

ANTIBACTERIAL ACTIVITY OF GREEN BETEL LEAF (*Piper betle. L*) EXTRACT PAPER SOAP AGAINST *Staphylococcus aureus* ATCC 25923

Komang Ayu Trianinda Putri Yuliantari¹, Ida Ayu Alit Widhiartini^{2*}, I Gusti Ayu Artini², I Made Jawi²

Departement of Pharmacology

¹. Bachelor of Medicine, Faculty of Medicine, Udayana University

². Department of Pharmacology, Faculty of Medicine, Udayana University

***Correspondence author:**

Ida Ayu Alit Widhiartini

Department of Pharmacology, Faculty of Medicine, Udayana University

E-mail: widhiartini@unud.ac.id

E-mail: ayutrianinda@gmail.com

ABSTRACT

Background: Green betel leaf (*Piper betle. L*) is a popular herb for its antimicrobial activity. Extract rich in secondary metabolites that have been proven to antibacterial properties. Paper soap formulations as an innovation of green betle leaf extract in a compact, convenient, and easy to use form. This study aims to explore the antibacterial activity of green betel leaf extract paper soap against *Staphylococcus aureus* ATCC 25923.

Methods: Antibacterial activity testing was conducted at the Microbiology Laboratory, Faculty of Medicine, Udayana University, using Kirby-Bauer method in triplicate. Paper soap was formulated with extract concentrations of 5%, 10%, and 20%, along with a negative control and a positive control using antiseptic soap. The paper soap samples were diluted with 2 mL of distilled water, applied onto sterile disks, and placed on agar plates previously inoculated with *Staphylococcus aureus* ATCC 25923 in triplicate.

Results: The results showed that the 20% concentration of green betel leaf extract paper soap produced an inhibition zone of 7 mm, which is categorized as moderate.

Conclusion: However, the inhibition observed was not statistically significant in suppressing the growth of *Staphylococcus aureus* ATCC 25923.

Keywords: Piper betle. L, paper soap, antibacterial activity, *Staphylococcus aureus* ATCC 25923

INTRODUCTION

Green betel leaf (*Piper betle. L.*) is a plant that has been scientifically and empirically proven to have therapeutic benefits, especially as an antibacterial agent.⁷ Its antibacterial activity is associated with the secondary metabolites in the leaf, including flavonoid, alkaloid, tannin, saponin, and steroid.¹⁰ One of the bacteria that can be inhibited is *Staphylococcus aureus*, which is a normal flora on the skin but can become pathogenic when present in excessive amounts.¹⁶ *Staphylococcus aureus* is highly contagious, especially through daily activities such as handshaking and direct contact.¹¹

The prevention of *Staphylococcus aureus* infection can begin with a simple measure, handwashing with soap. However, the use of chemical-based soaps can have side effects such as skin irritation and inflammation.⁶ Therefore, the development of soaps made from natural ingredients has

increasingly been carried out to minimize the side effects of chemical-based soaps. Various forms of natural soap products are already available on the market, but they are considered impractical to carry and require a larger volume of water for use. Thus, innovations based on advances in technology and scientific knowledge are needed to improve efficiency; one such innovation is paper soap.¹⁵

The development of paper soap as an antiseptic has been widely researched. Various studies have demonstrated the effectiveness of paper soap formulations containing natural ingredients in inhibiting bacterial growth.^{1,2,8,14} Nevertheless, the antibacterial efficacy of paper soap incorporated with green betel leaf extract necessitates more comprehensive examination. The present study was conducted to develop a formulation and assess the inhibitory potential of green betel leaf extract paper soap against *Staphylococcus aureus* ATCC 25923.

MATERIAL AND METHOD

Tools and Instruments

The tools used in this study included set of maceration, rotary evaporator, scale, knife, extraction container, measuring cup, beaker glass, stirring rods, strainer, chopper, incubator, inoculation loop, paper cutter, petri dishes, blender, dehydrator, sterile tweezers, scissors, micropipette, and plastic wrap. The instruments used in this study are analytical balance, caliper, pH paper, and biosafety cabinet (BSC).

