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ANTIMICROBIAL AND ANTIVIRAL PROPERTIES OF DIFFERENT TYPES OF PROPOLIS

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Summary. Propolis is produced by bees; it is a viscous, resinous substance mainly derived from trees, shrubs, and flower buds, enriched in wax, essential oils, pollen, and bee saliva. The composition of propolis varies widely depending on the region of origin and vegetation that occurs in the area, the climate, and the season in which it is produced. So far, more than 400 substances have been identified in propolis and among them are phenols, flavonoids, phenolic acids and their esters and flavones. Propolis is divided into several types; the most common types are poplar, European, Brazilian, and pacific propolis. Antimicrobial, antifungal, and antiviral effects of propolis have been demonstrated. Propolis inhibits Gram-positive bacteria and, to a lesser extent, Gram-negative bacteria. Propolis also inhibits the growth of mold (*Aspergillus* and *Penicillium*) and yeast (*Candida*). The antiviral activity of propolis against poliovirus, influenza A and B viruses, reoviruses, and HIV has been demonstrated.

Key words: antimicrobial propolis activity, antiviral propolis activity, chemical composition, propolis

INTRODUCTION

Propolis, also called bee putty or bee glue, is a natural product of plant origin, collected and processed by bees (*Apis mellifera*) [Burdock 1998, Bankova 2005, Graikou et al. 2016]. It is made of resinous substances derived from trees, bushes, and flower buds; beeswax, pollen, bee pollen; and enzymes derived from saliva of bees. Resins from which propolis is formed are found on the plant parts that are most exposed to the activity of microorganisms, serving to protect them against microbial contamination [Bankova et al. 2016]. The composition of propolis depends on the region and climate in which it is produced and the vegetation that occurs in the area. For the production of propolis, bees

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use various species of poplar and alder in temperate climates [Burdock 1998]. Propolis is a substance with the following characteristics: has a malleable consistency; is adhesive; creamy in color, dark yellow, orange or brown, and sometimes even green; and has a very intense odor [Ertürk et al. 2016]. Propolis is hard at 15°C, is plastic and soft at 36°C, and is liquid in the range 70–80°C. Bees use it to seal the hive and to hygienize its interior [Waldner-Tomic et al. 2014]. It is used in the pharmaceutical, cosmetic, and food industries.

The aim of the study was to present the latest results of antimicrobial and antiviral properties of propolis. The chemical composition, types of propolis, as well as antibacterial, antifungal and antiviral properties of propolis have been discussed.

CHEMICAL COMPOSITION OF PROPOLIS

Propolis consists of resinous substances of plant, pollen, bee pollen, beeswax, and secretions of bee glands. Propolis may contain mechanical elements such as frame fragments and dead bee parts. In general, the chemical composition of propolis comprises the following: resin substances accounting for about 50%, 30% of beeswax, 10% of aromatic substances and oils, and 5% of pollen and mechanical admixtures [Burdock 1998].

Research on the chemical composition of propolis has been going on for many years. Almost 420 chemicals, including those from agricultural chemicals [De Groot et al. 2014], have been identified in the samples of propolis from different geographic regions of the world [Milojkovic-Opsenica et al. 2016].

The following are the chemical compositions of propolis: aromatic acids, such as cinnamic acid, caffeic acid, ferulic acid, benzoic acid, salicylic acid, 2-amino-3-methoxybenzoic acid, and aromatic esters, such as cinnamic and caffeic acid ether ester, benzoic acid phenylmethyl ester. In addition, volatile compounds, such as geraniol, nerol, farnesol, β-eudesmol, caryophyllene, aromatic compounds, such as vanillin, hydrocarbons, such as eicosane, tricosane, pentacosane, hexadecanol, steroids, such as cholinasterol, fucosterol, stigmasterol, enzymes, such as α-amylase, β-amylase, α-lactamase, β-lactamase, maltase, esterase, and transhydrogenase, have been identified. The presence of flavonoids such as tectochrysin, pinocembrin, chrysin, galangin, genkwanin, apigenin, kaempferol as well as 5-hydroxy-4',7-dimethoxyflavone, pinostrobin chalcone, pilloin was identified [Haung et al. 2014, Kubiliene et al. 2015].

