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Anti-microbial effect of Pudina extract on streptococcus mutans: In–vitro study

Nisarg J Chaudhary* Ajit Krishnan CG[†] Thanveer K[‡] Harsh Shah[€]

*MDS, Lecturer, Department of Public Health Dentistry, Karnavati School of Dentistry, Gandhinagar, Gujarat, India. [†]MDS, Professor and Head, [‡]MDS, Professor, Department of Community and Preventive Dentistry, KM Shah Dental College, Vadodara, [€]MDS, Lecturer, Department of Public Health Dentistry, Ahmedabad Dental College & Hospital, Ahmedabad, India. Email: nisargjc@yahoo.co.in

Abstract:
Objective: To evaluate the efficacy of alcoholic extracts of Pudina (Mint) on Streptococcus mutans.
Methods: An Experimental design, in vitro study, Lab setting. Methanolic extract of Pudina was prepared by cold extraction method. The extract was then diluted with an inert solvent, dimethyl formamide, was to obtain 3 different concentrations (5%, 10%, 50%) of the extract. Chlorhexidine (0.2%) was used as a positive control and dimethyl formamide was used as a negative control. The extract, along with the controls, was then subjected to microbiological investigation to determine which concentration gave a wider inhibition zone against Streptococcus mutans. The zone of inhibition was measured in millimeters.
Results: At the 50% concentration of Pudina extract, a zone of inhibition of 10 mm was obtained. This was widest zone of inhibition observed among all three different concentrations of Pudina that were investigated.
Conclusion: Pudina extract demonstrated an antimicrobial property against Streptococcus mutans.
Keywords: Antimicrobial activity, Mint, Pudina, Streptococcus mutans.

Introduction:

Dental diseases are recognized as major public health problems throughout the world. Numerous epidemiological studies showed that tooth decay is the most common affliction of

mankind.[1] Dental caries is steadily increasing in the underdeveloped and developing countries. Treatment is expensive and not a realistic option for the poor. Hence, there is an urgent need to promote traditional preventive measures that are acceptable, easily available, and cost effective.[2]

Ayurveda is a science of life. Ayurveda is an ancient Indian medical science, the origin of which can be traced back to the Vedas, which are the oldest available classics of the world. Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind. The World Health Organization reported that 80% of the world's population rely chiefly on traditional medicine and a major part of the traditional therapies involve the use of plant extracts or their active constituents.[3]

Pudina (Mentha arvensis), a perennial aromatic herb belonging to the family Labiatae and genus Mentha is an important culinary plant with immense medicinal use. Today, Pudina is cultivated in North America, Africa, Australia, and Asia mainly for its pharmaceutical, medicinal, and culinary uses.[4]

The entire plant is antibacterial, anti-Fibrile. It yields an essential oil and menthol which exert, through their rapid evaporation, lightly anesthetic and anodyne local effect. It is effective in headache, rhinitis, cough, colic and vomiting. Pudina contains menthol as antioxidants, as a form of biologically active component.[5]

The impetus for the study was the non-availability of literature about the antimicrobial activity of Pudina against caries-causing microorganisms like Streptococcus mutans. Chlorhexidine is used as a gold standard against which other antimicrobial agents are compared. It has been studied extensively and is currently the most potent chemotherapeutic agent against Streptococcus mutans and dental caries.[6-9] Therefore, an attempt is made to compare the antimicrobial activity of Pudina with 0.2% chlorhexidine against Streptococcus mutans.

Material and methods:

Ethical clearance:

The study protocol was reviewed and approved by the research cell of K. M. Shah Dental College and Hospital i.e. Human Research Review Board and ethical approval was obtained by the Ethics committee, Sumandeep Vidyapeeth, Piparia, Vadodara.

Preparation of Pudina extracts

Pudina leaves were obtained from market and were dried in sunlight. The dried leaves were then a powdered finely (figure 1). 100 gram of finely powdered Pudina was then macerated with 500 ml of 100% methanol. It was then subjected to filtration with Whatman filter paper to obtain a clear filtrate (figure 2). The filtrate so obtained was reduced at a low temperature of less than 60º C to obtain a solid residue of Pudina extract. From 100 grams of Pudina powder dissolved in 500 ml of methanol, 5 grams of residue (extract) was obtained, so the yield was 5% w/w (figure 3).

Preparation of different concentrations of Pudina extract:

Half(0.5) gram of extract was dissolved in 10 ml of dimethyl formamide to obtain 5% concentration of extract. One gram of extract was dissolved in 10 ml of dimethyl formamide to obtain 10% concentration of extract and with same procedure 50% concentration was made. 0.2% of Chlorhexidine was used as a positive control and dimethyl formamide was used as a negative control.

Microbiological procedures:

Pure strains of Streptococcus mutans (MTCC 890) were obtained from microbial type culture collection (MTCC), Chandigarh.

Streptococcus mutans was added to nutrient broth which was incubated at 37^0 for 24 hours. It was then sub-cultured onto nutrient agar plate and incubated at 37^0 for 24 hours. The inoculum for antimicrobial activity was prepared by adjusting the density of organism to approximately 10^8 colony forming units/ml with the help of 0.5 Mcfurland opacity standards. Lastly it was inoculated on blood agar plate by lawn culture method.

Ditch plate method:

Ditches will be made in Petri-dishes by using punch. These ditches will be filled with the equal amount of prepared extract of Pudina. This procedure will be repeated for the three concentrations of Pudina extract. Plates will then be incubated at 37°C for 48 hours, after which zone of inhibition will be measured by Ditch plate method (figure 4).

