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Original Research Article

A comparative evaluation of antimicrobial efficacy of extracts of *Rosa Indica*, *Jasminum grandiflorum* L and *Hibiscus rosa-sinensis* L against *Streptococcus Mutans*, *Lactobacillus acidophilus* and *Staphylococcus aureus*: an *in vitro* study

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ABSTRACT

Background: Early humans were driven to explore their immediate natural surroundings for remedies to relieve pain and discomfort by using many plants and animal products. Despite advances in medicine, plants play a vital role in contributing to health care. However, scientific knowledge of the antimicrobial properties of plant species may contribute to the development of drugs against microorganisms of medical interest, particularly in dentistry. Aim was to evaluate the antimicrobial efficacy of Jasmine, Hibiscus and Rose leaf extracts on oral microorganisms versus the gold standard, Chlorhexidine.

Methods: Ethanol extracts prepared from Jasmine, Hibiscus, and Rose leaves were screened for antimicrobial activity against *S. mutans*, *L. acidophilus*, and *S. aureus* using the agar well diffusion method. The minimum inhibitory concentration (MIC) was determined for the test organisms. Statistical analysis was performed with one-way analysis of variance (ANOVA) followed by post hoc and Kruskal-Wallis tests.

Results: At low concentration, 0.2% chlorhexidine (5 µg/ml) showed the maximum zone of inhibition, followed by Rose, Jasmine, and Hibiscus leaves. Rose leaves (10 µg/ml, 25 µg/ml, 50 µg/ml, 75 µg/ml) showed the maximum zone of inhibition, followed by 0.2% chlorhexidine, hibiscus, and jasmine leaves against *S. aureus*. Extracts were found to have statistically significant ($p \leq 0.05$) antimicrobial activity against *S. mutans* and *S. aureus* with MIC values of 0.2 µg/ml and 3.125 µg/ml respectively. Whereas no significant value was seen against *L. acidophilus*. A proportional increase in their antimicrobial activity (zone of inhibition) was observed.

Conclusions: Chlorhexidine continues to remain as the gold standard; however, a highly significant difference was noted between the groups. Thus, confirming the presence of active constituents in medicinal plants.

Keywords: Antibacterial, Jasmine, Hibiscus, Rose leaves, Chlorhexidine

INTRODUCTION

Oral diseases, particularly dental caries, gingivitis, and periodontitis, remain among the most prevalent chronic diseases affecting populations worldwide and significantly

impair oral function, quality of life, and overall well-being.^{1,2} Dental plaque, a complex microbial biofilm, plays a central role in the initiation and progression of these diseases through the interaction of pathogenic microorganisms and host inflammatory responses.³

Among the microorganisms frequently associated with oral infections are *S. mutans*, *L. acidophilus*, and *S. aureus*, which contribute to biofilm formation, acid production, and tissue destruction.^{4,5}

Mechanical plaque control measures such as tooth brushing, flossing, and interdental cleaning are effective in maintaining oral hygiene. However, inadequate patient compliance often limits their effectiveness, necessitating the adjunctive use of chemotherapeutic agents.⁶ Chlorhexidine is widely regarded as the gold standard antiplaque and antimicrobial mouth rinse because of its broad-spectrum activity.⁷ Nevertheless, its prolonged use is associated with undesirable side effects, including tooth staining, taste alteration, and mucosal irritation, which may reduce patient acceptance.^{7,8}

Although a number of new antibiotics have been introduced in the last three decades, their clinical efficacy is being threatened by the emergence of multidrug-resistant pathogens. Thus, there is a renewed interest in traditional medicines and an increasing demand for more drugs from plant sources, mainly due to the current widespread belief that 'green medicine is safe'.^{1,2}

Medicinal plants have been used for centuries in traditional healthcare systems and are recognized as valuable sources of bioactive compounds with antimicrobial, antioxidant, and anti-inflammatory properties.⁸ The World Health Organization has emphasized the importance of investigating medicinal plants for the development of safe and effective therapeutic agents.⁸

Among the medicinal plants traditionally used for oral and systemic health, *Jasminum grandiflorum* L. (Jasmine), *Hibiscus rosa-sinensis* L. (Hibiscus), and *Rosa indica* (Rose) have attracted attention because of their reported antimicrobial, antioxidant, and anti-inflammatory activities.⁸⁻¹⁰ *Jasminum grandiflorum* has been traditionally used in the management of toothache, stomatitis, gingivitis, ulcers, and wound healing.⁸ *Hibiscus rosa-sinensis* possesses antimicrobial, antifungal, anti-inflammatory, and antioxidant properties,⁹ while *Rosa indica* has demonstrated antimicrobial, antiseptic, and anti-inflammatory effects in various studies.¹⁰

Although several investigations have reported the medicinal benefits of these plants, limited evidence is available regarding their comparative antimicrobial efficacy against common oral microorganisms.

