ORIGIN, PHYSIOCHEMICAL COMPOSITION AND BIO-MEDICAL IMPORTANCE OF PROPOLIS

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Summary: Current studies were performed to evaluate the medicinal importance of propolis and its origin and physiochemical composition depending upon the nature of vegetations present in various localities. The propolis contains a myriad of chemical compounds such as flavonoids, esters, phenolic acid, amino acids and terpenoids. Different analytical techniques (e.g., TLC, GC, HPLC, MS, NMR and GC-MS) are used for the characterization of various constituents of Propolis. Propolsi finds biomedical importance and numerous clinical applications as it possesses large quantities of anti-oxidants, anti-bacterial, anti-fungal, anti-viral and anti-inflammatory agents and also displays anticancer potential.

Keywords: propolis, analysis, medicinal, nutritional, physicochemical

INTRODUCTION

Propolis (bee glue) is a natural resinous product which is collected from plants by bees for the adaptation and construction of their nests (de Groot, 2013). It majorly consists of honeycomb paraffin and plant extracted stuff like spermaceti and evaporative composite (Bankova et al., 2000). It has been reported that honeybee hives serve as a "treasure island" and rich source of honey, propolis, royal jelly, bee pollen, beeswax, and bee venom. Increasingly, it is being realized that honey and its bioactive components are involved in prevention and inhibition of cancer by targeting multiple proteins in cancer cells (Farooqi et al., 2019). In the past decade much research has been focused on the physical and chemical constellation of propolis and its significance. The resin of green propolis has important constituents such as cinnamic acids and chemical compounds containing phenyl groups (Gambichler et al., 2004; Salatino et al., 2005). Above 68 °F (20°C), propolis becomes sticky but below this temperature, it is found fragile (El Sohaimy & Masry, 2014). Propolis is used as a resin ingredient (de Groot, 2013), in musical instruments (Burdock, 1998), for sealing the surfaces of bridges (Nair et al., 2008), in chewing gum (Ercan et al., 2015) and also for conversion of oils and fats into automobile wax (Ahuja & Ahuja, 2011).

Keeping in view the importance of propolis and its large number of applications, current studies were performed to review its chemical composition and biomedical importance.

ANALYTICAL TECHNIQUE FOR CHARACTERIZATION OF PROPOLIS

Propolis can be characterized by different analytical techniques which involve purification and separation of chemical constituents of propolis. They include TLC (thin-layer chromatography), GC (gas-chromatography) and HPLC (high--performance liquid chromatography), MS (mass spectroscopy), NMR (nuclear magnetic resonance) and GC-MS (gas chromatography-mass spectrometry). These techniques help to determine the compounds of propolis, including flavonoids, phenols, terrenes, sugars, esters, hydrocarbons and minerals (Muscat, 2013).

CHEMICAL COMPOSITION

The studies on chemical composition studies of 100g propolis extract in 900mL of 70% ethyl alcohol have shown that the propolis sample contains huge quantities of aromatic acids, esters and the components which responsible for the

anti-fungal ,anti-bacterial, anti- inflammatory, anti-viral, and anti-cancer activities of propolis (Sahinler & Kaftanoglu, 2005). Propolis medication has shown a great improvement in the recovery of the body weight loss and treatment of kidney weight in diabetic patients. It has also strong automotive effect on control of metabolism(Abo-Salem et al., 2009). The propolis extract has also been reported to contain flavonoids, amino acids, terpenes and derivatives of cinnamic acid (Khayyal et al., 1993). Most phenolic compounds were indicated during using ultraviolet and IR spectroscopic analyses (Cabral, 2016; Gonzalez et al., 2019); pharmacological flavonoids were also identified in propolis (Bonvehí & Coll, 1994). The basic structure of flavonoids has been displayed in figure 1. Many chemical contents of propolis were characterized by using high-performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS) (Zeitoun et al., 2019). The propolis contains 156 components of which phenolics are most important owing to their participation in various biochemical activities (V Bankova et al., 1994). Flavonoids without β-ring substituents are the peculiar property of temperate zone of propolis; they include chrysine, pinocembrin, galangin (a flavonol) and pinobanksin (an antioxidant). Caffeic acid phenethyl ester is a major component of temperate propolis having versatile biochemical activities, such as inhibition of cell proliferation, hindrance of nuclear factor κ-B (kappa-light-chain-enhancer of activated B cells), induction of apoptosis and cell cycle arrest (Huang et al., 2014). Figure 2 shows the important chemical ingredients of propolis. Table 1 displays the components present in various extracts of propolis.

