Review Article

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# Role and the importance of green approach in biosynthesis of nanopropolis and effectiveness of propolis in the treatment of COVID-19 pandemic

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**Abstract:** The most fascinating product of honeybee is propolis. It has an immense role in dentistry, dermatology, and otorhinolaryngology. The increased popularity of propolis as an important remedy is due to its constituents, which have anti-inflammatory, immunomodulatory, antihepatotoxic, anti-cancerous, antifungal, antioxidant, antidiabetic, and antiviral activities. The diverse biological and pharmacological activities of propolis have piqued the interest of many scientists. Many techniques like gas chromatography-mass spectrometry, chromatography, and spectroscopy are being used to identify different propolis constituents. Flavonoids, phenolic acids, and their esters are the most pharmacologically active molecules of propolis and are known to disrupt the replication machinery of the virus corroborating the anti-coronavirus activity of propolis. The main aim of this article is to provide an insight of the increasing theragnostic uses of propolis and its nanoparticles, including their chemical analysis, diverse biological activities, and the necessity for chemical standardization. In this review, we have focused at the promising effects of propolis, its optimization, and its liposomal formulation as a therapeutic intervention for COVID-19 and its accompanying comorbidities.

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## 1 Introduction

Bees evolved from *Apoidea* approximately 140 million years ago. In earlier times, the role of bee products was considered essential only for the survival and development of the bees. With time, the role of bee products in medicines, various technologies, and in cosmetics got popularized. The major bee products are honey, bee wax, royal jelly, and propolis. Term apitherapy is very popular nowadays and is used to treat illness, pain from acute and chronic injuries by using honey bee products [1–3]. Illness like multiple sclerosis and rheumatoid arthritis can be treated with apitherapy [4]. One of the most innovative and rapidly evolving field is nanotechnology, which has the potential to increase the efficacy of the bee products. Synthesis of nanoparticles involves two main approaches, i.e. top-down approach (chemical method and physical method) and bottom-up approach (biological method) as given in Figure 1. Plants, bacteria, algae, and fungi are the main agents which are employed in the biological method for nanoparticles synthesis. Biogenetic synthesis of the nanoparticles is now gaining popularity leading toward the green chemistry approach [5]. In this article, the role of propolis and its nanoparticles, composition, analytical methods, possible research areas, and its chemical standardization is discussed.

### 1.1 What is propolis?

The Greek word propolis means pro, for or defense, and polis, the city, i.e., “defense of the hive” [3,6–9].

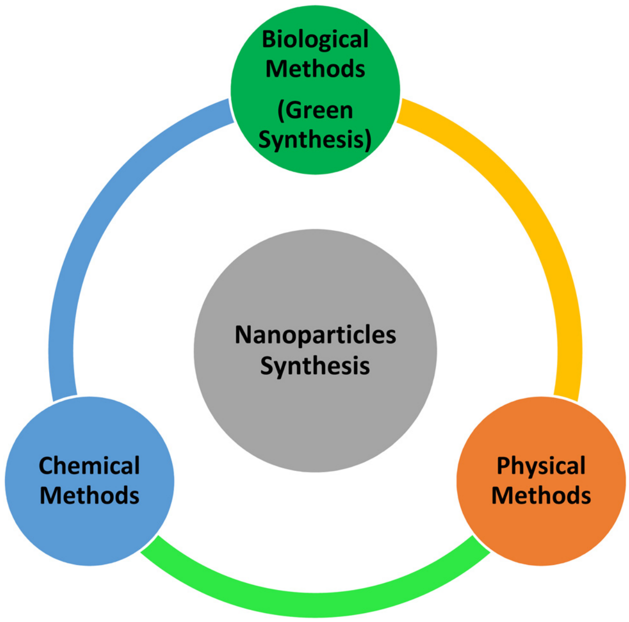


**Figure 1:** Methods for the synthesis of nanoparticles.

The word propolis was coined by Aristotle [10]. It is also referred as "Bee Glue" [7]. Propolis is a resinous mixture and it is produced from honeybees by mixing saliva and beeswax with exudate collected from the tree buds [11]. Propolis is lipophilic in nature [12]. Propolis is collected by the worker bees and then carried back to their colony packed on their hind leg [13]. The United States Department of Agriculture's United States Standards for Grades of Extracted Honey, effective May 23, 1985 describes propolis as "Propolis means a gum that is gathered by bees from various plants. It may vary in color from light yellow to dark brown. It may cause staining of the comb or frame and may be found in extracted honey" [7]. Variation of colour is due to different plant sources [9]. The melting point of propolis is 25–45°C. Propolis is a soft, pliable, and sticky substance at room temperature. Above 45°C, it becomes sticky and gummy, and from 60°C to 70°C, it becomes liquid. In frozen conditions, it becomes hard and brittle [12,13]. The viscosity of propolis decreases upon heating to about 40°C, and hardening is observed below 10°C [6,8]. Propolis is used by the bees to seal the holes in their honeycombs, and also to cover carcasses of intruders who die inside the hive so that the decomposition of dead organisms does not harm or lead to any bacterial or other infections in their hives [6,11,14]. Against pathogenic microorganisms propolis also acts as a chemical weapon of bees [10]. Antiseptic and antimicrobial properties of propolis help bees to protect the colony from diseases [2,3,6,7,15]. Bees also use propolis to reduce air flow into the hive to retain heat [16]. *Apis dorsata* (giant honey bee) uses propolis to reinforce the hive's adhesion, although *Apis cerana* does not use it at all. Maximum use of propolis is mostly made by *Apis mellifera* [17]. Activity of propolis gets affected by the methods of extraction. Solid–liquid extraction is the most common method in which different concentrations of ethanol, methanol, or water are used [18]. It is necessary to purify propolis by using extraction with solvents as it is not possible to commercialize them as raw materials [19]. The most common solvent is absolute ethanol, which is used to prepare propolis extracts; also extraction with aqueous ethanol (70–95%) can result in wax-free tinctures containing higher amounts of phenolic substances [20,21]. Some other solvents which are used for extraction are water, methanol, chloroform, dichloromethane, acetone, and ether [12,18]. About 70% of ethanol extract of propolis is also called propolis balsam, which includes phenolic acids (caffeic acid and ferulic acid), flavonols (galangin, kaempferol, and quercetin), flavones (chrysin, apigenin, luteolin, and tectochrysin), and flavanones (pinocembrin) [22].

