

Propolis an over view

Ahmed G. Hegazi, *International Congress of propolis, Buenos Aires, Argentina, 2000*



Propolis, or "bee glue," is a well-known substance that beekeepers find in their hives. Propolis according to research has shown to be effective against a variety of bacteria, viruses, fungi, and molds. It has been shown to be a non-specific immunostimulant. Propolis is a natural brownish-green resinous product collected by honey bees. The word is derived from the Greek pro (before) and polis (city). Propolis was being used to make the protective shield at the entrance of Beehive. Also it used to fill the cracks in the hive, to attach the corners of frames to the grooves in the hive, and also to polish the cells of the honeycomb. The bodies of dead lizards, snakes and mice that have entered hives are sealed into the walls with bee glue, thereby protecting the colonies against the unpleasant odor and bacterial flora of the putrefying corpses (Ghisalberti et al., 1978).

Origin:

Propolis is made from substances collected by bees from the buds of trees (Willow, Poplar, Birch, Fir, Pine, Horse, Chestnut, ..etc). It is prepared from pollen. Researchers stated that propolis has originated either internal and external origins.

1- An Internal origin:
Propolis might be a resin residue forming from the first phase of pollen digestion in small organ placed between the sac and lower gut. All cells and specially the newly built ones are varnished with this internal propolis before the queen lays eggs in them (Caillas, 1978).

2- An External origin:
Bee forager harvested propolis only from the buds particularly from polar and older. It has been found however, that they harvest it also from other trees science propolis has been found in hives in places where there are neither older nor polar. It is well known by all apiarists that the hives placed in forests have more propolis in them than those on the plain.

History:

Propolis was used specially in antiquity, in Egypt. There some thousand years BC propolis was very well known to the priests who had monopolized medicine, chemistry and art of mummifying corpses. The fact that propolis was also known to the old Greeks is demonstrated by the very Greek name of it (Makashvili, 1978). The first held the opinion that bees harvest propolis from resin of willow buds, of poplar, wild chestnut and other plants and other writer assumed that bees harvest it from Styx (Makashvili, 1978).

The Holy Qur'an has a long Sorat with the name of bees (Al Nahl). The Ayahs number 68-69 In the name of God Most Gracious, Most Merciful (68) "And thy Lord taught the Bee to build cells in hills, On trees and in (men's) habitations; (69) Then to eat of all The produce (of the earth), And find with skill the spacious Paths of its Lord: there issues From within their bodies A drink of varying colors, Wherein is healing for men: Verily in this is Sign For those who give thought". Abu Ali bin Sina (Avicenna) distinguishes two kind of wax in his well known work The Canon Medical Science the clean and the black wax. The clean wax is that which composes the comb cells where the bees rear the brood and store the honey and the black wax is the filth the hive. It is clear enough that the black wax is propolis that after Avicenna's testimony has the characteristic of eliminating the spikes. It also rarefies, cleans and Soaks. He also writes that by its strong smell, the black wax makes you sneeze.

In Folk Georgian medicine, they used ointments with propolis to cure some diseases. There was the custom of placing a propolis cake on the belly button of the newborn baby and also they rubbed children's toys with propolis. Also in folk medicine, the use of propolis is widely known especially for the treatment of corns. People inhale propolis in case of affections of respiratory tracts and of the lungs. It is also efficient for burns and angina. The therapeutic characteristics of the propolis have been well known for a very long time. This is explained by its very pronounced anti-microbial characteristics. Propolis was used effectively on wounds by doctors during the Anglo- Boer war and during The World War II. It was also used in hospitals. From 1969 Orthodox medicine in USSR accepted the use of propolis 30 % (30 % alcoholic solution of propolis). It is produced by the pharmaceutical product plant in Tallinn (Makashvili, 1978).