Propolis is rich in the following micro- and macro-elements: calcium, magnesium, potassium, sodium, iron, zinc, manganese, aluminum, barium, strontium, and chlorine. The bioelement content in propolis is about 0.6%. In Argentine propolis, 8 trace elements (Br, Co, Cr, Fe, Rb, Sb, Sm, and Zn) have been isolated, the presence of which is correlated with the region of propolis production. Vitamins B1, B2, B6 as well as vitamin C and vitamin E were also identified in propolis samples [Cantarelli et al. 2011].

Propolis also contains non-oil substances, waxes insoluble in ethanol, and beeswax derivatives, ranging 4.8–19.3%. The composition of beeswax and their derivatives consists of, among others, organic acids, hydrocarbons, esters, monohydric alcohols, hydroxy acids, and dihydroxy alcohols. The following are the most common acids found in propolis: palmitic acid, melissic acid, cerotic acids. Beeswax also contains small amounts of alcohols, carotenoids, cholesterol ester lactones, and flavonoids (chrysin). The composition of beeswax may vary depending on the species of bees, their genetics, and the external environment. Approximately 3–5% of the chemical composition of propolis consists of essential oils, mainly monoterpenes and sesquiterpenes [Negri et al. 2000, Kalogeropoulos et al. 2009].

TYPES OF PROPOLIS

Referring to literature data, there are several types of propolis, depending on its botanical and geographical origin and chemical composition. The most common is poplar, European, Brazilian, and pacific propolis [Bankova et al. 2005]. Poplar type of propolis occurs in Europe, North America, and non-tropical regions of Asia. It is made from various species of poplars (*Populus* spp.), most commonly from *P. nigra*. The chemical composition of poplar propolis mainly comprises flavones, flavanones, cinnamic acids and their esters [Bankova et al. 2005], and mono and sesquiterpenes dominate the composition of essential oils [Ristivojević et al. 2015]. Two further subtypes of poplar propolis, i.e. blue and orange, are distinguished, which differ in chemical composition, because of the intensive development of chromatographic methods and their application in the chemical characteristics of propolis [Morlock et al. 2014, Ristivojević et al. 2015].

Some researchers also distinguish the green type of poplar propolis [Chasset et al. 2016]. Biomarkers present in the blue propolis are kaempferol, caffeic acid, luteolin, while flavonoids such as quercetin and pinocembrin, tectochrysin, and pinobanksin are present in orange propolis.

In the chemical composition of poplar propolis, compounds such as coumaric acid, chrysin, galangin, pinobanksin-3-acetate are also present in both subtypes. The following are biomarkers of green poplar propolis: apigenin, galangin, apigenin-methyl-ether, and galangin-methyl-ether [Morlock et al. 2014, Ristivojevic et al. 2015, Chasset et al. 2016]. Europe is dominated by orange type of propolis [Opsanica et al. 2016]. However, besides the geographical origin of the region, the chemical composition of propolis is influenced by several other factors as follows: accumulation of phytochemicals, sunlight, humidity, and even soil mineral components [Opsanica et al. 2016].

Red propolis (clusia propolis) comes from Cuba and Venezuela, and its plant source is clusia (*Clusia* spp.). The chemical composition of red propolis consists of formononetin, biochanin A, pinocembrin as well as a high content of propyl gallate, catechin, epicatechin, methyl eugenol, *trans*-anethole, *trans*-methyl isoeugenol, triterpenic alcohols, isosativan, and xanthochimol. Red propolis has proven antimicrobial and antifungal properties, as well as liver protecting, analgesic, and anti-inflammatory activities. It is considered a nutriceutic that can be used to protect food from spoilage [Bankova 2005, Lopez et al. 2015, Mendonça et al. 2015].

