

EVALUATING OF THE ANTIBACTERIAL EFFICACY OF PROSOPSIS JULIFLORA AND THREE COMMERCIALLY AVAILABLE MOUTHRINSES: AN IN VITRO STUDY

Dentistry

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ABSTRACT

Immunocompromised individuals are more likely than the general population to get oral and periodontal infections. These conditions, which impact the tissues around teeth, are typically brought on by inadequate dental hygiene, which results in the accumulation of plaque, which harbours a number of bacteria. Although many medications and synthetic compounds on the market appear promising, it is well recognised that continuous usage of these medicines might result in adverse side effects. For this reason, Prosopis juliflora leaf extract is used instead. Unlike commercial mouthrinses, the goal is to test for and evaluate the antibacterial effectiveness of P. juliflora crude extract against periodontal and oral infections. Instat Plus was used to calculate the data, and MIC was used to measure the antibacterial activity. It was shown that, in comparison to other commercial mouthrinses, P. juliflora was the most effective mouthrinse against particular microorganisms. The findings of this investigation unequivocally show that P. j. crude extract

KEYWORDS

INTRODUCTION:

A number of systemic illnesses in humans, including pneumonia, septicaemia, and endocarditis, can be caused by opportunistic bacteria with strong virulence features that are harboured in the oral cavity by periodontal disorders [1, 2]. The microbiota associated with localised aggressive periodontitis is composed of anaerobic, gram-negative, capnophilic bacteria [3]. Enterococcus faecalis, the most often identified species from endodontic infections, is responsible for the subsequent failure of teeth that have had endodontic treatment when it is associated to re-infection [4]. The majority of periodontal infections are also caused by anaerobes. Treponema denticola, Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum, Porphyromonas gingivalis, and Prevotella intermedia are some of the microbes linked to periodontal illnesses [3]. Furthermore, there was a weak association [5] between periodontitis and cardiovascular disease, atherosclerosis, and stroke. Mouthrinses are typically recommended for patients who do not respond well to mechanical oral hygiene methods for plaque management. This study provides more proof of mouthrinses' value in effectively controlling oral bacteria. The ability of any mouthrinse to inhibit or eliminate a wide range of harmful oral and periodontal germs is what determines how effective it is.

Chlorhexidine, the most common component of mouthrinses, has demonstrated efficacy as an antibacterial. This is sold along side a variety of chemical under multiple brand names. However, prolonged use of chlorhexidine can cause a variety of negative side effects, including drymouth, gingivitis, gastrointestinal problems, tooth discoloration, and many more [6]. There are many natural treatment options for periodontal diseases. Herbal remedies like aloe vera and clove oil have been demonstrated to lessen gum pain and irritation when applied topically [7]. Compared to traditional antibiotics, which have the disadvantage of low benefit to high risk, herbal remedies have a high benefit to low risk ratio. [8] Prosopis juliflora is a handy local plant that has been used for centuries as a traditional remedy. It has antibacterial, antifungal, hemolytic, anti-inflammatory [9, 10], and wound-healing properties, according to pharmacological characteristics tested in vitro. Antitumor activity was also observed against MDA-MB-231 breast adenocarcinoma cells [22] and a number of human epithelial and hepatic tumor cells [12]. Previous research has shown that P. juliflora leaf extract contains the flavonoid squerectin and apigenin, which have antibacterial properties [13]. Additionally, it is used to treat headaches, painful gums, and bladder infections [14, 15]. The current study assessed P. juliflora leaf crude extract's in vitro antibacterial activity against three widely used and commercially

available mouthrinses. S. aureus, E. faecalis, A. actinomycetemcomitans, P. intermedia, and P. gingivalis were selected as the pathogenic bacteria. These microorganisms are principally responsible for periodontal and oral infections that result in bleeding when the pocket is probed and deepened [25]. They have a strong correlation with the onset of cardiovascular diseases (CVDs), endothelial damage, and cholesterol plaque [15].

