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Antibacterial and antioxidant activity of mangrove plant (*Avicennia Marina*) extract against some specific oral pathogens

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Abstract---In the Present study, the leaf extracts of mangrove plant *Avicennia marina* revealed the presence of some bioactive components which showed remarkable antibacterial properties. The leaves were dried and extracted thoroughly with methanol. The effects of methanol extracts were found to be inhibiting the growth of some bacterial pathogens such as *Streptococcus mutans*, *Streptococcus oralis* and *Streptococcus rattus*. It also showed that the plant parts can be used to treat infections caused by these bacteria. This study also attests its use in traditional medicine to treat various dental infections. The methanolic extracts were highly effective against the isolated bacterial strains and the antioxidant property highlights the potent activity and it is also considered as a natural antioxidants.

Keywords---antibacterial, antioxidant, mangroves, pathogens, reducing power.

Introduction

Mangrove is an estuarine plant living in a high salinity habitat, influenced by temperature, wind, wave, and ocean current. Mangroves are circum tropical distribution, occurring in 112 countries and territories. Global coverage has been variously estimated at 10 million hectares (Bunt, 1992). The secondary metabolite compounds contained in these mangrove plants can potentially be developed as antibacterial, antiviral, and antioxidant agents (Spalding et al., 2001). Pichavaram forms a great wealth of biological diversity in mangrove ecosystem in India. The mangroves are distributed in varying degree of abundance, in which *Avicennia marina* (Forssk.) Vierh (*A.marina*) is the most common species followed by *Rhizophora apiculata*, *Rhizophora mucronata*, *Bruguiera cylindrica* and *Aegicerascorniculatum*.

Mangroves are highly resistant to salinity and tidal fluctuations and they are known to be a source of several bioactive compounds and secondary metabolites like alkaloids, phenolics, tannins, flavonoids, steroids and terpenoids with toxicological, pharmacological and ecological importance (Ravikumar et al., 2010). Mangrove plants are having biological activity such as anti-oxidant, -bacterial, -fungal, -ulcer, -cancer, -plasmodial and -tumor properties (Afzal et al., 2011). Use of mangroves as potent source of natural antioxidant and antimicrobial agents (Abeysinghe et al., 2002). Mangrove extracts can also be possible sources of mosquito larvicides, antifungal, antiviral, anti-cancer and anti-diabetic compounds (Wu et al 1997). The mangrove plants have also been proved for antiviral, antibacterial and anti-ulcer properties. Based on the above said this study was aimed to test the potency of methanolic extracts of *A.marina*.

Materials and Methods

Preparation of leaf extracts

Raw *A. marina* leaves were collected from the Pichavaram mangrove environment. The fresh samples were washed with running tap water followed by distilled water to remove the debris, impurities and soil. 100 grams of the powder were soaked in 500 ml of 90% methanol in a conical flask for 72 hours on the shaker at room temperature (27°C). After that, the mixture was filtered using fine muslin cloth and later by Whatman TM No. 1 qualitative filter paper followed by distillation process in rotary evaporator.

Isolation of bacterial strains

The human dental bacterial pathogens *Streptococcus mutans*, *Streptococcus oralis* and *Streptococcus rattus* were isolated from the infected patients. All the strains were isolated and cultured in sterile media from Rajamuthaiah Dental College, Chidambaram.

Identification of isolated pathogen

The isolated bacterial pathogens were identified by using biochemical methods according to (Holding and Colee, 1971).

Antioxidant activity

1 mg of the dried leaves were dissolved in 1 ml dimethyl sulfoxide (DMSO) to produce the final concentration, 1 mg/ml. The standards used in this test were ascorbic acid at the same concentration. For DPPH reagent (light sensitive), the concentration used was 2.5 mg/ml. The DMSO solution was added to each 96-well plate followed by the addition of leaf extract on first row wells. Once suspended, the mixture of leaf extract was diluted from first row to second row and the dilution process was continued until the last row. The DPPH reagent was added to each well in dark environment. Then, the plate was wrapped in aluminum foil and incubated on the shaker at room temperature for 20 minutes. The absorbance was recorded at 517 nm.

Antibacterial activity

Antibacterial activity were examined for methanolic extracts of *A. marina* leaves against some dental pathogens which includes *Streptococcus mutans*, *Streptococcus oralis* and *Streptococcus rattus*. A standard concentration of (25µg/ml 50 µg/ml 75µg/ml 100 µg/ml) of leaf extracts were prepared and tested against the pathogenic bacteria with control. The zone of inhibition was measured after 24 hours of incubation at 37°C.

