

ANTIBACTERIAL POTENTIAL OF EGGPLANT FRUIT (*Solanum melongena* L) ETHANOL EXTRACT AGAINST *Propionibacterium acnes* BACTERIAL GROWTH

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ABSTRACT

Acne is one of the skin problems that, without our realizing it, can reduce self-confidence, despair, and even depression among teenagers and young adults. The incidence of bacterial infections is increasing with the increasing cases of resistance of bacteria, such as *Propionibacterium acnes*, to the side effects of anti-acne drugs. Indonesia has many plants that are beneficial to humans and have properties for treating and improving health, one of which is the eggplant. The purpose of this study is to test whether eggplant has antibacterial activity against *P. acnes*. The eggplant was extracted using a maceration extraction method. For antibacterial activity, well diffusion agar was used with extract concentrations of 60%, 70%, 80%, and 90%. The inhibition zones of the ethanol extract of *S. melongena* at 60%, 70%, and 80% concentrations were 13,76 mm, 18,63 mm, and 20,79 mm, respectively, with the highest inhibition zone at 90% concentration at 20,64 mm. The ethanolic extract of *S. melongena* has a strong inhibitory effect on *P. acnes*.

Keywords: antibacterial activity, *Solanum melongena* L, maceration, *Propionibacterium acnes*.

INTRODUCTION

Acne is one of the skin problems that, without our realizing it, can reduce self-confidence, despair, and even depression among teenagers and young adults. Acne is not a dangerous disease, but it can reduce a person's self-confidence (Togatorop *et al.*, 2022). Factors of self-confidence and social activity are very important. Even though the problem of acne is considered mild and can be self-treated, it has physical and emotional effects (Puspitasari, 2021).

Acne cases in Southeast Asia occur around 40-80%. Acne cases in Indonesia generally occur around 80-100%. Acne is often found in women aged 14-17 years and in men aged 16-19 years. Acne is influenced by several factors, such as genetic, endocrine (pituitary androgenic sebotropic), dietary factors, sebaceous gland activity, psychological factors, season or weather, stress, cosmetics, pollution, and bacterial infections (Manarisip, Kepel, and Rompas, 2015).

Bacteria that commonly infect acne are *Propionibacterium acnes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*. *P. acnes* is a Gram-positive bacterium that is capable of destroying polysebaceous follicles by releasing hydrolytic enzymes that produce lipase, hyaluronidase, protease, lecithinase, and neuramidase, which play a role in inflammation. *P. acnes* converts unsaturated fatty acids into saturated fatty acids, which can cause sebum to become solid (Hafsari *et al.*, 2015). The mechanism of acne is *P. acnes* bacteria damage the stratum corneum and startum germinativum by damaging the pore

walls. This condition can cause inflammation, clogging, and the hardening of fatty acids and skin oils (Nirmal and Panichayupakaranant, 2014).

The incidence of bacterial infections is increasingly widespread with the increasing cases of resistance of bacteria such as *P. acnes* as the side effects of anti-acne drugs. Therefore, to reduce the occurrence of side effects, natural alternative ingredients are needed that can overcome the problem of acne, which acts as an antibacterial (Rodiah et al., 2017). Indonesia has many plants that are beneficial to humans and have properties for treating and improving health, one of which is the eggplant (Rimadhani and Rahmadewi, 2015). The development of natural antibacterial research continues to increase. The eggplant contains natural compounds such as vitamins A, B1, B2, C, D, phosphorus, carotenoids, anthocyanins, and alkaloids. Anthocyanins are part of the flavonoids synthesized via the phenylpropanoid pathway, which work as antibacterials (Priska et al., 2018).

Previous studies have been done to test the inhibitory power of the eggplant peel ethanol extract against *S. aureus* and *E. coli* using the disc diffusion method (Purnamasari, Vifta, and Susilo, 2018). In previous studies, the selection of solvents and extraction methods in extracting the eggplant fruit provided inhibition as an antibacterial using the disc diffusion method (Kalimuthu et al., 2017; Salamatullah et al., 2021).

Based on the background described above, there has been no research using the well method in antibacterial testing, so researchers are interested in knowing the effectiveness of the ethanol extract of the eggplant as an anti-acne candidate against *P. acnes* bacteria using the well method.

RESEARCH METHODS

Equipment and Materials

The tools used in this study included autoclaves (American®), ovens (Mettler®), incubators (Mettler®), microwaves (Sharp-R-21D0(S)IN), analytical balances (Ohaus), micropipettes, calipers, L rods, well tools, petri disk, and other glassware.

The materials used in this study were eggplant (*Solanum melongena* L), 96% ethanol (Brataco®), DMSO (Merck, Germany), physiological NaCl, nutrient agar (Oxoid®), *P. acnes* ATCC 11827 bacteria, Dragendorff, Mayer, AlCl₃, FeCl₃, and H₂SO₄.

