(International Print/Online Journal)

SJIF IMPACT FACTOR: 6.829
PUBMED-National Library of Medicine ID-101739732

ISSN (Print): 2209-2870
ISSN (Online): 2209-2862

International Journal of Medical Science and Current Research (IJMSCR)
Available online at: www.ijmscr.com
Volume 9, Issue 2 , Page No: 135-142
March-April 2026

IJMSCR

Antimicrobial Efficiency of Pomegranate and Jasmine Leaf Extract on Streptococcus mutans: An In Vitro Study

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Type of Publication: Original Research Paper
Conflicts of Interest: Nil

Abstract

Background:

Dental caries remains one of the most common chronic infectious diseases affecting individuals worldwide and is a major public health concern. Among the various microorganisms associated with the initiation and progression of dental caries, *Streptococcus mutans* plays a crucial role due to its ability to adhere to tooth surfaces, produce extracellular polysaccharides, and generate organic acids that cause enamel demineralization.The aim of the present study was to evaluate and compare the antimicrobial efficacy of pomegranate (*Punica granatum*) fruit juice extract and jasmine (*Jasminum officinale*) leaf extract against *Streptococcus mutans* using standard in vitro microbiological methods.

Materials and Methods:

Fresh pomegranate juice extract and jasmine leaf extract were prepared under sterile conditions using conventional aqueous extraction techniques. The antimicrobial activity of the extracts was evaluated against *Streptococcus mutans* using the agar well diffusion method and minimum inhibitory concentration (MIC) determination by broth dilution. Extract concentrations of 25%, 50%, and 75% were tested. Chlorhexidine gluconate 0.2% was used as a positive control, while distilled water served as a negative control. The zones of inhibition were measured in millimeters, and MIC values were determined as the lowest concentration inhibiting visible bacterial growth.

Results:

Both pomegranate and jasmine leaf extracts demonstrated antimicrobial activity against *Streptococcus mutans* in a concentration-dependent manner. Pomegranate extract consistently showed greater antimicrobial efficacy compared to jasmine leaf extract at all tested concentrations. The highest antimicrobial effect was observed with 75% pomegranate extract, which produced a mean inhibition zone of 17.8 ± 1.1 mm. The MIC values were 125 µg/mL for pomegranate extract and 250 µg/mL for jasmine leaf extract, indicating greater potency of pomegranate. Chlorhexidine demonstrated the highest antimicrobial activity among all tested agents.

Conclusion:

Within the limitations of this in vitro study, both pomegranate and jasmine leaf extracts exhibited significant

antimicrobial activity against *Streptococcus mutans*. Pomegranate extract showed superior efficacy compared to jasmine leaf extract. These findings suggest that these herbal extracts, particularly pomegranate, have potential applications as natural antimicrobial agents in preventive oral healthcare products such as mouthwashes and dentifrices.

Keywords: Streptococcus mutans, pomegranate, jasmine leaf, herbal extract, antimicrobial activity, dental caries

Introduction

Dental caries is one of the most prevalent chronic infectious diseases affecting individuals of all age groups worldwide and remains a significant public health concern. It is a multifactorial disease resulting from the interaction between host factors, dietary carbohydrates, oral microorganisms, and time. The process begins with the adherence of cariogenic bacteria to the acquired pellicle on the tooth surface, leading to the formation of dental plaque, which acts as a reservoir for pathogenic microorganisms. These bacteria metabolize fermentable carbohydrates, particularly sucrose, glucose, and fructose, producing organic acids such as lactic acid. The repeated acid attacks result in the gradual demineralization of enamel and dentin, disrupting the balance between demineralization and remineralization. If this process continues unchecked, it leads to irreversible cavitation, tooth sensitivity, pain, infection, and eventual tooth loss, significantly affecting oral health and quality of life.

Among the various microorganisms implicated in dental caries, *Streptococcus mutans* is considered the principal etiological agent due to its unique virulence characteristics. It possesses the ability to adhere firmly to tooth surfaces through the production of extracellular polysaccharides synthesized by glucosyltransferase enzymes. These polysaccharides facilitate biofilm formation and enhance bacterial colonization. Furthermore, *S. mutans* is highly acidogenic, meaning it efficiently produces acids from carbohydrate metabolism, and aciduric, meaning it can survive and thrive in acidic environments that inhibit other bacterial species. This dual capability enables *S. mutans* to dominate the oral microbial ecosystem and play a critical role in the initiation and progression of dental caries.

