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Chapter

Volatile Compounds, Chemical Composition and Biological Activities of *Apis mellifera* Bee Propolis

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Abstract

Propolis is a wax-like resin collected by bees from tree shoots and/or other botanical sources that is used as glue to seal cracks or open spaces in the hive. Its color varies from green to brown and reddish, depending on its botanical origin. Among the substances that can be found in propolis, low molecular weight compounds, such as monoterpenes and sesquiterpenes are the most common. Several biological activities are attributed to these classes of substances, such as antifungal, antibacterial, and others. The objective of this work was to evaluate the chemical composition of volatile compounds present in propolis samples and to analyze their correlation with biological activities.

Keywords: essential oils, Africanized bees, bioactive compounds

1. Introduction

Propolis is formed by vegetable oils and resins, mixed with salivary secretions from bees, and may be in the form of isolated accumulations or combined with waxes. It is constituted by a complex mixture of various compounds and looks similar to a resinous wax collected by bees from tree shoots or other botanical sources. It is also used as glue to seal cracks or open spaces in the hive. Its color varies from green to brown and reddish, depending on its botanical origin and chemical composition. Bees can also use it to prevent diseases and parasites in the hive. In terms of chemical composition, it is generally composed of resin, wax, essential oils, phenolic acids, flavonoids, terpenes, aldehydes, alcohols, fatty acids, and phytosterols [1–4].

In this sense, propolis may represent a natural alternative in the search for bioactive compounds [5], since the use of secondary metabolites is increasing and represents a very broad field of research that can still be explored [6]. In addition, the wide variety of natural substances that can be found in organic matrices can provide key substances for the treatment of various pathologies [7]. The main substances present in propolis are low molecular weight, nonpolar, and volatile compounds [8].

The chemical composition of volatile substances present in propolis is very varied. Several compounds can be found, such as: nerolidol, α-pinene, β-pinene, cedrol, 3-methyl-2-buten-1-ol, octane, tricyclene, β-caryophyllene, spatulenol, δ-cadinene, selina-3,7(11)diene, nerolidol, benzenepropanoic acid, allyl benzyl ether, 1,8-epoxy-p-menth-2-ene, γ-terpinene, mentha-3(8),6-diene, cis-sabinol, 2,3-dehydro-1,8-cineole, α-copaene, p-ethylguaiacol, β-copaene, junipene, γ-cadinene, (3e)-6-phenyl-3-hexen-2-one, p-mentha-1(7),2-dien-8-ol, 4-terpineol, β-fenchyl alcohol, sabinene, δ-3-carene, limonene, α-thujene, α-terpinene, α-terpinolene, trans-verbenol, camphene, verbenene, o-cymene, and α-phellandrene. Moreover, geographical origin and seasonality may influence this composition [9, 10].

Authors have been studying volatile compounds and their applications [11–13] and have seen how these secondary metabolites can be promising in treating various diseases, such as neurodegenerative syndromes [14, 15] and infections caused by microorganisms [16, 17]. Considering the importance of the search for volatile substances present in propolis that may be beneficial for the maintenance of human health, this work aims to perform a literature review in order to address the main biological activities of these metabolites.

2. Main methods of essential oil (EO) extraction

EOs can be extracted from different plant parts and by different methodologies, which generally depend on the botanical material used, and may have a direct relation to the quality of the extracted oil. Therefore, choosing an inappropriate procedure can cause changes in its composition [18, 19]. EO extraction methods are divided into two categories: conventional methods and innovative methods. Traditional methods include hydrodistillation and steam distillation, and among the innovative ones, supercritical fluid extraction [20].

2.1 Hydrodistillation

Hydrodistillation (HD) is the most traditional, simple, and versatile technique used in the extraction of EOs [21, 22]. The basic principle of this type of extraction is azeotropic distillation (substances behave as if they were pure in relation to the boiling point), and to occur, a heating source, a container to place the vegetal biomass (for example, a volumetric flask), a condenser, and a decanter for collecting the oil and water mixture are necessary. HD is considered a multilateral method and, although simple, can be used in small or large industries because of its selectivity and low installation cost [20, 23, 24]. Hydrodistillation process is originated in alembics, however, since the third edition of the European pharmacopeia, its use along with the modified Clevenger system has been recommended, as this system enables the condensate recycling [20].

