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EXTRACTION, PHYTOCHEMICAL SCREENING, TOTAL PHENOLIC CONTENT, AND ANTIBACTERIAL ACTIVITY OF MISTLETOE ON OIL PALM (Ficus heteropleura Blume) LEAVES

Fauzia Hamiro¹, Fitra Perdana^{1,2*}, Jufrizal Syahri¹

¹Chemistry Study Program, Universitas Muhammadiyah Riau, Pekanbaru, Indonesia ²Department of Chemistry, Universitas Riau, Pekanbaru, Indonesia *Email: fitra.perdana@lecturer.unri.ac.id

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ABSTRACT

Ficus heteropleura Blume, commonly known as mistletoe on oil palm, is a semi-parasitic plant that grows on oil palm trees. This study aimed to investigate the secondary metabolite profile, determine of total phenolic content, and evaluate antibacterial activity of ethyl acetate extract of F. heteropleura leaves. Extraction was performed using a grade maceration method. Qualitative phytochemical screening was conducted to identify major classes of secondary metabolites, and total phenolic content was quantitatively determined using the Folin-Ciocalteu method with gallic acid as the standard. Ethyl acetate extraction yielded 19.27 g of crude extract. Phytochemical analysis revealed the presence of steroids, flavonoids, saponins, and tannins. The total phenolic content was determined to be 35.730 mg GAE/g. UV-Vis spectrophotometric analysis showed characteristic absorption peaks at 475.0 nm and 432.8 nm, indicating the presence of phenolic pigments with conjugated C=O groups and a peak at 666.3 nm associated with chlorophyll, contributing to the green coloration. FTIR analysis confirmed the presence of functional groups such as O-H, C=C, C=O, C=C, and C-O. Antibacterial activity demonstrated that the ethyl acetate extract exhibited strong inhibitory effects against Staphylococcus aureus and Escherichia coli, with average inhibition zone diameters ranging from 5 to 10 mm, indicating strong antibacterial activity.

Keywords: mistletoe on oil palm, *Ficus heteropleura* Blume, total phenolic, antibacterial

INTRODUCTION

Indonesia is well known for its rich biological diversity, especially the wide variety of indigenous plant species that exhibit promising medicinal properties. This rich biodiversity is primarily supported by Indonesia's favorable natural environment, characterized by its tropical climate and nutrient-rich soils (Tahar et al., 2023). The use of herbal remedies in traditional medicine has demonstrated therapeutic efficacy and is generally associated with fewer adverse

effects compared to synthetic pharmaceuticals. In addition, herbal treatments are often more affordable and accessible. Medicinal plants are commonly used by local communities to manage minor ailments such as pain, headaches, gastritis, diarrhea, allergies, and skin conditions (Nursanti et al., 2023). With over 30.000 medicinal plant species identified in Indonesia-out of approximately 40.000 known globally-around 90% are utilized within Asia, and approximately 7.500 species (25%) possess documented medicinal properties. Nonetheless, only about 1.200 of these have been developed into herbal medicinal products (Udayani et al., 2023).

Plants naturally produce secondary metabolites, which are widely utilized in the pharmaceutical industry. Major classes of these compounds include alkaloids, flavonoids, saponins, steroids, and tannins. Secondary metabolites are low molecular weight compounds that play essential roles in protecting plants against insect attacks, microorganisms, and herbivorous animals. Additionally, these compounds aid plants in adapting to unfavorable environmental conditions. In nature, secondary metabolites function as antibacterial, antioxidant, antifungal, and antiviral agents, and they also show potential as herbicides and insecticides (Ningsih et al., 2020).

The increasing public interest in natural remedies has led to greater recognition of plant-based medicinal products. The use of medicinal plants in traditional healthcare continues to grow and is regarded as a practical approach to meeting community health demands. This is largely due to the advantages offered by traditional medicine, including lower cost, greater accessibility, and fewer adverse effects. Herbal treatments are particularly valued for their reduced risk of side effects, unlike many synthetic drugs which are often associated with negative reactions (Rizkayanti et al., 2017).

