Althea Medical Journal
ISSN Online: 2337-4330
https://doi.org/10.15850/amj.v10n4.2941

Original Article

Antibacterial Activity of *Terminalia mantaly* Stem Ethanol Extract as Hand Sanitizer Gel

Evi Sovia,[1] Hernandi Sujono,[2] Rinaldi Farizi,[2] Sutrisno,[3] Adilfi Risma Nurhadianti,[2] Yenny Febriani Yun[2]

[1]Department of Pharmacology, Faculty of Medicine, Universitas Jenderal Achmad Yani, Cimahi, Indonesia, [2]Department of Chemistry, Faculty of Sains and Informatics, Universitas Jenderal Achmad Yani, Cimahi, Indonesia, [3]Department Bioethic and Medicolegal, Faculty of Medicine, Universitas Jenderal Achmad Yani, Cimahi, Indonesia

Abstract

Background: Hand sanitizers generally contain alcohol. However, it can cause dry and irritated skin. Therefore, it is necessary to find antibacterial alternatives that are safe for the skin. One of the plants that has antibacterial activity is *Terminalia mantaly* (*T. mantaly*). This study aimed to investigate the antibacterial activity of ethanol extract and hand sanitizer gel of *T. mantaly*.

Methods: The antibacterial activity test against gram-positive (*Staphylococcus aureus* and *Streptococcus pyogenes*) and gram-negative bacteria (*Escherichia coli* and *Shigella dysenteriae*) was carried out using the liquid micro dilution method. The phytochemical tests were also performed. This study was conducted at the Chemistry Laboratory, Faculty of Sains and Informatics, Universitas Jenderal Achmad Yani, Cimahi, Indonesia from April to June 2022.

Results: The ethanol extract of *T. mantaly* stem contained secondary metabolites of alkaloids, tannins, quinones, saponins, steroids, and flavonoids. The minimum inhibitory concentration (MIC) values of *T. mantaly* stem ethanol extract against *Escherichia coli*, *Shigella dysenteriae*, *Staphylococcus aureus, Streptococcus pyogenes* were 0.63%, 0.63%, 0.16%, and 0.16%, whereas the hand sanitizer gel gave MIC values of 0.63%; 0.31%; 0.16%; and 0.16% respectively. The minimum bactericidal concentration (MBC) values of *T. mantaly* stem ethanol extract and hand sanitizer gel had the same results, namely 2.50%, 0.16%, 1.25%, and 1.25%. The physical stability of the hand sanitizer gel from the ethanol extract of *T. mantaly* stem met the physical stability standards of the gel.

Conclusion: The ethanol extract of the *T. mantaly* stems has stronger antibacterial activity against gram-positive bacteria than against gram-negative bacteria.

Keywords: Antibacterial, hand sanitizer gel, secondary metabolites, *Terminalia mantaly*

Althea Medical Journal.
2023;10(4):234–241

Received: September 19, 2022
Accepted: Juni 17 2023
Published: December 31, 2023

Correspondence:
Dr. Evi Sovia, dr., MSi
Department of Pharmacology, Faculty of Medicine, Universitas Jenderal Achmad Yani, Cimahi, Indonesia,
Jl. Terusan Jenderal Sudirman, Cimahi, Indonesia
E-mail:
evi.sovia@lecture.unjani.ac.id

Introduction

Infectious diseases are one of the types of diseases that most commonly affect Indonesian people. One of the causes of infectious disease is bacteria.[1] To prevent infection, alcohol is generally used as an antiseptic. Currently, the most effective hand sanitizer products are alcohol-based formulations containing 62–95% alcohol.[2] However, excessive use of alcohol can cause dry and irritated skin. Therefore, it is necessary to use antibacterial ingredients that are safe for the skin. The use of natural ingredients derived from plants is an alternative solution today. One of the plants that has the potential to have antibacterial activity is ketapang (*Terminalia*).[3]

