CHAPTER 2

# Latest Developments in Propolis Research: Chemistry and Biology

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**Abstract:** Propolis is a plant derived bee product which serves dual purposes in the honeybee colony: building material and protective substance. Propolis has been used as remedy in the traditional medicine of numerous nations, because of its antimicrobial, antioxidant, and many other beneficial pharmacological actions. In this chapter, the results of the newest (in the last 5 years) chemical studies of propolis from different geographic and plant origin are reviewed, together with the new identified source plants: 152 new constituents of propolis, being 57 new chemical entities, and 12 new chemical types of propolis are listed. The importance of propolis for the bee colony is discussed, with special attention to the activity of propolis and its constituents against bee pathogens and parasites. The review of recent propolis literature demonstrates its potential to serve as a source of new chemical structures and new bioactive compounds due to its chemical diversity. It also reveals the potential of propolis to be used for development of innovative products, mainly in the field of food industries, animal husbandry, and beekeeping. For this to happen, the combined efforts of researchers and technologists from different areas are necessary, in order to make better use of bee glue.

**Keywords:** *Apis mellifera*, *Ascophaera apis*, Bee health, New constituents, New sources, *Paenibacillus*, Plant sources, Propolis, Social immunity, *Varroa destructor*.

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## 1. INTRODUCTION

Propolis (bee glue) is a plant derived bee product that serves dual purposes in the honeybee colony. It is a building material, used by worker bees to cover the internal surface of the cavity they inhabit (the so-called "propolis envelope" in the nests of feral bees [1]), to block holes and cracks, to tighten the hive entrance in cold season, etc. On the other hand, propolis is a protective material, guarding the colony against infections: it contains putrefaction of insect, small animals – intruders killed in the hive and too large to be carried out. It is an important element of the social immunity of honeybees [2].

Bees collect resinous materials from different plant parts (lipophilic materials on leaves and leaf buds, mucilages, gums, resins, latices, etc.) [3], and mix these materials with wax to produce propolis. In order to come to the resinous materials, in some cases bees cut fragments of vegetative tissues to release the resin [4]. Bees incorporate in propolis protective plant excretions, which prevent vulnerable plant tissues from infection by harmful microorganisms. They make use of the biosynthetic potential of plants and apply the secondary plant metabolites in the resins for the same purpose as the source plant: for protection. Thus, the action against microorganisms is an essential property of propolis, and this fact has been recognized by human beings since times immemorial. Propolis has been used a remedy in the traditional medicine of numerous nations, mainly to treat burns and wounds, sore throat, stomach ulcer, *etc*. [5]. Modern science has revealed many other beneficial pharmacological properties of bee glue [6, 7].

The chemical composition of propolis varies significantly depending on the particular resin used by the honeybees in propolis production; at different locations with specific climatic and phyto-geographic conditions the chemistry of propolis differs dramatically. However, it took some time for researchers to realize this peculiarity of propolis. The paradigm shift occurred about the turn of the 21$^{st}$ century. It became clear that unlike beeswax or bee venom, from chemical point of view there is no single product that is "just propolis". This resulted in a new approach to propolis research, and especially in studying the biological activity of propolis. It was understood that it was not enough to report that experiments were performed with propolis, but that it was necessary to chemically

characterise the particular propolis used in the experiments [8].

Although of different chemical composition, propolis from different locations always demonstrates considerable biological activity [9, 10]. For this reason, the chemical diversity of different propolis samples has the potential to provide valuable leads to active components. The study of bee glue in unexplored regions offers possibilities to uncover new biologically active compounds with important pharmacological effects, especially antibacterial, antioxidant and anticancer substances. In this chapter, the recently discovered new propolis constituents (since 2009), including such coming from newly found source plants will be discussed.

## 2. GENERAL PHYSICO-CHEMICAL PROPERTIES OF PROPOLIS

Propolis is a sticky material; its colour is variable and depends on the plant source: brown, brown-yellow, yellow, green, brown-orange, red. Its smell also varies with the botanical origin but is in general pleasant, balmy or resinous; the taste is bitter and pungent. At temperatures lower than 15°C propolis is hard and brittle, while over 30°C it is soft, flexible and very sticky. Important characteristic of propolis is the amount of substances soluble in 70% ethanol, called “balsam”. Its content can be between 20 and 80%. The balsam contains mainly the plant derived bioactive compounds [5]. Besides the resins collected from plants, propolis contains also variable amounts of beeswax, pollen, mechanical impurities, up to 2% minerals.

The percentage of beeswax varies significantly: values between 5 and 49 % have been reported in the literature [11, 12]. Moreover, the wax amount depends on the harvesting method: the use of a propolis collector usually reduced the wax content below 20% [13].

Mechanical impurities consist of wood particles, remains of dead bees, moth cocoons, plant parts, etc. Their amount is variable too; differences in plant sources could explain the variations. In general, green Brazilian propolis is characterized by much higher percentage of mechanical impurities, up to 40%, compared to poplar type propolis (usually less than 10%). This could be due to the way bees collect resins from *Baccharis dracunculifolia* plants to produce green propolis:

cutting young leaves and buds in order to liberate the resinous material from them [14]. As a result, secretory hairs belonging to the genus *Baccharis* have been found in green Brazilian propolis in high quantities [15].

The density of propolis is about 1.11 – 1.14, its melting point between 80 – 105°C [16]. Propolis has very low solubility in water, even by boiling; it is better soluble in organic solvents such as ethanol, acetone, or ethanol-chloroform mixtures [16]. Further properties, such as antioxidant capacity, total phenolics and flavonoids content, different types of biological activities, depend to a large extent on the specific chemical composition of the particular propolis sample.

## 3. NEW PROPOLIS CONSTITUENTS AND NEW PROPOLIS SOURCES, IDENTIFIED SINCE 2009

In the 5 year period 2009 – 2014, a significant number of new propolis constituents have been reported. Besides the well known propolis types, originating from "conventional" source plants, such as *Populus nigra, P. tremula, Betula* spp*., Baccharis dracunculifolia, Macaranga tanarius, Mangifera indica,Dalbergia* spp., new propolis types have been reported. The new constituents are listed in (Table **1**). In this table, only isolated compounds, properly characterized by spectral methods, are included.

From all the 152 new constituents of propolis, 57 are new chemical entities. This large number is a substantial confirmation of the chemical diversity of propolis and its potential as source of new biologically active compounds.

There are several further articles reporting new propolis constituents, which are not listed in (Table **1**). The reason is that these compounds have not been isolated and identified by spectral methods. They have been only tentatively identified by LC-UV-MS and ESI-MS-MS spectra. Among them, a number of flavonoid glycosides, including one *C*-glycoside, have been detected by LC-UV-ESI-MS in Brazilian propolis from different regions [48, 49]. In propolis from Portugal, over 10 glycosides of quercetin and kaempferol were found using LC-DAD-ESI-$MS^n$ [50]. Nevertheless, their positive identification after isolation as individual compounds should be the ultimate proof for their presence in propolis.

