTERISTICS OF TERPENOIDS IN FOOD SYSTEMS

Terpenes have great chemical diversity and various applications in the pharmaceutical, food and fine chemical industries [19]. These compounds are produced in nature by some animals, microorganisms and mainly by plants, responsible for aromas and flavors characteristic of fruits and spices [20]. One of the great challenges of the food industry is to mask the unpleasant taste and odor of anti-oxidants, vitamins, minerals and other substances present in nutraceuticals and fortified food [21]. For this, several strategies are used, among them, the use of compounds of natural origin that give the food a more pleasant aroma and flavor [22].

Essential oils obtained from leaves are mainly found in monoterpenes α- and β-pinene, limonene, 3-carene, α-phellandrene and myrcene. Monoterpenes with floral and fruity aromas are most commonly found in seeds and flowers [23, 24]. Woody and balsamic aromas are characteristic of sesquiterpenes and sesquiterpenols found in woody oils [25]. Carotenoids have great anti-oxidant activity because they are able to suppress free radicals produced by chemical reactions in the human body. Other terpenes, such as lutein, γ-carotene, lycopene and β-carotene, have been linked to the fight against breast, colorectal, prostate, lung and uterine cancers [26, 27]. In these studies, carotenoids were investigated for these properties due to the protective power of human tissue when ingested in food or drinks. In addition to these functions, they help to protect the skin against UV rays and improve the immune response. Carotenoids can be found in fruits and vegetables such as sweet potatoes, squash, beets, papaya, mango, broccoli and spinach [28, 29].

CHAPTER 11

Potential Use of Terpenoids for Control of Insect Pests

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Abstract: Essential oils (EOs) have diverse chemical compositions depending on the plant species used, but the most common constituents present in EOs are mono- and sesquiterpenoids. Such volatile terpenoids have different functions in plant ecology, acting, for example, as chemical defenses against fungi, bacteria, and insects, attracting pollinators, inhibiting germination, and mediating intra- and interspecific plant communication. Mainly terpenoids present the ability to inhibit the main families of detoxifying enzymes of insects, allowing the formulation of botanical insecticides, and using blends of EO compounds considered synergists among themselves. In this case, both combinations of essential oils from different plants and the enrichment of essential oils and/or their fractions with compounds with proven synergistic effects can be considered. This chapter presents research results that indicate synergistic, additive, and antagonistic interactions between terpenoids, indicating that this is one of the main properties considered when formulating insecticides based on commercially available EOs. Considerable advances are still necessary for large-scale production, and limitations related to raw material supply, registration, and, mainly, adequacy of formulations for the control of different targets without phytotoxic effects, are the main challenges to be overcome in the short-term.

Keywords: Additivism, Antagonism, Agrochemical Industry, Aromatic Plants, Bioinsecticides, Biological Interference, Botanical Insecticides, Cytochrome P450, Enzyme Inhibition, Esterases, Essential Oils, Glutathione S-Transferase, Insecticide Formulations, Integrated Pest Management, Insect Toxicology, Insecticidal Plants, Microsomal Monooxygenases, Pest Control, Synergism, Terpenoid Blends.

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INTRODUCTION

Essential oils (EOs) are products obtained from plants by dry distillation, steam distillation, or, in the specific case of citrus, fruit pressing [1]. Their chemical composition varies greatly depending on the plant species used, but the most common constituents present in EOs are mono- and sesquiterpenoids. These volatile terpenoids have different functions in plant ecologies, such as chemical defenders against fungi, bacteria, and insects, pollinator attractors, germination inhibitors, and mediators of intra- and interspecific plant-plant communication [2].

Isolation, identification, and synthesis techniques lead to the obtaining of several volatile terpenoids in their pure form, allowing the investigation and use of specific metabolites originally present in EOs. These substances exhibit several applications and have drawn attention due to their potential use as alternative pesticides [3]. The toxicity of terpenoids and essential oils is reported against many pest insects of agricultural importance, such as *Diabrotica undecimpunctata* howardi Barber (Coleoptera: Chrysomelidae) [4]; *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) [5]; *Rhyzopertha dominica* (Fabricius) (Coleoptera, Bostrichidae); *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae); *Sitophilus oryzae* (Linnaeus) (Coleoptera: Curculionidae) [6]; and *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) [7].

