

Impact of Propolis on the Oral Health

SUMMARY

Propolis is a natural resinous substance collected by honey bees from buds and exudates of plant species, mixed with bee enzymes, pollen and wax. It has a complex composition with a wide range of effects, including antibacterial, antiviral, antifungal, antiflogistic, antioxidant, hepatoprotective, carcinostatic and immunomodulatory properties. It is often applied in the treatment of diseases involving the oral cavity and gums. The aim of this paper is to describe the therapeutic properties of propolis, chemical composition and its application in the oral cavity. Literature and systematic information on the composition and the effects of propolis on health were collected, with particular reference to the use in the treatment of oral cavity diseases. The chemical composition of propolis is very complex. The health impact depends on the biologically active components it contains. A particularly important application is in the treatment of diseases of the oral cavity. Studies show that propolis can help prevent dental caries and control gingivitis and plaque. It reduces halithosis (bad breath) and symptoms of periodontosis. It is also effective in fighting viruses. It can have significant application in orthodontics and restorative dentistry. A wide range of effects allows the multiple uses of propolis-based products. Recent research has been increasingly focused on diseases of the oral cavity. The development of novel propolis-based pharmaceutical forms could significantly reduce the use of antibiotics in conventional treatment of diseases of the oral cavity.

Key words: Propolis, Oral Health, Apitherapy, Oral Cavity

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Introduction

Propolis, also referred to as bee glue, is a natural, non-toxic, resinous and sticky substance produced by honey bees through the mixing of hypopharyngeal gland secretions with digestive resin products collected from buds and bark of trees, flowers, leaves and other botanical sources^{1,1}. Propolis most often originates from trees such as poplar, willow, beech and wild chestnut². Bees use propolis to protect and strengthen their hive. With this natural product, the hive is protected from rain and pests such as insects and rodents. Propolis maintains aseptic conditions and the appropriate temperature within the hive. Propolis acts as a biocide that successfully fights bacteria, mushrooms and larvae of certain pests³. In order

to eliminate the potential infection within the hive, the bodies of dead pests are covered with propolis and thus prevent their decomposition⁴.

Propolis is a lipophilic material that is firm and fragile at lower temperatures, while flexible and sticky at higher temperatures. Its melting point is between 60°C and 70°C, and for some samples the temperature can go up to 100°C⁵. The best solvent used for the preparation of propolis is ethanol. Other solvents such as ethyl ether, water, methanol and chloroform can be used for the extraction and identification of propolis components⁶. Gloverin and propylene glycol are used in the preparation of propolis for the pharmaceutical and cosmetic industries⁷. It has a pleasant aromatic scent and can be, depending on origin and botanical origin, red, brown, yellow or green in colour^{8,9,10}.

Chemical composition of propolis

The chemical composition and content of the biologically active compounds of propolis depends on the geographical and botanical origin, the type of bees, and the seasons in which propolis is collected¹¹. Raw propolis consists of about 50% resin, 30% wax, 10% essential oils, 5% pollen and 5% of various organic compounds^{12,13,14}. Techniques for separating and purifying the mixture, such as high pressure liquid chromatography - HPLC, thin layer chromatography - TLC¹⁵, gas chromatography-GC, as well as identification techniques such as mass spectroscopy - MS¹⁶, nuclear magnetic resonance-NMR, gas chromatography in combination with mass spectroscopy- GC-MS¹⁷, has identified several compounds within propolis including flavonoids, terpenes, phenols and their esters, sugars, hydrocarbons and mineral elements. In contrast, relatively frequent phytochemicals such as alkaloids and iridoids have not been detected¹⁸.

The action of propolis on human health

Propolis exhibits a wide range of biological properties, and one of the most significant characteristics is its antimicrobial activity. This is supported by a large number of publications¹⁹. Table 1 below describes the biological activity of propolis components.

Research has shown that propolis extracts, which are composed of many polyphenols, have a strong antioxidant activity^{20,21,22}. Geographically and botanically different propolis samples have a different chemical composition, which directly affects their effect as antioxidants²³. Fabris *et al.* (2013) showed that ethanolic extracts of Russian and Italian propolis have a similar antioxidant effect because they have a similar polyphenol composition, while, on the other hand, the ethanolic extract of Brazilian propolis exhibits significantly less antioxidant effect because it contains a smaller amount of polyphenols in its composition. Phenolic acids and flavonoids exhibit a strong antioxidant effect, which is closely related to the chemical structure of the components²⁵.

