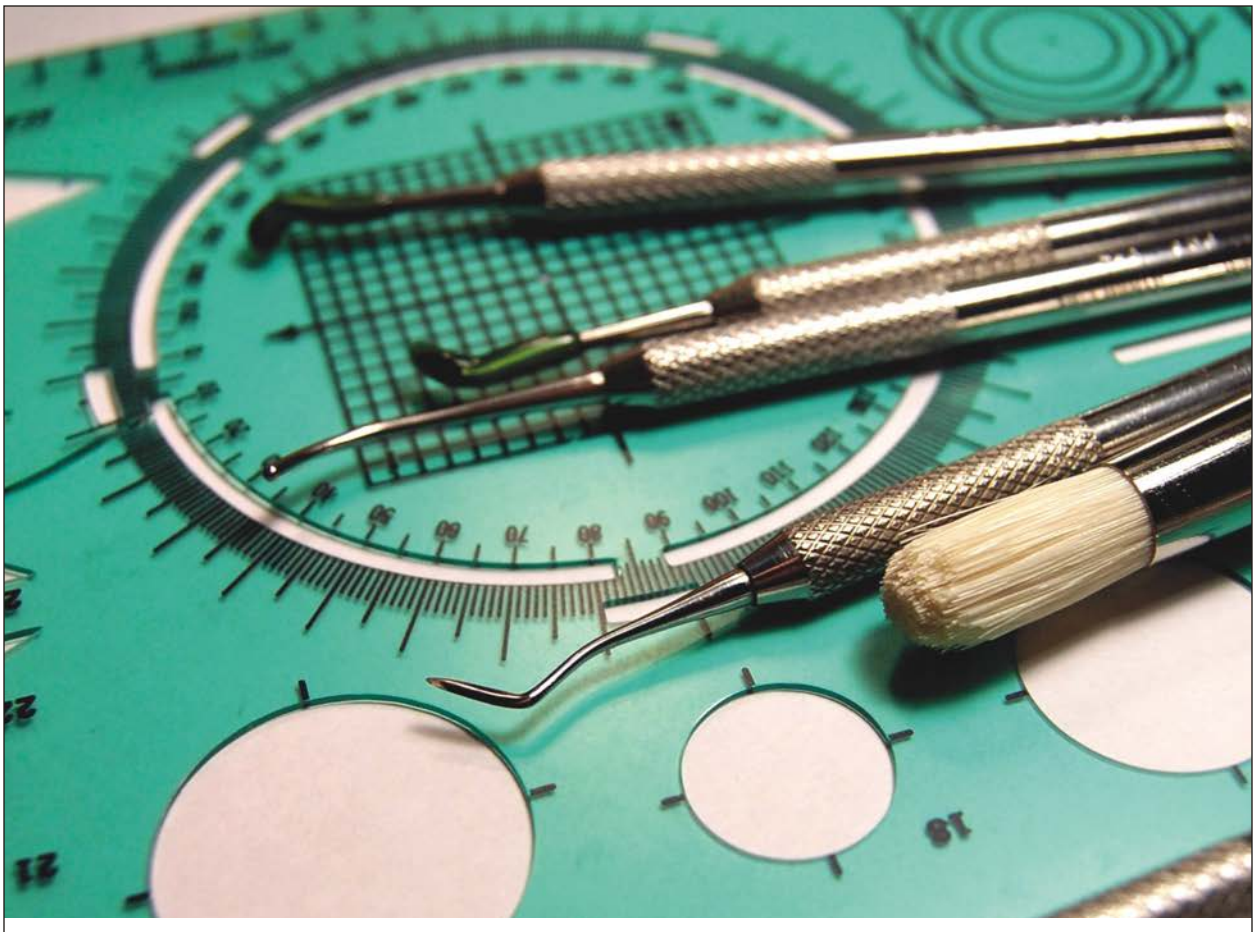

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# Research Article
# Anticariogenic Activity of Some Indian Traditional Medicinal Plants

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## Abstract

**Background and Objective:** Today, dental caries is still one of the most common diseases in the world. The causes of tooth decay in most cases are the poor oral hygiene and high sugar consumption. Despite several dental caries agents being available in the market, the search for an effective agent still continues. Several undesirable side effects associated with these agents stimulated the search for alternate agents. The objective of this study was than to investigate the inhibitory effect of the crude seed extracts from some traditional medicinal plants on anticariogenic organisms under *in vitro* condition for their use for controlling dental caries. Attempt was also made to characterize bioactive compound at primary level. **Materials and Methods:** The anticariogenic activity was assessed by agar well diffusion method. MIC against six dental microorganisms and the radial zone of inhibition was measured. Phytochemical evaluation of all the selected plants showed good MIC values and TLC-Bioautography. The data was statistically analyzed by one way analysis of variance (ANOVA) using SPSS ver. 16.0. **Results:** The results revealed that the hexane extract of plant seeds has more potent anticariogenic activities against oral microorganisms. Maximum zone of inhibition (18 mm) was found when seed extracts of *Mimusops elengi* L. tested against *Candida albicans*. The MIC of hexane extract of *Mimusops elengi* L. and *Punica granatum* L. seed against CA and SP was 0.075 and 0.3, respectively. The MIC value of 0.3 was found for SP using *Punica granatum* L. The result of bioautography showed that the compound was active against *Candida albicans* and *Streptococcus pyogenes*. **Conclusion:** Hence, the study concludes that the very good activity of methanol extract of seed of *Mimusops elengi* L. and *Punica granatum* L. will be useful in the future development of effective toothpaste or mouth washer against oral microorganisms.

