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EVALUATION OF ANTIMICROBIAL PROPERTY OF EXTRACT OF *PUNICA GRANATUM* (L.) ON ORAL PATHOGENS

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ABSTRACT

The aim of the present research was the examination of the potential activity of various organic solvent extracts of *Punica granatum* on the important microbiota harbouring in the oral cavity. The dried peels and seeds of *P. granatum* were extracted using chloroform, methanol and n-butanol and their potential activity were studied. The inhibitory effect of crude extracts were observed on various microbial plates which were incubated with different bacterial species in terms of zone of inhibition as mm in diameter. The data was analysed by One Way Analysis of Variance for significance. The peel extract was more potent in inhibiting the microbial population growth as compared to seed extract. While all the four solvents were effective in case of peel extracts, the bioactivity was much higher in species such as *Streptococcus mutants*, *Streptococcus sanguis*, *Staphylococcus aureus*, *Lactobacillus acidophilus* and *Actinomyces viscosus*, among 8 species evaluated in the present investigation. The peel extract of *P. granatum* contains active biomolecule(s) against important oral microbial pathogens and thus holds promise as a potential bioactive molecule against Dental Caries. Based on the bioactivity analysis, the peel extract of *P. granatum* may further be subjected to purification and characterization of the bioactive compound(s) for regulating oral microbial contamination.

KEYWORDS: ***Punica granatum, peel extract, antimicrobial activity and zone of inhibition.***

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INTRODUCTION

Polymicrobial flora consisting of bacteria and yeast communities are primary causatives of dental caries and related oral diseases. The oral pathogens play a vital role in fermenting sugars into acidic metabolites which leads to mineralization of enamel. The bacteria and yeast such as *Streptococcus sp.*, *Staphylococcus sp.*, *Lactobacillus sp.* and *Candida sp.* are found to be the dominant oral pathogens. There has always been a search for the replacement of natural therapeutic drugs to treat the various health hazards. Pomegranate (*Punica granatum*) is known for its medical properties, since it is popularly used in various medical therapies. According to Greek mythology the fruit represents regeneration. *Punica granatum* belongs to the genus *Punica* and family *Lythraceae*.[1] Extensive studies have been conducted on the antioxidant, anti-inflammatory and anti-carcinogenic properties of pomegranate extracts in addition to prevention from diabetic, neuronal and cardiovascular diseases.[2] The active components in *P. granatum* include ellagitannins, punicic acid, flavanoids and anthocyanins. The seed of *P. granatum* also contains anthocyanins, glucose, ascorbic acid, catechins, gallic acid and caffeic acid. The peel contains phenoic punicalagin.[3] *Punica granatum* exhibits antibacterial action through bacteriostatic and bacteriocidal effect.[4] Research has also proved that the presence of ellagitannins and punicalagin is to be responsible for the antibacterial effect and the peel is often reported to be more effective than the seed extract.[5-6] However, studies related to antimicrobial activity of the *P. granatum* extract on most common human oral pathogenic microbes which persist in the oral cavity are scanty. In this study, an attempt has been made to evaluate the antimicrobial activity of seed and peel extracts of *P. granatum* on some common human oral microbial pathogenic species.

MATERIALS AND METHODS

Preparation of extracts

Fresh *P. granatum* (500 g) were cleaned with water and air dried. The organic solvent extracts were prepared by following a standard protocol of Abegunde and Ayodele-Oduola.[6] Briefly, the peels and seeds were manually separated and shade dried. The dried samples were powdered (250 g each) and were soaked in ethyl acetate 100 ml for 24 h. The extract obtained was filtered with Whatmann filter paper No.1 and the filtrate was evaporated in vacuum to give a residue of ethyl acetate extract (1 g). This procedure was repeated successively with chloroform, methanol and n-butanol to yield chloroform extract of 0.75 g, methanolic extract of 0.6 g and butanolic extract of 0.212 g, respectively.

In vitro antimicrobial activity

The agar well-diffusion method was carried out to evaluate the inhibitory activity spectra of different extracts against test bacterial strains.[7] Lag phase culture 100 µL was diluted (106 colony forming units, CFU) and was spread onto surface of media (*Mueller Hinton Agar* –MHA).Wells (5 mm in diameter) were made in media using a sterile borer and were filled with 50 µl of crude extracts. Bacterial plates were incubated at 37°C for 24–48 h, until growth of test microorganisms was evident in control plates. The zones of inhibition (including well diameter) around wells were measured in millimetre (mm) using Himedia inhibition scale. The antimicrobial activity was expressed as the diameter of inhibition zones produced by the respective extracts against test microorganisms, such as *Streptococcus mutants* MTCC 497, *Streptococcus sanguis* MTCC 442, *Streptococcus salivarius* MTCC 1938, *Streptococcus mitis* MTCC 2695, *Staphylococcus aureus* MTCC 87, *Staphylococcus epidermidis* MTCC435, *Lactobacillus acidophilus* MTCC 447 and *Actinomyces viscosus* MTCC 7345. Ciproflaxacin (10 µg/ml) was used as control. The experiment was repeated in triplicates by following a standard protocol of Abegunde and Ayodele-Oduola.[6] The data on bioactivity were tested by One Way Analysis of Variance.

