

ANTIBACTERIAL ACTIVITY OF *Apis mellifera* L. PROPOLIS COLLECTED IN THREE REGIONS OF KENYA

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ABSTRACT: The present study aimed at investigating the susceptibility of the microorganisms *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis* to ethanolic extracts of propolis (EEP) from three regions of Kenya (Taita, Tana and Samburu). Propolis was extracted using four different concentrations of ethanol: pure, 70%, 50%, and 30%. Ethanol (70%) and Streptomycin were used as controls. The agar diffusion method using filter paper disks was employed. Antibacterial activity was determined as an equivalent of the inhibition zones diameters (in millimeters) after incubation at 37°C for 24h. Significant differences in the antibacterial activities of propolis were observed among the three regions, depending on the test microorganisms and on the procedure used for the preparation of propolis extract. *Bacillus subtilis* and *Staphylococcus aureus* were the most susceptible bacteria and 70% EEP had the best antibacterial effect.

KEYWORDS: *Apis mellifera*, propolis, antibacterial agents, disk diffusion antimicrobial tests.

CONFLICTS OF INTEREST: There is no conflict.

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INTRODUCTION

Propolis is a complex resinous mixture collected by honeybees from plant exudates and mixed with hypopharyngeal secretions, beeswax and pollen. It is used for honeycomb construction and polishing, for maintenance of the aseptic conditions in hive environment, and for protection and adaptation of nests (7, 8, 13, 14, 28). Propolis has a complex chemical composition, depending on the diversity of plants and geographical locations from which bees collect it (1, 3). Similarly, the biological properties of propolis may vary according to different plant sources (6, 14, 29). These biological activities such as antibacterial, antiviral, antifungal, among others, continue to attract the researchers interest (1, 4, 9, 11, 12, 14, 23, 36). Most of the antibacterial, antifungal and anti-inflammatory activity of propolis is due to the presence of polar compounds, mainly phenols (flavonoids, phenolic acids and their esters) and aromatic acids (caffeic acid and p-coumaric acid) (15, 24). Propolis has also been reported to be protective against radiation-induced damage (10) and to have antimutagenic effect (38) and anti-inflammatory activity (22, 33).

Though the antibacterial activity of propolis has been extensively reported, literature on the antimicrobial activity of Kenyan propolis is scarce. The present work aimed at investigating (i) the antibacterial activity of EEP collected from three regions of Kenya and (ii) the effect of extraction procedures on the antibacterial activity of EEP. Five bacteria strains were used for the study.

MATERIALS AND METHODS

Propolis Extracts Preparation

Propolis samples were collected from five colonies in each of the following three regions of Kenya: Taita District (between 37°35'E, 4°8.2'S and 39°13'E, 2°40.8'S), Tana River District (between 38°26'E, 3°4.4'S and 40°43.8'E, 0°0.9'S) and Samburu District (between 36°17'E, 0°33.9'N and 38° 4.9'E, 2°30.9'N). Propolis samples from each place were ground to powder and subjected to extraction with varying ethanol concentrations. Thirty grams (30g) of ground crude propolis from each place were added to 100ml of each of the following solvents: pure absolute ethanol (Sigma-Aldrich, Germany); mixtures of absolute ethanol and distilled water containing 70%, 50% and 30% ethanol (v/v). The solutions were kept at room temperature in the absence of light for 20 days and shaken once daily. After 20 days, filtration was done and resultant solvents were totally evaporated using a water bath at temperatures not

exceeding 50°C. The dry extracts were then re-dissolved in 70% ethanol in order to obtain solutions containing 10% (w/v) propolis extracts (2).

Bacterial Cultures

Five bacteria strains obtained from Inocline International, Nairobi, and *Pseudomonas aeruginosa* (American Type Culture Collection [ATCC] 27853), *Salmonella typhi* (ATCC 2202), *Escherichia coli* (Standard Culture 25922), *Staphylococcus aureus* (ATCC 20591), and *Bacillus subtilis* (ATCC 6633) strains were used.

