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Research Article

Fractination and Characterization of Pandan Jeronggi Fruit (*Iris Domestica (L.) Goldblatt & Mabb*) Ethanol Extracts As Well As Antibacterial Activities from Each Fraction

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ABSTRACT

Objectives: The research aims to find out antibacterial activity of ethanol extract, n-hexane fraction, ethylacetate fraction and residual fraction (water) of Pandan Jeronggi fruit against several types of bacteria.

Methods: Antibacterial activity test was carried out using the agar diffusion method by measuring the diameter of the clear zone around the paper disc.

Results: The results of the simplicia characterization and ethanol extract obtained water content of 9.23% and 19.13%. Phytochemical screening test results in thick extract there are alkaloids, flavonoids, glycosides, saponins, and tannins. Antibacterial activity test results shows the inhibition of gram-positive and gram-negative bacteria. Ethyl acetate fraction and ethanol extract have the highest antibacterial activity with a concentration of 500 mg/ ml inhibitory diameter of 13.68 mm for Sthaphylococcus aureus and 11.44 mm for pseudomonas aeruginosa bacteria. Ethanol extract in Sthaphylococcus aureus 12.12 mm and 10.76 mm for pseudomonas aeruginosa bacteria, while the remaining fraction shows not too high inhibitory properties such as ethanol extract and ethylacetate fraction which is 10.42 mm in Sthaphylococcus aureus and 9.24 for pseudomonas aeruginosa bacteria in the n-hexane fraction has no inhibitory power. The KKt results obtained from the ethyl acetate fraction contained three visual stains, with $AlCl_3$ spray two are also three stains, with ammonia vapor there are three stains, the compound contained in the ethyl acetate fraction suspected as an antibacterial activity is fenolik.

Keywords: Pandan Jeronggi Fruit, Antibacterial, Staphylococcus aureus, Staphylococus epidermis, Klebsiella pneumonia, Pseudomonas auroginosa, Salmonella tiphy

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INTRODUCTION

North Sumatra is a fertile area and rich in various plants. Some of these plants have been used traditionally by the community as medicinal materials. One of them is Pandan Jeronggi (Iris Domestica (L.) Goldblatt & Mabb). These plants can reach up to 150 cm in height and grow on hillsides and swampy areas in young forest areas. Traditional use of Pandan jeronggi fruit to stop bleeding on wounds and to treat wounds and to be used as a medicine for boils and to accelerate the maturation of boils, anti-bacteria and other skin diseases [1].

At present, the research and development of medicinal plants both domestically and abroad is multiplying. The development of research, especially in terms of pharmacology, phytochemistry and microbiology based on indications of medicinal plants that have been used by some people with empirically proven efficacy. The results of this study further strengthen the users of medicinal plants for their efficacy and usefulness [2].

Infection can cause various microorganisms, such as bacteria, viruses, fungi, and protozoa [3]. The bacterium that causes the infection is Staphylococcus aureus. Infection caused in the

form of local abscesses (ulcers and acne), bacteremia, endocarditis, pharyngitis, pneumonia [4]. meningitis, and empyema [5]. Bacteria Staphylococcus aureus is a bacterium that is a healthy flora found in the skin, nose, and respiratory tract, diseases that appear such as acne, ulcers, ulcers, and pneumonia [6]. Bacteria Staphylococcus aureus, Escherichia coli bacteria cause diarrhea in humans. The way to prevent the growth of these bacteria is to utilize active ingredients from plants that can use as antibacterial [7]. Previous research has carried out on the Iris Nigricans species. The test carried out was an antioxidant and antimicrobial effectiveness test of the methanol extract of Iris nigricans (rhizomes, leaves, and flowers). The antimicrobial activity of rhizome extract, leaf extract, and flower extract is carried out by the disk diffusion method on various types of bacteria where the bacteria are Escherichia coli, Bacillus subtilis, Staphylococcus aureus, and Klebsiella pneumonia [8].

According to previous researchers of the Iris nigricans species conducted by [9] by testing rhizome, leaf, and flower extracts using methanol as a solvent. The researchers used the disk diffusion method, according to the results of the study which obtained anti-bacterial activity from various parts Iris nigricans extracts of leaves and rhizomes showed vigorous anti-bacterial activity of the categories associated with the presence of phenolic components (flavonoids and xanthones) which can interfere with bacterial cell membranes.