Therefore, the present study was undertaken to evaluate and compare the antimicrobial activity of leaf extracts of *Jasminum grandiflorum*, *Hibiscus rosa-sinensis*, and *Rosa indica* against *S. mutans*, *L. acidophilus*, and *Staphylococcus aureus*, using chlorhexidine as the reference standard. The findings of this study may contribute to the development of plant-based alternatives for the prevention and management of oral diseases.

METHODS

Study design

This in vitro experimental study was conducted in the Department of Public Health Dentistry, College of Dental Sciences, Davangere, in collaboration with the Department of Pharmacognosy, Bapuji College of Pharmacy, Davangere, and the Departments of Microbiology at JJM Medical College, Davangere, and Maratha Mandal NGH Institute of Dental Sciences and Research Centre, Belagavi, Karnataka, India.

The antimicrobial efficacy of ethanolic leaf extracts of *Jasminum grandiflorum* (Jasmine), *Hibiscus rosa-sinensis* (Hibiscus), and *Rosa indica* (Rose) was evaluated against *S. mutans*, *L. acidophilus*, and *S. aureus* and compared with 0.2% chlorhexidine mouthwash.

Preparation of plant extracts

Fresh leaves of *Jasminum grandiflorum*, *Hibiscus rosa-sinensis*, and *Rosa indica* were procured locally and authenticated by a botanist. The leaves were shade-dried for 7 days and pulverized into coarse powder.

For preparation of ethanolic extracts, 10 g of powdered leaves from each plant were separately soaked in 100 mL ethanol for 24 h. The extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure below 40°C using a rotary evaporator. The dried extracts were sterilized under ultraviolet light for 24 h, tested for sterility on nutrient agar, and stored at 4°C until further use.

Test materials

The study groups included: Group I: 0.2% Chlorhexidine mouthwash (control), Group II: *Rosa indica* leaf extract, Group III: *Jasminum grandiflorum* leaf extract and Group IV: *Hibiscus rosa-sinensis* leaf extract.

Qualitative analysis of phytochemical constituents (Pathak et al)

Test for alkaloids

In the qualitative test of alkaloids, alkaloid-detecting reagents were used. Five grams of fresh leaf were chopped and ground with a mortar and pestle to moisten with 10 ml of 2% HCl. Then the mixture was heated in a water bath at 60°C for one hour, and then the extract was filtered through filter paper. To detect alkaloids, we mixed 2 ml of each extract with 1 mL of 1% HCl in five test tubes, then added 6 drops of Meyer's, Wagner's, Dragendorff's, Hager's, and Tannic Acid reagents to each test tube, and observed yellowish, brownish red, orange, yellow, and reddish precipitation, respectively. The reagents were prepared by following standard methods (Cromwell 1955). If the plant extract reacts with different chemical reagents and

produces the described colors, it is considered the presence of alkaloids.

Test for flavonoids

A portion of the powdered plant sample was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered, and 4 ml of the filtrate was shaken with 1 mL of dilute ammonia solution. A yellow coloration was observed, indicating a positive test for flavonoids.

Test for saponins

About 2 g of crude powder was boiled with 20 ml of distilled water in a water bath and filtered. Ten ml of filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable, persistent froth. The persistent froth indicates the presence of saponins.

Test for tannins

If a dark green or deep blue color develops in a mixture of 5 ml of extract and 1 ml of 5% ferric chloride, it indicates the presence of tannin in the extract. Phenols: The presence of phenols was revealed by the emergence of a blue or green color after a few drops of 10% aqueous ferric chloride were added to the plant extract.

Test for carbohydrates

Molisch's test was used to detect the presence of carbohydrates. One drop of concentrated sulphuric acid was added to about 1g of each herbal extract, and then three drops of 1% α -naphthol in 80% ethanol were added to the mixture without mixing to form an upper phase. Formation of a brown or purple ring at the interphase indicated the presence of carbohydrates.

Test for terpenoids

The 0.5 g of crude extract was diluted in 5 mL of methanol and combined with 2 mL of chloroform. Seven mL of concentrated H₂SO₄ was added to the mixture, resulting in the formation of a reddish-brown interface, which denotes the presence of terpenoids. Steroids: Five mL of methanol was added to 0.5 g of crude extract. One ml of this solution was taken into a test tube. Ten ml of chloroform was added to it, and then an equal amount of concentrated H₂SO₄ was added from both sides of the test tube. The presence of steroids was determined by the yellow-green fluorescence of the H₂SO₄ layer and the red-colored upper layer.

