ORIGINAL ARTICLE

Essential oils from *Homalomena* aff. *pendula* (Blume) Bakh F. and Its Antioxidant Activities

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ABSTRACT

Homalomena is a genus from the family Araceae that is characterized by scent. Most Homalomena species from Indonesia have limited information on the chemical components of their essential oils, including those from West Sumatra Province. The species of *Homalomena* aff. *pendula* (Blume) Bakh. F. is found in large amounts in Padang city, and it has the potential to explore its profile of essential oil substances in its leaves, petioles, and rhizomes. In this research, the antioxidant activity of the essential oils was screened using three methods: DPPH, ABTS, and FRAP. The essential oils are extracted by hydrodistillation, and their components are analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). The results show that the three major components in the leaves and petioles were β-bisabolene, α-selinene, and α-bergamotene, each present at different percentages. The rhizomes had the same higher component as β-bisabolene, followed by trans-caryophyllene and α-bergamotene. The antioxidant activity, as measured by DPPH and ABTS assays, showed IC_{50} values greater than 250 µg/mL. That result is also supported by the FRAP value of leaves, petioles, and rhizomes, 0.165±0.0081; 0.168±0.0019; 0.191±0.0003 µmol Fe(II)/mg sample and 0.013±0.0006; 0.014±0.0001; 0.015±0.0001 µmol trolox/mg sample, respectively. Although the essential oils from *H.* aff. *pendula* do not exhibit antioxidant activity, the primary compound, β-bisabolene, has been reported to possess other potential activities.

Keywords: Antioxidant activity, Araceae, Essential oils, *Homalomena* aff. *pendula*

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Access This Article

Introduction

Homalomena is a genus from the family Araceae that is distributed in tropical Asian Countries, with a key determinant of its aroma. Some of its species have been used as ethnomedicines in Southeast Asia [1]. Approximately 310 Homalomena species have been identified, eachwith a distinct aroma [2]. West Sumatra is home to several endemic species of the genus Homalomena, including *Homalomena rusdii*, *Homalomena padangensis*, and *Homalomena gadutensis* [3]. However, *Homalomena* aff. *pendula* (Blume) Bakh. f. is a wild species in Padang city, which can be collected in large amounts for gaining its essential oils. This plant grows well in hilly areas, on riverbanks, and along lakefronts. Homalomena species ares also used in traditional medicine for fever, cough, and influenza [4], [5], [6].

Essential oils from the genus Homalomena have been reported for *H. cochinchinensis* and *H. pendula* [5]. A group of compounds from these essential oils was identified as oxygenated monoterpenes, accounting for 45.3% and 71.9%, respectively. Both Homalomena species had the same principal component, linalool, at approximately 56.3% in *H. cochinchinensis* and 31.6% in *H. pendula*. The percentage of the compound can vary due to geographic factors, soil nutrient availability, and plant growth [7].

Screening antioxidant activity in essential oils has several advantages. The antioxidant mechanism can be utilized in medicine, cosmetics, and food preservatives. Several reports on essential oils have demonstrated their ability to reduce the toxic effects

through radical scavenging. The degenerative diseases can also be treated with antioxidant substances [8], [9], [10]. The potential of essential oils with antioxidant activities could be applied to human health. However, the limited information on chemical components from *H.* aff. *pendula* in Sumatra had initiated this study to identify its essential oil substances.

This study extracted essential oils from various parts of *H.* aff. *pendula*. The plant material included fresh leaves, petioles, and rhizomes. Each essential oil component will be analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). The activity will be screened for its antioxidant properties using the DPPH, ABTS, and FRAP assays.

METHODS

Plant Materials

The plant sample was collected in Gunung Padang, Padang City, West Sumatra (50-80 m asl, 0°55'32.8"S, 100°21'46" E). The sample was divided into leaves, petioles, and rhizomes. Identification of the plant sample was conducted and stored at the Herbarium ANDA, Universitas Andalas with number collection ANDA-0053453.