Volatile terpenoids interfere in several physiological and behavioral processes of insects, and their insecticidal action is widely reported in the literature [8 - 10]. Insectistastical properties, such as repellency, feeding inhibition, and growth reduction, are more frequent than insecticidal effects in the more than 2,000 bioactive plant species used to control pest arthropods [11].

EOs can also cause toxicity by contact or fumigation, but generally, they do not have a specific mode of action [2]. These compounds can cause cytotoxic effects due to their ability to damage cell membranes [8]. Against insects, commonly, the toxicity of essential oils and terpenoids may be related to neurotoxic effects and growth-regulating action [2, 12].

Many compounds present in essential oils, including terpenoids, are able to inhibit the main families of detoxifying enzymes in insects, and can be used in synergistic formulations with chemical insecticides in order to increase their ability to control pests, using lower doses of active ingredients with proven resistance evolution [13].

Due to these properties, so-called botanical insecticides can also be formulated by blending EO compounds considered synergistic with each other. In this case, both combinations of essential oils from distinct plants and enrichment of EOs and/or their fractions with compounds known to have proven synergistic effects can be considered.

Particularly in the USA, the development and marketing of insecticides with active ingredients obtained from EOs are facilitated and encouraged due to the relative rapidity of registration compared to conventional synthetic insecticides [14]. In fact, many formulations have been developed by American and European companies for the control of household and garden pests, and ectoparasitic mites in bees [15].

Considerable advances are still required for the large-scale production of EO-based insecticides. Limitations related to raw material production, registration, and especially adequacy of formulations to control different targets without negatively affecting host plants, are the main challenges to be overcome in the short term.

Some essential oils, although containing lower proportions of terpenoids, still cause phytotoxicity to plants [16].

MECHANISMS OF INSECTICIDAL ACTION OF TERPENOIDS

In recent years, the use of essential oils obtained from aromatic plants as low-risk insecticides, has increased considerably due to consumer demands and market restrictions. The main plant families from which EOs can be extracted are Apiaceae, Asteraceae, Cupressaceae, Hypericaceae, Lamiaceae, Lauraceae, Myrtaceae, Pinaceae, Piperaceae, Rutaceae, Santalaceae, and Zingiberaceae [17].

Essential oils containing terpenoids as major compounds can exhibit insecticidal, repellent, and growth-regulating effects on various pest insects, effectively controlling pre- and post-harvest phytophagous species. They can also present a repellent effect on disease-causing pathogen vectors such as mosquitoes, household insects, and pests of ornamental plants. With few exceptions, their toxicity in mammals is low, with short persistence in the environment [18].

Few studies have evaluated in detail the toxicology of the major compounds of EOs. However, there is evidence of the negative effects of terpenoids on neurological processes in insects, making their use promising for insect pest control.

CHAPTER 12

Potential Antimicrobial Activities of Terpenoids

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Abstract: The antimicrobial effect of essential oils and their main constituents, the terpenoids, has been generally reviewed in this article, with a comparative investigation of the structure-activity relationship. Terpenoids are widespread metabolites in plants belonging to different chemical classes, whereas oxygenated derivatives constitute the predominates. They could be classified as diterpenes, triterpenes, tetraterpenes, or hemiterpenes and sesquiterpenes. As crude materials, terpenoids are also broadly utilized in drug, food, and beauty care product ventures. Terpenoids have antitumor, anti-inflammatory, antibacterial, antiviral, antimalarial effects, promote transdermal absorption, prevent and treat cardiovascular diseases, and hypoglycemic activities. Moreover, terpenoids have many critical uses as insecticides, immunoregulators, antioxidants, antiaging, and neuroprotection agents. Terpenoids have a complicated construction with assorted impacts and various components of activity. Using plants – containing – terpenoids as neutraceuticals in the nutrition of humans and animals also constitutes a potential issue as natural inhibitors for microbes. These phytochemicals are generally conveyed in soil products and are particularly helpful in food protection as microbial development inhibitors.

Keywords: Terpenoids, Essential oils, Antimicrobial activities, Mode of action, Progress of research.

