

ULUDAĞ ARICILIK DERGİSİ / ULUDAG BEE JOURNAL

THE HISTORICAL USES OF PROPOLIS IN FOLK MEDICINE, WITH ITS BIOLOGICAL ACITIVITES AND CHEMICAL COMPOSITION

Halk ilaçlarında Propolisin Tarihi Kullanımı ile Onun Biyolojik Aktivitesi ve Kimyasal Kompozisyonu

(Genişletilmiş Türkçe Özet Makalenin Sonunda Verilmiştir)

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Geliş Tarihi: 21.03.2012; Kabul Tarihi: 14.11.2012

ABSTRACT: Propolis is alike of gum material produced by honey bees in the construction and maintenance of their hives. Here the antifungal, antibacterial, antiviral, insecticidal and antioxidant activities of propolis, among other attributes of propolis and the chemical composition are reviewed. In recent years the biological activity research on propolis has become increasingly very attractive to many scientists in various fields of research. The comparative biological studies of propolis of different origin and the chemical composition analyses are becoming attractive. Similar types of studies are extremely valuable with the help of information about propolis standardization and practical applications in scientific fields.

Keywords: Propolis, *Apis mellifera*, bioactive compound, beeswax, plant.

Anahtar kelimeler: Propolis, *Apis mellifera*, biyoaktif bileşim, balmumu, bitki.

INTRODUCTION

Propolis, collected by *Apis mellifera* bees from various plant sources and mixed with secreted beeswax, is a multifunctional material used by bees in the construction, maintenance, and protection of their hives (Burdock, 1998; Ghisalberti, 1979). Bees have been observed scraping the protective resins of flower and leaf buds with their mandibles and then carrying them to the hive like pollens on their hind legs. These resins are used by worker bees to line the inside of nest cavities and all brood combs, repair combs, seal small cracks in the hive, reduce the size of hive entrances seal off inside the hive any dead animals which are large to be carried out and perhaps most important of all, to mix small quantities of propolis with wax to seal brood cells (Anon, 1965; Asis, 1989; Ask-Upmark, 1967; Andrich et al., 1987).

The literal meaning of propolis is derived from the Greek *pro-*, for or in defense, and *polis-*, the city, that is, defense of the city (or the hive) (Ghisalberti 1979). Propolis is a strongly adhesive, resinous substance collected, transformed and used by bees

to seal holes in their honeycombs, smooth out the internal walls and protect the entrance against dangers from the outside. Honeybees (*Apis mellifera* L.) collect the resin from the cracks in the bark of trees and leaf buds. Resine is masticated and then salivary enzymes added and the partially digested material is mixed with beeswax and used in the hive (Ghisalberti, 1979; Marcucci, 1995). Although propolis may contain some pollen, it is not pollen. Although definitive studies of the metabolism of propolis do meet in the literature, the metabolism of many components of propolis is well known. The biologically most active component of propolis, the flavonoids, is known to be metabolized with no residues of flavonoids accumulating in the body (Havsteen, 1983). Acute toxicity tests of raw, unprocessed propolis would not be expected and because the method for propolis extraction remains unstandardized, variability in reported toxicity would be expected. Nonetheless, a useable body of data exists. For example, Arvouet-Grand et al. (1993) reported the oral LD₅₀ of propolis extract in the mouse to be greater than 7340 mg/kg, while reported an LD₅₀ of 2050 mg/kg and an LD₁₀₀ of

2750 mg/kg. As the most important chemical weapon of bees against pathogenic microorganisms, propolis has been used as a medicine by human being since ages ago for treatment of wounds, burns, sore throats and stomach ulcers. For this reason, propolis has become the subject of intense pharmacological and chemical studies for the last 30 years. Numerous studies carried out with combined efforts of phytochemists and pharmacologists, led on recent years to the idea that different propolis samples could be completely different in their chemistry and biological activity (Bankova, 2005). The propolis is used traditionally in folk medicine mainly because it's antibacterial effect. Other properties, as antifungal, antiviral, anti-inflammatory and immuno-stimulating have also been described to this resin (Beyer and Melzig, 2005).

In this article, the available information concerning the antifungal, antiviral, insecticidal, antioxidant biological activity of propolis, among other attributes of propolis and the chemical composition of propolis are summarized.

