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CHEMICAL COMPOSITION OF ESSENTIAL OILS AND ITS LOCOMOTOR ACTIVITY FROM THE BARKS OF *CINNAMOMUM SINTOC* BL. OF TWO DISTRICTS IN MIDDLE JAVA

MUCHTARIDI MUCHTARIDI^{1*}, SRI A SUMIWI², RINA F NUWARDA¹

¹Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Padjadjaran University, Jatinangor, Bandung Sumedang, West Java, Indonesia. ²Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Padjadjaran University, Jatinangor, Bandung Sumedang, West Java, Indonesia. Email: muchtaridi@unpad.ac.id

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ABSTRACT

Objective: The objective of this study was to determine of volatile compounds of essential oils from *Cinnamomum sintoc* Bl (Sintok) barks belongs to *Lauraceae* of two districts of Middle Java.

Methods: Analysis of essential oil components from the barks of *C. sintoc* Bl was performed by confirmation of the linear retention indices, following by the comparison of NIST library peak and mass spectrum peak with literature data.

Results: The essential oils from Yogyakarta and Jember of *C. sintoc* Bl obtained by steam distillation with the percentage of 1.10% and 1.15% (w/w), respectively, with eugenol and methyl eugenol having higher percentage, compare to other components, 35-38% and <10%, respectively. The sintok barks oil of Yogyakarta at 0.1 mL a dose decreased the locomotor activity as much as 11.33%, while at 0.3 and 0.5 mL doses increased the locomotor activity as much as 35.83% and 51.13%.

Conclusion: It can be concluded from this study that the inhalation of sintok barks oil gave different influence to locomotor activity depends on the doses given.

Keywords: Cinnamomum sintoc Bl, Methyl eugeunol, Linear retention indices, Eugenol.

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INTRODUCTION

Some fragrance components of aromatic plants used for cooking are known to influence locomotor activity [1]. The first study on aromatherapy was performed by Kovar and coworker who investigated the effect of inhalation of essential oil toward the motor behavior of mice. Kovar and coworker were intended to prove the benefit of essential oil and its single component which administered by inhalation. Essential oils from rosemary and dwarf pines have been proven to increase the motoric activity, while those from Melissa and Valerian showed the opposite effects [2,3]. Muchtaridi *et al.* investigated that some essential oils of Indonesian aromatic plants inhibit the locomotor activity of mice [1,4]. Sintok is an aromatic plant that grows in Indonesia, Malaysia, and Thailand with a woody stem extending involved in *Lauraceae* tribe.

Empirically, sintok is utilized as outer or inner medicines. It is used to treat worms in the belly, amoebic dysentery, and swelling (inflammation). Sintoc bark (*Cinnamonum sintoc* BI) belongs to *Lauraceae* family. It has been used as common treatment for swelling caused by insects' bites [5].

Research on chemical contents of sintoc bark has been conducted not only in Indonesia but also other countries. Jantan *et al.* examined chemical contents in sintoc bark form Malayan peninsula using gas chromatography and mass spectroscopy [6]. They found that sintoc barks consist of safrole (23.4%), murolen (13.5%), along with adequate amounts of eugenol, linalool, germakren, kadinen, terpinol, and the other terpenes.

Currently, the study to determine anti-inflammation activity from ethanol extract and the fraction solvent of sintok barks has been carried out. However, further research in finding an active compound of sintok still needs to be done [7]. Research on the activity of essential oil of sintoc bark from Malaysia showed that the essential oil has an *in vitro* anti-inflammatory activity by antagonizing lipoxygenases and platelet activating factor while *in vivo* it inhibits edema in mice's ear induced by tetradecanoylphorbol acetate [8]. However, the locomotor activity of essential oils of *C. sintoc* Bl (Sintok) barks has been yet reported. Therefore, the aim of this study was to examine the effect of essential oils from sintok barks on locomotor activity of mice.

MATERIALS AND METHODS

Materials

Plant materials

C. sintoc Bl plants were collected in July 2005 from Yogyakarta and Jember of Central Java. Specimens were identified by the Herbarium Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran and then stored in the Herbarium Biology Department, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran. 5 kg of sintok barks were dried in the open air for 14 days at room temperature then weighed 1 kg each for distillation.

