Propolis and Human Health

Propolis is a waxy resin, with a complex composition and viscous consistency, elaborated from different resinous vegetable particles by bees and used in the construction, repair and protection of their hives. Since ancient times propolis has been widely used for diverse purposes. Currently, the activity, effects and possible applications of propolis in biology and medicine are being investigated, with an emphasis on their use as a dietary supplement, as well as their possible applications within the pharmaceutical industry.

In this work a review on the properties of propolis in the treatment and prevention of different types of diseases has been carried out. After a brief review of the origin and composition of propolis, an exhaustive bibliographical search of its properties, the parameters used to evaluate its quality, and its possible indirect effects has been carried out. According to the studies reviewed, the effectiveness of propolis lays mainly in its antioxidant, anti-inflammatory and anti-microbial activities

INTRODUCTION

The term propolis originates from Greek and means defender of the city. With respect to bee- hives, it is understood as being synonymous with a wax like resin with a complex composition of viscous consistency, produced by bees for use in the construction, repair, insulation and protection of the hive . Bees (Apis mellifera), collect resinous particles from leaf buds, shoots and petioles of leaves from different vegetables (elm, poplar, willow, birch, hoarse chestnut, pine, fir, oak trees and some herbaceous plants) with their mandibles, which once introduced into the hive are mixed with wax and salivary secretions, in order to obtain propolis. Annual production (10-300g/hive) varies in accordance with the variety of bee, the climate, the flora and the collection mechanism used .

Thanks to its content in essential oils, propolis is usually aromatic and, in accordance with its botanical origin and the season in which it is harvested, varies in colour (from light yellow to dark chestnut), taste (bitter, slightly spicy or insipid), or consistency. Propolis is collected from beehives through the use of traps or through scraping methods. However, it is the use of traps from which the highest quality propolis is obtained, due to a lower degree of contamination .

Harvesting is carried out before the arrival of the winter season in the temperate climates, while in tropical zones, harvesting takes place on commencement of the rainy season, which is the period when propolis production appears to be more active. As much in the collection, as well in processing, rigorous standards of hygiene should be applied, in order to avoid contamination of the product and the subsequent loss of its commercial value.

As in the case of honey, propolis has been known to mankind from the remotest of ancient times and has been widely used by different cultures for differing purposes, among which its use in medicine is included . However, since the subsequent development of pharmaceutical chemistry, as has equally occurred in case of phytotherapeutic treatments, propolis practically fell into disuse. However, more recently there has been a resurgence in its use, and currently, research

is being carried out on its activity, effects and possible uses in biology and medicine. The most prominent are its application as a dietary supplement and its use in the pharmaceutical industry .

In the present work, the evidences available on the properties of propolis in the treatment and prevention of varying types of disorders are revised, and in order to do so, a brief revision of the origin and composition of propolis, as well as a more exhaustive appraisal of the literature nformation available on its properties, has been carried out.

BOTANICAL ORIGIN AND COMPOSITION

In temperate zones, propolis mainly comes from the exudate from the black poplar buds or from poplars belonging to the genus Populus spp; in the northern area of Russia from birch buds (Betula verrucosa) and P.tremula; in Mediterranean regions from black poplars and from the leaves of Cistus spp; in Brazil from the eaves of some species of Baccharis dracunculifolia in Venezuela and Cuba from the floral resin of the genus Clusia, and in more tropical

areas it is obtained from other plants . Propolis may be classified on the basis of its geographical origin, and although data on the flavonoids and phenolic esters of European and North American propolis are incomplete, it is known that the propolis from the genus Populus spp contains a mixture of flavonic aglycones, hydroxycinnamic acids and its esters; and that the Russian variety basically contains flavonoid aglycones, while the Brazilian variety prenylated carbon derivatives of p-coumaric acid . In fact, the composition of propolis, which is complex and varies in accordance with the phytogeographical diversity of the areas from which it is collected, gives useful information on its properties, botanical origins and geographical situa- tion Although the main components of propolis are the flavonoids and the phenolic acids and their esters, the methods to be detected. However, the variability in composition is very high, and for this reason further studies are required, in order to obtain a greater knowledge of its components .