According to Przybyłek and Karpiński [14], propolis is composed of flavonoid group (chrysin, pinocembrin, apigenin, galangin, kaempferol, quercetin, tectochrysin, and pinostrobin), aromatic acids (ferulic acid, cinnamic acid, caffeic acid, benzoic acid, salicylic acid, and $\rho$-cumaric acids), terpenes (terpineol, camphor, geraniol, nerol, and farnesol), and some micro- and macroelements (Mn, Fe, Si, Mg, Zn, Se, Ca, K, Na,) along with vitamins (B1, B2, B6, C, E). Propolis, which is mostly made up of plant secretions, is a good source of cinnamic acid and esters [14]. Raw propolis is typically composed of 50% plant resins, 30% waxes, 10% essential and aromatic oils, 5% pollens, and 5% other organic substances [10,23,24]. In six Indian propolis samples observed by authors of ref. [25], 93 compounds were studied out of which 14 were new for propolis. Indian propolis samples were characterized by the presence of significant amount of carboxylic acid (20.4%), terpenoids (15.0%), steroids (11.5%), hydrocarbons (9.6%), sugars (6.4%), alkaloids (6.4%), flavonoids (4.3%), phenols (3.2%), ketones (2.1%), amino acid (2.1%), vitamins (2.1%), and other compounds (15.0%) [25] (Figure 2). Variations in chemical compositions of propolis are observed due to different geographical regions. Chemical composition of propolis for Africa and Europe was given by Bankova et al. [26] as shown in Figure 2.

## 1.2 Botanical sources

Quality and composition of propolis depend on the source plants or the flora at the site of collection [1,8,27]. Bees use

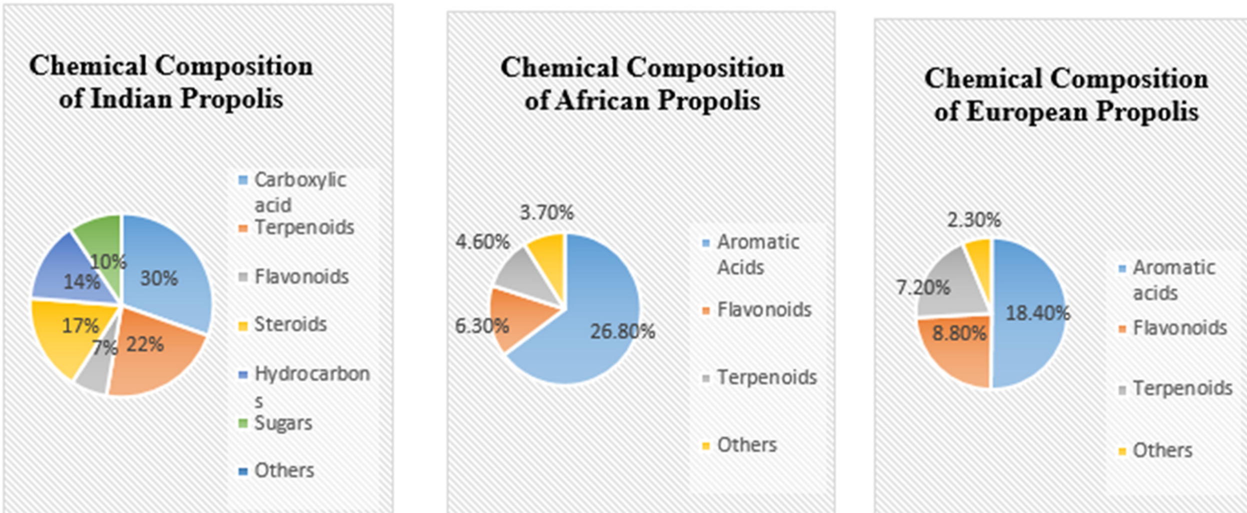


**Figure 2:** Chemical composition of propolis from different geographical regions.

variety of plants as a source of propolis, which includes poplars, resins of conifers, birch, alder, willow, palm, *Dalbergia ecastaphyllum*, etc. [27].

## 1.3 Types of propolis

Catchpole et al. [16] stated that there are around seven types of propolis. Mountford-McAuley et al. [15] and Catchpole et al. [16] categorized propolis on the basis of their geographical distribution and on the basis of plant from which resin is collected (Table 1).

