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IDENTIFICATION OF ANTIBACTERIAL COMPOUNDS FROM ENDOPHYTIC BACTERIAL EXTRACT OF GREEN GRASS CINCAU PLANT (PREMNA OBLONGIFOLIA MERR)

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Abstract.

Green grass jelly (*Premna oblongifolia Merr*) is a plant that belongs to the Verbenaceae family with the Premna genus. The genus Premna has about 200 species in the Verbenaceae family which are spread in tropical to subtropical areas such as Asia, Africa, and Australia. This plant has potential as antibacterial, antipyretic, anti-inflammatory and antioxidant. This research was conducted to make an extract of endophytic bacteria obtained from green grass jelly plants by maceration method. The extract obtained was tested for its antibacterial activity by disc diffusion method. Extracts with the highest antibacterial activity were identified by FTIR and GCMS. The results showed that the methanol extract of endophytic bacteria of green grass jelly plants had broad spectrum antibacterial activity because it was able to inhibit the growth of Gram-positive bacteria (*Bacillus subtilis*) and Gram-negative bacteria (*Pseudomonas aeruginosa*). The results of FTIR and GCMS showed that the active compounds contained in the endophytic bacteria of green grass jelly plants were thought to be Nerolidol compounds or 3,7,11-trimethyl-1,6,10-dokatrien-3-ol which are secondary metabolites of the sesquiterpene alcohol group and play a role in as an antibacterial.

Keywords: grass jelly (*premna oblongifolia merr.*); antibacterial; endophytic bacteria

1. INTRODUCTION

Green grass jelly (*Premna oblongifolia Merr*) is a plant that belongs to the Verbenaceae family with the Premna genus. The genus Premna has about 200 species in the Verbenaceae family which are spread in tropical to subtropical areas such as Asia, Africa, and Australia (Rekha **[1]**). The grass jelly plant is often used by the people of Indonesia as a febrifuge (*antipyretic*), high blood pressure (*antihypertensive*) medicine and its sap is efficacious as a stomach ulcer medicine. The leaves and roots of this green grass jelly plant contain saponins, flavonoids, alkaloids and contain polyphenols (Hutapea **[2]**). Literature review shows that the green grass jelly plant has bioactivity as an antioxidant, antibacterial, antidiabetic and anticancer.

Endophytic bacteria are microbes that live in plant tissues symbiotically by forming colonies during certain periods of their life cycle. According to Brader **[3]** Endophytic bacteria promote plant growth and health and provide beneficial effects characterized by metabolic interactions. Living organisms are a source for a diversity of different metabolites, most of these metabolites have been found in plants but microorganisms are a rich source of more than 20000 biologically active compounds. The active compounds from endophytic microbes of medicinal plants will have greater activity than the activity of the active compounds of the host plant. The development of research on endophytic microbes needs to be done because in terms of efficiency **[4]**, This is very beneficial because the life cycle of endophytic microbes is shorter than the life cycle of the host plant so that it can be made on a large scale without using a large area. Endophytic bacteria grow in the vascular tissue of their host plants (Stone **[5]**). Vascular tissue (vessels) is found throughout the plant body, transporting substances between roots and shoots (Campbell **[6]**). Each level plants. It can contain some endophytic microbes capable of producing secondary metabolites which are thought to be the result of genetic recombination from their host plants into endophytic microbes (Tan & Zou **[7]**).

Several types of endophytic bacteria are known to produce active compounds that are antifungal (Zhang **[8]**; Qiao **[9]**), antibiotics, antioxidants and have cytotoxic activity (Qiao **[9]**). According to Radji **[10]** endophytic bacteria produce bioactive compounds whose characteristics are like or the same as compounds produced by their host. This research was conducted to determine the antibacterial activity in the endophytic bacterial extract of green grass jelly plants and to identify the active compounds in the extract using FTIR and GCMS.