Test for glycosides

In 5 mL of methanol, the crude extract (0.5 g) was dissolved. Two ml of methanolic extract were combined in a test tube with ten ml of 50% HCl. It was then heated for 30 minutes in a water bath. Five ml of Fehling's reagent were added to the mixture, and it was then boiled for five

minutes. The presence of glycosides was assumed based on the brick-red color of the precipitate.

Test for cardiac glycosides

Five mL of plant extract was added along with 2 mL of glacial acetic acid, one drop of ferric chloride solution, and 1 mL of concentrated H₂SO₄. A brown or green ring indicates the presence of cardiac glycosides.

Test for quinine

One ml of plant extract was mixed with 1 ml of H₂SO₄, and the mixture was left to stand for a while to acquire color. The appearance of a red color indicates the presence of quinine.

Test for coumarin

One mL of extract was added to one mL of 10% NaOH, and the mixture was left to stand for a while. The emergence of a yellow color indicates the presence of coumarin.

Microorganisms and culture conditions

Standard strains of *S. mutans*, *L. acidophilus*, and *S. aureus* were obtained from the Department of Microbiology. The microorganisms were cultured using appropriate selective media: Brain Heart Infusion (BHI) agar for *S. mutans*, de Man Rogosa Sharpe (MRS) agar for *L. acidophilus*, and Mueller-Hinton agar for *S. aureus*.

Antimicrobial assay

Agar well diffusion method

Antimicrobial activity was evaluated using the agar well diffusion method. Brain Heart Infusion agar plates were brought to room temperature before use. Bacterial inoculate were prepared by suspending colonies in broth and adjusting the turbidity to 0.5 McFarland turbidity standard. Within 15 minutes of preparation, sterile cotton swabs were used to uniformly inoculate the agar surface. The inoculated plates were allowed to stand for 3-15 minutes, after which five wells of 5 mm diameter were aseptically prepared in each plate. Stock solutions of the test extracts were prepared by dissolving 10 mg of each extract in 1 mL dimethyl sulfoxide (DMSO). different concentrations of the test extracts were dispensed into the wells. (i. e, 5, 10, 25, 50, and 75 μ L) were dispensed into the wells Chlorhexidine served as the positive control. The plates were incubated at 37°C for 18-24 hours, and the antimicrobial activity was determined by measuring the diameter of the zones of inhibition (mm) using a digital vernier caliper.

Determination of MIC

The MIC of each extract was determined using the broth

serial dilution method in thioglycollate broth. Serial ten-fold dilutions of each extract were prepared, inoculated with standardized bacterial suspensions, and incubated at 37°C for 48-72 h under appropriate atmospheric conditions. MIC was defined as the lowest concentration of the extract that showed no visible bacterial growth.

Procedure

Nine dilutions of each drug have to be made with Thioglycolate broth for MIC. In the initial tube, 20 microliters of drug was added to the 380 microliters of thioglycolate broth. For dilutions, 200 microliters of Thioglycolate broth was added to the next 9 tubes separately. Then, from the initial tube, 200 microliters was transferred to the first tube containing 200 microliters of Thioglycolate broth. This was considered a 10^{-1} dilution. From the 10^{-1} diluted tube, 200 microliters were transferred to the second tube to make 10^{-2} dilution. The serial dilution was repeated up to 10^{-9} dilutions for each drug. From the maintained stock cultures of required organisms, 5 microliters were taken and added to 2 mL of Thioglycolate broth. In each serially diluted tube, 200 microliters of the above culture suspension was added. The tubes were incubated for 48-72 hours in an anaerobic jar at 37°C and observed for Turbidity to determine MIC.

Statistical analysis

Data were entered into Microsoft excel and analyzed using Statistical Package for the Social Sciences (SPSS) version 26.0 (IBM Corp., Armonk, NY, USA). Mean zone of inhibition and MIC values were calculated for all study groups. Intergroup comparisons were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. For MIC analysis, the Kruskal-Wallis test was used where applicable. A $p < 0.05$ was considered statistically significant.

Ethical consideration

Ethical approval to carry out the research was sought from the Institutional Research and Ethics Committee (IREC) of the College of Dental Sciences, Davangere. Permission and authorization to carry out the research study were sought from both the Department of Pharmacognosy, Bapuji College of Pharmacy, Davangere, and the Departments of Microbiology at JJM Medical College, Davangere, and Maratha Mandal NGH Institute of Dental Sciences and Research Centre, Belagavi, Karnataka, India.