Mistletoe on oil palm (*Ficus heteropleura* Blume) is a parasitic plant that grows by attaching to a host. Despite its parasitic nature, mistletoe holds potential as a medicinal plant. The secondary metabolites it contains can vary depending on the host, as the plant absorbs nutrients and compounds from its host to sustain its survival. Almost all parts of *Ficus* species can be utilized in various ways, ranging from traditional medicine to simple tools and lightweight boat frames. Overall, plants of the *Ficus* genus are recognized for their potential as sources of food and medicine (Thoyyibah & Angio, 2023).

The exploration of *Ficus* species for pharmacological applications has gained attention due to their rich content of polyphenols and flavonoids, which are known to possess antibacterial properties (Sukmawati, 2019). Flavonoids exert antimicrobial effects by inhibiting Lantanida Journal, 13(2): 188-202

nucleic acid synthesis, disrupting bacterial cell membrane integrity, and interfering with oxygen utilization, ultimately suppressing bacterial proliferation. Similarly, phenolic compounds exhibit antibacterial activity by compromising the bacterial cell wall and deactivating essential enzymes (Ramayani et al., 2021). These phenolics are widely distributed in plants and encompass a broad range of structural classes, including flavonoids, simple monophenols, polyphenols, phenylpropanoids, and phenolic quinones (Cahya et al., 2021).

Antibacterial compounds are chemical agents or drugs designed to kill or inhibit the growth of bacteria, particularly pathogenic species that pose a threat to human health. These agents belong to the broader category of antimicrobial substances, with specific activity against bacterial cells. Among the most common human pathogens are *Staphylococcus aureus* and *Escherichia coli*. *E. coli* is known to produce enterotoxins that can lead to acute foodborne illness, while *S. aureus* can trigger inflammatory responses, particularly in skin tissues (Yevani et al., 2023).

In general, pathogenic bacteria that commonly infect humans and other mammals include *S. aureus* and *E. coli. S. aureus* is a Gram-positive bacterium that can produce harmful toxins when its population reaches concentrations of approximately 10⁵ CFU/mL. In contrast, *E. coli* is a Gram-negative, rod-shaped bacterium that can grow under both aerobic and facultative anaerobic conditions. When *E. coli* populations reach levels of 10⁶ CFU/mL, they are capable of producing toxins that may exert toxic effects on the host (Azkiyah, 2020).

This study aimed to extract the leaves of mistletoe on oil palm using a grade maceration method with solvents of increasing polarity: *n*-hexane, ethyl acetate, and ethanol. Phytochemical screening, determination of total phenolic content, and antibacterial activity assays against *E. coli* and *S. aureus* were conducted on the ethyl acetate extract. This research is expected to contribute to the growing body of knowledge on the antibacterial potential of mistletoe on oil palm leaves and support their development as a natural therapeutic agent.

RESEARCH METHODS

Materials

The materials used in this study included mistletoe on oil palm (*Ficus heteropleura* Blume) leaves, distilled water, FeCl₃ (Merck), ethanol (Merck), concentrated HCl (Merck), Mg powder (Merck), H₂SO₄ (Merck), Mayer's reagent (Merck), Wagner's reagent (Merck), Dragendorff's reagent (Merck), Folin-Ciocalteu reagent, *Aqua DM*, Na₂CO₃, *Nutrient Agar*

(NA) (Merck), *Nutrient Broth* (NB) (Merck), *Mueller Hinton Agar* (MHA), DMSO (Merck), 0.9% NaCl (Merck), and suspensions of *S. aureus* and *E. coli*.