Table 1. Biological activity of different propolis components²⁶

| Component, propolis type | Biological activity | References |
|--|---|---|
| Polyphenols and flavonoid (in all types of propolis) | Antibacterial, antiviral, antiallergic antimicrobial, antitumor, antioxidant, immunomodulatory, hepatoprotective, cardioprotective. | Almeida and Menezes, 2002 ²⁷ ; Havsteen, 2002 ²⁸ , Ghisalberti, 1979 ² |
| Caffe-phenethyl ester acids (Topola, Baccharis) | Antioxidant, antitumor, antiinflammatory, antibacterial, antiviral, fungicidal, cardioprotective immunomodulatory. | Bankova, 2009 ²⁹ , Bankova <i>et al.</i> , 2007 ³⁰ |
| Caffeic acid (Topola, Baccharis). | Antiviral, antioxidant, antitumor | Farooqui T. and Farooqui A., 2010 ³¹ |
| Artepelin C (Baccharis) | Antioxidant, antiinflammatory, antitumor | Bankova, 2009 ²⁹ ; Bankova <i>et al.</i> , 2007 ³⁰ |
| Terpene (terpens) (Greece, Croatia, Brazil) | Antibacterial, antimicrobial | Bankova, 2009 ²⁹ ; Bankova <i>et al.</i> , 1995 ³² |
| Essential oils (Brazil, Poland) | Antibacterial | Bankova <i>et al.</i> 1995 ³² ; Zwolan and Meresta, 2000 ³³ |

According to research, Propolis is much more effective in combating Gram-positive bacteria than against Gram-negative bacteria^{34,35}. This is due to the fact that the membrane of Gram-negative bacteria exhibits greater complexity in the material than the Gram-positive bacteria. In the suspension of bacteria, certain components of propolis can be destroyed by hydrolytic enzyme of the bacteria. Some of the mechanism of action by which propolis exhibits its' antimicrobial effect, is through the suppression of permeability of the bacterial membrane and inhibition of bacterial mobility. Propolis can affect the flow of ions through the inner bacterial membrane and lead to disturbance of the membrane potential, that affect the electrochemical gradient that is necessary

for the production of adenosine triphosphate (ATP) required for the smooth flow of the membrane transport and for maintaining its mobility³⁶. The polypeptide directly affects the organisms in vitro. On the other hand, propolis can indirectly participate in the destruction of microorganisms by stimulating the in vivo immune system and activating the mechanisms responsible for killing microorganisms³⁷. The propolis can be combined with antimicrobial drugs because it has been proven that propolis reduces bacterial wall resistance on antibiotics and has a synergistic effect with antibiotics that work on ribosome's, but does not show interaction with antibiotics that affect DNA or folic acid^{38,39,40}.

Propolis has also been shown to have a significant antiviral effect. It acts at different levels and impedes the replication of certain viruses such as herpes simplex type 1 and 2, adenovirus type 2, influenza virus, human immunodeficiency virus (HIV), and others. By research, propolis can exhibit antiviral activity by causing partial blocking of viral penetration into the cell, affecting

the steps in the viral replication cycle, and leading to degradation of the RNA virus before penetration in a cell or after its release into the supernatant⁴³. Certain clinical trials in the male and female populations have shown that application of fat containing propolis can result in faster healing in genital herpes compared to conventional acyclovir treatment⁴².