Ketapang kencana (*Terminalia mantaly*) is a versatile tree, and its wood is known to be durable, hard, strong, and suitable for

construction that requires strength such as bridges, boats, ships, floors, frames and doors as well as all useful parts of the plant such as roots, bark, leaves and flowers as a medicinal ingredient. *Terminalia mantaly* has a shady and beautiful heading so it is good to plant in the yard or in a roadside park.[4] *Terminalia mantaly* has been known to have antifungal, antiplasmodial, and antibacterial effects.[5–7] The leaves of *Terminalia mantaly* contain chemical compounds such as flavonoids, alkaloids, tannins, saponins, cyanogenic glycosides and phenols. *Terminalia mantaly* leaf extract water can kill gram-negative bacteria (*Eschericia coli* and *Salmonella typhi*), as well as gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*).[8] Previous research reported that the ethanolic extract of Ketapang Kencana leaves had better antibacterial activity than the aqueous extract against *Eschericia coli* and *Staphylococcus aureus*.[9] The hydro ethanol extract of *Terminalia mantaly* is known to inhibit the growth of eight strains of enterobacteria, and it is predicted that the secondary metabolites in the polar extracts are antibacterial.[10] However, study on *Terminalia mantaly* stem as an antibacterial is still limited.

Several studies have shown that the use of hand sanitizer is effective in inhibiting bacterial growth and has bactericidal activity.[11] Hand sanitizer in gel preparation is deemed more in demand than liquid preparation. This is due to several important desirable characteristics, such as fast absorption, soft and moist hand sensation, clean, non-sticky texture, and low odor.[12] In addition, gel has the advantages of being easily washed off with water, high adhesion, cooling the skin, and good drug release.[13]

The antibacterial activity on other strains needs to be studied, especially its activity on gram-positive bacteria. This study aimed to investigate the antibacterial activity of *Terminalia mantaly* stem ethanol extract. The development of this research can then be applied to produce hand sanitizer products in gel form made from natural ingredients which will be beneficial for community.

Methods

Stems of *Terminalia mantaly* (*Combretaceae*) were collected in 2020 from Kota Baru Parahyangan Regency, West Java, Indonesia. Voucher specimen number 137/11.CO2.2/PL/2020 was identified at the College of Biological Sciences and Technology, Bandung Institute of Technology as *Terminalia mantaly H. Perrier*. Ethical approval was obtained from the research ethics commission of Universitas Jenderal Achmad Yani No.059/M/KEPK/2022.

The chemicals used in the extraction process were technical solvents (redistilled) of n-hexane, ethyl acetate, and ethanol. Silica GF254 was used in thin layer chromatography (TLC) and H_2SO_4 solution (10% in ethanol) as a stain-display reagent.

Stem samples (without bark) of *Terminalia mantaly* were dried at room temperature and crushed. *Terminalia mantaly* powder (5 kg) was macerated with 96% ethanol solvent for 24 hours gradually with a sample and solvent ratio of 1:10 (3x24 hours) and 1:5 (3x24 hours). Extraction was the withdrawal of the desired substance from the raw material using a selected solvent where the main substance was dissolved. The solvent used in extraction was selected based on its ability to dissolve the active substance maximally and minimize unwanted compounds.[14] The macerate was filtered and then concentrated using a vacuum rotary evaporator. The concentrated extract was heated in an oven at 50˚C for 48 hours.

Phytochemical tests were carried out on the *Terminalia mantaly* ethanol extract to qualitatively determine the phytochemical constituents as a standard procedure.15 The phytochemical constituents tested were alkaloids, flavonoids, quinones, tannins, steroids, and saponins.