MATERIALS AND METHODS:

Extract Preparation

Using leaves collected from various locations in and around Melmaruvathur, the Botany Department of Lakshmi Bangaru Arts and Science college in Melmaruvathur, India, conducted the taxonomy of P. juliflora. The leaves were carefully chosen to ensure they were disease-free. 100 g leaf samples were surface sterilized twice using autoclaved distilled water. After letting them air dry, we wiped them to remove any remaining water. To create a homogenous mixture, this was now macerated in 100 milliliters of sterile distilled water and mixed for ten minutes. This mixture was centrifuged at 4000 g for 30 minutes after being filtered through two layers of muslin cloth to produce a pure solution. Next, Whatmann No. 1 filter paper was used to filter the supernatant [17]. This functioned as a stock solution with the designation "D" as well as a plant extract in water that contained 1g/ml of the extracted material.

Sample Preparation:

In order to create an array of mouthrinses, three commercially available mouthrinses with distinct chemical compositions were assigned the designations "A," "B," and "C." Chemically, mouthwash 'A' contained 0.09% w/v of zinc chloride IP, 0.2% w/v of chlorhexidine gluconate, and 0.09% w/v of sodium fluoride. Mouthrinse 'B' was made using a 0.2% w/v diluted IP chlorhexidine gluconate solution. Benzoic acid, sodium saccharin, methylsalicylate, nicopropanol, poloxamer 407, thymol, menthol, and eucalyptol were all present in mouthwash 'C'. There was also water that had been purified. A 5 µg Ciprofloxacin (CF) Disc from Hi-Media Laboratories in India served as the control.

Microbiological Intervention Preparations:

The Department of Public Health Dentistry at Adhiparasakthi Dental College and Hospital in Melmaruvathur, Tamil Nadu, provided clinical microbiological strains of Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Staphylococcus aureus, Enterococcus faecalis, and Prevotella intermedia.

The sub cultures were placed on the appropriate, carefully thought-out media. In conclusion, Hi-media laboratories in India provided all of the culture medium. *A. actinomycetemcomitans*, *P. intermedia*, *P. gingivalis*, *E. faecalis*, and *S. aureus* were cultivated on Baird-Parker agar, MacConkey's agar, and BHI and TSBV.

Methodology:

The antibacterial activity of aerobes on Muller Hinton Agar (Hi-Media Laboratories, India) and anaerobes on Wilkins Chalgren Blood Agar was assessed using the disc diffusion method in accordance with CLSI guidelines [18]. The media was put into Petridishes when it was ready. A homogenous 0.1 ml test organism with 105 CFU/ml was applied to the media's surface. Each organism went through the same process [19].

Ten minutes later, 10 µl volumes of each mouthrinse (A, B, and C), plant extract (D), and aseptic dried discs that had been dried overnight at 37.0°C were added to the medium. Ciprofloxacin disc (CF), at a concentration of 5 µg, served as the control. The aerobes were cultivated at 37.0°C in an incubator while the anaerobic plates were kept in an anaerobic jar (Hi Media Anaerobic System-Mark V, with Anaerocult gas pack, MERCK). The antibacterial activity of the plates was assessed by measuring the zone of inhibition surrounding each disc in each plate in millimeters (mm) using the Hi Antibiotic Zone scale (HARMAN RESEARCH LAB TANJORE) following a 48-hour incubation period. The results showed that mouthrinses and crude oil inhibited the test organisms.

Statistical Analysis:

Based on statistical analysis, the Minimum Inhibitory Concentration (MIC) for each organism was determined using the Mean ± SD values of all six replicates for each test sample. The Version 20.0 SPSS.IBM.USA tool was used to conduct a one-way ANOVA.

RESULTS:

The result of this study unequivocally demonstrated that *P. juliflora* leaf extract exhibited superior inhibitory action against test bacteria that are common in oral and periodontal tissues compared to commercially available mouthrinses A, B, and C [Figures 1, 2, 3, 4]. Chlorhexidine, which was present in Sample "B", outperformed the plant extract. "A" was the most active sample, and "C" was the least active. The crude extract of *P. juliflora* was most effective against *A. actinomycetemcomitans* and *E. faecalis*, according to the MIC values against various pathogens. Mouthrinse "B" was nearly as effective against *S. aureus*, *P. gingivalis*, and *E. faecalis*.

