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Essential Oils of *Alpinia monopleura* and Their Antibacterial and Antioxidant Activity

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ABSTRACT. *Alpinia monopleura* is an endemic plant which widespread and abundant in Sulawesi. Nevertheless, previous studies have not studied the chemical compound and biological activities. This study describes the chemical compounds and antibacterial and antioxidant properties of the essential oils isolated from *A. monopleura*. The essential oil was obtained by the hydro distillation method and interpreted by Gas Chromatography and Mass Spectroscopy (GCMS). Antibacterial properties were determined by the agar diffusion method, while 2,2 diphenil-1-picrylhidrazil (DPPH) and 2,2-Azinobis 3-ethyl benzothiazoline 6-sulfonic acid (ABTS) determined antioxidant activity. The most important components are α-caryophyllene, β-pinene, limonene, α-pinene, β-caryophyllene and caryophyllene oxide. Essential oils of leaves and fruit showed excellent antibacterial properties with a MIC strength of 31.3 µg/mL against *Staphylococcus aureus* ATCC 25023 and *Escherichia coli* ATCC 35218. The highest antioxidant effect was also shown by inhibiting ABTS and DPPH radicals, with IC_{50} strength of essential oil obtained from the leaf being 15.60 and 19.42 µg/mL, respectively, while from the fruit were 10.44 and 11.93 µg/mL. These antibacterial and antioxidant properties indicate that essential oils have advantages in their use in the food and pharmaceutical industries.

Keywords: *Alpinia monopleura*, antibacterial, antioxidant, essential oil

INTRODUCTION

Alpinia has approximately 250 species spread across the tropics and subtropics and is the genus with the largest species of Zingiberaceae (Zhang et al., 2016). Alpinia genus is mainly known as an ethnomedicinal plant and herb in many countries such as Indonesia, India, Vietnam, China, and Japan (Van et al., 2021). Several pharmacological studies of *Alpinia* have been reported, and they have various bioactivities such as anticancer activity (Ibrahim, 2022; Lintao & Medina, 2021), antioxidant activity, antimicrobial activity (Cruz et al., 2020; Ferdous et al., 2018), antiviral activity (Hatanaka et al., 2021; Narusaka et al., 2021; Zubair et al., 2021), anti-inflammatory activity, anti-nociceptive activity (Yu et al., 2020), antiparasitic activity (Sulistyowaty et al., 2021), neuroprotection activity (Hashim et al., 2021; Mundugaru et al., 2018), antihypertensive activity (Moura et al., 2005) and analgesics activity (Ahmed et al., 2015).

Alpinia is also an aromatic herb because it contains several essential oils in most parts of the plant. *Alpinia* essential oil has known chemical content, such as camphor, 1,8-cineole, β-myrcene, α-pinene, γ-terpinene, α-fenchyl acetate, ρ-cymene, ocimene, geraniol, methyl cinnamate, β-caryophyllene and β-pinene as the major compound (Van et al., 2021). Several essential oils from the *Alpinia* plant have been known to have antibacterial (Zhou et al., 2021), antifungal (Sujono et al., 2019), antioxidant (Kawai et al., 2021), anticancer (Elgamal et al., 2021), antihypertensive (Lahlou et al., 2003), pesticidal (Nguyen et al., 2022) and immunomodulatory (Sahoo et al., 2018).

Alpinia plants, especially *Alpinia monopleura*, can be found easily in the Sulawesi. This plant is an endemic *Alpinia* in Sulawesi, a broad and abundant distribution and is widely used in the community (Rugayah et al., 2019). However, the composition and pharmacological studies of this plant are entirely unknown. In addition, variations in climate and geographical location cause essential oils to have different chemical content and concentration so that they have the potential to have different biological activities (Mahdavi et al., 2017). Thus, it is undoubtedly a fascinating thing to study and explore as one of the efforts to develop alternative medicinal ingredients, especially the development of antibiotics.

