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SCREENING OF *ACCACIA ARABICA* POD EXTRACTS AS ANTI-BACTERIAL AGENTS AGAINST DENTAL PATHOGENS

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ABSTRACT

Several bacteria have now become antibiotic-resistant. This increases the importance of ayurvedic medicine. We report, here, the activity of different extracts (petroleum ether, chloroform, methanol and water) of Accacia arabica Pod against dental pathogens – Streptococcus mutans, Streptococcus salivarius, Staphylococcus aureus, Lactobacillus acidophilus and Streptococcus sanguis. The cup-plate method was used in anti-bacterial activity of the extracts at concentration of 200 mg/ml against dental pathogens. Minimum inhibitory concentration (MIC) values of most effective extracts against the most susceptible bacteria were determined using a two-fold serial micro dilution method. Methanolic extract showed maximum anti-bacterial activity against all the bacteria. The most susceptible bacteria were S. sanguis followed by S. aureus, S. mutans, S. salivarius and L. acidophilus. The MIC values showed that methanolic extract was more effective than water extract.The plant has the potential to generate herbal metabolites. The crude extracts demonstrating anti-dental caries activity could result in the discovery of new chemical classes of antibiotics. These chemical classes of antibiotics could serve as selective agents for the maintenance of human health and provide bio-chemical tools for the study of infectious diseases

Key words: *Anti-bacterial activity, cup-plate method, minimum inhibitory concentration, Accacia arabica*

INTRODUCTION

Accacia arabica (Mimosoideae) is a perennial tree. The pods arise on young branches of this tree at June to September (Prabat 2006). The plant is known as Babool in Hindi. *Streptococcus mutans, Streptococcus sanguis, Streptococcus salivarius* and *Lactobacillus acidophilus* play a major role in dental plaque formation. *Staphylococcus* spp. also ferments many sugars and the produced

product is utilized by the dental plaque bacteria.(Kornman and Robertson.1985) A large number of *Streptococcus* spp. And *Lactobacillus* spp. are involved in root caries and periodontal diseases.(Archa Vermani 2009, Schiipbach *et al.* 1995 and Marsh 1992) In recent times micro- organisms developed resistance to many antibiotics due to the indiscriminate use of anti-microbial drugs in the treatment of infectious diseases. Therefore, developing of alternative antimicrobial agents is required, and local medicinal plants are considered the important sources of novel anti- microbial agents.(Cowman 1999) This study was carried out to investigate the anti-bacterial properties of the pods of *A. arabica*, extracted by four solvents of different polarity against dental pathogens.

MATERIALS AND METHODS

The Pods of *A. arabica* used in this study were collected from the foothill of shivalik mountain in region Haridwar. They were identified at the Department of Botany and Microbiology, Gurukul Kangri University, Haridwar and certified by Botanical Survey of India, Dehradun. The Pods were dried under shadow and crushed to small pieces using pestle and mortar and then powdered in an electric grinder. The extracts were prepared by immersing 200 gm of dried powdered material in 600 ml of solvents i.e. petroleum ether, chloroform, methanol and water using the Soxhlet apparatus. Crude extracts were obtained by removing the solvent in vacuum evaporator at 30°C and stored in sterile bottles at 4°C until further use. The strains of dental infection-related bacteria used in this study *Staphylococcus aureus, S. mutans, S. salivarius, L. acidophilus* and *S. sanguis* were isolated from Aggarwal Dental Clinic, Haridwar. The isolates were identified according to published guidelines.(Burneti *et al.*1994) All the bacterial strains were grown and maintained on nutrient agar slants at 4° C. The extracts were dissolved in the same solvent with which it has been extracted (petroleum ether, chloroform, methanol and water) to a final concentration of 200 mg/ml for cup-plate method.(Perez *et al.1990*) The cup-plate method was used to evaluate the anti-bacterial activity. This method depends upon the diffusion of the tested material to such an extent that growth of the added micro-organism is prevented entirely in a zone around the hole containing a solution of tested material.(Ahmad *et al. 1998* and Prabhat 2005) One hundred microliters of diluted inoculums of 10^5 CFU/ml(Indian Pharmacopoeia 1996) of 24 hours old cultures of test organisms were mixed in Mueller Hinton Agar media and shaken. Then media was poured (25-30 ml) in sterilized petridishes (20 × 90 mm).

