

COMPARATIVE STUDIES ON ANTIMICROBIAL EFFICACY OF *Punica granatum* Linn. AND *Mimusops elengi* Linn. AGAINST SOME COMMON ORAL PATHOGENS

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Abstract: Dental caries, biofilms of microorganisms on tooth surface and dental plaques lead to the development of many oral and periodontal diseases. The aim of this study was to evaluate the antimicrobial efficacy of *Punica granatum* and *Mimusops elengi* extracts against some common oral pathogens like *Streptococcus mutans*, *Lactobacillus fermentum* and *Pseudomonas aeruginosa*. Ethanolic, methanolic and acetone extracts were prepared using Soxhlet apparatus and rotaevaporator and were examined against some of the oral pathogens by disc diffusion method and mean of diameters of inhibition zone of each bacterium in different dilutions was reported. The result indicated that the extracts obtained from *P. granatum* exhibited antimicrobial activity against all organisms. Among all three bacterial strains examined, it was more effective against *P. aeruginosa*, less effective against *L. fermentum* and least effective against *S. mutans* in different extracts of methanol, ethanol and acetone. Further research could study the antimicrobial efficacy of this herb against other pathogens in greater depth with more possible useful results. Another herb, a common Indian native plant is *Mimusops elengi* Linn, for a long time in the traditional ayurveda system of medicine, *Mimusops* has been described and used for its potential medicinal values. All the different parts of this plant such as leaves, twig, bark, stem, root, fruits, seeds, and flowers are used in different concentration or in combination with other plant materials to improve oral health. Ethanolic extract of bark shows anti-inflammatory, analgesic as well as antipyretic effect. The phytochemical agents such as Lupeol, saponin present in the bark show anti-inflammatory activity and tannins show antibacterial as well as antioxidant properties. The extract of bark in different materials has shown significant antioxidant potential and scavenging property. The ethanolic, methanolic, acetone, petroleum and water extract of bark showed antibacterial property against different strains of *Streptococcus* species, *Staphylococcus* species and against *E. fecalis*. This plant should be explored and further research work should be carried out in periodontal field for new valuable drug formulation, which will be economically more suitable to the majority of populations in developing countries.

Keywords: Dental caries, dental plaque, *Punica granatum*, *Mimusops elengi*, disc-diffusion, *Streptococcus mutans*, *Lactobacillus fermentum*, *Pseudomonas aeruginosa*.

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Introduction

Oral diseases are major health problems with dental caries and periodontal diseases among the most prominent global infectious diseases. These dental problems are most common in the human communities and is prominent among children and adolescents (Gonzalves W., 2008). The general quality of life is badly affected by poor Oral health and is linked to chronic conditions and systemic diseases. The development of dental caries involves mainly two groups of bacteria- Gram positive bacteria like *Streptococcus mutans* & *Lactobacillus fermentum* and Gram negative bacteria like *Pseudomonas aeruginosa*. Although antibiotics are used for treating dental problems but microorganisms develop a resistance to withstand the effects of an antibiotic. Therefore a renewed effort is needed to be made to seek antibacterial agents effective against pathogenic bacteria. This study was conducted to check the antibacterial effect of *Punica granatum* and *Mimusops elengi* against some of the dental bacterial flora as well as to make comparative studies.

Punica granatum Linn (Pomegranate) belongs to family puniceae, has long been recognized as food as well as medicine especially for the treatment of like periodontal diseases like dental caries and dental plaques. Gastro-Intestinal (GI) diseases. Pomegranate is a fruit of great antiquity and is known to have been cultivated in the Middle East more than 5,000 years ago. The plant is found all over India. Pomegranate has been considered important since prehistoric times as an agency of longevity (Ram, 1998). Besides a remedy for dental problems, the fruit is good for dysentery, diarrhoea and gastralgia (Warrier, *et al.*, 2002). Also *Mimusops* has been described and used for its potential medicinal values. All the different parts. Ethanolic extract of bark shows anti-inflammatory, analgesic as well as antipyretic effect (Manjeshwar *et al.*, 2011, Kalitha *et al.* 2004). The phytochemical agents such as Lupeol, saponin (Phogat M. *et al.* 2014.) present in the bark show anti-inflammatory activity and tannins show antibacterial as well as antioxidant properties of this plant such as leaves, twig, bark, stem, root, fruits, seeds, and flowers are used in different concentration or in combination with other plant materials to improve oral health (Gami *et al.* 2010).

MATERIALS & METHODS

Selection of Bacterial Strains

Bacterial strains of different species (*Streptococcus species*, *Lactobacillus fermentum* *Pseudomonas aeruginosa*, *Staphylococcus* and *Candida albicans*) with enhancing activity in caries formation were selected from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology(MTCC), Chandigarh.

