2nd UPhIC
UNISSULA PHARMACY INTERNATIONAL CONFERENCE

WORLD CLASS ISLAMIC CYBER UNIVERSITY
UNISSULA
SULTAN AGUNG ISLAMIC UNIVERSITY

Article

Antibacterial Activity of 96% Ethanol Extract of Green Eggplant (*Solanum Melongena* L.) Fruit Against *Staphylococcus Aureus* Atcc-29213 Using Well Diffusion Method

Rose Malinda Andamari Wahyu Utami [1]*, Tri Nur Azizah[1], Ferina Damayanti[1], Hogi Noni Saputro[1], Nurul Syazwani [1], and Lucky Restyanti Wahyu Utami[2]

[1] Sekolah Tinggi Ilmu Kesehatan Kendal; rosemalinda1995@gmail.com; trinur.azizah4@gmail.com; apt.ferinadamayanti@gmail.com; hoginonisaputro@gmail.com; syazwanin27@gmail.com
[2] Universitas Widya Husada Semarang; lucky.restyanti@uwhs.ac.id
* Correspondence: rosemalinda1995@gmail.com; Tel.: 085742799122

Abstract: This study aims to evaluate the antibacterial activity of 96% ethanol extract of green gelatik eggplant (*Solanum melongena* L.) fruit against Staphylococcus aureus ATCC-29213 bacteria using the well method. The research process includes the preparation of simplicia, ethanol extraction through maceration, qualitative tests with phytochemical screening to identify active compounds, and antibacterial tests using the well method using Staphylococcus aureus ATCC-29213 bacteria as the test material and 96% ethanol extract of green gelatik eggplant (*Solanum melongena* L.) fruit made in concentrations of 5%, 10% and 15% for comparison. The results showed that the extract contains flavonoids, alkaloids, tannins, and steroids that have the potential as antibacterials. Antibacterial activity test at 5% concentration obtained an average inhibition zone diameter of 0.0033 cm, a concentration of 10% obtained an average inhibition zone diameter of 1.17 cm, and a concentration of 15% obtained an average inhibition zone diameter of 1.58 cm. The data shows an increase in the diameter of the inhibition zone as the concentration increases, although it is still lower than the positive control (amoxicillin). ANOVA analysis showed a significant difference between treatment groups ($p = 0.000$), indicating a positive relationship between extract concentration and inhibitory power against S. aureus.

Keywords: Green Eggplant, Staphylococcus aureus, Well Diffusion Method

1. Introduction

Bacterial infections remain a major health problem worldwide, particularly those caused by Staphylococcus aureus. This bacterium is a gram-positive pathogen frequently found on the skin, respiratory tract, and mucosa of humans, and can cause various diseases, including skin infections, pneumonia, and sepsis [1]. The increasing resistance of S. aureus to antibiotics, such as methicillin (methicillin-resistant Staphylococcus aureus or MRSA), is a serious health concern because it limits therapeutic options [2].

To address this issue, the search for new antibacterial sources from natural ingredients presents a promising alternative. One plant with potential use is the green gelatik eggplant (Solanum melongena L.), a member of the Solanaceae family. In addition to being consumed as a vegetable, various parts of this plant are reported to contain secondary metabolites such as flavonoids, alkaloids, tannins, and saponins, which have antibacterial potential[3].

Several previous studies have shown that ethanol extract of eggplant contains active compounds that can inhibit the growth of gram-positive bacteria, including S. aureus.

However, specific research on the antibacterial activity of 96% ethanol extract of green gelatik eggplant against Staphylococcus aureus ATCC-29213 using the well diffusion method is still limited. The well diffusion method is a simple and effective antibacterial test method for evaluating the potential of antimicrobial substances against the growth of microorganisms [4].

