

# Chemical Composition and Locomotors Activity of Essential Oils from the Rhizome, Stem, and Leaf of *Alpinia malaccensis* (Burm F.) of Indonesian Spices

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## ABSTRACT

The essential oils of three parts of the plant *Alpinia malaccensis* (Burm f.) (Zingiberaceae) collected from Sumedang, West Java Province has been studied. Determination of essential oil components from the rhizome, stem, and leaf of *Alpinia malaccensis* (Burm f.) was performed by confirmation of the linear retention index (LRI) and comparing the NIST library peak with data reported in the literature, the mass spectrum peaks with literature data. The essential oils obtained from rhizome, stem, and leaf of *Alpinia malaccensis* (Burm f.) by steam distillation was 1.22%, 0.25%, and 0.7%, (w/w), respectively. Rhizome oil composition was almost similar with stem oil with methyl cinnamate as the major component. Composition of leaf oil is different from rhizome and stem oil which dominated  $\alpha$ -pinene (30.57%). The rhizome in doses 0.1 ml (47.09 %) had stronger locomotor inhibition activity compare than stem (26.97 %) and leaf oils (32.23 %) in doses 0.1 mL.

## INTRODUCTION

*Alpinia malaccensis* (Burm f.) (*AM*), Zingiberaceae, which in West Java that called Laja Gowah is a rhizomatous herbs that grow wild in Java and the island of Ambon of Indonesia. It also grows in Bangladesh, Malaysia, India, Myanmar, and Thailand. In Sumedang and Subang, West Java, the rhizomes are used in traditional medicine as an antiemetic or as a seasoning for meat processing, and sent to Central Java as an active ingredient in cosmetics (Oyen, 1999).

*AM* is commonly cultivated and grows wild in the forests of teak, bamboo groves and forests. It grows at a height 2-1500 m above sea level and enjoy under the shade, and the tall of *AM* reach to 1-4 meters. All part of *AM* has aroma, especially the rhizome which has a stronger fragrance (Sastrapradja and Setijati, 1977; Heyne, 1987). Heyne (1987) explains that the fresh rhizome of *AM* contain 0.25% volatile oil. Laja gowah fruit often used traditionally as an antiemetic drug, whereas in Ambon leaf and fruit are used to freshen the mouth and perfect sound.

Laja gowah fragrant rhizome oil used for hair (van Valkenburg and Bungapraphatsara, 2001).

In Sumedang and Subang, West Java, the leaf is used in children as an anti-vomiting, and rhizome oil used as massage oil. According to the literature (Heyne, 1987), wet rhizome contains essential oils of 0.25%.

Fragrant essential oils of the rhizome is used as a cosmetic in traditional medicine. Dried and fresh rhizome contain essential oil to the extent of 0.25% and 1.33%, respectively. The essential oil the rhizome contains methyl cinnamate as the major component, 60 % (Muchtaridi *et al.*, 2003).

All parts of the rhizome is a fragrance, but the interesting results of this study is that almost every part to provide a unique scent. Here we also show that our study is significantly different from previous studies from other countries (Malaysia) (Azah *et al.*, 2005), Thailand (Nuntawong and Suksamrarn, 2008), and Bangladesh (Bhuiyan *et al.*, 2010). Therefore, the purpose of this study was to determine differences in the composition of essential oils from plants obtained from the Sumedang-Subang, West Java - Indonesia.

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## MATERIAL AND METHODS

### Materials

#### Plant Material

All parts (stems, leaf and roots) from AM were collected in November 2005 (rainy season) from Batugara, Ganeas, Sumedang (F 6 ° 44'-70 ° 83 ', 1,000 m) district. Specimens are identified by the Herbarium Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran and voucher specimens are stored in the Herbarium Biology Department, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran. Rhizome (6 kg), leaf (3 kg) and stems (3 kg) was dried in air for 14 days at room temperature to produce dry samples, each weighing 1 kg of rhizome, 0.5 kg of stems, and 1 kg of leaf, prepared for the distillation.

#### Animals

Male mice weighing 25 to 30 g and 2 to 3 months old were used. The mice were adapted for one week to the laboratory in which locomotor activity experiments were conducted and were selected for wheel rotations of between 150 to 300 rpm before the experiments were started.

#### Methods

##### Isolation of essential oil

All dry samples (1 kg roots, stems 0.5 kg, and 1 kg of leaf) on steam-distillation in Monaco Lembang, West Java, for 3 hours to isolate the essential oil fraction. Oil stored at -20 ° C after the addition of sodium sulfate.

