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Research Article

Antibacterial Test of Ruku-Ruku Leaf Extract (*Ocimum tenuiflorum* L.) Against the Growth of *A. actinomycetemcomitans*

Hanifah Ramadhanty[1], Aria Fransiska[2*], Rahmi Khairani Aulia[2]

[1] Faculty of Dentistry, Andalas University, Padang, Indonesia
[2] Departement of Dental Material, Andalas University, Padang, Indonesia

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Abstract

A. actinomycetemcomitans bacteria is the main pathogenic microorganism identified as the cause of aggressive periodontitis, with a prevalence of 90%. Periodontitis treatment is generally done mechanically by scaling root planning (SRP) and chemically by administering antibiotics as antibacterial agents. Specifically, ruku-ruku leaves contain several natural compounds with potential as antibacterials. This study, thus, aims to determine the potency of ruku-ruku leaf extract in inhibiting the growth of *A. actinomycetemcomitans* bacteria. This study employed a true experimental method in the form of a post-test-only control group design. Ruku-ruku leaf extract was prepared by a maceration method using 96% ethanol solvent and diluted with DMSO (Dimethyl Sulfoxide) to obtain concentrations of 10%, 20%, 40%, and 70%. The inhibition test was conducted using the Kirby-Bauer method, which utilized paper discs on Mueller Hinton Agar media. The zone of inhibition formed around the discs was measured. Data analysis was then performed using the One-Way ANOVA test, and the Post Hoc LSD (Least Significant Difference) test was continued. The results of this study revealed significant differences in inhibition from each treatment group. The concentration of 70% ruku-ruku leaf extract is more effective in inhibiting the growth of *A. actinomycetemcomitans* with an inhibition zone of 7.49 mm. Post Hoc LSD results uncovered *p* values of 0.001 and 0.003 ($p<0.01$). In conclusion, ruku-ruku leaf extract has potential antibacterial properties against the organization of *A. actinomycetemcomitans*.

Keywords: aggressive periodontitis, *Aggregatibacter actinomycetemcomitans*, antibacterial agent, *Ocimum tenuiflorum*, ruku-ruku

INTRODUCTION

Aggressive periodontitis is a periodontal disease due to a complex dynamic interaction between specific pathogenic bacteria, immune response, and environmental factors.[1,2] Aggressive periodontitis can develop early in childhood or adolescence in younger individuals under 30 years of age and can also affect individuals with a systemic predisposition.[3] Aggressive periodontitis cases are estimated to be 0.1% in developed countries, 5% in developing countries, and 8% in Indonesia.[4] The prevalence of aggressive periodontitis in subjects aged ≤35 years ranges from 1% to 15%.[5] The prevalence of aggressive [*]periodontitis is rare, but the disease can lead to more rapid tooth loss in affected individuals if not diagnosed early and treated properly.[3,6]

The main pathogenic microorganism identified as the cause of aggressive periodontitis is *A. actinomycetemcomitans*, having the highest prevalence in patients with aggressive periodontitis at 90% compared to only 21% of patients with chronic periodontitis and about 17% in healthy individuals.[7] These bacteria have a defense mechanism by forming biofilms and causing damage to periodontal tissues.[7,8]

Early-stage treatment in periodontal cases can be done mechanically by scaling root planning and curettage, while

[*]Corresponding author, e-mail: aria.fransiska@dent.unand.ac.id

chemically, it provides antimicrobial therapy. Antimicrobial therapy can be performed locally or systemically.[8,9] Local administration of antimicrobials has the advantage of reducing continuous drug consumption, allowing long-term drug administration, and can be applied directly to the area of disease activity.[10] Local administration of antimicrobials with gel preparations is more widely used for treating oral mucosa, as it can penetrate the tooth socket well and does not leave a sticky residue. Metronidazole gel is one of the most commonly used local antibiotics in periodontal treatment since it has a good ability to overcome anaerobic bacteria with minimal side effects. However, the circulation of this gel remains small in the market because it is difficult to obtain and relatively expensive.[9–11]

Hence, the development of herbal natural materials such as plant extracts can be used as an alternative antimicrobial agent in local antibiotic therapy with gel preparations.[8] Using herbal ingredients has no side effects, is affordable, and is easy to get.[12,13] Moreover, Indonesia is a country that has contributed to world medicine with herbal plants and is one of the largest users of herbal plants in the world.[12,14] Herbal plants have been used for thousands of years as raw materials for medicines.[12]