Extraction of Essential Oils

The sample of leaves, petioles, and rhizomes from *H.* aff. *pendula* were used 24.4, 52.9, and 43.8 Kg, respectively. All parts of the sample were extracted by hydro-distillation with a Clevenger-type apparatus for 6 hours. The essential oils are then mixed with sodium sulphate anhydrate to remove residual water, and kept in the fridge at 4-5ºC before further use [11].

Essential oils Characterization

Characterization of essential oils included organoleptic, yield, specific gravity, refractive index, and analysis of chemical substances of essential oils using GC-MS [12]

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

Chemical analysis of the essential oils was performed as described by Nguyen et al. (2023) [5]. Each essential oil was diluted at a 1:100 ratio with dichloromethane and 1 µL was injected into a GC-MS Shimadzu QP2010 SE (Kyoto, Japan) equipped with a Rtx-5MS column (5% diphenyl/95% dimethyl polysiloxane). Helium was utilized as the carrier gas at 1.5 mL, min. The oven temperature profile was set as follows: initial hold at 60°C for 2 min, a ramp to 240°C at 3°C/min with a 10 min hold, and a final increase to 280°C at 5°C/min, maintained for 40 min. For the mass detector, the interface temperature was maintained at 280°C, and data were acquired across a mass range of 40-500. The GC-MS data were matched to Wiley 7 based on their mass spectra.

Antioxidant assay using 2,2-diphenyl-picrylhydrazyl (DPPH)

The antioxidant method, an established approach from Hidayat et al. (2017) [13], was employed with slight modifications [13,14,15]. Ten mg DPPH powder was dissolved in 100 mL of p.a methanol. The sample was prepared at a concentration of 10 mg/mL in p.a methanol and then serially diluted to 250, 125, 62.5, 31.25, 15.625, 7.8125, and 3.90625 µg/mL. The positive control was vitamin C, and the negative control was p.a methanol. Each sample and control were diluted to 50 µL and mixed with 150 µL of DPPH solution. The mixture was incubated for 30 min in the dark and measured at 517 nm using a microplate reader.

The IC_{50} value was determined using a linear regression analysis of % inhibition versus concentration. The % inhibition is calculated following this formula:

$$(\%)\ \text{inhibition} = (A-B)/A \times 100\%$$

A = the absorbance of the negative control

B = the absorbance of the sample

The lower IC_{50} value means the better the antioxidant activity of a sample [16,17].

Antioxidant assay with 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) [16,17]

The ABTS assay used a seven mM ABTS solution in aquabidest and a 2.45 mM potassium persulfate ($K_2S_2O_8$) in aquabidest. The mixture of those solutions (1:1) was incubated in a dark room for 16 hours. The same concentration as used in the DPPH assay was employed. The sample and the positive control were dissolved in analytical grade methanol. The positive control used Trolox with various concentrations of 1.5, 2, 2.5, 3, and 3.5 µg/mL. The incubation ABTS solution was diluted with aquabidest (1:49) and measured at 734 nm, with an absorbance range of 0.7-0.9 nm. The diluted ABTS reagent was added to a 96-well plate containing 20 µL of the sample and 180 µL, then incubated for 6

minutes. The measurement was performed at a wavelength of 734 nm, and each sample was measured three times.

The sample was analyzed by calculating the percentage inhibition for each sample using the same formula as in the DPPH method. The inhibition and concentration data were used to calculate the IC_{50} value via linear regression.

Antioxidant Assay with Ferric Reducing Antioxidant Power

The FRAP method used an acetate buffer at 300 mM at pH 3.6. The 2,4,6-tripyridyl-s-triazine (TPTZ) at 10 mM was dissolved in 40 mM hydrochloric acid (HCl). The 20 mM$FeCl_3.6H_2O$ solution was prepared in aquabidest. The FRAP reagent was prepared by combining acetate buffer, TPTZ solution, and $FeCl_3 \cdot 6H_2O$ solution in a 10:1:1 ratio [15]. Essential oil samples and Trolox were dissolved in methanol p.a. Standard curves were prepared for Trolox and $FeSO_4 \cdot 7H_2O$ with various concentrations. The assay was performed by mixing the FRAP solution and the sample in 140- and 70-µL aliquots, respectively, in a 96-well plate. Afterwards, the mixture was incubated for 30 min and measured at 593 nm. All samples were prepared and measured in three replicates.

