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ANTIBACTERIAL POTENTIAL OF GREEN MENIRAN (*Phyllanthus niruri* L.) LEAVES EXTRACT AGAINST *STAPHYLOCOCCUS AUREUS* ATCC25923 : AN IN VITRO STUDY

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ABSTRAK

Meniran hijau (*Phyllanthus niruri* L.) mengandung metabolit sekunder yang bermanfaat sebagai antibakteri. Tanaman ini bisa menjadi kandidat solusi terhadap fenomena resistensi antibiotik, salah satunya bakteri *Staphylococcus aureus*. *S. aureus* merupakan bakteri gram-positif yang menyebabkan infeksi bernanah dan umumnya menyerang organ pernapasan, kulit, dan saluran pencernaan. Tujuan dari penelitian ini adalah untuk mengetahui efektivitas ekstrak tumbuhan ini sebagai antibakteri terhadap *S. aureus*, konsentrasi ekstrak yang paling efektif dalam menghambat bakteri tersebut dan juga efisiensi ekstrak yang dihasilkan. Metode yang digunakan adalah eksperimen dengan pendekatan kuantitatif menggunakan Rancangan Acak Lengkap (RAL) non-faktorial yang terdiri dari 7 perlakuan dengan masing-masing 3 ulangan. Analisis data dilakukan dengan menggunakan uji non-parametrik Kruskal Wallis dengan tingkat signifikansi 0,05 dan uji lanjutan Dunnet. Hasil penelitian menunjukkan terdapat efisiensi ekstraksi sebesar 22,5% pada penelitian ini dan mempunyai efek antibakteri terhadap S. aureus secara in vitro. Dalam hal ini konsentrasi 80% ditetapkan sebagai konsentrasi yang paling efektif dalam menghambat pertumbuhan bakteri *S. aureus* dengan rata-rata diameter zona hambat sebesar 24,27 mm.

Kata Kunci : Antibakteri, Daun Meniran Hijau, *S. aureus*

ABSTRACT

Green meniran (*Phyllanthus niruri* L.) contains secondary metabolites which are useful as antibacterials. This plant could be a candidate for a solution to the phenomenon of antibiotic resistance, one of which is the *Staphylococcus aureus* bacteria. *S. aureus* is a Gram-positive bacteria that causes purulent infections and commonly affects the respiratory organs, skin and digestive tract. The purpose of

this study was to determine the effectiveness the extract of this plant as an antibacterial against *S. aureus*, the most effective concentration of the extract in inhibiting this bacteria and also the efficiency of the extract produced. The method used was experimental with a quantitative approach using a non-factorial Completely Randomized Design (CRD) consisting of 7 treatments with 3 replications each. Data analysis was performed using the Kruskal Wallis non-parametric test with a significance level of 0.05 and the Dunnet follow-up test. The results of this study indicated there was 22.5% extraction efficiency in this study and had an antibacterial effect on *S. aureus* in vitro. In this case, a concentration of 80% was determined as the most effective concentration in inhibiting the growth of *S. aureus* bacteria with an average diameter of the inhibition zone of 24.27 mm.

Keywords: Antibacterial, Green Meniran Leaves, *S. aureus*

INTRODUCTION

Staphylococcus aureus is a Gram-positive bacteria that attacks respiratory organs, skin and digestive tract accompanied by purulent abscesses [18]. This infectious disease is commonly treated with therapy in the form of the use of antibiotics such as penicillin, cloxacillin, dicloxacillin and erythromycin. However, inappropriate use of antibiotics, adaptation, evolution and mutation of bacteria lead to cases of resistance. This complicates healing, many medical procedures fail and are very risky and increase the cost of treatment [5;15], so until now there is no effective antibiotic against *S. aureus.*

There is a need for drugs that have strong power in tackling infections but have low side effects. The development of alternative antibiotics from herbal plants is one solution to this phenomenon [6]. One of the plants that is currently of interest to researchers is plants of the genus Phyllanthus [8]. One of the plant members of this genus is green meniran (*Phyllanthus niruri* L.).

