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Antibacterial Activity of Stingless Bee (*Dactylurina studingeri*) Propolis on Bacteria Isolated from Wound

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Abstract

With the high rate of antibiotics resistance observed in bacteria isolated from wounds, there is need for alternative therapies in the treatment of secondary infections caused by these bacteria. The antibacterial activity of Stingless bee propolis was determined using four test organisms. Concentrations of Ethanolic Extracted propolis (EEP) ranging from were assayed for the sensitivity tests using the agar diffusion method. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined using the tube dilution method and streak-plate method respectively. Concentrations of Ethanolic Extracted propolis greater than 6.25mg/ml inhibited the growth of all test organisms but the rate of inhibition varies,

a higher activity of the propolis was observed on Gram positive bacterium while a lower activity of the propolis was observed on Gram negative bacteria The Ethanolic Extracted propolis(EEP) exhibited both bacteriostatic and bactericidal activities. The results revealed that Ethanolic Extracted propolis has antibacterial effect on the investigated organisms.

Keywords: Propolis, Wound infection, Antibacterial activity, Resistance

Introduction

Due to the increasingly alarming rate of antimicrobial resistance exhibited by wound-borne pathogens, wound infections have taken a new turn in fatality and chronicity [1]. Wound infection is one of the most frequent nosocomial complications especially among surgical patients. *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli* are Gram negative bacteria frequently isolated from wound. These bacteria are popular for the alarming level of multi-drug resistance they exhibit to conventional antibiotics [2-5]. These findings established the need for other possible sources of antimicrobial agents that could show efficacy by inhibiting the growth of these organisms.

Propolis or bee glue, is a natural resinous mixture produced by honeybees (*Apis mellifera*) from substances collected from parts of plants, buds and exudates. Propolis has been used in traditional medicine since ancient times as remedy for ailments because of its pharmacological properties. A couple of researches have also reaffirmed its activity as an antibacterial [6], antifungal, antiprotozoan, antiparasitic and antiviral agent [7]. Interestingly, besides its antimicrobial potential, propolis has also been shown to inhibit the adherence of microorganisms to host surfaces especially in biofilm formation and establishment [8]. Thus, the aim of this research was to evaluate and reaffirm the antimicrobial efficacy of the ethanolic extracts of stingless bee propolis against selected bacteria isolated from wound.

Materials and Methods

Collection and Maintenance of test Organisms

Pure culture of the test organisms; *Pseudomonas aeruginosa, Staphyloccocus aureus, Klebsiella sp. and Escherichia coli* were collected from the department of Microbiology and Parasitology of the University of Ilorin Teaching Hospital (UITH), Ilorin. The pure isolates were maintained on agar slants and stored at 4^0C until use.

Collection and Maintenance of Propolis

The propolis of stingless bee (*Dactylurina studingeri*) was collected from the Teaching and Research Farm, Faculty of Agriculture, University of Ilorin, Ilorin. The propolis was sourced from a tree close to the University of Ilorin dam. The propolis was extracted using a known volume of 70% ethanol and the ethanolic extracted propolis (EEP) was stored in a glass bottle and kept in a dark cupboard.

Preparation of Ethanolic Extracted Propolis (EEP)

The exact concentration of the desired extract was first decided. The required quantity of propolis was weighed and the right volume of alcohol measured. The alcohol and propolis were poured into a container, sealed and placed on a shaker for a week. After one week, the mixture was filtered through a whatman filter paper. The filtrate was was kept in clean, dark, airtight bottles. The filtrate was concentrated by using a rotary evaporator to evaporate the ethanol, thus leaving behind the extract which was a reddish viscous liquid.

Antibacterial Sensitivity of Propolis

The agar well diffusion method was employed. Overnight broth culture of the test organims were seeded onto solidified Mueller Hinton agar plates using a sterile spreader and for 15 minutes for proper adsorption. A sterile cork- borer (6mm in diameter) was used to bore 6 holes at equidistance to each other on the solidified agar plates. Different concentrations of the

propolis: 6.25mg/ml, 12.50mg/ml, 25.0mg/ml, 50mg/ml, and 100mg/ml were introduced into the wells. One of the holes was made to serve as the control containing only ethanol. The plates were then left for 1hour to allow the extract diffuse in the medium before incubating at 37^0C for 24 hours. After incubation, the zones of inhibition were observed and measured along two axes at 90^0C to each other and the calculated mean was recorded.