Propolis can be analyzed for the presence of some mineral contents and toxic trace elements by near infrared spectroscopy with a remote reflectance fibre-optic probe. This procedure can be applied to a variety of samples for the determination of Pb, Cu, Ni, Cr, Zn, P, Mg, K, Fe, Ca, Al and is able to detect the toxic elements e.g., Pb, Cu, Zn in a very short time of 3-4 minutes. The method finds applications in the commercialization of propolis in order to ensure its safety before use (González-Martín et al., 2015).

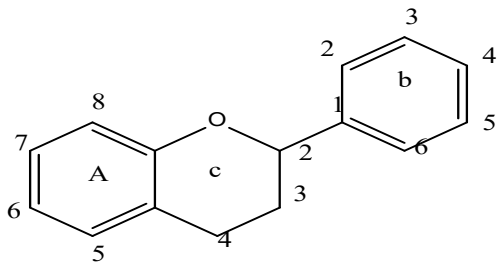

FIGURE 1. Basic structure of flavonoids (Russo & Speranza Sánchez, 2006)

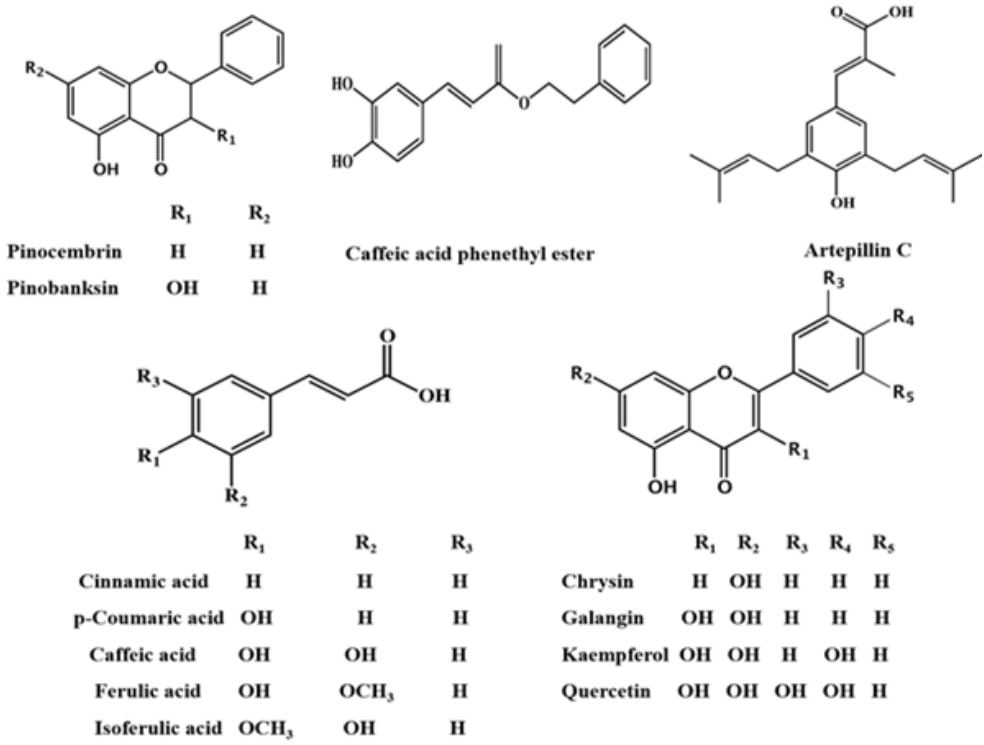

FIGURE 2. Chemical components in propolis (Cui-ping et al., 2014)

TABLE 1. Chemical components present in various solvent extracts of propolis (Cauich-Kumul & Campos, 2019)

Methanol	Water	Ethanol	Chloroform	Ether	Dichlorome-thane	Acetone
Phenols	Saponins	Tannins	Terpenoids	Alkaloids	sterols	Flavonoids
Anthocyanin	Anthocyanin	polyphenols	Flavonoids	coumarone	Terpenoids,	-
lactones, flavones	polypeptides	polyacetylene	-	terpenoids	tannins	-
xanthoxylin	tannins	Terpenoids	-	-	polyphenols	-
terpenoids	lectins	alkaloids	-	-	alkaloids	-
polyphenols	terpenoids	sterols	-	-	polyacetylene	-

BIOMEDICAL POTENTIAL OF PROPOLIS

Propolis has been used in traditional medicine (Simone-Finstrom & Spivak, 2010). Propolis is largely investigated for the possible development of new medications integrated with control of *Candida albicans* and modulation of immune system (Gavanji & Larki, 2017). It has a large number of beneficial biological activities including antiviral, antifungal and antibacterial potential. Its use as a dietary supplement helps in the prevention of diseases and is used as a part of numerous biopharmaceuticals (de Groot, 2013).