**Table 1. Compounds found for the first time in propolis since 2009.**

| Geographic origin | Plant source of the compound | Structure type | Compound | Major/minor compound | References |
|---|---|---|---|---|---|
| **Africa** | | | | | |
| Kenya, Mwingi | Unknown | Arylnaphtalene lignane | Tetrahydrojusticidin B* | Minor | [17] |
| | | Arylnaphtalene lignane | 6-methoxydiphyllin* | Minor | |
| | | Arylnaphtalene lignane | Phyllamyricin C | Minor | |
| Kenya, Voi | *Macaranga schweinfurthii* | Geranyl flavonol | Macarangin | Major | [17] |
| | | Geranyl stilbene | Schweinfurthin A | Minor | |
| | | Geranyl stilbene | Schweinfurthin B | Minor | |
| Egypt | Unknown | Prenyl flavonoid | Isonymphaeol D* | Minor | [18] |
| Algeria | Unknown | Flavone | Ladanein | Minor | [19] |
| | | Flavone | Pilosin | Minor | |
| Ghana | Unknown | Prenylated stilbene | (*E*)-5-{2-[8-hydroxy-2-methyl-2-(4-methylpent-3-en-1-yl)-2*H*-chromen-6-yl]vinyl}-2-(3-methylbut-2-en-1-yl)benzene-1,3-diol* | Minor | [20] |
| | | Prenylated stilbene | 5-[(*E*)-3,5-dihydroxystyryl]-3-[(*E*)-3,7-dimethylocta-2,6-dien-1-yl]benzene-1,2-diol* | Major | |
| Cameroon | Unknown | Prenylated phloroglucinone | Deperoxidised derivative of plukenetione C* | Minor | [20] |

***(Table 1) contd.....***

| Geographic origin | Plant source of the compound | Structure type | Compound | Major/minor compound | References |
|---|---|---|---|---|---|
| Cameroon | *Mangifera indica* | Alkyl phenol | 3-Undecylphenol | Minor | [21] |
| | | Alkyl phenol | 3-Tetradecylphenol | Minor | |
| | | Alkyl phenol | 3-Pentadecylphenol | Minor | |
| | | Alkyl phenol | 3-Hexadecylphenol | Minor | |
| | | Alkyl phenol | 3-Heptadecylphenol | Minor | |
| | | Alkyl phenol | 3-Nonadecylphenol | Minor | |
| | | Alkyl phenol | 3-(10'Z-Pentadecenyl)phenol | Minor | |
| | | Alkyl phenol | 3-(12'Z-Pentadecenyl)phenol | Minor | |
| | | Alkyl phenol | 3-(8'Z-Heptadecenyl)phenol | Minor | |
| | | Alkyl phenol | 3-(12'Z-Heptadecenyl)phenol | Minor | |
| | | Alkyl phenol | 3-(14'Z-Heptadecenyl)phenol | Minor | |
| | | Alkyl phenol | 3-(13'Z-Nonadecenyl)phenol* | Minor | |
| | | Alkyl phenol | 3-(14'Z-Nonadecenyl)phenol* | Minor | |
| | | Alkyl resorcinol | 5-Hexadecylresorcinol | Minor | |
| | | Alkyl resorcinol | 5-(10'Z-Pentadecenyl)resorcinol | Minor | |
| | | Alkyl resorcinol | 5-(12'Z-Heptadecenyl)resorcinol | Minor | |
| | | Alkyl resorcinol | 5-(14'Z-Heptadecenyl)resorcinol | Minor | |
| | | Alkyl resorcinol | 5-(14'Z-Nonadecenyl)resorcinol* | Minor | |
| Algeria, North | *Populus* spp. | Dihydroflavonol | Pinobanksin 3-(*E*)-caffeate | Major | [22] |
| Algeria | *Cistus* spp. | Clerodane diterpene | Cistadiol | Major | [22] |
| | | Clerodane diterpene | 18-Hydroxy-*cis*-clerodane-3-ene-15-oic acid | Major | |
| Cameroon | Unknown | Oleanane triterpene | Olean-12-ene-3в,28-diol | Minor | [23] |
| **Asia** | | | | | |

***(Table 1) contd.....***

| Geographic origin | Plant source of the compound | Structure type | Compound | Major/minor compound | References |
|---|---|---|---|---|---|
| Korea, Jeju island | *Angelica keiskei* | Chalcone | Jejuchalcone A* | Minor | [25] |
| | | Chalcone | Jejuchalcone B* | Minor | |
| | | Chalcone | Jejuchalcone C* | Minor | |
| | | Chalcone | Jejuchalcone D* | Minor | |
| | | Chalcone | Jejuchalcone E* | Minor | |
| | | Chalcone | (-)-Jejuchalcone F* | Minor | |
| | | Chalcone | (+)-Jejuchalcone G* | Minor | |
| | | Chalcone | (-)-Jejuchalcone H* | Minor | |
| | | Chalcone | 4-Hydroxyderricin | Major | |
| | | Chalcone | 2',4,4'-Trihydroxy-3'-(6,7-dihydroxy-3, 7-dimethyl-2-octaenyl)chalcone ((±)-TB5) | Minor | |
| | | Chalcone | (±)-Xanthoangelol B | Minor | |
| | | Chalcone | Xanthoangelol | Major | |
| | | Chalcone | Xanthoangelol F | Minor | |
| | | Chalcone | (±)-Bavachromanol | Minor | |
| | | Chalcone | 2',3'-[2-Methyl-2-(4-methylpenten-3-yl)-3-hydroxy-dihydropyrano]-4,4'-dihydroxychalcone ((+)-TB2) | Minor | |
| | | Chalcone | (±)-Lespeol | Minor | |
| | | Chalcone | (+)-TB1 | Minor | |
| | | Coumarin | (*S,S*)-(+)-Laserpitin | Major | |
| | | Coumarin | (*S,S*)-(-)-Isolaserpitin | Major | |
| | | Coumarin | (*S*)-(-)-Selinidin | Minor | |
| | | Coumarin | (*R,R*)-(+)-Khellactone | Major | |
| | | Coumarin | (*R,R*)-(+)-3'-Senecioylkhellactone | Minor | |
| | | Coumarin | (2'*S*,3'*R*)-(+)-Vaginidiol | Minor | |
| | | Coumarin | (2'*S*,3'*R*)-(+)-Daucoidin A | Minor | |
| | | Coumarin | (*S*)-(-)-Oxypeucedanin hydrate | Minor | |
| | | Coumarin | (*S*)-(+)-Marmesin | Minor | |

