Chapter

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

**Jorddy Neves Cruz, Adriane Gomes da Silva,**
**Wanessa Almeida da Costa, Ely Simone Cajueiro Gurgel,**
**Willison Eduardo Oliveira Campos, Renan Campos e Silva,**
**Marcos Ene Chaves Oliveira, Antônio Pedro da Silva Souza Filho,**
**Daniel Santiago Pereira, Sebastião Gomes Silva,**
**Eloisa Helena de Aguiar Andrade**
**and Mozaniel Santana de Oliveira**

**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, α-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
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$_2$SO$_4$ [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
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 thermalabile 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
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.

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.

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.

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. 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. 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. 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.

### 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] |
| Portugal | Petrolatum 37 (11)-diene | | |
| Turkey | Ethyl phenyl alcohol (77%), benzyl alcohol (74%), 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 (8.3%), 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. 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.

Acknowledgements

Jorddy Neves Cruz appreciates the support of the Eliseu Alves Foundation (SAIC/Embrapa: 22500.18/0015-7).

Conflict of interest

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

Jorddy Neves Cruz\textsuperscript{1,2*}, Adriane Gomes da Silva\textsuperscript{2}, Wanessa Almeida da Costa\textsuperscript{3}, Ely Simone Cajueiro Gurgel\textsuperscript{4}, Willison Eduardo Oliveira Campos\textsuperscript{2}, Renan Campos e Silva\textsuperscript{3}, Marcos Ene Chaves Oliveira\textsuperscript{2}, Antônio Pedro da Silva Souza Filho\textsuperscript{2}, Daniel Santiago Pereira\textsuperscript{2}, Sebastião Gomes Silva\textsuperscript{1}, Eloisa Helena de Aguiar Andrade\textsuperscript{3} and Mozaniel Santana de Oliveira\textsuperscript{1,4*}

\textsuperscript{1} Adolpho Ducke Laboratory, Emílio Goeldi Paraense Museum, Belém, PA, Brazil
\textsuperscript{2} Laboratory of Agro-Industry, Embrapa Eastern Amazon, Belém, Pará, Brazil
\textsuperscript{3} Program of Post-Graduation in Chemistry, Federal University of Para, Belém, PA, Brazil
\textsuperscript{4} Postgraduate Program in Biological Sciences - Tropical Botany, Federal Rural University of Amazonia and Emílio Goeldi Paraense Museum, Belém, PA, Brazil

*Address all correspondence to: jorddynevescruz@gmail.com and mozaniel.oliveira@yahoo.com.br

IntechOpen

© 2020 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
References

[1] Aygun A. Effects of Propolis on eggshell. In: Hester PY, editor. Egg Innov. Strateg. Improv. 1st ed. Cambridge, Massachusetts, EUA: Elsevier/Academic Press; 2017. pp. 145-156. DOI: 10.1016/B978-0-12-800879-9.00014-7

[2] Kocot J, Kiełczykowska M, Luchowska-Kocot D, Kurzepa J, Musik I. Antioxidant potential of propolis, bee pollen, and royal jelly: Possible medical application. Oxidative Medicine and Cellular Longevity. 2018; 2018:1-29. DOI: 10.1155/2018/7074209

[3] Daffalla K, Mahmoud A. Propolis as a natural remedy. Journal of International Oral Health. 2016;8: 646-649. DOI: 10.2047/jioh-08-05-24

[4] Silva-Carvalho R, Baltazar F, Almeida-Aguiar C. Propolis: A complex natural product with a plethora of biological activities that can be explored for drug development. Evidence-based Complementary and Alternative Medicine. 2015;2015:1-29. DOI: 10.1155/2015/206439

[5] Rufatto LC, dos Santos DA, Marinho F, Henriques JAP, Roesch Ely M, Moura S. Red propolis: Chemical composition and pharmacological activity. Asian Pacific Journal of Tropical Biomedicine. 2017;7:591-598. DOI: 10.1016/j.apjtjb.2017.06.009