RESULTS

The present study clearly shows a significant antimicrobial activity exerted by the seed and peel extracts of *P. granatum* (Tables 1 and 2). Overall a remarkable degree of antimicrobial property was noticed in both seed and peel extracts. However, the extract from peel showed broader antimicrobial activity as compared to the extracts of the seed. Similarly the bioactivity testing was also much higher in case of peel extracts as compared to seed extracts. Although the ethyl acetate and butanolic extracts from seeds did not show any antimicrobial activity, the chloroform and methanolic extracts of seeds indicated considerable antimicrobial property (Table 1). All the eight species examined in the present investigation were totally inactive to the seed extract obtained using ethyl acetate and n-

butanol. While the species like *Streptococcus mutants* MTCC 497, *Streptococcus sanguis* MTCC 442 and *Streptococcus salivarius* MTCC 1938 were sensitive showing moderate inhibition ($p< 0.05$) to the seed extract of chloroform among the eight species tested, *Staphylococcus epidermidis* MTCC 435 alone was sensitive and showed similar level of inhibition ($p< 0.05$) in case of methanolic extract. The results of this investigation showed that peel extract have yielded better results in bioactivity testing as compared to seed extracts (Table 2). All the four solvents (ethyl acetate, n-butanol, chloroform and methanol) extracts showed very high degree of ($p< 0.01$) antimicrobial property. Among the eight species tested, the three species (*Streptococcus salivarius* MTCC 1938, *Streptococcus mitis* MTCC 2695 and *Staphylococcus epidermidis* MTCC 435) were mildly resistant at very high level ($p< 0.05$) to the peel extract. The peel extracts obtained using ethyl acetate, n-butanol, chloroform and methanol had a greater inhibitory ($p< 0.01$) impact on the species such as *Streptococcus mutants* MTCC 497, *Streptococcus sanguis* MTCC 442, *Staphylococcus aureus* MTCC 87, *Lactobacillus acidophilus* MTCC 447 and *Actinomyces viscosus* MTCC 7345.

Table 1
Efficacy of seed extract of Punica granatum on various oral microbial strains by well diffusion method

Microbial strains	Inhibition of various organic solvents extracts (mm)				
	Ciprofloxacin	Ethyl acetate	n-Butanol	Chloroform	Methanol
Streptococcus mutants MTCC 497	20.5±0.4	ND	ND	10.9±0.6*	ND
Streptococcus sanguis MTCC 442	20.2±0.6	ND	ND	6.4±0.4*	ND
Streptococcus salivarius MTCC 1938	20.0±0.7	ND	ND	6.7±0.3*	ND
Streptococcus mitis MTCC 2695	20.5±0.5	ND	ND	ND	ND
Staphylococcus aureus MTCC 87	20.7±0.3	ND	ND	ND	ND
Staphylococcus epidermidis MTCC 435	20.1±0.5	ND	ND	ND	8.7±0.5*
Lactobacillus acidophilus MTCC 447	19.4±0.6	ND	ND	ND	ND
Actinomyces viscosus MTCC 7345	18.8±0.4	ND	ND	ND	ND

(Values are Mean ± S.E.M.; N = 3; *p< 0.05).

Table 2
Efficacy of peel extract of Punica granatum on various oral microbial strains by well diffusion method

Microbial strains	Inhibition of various organic solvents extracts (mm)				
	Ciprofloxacin	Ethyl acetate	n-Butanol	Chloroform	Methanol
Streptococcus mutants MTCC 497	20.5±0.5	15.0±0.5**	14.9±0.6**	18.5±0.4**	17.7±0.4**
*Streptococcus sanguis*MTCC 442	20.1±0.4	16.0±0.4**	16.7±0.4**	18.8±0.3**	14.9±0.5**
Streptococcus salivarius MTCC 1938	20.2±0.6	ND	ND	ND	ND
Streptococcus mitis MTCC 2695	20.2±0.3	ND	ND	ND	ND
Staphylococcus aureus MTCC 87	20.3±0.4	20.0±0.2**	12.8±0.3**	22.6±0.4**	16.4±0.6**
Staphylococcus epidermidis MTCC 435	20.1±0.5	ND	ND	ND	ND
Lactobacillus acidophilus MTCC 447	19.6±0.6	14.4±0.2**	11.2±0.4**	12.1±0.4**	10.3±0.7**
*Actinomyces viscosus*MTCC 7345	18.9±0.5	18.7±0.3**	17.7±0.3**	20.3±0.3**	16.2±0.4**

(Values are Mean ± S.E.M.; N = 3; **p< 0.05).