Equipments

The equipment used in this study included filter paper (Whatman), vacuum rotary evaporator (Büchi Rotavapor R-3), glassware (Iwaki), micropipettes (Dragonlab), analytical balance (Shimadzu), hot plate (Nesco), oven (B-One), microwave (Cosmos), incubator (Memmert), shaker incubator (Argo Lab), colony counter (Bexco), autoclave (All American), vortex mixer (Star Lab), UV-Vis spectrophotometer (Shimadzu), and FT-IR spectrophotometer.

Sample Preparation and Extraction

The collected mistletoe on oil palm leaves were first washed with running water to remove any adhering dust or impurities. After cleaning, the leaves were air-dried for approximately two weeks to reduce moisture content. Once dried, the leaves were ground into a fine powder using a blender to obtain simplicia powder and stored in a closed container at room temperature.

The extraction process was carried out by weighing 800 g of dried powdered mistletoe on oil palm leaves and performing grade maceration. The solvents used were *n*-hexane, ethyl acetate, and ethanol. Initially, the powdered simplicia was macerated with *n*-hexane, followed by ethyl acetate extraction of the resulting residue. The residue from the ethyl acetate extraction was then extracted with ethanol. Each maceration was conducted for 3x24 hours with occasional stirring. The soaking process was repeated until the sample became clear. The mixture was then filtered to separate the filtrate from the residue. The ethyl acetate filtrate was concentrated using a rotary evaporator at the appropriate solvent temperature until a thick extract was obtained (Norhaslinda et al., 2023).

Phytochemical Test of Ethyl Acetate Extract

Qualitative phytochemical screening was based on the method described by Rati et al. (2024) with modifications. Tannin test was conducted by 0,5 grams of the extract were dissolved in 10 mL of hot water, followed by the addition of 1% FeCl₃ solution. The formation of a dark green coloration was indicative of tannin content in the extract.

Flavonoid test was performed by mixing 0,5 grams of the extract with 5 mL of ethanol, followed by heating for approximately 5 minutes. Subsequently, 10 drops of Lantanida Journal, 13(2): 188-202

concentrated HCl and 0,2 grams of magnesium powder were added. The appearance of a reddish-black, yellow, or orange coloration indicated a positive result for the presence of flavonoids.

Saponin test was carried out by placing 0,5 grams of the extract into a test tube, followed by the addition of 10 mL of hot distilled water. The mixture was vigorously shaken for approximately 1 minute and then allowed to stand for 10 minutes. The formation of stable froth or foam indicated a positive result for the presence of saponins.

Steroid and terpenoid tests were conducted by placing 0,5 grams of the extract into a test tube, followed by the addition of 2 mL of concentrated H₂SO₄. The mixture was gently shaken and allowed to stand for several minutes. The appearance of a blue to green coloration indicated the presence of steroids, while a reddish-brown to purple coloration signified the presence of terpenoids.

Alkaloid test was performed by dissolving 2 grams of the extract in 5 mL of 2 N hydrochloric acid, followed by gentle heating and subsequent cooling. The resulting solution was evenly distributed into three separate test tubes, each containing 1 mL. To each tube, one of the following reagents was added: Mayer's, Wagner's, or Dragendorff's. A positive indication of alkaloids was observed through the formation of distinct precipitates: a white or yellow precipitate with Mayer's reagent, a brown precipitate with Wagner's reagent, and an orange precipitate with Dragendorff's reagent. These reactions collectively confirmed the presence of alkaloid constituents in the extract.

Total Phenolic Content Determination

A total of 0,3 mg of the extract was dissolved in 3 mL of distilled water until homogeneous. Then, 0,1 mL of the extract solution was pipetted and mixed with Folin-Ciocalteu reagent, followed by thorough shaking and incubation for 3 minutes. Subsequently, 1,2 mL of 7% sodium carbonate (Na₂CO₃) solution was added, and the mixture was incubated at room temperature for 60 minutes. The absorbance was measured using a UV-Vis spectrophotometer at a maximum wavelength of 759 nm.