Based on the above background the authors are interested in researching different species by testing the effect of extracts and fractions of Pandan jeronggi fruit fruit (Iris Domestica (L.) Goldblatt & Mabb) on the antibacterial activity and the Paper chromatography (KKt) profile of the most effective fractions.

METHODS AND MATERIALS

Plant material

The samples used were pandan jeronggi obtained from Padang Pulau village, Bandar Pulau District, Asahan Regency, North Sumatra Province, and purposive sampling. Plant identification carried out at the Institute of Sciences Indonesia (LIPI) Jl. Raya Jakarta – Bogor Km. 46 Cibinong 16911 Bogor – Indonesia.

Ethanol extract preparation

As much as 500 g of Simplicia powder put into a closed container, added 3.75 liters of 96% ethanol, then the container was closed and left for five days protected from light while occasionally stirring. Then filtered and collected in a dark-colored bottle (Maserati I). To the same simplicia powder, add 1.25 liters of 96% ethanol, then the container is closed and left for three days protected from light while stirring occasionally. Then filtered and collected in a dark-colored bottle (Maserati II). Then, Maserati, I, and II were poured in a manner of silence for two days, then poured and taken clear liquid at the top. The extract concentrated using a rotary evaporator and then evaporated in a water bath [10].

Fractination of ethanol extract

As much as 20 g of ethanol extract was dissolved in 96% ethanol until dissolved then 40 ml of distilled water were added, put in a separating funnel, then added 100 ml of n-hexane, then shaken, and allowed to stand until there were two separate layers (± 30 minutes). The n-hexane layer (upper layer) is taken by flowing the liquid in a separating funnel, and fractionation carried out until the n-hexane layer gives negative results with LB reagents. The n-hexane layer collected was concentrated with a rotary evaporator so that the n-hexane fraction obtained. Then the residue (residue) is added 100 ml of ethyl acetate, then shaken, allowed to stand until there are two separate layers (± 30 minutes), the layer of ethyl acetate (top layer) taken by flowing the liquid in the separating funnel, and the fractionation carried out until the ethyl acetate layer gives negative results with FeCl3 reagents. The ethylacetate layer collected was concentrated with a rotary evaporator so that the ethylacetate fraction was obtained. A layer of water (residual) is taken and concentrated with a rotary evaporator so that a water fraction is obtained (Bassett et al., 1994).

Antibacterial test

Testing the antibacterial activity of ethanol extract, n-hexane fraction, ethyl acetate fraction and the residual fraction was carried out by diffusion method using paper scavenging, bacteria used by Gram-positive bacteria (Staphylococcus aureus ATCC 25923, Staphylococcus epidermis ATCC 12228) and Gram-negative bacteria (Salmonellathypi ATCC 14028, Pseudomonas aeruginosa ATCC 9027 and Klebsiella pneumonia).

RESULT AND DISCUSSION

Authentication Plant

The results of the identification of plants carried out at the Indonesian Institute of Sciences (LIPI) Bogor - Biological Research Center are plants of Jeronggi (Iris Domestica (L.) Goldblatt & Mabb) Iridaceae family

Phytochemical Screening Result

Phytochemical screening of Simplicia powder, ethanol extract, n-hexane fraction, ethylacetate fraction, the remaining fraction of Pandan jeronggi fruit. The results of phytochemical screening can see in table 1 below.

Table: 1. Phytochemical screening result of Pandan jeronggi fruit.

	Phytochemical Screening	Ethanol extract	Hexane Fraction	Etylacetate Fraction	Residual Fraction
	Alkaloid	+		+	-
	Flavonoid	+	-	+	+
	Glycoside	+	-	+	+
	Saponin	+	-	-	+
	Tanin	+	-	+	-
	Triterpenoid/Steroid	-	-	-	-

Based on the results of phytochemical screening tests for simplicia powder and ethanol extract of Pandan jeronggi fruit, there are chemical compounds containing alkaloids, flavonoids, glycosides, saponins, and tannins. The results of phytochemical screening examination for a fraction of hexane, ethyl acetate fraction, and a fraction of residual leaves of pandan jeronggi showed different results. In the n-hexane fraction no chemical compounds were examined, in the ethylacetate fraction, the content of the chemical compounds were alkaloids, flavonoids, glycosides, and tannins, and the remaining fractions contained flavonoids, glycosides, and saponins.

