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Research Article

CHEMICAL COMPOSITION AND LOCOMOTOR ACTIVITY OF *ANDALIMAN* FRUITS (*ZANTHOXYLUM ACANTHOPODIUM* DC.) ESSENTIAL OIL ON MICE

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ABSTRACT

Introduction: *Andaliman* (*Zanthoxylum acanthopodium* DC.) is spices from North Sumatra that have potent as aromatherapy material.

Objective: The objective study is to evaluate the inhibitory effect of *Andaliman* (*Zanthoxylum acanthopodium* DC.) fruits essential oil on the locomotor activity of mice in a wheel cage

Methods: The study on analysing of essential oil compounds of *andaliman* fruits using GC-MS method and locomotor activity on mice has been carried out. The locomotor activity was tested by wheel cage methods.

Results: The results of GC-MS analysis showed that there were 29 compounds identified in fruits of *andaliman*'s essential oil with some major compounds such as geranyl acetate, citronella, β-citronelol, nerol, limonene, geraniol, caryophyllene, citronellyl acetate, and α-pinene by LRI index confirmation. The locomotor activity showed that the inhalation of essential oil andliman fruits at 0.1 mL a dose decreased the locomotor acivity as much as 9.67 %, while at 0.3 and 0.5 mL doses increased the locomotor activity as much as 0.62 % and 3.95 %.

Conclusion: This study could conclude that inhalation of *andaliman* fruits essential oil gave different influence to locomotor activity depended given doses.

Keywords: *Andaliman*, *Zanthoxylum acanthopodium* DC, Locomotor Activity, Aromatherapy.

INTRODUCTION

Aromatherapy is one of many ways to cope with depression which triggered by many problems in everyday life [1-5]. The first research on aromatherapy was performed by Kovar and colleagues (1987) which shown the effect of inhalation of essential oil towards the motor behaviour of mice. Kovar and his team were intended to prove the benefit of essential oil and its single component which given by inhalation. Essential oil from rosemary and dwarf pines has been proven in increasing the motoric activity, while those from melissa and valerian decreasing it [2, 6].

Buchbauer's research group had analysed the motility of mice which inhaled by essential oil and its single component. Buchbauer (1993) had investigated 44 types of fragrances which indicated to have sedative effects on human, through experiment on mice with and without caffein induction. As results, essential oils of lavender, linalool and linalyl acetate (major component of lavender oil), neroli's oil, citronelal, α-terpineol, benzaldehyde, and sandalwood's oil decreased 40-78 % of mice motility compared to its control [7]. Essential oil from seed of nutmeg have also been proven in decreasing the motoric activity of mice up to 68,62 % [8], whereas Hongratanaworakit (2010) studied that jasmine oil demonstrated to give stimulating effect and provide evidence for aromatherapy to reduce of depression and raise mood in humans [9].

One of the spices which still used as a primary commodity is *andaliman*. The use of *andaliman* (*Zanthoxylum acanthopodium* DC.) as seasoning to improve the food taste has been long time used by Batak Toba people. There are a lot of specialty food from Batak which use *andaliman* as their seasoning, e.g. naniura, naniarsik, nanitombur, nandipadar, and sang-sang which usually served to guests during traditional events. A bite of *andaliman* fruit will give a spicy-bitter taste and smell of essential oils that can increase saliva production. Moreover, some of other plants from genus Zanthoxylum have been used as an aromatherapy preparations [10-12]. *Andaliman*'s relative plants such as *Zanthoxylum schinifolium* from Korea has been used as aromatherapy preparations as well [11]. Therefore, the aim of this research is to study the effect of essential oil from *andaliman*'s fruit on locomotor activity of mice.

MATERIALS AND METHODS

Materials

Plant Materials

Materials used in this study is the fruits of *andaliman* which obtained from a traditional market in Medan on July 2005. The plant was determined in the Laboratory of Herbarium Plant Taxonomy, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran. *Andaliman* fruit was initially separated from its stalk, then washed in running water until all dirt were removed. Furthermore, the fruit was dried and aerated for a day. Then, the fruit was dried for three to four days with 10 hours each day using indirect sunlight.

Chemicals

Pure lavender oil from species of *Lavandula officinalis* (Body Shop), standard alkane C_8 – C_{20}, and C_{21} – C_{40} (Sigma).

Animal Experiments

Animals used are white-male-mice Swiss westar strain, 20-30 gram of body weight. The animals were obtained from Laboratory of Animal Development, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran.