2. RESEARCH METHODS

In this study, the first stage was the isolation of endophytic bacteria from parts of the green grass jelly (*Premna oblongifolia Merr*) plant, namely the breath roots, stems and leaves, then screening/screening of potential bacterial isolates by looking at the inhibition zone formed from the endophytic bacterial isolates. grown on agar media containing the test bacteria. The second extraction of endophytic bacteria isolates with methanol solvent after which the viscous extract was tested for activity antibacterial. Furthermore, the most active extracts were

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identified using FTIR and GC-MS (Gas Chromatography-Mass Spectrophotometer) instruments.

2.1. Endophytic Bacteria Isolation

Samples in the form of the respiratory roots, stems and leaves of the green grass jelly plant (Premna oblongifolia Merr) were surface sterilized. Samples were washed with running water until clean and then cut each piece into 1-3 cm in size. The sample pieces were surface sterilized gradually, the sample was first immersed in 96% ethanol for 1 minute, followed by 5.25% Na-hypochlorite for 5 minutes, then rinsed again in 96% ethanol three times. The sterilized samples were then planted on Tryptic Soy Agar (TSA) isolation media and then incubated in a dark room at room temperature and observed for the growth of endophytic bacterial colonies. Purification was carried out by transferring bacterial colonies into new TSA media.

2.2. Rejuvenation and Screening of Endophytic Bacterial Isolates

Rejuvenation was carried out on isolates of endophytic bacteria and test bacteria. Rejuvenation was carried out by inoculating endophytic bacteria and test bacteria isolates into TSA media and then incubated at 28-30oC for 24 hours. The test bacteria used were Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa and Salmonella typhusa. Screening is done first by making a suspension of the test bacteria with turbidity according to the McFarland standard of 1x108 cfu/mL. The growing test bacterial colonies were transferred to 5 mL of BSCP media solvent and then vortexed and compared for turbidity with the McFarland standard. Then poured into 45 mL of BSCP media solvent, homogenized. A total of 1 mL of the test bacterial culture was put into a sterile petri dish and then ± 20 mL of TSA medium was poured with a temperature of ± 40 C, then homogenized. The endophytic bacterial isolates were inoculated into the media containing the test bacteria using an ossicle, then incubated at 28-30ºC for 24-24. 48 hours. Clear zone formed This indicates the presence of antibacterial compounds produced by endophytic bacteria.

2.3. Secondary Metabolite Extraction (Nasution [11])

After obtaining potential endophytic bacteria from the screening results, the next step is the extraction of potential endophytic bacteria to obtain antibacterial active compounds. Extraction was carried out by making a suspension of potential endophytic bacterial isolates with turbidity according to McFarland standards (1x108 cfu/mL) then the suspension was pipetted 1 mL into a petri dish and then poured with TSA media, homogenized, and incubated at 30-35oC for 24 hours. Then the media that has been overgrown with endophytic bacterial isolates is macerated with methanol for 6 days. The maceration results are filtered to separate the solvent from the agar medium. Then the filtrate was centrifuged at 3600 rpm for 10 minutes to separate the pellet and supernatant portions. The extract obtained was then evaporated using a rotary evaporator to obtain a thick extract which was used for further analysis.

2.4. Antibacterial Activity Testing (Novriza [12])

Antibacterial activity testing was carried out using the disc diffusion method. The test was carried out by making an extract solution with 3 different concentration variations, namely 128 ppm, 64 ppm and 32 ppm. The methanol extract of endophytic bacteria was dissolved using DMSO as a solvent. Then 0.1 mL of the test bacterial suspension (1x108 cfu/mL) was pipetted into a sterile petri dish and poured with sterile TSA media. ± 20 mL, homogenized and allowed to solidify at room temperature. Then a paper disc with a diameter of 6 mm was taken, then 50 L of methanol extract solution was pipetted and dripped onto the paper disc and then planted on TSA media containing the test bacteria and incubated at 30-35oC for 24 hours. The negative control used DMSO solvent while the positive control used amoxicillin. The antibacterial power of each treatment was indicated by the diameter of the clear zone formed around the paper disc.

