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Screening of Antibiotic Resistant Inhibitors from Indian Traditional Medicinal Plants Against *Streptococcus mutans*

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Abstract: Aqueous and ethanolic extracts of ten plants used in traditional Indian medicine for treatment of an infectious nature were screened for antibacterial activity by disc diffusion method against *Streptococcus mutans*. Among them the ethanol extracts of *Allium sativum* bulb exhibited high degree of inhibition followed by *Azadirachta indica* leaf extracts. The aqueous extracts of plants did not show any antibacterial activity against *Streptococcus mutans*.

Key words: Antibacterial activity, *Streptococcus mutans*, traditional medicine, high degree of inhibition, dental caries

INTRODUCTION

Human infections caused by Gram-positive bacterial pathogens are increasingly difficult to treat, predominantly due to emergence of drug resistant bacterial strains. One such Gram-positive bacterial organism is *Streptococcus mutans* causing dental caries in humans (Hamada and Slade, 1980; Akhtar and Bhakuni, 2004). This bacterium adheres firmly to the smooth tooth surfaces and produces sticky water insoluble dextran from dietary sucrose, forming plaque which facilitates the accumulation of microorganisms. *Streptococcus mutans* and other organisms in the plaque produce organic acids such as lactic acid that gradually destroy the enamel and form a cavity (Bhattacharya *et al*., 2003). Elimination of *Streptococcus mutans* would reduce the incidence of dental caries. Extensive efforts have been made to find an active agent against 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; Bhattacharya *et al*., 2003). In addition they may lead to side effects including gastrointestinal problems (Craig, 1998). These drawbacks justifies further research and development of natural antimicrobial agents that are effective and safe for the host.

It has been well documented that traditional medicinal plants confer considerable antibacterial activity against various microorganisms (Jonathan *et al*., 2000). Many plants were reported to inhibit the growth of many oral bacteria (Pack *et al*., 1998), particularly *Streptococcus mutans* and control plague and thus prevent caries have been investigated (Jagtap and Karkera, 2000; Margan *et al*., 2001). Use of plant based alternatives for oral health has been successfully promoted for example, the use of antibacterial chewsticks has been widely advocated by health agencies where their use is culturally acceptable (Almas, 1999; Jagtap and Karkera, 2000). In the present study an attempt has been made to enrich the knowledge of antibacterial activity of ten Indian traditional medicinal plants that have been screened for antistreptococci activity.

MATERIALS AND METHODS

Ethnobotany and Plant Collection

The ethnobotanical survey was conducted in Shevaroy and Kolli hills of Eastern Ghats of Tamil Nadu (Fig. 1), South India from January to July 2000. The comparison between medicinal and

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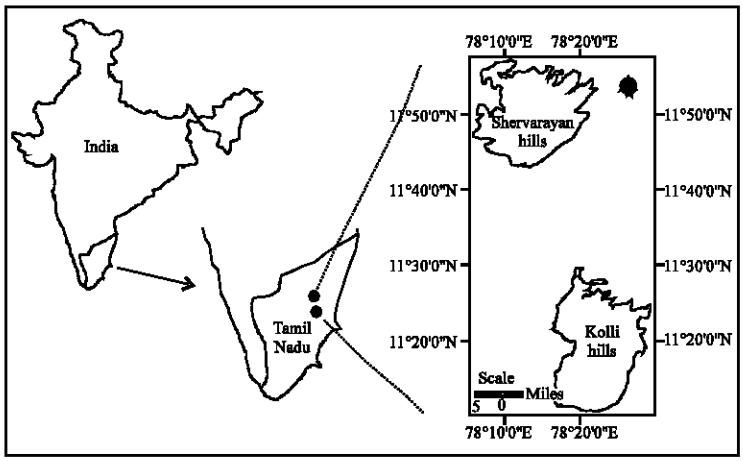

Fig. 1: Location map of Kolli and Shevaroy hills

non-medicinal plants is based on research in the community namely Malayalis of the same areas. Based on their information and literature we have collected 12 medicinal plants for antibacterial activity against *Streptococcus mutans*.

Extraction of Plant Materials

The collected plant materials were separated into leaf, root, bulb and bark and shade dried at 37°C for 7 days. The extracts were prepared using the solvents viz., water and ethanol. Dried plant materials (20 g) were extracted with 100 mL of water and ethanol for 72 h. Each mixture was stirred every 24 h using a sterile glass rod. At the end of fraction each extract was passed through Whatman No.1 filter paper. The filtrate obtained was concentrated in vacuo using rotary evaporator.

