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RESEARCH PAPER

Extraction and total phenolic content from mistletoe on oil palm (*Ficus heteropleura* Blume) leaves and evaluation of their antibacterial and antioxidant activities.

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Abstract: Ficus heteropleura Blume, commonly known as mistletoe, is a semi-parasitic plant frequently found on oil palm plants. This study aimed to evaluate the secondary metabolite content, total phenolic concentration, and the antibacterial and antioxidant activities of the ethanol extract of Ficus heteropleura leaves. Extraction was conducted via grade maceration and total phenolic content was determined using the Folin–Ciocalteu method with gallic acid as the standard. Antibacterial activity was tested using the disc diffusion method against Staphylococcus aureus and Escherichia coli, while antioxidant potential was evaluated through the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. Phytochemical screening confirmed the presence of flavonoids, terpenoids, saponins, tannins, and phenolics with a total phenolic content of 25.500 mg GAE/g. The extract exhibited moderate antibacterial activity with inhibition zones ranging from 7.5 to 10.3 mm. The antioxidant activity was classified as very strong with an IC_{50} value of 0.67 µg/mL, compared to 0.45 µg/mL for ascorbic acid.

Keywords: Ficus heteropleura Blume, Total Phenolic, Antibacterial, Antioxidant

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INTRODUCTION

The human body is highly susceptible to microbial exposure, with the skin, as the outermost organ, being particularly vulnerable [1]. Various groups of microorganisms are responsible for causing infections in humans, among which pathogenic bacteria, such as *Staphylococcus* and *Streptococcus*, are significantly implicated in skin infections. Meanwhile, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella*, and *Vibrio* have been reported as causative agents of foodborne illnesses [2]. *S. aureus* and *E. coli* are two bacterial species that function as part of the normal human microflora but are also among the most common pathogens affecting. Infections caused by *S. aureus* include boils, impetigo, folliculitis, furuncles, osteoarticular infections, pneumonia, thrombophlebitis, cellulitis, conjunctivitis, and *staphylococcal scalded skin syndrome* (SSSS). Meanwhile, *E. coli* is known to cause urinary tract infections, sinusitis, gastrointestinal infections, meningitis, pneumonia, and gastroenteritis [1]

Phenolic compounds have demonstrated effective antibacterial activity against *E. coli*, a major causative agent of diarrhoea and extraintestinal infections. Additionally, these compounds also exhibit antimicrobial activity

against other intestinal pathogens such as *Salmonella* and *Campylobacter*. As secondary metabolites, phenolics not only inhibit microbial pathogens but also contribute to the prevention of chronic diseases. Numerous plants and fruits rich in phenolics have been reported to exhibit protective effects against cardiovascular diseases, Alzheimer's disease, cancer, inflammation, ageing, viral infections, allergies, and skin damage, including carcinogenesis. These effects are attributed to the presence of phenolic compounds with high antioxidant activity in these plants and fruits [2].

Antioxidants are widely used by the public for therapeutic purposes, as adjunct treatments, as prophylactic agents, and as anti-ageing supplements. Furthermore, numerous studies have highlighted their multifaceted roles in mitigating various pathological conditions [3]. Antioxidants are substances that terminate early-chain reactions and propagation, thereby inhibiting or delaying molecular oxidation. The human body requires exogenous antioxidants when exposed to excessive free radicals due to the insufficiency of its endogenous antioxidant defence systems [4].

Mistletoe on oil palm (*Ficus heteropleura* Blume) has been long utilized in traditional medicine for the treatment of various ailments, including postpartum care, smallpox, diabetes, as a diuretic, for cough, cancer, and peptic ulcers [4]. *Ficus heteropleura* is a parasitic plant that derives nutrients from its host. Despite its parasitic nature, mistletoe has shown potential as a medicinal plant. The composition of secondary metabolites in mistletoe varies depending on the host species, as mistletoe acquires nutrients and bioactive compounds from its host to sustain its growth [5]. The leaves are the most commonly used part of the plant in traditional herbal preparations and are believed to possess medicinal properties [6]. Plants of the *Ficus* genus have been reported to contain various secondary metabolites such as flavonoids, polyphenols, tannins, alkaloids, and saponins [7].

