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Research Article

Antibacterial Effectiveness of Belimbing Wuluh Leaf Extract (*averrhoa bilimbi* l.) and Meniran Herb (*phyllanthus niruri* l.) Against S*taphylococcus Epidermidis*

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ABSTRACT

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Acne is an inflammatory condition of the skin which is commonly called polysebaceous which is caused by the *Staphylococcus epidermidis* bacteria. Meniran herbs and starfruit leaves contain compounds that are used as antibacterials, namely tannins, flavonoids and saponins. The purpose of this study was to determine the effectiveness of the antibacterial combination of star fruit wuluh leaf extract (*Averrhoa bilimbi* L.) and meniran herb (*Phyllanthus niruri* L.) as an antibacterial in inhibiting the growth of the *Staphylococcus epidermidis* bacteria that causes acne. The research was an experimental study by conducting an antibacterial effectiveness test against *Staphylococcus epidermidis* bacteria. The results of this research are that the combination of meniran herb extract and starfruit can inhibit the growth of *Staphylococcus bacteria*. A 1:3 combination of starfruit leaf extract (*Averrhoa bilimbi* L.) and meniran herb (*Phyllanthus niruri* L.) is effective in inhibiting the growth of *Staphylococcus epidermidis* bacteria 8.568 mm (Medium).

1. Introduction

Acne vulgaris or acne is an inflammatory condition of the skin which is usually called polysebaceous which occurs in teenagers and adults characterized by the presence of blackheads. Even though it doesn't have a fatal impact, acne is quite disturbing because it can reduce self-confidence. An example of bacteria that causes acne is *Staphylococcus epidermidis* (Saragih et al., 2016). *S. epidermidis* produces a kind of toxin or toxic substance. These bacteria also produce mucus which makes it easier for the bacteria to stick and this mucus also makes the bacteria more resistant to phagocytosis and certain antibiotics. *S. epidermidis* can generally cause inflammatory diseases such as acne, skin infections (Wulandari, 2017). The mechanism for causing acne is that bacteria damage the stratum corneum and germinative stratum by secreting chemicals that are destroyed by the pore walls. This condition can cause inflammation. Fatty acids and skin oils become blocked and harden. If the pimple is touched, the inflammation will spread until the fatty acid solids and hardened skin will enlarge.

Antibiotics are drugs from microorganisms that are used to treat bacterial infections. Not only kill microorganisms or prevent bacterial growth, but it can also help the body's natural defense system get rid of bacteria. Improper use of antibiotics can cause resistance (Yulia et al., 2020). Antibiotic resistance makes the body immune to the same type of bacterial infection. The ability of the drug's active ingredients to kill bacteria decreases due to overdose (Andiarna et al., 2020) One way to avoid synthetic antibiotic resistance is to use natural antibiotics and herbs (Saleh & Pasanda, 2019) Examples of plants that have antibacterial activity are meniran herb and starfruit leaves .

Meniran herb (*Phyllanthus niruri* L.) is a medicinal plant that has many benefits and has the potential to be used as a raw material for natural medicine. This plant grows wild but its benefits are not widely known by the public, especially its antibacterial effects (Hatur Rahman, 2021). Meniran herb contains many bioactive components including alkaloids, flavonoids, tannins and saponins which function as antibacterials. (Adrianto et al., 2021).

Another plant that is useful as an antibacterial is starfruit (*Averrhoa bilimbi* L.). Wuluh starfruit or also called vegetable starfruit or sour starfruit is usually also used as a cooking spice and herbal medicine (Suryaningsih, 2016). The chemical compounds contained in starfruit leaves include tannins, flavonoids, saponins, glucose sulfur, formic acid, peroxide and triterpenoids. The antibacterial ability of starfruit is due to the presence of flavonoid and tannin compounds (Afifi et al., 2018) .

2. Materials and Methods

Material

The equipment used in this research was a cup, test tube, cotton, falcon, micropipette (Socorex), micropipette tips, tube needle, sprider, tweezers, disc, Bunsen, vortex (Gemmy VM-300), Laminar air flow (Trimas Mitra Nusantara), autoclave (HVE-50 Hirayama), incubator (Memert), weighing scale analytical (Fujitsu), Erlenmeyer, dropper pipette, caliper, and rotary evaporator (Eyela A-1000S).

The materials used in this research were meniran herb extract, wuluh starfruit leaf extract, sterile distilled water, 70% ethanol, DMSO, Wagner, Mayer, Dragendrof, H_2SO_4, $FeCl_3$, HCl, Magnesium, Anhydrous Acetic Acid, Hydrochloric Acid, Chloroform, Nutrient Agar (NA), sterile NaCl, clindamycin, Mc standard solution. Farland, *Staphylococcus epidermidis* bacteria.