Characterization

Ethyl acetate extract of mistletoe on oil palm leaves was characterized using a UV-Vis spectrophotometer (Shimadzu) to observe wavelength shifts associated with color changes, and an FTIR spectrophotometer to identify alterations in functional groups.

Antibacterial Activity

Antibacterial activity was carried out *in vitro* using the agar diffusion method with 6 mm diameter paper disks. Sterile *Mueller Hinton Agar* (MHA) medium was heated to a temperature of 40°C-45°C. A 1 mL aliquot of bacterial suspension was aseptically transferred into a petri dish, followed by the addition of 12-15 mL of MHA medium. The mixture was homogenized and allowed to solidify. Paper disks impregnated with ethyl acetate extract at concentrations of 20%, 40%, and 60% were then placed onto the agar surface. A positive control was prepared using the antibiotic chloramphenicol, while DMSO served as the negative control. All petri dishes were wrapped with paper and incubated at room temperature or 37°C for 24 hours. The inhibition zones were measured using a caliper. The antibacterial effectiveness of the ethyl acetate extract was assessed by comparing the inhibition zones with those produced by chloramphenicol against *S. aureus* and *E. coli*.

RESULTS AND DISCUSSION

Sample Preparation and Extraction

The extraction process was carried out to isolate the bioactive compounds present in mistletoe on oil palm leaves. The result of the extraction using ethyl acetate as the solvent is presented in Table 1.

Table 1. Results of Extraction

Sample	Extract weight (g)	Yield (%)
Fresh leaves	2400.00	-
Dried leaves	800.00	33.300
Ethyl acetate extract	19.27	2.404

Samples of mistletoe on oil palm leaves used in this study were collected from Sabak Auh, Siak Regency, Riau Province. A total of 2.400 grams of fresh (wet) leaves were obtained after separating them from the stems. The leaves were then dried at room temperature without direct sunlight exposure to prevent the degradation of active compounds contained within. This drying process aimed to reduce the moisture content in the samples, inhibit microbial growth, and halt enzymatic activities that could potentially break down bioactive compounds (Budiana, 2023).

The dried mistletoe on oil palm leaves were then ground into powder, yielding 800 g of powdered material. The purpose of powdering the dried leaves was to reduce particle size and increase the surface area contact between the plant material and the solvent, facilitating optimal Lantanida Journal, 13(2): 188-202

penetration and extraction of phytochemical compounds. The powdered samples were stored in dark bottles for subsequent stepwise maceration. The maceration process involved soaking the samples in *n*-hexane and ethyl acetate solvents, with repeated extractions. The resulting macerates were filtered, and the filtrates were concentrated using a rotary evaporator. The thick ethyl acetate extract obtained from the fractionation process was then weighed to determine its mass and calculate the yield percentage. This yield calculation served to assess the efficiency of ethyl acetate in extracting secondary metabolites from the sample, although it does not provide information on the specific types of metabolites extracted. The thick ethyl acetate extract of oil palm mistletoe leaves was then used in subsequent stages, including secondary metabolite screening, characterization, and antibacterial activity testing.

Phytochemical Test

Phytochemical tes was performed on ethyl acetate extract to identify groups of compounds present in the extract. The results of the screening are presented in Table 2.

Table 2. Results of Phytochemical Test

Groups of of	Reagent	Change	Result
compound			
Alkaloids	- HCl 2N + Mayer	- No white or yellow precipitate was formed	-
	- HCl 2N + Dragendorff	- No orange precipitate was formed	-
Terpenoids	H_2SO_4	A reddish-brown to purple color was formed	-
Steroids	H_2SO_4	No blue to green coloration was observed	+
Flavonoids	Etanol, concentrated HCl, 0,2 g Mg	A reddish-black, yellow, or orange coloration was observed	+
Saponins	Hot distilled water	Foam was observed.	+
Tannin	Hot distilled water + FeCl ₃ 1%	A dark green coloration was observed	+

Note: (+): Identified; (-): Not identified

Total Phenolic Content Determination

In this study, quantitative analysis was conducted to determine the total phenolic content in the ethyl acetate extract of mistletoe on oil palm leaves. The results of the analysis are presented in Table 3.