Antimicrobial susceptibility testing:

The ditch plate method was used to test the antimicrobial activity. Ditches were prepared on blood agar plates with the help of punch having 6-mm diameter. On each petri dish, four ditches were made and labeled for various concentrations of Pudina extract. 50 micro liter each of 5%, 10% and 50% Pudina extracts were introduced into equal sized ditches made on petri dishes.

Fig 1: Pudina powder

Fig 2: Filtration of pudina powder

Fig 3: Pudina powder macerated with methanol

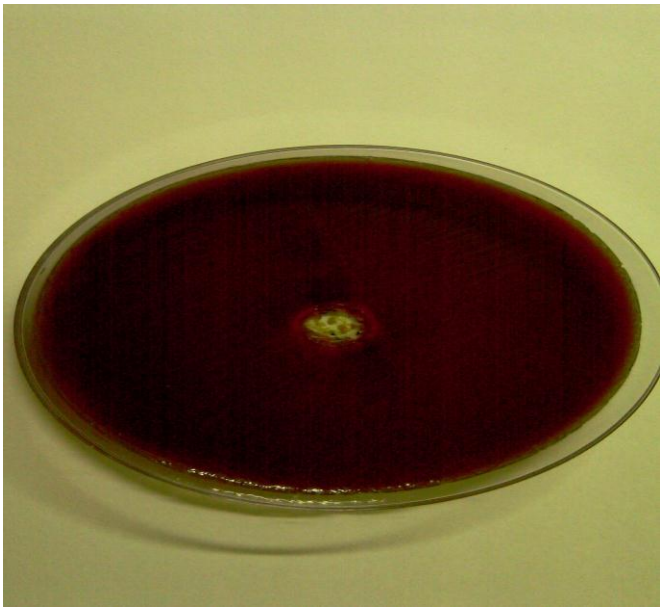

Fig 4: Petri dish showing zones of inhibition by ditch plate method

The plates were left for 1 hour at room temperature and then incubated at 37°C for 48 hours and examined for zone of inhibition. The average of those zones was recorded in millimeters. No statistical tests were performed as they were not required and the data obtained were appraised observationally.

Result:

There was no zone of inhibition observed with 5% Pudina extract. Zone of inhibitions of 8.0, 7.0, 8.0, 8.0 mm were observed with 10% extract with mean zone of inhibition of 7.7 mm. Zone of inhibitions 11.0, 10.0, 9.0, 10.0 mm were observed with 50% Pudina extract with mean zone of inhibition of 10.0 mm (Table 1).

Zones of inhibition for 0.2% Chlorhexidine and Dimethyl formamide was seen that with 0.2% Chlorhexidine (positive control) a much wider zone of inhibition of 20 mm, 24 mm and 32 mm for 5%, 10% and 50% respectively. Streptococcus mutans were resistant to the action of dimethyl formamide (negative control).

Discussion:

Medicinal plants continue to be an important therapeutic aid for alleviating the ailments of humankind. In India, the use of different parts of several medicinal plants to cure specific ailments has been in vogue from ancient times. The chemical composition of Pudina is highly complex, containing many nutrients and other biologically active

Table 1: Effect of various concentrations of Pudina extracts on streptococcus mutans

Concentration	Zone of inhibition (mm)				
	Z_1	Z_2	Z_3	Z_4	Mean ±SD
5%	0	0	0	0	0
10%	8.0	7.0	8.0	8.0	7.7 ± 0.5
50%	11.0	10.0	9.0	10.0	10.0 ± 0.8

compounds, the proportion of which may vary considerably between strains and even between the plants of the same field. Fresh Pudina is frequently consumed in India in various food and also used as a flavoring agent.

Menthol ($C_{10}H_{20}O$), the active constituent present in Pudina, perhaps is largely responsible for the therapeutic potentials of Pudina.[10] It is used to treat liver and spleen diseases, asthma and jaundice. The oil is antiseptic, carminative, refrigerant, stimulant and diuretic. Menthol is used in medicine for stomach disorders and in ointments for headache. But the effect of this plant on streptococcus mutans was not assessed.

The other important constituents include 4.5 to 10% esters-menthyl acetate and 15 to 20% of ketones. The antimicrobial activity of Pudina can be attributed to these constituents. Methanol was used as a solvent because the essential oils in Pudina are more soluble in alcohol when compared to distilled water.[10]

Dimethyl formamide, an inert solvent was used to dilute the extract to neutralize the effect of alcohol which itself is an antiseptic attributing the result solely to Pudina.

In the present study, various concentration of Pudina extract are made like 5%, 10% and 50%. Minimum 5% concentration is made to check the effect on streptococcus mutans and maximum 50% for the same. In this study, no zone of inhibition observed with 5% Pudina extract and it was seen in 10% and 50% of concentration. It's effect is might be more in more than 50% concentration but it could be confirm by further studies.

In the present study Chlorhexidine was found to be more effective when compared to Pudina extract, however the well-known side effect of Chlorhexidine i.e. staining of teeth and restoration, alteration of taste sensation, development of resistant microorganisms, may limit the long term use of Chlorhexidine. In comparison herbal medicines like Pudina are abundantly available, easily accessible, economically feasible, and culturally acceptable and may possess minimal side effects and hence can be recommended for long term use.

Effect of Pudina extract was better as compare to other herbal product like neem, mango and tulsi.[5,11]

The results of the current study could not be compared with other studies as this is the first study of its kind. Further the composition of Pudina extract at 50% might be such that it has a maximum antimicrobial potential, perhaps this needs to be confirmed by further studies.

Conclusion:

Pudina extract demonstrated an antimicrobial activity against Streptococcus mutans. It has the maximum antimicrobial potential at the 50% concentration level.

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