Research Procedures

1. Sample Preparation

The research sample used was the eggplant that was obtained directly from the eggplant farmers in Tabing Rimbah Village, Mandastana District, Barito Kuala Regency, South Kalimantan. The eggplants that had been picked were then cleaned of foreign objects or other impurities. Eggplant fruit that has been cleaned and then chopped to facilitate the drying process. Eggplant samples were dried using a drying cabinet at 50°C. The dried samples were then powdered using a blender to obtain simplicia powder (Zamzani and Triadisti, 2021).

2. Standardization of Simplicia

Standardization of simplicia was carried out to determine the quality of simplicia. The following is the standardization of simplicia on eggplant samples.

3. Determination of water content

The water content was determined by the toluene distillation method. The toluene used was saturated with water first, then 5 grams of simplicia were weighed and put into a round bottom flask, and the saturated toluene was added. The flask was heated for 15 minutes. After the toluene started to boil, the distillation was adjusted to 2 drops per second, then 4 drops/second. After all the water was distilled, heating was continued for 5 minutes. The receiving tube was allowed to cool down to room temperature. The volume of water was read after the toluene and water had completely separated.

$$\text{Water Content} = \frac{\text{Water volume (ml)} \times \text{Water density (g/ml)}}{\text{Initial weight of simplisia}} \times 100\%$$

4. Determination of level of soluble extract in ethanol

Approximately 5 grams of simplisia powder that had been dried in air were put into a corked flask, and then 100 mL of ethanol was added. Shaken several times for the first 6 hours and then left for 18 hours. The mixture was filtered immediately to avoid ethanol being evaporated. As much as 20 mL of the filtrate was evaporated to dry in a flat-bottomed cup that had been tare, and the residue was heated at 105 °C to a constant weight. The content was calculated as the percent of the extract that was soluble in ethanol against the material that had been dried in air.

$$\text{Level of soluble extract in ethanol} = \frac{\text{Residual weight}}{\text{Sampel initial weight}} \times \text{DF} \times 100\%$$

Description:

DF: Dilution Factor

5. Determination of soluble extract In water levels

Approximately 5 grams of simplisia powder that had been dried in air were put into a plugged flask, and then chloroform saturated water was added. Shaken several times for the first 6 hours and left for 18 hours. The mixture was filtered and 20 mL of the filtrate was evaporated to dry in a flat-bottomed cup that had been tipped. The residue was heated at 105°C to a constant weight. The content was calculated as the percent of the extract that was soluble in ethanol against the material that had been dried in air.

$$\text{Level of soluble extract in water} = \frac{\text{Residual weight}}{\text{Sampel initial weight}} \times \text{DF} \times 100\%$$

Description:

DF: Dilution Factor

6. Determination of drying shrinkage

As much as 2 grams of eggplant simplisia were put into an evaporating cup and then put in the oven at 105°C for 30 minutes. The weight of the drying shrinkage content in g/g was calculated for materials that have been dried in air using the following formula ([Suryadini, 2011](#)):

$$\text{Drying shrinkage} = \frac{\text{Weight before drying} - \text{Weight after drying}}{\text{Sampel initial weight}} \times 100\%$$

7. Extraction process

As much as 300 grams of eggplant fruit simplisia were put into a maceration vessel, and 96% ethanol solvent was added until the powder was completely soaked. Extraction results obtained with a ratio of 1:10 of 400 grams of eggplant simplisia powder in 4000 mL of ethanol were 96% . The mixture was left for 3 days, protected from sunlight, and stirred as often as possible.

8. Phytochemical Screening

Phytochemical screening aimed to identify classes of secondary metabolite compounds contained in samples, including alkaloids, flavonoids, saponins, terpenoids, and tannins ([Sari, Muhani, and Fajriaty, 2017](#)).

9. Tools sterilization

Glassware was washed first with detergent and then rinsed thoroughly. Glassware that was heat-resistant was sterilized in an oven at 180°C for 2-3 hours. Equipment that is not heat resistant can be sterilized using an autoclave at 121 °C for 15 minutes.

Inoculating loops and needles, as well as tweezers, were sterilized by heating over a Bunsen flame.

10. Medium preparation

4 grams of nutrient agar (NA) were dissolved in 200 mL of distilled water using an Erlenmeyer. The solution was put in the microwave to be boiled, then stirred until homogeneous. The solution was then sterilized in an autoclave at 121 °C for 20 minutes.

11. Pure bacteria culture rejuvenation

P. acnes bacteria were taken in as much as 1 inoculating loop, then inoculated by streaking on the surface of the NA media, and then incubated for 24 hours at an incubator temperature of 37 °C.

