

Effectivity of Mengkudu (*Morinda citrifolia*) Fruit Extract in Inhibiting the Growth of *Streptococcus viridans* Causing Dry Socket Post Extraction

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ABSTRACT

Background: Post-extraction wound healing usually proceeds but sometimes has problems and causes complications in the form of a dry socket. *Streptococcus viridans* bacterial infection is one of the causes of dry socket, therefore prevention of infection can be done by providing antibiotic or antibacterial (antiseptic) therapy. Noni fruit (*Morinda citrifolia*) contains many phenols, saponins, and alkaloids that can potentially be antibacterials. **Objective:** This study aims to determine the effectiveness of noni (*Morinda citrifolia*) extract against *Streptococcus viridans* bacteria in post-extraction sockets. **Methods:** This type of research is a laboratory experiment with Post test only control group design, where this study uses the Kirby-Bauer diffusion method with *Streptococcus viridans* bacteria samples and uses noni fruit extract (*Morinda citrifolia*) with concentrations of 3.125%, 6.25%, 12.5%, 25%, and 50%; Positive control Chlorhexidine digluconate 0.2%; And negative control DMSO. **Results:** The data analysis using Kruskal-Wallis showed a p-value of 0.000 on inhibition, which showed that noni fruit extract could inhibit the growth of *Streptococcus viridans* bacteria. **Conclusion:** Noni fruit extract (*Morinda citrifolia*) effectively inhibits the growth of *Streptococcus viridans* bacteria with an effective dose of 50%, but the antibacterial activity is still below the activity of Chlorhexidine gluconate 0.2%.

Keywords: Noni fruit extract, *Streptococcus viridans*, Antibacterial

ABSTRAK

Latar Belakang: Penyembuhan luka pasca ekstraksi pada umumnya berjalan dengan normal, akan tetapi terkadang mengalami masalah dan menimbulkan komplikasi berupa dry socket. Infeksi bakteri *Streptococcus viridans* menjadi salah satu penyebab dari dry socket, untuk itu pencegahan infeksi dapat dilakukan dengan memberikan terapi antibiotik atau antibakteri (antiseptik). Buah Mengkudu (*Morinda citrifolia*) banyak mengandung fenol, saponin dan alkaloid yang berpotensi sebagai antibakteri. **Tujuan:** Penelitian ini bertujuan untuk mengetahui efektivitas ekstrak mengkudu (*Morinda citrifolia*) terhadap bakteri *Streptococcus viridans* pada soket pasca ekstraksi. **Metode:** Jenis Penelitian adalah eksperimental laboratorium dengan rancangan Post test only control group design, dimana penelitian ini menggunakan metode difusi Kirby-Bauer dengan sampel bakteri *Streptococcus viridans* dan menggunakan ekstrak buah mengkudu (*Morinda citrifolia*) dengan konsentrasi 3,125%, 6,25%, 12,5%, 25% , dan 50%; Kontrol positif Chlorhexidine diglucunate 0,2%; Dan kontrol negatif DMSO. **Hasil:** Hasil analisis data menggunakan Kruskal-Wallis menunjukkan nilai p-value 0,000 terhadap daya hambat yang menunjukkan ekstrak buah mengkudu mampu menghambat pertumbuhan bakteri *Streptococcus viridans*. **Kesimpulan:** Ekstrak buah mengkudu (*Morinda citrifolia*) efektif menghambat pertumbuhan bakteri *Streptococcus viridans* dengan dosis efektif 50%, akan tetapi aktivitas antibakteri masih dibawah aktivitas Chlorhexidine glocunate 0,2%.

Kata Kunci: Ekstrak buah mengkudu, *Streptococcus viridans*, Antibakteri

1. Introduction

Tooth extraction will leave a wound in the form of an open tooth socket. Wound healing after tooth extraction typically proceeds. Still, sometimes, it experiences problems and causes complications in the form of alveolar osteitis and osteomyelitis, which provide discomfort for patients, such as pain that interferes with masticatory function and speech function (1). The role of microorganisms supports

the disruption of the wound healing process because the endotoxins produced cause prolongation of the inflammatory phase.