The data analysis was performed using standard curves of Trolox and $FeSO_4 \cdot 7H_2O$. The FRAP value was reported in relation to the two standards used.

RESULT AND DISCUSSION

Homalomena aff. *pendula* (Blume) Bakh. f. (**Figure 1**) has been identified as the original species, not *H. pendula* (Blume) Bakh. f. After carefully searching for the *H. pendula* from Padang city, here is the *Homalomena* aff. *pendula* description: erect herbs; leaves herbaceous 23–28 cm x 24.5–31 cm, based cordate, apex shortly acuminate, primary nerves 8-9 pairs; petiole stout, in lower half widened into firm sheath, 52–78 cm long, 1–1.5 cm diam.; sheath 13–23 cm long, margin slightly red; spathe elongate elliptic, mucronate, 5–5.5 cm long, 1–1.2 diam., dull greenish yellow, peduncle stout pendulous, 10–15 cm long, 0.5–0.6 cm diam.; spadix female 1.5–2.0 cm long, 0.7–0.8 diam., cream to citrine, male 2–2.2 cm long, 0.7–0.8 diam., apex obtuse, ivory to cream; berry

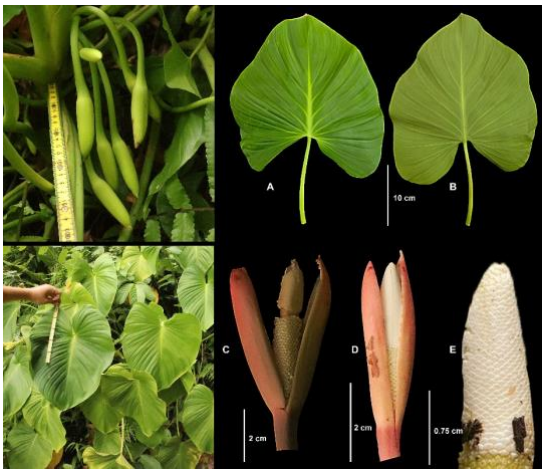

Figure 1. The *Homalomena* aff. *pendula* picture, front leaf (A), back leaf (B), flowers (C,D,E)

irregular shape, many seeds, dull greenish yellow [18].

The characterization of the essential oils includes organoleptic, yield, refractive index, and specific gravity **(Table 1)**. The yield of hydro-distillation of essential oils is relatively low, respectively, from leaves, petioles, and rhizomes (0.0084, 0.0072, and 0.0029% w/w). The aroma of the petiole is stronger than that of the other parts. The essential oils from leaves, petioles, and rhizomes are primarily composed of sesquiterpenes, with percentages of 92.56, 90.36, and 77.69%, respectively. The primary compound from leaves, petioles, and rhizomes was also the same, β-bisabolene, with percentages of 35.75, 36.13, and 21.79%, respectively. This compound was reported to have anticancer and antibacterial properties [19], [20]. The α-selinene compound was also present in each part of *H.* aff. *pendula* with percentages of 16.68, 12.98, and 7.12 from leaves, petioles, and rhizome, respectively. This second compound was potentially antibacterial [21]. Bergamotene accumulation was also detected in each plant part, with concentrations ranging from 7.54% to 8.86% **(Table 2)**. The compound is also reported to be anti-inflammatory [22]. The potential compound for antioxidant and antimicrobial activity was detected as δ-cadinene, which was available in each part of the plant, with a range from 4.12 to 5.72 per cent [23], [24]. The last five major compounds in this plant were trans-caryophyllene, which was reported as anti-inflammatory, neuroprotective, and muscle relaxation [25], [26].