Determination of the Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration was determined using the tube dilution method. Seven test tubes were used for each test organism. Each tube contained 10ml of sterile peptone water and 1ml of overnight broth culture of the test organism. One milliliter of the different concentrations of propolis (6.25mg/ml-100mg/ml) was introduced into each test tube. There was a positive control tube containing only the broth and inoculum and a negative control tube containing the broth and propolis only. The tubes were then incubated at 37^0C for 24 hours. After incubation, each of the test tubes was observed for presence or absence of growth which was indicated by the visible turbidity of the solution in the tubes. The least concentration of propolis that produced no growth was taken as the Minimum Inhibitory Concentration (MIC).

Determination of the Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration was determined by streaking samples from the tubes which were used for the determination of MIC that showed no visible turbidity as compared to its control. The plates were then incubated at 37^0C for 24 hours. The lowest concentration of the propolis that showed no growth was then recorded as the MBC.

Results

The results of this research are illustrated in Tables 1, 2 and Figure 1. Table 1 shows the Antibacterial sensitivity of different concentrations of the propolis samples against the test organisms; Table 2 shows the Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of the propolis samples on the test organisms and Figure 1 is a bar chart showing a

plot of the diameter of the zone of inhibition against the activity of the test organisms at different concentrations of the propolis.

Table 1: Antibacterial sensitivity of different concentrations of the propolis samples against the test organisms.

	CONCENTRATIONS OF PROPOLIS SAMPLE(mg/ml)/ZONES OF INHIBITION(mm)					
TEST ORGANISMS	100	50	25	12.5	6.25	CONTROL
Pseudomonas aeruginosa	13.5	12.0	11.5	10.0	8.0	4.0
Staphyloccocus aureus	17.0	14.5	13.0	12.5	9.0	6.0
Escherichia coli	15.0	13.0	12.0	11.5	8.5	5.0
Klebsiella sp.	14.0	12.5	11.0	9.0	7.0	4.0

Table 2: Minimum Inhibitory Concentration and Minimum Bacteriocidal Concentration of the propolis samples on the test organisms.

	MIC (mg/ml)	MBC(mg/ml)	
TEST ORGANISMS		BACTERIOSTATIC	BACTERICIDAL
Pseudomonas aeruginosa	6.25	12.5	12.5-100
Staphyloccocus aureus	6.25	6.25	12.5-100
Escherichia coli	6.25	12.5	12.5-100
Klebsiella sp.	6.25	12.5	12.5-100

