LUCRĂRI ŞTIINŢIFICE MEDICINĂ VETERINARĂ VOL. XL, 2007, TIMIŞOARA

ANTIBACTERIAL ACTIVITY OF HONEY AND PROPOLIS MELLIFERA AGAINST *STAPHYLOCOCCUS AUREUS*

IULIA KRISZTINA RINDT, MIHAELA NICULAE, F. BRUDAŞCĂ

University of Agricultural Sciences and Veterinary Medicine,
Faculty of Veterinary Medicine
Str.Manaştur No.3-5, Cluj-Napoca, Romania

Summary

The antibacterial activity of honeydew honey and propolis was evaluated *in vitro* against *Staphyococcus aureus* strains isolated from canine patients with dermatitis.

Performing a broth microdilution protocol we determined the antimicrobial potential of honeydew honey and propolis and obtained data regarding the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). The sensitivity of *Staphylococcus aureus* strains to honeydew honey was compared with an artificial honey solution, while the sensitivity to propolis was compared to 96º alcohol.

Honeydew honey showed bactericidal effects against the bacterial tested *S. aureus* stains, but was less efficient then the propolis at certain concentrations. Artificial honey also displayed antimicrobial activity to a certain extent.

In conclusion, our results show that honeydew honey and propolis could be considered as an alternative treatment in canine recurrent dermatitis.

Honey and propolis are bee products that have been used for centuries in folk medicine (Ghisalberti 1979; Zumla and Lulat 1989). These products have received increased attention from the general population because of their health claims (Dobrowolski et al. 1991). The antimicrobial activity of honey is mainly credited to its acidity, osmolarity, and enzymatic generation of hydrogen peroxide via glucose oxidase (Dustmann, 1979; Molan, 1992a). Additional honey components, such as aromatic acids or phenolic compounds, may also contribute to the overall antimicrobial activity (Weston, 1999).

Propolis is a resinous substance collected by *Apis mellifera* from various tree buds. Its composition is directly related to that of bud exudates collected by bees from various trees; poplar (*Populus* spp.), birch (*Betula alba*), beech (*Fagus sylvatica*), horse chestnut (*Aesculus hippocastanum*), alder (*Alnus glutinosa*) and various conifers (Ghisalberti, 1979; Bankova et al., 2000). More than 300 constituents have been identified in different propolis samples (Bankova et al., 2000). Flavonoids, aromatic acids, diterpenic acids and phenolic compounds appear to be the principal components responsible for the biological activities of propolis samples. Bees use propolis for coating hive parts and also to seal cracks and crevices in the hive.

Materials and methods

Honey samples: were: 1) ***Artificial honey*** (100 g) was prepared by dissolving 1.5g sucrose, 7.5g maltose, 40,5g fructose and 33,5g glucose in 17 ml sterile saline solution. This solution presents the proportions of the four predominant sugars in natural honey samples (Cooper et al. 2000). 2) ***Honeydew honey***, a special kind of honey collected by bees from the hig sugar content liqid excrestions of aphids.

Propolis sample: natural propolis 30 g. and ethylic alcohol 96º up to 100 ml.

Bacterial strains: The honey samples and the propolis were tested against *Staphylococcus aureus* strains: reference strain *Staphylococcus aureus* ATCC 25923 as well as four strains isolated from clinical cases of canine pyodermitis. All these strains were totally resistant to penicillin, tetracyclin, cephalotine, oxacilline, enrophloxacine, gentamicin, amoxycillin with clavulanic acid and cyprophloxacine. All bacterial strains were cultivated for 24h at 37°C on agar plates (Merck). 24h old bacterial cultures were diluted in sterile saline and the optical density was adjusted to that of tube 0.5 in McFarland' scale, to standardize the inoculum (1.5x 10^8cfu/ml).

Antimicrobial assay: Three different methods were used to evaluate the antimicrobial activity of honey: broth microdilution method, well and disc diffusions, respectively. The broth microdilution method, established previously by Carson *et al.* (1995) was finally used to determine the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC).