Key words: Anticariogenic activity, dental caries, dental organisms, seed, HPTLC, bioautography, phytochemistry

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Dental caries are still one of the most common diseases in the world. The link between oral diseases and the activities of microbial species that form part of the microbiota of the oral cavity is well established[1]. Over 750 species of bacteria inhabit the oral cavity (~50% of which are yet to be identified) and a number of these are implicated in oral diseases. The dental caries development of the involves acidogenic and aciduric Gram-positive bacteria. The primarily dental caries present the mutans streptococci (*Streptococcus mutans* and *S. sobrinus*, lactobacilli and actinomycetes). The metabolize sucrose to organic acids (mainly lactic acid) that dissolve the calcium phosphate in teeth, causing decalcification and eventual decay. Dental caries is thus a supragingival condition[2]. In contrast, periodontal diseases are subgingival conditions that have been linked to anaerobic Gram-negative bacteria such as *Porphyromonas gingivalis*, *Actinobacillus* sp., *Prevotella* sp. and *Fusobacterium* sp. In dental diseases, the areas at or below the gingival crevice become infected caused by cellular inflammatory response of the gingival and surrounding connective tissue[2]. Dental caries, a localized, progressive decay of the teeth, results from colonization of the vulnerable surfaces of the teeth by a characteristic group of bacterial ions diseases. The control of dental caries present one of challenges that must be met today by dental profession that treatments of disease has been overemphasized and prevention minimized[3].

*Streptococcus mutans* is the most destructive bacterial strain in the mouth as it attaches easily to teeth and produces a lot of acid. Other common but less destructive acid-producing bacteria are lactobacillus and actinomyces. After all the sugars are consumed by the bacteria, acid production eventually stops and the tooth has a chance to repair itself (remineralisation) helped by the minerals of saliva and toothpaste's fluoride. If dental plaque is not removed regularly, or if sugar is consumed too often, then the demineralization periods are not enough to repair the damage. Eventually a small cavity appears on the tooth enamel. The continuous exposure of the tooth to acids is what causes tooth decay. Tooth decay can then penetrate through the protective enamel down to the softer, vulnerable dentine and continue to the soft tooth pulp and the sensitive nerves within it. Although, the metabolic activity of plaque bacteria in our mouth is what actually causes dental caries, the underlying causes of tooth decay are in most cases the poor oral hygiene and high sugar consumption[3]. *Streptococcus mutans* utilized the sucrose and produce the gummy, extracellular, dextran-based polysaccharide that allows them to bind each other forming bacterial film. Dextran produces by the *S. mutans* via the enzyme dextran sucrase using sucrose use as a substrate.

Many modern drugs have been isolated from natural sources based on their use in traditional medicine. Many medicinal plants have been used for the treatment of the controlling and curing the disease through out the world. Several plant phytochemical constitutes have been evaluated with respect to their antimicrobial efficacy against pathogenic bacteria. Many researchers reported the biological activities of plants and their natural product derivatives. The antimicrobial activity of the oil and the major components was tested against oral pathogenic microorganism's species. The most sensitive microorganisms were *Candida albicans* and *Streptococcus mutans*. Thus, it's likely usefulness to combat oral microbial growth. Essential oils such as *Eucalyptus globules* and their derivatives of some plants are effective against dental caries. Devi *et al.*[4] reported the 62 plant species of different plants part are useful in dental caries.

The objective of this study was then to investigate the inhibitory effect of the crude extracts from some traditional medicinal plants on anticariogenic organisms under in vitro condition. The reason for this is to provide a scientific validity for their use for controlling dental caries. There is a less information regarding bioactivity of seed extracts against cariogenic microorganisms. The seed extracts were tested for their effect on anticariogenic organisms under *in vitro* condition. Attempt was also made to characterize bioactive compounds at primary level.

## MATERIAL AND METHODS

**Plant materials:** The different plant species were selected and collected between November to December, 2016 form different part of Gujarat and surroundings of Vallabh Vidyanagar (Table 1). The seeds of healthy and disease free plants were used to test the anticariogenic activity. The plant specimens were identified by Dr. Kalpesh Ishnava (Plant Taxonomist) at Ashok and Rita Patel Institute of Integrated Study and Research in Biotechnology and Allied Sciences (ARIBAS), New Vallabh Vidyanagar, Gujarat, India. All the chemicals used the analytical grade in the experiment.