In HD, plant material, which can be any plant organ, is immersed in boiling water [19, 25]. In summary, the hydrodistillation system (**Figure 1**) consists of a container, usually a volumetric flask, which is connected to a Clevenger-type apparatus attached to a refrigeration system, with temperature ranging from 10 to

Figure 1.
Hydrodistillation system.

15°C. The solid-liquid mixture is heated, at atmospheric pressure, until it reaches water boiling temperature, allowing the odorous molecules to evaporate along with the water, forming an azeotropic mixture, which is drawn into the condenser, where it liquefies and is collected at the end of the extraction. Due to its hydrophobic character, the oil does not mix with water, so it can be separated by decantation. After separation, the oil is completely dehydrated using anhydrous Na_2SO_4 [19, 26, 27].

Hydrodistillation has some drawbacks that can qualitatively and quantitatively interfere in EOs, such as prolonged extraction time and chemical changes in terpene molecules, caused by hydrolysis and cyclization reactions. These are due to excessive contact time with water and loss of some polar molecules [20, 26].

2.2 Steam distillation

Steam distillation (SD) is another traditional method for EO extraction widely used for commercialization and can be used on laboratory and/or industrial scale for its simplicity and low cost, compared to other more sophisticated methods [28, 29]. The fundamental difference between steam distillation and hydrodistillation lies in the fact that, in SD, plant material is not in direct contact with water [20].

Steam distillation is divided into two basic types: direct (or wet) steam distillation and indirect steam distillation. In direct steam distillation (**Figure 2A**), the plant material is placed in a grid above the hot water, and steam passes through it. The leaves should be carefully distributed on the grid to allow uniform extraction and vaporization. Indirect steam distillation (**Figure 2B**) is the most common method for extraction of essential oils. In this process, no water is poured into the distillation tank. Instead, steam is directed to the tank from an external source. Volatiles are released from plant material when steam breaks the glands containing the oil molecules. From this stage, condensation and separation processes are the same [30].

Generally, the time in steam distillation extraction is reduced, which, together with the lack of contact between plant biomass and water, minimizes the chemical changes in EO's constituent molecules [20]. In addition, this technique is appreciated for generating high oil yield and being energy efficient [31].

2.3 Supercritical fluid extraction

Although traditional methods are still widely used in EO extraction, supercritical fluid extraction has become widespread as an alternative to conventional

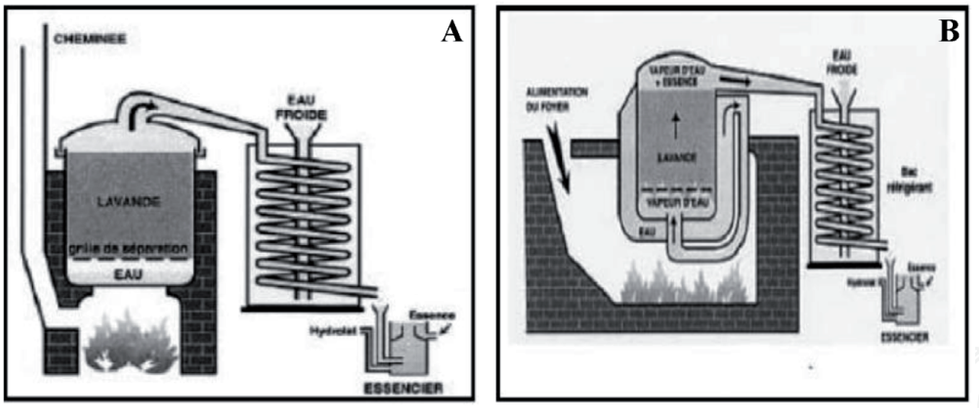

Figure 2.
Representative schemes of direct (wet) steam distillation (A) and indirect steam distillation (B) [20].

extraction methods [32, 33]. Supercritical fluid extraction arose from the need of new techniques that could minimize chemical changes and optimize extraction time [34]. This technique is considered an innovative "green" separation process to obtain natural products, such as EOs, and presents a prominent role in food and pharmaceutical industries [35, 36]. Among many possible supercritical fluids, CO_2 is the most widely used. Its critical point is reached at pressure of 72.9 atm and temperature of 31.2°C, which makes it not harmful to EO thermolabile molecules, thus preventing the chemical changes that occur in classic extraction processes [20, 36]. In addition, CO_2 is an inert gas, which means it is not reactive and can be eliminated simply by pressure decrease at the extractor outlet [20].