Materials

The materials used in this study included green betel leaves (*Piper betle* L.) collected in the morning before sunrise from Bajera Village, Selemadeg District, Tabanan Regency; 96% ethanol; Whatman paper 0,1; filter paper; distilled water (aquadest); and *Staphylococcus aureus* cultured on Mueller–Hinton agar. The soap formulation materials included green betel leaf extract, olive oil, KOH, HPMC, propylene glycol, and soluble paper.²

Extract Preparation

Green betel leaves were collected, sorted, washed with running water, and then drained. The leaves were dried in a dehydrator at 40 °C. After drying, the leaves were weighed and ground into powder using a chopper. The powder was then sieved and weighed again. All of the green betel leaf simplicia were placed into a maceration container. The maceration process was conducted over a 72-hour period (3 × 24 hours) under periodic agitation in a sealed, light-protected container. The clear liquid obtained was filtered, and the filtrate was evaporated using a rotary evaporator to yield a viscous extract. No further re-extraction was performed on the remaining residue.

Paper Soap Preparations

The preparation of paper soap containing green betel leaf extract began with the preparation of a gel base by dissolving HPMC in hot water and stirring until a transparent gel was formed. In a separate beaker, olive oil was heated and then mixed with propylene glycol and stirred until homogeneous. A 30% KOH solution and distilled water were added to the olive oil mixture and stirred until the saponification process was complete, followed by the addition of the HPMC gel. After the saponification process was completed, green betel leaf extract was added to the soap mixture. The paper soap was cut into 3×3 cm pieces, then 0.15 mL of liquid soap containing green betel leaf extract was applied to each piece and dried in a dehydrator at 40 °C for 20 minutes.

Organoleptic Test

The organoleptic test was performed objectively based on the physical appearance of the paper soap. The parameters tested included color, consistency, and aroma.

pH Test

The pH test was performed to ensure that the acidity level of the paper soap was safe for contact with the skin. The pH testing procedure was performed by placing one sheet of paper soap into a beaker and dissolving it in distilled water. Subsequently, pH paper was dipped into or moistened with the solution, and the color change on the pH paper was observed, then compared with the color reference chart provided on the pH paper box. According to the Indonesian National Standard (SNI) 2588:2017, the ideal pH range for handwashing soap is 4–10.

Bacterial Sample Preparation

The *Staphylococcus aureus* ATCC 25923 strain utilized in this research was sourced from the Medical Microbiology Laboratory, Faculty of Medicine, Udayana University. A single loopful of bacterial colonies was harvested from the agar medium and subsequently suspended in a 0.9% NaCl solution. The suspension was homogenized and calibrated to the 0.5 McFarland turbidity standard, corresponding to a concentration of approximately 1.5×10^8 cells/mL. Finally, the standardized inoculum was inoculated onto the agar surface via streak plate method to facilitate bacterial growth.

Antibacterial Activity Test

Five test discs containing the positive control, negative control, and three different concentrations were placed on the agar medium that had been streaked with the bacterial suspension. The antibacterial activity of the paper soap was evaluated by adding 2 mL of aquadest to each extract concentration, alongside positive and negative control. The addition facilitated the dissolution of the paper soap, ensuring the resulting solution completely saturated the surface of each sensitivity disc. Consequently, this procedure led to a proportional reduction in the final concentration of the soap. The agar media were subsequently incubated at 37°C for 22 hours. Antibacterial activity was quantified by measuring the diameter of the inhibition zone (clear zone) surrounding the discs using a vernier caliper.

RESULT

Based on the conducted study, the results are presented in the following tables:

Table 1. Yield of Green Betel Leaf (*Piper betle*. L)

Fresh leaves	Dry leaves	Dried simplicia	Viscous extract	Yield
1.892 gr	519 gr	504 gr	11.49 gr	2.21%

Based on the table shows a gradual decrease in weight from the fresh leaves, after drying, grinding and

standardizing, until they become a viscous extract as the final result of the extraction process.

Based on the calculations, the yield was 2.21%. This relatively low yield indicates that the amount of compounds

that can be extracted from green betel leaves using 96% ethanol is not very high.

