The study on the efficacy of some herbal extracts for the control of dental caries pathogen-Streptococcus mutans

Prajapati R. A.#1. and Raol B. V. #2

#1 Department of Biotechnology, Smt. S.S.P Nootan Science and Commerce college, Visnagar, District-Mehsana, Gujarat-384315, Contact: 989839935,

#2 Department of Microbiology, Shri P.H.G. Muni. Arts and Science College, Kalol District: Gandhinagar, Gujarat-382721 Contact: 9898855432,

ABSTRACT

Streptococcus mutans plays leading role in development of dental caries-a most common chronic infectious disease in the human race. The need of the time is to execute more and more screening of herbal plant products and extracts for their pharmacological studies. A systematic investigation should be undertaken to screen the antimicrobial potentiality of herbal plant and their products against S.mutans. It was carried out using 20 herbal extracts prepared with water, methanol, acetone and petroleum ether. The antimicrobial effect of each types of extract was determined by agar well diffusion method against the standard strain (S.mutans MTCC 890) and clinically isolated strain.

Out of four types of extracts of twenty herbs, a S.mutans MTCC 890 strain showed sensitivity towards aqueous extract of 5 herbs (25%), methanol extract of 8 herbs (40%), acetone extract of 6 herbs (30%) and petroleum ether extract of 4 herbs (20%). Whereas the clinical isolate was remarkably inhibited by aqueous extract of 5 herbs (25%), methanolic extract of 8 herbs (40%), acetone extract of 6 herbs (30%) and petroleum ether extract of 3 herbs (15%).

An investigation on natural products to cure diseases may create an alternative source of promising medicines. This study may open the possibilities of finding new clinically effective herbal remedy for dental caries.

Key words: Dental caries, Streptococcus mutans, Herbal extracts,

Corresponding Author: Prajapati R. A.
INTRODUCTION

Dental caries is one of the most common chronic infectious diseases in the world \cite{1,2} A very numerous and various microbial flora play main role in the etiology of the caries but \textit{Streptococcus mutans} (\textit{S.mutans}) plays leading role in caries formation. It produces an enzyme dextran-sucrase, which converts the sucrose of food to dextrin polymer, and dextrin then combines with salivary proteins to create a sticky, colorless film (plaque) on tooth surfaces. The plaque provides the asylum for the activities of Lactobacilli to produce lactic acid, which dissolves the enamel by decalcifying it. Hypothetically, the control of dental caries is hidden in the inhibition of each step in the process of caries formation \cite{3}. The use of herbal plant as medicine is an ancient practice common to all societies. Such herbal plants are used medicinally worldwide and supply many potential and quality drugs. Herbal plants represent a rich source of antimicrobial agent. A wide range of herbal plant part extracts are used as pink drugs as they show medicinal significance. A systematic investigation was undertaken to screen the antimicrobial potentiality of herbal plants and their products against \textit{S.mutans}.

MATERIALS AND METHODS

Collection of plant material

The twenty herbal plant materials was collected locally from either botanical garden, farm or from pharmaceutical store (Table-1). Authentication was made by Dr. Y. M. Patel, Professor, Department of Botany, M. N. Science College, Visnagar, Gujarat, India.