Brazilian green propolis is another described type (alecrim propolis) obtained from *Baccharis dracunculifolia*, which is characterized by a particularly high content of prenylated phenylpropanoids (e.g. artepillin C) as well as 3-prenylcinnamic acid allyl ester, sesquiterpenes (e.g. farnesol), diterpenes (isocupressic acid derivative), propolis-benzofurans A and B, and agathic acid. In addition, kaempferol, kaempferide, cinnamic acid, caffeic acid, and ferulic acid were detected in this propolis [Weinstein et al. 2005, Fernandes-Silva et al. 2013].

In Russia, birch propolis is distinguished. Birch (*Betula verrucosa*) is used in the production of this propolis. The main components of birch propolis consist of flavones and flavonoids, but other than poplar propolis [Bankova 2005].

In 2015, Mediterranean was another type of propolis that was described. It is obtained from the conifers of *Cupressaceae* and *Pinaceae* family. Its composition is mainly diterpene. Propolis from Greece and Cyprus contains a lot of terpenes and aromatic compounds, which have strong anti-microbial properties [Graikou et al. 2016].

ANTIBACTERIAL PROPERTIES OF PROPOLIS

Propolis has a broad spectrum of action against bacteria, although its effect on microorganisms is not the same. Bactericidal and bacteriostatic effects are caused by the synergistic action of many propolis components [Mirzoeva et al. 1997]. Gram-positive bacteria are much more sensitive to propolis than Gram-negative bacteria. This is probably because the outer membrane of Gram-negative bacteria exhibits a special protective mechanism against the intrusion of propolis components into the cell [Tegos et al. 2002]. Some components of propolis, especially cinnamic derivatives (caffeic acid and caffeic acid phenethyl ester – CAPE) and flavonoids (quercetin and naringenin), negatively affect the potential of bacterial cell membrane, increasing its permeability and causing decreased motility. Denatured proteins (e.g. enzymes) were also shown after high doses of phenolic compounds contained in the ethanol extract of propolis (EEP). It was also found that caffeic acid phenethyl ester (CAPE) inhibited the cell division of *Streptococcus agalactiae*, thereby creating pseudo-multicellular structures [Takasi et al. 1994]. Ingredients of propolis, mainly flavonoids, act on virulence factors of Gram-positive bacteria, inhibit coagulase activity and decrease lipase activity, and impede the formation of biofilms [Scazzocchio et al. 2006]. Research indicates structural rather than functional EEP. Propolis extracts affect the cell membrane damage, resulting in cell lysis and bacterial death [Bryan et al. 2015].

Comparing Brazilian propolis with Korean propolis showed stronger antimicrobial properties of Korean propolis, probably due to the higher content of polyphenols and flavonoids. The disc diffusion method showed larger inhibition zone diameter for *S. aureus* (5.2–5.8 mm) caused by Korean propolis and smaller (2.0 mm) caused by Brazilian propolis. Likewise, *Bacillus subtilis* and *Salmonella* ser. Typhimurium were more strongly inhibited by Korean propolis (4.0–6.1 mm) than Brazilian (3.0 mm). The growth of *Pseudomonas aeruginosa* was not inhibited by both types of propolis [Choi et al. 2006].

Antibacterial activity of propolis also depends on the climatic conditions prevailing in a given region of the world. Propolis from five regions with varied climates of western Algeria (from dry and cold to hot and then to sunny and high humidity) was investigated [Benhanifia et al. 2014]. Better antimicrobial properties showed propolis produced in hot and sunny climates and weaker in dry and cool. Propolis from hot and sunny climate most strongly inhibited the growth of *Bacillus subtilis* and *B. cereus* and bacteria isolated from cows suffering from mastitis (*S. aureus* and *P. aeruginosa*), which was imaged with large diameter zones of inhibition. Regardless of climatic conditions, ethanol extracts from propolis did not inhibit the growth of *Escherichia coli* and *P. aeruginosa*.