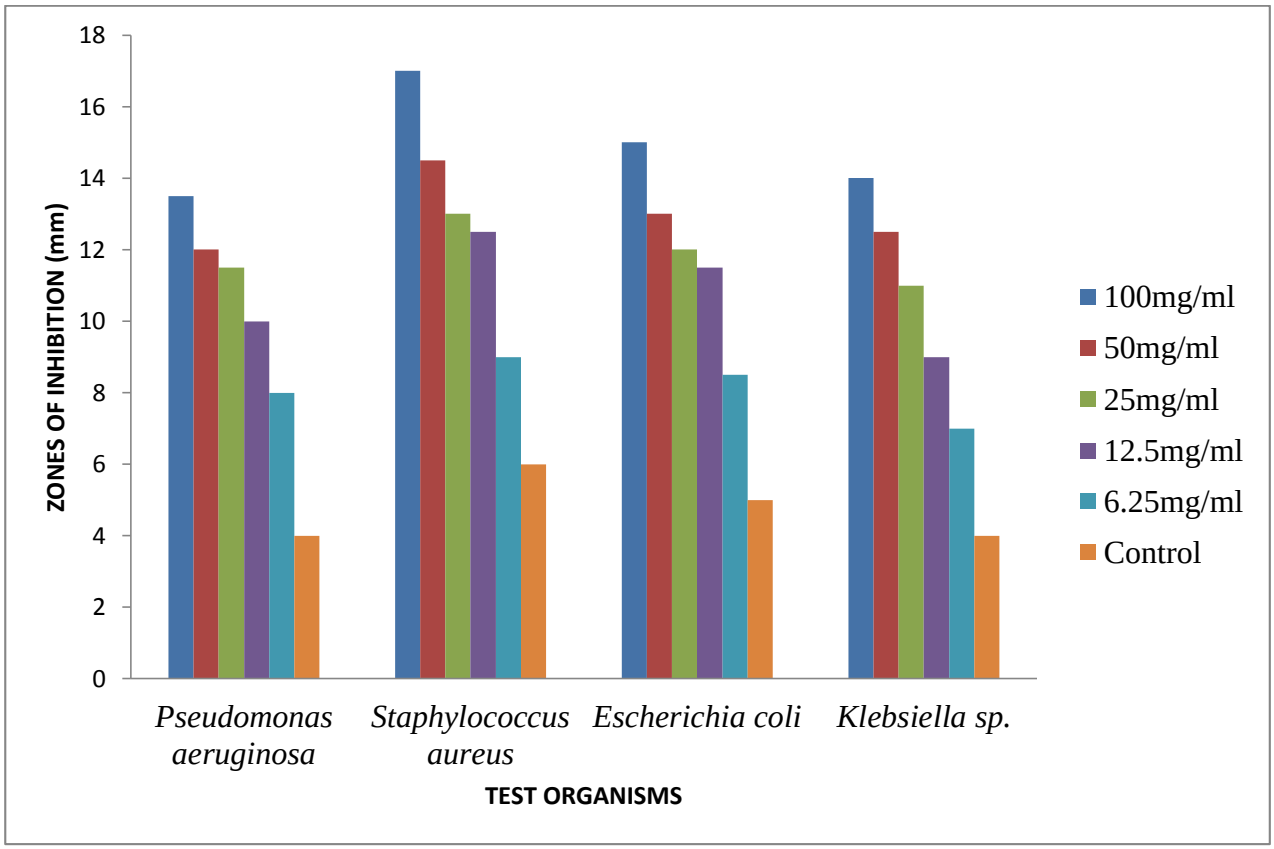

Fig 1: Bar chart showing activity of the test organisms to different concentrations of the propolis.

Discussion

Results from this study reaffirm previous reports on the presence of pharmacological constituents with antibacterial efficacy in propolis. This study has shown that wider zone of inhibition was observed with higher concentration of propolis and the values of the zones of level of sensitivity of the bacteria decreases as the concentration of the propolis reduces. All the test organisms were inhibited at concentrations ranging from 100mg/ml (highest) to 6.25 mg/ml (lowest).

In this study, propolis was considerably active against both Gram-positive and Gram-

negative bacteria at concentrations ranging from 6.25mg/ml to 100mg/ml. *E. coli* was observed to be sensitive to EEP with zone of inhibition ranging from 8.5- 15.0mm. This finding deviates from the reports of Vivieros *et al.* [10] and Bessa *et al.* [1] that *E. coli* showed strict resistance to ethanolic extracts of propolis.

According to the results of this study, ethanolic extracted propolis showed a higher activity against the Gram positive bacterium used (*Staphylococcus aureus*) compared to Gram negative bacteria (*Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella* sp.). This corresponds with the report of the previous research work on propolis [1; 2; 6; 8; 18]. In this study, the value of MIC of the Ethanolic Extracted Propolis (EEP) against *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli* and *Klebsiella sp*. was 6.25mg/ml; while the results of the MBC ranged from 12.5-100mg/ml against the test organisms. The relatively low values of the MIC and MBC further establish the efficacy of the propolis extract. The antimicrobial efficacy of propolis has been attributed to the presence of flavonoids and phenolic acids [19]. These phytochemicals have been reported to possess pharmacological properties that confer therapeutic abilities on propolis [20]. Hence, EEP has been reaffirmed to be a good source of antibiotics with promising therapeutic values.

Conclusion

In conclusion, this study has shown that propolis has a very good antibacterial activity. Therefore, it is suggested that the industrial processing of propolis into usable forms and prescribing it for clinical use should be encouraged

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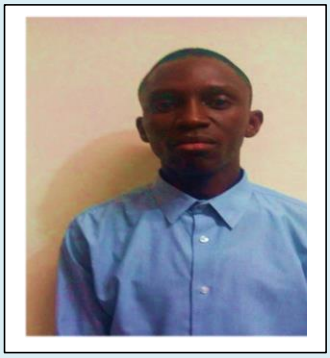

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