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Bactericidal Effect of Certain Plant Extracts on Dental Caries causing Organism- *Streptococcus mutans*

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The antagonistic and synergistic effect of four selected plants used in traditional Indian medicine against the caries causing *Streptococcus mutans* was studied. The phytochemical composition reveals the presence of flavonoids, terpenoids, lignins and saponins invariably in the selected plants. The aqueous extract of clove at different concentration showed effective inhibition of growth followed by eucalyptus, banyan and neem solvent extracts. Further the synergistic effect of clove with other extracts showed best results in clove + eucalyptus combination which was effective in controlling the growth of the isolate.

Key words: *Streptococcus mutans*, Antimicrobial activity, Antagonistic, Synergistic, Dental caries.

Nature has been bestowed human with thousands of natural drugs which serves as effective ailments for a number of diseases. The practice of tooth cleaning by chewing sticks have been known since antiquity. The use of chewing stick persists today among many African and southern Asian communities as well as in isolated areas of tropical America and southern United states (Lewis and lewis, 1977).

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The notion that dental caries in animals is an infectious transmissible disease was first demonstrated by Keyes (1960). The etiology of the disease is multifactorial life habit and mutans *Streptococcus* infection being the most important factor (Johnson, 1991). It includes the breakdown of enamel, the hardest material in the human body, and a subsequent breakdown of the underlying dentin (Tanzer, 1992).The tooth surface is unique among all body surfaces in two ways. First, it is a non shedding hard surface, and second this surface is introduced into the human mouth during the first years of life. The earliest point at which the cariogenic mutans Streptococci may become established is when the first teeth erupt. Solid surfaces are required for both streptococcal colonization and multiplication (Loesche, 1986). Among the oral Streptococci, *S. mutans* has a high degree of surface hydrophobicity (Olson and

Westergren, 1982) and its adhesion to saliva coated hydroxyapatite is dependent on hydrophobic interaction (Westergren and Olson, 1983). This bacterium adheres firmly to the smooth tooth surfaces and produces sticky water insoluble dextran from sucrose, forming plaque which facilitates the accumulation of microorganisms (Bhattacharya *et al.,* 2003). This is followed by the fermentation of glycoproteins by cariogenic bacteria producing organic acids. When fermentable carbohydrates are present, the main organic acids produced are lactic, formic and acetic acids (Geddes, 1975).These acids coincides with a pH drop in plaque, resulting in demineralization of the tooth (Loesche, 1986) and creating an environment which is advantageous for further growth of *Streptococcus mutans* (Bradshaw *et al.,* 1989). It is not however, the only cause of dental decay, after initial weakening of the enamel, various oral bacteria such as *Lactobacilli, Actinomyces* gain access to interior regions of the tooth.

Elimination of *Streptococcus mutans* would reduce the incidence of dental caries. However *Streptococcus mutans* was found to be resistant to many of the antibacterial agents viz., penicillin, amoxicillin, cefuroxin, tetracycline and erythromycin (Jarvinen *et al.,* 1993). Therefore relying on the natural antimicrobial agents that are effective and safe for host will turn to be the solution in future. In the present study an attempt has been made to enrich the knowledge of antimicrobial activity of four Indian traditional medicinal plants that have been screened for antistreptococci activity.

MATERIAL AND METHODS

Collection of Plants

Four medicinal plants such as neem, banyan, eucalyptus and clove were collected from in and around Gandhigram Rural University, Gandhigram, Dindigul, Tamil Nadu, India.

Preparation of Aqueous solution

The collected four plant materials were surface sterilized using distilled water and ground in mortar and pestle with sterile distilled water. Kirby- Bauer disc diffusion method was used (Cappucino and Natalie, 1999) for analyzing the biocidal activity of extracts. Susceptibility of the *Streptococcus mutans* to the extracts were analyzed by measuring the zone of inhibition formed after 24 hours of incubation.