RESULTS

The present in vitro study evaluated the antimicrobial efficacy of Jasmine, Hibiscus, and Rose leaf extracts against oral microorganisms, namely *Lactobacillus acidophilus*, *Streptococcus mutans*, and *Staphylococcus aureus*, and compared their activity with 0.2% chlorhexidine mouthwash. Antimicrobial efficacy was

assessed using minimum inhibitory concentration (MIC) and zone of inhibition assays (Figure 1-4).

MIC

The MIC values of the test agents against *Lactobacillus acidophilus* were evaluated at concentrations ranging from 100 µg/ml to 0.2 µg/ml using the Kruskal-Wallis test. No statistically significant difference was observed at any concentration tested ($p > 0.05$). The mean value remained 2.00 across most concentrations, indicating that no definitive MIC could be established for *L. acidophilus* within the tested concentration range.

For *S. mutans*, statistically significant differences were observed at concentrations of 1.6 µg/ml ($p = 0.003$), 0.8 µg/ml ($p < 0.001$), 0.4 µg/ml ($p < 0.001$), and 0.2 µg/ml ($p = 0.005$). Based on the observed inhibitory activity, the MIC was determined to be 0.2 µg/ml.

For *S. aureus*, significant antimicrobial activity was observed at concentrations of 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, and 3.125 µg/ml ($p < 0.001$). The MIC was determined to be 3.125 µg/ml, indicating greater susceptibility of *S. aureus* at comparatively higher concentrations than *S. mutans* (Table 1 and Figure 1).

Zone of inhibition assay

Antimicrobial activity against *Lactobacillus acidophilus*

The Mean Zone of inhibition values of test groups were evaluated at 75, 50, 25, 10, 5 µl/ml against microorganisms using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test.

At 75 µl/ml concentration, chlorhexidine exhibited the highest mean zone of inhibition (25.8 ± 0.42 mm), followed by Rose extract (15.6 ± 1.71 mm), Jasmine extract (14.8 ± 1.55 mm), and Hibiscus extract (1.0 ± 0.00 mm). The differences among the groups were statistically significant ($F = 752.613$, $p < 0.001$).

At 50 µl/ml concentration, chlorhexidine demonstrated the greatest inhibitory effect (23.8 ± 0.42 mm), followed by Rose (12.9 ± 1.45 mm), Jasmine (11.7 ± 1.25 mm), and Hibiscus (1.0 ± 0.00 mm). The intergroup difference was statistically significant ($F = 903.988$, $p < 0.001$).

At 25 µl/ml concentration, chlorhexidine produced the largest inhibition zone (20.4 ± 0.70 mm), followed by Rose (10.6 ± 0.97 mm), Jasmine (8.5 ± 1.35 mm), and Hibiscus (1.0 ± 0.00 mm). A statistically significant difference was observed among the groups ($F = 785.150$, $p < 0.001$).

At 10 µl/ml concentration, chlorhexidine showed the maximum inhibition zone (15.8 ± 0.42 mm), followed by Rose (3.4 ± 3.10 mm), while Jasmine and Hibiscus demonstrated minimal activity (1.0 ± 0.00 mm). The difference was statistically significant ($F = 205.691$,

p<0.001).

At 5 µl/ml concentration, chlorhexidine maintained the highest inhibitory effect (14.9±0.32 mm), whereas Rose, Jasmine, and Hibiscus exhibited negligible inhibition (1.0±0.00 mm). The difference among the groups was statistically significant (F=19321.000, p<0.001) (Table 2) (Figure 2).

Antimicrobial activity against *Streptococcus mutans*

At 75 µl/ml concentration, Rose extract demonstrated the greatest inhibitory activity (24.1±0.88 mm), surpassing chlorhexidine (22.9±0.32 mm). Hibiscus exhibited moderate activity (11.5±1.35 mm), while Jasmine showed negligible inhibition (1.0±0.00 mm). The intergroup difference was statistically significant (F=1745.222, p<0.001).

At 50 µl/ml concentration, Rose extract again exhibited the highest inhibition zone (20.6±0.97 mm), followed closely by chlorhexidine (19.9±0.32 mm). Hibiscus showed moderate activity (7.9±4.84 mm), whereas Jasmine remained ineffective. The difference was statistically significant (F=149.150, p<0.001).

