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Antimicrobial efficacy of some medicinal plant extract against *Streptococcus mutans* causing dental caries

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Abstract
Aim: To compare the antimicrobial efficacy of five Medicinal plant extracts against two strains of *Streptococcus mutans* causing dental caries using Agar well diffusion method.
Materials and methods: Five plant extracts were prepared by soxhlet extraction method using organic solvent. These extracts were tested against *Streptococcus mutans* MTCC 497& MTCC 890 causing dental caries *by* Agar well diffusion method.
Result: All the five plants extract are evaluated against both the strains of *Streptococcus mutans*. The highest zone of inhibition has been observed in the organic solvent based extract of *Pongamia pinnata* and *Acacia catechu* against the strain of *S. mutans* MTCC 890 is 15mm and 17mm which is same as the antibiotic (amoxicillin) inhibition zone and against MTCC 497 is 14mm and 16mm at concentration of 200µg/ml.
Conclusion: This study concluded that the organic solvent based extract of *Acacia catechu, Pongamia pinnata, Putranjiva roxburghii, Zanthoxylum armatum,* and *Zanthoxylum nitidum* are effective against both the strains of *Streptococcus mutans* (MTCC 497& MTCC 890) and can be used as a potential source for making herbal medicines or tooth paste that can be used to cure dental caries.

Keywords: Medicinal plants, dental caries, *Streptococcus mutans*, antimicrobial activity, agar well diffusion method

1. Introduction
Dental caries, more commonly known as tooth decay, is the most widespread disease in human being which is associated with our oral hygiene. Oral hygiene is an integral part of health of a person. Oral health when neglected, results in different types of oral ailments like dental caries and periodontal disease [1]. Dental caries is a disease caused by specific type of bacteria live in human mouth. These bacteria produce acid that destroys tooth enamel and results in cavities on its surface. The principle causal agent of dental caries are a group of Streptococcal species of which *Streptococcus mutans* are the most important agents for human dental caries [2].
Microbial resistance to most of the antibiotics commonly used to treat oral infections (penicillins and cephalosporins, erythromycin, tetracycline and derivatives and metronidazole) has been documented [3]. The resistance of microorganisms against the traditional antibiotics needs urgent attention for the development of the new drug molecules. It is well documented from ancient times that active principles from plant origin have been used as medicines for various diseases and microbial infections [4]. A wide variety of medicinal plants used traditionally have not yet been systematically investigated against various microbial pathogens [5]. The effect of plant extracts on bacteria has been studied by a large number of researchers in different parts of the world [6]. The antimicrobial activity of plant extracts and phytochemicals was evaluated with antibiotic susceptible and resistant microorganisms. The synergistic effects associated with antibiotics were also studied [7].
In this study *a comparative* evaluation of organic solvent based extract of five Medicinal plants viz *Acacia catechu, Pongamia pinnata. Putranjiva roxburghii. Zanthoxylum armatum, Zanthoxylum nitidum* against *Streptococcus mutans* MTCC 497& MTCC 890 Causing Dental Caries has been done.

2. Materials and Methods
The study was conducted in the institute of The Science Foundation for Tribal and Rural Resource Development (SFTRRD). The selected plant product (bark of *Acacia catechu,*

Pongamia pinnata. Putranjiva roxburghii. Zanthoxylum armatum, Zanthoxylum nitidum) were collected from RPRC (Regional plant resource centre) Bhubaneswar and NIEST (North East Institute of Science and Technology) Jorhat, Assam.

2.1 Microorganism used
Two pathogenic strains of *Streptococcus mutans* MTCC 479 and MTCC 870 are obtained from IMTECH Chandigarh in lyophilized dry powdered form and sub cultured on Brain heart Infusion agar at North East Institute of Science and Technology (NIEST) Jorhat, Assam. The culture of these strains for this study were procured from (NIEST) and were again sub cultured in Mueller Hinton Agar at SFTRRD at 4º C for further experiments.

