Emir. J. Food Agric. 2014. 26 (7): 639-642
doi: 10.9755/ejfa.v26i7.18198
http://www.ejfa.info/

SHORT COMMUNICATION

Chemical composition of essential oil of *Apis mellifera* propolis from Falcón State, Venezuela

Nurby Rios[1*], Carlos Yánez[2], Luis Rojas[3], Flor Mora[1], Alfredo Usubillaga[3] and Patricia Vit[4]

[1]*Departamento de Farmacognosia y Medicamentos Orgánicos, University of the Andes, Mérida, Venezuela*
[2]*Departamento de Toxicología y Farmacología, University of the Andes, Mérida, Venezuela*
[3]*Instituto de Investigaciones, Sección Productos Naturales, University of the Andes, Mérida, Venezuela*
[4]*Apiterapia y Bioactividad, Departamento de Ciencias de los Alimentos, Facultad de Farmacia y Bioanálisis, University of the Andes, Mérida, Venezuela*

Abstract

Apis mellifera bees obtain propolis by adding wax and salivary secretions to resinous, gummy or balsamic materials collected from various plant sources. Its chemical composition depends on many factors (type of bee, flora accessed, environment, management, season, vegetation and geographical area of collection). In the hive, the bees use propolis to consolidate structural components, varnish inside the cells and prevent vibrations. Propolis is known for its antibacterial, fungicidal, antiviral, anesthetic, antiulcer, immunostimulating, hypotensive, cytostatic, and antioxidant properties, the essential oil of *Apis mellifera* propolis, collected in Falcon state, was obtained by hydrodistillation using a Clevenger trap (0.06% yield). The oil was analyzed by gas chromatography coupled to mass spectrometry (GC / MS) in an HP GC-MS System Model 5973. Twenty-three compounds were identified (93.6%), of which the three major compounds were germacrene D (26.5%), β-caryophyllene (10.2%) and β-elemene (8.1%).

Key words: Essential oil, *Apis mellifera*, Propolis

Introduction

Propolis is a complex resinous material produced by bees from several plant exudates. *Apis mellifera* species obtain their propolis by addition of waxes, salivary secretions, or gummy and balsamic material collected from various plant species (De Albuquerque et al., 2008). The bees transport the propolis within the pollen racks, in the form of small and bright droplets. They use it as a filler, cement, or balsam to sold wall panels, close cracks and crevices in the hive to avoid the entry of foreign bodies such as insects, dust and water. It is also used to coat strange surfaces forming an antiseptic layer that prevents the formation or spread of any form of infection caused by insects or mice. Finally, propolis consolidates structural components and varnish inside the cells with disinfecting purposes and prevent vibrations (Oliveira et al., 2010; Mendizabal, 2005). This product beekeeping has a color ranging from brown to dark red depending on the plant or area origin, has a sweet smell, wet, bitter or strong, hard and brittle, insoluble in water, but soluble in alcohol, ether, ammonia, benzene-chloroform (Chaillou, 2004). Its chemical composition depends on many factors (type of bee, flora accessed, environment, management, season, vegetation and geographical area of collection). For this reason it does not have a specific chemical formula, some propolis analysis indicate that it contain mainly: resin (55%), wax (35%), oil (5%), organic material, minerals such as aluminum, cobalt, iron, nickel, calcium, silicon, zinc (5%), pollen and mechanical impurities. Their ratio thereof is variable and depends on the time of collection as well as the resin plants and bee (De Albuquerque et al., 2008; Lesser, 1987).

Since ancient times in many parts of the world, propolis has had various applications. It is used in the manufacture of cosmetics, varnishes and paints. In traditional medicine is used to improve and preserve health. Aristotle described that propolis cures skin diseases, heal wounds and fight infections effectively. Currently it is considered among the categories of natural therapy without toxicity and plays an important role in apitherapy.

