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Inhibitory Properties of Aqueous Extracts of Selected Indigenous Medicinal Plants Against Dental Caries Causing *Streptococcus mutans* and *Streptococcus mitis*

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Abstract: Inhibitory properties of aqueous extracts of 7 different plant species were tested against *S. mitis and S. mutans*. The determination of the antibacterial activity by agar well diffusion method showed that 5 plant extracts tested exhibited antibacterial activity against *Streptococcus mitis* and *S. mutans.* The *Coriandrum sativum, Mentha arvensis* plant extracts showed antimicrobial activity against *S. mutans* only. The determination of the MIC by means of the liquid dilution method showed that all the plant extracts exhibited an antimicrobial effect against *Streptococcus mitis* and *S. mutans*. In inhibition ELISA *Plmenia dioica* plant extract showed maximum of 91% of inhibition against *S. mutans* and 86.4% of inhibition against *S. mitis*. The above observations confirmed that some of these plant extracts possess bactericidal compounds, which inhibit the growth of dental caries.

Key words: *S. mitis* · Plant extracts · ELISA

INTRODUCTION

Dental caries is a disease caused by specific type of bacteria live in human mouth. These bacteria produce acid that destroys tooth enamel and results in cavities on its surface. Among the oral micro flora pathogens Streptococci are the main causative agent for dental caries. The principle causal agent of dental caries are a group of Streptococcal species of which *Streptococcus mutans* and *Streptococcus mitis* are the most important agents of human dental caries. Viridans streptococci are among the first bacteria to colonize the human mouth after birth. Generally, the predominant colonizing strains are *S. mitis* and *S. oralis*. The *S. mitis* is also one of the most important pathogens in bacterial endocarditis [1, 2]. Different species of streptococcus are dominate in different parts of the oral cavity and cause different clinical symptoms, therefore the identification of the species is important for perfect treatment [3]. Identifying effective antimicrobial agents against these oral pathogens are important in prevention of dental caries. Antibiotics such as penicillin and erythromycin are reported as effective drugs to prevent dental caries in animal but these are never used clinically because of hypersensitivity reactions [4]. Herbal medicines have been important sources of products for the developing countries in treating common infectious diseases and overcome the problems of resistance and side effects of the currently available antimicrobial agents. The World Health Organisation (WHO) estimates that 80% of the people living in developing countries almost exclusively use the traditional medicines. Medicinal plants used in traditional medicine should therefore be studied for safety and efficacy. Hence in our present study we investigate the antibacterial activity of the aqueous extracts of selected indigenous medicinal plants against *S. mutans* and *S. mitis*.

MATERIALS AND METHODS

Bacterial Strains: *Streptococcus mutans (MTCC890) and Streptococcus mitis* (MTCC 2696) were procured from IMTECH Chandigarh in lyophilized form and was used in our present study. The Isolates were brought to pure culture on Blood agar plates and maintained at 5°C.

Preparation of Plant Extracts: Medicinal plants were obtained from Kottakal aryavaidyasala, Coimbatore and the extracts were prepared by using distilled water as the solvent [5]. A 20 g of each powdered sample of the herbs

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were extracted by soaking in 180 ml of distilled water in a beaker, stirred for about 6 min and left overnight. Thereafter, the solutions were filtered using filter paper (Whatman No. 1) and the filtrates were lyophilised. Weighed and dissolved in appropriate volume of water.

Antimicrobial Activity of Various Plant Extracts Against *S. mutans and S. mitis* Testing for Antibacterial Activity: The agar well diffusion method was performed to assess the antibacterial activity of various plant extracts. Briefly, 0.3 ml of standardized bacterial stock suspensions 10^6 colony-forming units per ml was thoroughly mixed with 60 ml of sterile nutrient agar. 20 ml of the inoculated nutrient agar were distributed into sterile Petri dishes. The agar was left to set and in each of these plates 4 wells were made by using a sterilized 6 mm cork borer and the agar discs were removed. The wells were filled with 50µl of the different plant extracts of 100mg/ml, 150mg/ml and 200mg/ml so that the final drug concentration was 5mg/well, 7.5mg/well and 10mg/well respectively and allowed it to diffuse into the solidified nutrient agar for about 45 minutes and then kept in an incubator at 37°C. 50µl of distilled water instead of extracts was considered as controls. Duplicates were carried out for each filtrate against *S. mutans and S. mitis*. After incubation the diameters of growth inhibition zones were measured, averaged and the mean values were tabulated.

Minimum Inhibitory Concentration (MIC)-Dilution Method: Various concentrations of plant extracts (1mg to 10mg/ml) were added into each test tubes containing 5 ml of sterile nutrient broth. To this 50µl of an overnight broth culture of *S. mutans* and *S. mitis* were inoculated and the tubes were incubated for 24 hours at 37°C. One tube contained 5 ml sterile nutrient broth with 50µl of overnight broth culture was kept at 4°C in a refrigerator overnight. This served as control for the determination of complete inhibition. MIC is expressed as the lowest dilution, which inhibited growth judged by lack of turbidity in the tube. Because very faint turbidity may be given by the inoculums itself, the inoculated tube kept in the refrigerator overnight may be used as the standard for the determination of complete inhibition.