2. Materials and Methods

The tools used in this research were analytical balance (Ohaus), 40 mesh sieve (Sieve), laboratory glassware (Pyrex), spatula, rotary evaporator (Heidolph), oven (Memmert), evaporator dish, blender, water bath. The sample used was green gelatik eggplant (Solanum melongena L) from Bawang Village, Bawang District, Batang Regency. The materials used were pro analysis materials, namely: 96% ethanol, ethyl acetate, N-hexane, concentrated HCL, Mayer and Dragendorf reagents, chloroform, anhydrous acetic acid, Mg powder, 1% FeCl3, 1% HCl, hot water, concentrated H2SO4 solution, distilled water, p.a. methanol, Staphylococcus aureus bacteria.

a) Sampling and Preparation of Simple Drugs

Eggplants were harvested whole. The green long-tailed eggplants were thinly sliced and then sun-dried, covered with a black cloth, until the eggplants were completely dry. The dried eggplants were ground using a blender to obtain a fine powder, which was then immediately sieved through a 40-mesh sieve until the powder was completely fine.

b) Preparation of 96% Ethanol Extract

Prepare 250 grams of each simple drug powder and macerate it in 1.5 L of 96% ethanol for 3 days, stirring for 1 hour each day. The macerated filtrate was then evaporated using a Bunsen burner and then in a water bath to obtain a thick extract. The resulting thick extract was then weighed to calculate its yield.

c) Phytochemical Screening

1) Flavonoid Test

0.1 gram of green long-tailed eggplant extract was weighed, dissolved in 2-3 mL of methanol, and heated in a water bath. Mg powder is added, then 2 mL of concentrated HCl is added. A positive result will be a color change to red, yellow, or orange [5].

2) Alkaloid Test

0.5 grams of green Java sparrow eggplant powder was added to 1 mL of 2N HCl and 9 mL of distilled water, heated in a water bath for 2 minutes, cooled, and filtered. The resulting filtrate was then used for the alkaloid test. Two test tubes were taken, and 0.5 mL of the filtrate was added to each tube. Two drops of Mayer's and Dragendorf's reagents were added to each tube. A positive result indicates the presence of alkaloids if the Dragendorf's reagent forms an orange to reddish-brown precipitate, and the Mayer's reagent forms a white precipitate [5].

3) Saponin Test

0.5 grams of green Java sparrow eggplant powder was placed in a test tube, 10 mL of hot water was added, cooled, and then shaken vigorously for 10 seconds. If a large amount of foam forms for at least 10 minutes, reaching a height of 1 to 10 cm, and persists when 2 drops of 2N HCl are added, the sample contains saponins [5].

4) Tannin Test

One gram of green jay eggplant powder is boiled for 3 minutes in 10 mL of distilled water, then cooled and filtered. The resulting filtrate is diluted until almost colorless, then 1-2 drops of FeCl3 reagent are added. A color change to blue-black or green-black indicates tannins [5].

5) Terpenoid Test

One gram of the herb is macerated with 20 mL of n-hexane for 2 hours, filtered, the filtrate is evaporated, and the remainder is added with acetic anhydride and

concentrated sulfuric acid reagents. If the test produces a purple or red color that changes to blue-violet or blue-green, it indicates a positive result in the presence of free steroids or terpenoids [5].

d) Sterilization of Equipment and Materials

Petri dishes, Erlenmeyer flasks, funnels, and graduated pipettes are washed first, then placed in an autoclave along with cotton swabs wrapped in aluminum foil to be sterilized at 121°C for 15 minutes.

e) Media Preparation

The seeding medium is prepared by weighing 7 grams of Nutrient Agar (NA), dissolving it in 250 ml of distilled water, then adding 5 grams of peptone and 5 grams of NaCl using an Erlenmeyer flask. The medium is then homogenized on a hot plate until it boils. The homogenized medium is sterilized in an autoclave at 121°C for 15 minutes, then cooled to approximately 45-50°C.

f) Bacterial Inoculation on Slant Agar

The test bacteria were collected with a sterile loop needle and then streaked onto the agar slant. The plates were then incubated at 37°C for 24 hours. The same treatment was applied to each type of test bacteria.

g) Preparation of Test Bacterial Suspension

The test bacteria, inoculated with a sterile wire, were then suspended in a tube containing 2 ml of 0.9% NaCl solution until the turbidity reached the standard turbidity of 0.5 McFarland solution.

h) Preparation of Test Media

The base layer was prepared by pouring 20 ml of NA from the base medium into six Petri dishes and allowing it to solidify. After solidification, a steel backing was placed on the surface of the base layer. The bacterial suspension was then mixed with the NA seeding medium. Afterward, 30 ml of the suspension and seeding medium mixture was poured into each Petri dish, placing the backing as a second layer. The backing was then aseptically removed from the Petri dishes, creating wells for use in the antibacterial test.

i) Antibacterial Activity Test

Amoxicillin was added to the positive control wells, and 50 µl of ethanol extract of green Java sparrow eggplant was added to the treatment group at various concentrations (5%, 10%, and 15%). The Petri dishes were incubated for 24 hours. After 24 hours, the diameter of the inhibition zone was observed and measured using a graduated ruler. The diameter of the inhibition zone was measured from the edge (breakpoint) to the opposite edge, passing through the center of the well. If no inhibition zone was found around the well, the inhibition zone diameter was declared 0 mm. The diameter of the inhibition zone was then categorized for its antibacterial activity based on the classification[6].