12. Bacteria suspension preparation

Bacteria that have been rejuvenated were taken with sterile inoculating loops and then suspended into a tube containing 10 mL of a sterile 0.9% NaCl solution, then homogenized. Until the turbidity standard was obtained, which was the same as the standard McFarland solution turbidity by mixing 0.05 mL 1% BaCl₂ and 9.95 mL 1% H₂SO₄, the turbidity of the bacterial suspension was compared to the McFarland standard, which was proportional to the number of bacterial cell colonies: $1,5 \times 10^8$ CFU /mL (Khafidhoh, Dewi, and Iswara, 2015).

13. Antibacterial activity test

NA media was put into a petri disk, and then 1000 µL of *P. acnes* bacteria suspension was added. The suspension was spread evenly with a spread stick and then allowed to solidify. Next, wells with a diameter of 10 mm were made, and then each test sample (positive control of tetracyclic antibiotics, negative control of 1% DMSO, purple eggplant fruit extract concentration: 60%, 70%, 80%, and 90%) as was inserted into the well. Then the petri dishes were incubated in an incubator at 37 °C for 24 hours. Inhibition zone measurements were done horizontally and vertically with calipers using the formula $(DV+DH)/2-DS$.

RESULT AND DISCUSSION

The determination of a plant was done to ensure the validity of the plant that was used. Based on the results of determination No.244/LB.LABDASAR/XII/2021, which was carried out at the ULM FMIPA Laboratory, the purple eggplant plant sample used has the Latin name *Solanum melongena* L. The extraction results obtained a thick brownish extract of 91,29 grams with a yield of 41,03%.



Figure 1. Purple Eggplant Sample

Note:

A) The eggplant fruit simplicia,

B) The eggplant fruit extract.

The results of simplicia standardization can be seen in Table I. It showed that the sample qualified the requirements of the simplicia standard. Simplicia standardization aimed to guarantee quality standards and product safety when used as a safe and good-quality medicinal ingredient.

Table I. Result of simplicial standardization

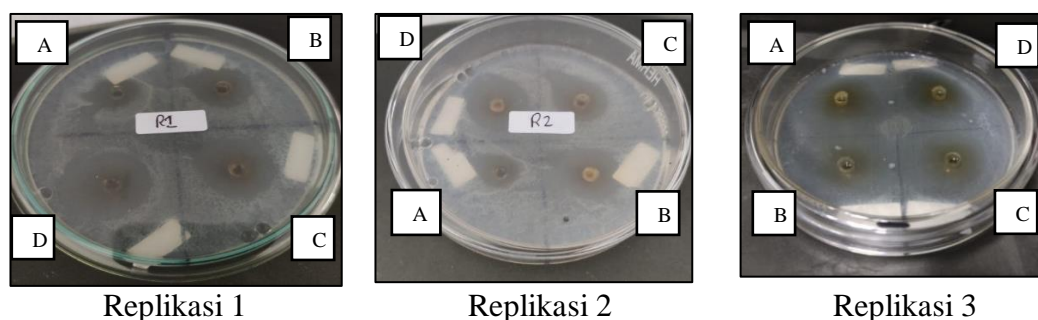
Parameters	Result	Standard
Water content	5%	<10%
Drying shrinkage	6%	<10%
Levels of soluble extract in water	31%	>24%
Levels of soluble extract in ethanol	40%	>6%

The results of the phytochemical screening can be seen in Table II. The phytochemical screening showed that purple eggplant fruit extract contained metabolite compounds, namely flavonoids, alkaloids, saponins, tannins, and terpenoids.

Table II. Phytochemical screening

Compounds	Reagent	Results	Description
Flavonoid	H ₂ SO ₄	(+)	Dark brown colour formed
Alkaloid	Mayer	(+)	White sediment formed
	Dragendorf	(+)	orange sediment formed
Saponin	Hot water	(+)	2 cm stable froth formed
Tannin	FeCl ₃	(+)	blackish green colour formed
Terpenoid	H ₂ SO ₄ +HCl	(+)	red colour formed

The presence of secondary metabolites in a plant is very important to help providing information in traditional medicine and modern medicine. Like other vegetables and fruits, eggplant has characteristic bioactive compounds, including phenolics, carotenoids, and alkaloids. In previous research, it was stated that the secondary metabolites contained in the purple eggplant plant (*S. melongena* L) were alkaloids, flavonoids, saponins, steroids, and tannins (Ashrafudoulla *et al.*, 2016). The presence of secondary metabolites in natural products provided antibacterial activity. The eggplant fruit was known to have antimicrobial properties (Mandy *et al.*, 2014; Kalimuthu *et al.*, 2017; Mbah *et al.*, 2019; Salamatullah *et al.*, 2021). The leaves and skin parts of the eggplant are known to have antibacterial properties (Purnamasari, Vifta, and Susilo, 2018; Bouhajib *et al.*, 2020). Based on the research by Salamatullah *et al.* (2021) which conducted an antibacterial test of eggplant fruit extract using the well method at a concentration of 1 mg/ml, it resulted in an inhibition of *E. coli* growth with a diameter of 16 mm. In the research by Hana, Gerung, and Antasionasti, (2021), which tested the antibacterial activity of *S. lycopersicum* L extract using the disc diffusion method and resulted in inhibition against *P. acnes*.