The antimicrobial effects are owed to chrysie, volatile compounds (coumaric acid), proto catechuic acid and terpenoids. The inter-action of ethanoic propolis along with calcium hydroxide is thought to be responsible for intracranial medication and antibacterial activity. In upcoming era, the effect of propolis would be focused on other anaerobic bacteria which are involved in endodontic infections.

During teeth injury, propolis is one of the best remedy for vitality of the periodontal ligament (PDL) cells; it also acts better than Hank's Balanced Salt Solution (HBSS), as more PDL cells survive milk and serum. Propolis works as a best storage medium for transport needed to carry the teeth to dental clinic. Propolis decreases the number of positive TRAP (Tartrate Resistant Acid Phosphatase) of giant cells and has an inhibitory effect on the initial phase of osteoclast genesis. Propolis increases osteoprotegerin expression and decreases the number of osteoclasts therefore inhibiting osteoclast genesis. This inhibitory effect is dose-dependent (Kousedghi et al., 2012).

ANTIMICROBIAL EFFECTS

Edible films based on ethanol extract (different concentrations) of propolis and hydroxypropylmethylcellulose were tested for antifungal studies against *Penicillium italicum* and *Aspergillus niger*. A significant antifungal potential of the composite films was observed especially against *Aspergillus niger* (Pastor et al., 2010).

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ANTIVIRAL POTENTIAL

For antiviral effects, specific esters derivatives of cinnamic acids were studied for their inhibitory potential against influenza virus. For this purpose, some substances were synthesized which are related with petroleum ether fractions (Serkedjieva et al., 1992).

ANTIOXIDANT ACTIVITIES

Antioxidant activities of alcoholic and aqueous extracts (EEP) of propolis from different origins have been studied extensively worldwide (Noureddine et al., 2017). Propolis in dry form have been found to show significant antioxidant properties (Marquele et al., 2006).

CYTOTOXIC ACTIVITY

There were investigations on breast adenocarcinoma MDA-MB-231 cells, glioblastoma U251 cells and Jurkat leukemic T-cells to see the effects of *Lebanese propolis*on the cell cycle distribution. It was concluded that significant anti-proliferative activity and cytotoxicity is exhibited by L. propolis (Noureddine et al., 2017).

SKIN TISSUE ENGINEERING AND IN WOUND DRESSING

Electrospinning of propolis/polyurethane blend solution can be used to prepare successfully biocompatible propolis loaded polyurethane (propolis/PU) nanofibers. Due to adhesive properties of propolis, its increasing amount in the blend can be used to point-bond the composite nanofibers. The mechanical strength and hydrophilicity of the fibrous membrane can be improved by incorporating small quantity of propolis through PU matrix. Fibroblast cells were seeded on the matrix in order to assay the cell behavior and cytocompatibility on the composite scaffolds. It was found that cell compatibility was improved by incorporating propolis into PU fibers. Furthermore, significant antibacterial potential was exhibited by composite nanofibers. So, a greater potential in skin tissue engineering and in wound dressing was observed by as-synthesized nanocomposite fibrous mat (Kim et al., 2014).There were investigations on the incorporation of the proplis extract into natural rubber matric. It was concluded that the extent of proplis incorporation plays a decisive role in antimicrobial activity, wettability and surface morphology. Also the possibility of obtaining a non-adhesive, translucent and flexible membrane was verified. It was suggested that such kind of a system may be less toxic and significantly affective in burn dressing and other clinical uses (Silva et al., 2014). Silver nanoparticles and ethanolic extract of proplis were delivered by using an electrospun biocompatible (non-water-soluble but hydrophilic) polylactic acid nonwoven material. This combination was found highly effective in wound healing and excellent antibacterial protection. Also the presence of propolis extract and/or silver nanoparticles have resulted in the formation of denser mats with thicker polylactic acid microfibers (average diameter = 168 ± 29). By the addition of 20% wt and 10% wt ethanol, there was increase in the average diameter of the microfibers to 370 ± 30 and 318 ± 40, respectively. The diameter was increased to 371 ± 25 and 282 ± 25 by adding 20% wt and 10% wt, respectively of proplisethanolic extract (Adomavičiūtė et al., 2017).

