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Evaluation of the Antibacterial Potential of Jatropha multifida Leaf Extract using Microwave-Assisted Extraction

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ABSTRACT
Oral diseases, such as dental caries and oral candidiasis, are often caused by a microbial imbalance. Conventional antifungal and antibacterial treatments, including Nystatin and Povidone-Iodine, face challenges like microbial resistance and side effects. Given Indonesia's rich biodiversity and the public's growing interest in herbal medicine. This study aims to explore the antimicrobial potential of Jatropha multifida leaf extract as a base for a new mouthwash formulation. Utilizing a post-test only control group experimental design, the study first extracted Jatropha multifida leaves with Microwave-Assisted Extraction. The agar disk diffusion method was then employed to screen various extract concentrations against *Staphylococcus aureus* to identify the most effective one. The collected data on microbial inhibition zones will be statistically analyzed using a One-Way ANOVA and the Dunnet Post Hoc test. We anticipate that the Jatropha multifida will exhibit significant antimicrobial activity, suggesting its potential as a natural and effective alternative to conventional antiseptics. This research provides a foundation for developing a novel herbal-based oral care product that addresses the current challenges of antimicrobial resistance and treatment side effects.

Keywords: *Jatropha multifida, microwave assisted extraction, antimicrobial activity, phytochemical analysis, in vitro study.*

INTRODUCTION
Oral diseases are a significant global health concern, frequently stemming from a disruption in the oral microbial balance (Yama et al., 2023). Pathogenic *Staphylococcus aureus* can also cause various oral problems, including angular cheilitis, endodontic infections, osteomyelitis, and denture stomatitis (Campos et al., 2023). While the nasopharynx is a common habitat, the oral cavity can serve as a starting point for auto-infection and disease transmission (Campos et al., 2023). Prevention and treatment for *S. aureus* infections often involve Povidone-Iodine (Çoğulu et al., 2025). Nevertheless, the use of Povidone/polyvinylpyrrolidone (PVP) is a concern due to potential allergic reactions and side effects such as chemical burns, cytotoxicity to soft tissues, and patient discomfort (Nair et al., 2023; Steins et al., 2023).

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Indonesia possesses one of the world's largest biodiversities (Adiyasa & Meiyanti, 2021). The public often prefers herbal medicines for prevention or treatment due to their perceived safety and lower cost (Saggar et al., 2022). *Jatropha multifida*, also known as the "Betadine plant," has been traditionally used to heal skin wounds (Mardiayanti siti, 2021). This plant has been studied for its antioxidant, anticancer, antibacterial, and anti-inflammatory activities (Vieira et al., 2021). Previous research on *Jatropha multifida* leaf extracts (96% ethanol) prepared via maceration and MAE has shown strong antifungal effects against *Candida albicans* with inhibition zones ranging from 18.9 mm to 24.7 mm (Aryunda et al., 2025). Other studies have also demonstrated its antibacterial effects against *Streptococcus mutans* (Kinasih et al., 2021). These activities are attributed to its phytochemical compounds, including flavonoids, tannins, and saponins (Senou et al., 2022). While a higher concentration of active ingredients generally provides a more potent antiseptic effect, the pure form of these compounds can be unstable over time (Rachmawati et al., 2022). Although *Jatropha multifida* is known as the “Betadine plant," there have been no studies comparing its antiseptic efficacy directly with Povidone-Iodine. Therefore, this study aimed to achieve two primary objectives: to analyze the phytochemical content of Jatropha multifida leaf extract and to observe the in vitro effectiveness of different concentrations of Jatropha multifida leaf extract in inhibiting the growth of common oral pathogenic microorganisms, comparing its efficacy to that of pure 1% Povidone-iodine.

METHODS

Research Design

The research design used in this study was laboratory experiment using a post-test only control group design. The disk diffusion assay was utilized to observe the antimicrobial response of microorganisms to various agents. The post-test only control group design was chosen to compare the outcomes between the treatment groups (various concentrations of the extract) and a control group (Povidone-iodine 1%) after the experiment was conducted. The antimicrobial activity was evaluated using the disk diffusion method. In addition to antimicrobial testing, the study will include a phytochemical analysis to detects the active compounds in the extract. Furthermore, the physical properties of the final mouthwash formulation will be assessed to ensure its stability, safety, and practicality for everyday application.

Location and Time

This research was conducted from July to August 2025. The initial stages, including the drying and extraction of leaves using the Microwave-Assisted Extraction (MAE) method, were performed at the Laboratorium Fitokimia in Universitas Sumatera Utara. The subsequent steps, such as extract concentration, antibacterial testing, and mouthwash formulation, were carried out at the Laboratorium Terpadu in Universitas Prima Indonesia. Finally, the phytochemical content detection of the *Jatropha multifida* leaf extract will be checked at the Laboratorium Terpadium in Universitas Negeri Padang.

