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Identification of Bioactive Compounds and Antibacterial Activity Fragrant Pandan Leaf Extract *(Pandanus Amaryllifolius Roxb.)* as an Antibacterial Growth Inhibitor *Pseudomonas Aeruginosa* and *Staphylococcus Aureus*

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ABSTRACT

Pandanus amaryllifolius Roxb. is recognized for its potential as a natural antibacterial agent due to its bioactive constituents. This study aimed to identify bioactive compounds in fragrant pandan leaves and evaluate their antibacterial activity against Pseudomonas aeruginosa and Staphylococcus aureus. Ethanol extracts were fractionated using n-hexane, ethyl acetate, and water. Bioactive compounds in the most active fraction were analyzed using GC-MS. Antibacterial activity was assessed by the disk diffusion method at concentrations of 20%, 40%, 60%, 80%, and 100%, and the data were analyzed using one-way ANOVA followed by Duncan's post-hoc test. GC-MS analysis of the n-hexane fraction tentatively identified 19 compounds with relative area percentages above 1%, predominantly terpenoids, with major constituents including 2(5H)-Furanone, 3-methyl- (6.82%), (-)-Globulol (4.87%), and Caryophyllene (4.12%). All extracts and fractions exhibited inhibitory activity against both test bacteria. The highest inhibition zone against *P. aeruginosa* (10.42 mm) was observed in the n-hexane fraction, while against *S. aureus* (10.00 mm) it was found in the ethanol extract. Statistical analysis showed that concentration and sample type significantly influenced inhibitory activity ($p < 0.05$). This study highlights the novelty of correlating GC-MS-identified compounds in the n-hexane fraction with antibacterial activity, suggesting its potential as a source of natural antibacterial agents.
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1. INTRODUCTION

Medicinal plants have become an important part of traditional medicine in various parts of the world, including Indonesia, which is known as a country with high biological diversity. Various types of plants are used by communities because of their secondary metabolite compounds, such as flavonoids, tannins, saponins, and alkaloids, which contribute to various important biological activities [1]. As scientific knowledge advances, the use of plants is no longer limited to cultural heritage but requires scientific investigation to identify the active compounds they contain and their potential applications in the fields of health and pharmacy. The leaves of the fragrant pandan plant (*Pandanus amaryllifolius Roxb*) are commonly used as a flavoring agent, fragrance, green colorant in dishes, and food preservative to prevent bacterial growth or contamination [2]

Based on the research conducted [3], ethanol extract of pandan wangi leaves (*Pandanus amaryllifolius Roxb.*) can inhibit the growth of bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa* at a concentration of 60%, with each having inhibition zones of 1.25 mm and 1.35 mm respectively, which are categorized as weak. It is recommended to test the antibacterial activity of pandan wangi leaf extract using other antibacterial methods and to evaluate other activities as well.

The extract of pandan wangi leaves is capable of inhibiting the growth of *Staphylococcus aureus* bacteria, as evidenced by the inhibition zones formed on the test medium: at a concentration of 20%, it can inhibit with an average zone of 3.16 mm (weak category); at 40%, an average of 4.16 mm; at 60%, an average of 4.83 mm; in

2023, at 80%, an average of 5.3 mm (moderate category); and at 100%, an average of 6.3 mm (moderate category). Therefore, it can be concluded that pandan wangi leaf extract can be used as an antibacterial agent.

In the study conducted [4], the well diffusion method was used. 10% DMSO served as the negative control, and variations in concentration were tested at 70%, 80%, 90%, and 100%. The average inhibition zone diameters of pandan wangi leaf extract at these concentrations were 9.85 mm, 11.47 mm, 12.05 mm, and 12.77 mm, respectively. The pandan wangi leaf extract exhibited moderate antibacterial activity at 70% concentration, and strong antibacterial activity at 80%, 90%, and 100% concentrations. At these higher concentrations, there was a significant difference in the inhibition zone diameters. The higher the concentration, the larger the inhibition zone diameter formed.

Based on the above background, this research was conducted to identify bioactive compounds in the n-hexane fraction of pandan wangi leaves using Gas Chromatography-Mass Spectrometry (GC-MS) method, as well as to evaluate the antibacterial activity of the extract and its fractions against Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (Pseudomonas aeruginosa). This study aims to obtain in-depth information regarding the potential active compounds contained in pandan wangi leaves and to assess their effectiveness as natural antibacterial agents based on solvent type, concentration, and test bacterial species.

