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Propolis as a Prospective Therapeutic Agent: A Review

[1]Meenu Khurana, [1]Tamanna Kaundal, *[1]Navneet Batra, [2]Rupinderjeet Kaur and [2]Anjali Sharma

[1]Department of Biotechnology, Goswami Ganesh Dutta Sanatan Dharma College, Sector- 32, Chandigarh
[2]Department of Biotechnology, DAV College, Sector-10, Chandigarh

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***Corresponding Author**
Navneet Batra
Email: batranavneet@gmail.com

ABSTRACT

Propolis is gummy substance made by honey bee used as a sealant in the hives which protects the hives from parasites as well as prevents bacterial and fungal growth. Different constituents known to be a part of Propolis mainly are the phenols, flavonoids, terpenes, cumaric acid and esters. The bioactivity of Propolis varies substantially depending on geographic region, plant sources, honeybee species, and extraction solvent. It is a multi-constituent bee product. Its advantages are easy availability, biodegradability and biocompatibility. It is anti-cancer, anti-microbial, anti-oxidant, has anti-biofilm potential and offers skin benefits.

Introduction

Propolis is a viscous compound formed by bee colonies by combining saliva, beeswax, and exudate from tree buds, flowers, and other botanical sources. For inappropriate open spaces, it is used for sealing and smoothing out the internal walls in the hive (Wagh, 2013). It provides the defense against the intruders like snakes, lizards and also protects the hive from rain, wind etc. Propolis is hard and brittle, but once heated, it becomes soft, gummy and sticky. Propolis can be yellow, green, red, or brown depending on its botanical source, although brown is the most frequent (Wagh, 2012).The melting point of Propolis is between 60ºC-70ºC but in some varities, it may be high as 100ºC (Fokt, 2010).It can be extracted using various solvents like water, methanol, chloroform, dichloromethane, ether and acetone (Trusheva, 2007). Its composition varies on the basis of vegetation, bee species, season of collection and geographical region. Resins, essential oils, vitamins, balsams, flavonoids, minerals, and pollens are the most common components of propolis. Alcohols, aldehydes, aliphatic esters, amino acids, aromatic acids, aromatic esters, chalcones, flavonoids, terpenoids, terpenes, ester, waxy acid, ketones, steroids, sugars are among the more than 300 components discovered in Propolis samples. Propolis also contains important vitamins, minerals and enzymes, such as dehydrogenases, phosphatases (Marcucci, 1995).

Mechanism of Action of Propolis

Propolis mode of action against *Streptococcus agalactiae* has been the focus of many researchers. By restricting cell division and the development of pseudo-multicellular streptococci, propolis prohibits bacteria from growing. It causes the disorganisation of cytoplasmic membrane and cell wall, causing the bacteriolysis, and also inhibits protein synthesis (Takaisi-Kikuni, 1994). The studies confer that antimicrobial action is mainly contributed by phenolic, cinnamic and flavonoid component which are responsible for the disunion of cytoplasmic membrane and hence inhibit the bacterial motility (Mirzoeva, 1997).

Biological Properties

Antioxidant Activity

Antioxidants are the substances that reduce the damage caused due to free radicals or oxidation. Propolis antioxidant scavenging activity has been widely investigated and evaluated utilizing a radical cation decolorization assay from diverse geographical regions.Among the different chemical constituents of Propolis, flavonoids mainly contribute to Propolis antioxidant activities. The predominant flavonoid fraction in Propolis is Quercetin glycosides (Zheng, 2017). Propolis antioxidant activities from various regions of the globe have already been compared. The antioxidant activity of an ethanolic extract of Propolis from Argentina, New Zealand and China was found to be significant, with kaempferol and phenethylcaffeate as ingredients, in addition to flavonoids and phenols, which mostly contribute to the antioxidant activity (Kumazawa, 2004).The Propolis extracted with water showed remarkably greater activity over ethanol extract as antioxidant. The reason behind could be the high polyphenol content and presence of flavonoids including pinocembrin and galangin (Laskar, 2010). Clinical assessment of effect of propolis on oxidative state and lipid regulation in humans in Talca, Chile, has shown benefits on oxidative status and improvement of high-density lipoprotein that contributes to less risk of heart diseases (Mujica, 2017). The effects of a 30-day commercial powdered Propolis extract

supplementation on antioxidant enzymes such superoxide dismutase, glutathione peroxidase, catalase, and the lipid peroxidation marker malondialdehyde were detected in healthy persons. After two weeks of Propolis treatment, there was decrease in malondialdehyde levels and rise in superoxide dismutase activity (Jasprica, 2007).