Using Mueller Hinton broth (Difco™), series of twofold dilutions of honedew honey and propolis were performed in sterile 96 wells microtitre plates. 100 µl of each resulted dilutions ranging from 1.563 (1/64) to 50% (1/2) (v/v) were mixed with equal volume of bacterial suspension. Positive (broth and inoculum) and negative (simple broth) growth controls were prepared. The honeydew honey effects were screened in comparison to artificial honey. The propolis effect was screened in comparison to alcohol 96°. The plates were incubated for 24h, at 37°C. The MIC was defined as the lowest concentration (highest dilution) of honey or propolis that inhibited the visible growth (no turbidity) compared to the control (simple broth). The results were read spectrophotometrically. After this, 10µl of each well was transferred to Mueller Hinton agar plates and incubated at 37°C for 24h.The MBC was considered as the lowest concentration of honey and propolis associated with no visible growth of bacteria on the agar plates. All samples were tested in duplicate. Mean values of growth inhibition were calculated.

Results and Discussions

The growth of numerous microorganisms associated with disease or infection is inhibited by honey (Molan, 1992a,b). Different researchers (Willix et al.,

1992; Cooper et al., 2002) suggest that honey is effective *in vitro* against wound-infecting bacteria including *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella enterica ser. typhimurium*. Azevedo et al. (1963) evaluated the antibacterial activity of bee-wax and propolis produced by *Apis mellifera* and by two Brazilian *Meliponinae* using the agar diffusion method. Propolis from *A. mellifera* inhibited *Bacillus subtilis*, *S. aureus* and *Mycobacterium smegmatis*. Wax from *Trigona ruficrus* inhibited *B. subtilis, S. aureus* and *Mycobacterium smegmatis*. Wax and propolis from *Trigona postica* inhibited *B. subtilis* and *S. aureus*. Collectively, these studies suggested that propolis has inhibitory activities against bacterial pathogens.

The ethanolic extract of propolis has been reported to possess various biological activities, such as antibacterial (Kujumgiev et al., 1999; Moreno etal., 1999; Sforcin et al., 2000), antifungal (Kujumgiev et al., 1999; Ota et al., 2001; Sawaya et al., 2002; Kartal et al., 2003), antiviral (Manolovan et al., 1985; Amoros et al., 1994), anti-inflammatory (Miyataka et al., 1997) local-anesthetic (Paintz and Metzner, 1979) antioxidant (Volpert and Elstner, 1993; Orhan et al., 1999) immunostimulating (Dimov et al., 1991) and cytostatic (Banskota et al., 1998).

In this research, five *Staphylococcus aureus* strains were tested in order to evaluate the antimicrobial potential of honeydew honey and propolis. The MICs values for honeydew honey and propolis, determined using a broth microdilution method, were presented in comparison to artificial honey in tables I and Fig. I.

Table I

Bacterial growth inhibition under honeydew honey, propolis and alcohol *in vitro* treatments

Dilution	Honedew honey		Mean	Propolis		Mean
1	0.084	0.067	**0.076**	0.199	0.193	**0.196**
2	0.058	0.06	**0.059**	0.035	0.044	**0.040**
3	0.075	0.081	**0.078**	0.023	0.032	**0.028**
4	0.055	0.049	**0.052**	0.152	0.158	**0.155**
5	0.036	0.043	**0.040**	0.112	0.139	**0.126**
6	0.003	0.001	**0.001**	0.124	0.123	**0.124**