[6] Harvey AL, Edrada-Ebel R, Quinn RJ. The re-emergence of natural products for drug discovery in the genomics era. Nature Reviews. Drug Discovery. 2015;14:111-129. DOI: 10.1038/nrd4510

[7] Huang S, Zhang C-P, Wang K, Li G, Hu F-L. Recent advances in the chemical composition of propolis. Molecules. 2014;19:19610-19632. DOI: 10.3390/molecules191219610

[8] Miguel MG, Figueiredo AC. Propolis and geopropolis volatiles. In: Bee Products - Chemical and Biological Properties. Cham: Springer International Publishing; 2017. pp. 113-136. DOI: 10.1007/978-3-319-59689-1_6

[9] Bankova V, Popova M, Trusheva B. Propolis volatile compounds: Chemical diversity and biological activity: A review. Chemistry Central Journal. 2014;8:28. DOI: 10.1186/1752-153X-8-28

[10] Jihene A, Karoui IJ, Ameni A, Hammami M, Abderrabba M. Volatile compounds analysis of Tunisian propolis and its antifungal activity. Journal of Biosciences and Medicines. 2018;06:115-131. DOI: 10.4236/jbm.2018.66009

[11] Pickett JA, Khan ZR. Plant volatile-mediated signalling and its application in agriculture: Successes and challenges. The New Phytologist. 2016;212:856-870. DOI: 10.1111/nph.14274

[12] Carvalho IT, Estevinho BN, Santos L. Application of microencapsulated essential oils in cosmetic and personal healthcare products - A review. International Journal of Cosmetic Science. 2016;38:109-119. DOI: 10.1111/ics.12232

[13] Gurgel ESC, de Oliveira MS, Souza MC, da Silva SG, de Mendonça MS, da Silva Souza Filhoe AP. Chemical compositions and herbicidal (phytotoxic) activity of essential oils of three Copaifera species (Leguminosae-Caesalpinoideae) from Amazon-Brazil. Industrial Crops and Products. 2019;142:111850. DOI: 10.1016/j.indcrop.2019.111850

[14] de Oliveira MS, da Cruz JN, Silva SG, da Costa WA, de Sousa SHB, Bezerra FW, et al. Phytochemical profile, antioxidant activity, inhibition of acetylcholinesterase and interaction mechanism of the major components of the Piper divaricatum essential oil
obtained by supercritical CO2. Journal of Supercritical Fluids. 2019;145:74-84. DOI: 10.1016/j.supflu.2018.12.003

[15] Silva SG, da Costa RA, de Oliveira MS, da Cruz JN, Figueiredo PLB, de Brasil DSB, et al. Chemical profile of Lippia thymoides, evaluation of the acetylcholinesterase inhibitory activity of its essential oil, and molecular docking and molecular dynamics simulations. PLoS One. 2019;14:e0213393. DOI: 10.1371/journal.pone.0213393

[16] Houdkova M, Rondevaldova J, DoskociI I, Koskoka L. Evaluation of antibacterial potential and toxicity of plant volatile compounds using new broth microdilution volatilization method and modified MTT assay. Fitoterapia. 2017;118:56-62. DOI: 10.1016/j.fitote.2017.02.008

[17] Rizzolo A, Bianchi G, Povolo M, Migliori CA, Contarini G, Pelizzola V, et al. Volatile compound composition and antioxidant activity of cooked ham slices packed in propolis-based active packaging. Food Packaging and Shelf Life. 2016;8:41-49. DOI: 10.1016/j.fpsl.2016.03.002

[18] Tongnuanchan P, Benjakul S. Essential oils: Extraction, bioactivities, and their uses for food preservation. Journal of Food Science. 2014;79:1231-1249. DOI: 10.1111/1750-3841.12492

[19] Aziz ZAA, Ahmad A, Setapar SHM, Karakucuk A, Azim MM, Lokhat D, et al. Essential oils: Extraction techniques, pharmaceutical and therapeutic potential - A review. Current Drug Metabolism. 2018;19:1100-1110. DOI: 10.2174/1389200019666180723144850