Table 2. Organoleptic Test Results of Green Betel Leaf (*Piper betle*, L) Extract Paper Soap

Preparation	Organoleptic		
	Aroma	Color	Consistency
Control ₍₋₎	Odorless	White	Not easily torn
5%	Characteristic betel leaf	Green	Not easily torn
10%	Characteristic betel leaf	Blackish green	Not easily torn
20%	Characteristic betel leaf	Blackish green	Not easily torn

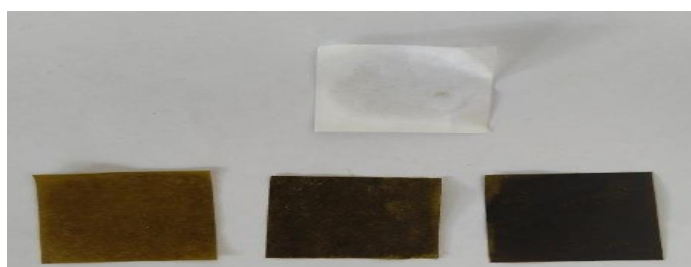


Figure 1. Organoleptic test

The organoleptic test results showed that all paper soap formulations containing green betel leaf extract had the characteristic aroma of betel leaf, with colors ranging from green to blackish green. All preparations, including the negative control

and paper soap with 5%, 10%, and 20% extract concentrations showed tear-resistant consistency and were physically suitable for use.

Table 3. pH Test Result of Green Betel Leaf (*Piper betle*, L) Extract Paper Soap

Formulation	Observation			Average pH
	I	II	III	
Control ₍₋₎	11	10	10	10.3
5%	10	9	9	9.3
10%	9	9	9	9
20%	9	9	9	9

The pH test results showed that the paper soap without extract had an average pH of 10.3, indicating that this preparation did not meet the Indonesian National Standard (SNI). Paper soap containing 5%, 10%, and 20% extract had

average pH values of 9.3, 9, and 9, respectively. These results indicate that increasing the extract concentration can lower the pH of the preparation.

Table 4. Result of the Measurement of Inhibition Zone Diameter of Green Betel Leaf (*Piper betle*, L) Extract Paper Soap

Concentration	Inhibition Zone Diameter (mm)			Median (Min-Max)
	I	II	III	
5%	0	0	0	0(0-0)
10%	0	0	0	0(0-0)
20%	6	7.5	7	7(6-7.5)
Control ₍₋₎	0	0	0	0(0-0)
Control ₍₊₎	19.5	20	20	20(19.5-20)

Antibacterial activity against *Staphylococcus aureus* ATCC 25923 was exhibited by the green betel leaf extract paper soap at a 20% concentration, yielding a median inhibition zone diameter of 7 mm. In contrast, no inhibitory effect was detectable at

concentrations of 5% and 10%, consistent with the observations in the negative control group. The positive control elicited the most pronounced response, characterized by a median inhibition zone diameter of 20 mm.

Table 5. Dunn Test Results for Green Betel Leaf (*Piper betle. L*) Extract Paper Soap

Treatment Group	p-value
Paper soap 10%	1.000
Paper soap 20%	0.635
Control ₍₊₎	0.054
Control ₍₋₎	1.000
Paper soap 10%	0.635
Control ₍₊₎	0.054
Control ₍₋₎	1.000
Paper soap 20%	1.000
Control ₍₊₎	0.192
Control ₍₋₎	0.033*

*Significant result (p<0,05)

Based on the Dunn test, no statistically significant difference was observed between the 20% concentration of green betel leaf extract paper soap and the positive control (p = 1.000). In contrast, a significant disparity was identified between the positive and negative control (p = 0.033). Conversely, the comparison between 20% paper soap formulation and the negative control did not reach statistical significance (p = 0.192).

DISCUSSION

pH testing was performed using pH paper in triplicate, and the average values were calculated. The results showed that paper soap without extract had an average pH of 10.3, indicating that this preparation did not meet the SNI requirements. Meanwhile, paper soap containing green betel leaf extract showed a decrease in pH value, with an average of 9.3 at 5% concentration. The 10% and 20% concentrations resulted in the same average pH of 9. These data indicate that the addition of extract can lower the pH to approach the SNI standards, with the most significant reduction observed at 5%, while 10% and 20% concentrations produced stable pH values.