Table 3. Results of Total Phenolic Content Determination

Sample	Replication	Absorbance	AVG Absorbance	Total Phenolic (mg GAE/g)
Extuset	1	0.3638	0.2629	35.730
Extract 2	0.3638	0.3638	33./30	

The absorbance values of the gallic acid standard produced a calibration curve with the linear regression equation y = 0.0025x + 0.0065 and a correlation coefficient $R^2 = 0.9954$. The total phenolic content in ethyl acetate extract is expressed as GAE (*Gallic Acid Equivalent*), which represents the amount of gallic acid (in milligrams) equivalent per gram of extract. The total phenolic content of ethyl acetate extract was found to be 35.730 mg GAE/g extract, indicating that each gram of extract contains phenolic compounds equivalent to 35.730 mg of gallic acid. The phenolic compounds present in the extract are secondary metabolites with potential as antibacterial agents and pharmaceutical raw materials.

Characterization

The UV-Vis spectrophotometric characterization of ethyl acetate extract of mistletoe on oil palm leaves provided the absorption wavelength of the extract. The data are presented in Table 4.

Table 4. UV-Vis Spectrum Wavelength of Extract

Monitoring	Wavelength (nm)	Note
	666.3	_
Extract	475.0	$\pi o \pi^*$
	432.8	

The UV-Vis spectrum of the extract obtained at 77°C revealed three absorption peaks within the visible region (400–800 nm). The appearance of these peaks indicates the presence of chromophore groups, particularly conjugated C=C double bonds and carbonyl (C=O) groups. The maximum absorbance detected within the 300–700 nm wavelength range reflects $\pi \to \pi^*$ electronic transitions, which are characteristic of conjugated double bonds such as C=C and carbonyl groups. Tannins, as natural polyphenolic compounds, typically contain phenolic hydroxyl groups, carboxyl groups, and chromophore moieties responsible for coloration. The presence of conjugated C=C and C=O chromophores supports the assumption that the brown coloration in the ethyl acetate extract may originate from its tannin content (Dewi & Ridlo, 2018). UV-Vis spectrophotometric analysis indicates the presence of pigmented compounds

such as conjugated phenolics and chlorophyll, both of which contribute to the greenish color of ethyl acetate extract of mistletoe on oil palm leaves.

Ethyl acetate extract of mistletoe on oil palm leaves was further subjected to characterization using Fourier Transform Infrared (FT-IR) spectroscopy. The resulting spectral data are illustrated in the following Figure 1.

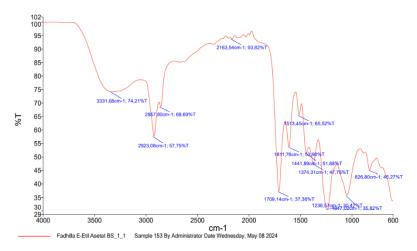


Figure 1. FTIR Characterization Result of Ethyl Acetate Extract

FTIR characterization results of mistletoe on oil palm leaves extract can be analyzed based on the data presented in Table 5.

Table 5. FTIR Characterization of Extract

Wavenumber (cm ⁻¹)	Functional Groups
3331.68	О-Н
2923.08	С-Н
2857.80	С-Н
2163.54	C≡C
1709.14	C=O
1611.76	C=C
1513.45	C=C
1441.89	C=C
1374.31	С-Н
1238.57	C-O
1047.02	C-O
826.80	С-Н

The FTIR spectrum of ethyl acetate extract of mistletoe on oil palm leaves shows an absorption band in the wavenumber region of 3200–3600 cm⁻¹, with a major peak observed at 3331.68 cm⁻¹. This band indicates the presence of stretching vibrations of hydroxyl (–OH) groups. In the range of 3300–3500 cm⁻¹, stretching vibrations of amine (–NH) groups may also

be detected; however, their signals are often overlapped by the broader –OH band. The –NH group typically gives rise to one or two sharp absorption bands with relatively low intensity. When –OH groups are present within the same region, the resulting absorption band tends to be broader, indicating the dominant contribution of hydroxyl group vibrations (Putri et al., 2023).