Antibacterial Activity Tests

The agar disk diffusion method (5) was employed to test the antimicrobial activity of EEP. Inoculum was prepared using fresh cultures of bacteria strains cultured on nutrient agar. A loop full of bacteria culture was inoculated into a nutrient broth medium and incubated for 24h at 37°C. The size was adjusted to 0.5 McFarland standard turbidity, approximately 10^8 colony-forming units (CFU/ml). Cell suspensions (100µl of target strain) were introduced into the nutrient agar plates and spread thinly on the plates using a glass spreader. Disks of 6mm diameter were impregnated with 25µl of each EEP and with ethanol 70% (control). The disks were then placed on inoculated agar plates, which were incubated at 37°C for 24h under aerobic conditions. The diameter of the inhibition zones (in millimeters) around the disks was measured after 24h. Tests were performed in duplicate.

Statistical Analysis

Results were analyzed using Analysis of Variance (ANOVA) and the probability $p=0.05$ was considered the critical value for all tests. Tukey's post-hoc test was used for separation of statistically significant means.

RESULTS AND DISCUSSION

Larger inhibition zones were verified for the Gram-positive bacteria *B. subtilis* and *S. aureus*, compared with the Gram-negative *P. aeruginosa* and *E. coli*. However, the inhibitory effects of EEP were similar between the Gram-negative *S. typhi* and the two Gram-positive bacteria strains.

Among the Gram-positive bacteria, *B. subtilis* had larger inhibition zones than *S. aureus* but such difference was not statistically significant ($p=0.05$). The antibacterial effect of Kenyan EEP on *S. aureus* and *B. subtilis* agreed with those reported by other authors. Detoma and Ozino (8), Krol *et al.* (25), Kujumgiev *et al.* (26), Park *et al.* (34), Ivan *et al.* (20) and Gonsales *et al.* (15), among others, have reported that *S. aureus* is susceptible to propolis effects. Varied inhibition zones by EEP have also been reported: 10–12mm by Massuda (30), 13mm by Brumfitt *et al.* (5), 10mm by Nieva *et al.* for Argentine propolis (31), and 0–11mm by Sato (35), suggesting there is variability in the biological activity of EEP, depending on its botanical origin and thus on its chemical composition. The antimicrobial activity of EEP was demonstrated by Ivan *et al.* (19, 20) against *B. subtilis* and by Orsi *et al.* (32) against *Salmonella sp.* In Gram-negative bacteria, the highest antibacterial activity was recorded for *S. typhi* compared with *E. coli* and *P. aeruginosa*. However, the differences in their inhibition zones diameters were not statistically significant ($p=0.05$). There are conflicting data on the susceptibility of *E. coli* to EEP. Shub *et al.* (37), Ivan *et al.* (20) and Gonsales *et al.* (15) reported that EEP were ineffective against *E. coli*. On the other hand, Grange and Davey (16), Fernandes *et al.* (11), Hegazi and Abd el Haddy 2002 (18) and Sato (35) reported complete or minimal susceptibility. Grange and Davey (16) and Ivan *et al.* (20) showed *P. aeruginosa* inhibition by EEP.

The antibacterial activity of EEP varied among the three regions studied; EEP from Tana and Samburu had better antibacterial activity than those from Taita (Table 1). Taita EEP extracted using pure ethanol showed no inhibition on any of the five bacteria strains; Samburu EEP extracted using pure and 70% ethanol had no inhibitory effect on *P. aeruginosa*. Taita and Samburu EEP extracted with 50% ethanol and Tana EEP extracted using 30% ethanol had no inhibitory effect on *P. aeruginosa*. Similarly, Tana and Samburu EEP extracted using pure ethanol had no inhibitory effect on *E. coli* and *S. typhi*, respectively. Taita and Samburu EEP extracted using 30% and 50% ethanol had no inhibitory effect on *E. coli*. Taita EEP extracted using pure ethanol as well as Samburu EEP extracted using pure, 30% and 50% ethanol had no inhibitory effect on *S. typhi*. Control (70% ethanol) had no inhibitory effect on *P. aeruginosa*, *E. coli* and *S. Typhi*. Streptomycin presented inhibitory effects on all five bacteria strains.