This study investigated *Ficus heteropleura* leaves for their potential as antibacterial and antioxidant agents. The extraction was performed using a graded maceration method, followed by quantification of total phenolic content, and evaluation of antibacterial and antioxidant activities. The antibacterial activity was assessed using the disk diffusion method against *S. aureus* and *E. coli*. Additionally, antioxidant activity was determined through free radical scavenging assays.

MATERIALS AND METHODS

Materials

The materials employed in this study comprised *Ficus heteropleura* leaves, ethanol 96%, *n*-hexane, ethyl acetate, HCl, Mayer’s reagent, pereaksi Dragendorff’s reagent, Mg, sulfuric acid, ferric chloride, distilled water, gallic acid, sodium carbonate, Folin-Ciocalteu, ascorbic acid, DPPH (2,2-diphenyl-1-picrylhydrazyl), Nutrient Broth (NB), Nutrient Agar (NA), Mueller Hinton Agar (MHA), NaCl 0,85%, alcohol 70%, dimethyl sulfoxide, chloramphenicol, and bacterial isolates (*S. aureus* and *E. coli*)

Equipment

The equipment utilized included a rotary evaporator (Buchi Rotavator R-3), a hotplate (Nesco), a microwave (cosmos), an autoclave (All American), an incubator (Memmert*)*, an incubator shaker (Argo Lab), a colony counter (Bexco), Mikcopipettes (Dragon Lab), a vortex (Starlab), a UV-Vis spectrophotometer (Shimadzu), petri dishes (iwaki), and standard laboratory glassware.

Sample Preparation and Extraction

The collected *Ficus heteropleura* leaves were rinsed with running water to remove any adhered dust particles. Subsequently, the leaves were air-dried for two weeks to reduce moisture content. Once dried, the leaves were finely ground using a blender and the resulting powder was stored in a sealed container at room temperature.

Plant extracts were prepared by pouring 800 g of dry leaf powder into separate, dark bottles, each containing 200 g of powder, and adding solvent until the sample was fully submerged. First, maceration was performed using *n*-hexane, followed by the extraction of the residue with ethyl acetate. The remaining residue from the ethyl acetate extraction was then extracted using ethanol. The mixture was allowed to soak for 72 hours with occasional stirring. The mixture was then filtered using Whatman filter paper to separate the solid components from the liquid extract. The obtained filtrate was then concentrated using a rotary vacuum to yield a concentrated distilled water extract. This extract was subsequently stored at 4°C for further use [8].

Phytochemical Test

The alkaloid test procedure involved adding 0.5 g of the sample to a test tube and

dissolving it in 5 mL of 2 N HCl solution. Subsequently, the filtrate was separated into three test tubes and tested with Meyer's reagent and Dragendorff's reagent. For this, 4 mL of the filtrate was transferred into a test tube, and 1 mL of each reagent was added. A positive test of Mayer's reagent result was indicated by forming a yellowish-white precipitate. A positive Dragendorff's reagent result was indicated by the formation of an orange precipitate [9].

The procedure of the terpenoids and steroids tests began by placing 0.5 g of extract into a test tube. Subsequently, 2 mL H_2SO_4 were added, and the mixture was gently homogenized and left to stand for several minutes. A positive steroid was indicated by the blue to green colour, and positive terpenoids were indicated by brownish-red.

The procedure of the flavonoids test began by preparing 0.5 g of extract, adding 5 mL of ethanol, and heating for ± 5 minutes in a test tube. Subsequently, ten drops of concentrated HCl were added to the mixture. Then, 0.2 g of Mg powder was added. A positive result was indicated by the appearance of a dark red, yellow, or orange colour, indicating the presence of a flavonoid.

The procedure of the sapponins test commenced with the addition of 0.5 g of extract into a test tube. Warm water was added to the mixture, and the contents were shaken for a minute. After allowing the mixture to stand for 10 minutes, the result was observed. If the foam remained constant, it indicated a positive outcome.

Tannin content was tested by placing 0.5 g of extract into a test tube and adding 10 mL of boiling distilled water. The mixture was mixed with a few drops of a 1% $FeCl_3$ solution. A positive test was indicated by the appearance of a blackish-green colouration in the solution.

The phenol test began by combining 0.5 g of the extract with three to four drops of $FeCl_3$ solution. A positive indication of phenolic compounds in the material was denoted by the formation of a bluish-black or dark black colour [10].

