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Effects of stingless bee and honey bee propolis on four species of bacteria

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ABSTRACT. We examined the antibacterial activities of several types of propolis, including Africanized honey bee green propolis and propolis produced by meliponini bees. The antibacterial activity of green propolis against *Micrococcus luteus* and *Staphylococcus aureus* was superior to that of *Melipona quadrifasciata* and *Scaptotrigona* sp propolis. Only two samples of propolis (green propolis and *Scaptotrigona* sp propolis) were efficient against *Escherichia coli*. *Melipona quadrifasciata* propolis was better than green propolis and *Scaptotrigona* sp propolis against *Pseudomonas aeruginosa*. We concluded that these resins have potential for human and veterinary medicine.

Key words: Propolis; Apitherapy

INTRODUCTION

Propolis is a resinous hive product collected from various plant materials by honey bees and is considered to be a protective barrier against the bees' enemies. The chemical composition of propolis includes flavonoids, aromatic acids, esters, aldehydes, ketones, fatty acids, terpenes, steroids, amino acids, polysaccharides, hydrocarbons, alcohols, hydroxybenzene, and several other compounds in trace amounts (Marcucci, 1995; Bankova et al., 1983, 2000). The composition of propolis varies according to the plants in a specific region. Propolis has been used as a folk medicine and has been reported to possess therapeutic or preventive effects against inflammation, heart disease, diabetes mellitus, microbes hepatotoxity, and cancer (Burdock, 1998; Banskota et al., 2001). The flavonoids in propolis (mainly pinocembrin) are considered to be responsible for its inhibitory effect on bacterial and fungus, but only traces of these compounds have been found in propolis of South American origin (Tomás-Barberán et al., 1993), indicating that this effect in propolis from this region could be due to a different class of compounds. Different microbiological tests have been used to evaluate this effect: serial dilution in tubes, broth dilution in plates and bioautography.

Propolis antimicrobial activity is one of the most extensively investigated biological actions; some factors may influence its inhibitory capacity (extract preparation, microorganisms tested, propolis origin, bee species, etc.). Several studies on its antimicrobial activity have been performed in various laboratories (Fernandes Jr. et al., 1997). Some authors attribute the complex composition of propolis as a reason for its antimicrobial activity, and some mechanisms of action have been proposed (Mirzoeva et al., 1997).

The antibacterial activity of propolis produced by Brazilian stingless bees was studied by Levy Jr. (1997), who reported a higher efficiency of propolis produced by *Apis mellifera* compared to that of some stingless bees. Kujumgiev et al. (1999) reported no differences in the antibacterial, antifungal, and antiviral activities of propolis from different geographic origins, including four samples from Brazilian *A. mellifera* and two stingless bees. Its antibacterial activity has been well documented but little is specifically known about its activity on dermatophytes. We examined the antibacterial activities of several types of propolis, including Africanized honey bee green propolis and propolis produced by meliponini bees.

MATERIAL AND METHODS

Propolis samples

The bee species are *Apis mellifera*, "Africanized honey bee" from Minas Gerais State; *Scaptotrigona* sp, "Tubi" from Maranhão State; *Melipona quadrifasciata*, "Mandaçaia" from São Paulo State.

Propolis samples were ground and ethanol extracts were prepared, as follows: 30 g propolis/100 mL ethanol (70%). The solutions were left at room temperature for 20 days and shaken once a day. After filtration, the solvents were totally evaporated in a water bath, at temperatures not exceeding 50°C.

Thin layer chromatography and bioautography

Thin layer chromatography plates (silica gel 60-GF254; Merck, Darmstadt, Germany) received 8 µL of the propolis solutions (containing 500 mg propolis extract), placed at a distance of 1.5 m from the lower edge of the plate. All solvents were purchased from Merck. Three mobile phases were tested, two based on Moreno et al. (2000) and pure chloroform. The mobile phase was hexane/ethylacetate/acetic acid (60:40:1, v/v). The plates were either visualized using sulfuric vanillin or biologically (bioautography) to evaluate the activity of the propolis extracts.