One example of a plant included in Indonesia's herbal commodities is the ruku-ruku plant.[14] This plant is classified as a horticultural plant in biopharma commodities based on the Decree of the Minister of Agriculture Number 511/Kpts/PD.310/9/2006.[15] Ruku-ruku (*Ocimum tenuiflorum* L.) belongs to the family of medicinal plants and is often used by the Indonesian people as a spice for cooking and traditional medicine.[16–19] As an example, the people of Rimbo Tarok, Kuranji Sub-district, Padang City, believe that ruku-ruku is a toothache medicine, especially for children who do not want to take medicine.[20] This plant contains alkaloids, terpenoids, saponins, tannins, steroids, and flavonoids, which are secondary metabolites supporting its pharmacological properties, such as antibacterial and antioxidant.[17]

Previous antibacterial activity research has shown that ethanol extract from ruku-ruku leaves can inhibit the growth of *Escherichia coli* bacteria with a strong category at concentrations of 40% and 70% compared to using concentrations of 10% and 20%.[21] Similar research also revealed that ethanol extract of ruku-ruku leaves at concentrations of 5%, 10%, and 20% against *Staphylococcus epidermidis* bacteria can be used as an antibacterial with a strong category.[22] Nevertheless, another similar study demonstrated that ruku-ruku leaf extract (*Ocimum tenuiflorum* L.) could not be proven to have inhibition against the growth of specific bacteria *Salmonella enterica serovar* Typhi.[17]

Based on the description above, this research aims to determine the inhibition of ruku-ruku leaf extract against the growth of *A. actinomycetemcomitans* as the dominant bacteria causing aggressive periodontitis with concentrations of 10%, 20%, 40%, and 70%.

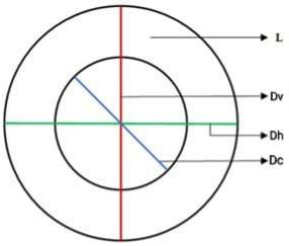
MATERIALS AND METHODS

Ruku-ruku (*Ocimum tenuiflorum* L.) was collected from the Kalumbuk area, Nanggalo District, Padang City, West Sumatra, and has been identified in Herbarium of Universitas Andalas. The ruku-ruku leaf extract has been qualitatively tested for phytochemicals. The results of phytochemical tests showed flavonoids, tannins, and steroid compounds. The bacterial isolate used in this study was a pure culture of *A. actinomycetemcomitans* ATCC: 29522, which is available at the Microbiology Laboratory of the Faculty of Medicine, Universitas Andalas with No. 17B/UN.16.2/Lab.Mikro/IV/2024. The medium utilized for the test was Mueller Hinton Agar (MHA). The control solution employed was DMSO (MERCK 102952, Germany).

This true experimental laboratory research applied a post-test-only control group design conducted on 30 samples. The extracts were prepared at the Organic Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Andalas, and the inhibition test was carried out at the Microbiology Laboratory, Faculty of Medicine, Universitas Andalas. The Ethics Committee of Medicine Faculty of Andalas University approved the study with No. 138/UN.16.2/KEP-FK/2024.

The extract was prepared using a maceration method. The fresh ruku-ruku leaves, as much as 4 kg, were cleaned with running water and then cut into small pieces and air-dried. Next, it was pulverized using a blender until 800 grams of powder were obtained. Ruku-ruku leaf powder was soaked with 96% ethanol solvent for 48 hours at room temperature with occasional stirring. After two days, the filtrate was filtered and continued by concentrating the extract with a rotary evaporator until a thick extract was obtained.[21] Extract concentrations of 10%, 20%, 40%, and 70% were diluted using DMSO solvent.

Preparation of bacterial suspensions began with taking the bacterial culture of *A. actinomycetemcomitans* on agar media using an Ose needle that had been incinerated, then scratching zigzag on all blood agar media evenly. They were incubated for 24 hours at 37ºC in an anaerobic atmosphere. Bacteria that had been incubated were taken colonies from blood agar media with a sterile Ose needle and then put into a test tube containing 0.9% NaCl solution until the turbidity was obtained according to the 0.5 McFarland standard or equivalent to (1.5 x 108 CFU/ml); afterward, with a sterile cotton swab, the bacterial suspension was inoculated on the top of the MHA media evenly.[23] The inhibition test was carried out using the Kirby-Bauer disc paper diffusion method by placing disc paper previously soaked in the extract and control solutions for 15 minutes. MHA media was then incubated at 37ºC for 24 hours in an anaerobic atmosphere in an incubator, and the inhibition zone formed, indicated by the clear zone around the disc, was observed.[21] Measurement of the inhibition zone formed around the disc paper was performed by measuring the vertical diameter, horizontal diameter, and disc diameter utilizing a caliper with mm units, and then the measurement results were calculated using the formula.[24,25] The data obtained were then analyzed with One-way ANOVA and Post Hoc LSD tests.



$$\frac{(Dv - Dc) + (Dh - Dc)}{2}$$

Note:
L = inhibitory zone
Dv = diameter of vertical
Dh = diameter of horizontal
Dc = diameter of the disc

RESULTS

The inhibition zones formed in ruku-ruku leaf extract with concentrations of 20%, 40%, and 70% can be seen in Figure 1. The inhibition zone was not formed in the ruku-ruku leaf extract with 10% concentration and DMSO control solution. The white areas formed around the discs with 10% concentration were extract precipitation or chemical interaction with the agar medium. The average results of the diameter of the inhibition zone can be seen in Table 1.