2. RESEARCH METHOD

Identification of bioactive compounds in pandan wangi leaves *(Pandanus amaryllifolius Roxb.)* was conducted using Gas Chromatography-Mass Spectrometry (GC-MS) technique. The pandan leaves samples were collected from the Cikupa area, East Jakarta, then dried, ground into powder, and extracted via maceration using 96% ethanol solvent for 6 × 24 hours. The macerate was sequentially fractionated with n-hexane and ethyl acetate to obtain fractions based on polarity. The fraction with the most prominent activity was analyzed for its compound content using GC-MS instrument at the Chemistry Laboratory of the National Research and Innovation Agency (BRIN), Serpong, South Tangerang. The instrument used was an Agilent 7890B GC (Gas Chromatograph) combined with a 5977A Mass Selective Detector (MSD).

The bioactive compounds from the pandan wangi leaf fractions with the highest antibacterial activity were analyzed using GC-MS (Agilent Technologies 7890) to identify the types of secondary metabolites suspected to be responsible for inhibiting the growth of Pseudomonas aeruginosa. The type of column used was HP Ultra 2-Capillary Column (30 m x 0.20 mm LD, 0.11 µm film thickness). The column temperature was set at 250°C, with helium as the carrier gas at a flow rate of 30 cm/sec, a ratio of 1/30, an ion source temperature of 230°C, and a surface ion temperature of 280°C.

Antibacterial activity testing was performed using the disk diffusion method against two bacterial test strains, namely *Staphylococcus aureus* (Gram-positive) and *Pseudomonas aeruginosa* (Gram-negative). The media used was Mueller-Hinton Agar (MHA), and the bacterial inoculum was adjusted to the McFarland 0.5 standard. Sterile filter paper disks (Ø 6 mm) were dipped into the extract and fraction solutions at various concentrations, then placed on the surface of the MHA plates inoculated with bacterial suspensions. Positive control disks containing a standard antibiotic (e.g., chloramphenicol) were used, while negative controls used the solvent (DMSO or 96% ethanol). The plates were incubated at 37°C for 24 hours. Antibacterial activity was assessed based on the size of the inhibition zone diameter (in mm) around the test disks.

3. RESULT AND DISCUSSION

The results of the identification using GC-MS of the chemical content of the N-hexane fraction of fragrant pandan leaves *(Pandanus amaryllifolius Roxb.)* are shown in Figure 1.

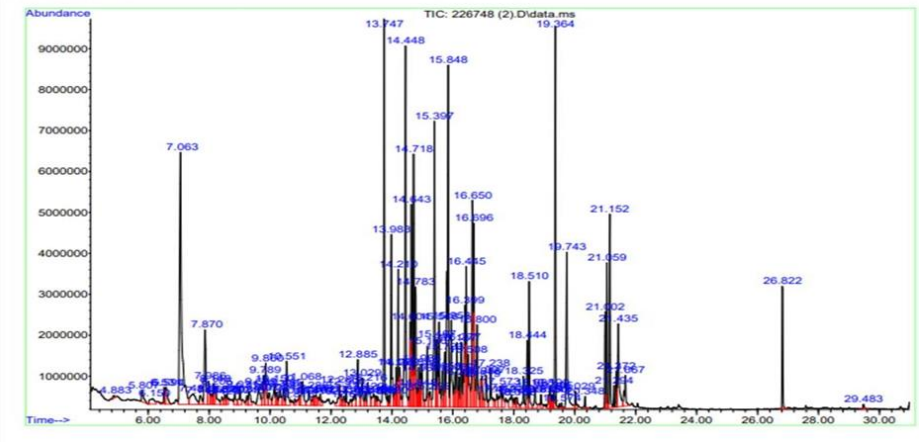


Figure 1. Chemical content of the N-hexane fraction of fragrant pandan leaves *(Pandanus amaryllifolius Roxb.)*

Based on the compound identification results shown in Figure 1, the GC-MS analysis of pandan wangi leaf extract *(Pandanus amaryllifolius Roxb.)* identified 19 chemical compounds with a concentration above 1%. The four compounds with the largest %Area are presented in Table 1. The compound with the highest concentration is 2(5H)-Furanone, 3-methyl- at 6.82%, followed by (-)-Globulol (4.87%), Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- (4.46%), and Caryophyllene (4.12%).