Carbon dioxide has characteristics that justify its use as supercritical fluid, such as low viscosity, high diffusivity, and density close to that of liquids [20]. Other factors related to CO_2 also help to understand the importance of using this gas as a supercritical fluid: non-toxicity, non-flammability, insipidity [20, 37], non-corrosivity, non-explosivity [35], great availability [36], and selectiveness [38]. It is also noteworthy that supercritical fluid extraction provides the purest EOs, as no trace of solvent remains after the end of the process, and no external substances are present in the extracted material [39].

In addition to providing a purer product, extraction using supercritical CO_2 is also more advantageous in relation to extraction time, as it is faster than conventional methods [35]. The low viscosity and high diffusivity of the supercritical fluid increase its penetration power based on the high mass transfer rate of solutes, allowing efficient extraction of compounds in the plant material. In addition, low viscosity contributes to lower fluid transport costs [36]. The efficiency of supercritical carbon dioxide extraction is due to the fact that it is a nonpolar solvent, similar to EO's constituents [35].

Despite being a very sophisticated, advantageous, and efficient method for the production of EOs, supercritical fluid extraction has some disadvantages regarding installation costs and equipment maintenance [20], besides high energy consumption to set pressure and temperature [26].

The supercritical fluid extraction system (**Figure 3**) is basically constituted by a carbon dioxide cylinder, cooling bath, high-pressure pump, oven, extraction container, vial, air compressor, flow meter, and flux control valves [35, 40, 41].

The process begins when the CO_2 contained in the cylinder is pumped into the cooling bath, in which it is liquefied and then pressurized by the high-pressure pump. The compressed CO_2 is then transferred to the main extraction cell, maintaining the required process temperature. These processes guarantee the ideal

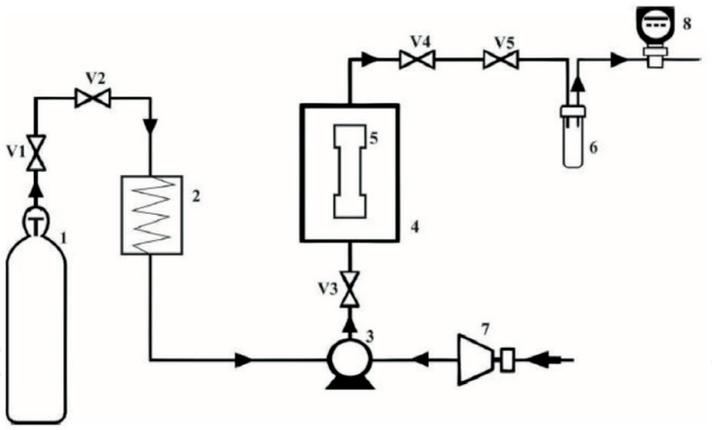

Figure 3.
Apparatus of supercritical fluid extraction of essential oils [40] (1) CO_2 tank; (2) cooling bath; (3) pump; (4) oven; (5) extraction container; (6) vial; (7) air compressor; (8) flow meter; V1-V5. Flux control valves.

thermodynamic conditions of the fluid that will pass through the material, thus extracting the essential oil.

In supercritical fluid extraction, there are two periods: the first is called static, at which the valve V4 is closed for about 30 minutes; then, the dynamic conditions are adjusted and V4 is opened, thus initiating the dynamic period and the extraction itself, because at this point, the essential oil begins to be poured into the collecting vial [35].

2.4 Factors that influence EO composition

Essential oils biosynthesized by aromatic plants can be directly influenced by multiple factors such as genetic, anthropic action, environmental conditions, geographical origin, circadian regime, seasonality, stage of development, and others [42–49].