Chemical composition of propolis:
The chemical composition is still insufficiently known. Propolis is a resin being dark green or brown in color with a pleasant flavor of poplar buds, honey, wax and vanilla but it can also have a bitter taste. When burnt, it exhibits a smell of aromatic resins of great value (Nikolaev, 1978). The chemical composition of propolis as well as its color and aroma are changed according to the geographical zones. Its color varies from yellowish-green to dark brown depending on its source and age (Ghisalberti, 1979). It can be likened to aromatic glue. It is hard and brittle when cold, but becomes soft and very sticky when warm. Ivanov (1980) studied the composition and physico- chemical properties of propolis. Also Kaczmarek, et al (1983) found B- Amylase in propolis. Bankova and co-workers (1982, 1983 & 1988) studied the chemical composition of ethanolic extract of propolis (EEP) and concluded that propolis contains mainly polyphenolic compounds, flavones, flavonones, phenolic acid and esters. Fatty acids in propolis were studied by Polyakov et al (1988). Hegazi and Abd el Hady (1997) was analyzed Egyptian propolis by gas chromatography mass spectrometry. 31 peaks were located of which 26 representing 25 compounds were identified, including 7 identified in Egyptian propolis for the first time. Abd El Hady (1994) and Abd El Hady & Hegazi (1994) found that the Egyptian propolis constituents present are phenolic acid esters (72.7 %); phenolic acids (1.1 %); aliphatic acids (2.4 %); dihydrochalcones (6.5 %); Chalcones (1.7 %); flavanones (1.9 %); flavones (4.6 %) and tetrahydrofuran derivatives (0.7 %). It was clear that phenolic acid esters are present in a major quantity

(72.7

%).

Christov et al., (1998) and Bankova et al., (1997) and my group with collaboration with Bulgarian group the chemical composition of Egyptian propolis sample by TLC and GC/MS. They identified 39 compounds, 8 being new for propolis. These results revealed that Egyptian propolis is characterized by the presence of unusual esters of caffeic acid with C12- C16 fatty alcohols, mainly saturated. These esters have not been found till now in propolis. Flavonoid aglycones and especially flavanones are typical components of poplar propolis. Till now there are no data about the presence of triterpenic alcohols in propolis. Recently we identified a series of triterpenes in Egyptian propolis, including the characteristic animal sterol precursor lanosterol. Propolis contain about: 55 % resins and balsams, 30 % waxes , 10 % etheric oils and 5% pollen. The components are rich in: Vitamins and mineral elements (Nikolaev,1978) Propolis contains some minerals such as Mg, Ca, I, K, Na, Cu, Zn, Mn and Fe as well as some vitamins like B1, B2, B6, C and E, and a number of fatty acids. Also, it contains some enzymes as succinic dehydrogenase, glucose-6-phosphatase, adenosine triphosphatase and acid phosphatase (Tikhonov and Momontova, 1987). Khayyal et al.(1993) suggested that propolis is a natural product produced by the honey bee. Its extract contains amino acids, flavonoids, terpenes and cinnamic acid derivatives. Abd

El-Hady and Hegazi (1994) isolated other substances from Egyptian propolis such as aliphatic and phenolic acids. Nagy et al (1985) investigated the chemical constituents, particularly the flavonoid components, of propolis by the GC/MS method. The presence of small amounts of vitamins has been reported for propolis obtained in the USA: Vitamin B1, Vitamin B2, Vitamin B6, Nicotinic acid, Pantothenic acid, Riboflavin, Vitamin A, Vitamin C and Vitamin E. Detection of Vitamins are in variable amount : Vitamin B1: 4.5 µg/g of fresh matter , 6.5 µg/g of dry matter, Vitamin A: 6.1 IU/g of fresh matter, 8.1 IU/g of dry matter, Riboflavin: 20 µg/g of fresh matter, 28 µg/g of dry matter, Vitamin B6 5 µg/g of fresh matter, Moreira (1986) studied the chemical composition of propolis specially vitamins and amino acids. Copper 26.5 mg/kg, Manganese 40 mg/kg . The ash residue of the propolis has been shown to contain iron, Calcium, Aluminum, Vanadium, Strontium, Manganese and Silicon. Walker and Crane (1987) investigated the chemical constituents of propolis. Also Greenaway et al (1990) studied the composition and plant origins of propolis. The biochemical aspects of propolis: Kleinrok et al. (1978) injected propolis intraperitoneally in mice and they found that ethanolic extract of propolis had a weak general effect on the experimental animals. Propolis has different pharmacological activities, Giurgea et al. (1981) reported that daily administration of 20 mg/100 g b.wt. standard propolis extract (SPE) to chicken for 15 days increased plasma total protein and gamma-globulin content. They suggested also that propolis has an anabolic effect and stimulated the immunologic processes. They also reported that daily administration of 20 mg propolis extract to chickens for 15 days changed the blood concentration of cholesterol, transaminase (ALT & AST), total proteins and amino acids. It also stimulated the immune system. In another study, Giurgea et al. (1982) reported that chicken fed on propolis extract showed a significant increase in serum total protein and a slight reduction in the glycogen level of lymphatic organs. Spectrophotometric study showed that propolis contains large amount of flavonoids and

proteins. Interaction of purified propolis in vitro with serum albumin or human serum proteins caused conformational changes in the protein and increased ceruloplasmin activity (Olinescu et al., 1982). Giurgea et al. (1984) found that daily administration of propolis extract to chickens caused a marked increase in the myofibril, protein fraction and muscle total protein when compared to corresponding control. They also stated that, propolis extract affects the levels of cholesterol, transaminase activity, total protein, gamma globulins and free amino acids. They found increases in gamma globulins and proteins and suggested that propolis had an anabolic effect, and that it stimulated the body's immune response.