Table-1. List of the selected herbal plant for the study

<table>
<thead>
<tr>
<th>Herbal plant</th>
<th>Vernacular name</th>
<th>Part</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Acacia arabica} L.</td>
<td>Babul</td>
<td>Twig</td>
<td>Farm</td>
</tr>
<tr>
<td>\textit{Acacia catechu} L.</td>
<td>Kheir</td>
<td>Bark powder</td>
<td>Local Market</td>
</tr>
<tr>
<td>\textit{Allium cepa} L.</td>
<td>Onian</td>
<td>Leaves</td>
<td>Farm</td>
</tr>
<tr>
<td>\textit{Allium sativum} L.</td>
<td>Garlic</td>
<td>Rhizome</td>
<td>Farm</td>
</tr>
<tr>
<td>\textit{Azadirachta indica} L.</td>
<td>Neem</td>
<td>Twig</td>
<td>Farm</td>
</tr>
<tr>
<td>\textit{Camellia sinensis} L.</td>
<td>Tea</td>
<td>Leaf</td>
<td>Local Market</td>
</tr>
<tr>
<td>\textit{Cinnamomum}</td>
<td>Taj</td>
<td>Bark</td>
<td>Local Market</td>
</tr>
<tr>
<td>\textit{Cinnamomum tamala} L.</td>
<td>Tamal patra</td>
<td>Leaves</td>
<td>Local Market</td>
</tr>
<tr>
<td>\textit{Curcumae longae} L.</td>
<td>Turmeric</td>
<td>Rhizome</td>
<td>Local Market</td>
</tr>
<tr>
<td>\textit{Emblica officinalis} L.</td>
<td>Amla</td>
<td>Fruits</td>
<td>Farm</td>
</tr>
<tr>
<td>\textit{Foeniculum vulgare} M.</td>
<td>Fennel</td>
<td>Seed</td>
<td>Farm</td>
</tr>
<tr>
<td>\textit{Glycyrrhiza glab} L.</td>
<td>Jethimadh</td>
<td>Rhizome</td>
<td>Local Market</td>
</tr>
<tr>
<td>\textit{Juglans regia} L.</td>
<td>Akhrot</td>
<td>Fruit</td>
<td>Local Market</td>
</tr>
<tr>
<td>\textit{Myristica fragrans} L.</td>
<td>Jayfal</td>
<td>Fruit</td>
<td>Local Market</td>
</tr>
<tr>
<td>\textit{Ocimum sanctum} L.</td>
<td>Tulsi</td>
<td>Leaves</td>
<td>Farm</td>
</tr>
<tr>
<td>\textit{Piper nigrum} L.</td>
<td>Kalamari</td>
<td>Beads</td>
<td>Local Market</td>
</tr>
<tr>
<td>\textit{Punica granatum} L.</td>
<td>Dadam</td>
<td>Seed</td>
<td>Farm</td>
</tr>
<tr>
<td>\textit{Syzygium aromaticum} L.</td>
<td>Cloves</td>
<td>Buds</td>
<td>Local Market</td>
</tr>
<tr>
<td>\textit{Triphala}</td>
<td>Trifala</td>
<td>Fruits</td>
<td>Pharmacy</td>
</tr>
<tr>
<td>\textit{Zingiber officinale} L.</td>
<td>Ginger</td>
<td>Rhizome</td>
<td>Farm</td>
</tr>
</tbody>
</table>
The samples were carefully washed under running tap water followed by two rinse of sterile distilled water. Readymade powders utilize directly. The materials were dried at room temperature (30°C) up to 2 weeks before extract preparation. They were cut into pieces with the help of scissors/knife and ground using a sterile electric herb grinding jar into fine texture powder/paste form and stored in air-tight bottles.

**Preparation of Extract:**

Aqueous extract: 50 grams of powdered/paste plant material was extracted with 200 ml cold sterile distilled water by maceration. The flasks were placed on rotary shaker at room temperature for 48 hours. Organic solvent extract viz. Methanol, Acetone and Petroleum ether (Merck, India). The same amount of pulverized plant material was extracted using Soxhlet extractor (Glassco, India) for 48 hrs with above mentioned solvents (Merck,) solvents. Each preparation was filtered through a sterile Whatman filter paper (Sigma-Aldrich, India) and the filtered extract was concentrated by keeping them in a hot air oven for 3-5 days at 40°C to completely evaporate the solvent and got in a crystal/powder/paste form. The obtained dried extracts were exposed to UV light for overnight and checked for sterility over the nutrient agar plates and stored in labeled sterile bottles in a freezer at 4°C until further use. The Stock crystal/powder/paste of different extracts were dissolved in 50% of Dimethyl Sulfoxide (DMSO) (Merck, India) to achieve 20 mg/ml concentration of each extract for further processing.

**Growth and Maintenance of S. mutans strains.**

A clinical strain of *S.mutans* was isolated from dental plaque sample of 12 years old school child who was significantly suffering from dental caries. The biochemical characterization had done on an automated microbial identification system -VITEK 2 (BioMérieux Canada, Inc.)\(^4\) followed by 16s rRNA sequencing (ABI 3500 XL Genetic Analyzer, USA). The standard reference strain of *S.mutans* (MTCC 890) was procured from MTCC, Chandigarh, India. For both the cultures were maintained on Mitis salivary bacitracin (MSB) agar medium and stored at 4 °C in refrigerator in laboratory. The purity of the cultures was checked periodically by colony morphology and Gram staining. The inoculums of test strains were adjusted to 1.5 x 10\(^8\) CFU/ml equal to that of the 0.5 McFarland standard by adding sterile distilled water.

**Antimicrobial sensitivity test**

The antimicrobial sensitivity of the test strains to 20 herbal extract was determined by agar well diffusion method [5]. 20 ml of Muller Hinton agar melted and cooled at 45 °C was poured into sterile petriplates and allowed to solidify completely. A lawn of both strains prepared by evenly spreading 100 µl inoculums with the help of a sterilized spreader onto the entire surface of agar plate. The 8 mm well were made within agar medium using stainless steel cup borer. A 100 µl each different extract was introduced into the wells. All the agar plates were incubated at 37 °C. The response of antimicrobial activity was measured by a zone of inhibition in millimeter after 24 hours using a scale. The experiments were conducted in triplicate for each extract against both the strains. The diameter of inhibition zones was measured. The mean value and standard deviation were calculated.
RESULT:

The aim of this investigation was to study the inhibition of *S. mutans* by the action of some herbal extracts. It carried out on clinically isolated strain and standard strain i.e. *S. mutans* MTCC 890.