Instruments

Instruments used in the experiments are distillation apparatus Stahl, pycnometer, GC-MS (Schimadzu-QP-5050A), rolling wheel kit, weighing scale equipment, inhalation box (made of fiber glass in size of 20x20x30 cm3, equipped with an electric fan)

Methods

Isolation of Essential Oil

Dried *andaliman* fruits (100 g) were water-distilled at Balitro, Monaco Lembang for 6 hours. The oil was stored at -20°C after addition of sodium sulphate.

Determination of Density of Essential Oils

The determination of density of essential oils was performed using pycnometer. An amount of essential oil was added into calibrated pycnometer, then weighed using analytical balance. Observation

temperature was noted. Weight of oil was calculated from the weight of pycnometer-oil-filled substracted with empty pycnometer. The density of oil was compared with the water density at observation temperature, resulted in specific gravity of essential oil.

Mouse Locomotor Activity Tests

Locomotor activity of mice was measured using a wheel cage, in which the mice ran and the number of rotations was recorded by a meter. Cage inhalators contained glass fibre (20 cm x20 cm x30 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 minutes; eligible mice were then divided into three groups: a control group, a lavender oil, positive control group (using 0.1, 0.3, and 0.5 mL/cage), and a nutmeg seed oil, treatment group (using 0.1, 0.3, and 0.5 mL/cage). Each group consisting of 5 mice was tested three separate times. After 30 minutes of inhalation, the mice were placed into the wheel cage and after 5 minutes, the number of rotations was recorded for 75 minutes in 15-minute intervals.

GC/MS analysis

Measurements were performed using a QP-5050A (Shimadzu) gas chromatograph coupled to a VG Autospect Mass Spectrometer at 70 eV, 40-550 amu with a fused silica capillary column (DB-5MS, 30m x 0.25 mm) using helium as a carrier gas and with 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. The MS was operated using an interface temperature of 240°C, and an electron impact ionisation of 70 eV with a scan mass range of 40-350 *m/z* (sampling rate of 1.0 scan/s).

Qualitative analysis

Identification of the compounds was conducted by comparing their linear retention indices (LRI) 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)[13]. Linear retention indices were calculated using GC data of a homologous series of saturated aliphatic hydrocarbons (C_8 to C_{40}) separated on the same column using the same conditions as for GC analysis of the essential oils and the blood samples. The blood samples were collected from the corner parts of the eyes using capillary tubes and placed in a heparin tube.

Quantitative analysis

Detailed analysis was performed using a modification of the methods of Jirovetz *et al*. [14, 15] and Kovar *et al*. [6]. The blood samples (500 to 600 µL), obtained according to [15], were centrifuged (1800 rpm/10 min) at room temperature and concentrated on a C18-column (100 mg Sep-Pak, Waters). Volatile compounds were separated using a mobile phase of the mixture of methanol – bidistilled water (60 : 40).

Five microlitres were injected to the GC-MS. Quantification of the volatile compounds in the blood samples was accomplished using 1,4-dichlorobenzene 0.5 % (500 µL) as an internal standard according to the following equation (1):

$$[C] = \frac{A}{IS} \; x \; \frac{IS\ weight\ (g)}{100\ mL} \; x\ \%\ EO\ x\ IS\ volume\ x\ 10^6 \qquad (1)$$

C= concentration (μg/g)

IS = GC peak area of Internal Standard

A = GC peak area of compounds of essential oils

% EO = yield of essential oils

Statistical analyses

All locomotor activity test data are presented as mean ± S.E.M. Data were analyzed by ANOVA followed by Newman Keuls *post hoc* test. Results were considered significant at $p < 0.05$. Data were analyzed using MINITAB® 13.5 software.

RESULTS AND DISCUSSION

Determination of of Essential Oil Level

The distilled *andaliman* essential oils has pale-yellow colour with lemon-like aroma. The concentration determination of *andaliman* fruit's essential oil using distillation Stahl apparatus are shown in Table 1

Table 1: Concentration of Essential Oils from *andaliman* Fruit

Experiment	Raw Materials Weight (gram)	Level of Essential Oil (%)
I	100	4.97
II	100	4.85
III	100	5.01
Means		4.94

Essential oils are easily getting oxidized. For this reason, essential oils have to be stored in dark bottle to avoid the sunlight. The isolation of *andaliman* fruits essential oil has yielded in 4.94 %, which means that in each 100 gram of *andaliman* fruits dried contained 4.94 mL of essential oils. This yield is more than what was reported by Ernitasari: 2.01 % [10], but in correspond with Katzer's statement of more than 4 % [11]. The yield of essential oil depends on many factors, such as the plant origin, plant age, harvesting time, and also from the isolation process itself.

