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# Impact of biodiversification on propolis composition, functionality, and application in foods as natural preservative: A review

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ABSTRACT

Despite the numerous studies conducted on the pharmaceutical applications and chemical composition of propolis, however, challenges are still there to utilize propolis in commercial drugs and food formulations. These challenges arise due to tremendous inconsistency in raw material related to geographic origin, collection season, bioactive compounds, gross composition, and botanical source authentication, which eventually create intricacy in the entire process of developing standardized formulations for commercial use. Thus, this review article has first-ever highlighted the biodiversification of propolis (such as Poplar, Aspen, Birch, Alecrim, Red, Brown, Caribbean, Mediterranean Propolis, Canarian etc.) and discussed their associated explicit bio-marker compounds which make it unique. Moreover, the review article also provided insights into the gross compositions and dietary components detected in propolis from different geographical regions. Thus, extensive studies and scientific data are collected to develop a robust platform for the broader utilization of propolis in the food and nutraceutical sectors. Furthermore, several evidences quoted propolis as a natural preservative due to its diverse bio-functionality in terms of antioxidant, antibacterial, and antifungal properties. By inhibiting oxidative agents and microbial growth, propolis can help to extend the shelf-life and improve the overall quality and stability of food products. This may offer a natural alternative to artificial preservatives, which can have potential health risks. Overall, the potential health benefits and natural preservative properties of propolis highlight its potential as a valuable ingredient for the food and nutraceutical industries. However, further studies are needed to fully understand its potential applications and develop standardized methods for its collection, processing, and quality control.

## 1. Introduction

Bee propolis is an extremely complex material of natural constituents possessing resinous consistency created by bees which exhibits tremendous variation based on its physical appearance, color, and consistency (Anjum et al., 2019). Its quality is majorly based on several factors such as geographical origin, types of vegetation source, time of collection, season, climatic conditions, and honeybees (Anjum et al., 2019; Neves, Silva, Lima, Cunha, & Oliveira, 2016; Pant, Thakur, Dar, Chopra, & Nanda, 2022). When bees procure propolis, they acquire resinous material (sprouts and exudates) with the help of their mandibles from different parts of various plants, such as leaf buds, shoots, and petioles of leaves which once introduced into the hive blended with wax and salivary secretions, produce propolis (Hsieh et al., 2019; Shahinozzaman, Obanda, & Tawata, 2021).

As per vast literature survey, propolis has generally studied in two categories: (i) detailed composition (deals with specific and individual components) and (ii) gross composition (deals with the major components). In several investigations based on its detailed composition, more than 500 phyto-chemical compounds have been detected in propolis worldwide till date (Anjum et al., 2019; Drescher, Klein, Neumann, Yañez, & Leonhardt, 2017; Pobiega, Kraśniewska, Derewiaka, & Gniewosz, 2019). The biological activity of propolis depends only upon the chemical composition that are phytochemicals and other plant secondary metabolites obtained from plant resins and exudates (Kasote, Bankova, & Viljoen, 2022; Salatino & Salatino, 2018). Resins and/or vegetable balsam comprise most of the propolis's bioactive constituents, such as polyphenolic compounds, and their derivatives (El- Guendouz et al., 2019). Over years, major attention has been drawn only towards its phytochemical composition or individual active ingredients such as

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phenolic acids, flavonoids and isoflavonoids, prenylated benzophenones, monoterpenes, diterpenes, terpenoids, hydrocarbons, volatile compounds, and their derivatives (Fangio, Orallo, Gende, & Churio, 2019; Galeotti et al., 2017; Papachristoforou, Koutouvela, Menexes, Gardikis, & Mourtzinos, 2019). Numerous investigations provide substantial evidence to show that it has antibacterial, antifungal, antiviral, anti-inflammatory, immunomodulatory, hepatoprotective, antiseptic, anticarcinogenic, antidepressant, and antioxidant properties. Due to such functionalities, propolis has been extensively used in traditional medicine, food supplements, nutraceuticals, and health care products (Galeotti et al., 2017; Pobiega et al., 2019). Nearby, several studies also quoted the application of propolis as a natural preservative, antioxidant, and functional food ingredient in the food system.

In aspect of gross composition, propolis is predominantly composed of plant resins and beeswax, whereas pollen and minerals are the minor constituents. Various researches quoted similar gross composition of propolis i.e., 70-40% resin and/or vegetable balsam, 10–30% waxes, 5–10% aromatic and essential compounds, 5–10% pollen, and other organic components (El-Guendouz Lyoussi, & Miguel, 2019; Galeotti et al., 2017; Pobiega et al., 2019). Although, limited investigations were conducted on aspects of the gross composition of propolis from different locations like, India, Indonesia, Malaysia, Morocco, Romania, Brazil (Kunrath et al., 2017; Duca, Sturza, et al., 2019; Fallah, Najafi, & Kavoosi, 2021; Mulyati, Sulaeman, Marliyati, Rafi, & Rokhmah, 2021; Pant et al., 2021). Moreover, few studies reported its nutrition composition related to its mineral, lipid, amino acid and vitamin profile from different locations (Kunrath et al., 2017; Duca, Sturza, et al., 2019; Fallah et al., 2021; Mulyati et al., 2021; Pant et al., 2021).

However, in accordance with a broad literature assessment, a review of propolis still requires exploring its gross composition, nutritional value, and functionality based on chemical composition. This mini-review will take researchers through a glimpse of the effect of biodiversification on propolis chemical composition, nutritional value, and functionality as a natural preservative, which will fortify the existing knowledge and application of propolis in the food system. Additionally, this paper may motivate researchers to focus more studies in these areas. Besides, the present study may draw huge attention of higher authorities to establish harmonized global regulations and standards for its chemical and gross composition based on propolis type.

## 2. Biodiversity in propolis composition

Propolis is an extremely complex substance that is recorded to contain hundreds of various compounds, diversifying in chemical composition and bioactivity based on two main factors: (i) botanical origin and (ii) geographical locations (Neves et al., 2016). Propolis is mostly recognized after the appellation of the source plant wherein anatomical attributes of plant tissues in the propolis can be acknowledged as an authentication marker of propolis origin (Kosalec, Bakmaz, Pepeljnjak, & Vladimir-Knezevic, 2004; Teixeira, Negri, Meira, & Salatino, 2005; Kasote et al., 2022). Propolis type and the source are generally identified by tracing bee collection sites and equating the chemical profiles of propolis and plant materials. In addition, species of honeybees, harvest season, method of harvesting, and post-harvesting processing of raw propolis also affect its gross composition and nutritive value (Anjum et al., 2019; Maldonado et al., 2020).

Propolis is generally categorized into various groups, known as Poplar, Birch, Green, Red, Pacific, Mediterranean, etc., based on the botanical sources and the existence of main constituents (El-Guendouz et al., 2019). These categories are directly associated with geographical zones such as temperate, Mediterranean, subtropical, and tropical global regions, as discussed below. and listed in Table 1 and shown in Fig. 1.

### 2.1. Temperate type propolis

The Poplar, Aspen, and Birch type propolis, also called temperate type propolis, are mainly found in North America, Europe, New Zealand, temperate zones of Asia (especially the northern region of Russia), Africa (mainly in the eastern Nile Delta region), China, Turkey, etc., (Huang, Zhang, Wang, Li, & Hu, 2014). The *Populus* species and their hybrids, such as *Aigeiros*, and *P. nigra* L, *P. alba* L are the main sources of resin exudates which are rich in flavonoids (i.e., Flavones, flavanones) but low in phenolics and their esters (Mohammadzadeh et al., 2007; Ristivojević, Trifković, Andrić, & Milojković-Opsenica, 2015; Biosard et al., 2019) (Figs. 1 and 2). In China, species such as pines, cypress, willow, and sumacs are the primary source of Poplar propolis (Lihong et al., 2009; Zhang et al., 2015). Whereas, in Turkey, species as pine trees, eucalyptus, and castanea are used by the bees for the same (Silici, Ünlü, & Vardar-Ünlü, 2007). Similarly, Aspen propolis is specifically obtained in the northern region of Europe, mountainous regions of Switzerland, North-Russia and *Populus tremula* L is recognized as a marker plant with a high concentration of phenolic acids (i.e., p-coumaric acid, ferulic acid, benzoic acid, benzyl p-coumarate), and phenolic glycerides (dicoumaroyl acetyl-, diferuloyl acetyl-, feruloyl coumaroyl acetyl and caffeoyl coumaroyl acetyl glycerol). as marker chemicals (Pavlovic et al., 2020; Popova et al., 2013). In Russia, *Betula verrucosa, Betula pubescens, and Betula litwinowii* species are the significant sources of Birch propolis characterized by flavones and flavonols (different from Poplar propolis) (Table 1). Nevertheless, flavonoids and phenolic acids are not confined to only temperate region propolis. Moreover, in regions where poplars *spp*. are not native plants, such as tropical and sub-tropical regions like equatorial regions of South America, Australia, etc., bees will pursue other vegetation to create propolis, which also comprises the flavonoids and phenolics like propolis produced from poplar spp. (Li, Awale, Tezuka, Esumi, & Kadota, 2010).

