Antimicrobial Properties of Propolis and Honey from the Kenyan Stingless bee, *Dactylurina Schimidti*

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Abstract

This study evaluated the antimicrobial activity of propolis and honey samples of stingless bee, *Dactylurina schimidti* collected from 4 colonies in Tana River district along the Kenyan Indian Ocean Coast. Ethanolic extract of Propolis (EEP) was extracted using 70 % ethanol. Pure honey and concentrations of 75 %, 50 % and 25 % honey in distilled water were prepared. These preparations were tested for antimicrobial activity against five different types of bacteria; *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* and two types of fungi; *Aspergillus niger* and *Candida albicans*. The disc diffusion method using filter paper discs was employed. Antimicrobial activity was determined as an equivalent of the inhibition zones diameters (in millimeters) after incubation of the cultures at 37^0C for 24 hours for bacterial species and 48 hours for fungal species. EEP exhibited highest inhibitory effect on Gram positive bacteria compared to all other bacterial and fungal strains. Pure honey had more effect in inhibiting bacterial growth than different dilutions of honey. Pure honey did not inhibit growth of *A. niger* and *C. albicans*. Generally, our findings indicate that propolis from *D. schimdti* had higher antimicrobial activity against the microbes compared to its honey.

Key Words: *Dactylurina schimidti,* extracts of propolis, honey, antibacterial activity, disc diffusion test

Introduction

The art and science of keeping bees by different cultures for purposes of harvesting honey, wax and other products dates back to many years. The medicinal properties of honey has been reported and documented by beekeepers and medical practitioners alike [1, 2]. Honey and propolis are bee products that have been used for centuries in folk medicine [3, 4]. Several studies have been conducted to authenticate this 'forklore' on medicinal properties of honey and there has been a renaissance in the use of honey and propolis as medicine in more recent times [5, 6, 4, 7]. The use of alternative therapies is mostly due to development of antibiotic resistance in bacteria and/or increasing awareness on the adverse side effects of many pharmaceuticals [8, 7].

Propolis is a complex resinous mixture collected by bees from plant exudates; and mixed with hypo-pharyngeal secretions, beeswax and pollen [9; 10, 11]. In the hive propolis is used for comb construction and polishing, to maintain aseptic hive environment and for protection and adaptation of bees nests. The chemical composition of propolis varies depending on the diversity of plants and geographic locations from which bees collect it [2]. The biological activities of propolis (antibacterial, antiviral, antifungal etc) vary according to its source [12]. Propolis has been used as a medicine since Egyptian times, but in common with other natural medicine, was forgotten for over 100 years. Just as is the case with honey, in recent times a renewed interest in propolis has been witnessed mainly

due to an increase in awareness and interest in all natural ways of combating disease and increasing concern on the side effects of chemical medicine.

The chemical composition of stingless bees honeys has been studied extensively [13]. Effects of species, locations and nest architecture on moisture content of stingless bees honey have also been reported [14] noted significant However, little work has been carried out to investigate the medicinal properties of stingless bees honey. No previous work has been reported on the antimicrobial properties of propolis and honey from the stingless bee, *Dactylurina schimidti.* There are no efforts to domesticate this species since it lives in open nests and is also highly defensive. Farmers in the study area burn its nests threatening the natural populations. The purpose of this study was to investigate the antimicrobial activity of Ethanolic Extracts of Propolis (EEP) and honey produced by *Dactylurina schimidti* a stingless bee found in open nests along the coastal forests of Kenya, East Africa.

Materials and Methods

Propolis Extracts preparation

Propolis and honey samples were collected from 4 colonies in Tana River District along the Kenyan Indian Ocean coast (lies between 38^0 26.0'E, 3^0 4.4'S and $40^0$43.8'E, 0^0 0.9'S). Propolis samples were ground to powder before extraction and subjected to extraction with ethanol. Thirty grams (30g) of ground crude propolis were dissolved in 100mL of 70% ethanol (Sigma-Aldrich – Germany). The solution was shaken daily and left at room temperature for 7 days. The

solutions were then filtered through Wattman paper No. 1 and placed in dark sample bottles.

Honey preparation

Honey was harvested straight from the nests using sterile syringes, filtered and stored in dark glass bottles. Honey solutions of concentrations 75%, 50% and 25% (v/v) were constituted by adding distilled water to honey.