Bioautography was carried out after airing the plates for over 8 h. The plates were covered with 20 mL sterile saline, antibiotic agar, inoculated with saline suspensions of *Pseudomonas aeruginosa*, *Micrococccus luteus*, *Staphylococcus aureus*, or *Escherichia coli*, and incubated for 24 h at 37°C. Inhibition zones were visualized as clear areas against a red background.

Bacterial strains and susceptibility tests

The bacterial strains (*P. aeruginosa*, *E. coli*, *M. luteus*, and *S. aureus*) were obtained from the Faculdade de Ciências Farmacêuticas de Ribeirão Preto da Universidade de São Paulo. The strains used in these tests were obtained from 24-h cultures and suspended in sterile saline solution to obtain concentrations of approximately 10^8 CFU/mL, by comparison with the McFarland tube. All tests were performed in duplicate.

Agar plate diffusion tests using paper disks were prepared with 20 mL sterile Müller Hinton agar (Merck, Darmstadt, Germany). The surface of the plates was inoculated using a sterile swab containing the saline suspension of bacteria and allowed to dry. The plates were incubated at 37°C and observed after 24 h, measuring clear inhibition zones around the disks.

RESULTS AND DISCUSSION

The composition of propolis primarily depends upon the vegetation of the area from where it was collected and secondarily upon the methods for its extraction (Marcucci, 1995). Based on the bioautography analysis, we found 1% ethanol extracts that inhibited the growth of *M. luteus* (Figure 1.1), *S. aureus* (Figure 1.2), *E. coli* (Figure 1.3), and *P. aeruginosa* (Figure 1.4).

The green propolis had a large area containing substances that inhibited the growth of *M. luteus* (Figure 1.1A), within the region containing the components with low, high and medium polarity. The *Scaptotrigona* sp propolis (Figure 1.1B) and *M. quadrifasciata* propolis (Figure 1.1C) had inhibitory substances with high and medium polarity.

Against *S. aureus* (Figure 1.2) the antibacterial activity of green propolis was better than *M. quadrifasciata* propolis and *Scaptotrigona* sp. The bioautography showed that green propolis, *Scaptotrigona* sp propolis, *M. quadrifasciata* propolis inhibited the growth of *S. aureus* (Figure 1.2) in the region containing the components with high and medium polarity.

Only two samples of propolis (green propolis and *Scaptotrigona* sp propolis) were efficient against *E. coli* (Figure 1.3). The bioautography of green propolis (Figure 1.3B) showed