Alkaloids were detected by mixing 1 gram of extract with 5 ml of dilute ammonia and then crushing in a mortar, then adding 20 ml of chloroform while continuing to crush. After filtering, the filtrate was put into a test tube and then 5 ml of 2N hydrochloric acid was added. The mixture was shaken vigorously until it forms 2 layers. The acid layer was separated and then divided into 3 parts. The first part was used as a blank. The second part was treated with 2–3 drops of Mayer reagent. Formation of white precipitate indicated the presence of alkaloids. The third part was treated with 2–3 drops of Dragendorf reagent, a red precipitate was formed, indicating the presence of alkaloids.

The presence of flavonoids was done by heating 1 gram of extract in a water bath, then filtering. A total of 5 ml of filtrate was put into a test tube then magnesium powder and 1 ml of 2N hydrochloric acid were added. The mixture was heated over a water bath, then filtered. After the resulting filtrate was orange, then put it in a test tube and added 5 ml of amyl alcohol. The mixture was then shaken vigorously and allowed to separate. The amyl

alcohol layer turned brownish red indicating that the extract contained flavonoids.

Meanwhile, quinones were detected by heating 1 gram of extract in a water bath, then filtering. The filtrate was treated with 2–3 drops of KOH solution. The formation of a solid red precipitate indicated the presence of quinones. The presence of tannins was carried out by heating 1 gram of extract in water bath, then filtering. The filtrate was retested by adding 5 drops of 1% gelatin. The formation of a white precipitate indicated the presence of tannins. Steroids were detected by treating the extract with chloroform and filtering. The filtrate was treated with a few drops of acetic anhydride, boiled and allowed to cool. Sulfuric acid was added. The formation of a brown ring at the junction indicated the presence of phytosterols (steroids).

The extract was stored in a test tube above a water bath, then mixed with water and heated for a while, then filtered. After the extract had cooled, the filtrate was shaken vigorously for 30 seconds. If the foam produced persisted for ten minutes, this indicated the presence of saponins.

Thin layer chromatography (TLC) analysis was also performed. The silica gel GF24 plate was prepared with dimensions of 5x3 cm and outlined 1 cm at the top and bottom using a pencil. At the bottom line of the plate, the diluted extract was smeared and put into a chamber containing the mobile phase which was a mixture of ethyl acetate solvent and n-hexane in a ratio of 3:7. Then the plate was observed under UV_{254} nm and UV_{365} nm lamps and sprayed with dilute H_2SO_4 stain reagent.

Minimum inhibitory concentration (MIC) was determined by preparing a 20% w/v extract solution with 10% dimethyl sulfoxide (DMSO) solvent and hand sanitizer gel with an extract concentration of 20% w/w. Into all wells, 100 µL Mueller's hinton broth was added using a micropipette. Into well number 2 lines 1–4, 100 µL of 10% DMSO solution was added. Into well number 3 rows 1–4, 100 µL of 70% alcohol was added, and 100 µL of hand sanitizer gel without extract for rows 5–8. Into well number 4, 100 µL of extract was inserted and then diluted using the spray pull technique into wells number 5 to number 12, the same thing was also done for the hand sanitizer gel with the hand sanitizer + extract sample. Test bacteria with standard of 0.5 McFarland as much as 100 µL were inserted into holes number 2 to 12 in each row on the plate. The plate was then incubated in an oven at 37 ˚C for 24 hours. The specific MIC value for each of the 55 antibiotics was the lowest concentration of an antibiotic that could kill certain microbes.[16] Each well in the plate was observed. If a precipitate appeared or the solution became cloudy, this indicated the growth of bacteria. The lowest concentration that was not overgrown with bacteria showed MIC. In this test, the liquid dilution method was used. For each concentration, the bacterial suspension was added to the media, whereas the solid dilution was mixed with agar media, then the bacteria were planted, incubated, and the concentration of antimicrobials that were able to inhibit or kill the test bacteria was observed.[17]

Minimum bactericidal concentration (MBC) was determined by preparing a petri dish containing Mueller's hinton agar, then makeing a barrier at the bottom of the petri dish using a marker. The MBC results that were not overgrown with bacteria were scratched using a round tip loop into a petri dish using the zig-zag technique. Put the petri dish in the oven at 37˚C for 24 hours to incubate. After 24 hours, bacterial growth was observed in the petri dish. The lowest concentration which was not overgrown with bacteria showed the minimum killing concentration. The higher the concentration of antibacterial substances given to the test bacteria, the higher the antibacterial power, meaning that the growth of the bacteria would be stunted if the concentration of antibacterial substances given was higher.[18] The procedure for determining the MIC and MIB was repeated 2 times.