Uses of Propolis

Bees have been about in existence for -125 million years and bees evolutionary success has allowed them to become perennial species that can exploit virtually all habitats on Earth (Bankova, 2005). East European countries have a lot studies. Western European and North American medical research has ignored this source of milder and useful material (Budavari, 1989; Chernyak, 1973; Ghisalberti 1979). The wound healing properties of propolis were known to the Egyptians, Greeks and Romans and in ancient times in Europe and North Africa. In 12th century, medicinal preparations with propolis are described for treating mouth and throat infections, as well as caries (Apimondia, 1975b; Apimondia, 1978; Apimondia, 1975a). Prevalent medicinal uses of propolis include treatment of the cardiovascular system and blood system (anemia), respiratory apparatus for various infections. (Apimondia, 1975b, Apimondia, 1978; Apimondia, 1975a). Propolis has been taken by mouth for bacterial infections such as tuberculosis; fungal infections such as oral candidiasis (thrush); parasitic infections such as malaria; and viral infections such as colds. Flavonoids in propolis have been shown to slow down or stop the division of bacterial cells in studies and also propolis is believed to have similar inhibitory effects on viral and fungal cells. (<http://natural-propolis.blogspot.com> 2012).

A chemical in propolis that it contains--caffeic acid phenethyl ester (CAPE) -- may interfere with the formation of substances that promote inflammation in the body and also propolis has been used in folk medicine to treat skin wounds. In a study of individuals with second-degree burns, cream with propolis and a prescription burn cream produced about equal results in preventing infection, but the cream with propolis promoted earlier healing and seemed to decrease inflammation more than the prescription (<http://www.chembalpharma.com> 2012). Ethanolic extracts of propolis have been shown to inhibit the activity of a mixture of crude glucosyltransferase (Gtf) enzymes in solution and also these enzymes are important for the formation of pathogenic dental plaque (Koo et al. 2000; Abd El Hady and Hegazi 2002; Akao et al. 2003; Ansorge et al. 2003; Borelli et al. 2002; Bretz 1998). In general, propolis is fairly stable, but suitable storage is important. Propolis and its extracts should be stored in airproof covers in the dark, preferably at less than 10°C-12°C and away from extreme and direct heat. Alike, very old propolis from the hive should not be mixed with fresher propolis. (Apimondia, 1975b; Apimondia, 1978; Apimondia, 1975a). After attach to other products, testing for propolis becomes even more complicated and overall product quality becomes important. Since there is much products in which propolis can be included, the standards for each type of product need to be considered (Budavari, 1989; Chernyak, 1973; Ghisalberti, 1979). One easy way to identify a different kind of quality, especially poor quality as a defect, is the homogeneity of products containing propolis extracts. Hand-mixed emulsions only stable for shorter periods. Thus special care needs to be taken to ensure the compatibility of the extraction method. Better mixing techniques and suitable emulsifiers, longer time, higher speed, different mixing sequences and warmer temperatures would have to be determined by testing (Budavari 1989; Chernyak 1973; Ghisalberti 1979).

Side Effects

Propolis may cause asthma attacks suffers from asthma. The exceptional side effects known to be associated with propolis are irritations of the skin or mucous membranes where it is applied (Burdock, 1998).

Physical characteristics of propolis

The colour of propolis ranges from yellow to dark brown depending on the origin of the resins. Rarely,

even transparent propolis has been reported. At temperatures of 25°C to 45°C propolis is a soft, pliable and sticky substance and propolis will become liquid at 60°C to 70°C, but for some samples the melting point may be as high as 100°C (Andrich et al., 1987).

The chemical and biological composition of propolis

Bees use materials resulting from a variety of botanical processes in different parts of plants for propolis (Bankova et al., 1987; Bankova et al., 1991; Bankova et al., 1998). These are materials actively secreted by plants as well as materials exuded from wounds in plants (Bankova, 2005).