### 2.2. Tropical types propolis

The Alecrim, Red, Brown, Caribbean, and Pacific-type propolis are mainly found in tropical regions, known as tropical type propolis. The Alecrim (green) propolis originated from *Baccharis dracunculifolia,* which contains prenylated phenylpropanoids (Artepillin C, Baccharin, Drupanin, etc.) and diterpenic acids as primary components (Olegário et al., 2019; Szliszka et al., 2013). In green propolis, Artepillin C is considered as a salient chemical compound which facilitates differentiation from other propolis (Righi et al., 2011; Silva-Beltrán et al., 2021) (Fig. 2). Brown propolis is mostly collected from the plant known as *Hyptis* spp. (such as *H. divaricata Pohl ex Benth)* high in prenylated benzophenones as a marker compound specifically in Brazil (Castro et al., 2009; Olegário et al., 2019). Whereas *Dalbergia* species such as *D. ecastaphyllum* are the primary source of red propolis, characterized by the existence of isoflavonoids (like pinobanksin, luteolin, rutin, formononetin, liquiritigenin, pinobanksin-3-acetate quercetin, pinocembrin, daidzein, isoliquiritigenin, isoflavones etc), triterpenoids (pterocarpans, Neovestitol, Vestitol, etc.) and some extent of prenylated benzophenones found mostly in Cuba and Mexico regions (Table 1 and Fig. 2) (Li, He, Awale, Kadota, & Tezuka, 2011; Neto et al., 2017; Salatino & Salatino, 2018; Kasote et al., 2022). Despite, in the Gulf Region of Mexico, green variety propolis is acquired predominantly, in contrast of the northern region, wherein the ecological system is more desertic than the other regions, and mesquite (*Prosopis laevigata*) is a major vegetable source (Silva-Beltrán et al., 2021; Vargas Sánchez, Martínez Benavidez, Hernández, Torrescano Urrutia, & Sánchez Escalante, 2020). Additionally, In Mexico, the Gulf and Pacific Coasts are the most remarkable locations with the diversity of tropical vegetation; due to which lot of variation have been observed in propolis color such as chestnut-green, yellow-red, red, dark yellow, dun, or black) (INEGI, 2020; Silva-Beltrán et al., 2021)

The Caribbean propolis collected from the flower of various *Clusia* species is mainly found in Venezuela, Amazon, and Cuba regions. Caribbean propolis contains high concentrations of prenylated benzophenones (such as Nemorosone, grcinielliptone I, hyperibone B,

**Table 1**
Various bioactive compounds detected in propolis based on biodiversification.

| Origins | Types and source | Category | Techniques | Identified compounds | Reference |
|---|---|---|---|---|---|
| Brazil | Tropical red propolis from *Dalbergia ecastaphyllum, Clusia* spp. | Isoflavonoids, prenylated benzophenones, and triterpenoids | | HyperiboneA, (6a*R*,11a*R*)-3,8-Dihydroxy-9-methoxypterocarpan, Moronic acid, Mucronulatol, Neovestitol, Vestitol | Kasote et al. (2022) |
| Jordan | Topical propolis | Polyphenols | HPLC-PDA | Gallic acid, Chlorogenic acid, Vanillin acid, Caffeic acid, Rutin, Lueolin-7-O-glucoside, Naringenin, Apigenin-7-O-glucoside, Rosmarinic acid, 4-hydroxy coumaric acid, Luteolin, Quercetin, Apigenin, Pinocembrin, Chrysin, CAPE Galangin, Carnosic acid, Hesperidin | Naik et al. (2021) |
| Brazil | green propolis From *Baccharis dracunculifolia* | prenylated phenylpropanoids and polyphenols | | Artepillin C, Baccharin, Caffeic acid, Chrysin, Cinnamic acids, *p*-Coumaric acid, Coniferyl aldehyde, Drupanin, Ferulic acid, Kaempferide, Quercetin 3,5,7-Trihydroxy-4′-methoxyflavanol | Olegario et al., 2019 |
| Pacific Ocean islands (Okinawa, Taiwan & Hawaii) | Macaranga-type Pacific propolis | Prenylated flavonoids | HPLC | Propolin C/Diplacone (Hymphaeol-A), Propolin D (Nymphaeol-B), Propolin G (Nymphaeol-C), Propolin F (Isonymphaeol-B), Propolin H (30 -Geranyl-naringenin), Prokinawan, Propolin A, Propolin B, Propolin E | Chen et al., 2018; Inui et al., 2014; Kumazawa et al., 2014; Huang et al., 2014; Shahinozzaman et al., 2021 |
| Brazil | Red propolis | Polyphenol | HPLC-DAD | Chlorogenic, Caffeic acid, Ellagic acid, Vitexin, Rutin, p-Coumaric acid, Quercetin, Luteolin, Apigenin | Neto et al. (2017) |
| Argentina | Temperate Propolis collected from *Populus alba, Salix* spp.*, Erythrina crista-galli, Acacia mearnsii, and Carya illinoinensis* | Polyphenols | HPLC | Caffeic acid, Coumaric acid, Ferulic acid, Quercetin, Cinnamic acid, Apigenin, Naringenin, Chrysin, Pinocembrin, Galangin, Pinocembrin derivatives | Busch et al. (2017) |
| Southeast European countries (Serbia, Bosnia and Herzegovina, and Bulgaria) | Poplar propolis from *Populus tremula, P. nigra, and P. x euramericana* | Phytochemicals | NMR | Chrysin, Galangin, Pinobanksin, Pinocembrin, Pinobanksin 3-O-acetate, Pinobanksin 3-O-methyl ether, Kaempheride, Apigenin, Naringenin, Caffeic acid, Benzyl caffeate, Caffeic acid phenethyl ester, Cinnamyl caffeate, p-Coumaric acid, Ferulic acid, Benzoic acid, Conyferyl benzoate, Conyferyl p-coumarate, Benzyl p-coumarate, Benzyl ferulate, 1,3-diferulyl-2-acetyl-glycerol, 1-p-coumaryl-2- acetyl-3-feruloyl-glycerol, 1,3-di-p-coumaryl - 2-acetyl-glycerol, | Andelkovic et al., 2017 |
| Polish | Birch propolis | Phytochemicals | TIC & GC-MS | Cinnamic acids (p-coumaric acid, ferulic acid, caffeic acid), Cinnamic acid esters (prenyl cinnamates, benzyl cinnamates, coniferyl cinnamates), Phenylpropenoid glycerides (1,3-di-p-coumaroyl glycerol, 2-acetyl-1,3-di-p-coumaroyl glycerol, 2-acetyl-1-p-coumaroyl-3-feruloyl glycerol, 2-acetyl-1,3-diferuloyl glycerol), Phenylpropenoid sesquiterpenols (6-Hydroxy-β-caryophyllene p-coumarate, 14-Hydroxy-β-caryophyllene p-coumarate 14-Hydroxy-β-caryophyllene ferulate), Flavonoids (apigenin, pinobanksin, pinobanksin 3-acetate, galangin chrysin, sakuranetin, homoeriodictyol pectolinaringenin), Sesquiterpenoids (birkenal & β-betulenal, 6-hydroxy-β-caryophyllene, 14-hydroxy-β-caryophyllene), Triterpenoids & sterols, Benzoic acid & other aromatics (Aliphatic C12–C30 acids, Aliphatic alcohol, Alkanes & alkene, unknown compounds) | Isidorov, Bakier, Pirożnikow, Zambrzycka, and Swiecicka (2016) |
| Ukraine & Slovak | Poplar type propolis | Phytochemicals | TIC & GC-MS | Cinnamic acids (p-coumaric acid, ferulic acid, caffeic acid), Cinnamic acid esters (prenyl cinnamates, benzyl cinnamates), Flavonoids (apigenin, pinobanksin, pinobanksin 3-acetate, galangin chrysin, sakuranetin), Sesquiterpenoids, Triterpenoids & sterols, Benzoic acid & other aromatics (Aliphatic C12–C30 acids, Aliphatic alcohol, Alkanes & alkene) | Isidorov et al. (2016) |
| Russia | Birch propolis | Phytochemicals | TIC & GC-MS | Cinnamic acids (p-coumaric acid, ferulic acid, caffeic acid), Cinnamic acid esters (benzyl cinnamates, coniferyl cinnamates), Phenylpropenoid glycerides (1,3-di-p-coumaroyl glycerol, 2-acetyl-1,3-di-p-coumaroyl glycerol, 2-acetyl-1-p-coumaroyl-3- feruloyl glycerol, 2-acetyl-1,3-diferuloyl glycerol), Phenylpropenoid sesquiterpenols (6-Hydroxy-β-caryophyllene p-coumarate, 14-Hydroxy-β-caryophyllene p- | |

