Antibacterial activity test of meniran herb extract (Phyllanthus Niruri L.) against staphylococcus epidermidis and klebsiella pneumoniae

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ABSTRACT

An antibacterial test of meniran herb extract (Phyllanthus niruri L.) against Staphylococcus epidermidis and Klebsiella pneumoniae has been carried out. Meniran herb powder was screened for phytochemicals to assess the secondary metabolites contained in it, then meniran herbs were extracted using the soxhletation method in stages with various solvents (n-hexane, ethyl acetate, and 70% ethanol). The disc diffusion method was used to test the inhibition zone diameter of n-hexane extract, ethyl acetate extract, and ethanol extract against Staphylococcus epidermidis and Klebsiella pneumoniae. Meniran herb extract was diluted with a concentration series using dimethylsulfoxide. Meniran herb extracts from the three types showed the best inhibition zone diameter when tested for minimum inhibitory concentration (MIC) by the solid dilution method. The results showed that meniran herb powder contained flavonoids, saponins, tannins, glycosides, and steroids/terpenoids. Antibacterial tests showed that ethyl acetate and 70% ethanol extracts had antibacterial activity only against Staphylococcus epidermidis. The antibacterial activity of the two meniran herb extracts had the largest inhibition zone diameter against Staphylococcus epidermidis at a concentration of 50%, which was 12.33 mm for the ethyl acetate extract and 16.00 mm for the ethanol extract. The MIC test of the ethanol extract of the meniran herb, which had the best antibacterial activity, found that at a concentration of 2.5% it was able to inhibit the growth of Staphylococcus epidermidis.

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INTRODUCTION

Meniran (Phyllanthus niruri L.) is a plant that has many properties and has been used as traditional medicine. The efficacy of these plants is associated with various active compounds such as alkaloids, flavonoids (quercetin, quercitrin), lignin, terpenoids, lupeol, and tannins (Elfahmi et al., 2014; Martinus & Rivai, 2015; MS et al., 2021). The meniran herb is traditionally used to treat inflammation,
ulcers, diabetes, kidney stones, gallstones, hepatitis, and to reduce blood pressure based on its diuretic activity (Bello et al., 2020; Hassim et al., 2019). Several previous studies exploring the potential of meniran as part of its antibacterial activity have been carried out, including on Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Candida albicans, Salmonella typhimurium, and Enterococcus faecalis (KH et al., 2016; MS et al., 2021). Staphylococcus epidermidis and Klebsiella pneumoniae are two other bacteria that can cause infection.

Staphylococcus epidermidis is a gram-positive, aerobic or facultative anaerobic, spherical or coccus-shaped irregular group. Staphylococcus epidermidis is found on the skin, mucous membranes, boils, and wounds. This bacterium can cause disease through its ability to multiply and spread widely in tissues. Staphylococcus epidermidis naturally lives on human skin and mucous membranes (Claudel et al., 2019; Otto, 2009). Klebsiella pneumoniae is one of the bacteria that includes gram-negative bacteria, non-motile bacteria, and facultative anaerobes. Klebsiella pneumoniae can cause extensive consolidation with hemorrhagic necrosis of the lungs. Klebsiella ranks second after E. coli for urinary tract infections in elderly people (Brisse et al., 2006).

Based on the description above, a study will be carried out to determine the antibacterial activity of meniran herb (Phyllanthus niruri L.) against Staphylococcus epidermidis and Klebsiella pneumoniae. The part used in this study is the whole meniran plant that is above ground. This meniran herb contains active compounds that are non-polar, semi-polar, and polar, so that in this study three solvents were used, namely n-hexane, ethyl acetate, and ethanol 70%.

**RESEARCH METHOD**

**Tool**

A set of soxhleting tools, filter paper, hotplate, round bottom flask (Pyrex), sieve no. 4 and 18, Blender (Miyako), Flannel cloth, Analyte balance (Sartonius), Stir bar, Beaker glass (Pyrex), Glass funnel, Rotary evaporator (Buchi), Waterbath, Measuring cup (Pyrex), Watch glass, Porcelain cup, Measuring flask (Pyrex), Test tube (Pyrex), Autoclave, Incubator (Binder), 5 mm sterile disc paper, Aluminum foil, Petri dish, Ose needle, Yellowtip, Micropipette (Socorex), Volume pipette (Fortuna), Oven (Memmert), Bunsen burner, Dropper pipette, Erlenmeyer (Pyrex), Beaker glass (Pyrex), Analytical balance (Sartonius), Laminar Air Flow (LAF) cabinet.