Amoros et al. (1994) investigated in vitro activity against herpes simplex virus type 1 of 3-methyl-but-2-enyl caffeate isolated from poplar buds. They found that this compound, as a minor constituent of propolis, reduce the virus titres by 3 Log₁₀ and viral DNA synthesis by 32 folds. Yamanchi et al. (1992) measured antioxidative activity of balsams by its inhibition of methyl linoleate autoxidation. The balsam from a simple of Chinese propolis was the most antioxidative and the main component responsible for this property was identified as benzyl caffeate. HPLC showed that the amounts of benzyl caffeate in the propolis balsams varied from nil (not detected) to 160 mg/g. Krol et al. (1990) suggested that antioxidative capacity of propolis is partly due to its high flavonoid contents. Hegazi et al. (1997) showed that, administration of Egyptian and Bulgarian propolis induces an antibacterial activity in vivo as well as in vitro. The ethanolic extract of propolis has a weak general effect on estimated parameters in normal rats and it is not a toxic substance. Both types of propolis exerted an anabolic effect for protein synthesis by liver cells. Both types of infections with *S. aureus* and *E. coli* caused an increase in the activity in serum AST and ALT and consequently decrease their activity in the liver. On the other hand, the activity of ALT and AST returned to the control level after administration of propolis in rats infected with *S. aureus* and *E. coli*. Propolis tends to normalize the serum total lipids in infected rats.

Biological activity of propolis: Propolis stimulated mammalian tissue regeneration, as it caused strong activation of mitosis of cells cultured in vitro and it enhanced protein biosynthesis (Scheller et al., 1977a, Popeskovic et al., 1977 and Gabrys et al., 1986). Extracts from propolis were investigated for cytostatic activity in vitro by Scheller et al. (1977b and 1978a). Chemical and pharmacological study of propolis from various locations were investigated by Papay et al. (1997). Experimental work of Stojko et al. (1978) and Haldon et al. (1980) showed that propolis accelerated osteogenetic process and the regenerative process of different tissues. Matsuno (1992 and 1997) isolated the tumoricidal substances from Brazilian propolis. Strehl et al. (1994) showed that ethanolic and aqueous extracts of propolis indicated substantial anti-inflammatory functions as well as antibiotic activities where the aqueous extract of propolis inhibits the enzyme dihydrofolate reductase, this inhibitory activity may at least partially be due to the content of caffeic acid. Krol et al. (1990) suggested that ethanolic extract of propolis (EEP) has remarkable medical properties, including protection of mice against gamma-irradiation. They said that this anti-oxidative effect is partially due to its high content of flavonoids. The diethyl ether fraction from Egyptian propolis methanol extract revealed the presence of phenolic ester present as a major constituent. The effect of Egyptian propolis on chicken body weight and lymphoid organs revealed

an increase in body weight after one week post injection and increase thymus weight after 14 days post injection up to the end of the experiment. While spleen weight slightly affected, but bursa and caecal tonsil weights were increased at the last two weeks of the experiment. The highest phagocytic activity was at 14 days post injection with propolis (65% vs 59%). Also, there was increase in the stimulation index of the peripheral lymphocytes as detected by lymphocyte transformation in case of propolis group (2.61 vs 1.92 and 1.31 vs 22% at 7th and 28th days post injection). The delayed hypersensitivity skin test using propolis as sensitizing antigen showed specific stimulation to propolis after 72 hours after inoculation with specific antigen. Egyptian propolis gave the typical delayed hypersensitivity when inoculated to the sensitized chickens. The thickness index was 0.90 mm thickness if compared with nonsensitized control group 0.12 mm thickness (Hegazi et al., 1996). Hegazi et al. (1995a) studied the effect of some bee products on immune response of chicken infected with virulent NDV. They found that, the mortality rate was reduced in-groups infected with virulent NDV and subsequently treated either with propolis or honey if compared with the infected groups only. It was clear that, propolis acts actively as antiviral agent than honey. The treatment with propolis and honey of NDV infected chicken groups induced increase in the antibody titres and phagocytic percentage. The inoculation of different antigens in the footpad of sensitized and non sensitized chickens induced different degrees of footpad thickness as well as cellular and vascular reaction depending on the type of inoculation with NDV antigen. The reaction was typical Arthus type. In propolis group inoculated with NDV antigen, the reaction was different and the lymphocytes appeared to play the main role in this reaction which became a delayed type of hypersensitivity. Honey and propolis as management of chronic skin ulcers were used by Tossoun et al (1997). Antimicrobial activity of propolis: The antimicrobial activity of propolis against a wide range of bacteria, fungi, yeasts and viruses has been investigated since the late 1940s and it showed variable activity against different microorganisms.