Table 1. Chemical compounds in N-hexane fraction of pandan leaves

No.	Peak	Retention	Area (%)	Compound	Qual
1	6	7.061	6.82	2(5H)-Furanone, 3-methyl-	91
2	51	13.741	4.12	Caryophyllene	99
3	53	13.98	2.13	Aromandendrene	99
4	56	14.207	1.49	Humulene	97
5	58	14.447	4.46	Benzene, 1-(1,5-dimethyl-4-hexenyl)	99
6	60	14.648	2.28	Naphthalene, decahydro...	99
7	61	14.724	2.97	Himachala-2,4-diene	93
8	62	14.787	1.74	(R)-1-Methyl-4-(6-methylhept-5- en-2-yl)	99
9	69	15.392	3.2	Nerolidol 2 / trans-Nerolidyl formate	91
10	71	15.543	1.4	Cyclopropa[e]azulen-4-ol (isomer)	99
11	74	15.846	4.87	(-)-Globulol	99
12	80	16.4	1.66	Humulenol-II	99
13	83	16.652	2.88	aR-Turmerone	98
14	84	16.69	2.07	Neointermedeol	99
15	98	18.505	1.25	2-Pentadecanone, 6,10,14-trimethyl-	97
16	105	19.362	3.47	Hexadecanoic acid, methyl ester	99
17	107	19.74	2.39	n-Hexadecanoic acid	99
18	111	21.063	1.33	9,12,15-Octadecatrienoic acid, methyl ester	99
19	112	21.152	2.08	Phytol	98

Note:
Pk (Peak) : Represents the chromatogram peak number detected during the GC-MS analysis process.
RT (min) : Retention time is the time required for a compound to exit the chromatography column and be detected by the detector.
Qual : Represents the percentage match between the mass spectrum of the detected compound and the reference spectrum in the database (e.g., NIST).

One of the important parameters in the fractionation process is yield, which describes the efficiency of separating compounds from the crude extract into a specific fraction. The yield of the n-hexane fraction of pandan wangi leaves is calculated based on the ratio of the weight of the obtained fraction to the weight of the extract used. The results of the n-hexane fraction yield calculation are presented in Table 2 below :

Table 2. Yield results of n-hexane fraction of fragrant pandan leaves *(Pandanus amaryllifolius Roxb)*.

Extract weight (grams)	Fractional weight	Fraction yield (%b/b)
10 gram	2,1 gram	21%

Phytochemical tests were conducted to determine the content of secondary metabolite compounds in the ethanol extract of pandan wangi leaves and its fractions. The compounds tested included alkaloids, flavonoids, tannins, saponins, terpenoids, and steroids. The phytochemical screening results showed variations in the active compound content in each fraction, which could influence their biological activity. The complete data of the phytochemical test results are presented in Table 3 below.

Table 3. Phytochemical screening of ethanol extracts from fragrant pandan leaves *(Pandanus amaryllifolius Roxb.)*, n-hexane fraction, and ethyl acetate fraction.

Compound	Extract	N-hexane fraction	ethyl acetate fraction
Alkaloid	+	+	+
Flavonoid	+	-	+
Tannin	+	+	+
Saponin	+	+	+
Terpenoid	+	+	-
Steroid	+	+	-

Description:
+ : Contains the compound
- : Does not contain the compound

The Minimum Inhibitory Concentration (MIC) of the ethanol extract and fractions from pandan wangi leaves against the bacteria *Pseudomonas aeruginosa* and *Staphylococcus aureus* are presented in Tables 4 and 5.