Received 17 December 2013; Revised 20 March 2014; Accepted 24 March 2014; Published Online 18 May 2014

*Corresponding Author

Nurby Rios
Departamento de Farmacognosia y Medicamentos Orgánicos, University of the Andes, Mérida, Venezuela

Email: nurbyrios@ula.ve

Propolis has has analgesic, anti-inflammatory, antibacterial, fungicidal, antiviral, anesthetic, antiulcer, immunostimulants, hypotensive, cytostatic, and antioxidant activities. It is used for the treatment of various diseases such as rhinitis, tonsillitis, bronchial asthma, ulcers, eczema, antiseptic, particularly eye infections, throat, urinary tract (Lesser, 1987; Graça and Antunes, 2011; Kusumoto et al., 2001).

The chemical composition of propolis essential oil has been little studied. Mainly Brazilian propolis has been explored. De Albuquerque et al. (2008), determined the chemical composition of the essential oil produced by *Apis mellifera* propolis from Minas Gerais. They found 17 compounds (91%) of which three were the major ones (E)-nerolidol (17.1%), β - caryophyllene (13.4%), and petrolatum – 3.7 (11) - diene (10.4%). Kusumoto et al. (2001), isolated and identified nine components from propolis essential oil, seven were known and two new 2,2-dimethyl-8-prenyl-6-vinilcromeno and 2,6- diprenil -4-vinylphenol. Also Oliveira et al. (2010) studied the essential oil collected by *Apis mellifera* in Rio de Janeiro, identifying 26 compounds (67.45%). The three major found were β - caryophyllene (12.69%), acetophenone (12.26%) and linalool (6.47%). Finally, Bancova et al. (1998), analized the chemical composition of the essential oil of Brazilian propolis in different seasons and the main components found were espatulenol (3.0 to 13.9%), (2Z, 6E)-farnesol (1.6 to 14.9%) benzyl benzoate (0.3 to 18.3%) and prenylated acetophenones (3.4 to 17.1%). These common compounds only differ in quantity in the different essential oils. From the chemical point of view, in Venezuela, propolis has been explored finding only some few chemical components presented in work of Tomás-Barberán et al. (1993), Trusheva et al. (2004) and Trusheva et al. (2004). Therefore, this work focuses on determining the chemical composition of the essential oil obtained from *Apis mellifera* propolis from Paraguana Peninsula, Falcon State, Venezuela.

Materials and Methods

The collection of propolis was performed using the scraping method, with a stainless steel spatula, without much edge to avoid dragging wood shavings. The propolis was obtained from the inner surface of the hive discarding what was in the background that usually is much polluted. Propolis was collected in 2010, in the Paraguana Peninsula, Falcón State, Venezuela.

Isolation of the essential oil

Oil extraction was performed from 800 g of propolis, which were extracted by the method of hydrodistillation using a Clevenger-type apparatus for 4 hours. The oil was dried over anhydrous sodium sulphate and stored at 4ºC.

Gas chromatography

GC analyses were performed using a Perkin-Elmer AutoSystem gas chromatograph equipped with flame ionization detector and data handling system. Two capillary columns of different polarities were used: a 5% phenylmethyl polysiloxane fused-silica column (AT-5, Alltech Associates Inc., Deerfield, IL) 60 m x 0.25 mm, film thickness 0.25 μm, and a polyethylene glycol fused-silica column (AT-WAX, Alltech Associates Inc., Deerfield, IL) of the same dimensions. For the AT-5 column an oven temperature of 60°C was used. It was then heated to 260°C at 4°C/min, the final temperature was maintained for 20 min. For the AT-WAX column an initial temperature of 50ºC was used. The oven was heated to 200ºC at 3ºC/min. The injector and detector temperatures were 200°C and 250°C, respectively. The carrier gas was helium at 1.0 ml/min. The sample was injected using a split ratio of 1:20. Retention indices were calculated relative to C8-C24 n-alkanes, and compared with values reported in the literature. The normalization method from the peak areas was used to calculate the percentage composition of the essential oil.