Research design

This research design begins with several stages, namely:

1) Simplicia Extraction of Wuluh Starfruit Leaves and Meniran Herbs

Making wuluh starfruit leaf extract with meniran herb using the maceration method with 70% ethanol solvent in a ratio of 1:10. Ethanol 70% is used as a solvent because it has the ability to filter compounds in a wide polarity range from polar to non-polar compounds. (Muthmainnah, 2017). 1.1 kg of starfruit leaves with 11 liters of solvent for 3 times 24 hours. Meanwhile, 2.5 kg of meniran herb with 25 liters of solvent. The maserate that has been obtained is then evaporated using a rotary evaporator until it thickens at a temperature of 50ºC with a speed of between 50-60 rpm.

2) Phytochemical Screening of Condensed Extracts of Starfruit Leaves and Meniran Herbs

Phytochemical screening of thick extracts of starfruit leaves and meniran leaves was carried out at the Phytochemical Laboratory, Binawan University. Phytochemical screening is carried out qualitatively to determine the compounds contained therein. Compound content checks carried out include:

1. Flavonoid Identification

   Identification of flavonoids is carried out by adding concentrated to the extract to be tested. The presence of flavonoids is indicated by a red color change in the sample (Yanti & Vera, 2019).

2. Alkaloid Identification

   Identification of alkaloids was carried out using Mayer's reagents (potassium tetraiodomercurate (II)), Wagner (ions in potassium iodide) and Dragendroff (bismuth nitrate in potassium iodide). Extract that will tested dissolved in a test tube with ammonia-chloroform. Then add 1 mL of 2 N sulfuric acid to the filtrate and shake until 2 layers are formed. The top layer was pipetted and filled in 3 test tubes. 3 drops of Mayer's reagent were added to the first tube, 3 drops of Dragendorff's reagent to the second tube, and 3 drops of Wagner's reagent to the third tube. The presence of alkaloid compounds was indicated by the presence of a white precipitate in the first tube and a brownish red precipitate in the other tube (Yanti & Vera, 2019).

3. Terpenoid/Steroid Identification

   Terpenoids/steroids were identified by dissolving samples in Lieberman-Burchard reagent (anhydrous acetic acid and concentrated sulfuric acid). Samples containing steroid compounds change color to greenish blue and compounds containing triterpenoids change color and form brown or purple rings (Yanti & Vera, 2019).

4. Saponin Identification

   Saponin was identified by dissolving the sample in distilled water, then heating it for 15 minutes and then shaking for 10 seconds. If a stable foam forms for more than 10 minutes and a few drops of 2 N hydrochloric acid are added, the sample is saponin positive. (Yanti & Vera, 2019).

5. Tannins Identification

   Identify tannins by 1 gram The extract was put in 10 mL of hot water, poured into a test tube and boiled for 5 minutes, and 3-4 drops of FeCl3 were added to the filtrate. If it is green-blue (green-black) it means it is positive for containing catechol tannin, whereas if it is blue-black it means it is positive for containing pyrogallol tannin (Muthmainnah, 2017).

6. Glycoside Identification

   Identification of glycosides was carried out by adding concentrated glacial acid FeCl3 and H2SO4 which was indicated by the presence of a purple ring in the test tube which indicated that the sample was positive for containing glycosides.

3) Phytochemical Screening of Condensed Extracts of Starfruit Leaves and Meniran Herbs

Testing of the antibacterial activity of starfruit leaf extract and meniran herb using *Staphylococcus epidermidis bacteria* which causes acne. The testing stage requires bacterial rejuvenation. The bacteria that have been rejuvenated are made into a bacterial suspension which is then grown on the surface of NA media using the streak method. The discs were soaked in 3 different ratios, namely 1:1, 1:2 and 1:3 with an increasing concentration, namely starfruit extract and a fixed concentration, namely Meniran herb extract. The test was carried out in 3 repetitions, the disc that had been soaked in the combination extract was then placed on the surface of the media that had been planted with bacteria and incubated at a temperature of 36-37ºC for 24 hours. The zone of inhibition resulting from each extract was measured using a caliper (Wijayanti & Safitri,2018).

3. Results and Discussions

Simplicia Extraction of Wuluh Starfruit Leaves and Meniran Herbs

Making wuluh starfruit leaf extract with meniran herb using the maceration method with 70% ethanol solvent in a ratio of 1:10. Ethanol 70% is used as a solvent because it has the ability to filter compounds in a wide polarity range from polar to non-polar compounds (Muthmainnah, 2017).