Test Bacteria

The test bacterium *Streptococcus mutans* was collected from Clinical Microbiology Laboratory, Sea Horse Hospital, Tiruchirappalli. Brain Heart Infusion (BHI, Difco) was used for the culture of *Streptococcus mutans*.

Antibacterial Assay

The disc diffusion assay of Iennette (1985) as described by Rosanaivo and Ratsimananga-Urverg (1993) and Rabe and Van Staden (1997) were used with modification to determine the growth rates ob bacterium with plants extracts. A diluted bacterial cultures (200 µL) was spread over Muller Hinton Agar Plates using sterile glass L-rod. Two hundred microliter of each extract was applied per filter paper disc (Whatman No.1, 6 mm diameter) and was allowed to dry before placing them on the agar plates in petriplates. Each extract was tasted in triplicate (3 discs/plate) and the plates were incubated at 37°C for 24 h and size of the growth zones were recorded. Calculations were carried out in triplicate with their mean values and standard deviation by using formula given by Gupta (1977).

RESULTS AND DISCUSSION

The ethanolic bulb extract of *Allium sativum* was the most active against *Streptococcus mutans*. It showed high degree of inhibition (25.6 mm) followed by *Azadirachta indica* leaf extracts exhibited significant inhibition (24.4 mm) against the test bacterium. The ethanolic extracts of *Embelia ribes* fruit

Table 1: Antibacterial activity of ten Indian Traditional medicinal plants

Family and Botanical name	Local name	Parts used	Anti-infective uses	Location/ Tribals	Solvent used	Inhibition zone Mean±SD
Liliaceae	Venkaya Poondu	Bulb	Antiseptic	Kolli Hills,	Aqueous	-
Allium sativum				Malayalis	Ethanol	25.6±0
Meliaceae	Veppam	Leaves	Skin infections,	Kolli Hills,	Aqueous	-
Azadirachta indica			oral infections	Malayalis	Ethanol	24.4±0
Myrsinaceae	Vizhalari	Fruits	Antiseptic	Shevaroy hills,	Aqueous	-
Embelia ribes				Malayalis	Ethanol	23.5±0
Papavaraceae	Bramma Thundu	Leaves	Skin infections	Shevaroy hills,	Aqueous	-
Argemone maxicana				Malayalis	Ethanol	22.7±0
Rubiaceae	Ezhumitchai	Roots	Asthma, Tumours,	Shevaroy hills,	Aqueous	-
Citrus medica			Skin infections	Malayalis	Ethanol	22.0±0
Mimosaceae	Shivakaay	Bark	Skin diseases	Kolli Hills,	Aqueous	-
Acacia nilotica			and Scabies	Malayalis	Ethanol	22.3±0
Asclepiadaceae	Erikku	Boiled leaves	Palalyses, Tumours	Kolli Hills,	Aqueous	-
Calotropis gigantica				Malayalis	Ethanol	22.0±0
Alangiaceae	Alangi	Leaf	Dog bite, fever,	Kolli Hills,	Aqueous	-
Alangium salviifolium			skin diseases	Malayalis	Ethanol	22.3±0
Lamiaceae	Tuzhasi	Leaf	Skin diseases,	Kolli Hills,	Aqueous	-
Ocimum sanctum			Dental caries	Malayalis	Ethanol	-
Menispermaceae	Chenthil Kodi	Leaf	Dyspepsia, Skin	Shevaroy hills,	Aqueous	-
Tinospora cordifolia			diseases, fever	Malayalis	Ethanol	-

showed moderate inhibition (23.5 mm). It was noted that the *Streptococcus mutans* was less susceptible to the extracts of *Citrus medica* roots (22 mm), *Acacia nilotica* barks (22.3 mm) and *Calotropis gigantica* leaves whereas the leaf residues of *Ocimum sanctum* and *Tinospora cordifotia* did not show any antibacterial effect on *Streptococcus mutans*. Complete absence of measurable inhibitory action was observed in the aqueous extracts of ten Indian Traditional medicinal plants (Table 1).

Among the plants tested *Allium sativum* was very promising and showed significant inhibition against the test bacterium. Numerous workers (Willis, 1956; Mantis *et al*., 1979; Jain, 1994) have obtained that garlic juice exhibits strong antibacterial properties and due to these properties garlic juice is very effective against various diseases. It is postulated that the antibacterial properties of garlic juice are due to the inhibition of succinic dehydrogenase via the inactivation of this groups (Srinivasan *et al*., 2001).

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