(*continued on next page*)

**Table 1** (*continued*)

| Origins | Types and source | Category | Techniques | Identified compounds | Reference |
|---|---|---|---|---|---|
| | | | | coumarate 14-Hydroxy-β-caryophyllene ferulate), Flavonoids (pinocembrin), Sesquiterpenoids (birkenal & β-betulenal, 6-hydroxy-β-caryophyllene, 14-hydroxy-β-caryophyllene), Triterpenoids & sterols, Benzoic acid & other aromatics (Aliphatic c12-c30 acids, Aliphatic alcohol, Alkanes & alkene, unknown compounds) | |
| Finland | Aspen type propolis | Phytochemicals | TIC & GC-MS | Cinnamic acids (p-coumaric acid, ferulic acid, caffeic acid), Cinnamic acid esters (benzyl cinnamates, coniferyl cinnamates), Phenylpropenoid glycerides (1,3-di-p-coumaroyl glycerol, 2-acetyl-1,3-di-p-coumaroyl glycerol, 2-acetyl-1-p-coumaroyl-3- feruloyl glycerol, 2-acetyl-1,3-diferuloyl glycerol), Phenylpropenoid sesquiterpenols (6-Hydroxy-β-caryophyllene p-coumarate, 14-Hydroxy-β-caryophyllene p-coumarate 14-Hydroxy-β-caryophyllene ferulate), Flavonoids (apigenin, sakuranetin, homoeriodictyol pectolinaringenin), Triterpenoids & sterols, Benzoic acid & other aromatics (Aliphatic C12–C30 acids, Aliphatic alcohol, Alkanes & alkene) | Isidorov et al. (2016) |
| Brazil | Green propolis | Polyphenol | LC-ESI MS | Caffeoylquinic acid isomers, Caffeic acid, p-Coumaric acid, Ferulic acid, Dicaffeoylquinic acid isomers, Coumaric acid prenyl ester, Caffeic acid derivatives, Tricaffeoylquinic acid, Dimethyl-dicaffeoylquinic acid, Naringenin, Kaempferol, Isorhamnetin, Hesperitina Caffeic acid derivative, 3,4-Dihydroxy 5-prenylcinnamic acid, 4-Hydroxy 3-prenylcinnamic acid (drupanin), Coumaric acid derivative, Sakuranetina, Izosakuranetina, Pinocembrina, Kaempferide & derivatives, Dicoumaric prenyl ester, Kaempferide derivatives, Coumaric acid derivatives, Artepillin C derivatives, 3-Prenyl-4-(dihydrocinnamoyloxy), cinnamic acid (baccharin) | Szliszka et al. (2013) |
| Argentina | Poplar type | Aromatic acids, epoxy lignans, flavones, and flavonols | | Chrysin, 2, 4-Dihydroxychalcone, Kaempferol, Pinocembrin, Quercetin, 2,4-Dihydroxy 3-methoxychalcone, | Aguero et al., 2011 |
| | Topical type propolis from *Dalbergia* spp. | flavanones, isoflavones, Diphenyl propanes, and pterocarpans | | (2R,3R)-3,5-Dihydroxy-7-methoxyflavanone-3-(2-methyl)butyrate, (2R,3R)-6-[1-(4′-Hydroxy-3′-methoxyphenyl)prop-2-en-1-yl]pinobanksin, (2R,3R)-6-[1-(4′-Hydroxy-3′-methoxyphenyl) prop-2-en-1-yl]pinobanksin 3-acetate, (7″R)-8-[1-(4′-Hydroxy-3′-methoxyphenyl)prop-2-en-1-yl] pinobanksin, (7″R)-8-[1-(4′-Hydroxy-3′-methoxyphenyl)prop-2-en-1-yl]galangin, Chrysin, (2R,3S)-8-[4-Phenylprop-2-en-1-one]-4′,7-dihydroxy-3′,5-dimethoxyflavan-3-ol | Li et al. (2011) |
| Northeast Portugal | *Apis mellifera* propolis | Phenolic acids, Flavones, Flavonols Flavanones and Dihydroflavonols | HPLC-ESI MS/MS | Caffeic acid, p-Coumaric acid, Ferulic acid, Isoferulic acid, p-Coumaric acid methyl ester, 3,4-Dimethyl-caffeic acid, p-Coumaric acid isoprenyl ester, Caffeic acid benzyl ester, Caffeic acid isoprenyl ester, Caffeic acid phenylethyl ester (CAPE), Caffeic acid cinnamyl ester, Apigenin, Chrysin, Chrysin-5-methyl-ether, Kaempferol-5-methyl-ether, Chrysin-6-methyl-ether, Pinocembrin, Hesperitin-5,7-dimethyl-ether, Pinobanksin, Pinobanksin-5-methyl ether, Pinobanksin-3-O-acetate, Pinobanksin-3-O-propionate, Pinobanksin-5-methyl-ether-3-O-pentanoate, pinobanksin-3-O-butyrate or isobutyrate, pinobanksin-3-O-pentanoate or 2-methylbutyrate, 5-Methoxy-3-hydroxy-flavanone | Falcao et al., 2010 |
| Greece | Mediterranean type Propolis from Conifer Tree *(Pinus* sp. *and Cupressus sempervirens)* | Diterpenes | GC-MS | Manoyl Oxide, Labda-8(17),12,13-Triene, 13-Epi-Manool, Communal, Sempervirol, Diterpenic Acid, Ferruginol, 14,15-Dinor-13-Oxo-8(17)-Labden-19-Oic Acid, Copalol, 18-Hydroxyabieta-8,11,13-Triene, Communic Acid, Totarol, 13-Epi-Torulosal, Neoabietic Acid, Pimaric Acid, Imbricataloic Acid, 13-Epi-Torulosol, Dehydroabietic Acid, Abietic Acid, 13-Epi-Cupressic Acid, Dihydroxyabieta-8,11,13-Triene, Ferruginolon, Isoagatholal, 2-Hydroxyferruginol, Hydroxydehydroabietic Acid, Agathadiol, Imbricatoloic Acid, Totarolon, Isocupressic Acid, | Popova et al. (2010) |

(*continued on next page*)

Table 1 (*continued*)

| Origins | Types and source | Category | Techniques | Identified compounds | Reference |
|---|---|---|---|---|---|
| | | | | 6/7-Hydroxyferruginol, Junicedric Acid, Acetylisocupressic Acid, 13(14)-Dehydrojunicedric Acid, Succinyloxyabietadiene, 18-Succinyloxyabieta-8,11,13-Triene,18-Succinyloxyabietadiene, 18-Succinyloxyhydroxyabietatriene | |
| Brazil | Temperate propolis | Essential oil | GC-MS | Cyclopentane, Propene, 2-Butenal, lR-Pinene, Benzaldehyde, Cyclohexene, 3-methylene-4-vinyl, Hexanoic acid, l-Hepten-6-one, 2-methyl, Limonene, 1,3,6-Octatriene, 3,7-dimethyl-, (Z)-Benzenemethanol, Trimethyl (3, 3-diflouro, 2-propenyl)silanea, Acetophenone, 1,4-Cyclohexadiene, l-methyl-4-(l-methylethyl), 2-Furanmethanol, 5-ethenyltetrahydro-5-trimethyl, Beta-linalool, Phenylethyl alcohol, 1,4-Hexadiene, 3,3,5-trimethyl- Lilac aldehyde, Phenol, 4-ethyl-50.9924 21 3-Cyclohexen-l-ol, 4-methyl-l-(l-methylethyl), Benzenepropanol, Benzaldehyde, 4-methoxy, 2,6-Octadien-l-ol, 3,7-dimethyl, dl-phenylalanine, N-formyl- Benzenepropanoic acid, methyl ester, 2-Methoxy-4-vinylphenol, Benzenepropanoic acid, ethyl ester, Caryophyllene, Benzenepropanoic acid, hexyl ester, Cubebene, Ylangene, Copaene, Naphthalene, l,2,3,5,6,8a-hexahydro-4, 7-dimethyl-l-(l-methyl)b, l,6,10-Dodecatrien-3-ol, 3,7, 11-trimethyl-, [S-(Z)], (−)-Spathuleno | Atungulu, Miura, Atungulu, Satou, and Suzuki (2007) |