a- Antibacterial activity :

Chernyak (1973) tested the bactericidal activity of propolis towards 20 staphylococcus, 10 streptococcus and 10 *E. coli* cultures using concentrations of 1.25-5 mg propolis/ml, it showed strong inhibitory activity against 25 of tested bacterial species. Many researchers had investigated the antibacterial activity of propolis and its extracts against Gram-positive and Gram-negative strains and found that propolis had antibacterial activity against a wide range of Gram-positive rods but had a limited activity against Gram-negative bacilli (Prado-Filho et al., 1962; Scheller et al., 1968; Vokhonina et al., 1969; Akopyan et al., 1970 and Grecianu & Enciu, 1976). The bactericidal effect of propolis was tested by Spataru and Frasinell (1963), who found that it had a bactericidal effect on *Staphylococcus* and *E. coli* strains at concentrations of 1.5-3 mg/ml, on *Shiga Bacillus* and *Pyocyanic Bacillus* at 6 mg/ml; on *Sonne Bacillus* at 1.5 mg/ml and on *Salmonella* strains at 3-5 mg/ml. Also, the minimum inhibitory concentration of propolis against 35 *S. aureus* strains and 92 other bacterial strains were determined by Gizmarik and Trupl (1976a). Scheller and co-workers (1977c) found 19 elements in propolis, 3 fractions were obtained and tested against *Staphylococcus*. In another experiment, Scheller et al. (1977d) found that the sensitivity of 90% of *Staphylococci* to ethanolic extract of propolis was lower than in

a standard strain of *S. aureus*. Shub et al. (1978) prepared ethanolic extracts from samples of propolis collected in 18 regions of the USSR. These extracts were serially diluted in agar, in petri dishes. The dishes were then inoculated with the bacteria *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, and the fungus *Candida albicans*, and incubated at 37 °C or 20-25 °C for 48h. Propolis at 125-500 µg/ml inhibited the growth of *B.Cereus* and *S. aureus*, but usually not that of the other 2 bacteria, or the fungus, even at concentrations higher than 1000 µg/ml. Kovalik, P.V. (1979) investigated 12 patients (35-62 years old) suffering from chronic sinusitis, caused by *Candida albicans*. In vitro tests the fungus was sensitive to propolis in 8 cases, weak by sensitive where 2 were and resistant. The patients were treated with an alcohol-oil emulsion of propolis. The emulsion (2-4 ml) was introduced into the sinuses after irrigation with isotonic saline (every day or every second day) . After 1-2 treatments with propolis, there was an improvement in the condition of patients after 5-8 treatments, clinical recovery occurred in 9 and improvement in 3 patients. Recovery occurred after 10-17 days. Ghisalberti (1979) tested propolis samples and concluded that it showed strong inhibitory activity against 25 of 39 tested bacterial species including *Bacillus* larvae which were most strongly inhibited. Other 24 strains were sensitive including Gram-positive cocci and acid-fast rods. Malimon et al. (1980) demonstrated a relationship between polyphenols content in alcoholic extracts of propolis (AEP) and their antimicrobial activity against *Bacillus cereus* 8035. In 91% of cases a high polyphenols content (59% or higher) was associated with significant antimicrobial activity. Spectrophotometric characteristics of AEP may serve as irrelevance index of the antimicrobial activity of raw propolis. Meresta et al (1980) determined minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) for 4 propolis extracts in various liquid and solid media, at pH values from 5.0 to 9.0. Between pH 6.0 and 6.8, the activity was stable and values were MIC, 60 Mg/ml, and MBC, 120 Mg/ml. Results were similar for all extracts. At higher values of pH, bactericidal activity decreased. Glinnik et al. (1981) found that, purified propolis in 5% solutions in ethanol inhibited the growth of *Staphylococcus aureus* and in vitro. Application of 10% solution to ear, nose and throat infections caused in human subjects by various bacterial pathogens gave highly effective therapeutic effects. Thus a 10% alcoholic solution of propolis is recommended for use in treating rhinolaryngeal infections. Glinnik and Gapanovich (1981) showed that, in chickens, propolis was effective against *S. aureus* and *S. epidermidis* in vitro. The activity of propolis against *Bacillus cereus*, *S. aureus*, *E. coli*, *P. aeruginosa* and *Candida albicans* was tested. Olivieri et al (1981) found that propolis (2-20%) Solution in isopropyl alcohol, equivalent to 4-40mg total flavone / ml) had inhibitory activity in vitro against a number of species of Gram positive bacteria and against some fungi and yeasts, but it was inactive against the Gram-negative bacteria tested. Shub et al (1981) tested 106 strains of *S. aureus* all of them were susceptible to 0.5-1.0 mg propolis/ml. strains resistant to benzyl/penicillin, tetracycline, and erythromycin were sensitive to propolis. Propolis had a synergistic effect when combined with any of the 3 antibiotics used against the antibiotic resistant strains. Pepeljnjak et al. (1982) found that, for pure propolis extracts, a concentration of 15-30 mg/ml was needed to inhibit the growth of *candida albicans*, *Aspergillus flavus*, *A. ochraceus*, *Penicillium viridicatum* and *P. notatum*. Pepeljnjak et al. (1982) found that, propolis concentrations of 0.25-2.0 mg/ml inhibited growth of *A.*

