

Antioxidant capacities of propolis - therapeutic effects and possible applications

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Abstract. Propolis is considered to be one of the most useful products of apitherapy, as the active principles are transmitted directly from the plant to humans, so its use has attracted quite a lot of supporters in human medicine. Due to its complex composition, which includes waxes, resins, balsams, volatile oils, vitamins, minerals, phenolic acids and flavonoids, the therapeutic benefits of propolis and its uses in various diseases are well known. In this paper aspects of the chemical composition of propolis, analysis methods of propolis and the way it can be used as a result of its therapeutic effects are presented.

Keywords: *propolis, antioxidant, therapeutic benefit, applications*

1. Introduction

The protective properties of the bioactives present in propolis make it a potential preventive and therapeutic agent, and it is considered a kind of natural antibiotic. The protective immune defence and antioxidant properties come from the constituents of the bioactive phytochemicals. The compounds in propolis also include, but are not limited to, phenolic acids, flavonoids, esters, aromatic aldehydes, amino acids, fatty acids, vitamins and minerals. Over time, both the efficacy and therapeutic properties of propolis have been demonstrated in more than 200 conditions, and propolis is already considered a powerful weapon in natural therapy. The therapeutic relevance of propolis is based on the discovery that propolis can successfully treat 30 types of viruses, nine species of parasitic fungi and 21 species of bacteria, following modern studies that refer to its therapeutic properties, being considered one of the most powerful natural anti-infectious drugs. [1]

Due to the presence of various phytochemicals, this bee product exhibits numerous biological activities, including antibacterial, antiviral, anti-inflammatory, antioxidant, immunostimulatory and antitumor benefits. Raw propolis of good quality will be recognisable by its lack of sheen, a good sign because if it is shiny then its properties will be diminished because there is too much wax in its composition, its strong specific smell will be bitter and sweet. Propolis is recognised as an aromatic resin-like substance, which is collected by bees from more than 20 species of trees from poplar bark, poplar buds, willows, poplar leaves, maple buds, conifers and plums. [2]

The therapeutic properties of propolis have been recognized since ancient times, these being: antifungal, anaesthetic, regenerative, bactericidal, analgesic, strong bioactive, antiviral, antitoxic and anti-inflammatory properties. Propolis has retained its position as a therapeutic adjuvant to date precisely because of its efficacy, only 0,2% of those who have used propolis have developed an allergy to the pollen found in its composition.

2. Chemical composition of propolis. Estimates of the antioxidant potential

The therapeutic action of different types of propolis is correlated with the presence of certain chemical compounds in their composition. An important clarification regarding the composition and

therapeutic effects of propolis is that there are differences in its composition, depending on the production technique, the plants supplying the resins, the evolutionary cycles, the flora of the area where the hives will be grown or climatic factors.

Regardless of area or climatic factors, the chemical composition of a raw propolis will include, but is not limited to, the following main components: amino acids, carbohydrates, vitamins, resins, waxes, volatile oils, enzymes, mineral salts and impurities.

Propolis is a complex nutrient with broad and varied physiological effects, which is why it has become the focus of much research due to its numerous biological and pharmacological properties.

The major groups of substances contained in propolis are as follows: [3,4]

- waxes are found in a percentage of 7,5 - 35%;
- impurities will be found in the range 4,4 - 19%;
- resins and balsams in 55%.

According to literature data, more than 300 compounds belonging to polyphenols, terpenoids, steroids, sugars, amino acids and others have been identified in propolis.

Balsams are in fact natural substances obtained by special methods from the bark of the aboriginal tree and are semi-liquid or liquid. There is no concrete expert data on how the resins in propolis were isolated, but their presence has never been questioned.

Among the constituents that are commonly found in resin and can also be identified in propolis are: cinnamic acid, ferulic acid, pentacyclic triterpenoids, benzoic acid - in concentrations ranging from 1,33 to 20%, coumarins and caffeic acid.

- volatile oils are found in a percentage of 10% in the composition of propolis, these compounds having as specific flavour characteristics.

- fatty acids are found in 5%;
- terpenes;

- free amino acids may be present in a number of 16 to 24, significant representatives of which are pyroglutamic acid, proline and arginine. The source of amino acids is the salivary secretions of bees and pollen.

- pollen in 5%;
- vitamins (pantothenic acid, A, B1, B2, B6, C, E, nicotinic acid);
- tannins.

The most important constituents of propolis, in addition to various aromatic and phenolic compounds are flavonoids (flavonols, flavonones, flavones).