Various preventive strategies have been developed to control dental plaque and reduce cariogenic bacteria, including mechanical plaque control through tooth brushing and flossing, and chemical plaque control using antimicrobial agents. Chlorhexidine gluconate is widely regarded as the gold standard antimicrobial agent in dentistry due to its broad-spectrum activity against Gram-positive and Gram-negative bacteria, substantivity, and ability to disrupt bacterial cell membranes. It is commonly used in mouthwashes, gels, and varnishes for caries prevention and plaque control. However, despite its effectiveness, prolonged use of chlorhexidine is associated with several undesirable side effects, including tooth staining, taste alteration, mucosal irritation, and increased calculus formation. Additionally, concerns regarding microbial resistance and patient compliance have highlighted the need for safer, natural, and more acceptable alternatives.

In recent years, there has been growing interest in the use of herbal and plant-based products in oral healthcare due to their therapeutic benefits, safety profile, availability, and cost-effectiveness. Medicinal plants contain a wide variety of bioactive phytochemicals such as polyphenols, flavonoids, tannins, alkaloids, and essential oils, which exhibit antimicrobial, anti-inflammatory, antioxidant, and immunomodulatory properties. These compounds can inhibit bacterial growth by disrupting cell membranes, interfering with enzyme activity, inhibiting bacterial adhesion, and preventing biofilm formation. As a result, herbal extracts are increasingly being explored as potential alternatives or adjuncts to conventional antimicrobial agents in dentistry.

Pomegranate (*Punica granatum*) is a well-known medicinal plant that has been used in traditional medicine for centuries. It is rich in bioactive compounds such as polyphenols, tannins, flavonoids, and ellagic acid, which possess strong antimicrobial and antioxidant properties. These compounds have

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been shown to inhibit the growth of various oral pathogens, including *Streptococcus mutans*, by damaging bacterial cell walls, inhibiting enzyme activity, and reducing bacterial adhesion. In addition, the antioxidant properties of pomegranate help neutralize free radicals and reduce inflammation, making it a promising candidate for oral healthcare applications.

Similarly, jasmine (*Jasminum officinale*) is a medicinal plant known for its therapeutic properties, including antimicrobial, anti-inflammatory, and antioxidant effects. Jasmine leaves contain phytochemicals such as alkaloids, glycosides, flavonoids, tannins, and essential oils, which contribute to their antimicrobial activity. These compounds can alter bacterial cell membrane permeability, inhibit bacterial metabolism, and prevent microbial proliferation. Jasmine has been traditionally used for the treatment of infections and inflammatory conditions, and recent studies suggest its potential application in oral healthcare.

Although several studies have independently demonstrated the antimicrobial effectiveness of pomegranate and jasmine extracts against oral microorganisms, comparative studies evaluating their relative efficacy against *Streptococcus mutans* are limited. Direct comparison is essential to determine which herbal extract provides superior antimicrobial activity and to assess their potential use in preventive dental products such as mouthwashes, gels, and dentifrices. Therefore, the present in vitro study was conducted to evaluate and compare the antimicrobial effectiveness of pomegranate fruit extract and jasmine leaf extract against *Streptococcus mutans* using agar well diffusion and minimum inhibitory concentration methods.

Materials and Methods

Study Design

The present study was designed as an in vitro experimental investigation to evaluate and compare the antimicrobial efficacy of pomegranate fruit extract and jasmine leaf extract against *Streptococcus mutans*. Standard microbiological techniques were employed to assess antimicrobial activity using agar well diffusion and minimum inhibitory concentration methods.

Preparation of Plant Extracts

Fresh pomegranate fruits were thoroughly washed with sterile distilled water to remove surface contaminants. The fruits were peeled, and the seeds were separated. The juice was extracted using a sterile mechanical juicer under aseptic conditions. The obtained juice was filtered using sterile gauze to remove particulate matter and stored in sterile containers for further use.