sulphureus for up to 10 days, but only the highest concentration showed definite fungicidal activity. Ochratoxin was detected in all cultures media, but its concentration was low at the first 10 days. Compared with the control culture, amounts of Ochratoxin A were directly proportional to the growth of *A. sulphureus*, and inversely to the propolis concentration. Pepeljnjak et al. (1982) analyzed ethanol extracts of 31 propolis samples by TLC. The same 15 components were identified in most samples. The results varied quantitatively in growth inhibition tests of the *B. subtilis*. The greatest inhibition was shown by extracts that had a comparatively high galangin content. Ibragimova et al (1983) used *Staphylococcus epidermidis* as a preventative sample of 14 types of staphylococci isolated from the milk of cows affected by mastitis. Sensitivity of microbial cultures to 6 types of antibiotic was compared for culture media containing propolis or no propolis. The presence of propolis prevented or reduced any gradual build-up in tolerance of staphylococci to antibiotics. Meresta and Meresta (1983) extracted propolis samples from apiaries in different regions of Poland using a combination of equal quantities of acetone, ethyl alcohol, ethyl ether and ethylacetate. Bacteriostatic and bactericidal actions of the extracts were tested using *Staphylococcus aureus*. The minimal inhibitory concentration of extracts ranged from 60 Mg/ml to 430 Mg/ml; minimal bactericidal concentration of extracts ranged from 110 Mg/ml to 1380 Mg/ml. Of the 149 propolis samples, 68% were classified as having good antibacterial activity. Giurgea et al. (1983) found that in rats fed with a standard propolis extract together with *Escherichia coli* antigen, there was an increase in the formation of antibodies compared with rats not receiving propolis. Jozwik et al. (1985) obtained propolis directly from beekeepers, or purchased from a shop, in Dublin, Poland. The biological activity of certain fractions of ethanolic propolis extracts, measured by inhibition of growth of 5 *Mycobacterium* species, was proportional to the concentration of flavonoids in the fraction. The strain *Mycobacterium* sp. 279 was the most sensitive to flavonoids and was therefore useful in comparative tests. The lowest concentration of flavonoids at which inhibition was observed was 0.00996 mg/ml. Meresta et al (1985) examined the sensitivity of 75 bacterial strains to propolis extracts. Of these, 69 were isolated from cows with mastitis, and were identified as *Staphylococcus* spp. and *Streptococcus* spp. All the strains displayed a high sensitivity to propolis extracts - usually of the same order or higher than that of the standard strain *Staphylococcus aureus* 209P (Oxford). Pepeljnjak et al. (1985) analyzed thirty-eight propolis samples, collected from various parts of Croatia, Yugoslavia. Considerable variants were found between samples - even from the same region in the number of constituents present, and their amounts. Some samples, which contained almost no 3,5,7-trihydroxy flavone (galangin) and relatively small amounts of 5,7 - dihydroxy flavone (pinocembrine), showed low inhibition of *Bacillus subtilis* growth. Samples with the highest pinocembrine content (about 80 Mg/ml ethanol extract) showed the highest inhibition and it was found that pinocembrine content and bacterial inhibitions were related. Such a relationship between galangin content and inhibition was also found but was less pronounced. Valdes Gonzales et al. (1985) stated that alcoholic extracts of propolis inhibited the growth of various bacteria, including strains of *Streptococcus* and *Bacillus*. Several UV absorbing substances inhibiting the DNA-dependent RNA polymerases of *Escherichia coli* and *Streptomyces aureofaciens*, as well as the restriction endonucleases Eco RI have been isolated from the water-soluble extract