Thereabouts, raw propolis is composed of 50% resin (polyphenolic fraction), 30% wax, 10% essential oils, 5% pollen and 5% various organic and inorganic compounds (Burdock 1998; Bankova et al., 2000). More than 200 compounds have been identified and so chemical composition of propolis is very complex (Burdock, 1998; Langner and Schilcher, 1999). Its biological activity depends on compounds from the polyphenolic fraction, flavonoids, aromatic acids, phenolic acid esters, lignans, triterpenes, etc. (Burdock 1998; Bankova et al. 1998). These groups of compounds are reported to have bactericidal (Pepeljnjak et al., 1982; Mirzoeva et al., 1997) fungicidal, antiviral (Kujumgiev et al., 1999), antiprotozoal (Bankova et al. 1998), antioxidant (Russo et al., 2002), anti-inflammatory (Borelli et al. 2002) and immunomodulatory (Orsolich and Basic, 2003) effects.

Scientific evidence

One of the most known and extensively tested properties of propolis is its *antibacterial activity* and so scientific tests have been conducted with a variety of bacteria. Many scientific articles related to the pharmacological properties of propolis mention its antihepatotoxic, antitumour, antioxidative, antimicrobial, and anti-inflammatory properties (Banskota et al., 2001; Król et al., 1990). Many of the studies tests have shown positive control of the organisms by various extracts and concentrations of propolis (Bankova et al., 1989).

Antifungal activity of propolis

Among the substances in propolis, the flavonoids are occupies a wide space, which are identified as being responsible for its anti-inflammatory, antimicrobial, and in particular its antifungal actions

(Sommez et al., 2005; Cushnie and Lamb 2005; Oliveira et al., 2006). The activity of European propolis against a broad range of some species of fungi has been associated to the presence of flavonoids and derivatives of caffeic acid (Kujumgiev et al., 1999; Bosio et al., 2000; Hegazi et al., 2000; Sawaya et al., 2002; Aliyazicioğlu et al., 2010). The results of Silici et al., (2003) showed that *Candida albicans*, *C.glabrata*, *Trichosporon* spp., and *Rhodotorula* sp. were susceptible to low concentrations of propolis, the latter showing a higher susceptibility and so relative to the other propolis tested, the propolis sample collected by *Apis mellifera caucasica* possessed the highest antifungal activity against all of the superficial mycoses. In addition, the results of Yavuz and Ertürk (2011) showed that *Candida albicans*, *Saccharomyces cerevisiae* and *Aspergillus niger* weren't susceptible to low concentrations of propolis and Propolis from Ordu, Turkey that the highest antifungal activity towards to *Aspergillus niger*.

Antimicrobial activity of propolis

The antimicrobial activity has been discovered on gram-positive and gram-negative bacterial species, including anaerobic bacteria of human oral cavity, mycobacteria, yeasts, parasites and viruses (Krol et al., 1993; Amoros et al., 1994; Harish et al., 1997; Bosio et al., 2000; Drago et al., 2000; Sforcin et al., 2000; Banskota et al., 2001; Huleihel and Isanu 2002; Koo et al., 2002; Santos et al., 2002; Santos et al., 2003; Santos et al., 2005; Stepanovic et al., 2003; Salomao et al., 2004; Orsi et al., 2005; Freitas et al., 2006; Aliyazicioğlu et al., 2010). The function of antimicrobial activity of propolis is complex. It could be attributed to the collective activity between phenolic and other compounds (Krol et al., 1993) primarily the galangin, pinobanksin flavonoids and pinocembrin (Burdock, 1998; Castaldo and Capasso, 2002) which disconnect the energy transducing cytoplasmic membrane and inhibit bacterial motility (Mirzoeva et al., 1997). The antibacterial activity of propolis has been correlated to the content of galangin, which has been indicated to be mainly responsible for antibactericidal activity (Pepeljnjak and Kosalec, 2004). *In vitro* interaction between propolis and antimicrobial drugs has been investigated (Krol et al., 1993; Scheller et al., 1999; Stepanovic et al., 2003) and combination of propolis with certain antibiotics and antifungals seem to be of potential medical interest (Stepanovic et al., 2003). Antibacterial and antiviral activity of Actichelated® propolis greater than hydroalcoholic

extract has been proved, it also able to step in bacterial adhesion to human oral cells (Drago et al., 2007). Propolis have shown especially good antimicrobial activity against many strains of bacteria have shown, above all *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*, but not against *Enterobacteriaceae* (Dzik et al., 2003). According to Yavuz and Ertürk (2011), propolis extracts collected from Van, Erzurum, Gümüşhane, Ordu, Rize and Muğla cities of Turkey showed highest antimicrobial activity against *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas auroginosa*, *Salmonella typhimurium*, *Shigella sonnei*, *Yersenia enterocolitica* but not against *Clostridium perfringens*, *Klebsiella pneumonia* and in addition, propolis from Rize against *Bacillus cereus* and propolis from Erzurum against *Listeria monocytogenes* showed the highest antibacterial activity than other bacteria.