The bacteriostatic effects of the orange and blue type of Serbian propolis with their dominant phenolic compounds, galangin, chrysin, and pinocembrin, were compared. Because of the higher content of phenolic components, there was a greater bacteriostatic activity of orange propolis than blue propolis. The strains of *Listeria monocytogenes* were most sensitive. MICs (minimal inhibitory concentration) of orange propolis ethanol extract were in the range of 0.4–1.9 $mg \cdot ml^{-1}$, and the MICs of the ethanol blue propolis extract were in the range of 0.4–10.6 $mg \cdot ml^{-1}$. The tested extracts showed a wide range of MIC values (0.3–16.1 $mg \cdot ml^{-1}$) against *B. subtilis*, *Enterococcus faecalis*, *Shigella flexnerii*, and *S. aureus* [Ristivojevic et al. 2016].

The antimicrobial activity of propolis extracts is affected by the solvent used and the extraction conditions. The effectiveness of inhibition of bacteria by aqueous, oily, and ethanolic extracts of propolis was compared. Extracts obtained by using water or olive oil and extraction at room temperature for 5 h showed no bacteriostatic effects. Adding polyethylene glycol (PEG) to water or olive oil and raising the extraction temperature to 70°C for 15 min caused the extracts to inhibit the growth of *B. cereus*, *S. aureus* as well as *Klebsiella pneumoniae*, *P. aeruginosa*, and *E. coli* at a similar level as ethanol extracts obtained at room temperature for 5 h. The chemical compositions of all extracts were as follows: caffeic acid, trans-*p*-coumaric acid, and ferulic acid, while naringenin, kaempferol, and galangin were also found in ethanol and oil extract with added PEG [Kubiliene et al. 2015].

The effect of ethanol extracts from propolis on bacteria isolated from human saliva was studied. The extract was more effective against *Lactobacillus* (MIC on the medium level of 0.7 $mg \cdot ml^{-1}$) than against *Streptococcus* (MIC average 1.1 $mg \cdot ml^{-1}$) [Dziedzic et al. 2013]. In turn, *L. rhamnosus* and *Bifidobacterium animalis* ssp. *lactis* are resistant to ethanol extracts of propolis [Baufadi et al. 2016]. The inhibition of *B. animalis* growth was observed only at 40% concentration of the extract and *L. rhamnosus* at 60%. *Streptococcus aureus* was very sensitive to the ethanol extract of propolis already at a concentration of 2%. Pathogenic bacteria are more sensitive to propolis than beneficial bacteria (*B. animalis* and *L. rhamnosus*). This allows the use of the ethanol extracts of propolis as a natural preservative in the food industry [Boufadi et al. 2016]. The minimum bactericidal concentration of the tested propolis extracts was 13.3 $mg \cdot ml^{-1}$ for *S. aureus* strains and 16 $mg \cdot ml^{-1}$ for *S. intermedius* isolates [Cardoso et al. 2010].

Antibacterial activity of propolis has also been reported against *Helicobacter pylori*, as well as *Campylobacter coli* and *C. jejuni* [Boyanova et al. 2003]. Propolis studies from different regions of Turkey showed high activity of EEP against *H. pylori*. The growth inhibition zones of these bacteria caused by the ethanol extracts of propolis were greater than those of Amoxicillin antibiotic and 70% ethanol (31–47, 40, and 10 mm, respectively). In addition, EEP also inhibits the activity of urease produced by *H. pylori*, and thus can provide effective support for the treatment of gastro-duodenal diseases [Baltas et al. 2016].

The innovative idea is to use propolis in combination with metal nanoparticles, which also have antimicrobial activity. An attempt was made to produce electrospun fast dissolving mats for wound dressings containing silver nanoparticles and ethanol extract from propolis, which can be used as wound dressings. This combination of active ingredients provides the strong antimicrobial effect of silver nanoparticles, on the one hand, and, on the other hand, support wound healing by propolis extract. The rapid release of silver nanoparticles from Electrospun PVP Mats and phenolic compounds from propolis has been demonstrated, which had significant impact on the inhibition of growth of microorganisms such as *S. aureus*, *S. epidermidis*, *E. faecalis*, *E. coli*, *P. aeruginosa*, *P. vulgaris*, *B. subtilis*, *B. cereus*, and *C. albicans* [Adomaviciute et al. 2016].