Results

The antibacterial activity was performed by using four different concentrations (25 µg/ml, 50 µg/ml, 75 µg/ml, 100 µg/ml) (Figure 1). Among the four concentrations, 100 µg/ml showed good inhibiting activity against dental bacterial isolates. The highest zone of inhibition was recorded in the 100µg/ml against *Streptococcus rattus* and the zone of inhibition is represented as (15mm) in diameter. The minimum zone of inhibition was found to be present in the 25 µg/ml concentration against *Streptococcus oralis*. The maximum concentration of 100µg/ml zone of inhibition tested against dental pathogen was shown in the (Figure 2).

The differences in the zone of inhibition of antibacterial activity of methanolic extract of mangrove *Avicennia marina* was analyzed using the Analysis of Variance (ANOVA) test. It showed significant differences between the concentrations (F=32.71, P=< 0.001). The results are shown in Table 1. To find out the differences in the zone of inhibition between different concentrations, pairwise t test was performed. The statistical results was showed that there was significant differences were found between all the concentrations (Table 2). To test the differences in the antioxidant bacterial activity of methanolic extract of mangrove *Avicennia marina* against oral pathogens, the Analysis of Variance (ANOVA) was performed. The results showed that there was significant differences present between the tested concentrations (F=989.25, P=< 0.001) (Table 3). To

find out the differences in zone of inhibition between different concentrations of extract of *Avicennia marina*, pairwise analysis test was performed (Table 4). The results showed that there was significant differences in antioxidant activity between the tested concentrations.

Discussion

The antibacterial activity of present study was comparable to similar research works of (Arivuselvan et al., 2011; Saad et al., 2011; Ravikumar et al., 2011). The antioxidant capacity of the mangrove extracts was measured by their ability to scavenge free radicals by DPPH assay. The crude extracts showed high antioxidant and free radical scavenging activity. Similar results were observed by (Proestos et al., 2013; Sylvie et al., 2014). The antibacterial activity has been attributed to the presence of some active constituents in the extracts. The antibacterial activity of cinnamon was probably due to their major component, cinnamaldehyde and their constituents is also known to inhibit bacterial acetyl-CoA carboxylase and responsible for major antibacterial activity (Blumenthal, 1998 and Meades et al., 2011). The similar trend has been observed in the present study. The work of (Rahul Singh, 2018) that the antibacterial compounds from natural resources would be the alternative to overcome the resistance problem was very much resembled with the present investigation that the importance of medicinal plants as herbal medicine used as resistant against many bacterial diseases.

Conclusion

This study has confirmed that crude extracts of *A. marina* possessed considerable antibacterial activity against some bacterial pathogens and it also showed remarkable antioxidant activity. Additionally, purified compound from the methanolic extracts of *A. marina* leaves can be used to treat various oral pathogens involved in tooth decay and dental related infection, provided the infections are caused by susceptible bacteria. The effect of this mangrove plant extract can be treated against a wider range of bacteria. The antioxidant results showed good reducing power activity. The results will be important to the scientific community.

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Appendix

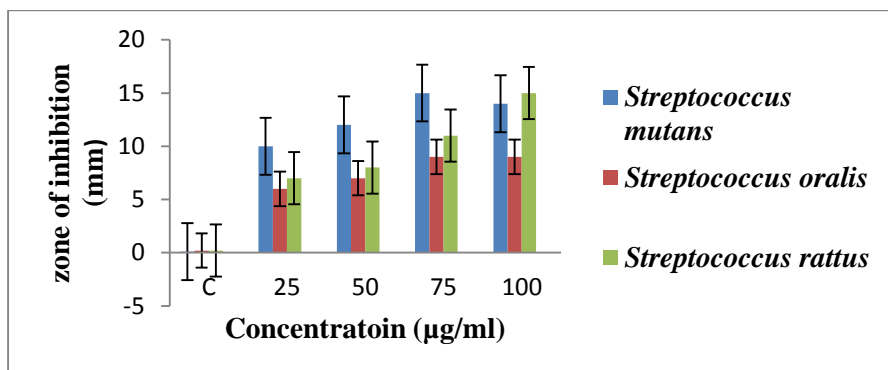


Figure 1. Antibacterial activity of methanolic extracts of *A. marina* leaf extracts against dental pathogens