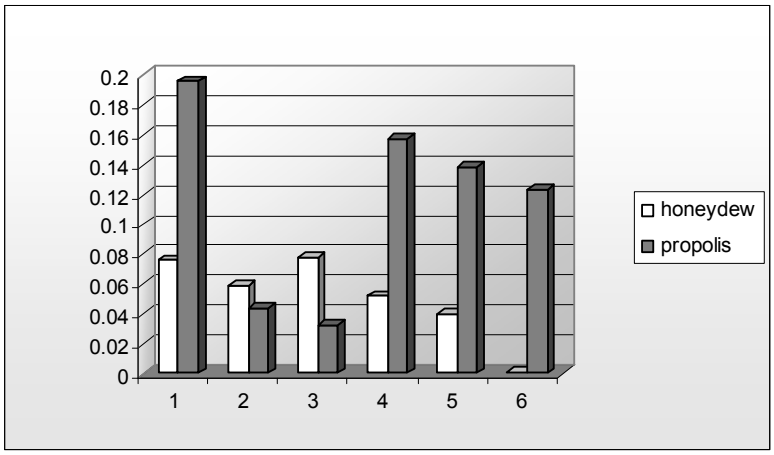

Figure I. Mean minimal inhibitory concentrations' values for honeydew honey and propolis against *S. aureus* strains

From both the table and the graph it is obvious that the honedew honey has a more constant effect, the most active at very low concentrations – a so called "homeopathic effect". Propolis was less effective at the highest concentration and became more active in dilutions 2 and 3, and lost its efficacy in the subsequent dilutions.

Therefore, a combination of the dilution 6 (1.563% or 1/64) of the honedew honey and dilution 3 (12.5% or 1/8) of the propolis could best be used in staphylococcal dermatitis in dogs.

Conclusions

The experiment proved that both honedew honey and propolis have bactericidal activity against totally antibioresistant *S. aureus* strains isolated from canine dermatitis. The differences recorded between the different efficacies of various dilutions used indicated that the two components could be usefully combined, elective concentrations being the most active ones for each product.

References

1. **Amoros, M., Simoes, C.M.O., Girre, L., Sauvager, F., Cormıer, M.,** 1994.Comparision of the anti *Herpes simplex* virus activities of propolis and 3-methyl-butyl-2-enyl caffeate. Journal of Natural Products 57, 644–647.

2. **Azevedo, J.L., Flechtmann, C.H.W. and Prado Filho, I.G.** (1963) Ocorreˆncias de substaˆncias antimicrobianas em produtos de alguns insetos sociais. Revista de agricultura 38, 129–36.
3. **Bankova, V.S., De Castro, S.L. and Marcucci, M.C.** (2000) Propolis: recent advances and plant origin. Apidologie 31, 3–15.
4. **Banskota, A.H., Tezuka, Y., Prasain, J.K., Matsushige, K., Saiki, I., Kadota, S.,** 1998.Chemical constituents of Brazilian propolis and their cytotoxic activities. Journal of Natural Product 61, 896–900.
5. **Carson, C.F., Cookson, B.D., Farrelly, H.D. and Riley, T.V**. Susceptibility of methicillin-resistant *Staphilococcus aureus* to the essential oil of Melaleuca alternifolia, (1995) Journal of Antimicrobial Chemistry 35, 421-424. 64, 235–240.
6. **Cooper, R.A., Molan, P.C., Harding, K.G.,** 2002. The sensitivity to honey of Gram-positive cocci of clinical significance isolated from wounds. Journalof Applied Microbiology 93, 857.
7. **Cooper, R.A., Wigley P. and Burton, N.F**. Susceptibility of multiresistant stains of *Burkholderia cepacia* to honey.(2000) Letters in Applied Microbiology 31,20-24
8. **Dimov, V., Ivanovska, N., Manolova, N., Bankova, V., Nikolov, N., Popov, S.,** 1991. Immunomodulatory action of propolis: influence on anti-infectious protection and macrophage function. Apidologie 22, 155–162.
9. **Dobrowolski, J.W., Vohora, S.B., Sharma, K., Shah, S.A., Naqvi, S.A.H. and Dandlya, P.C.** (1991) Antibacterial, antifungal, antiamoebic, antiinflammatory and antipyretic studies on propolis bee products. Journal of Ethnopharmacology 35, 77–82.
10. **Dustmann, J.H.,** 1979. Antibacterial effect of honey. Apiacta 14, 7–11.
11. **Ghisalberti, E.L.** (1979) Propolis: a review. Bee Word 60, 59–84.
12. **Kartal, M., Yıldız, S., Kaya, S., Kurucu, S., Topc¸u, G.,** 2003. Antimicrobial activity of propolis samples from different regions of Anatolia. Journal of Ethnopharmacology 86, 69–73.
13. **Kujumgiev, A., Tsvetkova, I., Serkedjieva, Yu., Bankova, V., Christov, R., Popov, S.,** 1999. Antibacterial, antifungal and antiviral activity of propolis of different geographic origin. Journal of Ethnopharmacology
14. **Manolovan, M., Maxımova, V., Gegova, G., Serkedjieva, Yu., Uzunov, S., Marekov, N., Bankova, V.,** 1985. On the influenza action of fractions from propolis. Comptes rendus de l'Academie de Bulgare de Sciences 38, 735–738.
15. **Miyataka, H., Nishiki, M., Matsumoto, H., Fujimoto, T., Matsuka, M., Satoh, T.,** 1997. Evaluation of propolis. Part I: evaluation of Brazilian and Chinese propolis by enzymatic and phsico–chemical methods. Biological and Pharmaceutical Bulletin 20, 496–501.
16. **Molan, P.C.**, The antibacterial activity of honey. 1. (1992a) The nature of the antibacterial activity. Bee World ;73:5-28