The urgent need for overcoming resistant bacteria is significant to discovering and developing novel antibiotics sourced explicitly from natural products, including essential oils. On the other hand, the demand for natural preservatives and natural antioxidants is also increasing. This is because the production of free radicals in the body is correlated with various human diseases. Thereby, the antioxidant activity of natural products is also interesting to study (Warsito et al., 2022). Given the potential medicinal properties of *Alpinia*, which have been studied extensively, the essential oil of *A. monopleura* could be a promising source of these properties, thus supporting its use locally in Sulawesi and the pharmaceutical and food industries. Therefore, this study aims to explain the essential oil and antibacterial and antioxidant properties of leaves and fruit the essential oils of the plant *A. monopleura*.

EXPERIMENTAL SECTION

Plant Materials and Determination

Leaves and fruit of *A. monopleura* were obtained from Andoolo, South Konawe Regency, Southeast Sulawesi (4°33'26"W, 122°20'19"S). The BRIN Research Center for Biology determined the sample and registered it with Number 1535/IPH.1.01/If.07/VIII/2019.

Essential Oil Extraction

Essential oil extraction was carried out by hydrodistillation of water. Each sample (500 grams) was wet-sorted, washed, and followed by chopping to increase surface contact with the extraction solvent. Then each sample was put into a 2 L measuring flask containing 1 L of aquadest and heated continuously for 3 hours until the essential oil was collected. The results were stored in dark bottles and cooled at 4°C for further chemical composition characterization and activity test (Lin et al., 2021).

The yield of essential oils produced from the leaves and fruit of *A. monopleura* was determined using equation (1) (Lunggela et al., 2022).

$$\text{Yield (\%)} = \frac{\text{Amount of oil produced}}{\text{Number of samples}} \times 100 \quad (1)$$

Identification of Chemical Compounds

The chemical compound of essential oils was characterized using GCMS (Agilent 7890B) combined gas chromatography with a mass spectrometer. Gas chromatography was performed on column (19091S-433) with dimensions of 3000 mm × 250 µm × 0.25 µm. The mass spectrum was set in electron energy (70 eV) with electron ionization mode. The detector and injector were operated at 325 and 250°C programmable temperatures from 40 to 300°C. The volatile oil sample was dissolved with n-hexane in the ratio of essential oil: n-hexane = 1:10 (v/v) and then injected with a volume of 1 µL (Kamal et al., 2022).

Antibacterial Properties Test

Antibacterial properties assays were conducted as previously reported (Musdalipah, et al., 2021; Salni & Marisa, 2020) with slight modifications. The essential oil of the leaves and fruit of *A. monopleura* was tested for its ability to inhibit the growth of *Staphylococcus aureus* ATCC 25023 and *Escherichia coli* ATCC 35218. The bacteria were rejuvenated in liquid medium (Nutrient Broth, Merck) and incubated (37°C). A colony was inoculated in physiological 0.9% NaCl solution (Merck) with shaking at 200 rpm until a new bacterial suspension (10^6–10^8 CFU/mL) was obtained for testing. Antibacterial properties test was carried out using the agar diffusion method. A total of 50µL of bacterial suspension was put into 15 mL of nutrient agar media (Merck) and allowed to solidify. A paper disc (6 mm) was placed on the surface of the media, dripped with 20µL of sample solution, allowed to spread for 30 minutes and incubated for 20 hours (37°C). Inhibition zone diameters (IZD) were measured based on forming a clear zone (zone of inhibition). Minimum inhibitory concentrations (MIC) are known by looking at the smallest concentration in providing inhibition at various concentrations of 500 to 15.6 g/mL. The positive control used was amoxicillin. The negative control and the solvent used to dissolve the essential oil was dimethyl sulfoxide.