Hand sanitizer gel was made by adding 1 gram of carbopol 940 and 78.3 gram of distilled water into a container, then stirring using a mixer for 15 minutes, and leaving the solution for 24 hours. After 24 hours, 5 gram of extract, 15 gram of propylene glycol, 0.18 gram of methyl paraben, 0.02 gram of propyl paraben were put into a container containing carbopol 940 solution and stirred using a mixer for 5 minutes. After stirring, 0.5 gram of triethanolamine was added to the mixture and then stirred again until the solution thickened. Then the hand sanitizer gel was transferred into a bottle.

The physical stability test of hand sanitizer gel was determined by examining pH, homogeneity, spreadability, and syneresis, then compared with standards. The hand sanitizer gel pH was measured by putting the gel into a beaker, then inserting the cathode into the pH meter. Homogeneity was determined by applying hand sanitizer gel to a transparent medium and then viewing the surface of the

Figure 1 Thin Layer Chromatography (TLC) Results of *T. mantaly* Stem Ethanol Extract under UV_{365} nm Light

media to determine whether the hand sanitizer gel was homogeneous or not. Meanwhile, spreadability was measured by placing 1gram of hand sanitizer gel on a glass medium, then placing another glass medium on top. A load weighing 125 gram was placed on the glass medium for 1 minute. The diameter of the hand sanitizer gel distribution was measured. To determine syneresis, samples were stored for 21 days and visually observed every 7 days to determine whether there was separation or not.[19]

Results

The results of phytochemical screening of the ethanol extract of *Terminalia mantaly* stems showed that it contained alkaloids, saponins, tannins, quinones, steroids and flavonoids. The results of TLC testing for the ethanol extract of *Terminalia mantaly* stems showed the presence of flavonoid compounds as indicated by the presence of a yellow stains after being sprayed with dilute H_2SO_4 staining reagent, and the presence of luminescence under UV_{365} nm light (Figure 1).

The results of MIC and MBC control showed that at 10% DMSO, the control media in the hand sanitizer section and control bacteria showed bacterial growth. In contrast, control media in the extract section and hand sanitizer without extract did not show bacterial growth (Table 1).

The results showed that the MIC of *Terminalia mantaly* extract against gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*) and gram-negative bacteria (*Escherichia coli*) was the same as the MIC of hand sanitizer. Meanwhile, the MIC of hand sanitizer against *Shigella dysenteriae* was 0.31%. The lowest MBC of *Terminalia mantaly* extract and hand sanitizer was found in gram-positive bacteria (*Staphylococcus aureus*),

Table 1 Results of MIC and MBC Control

Bacteria	Extract			Hand sanitizer		
	Control Media	10% DMSO	70% Alcohol	Control Media	Control Bacteria	Hand Sanitizer without Extract
Escherichia coli	-	+	+	-	+	-
Staphylococcus aureus	-	+	-	+	+	-
Streptococcus pyogenes	-	+	-	+	+	-
Shigella dysenteriae	-	+	-	+	+	-

Note : (+) there is bacterial growth; (-) there is no bacterial growth, MIC= Minimum inhibitory concentration, MBC= Minimum bactericidal concentration, DMSO= Dimethyl sulfoxide