3. Results

3.1. Phytochemical Screening

Table 1. Phytochemical Screening Results of Green Gelatik Eggplant Extract.

Class of compounds	Reagent	Positive results	Picture	explanation
Flavonoid	Dragendorf reagent	Red, yellow or orange		+ (yellow)

Alkaloid	Mayer's reagent and Dragendorf's reagent	orange to reddish brown sediment is formed		+ (orange to brown)
Tannin	FeCl3 reagent	A dark blue or blackish green color is formed		+ (blackish green)
Saponin	HCl 2N	1-10 cm foam is formed		- (no foam formed)
Steroid	anhydrous acetic acid and concentrated sulfuric acid reagents.	Terpenoid: reddish brown Steroid : green-blue		+ (reddish brown)

Table 2. Antibacterial Activity Test

Diameter	Concentration 5%	Concentration 10%	Concentration 15%	Positive Control
1	0	1,03	1,35	2,09
2	0	1,19	1,36	2,30
3	0,01	1,29	2,03	2,45
Average (cm)	0,0033	1,17	1,58	2,28

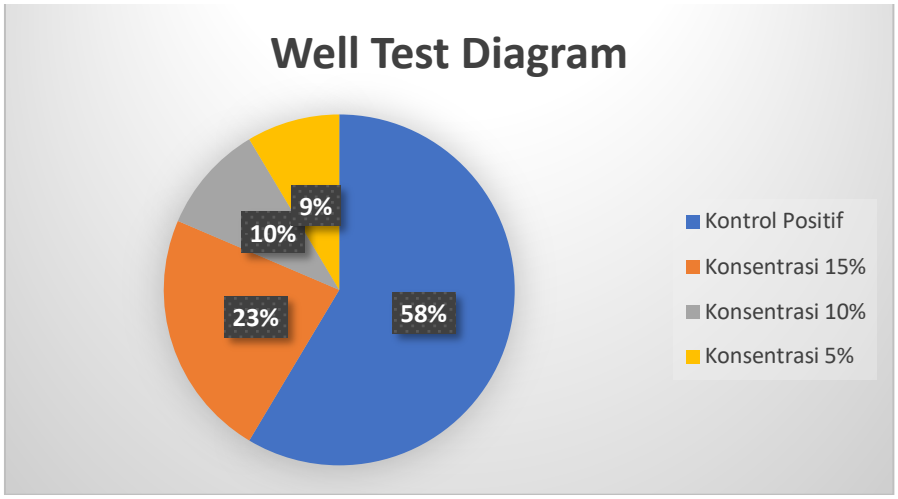

Figure 1. This is a figure. Diagram Antibacterial Activity Test

4. Discussion

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

4.1. Extract Yield Results

The yield of 96% ethanol extract from green gelatik eggplant fruit of 12.5% indicates that from 10 kg of fresh fruit, approximately 0.8 kg of dry powder is produced. This yield value is quite good, considering the high water content of fresh fruits such as eggplant. High yields generally indicate that the extraction process is effective and the secondary metabolites are successfully extracted by the solvent. Ethanol 96% is known to be a polar and semi-polar solvent capable of extracting various active compounds such as flavonoids, alkaloids, tannins, and steroids[7].

4.2. Phytochemical Screening Results

Phytochemical screening results showed that the extract contained flavonoids, alkaloids, tannins, and steroids, while saponins were not detected. Flavonoids work by disrupting cell membrane integrity and inhibiting bacterial enzymes [8]. Alkaloids can intercalate DNA and inhibit bacterial topoisomerases [9]. Tannins can denature cell wall proteins and essential bacterial enzymes [10]. Steroids contribute to reducing bacterial cell membrane permeability.