Antioxidant Properties Test

Antioxidant properties were carried out based on those previously reported, the 2,2 diphenil-1-picrylhidrazil (DPPH) method (Imran et al., 2022; Metasari et al., 2020) and 2,2-Azinobis 3-ethyl benzothiazoline 6-sulfonic acid (ABTS) method (Musdalipah et al., 2021; Wahyuni et al., 2021) with slight modifications. Samples were diluted in ethanol solvent at various concentrations (100 to 3.3 g/mL). DPPH radical solution (HIMEDIA) 0.05 mM (1.98 mg DPPH) was prepared in 100 mL ethanol, while ABTS radical was prepared (2.45 mmol/L $K_2S_2O_8$ (Merck) and 7.0 mmol/L ABTS (Sigma Aldrich)) for 16 hours (room temperature). A total of 4 mL of radical solution was mixed with 1 mL of the sample at all concentrations, shaken and incubated for 30 minutes for DPPH and 10 minutes for ABTS. Absorbance was determined using a UV-Vis spectrophotometer (Thermo Scientific) at 517 nm (DPPH) and 734 nm (ABTS) against the blank. The inhibitory activity of DPPH is expressed according to equation 2 below:

$$\text{Radical inhibitory activity (\%)} = \frac{\text{AC}-\text{AS}}{\text{AC}} \times 100 \quad (2)$$

where AC: absorbance of the negative control (DPPH and ABTS), and AS: absorbance of the sample in radical. The IC_{50} value of each essential oil sample was determined by: (a) Inhibitory activity (y) plotted against concentration (x) at six points (100, 50, 25, 15.5, 6.3, and 3.3 g/mL), (b) regression line equation is determined (y = ax+b), and (c) sample concentration (x) is calculated by substituting y=50 in the regression equation (b) (Wahyuni et al., 2021).

RESULTS AND DISCUSSION

Essential Oil Levels

The yield of *A. monopleura* oils resulting from this study was determined from the ratio between the weight of the essential oil extracted from the distillate and the weight of the sample that was distilled. Table 1 shows the amount of essential oil obtained from the hydro distillation process with different yields. This yield compares the quantity of essential oil produced from several processed simplicial. The higher the yield value, the higher the quality obtained (Lunggela et al., 2022). This is related to the type of essential oil content produced and the amount of content obtained.

Chemical Compound of Essential Oils

The essential oil that has been separated from the water was analyzed using GC-MS to determine the components contained in the essential oil. GC-MS analysis of essential oils exhibited 19 compounds from leaves representing 93.32% and 22 compounds from fruit representing 96.46% of total essential oils, as shown in **Table 2**. The compound that comes out first is methyl cyclopentane, which has the same properties as the mobile phase, which is nonpolar, while the last compound that comes out of longiborneol has the same properties as the stationary phase, which is polar. Peaks with different retention times indicate the number of components contained in the essential oil, while the area shows the abundance of a composition at each retention time.

The major composition of essential oil in leaves was caryophyllene oxide (22.6%), β-caryophyllene (16.5%), α-pinene (14.7%), limonene (13.4%), β-pinene (10.2%) and α-caryophyllene (7.2%). The major composition of fruit showed similar essential oil yet provided different percentages, namely caryophyllene oxide (24.1%), β-caryophyllene (18.3%), β-pinene (12.9%), limonene (11.5%), α-pinene (10.4%) and α-caryophyllene (7.6%). Chemically, essential oils are composed of a complex mixture of various compounds, but a particular compound is usually responsible for a particular aroma and activity. Most of the essential oils belong to the class of terpenoid organic compounds that are soluble in oil (lipophiles). The chemical structure of the major compounds contained in essential oils can be seen in **Figure 1**.

The essential oil of the same genus, *A. galanga*, showed the similarity of the compounds in *A. monopleura* yet provided different concentrations. The same compounds with the highest concentrations ever isolated from *A. galanga* were β-caryophyllene (40.5%), β-pinene (23.5%), caryophyllene oxide (20.3%), α-pinene (6.6%) and α-caryophyllene (5.1%) (Nguyen et al., 2021). The different levels of compound content between the two can be caused by climatic conditions and the type and level of soil fertility where the plant grows. The amount of concentration identified shows and proves that the compound is the main component of the genus *Alpinia*. Different concentrations of compounds in essential oils have the potential to show different pharmacological activities (Mahdavi et al., 2017).