**A preparation of seed extracts:** First of all the seeds of respective plants were thoroughly washed with running tap water, blotted and dried under sunlight and transferred immediately to the laboratory. After drying the plant materials were used for experiments. The shade dried plant material was powdered with the help of mixer grinder (Maharaja Mixer Ltd). The fine particles were separated and stored in clean container. The fine powered used for further analysis.

Table 1: Details of selected plant seeds against cariogenic microorganisms

| Botanical names | Family | Local Name | Collection site |
|---|---|---|---|
| *Acacia nilotica* subsp *indica* (Benth.) | Mimosaceae | Baval | V. V. Nager |
| *Achyranthus aspera* L. | Amaranthaceae | Adhedi | Karamsad |
| *Cassia occidentalis* L. | Caesalpiniaceae | Kasidro | V. V. Nager |
| *Mimusops elengi* L. | Sapotaceae | Borsali | New V.V. Ngar |
| *Moringa oleifera* Lam. | Moringaceae | Sargavo | Gana |
| *Mucuna pruriens* Baker. | Fabaceae | Kocha | Surkuva |
| *Pongamina pinnata* (L.) Pierre | Fabaceae | Karaj | V.V.Nagar |
| *Psoralea corylifolia* L. | Fabaceae | Bavachi | Anand |
| *Punica granatum* L. | Puniaceae | Dadam | V. V. Nager |
| *Sesamum iundicum* L. | Pedialiaceae | Kalatal | V. V.Nager. |
| *Simmodisa chinesdis* (Link) C.K. Schneider | Simmondsiaceae | Jojoba | AAU, Anand |

Soxhlet apparatus was fixed in suitable protective place. The water flow was regularly checked. The solvent chloroform was taken in Soxhlet flask and warmed up at suitable temperature. The seeds powder was 25 g weighed and filled in the Soxhlet extraction tube. The process was continued for 4 hrs after the extract was prepared. The extract was filtered with the help of Whatman Filter paper No. 1. The filtrate was collected in petridish and dried at room temperature. The dried extract from petridish was scraped and transferred to eppendorf tube. The same procedure used the different solvent like methanol, ethyl acetate, hexane and distilled water respectively.

**Oral pathogenic strains:** A group of bacteria known to cause tooth decay were selected and purchased from Microbial Type Culture Collection bank, Chandigarh as a freeze dried pure culture. The bacterial cultures were revived by using MTCC specified selective growth medium and preserved as glycerol stocks. The bacteria responsible for dental caries used the *Candida albicans* (CA) (MTCC-186), *Lactobacillus acidophilus* (LA) (MTCC-*447), *Lactobacillus casei* (LC) (MTCC-1423), *Streptococcus mitis* (SM) (MTCC-2696), *Staphylococcus aureus* (SA) (MTCC-96) and *Streptococcus pyogenes* (MTCC-442) for the study.

**Preparation of inoculums:** Fresh microbial cultures were prepared by streaking loopful of bacterial suspension in to organism specific selective media (Hi-media) and incubated at optimal temperature in order to maintain approximately uniform growth rate of each organism. The bacterial cultures from fresh media was compared with 0.5 McFarland turbidity standard, which is equivalent to approximately $1\times10^8$ bacterial cell count per mL, was maintained throughout the experimentation.

### Bioassay for antimicrobial activity

**Agar well diffusion method:** In the present study, to test antibacterial activity, 11 different plant seed extracts were used. The antibacterial activity was studied by agar well diffusion method. From the stock, 100 mg of each plant extract were suspended in 1 mL of dimethyl sulfoxide (DMSO). Antibiotic, ampicilin, amoxyilin and tetracycline were used as standard at a concentration of 100 μg $mL^{-1}$ and 100% DMSO were used as positive control and negative control, respectively. Bioassay was performed in duplicate and repeated twice.

**Minimum Inhibitory Concentration (MIC):** Minimum inhibitory concentration was evaluated by the two fold serial broth dilution method. Plant extracts showing more than 10 mm inhibition zone were selected for MIC. Selective broth medium was used for dilutions as well as preparing inoculums. The MIC was tested in the concentration range between 4.0-0.0031 mg $mL^{-1}$. Each assay was repeated thrice by using DMSO and selective medium as control.

### Phytochemical analysis

**Preliminary phytochemical analysis:** Qualitative phytochemical analysis of all the selected plant seeds extracts selected, based on MIC value was perform as per the methodology of Parekh and Chanda[5].

**Analytical thin layer chromatography:** Analytical TLC was performed to find out suitable solvent system for the development of chromatogram. The following solvent mixtures were tried on precoated TLC plates (Merck, silica gel 60 F254 plate, 0.25 mm). The mobile phase use the Toluene: ethyl acetate (5:5).