Table 1 represents comparison of antibacterial efficacy of various mouthrinses with *P. juliflora* leaf extract in which the zones of inhibition measured in millimeters were analysed using ANOVA test among the samples ABCD and CF. It was denoted that the very high statistical significance difference in all the bacterial strains *S. aureus*, *E. faecalis*, *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*. In *S. aureus* group sample D showed good zone of inhibition 9.12 ± 0.23 the least among group C 2.20 ± 0.17 . Likewise *E. faecalis* group highest Zone Of Inhibition was seen in sample B 11.23 ± 0.23 and least among group C 1.10 ± 0.08 . In *A. actinomycetemcomitans* group higher Zone Of Inhibition group B 14.15 ± 0.21 and least among group A 3.00 ± 0.08 . *P. gingivalis* group highest Zone Of Inhibition group D 11.06 ± 0.22 and least among group C 2.50 ± 0.10 . *P. intermedia* group high Zone Of Inhibition seen among group D 11.21 ± 0.17 and the least among group A 3.10 ± 0.13 . Overall Zone Of Inhibition with control factor ciprofloxacin group shown very high MIC among all the bacterial strains. Since it is a standard control for checking MIC among different bacteria, overall result states that when compared to the commercially available mouthrinses *P. juliflora* extract also had antimicrobial efficacy equivalent to others.

DISCUSSION

Since periodontal diseases are bacterial infections, treating the infection with an antibacterial seems like a wise way to slow down the disease's progression. The most frequent concern with traditional treatment, however, is systemic drug administration, which may lead to toxicity problems. Local drug administration thus turns out to be a better choice. Examples of local delivery methods include mouthwashes, irrigating solutions, dentogels, and sustained release devices [20].

Mouthwashes are crucial for maintaining clean teeth, according to

studies. As a result, their significance has increased dramatically in the contemporary world. When used in accordance with oral hygiene regimens, chlorhexidine, the active ingredient in the majority of mouthrinses, effectively fights a variety of bacteria, preventing and eliminating supragingival plaque and several other issues [21, 22].

Antibiotics are necessary for bacterial infections. Although many medications have been developed and used up to this point, it is generally acknowledged that long-term use of these medications results in resistance to the microbes that cause them. Consequently, natural materials like plant extracts are utilized in their stead. In order to inhibit a variety of microorganisms, the current study evaluated the antibacterial activity of *P. juliflora* leaf extract and discovered that a minimum concentration of 100 µg/10 µl leaf extract was sufficient.

The microbes used in the study were found to be clinical isolates of the most prevalent and powerful infections associated with periodontal and oral diseases [37]. *Staphylococcus* species are generally thought of as transitory bacteria and are not isolated from the oral cavity. Because they are frequent causes of oral and periodontal infections, *P. intermedia*, *P. gingivalis*, and *A. actinomycetemcomitans* were included in the study [23].

Although earlier studies have shown that certain compounds, such as quercetin and apigenin, have antibacterial properties [11, 13], no research on oral and periodontal infections has been done so far. The presence of a pelin, which has anti-allergic, antibacterial, anti-dermatitic, anti-inflammatory, and antiviral properties, and quercetin, which has anti-diabetic, analgesic, antibacterial, anti-inflammatory, and antiviral properties, may be the cause of *P. juliflora*'s bactericidal action [13]. The majority of commercial mouthrinses contained chlorhexidine as the active ingredient in the form of 0.2% w/v chlorhexidine gluconate.

Nevertheless, even without any other ingredients, the *P. juliflora* crude extract showed antibacterial efficacy against the organisms that were examined. It outperformed mouthrinses and outperformed the brand-name product in terms of zone of inhibition. Mouthwash "B", which has chlorhexidine in it, is probably that compounds with well-known antibacterial properties, such as apigenin and quercetin, also had a significant role in suppressing these infections.

