**Indonesian Journal of Pharmaceutical and Clinical Research**
Journal homepage:https://idjpcr.usu.ac.id

# Antibacterial Activity Test of Malacca Leaf Extract (Phyllanthus emblica) Against *Staphylococcus aureus* and *Staphylococcus epidermidis* Bacteria

Ridwanto Ridwanto[1*], Hijjatun Aprilia[1], Zulmai Rani[1], Ziza Putri Aisyia Fauzi[1], Vera Estefania Kaban[2], Nasri Nasri[3]

[1]*Faculty of Pharmacy, Universitas Muslim Nusantara Al-Washliyah, Medan,*
[2]*Department of Clinical Pharmacy, Faculty of Medicine, Dentistry and Health Sciences, Universitas Prima Indonesia, Medan 20118 Indonesia*
[3]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20155, Indonesia.*
*Corresponding Author:rid.fillah66@gmail.com

**ARTICLE INFO**
**Article history:**
Received 19 August 2024
Revised 04 September 2024
Accepted 05 October 2024
Available online 16 November 2024

E-ISSN: 2620-3731
P-ISSN: 2615-6199

**How to cite:**
Ridwanto, R., Aprilia, H., Rani, Z., Fauzi, Z. P. A., Kaban, V. E., & Nasri, N. (2024). Antibacterial activity test of malacca leaf extract (*Phyllanthus emblica*) against *Staphylococcus aureus* and *Staphylococcus epidermidis* bacteria. *Indonesian Journal of Pharmaceutical and Clinical Research*, 7(1), 22-27.


**http://doi.org/10.32734/idjpcr.v7i1.17912**

**ABSTRACT**
Antibacterials are substances that can interfere with the growth or even kill bacteria and have the side effect of antibacterial resistance. One effort to reduce resistance is choosing to use natural bacterial ingredients. One plant that can potentially contain antibacterial activity is malacca leaves (Phyllanthus emblica). The contents of this plant include flavonoids, alkaloids, tannins, saponins, and steroids/triterpenoids. This research aimed to prove the antibacterial activity test of malacca leaf extract. The stages in this research included phytochemical screening and testing the antibacterial activity of ethanol extract from Malacca leaves for Staphylococcus aureus and Staphylococcus epidermidis bacteria using the diffusion method using paper discs. The characteristics of malacca leaf simplicia obtained were water content of 4.6%, water-soluble content of 28%, ethanol-soluble essence content of 23.33%, total ash content of 2.00%, and insoluble ash content of 0.5%. The results of phytochemical screening showed the presence of flavonoids, saponins, tannins, glycosides, and steroids/triterpenoids. The antibacterial test results of malacca leaf extract at concentrations of 500 mg/ml, 400 mg/ml, 300 mg/ml, and 200 mg/ml have an antibacterial effect against Staphylococcus aureus and Staphylococcus epidermidis, and there is no difference in the effectiveness of the inhibitor against gram-positive and negative grams.

**Keywords**: Malacca leaf (*Phyllanthus emblica*), *Staphylococcus aureus*, *Staphylococcus epidermidis*

**ABSTRAK**
Antibakteri adalah zat yang dapat mengganggu pertumbuhan atau bahkan membunuh bakteri dan memiliki efek samping berupa resistensi antibakteri. Salah satu upaya untuk mengurangi resistensi adalah memilih menggunakan bahan antibakteri alami. Salah satu tanaman yang berpotensi mengandung aktivitas antibakteri adalah daun malaka (*Phyllanthus emblica*). Kandungan tanaman ini meliputi flavonoid, alkaloid, tanin, saponin, dan steroid/triterpenoid. Penelitian ini bertujuan untuk membuktikan uji aktivitas antibakteri dari ekstrak daun malaka. Tahapan dalam penelitian ini meliputi skrining fitokimia dan pengujian aktivitas antibakteri dari ekstrak etanol daun malaka terhadap bakteri *Staphylococcus aureus* dan *Staphylococcus epidermidis* menggunakan metode difusi dengan cakram kertas. Karakteristik simplisia daun malaka yang diperoleh memiliki kadar air sebesar 4,6%, kadar larut air sebesar 28%, kadar esens larut etanol sebesar 23,33%, kadar abu total sebesar 2,00%, dan kadar abu tidak larut sebesar 0,5%. Hasil skrining fitokimia menunjukkan adanya flavonoid, saponin, tanin, glikosida, dan steroid/triterpenoid. Hasil uji antibakteri ekstrak daun malaka pada konsentrasi 500 mg/ml, 400 mg/ml, 300 mg/ml, dan 200 mg/ml memiliki efek antibakteri terhadap *Staphylococcus aureus* dan *Staphylococcus epidermidis*, serta tidak ada perbedaan efektivitas penghambatannya terhadap bakteri gram positif dan gram negatif.