**Material**

Meniran herb (Phyllanthus niruri L.) obtained from the area around Bantul, Yogyakarta. The chemicals used include ammonia, amylalcohol, distilled water, acetic acid anhydrous [(CHCO)2]O, hydrochloric acid (HCl), sulfuric acid (H2SO4), chloroform (CHCl3), FeCl3, NaOH (Brataco), Dragendorf reagent, Mayer's reagent, ether, n-hexane, ethyl acetate, 70% ethanol (Brataco), 0.9% NaCl, dimethylsulfoxide (DMSO) (Merck), Aquabidest (Ikapharmindo). The test bacteria used were Staphylococcus epidermidis for gram positive and Klebsiella pneumoniae for gram negative obtained from the Microbiology Laboratory, Faculty of Medicine, University Indonesia, Salemba, Jakarta. The media used for bacterial growth and antibacterial testing were Nutrient Agar (NA) and Mueller Hinton Agar (MHA) media (Merck). The controls used were dimethylsulfoxide (DMSO) as a negative control and 0.025% ciprofloxacin as an antibiotic control.

**Sample Preparation**

Meniran herb simplicia was refined with a blender and sieved with a fineness degree of 4/18 (Sembiring et al., 2006).
Phytochemical Screening

A phytochemical screening of meniran herbal powder was carried out, including analyses for alkaloids, flavonoids, saponins, tannins, glycosides, and steroids/terpenoids (Mangunwardoyo et al., 2009).

Meniran Herb Extraction

The extraction of meniran herb powder was carried out in a multilevel manner (n-hexane, ethyl acetate, and ethanol 70%) with the soxhletation method based on the method from (Ulfa et al., 2014) with modifications. The powder was extracted with n-hexane, then the residue extracted from n-hexane was again soxhleted with ethyl acetate to obtain ethyl acetate extract. The residue from the extraction of ethyl acetate was again soxhleted with ethanol 70% to obtain ethanol extract. Soxhletation results of each n-hexane, ethyl acetate and 70% ethanol meniran extract were concentrated with a rotary evaporator and a water bath.

Extract Dilution and Control Preparation

Extracts of n-hexane, ethyl acetate and 70% ethanol of meniran herbs were made in a series of concentrations using dimethylsulfoxide (DMSO) with a concentration of 62.5% - 50%. The negative control used was DMSO. The control antibiotic used was ciprofloxacin with a concentration of 0.025%.

Gram Staining for Bacteria

Gram staining of the test bacteria was carried out using crystal violet reagent, iodine solution, 96% alcohol, and safranin, then emulsion oil was added and observed under a microscope with 1000x magnification (Becerra et al., 2016).

Bacterial strains and growth conditions

The cultivation/assay medium for Staphylococcus epidermidis and Klebsiella pneumoniae was Nutrient Agar (NA). Bacterial cultures for antimicrobial testing were prepared by picking colony 1-2 ose of test bacteria diluted in a 0.9% NaCl solution. The turbidity of the bacterial suspension was compared to that of a 0.5 MacFarland standard solution, resulting in bacteria with an amount of 1.5 x 10^8 CFU/mL. The bacterial suspension was then diluted to a concentration of 10^6 CFU/mL. The adjusted suspension was used as an inoculum within 15 minutes (Matuschek et al., 2014; Semeniuc et al., 2017).

Antibacterial Testing of Meniran Extract

Antibacterial testing of meniran herb extract was carried out using the disc diffusion (streak) method of (Nurhayati et al., 2020) with modifications. The test bacterial suspension was spread onto the surface of the MHA medium, then the disc containing the test solution was placed in the inoculum (the test bacterial suspension was inserted into the media) and incubated at 37 °C for 24 hours. The inhibition zone diameter formed on the discs was then measured for each extract.