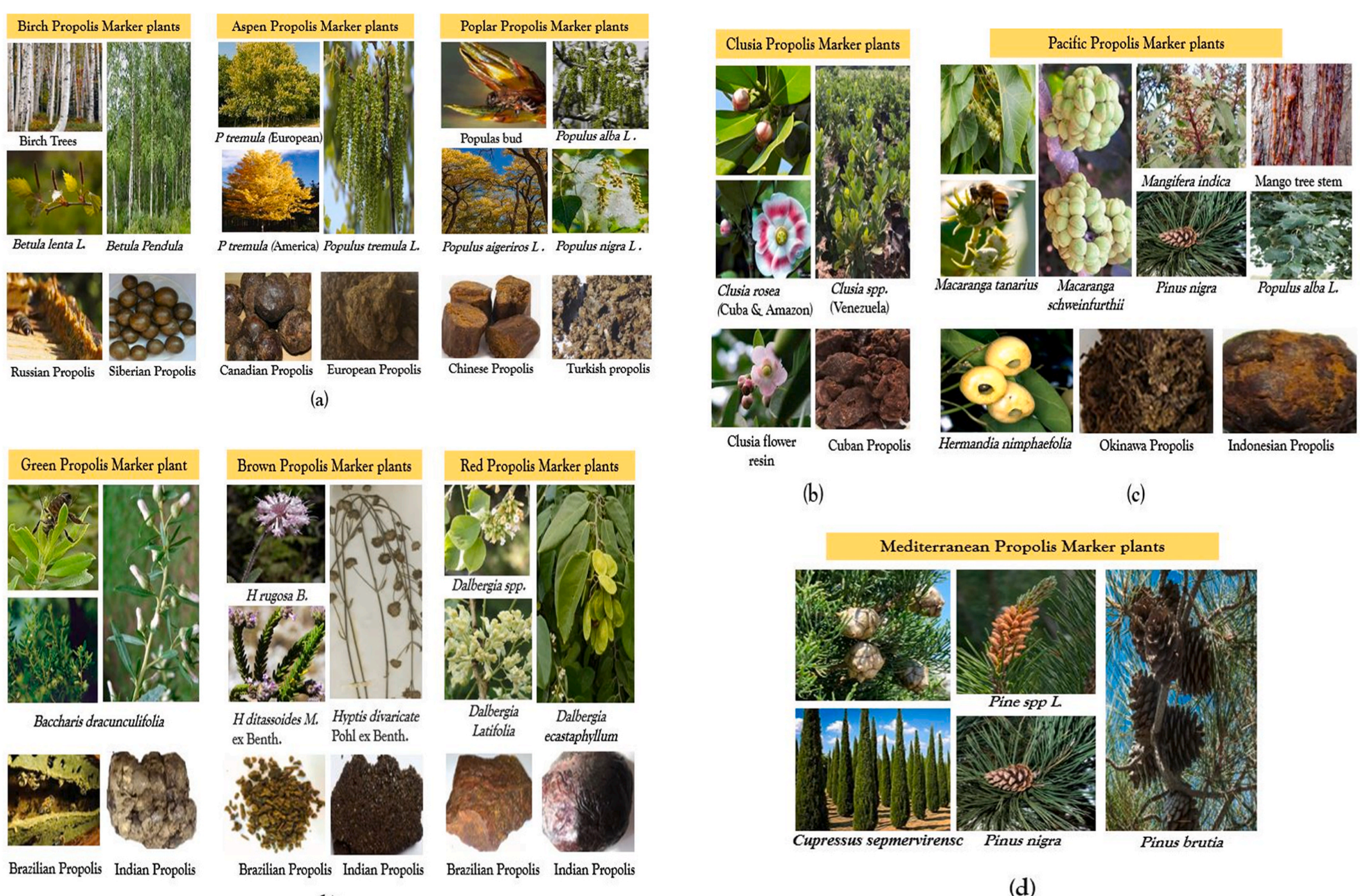


**Fig. 1.** Varieties of propolis and marker botanical sources based on different geographical zones: (a) temperate type propolis; (b and c) topical type propolis; and (c and d) sub-topical type propolis.

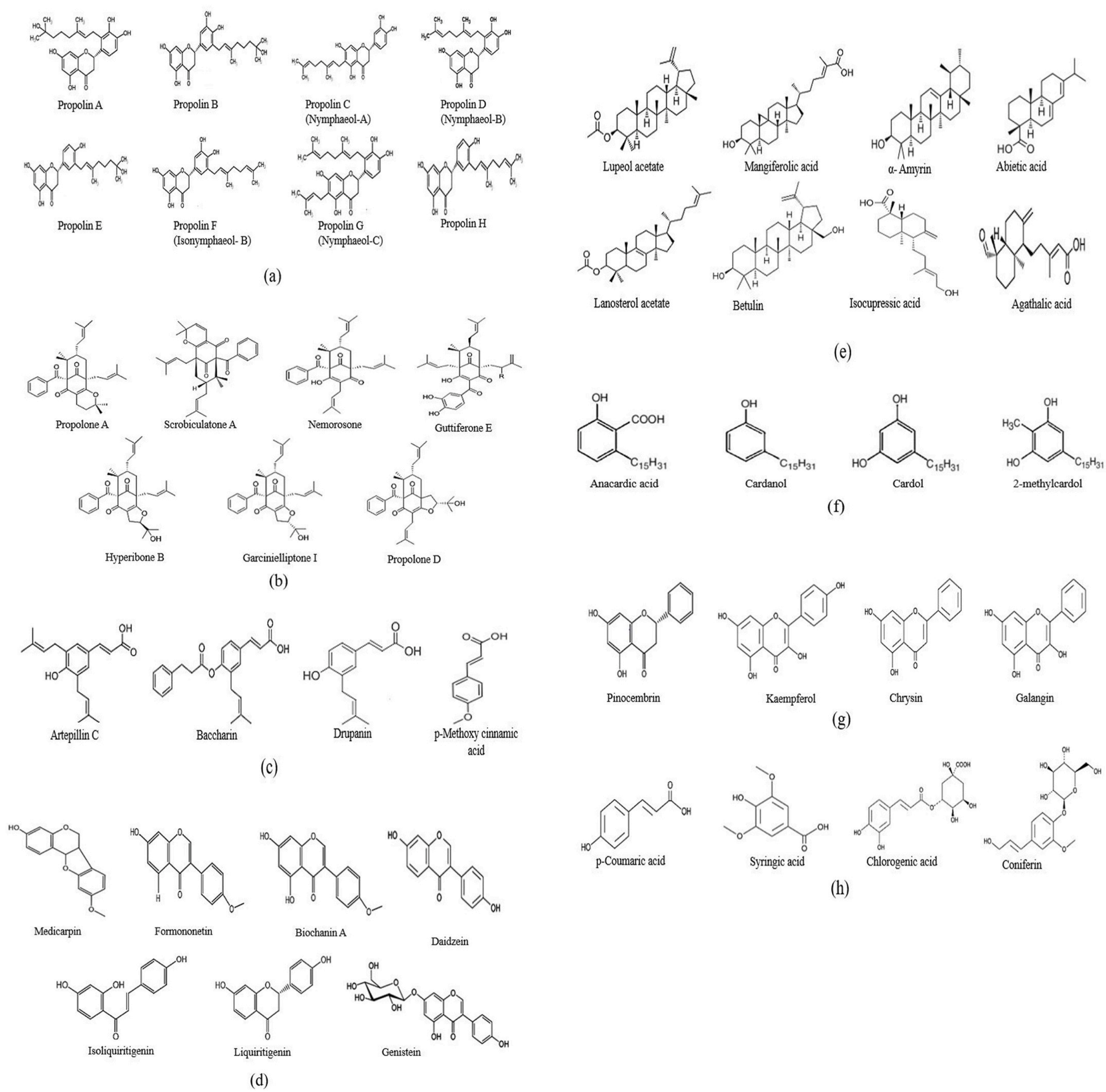


**Fig. 2.** Chemical structures of Marker compounds detected in different varieties of propolis globally. (a) Prenylated Flavonoids; (b) Prenylated Benzophenones; (c) Prenylated Phenylpropanoids; (d) Terpene, Terpenoids and Diterpenoids; (e) Iso-flavonoids; (f) Phenolic lipids; (g) Flavonoids; (h) Phenolic acids.

propolones B-D, etc.) (Hernandez, Fernandez, Cuesta-Rubio, Piccinelli, & Rastrelli, 2005; Kasote et al., 2022).