The FTIR spectrum of ethyl acetate extract of mistletoe on oil palm leaves reveals the presence of C=C stretching vibrations characteristic of alkene functional groups, observed within the wavenumber range of 1625–1440 cm⁻¹, with notable peaks at 1611.76, 1513.45, and 1441.89 cm⁻¹. Additionally, absorption bands appearing at 2923.08 and 2857.80 cm⁻¹ fall within the 2850–2970 cm⁻¹ range, indicating the stretching vibrations of aliphatic C–H bonds associated with alkane groups. These sp³ C-H stretching vibrations are typically observed below 3000 cm⁻¹. Furthermore, a distinct absorption at 2163.54 cm⁻¹ suggests the presence of a terminal alkyne (C≡C) group, supporting the identification of unsaturated hydrocarbon functionalities in the extract (Wahdaningsih et al., 2022).

The absorption bands within the wavenumber range of 1000–1300 cm⁻¹ indicate stretching vibrations of ester functional groups (C-O). In the FTIR spectrum of ethyl acetate extract of mistletoe on oil palm leaves, ester groups are identified by absorption peaks at 1238.57 cm⁻¹ and 1047.02 cm⁻¹. Additionally, absorption in the 675–995 cm⁻¹ region corresponds to bending vibrations of C-H bonds from aromatic compounds. Although the stretching vibrations of aromatic C–H typically appear above 3000 cm⁻¹ (between 3010–3050 cm⁻¹), this region often lacks specificity for distinguishing aromatic compounds from alkenes. Therefore, the observed aromatic C-H bending vibration at 826.80 cm⁻¹ in the ethyl acetate extract spectrum serves as an indicator of the presence of aromatic rings in the sample (Sunardi, 2023).

Based on Figure 1, the FT-IR spectrum of ethyl acetate extract of mistletoe on oil palm leaves indicates the presence of several classes of secondary metabolites, including flavonoids, tannins, saponins, and steroids. This is supported by the appearance of strong absorption bands in the wavenumber regions associated with stretching vibrations of hydroxyl groups (-OH). Although the presence of -OH groups may suggest phenolic or flavonoid compounds, the identification of specific compounds cannot be confirmed solely based on this functional group. The detection of ether (C-O) groups at wavenumbers 1238.57 cm⁻¹ and 1047.02 cm⁻¹ further supports the presence of phenolic and flavonoid constituents in the extract. These C-O stretching vibrations also suggest that polar compounds are present in ethyl acetate

extract. Moreover, the absorption band at 1709.14 cm⁻¹, associated with the stretching vibration of carbonyl groups (C=O), indicates the presence of tannins in the extract.

The presence of C–H group vibrations supports the assumption that C–H bonds exhibit sufficient intensity to induce the appearance of nonpolar compounds, such as terpenoids and steroids, as observable vibrational bands. The detection of C–H stretching bands indicates that these compounds are present in the ethyl acetate extract. The presence of C–H groups also reflects the significant absorption of nonpolar compounds, such as terpenoids and steroids, which are characterized by high carbon content and long-chain structures.

Antibacterial Activity

The results of the antibacterial activity test of *E. coli* and *S. aureus* using ethyl acetate extract at concentrations of 20%, 40%, and 60%, conducted through the disk diffusion method, are presented in Table 6.