Table 1. Essential oil levels from the leaves and fruit of *A. monopleura*

Sample	Amount (g)	Essential oil Amount (g)	Yield (%)
Leaf	500	5.8	1.16
Fruit	500	6.7	1.34

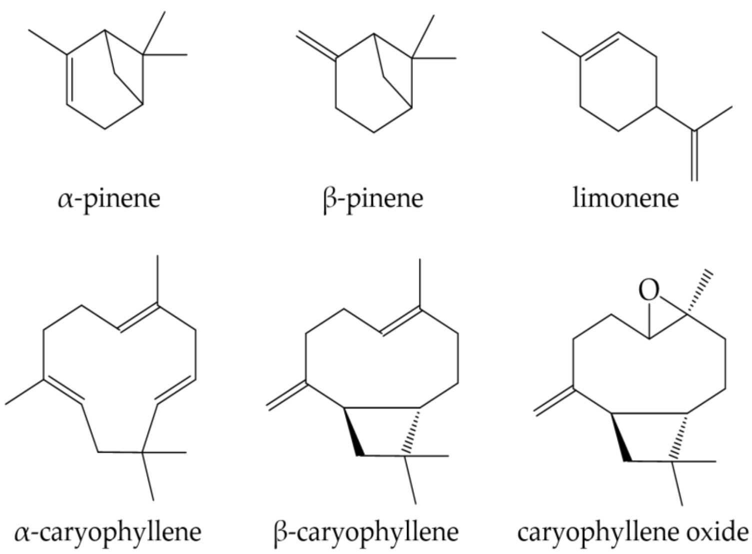

Figure 1. Chemical structures in A. monopleura

Table 2. The chemical compounds of the essential oil as found by GC-MS from the leaves and fruit of *A. monopleura*

No	R_t (min)	Compound	% Area	
			Leaves	Fruit
1	1.596	Methyl cyclopentane	1.08	1.188
2	3.641	α- pinene	14.688	10.362
3	4.156	Sabinene	-	0.594
4	4.219	β-pinene	10.188	12.969
5	4.352	β-mycrene	-	0.198
6	4.676	D^3-karen	0.396	0.396
7	4.955	Limonene	13.500	11.517
8	7.214	Pinocarvone	0.324	0.429
9	11.472	β-caryophyllene	16.488	18.249
10	12.014	α-caryophyllene	7.236	7.557
11	12.437	Germacrene	0.288	0.396
12	14.041	Caryophyllene oxide	22.608	24.057
13	14.25	β- selinene	0.468	0.33
14	14.425	2,5,9-trimethyl-cycloundeca-4,8-dienone	0.432	0.297
15	14.717	Bombykol	0.396	0.495
16	15.071	t-Muurolol	1.728	1.947
17	15.35	Longifolenaldehyde	1.296	0.363
18	15.529	1,3,3-Trimethyl-2-oxabicyclo [2.2.2]octan-6-ol	0.576	0.429
19	17.091	6-(2,6,6-dimethyl-1-cyclohexene)-4-methyl-1-ol	1.116	1.584
20	21.022	Retinal	-	1.65
21	22.117	2,4,7,14-tetramethyl-4-vinyl-tricyclo 5.4.3.0(1,8)-tetradecane-6-ol	0.612	0.528
22	22.438	Longiborneol	1.44	0.924
Total			93.132	96.459

Antibacterial Properties

The zones of bacterial inhibition by leaves essential oils and fruit essential oils from the *A. monopleura* are shown in **Table 3** and **Table 4**. The results of the antibacterial activity test showed that the bacteriostatic properties of essential oils against *S. aureus* bacteria were greater than those against *E. coli*. This is because *S. aureus* is a gram-positive bacterium with a simpler cell wall structure compared to *E. coli* which is a gram-negative bacterium. The difference in the zone of inhibition is caused by differences in the composition and structure of the cell wall between Gram-positive and Gram-negative bacteria. These differences cause different responses to the administration of essential oils. The cell wall structure of gram-negative bacteria *E. coli* consists of three layers, namely the cytoplasmic membrane, the outer membrane, and the layer between the two is a thin peptidoglycan (Miksusanti et al., 2012).