INTRODUCTION

Terpenoids are one of the natural bioactive classes classified according to the isoprene units. According to the number of isoprenes, terpenoids could be categorized into monoterpene (C_{10}), sesquiterpene (C_{15}), diterpene (C_{20}), triterpene (C_{30}), tetraterpene (C_{40}), and polyterpenoid ($C > 40$). Another well-known classification in the literature is based on oxygenated derivatives like carboxylic acids, esters, aldehydes, alcohols, and glycosides. The interconvertible of C_5 precursors isopentenyl diphosphateproduced *via* mevalonate (MVA) and themethylerythritol phosphate (MEP) pathways is responsible for the natural

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synthesis of terpenoids. The MVA pathway exists in the cytosol with the formation of metabolites such as sesquiterpenes, sterols, and triterpenes, while the MEP pathway is primarily present in plastids through many enzymes that lead to the generation of monoterpenes, diterpenes, and tetraterpenes [1].

Terpenoids represent the major bioactive constituents of the oils found in higher medicinal plants belonging to families like composite, Ranunculaceae, Araliaceae, Oleaceae, Magnoliaceae, Lauraceae, Aristolochiaceae, Rutaceae, Labiatae, Pinaceae, Apiaceae, Celastraceae, Acanthaceae, Taxaceae, and so on. Monoterpenes and sesquiterpenes are predominantly found in essential oils of the medicinal plant, while higher terpenes as triterpene, are primarily found in amber and gum.

The terpenoids are bioactive classes with antimicrobial properties against many microorganisms (Fig. **1**) [2]. Generally, terpenoids showed higher antimicrobial activity than terpenes. For example, the terpenoid fraction of *Helichrysum italicum* essential oil was higher than its terpene fraction against *S. aureus* and *Candida albicans* development [3]. Functional groups of the terpenoids structure play a key role in their antimicrobial activity [2, 4], whereas alcoholic and aldehydic terpenoids, *e.g*., such as terpinene-4-ol and cinnamaldehyde, have a crucial role in their antimicrobial activity, higher antimicrobial efficiency than others containing carbonyl group only. Moreover, geranyl acetic acid has been shown to have a higher antimicrobial activity than geraniol cause of carbonyl and hydroxyl moieties in its structure [2]. Eugenol and cinnamaldehyde are popular terpenoids widespread in the essential oils of many plants with remarkable bioactivity against a broad spectrum of microbes. After a survey of 30 strains of *H. pylori*, a significant human microbe associated with gastric and duodenal ulcers, Ali *et al*. [5] revealed that eugenol and cinnamaldehyde could inhibit H. pylori strains development without any reinforcement. Eugenol additionally has shown striking bioactivity against enterotoxins and biofilms of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-susceptible *S. Aureus* (MSSA) clinical strains [6]. According to Yadav *et al*. [6], eugenol depresses biofilm development, interferes with cell correspondence, destroys the pre-setup biofilms, and kills the microorganisms in biofilms, similarly to MRSA and MSSA mechanisms. These eugenol effects were due to the hindrance of the bacterial cell film and the spillage of the cell substance. In the study of Rathinam *et al*. [7], eugenol displayed practically identical impacts on biofilm development and the harmfulness factor combination of *P. aeruginosa*. A review on the cinnamaldehyde activity against *E. coli* and *S. aureus* using electron microscopy showed damage to the integrity of the bacterial membrane decreased the membrane potential and affected the metabolic activity, thus inhibiting bacterial growth [8]. The hydrogen bonding parameters and the solubility of terpenoids proved to affect their antimicrobial activity by Griffin and colleagues [9] during their study against *P. aeruginosa*, *E. coli*, *S. aureus*, and *C. albicans*.

