Research Paper

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Antimicrobial Efficacy of *Punica Granatum* Against Some Common Oral Pathogens

Botany

KEYWORDS : Dental caries, dental plaque, *Punica granatum,* disc-diffusion, *Streptococcus mutans, Lactobacillus fermentum, Pseudomonas aeruginosa*

Preety Prasad — Research Scholar, Botany Dept. Head, St. Xavier's College Ranchi

Ajay Kumar Srivastava — Botany Dept. Head, St. Xavier's College Ranchi,

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 extracts against some common oral pathogens like Streptococcus mutans, Lactobacillus fermentum and Pseudomonas aeruginosa. Ethanolic, methanolic and acetone extracts were prepared using soxhelet apparatus and rotaevaporator and were examined against the three selected 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.

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 (Jurenka, 2008.) against some of the dental bacterial flora.

Punica granatum Linn (Pomegranate) belongs to family Punicaceae, 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 antiguity 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).

MATERIALS & METHODS

Selection of Bacterial Strains

Bacterial strains of two different species (*Streptococcus mutans, Lactobacillus fermentum* and *Pseudomonas aeruginosa*) 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 sample was collected from the Ranchi region of Jharkhand. 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 (Nadkarni et al. 2000) .

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 soxhelet apparatus and rotaevaporatory system (Blatter et al. 2001). All extracts were kept at -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* 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.

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

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

Conclusion

From the table, it is clear that extracts prepared from flowers showed better results than those of leaves (Prashanth, 2001.). 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, terpinoids 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).

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