[20] El Asbahani A, Miladi K, Badri W, Sala M, Addi EHHA, Casabianca H, et al. Essential oils: From extraction to encapsulation. International Journal of Pharmaceutics. 2015;483:220-243. DOI: 10.1016/j.ijpharm.2014.12.069

[21] Azmir J, Zaidul ISM, Rahman MM, Sharif KM, Mohamed A, Sahena F, et al. Techniques for extraction of bioactive compounds from plant materials: A review. Journal of Food Engineering. 2013;117:426-436. DOI: 10.1016/j.jfoodeng.2013.01.014

[22] Jain SH, Ravikumar G. A brief review on essential oil extraction and equipment. Chemical Technology: An Indian Journal. 2010;5:19-24

[23] Moridi Farimani M, Mirzania F, Sonboli A, Moghaddam FM. Chemical composition and antibacterial activity of Dracocephalum kotschyi essential oil obtained by microwave extraction and hydrodistillation. International Journal of Food Properties. 2017;20:306-315. DOI: 10.1080/10942912.2017.1295987

[24] Rassem HHA, Nour AH, Yunus RM. Techniques for extraction of essential oils from plants: A review. Australian Journal of Basic and Applied Sciences. 2016;10:117-127

[25] Nora FMD, Borges CD. Ultrasound pretreatment as an alternative to improve essential oils extraction. Ciência Rural. 2017;47:1-9. DOI: 10.1590/0103-8478cr20170173

[26] Li Y, Fabiano-Tixier A-S, Chemat F. Essential Oils: From Conventional to Green Extraction. Cham: Springer International Publishing; 2014. pp. 9-20. DOI: 10.1007/978-3-319-08449-7_2

[27] Maia JGS, Andrade EHA. Database of the Amazon aromatic plants and their essential oils. Quimica Nova. 2009;32:595-622. DOI: 10.1590/0100-40422009000300006

[28] Muhammad Z, Yusoff ZM, Nurhans K, Nordin MNN, Taib MN, Fazalul Rahim MH, et al. Steam distillation with induction heating system: Analysis of kaffir lime oil compound and production yield at various temperatures. The Malaysian
[29] Cassel E, Vargas RMF. Experiments and modeling of the Cymbopogon winterianus essential oil extraction by steam distillation article. Chemical Society. 2006;50:126-129

[30] Chemat F, Boutekedjiret C. Extraction // Steam Distillation. In: Reedijk J, editor. Reference Module in Chemistry, Molecular Sciences and Chemical Engineering. Amsterdam, Netherlands: Elsevier; 2015. pp. 1-12. DOI: 10.1016/b978-0-12-409547-2.11557-4

[31] Yadav AA, Chikate SS, Vilat RB, Suryawanshi MA, Student UG, Mumbai N, et al. Review on steam distillation: A promising Technology for Extraction of essential oil. International Journal of Advanced Research and Development. 2017;4:667-671. DOI: 10.21090/ijaerd.33095

[32] Bhusnure O, Gholve SB, Giram PS, Borsure VS, Jadhav PP, Satpute VV, et al. Importance of supercritical fluid extraction (SFE) in hair analysis. Indo American Journal of Pharmaceutical Research. 2015;5:3785-3801

[33] de Oliveira MS, da Cruz JN, Mitre GP, da Costa WA, da Silva Kataoka MS, Silva SG, et al. Antimicrobial, cytotoxic activity of the Syzygium aromaticum essential oil, molecular docking and dynamics molecular studies of its major chemical constituent. Journal of Computational and Theoretical Nanoscience. 2019;16:355-364. DOI: 10.1166/jctn.2019.8108

[34] Uquiche E, Cirano N, Millao S. Supercritical fluid extraction of essential oil from Leptocarpha rivularis using CO₂. Industrial Crops and Products. 2015;77:307-314. DOI: 10.1016/j.indcrop.2015.09.001