Table 1 Simplicia Extraction Results

Extract	Parameter	Results
Starfruit leaves	Sample Weight	1,180 g
	Extract Weight	147.1 g
	% Yield	14.96 %
Meniran Herb	Sample Weight	2,500 g
	Extract Weight	160.5g
	% Yield	8.02 %

The extraction results in **Table 1** can be seen at a temperature of 40-50ºC to obtain the yield of herbal extracts meniran of 8.02%. According to the Indonesian Herbal Pharmacopoeia II edition, it is known that the yield of meniran herb (*Phyllanthus niruri* L.) is not less than 19.0% and for starfruit extract the yield is 14.96%.

Low yield results can be influenced by temperature, time, solvent and sample size. The smaller the sample particle size, the wider the area between the sample and the solvent so that the solvent can be maximized for extraction (Alegantina et al., 2015).

Phytochemical Screening of Starfruit Leaf Extract and Meniran Herbal Extract

The phytochemical screening results tested for each extract were flavonoids, alkaloids, saponins, tannins, steroids and glycosides. The results of the phytochemical extract screening can be seen in **Table 2.** Examination of the meniran herb extract showed negative for steroids and glycosides and for starfruit showed negative results for alkaloids, steroids and glycosides. Undetected alkaloids can occur due to high temperatures and differences in polarity between the alkaloid compound and the solvent (Putri & Lubis, 2020). Simple extraction cannot destroy the cell wall and cannot separate the alkaloids from the cell walls, because the alkaloids are in a bound form that cannot be released by simple extraction. The steroid results showed negative because the solvent used in the extraction process was a polar solvent. Because steroids are non-polar compounds, the compounds are not completely extracted in this solvent (Ergina et al., 2014).

Table 2 Results of Extract Phytochemical Screening

Compound	Reagent	Results	
		Meniran Herbal Extract	Starfruit Leaf Extract
Flavonoids	Ethanol 70% + Mg + concentrated HCl	(+)	(+)
Alkaloids	Mayer	(+)	(-)
	Wagner	(+)	(-)
	Dragendorff	(+)	(-)
Saponins	Aquadest + HCL 2N	(+)	(+)
Tannin	$FeCl_3$	(+)	(+)
Steroids	Lieberman-Burchard	(-)	(-)
Glycosides	Glacial acetic acid, $FeCl_3$ and H_2SO_4	(-)	(-)

Antibacterial Activity Test of Combination of Starfruit and Meniran Leaf Extracts

Based on the test results, the combination of starfruit leaf extract and meniran extract in **Table 3** can inhibit bacterial growth. This can be shown by the presence of a clear zone produced in the media where *Staphylococcus epidermidis bacteria are grown* . Clindamycin used as a positive control can inhibit bacterial growth with an average of 12.86 mm. The combination of starfruit leaf extract and meniran herb showed positive results where each concentration resulted in an increase in the diameter of the inhibition zone. The combination of extract ratio 1:1 produces an average diameter of 4.538 mm, for a ratio of 1:2 it produces an average inhibition zone of 8.251 mm and for a concentration of 1:3 it produces an average inhibition zone of 8.568 mm for *Staphylococcus epidermidis bacteria* . Clindamycin was chosen because it is often used in oral therapy to cure acne. DMSO solvent is a type of solvent that is capable of dissolving all types of compounds and was used for negative control in this study.

Table 3 Combination Antibacterial Test Results

Extract	Concentration	Inhibition Zone Diameter (mm)			Average (mm)
		1	2	3	
Combination	1:1	4.32	5.58	3.805	4.538
	1:2	8.375	8.9	7.48	8.251
	1:3	8.435	9.18	8.09	8.568
Positive Control	2 ppm	12.14	13.9	12.56	12.86
Negative Control	67%	-	-	-	-

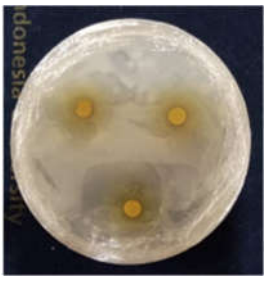

Figure 1. Combination Antibacterial Test

The bacterial inhibitory activity of starfruit leaf extract and meniran herb is influenced by the presence of compounds contained in the extract such as alkaloids, flavonoids and tannins. Flavonoids can inhibit nucleic acid synthesis, inhibit antibacterial metabolism and interfere with bacterial cell wall synthesis. Alkaloids change the composition of bacterial cell walls and bacterial DNA. Tannins denature proteins and destroy genetic material in bacterial cells (Dewangga & Qurrohman, 2019).

4. Conclusions

The conclusions of this research are the combination of starfruit leaf extract (*Averrhoa bilimbi* L.) and meniran herb (*Phyllanthus niruri* L.) is effective in inhibiting bacterial growth *Staphylococcus epidermidis*. The most effective combination of starfruit leaf extract and meniran herb at a concentration of 1:3 resulted in an inhibitory zone diameter of 8.568 mm.

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