Table 1. Characteristics of essential oils from each different parts of *Homalomena* aff. *pendula*

Parameter	Plant part		
	Leaves	Petiole	Rhizome
Color	Pale yellow	Pale yellow	Pale yellow
Scent	Specific aroma	Specific aroma	Specific aroma
Form	Oily	Oily	Oily
Yield (% w/w)	0.0084	0.0072	0.0029
Amount in volume unit	2.5 mL	4.7 mL	1.6 mL
Specific gravity (g/ml) (w/v)	0.8239±0.0003	0.8161±0.0001	nd*
Refractive index	1.4954	1.4953	1.4948

*nd = not calculated since the amount is not sufficient

Table 2. The chemical substances found in the essential oils of leaves, petioles, and rhizomes of *Homalomena* aff. *pendula*

No	tR	Compound	SI (%)	Area (%)			Molecular Formula/ Molecular Weight
				Leaves	Petiole	Rhizome	
1.	18.694	α-Cubebene	96	-	3.17	1.50	$C_{15}H_{24}$/ 204
2.	19.745	α-Copaene	96	2.20	2.75	1.98	$C_{15}H_{24}$/ 204
3.	20.519	α-Bergamotene	92	8.44	8.86	7.51	$C_{15}H_{24}$/ 204
4.	21.535	trans-Caryophyllene	96	0.51	1.23	8.39	$C_{15}H_{24}$/ 204
5.	22.342	Bicyclo [3.1.1] hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	96	-	-	3.15	$C_{15}H_{24}$/ 204
6.	22.669	β-Sesquiphellandrene	93	-	4.00	4.20	$C_{15}H_{24}$/ 204
7.	22.907	α-Humulene	98	0.69	1.13	1.87	$C_{15}H_{24}$/ 204
8.	23.187	Alloaromadendrene	94	0.60	0.58	0.58	$C_{15}H_{24}$/ 204
9.	23.355	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-	92	3.49	-	2.69	$C_{15}H_{24}$/ 204
10.	23.379	β-Farnesene	93	3.49	-	2.69	$C_{15}H_{24}$/ 204
11.	23.474	Unknown	88	0.36	-	1.53	$C_{15}H_{24}$/ 204
12.	23.890	2-Isoprophenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	95	0.61	0.78	-	$C_{15}H_{24}$/ 204
13.	24.061	Germacrene-D	94		1.05	1.75	$C_{15}H_{24}$/ 204
14.	24.261	β-Selinene	91	-	1.92	-	$C_{15}H_{24}$/ 204
15.	24.331	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	94	6.46	2.34	2.14	$C_{15}H_{22}$/ 202
16.	24.661	α-selinene	95	16.68	12.98	7.12	$C_{15}H_{24}$/ 204
17.	24.945	α-Muurolene	91	-	-	0.75	$C_{15}H_{24}$/ 204
18.	25.616	β-Bisabolene	96	35.75	36.13	21.79	$C_{15}H_{24}$/ 204
19.	25.959	δ-Cadinene	93	4.12	4.57	5.72	$C_{15}H_{24}$/ 204
20.	26.204	Naphthalene	96	-	0.39	0.61	$C_{15}H_{24}$/ 204
21.	27.633	Nerolidol	95	0.97	1.19	0.99	$C_{15}H_{26}O$/ 222
22.	27.919	Spathulenol	94	-	0.70	-	$C_{15}H_{24}O$/ 220
23.	28.136	Globulol	91	-	0.49	0.60	$C_{15}H_{26}O$/ 222
24.	28.864	d-Ledol	93	0.28	-	-	$C_{15}H_{26}O$/ 222
25.	29.915	Unknown	81	-	1.02	-	$C_{15}H_{24}$/ 204
26.	30.439	Unknown	89	-	1.05	2.94	$C_{15}H_{26}O$/ 222
27.	30.875	Unknown	88	0.49	2.01	-	$C_{15}H_{26}O$/ 222
28.	30.926	Unknown	89	-	-	2.77	$C_{15}H_{26}O$/ 222
29.	31.666	Unknown	86	1.16	1.62	2.74	$C_{15}H_{26}O$/ 222
30.	32.155	α-Bisabolol	95	0.69	1.01	5.38	$C_{15}H_{26}O$/ 222
31.	32.376	Unknown	86	0.32	-	1.00	$C_{15}H_{22}O$/ 218
32.	41.480	Unknown	85	2.09	-	0.53	$C_{20}H_{32}$/ 272
33.	43.789	Kaur-16-ene	93	1.14	0.47	0.68	$C_{20}H_{32}$/ 272