**Figure 2. Antibacterial activity test results using the diffusion method**

Note:

- A) Extract Concentration 60%,
- B) Extract Concentration 70%,
- C) Extract Concentration 80%,
- D) Extract Concentration 90%.

The diameter of the vertical inhibition zone with the abbreviation VD, the diameter of the horizontal inhibition zone with the abbreviation HD, and the abbreviation WD were the diameters of the well.

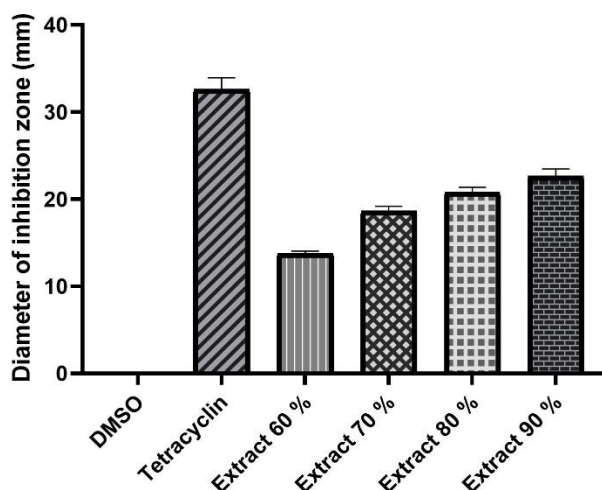


Figure 3. Inhibition zone of eggplant ethanol extract against the growth of *P. acnes*

The results of the research showed that eggplant (*S. melongena* L) ethanol extract tested by the well diffusion method has antibacterial properties. Antibacterial activity was shown by the presence of an inhibition zone. At a concentration of 60%, it has an inhibition zone value of 13,77 mm, indicating a relatively strong inhibition zone. At a concentration of 70%, it has an inhibition zone value of 18,63 mm, indicating a relatively strong inhibition zone. At a concentration of 80%, it has an inhibition zone value of 20,79 mm, indicating a very strong inhibition zone. At a concentration of 90%, it has an inhibition zone value of 22,64 mm, indicating a very strong inhibition zone (Figure 2). The antibacterial activity of eggplant is due to the presence of bioactive compounds belonging to the class of alkaloids, flavonoids, saponins, steroids, tannins, and terpenoids. Flavonoids are significant phenolic compounds in eggplant. Eggplant leaves and fruit have different flavonoid profiles. Eggplant fruit contained minor compounds such as kaempferol, quercetin, apigenin, and isorhamnetin (Huang et al., 2007); kaempferol was identified in the leaves (Piao et al., 2014). The mechanism of action of flavonoids in inhibiting bacterial growth is by inhibiting the synthesis of nucleic acids. Flavonoid plays a role in the process of intercalation or hydrogen bonding by accumulating nucleic acid bases that inhibit the synthesis of DNA and RNA (Rahman, Haniastuti, and Utami, 2017). Glycoalkaloids are nitrogen-containing steroid glycosides which are found abundantly in the *Solanum* genus. Research has informed derivative compounds from steroid glycoalkaloids, namely α -solamargine and α -solasonine (Sánchez-Mata et al., 2010). The mechanism of action of alkaloids in inhibiting bacterial growth is through inhibition of cell wall synthesis, which will cause bacterial cell death (Ramonah, Rahardhian, and Putri, 2020). The presence of aromatic groups in alkaloids allows them to interact with DNA. The presence of saponins contained in eggplant provides an antibacterial role by interfering with the surface tension of the cell wall. When the surface tension of the cell wall is disturbed, the antibacterial substance can easily enter the cell and disrupt metabolism until bacterial death occurs (Hemthanon and Ungcharoenwiwat, 2022).

Based on the negative control results, 1% DMSO did not inhibit of *P. acnes* growth. This indicated that DMSO had no effect on the extract test results, while the tetracycline drug treatment control produced an inhibition zone of 32,56 mm, indicating a very strong inhibition zone.

CONCLUSION

The antibacterial activity test of eggplant fruit (*S. melongena* L.) ethanol extract using the maceration method at concentrations of 60%, 70%, 80%, and 90% showed antibacterial activity against *Propionibacterium acnes* with inhibition zone values of 13,74% mm, 18,87% mm, 21,06% mm, and 23,18% mm.

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