In Gram negative bacteria, the peptidoglycan layer on the cell membrane is thinner than in Gram positive bacteria. The outer membrane of Gram-negative bacteria is composed of phospholipids and lipopolysaccharides so that antibacterial substances that interfere with the integrity of the cell membrane will more easily attack Gram-negative bacteria by dissolving phospholipids. Phospholipids will decompose into glycerol, carboxylic acid, and phosphoric acid so that the membrane cannot maintain its shape, as a result the membrane leaks, substances can enter and leave the cell uncontrollably so that metabolism is disrupted and bacteria lysis (Dewi et al., 2014). Gram positive bacteria have a thicker peptidoglycan layer than Gram negative bacteria. This thicker peptidoglycan layer causes the cell wall permeability of Gram-positive bacteria to be lower than that of Gram-negative bacteria (Al-Kobaisi, 2007). Therefore, the active substance of essential oils will be more difficult to penetrate the cell membrane of Gram-positive bacteria so that the antibacterial effect is less than optimal. Based on its effectiveness, *A. monopleura* essential oil is classified as a broad-spectrum antibacterial because it can act on gram-positive and gram-negative bacteria.

Table 3. IZD and MIC of essential oil of *A. monopleura* against *Staphylococcus aureus*

Essential oil	Concentration (μg/mL)	IZD (mm)	MIC (μg/mL)	Amoxicillin (10 μg/mL)
Leaves	500.0	17.56	31.3	12.54
	250.0	14.35		
	125.0	12.27		
	62.5	10.43		
	31.3	8.56		
	15.6	0.00		
Fruit	500.0	18.19	31.3	12.62
	250.0	15.21		
	125.0	13.04		
	62.5	11.41		
	31.3	8.65		
	15.6	0.00		

Table 4. IZD and MIC of essential oil of *A. monopleura* against *Escherichia coli*

Essential oil	Concentration (μg/mL)	IZD (mm)	MIC (μg/mL)	Amoxicillin (10 μg/mL)
Leaves	500.0	21.19	31.3	14.02
	250.0	17.93		
	125.0	14.67		
	62.5	11.41		
	31.3	8.68		
	15.6	0.00		
Fruit	500.0	22.82	31.3	14.21
	250.0	19.56		
	125.0	15.64		
	62.5	13.04		
	31.3	9.45		
	15.6	0.00		

Based on **Table 3** and **Table 4**, it is known that the MIC values for *E. coli* and *S. aureus* bacteria were 31.3 μg/mL each. At this concentration, the essential oil could inhibit the growth of the test bacteria with an inhibition zone diameter of 8.0–14.0 mm. Inhibition zone diameters (IZD, mm) ≥20.0 mm were classified as very sensitive to essential oils; 14.0–20.0 mm as an sensitive; 8.0–14.0 mm as an quite sensitive; and diameter ≤8.0 mm as an insensitive (Lin et al., 2021), this means that essential oil of *A. monopleura* has MIC activity which is quite sensitive. It is known that essential oils of the genus *Alpinia* have antibacterial activity (Zhou et al., 2021).

The activity is undoubtedly closely related to the composition of the compounds in it. The antibacterial activity shown by the essential oils from *A. monopleura* is very relatable to caryophyllene oxide, β-caryophyllene and limonene, which are confirmed to have antibacterial activity. Inhibition of the growth of *E. coli* and *S. aureus* bacteria in limonene showed MIC values of 16 μL/mL (Gupta et al., 2021) and 20 μL/mL (Han et al., 2021), β -caryophyllene, respectively, showed MIC values of 9 μM and 3 μM, respectively (Dahham et al., 2015). Caryophyllene oxide showed MIC values of 60 μg/mL (Schmidt et al., 2010) and 15.63 μg/mL (Alagasamy et al., 2021).