The Pacific propolis originates in the tropical islands (like Indonesia, Okinawa, and Taiwan, etc., situated in the Pacific Ocean), and contains prenylated flavonoids (such as propolin A, C, E, etc.) as chief compounds due to *Macaranga tanarius* and its other species as the main source of raw material (Chen et al., 2018; Inui, Hosoya, & Kumazawa, 2014; Kumazawa, Murase, Momose, & Fukumoto, 2014; Shahinozzaman et al., 2021). In the coastal region of Kenya stilbenes, are not very common in plants. Two geranylstilbenes such as schweinfurthin A and schweinfurthin B have been identified in propolis that originated in Kenya where *Macaranga schweinfurthii* is the only plant source of these two geranylstilbenes till now (Petrova et al., 2010; Labska, Plodkova, Pumannova, & Sensch, 2018)

### 2.3. Mediterranean propolis

The Mediterranean propolis type is also called Subtropical propolis, however such propolis is mostly collected from the coniferous plants. Plants belonging to Cupressaceae (*Cupressus sepmervirens*), genus *Cistus* (*C. ladanifer*), are marker vegetations of Sicilian, Cretan propolis, and Maltese propolis whereas, Pinus spp. are for Greek propolis (El-Guendouz et al., 2019; Melliou & Chinou, 2004; Popova, Chinou, & Bankova, 2009; Popova et al., 2011). The Mediterranean propolis obtained from Italy, Croatia, Malta, Greece, Turkey, Cyprus, Egypt, Libya, Algeria and Morocco primarily characterized based on the high amount of diterpenes (For example, Communal, Sempervirol, Diterpenic Acid, Ferruginol, Copalol, Communic Acid, Isocupressic Acid, totarol, agathadiol, isoagatholal, pimaric acid, etc.) (Popova, 2009; Popova, Graikou, Chinou, & Bankova, 2010; El-Guendouz et al., 2019). Mediterranean propolis generally does not contains phenolic acids and flavonoids (Popova et al., 2011).

### 2.4. Subtropical type propolis

Several types of propolis are found in the subtropical region, such as Canarian propolis, Mangifera indica propolis, Mixed propolis type, etc. Canarian-type propolis is specifically found in the Canary Islands and is distinguished by its high amount of Furofuran and lignanes (Kujumgiev et al., 1999; Miguel & Antunes, 2011). Other side, epoxy lignans are also found in significant amounts in Andean Argentinian propolis from the plant source named *Larrea nitida* (Agüero et al., 2011). However, the vegetation source of Canarian propolis is still unknown. On the contrary, *Mangifera indica* propolis is recognized by its main vegetation source, *Mangifera indica. L.* (common name is Mango). Such propolis contains a high amount of phenolic lipids like cardols, cardanols, and anacardic acid derivatives. It is mainly found in subtropical and tropical regions, for example, Thailand, Indonesia, India, Myanmar, etc. (Knödler et al., 2008; Trusheva et al., 2011). Furthermore, mixed propolis type originates from a mixture of two or three marker plant sources, e.g., Pacific-*Mangifera indica* propolis, aspen-poplar propolis, Cupressus-poplar propolis due to the variety of grown *spp* in subtropical regions. like *Macaranga tanarius, Mangifera indica. L., D. ecastaphyllum, Cupressus sepmervirens*, *P. nigra* L, *P. alba* L etc. (Trusheva et al., 2011)

Additionally, propolis collected from Myanmar, Solomon Island, and Japan is categorized by the existence of prenylated and geranylated flavonoids. In South Anatolia (Turkey) and Australia (Silici et al., 2007). Eucalyptus species have been considered the marker plant source; however, no scientific evidence has yet been published.

## 3. Propolis and its gross composition

In consonance with the literature, most of the data have been published related to the detailed composition of propolis, which is directly linked to the characterization of phenolic compounds, flavonoids, terpenoids, volatile compounds, and relevant constituents (Santos, Estevinho, de Carvalho, da Silva Conceição, & de Castro Almeida, 2020). In contrast, limited data is available on propolis's proximate/gross composition (Salatino & Salatino, 2021). The gross composition of propolis generally refers to the moisture, ashes, waxes, crude fat, crude protein, resins or vegetable balsam, pollen content, free sugar or carbohydrate content, essential and aromatic oil, and insoluble residues of propolis. Mostly cited and commonly listed gross composition of propolis in research papers are: "vegetable balsam and resin (50%), bee wax (30%), pollen (5%), essential and aromatic oils (10%)" (Anjum et al., 2019). For the past two decades till the present, similar or identical data has been quoted several times associated with the gross composition of propolis which is controversial and questionable (Anjum et al., 2019; Salatino & Salatino, 2021). Despite this, the nutritional composition of propolis is mainly influenced by the gross composition of propolis listed in Tables 2 and 3.

## 4. Propolis as a natural preservative in food

The utilization of propolis has been increasing as a functional food ingredient because nowadays, consumers are consistently shifting towards functional foods. At the present time, people are more aware and conscious about their food options, thus they usually look for food which is fresh, minimally processed, and augmented with naturally occurring superfoods or enhanced nutritional value which fetch maximum health benefits to them with maintained organoleptic properties (Irigoiti et al., 2021; Sharma, Pant, Brar, Thakur, & Nanda, 2022). For that reason, food industries are more focused towards the application of natural preservative over synthetic preservative due to their carcinogenic and teratogenic tendencies which have been observed after their prolong consumption (Viera et al., 2016). Over last two decades, Propolis has been regarded as a natural product that can be served as a functional food ingredient due to their rich chemical composition (i.e., phytochemicals) and bio-functionalities for example antibacterial, antioxidant, anti-inflammatory, antiseptic, antiulcer etc. (Azemin et al., 2017) (discussed in Table 4). Therefore, many food industries are focusing to use propolis as microbial inhibitor and anti-oxidant agent, specifically in bakery items, edible oils, fish and animal products. Since, these commodities are highly susceptible to lipid auto-oxidation which eventually lead to deterioration of organoleptic properties in such foods (Pobiega et al., 2019). Consequently, it can be incorporated as natural food preservative to entirely replace artificial preservative in processed food items to promote immunity and well-being (Fig. 3).

Furthermore, certain issues are also associated with raw propolis such as slightly bitter or an insipid taste, resinous flavor and low water solubility, contaminated with waxes or dirt. etc. to be consumed raw or used as a functional ingredient in processed food. Therefore, propolis must initially subjected to various purification processes to remove waxes and dirt followed by various extraction techniques to be utilized in various food, and pharmaceutical preparations. Solvent extraction is one of the majorly applied extraction techniques used for propolis

**Table 2**
Gross composition of propolis from different geographical locations.

| Country | Propolis type | Resin % | Gross composition | | | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | Moisture % | Lipid% | Protein% | Carb% | Ash % | Fiber% | |
| India | Brown | 40.90–47.66 | 4.98–7.37 | 53.62–68.89 | 7.28–9.41 | – | 3.01–4.71 | 1.94–3.15 | Pant et al. (2021) |
| Malaysia | Stingless bee propolis | – | – | 4.67–42.56 | 0.53–0.7 | 4.67–42.36 | – | – | Salleh, Hanapiah, Johari, Ahmad, and Osman (2021) |
| Iran | – | – | 15.45 | 13.34 | 11.00 | 57.15(s) | 2.76 | – | Fallah et al. (2021) |
| Korea | – | | 1.5–178 | 81.37–86.63 | 1.70–2.22 | | 2.12–2.34 | 0.24–0.31 | Jeong et al. (2012) |
| Indonesia | *Trigona* sp propolis | – | 7.44–8.76 | 38.67–61.64 | 2.02–3.05 | 25.8–64.91 | 1.31–3.42 | 45.02–58.72 | Mulyati et al. (2021) |
| Brazil | – | – | 7.46 | – | – | – | 2.04 | – | Kunrath et al., 2017 |
| Pakistan | – | – | 2.22 | 85.59 | 1.84 | – | 1.03 | 0.31 | Shahbaz et al. (2021) |
| Brunei Darussalam | *Heterotrigona itama* propolis | – | – | 45.60 | 0.18 | 0.43 | – | 0.3 | Abdullah et al. (2019) |
| Korea | – | – | 3.6–3.9 | 81.1–86.9 | 2–2.5 | 2.5–7.4 | 1.1–1.5 | 3.5–4.0 | Song & Gil, 2002 |
| Morocco | – | 59.01 | 1.01 | – | – | – | 4.87 | – | Touzani et al. (2019) |
| Morocco | – | 46-81-75.19 | 3.30–5.16 | – | 1.65–6.18 | – | 1.55–2.29 | – | Laaroussi et al. (2021) |
| Western Romania | *Geniotrigona thoracica* Propolis | – | – | 20.21–37.73 | – | – | – | | Duca, Sturza, et al. (2019) |
| Greece | | – | – | 1.1–7.1 | – | 6–21.1 | – | – | Popova et al. (2010) |