Fresh jasmine leaves were collected and washed thoroughly with distilled water to remove dirt and impurities. The leaves were shade-dried at room temperature to preserve their active constituents. After complete drying, the leaves were ground into a fine powder using a sterile grinder. The powdered leaves were subjected to aqueous extraction using sterile distilled water, followed by filtration to obtain the extract. All extract preparation procedures were conducted under sterile conditions to prevent contamination.

Test Microorganism

A standard strain of *Streptococcus mutans* was used as the test microorganism. The bacterial culture was maintained on Brain Heart Infusion (BHI) agar and incubated at 37°C under appropriate laboratory conditions. Fresh cultures were prepared prior to antimicrobial testing.

Agar Well Diffusion Method

The antimicrobial activity of the extracts was evaluated using the agar well diffusion method. Mueller-Hinton agar plates were prepared, and the surface of each plate was uniformly inoculated with *Streptococcus mutans* using a sterile cotton swab. Wells of 6 mm diameter were created in the agar using a sterile cork borer. Different concentrations of pomegranate and jasmine leaf extracts (25%, 50%, and 75%) were carefully introduced into the respective wells.

Chlorhexidine gluconate 0.2% was used as a positive control to compare antimicrobial efficacy, while sterile distilled water served as a negative control. The plates were incubated at 37°C for 24 hours. After incubation, the zones of inhibition surrounding each well were measured in millimeters using a calibrated ruler. The mean and standard deviation were calculated.

Minimum Inhibitory Concentration (MIC)

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The minimum inhibitory concentration of each extract was determined using the broth dilution method. Serial dilutions of the extracts were prepared in sterile broth media, and each dilution was inoculated with *Streptococcus mutans*. The tubes were incubated at 37°C for 24 hours. The MIC was defined as the lowest concentration of extract that showed no visible bacterial growth.

Statistical Analysis

The data obtained were expressed as mean and standard deviation. Since the study was conducted in vitro, the results were analyzed descriptively to compare the antimicrobial efficacy of the different extracts and controls.

Results

Agar Well Diffusion Test

Both pomegranate and jasmine leaf extracts demonstrated clear zones of inhibition against *Streptococcus mutans*, indicating effective antimicrobial activity. The antimicrobial effect was found to be concentration-dependent, with higher concentrations producing larger zones of inhibition.

Pomegranate extract consistently showed greater antimicrobial activity than jasmine leaf extract at all tested concentrations. At 25% concentration, pomegranate extract produced a mean inhibition zone of 10.5 ± 0.7 mm, while jasmine leaf extract produced 9.3 ± 0.6 mm. At 50% concentration, pomegranate extract showed an inhibition zone of 14.2 ± 0.9 mm compared to 12.6 ± 0.8 mm for jasmine extract. The highest antimicrobial activity was observed at 75% concentration, where pomegranate extract produced a zone of inhibition measuring 17.8 ± 1.1 mm, while jasmine extract produced 16.1 ± 1.0 mm.

The positive control, chlorhexidine 0.2%, showed the highest antimicrobial efficacy with a mean inhibition zone of 20.5 ± 0.5 mm. No inhibition zone was observed with the negative control (distilled water), confirming that the antimicrobial activity was due to the plant extracts.

Minimum Inhibitory Concentration

The MIC results further confirmed the antimicrobial effectiveness of both extracts. Pomegranate extract showed a MIC value of 125 µg/mL, indicating strong antimicrobial activity. Jasmine leaf extract showed a MIC value of 250 µg/mL, indicating comparatively lower antimicrobial potency. Chlorhexidine demonstrated the lowest MIC value of 62.5 µg/mL, confirming its superior antimicrobial efficacy as a standard reference agent.