Anti-inflammatory effect

Anti-inflammatory properties of propolis have long been known. Propolis extract has been reported to reduce platelet aggregation and eicosanoid production in vitro, implying that it may have potent anti-inflammatory actions (Marcio, 2012). A 13 percent aqueous extract was evaluated orally in rats and guinea pigs with a carrageenan rat paw edoema model and adjuvant-induced arthritis at three dosage levels (1, 5 and 10 ml/kg). In both models, the extract shown strong dose-dependent anti-inflammatory effects. Propolis extract was found to have significant anti-inflammatory effects *In vivo*. Its activity was linked to its effects on the production of inflammatory mediators such as prostaglandins, leukotrienes, and histamine. Another study looked at the effects of a Propolis-containing dental material combined with chlorhexidine at lower concentrations on humans (Naito, 2007). The results showed that combining Propolis with chlorhexidine improves antigen recognition by activating the NF-B signalling pathway somewhat and increasing the bactericidal activity of human monocytes against *S.mutans* (Santiago, 2016). Caffeic acid phenyl ester and galangin were tested for anti-inflammatory efficacy in rats in a similar study. The anti-inflammatory effect of two ethanolic extracts with and without caffeic acid and phenethyl ester (CAPE) was examined. The studies showed that carrageenineoedema, carrageenine pleurisy and adjuvant arthritis were substantially inhibited by CAPE alone. In acute and chronic inflammation, galangin has not demonstrated anti-inflammatory effects. The findings indicated that CAPE was responsible for the anti-inflammatory function of Propolis (Borrelli, 2002).

Anticancer activity

Propolis stimulates the apoptotic pathway in cancer cells, which reveals its anti-tumor properties in addition to antioxidant and anti-inflammatory properties. The anticancer activity of Indian stingless bee Propolis ethanolic extract has been studied in four cancer cell lines, including MCF-7 (human breast cancer) and HT-29 (human colon cancer), to determine cytotoxicity and apoptosis (Choudhari, 2013).

In another study Brazilian green Propolis has been demonstrated to activate p53 and inhibits the cancer cells from growing. Higher cytotoxicity was indicated by nuclear translocation and p53 activation in cell viability experiments utilising Artepillin C and green Propolis-supercritical extract. Using mice *In vivo* tumour suppression experiments, green Propolis supercritical extract and γ cyclodextrin (γCD) was shown to possess more vigorous anticancer activity than purified Artepillin C alone(Paulino, 2008). The constituents such as CAPE and chrysin present in Propolis mainly induce the apoptosis pathways by suppressing the complexes of cyclins, resulting in cell cycle arrest. Propolis can be explored as a potential chemotherapeutic and chemopreventive anticancer agent. Chrysin was the active anticancer agent extracted and found in a Chinese Propolis sample against MDA-MB-231 breast cancer cells. The phenethyl ester of caffeic acid is another powerful active component detected in small levels (Sarteshnizi, 2015). In MDA-MB-231 cells, chrysin, a histone deacetylase inhibitor, greatly inhibited cell proliferation and encouraged differentiation, according to a study (Sun, 2012).

Skin Benefits

Propolis' dermatology activity has been shown to assist in the healing of mild burns.The researchers compared a Propolis cream and silver sulfadiazine, a drug which is used to treat burns. Studies showed Propolis was effective in treating second degree burns. The anti-bacteria and anti-fungal characteristics provide many benefits in cosmetics (Gregory, 2002). The investigations also confirmed that burn wounds treated with Propolis had decreased levels of free radicals (Olczyk, 2013). Propolis-based skin cream was discovered to be effective for oily skin (Park, 2013). Because of its antibacterial activity, moisturization and rejuvenation capacity, and ability to promote skin suppleness, propolis in cosmetics has a lot of benefits.