Antibacterial test results of ethanol extract of Pandan Jeronggi

Antibacterial test results of ethanol extract of Pandan Jeronggi against Gram positive bacteria (Staphylococcus aureus ATCC 25923, Staphylococcus epidermis ATCC 12228) provide inhibitory power at concentrations of 500 mg / ml, 400 mg / ml, 300 mg / ml, 200 mg / ml, 100 mg / ml, 100 mg / ml, 100 mg / ml / ml, 50 mg / ml, 25 mg / ml, all of these bacteria provide inhibition except at a concentration of 12.5 mg / ml. The antibacterial test results of the Pandan Jeronggi Ethanol extract against Gram negative bacteria (Klebsiella pneumonia, Pseudomonas aeruginosa ATCC9027 and Salmonella thypi ATCC 14028) provide inhibitory power at concentrations of 500 mg / ml, 400 mg / ml, 300mg / ml, 200 mg / ml, 100 mg / ml, 100 mg / ml mg / ml, 50 mg / ml, except at concentrations of 25 mg / ml and 12.5 mg / ml in Klebsiella pneumoniae bacteria do not provide inhibitory power and at a concentration of 12.5 mg / ml in Salmonella typhi bacteria.

Table: 2 The results of the antibacterial activity test of ethanol extract from pandan jeronggi

No	Concentration (mg/mL)	Clear zona diameter (mm)				
		S.aureus	S.epidermidis	P.aeruginosa	S.typhi	K.pneumonia
1	500	12,12	11,80	10,76	10,32	9,94
2	400	11,10	10,26	9,66	9,12	9,26
3	300	10,28	9,04	9,34	8,24	8,66
4	200	8,96	8,50	8,48	7,58	8,66
5	100	8,32	8,10	7,66	6,84	6,92
6	50	7,36	7,34	7,00	6,62	6,24
7	25	6,78	6,66	6,74	6,32	6,00
8	12,5	6,00	6,00	6,00	6,00	6,00

At a concentration of 25 mg/ml, inhibition was obtained in all bacteria, both Gram-positive bacteria and Gram-negative bacteria (except in Klebsiella pneumonia). For blanks using dimethyl sulfoxide (DMSO) does not provide inhibition to Gram-positive and Gram-negative bacteria. It can see in Figure 1 that the anti-bacterial effect of ethanol extract on Gram-positive bacteria on average is higher than that of Gram-negative bacteria. Previously phytochemical screening has been carried out for simplicia, and the results obtained are groups of chemical compounds that are suspected to be anti-bacterial, such as alkaloids, flavonoids, saponins, and tannins. Extraction is done by using remaceration using ethanol solvents, which expected to attract these compounds. Secondary metabolites that are more polar may be present in more significant quantities than lamia compounds, which are more non-polar in the extract so that the extract is more influential on Gram-positive bacteria whose outer membrane consists of layers. more peptidoglycan than Gram-negative bacteria whose outer membrane consists of a layer of lipopolysaccharide consisting of lipids, polysaccharides and protehi [12]. Besides, Gram-positive bacterial cell walls contain teichoic acid-containing alcohol (glycerol or ribitol) [13].

At this time, chemical drugs are prevalent in the community. However, herbal medicines continue to practice because of the richness of individual plants in secondary metabolite varieties such as alkaloids, flavonoids, tannins, terpenoids, which have a study to have anti-bacterial activity [14]. Several studies have supported the antimicrobial activity of plant extracts due to the presence of saponins and quinone and coumarin compounds, which have also investigated as having an anti-bacterial activity (Khanna and Kannabiran, 2008).