The responses of 20 herbal extract were determined in terms of diameter (in mm) of inhibition zone. The average zone diameter of each type of extract against both strains with standard deviation are shown in Figure-1,2,3 and 4. An extract was interpreted as highly susceptible if the diameter of inhibition zone was more than 18 mm and resistant if zone size was less than 13 mm. Otherwise considered as intermediate response [6]. According to the above cited criteria, the evaluation of the 20 herbal plant extract efficacy for both the type of strain is shown in table-2. The results showed that minimum 3 and maximum 8 herbal extracts showed remarkable antimicrobial activity on both the type of *S. mutans*.

Table-2. Number of plants (percentage) showing the sensitivity against the both strains

<table>
<thead>
<tr>
<th>Response (Inhibition zone)</th>
<th>S. mutans -Clinical isolate</th>
<th>S. mutans MTCC 890</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aqueous</td>
<td>Methanol</td>
</tr>
<tr>
<td><strong>Sensitive</strong> (&lt;18 mm)</td>
<td>05 (25%)</td>
<td>08 (40%)</td>
</tr>
<tr>
<td><strong>Moderate</strong> (18 to 13 mm)</td>
<td>07 (35%)</td>
<td>05 (25%)</td>
</tr>
<tr>
<td><strong>Resistant</strong> (&lt;13 mm)</td>
<td>08 (40%)</td>
<td>07 (35%)</td>
</tr>
</tbody>
</table>

Figure. Effect of aq. extracts on *S. mutans* strains

![Effect of Aqueous Extracts on *S. mutans*](image)

Figure: 2 Effect of methanolic extracts on *S. mutans* strains
Figure: 3 Effect of acetonic extracts on *S. mutans* strains

Figure: 4 Effect of Petroleum ether extracts on *S. mutans* strains
DISCUSSION

The oral cavity is a habitat for temporary and permanent microorganisms. Some of these microorganisms play a main role in the development of the dental caries. The occurrence of dental caries in industrialized countries like India is increasing day by day [7]. To cope up this situation, a good level of dental caries treatment is required and may be costly. To keep the treatment economically feasible, the prevention should be done own selves at the doorstep level using herbal remedy, is the solution of choice. Chopra [8] has described that many plants being used in prevention of dental caries.

However, many efforts have been done to control dental caries Though, Natural products have been used for thousands of years in folk medicine for several purposes, but there were no practical use up to the present. As most of the oral diseases are due to bacterial infections and it has been well documented that medicinal plants confer considerable antibacterial activity against various microorganisms including bacteria responsible for dental caries [9]. In India herbal therapy is greatly exploited for its therapeutic potential and medicinal efficacy to cure dental caries.

A. cepa (Onion) and A. sativum (Garlic) are the common part of Indian food. A limited number of studies have investigated the antibacterial effects of onion and garlic extract against S.mutans. One previous study has reported the significant effect of 10% garlic solution in decreasing levels of oral microorganisms [10]. S.mutans were responsive to garlic extract with the minimum inhibition concentration ranging from 4 to 32 µg/ml [11] and our results were in total agreement with this study

Both, Acacia arabica (Babul) and A.indiaca (Neem) chew sticks have been used for dental problems since hundred years [12] and their extract can reduce the ability of some Streptococci to colonize tooth surfaces [13]. Ethanol extract of Acacia bark showed 22.3 mm inhibition zone against S.mutans [14] and aqueous extracts of chewing stick were effective at 50% concentration on S.mutans [15]. In earlier studies, the profound effect of chew sticks have been reported on the growth of S.mutans and at this juncture all types of the extracts of both plants also showed identical response.
The bark of the A. catechu commonly known as Khair, is used in traditional medicine and possesses antimicrobial potency. The ethanolic leaf extract displayed antibacterial activity against S. mutans and S. mitis with MIC of 62μg/ml and 5mg/ml respectively [16]. Here, S. mutans was inhibited with 2% extracts of all type and opted best zone (> 18 mm) with methanolic extract.

C. tamala belongs to family Lauraceae. The presence of certain volatile oil components such as cinnamic aldehyde and eugenol provide them antibacterial potency [17]. S. mutans MTCC 890 was inhibited against all the extracts of C. tamala and Cinnamomum bark extract with moderate to higher zone of inhibition.

