

Antibacterial effects of plants from lembang highland towards skin-infecting microorganisms

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ABSTRACT

Bacterial skin infection is a common infection in Indonesia. *Staphylococcus aureus* cause skin and soft tissue infections and *Propionibacterium acnes* causes acne (acnes vulgaris). *Moringa oleifera*, *Piper betle*, and *Leucaena leucocephala* leaves traditionally use as. This study aims is to evaluate antimicrobial activity of the ethanol extract of the leaves from Lembang highland against *S. aureus* and *P. acnes*. The extract of each leaves prepared using reflux in 50% ethanol and examination of antibacterial activity of the ethanol extract using the microdilution method *in vitro*. The results showed that the ethanol extracts of *M.oleifera*, *P.betle*, and *L.leucocephala* leaves had antibacterial activity against *S. aureus* and *P. acnes* with different minimum inhibitory concentrations. The greatest antibacterial activity against *S.aureus* was shown by the ethanol extract of moringa leaves (MIC = 1024 µg/ml), where the greatest antibacterial activity against *P. acnes* was possessed by the ethanol extract of betel leaves (MIC = 512 µg/ml).

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INTRODUCTION

Skin diseases are caused by bacterial, fungal, parasitic infections, basic allergic diseases and degenerative factors. Besides the differences in causes, other factors such as climate, habits and environment also contribute to differences in the clinical picture of skin diseases. (Hidayat, 2018) In Indonesia, bacterial skin infection is a common occur infection. Pathogenic bacteria that cause infection in humans is *Staphylococcus aureus*. *S.aureus* is the main cause of skin and soft tissue infections in the world. Another causative bacteria is *Propionibacterium acnes*. This bacterium is a bacteria that causes acne (acnes vulgaris). The prevalence of acne sufferers in Indonesia ranges from 80-85% of adolescents. (Sibero et al., 2019)

Antibiotics are the most effective treatment used as antimicrobials, but inappropriate use of antibiotics can lead to resistance. Therefore it is necessary to have alternatives from plants that have the potential as antimicrobials. As many as 51 of 79 vascular plants are effective as antibiotics, particularly those belonging to the Lamiaceae, Fabaceae, and Asteraceae families. Leaves, aerial parts, roots, bark, fruit, seed, stem, rhizome, and flower are among the analyzed plant parts with antibacterial effects.(Chassagne et al., 2021)

Plants that are traditionally used in Indonesia as antibiotics are *Moringa oleifera*, *Piper betle*, and *Leucaena leucocephala* leaves. The activity of a single *Moringa oleifera* extract as an antibiotic from various location has been studied. The extract of *M.oleifera* leaves from Oaxaca (Mexico) shown antibiotic effect against *Staphylococcus epidermidis* and *Enterococcus faecalis*.(Jiménez et al., 2022) Freeze dried extract of *M. oleifera* from Padang (Indonesia) also shown antibiotic effect against *Staphylococcus aureus*.(Angestia et al., 2020) Study of *M. oleifera* leaf extracts against bacteria isolated from patients in Damaturu hospital (Nigeria) showed antibiotic effect against *E. coli*, *Shigella spp*, *Salmonella typhi*, *E. faecalis* and *S. aureus*.(Abadallah & Ali, 2019) Study on *Piper betle* leaf from various places Nganjuk, Sidoarjo and Batu (Indonesia) showed that ethanol extract may inhibit *E. coli*, *Salmonella sp.*, *S. aureus*, and *Pseudomonas aeruginosa*.(Ermawati et al., 2021) *P.betle* extract is also effective against methicillin-resistant and methicillin-susceptible *Staphylococcus* strains.(Phensri et al., 2022) The leaves of *Leucaena leucocephala* shown antibacterial action against *E. coli*.(Rosida et al., 2018)

Variation in the habitat in which plants grow will cause differences in the secondary metabolite content and, in turn, their effects.(Katuuk et al., 2019) In this study, the antimicrobial activity of ethanol extract on *Moringa oleifera*, *Piper betle*, and *Leucaena leucocephala* leaves from Lembang Highland West Java was tested on microbes that cause skin infections *S. aureus* and *P. acnes*. This use is related to the activity of its chemical compounds as an antibacterial.

