Phytochemical Screening Of *Phaleria Macrocarpa* (Scheff.) Boerl.) And Antibacterial Activity Test Of Ethanol Extract Against *Staphylococcus Aureus* Bacteria

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Abstract.
Phaleria macrocarpa is known as one of the medicinal plants in Indonesia. Almost all parts of the plant have chemical content that is useful for being used as medicine. The growing use of crown plants by the community for various diseases requires a series of tests to obtain traditional medicine preparations that are safe for human use. The purpose of this study was to identify the content of secondary metabolite compounds (alkaloids, flavonoids, saponins, tannins, steroids, triterpenoids, and glycosides) and the antibacterial activity of Staphylococcus aureus with the good method because the good method has the advantage that it is easier to measure the area of the inhibition zone formed because an antibacterial activity is not only on the upper surface of nutrient agar but also at the bottom. The results of phytochemical screening showed that the flesh of Phaleria macrocarpa (Scheff).The results of testing the antibacterial activity of an ethanol extract of crown Phaleria macrocarpa (Scheff) fruit flesh (Boerl) were positive for alkaloids, flavonoids, glycosides, saponins, tannins, and steroids. Boerl.) showed effective results at a concentration of 40 mg/ml against Staphylococcus aureus bacteria with an inhibition zone diameter of 14 mm; the antibacterial activity was categorized as strong.

Keywords: *Phaleria macrocarpa (Scheff), Boerl.) and Staphylococcus aureus.*

I. INTRODUCTION

Infection is a disease caused by microbes that can easily infect humans, especially if the immune system is compromised. Infections can be caused by bacteria, viruses, or fungi [1]. Some of the infecting bacteria include Pseudomonas aeruginosa, Klebsiella pneumoniae, and Escherichia coli, which cause everything from skin infections to upper and lower respiratory tract infections. E. coli is also implicated in causing gynecology. Staphylococcus aureus causes intra-abdominal infections, bone and joint infections, lower respiratory tract infections, and skin infections. Proteus vulgaris is an opportunistic pathogen responsible for causing urinary tract infections and wound infections [2].The bacteria Staphylococcus aureus infects the upper respiratory tract, skin tissue, skin infections, and pneumonia. One of the widely used antibiotics to treat infections caused by Staphylococcus aureus is erythromycin [3].

Handling problems related to bacterial infections requires an effective and safe antibacterial agent that needs to be studied more deeply. In comparison to synthetic drugs, the advancement of natural medicine now provides a great opportunity for the treatment of infectious diseases. The discovery of antibacterial compounds derived from natural materials continues to be studied and developed in order to deal with the problem of antimicrobial resistance due to the irrational use of antibiotics. Plants rich in secondary metabolites such as tannins, lignins, carotenoids, flavonoids, and alkaloids are believed to have antimicrobial activity by inhibiting the growth of pathogenic bacteria. [4], [5]The Phaleria macrocarpa (Scheff) (Boerl.) is a medicinal plant found in the Pidie district of Indonesia. Almost all parts of the plant have chemical content that is useful for being used as medicine.Based on the above, the researchers will conduct research on Phaleria macrocarpa (Scheff). Boerl.) by examining the effect of ethanol extract on the flesh of the Phaleria macrocarpa, Schaff) Boerl.) on antibacterial activity using the pitting method against Staphylococcus aureus.

II. METHODS

2.1 Tools

The tools used in this research are glassware (Petri dish, beaker glass, Erlenmeyer, measuring cup), an autoclave (Tomy), an incubator (Memmert), a vernier sigma, and a laminar airflow cabinet (BioBase).

2.2 Materials

The materials used in this study are Phaleria macrocarpa (Sheff.) Boerl and Staphylococcus aureus ATCC 25923 bacterial cultures. The media used were Muller-Hinton Agar (MHA) (Oxoid), Muller-Hinton Broth (MHB) (Oxoid), and dimethylsulfoxide (DMSO) (SmartLab).

2.3 Plant Identification

Plant identification was carried out at the plant laboratory "Herbarium Medanense," Faculty of Mathematics and Natural Sciences, Department of Biology, University of North Sumatra.

2.4 Preparation of simplicity Powder of *Phaleria macrocarpa* (Scheff) Boerl.) Meat

Phaleria macrocarpa flesh is collected and washed thoroughly with running water, drained, then weighed as wet weight. The results were then dried in a dryer at 40 °C, the dried results were weighed as dry weight, and they were pollinated using a blender [6].