Table 4. Minimum Inhibitory Concentration (MIC) *Pseudomonas aureginosa*

Sample	Concentration (%)				
	20	40	60	80	100
Ethanol	++	++	+	+	-
Ethyl acetate	+++	++	++	+	-
N-hexane	++	++	+	-	-

Based on McFarland comparison (visual)
- – : turbidity << McFarland 0.5 (clear)
- + : approximately equivalent to McFarland 0.25 – 0.5 (weak turbidity)
- ++ : approximately equivalent to McFarland ~0.5 – 1.0 (moderate turbidity)
- +++ : approximately equivalent to McFarland ≥ 1.0 – 2.0 (high turbidity)

Table 5. Minimum Inhibitory Concentration (MIC) of *Staphylococcus aureus*

Sample	Concentration (%)				
	20	40	60	80	100
Ethanol	++	+	+	-	-
Ethyl acetate	++	++	+	+	-
N-hexane	+++	++	+	+	-

Based on McFarland comparison (visual)
- – : turbidity << McFarland 0.5 (clear)
- + : approximately equivalent to McFarland 0.25 – 0.5 (weak turbidity)
- ++ : approximately equivalent to McFarland ~0.5 – 1.0 (moderate turbidity)
- +++ : approximately equivalent to McFarland ≥ 1.0 – 2.0 (high turbidity)

Table 6. Inhibitory zone of fragrant pandan leaves *(Pandanus amaryllifolius Roxb.)* against the growth of *Pseudomonas aeruginosa* bacteria

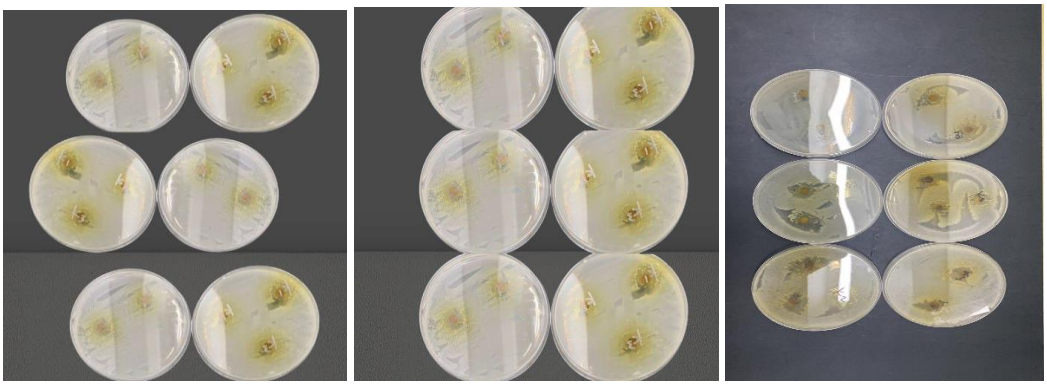
Concentration (%)	Extract (mm)	N-hexane fraction (mm)	ethyl acetate fraction (mm)
20	5,5	7,16	4,3
40	6,78	7,86	5,25
60	6,85	9,35	5,58
80	8,86	9,76	6,93
100	9,21	10,42	7,58
Positive Control	16.03	16.58	15.73
Negative Control	-	-	-

Description:
Positive Control : Chloramphenicol 30 µg/disc
Negative Control : DMSO 5%

Table 7. Inhibitory zone of fragrant pandan leaves *(Pandanus amaryllifolius Roxb.)* against the growth of *Staphylococcus aureus* bacteria

Concentration (%)	Extract (mm)	N-hexane fraction (mm)	ethyl acetate fraction (mm)
20	3,66	2,9	3,37
40	4,89	3,54	4,33
60	5,99	4,16	4,73
80	7,96	4,61	6,22
100	10	5,49	7,36
Positive Control	24.6	20.3	22.6
Negative Control	-	-	-

Description:
Positive Control : Chloramphenicol 30 µg/disc
Negative Control : DMSO 5%



A B C

Figure 2. Inhibition zone results of 96% ethanol extract and fractionation of pandan leaves *(Pandanus amaryllifolius Roxb.)*

Description:
- Image A: Ethanol Extract of Pandan Leaves
- Image B: N-Hexane Fraction
- Image C: Ethyl Acetate Fraction

Discussion

a. GC-MS Analysis

Chemical analysis using Gas Chromatography-Mass Spectrometry (GC-MS) of the n-hexane fraction of pandan wangi leaves (Pandanus amaryllifolius Roxb.) successfully identified 19 bioactive compounds with a content of over 1%, predominantly consisting of terpenoids, aromatic compounds, and saturated fatty acids. The six compounds with the largest %area include: 2(5H)-Furanone, 3-methyl- (6.82%), (-)-Globulol (4.87%), Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- (4.46%), Caryophyllene (4.12%), Hexadecanoic acid, methyl ester (3.47%), and Nerolidol (3.20%). The presence of these compounds is closely correlated with the antibacterial activity exhibited by the n-hexane fraction.