At 25 µl/ml concentration, Rose extract showed the maximum inhibition zone (19.4±0.97 mm), followed by chlorhexidine (18.1±0.32 mm), while both Jasmine and Hibiscus showed negligible activity. A statistically significant difference was observed (F=4076.220, p<0.001).

At 10 µl/ml concentration, Rose extract demonstrated marginally greater inhibition (17.1±1.45 mm) than chlorhexidine (16.9±0.32 mm), while Jasmine and Hibiscus showed no appreciable antimicrobial activity. The difference was statistically significant (F=1551.636, p<0.001).

At 5 µl/ml concentration, chlorhexidine exhibited the highest inhibition zone (14.9±0.32 mm), followed closely by Rose extract (13.5±1.08 mm). Jasmine and Hibiscus showed minimal inhibition. The difference among groups remained statistically significant (F=1844.421, p<0.001) (Table 2 and Figure 2).

Antimicrobial activity against *S. aureus*

At 75 µl/ml concentration, chlorhexidine produced the largest zone of inhibition (18.9±0.32 mm), followed by Rose extract (15.6±0.52 mm). Jasmine and Hibiscus showed negligible antimicrobial activity. The intergroup difference was statistically significant (F=9800.273, p<0.001).

At 50 µl/ml concentration, chlorhexidine showed the greatest inhibitory activity (15.1±0.32 mm), followed by Rose extract (14.3±0.48 mm), whereas Jasmine and Hibiscus demonstrated minimal activity. The difference was statistically significant (F=7520.400, p<0.001).

At 25 µl/ml concentration, chlorhexidine exhibited a zone of inhibition of 14.0±0.00 mm, followed by Rose extract (12.9±0.32 mm). Jasmine and Hibiscus remained ineffective. A statistically significant difference was observed among the groups (F=20747.667, p<0.001). At 10 µl/ml concentration, chlorhexidine demonstrated the highest inhibition zone (13.9±0.32 mm), followed by Rose extract (12.8±1.03 mm), while Jasmine and Hibiscus showed negligible activity. The intergroup difference was statistically significant (F=1750.029, p<0.001).

At 5 µl/ml concentration, chlorhexidine showed the maximum inhibitory effect (11.9±0.32 mm), followed by Rose extract (9.4±5.80 mm). Jasmine and Hibiscus exhibited minimal inhibition. The difference among groups was statistically significant (F=38.080, p<0.001) (Table 2 and Figure 2).

Overall comparison of antimicrobial agents

Comparative analysis across all tested microorganisms revealed that chlorhexidine consistently demonstrated the highest antimicrobial activity, especially against *L. acidophilus* and *S. aureus* at all tested concentrations. Among the herbal extracts tested, Rose leaf extract demonstrated the greatest antimicrobial activity, particularly against *S. mutans*, where it showed inhibition comparable to or greater than chlorhexidine at higher concentrations (10,25,50 and 75µl). Jasmine and Hibiscus extracts exhibited comparatively lower antibacterial efficacy than rose extract and chlorhexidine.

Table 1: MIC of test agents against oral microorganisms at different concentrations using Kruskal-Wallis test.

Concentration (µg/ml)	<i>L. acidophilus</i>	P value	<i>S. mutans</i>	P value	<i>S. aureus</i>	P value
100	2.000±0.000	1.000	2.000±0.000	1.000	2.000±0.000	1.000
50	2.000±0.000	1.000	2.000±0.000	1.000	2.000±0.439	1.000
25	2.000±0.000	1.000	2.000±0.000	1.000	1.750±0.464	<0.001*
12.5	2.000±0.000	1.000	2.000±0.000	1.000	1.700±0.496	<0.001*
6.25	2.000±0.000	1.000	2.000±0.000	1.000	1.400±0.474	<0.001*
3.125	2.000±0.000	1.000	2.000±0.000	1.000	1.325±0.000	<0.001*
1.6	2.000±0.000	1.000	1.725±0.452	0.003*	1.000±0.000	1.000
0.8	2.000±0.000	1.000	1.625±0.490	<0.001*	1.000±0.000	1.000
0.4	2.000±0.000	1.000	1.450±0.504	<0.001*	1.000±0.000	1.000
0.2	1.575±0.501	0.594	1.100±0.304	0.005*	1.000±0.000	1.000

*P<0.05 is considered statistically significant.