In European propolis, the total content of phenols, which are mainly responsible for most of its pharmacological properties, represent over half of the 160 different compounds that have been indentified . However some Swiss and Italian samples show unusual compositions (Table 1), with a high bencyl p-coumarate and bencyl ferulate content (5%), a relative scarcity of phenolic compounds and an absence of some ypical compounds (pinobankosin and prenyl caffeate) in propolis from Pnigra Turkish show varied compositions, and in some cases characteristic compounds which could be useful in the identification of origin.

TABLE 1: Composition of numerous European propolis samples[12].

COMPONENTE COMPOUND	Própolis *P. Nigra* (media) (%) Própolis *P. Nigra* (average) (%)	Própolis Suizo Italian propolis (%)	Própolis Italiano Italian propolis (%)
Pinocembrina Pinocembrin	7.2	0.3	0.2
Pinobanksina Pinobanksin	3.7	-	-
O-acetato de pinobanksina Pinobanksin O-acetate	8.0	0.5	0.4
Chrysina Chrysin	8.4	-	0.5
Galangina Galangin	7.8	0.3	0.2
Pentenil cafeatos Pentenyl caffeates	3.3	0.2	-
Bencil cafeato Bencyl caffeate	3.0	-	0.9
Prenetil cafeato Prenethyl caffeate	2.8	-	0.2
Glicéridos fenólicos Phenolic glycerides	1.1	23.1	-
Ácidos diterpénicos Diterpenic acids	-	-	53.2

In propolis (Table 2) over 180 compounds have been isolated. Its main components are resins and balsams, which contain flavonoids and phe- nolic acids or their esters (50%); highly variable wax contents (7.5-35%), which affect the corres- ponding remaining components; volatile oils (10%); pollen (5%) and impurities (4.4-19.5%). Additionally, they contain small quantities of terpens, tannins, traces of secretions from the salivary glands of bees and possible contami- nants. The active compounds are the flavanoids which include flavons, flavanols, flavonons and flavononols. It should be pointed out that most of the studies carried out have not been aimed at determining a complete chemical composition, but have merely determined some of the components of interest, particularly the flavonoids

TABLE 2: Average composition of propolis[3,27]

COMPOSITION	(%)	COMPOUNDS, CHARACTERISTICS AND COMMENTS
Resins	45-55	Flavonoids, phenolic acids and esters.
Waxes	7.55 to 35	Mostly beeswax, but also of vegetable origin
Essential oils	5-10	Volatile
Fatty acids	5	Mostly from wax and rest depending on botanical origin
Pollen	5	Pollen proteins and free aminoacids. Arginine and proline predominate.
Other organic compounds and minerals	5	14 trace elements of which Fe & Zn are the most abundant, others: Au, Ag, Cs, Hg, K, Sb.. Cetons Lactones Quinones Steroids Benzoic acid and esters Vitamins: B_1, B_2, B_3, B_6. Small quantities mainly from pollen. Sugars.

PROPERTIES AND BIOLOGICAL ACTIVITY

Propolis, is a product of extraordinary interest, as much in the field of medicine as the pharmaceutical industry. It is attributed with numerous properties: it is an anti-flammatory agent, an immunostimulant, a hepatoprotector, a carcinostatic, it has anti-microbial, anti-viral, anti-fungal, anti-protozoan properties, it is an anesthetic and a tissue regenerator. The flavonoids (quercitin, apigenin, galangina, etc.) and the phenolic acids (caffeic, isoferulic, cynammic and benzoic), in addition to being toxic to yeasts , inhibit the enzymatic activity of the hyaluronidase . Additionally, caffeic acid and the activity of dihydropholate reductase could explain the similarity between some of its effects and those of some non-steroid anti-inflammatory properties