##### Analytical Condition

Essential oils were analyzed on QP5050A GC/MS- (Shimadzu) equipped with a fused silica capillary DB-5ms 30 mm x 0.25 mm, 0.25 µ m, carrier gas helium 95.3 kPa, flow rate 1.7 ml / minutes. Temperature has been setup with the program as follows: 60 ° C for 5 minutes and then 250 ° C to 10 ° C / min, ending with 5 minutes at 300 ° C. Port Injector and detector temperature was 250 ° C and 280 ° C, respectively. The sample is injected by split and split ratio 1:20. MS operating conditions were: interface temperature 240 ° C; electron impact ionization at 70 eV by scanning the mass range (m / z) of 40-350 daltons with a sampling rate of 1.0 scans / s. Identification of compounds is done by searching on a computer using a digital library of mass spectral data by a Class-5000 software and by comparison of retention indices and mass spectra library authentic (Strehmel *et al.*, 2008; Ausloos *et al.*, 1999; Babushok *et al.*, 2007; Adams, 1995), relative to the C8-C20 and C21-C40 n-alkane series (Sigma) (Mijin *et al.*, 1999) in temperature-programmed run.

##### Mouse Locomotor Activity Tests

Locomotor activity of mice was measured using a wheel cage, in which mice ran and the number of rotations was recorded by a meter. Cage inhalators contained a glass fiber (20 cm x 20 cm x 30 cm) and were equipped with an electric fan for the

evaporation and distribution of volatile compounds. The mice were selected by weight (25 to 30 g) and by their ability to rotate the wheel cage up to 300 times in 30 min; eligible mice were then divided into three groups: a control group, a lavender oil group as positive control group (using 0.1, 0.3, and 0.5 mL/cage), and a essential oils tested as treatment group (using 0.1, 0.3, and 0.5 mL/cage). The application of the doses were based on the preliminary examination in which those doses were reasonable to be used and based on Kovar *et al.* (1987). Each group consisting of five mice was tested three separate times. After 30 min of inhalation, the mice were placed into the wheel cage and after 5 min; the number of rotations was recorded for 75 min in 15-minute intervals.

**Table 1:** Constituents of rhizome essential oil from AM (Burm f.).

No	LRI <sup>1</sup>	LRI <sup>2</sup>	Compounds name	Percentage (%)
1.	601	600	hexane	0.93
2.	930	939	$\alpha$ -pinene	14.90
3.	982	980	$\beta$ -pinene	12.44
4.	1031	1033	1,8-cineole	9.89
5.	1063	1062	$\gamma$ -terpinene	3.78
6.	1074	1088	terpinolene	0.45
7.	1077	1087	fenchone	0.43
8.	1089	1098	linalool	0.75
9.	1097	1097	sabinene	0.28
10.	1144	1143	camphor	6.22
11.	1187	1189	$\alpha$ -terpineol	6.00
12.	1380	1379	methyl cinnamate	64.4
13.	1525	1534	nerolidol	0.01
14.	1555	1561	$\gamma$ -caryophyllene	0.25
15.	1537	1590	viridiflorol	0.22
16.	1583	1593	hexadecane	0.17
17.	1604	1594	carotol	0.21
18.	1635	1653	$\alpha$ -cadinol	0.58
19.	1624	1659	patchouli alcohol	0.30
20.	1667	1683	$\alpha$ -bisabolol	1.03
21.	1659	1691	juniper camphor	0.38
22.	1683	1676	tetradecanal	0.03
23.	1782	1800	kctadecane	0.23
24.	1803	1778	pentadecanone	0.01
25.	1846	1879	hexadecanol	0.03
26.	1860	1900	nanodecane	0.09