Minimum Inhibitory Concentration (MIC) Determination

Bacterial suspensions were then inoculated on solid MHA. The bacterial medium was mixed with meniran herb extract, which had the best antibacterial activity, and then the test bacteria were added to the surface of the media and incubated at 37 °C for 24 hours. The MIC test was carried out using the solid dilution method, defined as the lowest concentration of meniran extract in solid media where no growth was observed after 24 hours (Mulyadi et al., 2017).

Data Analysis

The measurement results of the inhibitory diameter formed around the hole are measured using a ruler. Data in the form of the diameter of the inhibition zone from the largest meniran herb
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RESULTS AND DISCUSSIONS

Meniran herbs were obtained from the Bantul area, Yogyakarta. Herba meniran was determined at the Biology Laboratory of Gadjah Mada University, Yogyakarta. The results of the determination proved that the meniran herb used was a species (Phyllanthus niruri L.) from the Euphorbiaceae family. Meniran herb powder that has been dried is carried out in a powder manufacturing process in order to obtain good and optimal extraction results. The reduced size of the meniran herb will expand the surface that interacts with the active compound, making it easier to attract (Hu et al., 2018).

Extracts were made using the soxhletation method, where the principle of continuous extraction with a relatively constant amount of solvent was due to back cooling. In this process, the solvent used is 1 times the volume of the extractor from the soxhlet apparatus. This is useful so that when the solvent is evaporated, the flask is not empty and the extraction runs perfectly (Zain et al., 2016). The soxhlet extraction was done in stages, with each solvent—n-hexane, ethyl acetate, and 70% ethanol—being used for 8 cycles. The results of the soxhlet extraction were filtered and then evaporated to obtain a thick extract. The yield of the solvent used in Figure 1.

![Figure 1. Yield of meniran herb extract](image)

The yield of the thick extract of meniran herb showed that extraction with a 70% ethanol solvent could extract higher amounts of the components of the compound than the solvents n-hexane and ethyl acetate. Extraction using three different types of solvents aims to obtain and compare compounds from meniran herbs that have active potential as antibacterial compounds. The non-polar solvent used was n-hexane, the semi-polar solvent was ethyl acetate, and the polar solvent was 70% ethanol. The selection of non-polar n-hexane based on its selectivity in attracting non-polar, stable, and volatile compounds (Zain et al., 2016). Ethyl acetate was chosen because it can attract polar and non-polar compounds, has low toxicity, and is easy to evaporate (Wulandari et al., 2013). The choice of 70% ethanol as a filter solution is because it can dissolve phytochemical compounds more optimally because 70% ethanol still contains quite a lot of water (30%), which helps the extraction process so that some of these compounds can be attracted to ethanol and some are interested in water. Amino acids, sugars, and several phytochemical compounds such as alkaloids, flavonoids, flavonoid glycosides, and chlorophyll are dissolved in polar solvents, so the compounds extracted with 70% ethanol solvent are quite large and produce high yields (Sani et al., 2014).

An examination of the content and phytochemical screening of the meniran herb powder was carried out to determine the class of chemical compounds contained in the meniran herb. The
results of the screening of chemical compounds on the meniran herb powder showed that it contained flavonoids, saponins, tannins, glycosides, and steroids/terpenoids. These results are the same as previous studies on the phytochemical screening of meniran herbs (Tambunan et al., 2019). The results of phytochemical screening are shown in Table 1.

Table 1. Phytochemical screening of meniran herb powder

<table>
<thead>
<tr>
<th>No</th>
<th>Group of Chemical Compounds</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoid</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Glicosyde</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Steroid/terpenoid</td>
<td>+</td>
</tr>
</tbody>
</table>

Description: (-) There is no chemical compound content  
(+) There is a chemical content

Gram staining showed that each isolate of bacteria was Gram positive for Staphylococcus epidermidis and Gram negative for Klebsiella pneumoniae characterized by violet colour in bacterial cells shown in Figure 2. Staphylococcus epidermidis is a Gram positive bacterium because, when washed with alcohol, the color remains purplish blue. The color produced is related to the cell wall in Staphylococcus epidermidis, which contains a lot of strong peptidoglycan, so that the crystal violet that enters the bacteria cannot be washed off by alcohol. Klebsiella pneumoniae is known to be Gram negative bacteria, upon observation after washing with alcohol, the purple color disappeared or faded and when added with safranin, the color became red. The missing purple color is related to the cell wall of Klebsiella pneumoniae, which contains a lot of lipopolysaccharide and can be washed off by alcohol, making the bacteria appear transparent and turn red after being given safranin (Rahayu et al., 2017).