So far, prevention of bacterial infections has been carried out using antiseptics such as Chlorhexidine gluconate 0.2%. Chlorhexidine is a broad-spectrum antibacterial agent that uses chemicals to control bacterial activity. Long-term use of Chlorhexidine will cause side effects such as a burning sensation due to changes in mucous membranes. Therefore, many studies have been conducted to find quality natural ingredients equivalent to the ability of Chlorhexidine gluconate with minimal side effects.

Plants that have potential as natural antibacterials and stimulate wound healing are noni fruit (*Morinda citrifolia*) because they contain compounds including phenols, saponins, alkaloids, flavonoids, tannins, and phiomol-iridoids. Noviana R (2021) shows that the antibacterial activity of noni fruit at a concentration of 2.5% inhibits the bacterial activity of *S. viridans* by 6 mm (2). This research did not carry out phytochemical screening, so it cannot be known what compounds play the most role. Maskoen, A. M., and Hernowo, B. S. (2013) stated that noni fruit (*Morinda citrifolia*) can stimulate the wound healing process aimed at regulating the expression of vascular endothelial growth factor (VEGF) and the formation of type III collagen (3).

The study of the antibacterial activity of noni fruit against *Streptococcus viridans* that cause dry sockets is still minimal. Therefore, the Author wants to further examine this research by identifying the profile of secondary metabolite compounds through phytochemical screening and determining the antibacterial effectiveness of noni fruit (*Morinda citrifolia*) with certain concentration variations against *Streptococcus viridans* bacteria in post-extraction sockets.

2. Material and Methods

This research has been approved by the Animal Research Ethics Commission (KEPK) at the Faculty of Mathematics and Natural Sciences, University of North Sumatra. The samples used were *Streptococcus viridans* bacteria cultured at the Microbiology Laboratory of the General Hospital of the University of North Sumatra. This research is a laboratory experiment with five different concentrations, namely 3.125%, 6.25%, 12.5%, 25%, and 50%; DMSO as a negative control and Chlorhexidine 0.2% as a positive control repeated four times. This study used a diffusion test with the Kirby-Bauer method.

2.1. Extract Preparation

Production and dilution of noni fruit extracts were carried out at the ASPETRI Medicinal Plants Research and Development Laboratory. Noni fruit extract is made through the maceration process of noni fruit powder (*simplisia*), which weighs as much as 80 grams. The extraction method is by adding 96% ethanol, as much as 250ml, stirring for the first 6 hours while stirring occasionally, then letting stand for 18 hours. Then, it is filtered using cotton, filter paper, and the filtrate to obtain the results of maserat I. Repeat the extraction process by mixing the dregs with 125 ml of 96% ethanol to obtain maserat II. Then, combine the two macerates. Then, the macerate is evaporated using a Rotavapor device at a temperature of 40°C to get a thick extract. After receiving the noni fruit extract, it was diluted using DMSO to obtain each extract with a concentration of 3.125%, 6.25%, 12.5%, 25%, and 50%.

2.2. Phytochemical Screening

Phytochemical screening is conducted to determine the presence or absence of chemical compounds or active ingredients in a plant extract. Specific compounds such as flavonoids, alkaloids, tannins, saponins, terpenoids, phenols, and steroids are detected using reagents. The content of these compounds in plant extracts is determined by observing the changes after the reagents are added to the extract being tested.

2.2.1. Quantitative phytochemical screening

The phytochemical tests revealed that the extract contains phenols, alkaloids, flavonoids, saponins, tannins, terpenoids, and steroids. A black color change with FeCl detected phenols, while alkaloids were identified using Mayer, Bouchardat, and Dragendorff reagents. An orange-to-red color indicated flavonoids after reacting with HCl, Mg, and amyl alcohol. Saponins were detected by stable

froth formation after adding HCl. Tannins were identified by a dark blue or blackish-green color change after reacting with FeCl₃ and a light yellow color change after adding gelatin. A reddish-brown or purple color change recognized terpenoids after adding acetic acid anhydride and H₂SO₄, while a blue-to-green color identified steroids after mixing with H₂SO₄.