## 1.4 Factors affecting the propolis production

There are different factors which affect the propolis production, viz. botanical sources, genetics of honey bee, structure of hive, seasons, food availability, environmental factors, diseases, and colony strength. Both the quantity as well as the quality of propolis get affected by these factors [11,15,28]. Variation in composition of European propolis was reported by Bankova et al. [26] as shown in Figure 3. It can be inferred from Figure 3 that in spring season maximum amount of aromatic acids was present in European propolis and terpenoids were completely absent in the propolis samples.

## 1.5 Harvesting of propolis

Collection of propolis samples can be done by direct scraping of the substance from the wooden hive parts [13]. At the end of every season beekeeper cleans up the boxes properly so that they can be used in next season also. Therefore, at the end of the season scraping propolis from the frame rest, edges, inside the boxes produces very good quantity of propolis and it can be collected in multiple seasons [11]. It has been concluded from different papers that nowadays propolis traps (thick plastic sheets having 1.6 mm grooved slits over the entire surface) or mats with grid slots are placed into the hives by beekeepers for collecting propolis to be used in commercial scale [16]. The extraction process has a direct impact on the yield and selectivity of particular compounds. It has been reported that the properties of same propolis from different extracts can vary [29]. The different methods used for the extraction of propolis were cold extraction method, supercritical fluid extraction method, Soxhlet extraction, and sonication extraction. Cold extraction method was most commonly used for the preparation of propolis extract to be used in the chemical analysis of propolis. Collected propolis samples were placed in a freezer at low temperature (from −20°C to 4°C). Using grinding device, frozen propolis was powdered to get a size of 10–80 μm, and further using different solvents (chloroform, methanol, ethanol, water, olive oil, and propylene glycol) successive solvent extracts were prepared. For preparing ethanol extract, 70% ethanol (1:30 w:v) was added and placed for 5–7 days in dark and shaken only once in a day. Further drying was done after filtering it using Whatman's filter paper. The obtained extract was ready to use in further experiments. The same method was followed for various solvents. Weigh the dried extract to determine the extract yield [18]. For the preparation of propolis extract the most commonly used solvent is

**Table 1:** Different types of propolis

| S. no. | Type of propolis | Botanical source | Main bioactive compound | Geographical origin | References |
|---|---|---|---|---|---|
| 1 | Poplar | *Populus* spp. (poplar)<br>*Populus nigra*<br>*Populus italica*<br>*Populus tremula*<br>*Populus alba* | Polyphenols | Europe, China, America, New Zealand, Albania, and non-tropic regions of Asia | [1,10,12,30,31] |
| 2 | Red propolis | *D. ecastaphyllum* (coin vine) | Isoflavonoids and pterocarpans | Cuba, Mexico, and Northern Brazil | [30] |
| 3 | Brazilian green | *Baccharis dracunculifolia* (*alecrim*), *Araucaria angustifolia, Araucaria heterophylla, Clusia minor*<br>*Eucalyptus citriodora*<br>*D. ecastophyllum*<br>*Hyptis divaricata* | Prenylated *p*-coumaric acids, diterpenic acids, and flavanoids | Brazil | [1,12,14,15,32–35] |
| 4 | Birch | *Betula* spp. (birch) | Polyphenols | Russia | [12] |
| 5 | Mediterranean | Conifers (e.g., pine) | Flavonoids, terpenes | Greece, Sicily, Crete, and Malta | [12] |
| 6 | Clusia/Cuban | *Clusia* spp.<br>*Clusia rosea*<br>*Clusia minor* | Polyprenylated benzophenones | Cuba and Venezuela | [1,7–9,12,15] |
| 7 | Pacific | | *c*-Prenylflavanones furofuran lignans | Okinawa, Taiwan, Indonesia, and parts of Japan | [12] |
| 8 | Taiwanese propolis | | Prenylflavones | Taiwan | [1,36] |
| 9 | Netherland propolis | — | CAPE | Netherland | [1] |

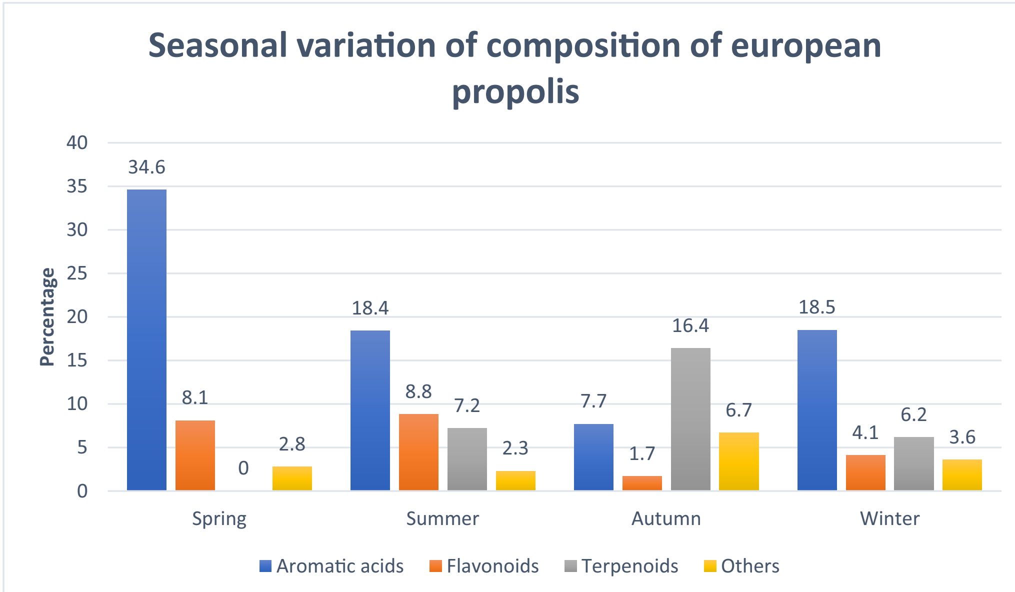


**Figure 3:** Seasonal variation of composition of European propolis.

ethanol but nowadays many cases have been reported of allergy to ethanol, which limits its use in extract preparation [24]. European (poplar type) propolis has an allergenic property due to the presence of 3,3-dimethylallyl caffeate [1,6]. Application of silicone barrier lotions before exposure to propolis is advised in cases of propolis allergies [6]. Propolis may inflict some immunological responses in few people. So further investigation is required before implementing their use in medical sciences.