Antiviral activity of propolis

Used for medicinal purposes, propolis has been shown in more recent times to possess broad spectrum antimicrobial activity since ancient times, including activity against many of the opportunistic pathogens associated with the acquired immunodeficiency syndrome (AIDS) (Banskota et al., 2001; Burdock 1998). Studies of its antiviral properties have concentrated principally on herpes simplex virus (Amoros et al. 1994; Vynograd et al., 2000) and another study, influenza virus (Serkedjieva et al., 1992). Using a cell line (CEM cells) propolis potently inhibited HIV-1 expression (Harish et al. 1997). The antiviral effects of propolis have generally showed very good results. The inclusion of a propolis extract in mouthwash and toothpaste helps to treat oral infections and even heal surgical wounds. Another clinical study also showed that the extract was particularly effective in shortening the symptoms of influenza (Serkedjiev et al., 1992). Propolis extracts clearly have virucidal properties as well (Amoros et al., 1992) investigated the *in vitro* effect of propolis on different DNA and RNA viruses including herpes simplex type 1, herpes simplex type 2, adenovirus type 2, vesicular stomatitis virus and poliovirus type 2. The inhibition of poliovirus propagation was clearly discovered through a plaque reduction test and a multistep virus replication assay with a selectivity index equal to 5. At the concentration of 30 µg/ml, propolis reduced the titre of herpes viruses by 1000, whereas vesicular stomatitis virus and adenovirus were less

sensitive. The effect of propolis on virus multiplication, propolis was also found to exert a virucidal action on the enveloped viruses HSV and VSV (Amoros et al. 1992).

Insecticidal activity of propolis

There is very limited literature on the acaricidal or insecticidal action of propolis. Considering that while plants produce compounds allure to bees, they need to protect other parts, generally the place where bees collect substances to make propolis, from herbivore attack. This defense is commonly made by repellents, deterrents or even by toxic chemical compounds (Harborne, 1988). Propolis contains substances of plant origin, and is reported to have medicinal, insecticidal, antimicrobial and phytotoxic properties. The different components of propolis are produced by plants in order to avoid infection of injured plant parts and help ward off and kill insects or mite pests (Ogren, 1990). Components of nectar, pollen and propolis may adversely affect the development of *V. destructor* in the hive of some bee populations (Amrin et al., 1996). Some authors suggested that some flavonoid components of propolis have insecticidal or at least insectstatic effects (König ve Dustmann 1988; Schkurat ve Poprawko 1980). Johnson, et al., (1994) researched the physical and chemical composition of North American samples of propolis and tested for bioactivity against larvae of the greater wax moth (*Galleria mellonella* L.), a common apiary pest (Johnson et al. 1994).

CONCLUSION

In this study; information on the antifungal, antiviral, insecticidal, antioxidant biological activity of propolis, among other attributes of propolis and the chemical composition of propolis were illustrated. This review could lead to increased production of propolis by farmers (the grower of honey and propolis) in rural communities and providing an additional source of income for them However this study could be used as a reference for scientists who study in the field.

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GENİŞLETİLMİŞ ÖZET

Propolis işçi baları tarafından üretilen reçinemi bir madde olup arı kovanında yapım, koruma ve bakım gibi çok amaçlı kullanılan bir materyaldir. İnsanoğlu tarafından kullanılışı balın keşfi kadar uzun bir tarihe dayanır. Bu çalışmada, propolisin antifungal, antibakteriyel, antiviral, insektisidal ve antioksidan aktiviteleri ve kimyasal bileşimi özetlenmiştir. Son zamanlarda propolisle ilgili biyolojik aktivite çalışmaları birçok farklı alandaki bilim adamı için giderek daha da ilgi çekici hale gelmektedir. Farklı orijinlerden propolisin karşılaştırmalı biyolojik aktivite çalışmaları ve kimyasal bileşim analizleri giderek daha önemli hale gelmektedir. Bu tür çalışmalar, özellikle propolisin standardizasyonu ve bilimsel alanlardaki pratik uygulamaları açısından bilgi verici olmaları sebebiyle son derece değerlidir.