Table 2 Results of MIC and MBC Extract and Hand Sanitizer Gel

Bacteria	MIC		MBC	
	Extract (b/v)	Hand sanitizer + Extract (w/w)	Extract (b/v)	Hand sanitizer + Extract (w/w)
Escherichia coli	0.63%	0.63%	2.50%	2.50%
Staphylococcus aureus	0.16%	0.16%	0.16%	0.16%
Streptococcus pyogenes	0.16%	0.16%	1.25%	1.25%
Shigella dysenteriae	0.63%	0.31%	1.25%	1.25%

Note: MIC= Minimum inhibitory concentration, MBC= Minimum bactericidal concentration

Table 3 Formulation of Hand Sanitizer Gel

Ingredients	Weight (grams)
Extract	2.50
	0.75
Carbopol 940	15.00
Propylene glycol	0.18
Methyl paraben	0.02
Propyl paraben	0.50
Triethanolamine	81.05

namely 0.16% (Table 2).

Table 3 showed the formulations used in manufacturing hand sanitizer gel. The most ingredient was triethanolamine (81.05 grams). The hand sanitizer gel produced from the ethanol extract of *Terminalia mantaly* stems was brown and smells like tea leaves.

The results of the physical test of the hand sanitizer gel included pH (5.89), homogeneity (homogeneous), syneresis (no separation), and spreadability (59.25) were in accordance with the physical stability standards of the gel (Table 4).

Discussion

Ethanol extracts from several *Terminalia* species are known to contain tannins, flavonoids, phenolic acids, triterpenes, triterpenoidal glycosides, lignans, and lignan derivatives. In addition, this genus has biological activities such as antidiabetic, antihyperlipidemic, antioxidant, antibacterial, antiviral, anti-inflammatory, anticancer, antiulcer, antiparasitic, hepatoprotective, and cardioprotective activities.[20] Methanol extract of *Terminalia mantaly* stem bark has been identified as a potential anti-yeast.[7,21] *Terminalia mantaly* also showed antiplasmodial activity.[22,23]

The content of flavonoid compounds in the extract of *Terminalia mantaly* stems showed negative results in phytochemical screening. However, in the TLC test, a yellow stain was obtained when diluted H_2SO_4 stain-seeking reagent was applied and lit at UV_{365}, indicating the presence of flavonoid compounds. This could be due to the relatively small amount of content so it does not show positive results during qualitative tests or phytochemical screening. The results of the secondary metabolites detected were in accordance with previous studies which stated that the *Terminalia mantaly* stem contained flavonoids, alkaloids, tannins, saponins, cyanogenic glycosides, and phenols.[2,24] Several secondary metabolites of terpenoids, tannins, sterols, and phenols have been studied to have antibacterial activity.[9]

The control media in the hand sanitizer section has been contaminated with bacteria from the environment during work because the work is not carried out in laminar air flow. Hence, bacterial contamination from the air can occur. The 10% DMSO solution was overgrown with bacteria because the 10% DMSO solution did not have antibacterial activity. Seventy percent alcohol could not inhibit the growth of *Escherichia coli* bacteria because the concentration was diluted after being mixed with the media and inoculum of the test bacteria, so that the bacteria could still grow. Hand sanitizer without extract can inhibit all test bacteria because the hand sanitizer contains several antibacterial ingredients such as methyl paraben and propyl paraben which function as preservatives.

Bacteria are divided into two groups based on the type of gram, namely gram positive and negative bacteria. Examples of gram-positive bacteria are *Staphylococcus aureus* and *Streptococcus pyogenes*, while *Shigella dysenteriae* and *Escherichia coli* are gram-negative bacteria.[25] *Staphylococcus aureus* and *Streptococcus pyogenes* can cause impetigo,[26] as well as digestive tract infections such as diarrhea caused by gram negative bacteria as *Escherichia coli*, and *Shigella dysenteriae*.[27] *Staphylococcus aureus* and *Escherichia coli* are two of the five major pathogens responsible for

Table 4 Results of The Physical Stability of Hand Sanitizer Gel

Test	Results	SNI Standard
pH	5.89	4.5–6.5
Homogeneity	Homogeneous	Homogeneous
Sinersis	No separation	No separation
Spreadability	59.25 mm	55.4–60.8 mm