**1 :** LRI experiment with DB5-MS column, **2 :** LRI reference in Adams (Adams, 1995) with DB5 column

## RESULTS AND DISCUSSIONS

### Chemical Composition of Essential Oils

Stem, leaf and rhizome oils AM contain 0.7%, 0.25% and 1.22% (w/w), respectively. In Table 1, 31 components of the rhizome oil dominates identified with methyl cinnamate (64.4%) followed by other components of  $\alpha$ -pinene (14.90%),  $\beta$ -pinene (12.44%), 1,8-cineol (9.89%), camphor (6.22%),  $\alpha$ -terpineol (6%),  $\gamma$ -terpinene (3.78%) and  $\alpha$ -bisabolol (1%). Components less than 1% were linalool (0.7%),  $\alpha$ -cadinol (0.58%),  $\alpha$ -terpinolene (0.4%), fenchone (0.4%), juniper camphor (0.4%), hexadecanoate acid (0.3%), sabinene (0.2%),  $\beta$ -caryophilene (0.2%), viridiflorol (0.2%), carotol (0.2%), and alcohol patchouli (0.1%). The stem oil, methyl cinnamate is still a major component with 30.24%, followed by 30 other compounds. 1,8-cineole (16%) in the rhizome oil exceeding the stem oil, followed by  $\alpha$ -pinene (13%),  $\beta$ -pinene (12%),  $\alpha$ -terpineol (6%), nerol (5%), camphor (1.3%),

and  $\alpha$ -ardinal (1.2%) as can be seen in Table 2. However, rhizome oil does not contain santalol, isoborneol, nerol, and phytol as in stem oil, but there are some substances contained in the rhizome is not in the stem such as  $\gamma$ -caryophyllene, nerolidol, viridiflorol, and carotol. On the other hand, essential oils do not contain flavone and diterpenoid as submitted by Nuntawong (Nuntawong and Suksamrarn, 2008).

**Table 2:** Constituents of stem essential oil from *AM* (Burm f.).

No	LRI <sup>1</sup>	LRI <sup>2</sup>	Compounds name	Percentage (%)
1	603	600	Hexane	0.03
2	942	939	$\alpha$ -pinene	13.05
3	979	980	$\beta$ -pinene	12.38
4	1032	1033	1.8 cineol	16.58
5	1140	1143	camphor	1.39
6	1149	1087	linalool	0.42
7	1153	1156	isoborneol	1.12
8	1191	1189	$\alpha$ -terpineol	6.23
9	1236	1240	nerol	5.50
10	1378	1379	methyl cinnamate	30.24
11	1645	1653	$\alpha$ -cadinol	1.20
12	1651	1683	$\beta$ -bisabolol	0.23
13	1657	1691	juniper camphor	0.29
14	1668	1659	patchouli alcohol	0.40
15	1679	1678	santalol	0.21
16	1685	-	stearaldehyde	0.16
17	1691	-	unknown	0.53
18	1702	1676	tetradecanol	0.19
19	1732	-	decanoic acid	0.27
20	1763	1700	heptadecane	0.14
21	1769	1700	heptadecane	0.21
22	1789	1667	citronellyl acetate	0.17
23	1805	-	Pentadecanone	0.20
24	1829	-	unknown	0.50
25	1854	1800	octadecane	0.10
26	1861	1900	Nanodecane	0.14
27	1875	-	pentadecatriene	0.24
28	1961	1961	hexadecanoic acid	0.80
29	1913	1944	isophytol	0.31
30	2112	-	dodocatretrianal	0.18
31	1880	1949	phytol	0.32

**1** : LRI experiment with DB5-MS column, **2** : LRI Adams (Adams, 1995) with DB5 column

There were significant differences in the composition of leaf oil composition compared to oil in the rhizome and stem  $\alpha$ -pinene (30%) is the main component in leaf oil, mint or donated coniferous smell while the rhizome and stem (Leffingwell, 1990) of *AM* has a balsamic odor.

This might be the reason the leaf are used as anti-emetics, and rhizomes are used as massage oil in Sumedang, where the sampling. However, these results differ significantly with that studied by Bhuiyan *et al.* (Bhuiyan *et al.*, 2010). They assume that the elements of the leaf oils *AM* from Bangladesh has been dominated by  $\alpha$ -phellandrene (31.80%), eucalyptol (13.76%), O-cymene (11.45%),  $\beta$ -pinene (11.34 %) and limonene (6.44%), even though methyl cinnamate has not been detected on leaf. Composition of leaf, stem and rhizome oils *AM* (Burm f.) in this study is significantly different from those reported by Azah *et al.* (Azah *et al.*, 2005), which indicates that the methyl cinnamate was dominant in leaf, stems and rhizome oils, with  $\alpha$ -pinene and 1.8-cineole accounted for less than 4%. However, our study used a different method of isolation of essential oils, steam distillation,

whereas they used distilled water, may affect the resulting compositions. In addition, the geographic and seasonal factors may be important in determining the chemical composition. Differences between the chemical compositions of essential oil of three parts of the plant can be seen in Table 4.