Table 2. Pathogenic bacteria, fungi, viruses and parasites on which propolis acts¹⁹

| | |
|--------------------------------|--|
| Gram-positive bacterias | <i>Bacillus cereus</i> , <i>Bacillus mesentericus</i> , <i>Corynebacterium sp.</i> , <i>Corynebacterium diphtheriae</i> , <i>Diplococcus pneumoniae</i> , <i>Enterococcus sp.</i> , <i>Mycobacteria sp.</i> , <i>Mycobacterium tuberculosis</i> , <i>Staphylococcus aureus</i> , <i>Streptococcus: critecus</i> , <i>epidermis faecalis</i> , <i>mutans</i> , <i>pyogenes</i> , <i>viridans</i> , <i>sobrinus</i> |
| Gram-negative bacterias | <i>Branhamella catarrhalis</i> , <i>E. coli</i> , <i>Helicobacter pylori</i> , <i>Klebsiella ozaemae</i> , <i>Proteus vulgaris</i> , <i>Pseudomonas aeruginosa</i> , <i>Salmonella: choleraesuis</i> , <i>dublin</i> , <i>enteritidis</i> , <i>exneri</i> , <i>gallinarum</i> , <i>pullorum</i> , <i>paratyphi-A</i> , <i>paratyphi-B</i> , <i>Shigella: dysintariae</i> , <i>sonnei</i> |
| Fungus | <i>Aspergillus sp.</i> , <i>Candida: albicans</i> , <i>guiliermondi</i> , <i>parapsilosis</i> , <i>tropicalis</i> ; <i>Cryptococcus sp.</i> , <i>Cyptococcus neoformans</i> , <i>Histoplasma encapsulatum</i> , <i>Madurella mycetomi</i> , <i>Microsporium: audoinini</i> , <i>canis</i> , <i>cepleo</i> , <i>distortum</i> , <i>ferrugeneum</i> , <i>gypseum</i> ; <i>Piedra hortae</i> , <i>Phialophora jeanselmei</i> , <i>Saccharomyces sp.</i> , <i>Trichophyton: sp.</i> , <i>Mentagrophytes</i> , <i>rubrum</i> , <i>Trichosporon cutaneum</i> |
| Viruses | <i>Adenovirus</i> , <i>Coronavirus</i> , <i>Hepres simplex</i> , <i>Infulenca A and B virus</i> , <i>virus Newcastle disease</i> , <i>Polio virus</i> , <i>Vaccinia</i> , <i>Rotavirus</i> ; <i>Vesicular stomatitis</i> , <i>Coronavirus</i> |
| Parasites | <i>Cholomonas paramecium</i> , <i>Eimeria: magna</i> , <i>media</i> , <i>perforans</i> ; <i>Giardia lambia</i> , <i>Giardia duodenalis</i> , <i>Trichomonas vaginalis</i> , <i>Trypanosoma cruzi</i> , <i>Trypanosoma evansi</i> |
| References | Banskota et al., (2001) ⁴³ ; Burdock (1998) ¹² ; David et al (2012) ⁴⁴ ; Ghisalberti (1979) ² ; Gressler et al., (2012) ⁴⁵ ; Marcucci (1995) ⁹ ; Tikhonov et al., (1998) ⁴⁶ |

Propolis have been shown to exhibit antifungal action against *C. albicans*, *C. tropicalis* and *C. krusei*⁴⁷. Propolis acts on the aflatoxicogenic types of *Aspergillus* such as *Aspergillus flavus* by inhibiting the condom germination⁴⁸. Propolis also acts on numerous parasites (Table 2).

Antiparasitic and immunomodulatory activity of Brazilian propolis on *Leishmania braziliensis* was investigated⁴⁹. Propolis directly affected the parasite and exhibited immunomodulatory effects on murine macrophages, although it has been shown that the parasite continues to affect the activation pathways of the cell. Components important for the antiparasitic action are phenolic compounds (flavonoids, aromatic acids, benzopyrene), di- and triterpenes; and essential oils found in the propolis sample⁴⁹.

Various in-vitro studies have demonstrated the cytotoxic effect of propolis on tumor cells. In-vivo studies have also shown that there is potential for the development of new antitumor drugs; showing no adverse effects when tested on rats⁵⁰. This natural product is able to block oncogenic signaling pathways, which in turn leads to decreased proliferation and cell growth. It also reduces the population of tumor stem cells by increasing apoptosis, preventing angiogenesis and modulating tumor micro-circulation^{51,52,53}. Caffe-phenethylether acids and artepelin C are distinguished as components possessing

antitumor properties. Other distinguished components include: chrysin, nemesone, galangin and cardanol⁵¹