In studies concerning the anti-bacterial (Staphylococcus aureus & Escherichia coli), anti- fungal (Candida albicans) and antiviral (Avia influenza) activity of propolis from different geographical regions, it was found that all were active against fungi and Gram (+) bacterial strains and additionally, many were also active against the influenza virus. In the samples from temperate zones, this activity is attributed to its ester and phenolic acid content, compounds which are not encountered in propolis from tropical zones. However, tropical zone propolis also shows a similar activity, due to its content in carbon-prenylated derivatives from p-coumaric acid . Different examples of propolis activity, on which studies are available to support such claims, are given below. Anti-oxidant activity Propolis is a natural source of anti-oxidants, that protect oils and serum lipoproteins from oxidation. Its anti-oxidant properties are due to its anti-radical activity (alcoxiradicals and to a lesser extent, superoxide) and an inhibiting effect on the cuprous ion, an initiator of the oxidation of low density lipoproteins. In Brazilian and chinese propolis, the aqueous extracts have shown greater anti-radical activity than the methanolic extracts. The contrary was found to be true of extracts from Dutch or Peruvian origin
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Propolis exerts anti-oxidant effects on the colon, reducing the concentration of lipid hydroperoxide concentration, and as some of its compounds are absorbed and enter into circulation, they act as

hydrophilic anti-oxidants and increase tissue concentration of vitamin C. In patients with ischemic episodes, propolis seems to reduce the risk of cerebal-vascular accidents .

Anti-microbial activity

Propolis is active against numerous microorganisms; Bacillus larvae, B. subtilis, B. de Koch, Staphylococcous aureus, Streptomyces sobrinus, S. mutans, S. cricetus, Saccharomyces cerevisiae, scherichia coli, Salmonella, Shigella, Giardia lambia, Bacteroides nodosos, Klebsiella pneumoniae even against one (Streptococcus piogenes), which is resistant to antibiotics. The cynammic and flavonic compounds of propolis, which alter the membranes and inhibit bacterial motility, probably contribute to this action and to the synergism observed with some antibiotics .

The anti-bacterial activity of European pro- polis is due to its flavonoid aglycones (galangin & pinocembrin) and phenolic compounds (pinobanksin, pinobanksin 3-O-acetate, bencyl-p-couma ate, caffeic acid esters, and ferulic and caffeic acids), and in propolis from the Canary Islands, lignan furofurans . The German variety, rich in phenylethyl-trans-caffeate, bencyl ferulate and galangin, is more effective against Staphylococcus aureus & Escherichia coli, than the French variety, rich in bencyl caffeate and pinocembrin The Mediterranean type (Bulgarian, Turkish, Greek and Algerian), composed of flavonoids, esters of caffeic and ferulic acids, diterpenes and hydroxydi- terpenes, also show significant bacteriostatic and bactericidal properties . In the case of Egyptian propolis, anti-micro- bial activity differs in accordance with its region of origin. In the South of the country, against Staphylococcus aureus & Escherichia coli, the Banisweif variety shows high activity, the Fayoum variety, moderate activity, and the Assiut and Souhag variety, low activity. In the Delta region, the Dakahlia and El-Saff variety are active against Escherichia coli, and the Sharkia and Ismailia variety against Staphylococcus au- reus. The last variety is also effective against Candida albicans

In Brazilian propolis, phenolic compounds have been identified, most notably 3,5-diprenyl-p-cou-maric acid, which posseses significant anti-bacterial activity and without seasonal differences against Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus faecalis On the whole, propolis presents a good dose dependent anti-microbial activity against Strep- tococcus pneumoniae, Haemophilus influenzae & Moraxella catarrhalis, but not against Entero- bacteriaceae· In vitro tests have demonstrated that propolis extracts are more effective against gram (+) coccii (Staphylococcus aureus, Streptococcus β-haemolyticus), but are only active against some gram –) bacteria, such as Escherichia coli or Pseudo- 50 inhibiting capacity . The solvent and the method of extraction used may modify the anti-microbial activity of propolis . Ethanolic extracts at 60-80% inhibit microbial growth, at 70-80% they have higher antioxidant activity and at 80% render hyaluronidase mainly inactive . Aqueous extracts induce a non- specific protection against gram (-) bacteria, such as Klebsiella neumoniae, Proteus vulgaris, Escherichia coli and Pseudomonas aeruginosa. Both the ethanolic and the aqueous extracts are active in bacterial periondontitis, probably due to the sinergic effect of various compounds .