Animals

Male mice weighing 25-30 g with age 2-3 months old were used. The mice were adapted for 1 week to the laboratory condition in which locomotor activity experiments were conducted and were selected for wheel rotations between 150 and 300 rpm before the experiments were started.

Methods

Isolation of essential oil

All dry samples (1 kg) were distilled using steam-distillation in Monaco Lembang, West Java, for 3 hrs to isolate the essential oil fraction. Oil stored at -20° C after the addition of sodium sulfate.

Analytical condition

Essential oils were analyzed based on a previous study [9]. The identification of compounds was conducted by screening a digital library of mass spectral data with a Class-5000 software and by comparison of retention indices and mass spectra library authentic (NIST and WILLEY) [10-13], relative to the C8-C20 and C21-C40 n-alkane series (Sigma) [14] in a temperature-programmed run.

Mouse locomotor activity tests

Locomotor activity was measured by recording the number of rotations of mice while running in a wheel cage using a meter. The locomotor evaluation condition methods refer to our previous study [1,15]. All experimental procedures for animal use have been approved by the Ethical Committee of Experimental Animals, Universitas Padjadjaran.

RESULTS AND DISCUSSION

Chemical composition of essential oils

Sintok barks oils from Yogyakarta and Jember contained 1.10% and 1.15% (w/w) of essential oils, respectively. Bark oil originated from Yogyakarta produced eugenol as the major component (38.38%) followed by myristicin (13.54%), safrole (10.17%), benzyl benzoat (4,66%), α -terpineol (4.40%), 4-terpineol (4.26%), methyl eugenol (4.14%), Δ -cadinol (1.28%), and isomyristicin (1.14%) (Table 1). The other components which were produced <1% were α -cadinol, Δ -cadinene, isopulegol, junifer camfphore, α -curcumene, borneol, L-linalool, α -copaene, camphore, bornyl acetate, α -calocorene, 1,8-cineol, tymol, gernacrene, caryophyllene oxide, globulol,

Table 1: Constituents of bark essential oil from sintok from Jogyakarta

S.No.	LRI ¹ LRI ² Compounds na		Compounds name	ne Percentage (%)		
1	1028	1031	L-Limonene	0.08		
2	1033	1039	1,8-cineol	0.27		
3	1095	1098	L-linalool	0.47		
4	1140	1146	Isopulegol	0.72		
5	1146	1143	Camphor	0.41		
6	1162	1165	Borneol	0.48		
7	1174	1177	4-terpineol	4.26		
8	1191	1189	α-terpineol	4.40		
9	1275	1285	Bornyl acetate	0.31		
10	1287	1285	Safrol	10.17		
11	1291	1290	Timol	0.25		
12	1358	1356	Eugenol	38.38		
13	1372	1376	α-copaene	0.47		
14	1400	1401	Metylceugenol	4.14		
15	1412	1408	Trans-caryophyllen	0.06		
16	1420	1418	β-caryophyllen	0.06		
17	1428	1439	Aromadendrene	0.17		
18	1444	1477	γ-murolene	0.17		
19	1450	1483	α-curcumene	0.50		
20	1486	1499	α-murolene	0.16		
21	1508	1503	Gernacrene	0.22		
22	1518	1524	∆-cadinene	0.78		
23	1520	1520	Myristicin	13.54		
24	1540	1542	α-calocorene	0.28		
25	1575	1576	Spatulenol	0.07		
26	1582	1581	Caryophyllene oxide	0.20		
27	1587	1583	Globulol	0.20		
28	1591	1590	Viridiflorol	0.14		
29	1620	-	Isomyristicin	1.14		
30	1638	1640	∆-cadinol	1.63		
31	1648	1653	α-cadinol	0.98		
32	1665	1691	Junifer camphor	0.69		
33	1700	-	Eugenic acid	0.24		
34	1760	1762	Benzyl benzoate	4.66		
35	1857	-	Eugenol derivative	0.28		
36	2176	-	Hexadecanoic acid	1.00		

¹LRI experiment with DB5-MS column, ²LRI Adams [13] with DB5 column. LRI: Linear retention indices

aromadendrene, γ -murolene, α -murolene, viridiflorol, L-limonene, spatulenol, t-caryophyllene, and β -caryophyllene.

