Application of SPE-GC/MS for Analysis Lead Compounds Aromatherapy in Blood Plasma of Mice of Essential Oils Materials from Insonesian Spice*

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ABSTRACT

Leads compunds is one of fulfilled in standardization of Indonesian drug medicine standardized, but essential oils of material of aromatherapy standardized don't find of essential oils material from Indonesia.

The result showed that the four essential oils gave an inhibitory effect on locomotor activity of the mice. Inhalation of essential oils of kemangi leaves, serai dapur herbs, pala seeds and ki lemo bark decreased locomotor with the percent inhibition up to 57,64 %, 55,72 %, 68,62 %, and 60,75 %, respectively. Identification and quantification of active compounds in the blood plasma were carried out with GC/MS analysis after the mice experienced half an hour, one hour, and two hours inhalation. The blood plasma of three mice were collected in heparin tube and the volatile compounds were isolated and concentrated by the C-18 column (100 mg-Sep-Pak) with methanol and bidistilled water mixture (60:40) as the eluent. The recovery analysis was increased up to 90 %.

Major volatile compounds identified from blood plasma of the mice after inhalation of the essential oil of kemangi leaves were linalool and linaly acetate, whereas myristicin, 4-terpineol, and esthers of chain length (methyl palmitate, methyl myristate, methyl oleate, and methyl stearate) were dominant in blood plasma of mice after inhlation of the the essential oil of nutmeg seeds, and citronellol and citronellal dominant only in the blood plasma of mice inhaled essential oil of ki lemo barks. Linalool, citronelal, citral, dan methyl cinnamate are cimpounds identified from blood plasma of the mice after inhalation of the essential oil of serai dapur herbs.

Keywords : Essential oils, kemangi, serai dapur, nutmeg, ki lemo, SPE-GC/MS

BACKGROUNDS

Sample preparation is one of the step in analysis wich able to determine efficacy of analysis, because it can establish reproducibility and recovery of the matrix interference. SPE (Solid Phase Extraction) is recent trends in sample preparation for reduction solvent volume and time. In tis research, application of SPE has carried out in determination of lead compounds aromatheraphy in blood plasma of mice after inhalation essential oil. Lead compounds is one of fulfilled in standardization of Indonseian traditional medicine (Kataoka, 2003).

Leading study of this research has been performed by Kovar et al. (1987). They investigated the efficiency of the essential oil of rosemary and its main constituent, 1,8-cineol. The locomotor activity of the test animal increased significantly when they inhaled this oil. The finding recently published by Buchbauer and his group (1993) had investigated 40 fragrance and 6 essential oils to observe their ability to decrease the locomotor activity of the test animal.

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The study carried out by Sangat (1996) in the fields etnopharmacology indicated that there were 49 species of Indonesia plants of 22 families ere tradisionals used aromatherapy, however the publication about active volatile compounds of aromatical plants were only few, especially Indonesia.

Materials and Methods

Materials of spices

The material of plants used were kemangi leaves (*Ocimum formacitratum L.*) obtained from The Cileunyi tradisionals market, *serai dapur* herbs (*Cymbopogon citratus* L.) from Tanjungkerta, Sumedang, nutmeg seeds (*Myristica fragrans* HOUTT) from Bogor, and Ki lemo barks (*Litcea cubeba* L.) from Lembang, West Java.
The materials were distilled in BALITRO, Monaco Lembang. Essential oils of kemangi leaves, *serai dapur* herbs, nutmeg seeds, and ki lemo barks got were of 0.07 %, 0.94 %, 6.85 %, and 1 % respectively.

Chemicals

Methanol 95% p.a (Merck) for eluen SPE, Heparin tube (Bohreinger), and *lavender* oils pure (Martina Bertho) of *Lavandula officinalis*, Alcane standart C_8-C_{20} (Sigma) and C_{21}-C_{40} (Sigma), and 1,4-dichlorobenzene (Sigma).

Apparatus and Procedure

Whell Cage; Locomotor activity was measured by the Wheel Cage method. In this system, the mice run and the number of moving was recorded by calculator machine.

Inhalator

Cage inhalator contained fiber glass (20x20x30 cm) which was completed by air electric fan for evaporation and distribution of volatiles compounds.