Anti-fungal activity

Central European propolis (Germany, France and Austria), with similar qualitative compositions and a predominance of trans-p-coumaric acid, show activity against Candida albicans[43], while Mediterranean varieties (Bulgaria, Turkey, Greece and Algeria), that contain flavonoids, esters of caffeic acid and ferulic acids, present anti- fungal activity to a lesser extent . Egyptian pro- polis from Dakahlia, with two caffeate esters and two triterpenoids, is more active against Candi- da albicans than the variety from Ismailia, whi- ch does not contain aromatic acids, esters, or flavonoids . Studies on the incidence of para- coccidiomycosis in Latin America suggest that, independently of geographical origin, macropha- ges stimulated with propolis increase fungicidal activity .

Propolis shows, in varying degrees, fungici- dal effects against numerous species such as Candida albicans, Aspergillus niger, Botrytis cinerea, Ascosphaera apis and Plasmopara Ars Pharmaceutica, monas aeuruginosa[48]. On the other hand, other studies , have indicated that the bacteriostatic or bactericidal effects of propolis depend on the dose and that gram (–) aerobic bacteria may also be inhibited at concentrations higher than 2.8mg/ ml. In summary, propolis extracts significantly inhibit all micro-organisms, especially the Acti- nomyces species[49]. The highest degree of inhibition on pathoge- nic fungi was observed in Trichophyton meta- grophytes, Candida albicans and Malassezia pachydermatis[42], and on the Candida genus. The effect of propolis depends on the species concer- ned, and in order of greatest to least effect is C. albicans, C. tropicalis, C. krusei and C. guillier- mondii Diluents of propolis; oil, ethanol, propilenglycol or glycerin, also influence its anti-fungal activity .

On comparison of anti-fungal activity of etha- nolic extracts of propolis with those of griseofulvin, against two varieties of Aspergillus flavus, it can be seen that both substances reduce dry micellar mass, the germination of conidia, the growth and production of aflatoxin B1, the greater the con- centration the greater the effect. However, to the same measure, griseofluvin is four times more effective than propolis[57]. On the other hand, against Candida albicans in oral mucose, the ethanolic extract of propolis at 20% has been shown to be as effective as nistatin. In comparison with other fungicides (clotrimazole, econazole & flucona- zole), to which fungi present resistence, propolis has been shown to be more effective .

Anti-viral activity

Propolis exerts inhibitory effects against bo- vine smallpox, influenza, Newcastle's disease, the herpes virus the rift valley fever, aviary in- fluenza, the bursal viral infection, the reovirus[23], the Hong Kong flu virus and consequently, could be of use in the prevention of such diseases Such an activity is attributed to its content in phenolic compounds, mainly caffeic acid, esters of caffeic and ferulic acid (3-methylbut-2-enyl caffeate, 3-methylbutil ferulate), and finally, fla- vonic aglycones, (luteoline and quercetin) which re highly active against herpes. The use of a Canadian propolis ointment, rich in flavonoids, reduced local symptoms and was more effective than acyclovir in the treatment of genital herpes. The aqueous extract of propolis inhibits the integration of provirus from the murine sar oma virus (MuSV-124) in the host genome, preventing the malignisation of NIH/3T3 cells.

Anti-protozoan activity

Four isolated compounds from Brazilian pro- polis, 3-prenyl-4-hydroxicinnamic, 2.2-dimethyl- 6-carboxi-ethenyl-2H-1-benzopiran, 3.5-diprenyl- 4-hydroxicinnamic and 2.2-dimethyl-6-carboxiethenyl-8-prenyl-2H-1-benzopiran, have been observed as being active against Trypano-soma cruzi[46]. From the study of an ethanolic propolis extract (100 µg/ml) against this same agent, it was observed that even though it is effective against the three parasitic forms, its maximum effect was obtained against trypomas- tigotes, which disappear from blood in 24 hours. It also inhibits protozoa infection of peritoneal macrophages and of myocardial cells, and for this reason, its possible use as an anti-trypanosomial agent is being studied .