2.5 Preparation of Ethanol Extract of *Phaleria macrocarpa (Scheff) Boerl.)* Meat

The maceration method was used to prepare an ethanol extract of flesh Phaleria macrocarpa at a 1:10 ratio. One part of the powdered simplistic of Phaleria macrocarpa was put into a container, and 10 parts of 96% ethanol solvent (pa) were added. Soaked for 6 hours, occasionally stirring, then set aside for 18 hours. Then filtered. The process was repeated once, and then all the macerates were collected and evaporated with a rotary evaporator until a thick extract was obtained [7], [8].

2.6 Phytochemical Screening of Ethanol Extract of *Phaleria macrocarpa* (Scheff) Boerl.)

Phytochemical screening was carried out on simplistic and extracts of Phaleria macrocarpa meat, including alkaloid examination, glycoside examination, saponin examination, flavonoid examination, tannin examination, and steroid/triterpenoid examination [9], [10].

2.6.1 Alkaloids Examination

A total of 500 mg of Phaleria macrocarpa pulp extract was added along with 1 mL of 2N hydrochloric acid and 9 mL of water, then heated in a water bath for 2 minutes, then removed and dried. Three drops of each filtrate were transferred to three test tubes. In the first tube, add 2 drops of Mayer reagent; in the second tube, add 2 drops of Buchardat reagent; and in the third tube, add 2 drops of Dragendrof reagent. If there is a white or yellow clumpy precipitate in Mayer's reagent, it indicates a positive alkaloid. If the solution produces a brown-to-black deposit on the Buchardat reagent, then the presence of tannins is positive, and the Dragondroff reagent produces a yellow-orange deposit [11].

2.6.2 Flavonoids examination

A total of 500 mg of Phaleria macrocarpa pulp extract was heated with water in a water bath, then dried. Add 100 mg of magnesium powder and 1 mL of 2N hydrochloric acid to the filtrate. Flavonoids are detected by the formation of a yellow-to-red color [12].

2.6.3 Saponins Examination

A total of 500 mg of Phaleria macrocarpa pulp extract was added to 10 mL of hot water; the mixture was then shaken vigorously for 10 seconds. The presence of saponin compounds in the sample is indicated by the formation of a froth for not less than 10 minutes that is 1–10 cm high and does not disappear with the addition of 1 drop of HCl 2N [13].

2.6.4 Tannins Examination

A total of 500 mg of Phaleria macrocarpa pulp extract was added to 20 mL of distilled water and heated. The solution is then evaporated, and a few drops of a 10% FeCl3 solution are added to see what color forms. The presence of tannins is indicated by the formation of a blue or blackish-green color [14].

2.6.5 Steroid/Triterpenoid Examination

A total of 500 mg of Phaleria macrocarpa of Heaven pulp extract was added to 2 mL of chloroform. Then I added 0.5 mL of anhydrous acetic acid. Furthermore, 2 mL of concentrated H2SO4 was added through the tube wall, and the color formed was observed. The presence of steroid compounds is indicated by the formation of a bluish-green color (the presence of steroid rings), while the formation of brownish or purple rings in the restriction of two dissolutions indicates positive triterpenes.

III. RESULT AND DISCUSSION

3.1 Phytochemical Screening Results

Extract of Phaleria macrocarpa contains flavonoids compound, alkaloids, saponins, tannins, and terpenoids. Flavonoids compounds contain antioxidants. The results of the phytochemical screening are shown in **Table 1**.

No	Chemical compounds	Result
1	Alkaloids	-
2	Flavonoids	+
3	Tannins	+
4	Saponins	+
5	Triterpenoids/Steroids	+
6	Glycosides	+

Table 1. Phytochemical Screening Results of *Phaleria macrocarpa*

Description: (+): Contains a group of compounds

(-): Does not contain a group of compounds

3.2 Antibacterial Activity Testing

A total of 0.1 mL of the test bacterial suspension was mixed with 15 mL of MHA media in a sterile petri dish, then left until the media solidified. On the media that has solidified, discs that have been soaked with the test solution are placed, and they are then incubated at 37 ℃ for 18–24 hours [15]. [16].

