Study of Bactericidal Properties of Propolis

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Abstract
The antibacterial activity of an alcoholic extract of propolis from Apis mellifera was investigated using a method of growth inhibition in the culture medium of microorganisms. For this study were been chosen bacterial species implicated in severe infections in animals: *Streptococcus suis, Staphylococcus aureus, Escherichia coli, Salmonella spp., Pseudomonas aeruginosa and Pasteurella haemolytica (Mannheimia haemolytica, 1999).* The propolis extract demonstrated to possess bactericidal properties against studied microorganisms in descending order as follows: *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes, Pasteurella haemolytica, Salmonella spp.* All these results demonstrates the possibility of using propolis in either treatment alone, as a natural alternative, be associated with an appropriate antibiotic for the potentiating effect in the event of serious infections in animals.

Key words: antibacterial activity, bactericidal properties, microorganisms, propolis.

1. Introduction

Propolis, also called "bee glue" or "Romanian penicillin" is an extremely valuable bee product. It consists of a mixture of resinous, sticky, greenish brown or brown, with pleasant aroma of resin and conditioners. Is processed by worker bees after collection of organic products at least 20 species of coniferous trees that produce softwood secretions buds of trees (poplar, birch, alder, chestnut, beech, ash, pine, fir), stems and young branches , petiole leaves and bark (willow, plum). This material resin bee added salivary gland secretions containing enzymes, wax and other biochemical compounds [1, 2]. Both colour and flavour of propolis and the chemical composition differ depending on the plant species from which the collected raw materials. Harvesting is done in warm days with temperatures above 20 ^{0}C, the finished product becomes plastic. From a hive, beekeepers can harvest a quantity wise 100-400 grams of propolis, depending on the area. The paper is scraped from the walls of the hive bee glue layer is maintained, then, in the form of little balls or in alcohol tincture. Healing effects of propolis have been known since ancient times. In ancient Egypt, priests used it to embalm the dead, ensuring preservation of mummies unaltered over the centuries [3]. The Greek philosopher Aristotle believes that propolis is a "cleaning wax" and recommended a remedy for bruises and suppurating wounds. Gradually preparation came into use folk medicine, although concrete explanations therapeutic effects reached insufficiently known by the general public being kept secret. Propolis contains a powerful germicide. The chemical composition was studied by many scientific institutions that have sought to explain the therapeutic properties and effects manifest in a variety of unhealthy conditions. Biochemical analysis revealed a complex mixture of substances derived from plant sources that were

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collected by bees. In propolis there are 55% resins and balsams, 25-30% waxes, 10% aromatic essential oils and 5% pollen. The lower amounts are amino acids, enzymes, vitamins (A, B, D, E, PP), the natural hormone, flavones, flavonoids, organic acids (cinnamic, caffeic, ferulic) and complex iron minerals are predominant , Zn, Cu, Co, Mn, Mo, Al, Ca, silicon, barium, vanadium, elements that are involved in physiological processes of the optimum development of human and animal bodies. It is worth recalling that ferulic acid is a powerful bactericide, destroying germs Gram-positive and Gram-negative. It was found that this preparation is used for lining walls bee hive and sealed with a glossy layer which prevents drafts inside [4]. This stopped all the cracks in the hive frames to increase juvenile disinfected and covered with a layer aseptic bodies of pests enter the hive and killed by injecting bee venom. Wax layer embalm corpses will keep uninvited guests hive infections and ensure perfect hygiene hive due to its antibiotic, antibacterial and healing. Incidentally, the word "propolis" derives from Greek and means "outside the city", linked to the fact that this substance acts as a shield against aggressors from outside the hive being likened to a real city.

Because of multiple chemical components, propolis is considered the most valuable bee product with a variety of therapeutic actions: antibacterial, antiseptic, antiviral, anti-toxic, anti-parasitic, epithelising, healing, anti-inflammatory, diuretic, analgesic, antitumor, anticancer, regenerating and stimulating immune system. With these properties in relieving and healing many positive morbid conditions, physical and mental.

Bactericidal and bacteriostatic action was proven by culture different bacteria (streptococci, staphylococci, Trichomonas, Klebsiella, Salmonella, Shigella, Proteus, Candida, Helicobacter pylori). It intervenes also in destroying intestinal parasites (Giardia). Modern studies to test the sensitivity of the 80 microorganisms to propolis showed that the 21 species of bacteria, 9 species of parasite fungus and 30 types of viruses are destroyed. For these effects, propolis is considered as the most powerful anti-infective medicine.

Note that propolis preserves their antimicrobial power, as opposed to synthetic antibiotics, to which the bacteria gradually develop resistance, which makes it necessary to periodically introduce new products. Other studies have shown the effects of anaesthetics exceptional 3.5 times higher than that of cocaine and 5.2 times higher than that of novocaine.