Microorganisms

Five bacteria strains: *Pseudomonas aeruginosa* (G+, ATCC 27853); *Salmonella* Typhi (*typhi*) (ATCC 2202); *Escherichia coli* (G-, STD 25922); *Staphylococcus aureus* (G+, ATCC 20591) and *Bacillus subtilis* (a local isolate); one strain of fungi, *Aspergillus niger* (a local isolate) and yeast, *Candida albicans* (EK 13A) were utilized to assay antimicrobial activity of honey and EEP.

Antimicrobial Activity Tests

The disc diffusion method was employed to test the antibacterial and antifungal activity of honey and ethanolic extracts of propolis (EEP). Bacteria were cultured on nutrient agar prior to transfer into nutrient broth while fungi were cultured on potato dextrose agar (PDA). For bacterial cultures, a loopful of culture was picked from the nutrient agar culture and inoculated into nutrient broth medium and incubated for 24 hours at 37^0C. Spores of fungi were prepared by washing the colonies of the fungal species in 5 ml distilled water. Density of bacterial cells or fungal spores was measured with McFarland's standard solution. The size was adjusted to 0.5 McFarland standard turbidity, approximately 10^8 colony forming units (CFU/ml). The cell or spore suspensions (100μl of target strain)

were introduced into the nutrient agar or PDA plates and spread thinly on the plates using a glass spreader. Discs of 6mm diameter were impregnated with 25μl of honey and EEP preparations. The discs were then placed on inoculated agar plates. The plates were incubated at 37^0C for 24 hours for bacterial cultures and 48 hours for fungal cultures under aerobic conditions. Streptomycin was used as control. The diameter of the inhibition zones around the discs was measured (in millimeters) after 24 hours and 48 hours respectively. Tests were performed in duplicate.

Statistical Analysis

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

Results

The EEP had the significantly higher inhibitory effect on the Gram positive bacteria, *B. subtilis* compared to all other bacterial and fungal strains *S. typhi*, *S. aureus, P. aeruginosa, E. coli*, and *A. niger.* The inhibition on *B. subtilis* however, was not statistically different to that observed for the yeast strain, *C. albicans* Among the Gram negative bacteria, EEP had higher inhibitory effect on *S. typhi* compared to *P. aeruginosa* and *E. coli.* These differences were not statistically significant (F=10.8; df 6,28; p=0.05). *S. aureus*, a Gram positive bacterial strain and the fungi, *A. niger* were not inhibited by the EEP (Table 1)

Undiluted honey showed high inhibitory effect on bacterial growth compared to diluted honey of 75%, 50% and 25% concentrations. Similarly, the 75% and 50%

concentrations had significantly higher inhibitory effects compared to the 25% concentration, however, there were no significant differences in the inhibitory effect of the 75% and 50% honey concentrations. (F=22.68; df 6,28; p = 0.05). Inhibitory effects of the honey were noted on *B. subtilis* and *S. typhi* in some instances. No inhibitory effect was noted on *E. coli, S. aureus, A. niger* and *C. albicans* (Table 2)

Table 1. Summary of the antimicrobial activity of Ethanolic Extracts of Propolis (EEP) from the stingless bee *Dactylurina schimidti* extracted with 70% ethanol against 5 bacteria strains (*B. subtilis, S. aureus, P. aeruginosa, E. coli,* and *S. typhi*), the fungal strain *Aspergillus niger* and the yeast strain *Candida albicans*.

Propolis Samples	**Inhibition zones (mm)**						
	B.subtilis	*E. coli*	*S.aureus*	*P.aeruginosa*	*S. typhi*	*A. niger*	*C.albicans*
A	6.0 aA	ND	ND	8.0bA	8.0bA	8.0bA	8.5aA
B	8.5 aB	9.5 bB	ND	9.0bB	8.5bB	7.0bB	10.5aB
C	9.5 aCD	7.5bCD	ND	8.0bcD	8.0bCD	7.0bCD	ND
D	9.0 aD	8.0 bD	ND	ND	ND	8.5bD	8.5aD

Means followed by the same small letter (a-c) within rows indicate there is no significant difference on the effect of EEP on the different test microbes ($p<0.05$).
Means followed by the same capital letter (A-C) within columns indicate there is no significant difference on the effect of EEP on the same test microbe ($p<0.05$).
ND = No inhibition was detected.