Discussion

The present in vitro study evaluated and compared the antimicrobial efficacy of pomegranate (*Punica granatum*) fruit extract and jasmine (*Jasminum officinale*) leaf extract against *Streptococcus mutans*, a primary cariogenic microorganism associated with dental caries. The results clearly demonstrated that both herbal extracts exhibited significant antimicrobial activity, as evidenced by measurable zones of inhibition in the agar well diffusion method and confirmed by minimum inhibitory concentration (MIC) values. Furthermore, the antimicrobial activity of both extracts was found to be concentration-dependent, with higher concentrations producing larger zones of inhibition. This finding suggests that the antimicrobial effect is directly related to the concentration of active phytochemical constituents present in the extracts. Increased concentrations likely provide greater availability of bioactive compounds capable of interacting with bacterial cell structures, disrupting metabolic processes, and inhibiting bacterial growth.

Among the two extracts evaluated, pomegranate extract consistently demonstrated superior antimicrobial efficacy compared to jasmine leaf extract at all tested concentrations. The largest zone of inhibition observed with pomegranate extract at 75% concentration indicates its strong inhibitory effect against *Streptococcus mutans*. This enhanced antimicrobial activity can be attributed to the rich presence of polyphenols, tannins, flavonoids, and ellagic acid in pomegranate. These phytochemicals are known to exert antimicrobial effects through multiple mechanisms, including disruption of bacterial cell wall integrity, alteration of membrane permeability, inhibition of essential bacterial enzymes, and interference with nucleic acid synthesis and energy metabolism. Tannins, in particular, can precipitate microbial proteins and inhibit bacterial adhesion, while flavonoids can damage microbial membranes and inhibit enzymatic activity. Additionally, polyphenols possess strong antioxidant properties that may indirectly inhibit bacterial survival by reducing oxidative stress conditions favorable for microbial

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growth. These mechanisms collectively contribute to the strong antimicrobial potential of pomegranate extract against cariogenic bacteria.

The findings of this study are consistent with previous research demonstrating the antimicrobial effectiveness of pomegranate extracts against oral pathogens, including *Streptococcus mutans*. Several studies have reported that pomegranate extracts can inhibit bacterial growth, reduce plaque formation, and interfere with bacterial adherence to tooth surfaces. The ability of pomegranate extract to inhibit glucosyltransferase activity is particularly important, as this enzyme plays a crucial role in extracellular polysaccharide synthesis and biofilm formation by *S. mutans*. Therefore, the results of the present study further support the potential use of pomegranate as a natural antimicrobial agent in preventive dentistry.

Jasmine leaf extract also demonstrated notable antimicrobial activity against *Streptococcus mutans*, although its effectiveness was comparatively lower than that of pomegranate extract. The antimicrobial properties of jasmine leaves may be attributed to the presence of phytochemical constituents such as alkaloids, glycosides, flavonoids, tannins, and essential oils. These compounds are known to exert antibacterial effects by disrupting bacterial cell membranes, altering membrane permeability, inhibiting protein synthesis, and interfering with bacterial metabolic pathways. Essential oils, in particular, have been shown to penetrate microbial cell membranes and cause leakage of intracellular contents, ultimately leading to bacterial cell death. Although jasmine extract exhibited lower antimicrobial activity compared to pomegranate, its effectiveness suggests that it still possesses significant antibacterial potential and may serve as a useful natural antimicrobial agent.

Chlorhexidine, used as the positive control in this study, demonstrated the highest antimicrobial activity, as expected, due to its well-established broad-spectrum antimicrobial action and substantivity. Chlorhexidine exerts its antimicrobial effect by disrupting bacterial cell membranes, causing leakage of intracellular components, and inhibiting bacterial enzyme systems. However, despite its effectiveness, chlorhexidine is associated with several undesirable side effects, including tooth discoloration, taste alteration, mucosal irritation, and increased calculus formation, particularly with long-term use. These limitations have encouraged the search for alternative agents that are safer, more biocompatible, and suitable for long-term use. The significant antimicrobial activity demonstrated by pomegranate and jasmine extracts in the present study highlights their potential as natural alternatives or adjuncts to conventional antimicrobial agents in oral healthcare. These herbal extracts could be incorporated into mouthwashes, toothpastes, gels, or varnishes to help reduce cariogenic bacterial load and prevent dental caries.