**Kata kunci**: Daun malaka (*Phyllanthus emblica*), *Staphylococcus aureus*, *Staphylococcus epidermidis*

## 1. Introduction

Bacteria are a group of organisms that do not have a cell nucleus membrane are very small (microscopic), and have a big role in life on earth (1). Several groups of bacteria can provide benefits or sources of disease. Infectious diseases are diseases caused by the entry and proliferation of microorganisms, namely bacteria, viruses, fungi, prions, and protozoa, into the body, causing organ damage (2). Indonesia is a country with a tropical climate with dusty conditions and warm and humid temperatures, which supports microorganisms to continue to reproduce and can ultimately cause infections (3). One area that is often infected is the skin, generally infected by the bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis* (4).

Antibacterials are substances that can interfere with the growth or even kill bacteria by interfering with the metabolism of harmful microbes (5). The mechanisms of action of antibacterial compounds include inhibiting cell wall synthesis, inhibiting the integrity of bacterial cell wall permeability, inhibiting the work of enzymes, and inhibiting the synthesis of nucleic acids and proteins (6). This will give rise to new problems, namely the challenge of the emergence of pathogenic bacteria that are resistant to antibacterials (7). One effort to reduce resistance is choosing a type of antibacterial using natural ingredients. One of the plants used as an antibacterial is the malacca plant (*Phyllanthus emblica*).

## 2. Method

### 2.1 Materials

The materials used in this research were samples of Malaca leaves. Chemicals used, 2 N sulfuric acid reagent, potassium iodide, bismuth (III) nitrate, concentrated nitric acid, mercury (II) chloride, alpha naphthol, 0.5 N nitric acid, anhydrous acetic acid, 2 N hydrochloric acid reagent, reagent sodium hydroxide 2 N, iron reagent (III), lead reagent (II), anhydrous sodium sulfate, chloroform, chloralhydrate 70 %, ethanol 96 %, distilled water, Nutrient Agar (NA), Eosin Methylen Blue Agar (EMBA), Mueller Hinton Agar (MHA), 0.9% NaCl solution, Mc.Farland standard suspension, *Staphylococcus aureus*, and *Staphylococcus epidermidis* bacterial cultures.

### 2.2 Preparation of Malacca Leaf Extract

The sample was put into a vessel (macerator) and then soaked with solvent until completely submerged add about 1-2 cm of solvent above the surface of the sample, then close the top to prevent the entry of impurities and evaporation of the solvent, but leave a small hole to prevent explosions due to evaporation solvent (8). Soaking is done for a certain period, for example for 24 hours with stirring every 1-2 hours (if you leave it at night, you don't need to stir it), the stirring process is not necessary. After 24 hours replace the solvent with a new solvent and then treat it the same as the first. Replacing the solvent is carried out to speed up the extraction process, because the first solvent may be saturated with the compound so it cannot re-dissolve the desired compound, and the replacement time depending on needs does not have to be 24 hours (9,10). Replacement of the solvent is stopped if the final solvent after being allowed to stand like the previous solvent shows the original color of the solvent which indicates that the compound has been completely extracted. The liquid extract from the first solvent and the next solvent are combined to be concentrated using a rotary evaporator (11).

### 2.3 Phytochemical Characterization and Screening

Extract characterization includes determining water content, determining water-soluble essence content, determining soluble essence content in ethanol, determining total ash content, and determining insoluble acid ash content (12). Phytochemical screening was carried out to determine the metabolite compounds found in malacca leaves, including screening for alkaloids, saponins, tannins, steroids/triterpenoids, and flavonoids (13).

