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Antimicrobial Activity of Propolis Extract Against Selective Pathogens

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Abstract: The present study is to investigate the antimicrobial activity of propolis extract against selective pathogens. The ethanol extract of propolis was prepared by 10 g of propolis in 50 ml of 90% ethanol. Antimicrobial activity of ethanol extracts were evaluated against two bacteria strain like *Staphylococcus aureus* and *Escherichia coli* and two fungal strains *Aspergillus niger* and *Candida albicans* were collected from Tuticorin Medical College and Hospital by disc diffusion method. The ethanol extract of propolis was found to be the most bacterial effective against *S. aureus* strains inhibition zone (12mm – 17 mm) and most fungal effective against *A. niger* inhibition zone (10mm-19mm). This result shows the ethanol extract of propolis is a good antifungal activity compared to bacterial activity.

Key words: Propolis · Ethanolic extract · Bacterial strains · Fungal strains

INTRODUCTION

Propolis is a resinous substance collected from the buds of certain trees by bees and used as a cement or sealant in the construction of their hives. Propolis has been used as folk medicine to cure infections [1]. The chemical composition, color and aroma of propolis change according to geographical zones. The colour of the propolis varies from yellowish-green to dark brown depending on its source and age [2]. Propolis has been shown to comprise over 300 constituents including flavonoids such as naringenin and quercetin as well as other phenolic compounds such as cinnamic acid. The exact composition of propolis varies according to variety and geographical source [3]. Generally pharmacologically active molecules in propolis are flavonoids and phenolic acids and their esters [4]. Propolis contain sticky plant substances mixed with bees wax and other bee secretion and its chemical composition is qualitatively variable depending on the vegetation in the green from which it was collected [5]. The mechanism of antimicrobial activity of propolis is complex and could be attributed to the synergistic activity between phenolic and other compounds [6]. Antibacterial activity of propolis on *Staphylococcus aureus* growth was higher when ethanol extracts were prepared with 60% - 80% ethanol solutions [7]. The major bioactive components of Propolis are aromatic acids, esters and the flavonoids galangin, quercetin, kaempferol, acacetin, pinocembrin and pinostobin [8]. The antimicrobial activity of propolis against a wide range of bacteria, fungi and virus has been investigated since the late 1940s and it shows variable activity against different microorganisms [9]. The flavonoids are through to be responsible for antibacterial, antifungal and antiviral actions of propolis [2]. Propolis contain some minerals such as iron, calcium vanadium, strontium, Manganese and silican as well as some vitamins like B1, B2, B6, C and E and a number of fatty acids. It also has antibacterial activity against *Streptococcus mutans* in the leading cause of dental cavities (tooth decay) worldwide and it considered being the most carcinogenic among the oral *Streptococc*i sp. The propolis antimicrobial activity against oral bacteria, as well as its action in inhibiting the production of polysaccharides, the application of propolis extract on rat molars reduced the severity of carious lesions in these

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animals [10]. In the present investigation, we aim to study the antibacterial activity of propolis extract against selective pathogens.

MATERIALS AND METHODS

Collection and extraction of Propolis: Propolis samples were collected from Tuticorin forest area during spring and summer season of 2013. The collected samples were cleaned, free of wax, paint, wood cut into small pieces and dried oven at 50 °C. The 10g of powder propolis were extracted with 50ml of 90% ethanol in dark brown container kept for 7 to 14 days at room temperature. The container was shaked 2 or 3 times per day and returned to warm dark place. Afire 14 days the extract was filtered through whatman No.1 Fitter paper and the solvents were concentrated by rotary evaporator (VC100A Lark Rota vapor® at 30°C) with reduced pressure to give a dark brown gummy mass. The resultant residues were stored at 4°C for further antibacterial screening.

Collection and Identification of Pathogens: The bacterial strains (bacteria strain like *S. aureus* and *E. coli*) fungal strains (*A. niger* and *C. albicans*) were collected from Medical College and Government Hospital, Tuticorin, Tamilnadu, India. The collected samples were platted on selective agar medium, the selective agar such as Manitol Salt Agar and Eosin Methylene blue Agar, Maconkey agar for bacteria. Potato Dextrose Agar (PDA) and Sabourauds Dextrose Agar (SDA) were prepared for fungal studies and milled heat for 5 minutes for bacterial medium and autoclave for the fungal medium. After solidification the samples were swabbed on petriplates. Then the plates were incubated at 37 °C for 24 hrs for bacterial medium and fungal medium for 3 days at 25 °C. The isolated bacterial and fungal organisms were stored in bacterial and fungal agar slants respectively and were subjected to biochemical analysis.

Biochemical Identification of Isolated Pathogens

Bacteria: The bacterial pathogens identified and confirmed by using conventional microbiological and biochemical procedures were followed from Department of Microbiology, Kamaraj College, Tuticorin. The various biochemical tests such as gram stainng Indole production test, Methyl red test, Voges-Proskauer test, Citrate utilization test and Catalase test, Coagulase, Haemolytic activity were used for the confirmation of the bacterial pathogens (Table 1).