Table 6. Results of Antibacterial Activities of Ethyl Acetate Extract

Bacterial	Concentration	Average of inhibition zone (mm)
E. coli	20%	8.29
	40%	9.20
	60	10.26
	Control +	20.35
	Control -	0.00
S. aureus	20%	7.51
	40%	8.12
	60%	10.19
	Control +	20.39
	Control -	0.00

Antibacterial activity test of the ethyl acetate extract of mistletoe on oil palm leaves was carried out using the disk diffusion method. The assay was performed in triplicate to ensure data consistency and to evaluate the effectiveness of the extract in inhibiting the growth of *E. coli* and *S. aureus*. Antibacterial activity was determined based on the diameter of the inhibition zone (clear zone) formed around the test disks on the medium.

Mueller Hinton Agar (MHA) was employed as the culture medium in this study, as it is widely accepted as a standard medium for evaluating antibiotic sensitivity due to its nutrient composition-particularly casein peptone and starch-which supports the growth of a wide range of bacterial species (Pertiwi et al., 2022). The extract was tested at concentrations of 20%, 40%, and 60% to assess the relationship between extract concentration and its potential antibacterial efficacy.

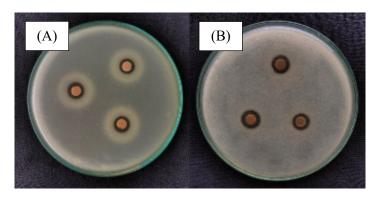


Figure 2. Antibacterial Activity of 60% Ethyl Acetate Extract of Mistletoe on Oil Palm Leaves Against (A) *E. coli* and (B) *S. aureus*

Antibacterial activity test results showed that the ethyl acetate extract of mistletoe on oil palm leaves produced inhibition zone diameters against E. coli of 8.29 mm, 9.20 mm, and 10.26 mm at concentrations of 20%, 40%, and 60%, respectively. As a comparison, the positive control using chloramphenicol (30 μ g) exhibited an inhibition zone diameter of 20.35 mm. Meanwhile, against E0. E1 aureus, the same extract produced inhibition zones of 7.51 mm, 8.12 mm, and 10.19 mm at 20%, 40%, and 60% concentrations, respectively, while the positive control resulted in an inhibition zone of 20.39 mm. Chloramphenicol (30 μ g) was used as the positive control in this study due to its broad-spectrum antibacterial properties, being effective against both Gram-positive and Gram-negative bacteria, thus serving as a standard reference for evaluating the antibacterial efficacy of the tested extract (Suryani et al., 2024).

The average diameter of the inhibition zones can be categorized based on their inhibitory strength. Antimicrobial activity is classified into four categories: weak activity with an inhibition zone < 5 mm, moderate activity with an inhibition zone of 5-10 mm, strong activity with an inhibition zone of 10-20 mm, and very strong activity with an inhibition zone of > 20-30 mm (Nabilla & Advinda, 2022). Based on these categories, the results of this study indicate that the ethyl acetate extract of mistletoe on oil palm leaves exhibits strong antimicrobial activity against *S. aureus* and *E. coli*, as the inhibition zones produced fall within the 5-10 mm range (Putri et al., 2023).

CONCLUSION

The ethyl acetate extract of mistletoe on oil palm leaves was found to contain secondary metabolites, including steroids, flavonoids, saponins, and tannins, as confirmed by qualitative phytochemical screening. Quantitative analysis revealed a total phenolic content of 35.730 mg GAE/g, expressed as gallic acid equivalents. UV-Vis spectrophotometric analysis indicated the presence of pigmented compounds, particularly conjugated phenolics, as evidenced by Lantanida Journal, 13(2): 188-202

absorption peaks at 475.0 nm and 432.8 nm, along with chlorophyll, which exhibited a characteristic peak at 666.3 nm and contributed to the extract's green coloration. Further FT-IR spectroscopic characterization revealed functional groups such as -OH, -CH, $C \equiv C$, C = O, C = C, and C = O, which are indicative of bioactive constituents. The antibacterial activity of the extract, evaluated against *S. aureus* and *E. coli*, demonstrated moderate inhibitory effects, with inhibition zone diameters ranging from 5 to 10 mm.

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