DISCUSSION

The substitution of natural therapeutic agent for chemically formulated drugs to achieve the inhibition of pathogenicity of a particular pathogen through a specific mechanism is the most important aspect of current research. The provocation of certain molecular complex in a particular herb through various pharmacological constituent activation methods is a widely used technology to check for the effect of the herb on the activity of the various pathogens. Most common commensals present in human oral cavity were selected in the study. In the present investigation, the seed of *P. granatum* did not show wide spectrum anti-microbial property among 8 different bacterial species examined. Considering the 4 different organic solvents employed, the extract derived from chloroform and methanol were moderately inhibited the microbial growth. The inhibitory activity was also very selective against only to the species such as *Streptococcus mutants* MTCC 497, *Streptococcus sanguis* MTCC 442 and *Streptococcus salivarius* MTCC 1938 for the extract derived from chloroform. Similar trend was also noticed in the case of *Staphylococcus epidermidis* MTCC 435 when exposed to methanoloic extract. It has also been well documented that the *P. granatum* peel extract action against S. aureus and *Pseudomonas aeruginosa* as a potent antimicrobial agent.[8] The pericarp extract of *P. granatum* possesses strong antibacterial activity against the multiple resistance of *Salmonella typhi*.[9] *P. granatum* is a well-known fruit for its therapeutic properties which has been evaluated due to its antimutagenic, antioxidant, anticancer antimicrobial, anti-inflammatory, antiatherosclerotic and antihypertensive effects.[10,11] In oral cavity *P. granatum* extract has positive therapeutic effect in reducing gingival bleeding in periodontal disease), recurrent aphthous stomatitis.[12, 13] Our results indicate that all the 4 different solvent systems examined in the present investigation were very effective and exerted a wide spectrum antimicrobial property. Among the 8 species examined *Streptococcus salivarius* MTCC 1938, *Streptococcus mitis* MTCC 2695 and *Staphylococcus epidermidis* MTCC 435 were only resistant and did not show any inhibitory activity. In this study, an attempt was also made to employ various solvents with different polarity indices and the expected order efficacy in terms of polar indices of the solvents used was methanol > ethyl acetate > chloroform > n-butanol. Overall the peel extract showed high efficacy with wide spectrum activity as compared with seed extract in inhibiting the various oral microbial strains. It may be due to the high level of anti-microbial compounds present in the peel than the seed of *P. granatum*. In the case of seed, a moderate inhibition was only possible by chloroform and methanolic extracts because of their high polarity index. However, this moderate inhibition was observed only in four pathogenic microbial strains *viz. Streptococcus mutants, Streptococcus sanguis, Streptococcus salivarius* and *Staphylococcus epidermidis.* In case of peel extract of *P. granatum*, *Streptococcus mutants, Streptococcus sanguis, Streptococcus aureus, Lactobacillus acidophilus* and *Actinomyces viscosus* showed highly significant inhibition irrespective of various solvents of different polarity indices used. However, the growth of species such as *Streptococcus salivarius*, *Streptococcus mitis* and *Staphylococcus epidermidis* was not at all inhibited by the peel extract of *P. granatum*, which may be due to high resistance to the anti-microbials present in the peel extract. The effect of peel as an antibacterial agent is better than the seed due to the presence of active components of tannins and polyphenols (punicalain and gallic acid.[14] that has its own toxicity and molecular structure. Tannins also carry out this activity by precipitating the cell wall and cell membrane.[14-15]Antimicrobial activity can also be due to the suppression of glycosyltransferase.[15,16] Previous researches have also demonstrated the antibacterial effect of gallic acid at low concentrations against tested sensitive strains.[16,17] *P. granatum* extract was used to regulate the adherence of various microorganisms present in the oral cavity and it was effective against the various species like *S. mutans*, *S. mitis* and *C. albicans.*[14] It has also been demonstrated to reduce carcinogenic bacterial metabolites responsible for tooth decay by using the peel extract of *P. granatum*.[18] Our study also supports the earlier findings that *P. granatum* is very rich in antimicrobial constituents and reiterates further research on the purification and characterization of various compounds in addition to their mode of action.

CONCLUSION

The peel extract obtained using different organic solvents of *P. granatum* is more effective than the respective seed extract. Although all organic solvents obtained from peel of *P. granatum* showed wide spectrum antimicrobial activity, the ethyl

acetate and chloroform extracts of peel showed significantly higher activity.

Clinical significance

The most neglected peel of *P. granatum* possesses interesting and novel biomolecules which could be further explored through purification and characterization for antimicrobial activity against many major microbial pathogens harbouring in the human oral cavity.

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AUTHOR CONTRIBUTION STATEMENT

The authors contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

CONFLICT OF INTEREST

Conflict of interest is declared none.

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