**TLC-bioautography:** Out of 11 plants seed extracts tested for anticariogenic activity, only three showing maximum growth inhibition against *Candida albicans*, *Lactobacillus casei* and *Streptococcus pyogenes* was selected and used for bioautography. By using capillaries 10 μL of aqueous extract of *Mimusops elengi* (Hexane and Distilled water extracts) and *Punica granarum* (Methanol extract) seed extract

(100 mg $mL^{-1}$ stock solution) was spotted on to 0.25mm thick precoated silica gel 60 F254 plate (Merck, Germany). The band length was 2 mm thick. After air drying the TLC plate was run using pre-standardized solvent system, Toluene: ethyl acetate: (5:5). The chromatogram was observed under UV illumination and used for bioautography. Organisms specific agar medium, seeded with specific bacteria *Candida albicans*, *Lactobacillus casei* and *Streptococcus pyogenes* was overlaid on to the silica gel plate loaded with sample and incubated at 37°C for 24 h. On the next day, the plate was flooded with 2, 3, 5-Tri phenyl tetrazolium chloride (0.1%) to visualize growth inhibition. The area of inhibition zone was appeared as transparent against reddish background (lawn of living bacteria).

**HPTLC analysis:** For chemical profile analysis, *Mimusop selengi* (Hexane and Distilled water extracts) and *Punica granarum* (Methanol extract) seed extracts (100 mg each) was mixed with 1 mL DMSO. The three different sample used for HPLC analysis (Camag system equipped with a sample applicator Linomat-5, twin development chamber, TLC scanner-3 and integration software, documentation system Reprostar-3 with G5 digital camera) (Camag, Switzerland). HPTLC aluminum sheet pre-coated with silica gel 60 (1.05547 E Merck) was used as the adsorbent. Toluene: Ethyl acetate (5.0:5.0) was used as the mobile phase. The chromatographic development chamber was saturated with mobile phase for 10 min prior to placement of the plates. The plates were run up to 8 cm height and derivatized (10% $H_2SO_4$ in methanol). The derivative plates were heated at 100°C for 4 min, bands were observed and scanned at 366 nm and photographs taken for record.

**Statistical analysis:** All the experiments were repeated three times. Data on the zone of the inhibition major by diameter were statistically analyzed using one way analysis of variance (ANOVA) using SPSS ver. 16.0[6]. The values are represented as mean and means of three observations were compared with Duncan's Multiple Range Test for determining the statistical significance. Probability level is $p \leq 0.5$.

## RESULTS AND DISCUSSION

In the present study, the anticariogenic activity assay of plants seed extracts against oral microorganism was carried out. The seeds of eleven selected traditional medicinal plants was extracted using Chloroform, Distilled water, Hexane and Methanol and used for anticariogenic activity assay. The result of anticariogenic activity assay of oral microorganisms was assessed by visualizing the presence or absence of inhibition zone and measuring the zone diameter. The results are summarized as under.

***Acacia nilotica* subsp *indica* (Benth.):** Hexane extract of this plant shows low activity against SP (10 mm) and LC (6 mm) no activity against CA, LA and SM (Table 2). Chloroform extract of this plant shows low activity against SP (8 mm) and LC and SA (3 mm) and no activity against CA, LA and SM (Table 2). Methanol extract of this plant shows moderate level activity against SP (8 mm) and LA (7 mm) and no activity against CA and LC (Table 2). Distilled water extract of this plant shows maximum activity against LA (12 mm) and no activity in CA, LC and SP (Table 2). Further MIC determination was done for distilled water extract of this plant and it was 1.2 mg $mL^{-1}$ against LA (Table 3). Omwenga *et al.*[7] reported the

Table 2: Antibacterial activity of different organic solvent extracts of seeds against cariogenic microorganisms (Zone in mm)

| Plantname and antibiotics | Hexane extracts | | | | | | Chloroform extracts | | | | | | Methanol extracts | | | | | | Distilled water extracts | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CA | LA | LC | SA | SM | SP | CA | LA | LC | SA | SM | SP | CA | LA | LC | SA | SM | SP | CA | LA | LC | SA | SMI | SP |
| *A. nilotica* | - | - | 6 | 2 | - | 10 | - | - | 3 | 3 | - | 8 | - | 7 | - | 2 | 4 | 8 | - | 12 | - | 3 | 3 | - |
| *A. aspera* | - | 8 | 7 | 3 | - | 9 | - | 5 | 7 | 4 | - | 9 | - | 5 | 3 | - | - | - | - | 2 | - | - | - | - |
| *C. occidentalis* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *M. elengi* | 18 | 8 | - | - | - | 9 | 5 | 4 | - | - | 2 | 10 | 4 | 3 | 8 | 5 | 6 | 14 | 16 | 4 | 12 | 7 | 5 | 15 |
| *M. oleifera* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *M. pruriens* | 10 | 4 | - | - | 6 | 10 | - | 3 | - | - | - | - | 3 | - | 4 | - | 13 | - | 2 | - | - | - | 15 | - |
| *P. pinnata* | - | - | - | - | - | 8 | 2 | - | - | - | - | - | 5 | - | 4 | - | - | 10 | - | 4 | 2 | - | - | - |
| *P. corylifolia* | - | 5 | 13 | 2 | 5 | 9 | - | 3 | 7 | 3 | - | 9 | - | - | - | 3 | - | 8 | - | - | - | - | - | - |
| *P. granatum* | - | - | - | - | - | 10 | - | 11 | 2 | - | - | - | 7 | 14 | 14 | - | 11 | 15 | - | - | 2 | 13 | 3 | - |
| *S. indicum* | - | - | - | - | - | - | - | - | - | 3 | - | - | - | - | - | 7 | - | - | - | - | - | 8 | - | - |
| *S. chinensis* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 2 | - | - | - |
| Amphotericin | 33 | 11 | 6 | 4 | 15 | 8 | 33 | 11 | 6 | 4 | 15 | 8 | 33 | 13 | 6 | 4 | 15 | 8 | 33 | 11 | 6 | 4 | 15 | 8 |
| Tetracycllin | 33 | 13 | 7 | 5 | 15 | 7 | 33 | 13 | 7 | 5 | 15 | 7 | 33 | 13 | 7 | 5 | 15 | 7 | 33 | 13 | 7 | 5 | 15 | 7 |
| Amphotericin | 28 | 13 | 5 | 3 | 19 | 5 | 28 | 13 | 5 | 3 | 19 | 5 | 28 | 11 | 5 | 3 | 19 | 5 | 28 | 13 | 5 | 3 | 19 | 5 |
| DMSO | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