[35] Sodeifian G, Sajadian SA, Ardestani NS. Experimental optimization and mathematical modeling of the supercritical fluid extraction of essential oil from Eryngium billardieri: Application of simulated annealing (SA) algorithm. Journal of Supercritical Fluids. 2017;127:146-157. DOI: 10.1016/j.supflu.2017.04.007

[36] Sovilj MN, Nikolovski BG, Spasojević MD. Critical review of supercritical fluid extraction of selected spice plant materials. Macedonian Journal of Chemistry and Chemical Engineering. 2011;30:197-220

[37] Wrona O, Rafińska K, Możeński C, Buszewski B. Supercritical fluid extraction of bioactive compounds from plant materials. Journal of AOAC International. 2017;100:1624-1635. DOI: 10.5740/jaoacint.17-0232

[38] Reverchon E. Supercritical fluid extraction and fractionation related products. Drying Oils and Related Products. 1997;10:1-37

[39] Parhi R, Suresh P. Supercritical fluid technology: A review. Journal of Advanced Pharmaceutical Technology & Research. 2013;1:13-36. DOI: 10.14302/issn.2328-0182.japst-12-145

[40] de Oliveira MS, da Costa WA, Pereira DS, Botelho JRS, de Alencar Menezes TO, de Aguiar Andrade EH. Chemical composition and phytotoxic activity of clove (Syzygium aromaticum) essential oil obtained with supercritical CO₂. Journal of Supercritical Fluids. 2016;118:185-193. DOI: 10.1016/j.supflu.2016.08.010

[41] Sapkale GN, Patil SM, Surwase US, Bhatbhage PK. Supercritical fluid extraction. International Journal of Chemical Sciences. 2010;8:729-743. DOI: 10.1111/j.1467-8306.1965.tb00516.x

[42] Stashenko EE, Martínez JR, Ruiz CA, Arias G, Durán C, Salgar W, et al. Lippia origanoides chemotype
differentiation based on essential oil GC-MS and principal component analysis. Journal of Separation Science. 2010;33:93-103. DOI: 10.1002/jssc.200900452

[43] Maia JGS, Taveira FSN, Andrade EHA, da Silva MHL, Zoghbi M. Essential oils of Lippia grandis Schau. Flavour and Fragrance Journal. 2003;18:417-420. DOI: 10.1002/ffj.1241

[44] Maia JGS, da Silva MHL, Andrade EHA, Carreira LMM. Essential oil variation in Lippia glandulosa Schauer. Journal of Essential Oil Research. 2005;17:676-680. DOI: 10.1080/10412905.2005.9699030

[45] Raposo JDA, Figueiredo PLB, Santana RL, da Silva AQ Jr, Suemitsu C, da Silva R, et al. Seasonal and circadian study of the essential oil of Myrcia sylvatica (G. Mey) DC., a valuable aromatic species occurring in the lower Amazon River region. Biochemical Systematics and Ecology. 2018;79:21-29. DOI: 10.1016/j.bse.2018.04.017

[46] Abdel-Hameed ESS, Salman MS, Fadl MA, Elkhateeb A, Hassan MM. Chemical composition and biological activity of Mentha longifolia L. essential oil growing in Taif, KSA extracted by hydrodistillation, solvent free microwave and microwave hydrodistillation. Journal of Essential Oil-Bearing Plants. 2018;21:1-14. DOI: 10.1080/0972060X.2018.1454343

[47] Gobbo-Neto L, Lopes NP. Plantas medicinais: Fatores de influência no conteúdo de metabólitos secundários. Quimica Nova. 2007;30:374-381. DOI: 10.1590/S0100-40422007000200026

[48] Hussain AI, Anwar F, Hussain Sherazi ST, Przybylski R. Chemical composition, antioxidant and antimicrobial activities of basil (Ocimum basilicum) essential oils depends on seasonal variations. Food Chemistry. 2008;108:986-995. DOI: 10.1016/j.foodchem.2007.12.010