GC/MS : It was performed using a QP-5050A (SCHIMADZU) gas chromatograph coupled to a VG Autospect Mass Spectrometer at 1 kV, 40-550 amu,.fused silica capillary column (DB-5MS, 30m x 0.25 mm), helium as carrier gas and temperature programming from 60°C/5 minutes to 300°C/1 min (10°C/ min) for blood plasma and 60°C/5 minutes to 300°C/2 min (10°C/ min) for essential oils. Identification of the substances was carried out by comparison of their retention indices (RI) with literature values and their mass spectral data with those from the MS data system Willey-229 lib., Nist-62 lib., and Nist-12 lib, (Adams 1995).

Retention Indices : Retention indices (RI) were calculated using GC data of a homologous series of saturated aliphatic hydrocarbons s within C_8 to C_{40}, performed in the same column and conditions as used in the GC analysis for the essential oils and the blood samples.

Collection of Blood : The blood samples were collected from corner parts of mice's eyes using capillary pipes, and placed in heparin tube.

Preparation and separation : Detail analysis was performed by modification of methods Jirovetz et al. (1992) and Kovar et al. (1987). The blood samples (500-600 µl), obtained according to Jirovetz and Buchbauer (1991), were centrifuged (1800 rpm/10

min) at room temperature and concentrated on a C18-column (100 mg-Sep-Pak). Volatile compounds were separated by eluen of methanol:bidistilled water (60:40). 5 µl was injected to GC-MS.

Quantification – Quantification of the volatile compounds in the blood samples was accomplished by the use of 1,4-dichlorobenzene as an internal standard.

Results and Discussion

Composition of Essential Oils

The accession essential oil of *kemangi* had a high content of E-citral (Geranial) (19.3 %) and linalool (8.17 %). The volatile compounds of essential oil of *serai dapur* herbs were α-citral (32.70 %), β-citral (28.99 %), linalool (1.6 %), citronellal (0.65 %) and methyl cinnamate (0.23 %). 4-Terpineol (13,92 %) and myristicin (13,57 %) were high content in essential oil of *nutmeg* seeds. On the other hand, alylbenzene or propylbenzene derivatives (myristicin, safrol, eugenol and derivatives) were dominant in *nutmeg* seeds. In ki lemo, 1,8-cineol (26.59 %) and citonellol (21.69 %) were the highest content.

Locomotor Activity and Active Compounds

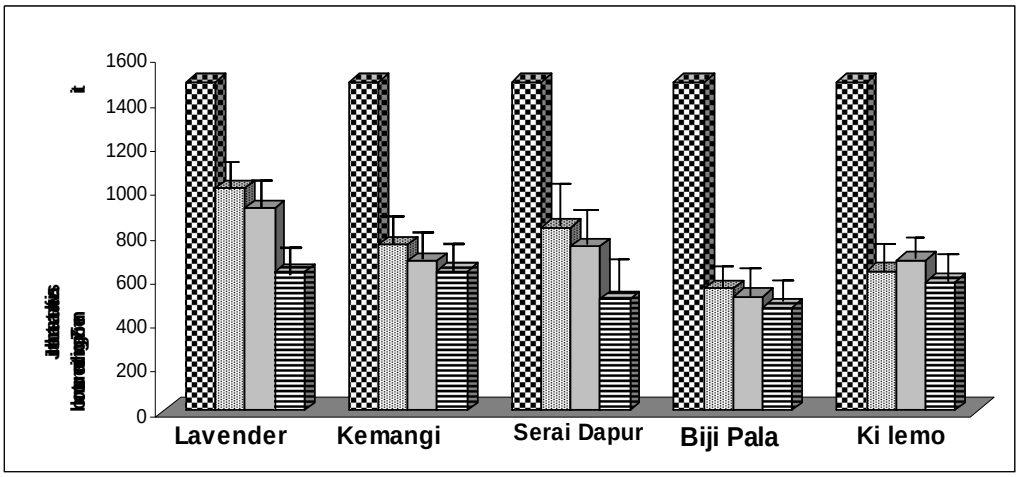
The numbers average of wheel cage of Mencit on duration 75 Minutes								% Inhibitory effect
		Dration (minute)						
		0-15	15-30	30-45	45-60	60-75	Numbers of average	
Controls	**0**	280,4±20,5	294,4±4,3	311,4±17,2	303,4±14,6	297,4± 9,7	1487,0	0
Lavender oils	**0,1**	217,6±18,1	200,4±12,3	195,6± 9,6	197,4±7,9	193,2±11,9	1004,2	31,14*
	0,3	187,8±19,0	181,4±20,5	186,2±15,0	180,4±16,4	178,4±19,4	914,2	38,52*
	0,5	139,6±11,2	128,8±10,9	116,8±10,2	123,2±8,9	118,2± 8,7	626,6	57,86*
Kemangi leaf	**0,1**	171,8±53,3	151,2±39,6	150,6±52,2	130,4±11,2	145,0±42,8	749,0	49,63*
	0,3	148,2±24,2	134,2±12,8	130,4±26,3	136,2±17,1	126,6±13,1	675,6	54,57*
	0,5	126,0±11,9	145,2±39,7	121,0±25,0	116,6±21,8	121,0±20,4	629,8	57,64*
Nutmeg seeds	**0,1**	120,0±35,5	113,6±32,8	114,2±22,7	105,0±22,3	100,2±22,1	553,0	62,81*
	0,3	121,4±41,8	119,2±37,0	110,8±36,4	81,8±23,2	82,2±18,5	515,4	65,33*
	0,5	107,8±27,2	104,0±26,1	84,4± 27,4	85,8±23,1	84,6± 23,2	466,6	68,62*
ki lemo BARKS	**0,1**	129,0±5,7	134,4±9,7	132± 23,7	122±19,7	115,4± 8,7	632,8	57,44*
	0,3	137,0±20,2	143,4±23,0	125± 12,5	139±11,5	136,6±19,8	681,0	54,20*
	0,5	115,0±9,2	123,8±8,4	112,8±10,7	113,8±8,8	118,2±11,2	583,6	60,75*
Serai Dapur Herbs	**0,1**	166,1±20,3	187,3±22,5	161,5±18,9	177,6±22,4	136,1±27,4	828,6	55,72*
	0,3	148,5±21,2	160,5±25,5	145,3±19,7	169,6+26,6	117,3±30,2	741,2	49,85*
	0,5	131,0±19,6	121,8±28,9	105,0±17,8	54,17±25,5	92,3±28,6	504,3	33,91*