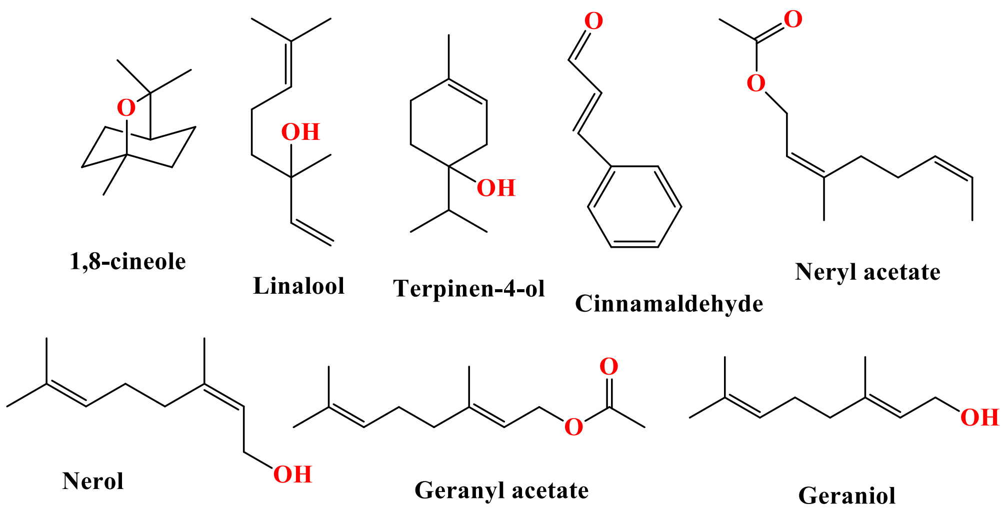

Fig. (1). Chemical structure ofmain antimicrobial constituents (terpenoids) in essential oils.

Antibacterial Activity

Other higher terpenoids like labdane diterpenoid (andrographolide) and pentacyclic triterpenoid (oleanolic acid) were showed a strong antibacterial effect and used as a therapeutic agent against diseases like tuberculosis [10 - 14] (Table **1**, Fig. **2**). Mentha family members are rich in monoterpenoids, which have a strong antimicrobial effect [15]. For example, menthol showed critical inhibitory action of biofilm on *Candida albicans* [16 - 20]. Patchouli liquor (PA) is a tricyclic sesquiterpenoid compound found in *Pogostemon cablin* (Blanco) Benth revealed an antioxidant efficiency against *Helicobacter pylori* activity *in vitro* and *in vivo* [21]. The exploratory information shows that the bactericidal impact of PA is time, pH, and concentration-dependent, whereas the minimal bactericidal concentrations were 25-75 μg/mL [21]. Many researchers discovered that *Artemisia annua* L. oil and extracts have diverse antibacterial activities against anaerobic microscopic organisms, facultative anaerobic microorganisms, microaerophilic microbes, and high-impact microorganisms [22 - 24].

The common use of antibiotics may lead to a lower efficiency toward clinically deadly pathogens like *Pseudomonas aeruginosa*. Cheng *et al*. [25] revealed the impressive inhibitory impact of andrographolide on the biofilm of P.aeruginosa and its synergistic antibacterial effect with azithromycin. Again, Banerjee *et al*. [26] showed an antibacterial activity for the labdane diterpenoid against the significant gram-positive microorganisms, among which *S. aureus* with a

CHAPTER 13

Terpenoids in Propolis and Geopropolis and Applications

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Abstract: Propolis is a resin, which comes from from bee colonies and is considered a natural antibiotic, without serious side effects, compared to synthetic treatments, and has several pharmacological properties. Geopropolis is a mixture of clay and propolis produced by species of stingless bees of the genus Melipona, hence the name geopropolis. It is formed in the same way as propolis produced by other bee species. In this review, we aim to address general aspects related to terpenoids present in propolis and geopropolis. Here, we report the main terpenoids, their chemical structure, and pharmacological and food industry applications.

Keywords: Bess, Food Industry, Pharmaceutical Properties, Stingless Bees, Terpenoids.

INTRODUCTION

Propolis is composed of approximately 50-60% of resins and aromatic balsams, 30-40% of waxes, 5-10% of essential oils, and up to 5% of other substances. Microelements such as aluminum, calcium, strontium, iron, copper, manganese,

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magnesium, silicon, titanium, bromine, zinc, and vitamins B1, B2, B6, C, and E are also present [1].

Its insoluble portion is composed of organic matter, plant tissues, pollen grains, and other substances. The soluble constituents of propolis, obtained using organic solvents, are divided into waxy materials (~30%), balsams, essential oils, and phenolic derivatives (~60%) [2].

According to Sforcin (2007) [3], bees use propolis to seal holes in their hives, smooth out the inner walls, and cover the remains of intruders, who have died inside the hive in order to prevent their decomposition. Propolis protects the colony from diseases due to its antiseptic efficacy and antimicrobial properties. Studies report that propolis's chemical composition can vary according to regional seasonality, which can influence its potential action [4].

The first records of the use of propolis by humankind date back to ancient Egypt (1700 B.C.), and it used to be employed as one of the materials to embalm the dead [1]. The Greeks, including Hippocrates, used it for internal and external cicatrization. The Roman historian Pliny refers to propolis as a medicine capable of reducing swelling and relieving pain. The term propolis was already described in the 16th century in France; and in 1908, the first scientific paper on its properties and chemical composition was published. Later in 1968, the first patent using Romanian propolis for the production of bath lotions was presented. Both works were indexed in *Chemical Abstracts* [2].