The examples of flavonoids found in propolis are presented in Figure 1. [5]

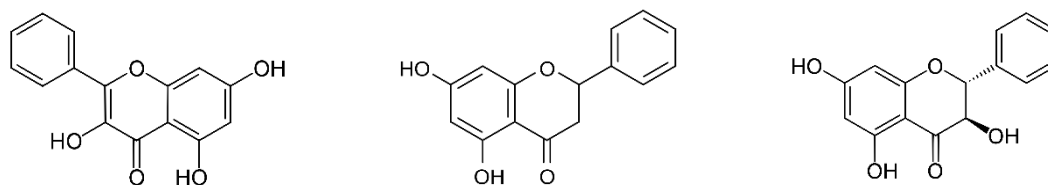


Fig.1. Structural formulas of galangin, pinocembrin and pinobanksin.

The mineral elements are found in different samples from different regions, such as Australia (0,68%), France (0,51-0,86%), USA (0,67%), China (0,55%).

The main representatives of mineral elements are vanadium, zinc, sodium, cadmium, magnesium, boron, barium, iron, strontium, aluminium, calcium, selenium, silver, silicon, lead, manganese, titanium, chromium, copper, nickel, molybdenum, cobalt and potassium.

Chemical composition - biological activity relationship

The multiple effects of propolis, such as antibacterial, antiviral, anti-inflammatory, antioxidant, immunostimulatory and anti-tumour effects are closely linked to its composition rich in beneficial substances such as flavonoids, phenolic acids, terpenoids, steroids and amino acids.

Thus, the antioxidant action is given by phenolic acids and their esters in the case of European propolis, respectively prenylated p-coumaric acids and flavonoids in the case of Brazilian propolis. Prenylated benzophenones are responsible for the antioxidant and antitumour action of Cuban propolis, and compounds such as prenylated flavanones have similar biological action in the case of Taiwanese propolis.

The examples of phenolic acids found in propolis are presented in Figure 2. [5]

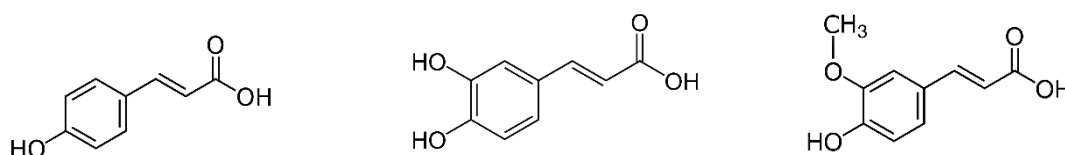


Fig.2. Structural formulas of p - coumaric acid, caffeic acid and ferulic acid.

The anti-tumour action of Brazilian propolis, considered one of the best bee products in the world, is due to benzofurans, prenylated p-coumaric acids and clerodane diterpenoids. This product also has hepatoprotective action given by flavonoids, prenylated p-coumaric acids, caffeoyl quinic acids and lignans.

With hepatoprotective action in European propolis are the compounds: caffeic acid and its esters and ferulic acid, while in Cuban and Taiwanese propolis no compounds with such biological action have been identified.

The anti-inflammatory and antibacterial action of European propolis is given by flavanones, flavones, esters of phenolic acids and phenolic acids.

Compounds such as prenylated p-coumaric acids, diterpenes and labdan are responsible for the antibacterial action of Brazilian propolis, and prenylated benzophenones confer antibacterial action to Cuban propolis. [6]

The chemical composition and biological activity of propolis depend on its botanical and geographical origin. The search for new bioactive substances, possible new constituents not yet identified in different types of propolis, is of great importance and is the subject of numerous specialist studies.

Analytical methods used for the identification and assay of biologically active constituents in propolis

The therapeutic qualities of propolis have been seriously highlighted over time, and depending on how propolis is standardised, purified and studied in the research, toxicological, technological, clinical and preclinical stages, it will be possible to make the transition from an empirical remedy to a medicine. The advertising of propolis must, however, be accompanied by clinical trials, in vivo or in vitro studies in order to be able to accurately determine its efficacy and pharmacovigilance, especially as its composition may differ depending on the area from which it is harvested, the species of bee that will be harvesting it and the season in which it is harvested.

Research on the chemical profile of propolis aims to propose a standard requiring quality parameters of propolis before its use in the pharmaceutical and cosmetic industry.

The measurement of parameters such as: wax content, polyphenol content, analysis of the flavonoid component of propolis, evaluation of the antioxidant and antimicrobial capacity are necessary to assess the quality of propolis and its use for therapeutic purposes being directly or indirectly responsible for the effects manifested by propolis.