**Table 3**
List of various nutritional compounds investigated in propolis based on different geographical origins.

| Origins | Types and source | Category | Techniques | Identified compounds | Reference |
|---|---|---|---|---|---|
| Northern India | Brown propolis collected by *Apis mellifera* | Fatty acid | GC-FID | Butyric acid, Caproic acid, Caprylic acid, Capric acid, Undecanoic acid, Lauric acid, Tridecanoic acid, Myristic acid, *cis*-10-Pentadecanoic acid, Myristioleic acid, Pentadecanoic acid, −10-Pentadecanoic acid, Palmitic acid, Palmitoleic acid, Heptadecanoic acid, *cis*-10-Heptadecenoic acid, Stearic acid, Elaidic acid, Oleic acid, Linoelaidic acid, Linoleic acid, α-Linolenic acid, γ-Linolenic acid, Arachidic acid, *cis*-11-Eicosenoic acid, *cis*-11–14-Eicosadienoic acid, *cis*-8,11,14-Eicosatrienoicacid, Heneicosenoic acid, Arachidonic acid, Eicosapentaenoic acid, *cis*-11,14,17- Eicosatrienoic acid, Behenic acid, Erucic acid, Eicosapentaenoic acid, *cis*-13,16- Docosahexaenoic acid, Lignoceric acid, Tricosanoic acid, Nervonic acid, Docosahexaenoic acid | Pant, Thakur, Chopra, Dar, and Nanda (2022) |
| Northern India | Brown propolis collected by *Apis mellifera* | Amino acid | HPLC | Histidine, Threonine, Lysine, Methionine, Valine, Isoleucine, Leucine, Phenylalanine, Arginine, Serine, Glycine, Aspartic acid, Glutamic acid, Alanine, Proline, Cystine, Tyrosine | Pant et al. (2022) |
| Northern India | Brown propolis collected by *Apis mellifera* | Mineral | MPAES | Calcium, Potassium, Magnesium, Phosphorus, Sodium, Iron, Manganese, Zinc, Copper, Aluminium | Pant et al. (2022) |
| Indonesia | *Trigona* sp propolis | Minerals | ICP-OES | Sodium, Potassium, Phosphorus, Copper, Zinc, Iron, Manganese, Calcium Magnesium | Mulyati et al. (2021) |
| Jordan | Topical propolis | Fatty acid | GC-FID | Butyric acid, Caproic acid, Caprylic acid, Capric acid, Undecanoic acid, Lauric acid, Myristic acid, Pentadecanoic acid, Palmitic acid, Stearic acid, Arachidic acid Lignoceric acid, Palmitoleic acid, cis-Oleic acid, *cis*-11-Eicosenoic acid, Lenoleic acid, α-Linolenic acid, *cis*-11,14-Eicosadienoic acid, *cis*-13,16-Docosadienoic acid | Nail et al., 2021 |
| Iran | – | Fatty acid | GC-MS | Octadecane, Pentacosane, Heptadecane, Myristic acid, Palmitic acid, Oleic acid, Linoleic acid, α-Linolenic acid, Behenic acid, | Fallah et al. (2021) |
| Indonesia | *Trigona* sp propolis | Vitamin | HPLC | Nicotinamide, Niacin, Pyridoxine, Folic acid | Mulyati et al. (2021) |
| Western Romania | *Geniotrigona thoracica* Propolis | Fatty acid | GC-MS | Lauric acid, Myristic acid, Palmitic acid, 14-Methylpalmitic acid, Stearic acid, Octadecanoic acid, −17-methyl, Cyclopropanepentanoic acid, 2-undecyl, Arachidic acid, Heneicosylic acid, Behenic acid, Lignoceric acid, Cerotic acid, Palmitelaidic acid, Hypogeic acid, Oleic acid, α-Linolenic acid | Duca et al., 2019 |
| Algerian | – | Fatty acid | GC-FID | Butyric acid, Capronic acid, Caprylic acid, Caprinic acid, Undecanoic acid Lauric Acid, Tridecanoic Acid, Myristic Acid, Myristoleic Acid, Pentadecanoic Acid, Ginkgolic acid, Palmitic acid, Palmitoleic acid, Heptadecanoic Acid, *cis*-10-heptadecenoic acid, Stearic acid, Oleic acid, Elaidic Acid, Linoleic acid, γ-Linolenic acid, α-Linolenic acid, Arachidic acid, *cis*-11-Eicosenoic acid, *cis*-11,14-Eicosadienoic acid, *cis*-11,14,17-Eicosatrienoic acid, Heneicosylic acid, *cis*-8,11,14-Eicosatrienoic acid, Arachidonic acid, *cis*-5,8,11,14,17-Eicosapentaenoic acid, Behenic acid Erucic acid, *cis*-13,16-Docosadienoic acid | Rebiai et al. (2017) |
| Turkey | *Caucasican* propolis | Amino acid | UFLC UV/VIS | Aspartic acid, Glutamic acid, Serine, Glycine, Histidine, Arginine, Threonine, Alanine, Proline, Tyrosine, Valine, Methionine, Isoleucine, Leucine, Phenylalanine, Lysine, Tryptophan | Eroglu, Akkus, Yaman, Asci, and Silici (2016) |
| Turkey | *Caucasican* propolis | Vitamin | HPLC-FD | Thiamine, Riboflavin | Eroglu et al. (2016) |
| South Spain | Topical type | Minerals | ICP-AES | Silver, Aluminum, Arsenic, Boron, Cadmium, Calcium, Cobalt, Chromium, Copper, Iron, Potassium, Magnesium, Manganese, Sodium, Nickle, Phosphorus, Lead, Sulphur, Antimony, Selenium, Silicon, Tin, Zinc, Mercury | Bonvehi et al., 2013 |

includes water, alcohol, polyethylene glycol etc. Extraction in alcoholic solution yields 10-fold higher phenolic content and bioactivity followed by polyethylene glycol and aqueous extraction (Pobiega et al., 2019). Several studies demonstrated the potency of hydro-alcoholic propolis extracts (in different concentrations) to retard fungal growth by topical application on various fruits like oranges, mandarin, grapes, apples etc. (Yang, Wu, Huang, & Miao, 2017) Fruits and vegetables can also be sanitized with propolis extract instead of using sodium hypochlorite. Likewise, a study conducted by Feas et al. (2014) to investigate the sanitization effect of propolis with sodium hypochlorite, where 2% concentration of propolis extract has been sprayed over the lettuce. The observations were similar to the effect of sodium hypochlorite over lettuce, where propolis 2% extract showed significant effect over the inhibit microbial growth. In another study from China, ethanol extract of propolis when coated on sweet cherry at a rate of 0.5–2% reduced the respiration rate and weight loss rate (Yang et al., 2020).