Despite the promising results, the present study has certain limitations that must be considered. As an in vitro investigation, the experimental conditions do not fully replicate the complex environment of the oral cavity, which includes the presence of saliva, fluctuating pH, mechanical forces, and interactions with other oral microorganisms. In the oral cavity, bacteria exist primarily in the form of biofilms rather than as free-floating planktonic cells. Biofilms provide enhanced protection to bacteria and increase their resistance to antimicrobial agents. The present study did not evaluate the effect of the extracts on biofilm formation or disruption, which is an important factor in caries prevention. Additionally, the cytotoxic effects of the extracts on oral tissues such as gingival cells and oral mucosa were not assessed. Safety evaluation is essential before clinical application of herbal extracts in oral healthcare products.

Furthermore, the study evaluated only aqueous extracts, and different extraction methods using solvents such as ethanol or methanol may yield different concentrations of active compounds and potentially greater antimicrobial activity. The stability, shelf life, and optimal formulation of these extracts for clinical use were also not evaluated. Therefore, further research, including in vivo studies, clinical trials, cytotoxicity assessments, and biofilm inhibition studies, is necessary to confirm the safety, efficacy, and practical applicability of these herbal extracts.

Overall, within the limitations of this study, both pomegranate and jasmine leaf extracts demonstrated significant antimicrobial activity against *Streptococcus mutans*, with pomegranate extract showing superior efficacy. These findings suggest that plant-based antimicrobial agents have promising potential in preventive dentistry and may serve as

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natural, safe, and effective alternatives to synthetic antimicrobial agents for the control of dental caries.

Conclusion

Within the limitations of this in vitro study, both pomegranate and jasmine leaf extracts demonstrated significant antimicrobial activity against *Streptococcus mutans*. The antimicrobial efficacy was found to increase with increasing extract concentration. Pomegranate extract showed greater antimicrobial potency compared to jasmine leaf extract, as evidenced by larger zones of inhibition and lower MIC values. Although chlorhexidine remained the most effective antimicrobial agent, the herbal extracts showed promising results and may serve as potential natural alternatives for caries prevention. These findings suggest that pomegranate and jasmine extracts could be incorporated into herbal oral healthcare products such as mouthwashes and dentifrices following further clinical validation.

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Table 1: Zone of Inhibition (mm) of Pomegranate and Jasmine Leaf Extracts Against Streptococcus mutans

Extract Type	Concentration (%)	Mean Zone of Inhibition (mm) ± SD
Pomegranate Extract	25%	10.5 ± 0.7
Pomegranate Extract	50%	14.2 ± 0.9
Pomegranate Extract	75%	17.8 ± 1.1
Jasmine Leaf Extract	25%	9.3 ± 0.6
Jasmine Leaf Extract	50%	12.6 ± 0.8
Jasmine Leaf Extract	75%	16.1 ± 1.0
Positive Control (Chlorhexidine 0.2%)	–	20.5 ± 0.5
Negative Control (Distilled water)	–	0.0 ± 0.0

Table 2: Minimum Inhibitory Concentration (MIC, µg/mL) of Pomegranate and Jasmine Leaf Extracts Against S. mutans

Extract Type	MIC (µg/mL)	Interpretation
Pomegranate Extract	125	Effective
Jasmine Leaf Extract	250	Effective
Chlorhexidine 0.2%	62.5	Reference Standard

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Figure 1: Zone of Inhibition (mm) of Pomegranate and Jasmine Leaf Extracts Against Streptococcus mutans