In South Africa, the war that occurred at the end of the 19th century was widely used due to its healing properties, and in World War II, it was used in several Soviet clinics. In the former USSR (Union of Soviet Socialist Republics), propolis received special attention in human and veterinary medicine, with applications in the treatment of tuberculosis, observing the regression of lung problems and recovery of appetite [5].

Propolis composition is mainly determined by the phytogeographical characteristics around the hive. However, it also varies seasonally in the same locality. The probable plant source, compared to its chemical composition, is the best indicator of the botanical origin of propolis [6].

Not only the chemical composition of propolis is determined by the vegetation characteristics but also by the pollen and honey deposits. As a consequence of this different chemical composition, there is also a variation in its pharmacological activities [7].

Propolis extract is a mixture of different components in different proportions, and it is not clear how these constituents interact and promote their effects on other organisms. Additionally, there is considerable variation in the composition of propolis extracts according to certain plant species and seasonality [8].

Hernandez *et al.,* (2010) [9] infer that at least one plant species contributes to the production of Cuban propolis. Therefore, although it is a product of animal origin, some chemical compounds of propolis are derived from the botanical source used by bees, especially those with biological action.

In Brazil, some types of propolis have already been characterized and classified by their coloration. According to Daugsch *et al.,* (2007) [10], a new type of red-colored propolis was verified in beehives found along the coast and rivers of Northeastern Brazil, showing physicochemical and biological characteristics different from the others already studied. However, this classification is still underestimated since bees can collect resin from a wide variety of plants.

Most papers in the literature refer to green propolis, and only in recent years has red propolis begun to be studied. Brazilian red propolis has new bioactive compounds never before found in the products already evaluated. It is an important source of substances with biological properties, including antioxidant activity [11].

The global interest in propolis has two justifications: the first is due to its panacea characteristics. In a certain way, these features also hinder its acceptance since doctors and other professionals tend to distrust its efficacy because dozens of biological activities are simultaneously attributed to it. The second reason is its high added-value, as a bottle of the alcoholic extract purchased in Brazil can cost up to 30 times as much in Tokyo. This high added value may justify, in part, their interest in propolis, especially the Brazilian propolis. Although Brazil produces 10 to 15% of the world's production, it supplies about 80% of the Japanese demand for propolis [12].

TERPENOIDS PRESENT IN PROPOLIS FROM *APIS MELLIFERA* BEES

Propolis contains a variety of different constituents, which include phenolic acids, esters, flavonoids, other phenolic molecules, terpenes, ketones, aromatic aldehydes and alcohols, proteins, fatty acids, waxy acids, amino acids, steroids, sugars, vitamins, minerals, and even enzymes [13 - 15]. Propolis has been studied for several applications, such as in human medicine, quality of life, cosmetics and food industries, aquaculture, and livestock, due to its antioxidant and antimicrobial properties [12].

CHAPTER 14

Terpenoids and Biotechnology

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Abstract: Terpenoids, or isoprenoids, represent a large and structurally diverse class of isoprene-based secondary metabolites that play a fundamental role in the organism of all living beings. In nature, terpenes are essential for the interaction of organisms with their environment, mediating antagonistic and beneficial interactions between organisms. In this chapter, we will cover the biotechnology production of terpenes, as well as their biosynthesis by micro-organisms. We will also investigate the various pharmaceutical applications of these compounds.

Keywords: Applications, Biosynthesis, Metabolites, Micro-organisms.

INTRODUCTION

Green plants, particularly angiosperms, exhibit a high number of terpenoids compared to other living organisms [1. It is estimated that more than 80,000 compounds belonging to this class are known, and many more are still unknown in all existing life forms [2].

The structural diversity of terpenoids results from a natural background marked by herbivore stress and other selectivity imposed by animals, resulting in a wide range of functionalized terpenoids preselected for their potent biological activities. It is also driven by stereospecific carbocation cyclizationrearrangement,

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and elimination reactions that transform some universal isopentenyl diphosphate precursors into core layers of numerous structurally distinct terpenoids [3 - 5]. Furthermore, reactions catalyzed by terpene cyclases from cryptic pathways are believed to be largely responsible for the expansive chemodiversity of terpenoid natural products [6].