CA: *Candida albicans*, LA: *Lactobacillus acidophilus*, LC: *Lactobacillus casei*, SM: *Streptococcus mitis*, SA: *Staphylococcus aureus*, SP: *Streptococcus pyogenes*

Table 3: MIC (mg $mL^{-1}$) of selected seed extracts against cariogenic microorganisms

| | Microorganisms | | | | | |
|---|---|---|---|---|---|---|
| Plant names | CA | LA | LC | SA | SMI | SP |
| *Punica granatum* D/W | - | - | - | 1.2 | - | - |
| *Mimusops elengi* D/W | 0.07 | 1.2 | - | - | - | 0.3 |
| *Psoralea corylifolia* Hex | - | - | 2.5 | - | - | - |
| *Punica granatum* Met | - | - | 0.6 | - | 5 | 0.3 |
| *Acacia nilotica* D/W | - | 1.2 | - | - | - | - |
| *Punica granatum* Chl | - | 5 | - | - | - | - |
| *Mimusops elengi* Hex | 0.3 | - | - | - | - | - |
| *Mimusops elengi* Met | - | - | - | - | - | 1.2 |
| *Mucuna pruriens* Met | - | - | - | - | 1.2 | - |
| *Mucuna pruriens* D/W | - | - | - | - | - | - |

CA: *Candida albicans*, LA: *Lactobacillus acidophilus*, LC: *Lactobacillus casei*, SM: *Streptococcus mitis*, SA: *Staphylococcus aureus*, SP: *Streptococcus pyogenes*, D/W: Distilled water, Hex: Hexane, Met: Methanol, Chl: Chloroform

Table 4: Phytochemical analysis of crude seed extracts of selected plants

| | Microorganisms | | | | | |
|---|---|---|---|---|---|---|
| Plant names | 1 | 2 | 3 | 4 | 5 | 6 |
| *Punica granatum* D/W | + | - | - | + | + | + |
| *Mimusops elengi* D/W | + | + | + | + | + | + |
| *Psoralea corylifolia* Hex | - | - | - | + | + | + |
| *Punica granatum* Met | + | + | + | - | + | + |
| *Acacia nilotica* D/W | - | - | - | - | + | + |
| *Punica granatum* Chlo | - | + | + | - | + | + |
| *Mimusops elengi* Hex | - | - | + | - | + | + |
| *Mimusops elengi* Met | - | - | + | - | - | + |
| *Mucuna pruriens* Meth | - | - | - | - | - | + |
| *Mucuna pruriens* D/W | - | - | + | - | + | + |

+: Present, -: Absent, 1: Saponins, 2: Cardiac glycosides, 3: Steroids, 4: Terpenoids, 5. Phenolic compound, 6: Alkaloid

antibacterial activity against some selected bacterial strain. This seed extracts more activity against LA (12 mm) and SP (10 mm). Extract show less activity compare to bark. This seed extract was not selected for further analysis.

***Achyranthus aspera* L.:** Hexane extract of this plant shows low activity against SP (9 mm) and LA (8 mm) no activity against CA and SM (Table 2). Chloroform extract of this plant shows low activity against SP (9 mm) and LC (7 mm) and no activity against CA and SM (Table 2). Methanol extract of this plant shows very low activity against LA (5 mm) and LC (3 mm) no activity against CA, SA, SM and SP (Table 2). Distilled water extract of this plants shows very low activity against LA (2 mm) and rest of organisms this plants shows no activity (Table 2). This seed extract was not selected for further analysis. Tullanithin *et al.*[8] reported antibacterial activity on leaf and stem against *E. coli*. This plants seed extracts compare the stem and leaf are more antibacterial activity. This plant extracts less effective against oral pathogen.

***Cassia occidentalis* L.:** Hexane, chloroform, methanol and distilled water extract of this plant shows no activity against all selected microorganisms. Vedpriya *et al.*[9] reported antimicrobial activity against human pathogenic microbes[9]. It is more activity in *S. aures* 12 mm and *Cadida albicans*-8 mm in methanol extract. This plant extract compare with other parts to seed extracts absent the antibacterial activity.