Minimum Bactericidal Concentrations (MBC): Dilutions and inoculations were prepared as described in determination of MIC. The control tube without plant extract is immediately sub cultured (Before incubation) on Todd Hewitt agar plates and incubated at 37°C overnight. The tubes were also incubated overnight at 37°C. The MIC of the control organism was read to check that the drug concentrations are correct. The tubes did not showing visible growth in the same manner as the control tube were subcultured on Todd Hewitt agar plates and incubated at 37°C overnight. The growth was compared with control, which represents the original inoculum. If similar number of colonies presents it indicates bacteriostasis only. A reduced number of colonies-indicating a partial or slow bactericidal activity, no growth-if the whole inoculum has been killed. The highest dilution showing at least 99% inhibition is taken as Minimum Bactericidal Concentrations (MBC).

Development of Antibodies for ELISA Inhibitory Studies: To determine the Inhibitory properties of plant extracts against *Streptococcus mitis and S. mutans by* indirect antigen capture ELISA, antibodies were generated against these microorganisms using layer hens. Briefly Formalin inactivated *Streptococcus mitis and S. mutans* cells were injected intramuscularly at the multiple sites of breast muscles of 24-week-old white leghorn chickens. The eggs were collected and the antibodies were extracted from egg yolk by using Polyethylene and ammonium sulphate precipitation method [6]. After dialysis, the crude fraction of IgY thus obtained was further purified by DEAE cellulose ion exchange column chromatography [7].

Inhibitory Studies by ELISA: Inhibitory properties of plant extracts against *Streptococcus mitis and S. mutans* were determined by indirect antigen capture ELISA [8]. Nunc polysorp ELISA plates were coated with *Streptococcus mitis and S. mutans* antigens using coating buffer (0.05M carbonate bicarbonate buffer, pH 9.6) and incubated at 4°C overnight. After coating plates were washed with PBST for 3 times. The empty sites blocked by 1% BSA (200 µl/well) and incubated at 37°C for 1 hour. Plates were subsequently washed and incubated with various plant extracts (100 µl/well), specific antibody (Chicken antibodies-IgY) served as controls. Wells were washed and incubated with antibodies (IgY) (100 µl/well). The wells were washed thrice with PBST and 100 µl of diluted (1:1000) rabbit antichicken immunoglobulin coupled to Horse Radish Peroxidase (Genei Pvt Ltd, Bangalore) was added and incubated. Then the plates were washed and 100 µl of TMB/H_2O_2 (Genei Pvt Ltd, Bangalore) was added. The plates were allowed to stand at room temperature in dark for 20 minutes. The reaction was stopped by adding 50 µl of 4N sulphuric acid and plates were read at 490 nm in an ELISA reader. All samples were tested in triplicates. The percentage of inhibition was calculated by comparing with controls (IgY).

RESULTS

Dental caries causing *S. mitis* and *S. mutans* were obtained from IMTECH, Chandigarh. The organisms were sub-cultured on Todd Hewitt agar and Blood Agar. Inhibitory properties of 7 different plant extracts were tested against both *S. mitis and S. mutans.* The determination of the antibacterial activity by agar well diffusion method showed that 5 plant extracts tested exhibited antibacterial activity against *Streptococcus mitis* and *S. mutans* (Table 1). *Coriandrum sativum, Mentha arvensis* plant extracts showed antimicrobial activity against *S. mutans* only. About 5mg of *Coriandrum sativum* and *Mentha arvensis* extracts produced 11mm and 10mm zone respectively. The determination of the MIC by means of the liquid dilution method showed that all plant extracts tested exhibited an antimicrobial effect against *Streptococcus mitis* and *S. mutans* (Table 2). In Inhibition ELISA *Plmenia dioica* plant extract showed maximum of 91% of inhibition against *S. mutans* and 86.4% of inhibition against *S. mitis*. *Mentha arvensis* which showed lowest inhibition of 25.3% against *S. mitis* (Fig. 1).