Wells of 8 mm diameter were punched into the agar medium and filled with 45 µl of plant extracts. All the solvents served as negative control. Each extract was assayed in triplicate and the mean values were observed. The plates were incubated at 37°C for 24 hours. The anti-bacterial activity was interpreted from the size of the diameter of zone of inhibition measured in millimeters (mm), it was observed as the clear zones surrounding the hole. The MIC of the most effective extracts (methanolic and aqueous) was determined for *S. aureus* and *S. sanguis*. MIC was determined by using the two-fold serial micro dilution method at a final concentration ranging from 10 mg/ml to 0.0195 mg/ ml.(Rwarinda 2015) The extracts were added to sterile Mueller Hinton Broth before bacterial suspensions with final inoculums of 10^5 CFU/ml. Each extract was assayed in triplicate. The extracts in broth were used as negative control and the bacterial suspensions were used as positive control. The turbidity of the wells in the microtiter plate was interpreted as visible growth of the microorganisms. The MIC values were taken as the lowest concentration of the extracts which showed no turbidity after 24 hours of inoculation at 37°C.

RESULTS

Table 1 represents anti-bacterial activities of all the extracts with respect to each of the test organism at concentration of 200 mg/ml. Anti-bacterial activity was found in all the extracts. The methanolic extract showed the maximum zone of inhibition against all the bacteria. The anti-bacterial activities of extracts were observed in increasing order petroleum ether is less than chloroform which is less than water which is further less than methanol. The methanolic extract showed the maximum zone of inhibition against *S. sanguis*. The minimum zone of inhibition was showed by petroleum ether extract against *L. acidophilus*.

The MIC values of the methanolic and aqueous extracts from the pods of *A. arabica* against *S. aureus* and *S. sanguis* are shown in Table 2. The MIC values of the methanolic and aqueous extracts were the same (0.1578 mg/ml) against *S. aureus*, whereas the MIC values of methanolic and aqueous extracts against *S. sanguis* were 0.0798 mg/ml and 0.1578 mg/ml.

Table 1: Anti-bacterial activity of *A. arabica* extractsprepared in different solvents

Plant extract	*S.aureus*	*S.salivarius*	*S.mutans*	*L.acidophilus*	*S.sanguis*
Petroleum Ether	13	10	12	11	16
Chloroform	18	15	16	16	20
Methanol	25	23	23	24	26
water	24	18	21	23	25

*After incubation of Mueller-Hinton agar plates, inhibition zone of bacterial growth around the well filled with each extract was measured (as mm). The entire test was done in triplicate and the mean of the values are shown in the Table

Table 2: MIC values (mg/ml) of extracts of pods of *A. arabica* against *S. aureus* and *S. sanguis*

Organism	Methanol extract	Water extract
S. aureus	0.158	0.158
S. sanguis	0.080	0.158

DISCUSSION

Streptococci and *staphylococci* ferment sugars and produce acids. These acids affect primary decalcification

of enamel which leads to total destruction and the decalcification of dentin. Major end-products of fermentation are lactic acid, dextrans and levans. These products are seen to cause dental caries.(Prabhat 2006) Present study has shown that the gall is potentially a rich source of anti-bacterial agents. All the four extracts inhibited the growth of all pathogens and methanolic extract was the most effective. The MIC value of methanol and water extracts against *S. sanguis* in comparison to *S. aureus* concludes that *S. sanguis* showed greater sensitivity towards the methanolic extract.

Successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Traditional healers use primarily water as the solvent.(Ahmad *et al* 1998) However, in the present study, we have found that plant extract in methanol provided more consistent antimicrobial activity as compared to those extracted in water.

It is probably because various organic compounds can be leached more in this solvent. Ethanolic extract of *A. arabica* also demonstrated significant activity against resistant bacteria.(Prabhat 2006). Prabhat *et al.* 2010) screened medicinal plants to detect anti-microbial activity and clearly demonstrated that alcohol is a better solvent as compared to water and petroleum ether. Prabhat *et al*.(Prabhat *et al.* 2005) reported good anti-bacterial activity of *Achyranthes aspera* (Apamarga) and *Mimusops elenigi* (Bakula) methanolic extracts against these dental pathogens. However, in this study, the pods extract showed better results than both the plants. The main constituents found in the pods of *A. arabica* are tannin (50-70%), gallic acid and ellagic acid.(Rwarinda 2015 and Osman 2017) Tannin is a phenolic compound that is soluble in water, alcohol and acetone. It gives precipitates with protein. [16] The anti-microbial activity seemed to depend on the contents of tannin and gallic acid in the extracts.(Irobi *et al.* 1994, Djipa *et al.* 2000 and Archa Vermani 2009)

Conclusion: The extracts of pods of *A. arabica* have high potential as anti-bacterial agent. This finding provides an insight into the usage of the pods of *A. arabica* as traditional medicine in dental powder and in the treatment of toothache and gingivitis.(Bhattacharjee 2001 and Prabhat *et al.* 2010)

The results of this study suggest that plant extracts possess compounds with antibacterial properties which can be used as anti-microbial agents in new drugs for the therapy of infectious diseases.

Further pharmacological evaluation of refined extracts of pods of *A. arabica* are needed before they can be used as therapeutic antimicrobials. Further, phyto-chemical studies are required to establish the types of compounds responsible for the anti-microbial effects of this medicinal plant.

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