Based on previous research by Sajee where tests conduct on several antimicrobials, the bacteria used were Escherichia coli, Bacillus subtitilis, Staphylococcus aureus, and Klebsiella pneumonia. In this case, the extract used is from rhizome extract, leaf extract, and flower extract from Iris nigricans using methanol as a solvent. Researchers used the disk diffusion method, according to the results of the study obtained anti-bacterial activity from various parts of Iris nigricans rhizome extract, leaf extract and flower extract showed anti-bacterial activity associated with the presence of phenolic components (flavonoids and xanthones) that can interfere with cell membranes in bacteria. Whereas in the Pandan jeronggi fruit plant in this study, the solvent used was ethanol,

which has proven to be very useful for anti-bacterial activity.

Antibacterial test results of hexane fraction of Pandan Jeronggi

The results of antibacterial testing of n-hexane fraction of Pandan jeronggi fruit against Gram-positive bacteria (Staphylococcus aureus ATCC 25923, Staphylococcus epidermis ATCC 12228) and Gram-negative bacteria (Klebsiella pneumonia, Pseudomonas aeruginosa ATCC 9027 and Salmonella thypi ATCC 14028) in figure 4.2 show fraction 4.2- hexane at a concentration of 500 mg / ml, 400 mg / ml, 300 mg / ml, 200 mg / ml, 100 mg / ml, 50 mg / ml, 25 mg / ml, 12.5 mg / ml does not provide inhibitory power.

Table 3. The results of the antibacterial activity test of hexane fraction from pandan jeronggi

No	Concentration (mg/mL)	Clear zona diameter (mm)				
		S.aureus	S.epidermidis	P.aeruginosa	S.typhi	K.pneumoniae
1	500	6,00	6,00	6,00	6,00	6,00
2	400	6,00	6,00	6,00	6,00	6,00
3	300	6,00	6,00	6,00	6,00	6,00
4	200	6,00	6,00	6,00	6,00	6,00
5	100	6,00	6,00	6,00	6,00	6,00
6	50	6,00	6,00	6,00	6,00	6,00
7	25	6,00	6,00	6,00	6,00	6,00
8	12,5	6,00	6,00	6,00	6,00	6,00

Table 3 data can see in Figure 2 graph that shows there is no inhibition in the n-hexane fraction. In the n-hexane fraction, there is usually a group of steroid/triterpenoid compounds, while the phytochemical screening results of Simplicia and ethanol extracts and the n-hex fraction of Pandan Jeronggi fruit are negative.

The n-hexane fraction showed no antibacterial activity at concentrations of 500 mg / ml, 400 mg / ml, 300 mg / ml, 200 mg / ml, 100 mg / ml, 50 mg / ml, 25 mg / ml, 12.5 mg / ml does not provide inhibition to gram-positive bacteria and gram-negative bacteria. Furthermore, after the phytochemical screening, the n-hexane fraction showed negative results. Based on biological activity, free triterpenoid compounds thought to be antibacterial. Test the antibacterial effects of the triterpenoid group isolates that have been done previously on the bacteria that cause diarrhea Escherichia coli and Staphylococcus aureus. Some terpenoid compounds that have investigated have antibacterial activity, including monoterpenoid linalool, diterpenoid (-) hardwicklic acid, phytol, triterpenoid saponin and triterpenoid glycoside. The terpenoid compounds are also known to be active against bacteria, but the antibacterial mechanism of triterpenoids is still not known. The terpenoid antibacterial activity thought to involve membrane breakdown by lipophilic components phenolic compounds and terpenoids have the main target of the cytoplasmic membrane, which refers to its hydrophobic nature.

Antibacterial test results of Ethylacetate fraction of Pandan Jeronggi

Test results for the antibacterial effect of the ethylacetate fraction for Gram-positive bacteria Staphylococcus aureus ATCC 25923, Staphylococcus epidermis ATCC 12228) and Gram-negative bacteria (Klebsiena pneumonia, Pseudomonas aeruginosa ATCC 9027 and Salmonella thypi ATCC 14028) where at concentrations of 500 mg / ml, 400 mg / ml, 400 mg / ml, 400 mg / ml, 400 300 mg / ml, 200 mg / ml, 100 mg / ml, 50 mg / ml, 25 mg / ml, 12.5 mg / ml.