**Table 3:** Constituents of leaf essential oil from *AM* (Burm f.)

No	LRI <sup>1</sup>	LRI <sup>2</sup>	Compounds name	Percentage (%)
1.	957	976	sabinene	0.44
2.	970	985	heptene	0.08
3.	989	939	$\alpha$ -pinene	30.57
4.	991	980	$\beta$ -pinene	11.41
5.	999	991	myrcene	1.95
6.	1023	1033	1.8-cineole	21.39
7.	1030	1001	octanal	0.13
8.	1036	1018	$\alpha$ -terpinene	0.005
9.	1042	1062	$\gamma$ -terpinene	0.53
10.	1055	1068	cis-sabinene	0.20
11.	1068	1088	$\alpha$ -terpinolene	0.09
12.	1070	1074	linalool oxide	0.02
13.	1083	1088	Linalool	0.84
14.	1086	1098	nonanal	0.14
15.	1102	1112	fenchol	0.03
16.	1120	1144	4-terpineol	0.28
17.	1126	1139	pinocarveol	0.58
18.	1132	1143	camphor	1.76
19.	1139	1124	octanol	0.15
20.	1146	1162	pinocarvone	0.60
21.	1158	1156	isoborneol	0.86
22.	1169	1179	naphthalen	0.07
23.	1173	1180	cymen-8-ol	0.19
24.	1181	1189	$\alpha$ -terpineol	2.42
25.	1191	1193	piperitol	0.19
26.	1218	1217	carveol	0.31
27.	1226	1290	siclohexane	0.07
28.	1204	1255	Geraniol	2.42
29.	1236	1252	myrtanol	0.008
30.	1247	1316	citral	0.08
31.	1266	1285	bornyl acetate	0.10
32.	1351	1383	geranyl acetate	0.15
33.	1371	1379	methyl cinnamate	9.24
34.	1389	1415	$\alpha$ -bergamotene	0.23
35.	1421	1426	Ionone	0.24
36.	1421	1453	geranyl acetone	0.16
37.	1424	1426	$\alpha$ -ionone	0.08
38.	1428	1418	$\gamma$ -caryophyllene	0.12
39.	1436	1440	$\alpha$ -humulene	0.04
40.	1459	1485	$\beta$ -ionone	0.22
41.	1470	1485	$\beta$ -selinene	0.37
42.	1476	1461	Aromadendrene	0.16

**1** : LRI experiment with DB5-MS column, **2** : LRI Adams (Adams, 1995) with DB5 column

### Locomotors activity Inhibition of Rhizome, Leaf and stem oil from *AM* (Burm f.)

Our previous study, we evaluate the effect of the essential rhizomes oils from *AM* on locomotors activity in mice and identify the active components that may be responsible for the activity (Mughtaridi *et al.*, 2011).

Here, we presented locomotors activity inhibition of the rhizome, stem, and leaf oils from *AM* as shown in Table 5. Inhibition of locomotors activity of rhizome oil showed stronger than leaf oil from *AM*. On the other hand, the third rhizome, stem, and leaf oils contain methyl cinnamate and 1,8-cineole that might be responsible on activity (Mughtaridi *et al.*, 2011).

**Table 4:** Chemical compositions of the rhizome, stem, and leaf oil in AM (Burm f.)

Compounds Name	Percentage (%)			LRI <sup>1</sup>
	Rhizome oil	Stem oil	Leaf oil	
$\alpha$ -pinene	14.8	13.1	30.6	939
$\beta$ -pinene	13.5	12.4	11.4	980
1.8-cineole	10.4	16.6	21.4	1033
$\gamma$ -terpinene	3.5	nd	0.5	1062
linalool	0.8	0.4	0.8	1087
camphor	6.3	1.4	1.8	1143
$\alpha$ -terpineol	6.3	6.2	2.4	1189
Methyl cinnamate	64.4	30.2	9.2	1379
Nerolidol	0.01	nd	0.2	1534
$\gamma$ -caryophyllene	0.25	nd	0.12	1418
$\alpha$ -cadinol	0.6	1.2	0.1	1653
patcouli alcohol	0.3	0.40	nd	1659
$\alpha$ -bisabolol	1.0	0.2	nd	1683
Juniper camphor	0.4	0.3	nd	1691
Decanoic acid	0.1	0.3	0.4	-
Isoborneol	nd	1.1	0.9	1156
nerol	nd	5.5	nd	1240
Sabinene	0.3	nd	0.4	1097
Myrcene	nd	nd	2.0	991
Hexadecanoic acid	0.01	0.8	nd	1961
Fenchone	0.4	nd	nd	1087
$\gamma$ -Terpinolene	0.4	nd	0.1	1088
Geraniol	nd	nd	2.4	1255
Camphene	nd	nd	1.7	
citronellyl acetate	nd	0.2	nd	1667

nd = no detection

1 : LRI Adams(Adams, 1995) with DB5 column

**Table 5:** Average number of mice wheel cage rotations within 75 min of inhalation of Rhizome and leaf essential oils from AM.