Gas Chromatography-Mass Spectrometry

GC-MS analyses were carried out on a Model 5973 Hewlett-Packard GC-MS system fitted with a HP-5MS fused silica column (30 m x 0.25 mm i.d., film thickness 0.25 μm, Hewlett-Packard). The oven temperature program was the same as that used for the HP-5 column for GC analysis; the transfer line temperature was programmed from 150ºC to 280ºC; source temperature, 230ºC; quadrupole temperature, 150ºC; carrier gas, helium, adjusted to a linear velocity of 34 cm/s; ionization energy, 70 eV; scan range, 40:500 amu; 3.9 scans/s. Sample (1.0 μL) was injected using a Hewlett-Packard ALS injector with a split ratio of 50:1. The identity of the oil components was established from their GC retention indices, by comparison of their MS spectra with those of standard compounds available in the laboratory, and by a library search (Nist, 05) (Adams, 1995; Skoog et al., 2001).

Results and Discussion

The Venezuelan propolis hydrodestilation produced 0.5ml of yellow essential oil (0.06%

yield). The analysis by gas chromatography - mass spectrometry allowed the identification of twenty compounds (93.6%) (Table 1) of which the three major ones were D-germacrene (26.5%), β-caryophyllene (10.2%) and β-elemene (8.1%) (Figure 1). The 60.86% are non-oxygenated sesquiterpenes and 39.13% are oxygenated sesquiterpenes. We found that the most abundant oxygenated sesquiterpene was α-cadinol. It is observed that similar to Brazil propolis; Venezuelan propolis contains β-caryophyllene in representative amount β-caryophyllene in representative amount (from 10% to 13%) (De Albuquerque et al., 2008; Oliveira et al., 2010; Kusumoto et al, 2001). However it is interesting to note, that as the essential oil composition of Brazilian propolis varies depending on the collection site, the Venezuelan propolis presents differences in composition according to the flora accessed from *Apis mellifera* (De Albuquerque et al., 2008).

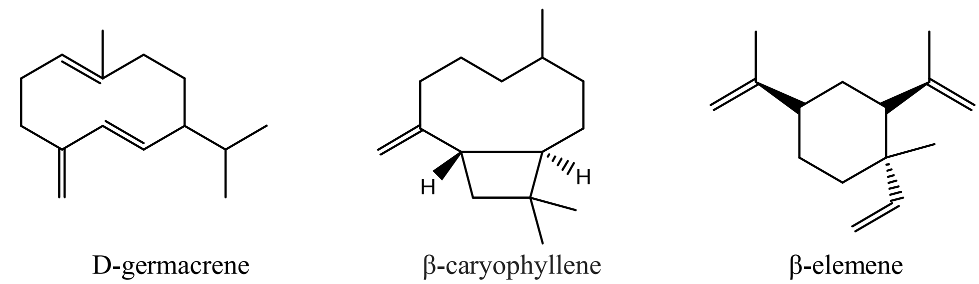

Figure 1. Major components of the essential oil of propolis

Table1. Chemical Composition of Propolis Essential Oil

N°	Compuesto	Área%	RT	Ik_{cal}	Ik_{lit}
1	α-copaene	0,8	18,451	1384	1379
2	β-bourbonene	0,8	18,738	1392	1388
3	β-elemene	8,1	18,966	1397	1389
4	β-caryophyllene	10,2	19,840	1429	1421
5	β-cubenene	1,1	20,123	1439	1440
6	α-guaiene	2,4	20,405	1450	1437
7	α-humulene	2,9	20,882	1466	1455
8	γ-muurolene	2,9	21,592	1490	1478
9	Germacrene D	26,5	21,760	1496	1479
10	β-silinene	2,2	21,891	1500	1489
11	Bicyclogermacrene	6,8	22,195	1510	1494
12	α-murolene	0,9	22,292	1514	1496
13	Germacrene A	4,4	22,461	1519	1503
14	γ-cadinene	1,2	22,702	1527	1513
15	δ-cadinene	5,0	22,980	1536	1522
16	Z-neronidol	1,5	24,137	1571	1564
17	Spathulenol	1,0	24,584	1584	1578
18	Isoaromadendrene epoxide	1,3	24,766	1590	1579
19	Viridiflorol	1,1	25,000	1595	1592
20	Guaiol	1,1	25,163	1602	1600
21	1-epi-cubenol	1,0	26,032	1639	1628
22	τ-muurolol	2,7	26,416	1655	1640
23	α-cadinol	7,8	26,775	1669	1652
	Total	93,6			