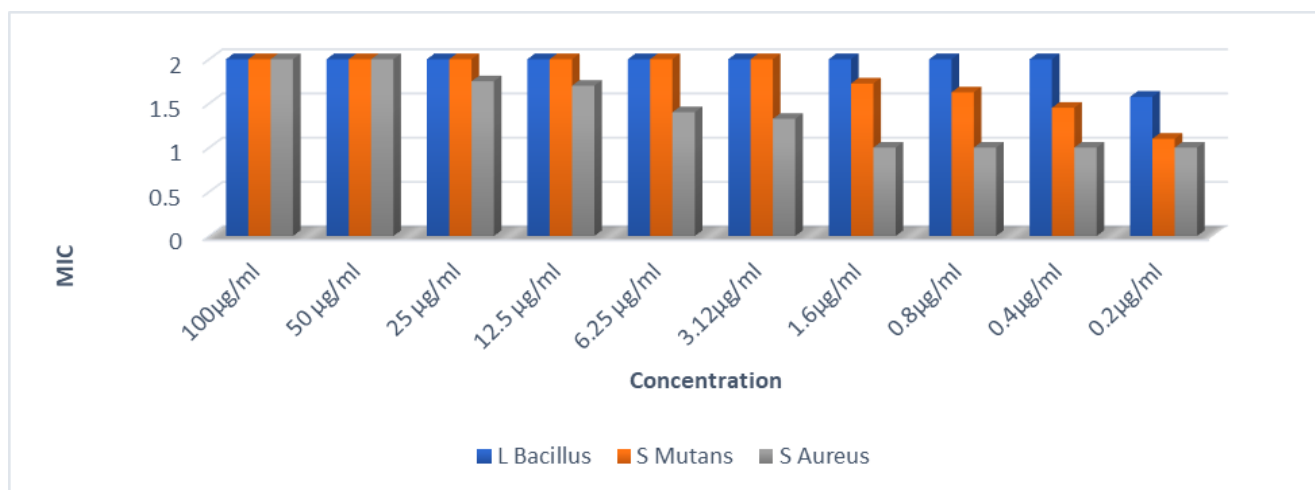


Figure 1: Mean MIC results of group 1 to group 4 on *L. acidophilus*, *S. mutans* and *S. aureus* at different concentrations.

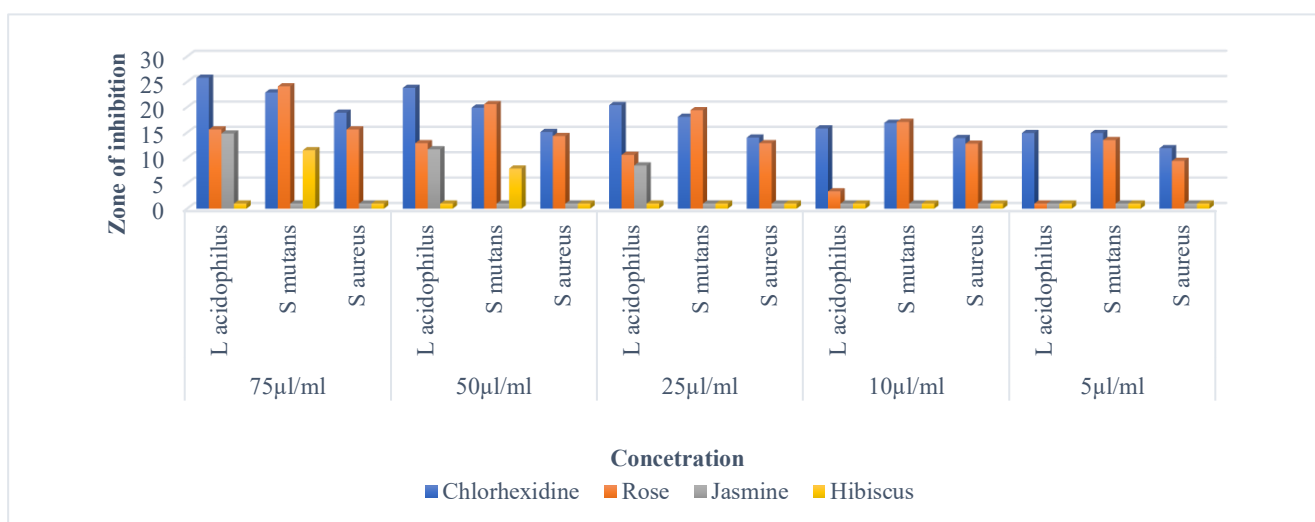


Figure 2: Comparison of antibacterial activity of group1 to group 4 against *L. acidophilus*, *S. mutans* and *S. aureus* at concentration 75 µl/ml, 50 µl/ml, 25 µl/ml, 10 µl/ml and 5 µl/ml.