Generally, the four essential oils gave an inhibitory effect on locomotor significantly. The results given in Table 1 indicated that inhalation of essential oil of kemangi leaves at doses of 0.1 ml, 0.3 ml, and 0.5 ml decreased locomotor with the percent inhibition of 49.63 %, 54.57 % and 57.64 %, respectively. While, the administration of essential oil of *serai dapur* herbs at doses 0.1 ml, 0.3 ml, and 0.5 ml decreased locomotor to 55.72 % , 49.85 % and 33.91 % respectively.

Essential oil of nutmeg seeds gave highest inhibitory effect than that of other essential oils. Inhalation at doses of 0.5 ml decreased locomotor to 68.62 %, whereas doses of 0.1 ml and 0.3 ml inhibited locomotor of 62.81 % and 65.33 % respectively.

A strong effect was observed when essential oil of ki lemo barks was given by inhalation at dose of 0.1 ml, 0.3 ml, and 0.5 ml. Doses of 0.1 ml inhibited locomotor activity of 57.44 %. The inhibitory effect decreased at doses 0.3 ml (54.20 %), but increased when essential oil ki lemo barks was inhaled at doses of 0.5 ml (60.75 %).

In the Figure 1, essential oil of nutmeg seeds afforded higher inhibitory effect. Increasing doses of essential oil of nutmeg, increased the inhibitory effect on locomotor. Sonavanne *et al.* (2001) explained that the nutmeg seeds reduce the number of head poking and potentiated pentobarbital-induced sleep after oral administration. These observations suggest that the nutmeg seeds gave sedative effect. Since the mechanism of anxiogenic activity is obscure, it is difficult to interpret the mechanism involved in potentiation of pentobarbital-induced sleep, but could be predicted that alybenzen or propylbenzen derivatives in nutmeg give role in sedative activity.



Picture 1. Graphic of numbers average on locomotors of mice after inhalation essential oils on duration 75 minutes

Explanation:

= Controls
= dosis 0,1 ml
= dosis 0,3 ml
= dosis 0,5 ml

Application SPE-GC/MS for analysis lead compound in blood plasma of mice after inhalation of essential oils of spices

Recovery of this analysis increased up to 90 %. The sample preparation got to reduction solvent volume and time. Lead compounds could be detected in blood plasma of mice after inhalation essential oils of spices using by application of SPE-GC/MS in preparation samples.