Antimicrobial activity

There are numerous reports for depreciation of antibacterial, antiviral, antifungal and antiprotozoal activity by Propolis. Various antimicrobial compounds have been isolated from propolis extracted from different geographical regions. The 2.3,5-diprenyl-4-hydroxycinnamic acid, 3-prenyl-4-dihydrocinnamoloxycinnamic acid, and 2,2-dimethyl-6-carboxyethenyl-2H-1-benzopyran were isolated and identified from Brazilian Propolis (Hajime, 1994). The high phenolic and flavonoid compounds in Iranain Propolis have been found to be responsible for its antimicrobial activity, which has already been demonstrated in previous studies. The mechanism and reason for inhibition of DNA gyrase, cytoplasmic membrane function, and that licochalcones A and C inhibit energy metabolism have also been discovered (Mohammadzadeh, 2007).The process of extraction has a big impact on Propolis' activity. The efficacy of three extracts (hydro-alcoholic, methanolic, and aqueous) was compared, and it was discovered that the hydro-alcoholic extract was more effective in extracting phenolic chemicals (Gregory, 2002). The antibacterial and antifungal activity of Propolis was examined after extracting it using several solvents, and it was discovered that the solvent used for extraction increased the strength of Propolis' antimicrobial activity. The combined action of ethanolic extract of propolis (EEP) and antimicrobial medicines that limit bacterial growth by interfering with

bacterial protein synthesis (Fer) has been demonstrated in vitro. (Fernandes, 2005).

The antibacterial action of sub-lethal dosages of EEP on pathogenic strains was found to be considerably enhanced for ampicillin, gentamycin, and streptomycin, moderately enhanced for choramphenicol, ceftriaxon, and vancomycin, and no impact for erythromycin (Scazzocchio, 2006). To analyse the synergic effect of Propolis and antimicrobials, antimicrobial properties of EEP samples were tested against 39 microorganisms from different regions of Serbia. The buildup of bacteria and extracellular polysaccharides generated from sucrose by *Streptococcus mutans* utilising the enzyme glucosyltransferase causes the formation of tooth cavities. Glucosyltransferase enzyme production is higher in oral organisms. EEP from Brazilian Propolis was found to inhibit both glucosyltransferase activity and *S. mutans* growth, but one of the Propolis samples from Rio Grande do Sul showed the greatest inhibition of enzyme activity and bacterial growth, as well as having the highest concentrations of pinocembrin and galangin (Koo H, 2002). Flavonoids such as quercetin, galangin, pinocembrine, and caffeic acid, benzoic acid, and cinnamic acid, which are contained in Propolis extract, act on the cell wall or membrane, causing structural damage. The virulence factor coagulase was fully repressed by EEP in a research on Staphylococcus, although lipase was reduced (Scazzocchio, 2006). Propolis' antibacterial power can be harnessed in combination with specific antibiotics, which will prove to be a medical blessing.

Antibiofilm activity

A biofilm is a clump of cells that attach to each other and to the surface by producing a slimy matrix made of lipids, polysaccharides, proteins, and DNA on the outside of the cells (Flemming, 2010). Biofilms pose a significant risk to invasive devices and can induce a variety of chronic infections. Biofilms are responsible for the majority of microbial illnesses in the body, according to the National Institutes of Health (NIH). For three reasons, biofilms innate antimicrobial resistance persists Antimicrobial drugs must diffuse into the extracellular polymeric substance (EPS) matrix to inactivate microorganisms within the biofilm. EPS reduces antimicrobial diffusion by interacting chemically with them or decreasing their transport rate. Second, biofilm-associated organisms have slower growth rates, which reduces the pace at which antimicrobial drugs enter the cell and so affects inactivation kinetics. Third, the environment immediately around the cells inside a biofilm may offer the organism with more protective conditions. Researchers have been compelled to develop new techniques to attack bacterial and fungal biofilms due to the limitations of antibiotic penetrance and resistance. As an antibiofilm agent, propolis has a lot of promise. A number of studies have been conducted to determine propolis anti-biofilm effectiveness against a variety of biofilm-forming species, including *Pseudomonas aeruginosa*, a prevalent pathogen in lung and skin diseases. *P. aeruginosa* biofilm formation, as well as swimming and swarming motility, were observed to be inhibited by propolis and bud poplar resin extracts (Marco, 2017). Propolis extracts were prepared and tested for antibiofilm activity against *Staphylococcus aureus* (MSSA) and methicillin resistant *Staphylococcus aureus* clinical strains in hexane, ethyl acetate, and ethanol. Ethyl acetate and ethanol extracts of propolis were shown to be more effective than hexane extracts in inhibiting biofilm formation in the bacteria investigated (Tosi, 1996).