The compound 2(5H)-Furanone, 3-methyl- is a volatile substance capable of disrupting bacterial communication systems (quorum sensing) and biofilm formation, which play a crucial role in bacterial resistance to conventional antibiotics [5]. This compound has also been reported to have growth-inhibitory effects on Pseudomonas aeruginosa, consistent with this study's results where the n-hexane fraction showed the highest antibacterial activity against this Gram-negative bacteria (zone of inhibition 10.42 mm at 100% concentration).

Compounds (-)-Globulol and Caryophyllene, also detected in significant amounts, are classified as sesquiterpenoids known to possess antibacterial activity through mechanisms such as disrupting cell membrane integrity, increasing ion permeability, and inhibiting essential metabolic enzymes. Caryophyllene has been extensively studied as a compound capable of causing physical damage to bacterial membranes, especially in Gram-negative bacteria with complex lipopolysaccharide (LPS) outer membranes ([5]; [6]).

The presence of fatty acids like Hexadecanoic acid, methyl ester (methyl palmitate) contributes to antibacterial properties by increasing bacterial membrane fluidity and inhibiting respiratory enzymes. This compound can induce morphological changes and cellular leakage in bacteria, leading to cell death ([7]).

These GC-MS results reinforce the phytochemical screening findings, which showed that the n-hexane fraction is rich in non-polar compounds such as steroids, saponins, and triterpenoids. These compounds are generally soluble in non-polar solvents and are known to have potent antibacterial effects against Gram-negative bacteria. The efficacy of the n-hexane fraction in inhibiting *Pseudomonas aeruginosa* is also due to its ability to penetrate the lipophilic outer membrane of the bacteria, a key characteristic of lipophilic compounds like terpenoids and furanone [4].

Previous research by Hanafi et al. (2023) demonstrated that pandan wangi leaf extract in nanoparticle form showed an inhibition zone of 11 mm against *Staphylococcus aureus* and Escherichia coli, whereas this study shows that the n-hexane fraction, even without nano formulation, can produce an inhibition zone of 10.42 mm against Pseudomonas aeruginosa. This difference suggests that fractionation using a non-polar solvent can increase the concentration of active compounds contributing to antibacterial effectiveness.

These GC-MS findings align with studies [8] and [4],which state that the antibacterial effectiveness of pandan wangi leaves increases with higher concentrations of active compounds and depends on the type of fraction used. This supports the hypothesis that non-polar fractions are more effective in extracting certain bioactive compounds that target Gram-negative bacteria. In conclusion, the compounds identified via GC-MS in the pandan wangi leaf n-hexane fraction are major contributors to the observed antibacterial activity and hold potential for development as natural antibacterial agents, especially against Gram-negative pathogens like Pseudomonas aeruginosa.

b. Antibacterial Activity

The antibacterial activity of ethanol extract and fractions of pandan wangi leaves (Pandanus amaryllifolius Roxb.) against *Pseudomonas aeruginosa* and *Staphylococcus aureus* shows promising results as candidates for natural antibacterial agents. Testing was performed using the disk diffusion method, with the zone of inhibition as an indicator of the extract or fraction's ability to inhibit bacterial growth. Chloramphenicol was used as a positive control because it is a broad-spectrum antibiotic, while DMSO served as a negative control, which has no antibacterial activity and functions as a neutral solvent.

Based on the results in Table 6, the ethanol extract and pandan wangi leaf fractions exhibited antibacterial activity against Pseudomonas aeruginosa, indicated by the formation of inhibition zones around the paper disks.

The n-hexane fraction showed the highest antibacterial activity compared to the ethanol extract and ethyl acetate fraction. At 100%, the n-hexane fraction produced an inhibition zone of 10.42 mm, while the ethyl acetate fraction was 7.58 mm, and the ethanol extract was 9.21 mm.