The roles of volatile compounds in locomotor activity are different. They are dependent compounds contained in essential oils. Compounds identified in blood plasma with the high bioavailability at 30 minutes until 2 hours are estimated to be responsible for pharmacological action and the compounds are called lead compounds (Buchbauer, 1993).

Compounds of essential oils of *kemangi* leafs detected in blood plasma of mice after inhalation with preparation SPE

In the Table 2, linalool , 1,8-cineole, 4-terpineole and α-Terpineol were major compounds identified . The compounds of essential oils of kemangi undetected in blod plasma of mice after inhalation ½ hours, but could be detected after inhalation 1 and 2 hours.

Table 2. Active volatile compounds identified after inhalation of essential oil of *kemangi* leaf at different duration of inhalation

Nama	Inhalation ½ hours (R^c = 80 %)		Inhalation 1 hours (R^c=88 %)		Inhalation 2 hours (R^c=88 %)		LRI Ref[a]
	LRI Eksp[b]	Conc. µg/ml	LRI Eksp[b]	Conc. µg/ml	LRI Eksp[b]	Conc. µg/ml	
1,8-Cineol	*nd*	*nd*	1035	0,8	*nd*	*nd*	1033
Linalool	*nd*	*nd*	1090	25,8	1090	5,9	1098
Borneol	*nd*	*nd*	1159	2,1	*nd*	*nd*	1156
4-Terpineol	*nd*	*nd*	1166	3,9	1165	1,9	1177
α-Terpimeol	*nd*	*nd*	1178	2,6	1178	1,3	1189
Linalil asetat	*nd*	*nd*	1216	9,6	1216	0,6	1257
α-Humulena	*nd*	*nd*	*nd*	*nd*	1446	2.2	1454

Explanation of Table 3.

nd = no detected , **a :** LRI reference in Adams (1995) with DB5 column, **b :** LRI experiment with DB5-MS column, **c :** Recovery (n=2) was calculated on the basis of comparation between 1,4-dichlorobenzene (methanol diluted) in blood plasma and 1,4-dichlorobenzene in methanol only

Compounds of essential oils of *serai dapur* herbs detected in blood plasma of mice after inhalation with preparation SPE

Citronellal was dominant compound in blood plasma of mice after inhalation of essential oil of *serai dapur* herbs, such as in the Table 3. Content of citronellal and methyl cinnamate in essential oils *serai dapur* herbs were small, but be found in blood plasma of mice.

Table 3. Active volatile compounds identified after inhalation of essential oil of *serai dapur* herbs at different duration of inhalation

No. Peak[c]	Name	LRI Exp[b]	Concentration (µg/ml)			LRI Ref[a]
			Inhalation ½ hours (R^c=82%)	Inhalation 1 hours (R^c=87%)	Inhalation 2 hours (R^c=90%)	
1.	*Linalool*	1098	*nd*	*8,7*	*3,4*	1098
2.	*Citronellal*	1153	*nd*	122,5	8,9	1156
3.	*Citral*	1238	*nd*	4,7	*4,6*	1240
4.	*Methyl cinnamate*	1388	*nd*	11,5	*3,2*	1379

Explanation of Table 3.

nd = no detected , **a :** LRI reference in Adams (1995) with DB5 column, **b :** LRI experiment with DB5-MS column, c **:** Recovery (n=2) was calculated on the basis of comparation between 1,4-dichlorobenzene (methanol diluted) in blood plasma and 1,4-dichlorobenzene in methanol only

Compounds of essential oils of nutmeg seeds detected in blood plasma of mice after inhalation with preparation SPE

In this research, myristicin and 4-terpineole were major compounds identified. Increasing duration of inhalation has increased the concentration of both myristicin and 4-terpineole in the blood plasma of mice (Figure 2). Table 3 indicates the results of identification of volatile compounds in blood plasma at different duration of inhalation.