A high content of polyphenols and flavonoids in alcoholic extracts of propolis is associated with significant microbial activity (16, 27). In a recent study, Volpi and

Bergonzini (39) demonstrated that EEP collected in Kenya had no identified flavonoids and was less rich in polyphenols; however, the specific place(s) from where the Kenyan propolis was collected is not mentioned. This probably explains the low inhibition zones or lack of inhibition recorded in the current study, compared with the EEP inhibitory effects reported by other researchers like Massuda (30), Brumfitt *et al.* (5), Nieva *et al.* (31) and Sato (35).

The present results allow the conclusion that Gram-positive bacteria (*B. subtilis* and *S. aureus*) are more susceptible than Gram-negative bacteria (*E. coli* and *P. aeruginosa*) to Kenyan propolis. These findings agree with earlier reports by Grange and Davey (16), Grecianu and Enciu (17), Ivan *et al.* (20), and Keskin *et al.* (21). However, susceptibility of Gram-negative bacteria to EEP is still an important subject for further investigations. It can also be concluded that the extraction procedures determine the EEP antibacterial activity. Probably, different extraction procedures lead to extraction of different compounds, which ultimately contribute to differences in the antibacterial activity.

Table 1. Summary of the antimicrobial activity of ethanolic extracts of propolis from three different regions of Kenya (Taita, Tana and Samburu) extracted with different ethanol concentrations (100%, 70%, 50% and 30%) against five bacteria strains (*Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella typhi*).

Region	Ethanol Concentration	Inhibition Zones (mm)				
		<i>B. subtilis</i> ATCC 6633	<i>S. aureus</i> ATCC 20591	<i>P. aeruginosa</i> ATCC 27853	<i>E. coli</i> STD 25922	<i>S. typhi</i> ATCC 2202
Taita	100%	NI (a, x)	NI (a, x)	NI (a, y)	NI (a, y)	NI (a, x, y)
Tana	100%	8.5 (b, x)	10.0 (b, x)	10.0 (b, y)	NI (a, y)	8.5 (b, x, y)
Samburu	100%	8.5 (b, x)	9.5 (b, x)	NI (a, y)	9.0 (b, y)	NI (a, x, y)
Taita	70%	9.0 (c, x)	8.0 (c, x)	10.5 (b, y)	9.0 (b, y)	11.0 (c, x, y)
Tana	70%	11.5 (c, x)	8.0 (c, x)	10.5 (b, y)	10.0 (b, y)	8.5 (b, x, y)
Samburu	70%	10.5 (c, x)	9.5 (c, x)	NI (a, y)	9.0 (b, y)	8.0 (b, x, y)
Taita	50%	8.5 (b, x)	9.0 (b, x)	NI (a, y)	NI (a, y)	9.5 (b, x, y)
Tana	50%	NI (a, x)	9.5 (b, x)	9.0 (c, y)	7.0 (c, y)	9.5 (b, x, y)
Samburu	50%	8.0 (b, x)	7.5 (c, x)	NI (a, y)	NI (a, y)	NI (a, x, y)
Taita	30%	10.5 (b, c, x)	8.5 (b, c, x)	7.0 (c, y)	NI (a, y)	11.0 (c, x, y)
Tana	30%	7.5 (b, c, x)	10.5 (b, c, x)	NI (a, y)	10.0 (b, y)	9.5 (b, x, y)
Samburu	30%	11.5 (b, c, x)	7.0 (c, x)	7.0 (c, y)	NI (a, y)	NI (a, x, y)
Control	70%	7.0 (b, x)	7.0 (c, x)	NI (a, y)	NI (a, y)	NI (a, y)
	Streptomycin 50%	10.5 (c, x)	11.0 (b, x)	22.5 (d, y)	18.0 (d, y)	23.5 (d, y)

Different letters within columns (a–d) and within rows (x–y) indicate significant difference ($p < 0.05$).

NI = No inhibition

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