2.2 Preparation of organic solvent based extract
As the secondary metabolites are highly soluble in organic solvents and many organic solvents are available for extraction, Methanol was chosen for extraction of active compounds. Air-dried bark of *Zanthoxylum nitidum, Zanthoxylum armatum, Pongamia pinnata*, *Putranjiva roxburghii*, *Acacia catechu* was taken in a Soxhlet apparatus (Biotechnics, India) and extracted with 96% Methanol for 8 hours. The extraction was done at 40 ºC. After the extraction extract was filtered by Watman filter paper no.1 and allowed to evaporate in water bath (Biotechnolab, Mumbai) at 50 ºC. The extract was taken out of the flask, measured and kept in tightly capped specimen tubes and were stored in 4 ºC refrigerator for further use.

2.3 Preparation of antibiotic stock solutions
The Antibiotics Amoxicillin taken for the study as tablets. The tablet was powdered using mortar and pestle, accurately weighed and dissolved in the distilled water in the same concentration as extract, using sterile glassware. Standard stock solution was made in aliquots of 5 ml volume and stored at room temperature.

2.4 Test for antibacterial assay
The solid agar was punched with 7mm diameter wells. The inoculums were spread on the agar plates using sterile swabs and then the wells were filled with plant extracts and antibiotic solution. The concentrations of the extracts employed were 50,100,150,200 µl. This solution was prepared by using DMSO (Dimethyl Sulfoxide) which doesn't have any effects against both the strains of *Streptococcus mutans*. The plates were then incubated at 37 ºC for 24 hours. After incubation, zone of growth inhibition for each extract was measured in millimeters using Himedia Antibiotic Zonescale PW096.

3. Results
All five extracts of the plant tested showed varying degree of antibacterial activities against the test bacterial species S.mutans (MTCC 497 & MTCC 890). The antibacterial activities of the Methanol plant extracts compared favorably with that of the antibiotics (Amoxicillin) in well diffusion method in table 1 and 2. Extracts of all plants showed inhibition of isolates of *S. mutans MTCC 497* but to varied extents. It was observed that bark extract of *Acacia catechu* and *Pongamia pinnata* bark extract of inhibit the strains of *S mutans MTCC 890* to higher extent. Least inhibitory activity was observed in case of *Puntranjiva roxburgi* and *Zanthoxyllum armatum*. Remaining plants showed moderate inhibition against the bacterial isolates. Inhibition caused by amoxicillin was higher than that of plant extracts against both the strains. But in the case of strain MTCC890 same the effect of bark extract *Pongamia pinnata* is same as the antibiotic.

Table 1

Plants Used	Plant Extract At Different Concentration Against *Streptococcus Mutans* Mtcc 497				
	Control	50µl	100µl	150ul	200µl
Acacia Catechu	NIL	4mm	6mm	8mm	14mm
Pongamia Pinnata	NIL	4mm	8mm	10mm	15mm
Putranjiva Roxburghii	NIL	1mm	1.5mm	2mm	2.7mm
Zanthoxylum Armatum	NIL	1mm	2mm	3.5mm	9mm
Zanthoxylum Nitidum	NIL	1mm	4.6mm	7mm	12mm
Amoxycillin	NIL	20mm	26mm	29mm	32mm

Table 2

Plants Used	Plant Extract At Different Concentration Against *Streptococcus Mutans* Mtcc 890				
	Control	50µl	100µl	150µl	200µl
Acacia Catechu	NIL	2mm	5mm	10mm	16mm
Pongamia Pinnata	NIL	8mm	10mm	14mm	17mm
Putranjiva Roxburghii	NIL	4mm	5mm	6mm	15mm
Zanthoxylum Armatum	NIL	1.5mm	2.3mm	4mm	10mm
Zanthoxylum Nitidum	NIL	2mm	4mm	8mm	16mm
Amoxycillin	NIL	1mm	8mm	12mm	18mm