[49] Moghaddam M, Mehdizadeh L. Chemistry of essential oils and factors influencing their constituents. In: Soft Chemistry and Food Fermentation. Elsevier; 2017. pp. 379-419. DOI: 10.1016/B978-0-12-811412-4.00013-8

[50] Castelo AVM, Del Menezzi CHS, Resck IS. Seasonal variation in the yield and the chemical composition of essential oils from two Brazilian native Arbustive species. Journal of Applied Sciences. 2012;12:753-760. DOI: 10.3923/jas.2012.753.760

[51] Liber Z, Carović-Stanko K, Politeo O, Strikić F, Kolak I, Milos M, et al. Chemical characterization and genetic relationships among Ocimum basilicum L. cultivars. Chemistry & Biodiversity. 2011;8:1978-1989. DOI: 10.1002/cbdv.201100039

[52] Brant S, Eduardo J, Pereira B, Kelly S, Bertolucci V, Silva A, et al. Teores do óleo essencial de cidrão [Aloysia triphylla (L'Hérit) Britton (Verbenaceae)] em diferentes horários de colheita e processamentos pós-colheita. Ciência e Agrotecnologia. 2009:2065-2068

[53] Pino JA, Marbot R, Delgado A, Zumárraga C, Sauri E. Volatile constituents of propolis from honey bees and stingless bees from yucatán. Journal of Essential Oil Research. 2006;18:53-56. DOI: 10.1080/10412905.2006.9699384

[54] Oliveira AP, França HS, Kuster RM, Teixeira LA, Rocha LM. Chemical composition and antibacterial activity of Brazilian propolis essential oil. Journal of Venomous Animals and Toxins Including Tropical Diseases. 2010;16:121-130. DOI: 10.1590/S1678-91992010005000007

[55] Anjum SI, Ullah A, Khan KA, Attaullah M, Khan H, Ali H, et al.
Composition and functional properties of propolis (bee glue): A review. Saudi Journal of Biological Sciences. 2019;26:1695-1703. DOI: 10.1016/j.sjbs.2018.08.013

[56] Lavinas FC, Macedo EHBC, Sá GBL, Amaral ACF, Silva JRA, Azevedo MMB, et al. Brazilian stingless bee propolis and geopropolis: Promising sources of biologically active compounds. The Brazilian Journal of Pharmacognosy. 2019;29:389-399. DOI: 10.1016/j.bjp.2018.11.007

[57] de Albuquerque IL, Alves LA, Lemos TLG, Dorneles CA, de Morais MO. Constituents of the essential oil of brazilian green propolis from Brazil. Journal of Essential Oil Research. 2008;20:414-415. DOI: 10.1080/10412905.2008.9700044

[58] Kusumoto T, Miyamoto T, Higuchi R, Doi S, Sugimoto H, Yamada H. Isolation and structures of two new compounds from the essential oil of Brazilian propolis. Chemical & Pharmaceutical Bulletin. 2001;49:1207-1209. DOI: 10.1248/cpb.49.1207

[59] Janes K, Bumba V. Composition of bee glue (propolis). Die Pharmazie. 1974;29:544-545

[60] Bankova VS, de Castro SL, Marcucci MC. Propolis: Recent advances in chemistry and plant origin. Apidologie. 2000;31:3-15. DOI: 10.1051/apido:2000102

[61] Pellati F, Prencipe FP, Benvenuti S. Headspace solid-phase microextraction-gas chromatography-mass spectrometry characterization of propolis volatile compounds. Journal of Pharmaceutical and Biomedical Analysis. 2013;84:103-111. DOI: 10.1016/j.jpba.2013.05.045

[62] Kujumgiev A, Tsvetkova I, Serkedjieva Y, Bankova V, Christov R, Popov S. Antibacterial, antifungal and antiviral activity of propolis of different geographic origin. Journal of Ethnopharmacology. 1999;64:235-240. DOI: 10.1016/S0378-8741(98)00131-7