2.5. Identification of Functional Groups with an FTIR Spectrophotometer

A total of 1-2 drops of the test sample were dripped onto the Salt plate/NaCl Window then the salt plate was pressed to form a thin, even layer. Make sure no bubbles form. Then the salt plate is placed in the sample holder on the FTIR spectrophotometer to be identified at a wavelength of 400-4000 cm^{-1}.

f. Identification of Antibacterial Compounds with GC-MS

The methanol extract of isolate AF2 was analyzed using GC-MS to identify antibacterial compounds in the extract. The most active fraction was dissolved in methanol pro-analysis then injected into GC-MS then interpreted its mass spectrum compared to the library bank/computer database.

3. RESULTS AND DISCUSSION

3.1. Endophytic Bacteria Isolation Results

The number of endophytic bacteria in plants cannot be determined with certainty, but these bacteria can be detected by isolating them on agar media (Bacon & Hinton, **[13]**). A total of 3 isolates of endophytic bacteria were isolated from the green grass jelly plant parts, namely stems and breath roots, while the leaves did not produce bacterial isolates but produced mold isolates. The results of morphological observations of bacterial isolates can be seen in table 1. In the morphology table of bacterial isolates, the three isolates have different morphology.

Table 1. Isolate Morphological Data

Morphology	Isolate Code		
	AF1	AF2	BT
Color	Beige	yellowish	White ivory
Form	Round	Round	Round
Elevation	arise	arise	Flat
periphery	Flat	Flat	Flat
Texture Surface	Fine	Rough	Fine

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3.2. Results of Rejuvenation and Screening of Endophytic Bacterial Isolates

Rejuvenation is a process to get a fresh and aged culture 24 hours. The results of rejuvenation are then used for the screening stage. Screening is the stage of selection and determination of endophytic bacteria of green grass jelly plants that have antibacterial activity.

The screening results showed that of the 3 isolates of endophytic bacteria that were isolated, only 2 isolates of bacteria had antibacterial activity, namely isolates AF2 (Root of breath-2) and BT (Stem) while isolate AF1 did not have antibacterial activity because it did not form a zone of inhibition on agar media. containing the test bacteria. Zinniel **[14]**) stated that usually the bacterial population was more in the roots and less in the leaves and twigs.

Table 2. Isolate Antibacterial Activity

Test Bacteria	Isolate Code		
	AF1	AF2	BT
BS	-	+	+
SA	-	-	-
EC	-	-	-
PA	-	+	+
ST	-	-	-

3.3. AF2. Isolate Metabolite Antibacterial Activity Test Results

The results of the methanol extraction were tested for antibacterial activity. This test was conducted to determine the antibacterial potential of the metabolite extract of the endophytic bacteria isolate in inhibiting the growth of the test bacteria. The results of the antibacterial activity test (Figure 1) showed that the methanol extract of the metabolite of endophytic bacteria had a broad spectrum because it was able to inhibit the growth of Gram-positive bacteria (Bacillus subtilis) and Gram-negative bacteria (Pseudomonas aeruginosa). According to Levinson **[15]** antibacterial compounds produced by bacteria have selective properties in inhibiting the growth of other bacteria. Broad range antibacterial compounds are antibacterial compounds capable of killing various types of microorganisms, while narrow range antibacterial compounds are antibacterial compounds capable of killing only a few types of microorganisms. Results Observations on the antibacterial activity of the metabolite methanol extract of AF2 isolates from various concentrations are presented in Table 3.