![Staphylococcus epidermidis (1000x)](image1)
![Klebsiella pneumoniae (1000x)](image2)

Figure 2. Gram stain on test bacteria

The antibacterial activity test of meniran herb extract was carried out using the solid diffusion method using discs against Staphylococcus epidermidis and Klebsiella pneumoniae. The principle of testing in this method is whether or not a clear zone is formed around the well after the agar medium planted with the test bacteria is incubated at 37 °C for 24 hours so that the amount of inhibition against the test bacteria can be clearly observed (Matuschek et al., 2014). DMSO solvent is used because it has properties as an aprotic polar solvent that can dissolve both polar and nonpolar compounds and is soluble in various organic solvents and water (Manik et al., 2014).

Ethyl acetate and 70% ethanol extracts showed from low to high concentrations had the ability to inhibit Staphylococcus epidermidis, but the n-hexane extract did not produce a clear area around the disc, as shown in table 2. The largest clear zone was observed in the growth of

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Staphylococcus epidermidis at 50% extract concentration in ethyl acetate extract and ethanol extract of meniran herbs. These results indicate that the greater the concentration of the two extracts, the greater the clear zone formed or its inhibitory power.

The inhibition produced by the 70% ethanol extract was greater than the inhibition produced by the ethyl acetate extract against *Staphylococcus epidermidis*. This difference in inhibition ability is probably caused by the active compound content extracted by ethyl acetate and ethanol solvents. Research conducted by Tambunan et al., showed that ethanol extracts contain flavonoids, saponins, tannins, gallocate quinones, terpenoids, coumarins, and essential oils (Tambunan et al., 2019). Based on statistical tests using the non-parametric Kruskal-Wallis test for ethyl acetate extract, it is known that Sig. was rejected (0.02 < 0.05), which indicated that the concentration series of meniran herb ethyl acetate extract affected the DDH results of *Staphylococcus epidermidis*. In the Kruskal-Wallis statistical test of ethanol extract, it is known that Sig. was rejected (0.012 < 0.05), which indicates that the concentration series of meniran herb ethanol extract affects the DDH results of *Staphylococcus epidermidis*.

### Table 2. Inhibition zone diameter of meniran herb extract against staphylococcus epidermidis

<table>
<thead>
<tr>
<th>Meniran Herb Extract</th>
<th>Concentration</th>
<th>Inhibition Zone rate (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-hexane</td>
<td>50%</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>25%</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>12.5%</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>6.25%</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>12.33</td>
</tr>
<tr>
<td>Ethyl Acetate</td>
<td>25%</td>
<td>10.00</td>
</tr>
<tr>
<td></td>
<td>12.5%</td>
<td>8.00</td>
</tr>
<tr>
<td></td>
<td>6.25%</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>16.00</td>
</tr>
<tr>
<td></td>
<td>25%</td>
<td>13.00</td>
</tr>
<tr>
<td>Ethanol</td>
<td>12.5%</td>
<td>12.00</td>
</tr>
<tr>
<td></td>
<td>6.25%</td>
<td>9.67</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.025%</td>
<td>13.00</td>
</tr>
<tr>
<td>DMSO</td>
<td>99.99%</td>
<td>0.00</td>
</tr>
</tbody>
</table>

DMSO control showed no clear zone. Antibiotic control with ciprofloxacin has a very strong category of inhibition against *Staphylococcus epidermidis* bacteria. Ciprofloxacin is a quinolone antibacterial that is sensitive to the growth of *Staphylococcus epidermidis* bacteria, with its mechanism of action inhibiting the action of the DNA gyrase enzyme on bacteria and being bactericidal (Sharma et al., 2010). The DDH produced by ciprofloxacin is very large, even though it has a concentration of only 0.025% compared to 70% ethanol extract and ethyl acetate, which must require large concentrations to get high inhibitory power. The largest inhibition zone was found at 50% extract concentration with an average of 16.00 mm. The inhibitory power of ethyl acetate extract at a concentration of 50% resulted in an average inhibition of 12.33 mm. The antibacterial effect of 70% ethyl acetate and ethanol extracts may be due to the chemical compounds contained in the meniran herb extract, namely flavonoid compounds and tannins.