Total Phenolic Content Determination

0.001 g of ethanol extract from *Ficus heteropleura* leaves dissolved in 1 mL of distilled water. From this solution, 0.1 mL was taken and mixed with 0.9 mL of distilled water and 0.5 mL of Folin-Ciocalteu reagent. The solution was vortexed and allowed to stand at room temperature for 5 minutes. Subsequently, 2.5 mL of a 7.5% Na_2CO_3 solution was added and vortexed until the mixture was homogeneous. The solution was then incubated for the required reaction time, and absorbance was measured at the maximum wavelength using a UV-Visible spectrophotometer. All measurements were performed in triplicate. The phenolic concentration was calculated by substituting the absorbance value into a linear regression equation obtained from a gallic acid calibration curve and expressed as total phenolic content in mg gallic acid equivalents (GAE) per gram of extract (mg GAE/g) or % w/w [11].

Characterization

The ethanol extract of *Ficus heteropleura* leaves was characterized using a UV-Visible Spectrophotometer to observe wavelength shifts associated with colour changes, and Fourier-Transform Infrared Spectroscopy (FTIR) to identify functional group transformations.

Antibacterial Activity

The antibacterial activity of the extract against *S. aureus* and *E. coli* was evaluated using the disc diffusion method with 6-mm-diameter paper discs. 1 mL of each bacterial suspension was transferred into a sterile Petri dish, followed by the addition of 15 mL of Mueller-Hinton Agar (MHA) at 45°C. The mixture was homogenized using a figure-eight motion and allowed to solidify. Sterile paper discs were impregnated with 15 µL of the extract at concentrations of 20%, 40%, and 80%. Each concentration was tested in triplicate. The discs were placed on the surface of the solidified agar using sterile forceps and incubated at 37°C for 24 hours. Chloramphenicol (30 µg) was used as the positive control, while 10% dimethyl sulfoxide (DMSO) served as the negative control. After incubation, antibacterial activity was determined by observing and measuring the clear zones (inhibition zones) that formed around the discs. The presence of a clear zone indicated that the extract or antibiotic exhibited inhibitory activity against the tested bacteria. The diameter of the inhibition zones was measured using a digital calliper [12]

Antioxidant Activity

A total of 2 mL of each concentration of the test solution was transferred into a test tube,

followed by the addition of 2 mL of 0.1 mM DPPH solution. The mixture was vortexed until homogeneous and incubated in the dark. Absorbance was measured at the maximum wavelength between 26 and 30 minutes of incubation [13].

The radical scavenging activity was expressed as the percentage of inhibition, calculated using the following equation:

$$\% \text{ Inhibition} = \frac{\text{Abs. control} - \text{Abs. extract}}{\text{Abs. control}} x100\%$$

The IC_{50} value was determined from the % inhibition using the following equation:

$$IC_{50} = \frac{50-a}{b}$$

Note:
a = x-value on the linear curve
b = y-value on the linear curve

RESULTS AND DISCUSSION

Sample Preparation and Extraction

Mistletoe on oil palm is a type of mistletoe that grows on oil palm trees as a semi-parasitic plant, deriving nutrients from its host. If left uncontrolled, the growth of mistletoe can hurt the productivity and development of oil palms [14]. The genus *Ficus* is classified as a soft-wooded plant that may be monoecious or dioecious, and can grow as trees, small trees, shrubs, or climbers. It often exhibits aerial roots, appearing as hemiepiphytes, epiphytes, or spreading root systems [15].

Figure 1. *Ficus heteropleura* Plant

In this study, *Ficus heteropleura* leaves were collected from oil palm plantations in Sabak Auh, Siak District, Riau Province, Indonesia, with a total of 2400 g of fresh leaves. The samples were subsequently dried, ground into powder, and extracted using a grade maceration method. The purpose of the extraction was to isolate compounds soluble in solvents from the *Ficus heteropleura* leaf simplicia using appropriate organic solvents. The results of the extraction are presented in Table 1.

Table 1. Results of *Ficus heteropleura* leaf extract

Sample	Extract weight (g)	Yield (%)
Fresh *Ficus heteropleura* leaves	2400	-
Dried *Ficus heteropleura* leaves	800	33,3
The ethanol extract	74	9,25

The maceration process yielded an extract weighing 74 g, yielding 9.25%. The extract exhibited a brown colour.