ANTIFUNGAL PROPERTIES OF PROPOLIS

Antifungal action is mainly attributed to flavonoids present in propolis [Freires et al. 2016]. The mechanism of action of propolis on yeasts is related to the disruption of cell wall instead of affecting the membrane and cell permeability [Pippi et al. 2015]. Pinocembrin is one of the components of propolis that plays a role in propolis' action on mold. Propolis inhibits hyphal cell respiration, which leads to the energy depletion and cell membrane damage, resulting in accelerated cell death [Peng et al. 2012].

Candida yeasts are one of the most commonly used fungi in propolis' research. The effect of propolis type on antifungal activity has been demonstrated. Stronger effects of red propolis than poplar (both from different regions of Brazil) were observed against *C. albicans*, *C. dubliniensis*, *C. glabrata*, *C. crozei*, *C. tropicalis*, and *C. parapsilosis*. The investigated propolis had the most potent inhibitory effect on the growth of *C. dubliniensis* (MIC of poplar propolis extract was 4.0 $\mu g \cdot ml^{-1}$ and of red propolis extract was 1.0 $\mu g \cdot ml^{-1}$). The weakest inhibitory effect was observed with *C. parapsilosis* (MICs were 31.3 and 4.0 $\mu g \cdot ml^{-1}$, respectively, for poplar propolis and red propolis extracts) [Freires et al. 2016]. Studies of the red propolis showed that MICs were equal to or higher than 1000 $\mu g \cdot ml^{-1}$ for *C. tropicalis* strains, *C. glabrata*, *C. dubliniensis*, and *C. albicans*, indicating the need to use high concentrations of extracts to inhibit the development of these yeasts [Lopez et al. 2015]. An activity was demonstrated by propolis from Turkey, with *C. albicans* growth inhibition zones of 15.33 mm in diameter [Ertürk et al. 2016].

The effects of various solvents (n-hexane, dichloromethane, ethyl acetate, and methanol) used for the extraction of Colombian propolis on the activity of extracts against *Colletotrichum gloeosporioides* and *Botryodiplodia theobromae* isolated from papaya, avocado, and mango were investigated. The strongest action against *C. gloeosporioides* had ethyl acetate-extracted propolis. At the same time, the extract exhibited the weakest effect on *B. theobromae* [Meneses et al. 2009].

The effects of ethanol in two different concentrations 70% and 96%) on the antifungal activity of propolis extracts against *Aspergillus niger*, *A. versicolor*, *Penicillium pinophilum*, *Paecilomyces variotii*, *Trichoderma virens*, and *Chaetomium globosum* was examined. There was no significant difference in the antifungal activity of the extracts, depending on the ethanol solution used, although in the case of *C. globosum* 96% extract was twice as potent (*MIC* 1.0 $mg \cdot ml^{-1}$) than 70% extract (*MIC* 2.0 $mg \cdot ml^{-1}$). Both ethanol extracts showed the same effect on *Aspergillus* strains (*MIC* $\geq$ 7.5 $mg \cdot ml^{-1}$) [Woźniak et al. 2015].

The use of propolis extracts prevents the production of aflatoxins by *Aspergillus flavus* [Ghaly et al. 1998]. EEP also inhibits the growth of *Saccharomyces cerevisiae* and *Penicillium chrysogenum* [Garedew et al. 2004], and its 1.0% 5.0%, and 10% extracts show inhibitory activity on *Aspergillus versicolor* and *P. aurantiogriseum* [Temiz et al. 2013].