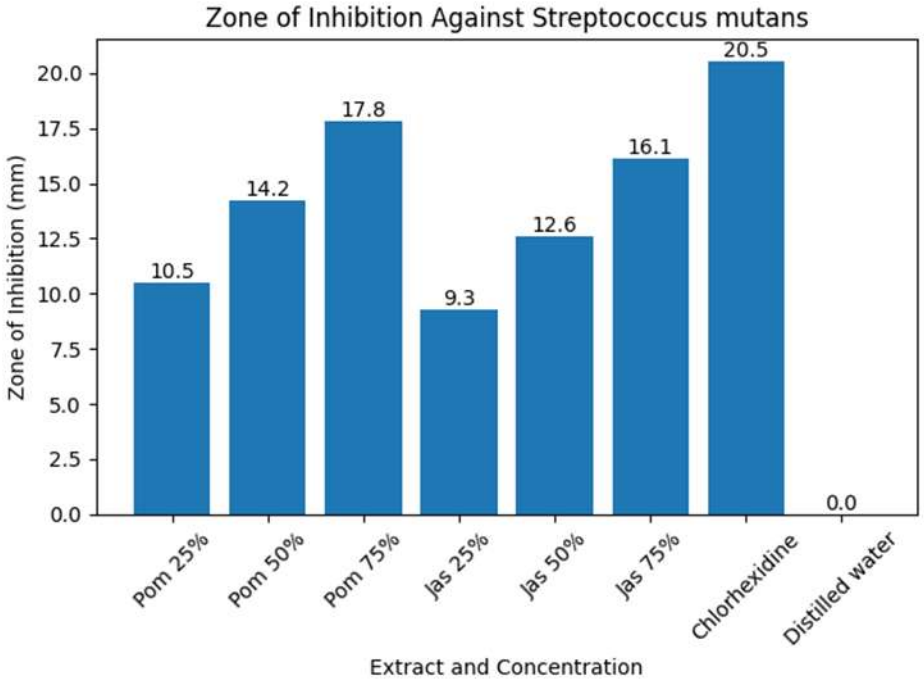
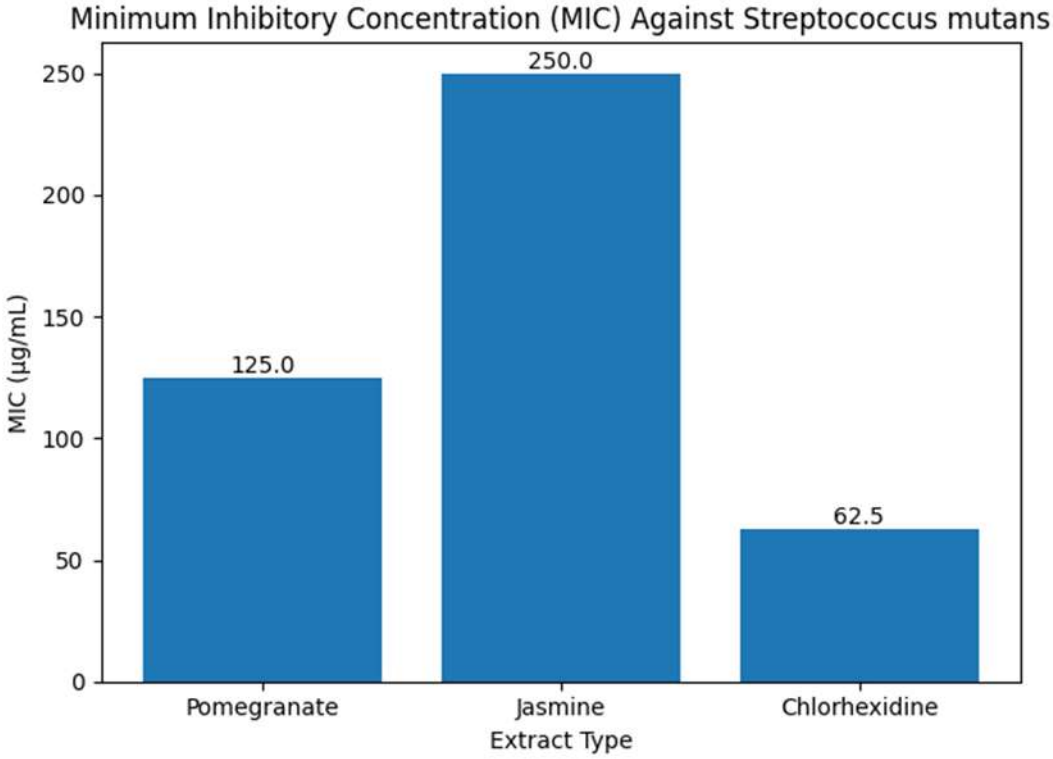


Figure 2: Minimum Inhibitory Concentration (MIC, µg/mL) of Pomegranate and Jasmine Leaf Extracts Against S. mutans



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