Propolis modifies non-specific immunity. Propolis and its constituents, such as cinnamic acid and its p-coumarinic derivative, stimulate the production of TNF- α (tumor-alpha necrosis factor) and interleukin (IL)-1 β in mouse macrophages^{54,55}. The expression of cellular receptors such as toll-like receptors TLR-2 and TLR-4 was also increased in peritoneal macrophages of propolis-treated mice⁵⁶. A study on the effect on reactive oxygen species also showed that propolis stimulates the formation of hydrogen peroxide (H₂O₂) through mouse macrophages while reducing the production of nitric oxide (NO)³⁸. In a second study however, the inhibitory effect on the production of superoxide anions by rabbit neutrophils was demonstrated by the propolis compounds⁵⁷. In humans, propolis can exhibit immunomodulatory effects on cellular receptors, as well as in the production of cytokines and the fungicidal activity of monocytes, depending on the concentration. It increases the expression of TLR-4 and CD80 receptors, influences the production of TNF- α and IL-10 and increases the fungicidal activity of monocytes⁵⁸. Cinnamic acid reduces the number and activity of TLR-2, HLA-DR and CD80 receptors, and increases the activity of TLR-4 receptors. High concentrations of cinnamic acid inhibit the production of TNF- α and IL-10, while the same concentrations encourage stronger fungicidal activity

against *C. albicans*⁵⁹. Propolis stimulates the production of antibodies; independent of the year of propolis collection and its origin. This was confirmed by a 2005 study in which scientists used Brazilian and Bulgarian propolis as an auxiliary in rats immunized with bovine serum albumin⁶⁰.

Propolis can exhibit pro-inflammatory and anti-inflammatory effects depending on the concentration, entry period and experimental conditions and can stimulate or inhibit certain processes. However there is still little evidence of the clinical efficacy of propolis in this manner⁶⁰.

Propolis also has a beneficial effect in the treatment of wounds due to its antifungal and antibacterial abilities in view of the presence of certain components such as: flavonoids, phenolic compounds, terpenes and enzymes. It reduces the amount of free radicals (reactive oxygen species) and thus facilitates the wound healing process. It participates in collagen metabolism by increasing the synthesis of collagen type I and type III collagen in tissues. Propolis is a potential apitherapy agent that has the ability to modify the metabolism of fibronectin. It develops a fibrous network of extracellular matrix and inhibits the fibronectin disintegration. Components such as quercetin and resveratrol inhibit the fibronectin biosynthesis and TGF- β (transforming growth factor β) dependent production of fibronectin in C2C12 myoblasts. Both components play an important role in the expression of fibronectin. Studies have shown that the mobility of epithelial cells depends on the reduced content of fibronectin in the extracellular matrix. Reduced amounts of this glycoprotein allow propolis to better cure wounds and produce granulation tissue⁶¹.

The oral cavity

The oral cavity represents the proximal part of the digestive system and plays a role in chewing and ingestion of food, as well as speech. The major components of the oral cavity are the teeth, tongue and the salivary glands. The mucous membrane of oral cavity has the role of protecting organs by allowing absorption and resorption of the substances, preventing the non-physiological change of substances and stimulating the secretion of harmful substances from the organism⁶².

The most common diseases of the oral cavity include:

1. Dental caries (tooth decay)
2. Gingivitis – inflammation of the gums
3. Periodontitis - inflammation of periodontium (tissue that supports the teeth)
4. Other diseases of the oral mucosa: angular cheilitis, oral herpes, oral candidiasis, exfoliative glossitis, prosthetic stomatitis (denture stomatitis), aphthous ulcer, and others⁶³.

Propolis and oral cavity health

Early animal studies have shown that propolis significantly reduces dental caries in rats as a result of multiple effects on the bacterial flora. It limits the number of microorganisms, slows down the synthesis of insoluble glucans, and slows down the activity of glucosyltransferase enzyme⁶⁴. This natural product has oral cavity activity due to its high fatty acid content such as oleic, linoleic, palmitic and stearic acids which slows the production of acid by *Streptococcus mutans* and reduces the tolerance of microorganisms to the acidic pH⁶⁵. Propolis also have a lower cytotoxic effect on fibroblasts (found in gums) compared to chlorhexidine (also used in caries prevention), suggesting that propolis can be used as an ingredient in mouthwash⁶⁶. Majority of studies uses propolis as a mouthwash in the form of aqueous and alcoholic solution^{67,68} or in the form of toothpaste⁶⁹. Propolis can be used in the form of a solution for the decontamination of fibers on the toothbrush⁶⁹.