Table 3. The results of antioxidant activities using three methods of antioxidant assays

No	Sample	IC50 (µg/mL)		FRAP Value (µmol standard/ mg sample)		Antioxidant category
		DPPH	ABTS	FeSO4.7H2O	Trolox	
1.	Leaves	>250	>100	0.165±0.0081	0.013±0.0006	Not active
2.	Petioles	>250	>100	0.168±0.0019	0.014±0.0001	Not active
3.	Rhizomes	>250	>100	0.191±0.0003	0.015±0.0001	Not active
4.	Vitamin C	5.32±0.03	-	-	-	Very strong
5.	Trolox	-	1.82±0.05	-	-	Very strong

The identified compounds from GC-MS data ware accepted if the similarity index (SI) value ≥ 90%, if the SI value below 90% indicated the compound as unknown. The identified compounds then compared with data of substances from *Homalomena pendula* and *Homalomena* aff. *pendula.* The data showed the differencethat *H. pendula* had a primary compound, linalool (31.6%), from its rhizome, followed by sabinene (6.2%), valerianol (5.6%), and δ-3-carene (5.1%). These various chemical substances can occur due to geographical, temperature, and soil nutrient differences [7], [27], [28]. In this case, the difference in secondary metabolites of the essential oils of *Homalomena* aff. *pendula* can be an indication that the species are different. It supports the identification of the *Homalomena* aff. *pendula,* which is called chemotaxonomy.

The antioxidant activity was screened for each essential oil from leaves, petioles, and rhizomes of *Homalomena* aff. *pendula.* Three methods of antioxidant assay are used to measure their activities (DPPH, ABTS, FRAP) [13,15,16,17]. **Table 3** displays the result of the antioxidant activity of essential oils from *H.* aff. *pendula.* The antioxidant activity of essential oils from *H.* aff. *pendula* were not active in DPPH and ABTS methods. It supported by lower value of antioxidant capacity measuring with FRAP method. Substances in essential oils also embraced these results. The β-bisabolene, as a primary compound, is a type of hydrocarbon. This group of compounds lacks a donor hydrogen or electron-donating group to neutralize the radical scavenging; the presence of this component is essential for antioxidant activities [29], [30], [31]. Most of the groups of compounds that can stabilize radical scavenging are phenolic, aliphatic, and aliphatic aldehyde groups. It is important to consider the solubility of essential oils, as the ABTS method has certain limitations in this regard. The maximum concentration of essential oil should not exceed 2000 ppm; exceeding this threshold can cause cloudiness when the essential oil solution is mixed with the ABTS reagent, ultimately affecting the absorbance measured by the microplate reader.

The primary compound of essential oil in *H.* aff. *pendula* have other potential activities. Several studies have reported the potential of β-bisabolene as an anticancer agent based on its toxicity effect on selected breast cancer cells, EpH4, 4T1, and MG1361, with respective IC_{50} values of >200, 48.99, and 65.49 µg/mL [19]. The *in silico* study on the biological activity of β-bisabolene demonstrated its potential as a nicotinic acetylcholine receptor (nAChR) subtype α4-β2 [32]. This bioactivity screening explored the potential of β-bisabolene in addressing neurological disorders, despite the antioxidant activity of essential oils from *H.* aff. *pendula* is weak, but other bioactivities are still possible to explore.

CONCLUSION

The research findings reveal that essential oils from *Homalomena* aff. *pendula* had β-bisabolene as a primary compound in leaves, petioles, and rhizomes. The antioxidant activities of the essential oils were weak, as the essential oil components were rich in sesquiterpene hydrocarbons. However, further study is needed to investigate the potential bioactivities of β-

bisabolene, as it may have applications in the treatment of anticancer and neurological disorders.

CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation.

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