Imuno stimulant activity

The effectiveness of propolis as an immunos- timulant has been checked by determining cyto- quine levels, before and after administration to ten healthy adults, who were given 500mg daily oral doses of propolis for thirteen days. Although their plasmatic values did not change throughout this period of time, their cytoquine secretion capacity increased significantly .

Analgesic and anti-inflammatory activity

Some of the phenolic compounds of propolis, such as caffeic acid and the caffeic acid phenyl- ethyl ester, quercetin and naringenin, posses anti- inflammatory effects and act upon the produc- tion of eicosanoids, as much in vitro, supressing the generation of prostaglandins and leukotriens in peritoneal macrophages, as in vivo, in acute peritoneal inflammation induced by zimosin .

Orally administered propolis significantly suppresses the lipoxigenase pathway in the ara- quidonic acid metabolism, and the caffeic acid phenyl-ethyl ester is, as one of the known pro- polis compounds, the most potent modulator of hearaquidonic acid cascade . Both products reduce cyclooxigenase activity in macrophages, measured on the basis of prostaglandin E_2 pro- duction, and protect cartilaginous tissue and human chondrocytes from the damage produced by interleukin-1ß .

Anti-inflammatory activity through free radical scavenging is mainly attributed to caffeic acid, to its prenethyl ester and to flavonoids such as, galangin, kaempferol and kaempferide . Additionally, although the action mechanism remains unknown, the anti-inflammatory effects of propolis and the inhibition that it exerts upon myloperoxidase activity of the lipooxigenase, and of the leukocyte NADPH-oxidase, could in part, be due to its excellent capacity to eliminate free radicals .

Propolis seems to posses potent analgesic and anti-inflammatory activity, given that its oral administration significantly inhibits paw oedema, induced in rats and mice by various agents (carrageenin, formalin, gamma radiation), just as it does in the case of the formation of granuloma and exudates, angiogenesis and the neurogenic response to pain .

Antitoxin activity

It has been proven that the aqueous extract of propolis inhibits the synthesis of interleukin-1β and nitric acid in human leukocytes, as well as the mutagenic power of daunomycin, benzopyrene, and aflatoxin-B1 on Salmonella. Propolis in rats, just as in the case of nicotinamide, probably due to its anti-radical activity, protects pancreatic β-cells from the destruction caused by streptozotocin , and the hepatocytes from the harmful effects of carbon tetrachloride. This may due to the fact that subsequent to treatment with orally administered propolis, the integrity of the bio membranes is conserved, and cellular glutathione re- duction, cytosolic loss of lactate dehydrogenase and the formation of lipo peroxide decreases.

On the other hand, galangin, an abundant flavonoid in propolis, with antioxidant properties, and capable of modulating enzymatic activity and suppressing the genotoxicity of many chemical products, has been proposed as an agent to be used in the chemoprevention of cancer .

Other activities

Remineralisation of the enamel of teeth: in “in vitro” tests, propolis solutions significantly increase values obtained in the Vickers hardness test . Estrogenics: “in vitro” studies with MCF-7 cells from human breast cancers, treated with ethanolic and ether extracts of propolis, show that both compete for estrogenic receptors and therefore, decrease cell proliferation. “In vivo” studies have shown that on a dose dependant basis, propolis significantly increases the weight of the uterus in rats. It has therefore been concluded that it is capable of activating estrogenic receptors. Phytoinhibitor: propolis extracts inhibit the germination of plants and seeds.

INDICATIONS

Traditionally, and since very ancient times, propolis has been used in Europe and North Africa in the treatment of wounds, mouth and throat infections, as well as in tooth cavities. In sub- Saharan Africa, it is still used for medicinal purposes and for other varying applications.

