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Research Article

ASSESSMENT AND COMPARISON OF THE ANTIBACTERIAL ACTIVITY OF PUNICA GRANATUM WITH CHLORHEXIDINE AGAINST PORPHYROMONAS GINGIVALIS, PREVOTELLA INTERMEDIA, AGGREGATIBACTER ACTINOMYCETEMCOMITANS: AN IN VITRO STUDY

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ABSTRACT

AIM: to assess and compare the antibacterial activity of punicagranatum extract with chlorhexidine gluconate 0.2% against prevotella intermedia, porphyromonas gingivalis and aggregatibacter actinomycetemcomitans

Materials and Method: In this in vitro study antibacterial activity of punica granatum extract was assessed on subgingival plaque samples which were collected from 30 subjects from which periodontal pathogens were isolated and incubated which were then treated with punica granatum extract (group 1) and chlorhexidine gluconate (group 2) for 10, 30 and 60 minutes respectively and incubated to check the zone of inhibition.

Results: punica granatum extract in the present study was found to be equally effective as chlorhexidine gluconate 0.2% and no significant difference was observed in the zone of inhibition between the two. Also, as the duration of exposure of plaque samples to punica granatum extract in vitro increased, the number of bacterial colonies reduced.

Conclusion: from the findings of the present study it may be concluded that punica granatum extract is effective against major periodontal pathogens.

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INTRODUCTION

Periodontal diseases are chronic bacterial infections that lead to gingival inflammation, periodontal tissue destruction, and alveolar bone loss. Acting as powerful allies in the fight against periodontal diseases, natural compounds can help safeguard against lethal age related diseases that emanate from our mouth.

Periodontal tissue destruction is a result of host immune-inflammatory process triggered by specific bacteria contained within the complex microbial plaque film.¹ Large number and variety of microorganism particularly gram negative bacteria find a suitable environment in periodontal pocket for their survival and proliferation, such as *Porphyromonas gingivalis*, *aggregatibacter actinomycetemcomitans* and *prevotella intermedia*.

The main therapeutic strategies of periodontal treatment are mechanical debridement by scaling and root planning, and periodontal surgery to remove dental plaque, and hopefully all

the associated bacteria at the sites of infection .However, one of the major problems associated with surgical procedure and wound healing processes is the potential risk of bacterial contamination and infection.² Such bacterial infection may impair wound healing and tissue regeneration, thus posing a great threat to the success of periodontal surgery. Hence, control of infection is therefore a prerequisite to successful wound healing and to avoid chronic state.

Antimicrobials of plant origin have numerous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. The beneficial effects of plant materials typically results from the combination of secondary metabolites such as alkanoids, steroids, tannins, and phenol compounds, which are capable of producing definite physiological action on the body.³

Punica granatum (Pomegranate) is an ancient fruit known for its multiple health benefits , including antimicrobial activity

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The fruit and its extracts, may serve as a natural alternatives due to their potency against a wide range of bacterial and viral pathogens. Every part of the plant has been tested for antimicrobial activities, including megranate peel/fruit juice, peel, arils, flowers, and bark.⁴ The major class of pomegranate phytochemicals is the polyphenols which includes flavonoids, condensed tannins and hydrolysable tannins. HTs are predominant polyphenols found in pomegranate juice and account for 92% of its antioxidant activity.

Hence our study aimed to focus on assessing and comparing the antibacterial activity of punica granatum extract with chlorhexidine against three periodontopathic pathogens.

MATERIALS AND METHODS

The present in vitro project was undertaken to study the efficacy of punica granatum pure extract in comparison with chlorhexidine gluconate (0.2%) against the periodontal pathogens.

The study was conducted in the Department of Periodontics KAHE's KLE Vishwanath Katti Institute of Dental Sciences and KLE's Dr. Prabhakar Kore Basic Science Research Centre Belagavi

Extraction procedure

Ripened punica granatum were obtained from local markets. The peels and seeds were manually separated. the seeds were washed with water for removal of adhering material.