Phytochemical Test

A phytochemical test was conducted on the ethanol extract of *the Ficus heteropleura* leaf to identify the secondary metabolites present in the sample qualitatively. The results of the phytochemical analysis of the ethanol extract are presented in Table 2.

Table 2. Results of the phytochemical test

Compound Class	Reagent	Change	Result
Alkaloids	HCl 2N + Mayer	No colour change and precipitate	-
	HCl 2N + Dragendorff	No colour change or precipitate	-
Terpenoids	H_2SO_4	Brownish red	+
Steroids	H_2SO_4	No colour change	-
Flavonoids	Ethanol, concentrated HCl, and 0.2 g Mg	Dark red colour	+
Saponins	Hot distilled water	Foamy	+

Compound Class	Reagent	Change	Result
Tannin	Hot distilled water + $FeCl_3$ 1%	Blackish green colour	+
Phenolic	$FeCl_3$	Dark black colour	+

Note: (+) : Identified
(-) : No identified

The phytochemical analysis of the ethanol extract revealed the presence of various compound groups. Terpenoids were identified by brownish-red colour, while a dark red colour indicated the presence of flavonoids. The continuous formation of froth or foam was detected, indicating the presence of saponins. Tannins were present blackish green color. Furthermore, phenolic compounds were identified by their dark black colour. However, the ethanol extract of *Ficus heteropleura* tested negative for alkaloid and steroid compounds. The presence of secondary metabolites, such as phenolics, suggests that the extract may have potential antibacterial and antioxidant activities.

Total Phenolic Content Determination

The results of total phenolic content determination are presented in Table 3.

Table 3. Results of total phenolic content Determination

Sample	Replication	Abs	AVG Abs	Total Phenolic (mg GAE/g)
Ethanol extract	1	0,2615	0,2615	25,500
	2	0,2615		

The total phenolic content was determined using the Folin–Ciocalteu method with gallic acid as the standard solution [16]. The absorbance values of the gallic acid standard yielded a calibration curve with the linear regression equation y = 0.0026x + 0.0065 and a correlation coefficient (R^2) of 0.9954. The total phenolic content in the ethanol extract of oil palm mistletoe leaves was expressed as GAE (Gallic Acid Equivalent), representing the amount of gallic acid equivalent in milligrams per gram of sample. The total phenolic content of the ethanol extract was 25.500 mg GAE/g.

Gallic acid, a stable natural phenol, is widely employed as a standard reference solution in phenolic assays. It belongs to the class of simple phenolic acids and is a derivative of hydroxybenzoic acid. As a low molecular weight triphenol compound, gallic acid has been widely recognized for its diverse biological and pharmacological activities, particularly as an antioxidant. In the assay, gallic acid reacts with the Folin–Ciocalteu reagent to produce a yellow coloration, indicating the presence of phenolic compounds in the tested extract. Upon the addition of Na_2CO_3 solution, the colour changes to blue. The purpose of adding Na_2CO_3 is to create an alkaline environment, allowing the phenolic compounds to react with the Folin–Ciocalteu reagent. Phenolic compounds will only react with this reagent under basic conditions, which facilitate the dissociation of protons from the phenolic compounds, forming phenolate ions that undergo further reactions with the reagent [10].

Characterization

The ethanol extract of *Ficus heteropleura* leaves was subsequently characterized using a UV-Visible spectrophotometer. The result of characterisation is presented in Figure 2.

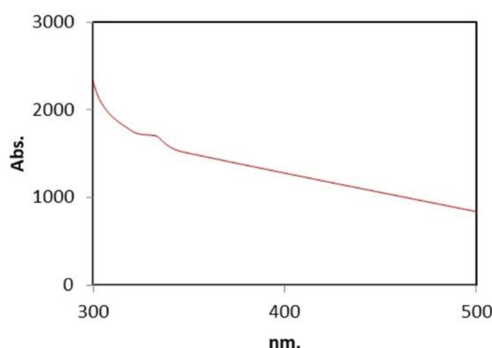

Figure 2. UV-Vis Spectrum

The UV-Vis spectrophotometer analysis of ethanol extract *from Ficus heteropleura* leaves revealed the absorbance wavelength of the extract, with the data presented in Table 4.