Biosynthesis of terpenoids occurs *via* mevalonate (MVA) or methylerythritol 4-phosphate (MEP) pathways to generate five-carbon isoprene units, dimethylallyl diphosphate (DMAPP) and isopentenyl diphosphate (IPP), which are coupled to isoprenyl diphosphates (Fig. **1**) and undergo cyclization reactions to produce a myriad of terpenoids [7 - 9].

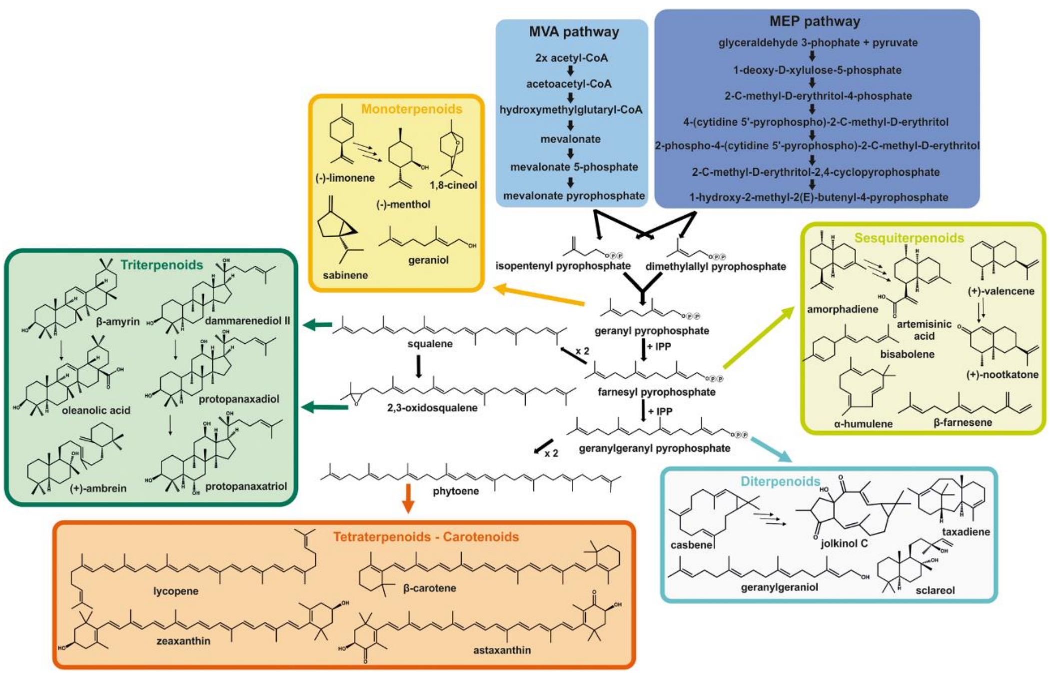

Fig. (1). Biosynthesis of terpenoids. Adapted from Moser; Pichler (2019).

Terpenes are responsible for defending many species of plants, animals, and micro-organisms against predators, pathogens, and competitors, and are involved in transmitting messages to co-species and mutualists about the presence of food, companions, and enemies [10]. For instance, there is much evidence that isoprenoids can act as chemical messengers that influence the expression of genes involved in mechanisms of plant defense or even influence the gene expression of neighboring plants [11].

Terpenes are of great industrial interest because they have promising pharmacological properties, which can lead to the identification of new pharmaceuticals, and can also be used in perfumery and food preservation [12]. For example, the taxol-based compound, Paclitaxel, is one of the most widely used drugs in the treatment of breast cancer [13], in addition to menthol, linalool, camphor, and limonene, which are used in the manufacture of essences, and also the natural rubber used mainly in the automotive industry [14]. Also, recent research has identified terpenes as potential materials for the production of specialty biofuels, since some compounds in this class meet current industrial and chemical requirements, including viscosity, flash and freezing points, high energy densities, and high net heat of combustion [15, 16].

Various methods of obtaining terpenes have been used, such as distillation or solvent extraction techniques, which are typically time-consuming and labor-intensive. In recent years, microextraction techniques (solid-phase microextraction - SPME and stir bar sorptive extraction - SBSE) have been developed, which aid in sample preparation and are environmentally friendly [17]. However, nowadays, supercritical CO_2 extraction, microwave-assisted extraction, and other solid-liquid extraction methods are the most common techniques to isolate and purify hydrophobic terpenes and other natural products from plant-derived raw materials [18].