of propolis by two dimensional paper chromatography. The inhibition of bacterial RNA-polymerase by the components of propolis was probably due to the loss of their ability to bind to DNA (Simuth et al.,1986). Kedzia, A. (1986) determined the minimal inhibitory concentrations (MIC) of ethanol extract of propolis to 112 strains of anaerobic bacteria. EEP showed the greatest effectiveness against strains of Bacteroids and peptostreptococcus; of 37 strains examined, 18 were inhibited at 0.01-0.50 mg/ml. EEP was slightly less effective against the Gram- positive rods of propionibacterium, Arachinia and Eubacterium (MIC 0.50-1.00 mg/ml). Strains of clostridium were the least sensitive to EED. Tothne and Papay (1987) examined the biological activities of samples of Hungarian propolis. They found propolis included anti-inflammatory, antibacterial, antifungal, and local anaesthetic effects. Propolis inhibited growth of resistant Gram-positive and Gram-negative bacteria and fungi. They used Hungarian propolis in producing pharmaceutical products. Petri et al. (1988) found that propolis sample from 20 locations in Hungary contained from 0.3 to 1.5-% essential oils. The 11 components of the oil fraction were the same for all samples but the ratios of the components differed. In microbiological tests, propolis oil showed good to moderate activity against Gram- positive and Gram-negative bacteria and against 3 fungi. Abdulsalam et al (1989) collected propolis sample from honey bee (*Apis mellifera*) colonies housed in Langstroth hives at Al- Hassa, Saudi Arabia. A propolis- ethanol mixture (1:15) was prepared. The unfractionated extract (PEE) was then used to find out its antimicrobial activities on 13 bacterial species (5 Gram- positive, 8 Gram-negative). Eight extract levels, 50-2000 ppm, were incorporated into dextrose-yeast extract medium. All Gram-positive species were inhibited by 100 ppm PEE in the medium (*Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus*, and *S. epidermis* and *Streptococcus pyogenes*) of the Gram negative species, *Enterobacter cloacae* and *Proteus vulgaris* were inhibited at 400 ppm, and *Pseudomonas aeruginosa*, *Serratia marcescens*, and *Serratia* sp. at 800 ppm. The other 3 species (*Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella typhimurium*) were inhibited at 1200 ppm of PEE. Meresta et al (1989) treated acute mastitis in 146 cows using propolis extract. Complete healing occurred in 125 cows, including all the cases caused by *Candida albicans*, 91% of cases caused by staphylococci, 85% caused by *Escherichia coli*. And 84.3% caused by streptococci. Propolis was very effective in the therapy of mastitis caused by microorganisms resistant to antibiotics. Milena et al (1989) reviewed the sources, and chemical, physical and biological properties of propolis. The activity of a 10% ethanolic extract of propolis against 17 fungal pathogens was compared with that of Mylyt, and E. German propolis- containing preparation. The propolis extract inhibited candida and all tested dermatophytes. Mylyt did not inhibit *Aspergillus*, *Fusarium*, *Penicillium notatum* or *scopulariopsis brevicaulis* of 7 components of propolis tested for antifungal activity, only benzoic acid, Salicylic acid and Vanillin were strongly fungistatic. Grange et al. (1990) tested an ethanolic extract of propolis on 21 bacterial strains. It showed antibacterial activity against a range of commonly encountered cocci and Gram-positive rods, including *Mycobacterium tuberculosis*, but only limited activity against Gram-negative bacilli. Grange and Davey (1990) found that propolis have antibacterial activity against a range of commonly encountered cocci and Gram-positive rods, including the human tubercle bacillus, but only limited activity against Gram-negative bacilli. These findings confirm that, the antimicrobial properties of propolis possibly attributed to its high flavonoid content.