COSMETIC APPLICATIONS

Propolis can be used as a useful constituent of biocosmetics (de Groot, 2013). From the rheological and texturalfeature's point of view, lipsticks having absence/presence of propolis were tested and found to be beneficial and productive for the purpose of make-up. No dyes, fragrances, preservatives or synthetic compounds were used in the production of investigated lipsticks. The propolis was found effective additive for the production of cosmetics. The viscoelastic properties of the lipsticks were significantly protected by the presence of propolis in lipstics. Moreover, the lipsticks with added propolis were found to be prone to crushing and more brittle. Low deformation conditions were used to test the properties of lipsticks from rheological point of view; some structural changes were indicated, probably due to the structure consolidation. The textural characteristics of these lipsticks were not significantly changed by increasing the temperature (30°C) (Goik et al., 2015). Viscoelasticity and texture of numerous pharmaceutical based gels based on propolis were studies. The construction of hydrogels is performed in such a manner that rate of formation of blood vessels and regeneration of tissues is taken place at a very accelerated rate (Osser et al., 2014). Propolishas a large number of varieties; the composition and variety of proplis depend upon the composition of plants (Park et al., 2000).

ANTI-DIABETIC EFFECTS

Conduction of tests in vitro as well as in vivo demonstrated that flavonoid may have anti-diabetic effects which are owed to the presence of a natural substance in propolis, which has become the cause of lowering postprandial glucose. The trend of occurrence of non-insulin dependent diabetes mellitus in the population of the developed county is increasing day by day (Kurek-Górecka et al., 2014).

NATUROPATHIC ALTERNATIVE TO ANTIBIOTICS

Propolis has become naturopathic medicineagainst antibiotics in treatment of minor burns (Gregory et al., 2002). Ethanolic extract of propolis (EEP), is well known for its remarkable anti-bacterial activity. It was prepared in culture medium with eight different antibiotics, having a fixed amount of a standard strain of bacteria (*Staphylococcus aurous*). Ethanolic extract of propolis (EEP) had a moderate effect on other, except ampicillin which has remarkable synergistic effect on the anti-bacterial activity of streptomycin and cloxacillin (Ikeno et al., 1991).

APPLICATION IN DENTISTRY

Propolishas been found to be advantageous in many aspects, including prevention ofdental caries (Parolia et al., 2010) and targeting chemotherapy-induced mucositis (Abdulrhman et al., 2012). The applications of propolis-based preparations in various specialties of dentistry and oral cavity diseases have been investigated (VK, 2014).

PHARMACEUTICAL APPLICATIONS

Propolis has been extensively used due to its antimicrobial and pharmaceutical properties, whereas its use as an antifungal agent in agriculture has been assessed recently (Curifuta et al., 2012). Much of work has been done for the feed mixture on selected parameters of mineral profile of broiler chickens. Many researches are focusing on the effects of other natural substances and phyto additives on mineral profiles of broiler chickens (Petruska et al., 2012). Propolis has been inclusively employed by myriad of civilizations for the treatment of wounds, colds, and ulcers owing to its local anesthetic and antiseptic properties. Taiwanese green propolis has been reported not only for the protection of liver from the pathogenesis of fibrosis but also for the renal tissue against toxicity. Propolis extract has great anti-parasitic as well as the antiprotozoal activity (Khazaei, 2016).

PHARMACOLOGICAL ACTIVITY

In the start of nineteenth centuries, pharmaceutical uses of propolis were reported. The propolis vaseline having antibacterial behavior, was prepared and used as medication in Anglo Boer war. It finds uses in healing of wounds, tissue regeneration in surgery, for securing dressing to wounds, for inhalations. Yellow beeswax is used as an additive for the preparations of styptic creams, a sterile mixture of beeswax can be used to prevent hemorrhage during cranial surgery; the treatment of rheumatism and gout by the use of ointments, have been reported recently. In the present studies, clinical treatment of mycotic infection and eczema was also investigated. Advancements in ointments activity, drugs, therapy were evaluated (Ghisalberti, 1979).