Table 2: Comparison of mean zone of inhibition (mm) of chlorhexidine, rose, jasmine and hibiscus extracts against *L. acidophilus*, *S. mutans* and *S. aureus* at different concentrations.

Microorganism	Conc. (µl/ml)	Chlorhexidine	Rose	Jasmine	Hibiscus	F value	P value
<i>L. acidophilus</i>	75	25.8±0.42	15.6±1.71	14.8±1.55	1.0±0.00	752.613	<0.001
	50	23.8±0.42	12.9±1.45	11.7±1.25	1.0±0.00	903.988	<0.001
	25	20.4±0.70	10.6±0.97	8.5±1.35	1.0±0.00	785.150	<0.001
	10	15.8±0.42	3.4±3.10	1.0±0.00	1.0±0.00	205.691	<0.001
	5	14.9±0.32	1.0±0.00	1.0±0.00	1.0±0.00	19321.000	<0.001
<i>S. mutans</i>	75	22.9±0.32	24.1±0.88	1.0±0.00	11.5±1.35	1745.222	<0.001
	50	19.9±0.32	20.6±0.97	1.0±0.00	7.9±4.84	149.150	<0.001
	25	18.1±0.32	19.4±0.97	1.0±0.00	1.0±0.00	4076.220	<0.001
	10	16.9±0.32	17.1±1.45	1.0±0.00	1.0±0.00	1551.636	<0.001
	5	14.9±0.32	13.5±1.08	1.0±0.00	1.0±0.00	1844.421	<0.001
<i>S. aureus</i>	75	18.9±0.32	15.6±0.52	1.0±0.00	1.0±0.00	9800.273	<0.001
	50	15.1±0.32	14.3±0.48	1.0±0.00	1.0±0.00	7520.400	<0.001
	25	14.0±0.00	12.9±0.32	1.0±0.00	1.0±0.00	20747.667	<0.001
	10	13.9±0.32	12.8±1.03	1.0±0.00	1.0±0.00	1750.029	<0.001
	5	11.9±0.32	9.4±5.80	1.0±0.00	1.0±0.00	38.080	<0.001

*ANOVA test; p<0.05 considered statistically significant. Values are expressed as Mean±Standard Deviation (mm).

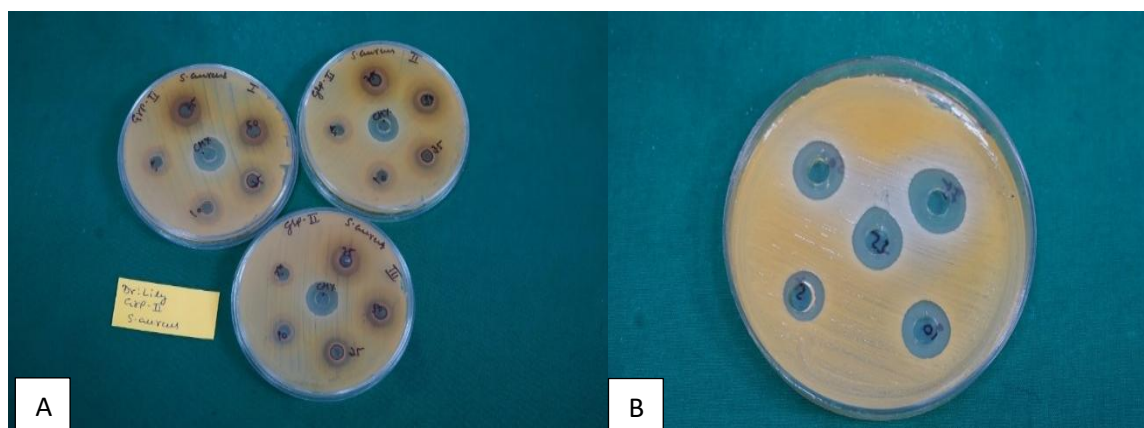


Figure 3 (A and B): Culture plates showing zone of inhibition against *S. mutans*, *L. acidophilus*, *S. aureus* and chlorhexidine.



Figure 4 (A-C): Estimation of MIC against. A. *S. mutans* B. *L. acidophilus* C. *S. aureus*.