Increasing concentrations from 20% to 100% corresponded with a proportional increase in the zone diameter, indicating a dose-dependent relationship where higher concentrations resulted in greater antibacterial activity. This suggests a linear correlation between the concentration of active compounds and their ability to inhibit bacterial growth. The ethyl acetate fraction and ethanol extract showed zones of inhibition, but their sizes were smaller than that of the n-hexane fraction. This can be explained by the solvent properties: as a non-polar solvent, n-hexane tends to extract lipophilic compounds such as terpenoids and steroids, which are known to have higher antibacterial potential against Gram-negative bacteria. Since *Pseudomonas aeruginosa* has an outer membrane rich in lipopolysaccharides (LPS), lipophilic compounds can more easily penetrate and disrupt the bacterial cell wall. Although the maximum zone of inhibition for the n-hexane fraction was still lower than the positive control chloramphenicol (16.03 mm), it demonstrated moderate activity and was more potent than the negative control (DMSO), which showed no activity.

Results in Table 7 show that ethanol extract, n-hexane fraction, and ethyl acetate fraction all inhibit the growth of *Staphylococcus aureus* at various concentrations. Increasing concentrations resulted in larger zones of inhibition. Ethanol extract exhibited the highest zone of inhibition at 10.00 mm at 100%, followed by the ethyl acetate fraction (7.36 mm) and the n-hexane fraction (5.49 mm). According to zone size classification, these values fall into the moderate antibacterial activity category (5–10 mm), with the ethanol extract showing the strongest activity.

The differences in effectiveness can be attributed to the polarity of the solvents and the composition of bioactive compounds in each fraction. Ethyl acetate and ethanol, being semi-polar to polar solvents, are capable of extracting compounds such as flavonoids, tannins, and saponins, which are known to inhibit bacterial growth through mechanisms like cell wall synthesis inhibition and pore formation. Conversely, the non-polar n-hexane tends to extract lipophilic compounds like steroids and terpenoids, which are generally more effective against Gram-negative bacteria. Although *Staphylococcus aureus* is a Gram-positive bacterium with a thick peptidoglycan cell wall but no outer membrane, it is relatively more sensitive to polar compounds. Therefore, it is not surprising that ethanol extract shows higher efficacy against S. aureus compared to the n-hexane fraction in this study.

Research [4], reported that ethanol extract of pandan wangi leaves produced a zone of inhibition of 12.77 mm against S. aureus at 100% concentration using well diffusion method. Although different testing methods were used, this indicates that pandan wangi leaves have potential as an antibacterial against Gram-positive bacteria. Compared to the positive control (chloramphenicol), which produced a zone of 24.6 mm, the activity of the extracts and fractions in this study is classified as moderate to low. The negative control (DMSO) showed no activity, confirming that the inhibition zones are solely due to the active compounds in the extracts and fractions.

This study suggests that n-hexane fraction of pandan wangi leaves has potential as a source of natural antibacterial compounds that could be further developed. Given the plant's relative ease of availability and traditional use, its application in pharmaceutical and health-related fields is promising, especially in developing herbal antiseptics, topical formulations, or active ingredients in sanitation products. Further research, including broader spectrum antibacterial testing, toxicity assessments on mammalian cells, formulation stability studies, and molecular mechanism investigations, are necessary. Isolating pure active compounds and evaluating their mechanisms of action will strengthen pandan wangi's position as a plant-based antibacterial candidate.

c. Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was determined using the broth dilution method. Concentrations tested included 20%, 40%, 60%, 80%, and 100%, added into Muller-Hinton broth medium with a total volume of 5 ml, followed by incubation at 37°C for 24 hours. Turbidity was observed, and the lowest concentration capable of inhibiting bacterial growth was recorded.

Results from Table 4 show that ethanol extract exhibited antibacterial activity starting at 80%, and was truly effective (no growth) at 100%. This indicates that ethanol extract is less effective against *Pseudomonas aeruginosa* at lower to moderate concentrations. Ethyl acetate showed a decreasing trend in bacterial growth, with no growth observed at 100%. Its effectiveness increased with higher concentrations. The n-hexane fraction demonstrated high efficiency, with no bacterial growth at 80% and 100%. This suggests that the non-polar compounds in the n-hexane fraction are more effective against Gram-negative bacteria like P. aeruginosa. The best MIC for P. aeruginosa was the n-hexane fraction at 80%, followed by ethanol at 100%, and ethyl acetate at 100%.

Results for *Staphylococcus aureus* (Table 4) show that ethanol extract inhibited growth at 60%, and was bactericidal at 80%. Ethyl acetate effectively inhibited growth at 80% and 100%, while n-hexane showed efficacy at 100%. The best MIC against S. aureus was ethanol and ethyl acetate at 80%, and n-hexane at 100%.

