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BIOACTIVITY OF BREADFRUIT LEAF EXTRACT (*ARTOCARPUS ALTILIS*)

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ABSTRACT

Breadfruit leaves (Artocarpus altilis) contain various secondary metabolites such as flavonoids, tannins, terpenoids, and alkaloids which are potential bioactive compounds for therapeutic purposes. This study is an experimental laboratory research aimed at identifying bioactive compound groups and evaluating the antibacterial and antioxidant activities of breadfruit leaf extract. Extraction was carried out using the maceration method with 96% ethanol p.a as solvent. Identification of bioactive compounds was analyzed by Gas Chromatography Mass Spectrometry (GC-MS) method, antibacterial testing by the disc diffusion method, and antioxidant evaluation using a modified Ferric Reducing Antioxidant Power (FRAP) assay. The test bacteria used were Klebsiella pneumoniae and Staphylococcus aureus, gentamicin and chloramphenicol as positive controls. Ascorbic acid was used as the positive control for the antioxidant assay. The GC-MS analysis revealed the presence of active compounds such as Tricyclo [4.3.0.0(7,9)] nonane, trans-11-tetradecenyl acetate, ethyl tridecanoate, α-farnesol isomer, vitamin E, and β-sitosterol. The extract showed antibacterial activity against both tested bacteria, with the smallest effective concentrations of 50 mg/mL for K. pneumoniae and 25 mg/mL for S. aureus, resulting in inhibition zones of 0.3125 mm and 1.5375 mm, respectively, although these values did not meet the sensitivity criteria. The antioxidant activity test showed a potential antioxidant value of 9.84 mg AAE/g extract, using ascorbic acid as the standard curve.

Keywords: antibacterial; antioxidant; artocarpus altilis; bioactive compounds; GC-MS

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INTRODUCTION

Breadfruit (*Artocarpus altilis*) is a tropical plant. Most breadfruit is used only for the edible fruit, while other parts, such as the leaves, are rarely utilized and often considered waste. *Artocarpus altilis* leaves are not widely consumed, but they are reportedly used in herbal therapy as infusions or decoctions (Jones et al., 2011; Mehta et al., 2023). Breadfruit leaves have long been used empirically in traditional medicine to treat various health problems, including respiratory, digestive, skin, and inflammatory conditions. Respiratory ailments, such as coughs, can be treated by drinking a decoction made from fresh breadfruit leaves. Breadfruit leaves are also used to relieve pain and inflammation (joint or muscle pain) by pounding and applying the leaves to the affected area. Breadfruit leaves are used to treat digestive problems, such as diarrhea, by boiling dried breadfruit leaves (the raw material) or brewing the leaf material as an herbal tea to relieve diarrhea. Meanwhile, breadfruit leaves are used for skin care, such as treating acne or irritation, by processing fresh breadfruit leaves into a paste and applying it to the affected skin (Silalahi, 2021).

Various in vitro and in vivo studies have shown the efficacy of breadfruit leaves in the field of medicine. In vitro studies have revealed various therapeutic activities of breadfruit leaf extract,

including antioxidant, antibacterial (Saraswaty et al., 2015), antimalarial (Maulida et al., 2024), and skin-lightening properties (Saad et al., 2021). Meanwhile, in vivo studies have demonstrated that breadfruit leaves possess anti-inflammatory, antidiabetic, immunosuppressant, antihypertensive, and cardioprotective properties (Affonso et al., 2016; Palupi et al., 2020). In vivo studies related to the toxicity of breadfruit leaves have reported that breadfruit leaves are safe for use or consumption (Fitrya et al., 2025). Based on these in vitro and in vivo tests, breadfruit leaves are an up-and-coming candidate for herbal ingredients to be developed in the future.

The bioactivity of breadfruit leaves is closely related to the active compounds contained in them. Phytochemical screening results show that breadfruit leaves contain compounds of the alkaloid, flavonoid, tannin, and triterpenoid/steroid groups (Yumni et al., 2021). These compounds act as antibacterial and antioxidant agents. The development of natural antibacterial agents is a potential alternative solution to reduce the risk of antibiotic resistance. Plants are a source of bioactive compounds that can be used in medicine, one of which is breadfruit leaves. Various studies have been conducted on the antibacterial and antioxidant activity of breadfruit leaves; however, in vitro studies related to respiratory tract and skin infections have not been widely reported. At the same time, the antioxidant potential of breadfruit leaves with Ferric Reducing Antioxidant Power (FRAP) has also not been widely reported. Therefore, the research conducted aims to identify active compounds and determine the bioactivity (antibacterial and antioxidant) of breadfruit leaves.