In the technology of foodstuffs, its antioxidant, anti-microbial and anti-fungal properties may offer a great variety of applications, with the advantage that its residues may be beneficial to human health. Its use; as a preservative in frozen fish, in the treatment of post-harvested foodstuffs, in the preserving of fruit, as well as its application as a pesticide and fungicide is at a study phase. However, insufficient studies have been carried out on the possible long term effects derived from its consumption.

In veterinary medicine, it is used to heal wounds and in the treatment of many pathologies, such as diarrhoeas, abscesses, burns, dermatosis, mas- titis, coccidiosis and eimeria in rabbits, etc. Additionally, it is used in weight gain programs for unweaned calves and egg-laying hens .

The healing of wounds

Together with its bactericidal and anti-inflammatory properties, propolis presents topical anaesthetic effects and favours healing, given that it stimulates epithelial regeneration and micro-circulation .

For this reason since ancient times, it has been frequently used with honey as a poultice or in occlusive bandaging, in the treatment of wounds and ulcer wounds of differing aetiologies, even for lepra .

Dermatology

Propolis is used in cosmetics mainly as a deodorant and preservative. Furthermore, it re- generates epithelial tissue through the stimulation of enzymatic systems, cellular metabolism, circulation and the formation of collagen, and due to the presence of arginine, it is useful in cases of acne and burns. On the comparison of the effects of propolis cream and silver sulfodiazine in the treatment of burns, both have been observed to inhibit microbial colonisation. However, areas treated with propolis show less inflammation and heal more rapidly Ethanolic extract of red Cuban propolis shows anti-psoriasis, anti-inflammatory and analgesic activity .

Neoplasia

It has been demonstrated that Korean propolis, like the commercial type (Sigma # p-1010), induces apoptosis of human hepatoma cell lines . The ethanolic extract of propolis is a good inhibitor of mutagenicity[85] and the methanolic extract presents cytotoxicity against murine co- lon 26-L5 carcinoma and human HT-1080 fibro- sarcoma. Additionally, several of its isolated compounds have shown anti-cancerogenic activity, associated with the inhibition of the cellular cycle and the induction of apoptosis, as in the case of 3-2 acid (2-dimethyl 8.3 methyl 2- butenyl) benzopyran-6-propenoic[86] or induced apoptosis without affecting the cellular cycle of cancerous cells, such as prenylflavanone propolin A, which also shows antioxidant activity .

It has been shown that the carbon prenylates of p-cummaric acid in Brazilian propolis act against hepatocarcinoma, that the prenethyl ester of caffeic acid and of methyl caffeate inhibit breast cancer and melanoma vitro" tests, artepilina C shows antibacterial activity and causes significant damage to solid and that in "in vitro" tests, artepilina C shows antibacterial activity and causes significant damage to solid tumours and leukemic cells . Its intratumoral injection (500 µg, three times per week) causes apoptosis, abortive mytosis and massive necro- sis in human malignant tumorous cells. Further- more, together with the suppression of tumoral growth, an increase in the quotient of T CD4/ CD8 cells and the total number of T-helper cells was seen to occur. This indicates that artepilina C acts upon the immunological system and pos- sesses direct anti-tumoral activity with cytotoxic effects and inhibits tumoral cell growth .

Both propolis and artepilina C inhibit lipid peroxidation and the development of pulmonary cancers, prevent the progression of adenomas and carcimonas[89] and prevent oxidation and carcinogenesis induced by nitrile ferric triacetate in mice . After having established, in several previous studies, that the caffeic acid esters in propolis inhibit tumoral growth, both in human colonic adenocarcinoma, as in pre-cancerous wounds induced in rat colons, the possible effect of a diet with added propolis (750 ppm of phenyle- thyl-3-methylcaffeate) on the carcinogenisis induced by subcutaneous azoxymethane in colonic mucose in male rats was studied. It was found that methylcaffeate significantly inhibits invasive and non-invasive multiplicity of colonic adenocarcinomas by reducing by half, with respect to control groups, colonic mucose activity, arachidonic acid production and the volume of he tumor . It is suggested that propolis exerts a protective effect in colonic carcinogenesis, preventing the development of preneoplasic lesions, given that a dose of 30 mg/kg of ethanolic

extract, administered after exposure to a cancerous agent (1,2 dimethylhydrazine), is strongly associated with a reduction in the number of aberrant crypts in the distal colon .