Sample Size

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The samples in this study are *Jatropha multifida* leaf extracts. An initial antimictobial test will be conducted to determine the antiseptic effect of the extract that is comparable to 1% povidone-iodine. The initial concentrations used in this initial stage would be 10%, 20%, 30%, 40%, and 50%. These will be compared to 1% povidone-iodine on two types of microbes. The preliminary study will use a total of 12 paper discs.
Once the concentration of *Jatropha multifida* extract with an antiseptic effect comparable to 1% povidone-iodine is identified, the treatment will be repeated to ensure the results are reliable and statistically significant (Charan & Kantharia, 2013). The sample size will be determined using Federer's formula. This study will use four treatment groups (t=4), each observing the zone of inhibition of different test materials against cultures of *Streptococcus mutans* and *Staphylococcus aureus*. The test materials will be 1% povidone-iodine and three types of *Jatropha multifida* leaf extracts with an efficacy equivalent to 1% povidone-iodine. A total of 48 paper discs will be used in the advanced stage.

Research Procedures

First, all metal and glass tools are thoroughly cleaned and then sterilized in an autoclave for one hour at 170°C (340°F). All plastic tools are cleaned, dried, and then rinsed with 70% alcohol. To create the concentrated extract of Jatropha multifida leaves, fresh leaves were dried at 400°C–500°C and then ground into a fine powder using food processor. The leaf powder is then mixed with a solvent in a 1:10 ratio and heated in a 450-watt microwave for 8 minutes. The mixture is then filtered using a vacuum filter to obtain a filtrate. This filtrate is evaporated using a rotary evaporator at 50°C, 80 rpm, and 100 mbar pressure until a concentrated extract is produced (Aryunda et al., 2025; Bagade & Patil, 2021). A phytochemical test of the Jatropha multifida leaf extract were conducted using the LC-MS and were identified by comparing the results with data from literature (Dah-Nouvlessounon et al., 2023). At the same time, the control solution for antimicrobial test were prepared by diluting 1 gram of povidone-iodine powder with distilled water to obtain a 1% povidone-iodine solution with a total solution weight of 100 grams. MHA (Muller Hinton Agar) medium is prepared by weighing 38g of MHA powder according to the composition on the package (2g beef extract; 17.5 g casein hydrolysate; 1.5 g starch; 17 g agar) and then dissolving it in 1 L of distilled water, with the help of heating if necessary. The medium is then sterilized using an autoclave at 121ºC for 20 minutes. The MHA medium is poured into sterile Petri dishes and left at room temperature to solidify. It is then stored at 4ºC (in a refrigerator) (Sharafuddin et al., 2023). A microbial suspension is prepared to a concentration of 1.215 mg/mL, then spread evenly on the Mueller Hinton Agar medium in a Petri dish. Sterile paper discs are dipped into the test solutions. On the initial stage of the test, Jatropha multifida leaf extracts at concentrations of 10%, 20%, 30%, 40%, and 50% and the control solution are used as the test solutions. On the advance stage pf the test, three extract concentrations that show similar inhibition activity to the control are used as the test solutions. These discs are then placed on the inoculated media and to be incubated at 37°C for 24 hours. After incubation, the diameter of the inhibition zone (the clear area around the disc) is measured using a caliper in millimeters (mm) indicating the antimicrobial effectiveness of each solution. After measuring the initial inhibition power, each type of

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solution is compared, and three types of *Jatropha multifida* solutions that have similar inhibitory power to the control are selected for advance stage of inhibition zone test.

RESULTS

Based on the study findings, the inhibition zone of control was 10.33 mm for Streptococcus mutans and 10.34 for *Staphylococcus aureus.* Among the tested concentrations of *Jatropha multifida* solution, the three with the nearest inhibitory effect to the control were found to be 30%, 40%, and 50%.The results of this test are summarized in Table 1.

Table 1. Results for measurement of early stage inhibition zone diameter assay

Treatment	**Inhibiton Zone Diameter (mm)**	
	Streptococcus mutans	*Staphylococcus aureus*
10	7.57	7.90
20	7.63	8.53
30	7.67	10.19
40	8.79	10.31
50	9.48	10.79
K+	10.33	10.34

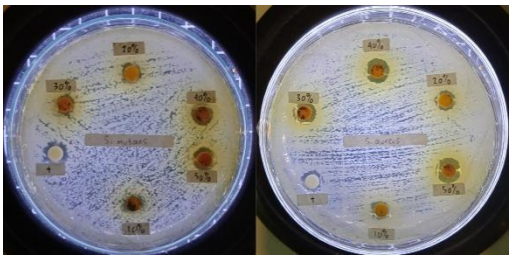

Figure 1. Early stage antimicrobial assay.

Table 2. Results for measurement of advance stage inhibition zone diameter assay.