2.2.2. Qualitative phytochemical screening

Total phenol, flavonoid, tannin, saponin, and alkaloid content was determined using various spectrophotometric methods. The Folin-Ciocalteu reaction was used for phenols with absorbance measured at a wavelength of 732 nm. Flavonoid content was determined by mixing the extract with ethanol, aluminum chloride, sodium acetate, and distilled water and then measuring the absorbance at 765 nm. Tannins were measured by reacting the extract with Folin-Ciocalteu reagent and Na₂CO₃, with absorbance observed at 765 nm. Saponins were measured after autoclaving the extract with H₂SO₄, followed by extraction and absorbance reading at 435 nm. Finally, alkaloid content was determined by extracting the ethanol extract with HCl and chloroform, neutralizing it, and measuring the absorbance at 430 nm.

2.3. Antibacterial Preparation of Noni Fruit

The study involved culturing *Streptococcus viridans* on blood agar media at the University of North Sumatra Microbiology Laboratory. Bacterial suspensions were prepared and spread on Muller Hilton Agar. Sterile paper discs soaked in various concentrations of noni fruit extract, a negative control (dimethyl sulfoxide), and a positive control (0.2% Chlorhexidine gluconate) were placed on the agar. After 24 hours of incubation at 37°C, the diameter of the clear zones around the discs was measured to assess antibacterial activity.

2.4. Data Analysis

The effect of antibacterial of *Streptococcus viridans* was analyzed by the Kruskal-Wallis test and Post Hoc test (Mann-Whitney). Also, the Significance is $p < 0.05$.

3. Result and Discussion

Phytochemical screening is performed to identify secondary metabolite compounds in noni fruit extract by adding specific reagents. Color changes indicate positive results for the presence of certain compounds, the formation of precipitates, or foam. The screening results confirm that noni fruit contains alkaloids, flavonoids, tannins, saponins, and triterpenoids (Table 1).

Table 1. Results Qualitative Phytochemical Screening of Noni Fruit (*Morinda citrifolia*)

No	Compound	Reagent	Result	%
1.	Alkaloid	a. Meyer	+	a. Yellow coloured solution
		b. Dregendorf	+	b. Red-brown coloured solution
		c. Bouchardat	+	c. Orange coloured solution
2	Flavonoid	HCl pekat + Serbuk Mg	+	Orange coloured solution
3	Tanin	Gelatin 1%	+	Clear yellow coloured solution
4	Saponin	Aquadest + HCl 2N	+	Foam formed
5	Triterpenoid	Asam asetat anhidrat + H ₂ SO ₄ Pekat	+	Brownish red solution
6	Steroid	Asam asetat anhidrat + H ₂ SO ₄ Pekat	-	Brownish red solution
7	Fenol	FeCl	+	Thick black coloured solution

Phenol has the highest level in noni fruit extract with a total of 32.8866 mgGAE/g extract (see Table 2). Phenol contained in noni fruit extract can denature the structure of bacterial proteins, causing fragility in the bacterial cell wall and disrupting the process of absorption of nutrients by bacteria. Compared to the results of phytochemical screening between noni fruit extract conducted in this study and that conducted by Triyasmono L.A.K (2016), it is almost the same as 14,44 mgGAE/g extract (4). In research, Sari T, et al. (2021) showed that the total phenolic compound obtained from the ethanol extract of noni fruit was 171.91 mg/L (5).

Table 1. Results Qualitative Phytochemical Screening of Noni Fruit (*Morinda citrifolia*)

Sample	Average alkaloids (mgGAE/g extract)	Average Tannins (mgGAE/g extract)	Average Flavonoids (mgGAE/g extract)	Average Saponins (mgGAE/g extract)	Average Fenols (mgGAE/g extract)
Repetition I	11,2540	16,7622	13,6327	30,9382	33,5148
Repetition I	12,7401	19,3891	17,7197	24,3774	32,0121
Repetition I	10,3374	16,1339	14,1110	29,7405	33,1330
Total	11,4438	17,4284	15,1545	28,3516	32,8866