The methods used in the qualitative and quantitative analysis of propolis extracts for the determination of biologically active compounds (polyphenols, flavonoids) are rapid, low-cost spectrophotometric methods for the determination of the main groups of bioactive substances in different types of propolis. An example is the quantification from poplar propolis of components such as flavones, flavonols, flavanones, dihydroflavonols and total phenolic compounds. [7]

Chromatographic techniques are also successfully used in the analysis of the polyphenolic profile of propolis and the determination of the contents of biologically active compounds such as the flavonoid component. A recent study analysed propolis samples from Bulgaria, Italy and Switzerland by gas chromatography-mass spectrometry (GC-MS), the results of which established a composition similar to the typical chemical pattern of aspen propolis. Compounds such as pinocembrin, pinobanksin -3-O-acetate, chrysin, galangin, prenyl esters of caffeic and ferulic acids were identified. In the mentioned study there were also two samples that differed significantly: one from an Alpine region of Switzerland, which was rich in phenolic glycerides, and one from Sicily, which contained only a limited number of phenolic substances and was rich in diterpene acids. [8]

This shows that there are major differences in the composition of propolis, depending on the plants supplying the resins, the evolutionary cycles, the flora of the area or climatic factors, which makes it difficult to standardise so that propolis extract for medicinal use can correspond to an optimal ratio between the quantity administered and the effect obtained.

Another chromatographic technique mentioned in the literature as being used with good results in determining the chemical profile of propolis is high-performance liquid chromatography coupled with mass spectrometry. [9,10]

Studies show that various chemical constituents of Brazilian propolis, including chroman, diterpenes and phenolic compounds, could be isolated and analysed by LC-MS using standardised methods. Dicafeoylquinic acids were detected in aqueous extracts of propolis, while diterpenes, flavonoids and prenylated phenolic compounds were found in their methanolic extracts. Based on the identified chemical constituents and their biological activities, the quality of the analyzed propolis can be evaluated and a direct link between the quality of Brazilian propolis and its phenolic constituents can be established. [11]

Analyzes of the mineral chemical composition of propolis can also be performed by near-infrared spectroscopy (NIR). In the literature mentioned is a study of propolis samples collected from Chile and Spain, in which the measured minerals were aluminum, calcium, iron, potassium, magnesium, phosphorus, and some potentially toxic trace elements such as zinc, chromium, nickel, copper and lead. The results obtained indicated that the NIR method is comparable to chemical methods. Another conclusion of the study was that the method is of interest in the rapid prediction of potentially toxic elements in propolis before consumption. There are also studies that prove the antioxidant capacity of propolis through near-infrared spectroscopy. [12, 13]

3. The uses of propolis in various pathologies

With the help of pharmacodynamic and chemical progress and pharmaceutical technology, it has been possible to develop rules for the preformulation and formulation of medicines, the most important rule, in fact, is that which refers to the relationship that the product in question and the effects have on a human organism, thus resulting in the term therapeutic dose. This term can also be used for products or medicines that are composed of a natural mixture of active substances with the help of a standardisation of the tincture and extract.

This standardisation term represents, in fact, a standardisation of the concentration of the active ingredient in the finished product and can be achieved by biological control or physico-chemical methods. Any experiment, whether clinical or laboratory, should be carried out only on standardised extracts so that the propolis extract for medicinal use can correspond to a ratio between the quantity administered and the effect obtained.

In the case of a standardised extract, the following possibilities may arise: individualisation of the treatment, lack of toxicity, administration of quantities containing controlled concentrations of active substances and maximum therapeutic efficacy.

The standardised propolis extract, although it may have side effects if the doses are exceeded, is not a toxic product.

Propolis tincture (propolis-based alcoholic solution) is the most widely used form of propolis and can be prepared as follows: 30 g of propolis, which is previously hardened in the refrigerator, should be added to every 100 g of 60° or 90° alcohol. It can be used to normalize blood pressure,

cure stomach pains, intestinal parasitosis, gastroduodenal ulcers, enteritis, cure internal infectious diseases, treat alcoholism, treat gynaecological, pulmonary, digestive, glandular or nervous system problems.

Locally, burns could also be treated, either with propolis tincture or with propolis-containing spray, which is used as an anaesthetic, antibacterial and stimulator of the regenerative process, covering the burned surface with a protective film and thus allowing the healing process to begin.

Propolis is very often used in food additives, beverages, medicines, supplements, the aim being to improve health and try to prevent conditions such as diabetes, cancer, inflammatory diseases, heart disease, liver disease.

Many studies and researches on propolis have been carried out over time, highlighting its beneficial effects with therapeutic action: antifungal, analgesic, epithelializing, immune system stimulator, diuretic, antioxidant, antimicrobial, antibiotic, antiparasitic, antitumor, anti-inflammatory and antiviral. [14,15]

The application of propolis in cosmetics and dermatology

In the cosmetic and dermatological industry, propolis is often used for its anti-inflammatory properties, and is exploited in products that will have a protective action against sunburn. In addition to sunburn it also protects the body against Roentgen and other types of radiation.

The ability to stimulate the healing process has been the therapeutic property that has been most noted of all the therapeutic properties of all bee products, most of which result in the regeneration of injured epithelium and tissue.