Microbe Type	Repetitions	Inhibition Zone Diameter			
		50	40	30	K+
Streptococcus mutans	1	8.36	7.75	7.9	9.76
	2	9.18	8.77	7.52	9.48
	3	10.35	10.16	7.14	10.48
	Mean	9.30	8.89	7.52	9.91
	SD	1	1.21	0.38	0.52
Staphylococcus aureus	1	10.69	9.46	9.15	8.68
	2	10.37	9.75	8.89	8.74
	3	10.7	9.54	9.15	8.83
	Mean	10.59	9.58	9.06	8.75
	SD	0.19	0.15	0.15	0.08

Table 3. One-Way ANOVA Test Results.

Bacterial test	Treatment	Repetition	Average ± SD	*p*-values
Streptococcus mutans	Control	3	8.8±0.45	p = 0.007
	Jatropha multifida 30%	3	7.52±0.38	

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	Jatropha multifida 40%	3	7.53±0.19	
	Jatropha multifida 50%	3	8.34±0.44	
Staphylococcus aureus	Control	3	8.75±0.08	p = < 0.001
	Jatropha multifida 30%	3	9.07±0.16	
	Jatropha multifida 40%	3	9.59±0.15	
	Jatropha multifida 50%	3	10.59±0.19	

These results are further detailed in Figures 2 and 3.effectivity observed *Jatropha multifida* leaf extract group (8.675 ± 0.5620 mm), while the highest was in the 50% group (12.725 ± 0.2500 mm).

A Shapiro-Wilk test was performed for normality, revealing that the data was normally distributed ($p>0.05$). Due to this normal distribution, the data underwent further statistical analysis using a one-way ANOVA test, which confirmed a significant difference ($p<0.05$) in the diameter of the inhibition zone among the 30%, 40%, 50%, and well as the positive (K+).

To pinpoint the specific differences in the inhibition of *Streptococcus mutans* and *Staphylococcus aureus* growth between all groups, a post hoc Dunnet test was conducted. The results of this test are summarized in Table 4.

DISCUSSION

This study primarily aimed to achieve two objectives: to analyze the phytochemical content of Jatropha multifida L. leaf extract by MAE and to observe its in vitro effectiveness in inhibiting the growth of common oral pathogens, *Streptococcus mutans* and *Staphylococcus aureus*. The research design, a post-test only control group laboratory experiment, was specifically chosen to effectively compare the antimicrobial efficacy of various concentrations of the plant extract against a standard antiseptic agent, Povidone-iodine 1%.

The results from the disk diffusion assay demonstrate that the Jatropha multifida leaf extract possesses significant antimicrobial properties against both Streptococcus mutans and *Staphylococcus aureus*. All tested concentrations (50%, 40%, and 30%) showed an inhibitory effect, with the 50% concentration proving to be the most effective. Notably, this concentration produced a mean inhibition zone of 10.59 mm against *Staphylococcus aureus*, which surpassed the positive control, Povidone-iodine 1% (K+), which had a mean inhibition zone of 8.75 mm. This is a particularly promising finding, suggesting the extract's strong potential as a natural alternative to conventional antiseptic agents.

These findings are consistent with, and expand upon, existing literature on Jatropha multifida. Our results align with the study by Listyaning (2021), which also found that this plant extract can inhibit the growth of *Streptococcus mutans*. Similarly, our findings corroborate the research by Sansetiyawati M (2015) and D. Anggita et al. (2018), which established the antibacterial effect of Jatropha multifida leaf extract on *Staphylococcus aureus*. The concentration-dependent increase in the inhibition zone observed in our study is directly supported by their findings, reinforcing that higher concentrations of the extract lead to greater inhibitory effects.

The observed antimicrobial activity is likely attributable to the presence of various secondary metabolites within the Jatropha multifida leaf extract. While the full results of the phytochemical analysis are forthcoming, it is anticipated they will confirm the presence of

compounds such as flavonoids, alkaloids, tannins, and saponins. These compounds are well-documented in scientific literature for their potent biological activities, including antimicrobial effects. The presence of secondary metabolites would provide a clear scientific basis for the extract's observed effectiveness. The results of this study suggest a viable and effective natural alternative to synthetic chemicals for combating oral pathogens. The demonstrated efficacy of the Jatropha multifida extract against both *S. mutans* and *S. aureus* indicates its potential for future development as a natural therapeutic agent.

Limitations and Directions for Future Research

Despite these promising results, several limitations must be acknowledged. This study's design as an in vitro experiment does not fully replicate the dynamic and complex conditions of the human oral cavity, such as variations in pH, temperature, and the presence of a diverse oral microbiome. Therefore, the findings may not directly translate to real-world efficacy.

Based on these limitations, several insightful directions for future research are recommended. In vivo studies using animal models and, eventually, clinical trials on human subjects are essential to validate the safety and efficacy of the extract in a real-world setting. Furthermore, future research should focus on isolating and purifying the specific active compounds from the extract to determine their individual contributions to the antimicrobial effect. This would allow for a deeper understanding of the mechanism of action and the development of more targeted and potent formulations.

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