Note: SNI= *Standar Nasional Indonesia*

54.9% of deaths due to infectious diseases.[28]

The results of the minimum inhibitory concentration show that the extract and the hand sanitizer containing the extract are able to inhibit all tested bacteria with different MIC. This extract has the highest MIC value against *Escherichia coli*, namely 0.63% (63 mg/ml) and has the lowest MIC value against *Staphylococcus aureus* and *Streptococcus pyogenes*, namely 0.16% (1.6 mg/ml). These results are in accordance with previous studies which stated that the MIC of *Terminalia mantaly* stems ethanol extract against *Escherichia coli* (0.3125 mg/ml) was higher compared to *Staphylococcus aureus* bacteria (0.078 mg/ml).[3] This is because gram-negative bacteria such as *Escherichia coli* have a layer of lipopolysaccharide which functions as a barrier to the entry of several substances including antimicrobial compounds, whereas gram-positive bacteria such as *Staphylococcus aureus* scantily have this layer.[29]

Differences in MIC value are caused by differences in test bacterial cultures, compound content in *Terminalia mantaly* stems and solvent during extraction. Previous study also proved differences in the MIC results of the aqueous extract of *Terminalia mantaly* leaves.[7,9] The MIC of Ketapang kencana leaf water extract against *Escherichia coli* was 0.625 mg/ml and 12.5 mg/ml.[7,9]

The MIC of the hand sanitizer+extract against *Shigella dysenteriae* is lower than the MIC of the extract alone. This could be due to the preservative content in hand sanitizers which can inhibit the growth of *Shigella dysenteriae* at lower concentrations. For *Escherichia coli, Staphylococcus aureus,* and *Streptococcus pyogenes* bacteria, MIC hand sanitizer+extract is the same as the MIC extract, this is because during the dilution process using the spray pull method, the concentration of the preservative contained in the hand sanitizer is also diluted, so it is less helpful in inhibits bacterial growth.

The results of MBC extract and hand sanitizer+extract are different for each bacterium. The extract has the highest MBC value against *Escherichia coli*, namely 2.5% (25 mg/ml) and has the lowest MBC value against *Staphylococcus aureus* 0.16% (1.6 mg/ml). The result of this study support previous research which stated that the MBC of ketapang kencana leaf water extract against *Escherichia coli* bacteria (25 mg/ml) was higher than against *Staphylococcus aureus* (6.25 mg/ml).[9]

The pH test results of the hand sanitizer gel obtained pH=5.89, this shows that the pH of the hand sanitizer gel meets the Indonesian National Standards, namely between 4.5–6.5. Using products with a pH below or above this range can cause irritation to the skin.

The homogeneity test is carried out to determine whether all the ingredients for making hand sanitizers have been mixed perfectly or not. A homogeneous gel, all the constituent ingredients can be dissolved and blended so that no ingredients form lumps. If there are still lumps of material, it is possible that the material has a different polarity or its solubility in the solvent used is small, so it is necessary to review the nature of the material and replace it with another material that is more suitable for use in the formulation.

The results of the 21-day synergism test on the hand sanitizer gel showed that there was no separation of components in the hand sanitizer gel. This indicates that the hand sanitizer gel emulsion is stable because the constituent components can dissolve homogeneously. If there is a separation, it means that the formulation used is not appropriate and the constituent components must be reviewed to obtain a gel preparation with a stable emulsion.

The spreadability test results obtained were 59.25 mm so it complies with the Indonesian National Standards, namely 55.4–60.8 mm.[19] The greater the spreadability of a product, the thinner the the product consistency. This dispersion is related to the viscosity of the product, the greater the spreadability, the smaller the viscosity.[30]

The limitation of the study is that the phytochemical content of the extract was not analysed quantitatively so it was less standardized. This study also did not examine the mechanism of action of the extract as an antibacterial agent.