METHOD

The research methods included preparation of medicinal plants, ethanol extraction, identification of active compounds using Gas Chromatography–Mass Spectrometry (GC-MS), antibacterial test using the disc diffusion method, and antioxidant test using the FRAP method. Breadfruit (*Artocarpus altilis*) leaf samples were obtained from Bekasi, West Java-Indonesia. The mature leaf blade (*lamina*) was used for medicinal plants, excluding the petiole.

Preparation of Simplisia and Extracts

Three kilograms of fresh breadfruit leaves were washed, drained, and then oven-dried at 37°C until the moisture content was less than 10% of the wet weight, resulting in a medicinal plant (simplisia). The medicinal plant was ground using a blender and then sieved through a 60-mesh sieve to obtain a fine powder. Twenty-five grams of the powdered medicinal plant was macerated using 96% ethanol (1:10) for three days at room temperature, with periodic stirring. The filtrate was filtered using Whatman No. 1 filter paper. 42, then evaporated using a rotary evaporator to obtain a crude breadfruit leaf extract.

Identification of Active Compounds

The composition of bioactive compounds in the ethanol extract of breadfruit leaves was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). Helium was used as the carrier gas with a flow rate of 1 mL/min. The column temperature was set at 50°C and gradually increased to 300°C in increments of 10°C per minute. The injector temperature was set at 250°C. The breadfruit leaf extract sample was first dissolved in n-hexane at a concentration of 1 mg/mL, then 1 µL of the sample was automatically injected using an autosampler in splitless mode to increase sensitivity. Analysis was performed in full scan mode over a mass range of m/z 50–500. The resulting chromatograms were compared with standard libraries from the National Institute of Standards and Technology (NIST) and the Wiley library. Compound identification was based on retention times and ion fragmentation patterns, while the relative composition of the compounds was determined by analyzing the peak areas in the chromatograms (TEKI, 2025).

Antibacterial Test

Antibacterial activity was tested using the modified diffusion method against the bacteria *Klebsiella pneumoniae* and *Staphylococcus aureus*, using Mueller-Hinton Agar (MHA) as the test medium.

Gentamicin and chloramphenicol served as positive antibacterial activity. The test was conducted by taking 500 µL of the test bacterial suspension and inoculating it onto the surface of the MHA medium using a sterile cotton swab. Sterile paper discs with a diameter of 6 mm containing breadfruit leaf extract at concentrations of 6.25, 12.5, 25, 50, and 100 mg/mL (dissolved in 20% DMSO) were placed on the surface of the agar medium. Discs containing 30 µL of 20% DMSO served as negative controls, and 30 µg antibiotic discs (chloramphenicol and gentamicin) served as positive controls. These discs were placed on top of the agar medium containing the test bacteria. All treated Petri dishes were incubated at 37°C for 24 hours. The diameter of the inhibition zone around the disc was then measured using a vernier caliper to determine the inhibitory power of the breadfruit leaves against the test bacteria. Each treatment was repeated four times.

Antioxidant Test

One mL of 1000 ppm breadfruit leaf extract (5 mg of extract dissolved in 5 mL of 96% ethanol) was mixed with 1 mL of 0.2 M phosphate buffer solution (pH 6.6) and 1 mL of 1% $K_3Fe(CN)_6$ solution. The mixture was incubated at 50°C for 20 minutes, then 1 mL of 10% TCA solution was added and centrifuged for 10 minutes at 3000 rpm. One milliliter of the supernatant was transferred to a test tube, to which 0.5 milliliters of 0.1% $FeCl_3$ solution and 1 milliliter of distilled water were added. The mixture was left for 10 minutes, and the absorbance was then measured. The absorbance of the breadfruit leaf extract was measured using a UV-Vis spectrophotometer at a wavelength of 720 nm. The standard curve was created from standard ascorbic acid solutions with concentration series of 60, 70, 80, 90, and 100 ppm, while the oxalate solution served as a negative control. The test results were expressed in units of mg ascorbic acid equivalents per gram of extract (mg AAE/g extract) (Rahmawati et al., 2024). This research has passed ethical approval with the number: No. 651/UKI.LPPM/PPM.00.00/ET.2024.

