

ANTIBACTERIAL ACTIVITY OF PROPOLIS COLLECTED IN DIFFERENT REGIONS OF BRAZIL

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ABSTRACT: The aim of this study was to investigate the antibacterial activity of propolis samples from Goiás, Paraná and São Paulo States, Brazil, and their flavonoids content. Ethanolic extracts of propolis (EEP) were prepared (30g of propolis in 70% ethanol), and the microorganisms *Staphylococcus aureus* and *Escherichia coli* were tested. The methodology employed was agar diffusion using filter paper discs. Ampicillin and tetracycline were used as controls. Antibacterial activity was determined by the reading of inhibition zone diameters (mm) after 24 hours incubation at 37°C. Results demonstrated that EEP inhibited the growth of *Staphylococcus aureus* but not that of *Escherichia coli*. Tetracycline and ampicillin showed an efficient action against both bacteria. Flavonoids content was variable, depending on the propolis sample. According to the results, it may be concluded that EEP showed effective action against Gram-positive bacteria, independently on their geographic origin, and a positive correlation between antibacterial activity and flavonoids content.

KEY WORDS: propolis, antibacterial activity, flavonoids.

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INTRODUCTION

Propolis has been used in folk medicine since ancient times. Recently, it has been the subject of several studies aimed at elucidating its biological properties, such as antibacterial, antiviral, anti-inflammatory, anticarcinogenic, immunomodulatory, among others (1, 3, 5, 14, 15, 16, 17, 21).

Propolis antibacterial activity has been widely investigated, showing a major effect on Gram-positive and a limited action on Gram-negative bacteria (6, 7, 9, 17, 19, 21, 22).

However, its biological properties may vary according to different plant sources (2). In Brazil, there are many plants that bees could visit as sources of propolis, and depending on the geographic location, its chemical composition may differ.

Based on these observations, the aim of this work was to investigate the antibacterial activity of ethanolic extracts of propolis (EEP) produced in different regions of Brazil against *Staphylococcus aureus* and *Escherichia coli* and to determinate the flavonoids content of propolis samples.

MATERIAL AND METHODS

Propolis samples

Propolis (twenty-two samples) was collected by *Apis mellifera* in different regions of Brazil: Goiás, São Paulo and Paraná States (Table 1). Propolis samples were ground and extracted (30g of propolis, completing the volume to 100ml with 70% ethanol) in absence of bright light, at room temperature, and after a week, extracts were filtered (15).

Flavonoids Content

Methanolic solutions of quercetin in the range of 4.0-12.0µg/ml were used as reference. Ethanolic extract of propolis (0.4ml), methanol (20ml), and 5% AlCl₃ (0.5ml) were added and the volume completed to 50ml with methanol at 20°C. After 30 min, absorbances were measured at 425nm (23).

Microorganisms

Staphylococcus aureus ATCC 25923 and *Escherichia coli* ATCC 35218 strains were obtained from the American Type Culture Collection, Rockville Md., USA.

Microbiological tests

Agar disc diffusion method was employed for the determination of antimicrobial activities of EEP (13). Suspensions of tested microorganisms (0.5 Mac Farland scale) were spread into solid media plates. Filter paper discs (6mm in diameter) were impregnated with 20 μ l of each EEP sample and with ethanol (control) and the inoculated plates were incubated at 37°C for 24 hours. Diameters of the inhibition zones were measured in millimeters. All the tests were performed in triplicate.

Statistical Analysis

Results were analyzed using Analysis of variance. The probability of 0.05 was chosen as the significant level (24). Pearson correlation was used in order to verify a possible correlation between EEP and flavonoids content.

RESULTS AND DISCUSSION

Flavonoids content (%) varied from 0.05 (sample 8 – Anhembi, São Paulo State) to 0.63 (sample 15 – Itaporanga, São Paulo State) (Table 2). They also varied according to the geographic region.

Massuda (12) mentioned 0.53%-1.49% range of flavonoids from propolis, and Sato (20) obtained from 0.0% to 7.04%. Marcucci *et al.* (2, 11) verified low flavonoids content in Brazilian propolis samples (0.84%). Gonzales & Bernal (8) suggested that tropic propolis is poor in flavonoids. On the other hand, Kujumgiev *et al.* (10) showed elevated values of flavonoids in Bulgarian propolis (42%).