Variability in content and composition of the EOs and other secondary metabolites is a way that the plant finds to better adapt to the exposed conditions, since the metabolic activity of the plants is a chemical interconnection between the plant and the environment it's inserted [47, 50].

The composition of EOs in plants of the same species that are living in the same place, but that have different chemical profiles, may be influenced by different genetic factors [51]. Other factors that significantly influence both quantitative and qualitative chemical variability of EOs are seasonal and circadian variations, which are related, respectively, to different periods of the year, and to day and night variations [52]. The chemical composition of the EO constituents obtained from the same plant organ may vary according to the species and extraction method used.

3. Chemical composition of *Apis mellifera* essential oil

Apis mellifera bees produce propolis by chewing resins collected from trees by adding salivary enzymes to them. The wax produced is used to cover hive failures, besides having antibacterial, antioxidant, antifungal, and antiviral activities, thus helping to protect the bees themselves.

Due to these properties, the extraction of propolis essential oil has gained prominence in the research field, being reported the presence of compounds such as terpenoids, alcohols, aldehydes, hydrocarbons, and aliphatic ketones in its chemical composition [53, 54]. And due to geographic factors, bee types, and

trees, volatile compounds of propolis essential oil have variable chemical composition [55, 56].

The volatile constituents of propolis are responsible for the pleasant aroma and contribute to its biological activity. These constituents may also play an important role as olfactory cues during resin collection by bees.

The chemical composition of propolis essential oil has already been studied, especially in Brazil. In the work of Albuquerque et al. [57], the chemical composition of propolis essential oil produced by *Apis mellifera* bees in Minas Gerais state was determined. Oliveira et al. [8], Kasumoto et al. [58], and Bancova et al. [10] also studied the chemical composition of propolis essential oil obtained in Brazil, in different regions, as can be seen in **Table 1**. The identification of each compound was performed by comparison with mass spectra and retention indices (RI).

In conducting the first study on propolis essential oil, Janas and Bumba [59] identified few constituents, such as benzoic acid, benzylic acid, vanillin, and eugenol. But later studies [60] show that the constituents of propolis essential oil are quite diverse, with variations in their polar constituents such as flavonoids, phenolic compounds, and phenolic acids, for example.

Frederica Pellati et al. [61] collected nine samples of propolis from *Apis mellifera* in different locations in Italy, extracted their essential oil through hydrodistillation, and identified them by gas chromatography coupled to mass spectroscopy and *headspace*. Then, 99 chemical components were identified.

Major compounds	Reference
(E)-nerolidol	[57]
β-caryophyllene	
Petrolatum 3.7 (11)-diene	
2,2-Dimethyl-8-renyl-6-vinylchromene	[54]
2,6-Diprenyl-4-vinylphenol	
Acetophenone	
Linalool	

Table 1.
Major volatile constituents of propolis in Brazil.

Country	Main compounds	Biological activity	Reference
Bulgaria	β-eudesmol (8.8%), δ-cadinene (5.3%), sesquiterpene alcohol (15.5%)	Antibacterial and antifungal	[62]
Turkey	Ethyl phenyl alcohol (7.7%), benzyl alcohol (7.4%), decanal (6.7%), ethyl benzoate (6.5%), nonanal (5%), cedrol (4.1%)	Antibacterial	[63]
Tunisia	α-Pinene (45.22%), cedrol (8.23%)	Antifungal	[10]
Brazil	Acetophenone (15.2%), nerolidol (13.3%), spatulenol (11.6%)	Antioxidant	[64]
India	Tricosane (13,6%), hexacosane (11.5%), palmitic acid (8.5%), linalool (6.7%), methyleugenol (6.0%)	Repellent activity against bees	[65]
Brazil	Longipinene (24.9%), α-eudesmol (6.9%)	Therapeutic effect	[66]

Table 2.
Main compounds and their biological activities in propolis.

Table 2 shows some important chemical constituents and their respective biological activities.

Geographic differences influence the chemical composition of essential oils extracted around the world, and as a result, these differences contribute significantly to the chemical properties and biological activities of all types of propolis. Its collection period also influences its oil composition, as it can be mixed with hive resins and wax.

In Venezuela [67], propolis essential oil produced by *Apis mellifera* had three main constituents: D-germacrene (26.5%), β-caryophyllene (10.2%), and β-elemene (8.1%), thus being similar to the chemical constituents of Brazilian propolis [64].