O**phthalmology**

Propolis, rich in antioxidants, prevents 70% of cases of cataract formation induced by expo- sure to selenites . After inducing corneal lesions with sodium hydroxide or silver nitrate in a group of rabbits, topical treatments with propolis extracts or dexamethasone were applied. Similar results were obtained for both substances. In postherpetic keratitis, the local application of thin propolis dressings for 10-15 days signi- ficantly accelerates corneal epithelisation, reduces by half recuperation, and doubles visual sharp ness in control patients .

Dentistry

Alcoholic extracts of propolis posses local anesthetic action, which is attributable to its content in pinocembrin and caffeate esters and composition dependent cariostatic effects. Ethanolic extracts of Brazilian propolis, rich in pinocembrin and galangin, have been found to inhibit glucosiltransferase activity and the growth of Streptococcus mutans Flavanones, some di- hydroflavanols and tt-farnesol sesquiterpene, which is the most antibacterial agent, inhibit the growth of St. mutans and St sobrinus in the oral cavity. Flavons and flavanols, in particular apigenin (4',5,7-trihydroxyflavon), inhibit glucosiltransfe- rase of St. mutans and St. sanguinis.

A study of cases and controls carried out on children has revealed the effects of propolis on acute and chronic inflammatory processes in the upper respiratory tracts. An aqueous extract of commercial propolis (NIVCRISOL®), with a high flavonoid content, was administered to both cases and control group and the effects were monitored through clinical observation and periodic analysis of viral, bacterial and fungal loads. The results indicated that this treatment reduces the number of symptomatic cases and the viral and microbial loads in the upper tract. Due to its effectiveness, good tolerance and low cost, its administration as a contributory remedy in the treatment of acute or chronic rhino-pharyngitis has been proposed.

Mineral metabolism

The effect of the addition of propolis in the diet of healthy rats and rats with ferropenic anemia to the digestive use of iron, calcium, phosphorus and magnesium has been studied. Propo- lis was found to produce, in both groups, weight gain. This would justify its use as a strengthe- ning additive, where in addition to improving the digestive use of iron, it serves to aid the phosphorus and calcium metabolism and to maintain suitable levels of magnesium. In anaemic rats it improves the efficiency of haemoglobin regeneration and the digestive use of calcium and magnesium, which moderates the adverse effects of iron deficiency on the metabolism of these minerals.

Alcoholism

In rats with experimental alcoholic hepatopathy through the oral administration of ethanol (3g/day) for one month, the administration of ethanolic extracts of propolis at a concentration of 10mg/kg administered three times per day for three days, was found to significantly reduce concentrations of transaminase (GOT & GPT), and serum and hepatic triacylglyceride. At 30mg/ kg significant increases in P-450 cytochrome and NADPH dependent-C-reductase concentrations, and lipid peridoxation are avoided, resulting in a notable reduction in hepatocellular fat degeneration and vacuolisation. At 100mg/kg the hepatic concentration of reduced glutathione diminishes and glutathione-S-transferase and γ-glutamylcystein sinthetase activity increases . Consequently, the antioxidants of propolis have a hepatoprotective effect that in propolis of European origin is attributed to the caffeic and ferulic acids and their esters, and to a lesser extent to the flavonic aglycones, while in the Brazilian variety, it is associated with two derivatives of dicaffeoylquinoic acid (3,4-dicaffeoylquinoic-acid and its methyl ester) .