The effects of potentially fungicidal substances, including propolis, on *Botrytis cinerea* molds, isolated from raspberry and cane fruit, were compared. Propolis has proved to be the most toxic to molds compared to biopesticides Bioczos S, Biosept 33 SL, Biochikol 020 PC, synthetic fungicide Signum 33 WG. Studies have shown that the use of propolis limits the formation of surviving forms of fungi (sclerotia), but only 7.5% of propolis has been shown to limit mycelial growth [Sapieha-Waszkiewicz et al. 2011].

ANTIVIRAL PROPERTIES OF PROPOLIS

Propolis also exhibits antiviral activity, thanks to the presence of flavonoids, caffeic acid, and esters of aromatic acids. The mechanism of action of these compounds consists in blocking the possibility of transmitting viruses to other cells, inhibiting their propagation and destroying the outer envelope of the virus [Marcucci 1995]. The activity of propolis against polio, influenza A and B viruses, retroviruses, and vaccinia virus [Gekker et al. 2005, Shimizu et al. 2008, Búfalo et al. 2009] as well as against HSV-1 and HSV-2 viruses (Herpes Simplex Virus) was observed. HSV-1 replication was attenuated after 24 h, while HSV-2 replicated weaker after 48-hour incubation. A significant decrease in the number of copies of viruses was noted [Yildirim et al. 2016]. HSV-1 activity is attributed to the two components present in propolis, i.e., galangin and chrysin [Schnitzler et al. 2010].

Particular attention should be paid to the study of propolis' activity against HIV. Melittin carried with bee saliva has a destructive effect on the external envelope of HIV, leading to its inactivation [Gekker et al. 2005].

CONCLUSION

Propolis is a substance composed of several hundred chemicals, including aromatic acids and esters, volatile compounds, enzymes, flavonoids, terpenes, and micro- and macro-elements. The composition of propolis is related to the geographic region of origin of the samples and vegetation occurring in a given area.

Propolis is divided into several types based on the origin of propolis, with the most common being Brazilian, European, and Pacific propolis. Propolis and its extracts exhibit a broad antimicrobial activity, in particular, not only for Gram-positive bacteria but also for Gram-negative bacteria, mold, and yeast. In recent years, antiviral and anticancer activities of propolis have also been reported. Increasingly, attempts are made to exploit the synergies of propolis with other substances, such as antibacterial metal nanoparticles. Research on the application of bee glue in the pharmaceutical, biomedical, and food industries is continuing.

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PRZECIWDROBNOUSTROJOWE I PRZECIWWIRUSOWE WŁAŚCIWOŚCI RÓŻNYCH TYPÓW PROPOLISU

Streszczenie. Propolis wytwarzany jest przez pszczoły w formie mazi głównie z substancji żywicznych pochodzących z drzew, krzewów oraz pąków kwiatowych, wzbogacony w wosk, olejki eteryczne, pyłek kwiatowy oraz ślinę pszczół (*Apis mellifera*). Skład propolisu jest bardzo zróżnicowany w zależności od regionu pochodzenia i roślinności występującej na danym terenie, klimatu oraz pory roku, w której jest produkowany. Do tej pory w składzie chemicznym propolisów zidentyfikowano ponad 400 substancji: fenole, flawonoidy, kwasy fenolowe i ich estry, flawony, dihydroksyflawony, chalkony oraz glicerydy fenolowe. Propolis podzielono na kilka typów. Najczęściej spotykane typy to propolis: topolowy, europejski, brazylijski i pacyficzny. Wykazano także jego przeciwbakteryjne, przeciwgrzybicze oraz przeciwwirusowe działanie, zwłaszcza etanolowych ekstraktów. Propolis działa hamująco na bakterie Gram-dodatnie, a w mniejszym stopniu na bakterie Gram-ujemne, hamuje również rozwój pleśni (*Aspergillus* i *Penicillium*) oraz drożdży (*Candida*). Dowiedziono także, że propolis ma właściwości przeciwwirusowe (względem wirusa polio, wirusa grypy typu A i B, reowirusów, a także wirusa HIV).

Słowa kluczowe: przeciwdrobnoustrojowe właściwości propolisu, przeciwwirusowe właściwości propolisu, skład chemiczny, propolis