RESULT

Identification of Bioactive Compounds

Identification of bioactive compounds contained in the ethanol extract of breadfruit leaves using the GC-MS method. The results of the analysis of the active compounds detected in the ethanol extract of breadfruit leaves are presented in Table 1.

Table 1.
GC-MS Analysis of Ethanol Extract of Breadfruit Leaves

No	*Retention Time (Minutes)*	Compound Name	Content (%)
1	30.234	trans-11-Tetradecenyl acetate	6.44
2	30.696	Z-8-Tetradecen-1-yl acetate	1.01
3	31.695	Ethyl tridecanoate	10.97
4	32.799	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	2.66
		Farnesol isomer a	
5	36.060	gamma-Tocopherol	9,41
6	37.874	gamma-Tocopherol	1,41
7	38.025	Oleic acid	1,00
8	38.515	Vitamin e	1,60
9	38.873	beta. -Sitosterol	6,66
10	41.514	1,6,10, 14-Hexadecatetraen-3-ol, 3,7,11,15-tetramethyl-, (E,E)-p-Camphorene	6,30
11	42.714	5H-3,5a-Epoxynaphth [2,1-c] oxepin, dodecahydro-3,8,8,1a- tetramethyl-, [3S- (3.alpha. 5a.alpha., 7a.alpha., 11a.beta.11b.alpha.)]-	1,04
12	43.410	Tricyclo [4.3.0.0 (7,9)] nonane, 2,2,5,5,8,8 hexamethyl-, (1.alpha., 6.beta., 7,alpha., 9.alpha)-	3,99
		2-(Pentadec-14-en-1-yl) furan	
13	43.783	Octadecanoic acid, propyl ester Oleic Acid 7,11-Hexadecadienal	4,08
14	44.900		27,89
15	45.079		2,96
16	45.845		1,38
17	46.169		1,23
18	57.229		1,95

Table 1, it is known that of the 18 compounds extracted by 96% ethanol solvent, 3 main compounds were detected in the breadfruit leaf extract, namely tricyclo [4.3.0.0 (7.9)] nonane (27.89%), ethyl tridecanoate (10.97%), and farnesol isomer α (9.41%). The peaks of these three compounds on the GC-MS are visible in Figure 1.

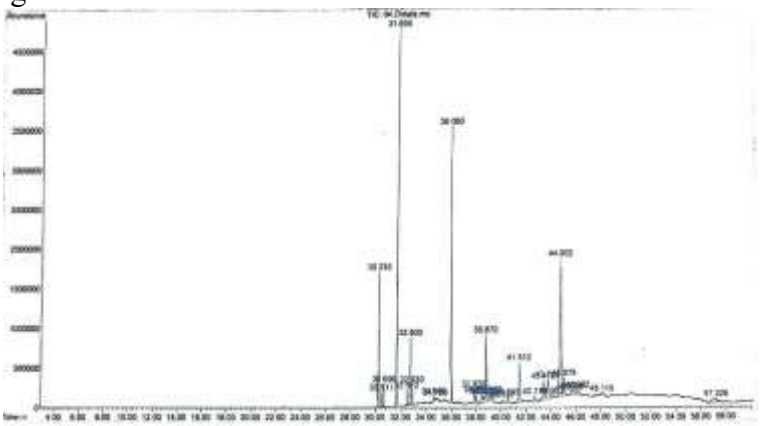

Figure 1. Peak GC-MS chromatogram of breadfruit leaf extract

Antioxidants

The antioxidant activity of breadfruit leaves was determined from the standard curve equation for ascorbic acid, which was used as a standard or positive control. The standard curve equation for ascorbic acid yielded a value of y = 0.0313x – 1.2828 with an R^2 value of 0.9992 (Figure 2).