With regard to propolis antibacterial activity, EEP inhibited *Staphylococcus aureus* growth, with inhibition diameters from 8 (sample 22 – Goiânia, Goiás State) to 13mm (sample 3 – Botucatu, São Paulo State). Sample 3 was statistically different from sample 22 ($p < 0.05$). Tetracycline and ampicillin showed an antibacterial activity with inhibition diameters of 23 and 33 mm, respectively (Table 2).

Park *et al.* (18) observed propolis action against *Staphylococcus aureus*, using the agar diffusion methodology. Derevice & Ozino (4), Fernandes Jr. *et al.* (7), Kujumgiev *et al.* (10), Sforcin *et al.* (21), and Marcucci *et al.* (11) verified susceptibility of *Staphylococcus aureus* to propolis.

Massuda (12) verified that propolis inhibited *Staphylococcus aureus* growth (10 to 14mm inhibition diameters), and Sato (20) obtained diameters varying from 0 to

11mm, suggesting variability in the biological activity of EEP. Differences in propolis biological activity may be related to its botanical origin, reflecting differences in its chemical composition.

With regard to *Escherichia coli*, EEP did not show any antibacterial activity. Tetracycline and ampicillin showed antibacterial activity, with inhibition diameters of 31 and 29mm, respectively (Table 2).

Sato (20) verified moderate antibacterial action of EEP against *Escherichia coli* (inhibition diameters from 8.0 to 8.8 mm), and Orsi *et al.* (17) demonstrated an elevated minimal inhibitory concentration of propolis against *Salmonella* sp, concluding that propolis shows limited action on Gram-negative bacteria. In this work, EEP was effective only against Gram-positive bacteria.

As a control of propolis solvent, 70% ethanol did not show antibacterial activity on the studied strains (Table 2). These results suggest that antibacterial action of EEP against *Staphylococcus aureus* was due to propolis constituents.

A positive correlation ($p < 0.01$) between the flavonoids content and the antibacterial activity of EEP against *Staphylococcus aureus* was observed (Table 2). Flavonoids are reported to be the most important group of compounds with propolis biological activity (2).

We can conclude from this work that EEP have antibacterial activity mainly on Gram-positive bacteria, showing a positive correlation with flavonoids content.

Table 1: Propolis samples.

Sample	Region	State	Town
1	Southeast	São Paulo	São Manoel
2	Southeast	São Paulo	Botucatu
3	Southeast	São Paulo	Botucatu
4	Southeast	São Paulo	Botucatu
5	Southeast	São Paulo	Botucatu
6	Southeast	São Paulo	Piracaia
7	Southeast	São Paulo	Piracaia
8	Southeast	São Paulo	Anhembi
9	Southeast	São Paulo	Santo Antônio de Sorocaba
10	Southeast	São Paulo	Pardinho
11	Southeast	São Paulo	Piapara
12	Southeast	São Paulo	Olímpia
13	Southeast	São Paulo	Olímpia
14	Southeast	São Paulo	Buri
15	Southeast	São Paulo	Itaporanga
16	Southeast	São Paulo	Itatinga
17	Southeast	São Paulo	Itatinga
18	Southeast	São Paulo	Itatinga
19	Southeast	São Paulo	Itatinga
20	South	Paraná	Campo Largo
21	South	Paraná	Curitiba
22	Middle West	Goiás	Goiânia

Table 2: Antibacterial activity of Ethanolic Extracts of Propolis (EEP), 70% ethanol, tetracycline and ampicillin (inhibition zone diameters, mm) against *Staphylococcus aureus* and *Escherichia coli* and flavonoids content (%) of each EEP.

Sample	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	
	diameter (mm)	diameter (mm)	Flavonoids (%)
1	10 ^a	-	0.62
2	12 ^a	-	0.59
3	13 ^{a,b}	-	0.58
4	10 ^a	-	0.60
5	12 ^a	-	0.55
6	10 ^a	-	0.57
7	11 ^a	-	0.55
8	-	-	0.05
9	10 ^a	-	0.62
10	12 ^a	-	0.55
11	12 ^a	-	0.54
12	11 ^a	-	0.53
13	11 ^a	-	0.59
14	11 ^a	-	0.57
15	12 ^a	-	0.63
16	12 ^a	-	0.57
17	12 ^a	-	0.55
18	10 ^a	-	0.53
19	11 ^a	-	0.59
20	10 ^a	-	0.49
21	10 ^a	-	0.53
22	8 ^{a,c}	-	0.24
Ethanol	-	-	-
Ampicillin	33	31	-
Tetracycline	20	29	-

Different letters show statistical difference between propolis samples ($p < 0.05$).

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