Antibacterial Comparative Study Between Extracts of Mexican Propolis and of Three Plants Which Use Apis mellifera for its Production

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Abstract: The aim of this study was to evaluate the antibacterial properties of extracts obtained from three Mexican plants (Callistemon citrinus Staf., Eucalyptus camaldulensis Dehnhard and Ricinus communis L) and of extracts of propolis produced by Apis mellifera in the same region comparing them by diffusion and plate dilution tests. Hexane and ethyl and methanol acetate extracts from each plant as well as propolis hexane and ethanol extracts were tested on 2 gram-positive and 8 gram-negative bacterial strains: Staphylococcus aureus, S. epidermidis, Shigella dysenteriae, Salmonella typhi, Yersinia enterocolitica, Enterobacter aerogenes, Enterobacter agglomerans (Pantoea agglomerans), Escherichia coli, Vibrio cholerae N. 01 and V. cholerae (clinical case). The propolis extracts was analyzed by HPLC chromatography. Propolis extracts were active against Staphylococcus aureus, S. epidermidis, Vibrio cholerae N. 01 and V. cholerae (clinical case). Callistemon citrinus Staf. extracts were effective against Staphylococcus aureus, S. epidermidis, Vibrio cholerae N. 01 and V. cholerae (clinical case), Eucalyptus camaldulensis Dehnhardt extracts acted against Staphylococcus aureus, S. epidermidis and Vibrio cholerae N. 01 and Ricinus communis L. extracts were effective against V. cholerae (clinical case), Shigella dysenteriae and E. agglomerans. In general, antimicrobial activity of propolis coincided with plant extract activity against Staphylococcus aureus, S. epidermidis, Vibrio cholerae N. 01 and V. cholerae (clinical case). The major compounds were the flavones, cinamic acid derivative and the caffeic acid derivative. These data corroborate the close relationship between propolis composition and the constitution of plants serving as source for some of its components. Thus, the antibacterial spectrum of propolis will vary depending on the geographical situation and the types of vegetation of a given region. On the other hand, plants used by Apis mellifera to produce propolis are being considered as source of secondary metabolites with antimicrobial action.

Key words: Propolis, Apis mellifera, mexican plant extracts, antibacterial properties, beehives, floral extracts

INTRODUCTION

Propolis is a multifunctional material used by bees to build and maintain beehives. It is a resinous, balsamic, rubbery substance of viscous consistency. Its color can be brownish green, chestnut or even black, it tastes bitter but has a sweet and pleasant odor. Color and composition largely depend on its botanical origin and the type of bee that produced it (Kujumgiev et al., 1999). Propolis samples show significant chemical differences related to its origin and have therefore become a matter of interest for chemists and biologists and have recently been used as source of new biologically active compounds (Bankova et al., 2002).

It has been widely reported that propolis is effective against bacteria, fungi, parasites and viruses, it shows antitumor, antioxidant, scar-forming and tissue-regenerating properties, as well as low toxicity in humans, among other properties (Kartal et al., 2003; Kujumgiev et al., 1999; Prytzyk et al., 2003). Propolis is
made by bees with substances collected from the leaf buds and stems of various plants, then mixed with wax and salivary secretions at the beehive. The complex chemical composition of this blend contains >160 active components. Its biological activity depends on various factors among them the type of plants used to collect the ingredients similarly to honey in which differences have been established according with the flowering features and type of nectariferous plants used by the bees to prepare it (Bankova et al., 2002; Sforin et al., 2000). Some researchers consider that sesquiterpenes, in particular bisabolol are some of the main components responsible for the biological activity of propolis as well as flavonoids, phenolic acids and their esters (Martins et al., 2002).

Along the last 3 decades, the frequency, etiology and epidemiology of systemic infections has changed as medical attention has evolved, particularly among intensive care patients, who require hospitalization. Many Gram positive cocci associated to nosocomial systemic infections are now resistant to commonly used antibiotics. At present, the three most common causes of nosocomial systemic infections in the United States are coagulase negative staphylococci, Staphylococcus aureus and enterococci (Karchmer, 2000).

Resistance to antibiotics of frequently isolated pathogens has jeopardized the clinical usefulness of several types of important antimicrobial compounds, including beta-lactamic antibiotics, macroloids, aminoglycosides, glycopenides and fluoroquinolones. Critical Gram-positive pathogens such as staphylococc, beta-hemolytic streptococci, enterococci and Gram-negative bacilli such as enterobacteria or Pseudomonas sp. have developed resistance to fluoroquinolones by prolonged exposure (Jones et al., 2008). This has rendered the search for new antibacterial agents critical.

Medicinal plants are natural resources providing valuable herbal products, they have their roots in antiquity (Chandrasekaran and Venkatesalu, 2004) and are presently still used in routine treatment against certain diseases among them, those caused by fungi (Rios and Racie, 2005).

However, even though propolis has been used for many years few studies have been performed in Mexico regarding its antibiotic activity. The aim of the present study was to evaluate and compare the antibacterial activity of propolis with that of three plants from which they collect the components to prepare it and other bee products.

**MATERIALS AND METHODS**

**Plants and propolis:** Samples from plants visited by bees and the samples of propolis used in the present study were collected at the Faculty of Higer Studies Cuautitlan facilities (FES-C), Campus 4 of the National Autonomous University of Mexico located in the municipality of Cuautitlan Izcalli, State of México, which is located in central México located geographically between parallels 18°21' and 20°17' North latitude and 98°36' and 100°36W, at 2,683 m above sea level in its highest plain.