Propolis from Manisa, Turkey, was tested against *Listeria monocytogenes*, a biofilm-forming bacteria, and Methicillin Sensitive Bacteria. The key compounds discovered in propolis extract responsible for the activity were *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas fluorescens*, *Micrococcus luteus*, and triacontyl acetate (Doganli, 2016). The most prevalent genital infection in women is vulvovaginal candidiasis (VVC)and the effect of a propolis extract on biofilm development by *Candida albicans* isolated from VVC patients has been studied (Capoci, 2017).The biofilms of vaginal isolates of *C.albicans* was exposed to propolis extract and tested for colony-forming units, proteins and carbohydrates content ,the studies revealed that reduction in cfu, carbohydrate and protein content in biofilm matrix and deformation of biofilm shown by scanning electron microscope (Capoci, 2015).Converting Propolis to nanopropolis form or manufacturing propolis nanoparticles can improve their release, anticancer efficacy, and antibiofilm efficiency. Nanopropolis is a nanosized propolis that has been joined together using various methods to improve its efficiency without changing its qualities. Nanoparticles are microscopic particles with at least one dimension smaller than 100 nanometers. The surface area to volume ratio of nanoparticles is very high. They can act as a powerful catalyst for diffusion. Human liver cancer (HepG2) and human colorectal cancer (HCT 116) cells were used to test the anticancer efficacy of Propolis loaded nanoparticles. The researchers discovered that propolis nanoparticles filled with propolis were more cytotoxic than free propolis. The propolis-loaded naoparticles cause apoptosis and drastically reduce the number of cells in the proliferative stages (Elbaz, 2016.

Enterococcus faecalis,a widespread bacterium that develops biofilm that are associated with hospital-acquired infections, that resist the penetration of anti-bacterial agents. The antimicrobial and anti-biofilm properties of Propolis, formed into nanoparticles with chitosan, were evaluated. E. faecalis biofilm *was reduced by 90%* at 200 µg/ml revealed by Scanning Electron Microscope confirmed the disruption of biofilms.Chitosan-propolis nanoformulation can be a potent anti-biofilm agent for curing infections involving biofilm formation .(Chitra, 2017).

Staphylococcus epidermidis is a common microbe found in the human body that can cause infections linked to indwelling devices. It is antibiotic-resistant, necessitating the use of naturally occurring antibacterial medicines. Malaysian propolis, has antibacterial and antibiofilm characteristics.

Malaysian propolis was used to make chitosan-propolis nanoparticles (CPNP), which were then tested against S. epidermidis. The cationic nanoparticles increased the net electric charge of S. epidermidis from -17 to -11 mV in a concentration-dependent manner, whereas the ethanol (Eth) and ethyl acetate (EA) extracts of propolis reduced the zeta potential from -17 to -20 mV. According to confocal laser scanning microscopy (CLSM), CPNP efficiently disrupted *S. epidermidis* biofilm formation and decreased viability to 25% compared to Eth and EA, which had viability of 60–70%. When compared to Eth and EA, CPNP was more effective at reducing the viability of both planktonic and biofilm microorganisms (Ramamurthy, 2019).The normal infections caused by planktonic cell can be combated by antibiotics but biofilms formed by sessile cell are very tough to be dealt with. The propolis is a strong scavenger for biofilm both as conventional form and nanoparticulate form which can open the ways for the effective use of propolis for combating the biofilms on in dwelling devices.

In the modern era of drug resistance, dearth of new antimicrobial compounds and search for eco-friendly alternatives to chemical compounds, Propolis is a promising alternative. A multi constituent, bee product, easily available, biodergradable, and biocompatible offers anti-microbial, anti-oxidant, anti-biofilm potential. The present article extensively reviews the different benefits of Propolis in laboratory and clinical settings, both in native and nanoparticulate forms, highlighting its diverse applications in future.

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