RESEARCH METHOD

Test Material Collection

Moringa oleifera, *Piper betle*, and *Leucaena leucocephala* leaves were collected from the Manoko, Lembang, West Java Province, Indonesia. Determination of each plant was carried out at the Herbarium Biology, University of Padjadjaran.

Simplicia and Extract Production

The ethanol extract was carried out by continuous extraction using a reflux with 50% ethanol as a solvent. The extract then evaporated until a thick extract, then the thick extract was dried in an oven at 60 °C.

Characteristics & Phytochemical Screening

Characteristics are carried out as plant identity and to find out impurities that may be present. The simplicia characteristics tested were organoleptic, water content, total ash content, water soluble extract content and ethanol soluble extract content.(Indonesia, 2017) Phytochemical screening was carried out to detect the presence of flavonoids, tannins, quinones, saponins, alkaloids, and steroids/terpenoids.

Preparation for Antibacterial Testing

Each of the test bacteria was inoculated in *Mueller Hilton Agar* (MHA) and incubated for 24 hours at 37°C. Incubated bacteria were taken with a round Ose needle and suspended in 5 mL of *Mueller Hilton Broth* (MHB), then incubated for 24 hours at 37°C. The following day, the test bacterial suspension was diluted with MHB to produce an absorbance of 0.08 - 0.13 using a UV-Vis spectrophotometer at a wavelength of 625 nm (equivalent to 0.5 McFarland). After obtaining absorbance in this range, the bacterial suspension was diluted again with MHB so that the final number of bacteria contained in each well of the plate was equivalent to approximately 5×10^5 CFU/mL (range $2-8 \times 10^5$ CFU/mL).(Choirunnisa & Sutjiatmo, 2017; Lorian, 2005)

Antibacterial Activity

Determination of Minimum Inhibition Concentration (MIC) method of the test extract was microdilution method. A total of 100 µL of sterile medium was added to the 96 wells microplate. Then, 100 µL of the test extract solution was added at position 12A (first row(A), 12th column) on

the microplate. This solution is stirred slowly using a micropipette until it is homogeneous, then 100 μL of this solution is pipetted and transferred to position 11A of the microplate. This dilution was continued up to position 3A. Dilution is carried out from right to left on the plate. The same thing was done for the dilution of the reference antibiotic. After dilution was carried out in all wells, 100 μL of the bacterial suspension that had been prepared was added to each microplate until the total volume of each well was 200 μL . The negative control was filled with 200 μL of media, while the positive control was filled with 100 μL of media and 100 μL of the test bacterial suspension. Microplates containing negative controls, positive controls, test extracts and reference antibiotics were then incubated for 18-24 hours at $35 \pm 2^\circ\text{C}$. MIC was observed as the lowest concentration where there was no bacterial precipitate at the bottom of the well (clear) which indicated inhibition of bacterial growth. The test was carried out in three times. (Choirunnisa & Sutjiatmo, 2017)

RESULTS AND DISCUSSIONS

Simplicia characteristic was determined to standardize and measure the quality of the simplicia. The results of the characteristic examination can be seen in Table 1.

Table 1. The results of the characteristics examination of *Moringa oleifera*, *Piper betle*, and *Leucaena leucocephala* leaves

Parameter	<i>Moringa oleifera</i>	<i>Piper betle</i>	<i>Leucaena leucocephala</i>
Organoleptic	Powder, brownish green, bitter	Powder, brownish green, bitter	Powder, brownish green, bitter
Water content (%v/w)	5.20	7.00	4.40
Total ash content (%w/w)	8.50	5.95	8.35
Water soluble extract content (%w/w)	30.90	23.41	33.41
Ethanol soluble extract content (%w/w)	22.29	15.29	9.26

Phytochemical screening was also carried out on simplicia and extracts which purpose to determine the content of secondary metabolites in simplicia and extracts, especially secondary metabolites which have the potential as antibacterial agents. The results of the phytochemical screening can be seen in Table 2.