Bacteria such as *Tannerella forsythensis*, *Porphyromonas gingivalis* and *Treponema denticol* make up a red complex of microorganisms that increases the depth of the periodontal pocket and causes bleeding of the gums. The propolis extract shows high efficacy in preventing the growth of bacteria belonging to the red complex⁶⁸. Clinical examination has shown that the 3% ethanolic propolis extract in the form of gel and paste slows down and ultimately prevents pathological changes in patients at an increased risk of occurrence of dental plaque gingivitis⁷¹. Based on a clinical trial of 25 patients, a non-alcoholic mouthwash containing 5% Brazilian green propolis proved effective in controlling plaque and gingivitis, suggesting its use in treatment and prevention periodontal diseases. However, a double-blinded randomized trial is needed before final clinical use in the dentistry industry⁷². The preventative effect of propolis on periodontal tissues implies a slowing down of the calcium phosphate precipitate formation process and can therefore be used as ingredient in mouthwash and toothpaste, hence limiting the accumulation of dental plaque⁷³.

Halitosis is a frequent or permanent existence of bad breath from the mouth, and is closely related to the hygiene of the oral cavity. By-products from the degradation of microorganisms in the oral cavity are one of the main causes of halitosis⁷⁴. The microbes most commonly responsible for the onset of aching and halitosis include *Prevotella intermedia*, *Porphyromonas endodontalis* and from the bacteria of the genus *Eubacterium*⁷⁵. By measuring the content of volatile sulfur compounds in the exhaled air through the halometer, it was concluded that propolis significantly reduces halitosis⁷⁶.

Propolis has been shown to affect certain etiological factors that lead to the development of periodontal

disease. Therefore, some researchers use it as part of their therapeutic protocol in the treatment of periodontitis⁷⁷. A micro-adhesive hydrophilic gel containing propolis, when applied to gingival pockets was also shown to be efficacious⁷⁸. Based on clinical and microbiological parameters, the subgingival flushing with propolis extract as an auxiliary agent in the treatment of periodontitis proved more effective than the conventional method of scraping and pollinating roots¹.

Studies have also been carried out on animals where propolis was given per os to determine if its systemic effect through circulation has positive action on oral cavity health. Morphological and histological pictures showed that oral propolis administration in rats prevents the loss of alveolar bone from periodontitis⁷⁹.

Herpes simplex type 1 is a virus that causes skin and mucous lesions on the membranes of the mouth. It is one of the most common human pathogens⁸⁰. Propolis is used locally in the treatment of oral cavity lesions caused by viruses, in studying its antiviral capabilities. As a propolis mixture, it is more effective in combating viral diseases compared to its individual components separately⁸¹. Propolis slows down changes in skin and virus growth in the early stage of infection with Herpes simplex type 1 and is not cytotoxic on healthy cells⁸².

Bee glue also is used in the treatment of recurrent aphthous stomatitis (canker sores). Although aphthous stomatitis is a relatively common disease whose symptoms are visible in the form of aphthous ulcers in the mouth; the etiology of the disease has not yet been established which significantly complicates the treatment⁸³. Propolis reduces the frequency of the disease and improves the quality of life in patients⁸⁴.

Dental avulsion is the traumatic displacement of a tooth from its socket in the alveolar bone. In oral surgery, propolis is used in the replantation of a broken permanent tooth and provides support in the healing process after surgery. Maintenance of periodontal cells is one of the key factors to determine success of tooth replantation. A research was carried out to determine the medium that provides the best protection during tooth replantation. Propolis as a transport medium showed positive results⁷⁷. According to the research by Ozan *et al.*, a 10% propolis solution showed better results compared to a 20% propolis solution, Hank's balanced saline solution (HBSS) or milk. A new study also showed the extraordinary effectiveness of propolis in not only reducing apoptosis of periodontal cells, but also increases metabolism and cell proliferation⁸⁶. Margo-Filho and Carvalho⁸⁷ have proven that locally applied propolis helps to heal wounds after surgery in the oral cavity, reduces inflammation and also acts as an analgesic. Propolis accelerates the epithelization and formation of granulation tissue in the area of healing⁸⁸.