These differences in MIC values are likely due to variations in bacterial cell wall characteristics: P. aeruginosa, a Gram-negative bacterium with an outer lipopolysaccharide membrane, is more susceptible to non-polar compounds from the n-hexane fraction than S. aureus, which has a thick peptidoglycan layer.

The capacity of each fraction to inhibit bacterial growth is influenced by the content of bioactive compounds extracted. Based on phytochemical screening (Table 3), the n-hexane fraction contains non-polar compounds such as steroids, saponins, and triterpenoids, known for their antibacterial activity. This supports the higher effectiveness of the n-hexane fraction against P. aeruginosa compared to ethyl acetate and ethanol extracts. Research by Hanafi et al. (2023) indicated that pandan wangi leaf extract nanoparticles at 60% concentration produced an inhibition zone of 11 mm against S. aureus. Although formulated as nanoparticles, antibacterial activity still depends on the presence of bioactive compounds in the extract.

The non-polar n-hexane fraction has an advantage in extracting lipophilic compounds like caryophyllene, globulol, and furanone, which are lipophilic and penetrate bacterial membranes easily. This correlates with findings [5], that terpenoid and aliphatic compounds work by destroying bacterial cell walls, especially in Gram-negative bacteria like P. aeruginosa.

d. Statistical Analysis

Data obtained from this study will be analyzed using Two-Way ANOVA to determine whether there is a significant effect of pandan wangi leaf extract on the antibacterial activity against P. aeruginosa and S. aureus. Before performing two-way ANOVA, normality and homogeneity tests will be conducted to ensure data validity. Normality tests check if the data follow a normal distribution. The analysis will be complemented with a Post Hoc Test at a significance level of $\alpha = 0.05$.

Results show that the factor of sample concentration has a sig value of $0.000 < 0.05$, indicating a significant difference in the inhibition zone data of pandan wangi leaves (Pandanus amaryllifolius Roxb.) against P. aeruginosa based on concentrations of 20%, 40%, 60%, 80%, 100%, and positive control. This also implies that the sample concentration influences the inhibition zone of pandan wangi leaves against P. aeruginosa.

The sample type factor has a sig value of $0.000 < 0.05$, indicating significant differences in the inhibition zone data based on the sample of extract, n-hexane fraction, and ethyl acetate fraction. This suggests that the type of sample affects the inhibition zone against P. aeruginosa.

Similarly, for S. aureus, the factor of sample concentration has a sig value of $0.000 < 0.05$, indicating significant differences based on concentrations of 20%, 40%, 60%, 80%, 100%, and positive control, with higher

concentrations resulting in larger inhibition zones. The sample type factor has a sig value of 0.001 < 0.05, showing significant differences based on extract, n-hexane, and ethyl acetate fractions.

Statistically, increasing the concentration of pandan wangi extract or fractions results in larger inhibition zones, indicating a positive dose-response relationship. This supports the theory that higher levels of bioactive compounds enhance antibacterial effectiveness.

Research by [9] demonstrated that pandan wangi extract showed a significant increase in antibacterial activity against S. aureus and E. coli with increasing concentrations (from 10% to 100%), consistent with the trend observed here.

The type of sample (extract, n-hexane fraction, ethyl acetate fraction) also significantly affects the inhibition zone, with ethyl acetate generally producing larger zones than n-hexane or crude extracts. This is likely due to the higher solubility of phenolic and flavonoid compounds, which are known to have antimicrobial activity, in ethyl acetate.

Research [10] supports this, indicating that the ethyl acetate fraction contains active compounds like phenolic acids, flavonoids, and fatty acid derivatives with high antibacterial activity, especially against S. aureus, consistent with this study's findings.

In comparing the effectiveness against P. aeruginosa and S. aureus, it is noted that S. aureus, a Gram-positive bacterium with a thick peptidoglycan layer, is generally more sensitive to polar compounds than P. aeruginosa, which has an outer lipopolysaccharide membrane making it more resistant. Nonetheless, pandan wangi leaves demonstrated significant inhibitory effects against both bacteria, with larger zones typically observed against S. aureus.

The statistical results and inhibition zone data support that the concentration and type of pandan wangi leaf fractions have a significant influence on antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The ethyl acetate fraction shows the highest activity, likely due to its higher content of polar bioactive compounds. These findings are consistent with recent literature that supports the use of specific fractions to enhance the antibacterial efficacy of herbal plant extracts.