Table 3. Active volatile compounds identified after inhalation of essential oil of nutmeg seeds at different duration of inhalation

Compound	Duration of Inhalation						LRI Ref[a]
	½ hours (R^d = 84 %)		1 hours (R^d = 90 %)		2 hours (R^d = 86 %)		
	LRI Exp[b]	Conc. µg/mL	LRI Exp[b]	Conc. µg/mL	LRI Exp[b]	Conc. µg/mL	
4-Terpineol	1181	1.5	1181	2.9	1183	6.3	1177
Safrole	*nd*	*nd*	*nd*	*nd*	1292	1.3	1285
Myristicin	1521	3.8	1521	5.2	1523	7.1	1520
Methyl myristate	1718	1.6	1719	1.4	1720	1.2	1726
Methyl palmitate	1919	67.8	1920	72.2	1922	58.7	1927
Palmitatic acid	1952	2.8	*nd*	*nd*	*nd*	*nd*	1961[c]
Methyl octadeca-10-oate	2094	23.3	2096	24.7	2097	18.9	-
Methyl oleate	2100	12.1	2102	13.8	2105	10.7	-
Methyl stearate	2129	13.1	2132	13.2	2134	10.8	2128

Explanation of Table 3.
nd = no detected , **a :** LRI reference in Adams (1995) with DB5 column, **b :** LRI experiment with DB5-MS column, **c :** LRI reference in King *et al*. (1993) with HP5 column, **d :** Recovery (n=2) was calculated on the basis of comparation between 1,4-dichlorobenzene (methanol diluted) in blood plasma and 1,4-dichlorobenzene in methanol only

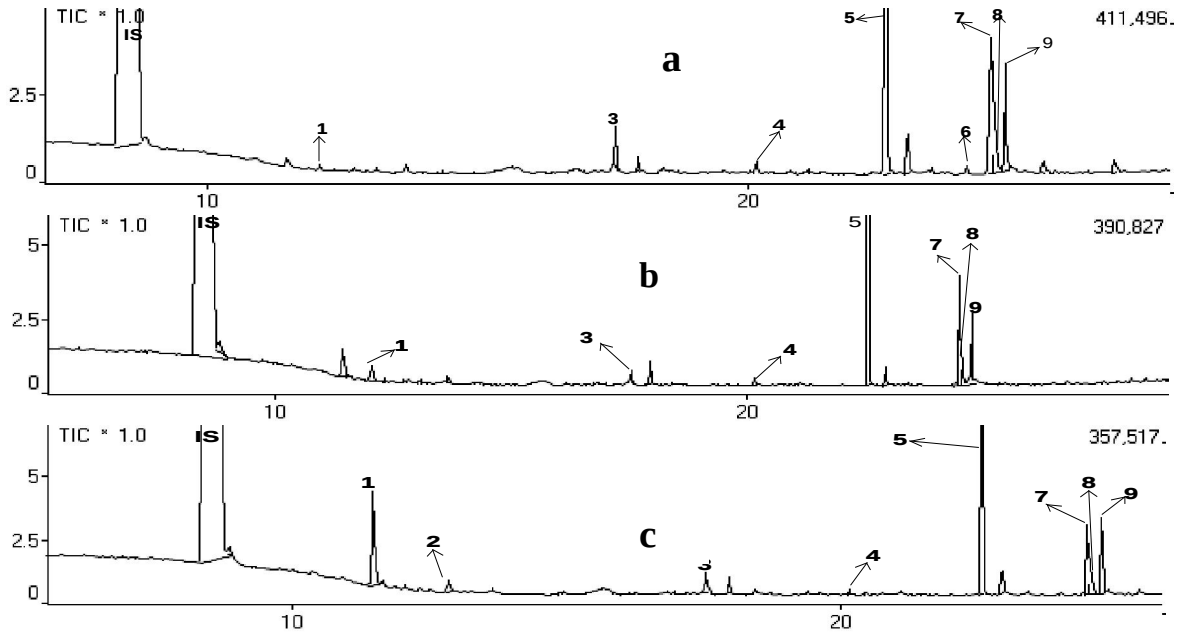


Figure 2. Total ion chromatogram of blood plasma sample after inhalation of nutmeg oil. Figure **a**: chromatogram of the sample after ½ hour inhalation, Figure **b** chromatogram of the sample after 1 hour inhalation, Figure **c**: chromatogram of the sample after 2 hour inhalation. **IS** : Internal standard (1,4-

dichlorobenzene), **1 :** 4-terpineol, **2 :** safrole, **3 :** myristicin, **4 :** methyl myristate, **5 :** methyl palmitate, **6:** palmitic acid, **7 :** methyl 10-octadecanoate, **8 :** methyl oleate, and **9 :** methyl stearate.