***(Table 1) contd.....***

| Geographic origin | Plant source of the compound | Structure type | Compound | Major/minor compound | References |
|---|---|---|---|---|---|
| China, Henan Province | Unknown | Flavanol | 8-[(*E*)-4-Phenylprop-2-en-1-one]-(2*R*,3*S*)-2-(3,5-dihydroxyphenyl)-3,4-dihydro-2*H*-2-benzopyra--5-methoxyl-3,7-diol* | Major | [25] |
| | | Flavanol | 8-[(*E*)-4-Phenylprop-2-en-1-one]-(2*S*,3*R*)-2-(3,5-dihydroxyphenyl)-3,4-dihydro-2*H*-2-benzopyran-5-methxyl-3,7-diol* | Major | |
| | | Flavanol | 8-[(*E*)-4-Phenylprop-2-en-1-one]-(2*R*,3*S*)-2-(3-methoxl-4-hydroxyphenyl)-3,4-dihydro-2*H*-2-benzopyran-5-methoxy--3,7-diol | Minor | |
| | | Flavanol | 8-[(*E*)-4-*p*-Henylprop-2-en-1-one]-(2*S*,3*R*)-2-(3-methoxyl-4-hydroxyphenyl)-3,4-dihydro-2*H*-2-benzopyra--5-methoxyl-3,7-diol* | Minor | |
| China, Hubei province | *Populus* spp. | Phenolic glyceride | 2-Acetyl-1-coumaroyl-3-cinnamoylglycerol* | Major | [26] |
| | | Phenolic glyceride | (+)-2-Acetyl-1-feruloyl-3-cinnamoylglycerol* | Major | |
| | | Phenolic glyceride | (-)-2-Acetyl-1-feruloyl-3-cinnamoylglycerol* | Minor | |
| | | Phenolic glyceride | 2-Acetyl-1,3-dicinnamoylglycerol* | Major | |
| | | Phenolic glyceride | (-)-2-Acetyl-1-(*E*)-feruloyl-3-(3’’(ζ),16’’)-dihydroxypalmitoylglycerol* | Minor | |
| Thailand | *Styrax* spp. | Flavanone | (7’’*S*)-8-[1-(4’-Hydroxy-3’-methoxyphenyl)prop-2-en-1-yl]-(2*S*)-pinocembrin | Minor | [27] |
| | | Cinnamic acid derivative | (*E*)-Cinnamyl (*E*)-cinnamylidenate | Minor | |

***(Table 1) contd.....***

| Geographic origin | Plant source of the compound | Structure type | Compound | Major/minor compound | References |
|---|---|---|---|---|---|
| Myanmar | *Mangifera indica* | Triterpene | (22*Z*,24*E*)-3-Oxocycloart-22,24-dien-26-oic acid* | Minor | [28] |
| | | Triterpene | (24*E*)-3-Oxo-27,28-dihydroxycycloart-24-en-26-oic acid* | Minor | |
| | | Triterpene | 28-Hydroxymangiferonic acid | Minor | |
| | | Triterpene | 27-Hydroxymangiferonic acid | Major | |
| | | Triterpene | (24*E*)-3-Oxo-23-hydroxycycloart-24-en-26-oic acid | Minor | |
| | | Triterpene | (24*E*)-3$\beta$-Hydroxycycloart-24-en-26-al | Minor | |
| | | Triterpene | (24*E*)-3$\alpha$,27-Dihydroxycycloart-24-en-26-oic acid | Minor | |
| | | Triterpene | (24*E*)-3$\beta$,27-Dihydroxycycloart-24-en-26-oic acid | Major | |
| | | Triterpene | (24*E*)-3$\alpha$,22-Dihydroxycycloart-24-en-26-oic acid | Minor | |
| | | Triterpene | (24*E*)-3$\beta$,23-Dihydroxycycloart-24-en-26-oic acid | Minor | |
| | | Prenylated flavanone | (2*S*)-5,7-Dihydroxy-4'-methoxy-8,3-diprenylflavanone | Minor | |
| | | Prenylated flavanone | (2*S*)-5,7,4'-Trihydroxy-8,3'-diprenylflavanone | Minor | |
| | | Prenylated flavanone | (2*S*)-5,7-Dihydroxy-4'-methoxy-8-prenylflavanone | Minor | |
| | | Prenylated flavanone | (2*S*)-5,7,4'-Trihydroxy-8-prenylflavanone | Minor | |
| Jordania | Unknown | Triterpenic acid | 24(*Z*)-1$\beta$-3$\beta$-Dihydroxyeupha-7,24-dien-26-oic acid* | Major | [29] |
| | | Triterpenic acid | 24(*Z*)-3$\beta$-Dihydroxyeupha-7,24-dien-26-oic acid | Major | |
| Saudi Arabia | *Psiadia* spp. | Kaurane diterpene | Propsiadin [(*ent*)-2-oxokaur-16-en-6,18-diol]* | Minor | [30] |
| | | Kaurane diterpene | Psiadin | Major | |
| | | Flavone | Psiadiarabin | Minor | |
| Iran | *Ferula* spp. | Prenylated coumarin | Suberosin | Minor | [31] |
| | | Terpene ester of aromatic acid | Tschimgin (bornyl *p*-hydroxybenzoate) | Minor | |
| | | Terpene ester of aromatic acid | Tschimganin (bornyl vanillate) | Minor | |
| | | Terpene ester of aromatic acid | Ferutinin (ferutinol *p*-hydroxybenzoate) | Minor | |
| | | Terpene ester of aromatic acid | Teferin (ferutinol vanillate) | Minor | |
| Oman | *Azadiracta indica* | Prenylated flavanone | 7-*O*-methyl-8-prenylnaringenin | Minor | [32] |
| | | Prenylated flavanone | 3',8-Diprenylnaringenin | Major | |
| Oman | *Acacia nilotica* | Flavanols | Mixture of 2,3-*trans*-3,4-*trans* and 2,3-*trans*-3,4-*cis*-mollisacacidins | Minor | [32] |

***(Table 1) contd.....***

| Geographic origin | Plant source of the compound | Structure type | Compound | Major/minor compound | References |
|---|---|---|---|---|---|
| **Australia and Oceania** | | | | | |
| Solomon islands | Unknown | Prenylated flavonol | Solophenol A* | Minor | [33] |
| | | Prenylated flavanone | Sophoraflavanone A | Minor | |
| Solomon islands | *Macaranga* spp. | Prenylated flavonol | Solophenol B* | Minor | [34] |
| | | Prenylated flavonol | Solophenol C* | Minor | |
| | | Prenylated flavonol | Solophenol D* | Minor | |
| | | Prenyl stilbene | Solomonin* | Minor | |
| Kangaroo island | *Lepidosperma viscidum* | Cinnamic acid ester | Methyl (*E*)-4-(4’-hydroxy-3’-methylbut-(*E*)-2’-enyloxy)cinnamate* | Major | [35] |
| | | Cinnamic acid ester | Methyl 4-(3’-methylbut-(*E*)-2’-enyloxy)cinnamate | Minor | |
| | | Prenyl stilbene | 5,4’-Dihydroxy-3’-methoxy-3-prenyloxy-(*E*)-stilbene* | Minor | |
| | | Prenyl stilbene | 3’,4’,5-Trihydroxy-3-methoxy-3-prenyloxy-(*E*)-stilbene* | Minor | |
| | | Prenyl stilbene | 3,5,3’,4’-Tetrahydroxy-2-prenyl-(*E*)-stilbene* | Minor | |
| | | Prenyl stilbene | 3,5,4’-Trihydroxy-3’-methoxy-2-prenyl-(*E*)-stilbene* | Minor | |
| | | Prenyl stilbene | 5,3’,4’-Trihydroxy-3-methoxy-2-prenyl-(*E*)-stilbene* | Minor | |
| | | Prenyl stilbene | 5,4’-Dihydroxy-3,3’-dimethoxy-2-prenyl-(*E*)-stilbene* | Minor | |
| | | Prenyl stilbene | 3,5,4’-Trihydroxy-4-prenyldihydrostilbene* | Minor | |
| | | Prenyl stilbene | 5,4’-Dihydroxy-3-prenyloxy-(*E*)-stilbene* | Minor | |
| | | Prenyl stilbene | 3’,4’-Dihydroxy-3,5-dimethoxy-(*E*)-stilbene | Minor | |
| | | Prenyl stilbene | 3,5,3’,4’-Tetrahydroxy-2,4-diprenyldihydrostilbene | Minor | |
| | | Prenyl stilbene | 3,5-Dihydroxy-2-prenyl-(*E*)-stilbene | Minor | |
| | | C-methylflavanone | Farrerol | Minor | |
| | | C-methylflavanone | Matteucinol | Minor | |
| Kangaroo islands | *Acacia paradoxa* | Chalcone | 2’,3’,4’-Trimethoxychalcone* | Major | [36] |
| | | Chalcone | 2’-Hydroxy-3’,4’-dimethoxychalcone* | Major | |
| | | Chalcone | 2’,4’-Dihydroxy-3’-methoxychalcone | Major | |
| | | Dihydroflavonol | 5,7-Dihydroxy-6-methoxy-2,3-dihydroflavonol 3-acetate* | Major | |
| Europe | | | | | |