Table 3. Antibacterial Activity Test Results of AF2. Isolate Methanol Extract

Concentration(ppm)	Zone Diameter Resistance (mm)	
	PA	BS
128	6.69	2.00
64	3.17	1.59
32	2.11	<1
16	<2	-

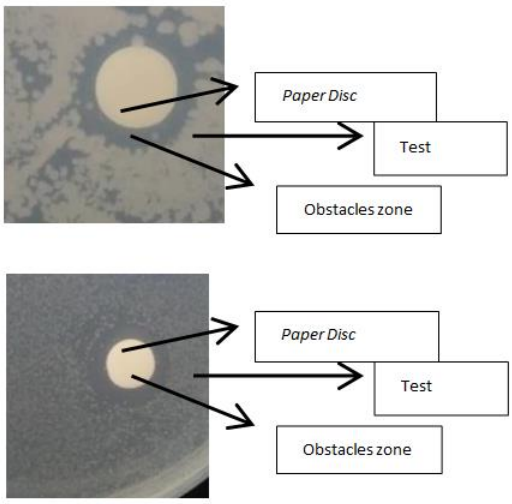

Figure 1. Inhibition Zone of AF2. Isolate Methanol Extract

Results observation the showed that the methanol extract of endophytic bacterial metabolites of green grass jelly plants had different susceptibility to the two types of test bacteria. The methanol extract of isolate AF2 against Bacillus subtilis is classified as an antibacterial that has low activity where the diameter of the inhibition zone formed is <5 mm at concentrations of 128 ppm and 64 ppm while at a concentration of 32 ppm no inhibition zone is formed. In contrast to the results shown for Pseudomonas aeruginosa, the methanol extract of isolate AF2 had moderate activity against these types of gram-negative bacteria with the diameter of the inhibition zone formed at a concentration of 128 ppm of 6.69 mm while at a concentration of 64 ppm & 32 ppm the diameter of the inhibition zone formed was 6.69 mm. <5 ppm.

3.4. Identification Results of AF2. Isolate Extract Functional Groups

3.4.1. FTIR Test

The FTIR test was carried out to determine the functional groups contained in the methanol extract of isolate AF2. The results of the functional group analysis are presented in Figure 2.

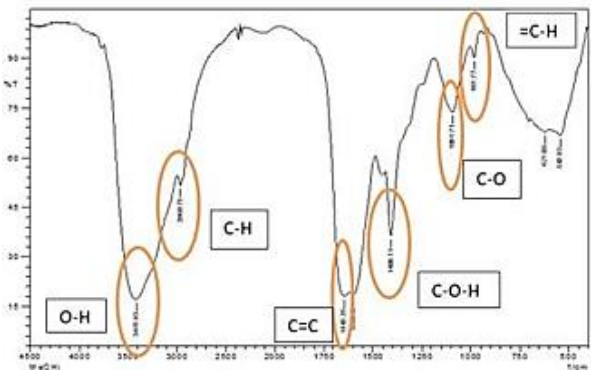

Figure 2. FTIR Spectrum of Isolate AF2

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Interpretation of the FTIR spectrum is presented in Table 4. The resulting spectrum shows the presence of functional groups that absorb at certain frequencies or wavelengths.

Table 4. Interpretation of FTIR

Frequency (cm^{-1})	Frequency Range (cm^{-1})	Functional groups	Compound Type
981.77	650-1000	=CH (oops)	Alkene
1091.71	1050-1300	CO	Alcohols, esters, ethers, carboxylic acids, anhydride
1406.11	1220-1440	COH	Alcohol,Phenol
1643.35	1600-1680	C=C	Alkene
2960.73	2850-3000	CH	Alkanes (strain)
3415.93	3200-3650	OH	Alcohol

3.4.2. GCMS Test

Gas Chromatography Mass Spectrophotometryis a gas chromatography using a mass spectrophotometer detector. Mass spectroscopy works to ionize molecules in the mobile phase, then sorts and identifies masses according to their molecular mass. In this study, GCMS was used to determine the content of active compounds in the methanol extract of AF2 isolates of endophytic bacteria. Results of analysis using GCMS can be seen in Figure 3 and the interpretation of the alleged compounds can be seen in Table 5.