[63] Betul D, Atac U, Fatih D. Volatile composition of Anatolian propolis by headspace-solid-phase microextraction (HS-SPME), antimicrobial activity against food contaminants and antioxidant activity. Journal of Medicinal Plants Research. 2013;7:2140-2149. DOI: 10.5897/jmpr2013.4470

[64] Sena-Lopes Â, Bezerra FSB, das Neves RN, de Pinho RB, de Silva MTO, SavegnagoL, etal.Chemical composition, immunostimulatory, cytotoxic and antiparasitic activities of the essential oil from Brazilian red propolis. PLoS One. 2018;13:e0191797. DOI: 10.1371/journal.pone.0191797

[65] Naik DG, Vaidya HS, Namjoshi TP. Essential oil of Indian propolis: Chemical composition and repellency against the honeybee Apis florea. Chemistry & Biodiversity. 2013;10:649-657. DOI: 10.1002/cbdv.201200165

[66] Li YJ, Xuan HZ, Shou QY, Zhan ZG, Lu X, Hu FL. Therapeutic effects of propolis essential oil on anxiety of restraint-stressed mice. Human & Experimental Toxicology. 2012;31:157-165. DOI: 10.1177/0960327111412805

[67] Rios N, Yánez C, Rojas L, Mora F, Usuibillaga A, Vit P. Chemical composition of essential oil of Apis mellifera propolis from Falcón state, Venezuela. Emirates Journal of Food and Agriculture. 2014;26:639-642. DOI: 10.9755/ejfa.v26i7.18198

[68] Awale S, Shrestha SP, Tezuka Y, Ueda JY, Matsushige K, Kadota S. Neoflavonoids and related constituents from nepalese propolis and their nitric oxide production inhibitory activity. Journal of Natural Products. 2005;68:858-864. DOI: 10.1021/np050009k
[69] Amaral TY, Padilha IG, Presídio GA, da Silveira EAAS, Duarte AWF, Barbosa APF, et al. Antimicrobial and anti-inflammatory activities of *Apis mellifera* honey on the helicobacter pylori infection of wistar rats gastric mucosa. Food Science and Technology. 2017;37:34-41. DOI: 10.1590/1678-457X.31016

[70] Alves RRN, Neta ROS, Trovão DMBM, Barbosa JEL, Barros AT, Dias TLP. Traditional uses of medicinal animals in the semi-arid region of northeastern Brazil. Journal of Ethnobiology and Ethnomedicine. 2012;8:34-36. DOI: 10.1186/1746-4269-8-41

[71] Han SM, Lee KG, Yeo JH, Baek HJ, Park K. Antibacterial and anti-inflammatory effects of honeybee (*Apis mellifera*) venom against acne-inducing bacteria. Journal of Medicinal Plants Research. 2010;4:459-464. DOI: 10.5897/JMPR09.427

[72] de Souza ECA, da Silva EJG, Cordeiro HKC, Lage Filho NM, da Silva FMA, dos Reis DLS, et al. Chemical compositions and antioxidant and antimicrobial activities of propolis produced by frieseomelitta longipes and *Apis mellifera* BEES. Quimica Nova. 2018;41:485-491. DOI: 10.21577/0100-4042.20170208

[73] Wiwekowati, Astawa P, Jawi IM, Sabir A. Antioxidant activity of *Apis mellifera* sp. Propolis extract from Java (Indonesia). International Research Journal of Engineering, IT and Scientific Research. 2017;3:18. DOI: 10.21744/irjeisv3i5.530

[74] da Cunha JSM, Alfredo TM, dos Santos JM, Junior VVA, Rabelo LA, Lima ES, et al. Antioxidant, antihyperglycemic, and antidiabetic activity of *Apis mellifera* bee tea. PLoS One. 2018;13:1-17. DOI: 10.1371/journal.pone.0197071