Eugenol was still a major component with 26.31% in sintok oil from Jember, followed by 35 other compounds. Yields of eugenol and methyl eugenol in sintok barks Yogyakarta oil more than the sintok barks Jember oil, while myristicin was presented in a similar level as shown in Table 2.

However, α -terpineol (5.64%) and 4-terpineol (5.67%) of sintok barks Jember oil had a higher percentage than sintok barks Yogyakarta oil. Eugenol derivatives were produced more in sintok barks Jember oil than sintok barks Yogyakarta oil. Eugenol and methyl eugenol were also found in cloves, basil, and nutmeg. Eugenol and ethyl eugenol are used commonly for medicine and agriculture.

Eugenol and methyl eugenol are found as the most active attractant for the oriental fruit fly, *Bactrocera dorsalis* [16]. As medicine, these compounds reduce pain and inflammation for dental treatment [17,18] and muscle arthritis.

The composition of barks sintok oil in this study were significantly different from those reported by Jantan *et al.* [6], which indicated that the linalool was dominant in barks oil (23.8%), followed tetradecanal (16%). Surprisingly, eugenol and their derivatives did not found in bark of sintok Pahang, Malaysia. Jantan *et al.* reported that the most abundant component of the bark oil was linalool (23.8%). Except for

Table 2: Constituents of bark essential oil from sintok from Iember

S.No.	o. LRI ¹ LRI ² Compounds		Compounds name	ame Percentage (%)	
1	1100	1098	L-linalool	0.89	
2	1100	1097	t-sabinen hidrat	0.11	
3	1140	1143	Camphor	0.25	
4	1160	1165	Borneol	0.78	
5	1174	1177	4-terpineol	5.64	
6	1181	1189	α-terpineol	5.71	
7	1280	1285	Bornil asetat	0.27	
8	1285	1285	Safrol	7.62	
9	1288	1290	Timol	0.05	
10	1350	1356	Eugennol	26.31	
11	1370	1376	α-copaene	0.90	
12	1402	1401	Methyl eugeunol	2.35	
13	1406	1402	Isoeugenol	1.07	
14	1410	1408	Trans-caryophyllen	0.69	
15	1412	1418	β-caryophyllen	0.66	
16	1427	1439	Aromadendrene	0.56	
17	1444	1477	γ-murolene	0.51	
18	1445	1483	α-curcumene	0.89	
19	1487	1499	α-murolene	1.38	
20	1503	1503	Gernacrene	0.70	
21	1517	1524	∆-cadinene	2.37	
22	1518	1520	Myristicin	13.00	
23	1592	1542	α-calocorena	0.94	
24	1570	1576	Spatulenol	1.96	
25	1575	1581	Kariofilen oksida	0.58	
26	1580	1583	Globulol	0.50	
27	1601	1590	Viridiflorol	1.47	
28	1610	-	Isomyristicin	0.58	
29	1622	1640	∆-cadinol	1.68	
30	1638	1653	α-cadinol	1.27	
31	1676	1691	Junifer camphor 2.16		
32	1680	-	Eugeunic acid	0.89	
33	1698		Methyl	0.60	
			octadecanoate		
34	1762	1762	Benzyl benzoat	0.75	
35	1857		Eugenol derivate	3.95	
36	2176		Hexadecanoic acid	0.32	

¹LRI experiment with DB5-MS column, ²LRI Adams [13] with DB5 column. LRI: Linear retention indices