2. Materials and methods

This study was been performed in June-October 2013, when propolis samples were been collected from beekeepers located in Timiş county. All propolis samples were stored at -20 ^{o} C until the collection was completed. In the view of ethanol extract preparing, all propolis samples were cut into small pieces and ground. Then, twenty-five grams of propolis was mixed with 250 ml of 70% ethanol at 25 ^{o} C for 24 hours [5, 6]. The obtained mixture was then filtered through filter paper and centrifuged at 400 rpm for 20 minutes. The alcoholic solution was restored to its initial volume of 250 ml with 70% ethanol and stored at -10 ^{o} C. For this study were been chosen bacterial species implicated in severe infections in animals: *Streptococcus suis, Staphylococcus aureus ATTC 6538P, Escherichia coli ATTC 10536, Salmonella spp., Pseudomonas aeruginosa ATTC 27853 and Pasteurella haemolytica type A (Mannheimia haemolytica, 1999)* provided from the Microbiology laboratory collection and inoculated in usually liquid culture medium. For each bacterial species were prepared Petri dishes with Mueller Hinton Agar. The used method was agar diffusion technique. Every Petri dish was inoculated in flood with a sterile pipette the studied species of bacteria, so that were been finally obtained six Petri dishes. Then, equal concaves were practiced with a matrix with 4 mm diameter, in the middle of Petri dish with agar gel and the agar discs were removed with a needle. In each Petri dish, in the concave practiced in the middle was been poured 1ml ethanol extract of propolis with an automatic pipette [7,8]. All inoculated Petri dishes remained at room temperature 30 minutes, after that were incubated at 37 ^{o} C for 24 hours.

3. Results and discussion

In Table 1 are presented the results of agar diffusion technique using the six bacterial species

mentioned. After 24 hours of incubation at 37 ^{o} C, around every bacterial species inoculated in culture medium formed inhibition areas by variable diameters. The lowest zone of inhibition was formed around *Pasteurella haemolytica* (15 mm), which shows that this bacterium manifested the maximal resistance to propolis. The largest inhibition zone developed around *Staphylococcus aureus* (27 mm) which manifested the greatest sensitivity to propolis, followed by *Escherichia coli* (23 mm), *Pseudomonas aeruginosa* (22 mm), *Salmonella spp.* (19 mm) and *Streptococcus suis* (18 mm), results who are in concordance with those obtained by other researchers. Important is that all bacterial species examined none showed resistance to propolis, this demonstrates again the reason for which propolis is called natural antibiotic.

Table 1. The results of agar diffusion technique

Bacterial species	Diameter of inhibition area (mm)
Streptococcus suis	18
Staphylococcus aureus	27
Escherichia coli	23
Salmonella spp	19
Pseudomonas aeruginosa	22
Pasteurella haemolytica	15

In the view of understanding how important the obtained results are, in table 2 are shown diseases in animals of economic interest which have etiologic agents each studied bacterial species. These serious diseases enough frequent in farms encountered great economic damages in growth sectors of animals concerned. The obtain results on the six bacterial species implicated in severe infections in farm animals demonstrate another time that there no doubt, propolis has a strong antibacterial effect as reported in previous studies [5-7].

Table 2. Diseases produced on animal species by studied etiologic agents

Bacterial species	Affected farm animal species	Diseases
Streptococcus suis	pigs, ruminants	polyarthritis, meningitis, septicaemia, abortions
Staphylococcus aureus	chickens, horses, sheep, cows, pigs	pododermatitis, mastitis, purulent infections
Escherichia coli	pigs, cows, poultry, horses, rabbits	severe diarrhoea, weight losses, dehydration
Salmonella spp	poultry, horses, ruminants, rabbits	abortions, severe diarrhoea, dysentery, lethargy
Pseudomonas aeruginosa	poultry, rabbits, pigs, ruminants	mastitis, pneumonia, otitis, keratitis, dermatitis
Pasteurella haemolytica (Mannheimia haemolytica, 1999	ruminants, poultry, rabbits	pneumonia, rhinitis, mastitis, septicaemia

Also propolis could be a promising antibacterial agent [1], reason for further in-vitro and in-vivo studies need to be performed. Based on these results and many other obtained in researches made on propolis activity it is possible to apply this miraculous natural product in an adequate solvent in cases of infections caused by studied bacteria with a positive outcome, making the application of propolis more and more successful [5,6]. Today the propolis extraction use solvents such toluol, acetone and chloroform, all toxic substances, with harmful effects on the animal and human organism, even when it is applied in minimal quantities [9]. For this reason it is necessary to evaporate the extracted propolis by means of a vacuum evaporator so that this extract is turned into a solid state, like powder, which contains all active components while the solvent responsible by toxic effects is eliminated through evaporation.

The obtained propolis powder can be used in pharmaceutical industry for production of tablets that are administered *per os* or ointments for external application both in animals in humans. Due to its antibacterial effects in many studies demonstrated also antifungal and antiviral effects, propolis can be successfully used as an alternative treatment.

4. Conclusions

Using a remarkable natural product of bees, propolis, with more health beneficial actions, including antibacterial effect, against six bacterial species responsible of severe infections in farm animals, the obtained results emphasized that no

one of studied bacteria manifested resistance to propolis. The largest diameter of inhibition area developed through agar diffusion technique and the great sensitivity to propolis manifested *Staphylococcus aureus* (27 mm), followed by *Escherichia coli* (23 mm), *Pseudomonas aeruginosa* (22 mm), *Salmonella spp.*(19 mm), *Streptococcus suis* (18 mm) and *Pasteurella haemolytica* (15 mm). All these results shows that there is possibility of including propolis in an alternative treatments, with the condition of obtaining propolis in a variant without toxicity caused by the extraction solvents. This natural therapy may prevent the spread and development of resistant bacterial strains, a not insignificant problem in the world that has become increasingly common in recent years.

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