***(Table 1) contd.....***

| Geographic origin | Plant source of the compound | Structure type | Compound | Major/minor compound | References |
|---|---|---|---|---|---|
| Greece (Crete) | *Cupressus sempervirens* | Diterpenic acid | 14,15-*Dinor*-13-oxo-8(17)-labden-19-oic acid* | Minor | [37] |
| | | Diterpenic acid ester | Labda-8(17),13*E*-dien-19-carboxy-15-yl oleate* | Minor | |
| | | Diterpenic acid ester | Labda-8(17),13*E*-dien-19-carboxy-15-yl palmitate* | Minor | |
| | | Triterpenic acid | 3,4-*Seco*-cycloart-12-hydroxy-4(28),24-dien-3-oic acid* | Minor | |
| | | Triterpenic acid | Cycloart-3,7-dihydroxy-24-en-28-oic acid* | Minor | |
| | | Diterpene | *Trans*-communal | Minor | |
| | | Diterpenic acid | Pimaric acid | Minor | |
| | | Diterpene | Totarolone (3-oxototarol) | Minor | |
| | | Diterpenic acid | 15-Oxolabda-8(17),13*E*-dien-19-oic acid | Minor | |
| | | Diterpenic acid | 15-Oxolabda-8(17),13*Z*-dien-19-oic acid | Minor | |
| | | Diterpenic acid | Junicedric acid | Minor | |
| | | Flavonoid | Isorhamnetin-3-*O*-rutinoside | Minor | |
| Malta | *Ferula communis* | Terpenyl ester of substituted benzoic acid | 2-Acetoxy-6-*p*-methoxybenzoyl jaeschkeanadiol | Minor | [38] |
| | | Terpenyl ester of substituted benzoic acid | 2-Acetoxy-6-*p*-hydroxybenzoyl jaeschkeanadiol | Minor | |
| Bulgaria | *Populus* spp. | Oxo-fatty acid | 9-Oxo-10(*E*)-12(*Z*)-octadecadienoic acid | Minor | [39] |
| America | | | | | |
| Mexico, Sonora State | Unknown | Phenylallylflavanone | (2*R*,3*R*)-6-[1-(4'-Hydroxy-3'-methoxyphenyl)pr-p-2-en-1-yl]pinobanksin; | Minor | [40] |
| | | Phenylallylflavanone | (2*R*,3*R*)-6-[1-(4'-Hydroxy-3'-methoxyphenyl)pr-p-2-en-1-yl]pinobanksin 3-acetate | Minor | |
| Mexico, Sonora State | Unknown *Populus* spp. (?) | Phenylallylflavanone | (2*R*,3*R*)-3,5-Dihydroxy-7-methoxyflavanone-3-(2-methyl)butyrate | Minor | [41] |
| | | Phenylallylflavone | (7''*R*)-8-[1-(4'-Hydroxy-3'-methoxyphenyl)prop-2-en-1-yl]chrysin | Minor | |
| | | Phenylallylflavonole | (7''*R*)-8-[1-(4'-Hydroxy-3'-methoxyphenyl)prop-2-en-1-yl]galangin | Minor | |
| Mexico | *Dalbergia* spp. | Phenol | 1-(3',4'-Dihydroxy-2'-methoxyphenyl)-3-phenylpropane | Minor | [42] |
| | | Phenol | (*Z*)-1-(2'-Methoxy-4',5'-dihydroxyphenyl)-3-phenylprop-2-ene | Minor | |
| | | Flavan | 3-Hydroxy-5,6-dimethoxyflavan | Minor | |
| Brazil, Bahia | Unknown | Prenylated benzophenone | Hyperibone A* | Minor | [43] |
| Brazil | Unknown | - | Tannins | Minor | [44] |

***(Table 1) contd.....***

| Geographic origin | Plant source of the compound | Structure type | Compound | Major/minor compound | References |
|---|---|---|---|---|---|
| Argentina, Andean region | *Larrea nitida* | Lignane | 3'-Methylnordihydroguaiaredic acid* | Major | [45] |
| | | Lignane | Nordihydroguaiaredic acid* | Minor | |
| | | Lignane | 4-[4-(4-Hydroxyphenyl)-2,3-dimethyl-butyl]benzene-1,2-diol* | Minor | |
| | | Lignane | *Meso*-(rel 7*S*,8*S*,7'*R*,8'*R*)-3,4,3',4'-tetrahydroxy-7,7'-epoxylignan* | Minor | |
| | | Lignane | (7*S*,8*S*,7'*S*,8'*S*)-3,3',4'-Trihydroxy-4-methoxy-7,7'-epoxylignan* | Minor | |
| Argentina, Tucuman | *Zuccagnia punctata* | Flavone | 3-Hydroxy-7,8-dimethoxyflavone | Minor | [46] |
| Honduras | *Liguidambar styraciflua* | Ester of cinnamic acid | (*E*)-Cinnamyl (*Z*)-cinnamate | Minor | [47] |

* New natural compound

Another new propolis constituent identified in the last 5 years is β-glucosidase. The β-glucosidase activity of propolis has been studied and it was found that glucose derivatives were rapidly hydrolyzed but not conjugates with other sugars [51,52].

Not only new chemical constituents but new chemical types of propolis have been found and respectively their sources have been identified as new propolis-bearing plants. They are listed in (Table **2**).

**Table 2. Newly identified propolis plant sources.**

| Species | Plant family | Geographic location | Reference |
|---|---|---|---|
| *Acacia nilotica,* (L.) Delile | Fabaceae | Oman | [32] |
| *Acacia paradoxa* DC | Fabaceae | Australia | [36] |
| *Angelica keiskei* Ito | Apiaceae | Korea, Jeju Island | [24] |
| *Azadiracta indica* A.Juss. | Meliaceae | Oman | [32] |
| *Cupressus sempervirens* L. | Cupressaceae | Greece, Malta | [37, 53] |
| *Ferula* spp., incl. *Ferula communis* L. | Apiaceae | Iran, Malta | [31, 38] |
| *Larrea nitida* Cav. | Zygophylaceae | Argentina | [45] |
| *Lepidosperma viscidum* R.Br. | Cyperaceae | Australia | [35] |
| *Liquidambar styraciflua* L. | Hamamelidaceae | Honduras | [47] |
| *Macaranga schweinfurthii* Pax | Euphorbiaceae | Kenya | [17] |
| *Psiadia* spp. | Asteraceae | Saudi Arabia | [30] |
| *Zuccania punctata* Cav. | Fabaceae | Argentina | [46] |

The remarkable chemical variability of propolis is, of course, due to its plant origin and to the fact that at different geographic locations the source plants vary with respect to the local flora at the site of collection. However, there is another important, but often neglected factor - the choices made by bees. It is obvious that bees choose sticky resinous materials because of their physical properties. On the other hand, this material is also their chemical defence against microorganisms, but it is yet not clear how exactly bees recognise these properties in the collected material. The new knowledge on propolis sources brings new insight into the complex relationship between honeybees and plants. Among the new sources, some belong to families already known to include propolis-bearing plants, *e.g.*

Fabaceae (*Dalgbergia*, the source of red South American propolis) and Asteraceae (*Baccharis dracunculifolia*), Euphorbiaceae. Plants of a number of the listed plant families have not been found to be sources of propolis till now: Apiaceae, Hamamelidaceae, Meliaceae. Obviously, bees do not limit their choices but have adapted to make the best of the available plants in any habitat.