Brumfitt et al. (1990) discovered that the material extracted from propolis by alkaline aqueous solvent or organic solvent showed weak inhibitory activity in vitro against certain species of Gram-positive bacteria. Focht, et al (1993) studied the bactericidal effect of propolis in vitro against agents causing upper respiratory tract infections. Krol et al (1993) incubated ethanolic extract of propolis (EEP) with 8 common antibiotics in culture medium containing fixed amount of standard strain of staphylococcus aureus. The antibiotic compounds used were: penicillin G, doxycycline, streptomycin, cloxacillin, chloramphenicol, ceffradine, ampicillin and polymyxin B. They were used in varying levels, ranging between 0.000005 and 125.0 Mg/ml or units, respectively. Minimal inhibitory concentrations were established in the absence of EEP, than EEP was added in concentrations up to 600 Mg/ml. EEP had marked synergistic effect on the antibacterial activity of streptomycin and cloxacillin, and cloxacillin, and a moderate synergistic effect on the others, except ampicillin. Rojas Hernandez et al. (1993) evaluated the antimicrobial activity of ethanolic extracts of propolis against 30 clinical strains of Mycobacterium and the inhibitory and bactericidal minimum concentrations (IMC and BMC, respectively) were determined for 1 and 7 days of treatment. All mycobacterium strains with the exception of M. tuberculosis were inhibited with 1.0 mg/ml propolis only 30% of the M. tuberculosis strains tested were inhibited by 2.0-5.0-mg/ml propolis. The difference in BMC for 1 and 7 days of treatment was not significant. Aga et al. (1994) isolated three antimicrobial compounds from Brazilian Propolis and identified them as 3,5 diprenyl-4- hydroxycinnamic acid, 3-prenyl - 4-dihydrocinnamoxycinnamic acid and 2,2- dimethyl -6- carboxyethenyl- 2H-1- benzopyran. Their respective antimicrobial activities, expressed as MIC in Mg/ml, against Bacillus cereus were 15.6, 31.3 and 125; against *Enterobacter erogenous*, 31.3, 62.5 and 125; and against *Arthroderma benhamiae*, 15.6, 7250, and 62.5 thus the first compound showed the highest activity and is likely to be one of the major antimicrobial compounds in Brazilian propolis. Takasi et al. (1994) stated that propolis inhibits bacterial growth by preventing cell division, thus resulting in the formation of pseudo-multicellular streptococci. In addition, propolis disorganized the cytoplasm, the cytoplasmic membrane and the cell wall, caused a partial bacteriolysis and inhibited protein synthesis. It was evidenced that the mechanism of action of propolis on bacterial cell is complex and a simple analogy can not be made to the mode of action of any classic antibiotics. Microcalorimetric and electron microscopic studies on the mode of the antibacterial

action of propolis inhibits bacterial growth by preventing cell division, thus resulting in the formation of pseudo-multicellular streptococci. In addition, propolis disorganized the cytoplasm, the cytoplasmic membrane and the cell wall, caused a partial bacteriolysis and inhibited protein synthesis. It was evidenced that the mechanism of action of propolis on bacterial cells is complex and a simple analogy cannot be made to the mode of action of any classic antibiotics (Takasi et al., 1994). Higashi and De Castro (1995), studied the ethanolic (EEP) and dimethyl-sulphoxide extracts (DEP) of propolis, for their anti- protozoan properties and found that they were active against the different forms of the parasite studied. Total lysis of blood stream trypomastigotes was observed after 24 hr in the presence of EEP at a concentration of 100 µg/ml. The effect was found to be temperature-dependent. Treatment of infected peritoneal macrophages and heart muscle

cells with EEP strongly inhibited infection levels. Grochowski et al (1996) carried out experiments on a group of 64 mice with burns that had been artificially infected with *P. aeruginosa*. After 24h, mice from the experimental group (A) were treated daily with 3% propolis ointment containing Soya oil, dehydrated butter, fresh dehydrated pork fat and beeswax. Mice in the control group (B) were left untreated. In-group A, the burns took 7-13 days to heal completely in 85% of the mice, and up to 16 days in 11% of mice. In-group B, the burns healed in 14-18 days in 84% of the mice; 4 mice died within that period. Other experiments showed that the ointment medium was important as the propolis; Vaseline and lanolin ointments containing propolis, Vaseline and lanolin ointments containing propolis were much less effective than the ointment used in A. Hegazi et al., (2000) investigated three propolis samples from Austria, Germany and France by GC/MS, where eleven compounds were being new for propolis. The samples showed some similarities in their qualitative composition. phenylethyl-trans-caffeate, benzyl ferulate and galangin were predominant in German propolis. Benzyl caffeate was predominant in French sample. pinocembrin was predominant in French and Austrian propolis and trans-p-coumaric acid was predominant in all samples. The antimicrobial activity against *Staphylococcus aureus*; *Escherichia coli*, and *Candida albicans* was evaluated. German propolis showed the highest antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. While Austrian propolis has the highest activity against *Candida albicans*. French propolis was effective against all pathogens but less than German and Austrian propolis.