# 2 Biological aspects of propolis

Propolis can be used extensively in pharmaceutical sciences due to its immense potential nutraceutical value and biological properties. There is a need for chemical standardization of propolis before considering its use in medical sciences as its constituents vary according to the geographical region and availability of botanical sources. Important constituents of propolis have increased the chances of its use against COVID-19, in nanomedicines, and in apitherapy. More clinical trials are needed to commercialize the use of propolis in its different forms.

In ancient times, propolis was famous for its general healing qualities, for the cure of some lesions of the skin, to heal sores and ulcers, and recently it is also used in tissue regeneration [6]. Due to the diversity in the chemical composition of propolis, it has been reported to possess various biological activities, namely anti-cancerous, antioxidant, anti-inflammatory, antibiotic, antifungal, antihepatotoxic, and antidiabetic [14,15,37,38].

## 2.1 Nutraceutical value of propolis

Propolis is supposed to have nutraceutical benefits due to its high amount of different amino acids, vitamins, and minerals [39]. There has been very less attempt to examine the nutraceutical potential of Indian propolis as of now [39]. Although propolis has less nutritional value, they have higher antioxidant effects than other bee products such as royal jelly and honey [40,41]. It contains about 1% amino acids, with arginine and proline accounting for nearly half of this quantity [40]. Literature surveyed for the nutritional analysis of propolis revealed that methanolic extract of propolis (MEEP) had $283.33 \pm 51.31\ g \cdot kg^{-1}$ lipids, $30.07 \pm 7.30\ g \cdot kg^{-1}$ fibres, $102.56 \pm 2.84\ g \cdot kg^{-1}$ proteins, and $389.36 \pm 57.50\ g \cdot kg^{-1}$ carbohydrates with a calorie content of $38{,}409.33 \pm 6{,}169.80\ kJ \cdot kg^{-1}$. MEEP had a greater carbohydrate content with $493.6 \pm 54.69\ g \cdot kg^{-1}$. It was likewise high in energy ($40{,}406.14 \pm 4{,}801.12\ kJ \cdot kg^{-1}$) [39]. It was observed that propolis sample was rich in calcium (19.2 ppm), zinc (4.72 ppm), iron (1.3 ppm), manganese (0.09 ppm), boron (0.19 ppm), rubidium (0.08 ppm), strontium (0.01 ppm), molybdenum (0.02 ppm), barium (2.84 ppm), aluminium (3.2 ppm), and lithium (0.03 ppm) [39]. An experiment was carried out to explore the impact of alcoholic extract of propolis on the performance of Ross (308) broiler chicks. A statistical comparison was done for weight gain, feed consumption, feed conversion ratio, and mortality rate. The results showed that average weight increase, feed consumption, and feed efficiency were significantly greater for propolis fed birds in all periods, and the presence of propolis also reduced the mortality rate in comparison to the control diet. Authors discovered that administering propolis extract on a daily basis altered chickens' blood concentrations of cholesterol, total proteins, and amino acids. It also activated the immune system, which resulted in lower mortality when compared to the control [42]. It was reported from different studies that effectiveness of bee propolis on physical fitness and other parameters was assessed in rats after adding propolis to their diet as a supplement. In rats, fed with propolis, the authors noted an increase in haemoglobin regeneration efficiency, calcium and phosphorus absorption, iron utilization, and weight gain [43].

## 3 Chemical composition

Composition of propolis depends upon the geographical region as well as on the methods of extraction [12]. More than 300 complexes have already been identified [44]. To know about the composition of propolis, propolis extracts or samples were analysed by gas-chromatography, gas chromatography-mass spectrometry (GC-MS), and thin layer chromatography (TLC). Total content of flavonoids in propolis was determined by complementary colorimetric aluminium chloride method [18,37,45,46]. Folin–Ciocalteu method and phenolic sulphuric acid method were used for the determination of total polyphenol content and total polysaccharide content, respectively [30,37,39,47–49].

It was reported from the literature surveyed that phenolic compounds were the main components of propolis, which included flavonoids, aromatic acids, and benzopyranes [2]. Propolis extract was composed of cinnimic acids, caffeic acid, terpenes, phenolic acids, amino acids, flavonoids, and phenolic acid esters. Authors in refs [15,23,26,50] stated that the species from which resin was collected can be identified by the chemical composition of propolis. Pinocembrin was present in highest amount among flavonoid group, i.e., 4.7% [14,45]. It was reported that ethanolic extracts of propolis had a greater content of artepillin C (3,5-diprenyl-*p*-coumaric acid, a phenolic compound) [14]. The most effective flavonoid agents noted were galangin, pinocembrin, and pinostrobin (Figure 4) [8].