Despite being considered a renewable source, plants normally produce low concentrations of terpenoids in their tissues. Furthermore, due to the complexity of these molecules, the chemical synthesis of terpenoids is inherently difficult, expensive, and produces relatively low yields. Thus, engineering metabolic pathways to produce large amounts of complex terpenoids in a treatable biological host represents an attractive alternative to extraction processes from environmental sources [19]. In this scenario, micro-organisms, such as *Escherichia coli* and *Saccharomyces cerevisiae* have emerged as a sustainable alternative for the production of industrially valued terpenes, by applying synthetic biology techniques. In addition, they provide a promising alternative to producing non-native terpenes because of the genetic tools available in metabolic engineering and genome editing [4].

Recently, the use of modern biotechnological techniques has increased considerably in order to achieve large-scale production of terpenes with vast structural diversity for applications in the pharmaceutical industry, using the heterologous expression method aided by metabolic engineering techniques [20]. They have been applied for agronomic purposes, producing more resistant plants and obtaining a higher yield of aromatic compounds through the manipulation of transcription factors [21]; or for biotransformation of terpenes into more powerful

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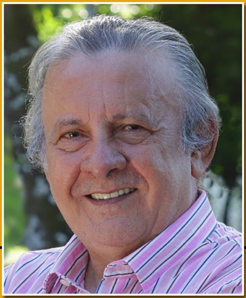
Mozaniel Santana de Oliveira

Mozaniel Santana de Oliveira graduated in Chemistry from the Federal University of Pará, Brazil. He obtained both a master's and Ph.D. in Food Science and Technology from the same university. He has 12 years of professional experience. From 2010 to 2014, he worked on the chemistry of natural products at the Empresa Brasileira de Pesquisa Agropecuária (Embrapa), and from 2014 to 2018, he worked in the Postgraduate Program in Food Science and Technology at the Federal University of Pará, specifically with essential oils. Since 2020, he has been a researcher for the Institutional Training Program - PCI, at the institution Museu Paraense Emilio Goeldi, linked to the Ministério da Ciência, Tecnologia e Inovações of Brazil (MCTI), with studies focused on extraction, characterization chemistry, and applications of essential oils in several industrial segments, among them the food industry. Specifically, Dr. Oliveira has experience in engineering, food science and technology, pharmacology and drug discovery, medicinal chemistry, ethnopharmacology and ethnobotany, phytochemistry, methods of extraction of bioactive compounds, biotechnology of natural products, and allelopathy to find new natural herbicides to control invasive plants. He also has experience in the area of essential oil extraction using supercritical technology and conventional methods. Since 2020, he has supervised and co-supervised master's and Ph.D. students in several graduate programs. Dr. Oliveira serves as a reviewer for thirty-one international scientific journals and is the academic editor of the journals Evidence-based Complementary and Alternative Medicine, Journal of Food Quality, Molecules, and Open Chemistry.



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Antonio Pedro da Silva Souza Filho is a Brazilian, graduated in Agronomic Engineering from the Universidade Federal Rural da Amazônia (UFRA-1977), with a Ph.D. in Animal Science from the Universidade do Estado de São Paulo (Unesp-1995) and Post-doctoral internship at the Institute of Chemistry of the Universidade de São Paulo (2001). He began his professional activities in 1978, at Embrapa, having worked over the years on several research projects, both as a research coordinator and project member, in the area of natural products, specifically in the line of prospecting chemical molecules with potential for use. in weed management, focusing on the bioactivity of essential oils. He also worked as a collaborating professor in the postgraduate courses in Chemistry of Natural Products and Animal Science, at the Universidade Federal do Pará (UFPA), having supervised several Master and Doctoral students. He also participated as co-advisor of masters and doctoral students from the Universidade do Estado de Paraná and Federal de Viçosa. He contributed to the development of doctoral thesis works at the Universidade Federal do Amazonas and the Universidade Federal do Maranhão. Currently, he is linked to Embrapa and throughout his scientific career, he has published numerous scientific articles in different specialized journals and has published several books and book chapters in the area of natural products with an emphasis on the chemical composition and bioactivity of essential oils. He was the President of the Brazilian Society for the Science of Weeds (SBCPD).