## 4. PROPOLIS AND BEE HEALTH

Propolis has been used since millennia as a remedy in traditional medicine systems all over the world. For this reason, it has been attracting the growing attention of researchers for the last 60 years. In the course of these studies, a number of propolis' diverse and useful pharmacological activities have been revealed but the studies have been directed almost exclusively to the potential health benefits for human beings, and later on for domestic animals such as cattle, pigs, poultry, dogs, etc. Only recently, an interest appeared in the capacity of propolis to act against bee pathogens and in the possibility to apply propolis or its constituents in beekeeping instead of pesticides and the banned in Europe antibiotics.

A number of these studies revealed the important role of propolis as an element of honeybees' social immunity. As resin-collecting bees (foraging for propolis) have no direct benefit from this activity, it can be considered a part of the bees' social immune system, providing the colony with some defence against infections and parasites [54]. Especially the research of Spivak and her group contributed significantly to this concept. Treating of beehives with propolis extract resulted in lower bacterial loads and in significantly lower expression of two honeybee immune-related genes in the propolis treated colonies [54]. The costs of an elevated immune system have been well-documented across bee species and include reduced life span under stressful conditions, and lowered colony productivity [54, 55]. The study [54] showed that propolis does not suppress the immune system; however it allows for this system to be downregulated and reduces the costs for an elevated immunity in the absence of pathogen challenge. Further, Simone-Finstrom and Spivak [56] observed a case of self-medication of bee colonies by propolis collection: there were an increased number of individuals that collect propolis in response to infection with chalkbrood *Ascophaera apis*.

Several research groups have demonstrated the ability of propolis to suppress the development of *Paenibacillus larvae*, the causal agent of American foulbrood (AFB) disease. *In vitro* inhibition of *P. larvae* was found for propolis samples from different states of Brazil, from Minnesota (USA) [57], Argentina [58], and for European poplar type propolis from Romania and Bulgaria [39,59]. *In vivo* antibacterial effect of propolis was also demonstrated: field assays showed effective treatment of hives affected with AFB by Egyptian propolis in concentrations 0.1 and 0.05% [60]. Antunez *et al.* [58] found significant decrease in the number of *P. larvae* spores/g of honey in naturally infected beehives treated with propolis extract. The authors suggested a mechanism of action including oral ingestion of the extract which facilitates the direct effect on *P. larvae*. They supposed that this mechanism could not prevent the infection of new larvae, but could inhibit the replication of vegetative bacterial cells in larval gut. In addition, they found that propolis extract had low oral toxicity to honeybees. Very recently, a number of propolis samples from different locations in the USA have demonstrated differential activity against the growth of *P. larvae* and *A. apis*. Differential activity was observed both among samples and between pathogens [61].

Of course the chemical constituents of propolis are the active principles that suppress *P. larvae*. Bilikova *et al.* [39] isolated and identified several constituents of poplar propolis with significant activity against *P. larvae* strains: pinocembrin, 3-*O*-acetylpinobanksin, and a well-defined mixture of caffeic acid esters, including CAPE (caffeic acid phenethyl ester). Mihai *et al.* [59] found that the concentration of phenolics and flavonoids in poplar type propolis affects its antimicrobial action against *P. larvae* but also that there is a significant interaction in this respect between flavones/flavonols and flavanones/dihydroflavonols in propolis extract.

In the last 30 years, *Varroa destructor* has become the most significant threat to beekeeping in Europe and all over the world. In the search for natural anti-varroa agents, propolis has also been studied. Garedew *et al.* [62, 63] showed a lethal and narcotic effect of propolis on varrroa mites. Propolis extract with 70% ethanol (10% w/v) resulted in 100% mortality with a brief contact time of 5 s. Even sublethal concentrations resulted in a significant decrease of the heat production

rate in the mites (detected by calorimetry, [62]), thus causing weakening of the mites. Damiani *et al.* [64,65] also demonstrated anti-varroa effect of Argentinean propolis from the Pampean region: spraying infested bees with a 10% propolis solution did not affect the bees but was lethal to 78% of the mites. Recently, Popova *et al.* [66] found some relationship between the chemical composition of propolis and the honeybee resistance against *V. destructor*. Propolis of varroa resistant colonies from Avignon, France, contained higher concentrations of caffeic acid and its pentenyl esters: 3-methyl-3-butenyl caffeate, 2-methyl-2-butenyl caffeate and 3-methyl-2-butenyl caffeate, compared to susceptible colonies. It is yet unknown how these compounds affect the ability of bee colonies to resist varroa infestation.

Obviously, the studies on the importance of propolis for the health and wellbeing of honeybees and their social immunity are in their early stage. The next years will bring new and insightful findings in this field which will support the efforts to better understand natural defence mechanisms of bees and help humans to improve honeybee health and, hopefully, stop colony collapse disorder (CCD).

## CONCLUSION

The interest in propolis research has been steadily growing since the 1970's. The annually published articles containing "propolis" in title, abstract and/or keywords increased from an average of 20 for the period 1975 – 1985 to 300 for the last five years (Scopus database). At the same time, it has become obvious that propolis is highly variable with respect to its chemical composition, and that many propolis chemical types exist, having distinct chemical profiles determined by their plant sources. For this reason, the chemical profiling and characterization of every propolis sample used in studying its potential applications in any possible field is of crucial importance. It is vital to stress that any research aiming propolis application, done with propolis without chemical characterization is irreproducible and irrelevant, and thus a waste of time and efforts.

The review of recent propolis literature demonstrates the potential of propolis to serve as a source of new chemical structures and new bioactive compounds due to its chemical diversity, resulting from the diversity of plant source used by bees in

different geographic regions. It also reveals the potential of propolis to be used for development of innovative products, mainly in the field of food industries, animal husbandry, beekeeping, etc. For this to happen, the combined efforts of researchers and technologists from different areas are necessary, in order to make better use of bee glue.

## CONFLICT OF INTEREST

The author confirms that author has no conflict of interest to declare for this publication.

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## REFERENCES

[1] Seeley, T.D.; Morse, R.A. The nest of the honey bee (*Apis mellifera* L.). *Insectes Soc.,* **1976**, *23*, 495-512.
[http://dx.doi.org/10.1007/BF02223477]

[2] Simone-Finstrom, M.; Spivak, M. Propolis and bee health: the natural history and significance of resin use by honey bees. *Apidologie (Celle),* **2010**, *41*, 295-311.
[http://dx.doi.org/10.1051/apido/2010016]

[3] Crane, E. *Beekeeping: Science, Practice and World Recourses*; Heinemann: London, **1988**.