Table: 4. The results of the antibacterial activity test of etylacetate fraction from pandan jeronggi

No	Concentration (mg/mL)	Clear zona diameter (mm)				
		S.aureus	S.epidermidis	P.aeruginosa	S.typhi	K.pneumoniae
1	500	13,68	13,2	11,44	10,36	10,24
2	400	12,4	12,06	10,76	9,66	9,86
3	300	11	10,94	9,82	9,44	8,64

4	200	10,04	9,98	9,58	8,14	8,2
5	100	9,62	9,24	9,3	7,82	7,64
6	50	8,88	8,26	8,7	7,2	6,72
7	25	7,98	7,2	7,68	6,94	6,46
8	12,5	7,22	6,54	6,76	6,42	6,26

The most significant antibacterial effect on Gram-positive bacteria was seen in Staphylococcus aureus ATCC 25923 with 13.68 mm diameter inhibition while Gram-negative bacteria were seen on Pseudomonas aeruginosa ATCC 9027 with inhibition diameter 11.4 mm. The ethyl acetate fraction showed antibacterial activity against all pathogenic bacteria tested, but the antibacterial activity against Gram-positive bacteria was higher than that of Gram-negative bacteria. The content of the group of chemical compounds contained in the ethylacetate fraction in the form of polar compounds, namely the flavonoid group, and tannins with a more considerable amount than in the ethanol extract and the properties of the outer membrane layer (outer wall layer) of Gram-positive bacteria according to Pratiwi (2008) are more polar due to the presence of a polysaccharide layer causes groups of compounds contained in the ethylacetate fraction more easily enter the Gram-positive bacterial cell wall membrane. Previous studies have shown the presence of antibacterial properties and subsequent mechanism of action in several classes of secondary metabolites. Several classes of polyphenols, such as phenolic acids, flavonoids, and tannins, serve as a means of plant defense against pathogenic microorganisms. Several hydroxyl groups on the phenol component increase toxicity to microorganisms. Antimicrobial effects of flavonoids form complexes with extracellular proteins and dissolve by cell walls [14] The antimicrobial effect of tannin is shown by involving different mechanisms, such as inhibition of extracellular microbial enzymes and inhibition of oxidative phosphorylation in the process of microbial metabolism [15].

The mechanism of action of tannin as an antibacterial is inhibiting the reverse transcriptase enzyme and DNA topoisomerase so that bacterial cells cannot form [16]. have an antibacterial activity that is related to their ability to activate microbial cell adhesion and also activate enzymes and interfere with protein transport in the inner layers of cells [17]. According to Sari [18]. tannins also have targets on cell wall polypeptides, so that cell wall formation is less than perfect. It causes the bacterial cell to become lysis due to osmotic and physical pressure so that the bacterial cell will die. Also, according to Akiyama et al. 2001, the complexation of iron ions with tannins can explain tannin toxicity. Microorganisms that grow under aerobic conditions require iron for various functions, including the reduction of DNA ribonucleotide precursors. Due to the strong binding capacity of tannins. main target of the cytoplasmic membrane, which refers to its hydrophobic nature.

Antibacterial test results of residual fractionof Pandan Jeronggi

The results of antibacterial testing of the remaining fraction of Pandan jeronggi fruit fruit against Gram-positive bacteria (Staphylococcus aureus ATCC 25923, Staphylococcus epidermis ATCC 12228) and Gram-negative bacteria (Klebsiella pneumonia, Pseudomonas aeruginosa ATCC 9027 and Salmonellathypi ATCC 14028) are seen in Figure 4.4 providing a small inhibitory power at concentrations of 500 mg/ml, 400 mg/ml, 300 mg/ml, 200 mg/ml, compared to ethanol extract, and ethyl acetate fraction. At the same time, concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml has no inhibition against gram-positive and gram-negative bacteria. For Gram-positive bacteria, the largest inhibitory diameter seen in Staphylococcus aureus bacteria with inhibition diameters of 10.42 mm at a concentration of 500 mg/ml. In contrast, for Gram-negative bacteria, the largest inhibitory diameter was seen in Pseudomonas aeruginosa bacteria with an inhibiting diameter of 9.24 mm at a concentration of 500 mg/ml. Dimethyl sulfoxide used in antibacterial testing of the residual fraction of all bacteria does not provide inhibitory power.