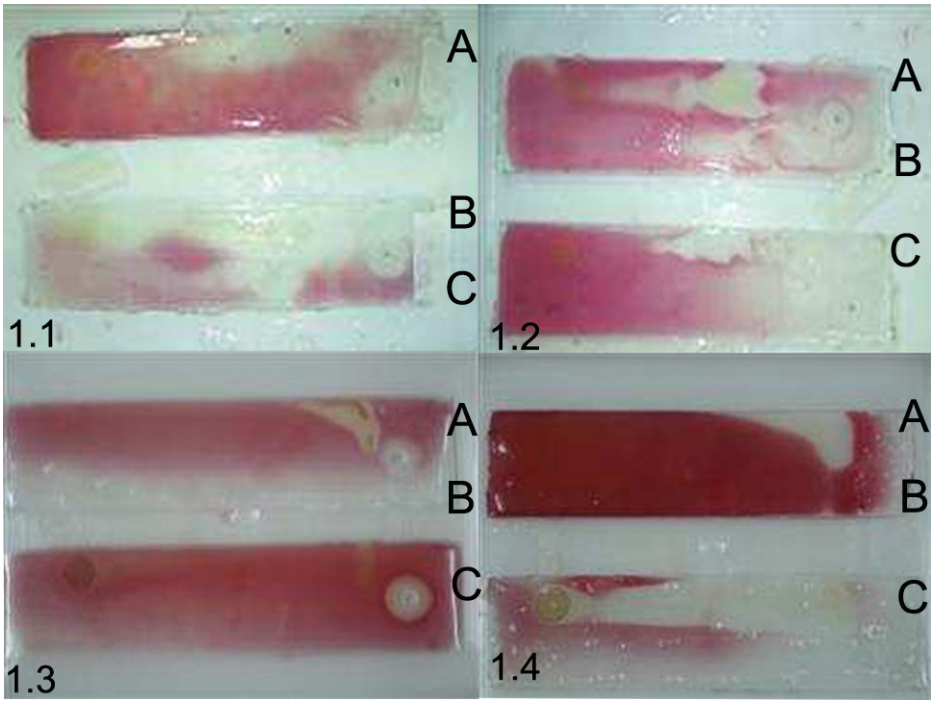

Figure 1. 1.1. Biotest against *Micrococcus luteus*: A = green propolis, B = *Scaptotrigona* sp propolis, C = *Melipona quadrifasciata* propolis. **1.2.** Biotest against *Staphylococcus aureus*: A = green propolis, B = *Scaptotrigona* sp propolis, C = *M. quadrifasciata* propolis. **1.3.** Biotest against *Escherichia coli*: A = *Scaptotrigona* sp propolis, B = green propolis, C = *M. quadrifasciata* propolis. **1.4.** Biotest against *Pseudomonas aeruginosa*: A = green propolis, B = *Scaptotrigona* sp propolis, C = *M. quadrifasciata* propolis.

antibacterial components with low, high and medium polarity and *Scaptotrigona* sp propolis (Figure 1.3A) had antibacterial components with high polarity.

Against *P. aeruginosa* (Figure 1.4), *M. quadrifasciata* propolis was better than green propolis and *Scaptotrigona* sp propolis. The bioautography of *M. quadrifasciata* propolis (Figure 1.4C) showed antibacterial components with low, high and medium polarity, and green propolis (Figure 1.4A) had antibacterial components with high polarity.

The bactericidal activity of these samples of Brazilian propolis was due to the combined effect of several components with antibacterial activity. The substances that were not completely identified, but eluted from the area showing bactericidal activity, could also be contributing to the bactericidal effect. Further studies will be necessary to determine these substances and their activity for a better understanding of this issue.

The use of flavonoids against bacterial and fungus infections has two purposes: 1) to kill the bacterial or fungal cells and 2) to counteract the spread and the effects of the bacterial toxins (McClure, 1975; Lopes et al., 1998). Many but not all, of the bacterial strains commonly encountered by humans are killed by flavonoids. However, the mechanism is not yet

known. Since eicosanoids do not appear to be formed by bacteria, the primary targets of the flavonoids, the PG COX, and the related enzyme lipoxygenase are not involved, since only eukaryotic cells, including plants, possess such enzymes. Nor is another important target, the cAMP PDE involved, since bacteria, like other prokaryotic cells, do not possess this enzyme. However, they do contain metalloenzymes, the heavy metal atoms that form strong ligand complexes with flavonoids, phosphatases. Therefore, the bactericidal effect of the flavonoids may be the result of a metabolic perturbation. Ion channels, which are components of both bacterial and animal cells, are regulated by phosphorylation/dephosphorylation reactions. Fungi, which often accompany bacterial infections, may be killed by flavonoids due to either of the two mechanisms mentioned above.

Apart from the active role that the flavonoids play in the destruction of infecting organisms, they strongly affect the connective tissues by inhibiting some of the enzymes that can hydrolyse their proteoglycan and protein meshwork. This mesh sterically hinders the diffusion of infective organisms through the tissue.

Although we did no chemical analyses of the propolis extracts, propolis composition should certainly differ among these samples and would be responsible for their differing antibacterial activity. This conclusion is supported by the findings of Bankova et al. (1998), who reported differences in propolis chemical composition produced by species of Brazilian stingless bees.

We conclude that, in general, green honey bee propolis is better than stingless bee propolis; we also conclude that these resins have potential importance for human and veterinary medicine.

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