Reçineler veya polenlerin sebep olduğu alerjik reaksiyonlar nedeniyle propolis, bazı insanlarda astıma neden olabilir. Rengi sarıdan koyu kahveregine doğru değişir. 25-30°C arasında yumuşak ve çok yapışkan haldedir, 60-70°C' ta sıvıdır, fakat bazı örneklerin erime noktası 100°C'a kadar çıkabilir.

Farklı propolis örnekleri farklı kimyasal bileşenlere sahiptir. Ham propolis genel olarak %50 reçine, %30 balmumu, %10 esansiyel yağlar, %5 polen ve çeşitli organik, inorganik içeriğe sahip olabilir. Biyolojik aktivitesi fenolik parçalar, flavonoidler, aromatik asitler, fenolik asit esterleri, triterpenler, lignin vb. maddelerden oluşur. Bu grupların bakteri, mantar, virüs ve protozoa öldürücü, aynı zamanda antioksidan, antiinflamatuvar ve immünomodülatör etkisi olduğu bulunmuştur. Propolisin antihepatotoksik, antitümör, antioksidatif, antimikrobiyal ve antiinflamatuvar özellikleri, virüsler ve diğer mikroorganizmalar üzerine etkileri ile ilgili

pek çok yayın vardır. Pek çok test bu özelliklerle ilgili olarak olumlu sonuç vermiştir.

Propolisin antibakteriyel ve antifungal özelliği flavonoidler ve kafeik asit türevlerinin varlığı ile ilişkili bulunmuştur. Ayrıca flavonoidlerin anti-enflamatuvar özelliği olduğu da tespit edilmiştir. Propolis özellikle *Streptococcus pneumoniae*, *Haemophilus influenzae* ve *Moraxella catarrhalis*'e karşı antimikrobiyal etki gösterirken Enterobacteriaceae türlerine karşı etki göstermemektedir. Son zamanlarda immün yetmezlik sendromu (AIDS) ile ilişkili birçok fırsatçı patojenlere karşı etkinliği de dahil olmak üzere, geniş spektrumlu antimikrobiyal aktiviteye sahip olduğu gösterilmiştir. Gargara ve diş macunu özü içerdiği, ağız enfeksiyonlarını önlediği ve hatta cerrahi yaraların iyileşmesine yardımcı olduğu bulunmuştur. Grip semptomlarını azaltma özelliği olduğu ile ilgili çalışmalar da mevcuttur. Virüs çoğalması üzerindeki etkisi yanı sıra, zarflı virüsler HSV (*Herpes Simplex* (Uçuk) Virüsü) ve VSV (*Vesicular Stomatitis Virus*) üzerinde bir virüsid tesir oluşturduğu da bulunmuştur.

Akarlar ve böcekler üzerine etkileri ile ilgili kısıtlı çalışma vardır. Propolisin yaralı ağaç parçalarının enfeksiyonu önlemek, böcekler veya akarları kovmak ya da öldürmeye yardımcı olmak için bitkiler tarafından üretilen farklı bileşenleri vardır. Bazı yazarlar propolisteki bazı flavonoid bileşenlerinin insektisidal ya da en azından insetstatik (böcek larvalarının gelişme inhibisyonu) etkisi olabileceğini ileri sürmüşlerdir ve büyük bal mumu güvesine (*Galleria mellonella* L.) olan etkisini test etmişlerdir.

Bütün bu olumlu özelliklerine bakıldığında, propolis ile ilgili çalışmaların önü oldukça açık görünmektedir. Özellikle bir arı ürününün daha ekonomiyeye katkısını sağlamak ve arı üreticilerini propolis üretimi için teşvik amacıyla bu makalenin faydalı olacağını düşünmekteyiz.