Table 4. UV Vis spectrum wavelength of ethanol extract *of Ficus heteropleura*

Monitoring	Wavelength (nm)	Note
Extract	333.8	π→π*

Based on the UV-Vis spectrophotometer analysis, the ethanol extract of *Ficus heteropleura* leaves exhibited a characteristic absorption spectrum. The maximum absorbance was observed at a wavelength of 333.8 nm, indicating a π→π* electronic transition, which is typically associated with the presence of conjugated C=C bonds and C=O chromophores. The occurrence of maximum absorbance within the 300–550 nm range suggests the presence of tannins—natural polyphenolic compounds containing phenolic hydroxyl and carbonyl groups. Furthermore, the presence of chromophoric groups contributes to the colouration of a compound. The conjugated C=C and C=O bonds, which function as chromophores, support the hypothesis that the brown colouration of the extract is attributed to the presence of tannins [17,18].

The ethanol extract of *Ficus heteropleura* leaves was subsequently characterized using Fourier-transform infrared (FTIR) spectroscopy. The results of the FTIR characterization are presented in Figure 3.

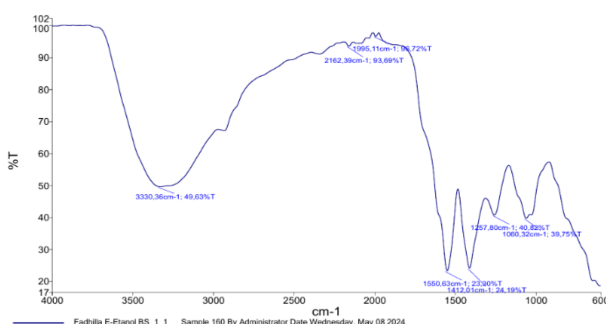

Figure 3. FTIR characterization result

The FTIR characterisation results of the ethanol extract of Ficus heteropleura leaves were analysed, and the data are presented in Table 5.

Table 5. FTIR characterization of ethanol extract *Ficus heteropleura*

Peak Wavenumber (cm^{-1})	Functional Group	Reference
3330,36	O-H	[19]
2162,39	C≡C	[20]
1995,11	C=O	[19]
1550,63	C=C	[21]
1412,01	C-H	[22]
1257,80	C-O	[23]
1060,32	C-O	[23]

The FTIR analysis provided information on several secondary metabolites present in the ethanol extract of Ficus heteropleura leaves. Various functional groups were identified based on absorption peaks at specific wavelengths. A broad peak at 3330.36 cm^{-1} indicated O–H stretching vibrations, commonly associated with alcohols and phenols. Secondary metabolites such as phenolic compounds typically possess hydroxyl groups that absorb at this wavelength [19]. A peak at 2162.39 cm^{-1} suggested the presence of a C≡C group, which, although rare in secondary metabolites, can occur in complex alkaloids or terpenoids [20].

Furthermore, a peak at 1995.11 cm^{-1} indicated the presence of a C=O group, often found in certain flavonoids [19]. The peak at 1550.63 cm^{-1} corresponded to C=C stretching, typically associated with terpenoids and saponins [21]. The peak observed at 1412.01 cm^{-1} reflected C–H bending in alkanes or O–H bending in phenols, commonly present in flavonoids, tannins, and other phenolic compounds [22]. A peak at 1257.80 cm^{-1} was associated with C–O stretching vibrations in esters or ethers, which may be found in terpenoids, flavonoids, and saponins. Lastly, the peak at 1060.32 cm^{-1} was attributed to C–O stretching vibrations, typically observed in alcohols, ethers, or esters, and also commonly found in flavonoids, saponins, and terpenoids [23].

Antibacterial Activity Test

The antibacterial activity assay against *S. aureus* and *E. coli* was conducted by measuring the inhibition zones formed around the paper discs. The results of the antibacterial activity test of the ethanol extract of *Ficus heteropleura* leaves at concentrations of 20%, 40%, and 60% are presented in Table 6.