In addition, coating based on polymers incorporated with propolis extract can be used to coat different food items. These multiple ways of adding propolis to the food product diminish the possibility of microbial spoilage. Furthermore, combined effect of propolis extract with thermal treatment or ultrasound has been studied by Alvarez, Ponce, Goyeneche, and Moreira (2017) on the freshly-chopped mixed vegetables for soup to determine impact on physicochemical, nutritional, microbiological, and sensory attributes. The researcher observed that use of propolis significantly inhibited the activity of enzyme associated with enzymatic browning, retard microbial proliferation and enhance quality characteristics throughout refrigeration storage period. In terms of food preservation, synergistic effect of ascorbic acid combined with propolis extract has been observed on the antioxidant activity in orange soft drinks (Vasilaki, Hatzikamari, Stagkos-Georgiadis, Goula, & Mourtzinos, 2019). In a study conducted by Mutton, Oliveira, Costa, Roviero, & Freita (2014) impact of ethanolic extract of brown and green propolis when incorporated in alcoholic fermented beverage resulted in the inhibition of bacterial contamination. Likewise, Ulloa et al. (2017) fused propolis extract in 0.25 g/L of concentration in beer and observed momentous reduction in oxidation stress through brewing process as well as upsurge the phenolic fraction of the product. In another research conducted in Turkey, demonstrated the level of patulin significantly decreased in apple juice when fused with ethanolic extract of propolis at a concentration of 0.1,1, 2 mg/ml (Silici & Karaman, 2014). Luis- Villarova et al. (2015) concluded that apple juice exhibiting a lower shelf life deteriorate at a faster rate for that reason apple juice in combination with poplar propolis extract at a concentration of 0.2 mg/ml minimized the growth of listeria monocytogenes. Moreover, from the above studies it could be concluded that same food product in varied concentrations of propolis have different effect on preservative action of apple juice. Luis-Villarova et al. (2015) found positive impact of propolis extract on the reduction of thermal processing time of heat sensitive food commodities.

**Table 4**
Application and action of propolis as a natural preservative in various food formulations.

| Origin | Propolis | Food product | Use | Concentration | Benefits | Reference |
|---|---|---|---|---|---|---|
| Brazil | Green propolis | – | Nano-emulsion | 1% propolis extract | Anti- bacterial activity against Gram positive bacteria (*Staphylococcus aureus, S. saprophyticus, Listeria monocytogenes, Enterococcus faecalis*) Anti- oxidant activity | Seibert et al. (2019) |
| China | Poplar propolis | Orange juice | Emulsion | 0.02 g/ml | Antioxidant capacity, Protected lightness, Good alternative to synthetic preservative | Yang et al. (2017) |
| Tuscany, Italy | Italian propolis | Milk and whey cheese | Ethanolic propolis extract | 2 & 5% | Inhibit the bacterial growth in milk and whey cheese | Pedonese et al. (2019) |
| Iran | Propolis | Chicken kebab | Propolis extract | 0–12% | Increased shelf life, Control the increase in pH, | Mahdavi-Roshan, Gheibi, and Pourfarzad (2022) |
| Brazil | Red propolis | Tilapia Salami | Propolis extract | 0.2–0.6% | Protective against chemical and microbial deterioration | Mafra et al. (2022) |
| Greece | Green propolis | Non- carbonated orange soft drink | Propolis green extract | 0.3 & 1.3% | Antioxidant activity & Inhibit the growth of bacteria, yeast & molds, | Vasilaki et al. (2019) |
| Braganca, Portugal | Propolis | Lettuce | Propolis extract | 2% | As an alternative to sodium hypochlorite to inhibit microbial growth | Feás, Pacheco, Iglesias, and Estevinho (2014) |
| Spain | Poplar propolis | Apple juice | Propolis dietary supplement | 0.2 mg/ml | Effective against *Listeria monocytogenes* | Luis-Villaroya et al. (2015) |
| Giza, Egypt | Propolis | Milk and Yoghurt | Propolis water extract | 1–2% | Improves the quality and microbial safety | El-Deeb (2017) |
| Portugal | Propolis | Meat sausage | Ethanolic extract | 0.15 mg/ml | Inhibited *Listeria innocua* | Casquete, Castro, Jácome, and Teixeira (2016) |
| Brazil | Propolis | Italian type Salami | Ethanol extract | 0.01 & 0.05% | Inhibition of oxidative action | Kunrath et al. (2017) |
| Egypt | Propolis | Egyptian sausage | Ethanol extract | 400 &600 mg | Control the lipid oxidation and microbial effects | EL-MOSSALAMI et al.,2013 |
| Mexico | Commercial and non-commercial propolis | Beef patties | Ethanol extract | 2% | Act as natural antioxidant and inhibit microbial activity | Vargas-Sanchez et al., 2014 |
| Egypt | Propolis | Ice cream | Soxhelet extracted propolis extract | 600 mg/L | Active against *Enterotoxigenic Methicillin* resistant *Staphylococcus aureus* | El-Bassiony, Saad,& El-Zamkan, 2012 |
| Spain | Propolis | Honey | Ethanol extract | 0.1–0.5% | Improves sensory attributes and bioactive properties of honey | Osés, Pascual-Maté, Fernández-Muiño, López-Díaz, & Sancho, 2016 |
| Turkey | Kayseri Propolis | Apple juice | Ethanol extract | 0.1,1,2 mg/ml | Results in the reduction of patulin concentration in apple juice | Silici et al., 2007 |
| Cyprus | Propolis | Pomegranate | Ethanol extract | 1drop/250 ml | Increase the shelf life upto 37 days | Kahramanoglu & Usanmaz (2017) |
| Brazil | Brown and green propolis | Alcoholic fermented beverage | Ethanol extract | 700µl//L | Inhibit bacterial contamination | Mutton, Oliveira Filho, Costa, Roviero, & Freita,2014 |
| China | Propolis | Sweet cherry | Ethanol extract | 0.5–2% | Reduces the respiration rate, reduce weight loss rate | Yang, Gong, Gao, Huang, Xu, & Xiong (2020) |
| Egypt | Propolis | Milk powder | Water extracted propolis nano emulsion, Propolis powder | – | Inhibitory action against Aflatoxin from *Aspergillus flavus* | Hassanien et al. (2021) |
| Korea | Propolis | ZnO nanoparticles -Chitosan-based film for meat packaging | Alcoholic extract | 0.2g | Improved antioxidant activity, Reduced aerobic microorganisms | Roy et al., 2021 |
| Brazil | Commercial propolis | Starch biscuits | Lyophilized propolis, Microencapsulated propolis | 750 mg/kg, 500 mg/kg | Inhibit lipid oxidation | Rodrigues et al. (2021) |

Further, the study emphasized the effect of propoylene glycol extracted propolis in apple juice, reduced the thermal processing time and temperature for the inactivation of 5 log cfu/ml of E.Coli by 75%. Pedonese et al. (2019) used Italian propolis ethanolic extract up to 2–5% in milk and whey cheese which retarded bacteria growth in products. Whereas, green propolis extract at concentration of 1% incorporated in nano-emulsion proved to be fatal against gram positive bacteria (such as *Staphylococcus aureus, S. saprophyticus, Listeria monocytogenes, Enterococcus faecalis*) and also possessed good anti-oxidant activity (Seibert et al., 2019). In a similar study, Egyptian propolis nano-emulsion used as a preservative in milk powder which showed inhibitory action against *Aflatoxin* from *Aspergillus flavus* (Hassanien, Shaker, El-Sharkawy, & Elsherif, 2021). El-Bassiony, Saad, and El-Zamkan (2012) studied the effectiveness of propolis extract (600 mg/L) as an antimicrobial agent in an ice cream and found that propolis supplemented ice cream was active against Enterotoxigenic Methicillin resistant *Staphylococcus aureus* and preserved ice cream from gram positive pathogenic bacteria. Apart being antimicrobial agent, propolis also studied as a powerful antioxidant agent in various food products. For example, Cottica et al. (2015) found decent antioxidant capacity of propolis extract in dairy beverages with the combination of conjugated linoleic acid. Recently, Santos, Estevinho, Carvalho, Conceicao, & Almeida (2020) added red propolis extract to commercial yoghurt as a natural preservative to substitute the potassium sorbate where they observed color alteration of the yoghurt with improved antioxidant activity. Consequently, it can conclude that after addition of propolis extract improved the retention of maximum nutrients throughout processing and storage. Additionally, it also enhances antioxidant capacity as well as confines the development of