Compounds of essential oils of *ki lemo* barks detected in blood plasma of mice after inhalation with preparation SPE

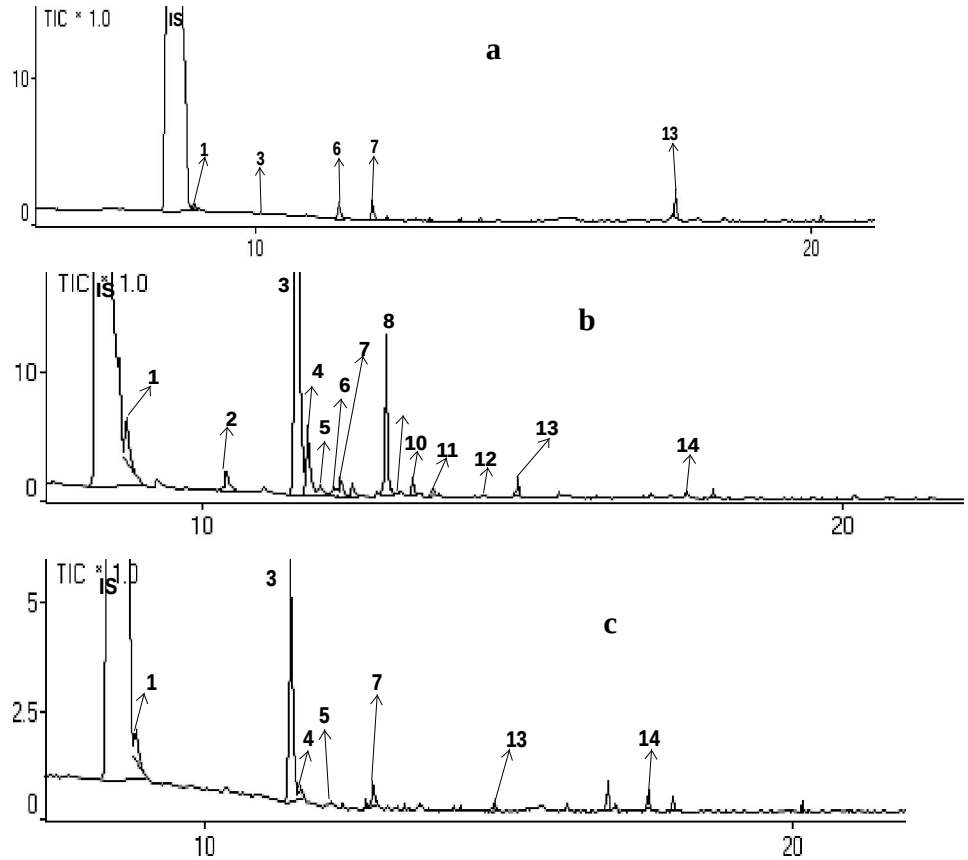
Citronellol, citronellal, α-terpineol, dan 1,8-cineole were identified in blood plasma of mice after inhalation of essential oil of *ki lemo* barks, such be showed Tabel 5 and Figure 3.

Table 2. Active volatile compounds identified after inhalation of essential oil of *ki lemo* barks at different duration of inhalation

No. Peak[c]	Name	LRI Exp[b]	Conc. (µg/ml)		
			Inhalaton ½ hours (R^c=82 %)	Inhalation 1 hours (R^c=85%)	Inhalasi 2 hours (R^c=83%)
1.	1,8-Cineol	1032	5,5	59,9	4,3
2.	*Linalool*	1098	*nd*	10,4	*nd*
3.	Citronellal	1153	14,9	39,3	37,1
4.	Neo-isopulegol	1161	*nd*	35,6	*nd*
5.	Isopulegol	1171	*nd*	6,9	1,5
6.	4-Terpineol	1180	*nd*	4,2	*nd*
7.	α-Terpineol	1196	8,1	5,6	*nd*
8.	Citronellol	1225	22,3	53,1	33,8
9.	**Neral**	1238	*nd*	4,7	*nd*
10.	Linalyl acetate	1246	*nd*	1,5	*nd*
11.	Nerol	1249	*nd*	6,5	*nd*
12.	Geranial	1267	*nd*	2,9	*nd*
13.	β-Terpenil acetate	1347	*nd*	5,7	0,9
14.	(E)-Caryophylena	1427	*nd*	1,0	0,6

Explanation:
nd = no detected , **a :** LRI reference in Adams (1995) with DB5 column, **b :** LRI experiment with DB5-MS column, **c :** Recovery (n=2) was calculated on the basis of comparation between 1,4-dichlorobenzene (methanol diluted) in blood plasma and 1,4-dichlorobenzene in methanol only

This research reports that the volatile compounds detected in blood samples were estimated to have association with depression of locomotor activity. It is suggested that the depression of locomotor activity by essential oils of nutmeg seeds is due, at least in part, to the direct pharmacological action of one or more of its constituents (Muchtaridi *et al*., 2004).