It was also observed that content of diterpenes was maximum in propolis extract from both Kazan and Marmaris regions of Turkey. Steroid compounds were absent in propolis extract of Marmaris, whereas it was 14.99% in propolis extract from Kazan. In the propolis extract from Marmaris, 20.44% of caffeic acid isomers was also observed [44]. In Indian propolis, maximum concentration of carboxylic acids was observed, i.e. 20.4% [25]. In Bulgarian propolis maximum amount of pinocombrin (23%) was observed among flavonoids, while traces of acetophenone derivatives were also found. In Mongolian propolis maximum amount of 3-methyl-2 butenyl caffeate (~24%) was observed [51]. When chemical analysis of red type Mexican propolis was carried out by Lotti et al. [52], they found three new compounds:

1. 1-(3′,4′-dihydroxy-2′methoxyphenyl)-3-(phenyl) propane,
2. (*Z*)-1-(2′-methoxy-4′,5′-dihydroxyphenyl)-2-(3-phenyl)propene, and
3. 3-hydroxy-5,6-dimethoxyflavan.

In Egyptian propolis, a total of 57 compounds were discovered [53]. In European propolis, among aromatic acids 3,5-diprenyl-*p*-coumaric acid was present in maximum concentration, i.e., 5.8%, and kaempferid was 2.8% among the flavonoids [26]. Chemical constituent of propolis varies according to the different regions (as shown in Table 2) and their varying components are responsible for different biological activities (Table 3).

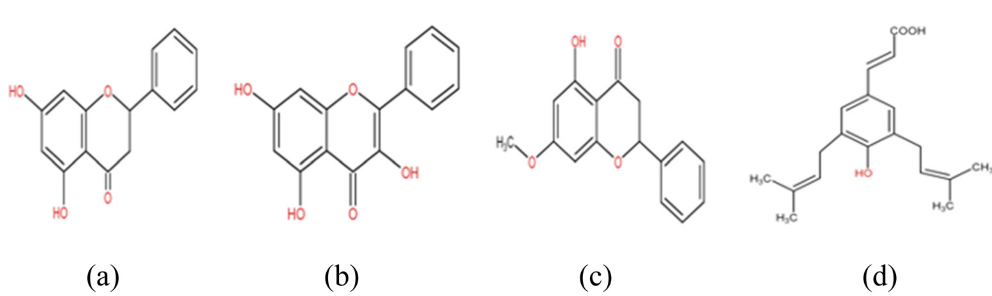


**Figure 4:** Chemical structures of important phenolic and flavonoid agents: (a) pinocembrin, (b) galangin, (c) pinostrobin, and (d) artepillin C.

**Table 2:** Propolis from different nations and their chemical constituents

| Propolis | Chemical constituents | References |
|---|---|---|
| Indian propolis | Carboxylic acids (20.4%)<br>Terpenoids (15%)<br>Steroids (11.5%)<br>Hydrocarbon (9.6%)<br>Sugars (6.4%)<br>Alkaloids (6.4%)<br>Flavonoids (4.3%)<br>Phenols (3.2%)<br>Ketones (2.1%)<br>Amino acids (2.1%)<br>Vitamins (2.1%)<br>Other compounds (15%) | [25] |
| African propolis | Aromatic acids (26.8%)<br>Flavonoids (6.3%)<br>Di and tri-terpenes (4.6%)<br>Others (3.7%) | [26] |
| *Turkey (Kazan) | Aliphatic acids (5.79)<br>Aromatic acids (0.83)<br>Sesquiterpenes (1.72)<br>Diterpenes (34.89)<br>Sugars (3.24)<br>Steroid compounds (14.99)<br>Other compounds (13.64) | [44] |
| *Turkey (Marmaris) | Aliphatic acids (5.78%)<br>Aromatic acids (21.03%)<br>Monoterpenes (0.23%)<br>Sesquiterpenes (0.52%)<br>Diterpenes (33.17%)<br>Sugars (1.14%)<br>Other compounds (0.78%) | [44] |
| *Bulgarian propolis | Flavonoids (~42%)<br>Derivatives of cinnamic acid (~31%)<br>Acetophenones derivatives (traces)<br>Unknown compounds (~15%) | [51] |
| *Mongolian propolis | Flavonoids (~25%)<br>Derivatives of cinnamic acid (~34%)<br>Unknown compounds (~4%) | [51] |
| Mexican propolis (red type) | 1-(3′,4′-Dihydroxy-2′ methoxyphenyl)-3-(phenyl) propane<br>(*Z*)-1-(2′-methoxy-4′,5′-dihydroxyphenyl)-2-(3-phenyl) propene<br>3-Hydroxy-5,6-dimethoxyflavan<br>Flavanones, isoflavans, and pterocarpans<br>Polyprenylated benzophenones | [30,52] |
| Egyptian propolis | Pterin-6-carboxylic acid<br>Cinnamic acid<br>Dodecanoic acid<br>Ascorbyl palmitate<br>Flavanones<br>Cinnamic acid derivatives | [53] |
| Haramaya propolis (Ethiopia) | Benzenamine<br>*N*,*N*-dibutyl (21.94%)<br>Paromomycin (9.74%)<br>4-Aminobutyramide, *N*-methyl-*N*-[4-(1-pyrrolidinyl)-2-butynyl] (9.26%)<br>DL-tryptophan, 5-methoxy (7.43%)<br>Imidazole, 2-fluoro-5-(2-carboxyvinyl) (4.66%)<br>2,7-Dioxatricyclo (4.4.0.0(3, 8) decan-4-amino (6.25%) | [10] |
| European propolis | Aromatic acids (18.4%)<br>Flavonoids (8.8%)<br>Di- and tri-terpenes (7.2%)<br>Phenolic acid esters (CAPE)<br>Others (2.3%) | [26,30,34] |
| Brazilian propolis (green propolis) | Prenylated phenylpropanoids (e.g., artepillin C)<br>Chlorogenic acid<br>Benzoic acids<br>Triterpenoids | [30] |
| Brazilian propolis (red propolis) | Pterocarpans<br>Isoflavonoids<br>Chalcones<br>Prenylated benzophenones<br>Phenyl propanoids | [30] |
| Anatolian propolis | Aromatic alcohols (5.5%)<br>Aromatic acids (3.65%)<br>Flavanones (36.08%)<br>Fatty acids (3.26%)<br>Linear hydrocarbons and their acids (1.34%)<br>Cinnamic acid and its esters (2.7%)<br>Others (24%) | [54] |
| Nigerian propolis | Phlobatannins<br>Glycosides<br>Tannins<br>Anthraquinones<br>Flavonoids<br>Alkaloids<br>Saponins | [43] |