Table 2. The results of phytochemical screening of *Moringa oleifera*, *Piper betle*, and *Leucaena leucocephala* leaves

Parameter	<i>Moringa oleifera</i>		<i>Piper betle</i>		<i>Leucaena leucocephala</i>	
	Simplicia	Extract	Simplicia	Extract	Simplicia	Extract
Alkaloid						
- Dragendorff	-	-	-	-	-	-
- Mayer	+	+	-	-	-	-
Flavonoid	+	+	+	+	+	+
Polyphenol	+	+	+	+	+	+
Tannin	+	+	+	+	+	+
Quinones	-	-	-	-	+	+
Saponnin	+	+	+	+	-	-
Mono and Sesquiterpenoid	+	+	+	+	+	+
Steroid and Triterpenoid	+	+	+	+	+	+
	triterpenoid	triterpenoid	triterpenoid	triterpenoid	triterpenoid	triterpenoid

+= containing the compounds, - = not containing the compounds

All of the simplicia and extract contained secondary metabolites of flavonoids, polyphenols, tannins and terpenoids. Flavonoids are known to damage bacterial cell membranes by forming complex compounds with extracellular proteins that damage the bacterial cell membrane and cause intracellular compounds to be released. In addition, flavonoids can inhibit the use of bacterial oxygen which can lead to inhibition of energy metabolism. Phenol can act as a poison by inhibiting the activity of bacterial enzymes and can also denature proteins so that the metabolic activities of bacterial cells die. (Sadih et al., 2022) Tannin toxicity for bacteria because tannin have mechanism include inhibition of extracellular microbial enzymes, deprivation of the substrates required for microbial growth or direct action on microbial metabolism through inhibition of oxidative phosphorylation. (Scalbert, 1991) Previous studies state that oxygenated terpenes (terpenoids) such

as phenolics exhibit better antimicrobial activity than hydrocarbons such as R-(-)-limonene, terpinene, camphene, and (+)- α -pinene, which agree with the present work, since these compounds presented weak antimicrobial action. (Guimarães et al., 2019)

The results of antibacterial activity using the microdilution method of ethanol extract can be seen in Figure 1 and Table 3. Determination of antibacterial activity in vitro can be performed using several methods, including the agar dilution method. The consideration for choosing this method is because the test method is simpler, the sample required is less, the sensitivity is higher and the results are quantitative. (Lorian, 2005) MIC testing was performed triplo for the tested plant extracts and duplo for antibiotics. The principle of the test is multiple dilution of the test substance in liquid medium which is performed on a 96-microwell-round-bottomed microdilution plate. In general, all the tested extracts had an effect on *S.aureus* and *P.acne*.

Table 3. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Moringa oleifera*, *Piper betle*, and *Leucaena leucocephala* leaves extract

Extract	<i>S. aureus</i>		<i>P. acnes</i>	
	MIC ($\mu\text{g/ml}$)	MBC ($\mu\text{g/ml}$)	MIC ($\mu\text{g/ml}$)	MBC ($\mu\text{g/ml}$)
<i>Moringa oleifera</i>	1024	4096	1024	2048
<i>Piper betle</i>	2048	2048	512	1024
<i>Leucaena leucocephala</i>	2048	> 4096	2048	> 4096
Tetracycline	8	16	8	32

The results showed that the extract that had the greatest antibacterial activity against *S.aureus* was *M.oleifera* extract (MIC = 1024 $\mu\text{g/ml}$), although the results were higher than tetracycline (MIC = 8 $\mu\text{g/ml}$). The greatest antibacterial activity against *P. acnes* was possessed by *P.betle* extract (MIC = 512 $\mu\text{g/ml}$). All extract gives smaller MIC than MBC, except for *P.betle* extract against *S.aureus* which had the same MIC and MBC value.