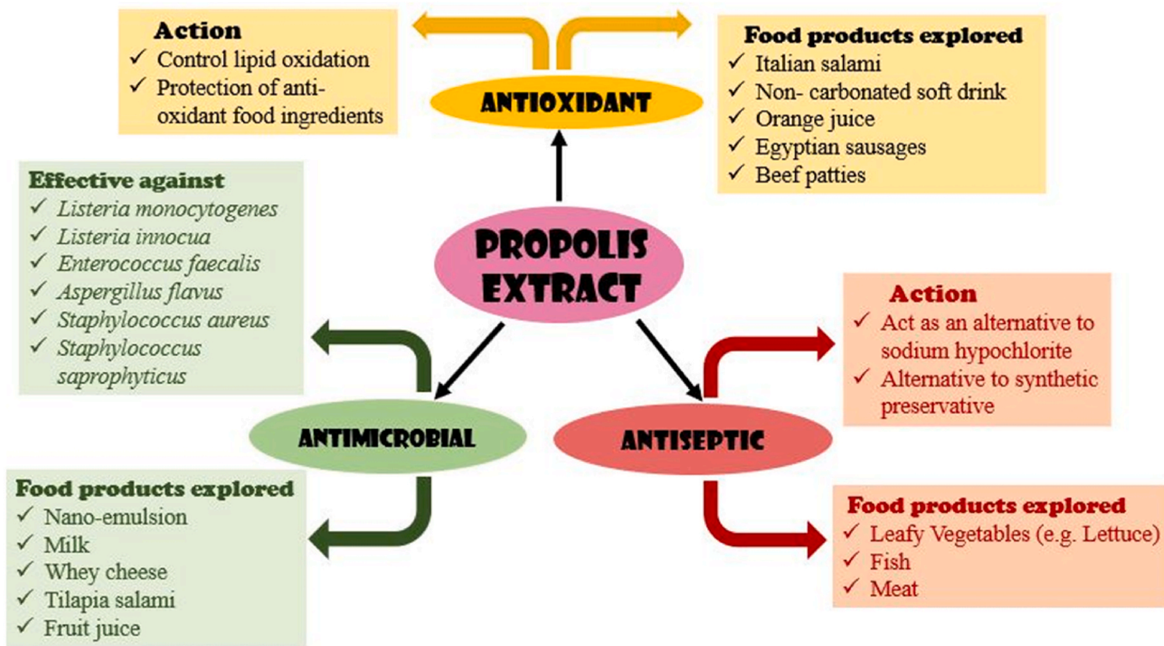


**Fig. 3.** Applications of propolis as a natural preservative in food systems.

aldehydes moieties which are responsible for off-flavouring a foul smell in dairy products. In the preservation of food, another factor of lipid oxidation is a matter of concern which impacts the early degradation of food products. In this regard, Rodrigues et al. (2021) studied the effect of propolis in inhibiting the lipid oxidation of biscuits. For this, lyophilized and microencapsulated propolis powder was added to biscuits in a concentration of 500 and 750 mg/kg and found that propolis had a positive effect in inhibiting the lipid oxidation of starch biscuits. Lipid oxidation in Egyptian meat sausage was controlled with the addition of 400 and 600 mg of ethanolic propolis extract (Ha EL-Mossalami, Hanaa, & Abdel-Hakeim, 2013). Chicken kebab up to 12% of propolis extract controlled the increase in pH, and improved storage stability (Mahdavi-Roshan, Gheibi, & Pourfarzad, 2022). Propolis extract also found its application in packaging films for the preservation and extended shelf life of the food products. Roy, Priyadarshi, and Rhim (2021) developed ZnO nanoparticles chitosan-based film integrated with 0.2 g alcoholic extract for the packaging of meat and concluded that packaging film with propolis as one of the ingredients helped in retaining antioxidant activity and reduced aerobic microorganisms.

Propolis, being a bee product, varies in its chemical composition from other bee products like honey, bee pollen, royal jelly, etc. Therefore, propolis together with honey and other bee products, can have a tremendous effect on its bioactive properties. One such study was carried out with honey, in which propolis extract being a rich source of bioactive compounds was added to honey in 0.1–0.5%. On addition of propolis to honey, improved sensory attributes along with a significant rise in its bioactive properties (Oses, Pascual-Mate, Fernandez-Muino, Lopez-Diaz, & Sancho, 2016). Therefore, it can be concluded that with the technological upright in the current era people turned over to healthier and safer foods. Propolis as natural preservatives are such components which can replace synthetic preservative available in the market.

## 5. Propolis dosage and safety considerations

Over the last two decades, propolis has been established as a dietary supplement, functional food, and natural preservative in the global food and nutraceutical Industry. Numerous clinical investigations have reported the safety of propolis both in mice and humans unless administered in high concentrations (Bazmandegan et al., 2017; Cao et al., 2015; Cornara, Biagi, Xiao, & Burlando, 2017). Moreover, it should be considered that the antagonistic effects and toxicity of propolis in human trials were found to be negligible, further supporting its overall safety profile. Although health supplements derived from natural sources, like propolis, are often regarded as "mere" herbal ingredients, consumers often overlook the possibility of adverse effects. One potential concern is the occurrence of allergic reactions to propolis, but it is important to note that documented cases of oral sensitization to propolis are infrequent. (Berretta et al., 2017).

On the other hand, allergic reactions resulting from local administration of propolis are significantly more common. Several investigations have been conducted to determine the safe dosages of propolis for human consumption. For instance, Burdock (1998) proposed a safe dosage of 1.4 mg/kg body mass per day, equivalent to approximately 70 mg/day for humans. Subsequently, Brätter, Tregel, Liebenthal, and Volk (1999) conducted a study where they administered 500 mg of propolis orally to healthy volunteers for 13 days, with a specific focus on evaluating the immune response (IL-6, IL-8, and TNF-α). They observed an enhanced ability in cytokine secretion; however, there were no significant changes in plasmatic levels. Similarly, Jasprica et al. (2007) investigated the antioxidant potential of propolis in the human diet, wherein the best outcomes were achieved using dosages around 500 mg/day successfully. In a study conducted by Cohen, Varsano, Kahan, Sarrell, and Uziel (2004), 430 children aged 1–5 years old participated. The treated group ($n = 215$) received the combination of echinacea (50 mg/ml), propolis (50 mg/ml), and vitamin C (10 mg/ml) orally for a duration of 12 weeks. In this study, children aged 1–3 years old were administrated a dose of 5.0 ml twice daily, while children aged 4–5 years received a dose of 7.5 ml. The findings revealed a significant reduction in the incidence and severity of respiratory tract infections, with a 55% reduction in the number of sick children and a 50% decrease in respiratory-related diseases.

de Barros, Sousa, Bastos, and de Andrade (2007) reported that the recommended safe dose of Brazilian propolis LD50 for humans is 17,073 mg/day. Applying a safety factor of 10, recommended by Food Drug Administration guidelines (United States FDA, 1994), a daily dose of 1700 mg or 1.7 g of propolis is considered a safe dosage for adult consumption. In the European Union (EU), propolis is classified as a food supplement, subject to regulation under Directive 2002/46/EC (European Parliament and Council of the European Union, 2002). European Food Safety Authority also documented some safer and recommended oral dosages of propolis in different formulations and purposes, such as (i) Consumption of 0.7–1.3 g per day, staying within the safe limits of consumption, which is up to 2 g of propolis/kg/day (Not for people who are allergic to the propolis), (ii) Minimum dose of propolis extract is at least 250 mg/day for children while for adults is 750 mg/day, (iii) The recommended daily dose in food supplement is 24–72 mg of propolis (iv) For an immune-boosting effect, subjects may be administered with 500 mg of propolis extract once to three times a day, as prescribed for the designated period. Furthermore, EFSA has also prohibited the consumption of propolis for pregnant and lactating women as well as children younger than three years old. (ESFA, 2010).

Based on the combined results of the previously mentioned studies, it is reasonable to propose that propolis dosages ranging approximately from 260 mg to 2 g, which have already been employed in human trials, can be considered safe however, the observed biological outcomes displayed significant variation. Based on the available evidence, it is reasonable to conclude that dosages within this range are likely safe, as none of the investigations indicated any harm or complications for the participants (Berretta et al., 2017). The findings indicate that the prophylactic use of propolis depends on the reactivity of the immune system and the duration of administration, with no adverse effects observed.

## 6. Conclusion

Nevertheless, in some instances, vegetation sources of propolis are just summarized by tracing bees foraging patterns. Still, no authenticated data is available based on comparing chemical profiling of marker plants and propolis. Therefore, further study is needed to compare chemical compounds in propolis and plants to confirm the exact botanical origin. However, seasonality, different bee species, their food supplementation, and apicultural practices may affect propolis's bioactive composition, which must be accounted for in further studies. The information regarding the chemical and nutritional composition of any food materials is crucial to optimize product formulation. So, there is also a strong need to conduct a systemic investigation and comprehensive study on propolis's gross or nutritional composition globally. Moreover, the application of propolis is widely explored in the pharmaceutical and skin care industries. Nonetheless, the present review extensively focused on its functionalities as a natural preservative as an edible coating, anti-microbial, and anti-oxidant agent in varieties of processed food commodities such as cheese, yoghurt, fruit juices, alcoholic beverages, bakery items, milk powder, etc. Henceforth, this may open doorways to expand the commercialization of propolis in developing countries, which may serve as a boon for beekeepers and the apicultural sector worldwide.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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