An antibacterial activity test was conducted to determine the microbial growth response to antibacterial substances. This study used the disc diffusion method with DMSO as a negative control because DMSO does not provide antimicrobial effects and Chlorhexidine gluconate as a positive control (6). The results of the antibacterial activity test were carried out by measuring the diameter of the clear zone formed around the disc paper after incubating at 37°C for 24 hours. The results showed that noni fruit extract with concentrations of 3.125%, 6.25%, 12.5%, 25%, and 50% and Chlorhexidine gluconate 0.2% (minosep®) as a positive control found a clear zone around the disc, indicating the inhibition of the growth of *Streptococcus viridans* bacteria with different inhibition diameters.

Table 3. Diameter value of inhibition of *Morinda citrifolia*

Treatment group	Repetition				Diameter mean (mm)	SD
	1	2	3	4		
Noni fruit extract (<i>Morinda citrifolia</i> L.)						
Concentration 3,125%	6,7	7,1	7,0	7,0	6,950	0,173
Concentration 6,25%	7,2	7,5	8,2	7,1	7,500	0,496
Concentration 12,5%	7,5	8,3	9,7	7,5	8,250	1,037
Concentration 25%	8,1	10,2	9,9	7,6	8,950	1,292
Concentration 50%	12,0	13,7	14,9	10,6	12,800	1,888
Positive control (<i>Chlorhexidine diglucunate</i> 0,2% (minosep®))	15,9	16,3	15,1	20,5	16,950	2,418
Negative control (DMSO)	0	0	0	0	0	0

Based on the research results, the effective concentration of noni extract against *Streptococcus viridans* is 50%, 12.80 ± 1.89 mm. The antibacterial ability produced by noni fruit extract concentrations of 3.125%, 6.25%, 12.5%, 25%, and 50% has not been able to be more effective than the antibacterial ability of Chlorhexidine gluconate 0.2% with an inhibition diameter of 16.95 ± 2.42mm (see Table 3).

Table 4. Results of statistical tests of Kruskal Wallis antibacterial effect of noni fruit (*Morinda citrifolia*)

Treatment group	N	Mean Rank	p-value
Extract C. 3,125%	4	6,63	
Extract C. 6,25%	4	11,63	
Extract C. 12,5%	4	14,75	
Extract C. 25%	4	17,00	
Extract C. 50%	4	22,50	
Positive control (<i>Chlorhexidine digluconate</i> 0,2%)	4	26,50	
Negative control (DMSO)	4	2,50	

The Kruskal Wallis test results showed a significant value of p=0.000 (p<0.005) (see Table 4). This states that Ho is rejected, which means *Morinda citrifolia* effectively inhibits the growth of *Streptococcus viridans* bacteria in vitro. David and Stout classify the criteria for antibacterial inhibition into four, namely weak (<5mm), moderate (5-10 mm), strong (11-20mm), and very strong (>20mm) (7). Based on the study's results, it is known that noni fruit extract with a concentration of 3.125%, 6.25%, and 12.5% has a zone in the medium category. Meanwhile, 25% and 50% concentrations are classified as strong categories. Very strong antibacterial activity was found in the positive control of Chlorhexidine gluconate 0.2% with an inhibition zone diameter of 16.95 ± 2.42mm (see Table 3).