4. Biological activities of *Apis mellifera*

The main chemical compounds isolated from *Apis mellifera* are aliphatic acids and esters, aromatic acids and esters, sugars, alcohols, aldehydes, fatty acids, amino acids, steroids, ketones, chalcones and dihydrochalcones, flavonoids (flavones, flavonols, and flavonones), terpenoids, proteins, vitamins B1, B2, B6, C, E, as well as various minerals. Although flavonoids are the most studied components, they are not the only responsible for its pharmacological properties. Several other compounds have been related to the medicinal properties of *Apis mellifera* [68, 69].

There are reports attributing to *A. mellifera* the most varied applications in folk and veterinary medicine, which corroborates its great therapeutic potential, especially in relation to anti-inflammatory, antimicrobial, antineoplastic, antidiabetic, and antioxidant activities [70].

4.1 Anti-inflammatory activity

Amaral et al. [69] evaluated the anti-inflammatory potential of *Apis mellifera* against stomach inflammation induced in healthy adult female Wistar rats infected with *Helicobacter pylori*. This bacterium may cause chronic irritation and increase the risk of developing gastric ulcers. They concluded that the administration of solutions of *Apis mellifera* increases the endogen prostacyclin in rats mucosa, incrementing cytoprotection, and reducing pathogen population. In addition, the high contents of phenolic compounds and flavonoids aid in the protection of the mucin producing cells of the stomach, also contributing to its therapeutic potential.

4.2 Antimicrobial activity

Han et al. [71] evaluated the response of *Apis mellifera* venom (BV) against *acne vulgaris,* in order to prove its antimicrobial potential. *Acne vulgaris* is a chronic inflammatory disorder of the sebaceous follicles. The authors incubated *P. acnes,* clindamycin-resistant *P. acnes*, *Staphylococcus epidermidis*, and *Streptococcus pyogenes*. In their results, BV proved to be bacteriostatic and exhibited low cytotoxicity at 10 μg/ml in human epidermal keratinocytes and monocytes. The authors state that BV can be an alternative for the treatment of *acne vulgaris*.

4.3 Antineoplastic activity

There are several studies that report the antineoplastic activity of *Apis mellifera*. Lee et al. [71] evaluated the anticancer potential of *Apis mellifera* venom (BV), which showed cytotoxicity in HL-60 cells and normal human lymphocytes. Hamzaoglu et al. [71] implanted cancer cells into mice wounds. A significant decrease in the tumors was observed in mice that were treated with *Apis mellifera*

coating before and after surgery. This property may be due to its hypertonicity, acceleration of epithelization, low pH, and the presence of inhibin and catalase.

4.4 Antioxidant activity

In the work of Souza et al. [72], *A. mellifera* extracts, obtained by hydrodistillation, exhibited high antioxidant activity evaluated by free radical DPPH sequestration and β-carotene/linoleic acid methods. The authors linked these results to the presence of the following compounds: prenylated benzophenones, epinemorosone, xanthochymol, gambogenone, and aristophenone A. Wiwekowati et al. [73] also attributed the high antioxidant potential of *A. mellifera* to the structure of its flavonoids and phenolic acids, which was evaluated by inhibition of free radical DPPH.

4.5 Antihyperglycemic and antidiabetic activities

Cunha et al. [74] evaluated, *in vivo*, the control of postprandial hyperglycemia by performing a test of oral glucose tolerance in normoglycemic mice. After glucose overload, the mice treated with *A. mellifera* showed, after 30 min, reduced hyperglycemia peak and blood glucose values, as well as normalization of water intake. These results are similar to that showed by metformin, a first-line medication for the treatment of type 2 diabetes. Control of postprandial hyperglycemia has been linked with reduced vascular damage in diabetic patients.

5. Conclusions

Propolis essential oil presents various biological properties, being active against microorganisms such as bacteria, fungi, and viruses. It is evident that climatic factors are able to influence the chemical composition of the *Apis mellifera* propolis essential oil. In addition, the extraction technique chosen may also influence its yield and chemical composition.

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Conflict of interest

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

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