In the case of mal-occlusion followed by narrowing of the upper jaw, it is necessary to use an orthodontic

device for the expansion of the palatal suture. During treatment, bone remodeling occurs in the area of palatal suturing⁷⁷. Research on rats showed that propolis solution helps in bone formation during treatment with orthodontic appliances that lead to the spread of palatal suture. The results of this study showed an increased amount of osteoblastic activity in rats who received propolis in treatment and faster bone remodeling⁸⁹.

In restorative dentistry, propolis is used to reduce the permeability of dentine and in direct overlapping of the pulp to form reparatory dentin⁷⁷. Ahangari *et al.*⁹⁰ have proven that propolis acts more efficiently in direct overlapping of the pulp compared to products of calcium hydroxide most commonly used for this purpose. It stops the inflammatory reaction, the infection of the microbes and the necrosis of the pulp and encourages the formation of high-quality tubular dentin by stem cell stimulation. The stimulatory effect on tooth pulp is conditioned by the presence of flavonoids in propolis extracts⁹¹.

One of the aims of endodontic treatment is the elimination of microorganisms in the root canals of teeth⁹². The efficacy of drugs is reflected in the *Enterococcus faecalis* test that is resistant to adverse conditions and can survive in the root canal system despite the use of certain medicines⁹³. The study has shown that it significantly reduces the number of cultured bacteria *Enterococcus faecalis*, but that it is not superior to chlorhexidine⁹⁴. However, due to the low level of periapical tissue and protective effect on periodontal cells, propolis can be used in the disinfection of the root canals of the teeth⁹⁵.

Prosthetic stomatitis is a common disease in people using dental prostheses. The etiological factors of the disease are: infection with *Candida albicans*, improper hygiene of the oral cavity and excessive use of prosthesis⁹⁶. Products based on propolis show strong anti-fungal effects on various types of *Candida*, and the most sensitive to propolis is *Candida albicans*⁴⁷. The most commonly used form of propolis for prosthetic stomatitis is as a mouthwash⁴⁷ or as a gel for local application. Acrylic resin is one of the materials used to make dental prostheses. Da Silva *et al.*⁹⁸ showed that propolis in the form of a gel can adversely affect the acrylic resin in a way that makes it rough and more prone to adherence to microorganisms.

Potential adverse reactions of propolis

Apart from being a resinous substance with multiple usages (as described above), propolis is also a known sensitizer; as highlighted by Menniti-Ippolito *et al.*¹⁰⁰ in their report. There were 18 suspected adverse reactions involving propolis-based products that were reported between April 2002 and August 2007 to the Italian National Surveillance System.

It is advised to not be used by patients with predisposition to allergies, especially towards pollen and honey, as well as by individuals with atopy or asthma. The study concluded that healthcare practitioners and the general public must be made aware of the potential risk of allergic reactions of consuming products derived from bees, and that a warning label should be visible on product packaging.

In addition, a case report by Hay and Greig^{101,102} also supported the antigenic property of propolis and further suggests to consider delayed contact sensitivity reactions by propolis as a differential of oral mucosal lesions.

Apart from the case report by Hay and Greig¹⁰¹, another case report by Budimir *et al.*¹⁰³ also noted adverse effects by individuals using self-prescribed propolis products. These adverse effects include oral mucositis, contact cheilitis and perioral dermatitis. However these case reports highlight the self-treatment with propolis-based products rather than through prescription.

It is concluded that despite several case reports stating the adverse reactions experienced by self-prescribing individuals, proper patient education, prescription and treatment monitoring of propolis-based products has a bigger benefit potential.

Conclusions

With the development of modern methods of analysis, new knowledge about propolis activity on human health has emerged. Its mechanisms of action are still being investigated, which will likely lead to the development of new products that affect the health of the oral cavity. Coupled with patient education, proper prescription and treatment monitoring, the benefits of propolis-based products, such as, antibacterial, antiviral, antifungal, anti-inflammatory, antioxidant and chemopreventive actions can be utilised. This may significantly reduce the use of conventional treatments and antibiotics, shifting towards the usage of propolis in the management of oral cavity conditions.

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