Table 2. Summary of the antimicrobial activity of varying concentrations of honey from the stingless bee *Dactylurina schimidti* against 5 bacteria strains (*B. subtilis, S. aureus, P. aeruginosa, E. coli,* and *S. typhi*), the fungal strain *Aspergillus niger* and yeast strain *Candida albicans*

Honey Concentration	**Inhibition zones (mm)**						
	B.subtilis	*E.coli*	*S.aureus*	*P.aeruginosa*	*S.typhi*	*A.niger*	*C.albicans*
Pure Honey	14.8 aA	ND	ND	6.8 bA	8.3 cA	ND	ND
75%	9.9 aC	ND	ND	ND	ND	ND	ND
50%	9.8 aC	ND	ND	ND	ND	ND	ND
25%	6.5 aB	ND	ND	ND	ND	ND	ND

Means followed by the same small letter (a-c) within rows indicate there is no significant difference on the effect of honey concentrations on the different test microbes ($p<0.05$).
Means followed by the same capital letter (A-C) within columns indicate there is no significant difference on the effect of honey concentrations on the same test microbe ($p<0.05$).
ND = No inhibition was detected.

Discussion

Propolis and honey has been found to possess antimicrobial activity and this has been attributed to specific chemicals in the propolis and honey [11, 9, 15, 3, 5, 16, 2, 17, 18].

The bacteria strains, *B. subtilis, P. aeruginosa, E. coli,* and *S. typhi* were susceptible to EEP from the Kenyan stingless bees (Table 1). Results on the antibacterial activity of propolis from stingless bees have been contradictory, probably owing to the preparation of the samples, different bee species and also the diversity of plants from which the propolis has been collected [19]. [20] has reported that *S. aureus* is susceptible to propolis from *Apis mellifera* and the Brazilian native bee, *Tetragonisca angustula.* In a related study, [19] reported that propolis from native Brazilian bees was active against *S. aureus* and *E. coli.* Interestingly, in this study there was no inhibition in the case of *S. aureus.* [19] noted higher Minimum Inhibitory Concentration (MIC) values for propolis extracts against *E. coli* compared to *S. aureus,* findings similar to those reported in this study. This is not surprising, however, since multiple antibiotic resistance by *S. aureus* has been reported [20, 1, 22]. It is likely that there is a variation in the protective mechanism of strains of *S. aureus*, leading to contradictory results in the various studies. On the other hand, [23] reported *S. aureus* as most susceptible to propolis extracts of the Brazilian native bee, *Tetragonisca angustula* compared to *B. subtilis, E. coli* and *C. albicans.* This contradicts results obtained in this study where we found the 2 strains to be susceptible to propolis extracts of *D. schimidti*. The EEP inhibited growth of *Aspergillus niger* and

Candida albicans and this corroborates the results reported by [19]. The inhibitory effect of propolis extracts of the native Brazillian bee (*Melipona quadrifasciata anthidiodes*) against *S. aureus, E. coli* and *Candida albicans* has also been studied by [24], who reported that the extracts were active against the test microbes.

Mixed observations were made regarding the susceptibility of the test microbes to the honey from *D. schimidti*. *B. subtilis* was susceptible to pure honey, 75 and 50% honey but its growth was not inhibited by 25% honey concentration. The growth of *S. typhi* was inhibited in pure honey and 75% honey concentration. None of the bacteria were susceptible to 25% honey concentration. All concentrations of the honey did not inhibit the growth of *S. aureus, P. aeruginosa, E. coli, C. albicans* and *A. niger*. [25] have reported that *S. aureus* is not susceptible to stingless bees honey. [20] reported that *S. aureus* was susceptible to honey from *Apis mellifera* and the stingless bee *Tetragonisca angustula.* [16] and [17] also reported that honey from *A. mellifera* inhibits the growth of *Candida sp*, *E. coli* and *A. niger* .

Collectively, our findings indicate that propolis from *D. schimdti* had higher antimicrobial activity against the tested microbes compared to its honey. There is need for characterization of the active components in the propolis extracts.

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REFERENCES

[1] Anand S, Shanmugam J. Anti-staphylococcal properties of natural Honey. Biomedicine 18 (1998) 15 – 18

[2] Bankova V, Marcucci M C and Catro S L. Propolis: recent advances in chemistry and plant origin. Apidologie 31 (2000) 3 – 15.