4.3. Antibacterial Activity Test Results

Antibacterial testing using the well diffusion method showed that the 5% extract concentration showed almost no inhibitory activity (0.003 cm). At a concentration of 10%, the inhibition zone increased (1.17 cm), and at a concentration of 15%, the activity was greater (1.58 cm). However, this inhibition was still lower than the positive control (amoxicillin), which reached 2.28 cm. This indicates that green gelatin eggplant fruit extract has concentration-dependent antibacterial activity but is not as effective as standard antibiotics [11].The well method also supports the even diffusion of antibacterial compounds from the extract in the medium, allowing for more accurate evaluation of inhibition zones [4].

4.4. Data Analysis

In the data analysis test, the Shapiro-Wilk test results showed that the data at a concentration of 10%, a concentration of 15 and the positive control were normally distributed ($p > 0.05$), but the data at a concentration of 5% were not normal ($p < 0.05$). The Levene's Test of homogeneity of variance showed a significance value of $0.018 > 0.05$, meaning that the variance between groups was homogeneous.ANOVA statistical analysis ($p = 0.000$) demonstrated a significant difference between treatment groups. Thus, the higher the extract concentration, the stronger the inhibitory effect against Staphylococcus aureus. These results align with other studies reporting that medicinal plant extracts exhibit antibacterial activity that increases with concentration [12].

5. Conclusions

A 96% ethanol extract of green gelatik eggplant contains antibacterial compounds such as flavonoids, alkaloids, tannins, and steroids that synergistically exert activity against Staphylococcus aureus ATCC-29213. Although its inhibitory power is not yet comparable to standard antibiotics, this extract shows potential as a natural antibacterial agent. Its effectiveness increases with concentration, which was statistically confirmed through ANOVA.

References

1. Cheung GYC, Bae JS, Otto M. Pathogenicity and virulence of Staphylococcus aureus ABSTRACT. *Virulence*. 2021;12(1):547-569. doi:10.1080/21505594.2021.1878688
2. Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler VG. Staphylococcus aureus Infections : Epidemiology , Pathophysiology , Clinical Manifestations , and Management. 2015;28(3):603-661. doi:10.1128/CMR.00134-14
3. Daud, M., Yuliani, F. S., & Wahyuni W. Aktivitas antibakteri ekstrak etanol buah terong ungu (Solanum melongena L.) terhadap bakteri Staphylococcus aureus dan Escherichia coli. *J Farm Galen*. 2020;6(1):78–84. doi:https://doi.org/10.22487/j24428744.2020.v6.i1.13481
4. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity : A review $. *J Pharm Anal*. 2016;6(2):71-79. doi:10.1016/j.jpha.2015.11.005
5. Qodri UL. Analisis Fitokimia Dan Penentuan Kadar Fenolik Total Pada Ekstrak Etanol Tebu Merah Dan Tebu Hijau (Saccharum Officinarum L .) Phytochemical Analysis and Determination of Total Phenolic Content in Ethanol Extract of Red Sugar Cane and Green Sugar Cane (Saccharum Officinarum L). 2023;4(2):91-102.
6. Assay A. Disc Plate Method of Microbiological Antibiotic Assay. 1971;22(4):659-665.
7. Abdallah, E. M., Khalid, A. S., & Ibrahim N. Antibacterial activity of extracts from Solanum melongena fruits. *J Med Plants Stud*. 2016;4(3):196-199.
8. Othman L, Sleiman A, Abdel-massih RM. Antimicrobial Activity of Polyphenols and Alkaloids in Middle Eastern Plants. 2019;10(May). doi:10.3389/fmicb.2019.00911
9. Mekonnen, T. W. et al. Plant-derived alkaloids as antibacterial agents. Frontiers in Pharmacology. *Front Pharmacol*. 2020;11. doi:https://doi.org/10.3389/fphar.2020.583868
10. Zhang, L. et al. (2019). Tannins and their impact on antimicrobial activity. *Compr Rev Food Sci Food Safet*. 2019;18(3):823-838. doi:https://doi.org/10.1111/1541-4337.12438
11. Moglad EH. Antimicrobial potential of medicinal plants against multidrug-resistant bacteria. *J King Saud Univ – Sci*. 2021;33(2). doi:https://doi.org/10.1016/j.jksus.2021.101240
12. Sulianti, I., Fitriana, R., & Nurhayati I. Aktivitas antibakteri ekstrak tumbuhan obat tradisional Indonesia terhadap Staphylococcus aureus. *J Biol Trop*. 2022;22(3):453-462. doi:https://doi.org/10.29303/jbt.v22i3.3959