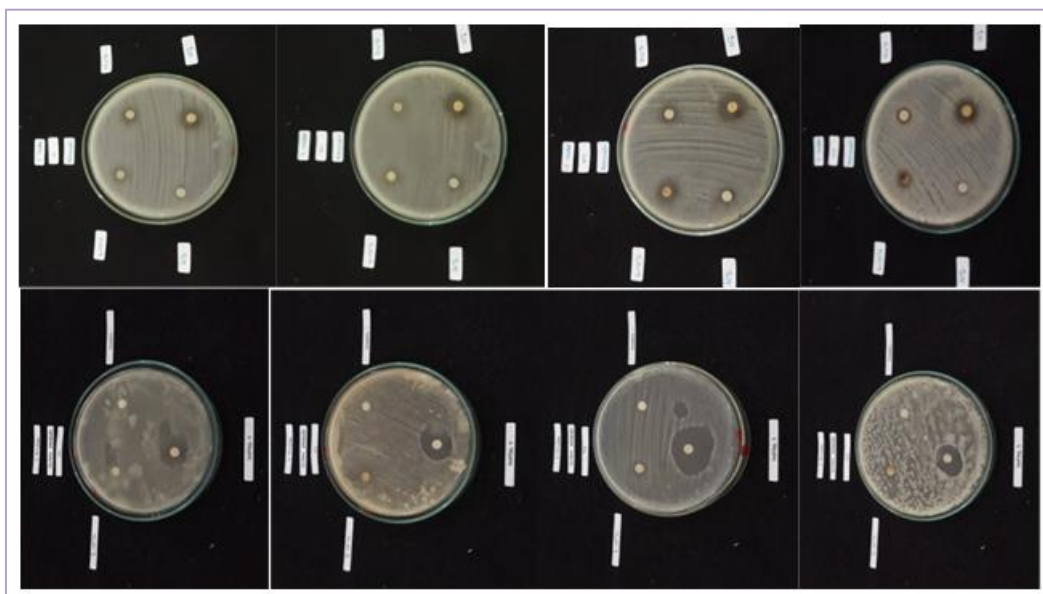


Figure 1. The inhibition zone formed for noni fruit extract, Chlorhexidine gluconate, DMSO

Several factors influence the inhibition of noni fruit extract or the formation of a clear zone around the disc. First, the content of metabolite compounds. The antibacterial power of noni fruit extract occurs because noni fruit contains saponins, flavonoids, tannins, triterpenoids, alkaloids, and steroids that can damage bacterial cell walls. Saponins reduce the surface tension of bacterial cell walls and damage membrane permeability. Saponins diffuse through the outer membrane and span cell walls, then bind to the cytoplasmic membrane, disrupting and destabilizing the cell membrane (8). The antibacterial activity of flavonoids denatures bacterial cell proteins. It destabilizes the cell wall and cytoplasmic membrane of bacteria, disrupting the control of the protein arrangement of bacterial cells so that bacterial cells lose their shape and lysis. The antibacterial ability of tannins can reduce cell permeability and cause cell wall damage. The mechanism of action of triterpenoids involves membrane damage by compounds that react with porins on the outer membrane of the bacterial cell wall, thereby limiting the entry of nutrient molecules into the cell so that bacteria will lack nutrients and their growth is inhibited and die (9, 10).

Second, the concentration of the extract. The concentration is increased or decreased from the initial concentration (significantly) based on the theory that the higher the concentration of a solution, the higher the viscosity so that the diffusion speed of passive antibacterials out of the culture media preparation (disc paper circle) thus killing the bacteria in the media (11, 12). Third, the quality of the extract is not homogeneous. The quality of noni fruit extracts whose solubility of antibacterial compounds is not optimal causes the resulting antibacterial activity is not optimal.

4. Conclusion

Noni fruit extract (*Morinda citrifolia*) has antibacterial effectiveness against *Streptococcus viridans* in vitro with an average concentration of 3.125%, 6.25%, 12.5%, 25%, and 50% showing an inhibition zone against bacterial growth of 6.95 ± 0.17 ; 7.50 ± 0.50 ; 8.25 ± 1.04 ; 8.95 ± 1.29 ; 12.80 ± 1.89 . The 50% concentration of noni fruit extract is the most effective, but it has not been able to equal the diameter of the inhibition zone produced by chlorhexidine gluconate by 0.2%.

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Authors Contribution

Contribution	Dohude, G. A	Riza, A	Siregar, I. B	Lestari, A
Concepts or ideas	√	√	√	√
Design		√	√	√
Definition of intellectual content	√			√
Literature search		√	√	√
Experimental studies	√	√	√	√
Data acquisition	√			√
Data analysis	√	√	√	
Statistical analysis	√			√
Manuscript preparation	√	√	√	√
Manuscript editing	√			√
Manuscript review	√	√	√	√



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