[4] Salatino, A.; Teixeira, E.W.; Negri, G.; Message, D. Origin and chemical variation of Brazilian propolis. *eCAM,* **2005**, *2*, 33-38.

[5] Ghisalberti, E.L. Propolis: A review. *Bee World,* **1979**, *60*, 59-84.
[http://dx.doi.org/10.1080/0005772X.1979.11097738]

[6] Burdock, G.A. Review of the biological properties and toxicity of bee propolis (propolis). *Food Chem. Toxicol.,* **1998**, *36*(4), 347-363.
[http://dx.doi.org/10.1016/S0278-6915(97)00145-2] [PMID: 9651052]

[7] Sforcin, J.M.; Bankova, V. Propolis: is there a potential for the development of new drugs? *J. Ethnopharmacol.,* **2011**, *133*(2), 253-260.
[http://dx.doi.org/10.1016/j.jep.2010.10.032] [PMID: 20970490]

[8] Bankova, V. Chemical diversity of propolis and the problem of standardization. *J. Ethnopharmacol.,* **2005**, *100*(1-2), 114-117.
[http://dx.doi.org/10.1016/j.jep.2005.05.004] [PMID: 15993016]

[9] Kujumgiev, A.; Tsvetkova, I.; Serkedjieva, Y.; Bankova, V.; Christov, R.; Popov, S. Antibacterial, antifungal and antiviral activity of propolis of different geographic origin. *J. Ethnopharmacol.,* **1999**,

*64*(3), 235-240.
[http://dx.doi.org/10.1016/S0378-8741(98)00131-7] [PMID: 10363838]

[10] Seidel, D.J.; Qiang, F.; Randel, W.J.; Reichler, T.J. Widening of the tropical belt in a changing climate. *Nat. Geosci.,* **2008**, *1*, 21-24.

[11] Popova, M.; Chen, C-N.; Chen, P-Y.; Huang, C-Y.; Bankova, V. A validated spectrophotometric method for quantification of prenylated flavanones in pacific propolis from Taiwan. *Phytochem. Anal.,* **2010**, *21*(2), 186-191.
[PMID: 19856482]

[12] Puker, A.; Abot, R.A.; Matias, R.; Rodrigues, S.R.; Pinto, A.M. Propolis produced by africanized honeybees in the Cerrado-Pantanal ecotone: Effects of seasonality in production and physico-chemical characteristics. *Sociobiology,* **2010**, *56*, 149-162.

[13] Stan, L.; Mărghitaş, L.A.; Dezmirean, D. Quality criteria for propolis standardization. *Animal Sci. Biotechnol.,* **2011**, *44*, 137-140.

[14] Weinstein Teixeira, E.; Negri, G.; Meira, R.M.S.A.; Message, D.; Salatino, A. Plant origin of green propolis: bee behavior, plant anatomy and chemistry. *eCAM,* **2005**, *2*, 85-92.

[15] Santos, F.A.; Bastos, E.M.; Maia, A.B.; Uzeda, M.; Carvalho, M.A.; Farias, L.M.; Moreira, E.S. Brazilian propolis: physicochemical properties, plant origin and antibacterial activity on periodontopathogens. *Phytother. Res.,* **2003**, *17*(3), 285-289.
[http://dx.doi.org/10.1002/ptr.1117] [PMID: 12672164]

[16] Bogdanov, S. Propolis. *http://www.apitherapie.at/ATdownloads/Bogdanov_Propolis.pdf,* 1999 [Accessed on 8 January 2015];

[17] Petrova, A.; Popova, M.; Kuzmanova, C.; Tsvetkova, I.; Naydenski, H.; Muli, E.; Bankova, V. New biologically active compounds from Kenyan propolis. *Fitoterapia,* **2010**, *81*(6), 509-514.
[http://dx.doi.org/10.1016/j.fitote.2010.01.007] [PMID: 20079813]

[18] El-Bassuony, A.; AbouZid, S. A new prenylated flavanoid with antibacterial activity from propolis collected in Egypt. *Nat. Prod. Commun.,* **2010**, *5*(1), 43-45.
[PMID: 20184018]

[19] Segueni, N.; Zellagui, A.; Moussaoui, F.; Lahouel, M.; Rhouati, S. Flavonoids from Algerian propolis. *Arab. J. Chem.,* in press
[http://dx.doi.org/10.1016/j.arabjc.2011.05.013]

[20] Almutairi, S.; Eapen, Ben.; Chundi, S.M.; Akhalil, A.; Siheri, W.; Clements, Carol.; Fearnley, J.; Watson, D.G.; Edrada-Ebel, R. New anti-trypanosomal active prenylated compounds from African propolis. *Phytochem. Lett.,* **2014**, *10*, 35-39.
[http://dx.doi.org/10.1016/j.phytol.2014.07.002]

[21] Kardar, M.N.; Zhang, T.; Coxon, G.D.; Watson, D.G.; Fearnley, J.; Seidel, V. Characterisation of triterpenes and new phenolic lipids in Cameroonian propolis. *Phytochemistry,* **2014**, *106*, 156-163.
[http://dx.doi.org/10.1016/j.phytochem.2014.07.016] [PMID: 25104230]

[22] Piccinelli, A.L.; Mencherini, T.; Celano, R.; Mouhoubi, Z.; Tamendjari, A.; Aquino, R.P.; Rastrelli, L. Chemical composition and antioxidant activity of Algerian propolis. *J. Agric. Food Chem.,* **2013**, *61*(21), 5080-5088.

[http://dx.doi.org/10.1021/jf400779w] [PMID: 23650897]

[23] Paul, S.; Emmanuel, T.; Matchawe, C.; Alembert, T.T.; Elisabeth, Z.M.; Sophie, L.; Luce, V.E.; Maurice, T.F.; Joel, Y.G.A.; Alex, A.D.T.; Joseph, M.T. Pentacyclic triterpenes and crude extracts with antimicrobial activity from Cameroonian brown propolis samples. *J. App. Pharm. Sci.,* **2014**, *4*, 1-9.

[24] Shimomura, K.; Sugiyama, Y.; Nakamura, J.; Ahn, M-R.; Kumazawa, S. Component analysis of propolis collected on Jeju Island, Korea. *Phytochemistry,* **2013**, *93*, 222-229.
[http://dx.doi.org/10.1016/j.phytochem.2012.02.018] [PMID: 22483235]

[25] Sha, N.; Guan, S.H.; Lu, Z.Q.; Chen, G.T.; Huang, H.L.; Xie, F.B.; Yue, Q.X.; Liu, X.; Guo, D.A. Cytotoxic constituents of chinese propolis. *J. Nat. Prod.,* **2009**, *72*(4), 799-801.
[http://dx.doi.org/10.1021/np900118z] [PMID: 19278239]

[26] Shi, H.; Yang, H.; Zhang, X.; Sheng, Y.; Huang, H.; Yu, L. Isolation and characterization of five glycerol esters from Wuhan propolis and their potential anti-inflammatory properties. *J. Agric. Food Chem.,* **2012**, *60*(40), 10041-10047.
[http://dx.doi.org/10.1021/jf302601m] [PMID: 22978445]

[27] Athikomkulchai, S.; Awale, S.; Ruangrungsi, N.; Ruchirawat, S.; Kadota, S. Chemical constituents of Thai propolis. *Fitoterapia,* **2013**, *88*, 96-100.
[http://dx.doi.org/10.1016/j.fitote.2013.04.008] [PMID: 23660244]