*Percentage of total ion current, GC-MS.

## 4 Chemical standardization of propolis and the problem of standardization

As the composition of propolis varies according to the source plant and local flora, it can create difficulty in its

**Table 3:** Different compounds of propolis and their biological activities

| Compounds | Biological activity | References |
|---|---|---|
| Ferulic acid | Antibacterial<br>Hepatoprotective<br>Antiproliferative | [1,6,8,30] |
| Pinocembrin | Antimicrobial<br>Antibacterial | [6,8] |
| Stilbenes | Fungicidal and fungistatic toward wood rotting fungi | [6] |
| Pterostilbene | Antifungal | [6] |
| Gelangin | Antibacterial<br>Antiproliferative | [6,8,38] |
| Caffeic acid | Antibacterial<br>Anti-tumor<br>Hepatoprotective<br>Antioxidant | [1,6–8,55] |
| Flavonoids | Antibiotic<br>Anti-inflammatory<br>Antiviral<br>Hepatoprotective<br>Antioxidant | [1,6,8,56] |
| Flavonones | Antibacterial<br>Anti-inflammatory | [1,34] |
| Flavones | Antibacterial<br>Anti-inflammatory | [1] |
| Phenolic acids and their esters | Antibacterial<br>Anti-inflammatory<br>Antioxidant | [1] |
| Prenylated *p*-coumaric acid | Antibacterial<br>Anti-tumor<br>Hepatoprotective<br>Antioxidant | [1,34] |
| Labdane diterpenes | Antibacterial | [1,34] |
| Clerodane diterpene | Anti-tumor | [1] |
| Prenylated benzophenones | Antibacterial<br>Anti-tumor<br>Antioxidant | [1] |
| CAPE | Anti-tumor<br>Antioxidant<br>Hepatoprotective<br>Anti-inflammatory<br>Antibacterial<br>Antiviral | [1,6–8,55] |
| Benzofuranes | Anti-tumor | [1] |
| Prenylated flavanones | Anti-tumor<br>Antioxidant | [1] |
| Lignans | Hepatoprotective | [1] |
| Caffeoylquinic acids | Hepatoprotective | [1] |
| Cinnamic acid | Antiproliferative | [38] |

chemical standardization and medical use [8,57]. Chemical standardization of propolis is very necessary in order to get accepted officially in the main streams of healthcare system. The most important active principle component in propolis is caffeic acid phenethyl ester (CAPE), which can be used for the standardization of propolis. But in tropical samples of propolis CAPE is not present. So, we need a universal analytical method for standardization of propolis. We can do this by categorizing the propolis into different types according to their plant source and their corresponding chemical profile [1,38].

### 4.1 Standardization of poplar-type propolis based on the biologically active substances

Identification of poplar-type propolis is necessary. According to the information given for the chemical composition of propolis by Bankova et al. [27], seven phenolic compounds were selected as markers and proceeded with TLC procedure. Three parameters have been used for the characterization of chemical profile of poplar propolis: total flavone, flavonol content, total flavanone and dihydroflavanol content, and total phenolic content. For the quantification of these three main groups spectrophotometric assay was done. For quantification of total flavones/flavonols ammonium chloride complex-based spectrophotometric assay is used. After that colorimetric method with 2,4-dinitrophenylhydrazine was applied for the quantification of flavanones and dihydroflavonols as their amount was high in poplar propolis. Folin–Ciocalteu procedure was used for the final measurement of phenolic content. Final results were verified by HPLC procedure [1].

## 5 Therapeutic applications of propolis

### 5.1 Traditional uses of propolis

Propolis has been employed as traditional medicine for thousands of years. Egyptians use propolis in the practice of mummifying corpses [58]. Propolis is being used from the ancient times in the treatment of gastroduodenal ulcers (internal use) and as an antiseptic (external use) [3]. Propolis was extensively used in several Soviet clinics during the Second World War for its antibacterial and anti-inflammatory properties [12]. Polyanthus, a perfume for Greeks featured propolis as a key component [59]. Wound healing and antibacterial properties were discussed in earlier times by Aristotle, Galen, and Pliny. Since the eighteenth century propolis has been categorized as an official medication by London pharmacopoeia.