Green meniran can be used as an alternative natural ingredient that has potential as an antibacterial. This plant grows wild in various habitats, its adaptability causes green meniran to be easily found almost throughout Indonesia. So that if produced as a natural antibiotic product, it will be of economic value.

Research has been conducted to test the phytochemical content of this plant, including [14;19] and [11] which concluded that meniran herb extract contains alkaloid, flavonoid, saponin, steroid, tannin, coumarin and

phenolic compounds that are believed to be able to inhibit bacterial activity. The part of the meniran plant that contains the most of these compounds is the leaves. However, scientific research proving that ethanol extract of green meniran leaves has the ability as an antibacterial against the growth of *S. aureus* is still not much done, so scientific references on this topic are still considered small.

Based on the description above, it is necessary to test the effectiveness of green meniran leaf extract (*Phyllanthus niruri* L.) as an antibacterial against *S. aureus* using in vitro method.

RESEARCH METHOD

Approach and Type of Research

This study used a quantitative approach with the type of experimental research and implemented a non-factorial Completely Randomized Design (CRD) consisting of 7 treatments and 3 replications. Antibacterial activity test was carried out by well diffusion method.

Location and Research Schedule

This research was conducted at the Science Fundament Laboratory Science Lab, Baitussalam District, Aceh Besar District from March to May 2023.

Procedure

1) Sterilization

Equipment sterilization was carried out by dry sterilization using an oven with a temperature of 160°C for 2 hours, while materials such as NB and MHA media were sterilized using an autoclave with a temperature of 121°C and a pressure of 1 atm for 15 minutes. During the research process, sterilization was also carried out with a Bunsen flame and 70% alcohol.

2) Preparation of Meniran Leaf

Meniran plants that have been taken are separated between the leaves and other parts that are not needed, then sorting is done, the leaves taken are dark green mature leaves that are healthy, not rotten and not damaged as much as 500 g. Then it is washed using clean running water and left for 3 days at room temperature until completely dry. Then the meniran leaves are mashed with a blender to form dry simplicia powder.

The simplicia powder was filtered using a 20 mesh sieve up to 300 g and then soaked using 96% ethanol solvent up to 1000 mL in a macerator container and stirring was carried out. The simplicia soaked was stored in a dark place for 5 days and stirred once a day for 10 minutes. Then the simplicia was filtered using Whatman No.1 filter paper in a beaker glass. The filtering results were evaporated using a Rotary Evaporator with a speed of 100 rpm and a temperature of 50o C for 3 hours to form a thick pure extract of 45 g.

3) Extract Dilution

Extract dilution is done by making a stock solution and followed by dilution according to the desired concentration. The stock solution is green meniran leaf extract with the highest concentration of 80% which will be diluted to obtain extracts of 40%, 20%, 10% and 5%. The required stock solution volume is 10 mL.

Thus, a stock solution with a concentration of 80% is obtained by adding distilled water to 8 g of concentrated extract up to 10 mL. After obtaining a stock solution of 10 mL, then dilution is made for another concentration of 2 mL using the dilution formula.

4) Nutrient Broth (NB) Media

0.26 g of NB powder was dissolved in 20 mL of distilled water and homogenized using a hot plate stirrer at a speed of 500 rpm until homogeneous. Then the media was sterilized using an autoclave at 121°C with a pressure of 1 atm for 15 minutes. After being sterilized, the media is cooled and then poured into a test tube.

5) Mueller Hinton Agar (MHA) Media

3.8 g of MHA powder was dissolved in 100 mL of distilled water and homogenized while heated using a hot plate stirrer at a temperature of 80o C and a speed of 500 rpm until homogeneous. Then the media was sterilized using an autoclave at 121°C with a pressure of 1 atm for 15 minutes. After being sterilized, the media is placed in a water bath shaker to keep the temperature stable before being poured into a petri dish.

6) Bacterial Rejuvenation

Test microorganisms from pure cultures were rejuvenated by incubating on NB media for 24 hours at 37ºC. Then the bacteria were taken at 10 hours and suspended in 0.85% physiological saline to adjust for turbidity with McFarland standard 0.5 which is equivalent to 1.5 x 108 CFU/mL. When appropriate, 1 mL of the bacterial suspension is poured into each petri dish and then the MHA media solution is added and homogenized, then wait until the media hardens.