DISCUSSION

Oral health has a strong correlation with the overall health of an individual. With the rising prevalence of oral disorders, the global demand for efficient and cost-effective products and treatments has grown.¹¹ This necessitates a better awareness of traditional practices and oral health beliefs, as well as a revitalized interest in using natural medicine to improve overall health.¹²

In 1924, Clarke first isolated a bacterial species from a human dental caries site; it was named *S. mutans* (*S. mutans*), and was shown to be capable of fermenting several sugars and producing a pH of 4.2 in glucose broth.¹³ Bunting et al and Jay identified *lactobacilli* in general and *Lactobacillus acidophilus* (*B. acidophilus*) in particular as possible candidates for causation of dental caries. Many studies carried out by them and thereafter by others have shown a frequent association between the presence of lactobacilli and the prevalence of dental caries, suggesting such a possibility.¹⁴ *S. aureus*, an opportunistic pathogen colonizing up to 30% of the human population, causes a variety of mild to severe diseases among humans and animals.¹⁵ This organism decreases susceptibility to several types of antibiotics every day and becomes more resistant, which is a growing source of concern in this era.¹⁵

Medicinal plants have been widely used in traditional healthcare systems for the treatment of infectious diseases, including oral infections, owing to their rich content of bioactive compounds such as alkaloids, flavonoids, and phenolic constituents that exhibit antimicrobial properties. In recent years, there has been growing interest in herbal therapeutics because of their safety, accessibility, and potential to combat antimicrobial-resistant microorganisms.¹⁶ In the present study, the ethanolic extracts of *Jasminum grandiflorum* L., *Hibiscus rosa-sinensis* L., and *Rosa indica* were evaluated for their antimicrobial efficacy against oral microorganisms. Antimicrobial activity was assessed using agar well diffusion and MIC methods, which are widely accepted techniques for determining microbial susceptibility and the inhibitory potential of plant extracts.

A study by Sekharan et al showed that jasmine leaf extract showed the highest zone of inhibition against *S. aureus*.¹⁷ Nagarajappa et al in their study showed that jasmine leaf extract showed the highest zone of inhibition against *S. mutans* and *Lactobacillus acidophilus*.¹ Literature survey reveals that many studies were published on antifungal, antibacterial drug, and antioxidant activities of roses. A study conducted by Pathak et al showed the antibacterial activity of rose extracts against different organisms.¹⁸ The present study demonstrated significant antimicrobial

activity of tested herbal extracts against oral microorganisms, with *S. mutans*, *Rosa indica* exhibited the highest zone of inhibition (24.1 mm) at 75 µl/ml, surpassing chlorhexidine (22.9 mm), while *Hibiscus rosa-sinensis* showed moderate activity. Highly significant difference was observed among the groups ($p < 0.001$). These findings are consistent with the study by Thaweboon et al who reported antimicrobial activity of *Jasminum grandiflorum* against *S. mutans*, *Lactobacillus casei*, *E. coli*, and *Candida* species.¹⁹

For *S. aureus*, chlorhexidine demonstrated the greatest antimicrobial efficacy across all tested concentrations, followed by rose, hibiscus, and jasmine extracts, with statistically significant intergroup differences ($p < 0.001$). Similar antibacterial effects of jasmine and hibiscus extracts against *S. aureus* have been reported by Suliman et al and Higginbotham et al highlighting the potential of these extracts as natural antimicrobial agents.^{20,21} Furthermore, the antibacterial activity of rose extracts against *S. aureus* and other pathogenic bacteria has been documented by Khan et al supporting the findings of the present study.²²

The growing concern of antimicrobial resistance and the adverse effects associated with synthetic drugs has increased interest in herbal alternatives. The antimicrobial efficacy demonstrated by the tested plant extracts highlights their potential as safe, natural, and cost-effective adjuncts for maintaining oral health and reducing the burden of oral diseases in the community.²³ The observed antimicrobial activity of the tested herbal extracts suggests their potential application as adjuncts or alternatives in oral healthcare. However, as the present investigation was conducted in vitro, further *in vivo* and clinical studies are required to validate their efficacy, safety, and potential role in the prevention and management of oral diseases.

CONCLUSION

In conclusion, the findings of the present in vitro study demonstrate that the ethanolic extracts of *Jasminum grandiflorum*, *Hibiscus rosa-sinensis*, and *Rosa indica* possess appreciable antimicrobial activity against the tested oral microorganisms. Among the herbal extracts, *Rosa indica* exhibited the greatest inhibitory effect against *S. mutans*, whereas 0.2% chlorhexidine showed superior antimicrobial efficacy against *L. acidophilus* and *S. aureus* across all tested concentrations. Although chlorhexidine remains the benchmark antimicrobial agent, the significant antibacterial activity exhibited by the herbal extracts highlights their potential as natural alternatives or adjuncts in the prevention and management of oral diseases. These findings support the growing interest in plant-based therapeutics and emphasize the need for further *in vivo* and clinical investigations to establish their efficacy, safety, and applicability in routine oral healthcare.

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