### 2.4 Antibacterial Activity Test of Malacca Leaf Extract

Pipet 0.1 ml of the bacterial suspension *Staphylococcus aureus* and *Staphylococcus epidermidis* with a concentration of 106 CFU/ml, put into a sterile petri dish. Next, 20 ml of liquid Mueller Hinton Agar (MHA) media was poured in, then homogenized and left to stand until the media solidified. Once the media is solid, insert a paper disc into which 0.1 ml of ethanol extract test solution of various concentrations has been dripped, then incubate at a temperature of 36-37ºC for 18-24 hours. Next, measure the diameter of the clear zone around the test solution using a caliper. The test was carried out three times (14,15).

## 3. Results

### 3.1 Malacca Leaf Extract

500 grams of malacca leaf simplicia powder was extracted by maceration using ethanol solvent96% then the extract was concentrated using a rotary evaporator and a thick extract of 102.5g was obtained.

### 3.2 Phytochemical Characterization and Screening

The results of the characterization and phytochemical screening of simplicia powder and malacca leaf extract can be seen in Table 1. The results of the characterization of simplicia powder and bay leaf extract include determining water content, water-soluble essence content, soluble essence content in ethanol, total ash content, and insoluble ash content Tamarind has fulfilled the requirements as a simple and good quality extract (16)a.

**Table 1.** Results of characterization and screening of simplicia powder and malacca leaf extract

| Characterization | | | Phytochemical Screening | | |
|---|---|---|---|---|---|
| No | Parameter | Check up result | Group | Results | |
| | | | | Powder | Extract |
| 1 | Water content | 4.6% | Alkaloids | + | + |
| 2 | Water soluble essence content | 28% | Tannin | + | + |
| 3 | Ethanol soluble essence content | 23.33% | Flavonoids | + | + |
| 4 | Total ash content | 2.00% | Saponin | + | + |
| 5 | Acid insoluble ash content | 0.5% | Glycosides | + | + |
| 6 | | | Triterpenoids / Steroids | + | + |

Information:
(+): Contains a group of compounds
(-) : Does not contain any compound class

The results of the phytochemical screening of malacca leaves showed that malacca leaves contain secondary metabolites of alkaloids, tannins, flavonoids, saponins, glycosides and triterpenoids/steroids. Phytochemical screening is carried out as a preliminary stage to provide an overview of the content of certain compounds in the natural ingredients to be studied (14,17).

### 3.3Antibacterial Activity of Malacca Leaf Extract

The results of the antibacterial activity test show that malacca leaf extract can inhibit the growth of *Staphylococcus aureus* and *Staphylococcus epidermidis* bacteria with test results data which can be seen in Table 2. The concentration of malacca leaf extract which provides the largest inhibitory diameter for the growth of *Staphylococcus aureus* bacteria is a concentration of 500 mg/mL with an inhibitory diameter of 11.43 ± 0.79 mm, while the growth of *Staphylococcus epidermidis* bacteria was 500 mg/mL with an inhibitory diameter of 11.63 ± 0.40.

The results of the antibacterial activity of the ethanol extract of malacca leaves were carried out using the diffusion method using disc paper. This test was carried out to see the activity of ethanol extract of malacca leaves with varying concentrations in inhibiting the growth of *Staphylococcus aureus* and *Staphylococcus epidermidis* bacteria as seen from the inhibitory power produced. Table 2 shows that the ethanol extract of malacca leaves has antibacterial activity against the bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis*. The higher the concentration of the ethanol extract of malacca leaves, the higher the inhibitory power obtained. The criteria for antibacterial strength are as follows: an inhibition zone diameter of 5 mm or less is categorized as weak, an inhibition zone of 5-10 mm is categorized as moderate, an inhibition zone of 10-20 mm is categorized as strong and an inhibition zone of 20 mm or more is categorized as very strong (8,18). Based on these criteria, it can be seen that the ethanol extract of malacca leaves with concentrations of 500, 400, 300 mg/ml, has a strong antibacterial, 200, 100, 50, 25, 12.5, 6.25 and 3,125 mg/ml has a weak antibacterial , namely against *Staphylococcus aureus* bacteria with an inhibition zone diameter of 11.4 ; 10.43 ; 10.23 ; 9.93 ; 9.8 ; 9.5; 9.26 ; 9.03 ; 9.06 and 8.93 mm and against *Staphylococcus epidermidis* bacteria with

concentrations of 500, 400, 300 mg/ml have strong antibacterial, 200, 100, 50, 25, 12.5, 6.25, 3.125 mg/ml, have weak antibacterial, namely 11.63; 11.06 ; 10.53 ; 9.86 ; 9.43 ; 9 ; 8.46 ; 8.73 ; 8.33 and 8.3 mm.