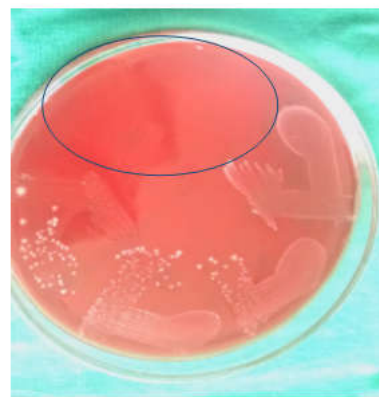
The seeds were grounded and the juice was extracted. The juice was then filtered using muslin cloth. The undiluted form of juice was used to check the antibacterial efficacy.



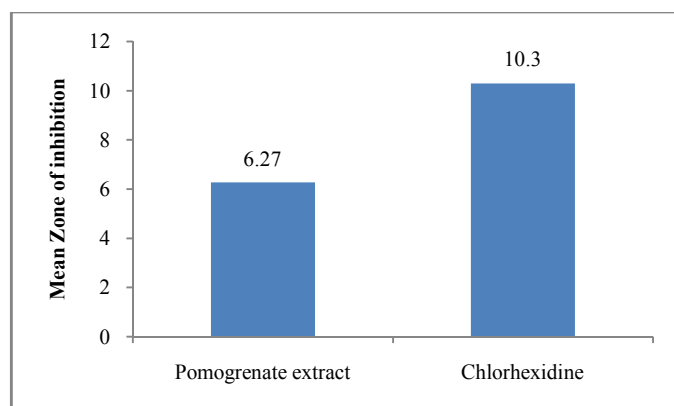
Fresh fruit juice extracted



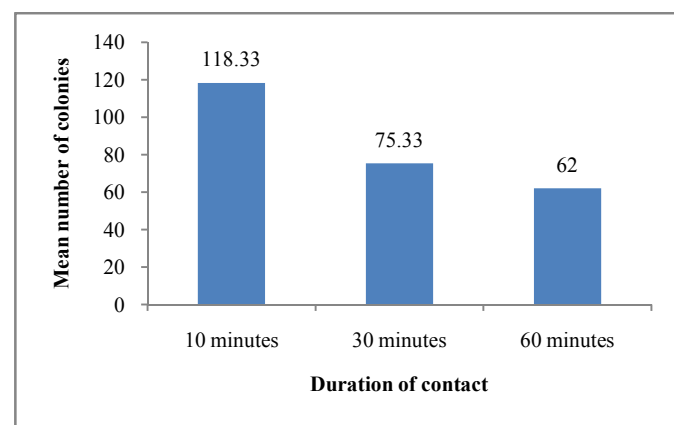
Filtration of juice done using filter paper



Minimum bactericidal concentration



Graph 1 Comparative assessment of zone of inhibition among two study group



Graph 2 Comparative assessment of mean number of colonies of micrororganisms according to duration of contact with extract before incubation

DISCUSSION

Pomegranates (*Punica granatum* L.) have a long history of antibacterial use dating back to biblical times. Egyptians used pomegranates to treat a number of different infections. It was utilized as a traditional remedy for thousands of years under the Ayurvedic system of medicine. Over the years there have been many small studies undertaken in different areas of the world on the bactericidal effects of pomegranates on a number of highly pathogenic and drug-resistant strains.

It has been shown that pomegranate contains agents, especially polyphenolic flavonoids, with pharmacological actions that could be considered conducive to good oral health, particularly in relation to gingivitis development. It has been suggested that reduction of oxidative stress, direct antioxidative activity, anti-

inflammatory effects, antibacterial activity, and direct removal of plaque from the teeth are the mechanisms involved in this action peel extract using *in vivo* models.