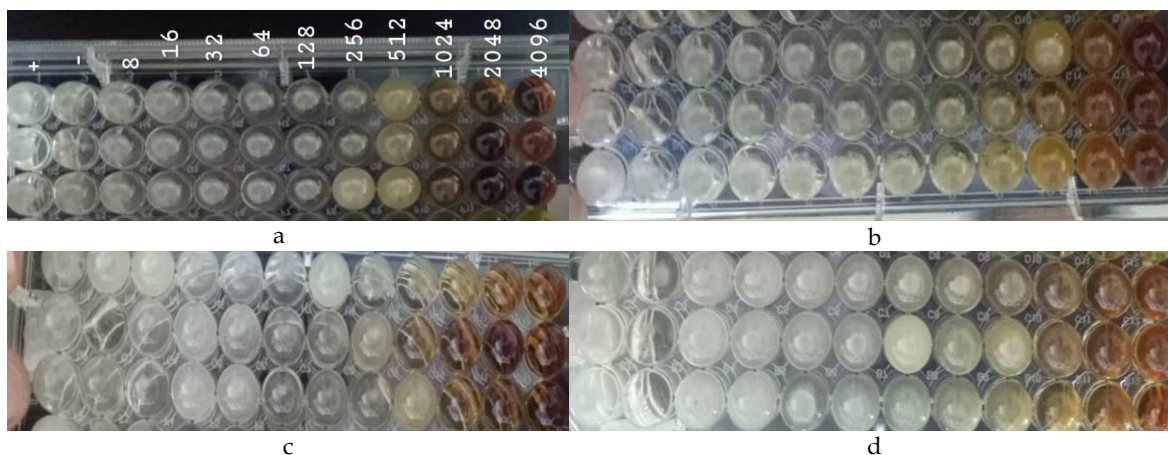


Figure 1. Minimum Inhibitory Concentration (MIC) using microdilution technique, a) *M.oleifera* extract against *S.aureus*; b) *M.oleifera* extract against *P.acnes*; c) *P. betle* extract against *S.aureus*; d) *P. betle* extract against *P.acnes*

The MIC results were different for each extract due to the varying levels of secondary metabolites. *Moringa* leaf extract had total flavonoid content of 0.45%. *P. betle* leaf extract has an average of total flavonoid 1.077%. *L. leucocephala* extract has total flavonoid content of 0.35%. (Hidayah et al., 2020; T. Hidayat et al., 2020; Kurniawan et al., 2021) The difference of total flavonoid correlate with the antibacterial effect. It can be seen from these results, the antibacterial activity of *P. betle* extract and *M.oleifera* extract has a better MIC value compared to *L. leucocephala* because they have a higher average total flavonoid content.

The chemical composition of gram-positive bacterial cells consists of a mucopeptide or peptidoglycan layer, this layer is non-polar, so that compound molecules with lipophilic properties will more easily penetrate the bacterial cell wall through interactions with proteins and the peptidoglycan layer causing damage to the cell wall structure and the bacteria experience lysis and finally the talent experienced death.(Ibrahim & Kuncoro, 2012) Another presumption is secondary

metabolites in the extract have a synergistic interaction, so that inhibit bacterial growth by the stability of proteins, lipids, salts and the level of acidity (pH) in the growth medium.

CONCLUSION

Ethanol extracts of *Moringa oleifera*, *Piper betle*, and *Leucaena leucocephala* leaves have antibacterial activity against *Staphylococcus aureus* and *Propionibacterium acnes* with different minimum inhibitory concentrations. The ethanol extract of Moringa leaves had the greatest antibacterial activity against *S.aureus* (MIC = 1024 µg/ml) and the greatest antibacterial activity against *P. acnes* was possessed by the ethanol extract of betel leaves (MIC = 512 g/ml). Because the antibiotic potential of the these extracts is very promising, further research is needed regarding the effects on other bacteria and their specific mechanism of action.

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