[3] Gonsales G Z, Orsi R O, Fernandes A, Rodrigues P and Funari S R C. Antibacterial activity of propolis collected in different regions of Brazil. J. Venom. Anim. Toxins incl. Trop. Dis 12 (2) (2006) (2) 276 – 284

[4] Zumla A and Lulat A. Honey – a remedy rediscovered. Journ. of Royal Soc. of Medicine 82 (1989) 384 – 385.

[5]. Molan P C. The antibacterial activity of honey: the nature of the antibacterial activity. Bee World 73 (1992) 5 – 28.

[6] Bogdanov S. Nature and origin of the antibacterial substances in Honey. Lebensm Wiss. Technol. 30 (1997) 748 - 753.

[7] Fearnley J. Bee Propolis: Natural Healing from the Hive. Souvenir Press Ltd. Great Russell Street, London WC1B 3PD (2001) pp 24-59

[8] Thomson W M. Propolis. Med. J. Austr.153 (1990). 654.

[9] Ghilsalberti E L. Propolis: A review. Bee World 60 (1979) 59 – 84.

[10] Garcia- Viguera C, Greenaway W and Whatley F R. Composition of propolis from two different Spanish regions. Z. Naturforsch 47c (1992) 634 - 637

[11] Burdock G A. Review of the biological properties and toxicity of bee propolis. Food Chem. Toxicol. 36 (1998) 347 - 63.

[12] Ghilsalberti E L. Propolis: A review. Bee World 60 (1960) 59 – 64.

[13] Sommeijer M J, Beetsma J, Boot W J, Robert E J and De Vries R. Perspectives for Honey Production in the Tropics, Proc. Symp. Organized by the Netherlands Expertise Centre for Tropical Apicultural Research (NECTAR), Utrecht, Netherlands (1995) Dec 18.

[14] Bijlsma L, Bruijn L. M, Martens E. P, Sommeijer M J. Water content of stingless bee honeys (Apidae, Meliponini): interspecific variation and comparison with honey of *Apis mellifera*. Apidologie 37 (2006) 480 - 486

[15] Rudnay J. A book of Honey: It's history and use, Budapest, Corvina (1987) 114p

[16] Efem S E. Recent advances in the management of fournier's gangrene: Preliminary observations: Surgery 113 (1993) 200 - 204.

[17] Radwan S, El-Essawy A, Sarhan M M. Experimental evidence for the occurrence in honey of specific substances active against microorganisms. Zentral Mikrobiol 139 (1984) 249 – 55

[18] Bankova V, Boudourova- Krasteva G, Sforcin J M, Frete X, Kujumgiev A, Maimoni Rodella R and Popov S. Phytochemical evidence for the plant origin of Brazilian propolis from Sao Paulo State. Z. Naturfosch 54 *(*1999) 401 – 5.

[19] Fernandes A, Leomill L, Fernandes A A H, Sforcin J M. The antibacterial activity of propolis produced by *Apis mellifera* and Brazilian stingless bees. J. Venom. Anim. Toxins incl. Trop. Dis 7 (2001) 73 - 182

[20] Miorin P L, Levy Junior N C, Custodio A R, Bteritz W A, Marcucci M C. Antibacterial activity of honey and propolis from *Apis mellifera* and *Tetragonisca*

angustula against *Staphylococcus aureus.* Journ. Appl. Microbiology 95 (5) 2003 913 – 920.

[21] Gulati R, Gulati B R, Sihag R C. Antibacterial activity of propolis and honey against Staphylococcus aureus and Escherichia coli. Indian Bee Journal 58 (1996) 128 – 130.

[22]. Cooper R A, Molan P C and Harding K G. Antibacterial activity of honey against strains of *Staphylococcus aureus* from infected wounds. Journal of Royal Society of Medicine 92 (1999) 283 – 285.

[23] Pereira A S, Bicalho B, De Aquino Neto F R. Comparison of propolis from *Apis melifera* and *Tetragonisca angustula*. Apidologie 34 (2003) 291 – 298.

[24] Velikova, M, Bankova, V, Tsvetkova I, Kujumgiev, V and Marcucci, M. C. Antibacterial ent-kaurene from Brazilian propolis of native stingless bees. Fitoterapia 71 (2000a) 693 - 696

[25] Demera J H and Angert E R. Comparison of the antimicrobial activity of honey produced by *Tetragonisca angustula* (Meliponinae) and *Apis mellifera* from different phytogeographic regions of Costa Rica. Apidologie 35 (2004) 411 - 417.