The pH value is influenced by the soap's ingredients, specifically the strong base (KOH). These pH test results are consistent with the study by Umayati et al. (2023), which showed that a higher concentration of extract leads to a lower resulting pH.¹³

To evaluate antibacterial activity, 2mL of aquadest was added to each treatment group to facilitate the dissolution of the paper soap. This procedure ensured that the resulting solution completely saturated the test discs, which were subsequently placed onto agar media previously inoculated with the target bacteria. The findings revealed that the paper soap formulated with green betel leaf extract exhibited no

inhibitory effect at concentrations of 5% and 10%. Inhibitory activity was exclusively observed at a 20% concentration, yielding a media inhibition zone diameter of 7 mm, which is categorized as moderate. In comparison, the negative control showed no zone of inhibition, while the positive control produced a median inhibition zone of 20 mm. Statistical analysis via the Dunn test indicated that a significant difference existed only between the positive and negative control (p = 0.033).

The diameter of the inhibition zone recorded in this study was notably smaller than the values reported by Anisaningrum, where a gel hand sanitizer containing green betel leaf extract yielded inhibition zones ranging from 23.16 to 25 mm against *Staphylococcus aureus*. Similarly, Maghfiroh reported that a serum formulation exhibited antibacterial activity against *Propionibacterium acnes* with inhibition zones of 11–12 mm. Furthermore, research conducted by Susanti et al. reported that shampoo formulations at concentrations of 15–25% resulted in an 80% mortality of *Pediculus humanus capitis*. Budiarta et al. (2024) reported that a deodorant spray formulations at 2.5–7.5% concentration produced inhibition zones of 11.94–17.48 mm against *Staphylococcus epidermidis*. The differences between these studies and other studies may be due to several factors, including differences in formulation type, solvent, extract concentration, test organism, and testing method.^{3,4,9}

Several factors potentially contribute to the low inhibition zones observed in the paper soap formulations, notably the dilution effect resulting from the addition of aquadest, which reduces the active concentration, as well as potential constituent degradation induced by pH variations. The first factor is dilution. During testing procedure, dissolving the paper soap in aquadest before applying it to the test disc directly reduces the concentration of active

compounds. According to the dilution principle ($C_1 V_1 = C_2 V_2$), adding more aquadest results in a lower concentration of the active ingredients, which consequently reduces the microbial inhibition zone.

The second factor affecting the inhibition zone is pH. Polyphenols are stable in acidic environments (low pH), while the alkaline environment created by soap can alter the chemical structure of polyphenols, resulting in reduced antibacterial activity.⁵

CONCLUSION AND SUGGESTION

In conclusion, that the green betel leaf extract paper soap at 20% concentration yielding a median inhibition zone diameter of 7 mm, which is categorized as a moderate inhibitory response. However, this effect did not reach statistical significance against the growth of *Staphylococcus aureus* ATCC 25923 when evaluated in vitro using Kirby-Bauer test.

The authors recommend further investigation of the active compounds in green betel leaf (*Piper betle L.*) that possess antibacterial effects. The addition of 2 mL of aquadest during the testing procedure should also be reconsidered, as it may reduce the efficacy of the paper soap.