Both honey and the combination of honey and propolis have proven to have very effective healing effects, working on both over-infected and normal wounds.

Propolis can be the treatment that will have beneficial and broad-spectrum results on some dermatological infections, successfully manifesting itself also on pathogenic staphylococcus through a bactericidal action, regardless of the sensitivity that this staphylococcus will have to antibiotics.

A notable advantage of propolis is the rapidity with which it exerts its effect when compared with other conventional medicines, thus reducing the healing period, and the best example to support this advantage is that of a scab or skin wound which are quite common in patients who are bedridden.

The proliferation of the epithelium will be enhanced when propolis-rich sprays or ointments are applied directly to the open sore or burn, while managing to limit their infection with viral, bactericidal, bacteriostatic and antifungal properties. These products also work successfully on *Pseudomonas aeruginosa*, a pathogen that is often resistant to common antibiotics and is a major pathogen in people with major burns. A number of studies have shown that propolis not only accelerates the healing process but also provides mechanical protection to the wound with a protective film. [16]

The benefits of propolis for oral hygiene and oral cavity diseases

Propolis can also be used for the oral cavity in the form of mouthwash or toothpaste containing propolis which, if used regularly, eliminates mouth ulcers, lesions and periodontitis. It is very important that oral hygiene is carried out correctly, propolis in tincture form can have an important haemostatic, antiseptic and oral bactericidal role. In the case of canker sores it is recommended to apply undiluted tincture, puncturing the canker sore before it ulcerates, allowing it to dry and thus avoiding its painful form.

According to studies propolis in mouthwash will significantly reduce the formation of insoluble polysaccharides, insoluble supragingival plaque. Tooth decay will be prevented and its occurrence will be slowed down by an inhibition of bacterial growth due to the antibacterial role of propolis. Chronic recurrent aphthous ulcers can also be removed or prevented by using propolis, which has four times the anaesthetic effect of procaine and thus succeeds in reducing pain and forming a protective film on the surface of the ulcerated lesion. [17]

The benefits of propolis as a natural remedy for respiratory affections

Due to its anti-inflammatory and antibacterial effects, apitherapy will be of particular importance for bronchopulmonary disorders, as the combination of propolis and honey has reliable results on chronic bronchitis, especially if eucalyptus or lime honey is used.

If used alone, propolis may not have the desired effects, but it can be an adjuvant to classical treatment of the respiratory system. Both Romania and Eastern European countries have conducted multiple clinical trials and have identified many propolis-based medicines that have had a good result on respiratory disorders, thus increasing the interest of Asian countries, South America and the United States in apitherapy. The biggest stumbling block to these studies has always been determining the therapeutic dose.

Propolis can be used to decrease the frequency and alleviate asthma attacks, as the immunoprotective properties of propolis help to increase the body's defence capacity. Propolis, used as an adjuvant, leads to an inhibition of tuberculosis bacillus development due to its support of the body against the Koch's bacillus and its ability to reabsorb infectious foci. Propolis often has stronger properties on respiratory conditions than medicinal substances that have been targeted at these conditions. [18, 19]

The role of propolis is to stimulate immunological factors, both non-specific and specific, so that imbalances in the bacterial flora will be avoided, and this will lead to an increase in the general resistance of the whole body to disease.

Although it is widely used, with therapeutic properties recognised worldwide, there have been cases where allergic reactions have occurred in people with known sensitivities. There is also a strong possibility that people who are hypersensitive to bee stings, have eczema or bronchial asthma may have allergic reactions to propolis, the cause of which is not yet known, and which may be due to some protein residue left in the propolis by bees or even small traces of bee venom. [20]

4. Conclusions

Thanks to the active ingredients transmitted to humans, propolis is considered to be a very effective product, with good results even where some natural or medicinal preparations have not led to the desired result. The positive effects of propolis have been highlighted by a series of research and studies carried out on it, and it is considered a surprising natural product with therapeutic effects such as anti-tumour, anti-inflammatory, anti-fungal, antibiotic, analgesic, antioxidant and antimicrobial. The beneficial effect of propolis can also be harnessed through the use of its volatile fraction, which includes aromatic substances, terpenes and flavones, which are essential oils, as active ingredients.

The analysis of the chemical profile of propolis, the analysis by appropriate instrumental techniques of the polyphenol content, the spectrophotometric or chromatographic determination of the flavonoid component allow the assessment of its antioxidant capacity. The determination of parameters related to the chemical composition is a necessity before using propolis in the pharmaceutical and cosmetic industry, especially as this can differ depending on the area where it is harvested, the species of bee that will harvest it, the season in which it is harvested and climatic factors. The evaluation of the antioxidant capacity is a necessary part of the assessment of the quality of propolis and offers the possibility of standardisation and therapeutic use, being responsible for the effects manifested by it.

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