b- Antiviral, antifungal and antiprotozoal activity :

loirich et al. (1965) showed that propolis had virulicidal action in vitro against influenza virus (type A). In 1975, Krivoruchka et al. suggested that, in an in vitro experiment, the aqueous extract of propolis sharply reduced the infectiousness of smallpox vaccine virus within 15 min in 20°C. Concentration of the virus was reduced by 10⁻⁵-10⁻⁴, and its infectivity was 21-129 times less than that of the control. The extract had a smaller effect in vivo and only when the extract was administered before infection. König and Dustmann (1986) reviewed the activity of the Propolis and viruses. Hegazi et al. (1997) reported that, propolis has antiviral, antibacterial and antifungal effect, and also it has a lot of chemical action in pharmacology. Serkedjieva et al. (1992) found that isopentyl ferulated (isolated from propolis) inhibited significantly the infectious activity of influenza virus A1 Honey Kony (H3N2) in vitro and the production of haemagglutinins in vitro by the use of diverse experimental patterns. It was found that the maximal inhibition of viral production was observed when test substances were present in the medium during the whole infectious process. Cizmarik and Trupl (1976) tested the inhibitory activity of propolis against *Aspergillus sulphureus* and against some fungi. The ethanolic extract of propolis inhibited 60 strains of yeasts and 38 strains of fungi. Kovalik (1979) used propolis in the treatment of patients with chronic fungal sinusitis. Milena, et al (1989) found fungistatic effect of propolis. Starzyk et al. (1977) found that propolis extract with certain concentrations was lethal to *Trichomonas vaginalis*, when applied clinically, it killed all active forms of the parasite within 24 hrs. Propolis, when injected simultaneously with concentrated tetanus antoxin, either once or under conditions for hyper-immunization, stimulated non-specific and specific immunity factors and increased the preventive propolis of immunizing sera and the resistance of animals to tetanus toxin (Kivalkina and Bodarova, 1975). Hegazi et al. (1993) studied the effect of propolis on different

NDV vaccinated strains. They found that the addition of propolis to NDV induced a significant reduction of infectivity titres. The effect of propolis was pronounced in Lasota, clone 30 and virulent NDV. The HI titres of kamarov and virulent NDV were reduced significantly. They suggested that propolis has antiviral activity against NDV. Investigations were performed by Ecsanu et al. (1981) on the effect of an aqueous extract of propolis on experimental infection with influenza virus A/PR8/34 (HONI) in mice. Propolis extract administered intranasal 3 hr before virus inoculation led to a reduction of the haemagglutination antibody (HA) titers recorded in the lung suspensions from infected mice, but no reduction in mortality or increase in mean survival length. When the extract was administered 3 hr after virus inoculation, the reduction in HA titers was accompanied by a slight decrease in mortality and increase in mean survival length, propolis caused an increase in both HA titre and mortality. Esanu et al., (1981) and Serkedjieva et al. (1992) studied the anti-influenza virus effect of propolis constituents, one of these inhibited the infectious activity of influenza virus in vitro and the production of haemagglutinins in vivo. Propolis displays strong antimicrobial activity. The anti-protozoan properties of different propolis extracts were studied regarding *Trypanosoma cruzi* and its interaction with host cells. Ethanolic and dimethylsulphoxide extracts were both active against the three forms of the parasite, with the former more active than the latter against the vertebrate forms, amastigotes and trypomastigotes (Higashi and De Castro, 1994). Hegazi et al. (1995a) studied the effect of some bee products on immune response of chicken infected with virulent

NDV. They found that the mortality rate was reduced in group infected with virulent NDV and subsequently treated either by propolis or honey if compared with the infected group only. Also, they stated that propolis act actively as an antiviral agent than honey. The treatment with propolis and honey for infected chicken groups induced increase in the antibody titres and phagocytic percentage. The inoculation of different antigens in the foot pad of sensitized and non sensitized chickens induced different degrees of foot pad thickness as well as cellular and vascular reaction depending on the type of the sensitizing antigens in foot pad. The most severe reaction was recorded in the honey and NDV group inoculated with NDV antigen. The reaction was typical to Arthus type. In propolis group inoculated with NDV antigen, the reaction was differed and the lymphocytes appeared to play the main role in this reaction which become a delayed type of hypersensitivity.

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