Table 6. Test of the antibacterial activity of ethanol extract of *Ficus heteropleura* against *S. aereus* and *E. coli*

Bacterial	Concentration	Average of inhibition

		zone (mm)
S. aureus	20%	8,9
	40%	9,5
	60%	10,3
	Control +	20,4
	Control -	0,0
E. coli	20%	7,5
	40%	9,9
	60%	10,3
	Control +	20,4
	Control -	0,0

The ethanol extract of *Ficus heteropleura* leaves was evaluated for antibacterial activity using the disc diffusion method against *S. aureus* and *E. coli*. This method was selected due to its practicality, ease of implementation, and rapid interpretation of results, making it a widely used approach for antibacterial screening. Mueller Hinton Agar (MHA) was employed as the culture medium in this study, as it is known to provide reliable and reproducible results. MHA is a standard medium for antibiotic susceptibility testing, particularly suitable for fast-growing bacteria such as *Staphylococcus* and contains casein peptone and starch as its primary components [24].

The antibacterial test was performed by applying 15 µL of the ethanol extract of *Ficus heteropleura* leaves into a sterile disc, which was then placed on the bacterial growth medium and incubated for 24 hours. Two control solutions were used for comparison: a positive control (30 µg chloramphenicol) and a negative control (10% DMSO). The negative control served to confirm that the solvent used for dilution did not interfere with the antibacterial activity of the test compound. The inhibition zone observed for the negative control against both test bacteria was 0.0 mm, indicating that the DMSO solvent did not affect the antibacterial results of the ethanol extract. The positive control, chloramphenicol, acted as a bacteriostatic antibiotic capable of inhibiting the growth of both Gram-positive and Gram-negative bacteria and served as a reference to evaluate the antibacterial activity of the ethanol extract. The extract was tested at concentrations of 20%, 40%, and 60% to determine whether increasing concentration would enhance antibacterial activity.

The average diameter of the inhibition zones at each extract concentration is presented in Table 6, showing variation across the concentrations tested. The ethanol extract of *Ficus heteropleura* leaves exhibited antibacterial activity against *S. aureus*, producing inhibition zones of 8.9 mm, 9.5 mm, and 10.3 mm at concentrations of 20%, 40%, and 60%, respectively. The inhibition zone for the positive control (30 µg chloramphenicol) was 20.4 mm. Similarly, against Escherichia coli, the extract produced inhibition zones of 7.5 mm, 9.9 mm, and 10.3 mm at 20%, 40%, and 60% concentrations, with the positive control showing an inhibition zone of 20.4 mm. The largest inhibition zones for both *S. aureus* and *E. coli* were observed at a 60% concentration, with an average diameter of 10.3 mm, while the smallest zones were observed at a 20% concentration, with average diameters of 8.9 mm and 7.5 mm, respectively.

The efficacy of an antimicrobial substance in inhibiting or killing microorganisms is concentration-dependent. The inhibition zone diameter observed at the 60% extract concentration was larger than that at 40% and 20%, indicating that higher extract concentrations result in greater antibacterial activity. Inhibition zones with diameters greater than 21 mm are categorized as very strong, those ranging from 11–20 mm as strong, 6–10 mm as moderate, and less than 5 mm as weak [25]. Based on the results obtained, the ethanol extract of *Ficus heteropleura* leaves showed moderate antibacterial activity against both *S. aureus* and *E. coli*, as indicated by average inhibition zone diameters ranging from approximately 7.5 mm to 10.3 mm.

The ethanol extract of *Ficus heteropleura* leaves exhibited a bacteriostatic effect against both *S. aureus* and *E. coli*. The observed antibacterial activity is attributed to the presence of secondary metabolites within the extract that possess antimicrobial properties, particularly flavonoids and saponins. The inhibition of bacterial growth is likely due to damage to structural components of the bacterial cell membrane. Such membrane disruption, induced by bioactive compounds, can impair nutrient and ion transport across the membrane, thereby inhibiting bacterial cell growth due to nutrient deficiency [26].

Antioxidant Activity

The ethanol extract of *Ficus heteropleura* leaves was evaluated for antioxidant activity using the DPPH radical scavenging assay, with absorbance measured by a UV-Visible spectrophotometer at a wavelength of 517 nm. The absorbance values obtained were used to calculate the percentage of inhibition. The absorbance measurements and corresponding inhibition percentages for each sample concentration are presented in Table 7.