**Table 2.** Data on the inhibitory diameter of malacca leaf extract against *Staphylococcus aureus* bacteria

| Concentration (mg/mL) | Mean diameter of inhibition zone (mm)±Std. Deviation | |
|---|---|---|
| | Inhibitory diameter (mm) *Staphylococcus aureus* | Inhibitory diameter (mm) of *Staphylococcus epidermidis* |
| 500 | 11.43 ± 0.79 | 11.63 ± 0.40 |
| 400 | 10.43 ± 0.66 | 11.06 ± 0.15 |
| 300 | 10.23 ± 0.57 | 10.53 ± 0.15 |
| 200 | 9.93 ± 0.49 | 9.86 ± 0.55 |
| 100 | 9.8 ± 0.39 | 9.43 ± 0.31 |
| 50 | - | - |
| 25 | - | - |
| 12.5 | - | - |
| 6.2 | - | - |
| 3,125 | - | - |

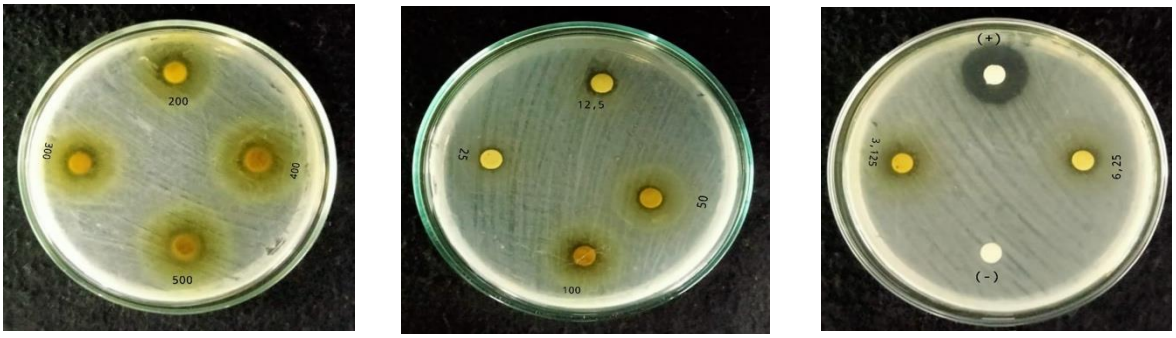


**Figure 1.** Inhibitory diameter of *Staphylococcus aureus* in various concentrations

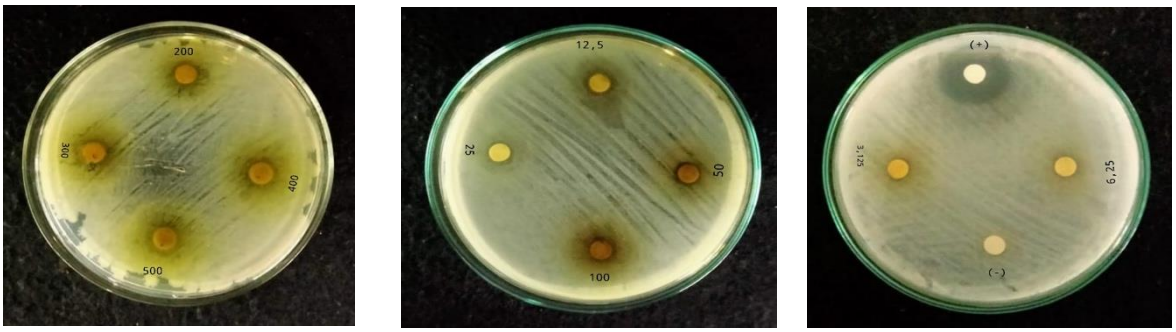


**Figure 2.** Inhibitory diameter of *Staphylococcus epidirmidis* in various concentrations

Based on table 2 from the results of the analysis using the diffusion method, it is known that the bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis* show that at all concentrations there is no difference in inhibitory power (19). This shows that malacca leaf extract has the same inhibitory activity against gram-positive and gram-negative bacteria. According to Siregar (2023), flavonoid metabolite compounds, saponins, tannins, and have antimicrobial activity (20). Flavonoid compounds which act directly as antibacterials work by denaturing bacterial cell proteins and damaging cell membranes irreparably (8,15). The mechanism of action of tannins is that they are able to inhibit the action of proteins in the cell walls and are astringent, that is, they shrink the bacterial cell walls, so that the cells lose their physiological activity and are destroyed (13,21). The mechanism of action of saponin has a mechanism of action as an antibacterial by reducing surface tension, resulting in increased permeability of cell leakage and causing bacteria to rupture or lyse (10,22). Based on table 2 of the results of the analysis using statistical methods, it is known that the bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis* show that at all concentrations their inhibitory

power is not much different. This shows that the ethanol extract of malacca leaves has the same antibacterial inhibitory activity against gram-positive bacteria.