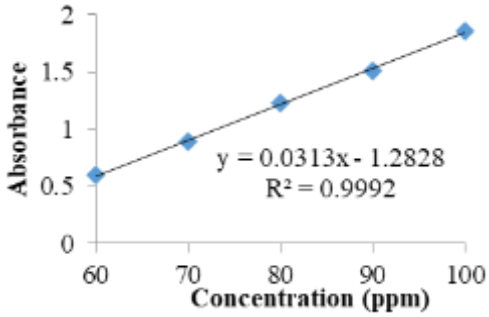

Figure 2. Standard Absorbance Value of Ascorbic Acid

Based on the equation of the standard curve line of ascorbic acid, it is obtained that y = 0.0313x – 1.2828, where y is the absorbance value and x is the antioxidant activity value of breadfruit leaves in each replication, as shown in Table 2.

Table 2.
Measurement of Absorbance Values And Antioxidant Activity of Breadfruit Leaves

Repeat	Absorbance value	Antioxidant activity (mgAAE/g extract)	Average antioxidant activity (mg AAE/g extract)
1	1.824	99.259	
2	1.729	96.224	98.386 ± 1.883
3	1.837	99.674	

Description: mgAAE/g extract = milligrams of ascorbic acid equivalent per gram of extract

Antibacterial

The antibacterial activity of breadfruit leaves against test bacteria was determined based on the inhibition zones formed around the treated paper discs. The antibacterial activity of breadfruit leaves against the test bacteria *K. pneumoniae* and S. aureus is shown in Figure 3. Figure 3, it is evident that breadfruit leaves exhibit inhibitory activity against the growth of the two test bacteria used. Breadfruit leaves produce the lowest inhibitory power on *S. aureus* at a concentration of 25

mg/ml, with an inhibition zone diameter of 1.5375 mm, and against *K. pneumoniae*, breadfruit leaves provide the lowest inhibitory power at a concentration of 50 mg/ml, with an inhibition zone diameter of 0.3125 mm. Meanwhile, gentamicin as a positive control against *K. pneumoniae*, produces an inhibition zone diameter of 18.3875 mm, and chloramphenicol as a positive control against *S. aureus*, produces an inhibition zone diameter of 10.1125 mm. 20% DMSO solution, used as a solvent for breadfruit leaves, which function as a negative control does not provide an inhibition zone against the two test bacteria. Antibacterial inhibitory power is grouped into four categories, as shown in table 3 (Walker et al., 2013).

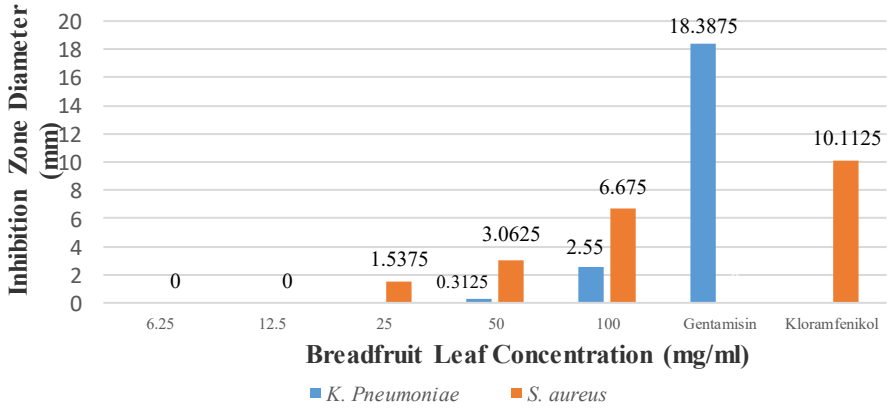

Figure 3 Diameter of the inhibition zone of breadfruit leaves against test bacteria

Table 3.
Grouping of Antibacterial Power

No.	Inhibition category	Inhibition zone diameter (mm)
1	Very Strong	>20
2	Strong	10-20
3	Moderate	5-10
4	Weak	< 5