Fungi: The fungal hypae were stained with lacto phenol cotton blue stain and they were observed microscopically.

Antimicrobial Activity of Propolis Extract by Disc Diffusion Method: The antimicrobial activity was tested against bacteria [11] and fungal pathogens [12] by agar well diffusion method.Twenty four hours old nutrient broth cultures of test bacteria and 72 hours old test fungi were aseptically swabbed on sterile Mullerhinton agar and Czapek dox agar plates respectively. The stock solutions of ethanol extracts were prepared at a concentration of 100mg/ml. The four different concentrations (20, 40 µL, 60 µL, 80 µL and 100 µL) of the extract were applied to 5mm well. Positive (control) well containing 50µL of tetracycline (1mg/ml) and as negative control containing 50µL of ethanol was used. The results were calculated by measuring the zone of inhibition in millimeters.

Statistical Analysis: Results were expressed as means ± standard deviation (SD). Data were statistically analyzed using ANOVA one-way analysis of variance. Duncan's Multiple comparisons among means were made by Duncan (1955), when significant F- values were observed ($P < 0.05$), using SPSS 17 statistical program.

Table 1: Biochemical characterization of test bacterial pathogens

S.No	Biochemical Test	*Escherichia coli*	*Staphylococcus aureus*
1	Gram Staining	-	+
2	Indole	+	-
3	Methyl red	+	+
4	Vogesprokaeur	-	+
5	Citrate	-	-
6	Catalase	+	+
7	Coagulase		+
8	Haemolysison Blood agar plate	-	B-haemolysis

Table 2: Antimicrobial activity of Propolis extract against Selective pathogens (Disc diffusion method)

		Zone of Inhibition (mm)			
S.No	Concentration (µL)	*Escherichia coli*	*Staphylococcus aureus*	*Aspergillus niger*	*Candida albicans*
1	20	8 ± 0.12[a]	12 ± 0.55[b]	10 ± 0.22[a]	8 ± 0.26[a]
2	40	9 ± 0.25[a]	13 ± 0.19[a]	12 ± 0.66[b]	15 ± 0.84[a]
3	60	11 ± 0.21[a]	15 ± 0.16[c]	14 ± 0.41[a]	13 ± 0.31[a]
4	80	11 ± 0.33[a]	16 ±0.51[a]	13 ± 0.21[b]	13 ± 0.25[a]
5	100	12 ± 0.41	17 ± 0.22[b]	19 ± 0.66[a]	12 ± 0.44[a]

[a,b] Values (Mean ± Standard Deviation) in the same row sharing the same superscript are Significantly different (P<0.05).

RESULTS

Antimicrobial activity of propolis samples against microorganisms (Bacteria and Fungi) depending on agar well diffusion test. (Table 2) showed the result of agar well diffusion test revealed propolis extract by the zone of microbial growth inhibition. The ethanol extract of propolis higher show inhibitory zone against of gram positive bacteria *S. aureus* from (12mm – 17mm) followed by *E.coli* (8mm-12mm) and fungi *A. niger* (10mm – 19mm), *Candida albicans* (8 – 15 mm) (Table.2).

DISCUSSION

Propolis has been found to possess antimicrobial activity and this has been attributed to specific chemical in the propolis. The present study shows that bacterial strains *E. coli* and *S. aureus* and fungi strain *A. niger* and *C. albicans* were susceptible to ethanol extract of propolis. Results on the antibacterial activity of propolis from stingless bees have contradictory probably owing to the prepared the sample from different bee species and also the diversity of plants from which the propolis has been collected has reported that *E. coli* and *S. aureus* susceptible to propolis from Tuticorin region. In this study there are lower inhibitory *E. coli* and higher inhibitory concentration *S. aureus.*Similar results also reported [13]. [14] Reported that ethanol extract of propolis had antibacterial activity against *S. aureus* and *P. aeruginosa.* The present study shows the maximum zone of bacterial inhibition of ethanol extract of propolis against *S.aureus* and fungal inhibition *A.niger.* The variation in the antimicrobial activity of propolis has been attributed to the differences in its chemical components likewise, generally the higher activity ethanol extract of propolis against Gram-positive were greater than Gram-negative bacteria, it may be due to differences in the structure and composition of Gram-negative and Gram-positive bacteria cell wall [15]. [16] Reported that propolis is rich in a wide variety of secondary metabolites such as phenolic compounds, tannins, terpenoids, alkaloids and flavonoids which have antimicrobial properties.

CONCLUSION

These results suggest that the propolis extract is excellent anti fungal, antibacterial activities and the propolis is one of the few natural remedies that have maintained its popularity over a long period of time.

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