Preparation of Acetone extracts

All the plant materials were powdered and kept soaked in acetone (1:4 w/v) for 24 hours and filtered through double layered muslin cloth. The extract was centrifuged at 5000 rpm for 10 minutes and the supernatant was used to assess the biocidal effect against *Streptococcus mutans*. The supernatant was kept at room temperature to evaporate the solvent (Kaushal Gautam *et al.,* 2003).

Preparation of test solution

100 mg of the each of the extracts was weighed and dissolved in 5ml of acetone, this was made upto 50ml using distilled water and using this as stock, various concentrations of solutions were prepared.

Isolation and identification of the bacterial isolate

Sterile cotton swabs were used for sample collection. Each swab was pressed in the infected area of the affected teeth and inoculated into the nutrient agar medium and incubated at 37 ± 2^0 C for 24 hours both in aerobic and anaerobic conditions. After the incubation period, the predominant isolate was picked and pure cultured for further analysis.

Identification of the isolate was carried out using various tests such as Gram staining, culturing on beef heart infusion agar, culturing on Mitis Salivarious agar, blood agar medium, sugar fermentation reactions and IMViC tests.

Antibacterial screening

Kirby- Bauer disc diffusion technique

Nutrient agar plates were swabbed with the culture broth evenly. The Whatmann filter paper discs of 6mm diameter were dipped in water extract for 20 minutes dried and was placed on the agar plates. The plates were incubated for 24 hours at 37^0 C. Similar procedure was followed for acetone extracts and the zone of inhibition was measured to decide the susceptibility or resistance of *Streptococcus mutans* to the extracts.

Plate dilution technique

The extracts in different concentrations (2000, 1000 and 500 ppm) were minced with Muller Hinton agar. The bacterial isolate was streaked on the plate and incubated for 24 hours

at 37°C. The change in growth pattern was observed by comparing with the control plate having 1ml acetone in Muller Hinton agar.

Phytochemical analysis

Four phytochemical tests such as Aurone's test, sulphuric acid test and Maule's test and saponin test were carried out to identify the families of chemical present in the extract that control the growth of *Streptococcus mutans.*

Synergistic effect of the plant extracts on bacterial isolate

Based on the performance of antagonistic activity of the bacterial isolate, the clove extract was selected and was mixed with the other plant extracts at the ratio of 1:1 and the zone of inhibition was observed.

RESULTS AND DISCUSSION

The experimental work indicates that the isolated bacterium is identified as *Streptococcus mutans* using various phenotypic and biochemical tests as given in table 1.

Antibacterial activity of plant extracts against *Streptococcus mutans*

The antibacterial activity of plant extracts against *Streptococcus mutans* in both aqueous and solvent extracts were evaluated. The acetone extract of *Syzyginm aromaticum* was the most active against *Streptococcus mutans.* It showed high degree of inhibition (22mm) followed by *Eucalyptus globules* extracts which exhibited significant inhibition (16mm) against the test bacterium. It was noted that *Streptococcus mutans* was less susceptible to the aqueous and acetone extract of *Ficus benhalensis* and *Azadirachta indica* compared to *Syzyginm aromaticum*. Measurable inhibitory action was observed in the aqueous extracts of *Syzyginm aromaticum* and *Eucalpyus globules*. The growth performance of the test bacterium at various concentrations of the acetone extracts shows inhibitory zone at the 1000ppm concentration of *Syzyginm aromaticum* and moderate growth at 500 and 1000ppm for *Eucalyptus globules* whereas growth performance of the test bacterium was good in almost all the concentrations of *Ficus benhalensis* and *Azadirachta indica.* (Table 2).

Analysis on the phytochemical composition of the plant extracts reveals the presence of various phytochemicals such as flavonoids, terpenoids, lignins and saponins etc.,. All the four group of compounds were present in *Syzyginm aromaticum.* The presence of these phytochemicals would have been implicated in the confernment of antimicrobial activities of *Syzyginm aromaticum* (Ingham, 1973).