DISCUSSION

Tricyclo [4.3.0.0 (7.9)] nonane is the compound contained in the most abundant breadfruit leaf extract. According to the NIST Webbook site and the PubChem website, tricyclo has a chemical formula of $C_{15}H_{26}$ with a molecular weight of 206.37 g/mol. The chemical structure of the compound consists of a stable tricyclic framework, as well as six methyl groups that provide high hydrophobic properties. Tricyclo is classified as a terpenoid hydrocarbon compound (NCBI, 2021). Terpenoid compounds are known to have many pharmacological effects, such as antioxidants, antibacterials, antifungals, antivirals, antiparasitics, anticancer, anti-inflammatory, analgesics, and hepatoprotective (Atriya et al., 2023). Other contents found in large quantities in the ethanol extract of breadfruit leaves are ethyl tridecanoate and farnesol isomer α. Ethyl tridecanoate has been reported to have antibacterial and anti-inflammatory activities (Shashikant et al., 2022). Meanwhile, several studies have shown farnesol to be an antimicrobial, anti-inflammatory, and anticancer agent (Calzada et al., 2025; Hartman et al., 2025; Maulida et al., 2024; Sun et al., 2025).

Based on Table 2, it is known that the average antioxidant value of breadfruit leaves is 98.386 ± 1.883 mgAAE/g extract. The results of the study are not too different from Maulida's 2024 study, which found breadfruit leaf activity of 118.46 ± 0.14 mgAAE/g extract (Maulida et al., 2024). The content of active compounds in a plant is greatly influenced by the place or location where the plant grows. It has been reported that increasingly stressed growing environments will produce plants with more vigorous or greater antioxidant activity (Wardani et al., 2020). The antioxidant activity of ethanol extract of breadfruit leaves is classified as strong with an inhibition concentration 50 (IC50) value of 86.305 µg/mL (Andriani et al., 2025). However, other studies show that methanol extract and breadfruit leaf fractions have very weak antioxidant activity with IC50 values of more than 200

mg/L (Misfadhila et al., 2019). The choice of method and solvent in extraction affects the antioxidant activity produced by the extract (Sasadara & Wiranata, 2022).

The antioxidant content of medicinal plants is highly dependent on the type of plant, plant variety, environmental conditions, climate and seasonal changes, geographic region where it grows, level of maturity, cultivation techniques, and other factors such as post-harvest handling and plant processing. In addition to these, other factors that influence the antioxidant content of medicinal plants are the content and concentration of phenolic compounds in a plant, which are one of the antioxidant marker compounds. In determining the appropriate antioxidant capacity, it is necessary to pay attention to and consider the extraction techniques, solvents, and test methods used for antioxidant analysis in medicinal plants (Škrovánková et al., 2012).

Table 3, it is known that the inhibitory power produced at the lowest concentration of breadfruit leaves against the two test bacteria used is classified as weak. The antibacterial activity of the ethanol extract of breadfruit leaves against Staphylococcus aureus is classified as weak. (Fiana et al., 2020). Other studies state that the ethanol extract of breadfruit leaves has a relatively weak inhibitory power against Escherichia coli, a Gram-negative bacterium (Maulida et al., 2024). The antibacterial activity of A. altilis leaf extract against B. cereus bacteria (Gram-positive) and E. coli bacteria (Gram-negative) produces a minimum inhibition concentration (MIC) value of 25 mg/mL of extract against B. cereus and E. coli with inhibition zones of 0.766 ± 0.06 cm and 1.27 ± 0.12 cm, respectively (Ahmad et al., 2020).

The 70% ethanol extract of breadfruit leaves, with 70% ethanol as a negative control, provides inhibitory power against K. pneumonia, with inhibition zone diameters of 15 mm and 6 mm, respectively. The 70% ethanol extract is often used as a disinfectant and antiseptic. The use of ethanol in maceration, which is still left in the extract, can produce inhibitory power against bacterial growth. (Dianda & Suharti, 2022). When using 70% ethanol as a solvent in extraction, it is essential to consider the ethanol residue left in the extract if you plan to perform an antibacterial test. Differences in research results can be attributed to variations in the type and concentration of solvents used in the extraction process. The use of solvents is adjusted to the material and purpose of the active compounds being extracted, so that the choice of solvent affects the active compounds contained in the extract.

CONCLUSION

Research indicates that the active compound identified in breadfruit leaves is tricyclo, producing an antioxidant activity of 98.4 mgAAE/g extract and possessing weak antibacterial activity against test bacteria. Based on this bioactivity, breadfruit leaves have the potential to be developed into a treatment.

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