Nowadays, propolis can be used for the treatment of many bacterial diseases like tuberculosis [60]. It is also known to possess antiviral, analgesic, and wound healing properties [8], and is used to treat various types of dermatitis, which are caused by bacteria and fungi. It is also available in the form of capsules (either in pure form or combined with aloe gel and *Rosa canina* or pollen), as an extract, as a mouthwash, and in powdered form [3]. Propolis is also known to lower blood pressure and cholesterol levels, and for the treatment of atherosclerosis [23]. Data given by Pillai et al. [61] also indicate that Indian propolis has ulcer-preventive as well as ulcer-curative characteristics. It is also known to show regenerative effects on biological tissues and exhibit anti-neoplastic activity against cancer cells [3]. Propolis helps to reduce the lung problems and in appetite recovery. Extracts or ointments of propolis are beneficial in the treatment of genital HSV infection [3]. One of the important components of propolis, CAPE, is known to provide protection against ischemic spinal cord injury after infrarenal aortic occlusion in rabbit. It has been reported that the use of an alcoholic solution of propolis and sulphopen (potassium penicillin G, benzene sulphonamide hydrochloride, and sulphonilamide) and Framykoin (neomycin with zinc bacitracin) can stimulate tissue regeneration [6]. Efficacy of nanopropolis is even more than propolis due to its smaller size.

## 5.2 Role of propolis as a functional food ingredient

Hundreds of nutritious products have entered the market, but contribution of propolis as a functional food ingredient is still largely insignificant. In many of the most recent studies, spray drying is recommended as an effective method for transforming the propolis ethanolic extract into a powder that can be used in food systems without changing its characteristics [62]. Propolis should be used as a nutritional supplement or functional food in the fight against the COVID-19 pandemic due to its diversified properties and inhibitory potential [63].

## 5.3 Role of propolis against COVID-19

Presence of the CAPE and other phenolic acids confirms the anti-inflammatory and antiviral properties of propolis. Chemical constituents of propolis especially flavonoids such as chrysin, kaempferol, quercetin, and its derivatives are known to inhibit different signaling pathways and *in vitro* virus replication. Investigational studies confirmed the anti-corona effects of propolis highlighting the need for clinical trials, further standardization, and investigation covering its prophylactic effects in high-risk groups. Potentially active components of propolis help to alleviate symptoms of COVID-19 [64]. The potential of propolis liposomes was revealed as the most promising approach for the treatment against COVID-19 [65]. As per the standards adopted by the World Health Organization, propolis is non-toxic and does not interact with the primary liver enzymes or with other important enzymes; as a result, propolis can be used alongside the main medications without running the risk of potentiation or inactivation [66,67]. Due to the biological properties of propolis, with some antibiotics it can exert a synergistic action in the prophylactic effect of COVID-19. Bioinformatics approaches can also provide fresh perspectives [67]. Trace elements such as zinc ions, selenium, vitamin A, vitamin B12, and retinoic acid present in propolis have an immense role in maintaining adaptive immune system. Zinc ions block viral enzymes necessary for the virus to replicate in the host cells.

**Table 4:** Role of propolis constituents in inhibiting COVID-19

| S. no. | Propolis constituent | Role | References |
|---|---|---|---|
| 1. | CAPE<br>Artepillin C | Inhibits PAK1 activity | [68–70] |
| 2. | Glyasperin A<br>Broussoflavonol F<br>Sulabiroins A<br>Isorhamnetin | Inhibits the binding of ACE-2 and SARS-CoV-2 | [71] |
| 3. | Phenolic compounds | Reduce the adsorption and entrance of the virus into the host cells | [72] |
| 4. | Flavonoids | Inhibits the activity of ACE | [69] |
| 5. | Genistin<br>Methylophiopogonanone A | Inhibits main protease and spike protein | [73] |