In conclusions, the ethanol extract of *Terminalia mantaly* stems contains alkaloids, saponins, tannins, quinones, steroids, and flavonoids. The ethanol extract of *Terminalia mantaly* stem has stronger antibacterial activity against gram-positive bacteria than against gram-negative bacteria. The physical stability of the hand sanitizer gel meets the physical stability standards of the gel. The results of this study can be used as a basis for further research to determine the active compound and mechanism of action of *Terminalia mantaly* as an antibacterial.

References

1. Kumar SV, Damodar G, Ravikanth S, Vijayakumar G. An overview on

infectious disease. Indian J Pharm Sci Res. 2012;2(2):63–74.
2. Jing JLJ, Yi TP, Bose RJC, McCarthy JR, Tharmalingam N, Madheswaran T. Hand sanitizers: A review on formulation aspects, adverse effects, and regulations. Int J Environ Res Public Health. 2020;17(9):1–17.
3. Zhang XR, Kaunda JS, Zhu HT, Wang D, Yang CR, Zhang YJ. The Genus Terminalia (Combretaceae): an ethnopharmacological, phytochemical and pharmacological review. Nat Prod Bioprospect. 2019;9(6): 357–92.
4. Marjenah M, Ariyanto A. Suitability of some species for intercropped with tropical almond (Terminalia catappa Linn) on some land system in east Kalimantan and its prospects as plantation forest. J Penelit Ekosist Dipterokarpa. 2018;4(2):57–70.
5. Majoumouo MS, Sibuyi NRS, Tincho MB, Mbekou M, Boyom FF, Meyer M. Enhanced anti-bacterial activity of biogenic silver nanoparticles synthesized from Terminalia mantaly extracts. Int J Nanomedicine. 2019;14:9031–46.
6. Jiatsa Mbouna CD, Tchatat Tali BM, Tsouh Fokou PV, Madiesse Kemgne EA, Keumoe R, Toghueo Kouipou RM, et al. Specific sub fractions from Terminalia mantaly (H. Perrier) extracts potently inhibit Plasmodium falciparum rings, merozoite egress and invasion. J Ethnopharmacol. 2022;285:114909.
7. Ngouana T, Mbouna C, Kuipou R, Tchuenmogne M, Zeuko'o E, Ngouana V, et al. Potent and synergistic extract combinations from Terminalia Catappa, Terminalia Mantaly and Monodora tenuifolia Against Pathogenic Yeasts. Medicines. 2015;2(3):220–35.
8. Dlama TT, Oluwagbemileke AS, Monday D. Comparative study of the quantitative phytochemical constituents and antibacterial activity of five tree species. Eur J Adv Res Biol Life Sci. 2016;4(1):29–38.
9. Kokora AP, Ackah JAAB, Nanga ZY, Kra MAK, Loukou GY, Coulibaly A, et al. Antibacterial activity of ethanolic and aqueous extracts of four medicinal plants on the in vitro growth of Escherichia coli and Staphylococcus aureus. J Drug Deliv Ther. 2013;3(3):113–6.
10. Abou O, Elisée KK, Vénérer MS, Bi S, Ibrahim K. Study of the antibacterial activity of bark extracts from *Terminalia mantaly* (Combretaceae) on the in vitro growth of eight (8) clinical enterobacteria strains. J Medicianal Plants Stud. 2018;6(2):101–5.
11. Manaye G, Muleta D, Henok A, Asres A, Mamo Y, Feyissa D, et al. Evaluation of the efficacy of alcohol-based hand sanitizers sold in southwest ethiopia. Infect Drug Resist. 2021;14:547–54.
12. Golin AP, Choi D, Ghahary A. Hand sanitizers: A review of ingredients, mechanisms of action, modes of delivery, and efficacy against coronaviruses. Am J Infect Control. 2020;48:1062–7.
13. Sugihartini N, Wiradhika RY. Gel formulation of ethanol extract of mangosteen peel (Garcinia mangostana L.) as a medication for burns in Wistar Rats. JKKI. 2017;8(2):110–7.
14. Ansel HC. Pengantar bentuk sediaan farmasi. 4th Ed. Jakarta: UI Press; 2011.
15. Tiwari, P., Kumar, B., Kaur, M., Kaur, G., Kaur H. Phytochemical screening and extraction: A Review. Int Pharm Sci. 2011;1(1):98–106.
16. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal. 2016;6(2):71–9.
17. Chikezie IO. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using a novel dilution tube method. African J Microbiol Res. 2017;11(23):977–80.
18. Venkateswarulu TC, Srirama K, Mikkili I, Nazneen Bobby M, Dulla JB, Alugunulla VN, et al. Estimation of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of antimicrobial peptides of Saccharomyces boulardii against selected pathogenic strains. Karbala Int J Mod Sci. 2019;5(4):266–9.
19. Febriani A, Maruya I, Sulistyaningsih F. Formulasi dan uji iritasi sediaan gel kombinasi ekstrak etanol rimpang kencur (*Kaempferia galanga L.*) dan ekstrak etanol herba pegagan (*Centella asiatica (L.) Urban*). Sainstech Farma. 2020;13(1):45–54.
20. Fahmy N, Al-Sayed E, Singab A. Genus Terminalia: A phytochemical and Biological Review. Med Aromat Plants. 2015;04(05): 1–21.
21. Tchuente Tchuenmogne M, Kammalac T, Gohlke S, Kouipou R, Aslan A, Kuzu M, et al. Compounds from Terminalia mantaly L. (Combretaceae) stem bark exhibit potent inhibition against some pathogenic yeasts