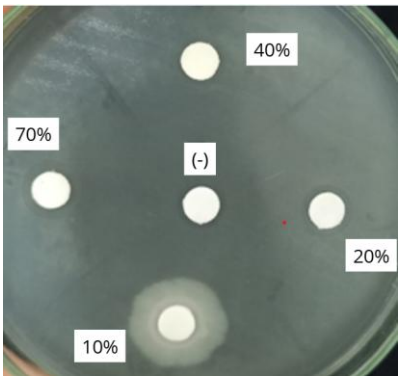


Figure 1. Inhibition zone test results by Kirby-Bauer disc diffusion method

Table 1. Average Diameter of Inhibition Zone of Treatment Group

Group	n	Inhibition zone diameter (mm)
		$\bar{x} \pm SD$
DMSO	6	0 ± 0
Ruku-ruku leaf extract 10%	6	0 ± 0
Ruku-ruku leaf extract 20%	6	4.91 ± 0.85
Ruku-ruku leaf extract 40%	6	6.43 ± 0.082
Ruku-ruku leaf extract 70%	6	7.49 ± 0.46

Information:
$\bar{x}$ = average diameter of the resistance zone
n = number of samples
SD = standard deviation

Table 1 reveals the average size of the inhibition zone formed in the leaf extract of ruku-ruku (*Ocimum tenuiflorum* L.). The difference in inhibition zones, according to David and Stout (1971), in measuring the response of bacteria to antibacterials based on the clear zone formed around the disk is grouped into four categories: the powerful category with an inhibition zone of >20 mm, the strong category with an inhibition zone between 10-20 mm, the medium category with an inhibition zone of 5-10 mm, and the weak category with an inhibition zone of <5 mm.[26] The concentration of 10% and the DMSO control solution had an average inhibition zone of 0.00 mm, meaning there was no inhibition against bacterial growth of *A. actinomycetemcomitans*. The inhibition zone formed at a concentration of 20% is classified as weak, while the concentration of 40% and 70% is classified as moderate.

The data from the univariate analysis were then processed statistically to analyze the inhibition of ruku-ruku leaf extract against the growth of *A. actinomycetemcomitans* bacteria. Bivariate analysis began with the Shapiro-Wilk normality test with a significance (p>0.01) to determine the normal distributed data. The results of the Shapiro-Wilk test obtained a value of p<0.01, indicating that the data were not normally distributed. Data transformation was carried out using natural logarithms to distribute the data normally (p>0.01). The Levene test was then conducted to determine whether the data tested had a homogeneous data variant. The results of the Levene test were obtained at p = 0.011, meaning that the data variant was homogeneous (p>0.01).

The data analysis was continued with the One-way ANOVA test with a p<0.01 result, which denotes that there was

a significant difference between the 20%, 40%, and 70% concentrations of Ruku-ruku leaf extract in inhibiting the growth of *A. actinomycetemcomitans*. A Post Hoc follow-up test analyzed the difference in barriers between treatment groups. The results of the Post Hoc LSD (Least Significant Differences) test can be seen in Table 2.

Table 2. Results of Post Hoc LSD Analysis

Treatment groups	Ruku-ruku leaf extract		
	20%	40%	70%
Ruku-ruku leaf extract 20%		0.001*	0.001*
Ruku-ruku leaf extract 40%			0.003*
Ruku-ruku leaf extract 70%			

** $p<0.01$ value = significant*

Table 2 exhibits a significant difference between the treatment groups of 20%, 40%, and 70% ruku-ruku leaf extracts with a $p<0.01$ value.