4. Discussion
Plants produce a wide variety of phytochemicals constituents, which are secondary metabolites and are used either directly or indirectly in the pharmaceutical industry. 'For centuries, man has effectively used various components of plants or their extracts for the treatment of many diseases, including bacterial infections [8]. Present study was done for five plant extracts such as *Acacia catechu, Pongamia pinnata, Putranjiva roxburghii, Zanthoxylum armatum,* and *Zanthoxylum nitidum* against *Streptococcus mutans* (MTCC 497& MTCC 890). Similar type of study was reported by many of the scientists before, the same plant extracts against different organisms causing different diseases.

Similar type of study was reported by many of the scientists before, the same plant extracts against different organisms causing different diseases. Methanolic extract of *Acacia catechu Willd* has Anti-microbial activity against pathogenic as well as nonpathogenic bacteria e.g *Bacillus subtilis,*

Staphylococcus aureus, Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa and *Candida albicans*. It is effective against gram positive as well as gram negative bacteria. It was found to be most effective against *Staphylococcus aureus* with about 20mm zone of inhibition at minimum bactericidal concentration (MBC) of the crude extract 1,000 g/ml [9]. Acetone extract of *Pongamia pinnata* contain tannins as the major constituent, the antimicrobial activity of the extract may be due to presence of tannins. The zone of inhibitions produced by 4mg, 6mg and 8mg disc concentration are 6±0.61 to 8±0.20, 8±0.11 to 10±0.19 and 11±0.41 to 14±0.16 respectively. It is evident from this study that the antibacterial effect of aqueous extract of *P. pinnata* twigs is concentration dependent as the zone of inhibition [10]. The antibacterial activities of *Z. armatum*, *R. anthopogon*, *C. glaucescens, A. calamus, A. vulgaris L, A. spectabilis* and *N. jatamansi* and microbial inhibition by essential oil from different plant species at different concentration *R. anthopogon, C. glaucescens and Z. armatum* are the most effective against *S. aureus, E. coli and K. pneumoniae*, respectively with MIC value of 125/20, 3.9/20 and 800 µg/20 µl, respectively. In addition, the lower concentration of all oils was found effective against the *E. coli and S. aureus. K. pneumoniae* was found to be strongly resistant even at very higher concentration of treated oil. *Z. armatum* oil was relatively found to be strongly efficient against all treated bacteria [11].

The antibacterial activity of ethanol and aqueous extract from *Z. nitidum* stem bark and root against seven bacterial strains was initially assessed by disk diffusion method. The extracts showed antibacterial activity against most of the tested bacteria mainly at higher concentrations. The ethanol extract of root exhibited moderate to feeble inhibition against all test bacteria with maximum against *B. cereus* (17 mm) and minimum against *K. pneumonia* (8.5 mm) at highest concentration (40 mg/ml). The activities decreased with decrease in concentration. This extract was found to possess maximum concentration [12].

In this study the highest zone of inhibition has been observed in the methanolic extract of *Pongamia pinnata* and *Acacia catechu* of 14mm and 16mm, 15mm and 17mm against both the strains of *S. mutans* MTCC 497 & MTCC 890 at concentration of 200µg/ml. And the lowest zone of inhibition has been observed in the extract of *Putranjiva roxburghii* and of 6mm against both only one strain of *S. mutans* MTCC 890 at concentration of 200µg/ml.

5. Conclusion

The conclusion of the study may prove that the methanolic extracts of as *Acacia catechu, Pongamia pinnata, Putranjiva roxburghii, Zanthoxylum armatum,* and *Zanthoxylum nitidum* are effective against both the strains of *Streptococcus mutans* (MTCC 497& MTCC 890). *Acacia catechu, Pongamia pinnata* are good bacterial inhibitor extracts. It recommended to mixing those plant extracts having potent antibacterial (anti-*S. mutans*) with tooth pastes.

6. References

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