7) Antibacterial Effectiveness Test

Bacteria that have been standardized for turbidity are taken as much as 1 mL and dissolved in MHA media in three petri dishes and then left to harden. Then, 7 holes were made using a sterile Cork Borer with a diameter of 6 mm in each petri dish and then filled with 50µL of solution into the hole, then incubated for 24 hours at 37°C. After incubation, the media for the wells was removed and observed. The clear zone formed around the wellbore was measured using a vernier caliper for each treatment.

8) Inhibition Zone Measurement

Inhibition zone measurements were carried out using a caliper with an accuracy of 0.05 mm. The inhibition zone can be marked by the clear area around the wellbore. The diameter of the inhibition zone measured includes the vertical inhibition zone and the horizontal inhibition zone. The diameter of the inhibition zone for each treatment is the average of the two inhibition zones.

The results of these measurements were adjusted to the criteria for the strength of antibacterial power by David and Stout in 1971.

9) Data Analyze

The data does not meet the assumptions of the normality test, then it is continued with the Non-parametric Kruskall Wallis analysis with the SPSS version 29 program, if the significance value (Asymp.Sig) <0.05 then it is continued with the Dunnet Non-parametric test.

RESULTS AND DISCUSSION

Results

Research results show the extraction efficiency is 22.5%. The results also showed that overall there was an inhibition zone formation around the wells which proved that there was antibacterial activity against *S. aureus* as shown in Table 1.

Table I. Diameter of the *S. aureus* bacterial inhibition zone

	Mean±SD (mm)	Inhibited Response
P0	0	No Response (<5)
P1	12.28 ± 0.27	Strong (10-20)
P2	13.01 ± 0.69	Strong (10-20)
P3	20.02 ± 1.42	Very Strong (>20)
P4	21.58 ± 0.74	Very Strong (>20)
P5	24.27 ± 1.28	Very Strong (>20)

Based on Table 1, the lowest concentration of green meniran leaf extract, namely 5%, was able to form an inhibition zone with a strong inhibitory response category. Even though all extract concentrations still formed a smaller inhibition zone compared to the positive control, starting from a concentration of 20% and so on have shown an inhibitory response in the same category as the positive control, which was very strong. Furthermore, the data from the test results were analyzed using SPSS with the Kruskall Wallis non-parametric test to draw conclusions about this study.

The results of the Kruskall Wallis statistical test analysis showed a significance value (Asymp. Sig) of 0.006 <0.05, which means that there was a significant difference between treatments in inhibiting the growth of *S. aureus*.

Data on the results of measurements of the inhibition diameter of all treatments and replicates were analyzed using the Dunnets Test as a follow-up test to determine which treatment had a significant effect as an antibacterial against *S. aureus.* Based on the results of the statistical analysis, it showed that P5 (80%) was significantly different from P1 (5%) and P2 (10%). The concentration of green meniran leaf extract had the most significant effect on inhibiting the growth of *S. aureus*.

Discussion

The extraction efficiency of green meniran leaves in this study was 22.5%. Based on this, the extraction in this study met the requirements according to the 2017 Indonesian

Herbal Pharmacopoeia which stated that the yield of the extract should not be less than 19%. So that it can be seen that the extraction results in this study are efficient so that they attract higher and maximum active compounds and have a fairly good dissolving power.

The yield in this study was higher compared to other studies including those by [16] obtaining the highest yield of 18.4%, while [3] obtained 8.04% and not much different from [10] namely 23.25%. The value is inversely proportional to the size of the extracted material, so the higher the yield, the smaller the particle size of the green meniran leaf extract. The small particle size allows for more contact between the material and the solvent, this results in more active compound content being obtained thereby increasing extraction efficiency.