***Mimusops elengi* L.:** Hexane extract of this plant shows high activity against CA (18 mm) and SP (9 mm)and no activity against LC, SA and SM (Table 2). Chloroform extract of this plant shows moderate activity against SP (10 mm) no activity against LC and SA (Table 2). Methanol extract of this plant shows highest activity against SP (14 mm) among the all extracts (Table 2). Distilled water extract of this plant shows maximum activity against CA (16 mm) and SP (15 mm) (Table 2). Further MIC determination was done for distilled water extract of this plant and it was 0.075 mg $m^{-1}$, l.2 mg $mL^{-1}$ and 0.3 against, respectively CA, LA and SP (Table 3). The MIC of Hexane and methanol extracts against CA (0.3 mg $mL^{-1}$) and SP (1.2 mg $mL^{-1}$), respectively. Phytochemical analysis of crude distilled water seed extracts presents the saponins, cardiac glycosides, steroids. terpenods, phenolic and alkaloid compound present (Table 4). Hexane extracts present the steroids, phenolic and alkaloid compound present (Table 4). Methanol extracts present the steroids and alkaloid compound present (Table 4). Methanol extracts present the steroids and alkaloid compound present conformation by analysis for bioautography against CA. The active compound mark in square. The active compound $R_f$ value is 0.65 and area (43.92%). Rahmatullah *et al.*[10] folk medicinal use in daudkandi sub district comilla district, Bangladesh. Rao *et al.*[11] report the *Streptococcus mutans* and *Staphylococcus aureus* were the most resistant strains. Most susceptible bacterial strains were *Lactobacillus fermemtum* and *Lactobacillus acidophilus*. *Moringa olifera* and *Mimusops elengi* were showed strong activity against all the tested bacterial strains[12]. This seed extracts exhibit more activity against CA, SM and SP compare to *Streptococcus mutans* and *Staphylococcus aureus*. Further, spectroscopic and chromatographic analysis are required for determination of structure of bioactive compound.

***Moringa oleifera* Lam:** Hexane, chloroform, methanol and distilled water extract of this plant show no activity against all selected microorganisms. Sabale *et al.*[13] reported the gum are very useful the dental caries. In this study, compare with other parts to seed extracts absent the anticariogenic activity.

***Mucuna pruriens* Baker:** Hexane extract of this plant shows activity against CA (10 mm) and SP (6 mm) no activity against LC and SA (Table 2). Chloroform extract of this plant shows low activity against LA (3 mm) and no activity against rest of microorganisms (Table 2). Methanol extract of this plant shows moderate level activity against SM (13 mm) and LC (4 mm) and no activity against LA, SA and SP (Table 2). Distilled water extract of this plant shows maximum activity against SMI (15 mm) and no activity in LA, LC, SA and SP (Table 2). Further MIC determination was done for methanol extract of this plant and it was 1.2 mg $mL^{-1}$ against SM (Table 3). Phytochemical analysis more active chemical present in the seed is alkaloid. Mastan *et al.*[14] reported the antimicrobial activity of various extracts of leaves[14]. *Mucuna pruriens* leaves extract against *S. araus*-80 mg $mL^{-1}$ reported. *Mucuna pruriens* leaves extract compare to more activity against *M. pruriens* seed extracts in the SM is minimum inhibitory concentration is 1.2 mg $mL^{-1}$ compare to the *S. aureus*.

***Pongamina pinnata* (L.) Pierre:** Hexane extract of this plant shows in only one activity against SP (8 mm) and no activity against rest of the oral microorganisms (Table 2). Chloroform extract of this plant shows low activity against CA (2 mm) and no activity against rest of the microorganisms (Table 2). Methanol extract of this plant shows moderate level activity against SP (10 mm) and CA (5 mm)and no activity against LA, SA and SM (Table 2). Distilled water extract of this plant shows low activity against LA (4 mm) and no activity in CA, SA, SM and SP (Table 2). This seed extract was not selected for further analysis. In this study, moderate level of activity against dental related organisms. Badgujar *et al.*[15] reported the traditional practices for oral health care. It is required the further analysis for this plant in seed extracts.

***Psoralea corylifolia* L.:** Hexane extract of this plant shows highest activity against LC (13 mm) and SP (9 mm) and no activity against CA (Table 2). Chloroform extract of this plant shows low activity against SP (9 mm) and LC (7 mm) and no activity against CA and SM (Table 2). Methanol extract of this plant shows low level activity against SP (8 mm) and SA (3 mm) and no activity against CA, LA, LC and SM (Table 2). Distilled water extract of this plant shows no activity (Table 2). Further MIC determination was done for hexane extract of this plant and it was 2.5 mg $mL^{-1}$ against LC (Table 3). Pytochemical analysis more active chemical present in the seed is terpenoid, phenolic compound and alkaloid. Cho *et al.*[1] reported the inhibited the artificial dental plaque formation and did not reduce the cell viability of human gingival fibroblasts. Chanda *et al.*[16] antibacterial compound from the seeds against Gram negative and Gram positive. It in this study, comparative hexane extracts are more effective to compare the other bacterial to oral microorganism.