4. CONCLUSION

This study successfully identified bioactive compounds in the n-hexane fraction of pandan wangi leaves (Pandanus amaryllifolius Roxb.) using GC-MS methodology, as well as evaluated their antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The GC-MS analysis revealed the presence of 19 bioactive compounds with a percentage area above 1%, including 2(5H)-Furanone, 3-methyl- (6.82%), (-)-Globulol (4.87%), and Caryophyllene (4.12%), which are known to possess antibacterial potential through mechanisms such as disrupting cell membranes and inhibiting bacterial metabolism.

The disk diffusion antibacterial activity test showed that all extracts and fractions could inhibit the growth of both test bacteria, with the highest activity against *Pseudomonas aeruginosa* demonstrated by the n-hexane fraction (zone of inhibition 10.42 mm at 100% concentration), while the highest activity against *Staphylococcus aureus* was exhibited by the ethanol extract (zone of inhibition 10.00 mm at 100%). Statistical analysis indicated that both the type of sample and concentration had a significant effect ($p<0.05$) on the antibacterial inhibition zones.

Based on these results, it can be concluded that the n-hexane fraction of pandan wangi leaves has the potential as a source of natural antibacterial compounds, especially against Gram-negative bacteria, and could be further developed as an active ingredient in phytopharmaceuticals or plant-based antiseptic products. Further studies are needed for toxicity testing, isolation of pure compounds, and the development of more applicable formulations.

5. ACKNOWLEDGEMENT

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6. REFERENCES

[1] Imansyah, R. The Potential of Secondary Metabolite Compounds in Medicinal Plants for the Development of Herbal Medicines. *Indonesian Journal of Herbal Pharmacy*, 6(2), 112–119. 2022

[2] Ismiyati, T., Nugroho, A., & Yuliana, D. Utilization of Pandan Wangi Leaves in the Food and Health Industry. Journal of Food Technology and Health, 3(1), 34–40.Finch, D. J., Hamilton, L. K., Baldwin, R., Zehner, M. (2013). *An exploratory study of factors affecting undergraduate employability*. 2021

[3] National Center for Biotechnology Information (NCBI). *Taxonomy Browser: Staphylococcus aureus*.

https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=1280. 2024

[4] Zuraida, R., Aulia, N., & Sari, D. Antibacterial Activity of Pandan Wangi Leaf Ethanol Extract against *Staphylococcus aureus. Journal of Pharmaceutical Sciences*, 7(1), 19–26. 2021

[5] Khan, A. U., Rehman, M. U., Zahoor, M., Shah, A. B., & Zekker, I. Biodegradation of Brown 706 Dye by Bacterial Strain Pseudomonas aeruginosa. *Water*, *13*(21), 2959. 2021

[6] Suryani, W., Handayani, R., & Malik, R. Antibacterial Activity of Pandan Leaves against Oral Pathogenic Bacteria. *Journal of Health Research*, 11(2), 117–122. 2023

[7] Nurwahida, A., Yuliani, N., & Tanjung, H. Effectiveness of Pandan Wangi Leaf Nanoparticle Extract on the Growth of *Staphylococcus aureus. Indonesian Journal of Pharmaceutical Chemistry*, 12(1), 23–29. 2023

[8] Karimah, D., Syafitri, N., & Rahmawati, D. Testing the Antibacterial Activity of Pandan Wangi Leaf Ethanol Extract against *Staphylococcus aureus* and Pseudomonas aeruginosa. *Indonesian Journal of Health Sciences*, 14(2), 75–82. 2024

[9] Puspitasari, D., Hanafi, M., & Wahyudi, D. Antibacterial Activity of Nano-Particles from Pandanus Amaryllifolius Roxb Leaf Extract Against Escherichia coli and *Staphylococcus aureus*. *Journal of the Aceh Health Polytechnic*, 3(1). https://doi.org/10.30867/jifs.v3i1.340. 2023

[10] Setiyanto, R., Amalia, A. R., & Christa, E. F. The Feasibility of Pandan Leaf Extract (Pandanus amaryllifolius) as a Formulation for Peel-Off Gel Mask. *Journal of Health Research "Forikes Voice,"* 15(3), 460–465. 2024