Table: 5. The results of the antibacterial activity test of resudial fraction from pandan jeronggi

No	concentration (mg/mL)	Clear zona diameter (mm)				
		S.aureus	S.epidermidis	P.aeruginosa	S.typhi	K.pneumoniae
1	500	10,42	9,48	9,24	9,02	8,18
2	400	9,62	8,24	8,1	8,44	8
3	300	8,78	7,3	7,08	7,4	7,16
4	200	6,84	6,62	6,50	6,62	6,58

5	100	6,00	6,00	6,00	6,00	6,00
6	50	6,00	6,00	6,00	6,00	6,00
7	25	6,00	6,00	6,00	6,00	6,00
8	12,5	6,00	6,00	6,00	6,00	6,00

The antibacterial effect of the residual fraction provides a small inhibitory effect between ethanol extract and the ethyl acetate fraction because in this fraction it is likely that the class of chemical compounds that are as antibacterials such as flavonoids, anthraquinones, and tannins in small amounts so that the antibacterial effect of the remaining fraction is not as strong, the ethyl acetate fraction and ethanol extract. According to Pratiwi (2008), one of the effectiveness of antimicrobials is influenced by the concentration or intensity of microbial agents. The higher the concentration of the substance, the more microorganisms that can be killed. However, at some point, increasing concentration does not increase the speed of killing. In several previous studies, it was noted that the antibacterial activity was higher in extracts extracted by solvents compared to water extracts. Also, there is an effect of varying sensitivity of pathogenic bacteria to extracts withdrawn by a certain organic solvent and extracts withdrawn with water because this is related to one or several active substances that have biological activity as antimicrobials [19]. (Kumaraswamy et al., 2008).

Based on the results of antibacterial testing of ethanol extract, n-hexane fraction, ethyl acetate fraction and residual fraction against Gram-positive bacteria (Staphylococcus aureus ATCC25923, Staphylococcus epidermis ATCC12228) and Gram-negative bacteria (Klebsiella pneumonia, Pseudomonas aeruginosa ATCC 902723 and Salmonthypi) can be seen that ATCC12228 was able to see that the typology was ATCC25923; ethyl acetate fraction is the most active fraction, followed by ethanol extract, residual fraction and n-hexane fraction. Results of chromatography profile The ethyl acetate fraction paper visually contained three stains with variations in red, yellowish-green and blue using AlCl3 stain viewers there were two stains one red stain, one yellowish blue stain using ammonia vapor there were three stains, one red stain 1 yellowish-green stain and one blue stain indicate the presence of phenolic compounds (flavonoids and tannins). In this ethyl acetate fraction, the red color is visible under UV-Visible light with a wavelength of 366 nm.

CONCLUSION

Phytochemical screening can be used to determine the class of chemical compounds contained in the powder simplicia and extracts; the test results found groups of alkaloid compounds, flavonoids, saponins, glycosides, and tannins. Ethanol extract of Pandan Jeronggi Fruit (Iris Domestica (L.) Goldblatt & Mabb). Can be anti-bacterial for Gram-positive and Gram-negative. The mean inhibitory diameter for Gram-positive bacteria (Staphylococcus aureus ATCC 25923, Staphylococcus epidermis ATCC 12228), were 12.12 mm, and 11.80 mm, respectively. The mean inhibitory diameter for Gram-negative bacteria (Klebsiella pneumonia, Pseudomonas aeruginosa ATCC 9027, Salmonella typhi ATCC 14028) was 10.76 mm, 10.32 mm and 9.94 mm, respectively. The fraction that has the most significant inhibition is the ethyl acetate fraction, followed by ethanol extract, the residual fraction and the n-hexane fraction do not provide any inhibitory effect on anti-bacterial activity. For Gram-positive bacteria, the anti-bacterial inhibition power at a concentration of 500 mg/ml ethyl acetate fraction was 13.68 mm and 13.20 mm, respectively, while Gram-negative bacteria were 11.44 mm, 10.36 and 10, respectively. 24 mm.

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