***Punica granatum* L.:** Hexane extract of this plant shows low activity against SP (10 mm) and no activity against rest of the microorganisms (Table 2). Chloroform extract of this plant shows moderate activity against LA (11 mm) and no activity against CA, SA, SP and SM (Table 2). Methanol extract of this plant shows highest level activity against LC (14 mm), SM (11 mm) and SP (15 mm) and no activity against SM (Table 2). Distilled water extract of this plant shows maximum activity against SA (13 mm) and no activity in CA, LA and SP (Table 2). Further MIC determination was done for distilled water and chloroform extract of this plant and it was 1.2 and 5 mg $mL^{-1}$ against SA and LC, respectively (Table 3). Methanol extracts of this plant against LC, SM and SP it was 0. 6, 5 and 0.3 mg $mL^{-1}$, respectively. Phytochemical analysis showed that more active chemical present in the distilled water seed is Saponins, terpenoid and phenolic compound and alkaloid. Methanol extracts present the terpenoids are absent and rest of the chemical are present in this extracts. The bioautigraphy against CA and LC performed. In bioautography, active compound $R_f$ value is 0.16 and 0.72 and area 32.14 and 50.75%, respectively. Devi *et al.*[17] reported the seed of ripened fruit maximum methanolic extracts against *Strptococcus* (25 mm). This study show the LC against 14 mm zone of inhibition is observed. Although, crude extract of Pomegranate was unable to inhibit *S. mutans* at low concentrations, it inhibits the growth of periodontal pathogens. *Punica granatum* "Pomegranate extract" have potential to be used to prevent periodontal diseases. Further, spectroscopic and chromatographic analysis are required for determination of structure of bioactive compound.

***Sesamum indicum* L.:** Hexane extract of this plant shows no activity against all selected microorganisms. Chloroform and methanol extracts of this plant shows low activity in SA (3 and 7 mm), respectively and rest of the microorganisms are absent (Table 2). Distilled water extracts shows less activity in SA (8 mm) and rest of oral bacterial absent the activity. Anand *et al.*[12] reported the oil pulling on dental caries causing bacteria. The *in vitro* antibacterial activity of sesame oil against dental caries causing bacteria was determined. *Streptococcus mutans* and *Lactobacillus acidophilus* were found to be moderately sensitive to the sesame oil.

Asokan *et al.*[18] reported the effect of oil pulling on *Streptococcus mutans* count in plaque and saliva using dentocult SM strip mutans test. In this study, seed extracts of different solvent are not effective for dental caries related organisms compare to oil.

***Simmodisa chinesdis* (Link) C.K. Schneider:** Hexane, Chloroform, methanol extract of this plant shows no activity against all selected microorganisms. Distilled water extracts of this plant shows less activity against LC (2 mm) and rest of the microorganisms shows no activity (Table 2).

There have been numerous reports of the use of traditional plants and natural products for the treatment of oral diseases. The different chemical compound present in the plants either inhibit the growth of pathogen or kill them and have no toxicity to host cell are consider for developing new antimicrobial drugs. Many plant-derived medicines used in traditional medicinal systems have been recorded in pharmacopeias as agents used to treat infections for their efficacy against oral microbial pathogens. The general antimicrobial activity of medicinal plants and plant products was determined such as essential oils and seed etc. In particular, traditional medicinal plant extracts or phytochemicals that have been shown to inhibit the growth of oral pathogens, reduce the development of dental plaque, influence the adhesion of bacteria to surfaces and reduce the symptoms of oral diseases. Despite widespread of toothbrushes and toothpastes of natural tooth cleaning method using plant derived product practiced for thousands of years in various countries. Natural products have been used to prevent oral diseases, especially dental plaque or caries.

Present study on anticariogenic activity of some traditional medicinal plants against oral microorganisms revealed that seed extracts are more powerful compared to other part of the plants extract as far as growth inhibition in cariogenic bacteria is consult. High degree of growth inhibition (10-18 mm) was observed in CA and SP by various extracts of three plants which are more than that of the standard antibiotic amoxycilin (8 mm). Majority of the selected plants are traditionally use from very long time in various human ailments. There are large numbers of reports on the antibacterial activity of ethanomedicinal plants. Badgujar *et al.*[15] reported the traditional practices for oral health care. Because of scarcity of literature on selected plants for their anticariogenic activity it is difficult to compare individual plants[15].

Preliminary phytochemical analysis revealed the presence of alkaloids in the various plants extracts. The other secondary metabolites like tannins, saponins, cardiac glycoside, steroids, terpenodiesetc, were present its trace amounts in some of the plant extracts (Table 4). Therefore, the variable antimicrobial effects of plant species are due to phytochemical properties and difference among species[14]. It is possible that some of the plants found ineffective against cariogenic bacteria. Because they have don't have antibiotic properties or insufficient quantity of anticariogenic substances. Some of the active constituents are insoluble in water. Change in the conformation during drying could also be possible and leads to in activity. Further, spectroscopic and chromatographic analysis is required for determination of structure of bioactive compound.

