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ORIGINAL RESEARCH ARTICLE

Phytochemical Analysis and Antimicrobial activity of *Salix alba* against Dental Biofilm forming Bacteria

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ABSTRACT

Objective: The objective of the present study was to find out the antimicrobial activity of *Salix alba* bark extract against the dental biofilm forming bacteria and to investigate the phytochemicals present in the bark extract of *Salix alba*.

Design: the dried bark of the plant *Salix alba* was extracted using methanol as solvent, Soxhlet apparatus was used for this purpose. Antimicrobial activity at different concentration was performed by using disc diffusion method. The minimum inhibitory concentration was carried out according to the method of National Committee for Clinical Laboratory Standards (NCCLS), phytochemical analysis was performed to know the phytochemicals present in the extract.

Results: Phytochemical analysis revealed the presence of Alkaloids, Flavonoids, and Tannins. This is the first report as we did not find any research article showing these results. The Minimum Inhibitory Concentration (MIC) shown by the bark extract was 125 µg/mL against *Streptococcus mutans* and 250 µg/mL against *Lactobacillus* respectively. The plant extract also showed a good activity against the *Staphylococcus aureus* the least activity was shown in *E. coli.*

Conclusion: Although, the medical importance of *Salix alba* was known from long ago, little attention has been paid to find the antimicrobial activity of this plant against the dental biofilm forming bacteria. The present study revealed that the presence of phytochemicals which have known antimicrobial activity. The extract of *Salix alba* can be used for oral hygiene and control of oral biofilm bacteria.

Key words: Phytochemicals, Oral Hygiene, Dental biofilm, Pathogenic bacteria and Chloramphenicol.

1. INTRODUCTION

Dental plaque is a thin whitish or pale yellow biofilm that builds up on the teeth. These biofilm are complex aggression of diverse microorganisms. Overgrowth or imbalance of the dental plaque microbial communities will lead to the growth of more pathogenic organisms deeper inside the bacterial matrix of plaque biofilm [1]. A number of microorganisms can produce enough acid to decalcify tooth structure. *Streptococcus mutans* has been implicated as one of the caries-producing organisms. Consequently *S. mutans* has been targeted in a large share of research [2]. The antimicrobial activity of medicinal plants towards oral bacteria has been well documented [3, 4]. Twigs of babul, Neem, clove oil and many others were used for brushing teeth in India [5, 6]. The literature survey of the folklore medicine revealed the use of *Psidium guajava* leaves to maintain oral hygiene, dried fruit of *Terminalia chebula* as an anticaries agent, stem of *Achyranthes aspera* for the treatment of toothache and stem of *Mimusop selengi* for strengthening the gums [7, 8]. Palambo has reported traditional medicinal plant extracts and natural products with activity against oral bacteria [9].

Hippocrates recommended chewing willow bark to patients suffering from fever, inflammation and pain [10]. In Nigeria and other parts of West Africa like Senegal, chewing sticks which are usually pencil-sized sticks of about 6 inches or 15mm long are used frequently during the day. The use of traditional agents in oral hygiene cuts across North Africa [11, 12]. Southern Africa [13, 14], and Asia as well [15, 16]. The potential of plant extracts from various plant parts have been evaluated against dental pathogens [17]. The present study was aimed to evaluate the phytochemical analysis and antimicrobial activity of bark of *Salix alba*

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against some dental biofilm forming bacteria. Increased resistance of oral bacteria to antibiotics has developed keen interest of researchers in herbal treatments. Thereby, in the present investigation our interests was focused towards the experimentation, evaluation and determination of efficacy of ethanolic extract of *Salix alba* against two dental biofilm causing pathogens.

2. MATERIALS AND METHODS

Plant material

The bark of the plant *Salix alba* was collected from Kashmir, India. It was identified and authenticated in the Department of Botany Annamalai University. The bark was washed with sterile water and dried at room temperature.

Test Microorganism

The bacterial species *Staphylococcus aureus*, *Streptococcus mutans*, *Lactobacillus* sp., *Escherichia coli* were obtained from Department of Microbiology, Raja Muthiah College and Hospital, Annamalai University. These pathogens were maintained on Nutrient Agar (NA) (Hi Media, India). Two bacteria *viz*., *Staphylococcus aureus* and *Escherichia coli* were used as internal control whereas *Streptococcus mutans* and *Lactobacillus* represent the dental plaque forming bacteria.

Preparation of plant extract

The stem bark of *Salix alba* was washed under running tap water. It was then dried under shade and ground into coarse powder in the electronic grinder. Fifteen grams of powder was then extracted in methanol [150 ml] by using Soxhlet method. Twelve cycles were done. The solvent was removed by evaporation at room temperature [28±20°C]. The extracts were kept in freeze until further use.

Phytochemial Analysis of Plant Extract [18]

Detection of Alkaloids

Solvent free extract [50 mg] was stirred with 2 to 3 ml of dilute hydrochloric acid and filtered.
The filtrate was tested carefully with various reagents a follows.

1) Mayer's test –To 2 to 3 milliliter of filtrate, a drop of Mayer's reagent is added by the side of the test tube. A white or creamy precipitate indicates the test as positive.
2) Wagner's test –To 2 to 3 milliliter of filtrate, few drops Wagner's reagent are added by the side of the test tube. A reddish brown precipitate confirms the test as positive.
3) Hager's test—to 2 to 3 milliliter of filtrate 1 or 2 ml of Hager's reagent is added. A prominent yellow precipitate indicates the test as positive.