Among the plants tested *Syzyginm*

Table 1. List of plants selected and their Vernacular name

S.No	Botanical name	Vernacular name	Family	Part used
1	*Azadirachta indica*	Neem	Meliaceae	Sticks
2	*Ficus benhalensis*	Banyan	Moraceae	Prop roots
3	*Eucalyptus globules*	Eucalyptus	Myrtaceae	Sticks
4	*Syzyginm aromaticum*	Clove	Myrtaceae	Buds

Table 1. Phenotypic and biochemical identification of the isolate

S.No	Test performed	Observation
1	Gram reaction	G +ve, cocci
2	Phenotype on Mitis Salivarius agar medium	+ve blue colored, small, irregularly margined, raised
3	Growth on a) 10°C b) 45°C	No growth1-89% +ve
4	Fermentation a) sorbitolb) mannitol and trehalose	+ve+ve with gas production
5	Blood agar medium	Gamma hydrolysis
6	Voges Proskauer	+ve

aromaticum was very promising and showed significant inhibition against the test bacterium. Based on the above mentioned data, *Syzyignm aromaticum* was selected for the synergistic activity evaluation. The comparative results on various combinations with *Syzyginm aromaticum* revealed a good synergism between *Syzyginm aromaticum* and *Eucalyptus globules* than when there were used individually (Table 3). The dried clove buds are being chewed to alleviate the pain of toothache and also widely used to disinfect root canals in temporary fillings and also as an oral anaesthetic (Zubaidah Haji and Hasnah, 2006).

Plants, as sources of medicinal compounds plays a dominant role in maintenance of human health since antiquities. Kerry, 2008 has reported the use of plants in the production of dentrifice and natural chewing gums for oral hygiene and to treat toothache, gingivitis and periodontal disease. Zubaidah and Hasnah (2006) performed a similar kind of work and reported that comparative effect of crude aqueous and solvent extract of clove on *Streptococcus mutans.* They observed both the extracts were found to reduce the synthesis of water insoluble glucan and both the extracts have an adhesion property due to the change on the cell surface of *Streptococcus mutans.* According to them crude clove extract has potential to influence the plaque inducing properties of *Streptococcus mutans* and

Table 2. Antibacterial activity of four Indian traditional medicinal plants against *Streptococcus mutans*

S. No	Name of the plant	Solvent used	Inhibition zone (mm)	Phytochemicals	Concentration of test solvent in parts per million (ppm)	Growth pattern
1	*Azadirachta indica*	Aqueous	R	Flavonoids	500	GG
		Acetone	8	Saponins	1000	GG
					2000	MG
2	*Ficus benhalensis*	Aqueous	R	Flavonoids	500	GG
		Acetone	14	Terpenoids	1000	MG
					2000	MG
3	*Eucalyptus globules*	Aqueous	14	Terpenoids	500	MG
		Acetone	16	Lignins	1000	MG
				Saponins	2000	PG
4	*Syzyginm aromaticum*	Aqueous	18	Flavonoids	500	MG
		Acetone	22	Terpenoids	1000	PG
				LigninsSaponins	2000	PG

R- Growth of organisms is not inhibited GG- Good Growth MG- Moderate Growth
PG- Poor Growth

Table 3. Synergistic effect of plant extract against *Streptococcus mutans*

S. No.	Mixed plant extracts(1:1)	Zone of inhibition in diameter on *Streptococcus mutans* (in mm)
1	Control	R
2	Clove + Banyan	20
3	Clove + Eucalyptus	30
4	Clove + Neem	18

R- Growth of organisms is not inhibited

subsequently affect to the caries inducing properties of bacterium. The factors that confer the antistreptococcal property of *Syzyginm aromaticum* may be due to the presence of 14-20% essential oil, (Zubaidah and Hasnah, 2006), antiadhesion of eugenol etc,. Results of the present study have implicated the buds of *Syzyginm aromaticum* as a potential candidate in such application.

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