Tannins in low concentrations are able to inhibit bacterial growth, while at high concentrations, tannins work as antimicrobials by coagulating or agglomerating bacterial protoplasm so that a stable bond is formed with bacterial proteins, and in the digestive tract, tannins are known to be able to eliminate toxins (Poeloengan & Praptiwi, 2010). The mechanism of action of flavonoids as antibacterials is to form complex compounds with extracellular and dissolved proteins so that they can damage the bacterial cell membrane, followed by the release of intracellular compounds. The mechanism of antibacterial action of steroid compounds is to damage the bacterial cell membrane (Manik et al., 2014).
Information
A = Ethanol extract
B = Ethyl acetate extract
C = Extract n-hexane

**Figure 3. Determination of** inhibition zone diameter meniran herbal extract against staphylococcus epidermidis

On the basis of observation of the growth of Gram-negative bacteria, *Klebsiella pneumoniae*, it is known that no clear area is formed around the disc of the three extracts of the meniran herb. This indicates that the n-hexane, ethyl acetate, and ethanol extracts of meniran herbs do not have antibacterial activity against *Klebsiella pneumoniae*, as shown in Figure 5.
The results of the DDH test obtained showed that the ethyl acetate and 70% ethanol extracts of meniran herbs had an inhibitory effect on gram-positive bacteria (*Staphylococcus epidermidis*) compared to gram-negative bacteria (*Klebsiella pneumoniae*). The difference in inhibition between Gram positive and Gram negative bacteria can be caused by differences in cell wall structure, binding, and activity of antibacterial compounds (Morse et al., 2013). Gram-positive bacteria have a lot of peptidoglycan, little lipid, and polysaccharides (teichoic acid) in their cell walls. Teichoic acid is a water-soluble polymer. This shows that the cell walls of gram-positive bacteria are polar (Morse et al., 2013).

Gram-negative bacteria with a more complex bacterial wall structure contain a lot of lipids, a little peptidoglycan, and an outer membrane consisting of phospholipids and lipopolysaccharides. The outer membrane serves as a selective defense against compounds that leave or enter the cell (semipermeable membrane). The presence of lipid content makes the bacterial cell membrane non-polar, so it is difficult for polar and semi-polar extracts to penetrate (Morse et al., 2013). The best DDH test in the study was the ethanol extract of the meniran herb against *Staphylococcus epidermidis*, then the Minimum Inhibitory Concentration (MIC) test was performed. The MIC test was carried out by reducing the concentration of the ethanol extract of meniran herbs to 2.5% and 2% and controlling bacteria, which only contained test bacteria and MHA media, as shown in Table 3 and Figure 6.

**Table 3. MIC Determination of Meniran Herb Ethanol Extract against Staphylococcus epidermidis**

<table>
<thead>
<tr>
<th>Sampel Uji</th>
<th>Konsentrasi</th>
<th>KHM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meniran Ethanol Extract</td>
<td>2.5%</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2%</td>
<td>+</td>
</tr>
<tr>
<td>Bacteri Control</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Description (-) = No bacterial growth  
(+ ) = There is bacterial growth
ACKNOWLEDGEMENTS

Thank you to Laboratory of the Center for Veterinary Research (BBalitvet) who has permitted it to be used as a research location.

CONCLUSION

Ethyl acetate and 70% ethanol extracts of meniran herb (Phyllanthus niruri L.) had antibacterial activity only against Staphylococcus epidermidis, and the Minimum Inhibitory Concentration (MIC) of an ethanol extract of meniran herb (Phyllanthus niruri L.) against Staphylococcus epidermidis was at a concentration of 2.5%. In the future, further research can be carried out to determine the chemical content of the extract, which is responsible for the antibacterial activity of the meniran herb.

References