17. Molan, PC., The antibacterial activity of honey. 2. (1992b) The nature of the antibacterial activity. Bee World ;73:59-76

18. Moreno, M.I.Y., Isla, M.I., Cudmani, N.G., Vattuone, M.A., Sampietro, A.R., 1999. Screening of antibacterial activity of Amaiche del Valle (Tucuman, Argentina) propolis. Journal of Ethnopharmacology 68, 97–102.

19. Orhan, H., Marol, S., Hepsen, ˙I.F., S¸ ahin, G., 1999. Effects of some probable antiooxidants on selenite-induced cataract formation and oxidative stress-related parameters in rats. Toxicology 139, 219–232.

20. Ota, C., Unterkircher, C., Fantinato, V., Shimuzu, M.T., 2001. Antifungal activity of propolis on different species of *Candida*. Mycoses 44, 375–378.

21. Paintz, M., Metzner, J., 1979. Zur lokalanasthetischen wirkung von propolis und einigen inhatsstoffen. Pharmazie 34, 839–841.

22. Sawaya, A.C.H.F., Palma, A.M., Caetano, F.M., Marcucci, M.C., Sılvaocunha, I.B., Araujo, C.E.P., Shimizu, M.T., 2002. Comparative study of in vitro methods used to analyze the activity of propolis extracts with different compositions against species of *Candida*. Letters in Applied Microbiology 35, 203–207.

23. Sawaya, A.C.H.F., Palma, A.M., Caetano, F.M., Marcucci, M.C., Sılvaocunha, I.B., Araujo, C.E.P., Shimizu, M.T., 2002. Comparative study of in vitro methods used to analyze the activity of propolis extracts with different compositions against species of *Candida*. Letters in Applied Microbiology 35, 203–207.

24. Sforcin, J.M., Fernandes Jr., A., Lopes, C.A.M., Bankova, V., Funari, S.R.C., 2000. Seasonal effect on Brazilian propolis antibacterial activity. Journal of Ethnopharmacology 73, 243–249.

25. Volpert, R., Elstner, F., 1993. Biochemical activities of propolis extracts. Part I: standardization and antioxidative properties of ethanolic and aqueous derivatives. Zeitschrift f˙ur Naturforschung 48c, 851–857.

26. Weston, R.J., Mitchell, Kevin R., Allen, Kerry L., 1999. Antibacterial phenolic components of New Zealand manuka honey. Journal of Food Chemistry 64, 295– 301.

27. Willix, D.J., Molan, P.C., Harfoot, C.G., A comparison of the sensitivity of wound-infecting species of bacteria to the antibacterial activity of manuka honey and other honey. 1992 The Journal Of Applied Bacteriology 73, 388– 394.

28. Zumla, A. and Lulat, A. (1989) Honey – a remedy rediscovered. Journal of Royal Society of Medicine 82, 384–385.