Collection of Medicinal Plants

The medicinal plant samples were collected from the Ranchi region of Jharkhand as well as from south Indian region (Bangaluru). The different parts such as leaves and inflorescence of *Punica granatum* were selected for testing its antibacterial effect and characterization of secondary metabolites of effective ones. All the different parts of *Mimusops elengi* such as leaves, twig, bark, stem, root, fruits, seeds, and flowers are used in different concentration or in combination with other plant materials to improve oral health.

Preparation of Plant Extracts

The air dried plant parts were powdered properly and extracts were prepared in different solvents systems (acetone, ethanol, chloroform, methanol and water) using a soxhlet apparatus and rotaevaporatory system. All extracts were kept in -20 degree centigrade. Before starting the antimicrobial assay, extracts were soluted in distilled water in proportion of 1/20% weight volume (w/v), this was the maximum concentration that could pass through the Millipore filter (30 mm in diameter), and then each solution was sterilized by the filter, gathered in sterilized tubes and kept in -20 degree centigrade.

Antibacterial Effect Checking of Medicinal Plant Extracts

Antibacterial effect of medicinal plant extracts were checked by Disc- diffusion method.

Disc Diffusion Method

The bacterial isolates were effectively swabbed on the prepared agar plates. After allowing the inoculums to dry at room temperature, four mm diameter sterile blank paper discs were aseptically put on agar surfaces and immediately impregnated with different dilutions of extracts. The first blank disc was impregnated with 100% w/v of the extracts and the other with 80, 40 and 20% w/v, respectively. Plates were incubated for 18 to 24 h, then the zones of inhibition measured and the average of diameters noted.

RESULTS AND DISCUSSION

Antimicrobial effect of *Punica granatum* *Mimusops elengi* extracts

The antimicrobial activity of *Punica granatum* acetone and methanol extracts against bacterial isolates (*Strptococcus mutans*, *Lactobacillus fermentum* and *Pseudomonas aeruginosa*) were tabulated using disc diffusion method. Punica had the most antibacterial activity at 100% w/vl. Also, it was observed that the extracts prepared from flowers gave better results than the leaves.

Prabhat et al in 2010 compared the petroleum ether, acetone, methanol and water extracts of bark, which were tested for their antimicrobial effect against five oral microorganisms

Staphylococcus aureus, *Streptococcus mutans*, *Streptococcus sanguis*, *Streptococcus salivarius*, *Lactobacillus acidophilus* and *Candida albicans*. Methanolic and water extract were found to be more effective against all strains. The Acetone and ethenol extracts of bark were tested in children for their antibacterial effect against salivary microflora by “paper disc diffusion method”. The extracts were found to be effective against different *Staphylococcus* species.

Different extracts of bark such as petroleum ether, benzene, chloroform, acetone, methanol and water (Mistry et al 2014) by saxchlation process have been prepared and were examined against gram positive and gram negative bacteria of dental plaque and calculus by ditch plate method. The best result was obtained by chloroform extract. Kulkarni AA et al. in their study stated, that aqueous and acetone extracts of bark act against salivary microflora, which was evaluated by paper disc diffusion method. The results were not significantly different for both the extracts, although extracts were active against the pathogen.

Table 1: Zone of inhibition (mm) of different extracts of *Punica granatum* against oral bacteria

Test organism	Acetone extract (leaves)	Methanol extract (leaves)	Acetone extract (flowers)	Methanol extract (flowers)
<i>Streptococcus mutans</i>	10	9.5	12	11
<i>Lactobacillus fermentum</i>	8	9	9	8.5
<i>Pseudomonas aeruginosa</i>	13	12	16	14

Conclusion

From the table, it is clear that extracts prepared from flowers showed better results than those of leaves. Also, acetone extracts had more antibacterial effect than the methanol extracts. This may be because acetone has better extracting capacity over methanol which may be attributed to the ability to extract the natural antimicrobial compounds such as alkaloids, flavanoids, terpenoids and phenolic compounds from the plant. The antibiotic activity of the extract of *P.granatum* is associated to tannin phytoconstituents and alkaloids found in leaves, roots, stems and fruits (Silva et al., 1991); there is a growing interest in using tannins as antimicrobial agents in caries prevention (Scalbert, 1991).The antimicrobial activity of *P granatum* has been widely investigated (Menezes et al., 2006; Pereira et al., 2006). Methanolic and acetone extracts showed strong antimicrobial activity in different

investigations done on both gram-positive and gram-negative non-oral bacteria (Haghighati et al., 2003; Machado Thelma et al., 2002).

Mimusops elengi Linn., on the other hand, has shown so many medicinal as well as periodontal properties. It has also been found to be more effective against various species of oral pathogens (Malhotra et al in 2011). Despite these studies and its easy availability in the society, it has not been extensively used in periodontal field (Rao KS, 2011). All the parts of the plant have been found useful with their medicinal value, but this plant should be explored more and further research work should be carried out in dental and periodontal field for new valuable and effective drug formulation, which will be economically and easily more suitable to the majority of populations in southern Asiatic countries.

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