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CONCLUCION

Lead compounds could be detected in blood plasma of mice after inhalation essential oils of spices using by application of SPE-GC/MS in preparation samples.

Recovery of this analysis increased up to 90 %. Major volatile compounds identified from blood plasma of the mice after inhalation of the essential oil of kemangi leaves were linalool and linaly acetate, whereas myristicin, 4-terpineol, and esthers of chain length (methyl palmitate, methyl myristate, methyl oleate, and methyl stearate) were dominant in blood plasma of mice after inhlation of the the essential oil of nutmeg seeds, and citronellol and citronellal dominant only in the blood plasma of mice inhaled essential oil of ki lemo barks. Linalool, citronelal, citral, dan methyl cinnamate are cimpounds identified from blood plasma of the mice after inhalation of the essential oil of serai dapur herbs.

REFERENCES

Adams, R.P. 1995. *Identification of Essential Oil Components by Gas Chromatography/ Mass Spectroscopy*. Allured Pub. Co. Carol Stream, USA.

Buchbauer, G., Jager, W., Dietrich, H., Plank, Ch., and Karamat, E. 1991. Aromatherapy: Evidence for Sedative Effects of Essential Oil of Lavender after Inhalation. *Journal of Biosciences*; 46c, 1067-1072.

Buchbauer, G., Jager, W., Jirovetz, L., Ilmberger, J., and Dietrich, H.1993. Therapeutic Properties of Essential Oil and fragrances. *American Chemical Society (ACS) Simposium* Series; 525, 160-165.

Buchbauer, G. 1993. Biological Effects of fragrances and Essential Oils. *Journal Perfumer and flavorist*; 18, 19-24.

Jirovetz, L., Buchbauer, G., Jager, W., Woidich, A., and Nikiforov, A. 1992. Analysis of Fragrance Coumpound in Blood Samples of Mice by Gas Chromatography, Mass Spectrometry, GC/FTIR, and GC/AES ater inhalation of Sandalwood Oil. *J. Bio. Chrrom*; 6, 133-134.

Jirovetz, L., *et al.* 1991. Investigation of Animal Blood samples after Fragrance Drug Inhalation by Gas Chromatography/Mass Spectrometry with Chemical Ionization and selected Ion Monitoring. *Biol. Mass Spect.*; 20, 801-803. (Short Communication)

Kataoka H. 2003. New rends in sample preparation for clinical and pharmaceutical analysis. *Trends in Analytical Chemistry*. 22(4): 232-243.

Kovar, K. A., Grooper, B., Fries, D., and Ammon, HPT. 1987. Blood Levels of 1,8-cineole and Locomotor Acivity of Mice after Inhlation and Oral administration of Rosemary Oil. *Journal of Planta Medicinal*; 53, 315-318.

King, M.F., Hamilton, B.L., Mathewas M.A., Ruhe D.C., and Field R.A,. 1993. Isolation and Identification of Volatiles and Condesnsable Material in Raw Beef with Spercritical Carbondioxide Extraction. *J. Agric. Food Chem.* 41: 1974-1981.

Muchtaridi, Anas, S., Antón, A., Slamet B. 2004. Analysis of Volatile Active Compounds of Essential Oils of Nutmeg seeds Possessing Inhibitory Properties on Mice Locomotor Activity. *Journal Natura Acta et mathematica*, 3 (3): 20-28

Sangat, H. dan Roematyo. 1996. Aromatherapy Plants: A Etnopharmacology Study. *Proceeding Simposium Nasional I Tumbuhan Aromatik* APINMAP; 22-23 Oktober

Sonavane, G., Vikram, S., Veena, K., Sanjay, B.K. 2001. Behavioural action of *Myristica fragrans* HOUTT seeds. *Indian Journal of Pharmacology.*, **33**: 417-424.