Detection of Saponins

Foam test – The extract [50 mg] was dissolved in 20 ml of distilled water. The suspension was shaken in a graduated cylinder for 15 minutes. A two centimeter layer of foam indicates the presence of Saponins.

Detection of flavonoids (Magnesium and hydrochloric acid reduction test)

The extract [50 mg] was dissolved in 5 ml of alcohol and few fragments of magnesium ribbon and concentrated hydrochloric acid [drop wise] were added. If pink to Crimson color develops presence of flavonoids was inferred.

Detection of Tannins (Ferric chloride test)

The extract [50 mg] was dissolved in 5 ml of distilled water. To this, few drops of 5% Ferric chloride were added. A dark green color indicates the presence of tannins.

Disc- diffusion method [19].

The petriplates were prepared with 20 mL of sterile Mueller Hinton Agar (MHA) (Hi-media) and the test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min. Three different concentrations (5 mg/disc, 2.5 mg/disc and 1.25 mg/disc) of the crude extracts were prepared and loaded on the sterile discs (Hi-media) which were placed on the surface of the solidified agar medium. Negative control (Solvent alone) was prepared using the respective solvent while chloramphenicol (0.01 mg/disc) was used as a positive control. The plates were incubated for 24 hours at 37^0C for bacterial growth. Zones of inhibition were recorded in millimeters and the experiment was repeated thrice for concordant results. All the data were statistically analyzed.

Determination of minimum inhibitory concentration (MIC):

The minimum inhibitory concentration was carried out according to the method of National Committee for Clinical Laboratory Standards [20]. The plant extracts were selected for the effective solvents (i.e., methanol) and was dissolved in water containing 4% dimethyl sulfoxide (DMSO). The initial test concentration of extract was 5 mg/mL. It was then serially diluted into two folds. Each tube containing 5 ml of bacterial broth was

inoculated with 5 mL of bacterial suspension containing 10^8 CFU/mL of bacteria. Chloramphenicol was used as positive control. The plates were incubated for 24 hrs at 37^0C. MIC was determined as the lowest concentration of extract showing no visible growth on the agar plate. All the data were statistically analyzed.

3. RESULTS AND DISCUSSION

The result for the phytochemical screening of the *salixalba* extract indicated that the extract tested positive for Alkaloids, Flavonoids and Tannins. The result was shown in (**Table 1**). The plant extract showed a good activity against all the tested organisms. The result for Disc Diffusion and MIC is shown in (**Table 2**).

Table 1: Phytochemical analysis of *Salix alba* ethanolic and methanolic extract

S .No	Phytochemicals	Extracts	
		Methanol	Ethanol
1	Alkaloids	+	-
2	Flavonoids	+	+
3	Saponins	-	-
4	Tannins	+	+

(+) indicates the presence and
(–) indicates the absence of the phytochemical

Table 2: Antimicrobial Activity of Methanolic Extract of *Salix alba* (Disc Diffusion Method).

S. No	Organism Tested	Concentration of extract			Negative control	Positive control
		1000 µg	500 µg	250 µg		
1	*Streptococcus mutans*	16	13	9	__	20
2	*Staphylococcus aureus*	20	15	11	__	24
3	*Lactobacillus* sp.	14	11	8	__	20
4	*Escherichia coli*	14	10	8	__	19

The size of the disc is in mm and includes the disc size of 6 mm.Positive control was chloramphenicol and the negative control was solvent without extract

Table 3: Minimum Inhibitory Concentration of methanolic extract of *Salix alba*

Concentration of Extract (µg/mL)						
Microorganism	**1000**	**500**	**250**	**125**	**100**	**MIC**
Streptococcus mutans	-	-	-	-	+	125
Staphylococcus aureus	-	-	-	-	+	125
Lactobacillus	-	-	-	+	+	250
Escherichia coli	-	-	+	+	+	500

Results of the present investigation signify that the methanolic extract of *Salix alba* has a good activity against the tested microorganisms. On the basis of the results obtained it was concluded that the zone of inhibition was due to the extract as there was no any inhibition zone observed against the solvent when used individually. The result of the phytochemical analysis of the plant revealed the presence of tannins, saponin, flavonoids and alkaloids. These compounds are known to be biologically active and have been shown to possess antimicrobial activity. The MIC for *Streptococcus mutans* was 125 µg/mL and for *Lactobacillus* was found to be 250 µg/mL. This is the first report of antimicrobial activity of *Salix alba* against dental plaque forming bacteria as per our knowledge [21, 22]. Extracts from *Salvadora persica* has been shown to possess antibacterial activity against *Streptococcus mutans* and controls plaque formation [23- 25].

The present findings were supportive towards the traditional use of the medicinal plants for oral hygiene and oral care. *Salix alba* could thereby be potentially used in the treatment of dental caries and for prevention of dental plaque as dental biofilm forming bacteria are currently imparting a strong multiple drug resistance to available antibiotics. Moreover, plant extract can be further studied to know the structure of the active compounds and for the isolation of the active compounds for developing an effective and natural therapeutic agent against dental biofilm.

4. CONCLUSION

The bark extract from the plant *Salix alba* was found to possess antimicrobial activity against the dental biofilm forming bacteria. The bark contains Tannins, Saponins, Flavonoids and Alkaloids which have been found to possess antimicrobial activity that support the usefulness of this plant as a good alternative for antiplaque agent.

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