The solvent used in this research is a polar solvent, namely 96% ethanol. The choice of solvent was based on the secondary metabolite compounds contained in green meniran leaf extract which are also polar. This affects the effectiveness of extraction where the compound will dissolve well in a solvent with the same properties. Apart from that, the length of maceration time also affects the yield value, a time that is too short causes the secondary metabolite compounds to not be completely dissolved, but a time that is too long will also cause damage to the extracted compounds.

Based on the results of the research that has been done, it also shows that green meniran leaf extract has a high antibacterial effect on *S. aureus*. This is thought to be due to the fact that green meniran leaves contain secondary metabolites, namely alkaloids, tannins, saponins, and flavonoids [1;14]. Various studies have proven that this compound plays an active role as an antibacterial against *S. aureus*.

Overall these compounds are thought to inhibit the growth of *S. aureus* by disrupting cell membranes but with different mechanisms. Alkaloid compounds act as antibacterial by destroying the peptidoglycan content in the bacterial membrane [4]. *S. aureus* is a gram-positive bacterium where 90% of its cell wall is composed of a thick peptidoglycan layer, the structure of the cell wall causes this compound to

more easily damage the cell wall which causes lysis of the bacteria. Likewise with flavonoid compounds that disrupt bacterial cell membranes by forming complex compounds so that intracellular fluids come out of cells, these compounds also inhibit the process of macromolecular biosynthesis [12]. Meanwhile, saponins and tannins reduce the surface tension of cell membranes, thereby disrupting membrane permeability and causing cytoplasmic fluid to leave the cell [13].

Another factor that is thought to cause compounds to easily enter cells is the cell membrane of Gram-positive bacteria which is composed of teichoic acid which is polar, as well as the bioactive compounds in the extract of green meniran leaves which are also polar, this condition causes the compounds to penetrate the cell wall more easily. Furthermore, the phenolic compounds in green meniran leaf extract, namely quercetin, nirurin, lignans, etc., can break bonds in the peptidoglycan layer of the bacterial membrane [7]. After successfully penetrating the cell wall, phenolic compounds will cause damage to hydrophobic bonds (such as proteins and phospholipids) as well as components that bind hydrophobically thereby increasing the permeability of the cell membrane, this will result in leakage of cell nutrients which will further interfere with the activity and biosynthesis of enzymes needed in reactions metabolism [9]. This is thought to cause growth retardation and even cause death in *S. aureus*.

The results of this study also showed that Tetracycline as a positive control formed the highest inhibition zone compared to all treatments. This is because these antibiotics have a broad spectrum which is able to inhibit the growth of various types of bacteria. This is because the antibiotic interferes with protein synthesis in the 30S ribosomal subunit and inhibits the attachment of aminoacyl-tRNA. As a result, the introduction of newly formed amino acids in the peptide chain is inhibited so that the protein synthesis process will be disrupted. Antibacterial with this mechanism has stronger antibacterial activity compared to other green meniran leaf extract treatments with mechanisms that tend to disrupt cell walls and cell membrane permeability.

Based on data from statistical analysis using Dunnet's advanced test, it was determined that 80% was the best concentration as an antibacterial against *S. aureus*. This is caused by the significant difference in the diameter of the inhibition zone produced by this treatment compared to other treatments. This is also influenced by the inhibitory diameter formed by this concentration, which is the highest compared to other green meniran leaf extract treatments.

Based on the overall research results found, the higher the extract concentration, the wider the inhibition zone formed. This is related to the level of extract contained in each concentration, the higher the concentration, the higher the level of green meniran leaf extract in the treatment so that the higher the active substance that diffuses into bacterial cells and inhibits their growth. This is in accordance with [2] who stated that an increase in extract concentration indicates an increase in the diameter of the inhibition zone formed, this is because there are more bioactive compounds in the extract which can interfere with bacterial growth. So based on this, it can be determined that a concentration of 80% is the best concentration of ethanol extract of green meniran leaves as an antibacterial against *S. aureus*.

CONCLUSION

This study indicated there was 22.5% extraction efficiency and had an antibacterial effect on *S. aureus* in vitro. concentration of 80% was determined as the most effective concentration in inhibiting the growth of *S. aureus* bacteria with an average diameter of the inhibition zone of 24.27 mm.

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