Effectiveness of the extract of pomegranate in inhibiting prostaglandin and leukotriene formation through inhibition of the eicosanoids enzymes, cyclooxygenase and lipoxygenase, raises the possibility of use of pomegranate derivatives in anti-inflammatory preparations. Abdollahzadeh *et al* (2011) assessed the effects of three different concentrations of a methanolic pomegranate peel extract at 4 mg/mL, 8 mg/mL, and 12 mg/mL on growth of dental bacteria.⁵ All concentrations of the pomegranate extract had antibacterial activity against *S. aureus* and *S. epidermidis*. Extract concentrations of 8 mg/mL and 12 mg/mL were effective against *L. acidophilus*, *S. mutans*, and *S. salivarius*. The extract did not inhibit *Actinomyces viscosus*. In another similar *in vitro* study, ethanol and water extracts of pomegranate both had inhibitory effects against *S. mutans* and *Porphyromonas gingivalis* (*P. gingivalis*) Brazilian *in vitro* study investigated the antimicrobial effect of a pomegranate-based oral gel (made from an extract of dried peel combined with Carbopol, water, and triethanolamine) against *Streptococcus sanguis*, *Streptococcus mitis*, and *S. mutans*. Several clinical trials have also explored the effectiveness of pomegranate peel extract rinses on reductions in oral plaque. Numerous *in vitro* and *in vivo* studies evidenced the efficacy of pomegranate peel extracts but till date not many studies have been conducted to assess the effectiveness of pomegranate fruit seed extract.

The present study was conducted to assess the *in vitro* effectiveness of pomegranate fruit seed extract against periodontal pathogens and to compare its efficacy with chlorhexidine.

Plaque samples were collected from 30 study subjects from which periodontal pathogens were isolated and incubated which were then treated with Pomegranate extract and chlorhexidine for 10, 30 and 60 minutes respectively and incubated. The study population was majorly of middle age group (Mean age: 41.13 ± 7.06) with most (36.7%) of them depicting *Porphyromonas gingivalis* in their plaque samples. The fair and poor plaque index status and moderate to severe gingivitis of the subjects suggest poor oral hygiene of the study population.

Present study revealed a significant difference ($p=0.001$) in the mean zone of inhibition of Pomegrenate extract (6.27 ± 6.94) and Chlorhexidine (10.3 ± 10.52). According to this finding, Pomegrenate fruit seed extract was found to be less effective than chlorhexidine mouthwash. This is in agreement with the study of Ahuja *et al.* (2011) in which pomegranate mouthwash was compared with chlorhexidine mouthwash.⁶ Pomegranate was effective in reducing plaque but not to the extent that chlorhexidine was. Also in an *in vitro* study conducted by Haffajee AD *et al* (2008), it was revealed that Pomegrenate extract exhibited less potency than chlorhexidine collaborating with the findings of our study.⁷ The present study also evidenced the association of number of bacterial colonies with the duration of exposure of plaque samples to pomegrenate extract *in vitro*. The mean number of colonies shown by plaque samples with 10, 30 and 60 minutes of exposure were 118.33 ± 66.2 , 75.33 ± 61.3 , and 62 ± 62.9 respectively. With increase in

duration of exposure there was a decrease in number of bacterial colonies which may be attributed to the increase in bacteriostatic or bactericidal activity of the extract with increase in exposure. There are numerous studies in literature depicting the antiplaque efficacy of plant extracts but to our knowledge none of the studies have assessed the antibacterial activity of extract according to duration of exposure. However, this finding is in collaboration with increase in antiplaque and antigingivitis efficiency of mouthrinses with increase in duration of usage. Singla *et al* (2017) observed in an *in vivo* study that there was a decrease in colony forming units when compared at the end of 48 hours and 7 days after using Pomegrenate mouthwash.

A study conducted at the Human Nutrition Center at Ohio State University in 2007 examined the effects of using a mouthwash containing pomegranate extract on the risk of gingivitis. Investigators noted that pomegranate's active components, including polyphenolic flavonoids (e.g., punicalagins and ellagic acid), are believed to prevent gingivitis through a number of mechanisms including reduction of oxidative stress in the oral cavity) direct antioxidant activity; antiinflammatory effects and direct removal of plaque from the teeth. They also noted that a published pilot study has already shown that pomegranate extract can reduce the clinical signs of chronic periodontitis. Hence the present study suggests the conduction of more *in vitro* and *in vivo* studies to assess the effectiveness of Pomegrenate fruit seed extract against periodontal pathogens. Though Pomegrenate fruit seed extract was not found to be as effective as chlorhexidine but further research comparing the efficacy of extracts prepared from various parts of Pomegrenate plant like peel, leaves, fruits, etc. is recommended.

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