DISCUSSION

The inhibition zone formed at a concentration of 20% is classified as weak, while the concentration of 40% and 70% is classified as moderate (Table 1). This differs from research conducted previously, showing that ethanol extract of ruku-ruku leaves has an inhibitory effect on the growth of *Staphylococcus epidermidis* bacteria with a weak category at a concentration of 10%, which is 9.8 mm and a strong category at a concentration of 20% with an inhibition zone of 12.1 mm.[22] This difference is also shown in the results of previous studies, revealing that ethanol extract of ruku-ruku leaves at concentrations of 10% and 20% have no inhibitory effect on the growth of *Escherichia coli* bacteria. In comparison, at concentrations of 40% and 70%, it has inhibition with a strong category with the formation of inhibition zones, namely 17 mm and 19 mm.[21]

Those differences can be influenced by the origin of the plant and its growth location because differences in geographical conditions and climate change can result in variations in the chemical compound content of a plant. The location of the growth is a biological factor that can affect the quality of the extract.[27,28] The location of the plant is also related to the height of the place where it grows, air temperature, air humidity, rainfall, and sunlight intensity. This aligns with previous research comparing simplisia from two different regions based on altitude, namely Bogor (at an altitude of 190-350 m above sea level) and Wonosobo (at an altitude of 250-2250 m above sea level), which showed that extracts with simplisia from Bogor contained better secondary metabolite compounds. For every 100 m rise above sea level, air humidity, rainfall, and sunlight intensity will increase, while the air temperature will drop by 0.50°C. This can affect the plant's morphology, genetics, and secondary metabolite compound content.[29]

The difference in the results of this study may also be due to the differences in the bacteria tested because each bacterium has a different sensitivity to the antibacterial compounds given.[30,31] The inhibition produced by the ethanol extract of ruku-ruku leaves with a concentration of 10% against *Staphylococcus epidermidis* bacteria in previous studies has a better ability than testing the same concentration of ruku-ruku leaf extract against *Escherichia coli* bacteria and *A. actinomycetemcomitans* bacteria tested in this study.[21,22] This is possible because *Escherichia coli* and *A. actinomycetemcomitans* are gram-negative bacteria, while *Staphylococcus epidermidis* bacteria are gram-positive. Gram-positive

bacteria tend to be more sensitive to antibacterial compounds than gram-negative bacteria, as gram-positive bacteria have a simpler cell wall structure, making it easier for antibacterial compounds to enter this gram-positive bacterial cell.[32,33]

Additionally, the difference in the inhibition zone formed in each research group agrees with previous studies, affirming that the higher the extract concentration, the greater the inhibition zone formed. This happens because the higher the concentration, the more active compounds are contained, so the effectiveness in inhibiting bacteria will increase and produce a wider inhibition zone.[22,34] The ability of ruku-ruku leaf extract to inhibit the growth of *A. actinomycetemcomitans* bacteria is because its active compounds have antibacterial abilities, such as flavonoid compounds, tannins, and steroids.

Flavonoids are plant defense compounds that inhibit insect appetite (antifeedant) and are toxic, but as antibacterials, flavonoids do not kill bacterial cells but induce the formation of bacterial aggregates, thereby reducing the number of colonies.[35] The flavonoid compounds in ruku-ruku leaf extract can damage the bacterial cell wall by altering organic components and nutrient transport from bacteria. This results in flavonoid compounds in this extract being able to enter the nucleus of bacterial cells and react with DNA, with the polarity difference between the lipids that make up the DNA and the flavonoids resulting in DNA lipids being damaged and bacteria lysing and cell death.[22,30,36,37]

Meanwhile, tannins are a group of polyphenols that are spread throughout the plant kingdom. The tannin compounds in this extract have properties that can shrink the bacterial cell membrane and cause cell permeability so that bacterial metabolism becomes disrupted, and eventually, the bacteria become lysed and die.[36–38] In addition, the steroid compounds in this extract can decrease the integrity of the cell membrane and disrupt cell membrane morphology so that bacterial cells become lysed.[39]

The largest inhibition zone was formed by ruku-ruku leaf extract with a concentration of 70%, and the smallest inhibition zone was formed by ruku-ruku leaf extract with a concentration of 20%. This result followed previous research on the concentration of ruku-ruku leaf extract on bacterial growth. It also indicates that increasing the concentration of ethanol extract from ruku-ruku leaves was directly proportional to its antibacterial power.[22] Research on the inhibition test of herbal plants with the potential to be natural antimicrobials states that plants that can be used as natural antibiotics are those with a very strong inhibition category (inhibition zone diameter ≥20 mm) against bacterial growth.[40] This proves that the 70% concentration of ruku-ruku leaf extract, showing the largest inhibition zone in this study with the inhibition power classified as medium, cannot be used as an antibiotic.

CONCLUSION

In conclusion, ruku-ruku leaf extract (*Ocimum tenuiflorum* L.) has antibacterial activity against the growth of *A. actinomycetemcomitans* bacteria. 70% ruku-ruku leaf extract is considered more effective in inhibiting the growth of *A. actinomycetemcomitans*.

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