## 4. Conclusion

From the research results it can be concluded that malacca leaf extract (Phyllanthus emblica) has antibacterial activity against *Staphylococcus aureus* with the largest inhibitory diameter, namely 11.43 ± 0.79 mm, while the bacteria *Staphylococcus epidermidis* has the largest inhibitory diameter, namely 11.63 ± 0.40 mm.

## 5. Acknowledgments

We appreciate everyone who helped with this study by participating and/or assisting.

## References

1. Sanjaya T. Penerapan Komputer Dalam Mengidentifikasi Virus dan Bakteri Penyakit Ichtyosis Menerapkan Algoritma Rete. J Pharm Health Res. 2020;1(2):43–8.

2. Rani Z, Nasution HM, Kaban VE, Nasri N, Karo NB. Antibacterial activity of freshwater lobster (Cherax quadricarinatus) shell chitosan gel preparation against Escherichia coli and Staphylococcus aureus. J Appl Pharm Sci. 2023;13(2):146–53.

3. Yuza M, Ridwanto R, Rani Z. Determination Of Total Flavonoid Content Of Yellow Wood (Arcangelisia Flava (L.) Merr) Extract And Antibacterial Activity Against Staphylococcus aureus. J Agromedicine Med Sci. 2023;9(3):140–5.

4. Nasution HM, Yuniarti R, Rani Z, Nursyafira A. Phytochemical screening and antibacterial activity test of ethanol extract of jengkol leaves (Archidendron pauciflorum Benth.) IC Nielsen against Staphylococcus epidermidis and Propionibacterium acnes. Int J Sci Technol Manag. 2022;3(3):647–53.

5. Syahputra RA, Fajrina R, Rani Z, Rahmadani A. Producing Polyurethane as Wound Plaster using Glycerol Transesterified of Waste Cooking Oil with Moringa Leaf Extract (Moringa Oleifera Lam.) as an Antimicrobial. Trends Sci. 2023;20(12):6963–6963.

6. Ridwanto R, Rosa VD, Rani Z, Fauzi ZPA. Utilization of Chitosan from Fresh Water Lobster (Cherax quadricarinatus) Shells in Anti-Acne Gel Preparations. Trends Sci. 2024;21(2):7243–7243.

7. Duppa MT, Firmansyah F, Syachrir S, Masri A. Efek Ekstrak Daun Sawo Manila (Manilkara zapota (L.) Van Royen) Sebagai Adjuvant Antibiotika Amoxicillin Terhadap Resistensi Escherichia coli Dan Staphylococcus aureus. J Mandala Pharmacon Indones. 2023;9(2):302–12.

8. Nasri N, Satria D, Kaban VE, Tania CG, Syahputra HD, Rani Z. Antibacterial Potential of Ethanolic Extract of Avocado Leaves (Persea americana mill.) against Clinical Isolate of Klebsiella pneumoniae and Proteus mirabilis. Trends Sci. 2024;21(7):7821–7821.

9. Vera VEK, Silalahi J. Testing Of The Cream Formula Turmina Right Extract Against The Inhibition Of The Development Of Melanoma Cells. Int J Sci Technol Manag. 2022;3(2):525–9.