REFERENCES

- Adlina S, Bachtar KR., Nurhasanah B, Susanti. Formulasi dan Uji Aktivitas Sediaan Sabun Kertas Ekstrak Etanol Daun Pandan (*Pandanus amaryllifolius*) Sebagai Antibakteri. *Pharmacoscrypt*. 2023;6(1); 22–30.
- Adri TA, Lololua KDE, Musawir. Formulasi dan Evaluasi Sediaan Paper Soap Kombinasi Ekstrak Etanol Daun Trembesi (*Samanea Saman (Jacq.)Merr*) dan Ekstrak Etanol Daun Miana (*Coleus Scutellarioides L.*) Sebagai Antiseptik. *Jurnal Ilmiah Fitomedia Indonesia*. 2023;2(1); 34.
- Anisaningrum S. Formulasi dan Aktivitas Sediaan Gel Handsanitizer Ekstrak Daun Sirih Hijau (*Piper betle L.*) Terhadap *Staphylococcus aureus* ATCC 25923 dan *Escherichia coli* ATCC 25922. 2022; 63.
- Budiarti M, Agustien GS, Fadilah NN. Uji Aktivitas Antibakteri Sediaan Deodoran Spray Dari Ekstrak Daun Sirih (*Piper betle L.*) Terhadap *Staphylococcus epidermidis*. *Perjuangan Nature Pharmaceutical Conference*. 2024;1(1); 112.
- Cao H, Saroglu O, Karadag A, Diaconeasa Z, Zocatelli G, Conte-Junior CA, et al. Available Technologies on Improving The Stability of Polyphenols in Food Processing. *Food Frontiers*. 2021; 2(2); 109–139. Available from: <https://doi.org/10.1002/fft2.65>
- Castanedo-Cázares JP, Cortés-García JD, Cornejo-Guerrero MF, Torres-Alvarez B, Hernandez-Blanco D. Study of the Cytotoxic and Irritant Effects of Skin Cleansing Soaps. *Gaceta Medica de Mexico*. 2020; 156(5); 419. Available from: <https://doi.org/10.24875/GMM.M20000430>
- Ermawati FU, Sari R, Putri NP, Rohmawati L, Kusumawati DH., Munasir, Supardi ZAI. Linn Leaves Extract From Nganjuk, Sidoarjo and Batu Against *Escherichia coli*, *Salmonella sp.*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Journal of Physics: Conference Series*. 2021; 1951(1). Available from: <https://doi.org/10.1088/17426596/1951/1/012004>
- Junita N, Awaluddin N, Nadjamuddin M, Wahid H. Formulasi dan Uji Aktivitas Antibakteri Sediaan Paper Soap Ekstrak Etanol Daun Bidara Arab (*Ziziphus spinachristi L.*) Terhadap Bakteri *Staphylococcus epidermidis*. *Journal of Experimental and Clinical Pharmacy (JECp)*. 2024;4(2); 82. Available from: <https://doi.org/10.52365/jecp.v4i2.1043>
- Maghfiroh A. Formulasi dan Evaluasi Sediaan Serum Ekstrak Daun Sirih Hijau (*Piper betle*) Terhadap Bakteri *Propionibacterium acnes* Secara In-Vitro. 2022.
- Putri AT, Suproborini A, Kusumawati D. Kandungan Metabolit Sekunder Ekstrak Etanol Daun Sirih Hijau (*Piper betle L.*). *Seminar Nasional Prodi Farmasi UNIPMA*. 2023;226–229.
- Samsudin D, Nopiyantri V, Nilawati A. Formulasi Dan Uji Aktivitas Antibakteri *Staphylococcus aureus* Paper Soap Ekstrak Daun Sirsak (*Annona muricata L.*) Dengan Variasi Gliserin Sebagai Plasticizer. *Journal of Pharmacy*. 2022;11(2); 2.
- Susanti MR, Musyarrafah M, Andriana A, Adnyana IGA. Formulasi dan Uji Efektivitas Sampo Ekstrak Daun Sirih Hijau (*Piper Betle L.*) Terhadap Mortalitas Kutu Kepala Manusia (*Pediculus Humanus Capitis*). *MAHESA: Malahayati Health Student Journal*. 2024;4(6). Available from: <https://doi.org/10.33024/mahesa.v4i6.14543>
- Umayati D, Nugraha D, Ramdan SRK. Formulasi dan Evaluasi Sabun Cair Ekstrak Daun Jambu Biji (*Psidium Guajava L*) dan Uji Iritasi Dengan Basis Minyak Zaitun (*Olive Oil*). *Pharmacy Genius*. 2023;2(2); 125–134. Available from: <https://doi.org/10.56359/pharmgen.v2i2.279>
- Wahid H, Sulaiman AW, Najamuddin M, Pratiwi EM. Formulasi dan Uji Aktivitas Sediaan Paper Soap Sabun Cuci Tangan Ekstrak Etanol Daun Jambu Biji (*Psidium guajava L.*) Terhadap *Staphylococcus aureus*. *EMPIRIS: Jurnal Sains, Teknologi Dan Kesehatan*. 2024;1(2);78–87. Available from: <https://doi.org/10.62335/cdcehp13>

16. Wati F, Priani SE, Darma GCE. Kajian Formulasi dan Aplikasi Sediaan *Paper Soap*. *Prosiding Farmasi*. 2020;6(2);456–460. Availbale from: <http://dx.doi.org/10.29313/.v6i2.23148>
17. Wijayanti TRA, Safitri R. Uji Aktivitas Antibakteri Ekstrak Daun Belimbing Wuluh (*Averrhoa Bilimbi Linn*) Terhadap Pertumbuhan Bakteri *Staphylococcus aureus* Penyebab Infeksi Nifas. *Jurnal Ilmiah Ilmu Kesehatan*. 2018;6(3);278. Available from: <https://doi.org/10.33366/cr.v6i3.999>