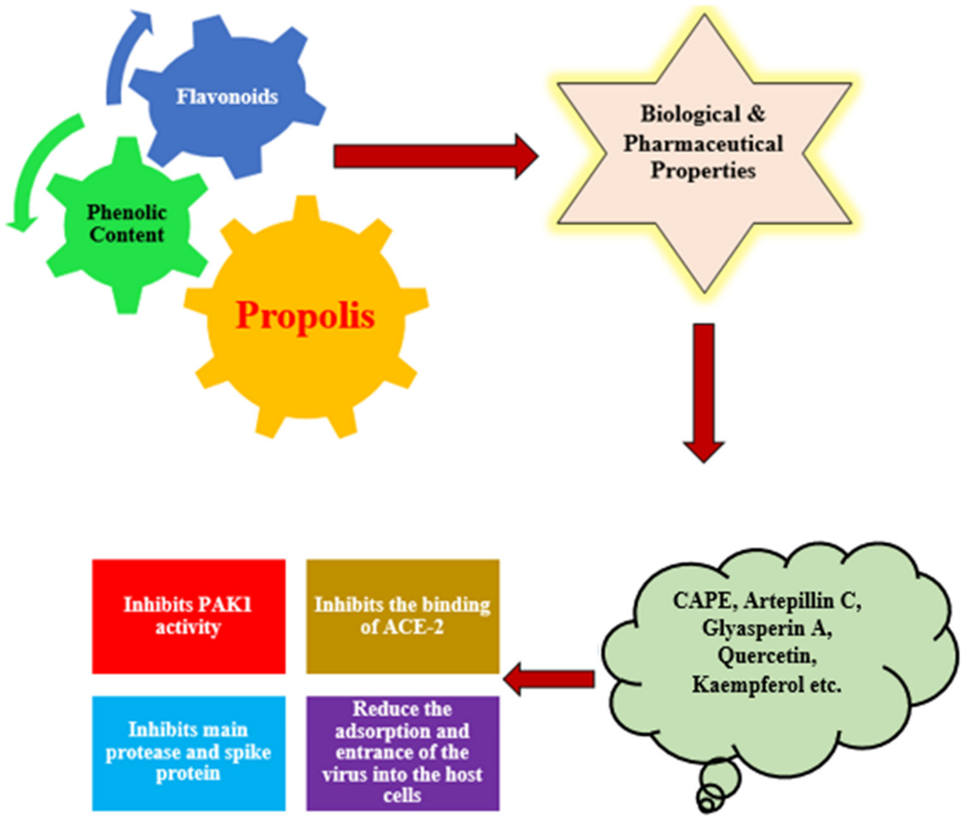


**Figure 5:** Role of propolis constituents in mitigating COVID-19.

Selenium inhibits proinflammatory cytokines [69]. It was revealed in many studies that propolis inhibits PAK-1 and interacts with ACE2 and TMPRSS2, which may aid in reducing or preventing SARS-CoV-2 host cell invasion as given in Table 4 [74]. It was revealed in a study that the main constituents of propolis such as caffeic acid, uercetin, kaempferol, and myricetin had a close interaction with the ACE2 protein and thus block the binding of COVID-19 virus with ACE2 protein as given in Figure 5 [63].

### 5.4 Role of nanotechnology and green chemistry in apitherapy

Nanotechnology has substantial impact in apitherapy. Utilization of free form of propolis is restricted due to its low absorption and low solubility. Nowadays, the production of nanopropolis by various nano-encapsulation technologies has become popular to increase the efficacy of propolis. Size of nanopropolis is 1–100 nm in diameter and due to this smaller size absorption of nanopropolis is easier by the body [75]. Nanopropolis exhibits more effective pharmaceutical properties than propolis. To treat the cancer, nanopropolis can be used as nanofood. More research is required on propolis prepared as nanoparticles to demonstrate their effectiveness in the treatment of diseases [76]. Considering the benefits of green chemistry propolis nanoparticles are being prepared. Propolis nanoparticles biosynthesized by green chemistry have promising effect in the treatment of human ailments. Green chemistry has several advantages, including cost effectiveness, requiring less energy, being non-hazardous, non-toxic, eco-friendly, and facilitating ease of procedures and adaptability [77]. Propolis nanoparticles exhibit remarkable antibacterial [75,78], antifungal [78], and antidiabetic [79] properties. Due to these properties they have broad range of applications such as in nanomedicine, pharmaceuticals, drug delivery, veterinary medicines, and cosmetics [75].

## 6 Conclusion

Propolis and its nanoparticles (nanopropolis) have been considered to be a possible source of natural antioxidants that can combat the effects of oxidative stress, which is implicated in the aetiology of many diseases. These have enormous nutritional and medicinal potential but still more research and standardization are required for its clinical and nutraceutical use. The complex, varied composition of propolis according to geographical region, as well as the variety of analytical methods employed to examine their antioxidant properties, are responsible for the broad range of results reported by various researchers. This emphasizes the need for chemical standardization of propolis. This review indicates a potential impact of propolis as an adjuvant therapy on COVID-19. For prevention and treatment of COVID-19 further investigations and clinical trials are required to emphasize the understanding of potential therapeutic benefits from propolis. Usage of propolis products as a health-promoting supplement in human medicine is restricted in many nations because they are not standardized and vary in their components and biological activity. Propolis has enticing therapeutic potential and can be employed to treat COVID-19. As the majority of the findings were non-clinical studies, or reviews, there is a necessity of more clinical trials. Nanoparticles generated with the green approach are eco-friendly, non-toxic, and have successfully been used in various applications.

## 7 Research possibilities

The use of propolis and its nanoparticles in alimentary supplements and bio-cosmetics is increasing day by day. This has driven the interest of researchers to work on the quality as well as quantity of propolis, and also on the chemical standardization of propolis [15]. More research is needed to better define the parameters for using propolis in food formulations or in the pharmaceutical industry [29]. The efficiency of mouth rinses containing propolis samples on oral bacteria was not shown to be as effective as chlorhexidine (CHX). Also, propolis samples were shown to be less cytotoxic on human gingival fibroblasts than CHX. At sufficient doses, standardized propolis formulations can be used as a mouth rinse. Advanced research is required to acquire a standardized chemical composition [80].

Use of propolis in medicines is a very popular topic of research worldwide. However, research into Indian propolis and its nanoparticles is still in its infancy. In India studies on propolis have not been reported extensively except in few states like Maharashtra, Madhya Pradesh, West Bengal, Tamil Naidu, and Gujarat, so its popularity among researchers also opens the door for research in other states also [12]. Role of CAPE in cancer treatment also requires more experimental studies [3].

Propolis helps to alleviate the pathophysiological effects of COVID-19 infection due to its antioxidant, antimicrobial, antiviral, and immunity enhancing properties. Additional research pertaining to its standardization and dosage, as well as an increase in its production, should be done [81]. Medical trials are required to use propolis and its nanopaticles in the medical sciences. The advancement of science and technology has resulted in a plethora of improvements to all facets of human life [82], which further can be enhanced by incooperating bee products with modern technologies like nanomedicine [83], biomarkers [84], and biosensors [85].

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