Samples of three plants from which bees take the components to make propolis and other bee products were collected. Aerial parts of the botanical material were identified by Ma. Edith Lopez Villafranco. Voucher specimens were deposited at the National Herbarium of Mexico (MEXU) at the National Autonomous University of México and at the herbarium of FES at the Faculty of Higer Studies Iztacala (FES-I) and a sample of each was saved for the ethnobotanic collection of this institution. Specimens were identified as Calistemon citrinus Stapf (IZTA 42143), Eucalyptus camaldulensis Dehnhardt (IZTA 42144) and Ricinus communis L. (IZTA 42146).

**Preparation of propolis extracts:** Propolis of Apis mellifera bees was macerated in 70% ethanol. It was left to rest at room temperature protected from the light for 2 weeks. Then, it was filtered and its fractions obtained by partition with hexane and ethanol. Both extracts were concentrated until dry and stored in a fresh and dry place, protected from light.

**Preparation of herbal extracts:** Flowers of the plant species visited by bees were collected at the FES-C premises, dried, ground and stored in a dry place. Extracts of this material were obtained by adding solvents in order of increasing polarity (hexane, ethyl acetate, methanol). Extracts were filtered and concentrated until dry by low-pressure distillation. Solvent remnants were eliminated by aeration.

**Microorganisms:** The strains used in this study were Staphylococcus aureus ATCC 12398, Salmonella typhi ATCC 19430, Enterobacter agglomerans (Pantoea agglomerans) ATCC 27155, Escherichia coli ATCC25922, Staphylococcus epidermidis, Enterobacter aerogenes, Shigella dysenteriae, Vibrio cholerae serotype 01 and V. cholera (a clinical isolate corresponding with group 01, producing enterotoxin, serotype Inha, biotype El Tor), which were obtained at the Phytochemistry Laboratory (FES-I) and Tersinia enterocolytica (donated by the Clinical Analysis Laboratory of University Hospital Campus Iztacala).

**Qualitative sensitivity tests (Agar diffusion test):** Bacteria were sown in Müller-Hinton growth medium (Bioxon,
pollen and its human use as food as a remedy for diseases or as a nutritional supplement. This research compares the antibacterial activity of propolis prepared by Apis mellifera with that of floral extracts from the plants visited by these bees. Table 1 shows antibacterial activity results for each extract.

Both, the propolis and the floral extracts showed antibacterial activity mainly against Gram-positive strains, as shown by their effects on the two species (S. aureus and S. epidermidis) tested here. Growth was inhibited by propolis extract in both, its hexane and ethanol partitions and by the hexane and methanol extracts of R. communis, the methanol extract of E. camaldulensis and the hexane, ethyl acetate and methanol extracts of C. citrinus.

The V. cholerae strains, V. cholerae Nr. 01 and the V. cholerae (clinical case) were both inhibited by the two propolis extracts and by the C. citrinus ethyl acetate and methanol extracts. Additionally, V. cholerae Nr. 01 was inhibited by the E. camaldulensis methanol extract and the V. cholerae (clinical case), by the R. communis ethyl acetate extract.

The highest sensitivity level was shown by S. aureus, S. epidermidis and the two V. cholerae strains treated with the propolis ethanol extract and the C. citrinus ethyl acetate extract, which gave MIC values <0.125 mg mL⁻¹.

The strains S. typhi, Y. enterococci, E. agglomerans (P. agglomerans) and E. coli were not inhibited by any of the extracts tested herein.

Two-factor ANOVA statistical analysis found that the extracts activity depend on the species of bacteria or the sensitivity of bacteria depends on the extract, the above is the interaction between bacterial strain and extracts (p<0.01).

Table 2 shows the composition of propolis extracts and its fractions. The major compounds in ethanol fraction were the flavones and cinnamic acid derivative and in the hexane fraction were cinnamic acid derivative and the caffeic acid derivative.

Propolis biological activity has been related to several factors including bee species, geographical region, type of vegetation, collection period and solvent used for extraction purposes. Besides, a combination of different compounds is known to be required for propolis to be biologically active (Bankova et al., 2002; Kujumgiev et al., 1999; Storacin et al., 2000).

About the species of plants used in this research, we found that C. citrinus is only used for ornamental purposes and there are few reports of its antibacterial activity (Cock, 2008; Melendez and Capriles, 2008). E. camaldulensis has been reported elsewhere to possess...
antibacterial activity (Martos et al., 2000) and the resin of *R. communis* has been traditionally used with due consideration of its high toxicity having shown that it has bactericide, insecticide and vermicidic activity among others (Challoner and McCarron, 1990).

**CONCLUSION**

Propolis extracts and extracts from the flowers of *C. citrinus, E. camaldulensis* and *R. communis* were found to possess antibacterial activity against *S. aureus, S. epidermidis, V. cholerae* 01 and *V. cholerae* (clinical case). These data underline the relationship between the antimicrobial activity of propolis and the plants from which bees take the components to make propolis. Thus, the antibacterial spectrum of propolis may vary depending on the geographical location and the surrounding vegetation. In Mexico, there is no much information that establishes this relation. Propolis extracts and extracts from the flowers of *C. citrinus, E. camaldulensis*
and R. communis could be used to prepare medicaments or directly can be probed as treatments. Further investigation is needed to establish the reach of these findings.

REFERENCES