CONCLUSION:

The study concludes, the antibacterial effectiveness of *P. juliflora* against tested oral and periodontal pathogens is reported in this in vitro investigation. It outperformed commercial mouthrinses even without any additional chemicals added. This increases the likelihood of further research on the pertinent subject. More purification may increase the extract's activity and increase its potency. However, prior to a thorough analysis of the active components in the *P. juliflora* leaf aqueous extract, several pharmacological and clinical research need to be carried out.

Figures

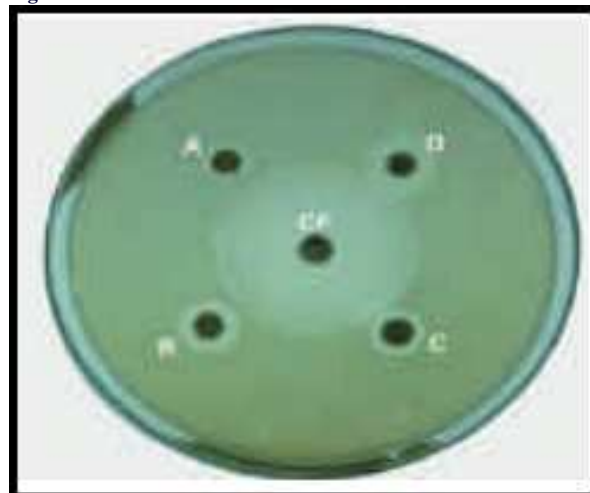


Figure 1. Zones of inhibition observed for *Enterococcus faecalis* on Muller Hinton media for Mouthrinse A, B, C, Ciprofloxacin CF and *P. juliflora* extract D.



Figure 2. Zones of inhibition observed for *Aggregatibacter actinomycetemcomitans* on Wilkins Chalgren Blood Agar media for Mouthrinse A, B, C, Ciprofloxacin CF and *P. juliflora* extract D.



Figure 3. Zones of inhibition observed for *Staphylococcus aureus* grown on Muller-Hinton media with Coomassie Brilliant Blue for Mouthrinse A, B, C, Ciprofloxacin CF and *P. juliflora* extract D.

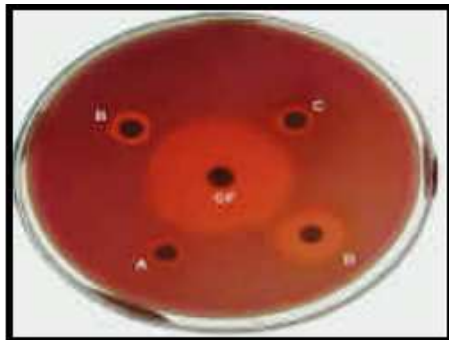


Figure 4. Zone of inhibition observed for *Prevotella intermedia* grown on Wilkins Chalgren Blood Agar media for Mouthrinse A, B, C, Ciprofloxacin CF and *P. juliflora* extract D.

Table 1. Comparison Of Antibacterial Efficacy Of Various Mouthrinses With *P. Juliflora* Leaf Extract

SAMPLER	ZONE OF INHIBITION in mm				
	<i>S. aureus</i>	<i>E. faecalis</i>	<i>A. actinomycetemcomitans</i>	<i>P. gingivalis</i>	<i>P. intermedia</i>
A	3.00±0.12	5.16±0.11	3.00±0.08	4.26±0.21	3.10±0.13
B	8.06±0.22	11.23±0.23	14.15±0.21	9.00±0.18	9.11±0.11
C	2.20±0.17	1.10±0.08	3.34±0.18	2.50±0.10	3.20±0.22
D	9.12±0.23	08.12±0.12	11.23±0.22	11.06±0.22	11.21±0.17
CF	21.23±0.21	25.06±0.32	18.00±0.18	27.93±0.32	18.12±0.13
SIG	0.01	0.00	0.00	0.00	0.00

The 'p' value is 0.001 indicates high significance, n = 6.

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