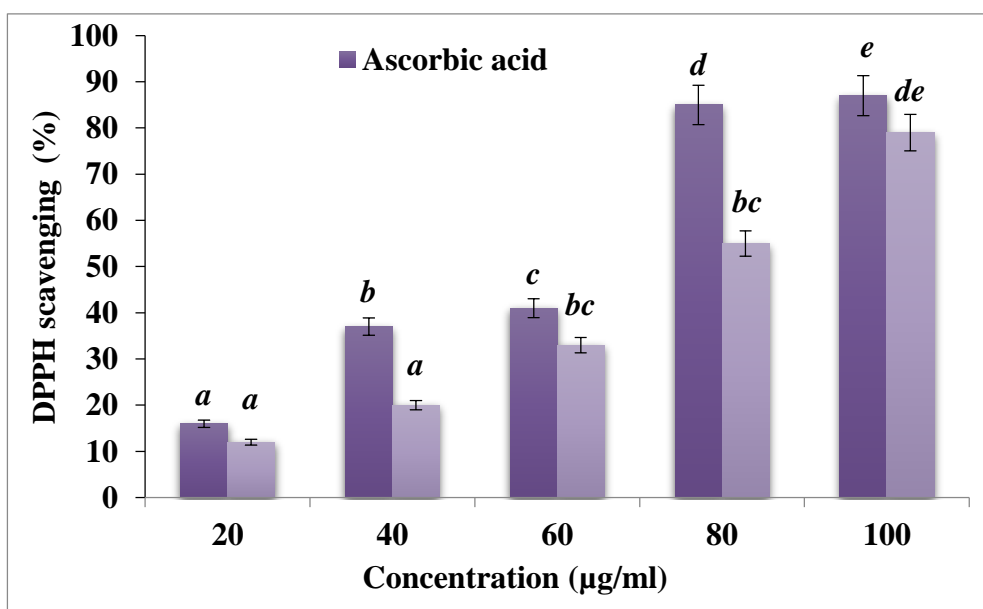


Figure 2. Antioxidant properties of leaf extracts of *A. marina*

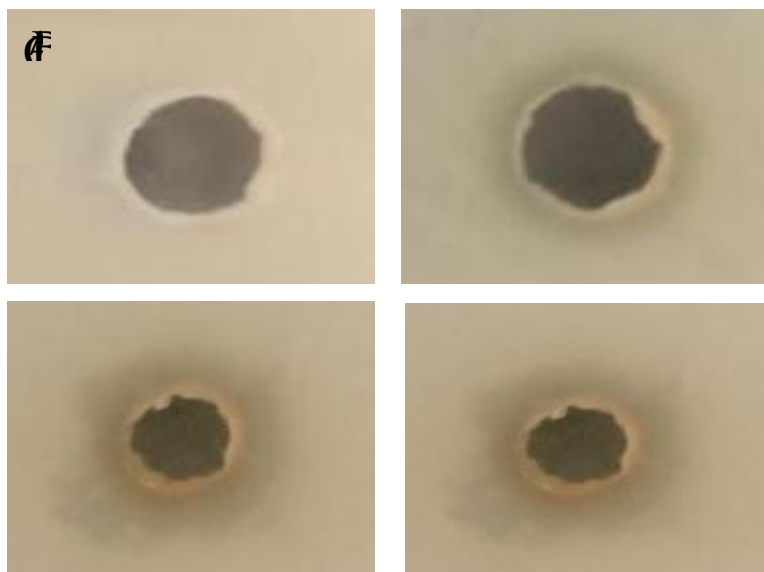


Figure 3. The Antibacterial activity against bacterial strains. A) control, B) *Streptococcus oralis* (C) *Streptococcus rattus* (D) *Streptococcus mutans*

Table 1
Analysis of variance for the differences in antibacterial activity between the different concentrations of extract of *Avicennia marina*

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	824.2685	4	206.0671	32.7087	2.22E-11	2.641465
Within Groups	220.5025	35	6.300071			
Total	1044.771	39				

Table 2
t values for the pairwise analysis done to find out the differences in antibacterial activity between the different concentrations of extract of *Avicennia marina*

Concentrations	Control ug/ml	25 ug/ml	50 ug/ml	75 ug/ml	100 ug/ml
Control	-				
25 ug/ml	10.14	-			
50 ug/ml	11.05	9.65	-		
75 ug/ml	12.81	15.93	13.09	-	
100 ug/ml	9.06	6.74	5.68	3.43	-

Table 3
Analysis of variance for the differences in antioxidant activity between the different concentrations of extract of *Avicennia marina*

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	13849.44	4	3462.36	989.2457	1.13E-22	2.866081
Within Groups	70	20	3.5			
Total	13919.44	24				

Table 4
t values for the pairwise analysis done to find out the differences in zone of inhibition between the different concentrations of extract of *Avicennia marina*

	20 ug/ml	40 ug/ml	60 ug/ml	80 ug/ml	100 ug/ml
20 ug/ml	-				
40 ug/ml	21	-			
60 ug/ml	20.4	17.68	-	-	-
80 ug/ml	45.72	44.41	11.4		
100 ug/ml	167.84	144.92	19.87	17.49	