[28] Li, F.; Awale, S.; Zhang, H.; Tezuka, Y.; Esumi, H.; Kadota, S. Chemical constituents of propolis from Myanmar and their preferential cytotoxicity against a human pancreatic cancer cell line. *J. Nat. Prod.,* **2009**, *72*(7), 1283-1287.
[http://dx.doi.org/10.1021/np9002433] [PMID: 19572611]

[29] Shaheen, S.A.; Zarga, M.H.; Nazer, I.K.; Darwish, R.M.; Al-Jaber, H.I. Chemical constituents of Jordanian propolis. *Nat. Prod. Res.,* **2011**, *25*(14), 1312-1318.
[http://dx.doi.org/10.1080/14786419.2010.509060] [PMID: 21732907]

[30] Almutairi, S.; Edrada-Ebel, R.; Fearnley, J.; Igoli, J.O.; Alotaibi, W.; Clements, C.J.; Gray, A.I.; Watson, D.J. Isolation of diterpenes and flavonoids from a new type of propolis from Saudi Arabia. *Phytochem. Lett.,* **2014**, *10*, 160-163.
[http://dx.doi.org/10.1016/j.phytol.2014.08.022]

[31] Trusheva, B.; Todorov, I.; Ninova, M.; Najdenski, H.; Daneshmand, A.; Bankova, V. Antibacterial mono- and sesquiterpene esters of benzoic acids from Iranian propolis. *Chem. Cent. J.,* **2010**, *4*, 8.
[http://dx.doi.org/10.1186/1752-153X-4-8] [PMID: 20350297]

[32] Popova, M.; Dimitrova, R.; Al-Lawati, H.T.; Tsvetkova, I.; Najdenski, H.; Bankova, V. Omani propolis: chemical profiling, antibacterial activity and new propolis plant sources. *Chem. Cent. J.,* **2013**, *7*(1), 158.
[http://dx.doi.org/10.1186/1752-153X-7-158] [PMID: 24053750]

[33] Inui, S.; Shimamura, Y.; Masuda, S.; Shirafuji, K.; Moli, R.T.; Kumazawa, S. A new prenylflavonoid isolated from propolis collected in the Solomon Islands. *Biosci. Biotechnol. Biochem.,* **2012**, *76*(5), 1038-1040.
[http://dx.doi.org/10.1271/bbb.120021] [PMID: 22738984]

[34] Inui, S.; Hosoya, T.; Shimamura, Y.; Masuda, S.; Ogawa, T.; Kobayashi, H.; Shirafuji, K.; Moli, R.T.; Kozone, I.; Shin-ya, K.; Kumazawa, S. Solophenols B-D and solomonin: new prenylated polyphenols isolated from propolis collected from the Solomon Islands and their antibacterial activity. *J. Agric. Food Chem.,* **2012**, *60*(47), 11765-11770.
[http://dx.doi.org/10.1021/jf303516w] [PMID: 23067056]

[35] Abu-Mellal, A.; Koolaji, N.; Duke, R.K.; Tran, V.H.; Duke, C.C. Prenylated cinnamate and stilbenes from Kangaroo Island propolis and their antioxidant activity. *Phytochemistry,* **2012**, *77*, 251-259.
[http://dx.doi.org/10.1016/j.phytochem.2012.01.012] [PMID: 22321386]

[36] Tran, V.H.; Duke, R.K.; Abu-Mellal, A.; Duke, C.C. Propolis with high flavonoid content collected by honey bees from *Acacia paradoxa. Phytochemistry,* **2012**, *81*, 126-132.
[http://dx.doi.org/10.1016/j.phytochem.2012.06.002] [PMID: 22784552]

[37] Popova, M.P.; Chinou, I.B.; Marekov, I.N.; Bankova, V.S. Terpenes with antimicrobial activity from Cretan propolis. *Phytochemistry,* **2009**, *70*(10), 1262-1271.
[http://dx.doi.org/10.1016/j.phytochem.2009.07.025] [PMID: 19698962]

[38] Popova, M.; Boryana, T.; Daniela, A.; Cutajar, S.; Mifsud, D.; Farrugia, C.; Tsvetkova, I.; Najdenski, H.; Bankova, V. The specific chemical profile of Mediterranean propolis from Malta. *Food Chem.,* **2011**, *126*, 1431-1435.
[http://dx.doi.org/10.1016/j.foodchem.2010.11.130]

[39] Bilikova, K.; Popova, M.; Trusheva, B.; Bankova, V. New anti-Paenibacillus larvae substances purified from propolis. *Apidologie (Celle),* **2013**, *44*, 278-285.
[http://dx.doi.org/10.1007/s13592-012-0178-1]

[40] Li, F.; He, Y.M.; Awale, S.; Kadota, S.; Tezuka, Y. Two new cytotoxic phenylallylflavanones from Mexican propolis. *Chem. Pharm. Bull. (Tokyo),* **2011**, *59*(9), 1194-1196.
[http://dx.doi.org/10.1248/cpb.59.1194] [PMID: 21881271]

[41] Li, F.; Awale, S.; Tezuka, Y.; Esumi, H.; Kadota, S. Study on the constituents of Mexican propolis and their cytotoxic activity against PANC-1 human pancreatic cancer cells. *J. Nat. Prod.,* **2010**, *73*(4), 623-627.
[http://dx.doi.org/10.1021/np900772m] [PMID: 20307087]

[42] Lotti, C.; Campo Fernandez, M.; Piccinelli, A.L.; Cuesta-Rubio, O.; Márquez Hernández, I.; Rastrelli, L. Chemical constituents of red Mexican propolis. *J. Agric. Food Chem.,* **2010**, *58*(4), 2209-2213.
[http://dx.doi.org/10.1021/jf100070w] [PMID: 20121106]

[43] Castro, M.L.; do Nascimento, A.M.; Ikegaki, M.; Costa-Neto, C.M.; Alencar, S.M.; Rosalen, P.L. Identification of a bioactive compound isolated from Brazilian propolis type 6. *Bioorg. Med. Chem.,* **2009**, *17*(14), 5332-5335.
[http://dx.doi.org/10.1016/j.bmc.2009.04.066] [PMID: 19497755]

[44] Mayworm, M.A.S.; Lima, C.A.; Tomba, A.C.B.; Fernandes-Silva, C.C.; Salatino, M.L.F.; Salatino, A. Does Propolis Contain Tannins? *eCAM,* **2014**, *2014*

[45] Agüero, M.B.; Svetaz, L.; Sánchez, M.; Luna, L.; Lima, B.; López, M.L.; Zacchino, S.; Palermo, J.; Wunderlin, D.; Feresin, G.E.; Tapia, A. Argentinean Andean propolis associated with the medicinal plant Larrea nitida Cav. (Zygophyllaceae). HPLC-MS and GC-MS characterization and antifungal

activity. *Food Chem. Toxicol.,* **2011**, *49*(9), 1970-1978.
[http://dx.doi.org/10.1016/j.fct.2011.05.008] [PMID: 21600954]