## CONCLUSION

Chloroform, Distilled water, Hexane and Methanol extract of plant seeds were analyzed for their anticariogenic activities against oral microorganisms. The activity was determined against *Candida albicans*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Staphylococcus aureus*, *Streptococcus mitis* and *Streptococcus pyogenes*. Plant seed extracts have great source as anticariogenic compound against oral pathogenic microorganisms, which can be used to treat infectious diseases. The very good activity of methanol extract of seed of *Mimusops elengi* L. and *Punica granatum* L. will be useful in the future development of effective for toothpaste or mouth washer against oral microorganisms.

## SIGNIFICANCE STATEMENTS

This study will help the researcher to uncover the critical areas of dental caries diseases problem solve by using medicinal plants that many researchers were not able to explore.

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## REFERENCES

1. Cho, Y.S., N.L. Schiller, H.Y. Kahng and K.H. Oh, 2007. Cellular responses and proteomic analysis of *Escherichia coli* exposed to green tea polyphenols. Curr. Microbiol., 55: 501-506.

2. Loesche, W., 2007. Dental caries and periodontitis: Contrasting two infections that have medical implications. Infect. Dis. Clin. North Am., 21: 471-502.
3. Barad, M.K., K.B. Ishnava and J.B. Chauhan, 2014. Anticariogenic activity and phytochemical studies of crude extract from some Indian plant leaves. J. Intercult. Ethnopharmacol., 3: 85-90.
4. Devi, B.P. and R. Ramasubramaniaraja, 2009. Dental caries and medicinal plants-An overview. J. Pharm. Res., 2: 1669-1675.
5. Parekh, J. and S.V. Chanda, 2007. *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turk. J. Biol., 31: 53-58.
6. SPSS., 2007. SPSS for Windows, Version 16.0. SPSS Inc., Chicago, IL., USA.
7. Omwenga, E.O., P.O. Okemo, P.K. Mbugua and C. Ogol, 2009. Ethnobotanical survey and antimicrobial evaluation of medicinal plants used by the samburu community (Kenya) for treatment of Diarrhorea. Phcog. Mag., 5: 165-175.
8. Tullanithi, K.M., B. Sharmila and T.S. Gnanendra, 2010. Preliminary phytochemical analysis and antimicrobial activity of *Achyranthes aspera* Linn. Int. J. Biol. Technol., 1: 35-38.
9. Arya, V., S. Yadav, S. Kumar and J.P. Yadav, 2010. Antimicrobial activity of *Cassia occidentalis* L. (Leaf) against various human pathogenic microbes. Life Sci. Med. Res., 2010: 1-11.
10. Rahmatullah, M., A. Momen, M. Rahman, D. Nasrin and S. Hossain *et al*., 2010. A randomized survey of medicinal plants used by folk medicinal practitioners in Daudkandi sub-district of Comilla district, Bangladesh. Adv. Nat. Applied Sci., 4: 99-105.
11. Rao, P.K., M. Anjana, L. Deepti, K. Madhavi and T.R. Rao, 2009. *In vitro* antibacterial screening of selected medicinal plant extracts against oral pathogens. Asian J. Exp. Sci., 23: 479-485.
12. Anand, T.D., C. Pothiraj, R.M. Gopinath and B. Kayalvizhi, 2008. Effect of oil-pulling on dental caries causing bacteria. Afr. J. Microbiol. Res., 2: 63-66.
13. Sabale, V., V. Patel, A. Paranjape, C. Arya, S.N. Sakarkar and P.M. Sabale, 2008. Plant review *Moringa oleifera* (Drumstick): An overview. Pharmacogn. Rev., 2: 7-13.
14. Mastan, S.A., P.J. Ramayya, L.M. Naidu and K. Mallikarjuna, 2009. Antimicrobial activity of various extracts of *Mucuna pruriens* leaves. Biomed. Pharmacol. J., 2: 55-60.
15. Badgujar, S.B., R.T. Mahajan and S.B. Kosalge, 2008. Traditional practice for oral health care in Nandurbar district of Maharashtra, India. Ethnobot. Leaflets, 12: 1137-1144.
16. Chanda, S., M. Kaneria and R. Nair, 2011. Antibacterial activity of *Psoralea corylifolia* L. seed and aerial parts with various extraction methods. Res. J. Microbiol., 60: 124-131.
17. Devi, A., V. Singh and A.B. Bhatt, 2011. Antibiotic sensitivity pattern of streptococcus against commercially available drugs and comparison with extract of *Punica granatum*. Int. J. Pharm. Bio Sci., 2: 504-508.
18. Asokan, S., J. Rathan, M.S. Muthu, V.P. Rathna, P. Emmadi, Raghuraman and Chamundeswar, 2008. Effect of oil pulling on *Streptococcus mutans* count in plaque and saliva using Dentocult SM Strip mutans test: A randomized, controlled, triple-blind study. J. Indian Soc. Pedodontics Prev. Dent., 26: 12-17.