* The compounds were identified by comparing the mass spectrum of each component with Wiley library database GC / MS data and logarithmic retention index (LRI). Area% was determined by GC-FID.
RT: retention time; Ikcal: Kovats index calculated; Ik lit: Literature Kovats Index

Conclusions

This work represents the first report of the chemical composition of essential oil obtained from *Apis mellifera* propolis from Venezuela. The essential oil is mainly composed of sesquiterpenes. A total of twenty three compounds (93.6%) were identified, the three major ones in the oil, are the D-germacrene (26.5%), β-caryophyllene (10.2%) and β-elemene (8.1%). There are significant differences with the chemical composition of the essential oils obtained from propolis from Brazil.

Acknowledgements

This work was funded by the Consejo de Desarrollo Científico, Humanístico, Tecnológico y de la Artes, Universidad de los Andes, Mérida, Project: FA-480-10-08-B). We thank Dr. Cesar Rengifo who supplied *Apis mellifera* propoplis sample.

References

Adams, R. 1995. Identification of essential oil components by gas chromatography/ mass spectroscopy. Allured Publishing. Illinois. p.804.

Bancova, V., B. Krasteva, S. Popov, J. Sforcin and F. Cunha. 1998. Seasonal variations in essential oil from Brazilian Propolis. J. Essent. Oil Res. 10:603-695.

Chaillou, L., H. Herrera and J. Maidana. 2004. Estudios de propóleos de Santiago del Estero, Argentina. Ciênc Tecnología Alimentos; 24:11-15.

De Albuquerque, I., L. Alves, T. Lemos, C. Dorneles and M. De Morais. 2008. Constituents of the essentials oil of brazilian green propolis from Brazil. J. Essent. Oil Res. 20:1-2.

Graça, M. and M. Antunes. 2011. Is propolis safe as an alternative medicine? J. Pharm. BioAllied Sci. 3:479-495.

Kusumoto, T., T. Miyamoto, R. Higuchi, S. Dot, H. Sugimoto and H. Yamada. 2001. Isolation and structures of two new compounds from the essential oil of brazilian propolis. Chem. Pharm. Bull. 49:1207-1209.

Mendizabal, F. 2005. Abejas. 1ra Ed. (Argentina): Albatros SACI. p. 65.

Lesser, R. 1987. Manejo y Crianza Practica de la Abeja. Como usar los productos de las abejas en la salud del hombre. 1ra Ed. (Chile): Andrés Bello. p. 122.

Oliveira, A., H. França, R. Kuster, L. Teixeira and L. Rocha. 2010. Chemical composition and antibacterial activity of Brazilian propolis essential oil. J. Venom Anim. Toxins Incl. Trop. Dis. 16:121-130.

Tomás-Barberán, F., C. García-Viguera, P. Vit-Olivier, F. Ferreres and F. Tomás-Lorente. 1993. Phytochemical evidence for the botanical origin of tropical propolis from Venezuela. Phytochemistry 34:191-196.

Trusheva, B., M. Popova, H. Naydenski, I. Tsvetkova, J. Rodriguez and V. Bankova. 2004. New polyisoprenylated benzophenones from Venezuelan propolis. Fitoterapia 75:683–689.

Skoog, D., J. Holler and T. Nieman. 2001. Principios de Análisis Instrumental. 5th Ed. (España): McGraw-Hill-Interamericana. P. 221.