MULTIFUNCTIONAL BEHAVIOR

The products containing propolis have increased uses as a dietary supplement and have extensive dermal contact. It has been largely reported as a natural remedy due to its antitumour, antiviral, antifungal and antibiotic potential. Although some allergic reactions of propolis have been reported, yet propolis is relatively non-toxic (Burdock, 1998).Investigation has revealed inhibitory effects on lymph proliferation and other prospective to the immune system (J. Sforcin, 2007). Increased numbers of cases of allergic contact dermatitis are reported especially in those individuals who use propolis in self treatment of numerous diseases or use in bio-cosmetics. The flavonoid aglycones and the other phenolic constituents present in bud secretion of poplar are effective for hypersensitivity (Hausen et al., 1987). Propolis extracts contain a complex mixture of natural ingredients so it displays a broad spectrum of biological potential. They are important from economical point of view and possess anticancer, antitumor, immunomodulatory, anti-inflammatory and antioxidant potential. They may be used for inexpensive treatment of cancer (Watanabe et al., 2011).Propolis shows inhibitory effects on protein synthesis

and cell division. It also demonstrates apoprtotic effect *via* inhibiting telomerase expression (Gunduz et al., 2005). In blood, various concentrations of propolis were determined for total leukocyte count and granulocytes. A decrease in level of aspartate aminotransferase and alkaline phosphatase was found. Hematological and biochemical protective effects of propolis were investigated. At the same time commercial demand of volatile components of propolis for its fragrance and biological activities played a vital role in food additives (Vassya Bankova et al., 2014). Many civilizations kept on using propolis because of being a natural drug. On industrial scale, it includes candies, shampoos, antiseptic mixtures, skin lotions, chocolate bars and toothpastes. In Second World War propolis emphasized a great role in treatment of wounds when no antibiotic was available at that time. Pharmacological properties like immune modulatory action, a low-cost potential anti-inflammatory agent, especially for muscles and articulations and other types of inflammations, infections, rheumatisms and torsions were studied and remedies were being provided with excellent results (Ramos & Miranda, 2007).

The importance of propolis extracts in different fields of medicines has been generally recognized (Talas & Gulhan, 2009). For the sake of making progress by using natural products in medicine, it is essential to take into account of restrictive factors. Propolis has been known for centuries as herbal remedy for curing infections. The benefits of propolis are more which are not ignorable. Researchers have started to study it at monotonous level. Synergies can be predicted and understood by studying the mechanism action ofactive constituents of propolis (Cheng & Wong, 1996). By use of propolis, the chances of detecting positive contact in medicaments, in cosmetics or in perfumes used are tested and results are recorded (Jappe et al., 2005). The most important function of propolis is to modulate costly immune system (Borba et al., 2015). The major constituents of propolis i.e. caffeic acid and phenethyl ester have good anti-inflammation and anti-tumor activities (Chang et al., 2017). Owing to anti-septic, anti-inflammatory and anti-oxidant properties of propolis, manufacturers are being attracted towards its outstanding benefits. The addition of honey in propolis contents is becoming more common in the food industry along with medicinal benefits. We believe that the grounds are set in terms of utilization of propolis as a useful product (Matos et al., 2015). For the explanation of propolis mechanisms of action *in vitro as well as* in *vivo, many* attempts had been carried out but the most of propolis' targets and actions are still not clear. Further investigation should be made to obtain new drugs (Sforcin, 2016).It is expected that scientists would become able to connect a peculiar chemical type with a specific type for biological activity. They will formulate directions for promoters. For the study of traumatic injuries, common people would be able to use the beneficial properties of propolis. Advanced studies on the active constituents of propolis are firmly needed in order to identify their biological effects. More tasks including bioavailability, effective doses for management of disease and stable preparation are needed (Martinotti & Ranzato, 2015).

CONCLUSIONS

Propolis is a natural compound with remarkable known characteristics since ancient Greek time. It comprises of a wide range of chemical compounds with fast biological actions. It is rich in phenolic components and is considered as a useful product for different traditional medicines including herbal medicines. Propolis extracts possess antimicrobial activity, antioxidant activity, anti-diabetic effects and commonly find applications in clinical field. The chemical composition of propolisis is co-related with biological activities; as a result this standard commodity should be used in complementary therapies.

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