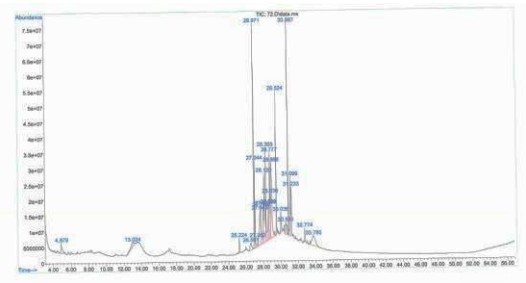

Figure 3. GCMS Spectrum

Table 5. Compound Content

Percentage of Similarities (%)	Compound	Content Percentage (%)
95	pentadecanoic acid methyl ester	10.81
50	Furazano[3,4- D]pyrimidine-5,7(4H,6H)dione	6.2
91	Methyl Palmitoleate	8.25
96	Methyl 15- methylhexadecanot	7.13
50	Pyrrolo[1,2]pyrazi n-1,4-dione, hexahydro- 3-(2-methyl,propyl)-(3S-trans)	12.23
87	Nerolidol	5.68
90	Ergotamine-GC Artifact I	6.94

Secondary metabolites that have been isolated include iridoids, diterpenoids, sesquiterpenes, triterpenoids, flavonoids, isoflavones, xanthones and others. The presence of phytochemical compounds from the genus Premna provides biological effects such as antioxidant, antibacterial, anti-inflammatory, and cytotoxic effects. These biological effects have been reported from the levels of plant extracts and from their pure compounds (Rekha **[1]**). Based on the results of the FTIR analysis test (Table 4) and the results of GCMS characterization, it can be assumed that the bioactive molecule contained in the methanol extract of AF2 isolate that act as an antibacterial is Nerolidol. This is also reinforced by previous research, namely Adjalian et al. (2015) reported that the results of the GCMS analysis of the leaf extract of Premna angolensis (a plant of the same genus) contained the compound (E)-Nerolidol. Nerolidol or 3,7,11-trimethyl-1,6,10-dokodetrien-3-ol is a class of natural sesquiterpene alcohol compounds found in various plants (Weng **[16]**) with the chemical structure shown in Figure 4. According to Sabine **[17]** nerolidol and its derivatives have antibacterial activity against shrimp microbes and antifungal tests have been carried out against plant pathogens.

The results showed that nerolidol has antibacterial activity against various strains of Staphylococcus aureus including MRSA (Multiple Resistance Staphylococcus aureus) by disrupting cell membranes (Hada **[18]**; Inoue **[19]**; Togashi **[20]**). The observed effect could be due to the presence of a long aliphatic chain in the chemical structure of nerolidol. This hypothesis may be in accordance with the findings of Togashi **[20]** that terpene alcohols with carbon chains C10 to C12 showed strong antibacterial activity against S. aureus FDA209P. Since the carbon chain length of nerolidol is C12, it was shown to cause damage to the cell membrane, which leads to leakage of macromolecules and eventually the cell will undergo lysis.

Neroridol compounds in plants are spread in several parts of the plant, including the leaves, seeds, tubers, and parts of the plant that are in contact with the air (aerial part of plants).

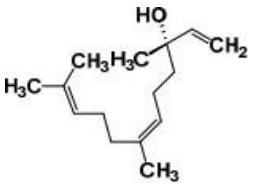

Figure 4. Structure of Nerolidol

4. CONCLUSION

Based on the results of the study, it can be concluded that Methanol extract of endophytic bacterial isolates of green grass jelly plants showed different potential as antimicrobial substances against the two types of test bacteria indicated by the MIC value of 32 ppm in inhibiting the pathogenic bacteria Pseudomonas aeruginosa and against Bacillus subtilis with an MIC value of 64 ppm.

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Results analysis FTIR and GCMS showed that the methanol extract of AF2 isolate of endophytic bacteria of green grass jelly plants contained compound Nerolidol or 3,7,11-trimethyl-1,6,10-dokatriene-3-ol which is compound metabolites secondary group of sesquiterpene alcohols and acts as an antibacterial.

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