The essential oil of *A. monopleura* is an oil from the terpenoid group, the mechanism of terpenoids as an antibacterial is sensors with porins (transmembrane proteins) on the outer membrane of the bacterial cell wall, forming strong polymer bonds resulting in damage to the porins. Damage to the porin, which is the entry and exit gate compound, will reduce the permeability of the bacterial cell wall which will result in a lack of nutrients in the bacterial cell, resulting in inhibited or dead bacterial growth (Cowan, 1999).

Table 5. The IC_{50} values of antioxidant activity of *A. monopleura*

Sample	IC_{50} (µg/mL)	
	DPPH Method	ABTS Method
Leaves	19.42 ± 1.00	15.60 ± 0.99
Fruit	11.93 ± 0.45	10.44 ± 0.26
Ascorbic Acid	8.29 ± 0.26	8.61 ± 0.22

Antioxidant Properties

The antioxidant properties of leaves essential oils the and fruits essential oils from the *A. monopleura* against DPPH and ABTS radicals through their ability to reduce radical compounds were exhibited in **Table 5**. The DPPH method is based on the antioxidant ability of a compound to donate hydrogen ions, whereas the ABTS method is seen based on the compound's ability to stabilize free radical compounds by donating proton radicals (Imrawati et al., 2017). The results showed that the ability of essential oils to scavenge ABTS radicals was better than DPPH. ABTS reacts more quickly with antioxidants, can dissolve in organic solvents and water, can be used at different pHs, and is more sensitive than the DPPH method which is only sensitive to acidic pH (Shalaby & Shanab, 2013).

The ability to reduce radicals is expressed as the IC_{50} value. The essential oil of *A. monopleura* showed an IC_{50} value of <50 µg/mL. IC_{50} value >200 µg/mL is no activity, >150-200 µg/mL is weak, >100-150 µg/mL is quite strong, >50-100 µg/mL is strong and <50 µg/mL is very strong (Yodha et al., 2021). It is known that essential oils of the genus *Alpinia* have antioxidant activity (Kawa et al., 2021). The essential oil from the leaves and fruit of *A. monopleura* has a very strong ability to provide antioxidant activity.

Based on the results of GCMS, the compounds in essential oils that have a role in providing antioxidant properties are most likely limonene, β-caryophyllene, caryophyllene oxide and α-pinene (Shah & Mehta, 2018; Dahham et al., 2015; Salleh et al., 2015; Wang et al., 2019).

This is supported by previous research data, that these compounds are able to inhibit free radicals. The activity of inhibiting DPPH and ABTS radicals in limonene showed IC_{50} values of 52.4 µg/mL and 82.2 µg/mL, respectively (Shah & Mehta, 2018), while β-caryophyllene, caryophyllene oxide and α-pinene showed IC_{50} values against DPPH radicals 12.5 µg/mL (Dahham et al., 2015), 10.25 µg/mL (Salleh et al., 2015) and 12.57 µg/mL (Wang et al., 2019), respectively.

The DPPH and ABTS radical scavenging abilities of essential oils show a significant positive correlation with essential oil concentrations and have a close relationship with their chemical components, especially their main components (Lu et al., 2018). The main components of essential oils are olefinic terpenoids which provide antioxidant activity depending on the number and position of C = C double bonds (Wojtunik et al, 2014). Double bonds can be easily analyzed with free radicals to carry out their antioxidant function. Terpenoids also have chain-breaking antioxidant activity similar to that of phenols (Gonzales & Gomez, 2012).

CONCLUSIONS

In this study, the hydro distillation method extracted two types of essential oils. Its chemical composition, as well as antibacterial and antioxidant properties, has been studied successfully. The major composition is caryophyllene oxide, β-caryophyllene, β-pinene, limonene, α-pinene and α-caryophyllene. Both oils exhibited antibacterial properties against *E. coli* and *S. aureus* and provided very strong activity in inhibiting DPPH and ABTS free radicals. This capability shows excellence in its use in the pharmaceutical and food industries.

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