Table 1: Antibacterial activity of aqueous extracts of various medicinal plants against *S. mitis* and *S. mutans*-Well cut method

		Zone (mm)	
Plant extracts	Conc. mg/50µl	*S. mitis*	*S. mutans*
Moringa oleifera	5.0	8	10
	7.5	12	12
	10	16	14
Pimenia diocia	5.0	13	13
	7.5	15	16
	10	17	18
Syzgium aromaticum	5.0	13	10
	7.5	15	12
	10	18	15
Mangifera indica	5.0	-	-
	7.5	7	6
	10	10	8
Psidium guajava	5.0	6	8
	7.5	10	11
	10	14	13
Coriandrum sativum	5.0	-	11
	7.5	-	14
	10	3	16
Mentha arvensis	5.0	-	10
	7.5	-	13
	10	3	15

Table 2: Minimal inhibitory concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) of various medicinal plants against *S. mitis and S. mutans* (in mg/ml)

	S. mitis		*S. mutans*	
Plant extracts	MIC	MBC	MIC	MBC
Moringa oleifera	3	5	4	6
Pimenia diocia	4	6	4	7
Syzgium aromaticum	4	7	3	7
Mangifera indica	5	7	5	8
Psidium guajava	5	8	5	8
Coriandrum sativum	7	10	4	7
Mentha arvensis	7	9	4	6

DISCUSSION

The oral ecosystem which contains a wide variety of microbial species, of which *S. mitis* and *S. mutans* play an important role in the development of dental caries in animals and humans.

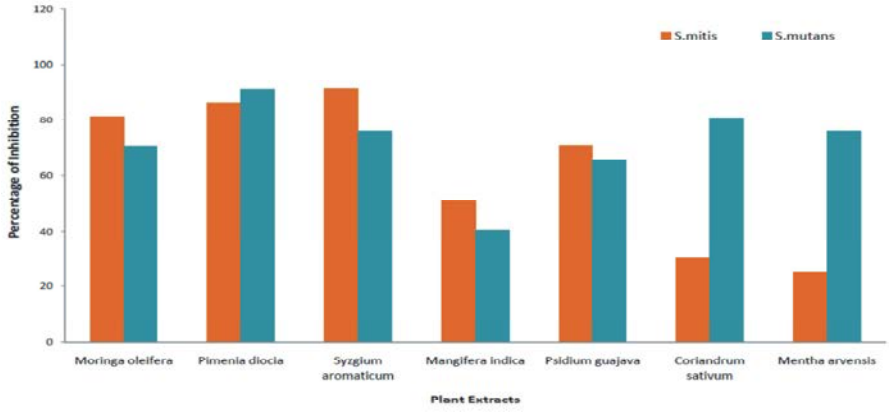

Fig. 1: Determination of Percentage of Inhibition by Inhibition ELISA. *Plmenia dioica* plant extract showed maximum of 91% of inhibition against *S. mutans Mentha arvensis* which showed lowest inhibition of 25.3% against *S. mitis.*

The *S. mutans* glucosyltransferase synthesize extracellular polysaccharides, mainly hydrophobic glycan from sucrose, colonize the tooth surface and initiate plaque formations [9]. Either inhibition of the above enzyme or elimination of *S. mutans* is essential for controlling dental plaque and prevention respectively. Viridans *Streptococci* have become increasingly resistant to antibiotics including penicillin, cephalosporin, erythromycin and tetracycline. Among the viridans *streptococci*, *Streptococcus mitis* has been considered the species with the highest level of resistance to penicillin. Although, use of plants against the various pathogens has been long recognized, more scientific attention has been given since last 20 years. Many Indian medicinal plants are recommended for the treatment of many diseases. In our present study we investigated the inhibition properties of aqueous extract of selected indigenous medicinal plants against *Streptococcal* dental caries. Dental caries causing *S. mitis* and *S. mutans* were obtained from IMTECH, Chandigarh. Aqueous extracts from 7 different plant species were investigated against these two dental pathogens. Each plant extracts were tested at three different concentrations (100, 150 and 200 mg/ml) to check their inhibitory potentials against these pathogens. The present experimental results indicates that out of 7 different plant species 5 plant extracts tested exhibited an antimicrobial effect against *S. mitis* and *S. mutans*. These results showed that the extracts from Moringa *oleifera, Syzgium aromaticum, Plmenia dioica, Mangifera indica, Psidium guajava* possessed some antimicrobial compounds which act against these two pathogens. The methanolic extracts of the *M. oleifera* which posses compounds with inhibitory properties against oral pathogens and can be used as oral medicine for dental caries [10]. Indirect antigen capture Assay (IACA) is highly sensitive and even detect some nano gram level proteins. We perform ELISA with slight modification, first we incubate antigen coated ELISA wells with plant extracts after washing steps we added specific antibodies (IgY). The bound antibodies were assayed with TMB/H_2O_2. In inhibition ELISA *Plmenia dioica* plant extracts showed maximum of 91% of inhibition against *S.mutans* and 86.4% of inhibition against *S. mitis*. *Mentha arvensis* which showed lowest inhibition of 25.3% against *S.mitis*. The result from this preliminary study indicates that these plant extracts could be used for therapeutic purpose in case of Streptococcal dental caries. Further investigations are needed for identification and purification of the specific antimicrobial components from these plants against *S. mitis* and *S.mutans*.

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