Since C. sinensis (Tea) is consumed regularly in the aqueous form. In this study, the effect of aqueous and organic extracts of tea on S. mutans growth was examined and found the 2% pure methanolic extracts of tea could restrict the growth of S. mutans. As a pilot study conducted earlier showed no effect on S. mutans growth with 1% and 2% crude aqueous extracts of black tea [3].

C. longa (Turmeric) has been evaluated for cariogenic properties against S. mutans. Its essential oil could inhibit the growth and acid production of S. mutans at concentrations from 0.5 to 4 mg/mL [18]. The zone of inhibition of aqueous extracts and organic extracts were obtained in the range between 11 mm and 18 mm.

E. officinalis is also named as Amla, or Indian gooseberry. It has various medicinal applications all aqueous and organic extracts of amla presented antimicrobial activity against S. mutans with the mean diameter of the highest zone of inhibition being 18.96 mm and an MIC of 50mg/ml in the previous study [19] and here 9 mm to 11 mm zone of inhibition were produced with 20 mg/ml of all extracts.

Antibacterial activity of F. vulgare Mill (fennel) seed essential oil against the growth of S. mutans was at the concentrations higher than 80 ppm. [20] but the aqueous and organic extracts provided poor response even at 2% concentration.

The extracts of the roots of G. glabra (Jethimadh) has shown magnificent antibacterial effect activity against the S. mutans [21]. All the extracts of J. regia were tested and exhibited a non trivial inhibitory effect on the growth of S. mutans. Antimicrobial activities of J. regia (Akhrot) bark extract against S. mutans have already been studied [22]. The results showed that akhrot extract exhibited a significant inhibitory effect only on the growth of S. mutans at a concentration of 1 mM. In present effort, the zones of inhibition of all extracts were obtained in the range between 15 mm and 21 mm.

The methanolic extract of M. fragrans had anti-plaque action [23]. Mean MIC values of ethanol extracts from mace of M. fragrans was 20 mg/ml and alike responses were acquire with all the extracts of M. fragrans against S. mutans.

Ocimum sanctum (Tulsi) is a time-tested leading medicinal herb and used in Ayurvedic medicine since early times and at the 4% concentration of Tulsi extract, was obtained Tulsi extract demonstrated an antimicrobial activity against S. mutans. It has the maximum antimicrobial potential at the 4% concentration level on the salivary streptococci levels and led a zone of inhibition of 22 mm [24] but O. sanctum in our study, worked at 2% concentration and gave the zone of inhibition in the range of 16 to 27 mm against S. mutans.
*P. nigrum* (Black pepper) has long been considered as a spice as well as a medicinal plant too. Its antimicrobial property has been tested against many common pathogens\(^{[25]}\) but not against *S. mutans* so far. *P. granatum* (Daadam) seed extract kills microorganisms isolated from the dental plaque of healthy adults. The hexane extract showed an antibacterial activity against *S. mutans* and may be a possible alternative for the treatment of dental plaque bacteria\(^{[26]}\). Here, all types of extracts of *P. nigrum* and *P. granatum* showed their potential antimicrobial activity with improved degree of inhibition among all the herbs tested in present study.

*S. aromaticum* belongs to the family Myrtaceae and its dried aromatic unopened floral buds are known as cloves. Its oil emerged as the potent anti-caries agent and exhibited superior antibacterial activity than the ciprofloxacin drugs\(^{[27]}\). The variety of bud extracts of clove also possess same strength of inhibition on *S. mutans*.

*Triphala* is the equal mixture of three dried fruits of medicinal plants *Terminalia chebula*, *Terminalia belerica* and *Phyllanthus embelica*. It may be effective agent to treat patients with dental caries. Biradar *et al.*\(^{[28]}\) reported that 6% triphala in a mouthwash formulation showed a 44% reduction of the salivary streptococci levels within 7 days. In our study, all types of extracts showed the greater degree of inhibition of *S. mutans*. Thus triphala was proven as an effective agent to Figureht against dental caries in the near future.

*Z. officinale* (Ginger) is used worldwide as a cooking spice, condiment and herbal remedy. Giriraju *et al.*\(^{[29]}\) examined that the 10% rhizome extract of ginger inhibits *S. mutans* and produced 8.0 mm zone by 1.25% ethanolic extract act. In present study 2.0 % acetonic extracts produce maximum 12.7 ± 1.2 mm size zones and our results were in accordance with this study.

**CONCLUSION**

From the above study and discussion, It was concluded that many of the studied herbal extracts have potential antimicrobial action against cariogenic pathogen. Such investigation on natural products to cure diseases may create an alternative source of promising medicines. This study might open the possibilities of finding new clinically effective herbal remedy for dental caries.

**REFERENCES**