and enzymes of metabolic significance. Medicines. 2017;4(6):1–12.
22. Samuel B, Adekunle YA. Isolation and structure elucidation of anti-malarial principles from Terminalia mantaly H. Perrier stem bark. Int J Biol Chem Sci. 2021;15(1):282–92.
23. Mbouna CDJ, Kouipou RMT, Keumoe R, Tchokouaha LRY, Fokou PVT, Tali BMT, et al. Potent antiplasmodial extracts and fractions from Terminalia mantaly and Terminalia superba. Malar J. 2018;17(1):1–9.
24. Brigitte OM, Bationo JH, Somda M, Ouattara A, Kiendrebeogo M, Nacoulma OG. Evaluation of acute toxicity, antioxidant and antibacterial potentials of leaves from two Combretaceae, Combretum micranthum G. Don. and Terminalia mantaly H. Perr. Middle-East J Sci Res. 2018;26(5):478–82.
25. Jawetz E, Melnick J, Adelberg E. Mikrobiologi Kedokteran. 27th Ed. 2017.
26. Bowen AC, Tong SYC, Chatfield MD, Carapetis JR. The microbiology of impetigo in indigenous children: associations between Streptococcus pyogenes, Staphylococcus aureus, scabies, and nasal carriage. BMC Infect Dis. 2014;14:727.
27. Aminshahidi M, Arastehfar A, Pouladfar G, Arman E, Fani F. Diarrheagenic Escherichia coli and Shigella with High Rate of extended-spectrum beta-lactamase production: two predominant etiological agents of acute diarrhea in Shiraz, Iran. Microb Drug Resist. 2017;23(8):1037–44.
28. Ikuta KS, Swetschinski LR, Robles Aguilar G, Sharara F, Mestrovic T, Gray AP, et al. Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet. 2022;400(10369):2221–48.
29. Mai-Prochnow A, Clauson M, Hong J, Murphy AB. Gram positive and Gram negative bacteria differ in their sensitivity to cold plasma. Sci Rep 2016;6: srep38610
30. Garg A, Aggarwal D, Garg S, Singla AK. Spreading of semisolid formulations: an update. Pharmaceutical Technology. 2002;26(9):84–105.