Table 7. Test of the antioxidant activity of ethanol extract of *Ficus heteropleura*

Sample	Concentration (ppm)	%Inhibition	IC_{50} (µg/mL)
Extract	15,625	75,19	0,67
	31,25	82,54	
	62,5	88,65	
	125	92,02	
	250	95,01	
	500	96,01	
Ascorbic Acid	15,625	72,94	0,45
	31,25	74,29	
	62,5	77,11	
	125	81,01	
	250	85,48	
	500	89,68	

The results of the antioxidant activity test showed that the extract has antioxidant properties. The method employed in this study was the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, which is commonly used to determine antioxidant activity based on the ability of antioxidants to scavenge free radicals by donating hydrogen atoms [27]. DPPH is a stable free radical at room temperature and is soluble in polar solvents such as ethanol and methanol [26].

In this study, ascorbic acid was used as a positive control to evaluate the relative antioxidant activity of the extract. Antioxidant activity was tested by measuring the decrease in the intensity of the purple colour of DPPH. When the DPPH solution is reacted with a compound capable of donating a hydrogen atom, the radical-scavenging compound reduces DPPH, which can be observed through a colour change from purple to yellow. This colour change occurs due to the formation of a bond between the nitrogen atom of the DPPH radical and the hydrogen atom donated by the antioxidant compound, thereby reducing DPPH to a more stable, non-radical form [27].

The DPPH radical scavenging activity of the ethanol extract of *Ficus heteropleura* leaves, along with ascorbic acid as a reference compound, was expressed in terms of IC_{50} (inhibition concentration), which is defined as the concentration of the test sample required to inhibit 50% of the DPPH free radicals. The IC_{50} value was determined from the linear regression equation derived from the relationship between the extract concentration and the percentage of DPPH radical scavenging activity at each concentration. Based on the data presented in Table 7, the regression equation for the extract was $y = 5.9458\ln(x) + 61.622$, and for ascorbic acid, $y = 4.975\ln(x) + 57.812$. These equations were used to calculate the respective IC_{50} values of the extract and ascorbic acid.

The results showed that the ethanol extract of *Ficus heteropleura* leaves exhibited very strong antioxidant activity, as indicated by an IC_{50} value of 0.67 µg/mL. As a comparison, ascorbic acid had an IC_{50} value of 0.45 µg/mL. The absorbance of both the extract and the ascorbic acid was measured at a maximum wavelength of 517 nm. According to Maitulung, an extract is classified as having very strong antioxidant activity when the IC_{50} value is less than 50 µg/mL, strong if the IC_{50} ranges from 50–100 µg/mL, moderate if between 100–250 µg/mL, and weak if between 250–500 µg/mL. Based on this classification, the ethanol extract of *Ficus heteropleura* leaves can be categorized as having very strong antioxidant activity, as its IC_{50} value was well below 50 µg/mL.

Flavonoids and phenolics are secondary metabolites found in plants and play a role as antioxidants. The higher the phenolic compound content, the greater the antioxidant activity. The phenolic structure contributes to antioxidant activity by influencing the number of hydroxyl (-OH) groups, which can neutralize free radicals. Phenolic compounds exhibit biological effects related to antioxidant activity through mechanisms such as metal chelation, reduction, electron donation, and free radical scavenging [26].

CONCLUSION

Ficus heteropleura leaves were successfully extracted, and based on the results of secondary metabolite content tests, it was found that the ethanol extract of palm mistletoe leaves contains several secondary metabolites, such as flavonoids, terpenoids, saponins, tannins, and phenolics. The total phenolic content was determined using the Folin-Ciocalteu method with gallic acid as the standard solution. The ethanol extract of palm mistletoe leaves contains a total phenolic content of 25.500 mg GAE/g. The antimicrobial ability of a substance to kill microorganisms depends on the concentration of the microbial agent used. Based on the results obtained, the ethanol extract of palm mistletoe leaves tested on both bacteria was categorized as moderate, as it produced an average inhibition zone diameter of approximately 7.5 mm to 10.3 mm. The results showed that the ethanol extract of palm mistletoe leaves (Ficus heteropleura Blume) exhibited very strong inhibition with an IC50 value of 0.67 µg/mL. At the same time, ascorbic acid, as a comparison, had an IC50 value of 0.45 µg/mL.

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