10. Satria D, Harahap U, Dalimunthe A, Septama AW, Hertiani T, Nasri N. Synergistic antibacterial effect of ethyl acetate fraction of Vernonia amygdalina Delile leaves with tetracycline against clinical isolate methicillin-resistant Staphylococcus aureus (MRSA) and Pseudomonas aeruginosa. Adv Pharmacol Pharm Sci. 2023;2023.

11. Nurmazela V, Ridwanto R, Rani Z. Antioxidant activity test of Barangan Banana Hump's ethanol extract (Musa Paradisiaca (L.)) with DPPH (1, 1 Diphenyl-2-Picrylhydrazyl) method. Int J Sci Technol Manag. 2022;3(5):1478–83.

12. Pulungan AF, Ridwanto R, Dalimunthe GI, Rani Z, Dona R, Syahputra RA, et al. Phytochemical screening and antioxidant activity testing of Porang (Amorphophallus Muelleri Blume) leaf ethanol extract from Kuta Buluh region, North Sumatera. Int J Health Pharm IJHP. 2022;3(1):1–7.

13. Kaban VE, Nasri N, Syahputra HD, Fitri R, Rani Z, Lubis MF. Formulasi Sediaan Gel dari Ekstrak Metanol Biji Alpukat (Persea americana Mill.) Sebagai Penyembuh Luka Sayat Pada Tikus Jantan (Rattus norvegicus). Herb Med J. 2022;5(2):48–54.

14. Nasri N, Kaban VE, Satria D, Syahputra HD, Rani Z. Mekanisme Antibakteri Ekstrak Etanol Daun Kemangi (Ocimum basilicum L.) terhadap Salmonella typhi. J Pharm Health Res. 2023;4(1):79–84.

15. Kaban VE, Nasri N, Syahputra HD, Lubis MF, Satria D. Uji Aktivitas Antibakteri Ekstrak Daun Karenda (Carissa carandas Linn.) Terhadap Bakteri Propionibacterium acne dan Staphylococcus epidermidis. J Pharm Health Res. 2023;4(1):91–6.

16. D Ardiansyah SA, Simbolon NS. Uji aktivitas antiobesitas dari ekstrak etanol daun malaka (Phyllanthus emblica L.) terhadap tikus putih jantan galur Wistar. J Sains Dan Teknol Farm Indones [Internet]. 2018 [cited 2024 Aug 17];7(1). Available from: https://ejournal.stfi.ac.id/index.php/jstfi/article/view/71

17. Dalimunthe A, Pertiwi D, Muhmmad M, Kaban VE, Nasri N, Satria D. The effect of extraction methods towards antioxidant activity of ethanol extract of Picria fel-terrae Lour. Herbs. In: IOP Conference Series: Earth and Environmental Science. IOP Publishing; 2022. p. 012040.

18. Nasution HM, Rani Z, Fauzi ZPA, Ridho AR. Antimicrobial Activity Test of Ethanol Extract of Senggani Leaves (Melastoma malabraticum L) Against Propionibacterium Acnes and Staphylococcus Epidermidis. J Sains Dan Kesehat. 2024;6(2):292–8.

19. Syahputra HD, Nasri N, Kaban VE. Pengujian Potensi Aktivitas Antibakteri dari Daun Cep-cepan (Saurauia cauliflora DC.) dalam Formulasi Sediaan Gel Terhadap Propionibacterium acnes. Herb Med J. 2022;5(1):28–32.

20. Siregar KB, Manalu K, Nasution RA. Aktivitas Antibakteri Ekstrak Kulit Batang Malaka (Phyllanthus emblica) Terhadap Pertumbuhan Escherichia coli Dan Staphylococcus aureus. BEST J Biol Educ Sains Technol. 2023;6(2):843–9.

21. Kusumastuti MY, Meilani D, Tawarnate S. Aktivitas Antibakteri Ekstrak, Fraksi Kloroform dan Fraksi n-Heksan Daun Kemangi terhadap Staphylococcus aureus dan Eschericia coli. J Indah Sains Dan Klin. 2021;2(1):17–22.

22. Rafita RY, Nasution HM, Rani Z, Fahmi F. The Buas Buas Leaf Utilization of Buas Buas Leaf (Premna pubescens Blume) Ethanol Extract as Liquid Soap With Anti-Bacteria Activity. Int J Sci Technol Manag. 2022;3(3):733–43.