		Mean Instead of Number of Average						% Inhibitory effect
		Minutes						
		0-15	15-30	30-45	45-60	60-75	Numbers of average	
<b>Controls</b>	<b>0</b>	280.4±20.5	294.4±4.3	311.4±17.2	303.4±14.6	297.4±9.7	1487	0
Lavender oils	<b>0.1</b>	217.6±18.1	200.4±12.3	195.6±9.6	197.4±7.9	193.2±11.9	1023.8	31.14*
	<b>0.3</b>	187.8±19.0	181.4±20.5	186.2±15.0	180.4±16.4	178.4±19.4	914.2	38.52*
	<b>0.5</b>	139.6±11.2	128.8±10.9	116.8±10.2	123.2±8.9	118.2±8.7	626.6	57.86***
Laja gowah leaf oil	<b>0.1</b>	239.1±23.4	228.5±22.2	291.5±35.3	237.1±28.9	250.6±27.3	1007.7	32.23*
	<b>0.3</b>	232.0±26.4	254.2±33.8	249.2±27.2	244.7±33.3	262.2±38.8	997.6	32.91**
	<b>0.5</b>	226.9±22.4	211.7±20.2	196.2±24.6	238.8±21.3	231.8±20.9	1095.4	26.33*
Laja gowah rhizome oil	<b>0.1</b>	180.4±24.8	174.3±33.2	157.2±29.7	146.8±34.7	128.0±22.9	786.7	47.09**
	<b>0.3</b>	203.6±40.1	196.5±30.5	188.8±19.7	171.9±36.6	147.2±38.5	908	38.94*
	<b>0.5</b>	246.6±28.9	259.6±38.7	198.7±34.6	173.5±30.9	183.4±40.1	1061.8	28.65*
Laja gowah stem oil	<b>0.1</b>	200.6±38.1	212.2±35.5	232.1±33.5	222.6±22.6	218.4±35.1	1085.9	26.97*
	<b>0.3</b>	186.2±30.9	178.7±38.2	180.2±28.9	173.4±26.5	165.8±40.2	884.3	40.53**
	<b>0.5</b>	188.2±25.2	190.2±22.3	216.7±22.3	189.8±40.1	177.6±33.5	962.5	35.27*

$F_{6,35} = 3.30$   $P < 0.05$ , as compared with the control treatment. (by ANOVA followed by Duncan post hoc test). \*\*  $F_{6,35} = 14.10$   $P < 0.01$ , as compared with the control treatment. (by ANOVA followed by Duncan post hoc test). \*\*\*  $F_{6,35} = 26.10$   $P < 0.001$ , as compared with the control treatment. (by ANOVA followed by Duncan post hoc test).

## Further Discussion

In Indonesia, this plant is not cultivated, and did not receive serious attention by the government of Indonesia. We recommend that AM (Burm f.) is given special attention because these plants have good prospects proved that all the major components in the essential oils of this plant has been used as a fragrance ingredient.

Methyl cinnamate can be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as product-non-cosmetic products such as household cleaners and detergents (Bhatia *et al.*, 2007), even these compounds have antibacterial activity (Huang *et al.*, 2009).  $\alpha$ -pinene is categorized as a local irritant, and is used as a counter irritant, insecticides, and mild antiseptic. Industry,  $\alpha$ -pinene have applications as a wax solvent, and raw materials for

camphor, perfumes, and pine oils. In addition, these compounds has anti browning in the food with inhibition monophenolase and diphenolase activity even it has antibacterial activity against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* and antifungal activity against *Candida albicans* (Xia *et al.*, 1999). 1.8-cineol is a fragrance ingredient that occurs in eucalyptus oil, with the smell of fresh and used in large quantities in the aroma and taste (Surburg and Panten, 2006).

Locomotor activity inhibition of the third part of the laja gowah were almost the same, however specifically inhibitory activity of leaf oil has a softer locomotor. Leaf oil is recommended to use for SPA, while the rhizome and stems oil is recommended for massage oil, balsamic odor because it is more dominant in both oils was compared leaf oils which predominant herbal odors.

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