QUALITY EVALUATION

The multiple and varied beneficial properties of propolis to health, which are dependent on its compounds, justify the necessity of an adequate evaluation of its quality. Although different countries have official parameters available to deter- mine such an evaluation, tests to measure its biological activity are scarce. In order for quality of propolis to be considered as high, the following requisites should be fulfilled·

1. **It should be free from toxic contaminants.**
2. **It should contain low percentages of wax, insoluble materials and ash.**
3. **Its botanical origin should be defined, in order to determine its active compounds.**
4. **It should have a high content in active principles.**

In the evaluation of the quality of propolis, in addition to a visual inspection of its physical and organoleptical characteristics (appearance, consistency, origin, colour or smell (see Table 3), which provide a subjective appreciation of the product and an indication of quality to a certain degree, the contents of its active principles should be determined, in order to provide a real and objective evaluation.

TABLE 3: Organoleptic characteristics that permit the quality evaluation of propolis

PARAMETER	QUALITY GOOD	QUALITY AVERAGE	QUALITY POOR
PRESENTA-TION	Flaky and granulated	In blocks or balls	Powder
APPEARANCE	On cutting differences in external and internal colouring	Slight differences in external and internal colouring	No differences in external and internal colouring
COLOUR	Green, yellow, orange or shades of the same colour	Brown	Dark
SMELL	Aromatic resin	Resinous	No smell
TASTE	Spicy or resinous	Slightly resinous	Insipid

In the characterization of raw samples of propolis, it is proposed that content of the following compounds should be determined: Total phenols, flavonoids, wax, ash, volatile compounds and dry residue. In the case of tinctures, total phenols, flavonoids, waxes, density and ethanol should be determined. In table 4, the analytical parameters that define the quality of propolis are shown.

TABLE 4. Analytical parameters that define the quality of propolis[105]

PARÁMETRO PARAMETER	• CALIDAD BUENA (A) GOOD QUALITY	(B) CALIDAD MEDIA (B) MEDIUM AVERAGE	(C) CALIDAD INFERIOR (C) LOW POOR
Test de identidad Identity test	+	+	+
Reacción de Shinoda Shinoda reaction	+	+	+
Índice de oxidación Oxidation index	>0-1.5 s	1.6-5 s	5.1-12 s
Resinas solubles en etanol 96° Soluble resins in ethanol 96°	70-85%	55-69%	40-54%
Cera + impurezas Wax + impurities	15-30%	31-45%	46-60%

The identity test consists of three simultaneous chemical reactions which check for the presence of active principles (total phenols) in resins and therefore, check the percentage of soluble resins in ethanol and establish the yield of propolis and its residue after extraction with solvents. The percentage of wax and mechanical impurities indicate the fraction of substances which are of no practical utility. The higher the percentage, the lower the quality of the product will be.

The Shinoda reaction, serves in the detection of flavonoids and the index of oxidation. Such a determination is included in the quality standards in numerous countries (Russia, Cuba and Rumania). The test evaluates antioxidant power against potassium permanganate, where the greater the velocity of the reaction, the higher the quality of the propolis will be . The index of oxidation is dependant on the phenolic compounds and to a lesser extent, the unsaturated long chain fatty acids. Samples of propolis with a content of phenolic compounds over 7%, present oxidation indices of less than 22 seconds . The difficulty in establishing common quali- ty control standards is a great impediment in the commercialisation of propolis.

SIDE EFFECTS

Propolis is relatively atoxic. Daily doses of 1400mg/kg have no negative effect in mice. However, chewing large quantities of raw propolis can produce nausea and digestive disorders. Apiarists often suffer from headaches while inspecting beehives. And allergic reactions are not un common , particularly to iso prenyl caffeate. Before initiating propolis treatment, an allergy test should be carried out, either through a topical application of the product on the forearm, or orally, after the appropriate precautions have been taken

CONCLUSIONS

Propolis has been used empirically in the treatment of multiple pathologies since ancient times. In the present day, subsequent to the advancement of analytical techniques, greater knowledge concerning its composition is available, permitting the study of the activity of its compounds to be carried out. The results obtained confirm its effectiveness, mainly, as an antioxidant, anti-inflammatory and anti-microbial agent.