3.3 Examination of simplicity Powder Characteristics

Examination of the quality characteristics of simplicity is the fulfillment of requirements for its use as a medicinal material and becomes a value determination for various product parameters. The parameters of simplisia used as medicinal raw materials must meet the requirements listed in the official monograph of Materia Medika Indonesia but the plant Phaleria macrocarpa is still not listed in Materia Medika Indonesia so that does not have a standard parameter reference that can be referred to. The results of the characterization examination of Phaleria macrocarpa simplicity powder can be seen in **Table 2.**

Table 2: Characterization of Phaleria macrocarpa (Scheff) Boerl.) simplicity Powder

No	Parameter	Result (%)
1	Water Content	7,66 %
2	Water soluble essence content	15,76%
3	Ethanol soluble juice content	11,32 %
4	Total ash content	6,96%
5	Acid insoluble ash content	0,25%

The water content, based on the requirements in general, is not more than 10%, according to the results of the characterization of the water content of Phaleria macrocarpa (Scheff). Boerl.) got a value of 7,66 % [17]. If the moisture content obtained in this examination exceeds 10%, it will be a good medium for the growth of microorganisms, especially fungi, so that the shelf life and quality of the product will be reduced and even damaged [18]. Determination of water-soluble juice content aims to determine the levels of chemical compounds from polar simplicity such as flavonoids, glycosides, and saponins [19]. Where the water-soluble juice content obtained was 15.76%. While ethanol-soluble juice content aims to determine chemical compounds that are polar, semipolar, and non-polar. The ethanol-soluble juice content obtained was 11.32%. The difference in levels in both examinations allows for differences in soluble compounds in each solvent, this allows for more solubility in polar compounds than others [20].

The examination of ash content aims to provide an overview of the internal and external mineral content derived from the initial process of raw materials to become simpler. Determination of total ash content is carried out to determine the level of inorganic compounds in simplicity, such as metals K, Ca, Na, Pb, and Hg [21]. The results of the examination of ash content obtained a value of 6.96%. This result shows the low ash content, where the internal mineral content in Phaleria macrocarpa (Scheff. Boerl.) is quite small. Acid insoluble ash content indicates the presence of mineral or metal contamination that is insoluble in acid.

The result of acid-insoluble ash content in simplicity was 0.25%. Acid-insoluble ash content indicates the presence of silicate content derived from soil or sand [22].

3.4 Antibacterial Activity Testing

The results of antibacterial activity testing against Staphylococcus aureus showed the formation of an inhibition zone around the disc at all variations of the test concentration. The results of the antibacterial activity test can be seen in **Table 3.**

Table 3. Activity Test Results of Ethanol Extract of Phaleria macrocarpa (Scheff. Boerl.) Against Staphylococcus aureus

NO	Extract Concentrations (mg/ml)	Diameter of Bacterial Growth Inhibition Area (mm)* Staphylococus aureus	Category
1	60	23,8	Very strong
2	55	20,4	Very Strong
3	50	18,8	Strong
4	45	17,5	Strong
5	40	14,3	Strong
6	30	11,9	Strong
7	20	10,8	Strong
8	15	8,5	Medium
9	10	7,8	Medium
10	5	3,4	Weak
11	K +	20,7	Very strong
12	K -	-	

The results of testing the antibacterial activity of ethanol extracts of Phaleria macrocarpa fruit flesh decreased along with a decrease in concentration so that the diameter of the inhibition zone formed was also getting smaller. The smallest concentration that still has antibacterial activity is at a concentration of 5 mg/ml, which is classified as weak. The inhibition zone limit is considered effective if it has an inhibition zone diameter of 13- 18 mm, is weak below 9 mm, moderately active 9- 12 mm, and very strong above 18 mm [23].The ethanol extract of a Phaleria macrocarpa meat contains compounds of flavonoids, terpenoids, alkaloids, saponins, and tannins. Flavonoids are polar compounds that more easily penetrate the peptidoglycan layer in gram-positive bacteria which are also polar, in addition, the cell wall of gram-positive bacteria also contains polysaccharides (teichoic acid) which is a water-soluble polymer.

This soluble nature also indicates that gram-positive cell walls are more polar) [24]. Terpenoids are able to dissolve lipids and agglomerate bacterial cell wall proteins so that the integrity of the bacterial cell wall is disrupted and reducing the permeability of the bacterial cell wall, and causing bacterial death [25]. Alkaloids work as antibacterials by damaging the constituent components of peptidoglycan so that the bacterial cell wall layer is not formed intact and causes cell death [26]. Saponins are active compounds that are like soap by reducing cell surface tension so that antibacterial substances easily enter the cell, disrupting metabolism until the bacteria die. Tannins are antibacterial where the bacterial cell wall that has been lysed due to saponin and flavonoid compounds makes it easier for tannin compounds to enter bacterial cells to coagulate bacterial cell protoplasm [27].

IV. CONCLUSION

The results of phytochemical screening of ethanol extract of fruit flesh Phaleria macrocarpa (Scheff). Boerl.) contains secondary metabolite compounds namely alkaloids, saponins, falvonoids, tannins, and triterpenoids which have antibacterial activity against positive bacteria (Staphylococcus aureus).

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