[46] Agüero, M.B.; Gonzalez, M.; Lima, B.; Svetaz, L.; Sánchez, M.; Zacchino, S.; Feresin, G.E.; Schmeda-Hirschmann, G.; Palermo, J.; Wunderlin, D.; Tapia, A. Argentinean propolis from Zuccagnia punctata Cav. (Caesalpinieae) exudates: phytochemical characterization and antifungal activity. *J. Agric. Food Chem.,* **2010**, *58*(1), 194-201.
[http://dx.doi.org/10.1021/jf902991t] [PMID: 19916546]

[47] Lotti, C.; Piccinelli, A.L.; Arevalo, C.; Ruiz, I.; Migliani, D.E.; Figueira, R. DeSáL.; Tessis, A.C.; Ferreira-Pereira, A.; Rastrelli, L. Constituents of Honduran Propolis with Inhibitory Effects on Saccharomyces cerevisiae Multidrug Resistance Protein Pdr5p. *J. Agric. Food Chem.,* **2012**, *60*, 10540-10545.
[http://dx.doi.org/10.1021/jf302578r] [PMID: 23004023]

[48] Righi, A.A.; Alves, T.R.; Negri, G.; Marques, L.M.; Breyer, H.; Salatino, A. Brazilian red propolis: unreported substances, antioxidant and antimicrobial activities. *J. Sci. Food Agric.,* **2011**, *91*(13), 2363-2370.
[http://dx.doi.org/10.1002/jsfa.4468] [PMID: 21590778]

[49] Righi, A.A.; Negri, G.; Salatino, A. Comparative Chemistry of Propolis from Eight Brazilian Localities. *eCAM,* **2013**, *2013*

[50] Falcão, S.I.; Vale, N.; Gomes, P.; Domingues, M.R.; Freire, C.; Cardoso, S.M.; Vilas-Boas, M. Phenolic profiling of Portuguese propolis by LC-MS spectrometry: uncommon propolis rich in flavonoid glycosides. *Phytochem. Anal.,* **2013**, *24*(4), 309-318.
[http://dx.doi.org/10.1002/pca.2412] [PMID: 23172843]

[51] Zhang, C.P.; Zheng, H.Q.; Hu, F.L. Extraction, partial characterization, and storage stability of β-glucosidase from propolis. *J. Food Sci.,* **2011**, *76*(1), C75-C79.
[http://dx.doi.org/10.1111/j.1750-3841.2010.01941.x] [PMID: 21535657]

[52] Zhang, C.P.; Liu, G.; Hu, F.L. Hydrolysis of flavonoid glycosides by propolis β-glycosidase. *Nat. Prod. Res.,* **2012**, *26*(3), 270-273.
[http://dx.doi.org/10.1080/14786419.2010.541877] [PMID: 21851328]

[53] Popova, M.; Trusheva, B.; Cutajar, S.; Antonova, D.; Mifsud, D.; Farrugia, C.; Bankova, V. Identification of the plant origin of the botanical biomarkers of Mediterranean type propolis. *Nat. Prod. Commun.,* **2012**, *7*(5), 569-570.
[PMID: 22799077]

[54] Simone, M.; Evans, J.D.; Spivak, M. Resin collection and social immunity in honey bees. *Evolution,* **2009**, *63*(11), 3016-3022.
[http://dx.doi.org/10.1111/j.1558-5646.2009.00772.x] [PMID: 19619221]

[55] Evans, J.D.; Pettis, J.S. Colony-level impacts of immune responsiveness in honey bees, *Apis mellifera. Evolution,* **2005**, *59*(10), 2270-2274.
[http://dx.doi.org/10.1111/j.0014-3820.2005.tb00935.x] [PMID: 16405170]

[56] Simone-Finstrom, M.D.; Spivak, M. Increased resin collection after parasite challenge: a case of self-medication in honey bees? *PLoS One,* **2012**, *7*(3), e34601.
[http://dx.doi.org/10.1371/journal.pone.0034601] [PMID: 22479650]

[57] Bastos, E.M.; Simone, M.; Jorge, D.M.; Soares, A.E.; Spivak, M. *In vitro* study of the antimicrobial activity of Brazilian propolis against *Paenibacillus larvae. J. Invertebr. Pathol.,* **2008**, *97*(3), 273-281. [http://dx.doi.org/10.1016/j.jip.2007.10.007] [PMID: 18054037]

[58] Antúnez, K.; Harriet, J.; Gende, L.; Maggi, M.; Eguaras, M.; Zunino, P. Efficacy of natural propolis extract in the control of American Foulbrood. *Vet. Microbiol.,* **2008**, *131*(3-4), 324-331. [http://dx.doi.org/10.1016/j.vetmic.2008.04.011] [PMID: 18508208]

[59] Mihai, C.M.; Mărghitaş, L.A.; Dezmirean, D.S.; Chirilă, F.; Moritz, R.F.; Schlüns, H. Interactions among flavonoids of propolis affect antibacterial activity against the honeybee pathogen *Paenibacillus larvae. J. Invertebr. Pathol.,* **2012**, *110*(1), 68-72. [http://dx.doi.org/10.1016/j.jip.2012.02.009] [PMID: 22386493]

[60] Kamel, A.A.; Moustafa, A.A.; Nafea, E.A. Propolis as a natural antibiotic to control American foulbrood disease in honey bee colonies. *Afr. J. Agric. Res.,* **2013**, *8*, 3047-3062.

[61] Wilson, M.B.; Brinkman, D.; Spivak, M.; Gardner, G.; Cohen, J.D. Regional variation in composition and antimicrobial activity of US propolis against *Paenibacillus larvae* and *Ascosphaera apis. J. Invertebr. Pathol.,* **2015**, *124*, 44-50. [http://dx.doi.org/10.1016/j.jip.2014.10.005] [PMID: 25450740]

[62] Garedew, A.; Lamprecht, I.; Schmolz, E.; Schricker, B. The varroacidal action of popolis: a laboratory assay. *Apidologie (Celle),* **2002**, *33*, 41-50. [http://dx.doi.org/10.1051/apido:2001006]

[63] Garedew, A.; Schmolz, E.; Schricker, B.; Lamprecht, I. Microcalorimetric investigation of the action of propolis on *Varroa destructor* mites. *Thermochim. Acta,* **2002**, *382*, 211-220. [http://dx.doi.org/10.1016/S0040-6031(01)00737-7]

[64] Damiani, N.; Fernández, N.J.; Maldonado, L.M.; Alvarez, A.R.; Eguaras, M.J.; Marcangeli, J.A. Bioactivity of propolis from different geographical origins on *Varroa destructor* (Acari: Varroidae). *Parasitol. Res.,* **2010**, *107*(1), 31-37. [http://dx.doi.org/10.1007/s00436-010-1829-7] [PMID: 20336318]

[65] Damiani, N.; Maggi, M.D.; Gende, L.B.; Faverin, C.; Eguaras, M.J.; Marcangeli, J.A. Evaluation of the toxicity of a propolis extract on *Varroa destructor* (Acari: Varroidae) and *Apis mellifera* (Hymenoptera: Apidae). *J. Apic. Res.,* **2010**, *49*, 257-264. [http://dx.doi.org/10.3896/IBRA.1.49.3.05]

[66] Popova, M.; Reyes, M.; Le Conte, Y.; Bankova, V. Propolis chemical composition and honeybee resistance against *Varroa destructor. Nat. Prod. Res.,* **2014**, *28*(11), 788-794. [http://dx.doi.org/10.1080/14786419.2014.881366] [PMID: 24483289]