Treatments	Mean instea	% Inhibitory effect					
	Minutes						
	0-15	15-30	30-45	45-60	60-75	Total of average	
Controls							
0	313.6±21	298.3±11	300.2±30	290.2±15	278.3±11	1480.6	0
Lavender oils							
0.1	144.2±33	142.2±31	142.3±12	135.9±19	131.3±18	695.9	52.994*
0.3	120.7±20	121.2±28	112.8±31	110.9±24	109.2±26	574.8	61.18*
0.5	115.6±25	114.8±30	116.8±41	100.2±11	90.2±11	537.6	63.69***
Laja gowah leaf oil							
0.1	268.9±22	263.2±23	268.9±41	258.9±34	245.2±10	1305.1	11.85*
0.3	216±42	210.3±22	205.5±30	200.5±22	199.9±19	1032.2	30.28**
0.5	140.3±06	135.3±15	131.1±08	124±23	125.5±17	656.2	50.68*
Laja gowah stem oil							
0.1	288.9±41	266.5±11	262.9±09	260.9±26	250.1±26	1329.3	10.22*
0.3	236.5±08	230.2±13	210.1±25	210.5±28	201.4±22	1088.7	22.45**
0.5	148.2±18	150.2±08	140.4±22	130.5±33	127.6±27	696.9	52.93*

Table 3: Average number of mice wheel cage rotations within 75 minutes of inhalation of rhizome and leaf essential oils from C. sintok oil

* $F_{6.35}$ =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).

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).

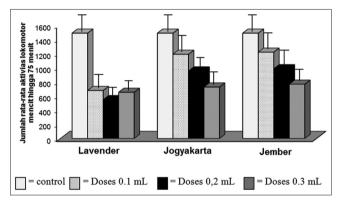


Fig. 1: Bar group of the average numbers of locomotors of mice after inhalation of essential oils within 75 minutes

terpinen-4-ol (2.4%) and p-cymene (1.3%), the other monoterpenes were present in <1% concentration in the oil [6].

This study was carried out using steam distillation for 3 hrs. The method was different from Jantan *et al.* who was using distilled water (or water distillation?) for 8 hrs. These differences might affect the yields of the components obtained. In addition, geographic and seasonal factors may also be important aspects in determining the chemical compositions [15].

Locomotors activity inhibition of bark oil from sintok (*C. sintoc***BI)** The effect of the bark essential oils from sintok on locomotors activity in mice was evaluated. As shown in Table 3, in general, the two sintok bark essential oils have a significant locomotor inhibitory activity compared to positive control (lavender oil). Locomotors activity of lavender oil as positive control showed higher inhibition than sintok bark oil. The inhibition locomotors activity of sintok bark oil from Yogyakarta higher than sintok bark oil from Jember as shown in Fig. 1.

Inhalation of sintok barks essential oil decreased mice locomotor activity in a dose-dependent manner. The essential oils of sintok bark at doses of 0.1, 0.3, and 0.5 mL decreased locomotor activity by 11.85%, 30.28%, and 50.68%, respectively, and those of Jember sintok oils at the same doses caused a locomotor activity decrease of 10.22%, 22.45%, and 52.93%, respectively. These dose-dependent effects might be due to higher concentrations of active components in the higher doses. On the other hand, the sintok bark oils showed strong but not dose-dependent inhibitory effects, in that the dose of 0.3 mL had a lower effect than that of 0.1 and 0.5 mL.

FURTHER DISCUSSION

In Indonesia, *C. sintoc* Bl is not cultivated and does not receive serious attention by the government. We recommend that this plant is given special attention as it has been proved that all the major components in the essential oils of this plant has been used as a fragrance ingredient and thus, has a good prospect in the future. In Central and West Java, sintok barks have still been purchased in the market whereas the plantation is very rare. Some Herbal Industries also use this plant for Jammu products.

Methyl eugenol can be found in fragrances and has been used extensively as a flavoring agent in many types of processed food, soft drinks, and sauces, perfumery, as an essential oil in aromatherapy, and cosmetics [19]. This compound has been used as an entomological perspective [20].

Eugenol exhibits pharmacological effects in almost all systems, such as penetration enhancer. It is and a very promising candidate for versatile applications, and the design of new drugs based on its pharmacological effects. α -terpineol, 4-terpineol, linalool, and 1.8-cineol are used as fragrance ingredients that have the smell of fresh and are used in large quantities in the aroma and taste [21,22].

The inhibition of locomotor activity by sintok barks oil from both Yogyakarta and Jember should be further investigated due to the toxic components which they contained (myristicin, safrole, and isomyristicin). In addition, eugenol and their derivatives can be considered as massage oils due to its effect to reduce acute pain.

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