Andaliman fruit in dried condition will cracked, hence its seeds is easily off from the rind. Pericarp of *andaliman* fruit, unlike its seeds, has a spicy-strong-bitter taste, and unique smell which similar to lemon [11].Therefore, it is indicated that majority of essential oils in *andaliman* fruits contained in the rind.The results showed that *andaliman* essential oil has density of 0.877 g/cm^3. Most of essential oils have density below 1 g/cm^3, which is less than water. Hence, the layers of essential oils are always above the water surface.

Analysis of Chemical Composition of Essential Oil

The chemical composition of *andaliman* essential oils was shown in Table 2.

Analysis of *andaliman* fruits essential oils using GC-MS showed 29 chemical components geranyl acetate (23,18 %), citronella (11,23 %), β-citronelol (10,64 %), nerol (8,20 %), limonene (5,81 %), geraniol (4,25 %), caryophyllene (3,05 %), citronellyl acetate (2,70 %), hexane (2,48 %), and α-pinene (2,15 %). These chemical components are predicted to be responsible for the locomotor activity of mice. Furthermore, few minor essential oils components were also determined, i.e. sopulegol (1.82 %), α-phellandrene (1.57 %), neryl acetate (1.53 %), germacrene (1.37 %), and aromadendrene (1.06 %). In this study, geranyl acetate is the major component in *andaliman* essential oil. Geranyl acetate (3,7-dimethyl-2,6-octadiene-1-ol acetate) classified into esther group, and an acetylation product of geraniol. The spectrum and chemical structure of geranyl acetate are depicted in Figure 2.

However, these results differ significantly with that studied by Zaman et al.[16]. They assume that the main components of *andaliman* fruits essential oil were linalool (14.3%), (23.3%), limonene (12.9%), α-terpineol (8.3%), α-pinene (7.9%) were the predominant monoterpenes of the fruit oil and the main

monoterpenes in the leaf oil were limonene (33.1%), geraniol (10.6%) and carvone (9.6%).

The inhalation effect of *andaliman* essential oil towards the amount of mice wheel's rotation are shown in Table 4 and Figure 2.

Table 2: Chemical composition of the Essential oil of *Andaliman* Fruit

No.	RetentionTime	LRI exp[a]	LRI ref[b]	Similarity (%)	Component name	Concentration (%)
1	5.962	961	939	98	α-pinene	2.15
2	7.500	974	-	89	α-phellandrene	1.57
3	7.812	985	985	84	6-methyl-5-heptene-2-one	0.33
4	8.894	1029	1031	96	limonene	5.81
5	9562	1058	1189	88	alpha-terpineol	0.82
6	9.846	1070	1074	86	linalool oxide	0.081
7	10.539	1099	1098	88	linalool	5.65
8	11.523	1149	1153	97	citronella	11.23
9	12.066	1176	1099	89	undecane	1.28
10	12.143	1180	1134	87	limonene-oxide	0.86
11	12.242	1185	1228	91	sopulegol	1.82
12	13.142	1234	1228	91	β-citronelol	10.64
13	13.492	1254	1255	91	nerol	8.20
14	14.000	1283	1354	96	geraniol	4.25
15	15.025	1346	1383	95	citronellyl acetate	2.70
16	15.297	1363	1365	95	geranyl acetate	23.18
17	16.204	1422	1418	88	neryl acetate	1.53
18	16.461	1440	1443	95	caryophyllene	3.05
19	16.568	1447	-	93	β-fernesene	0.68
20	16.801	1463	-	93	α-caryophyllene	0.72
21	16.920	1471	1503	82	hydroxy-linalool	0.38
22	17.084	1482	1439	91	germacrene	1.37
23	17.273	1495	1534	91	aromadendrene	1.06
24	17.820	1534	1441	87	nerolidol	0.58
25	19.071	1626	1713	86	1-napthalenol	0.86
26	19.812	1683	1722	92	tetradecanal	0.18
27	20.320	1723	1818	80	e-myrtenol	0.14
28	21.228	1723		96	farnesol	0.39
29	21.228	1796		96	farnecyl acetate	0.40

a LRI reference in Adams (1995) with DB5 column

b LRI experiment with DB5-MS column

Table 3: Average number of mice wheel cage rotations within 90 minutes of inhalation of *andaliman* and lavender essential oils.

Treatment Group	The average of wheel's rotation Time (minute)					
	0-15	**15-30**	**30-45**	**45-60**	**60-75**	**75-90**
NC	254,3	259,7	254,0	249,0	261,7	273,3
LV_1	213,7	209,3	208,3	206,0	220,7	205,0
LV_2	158,3	162,3	166,0	176,7	159,0	160,0
LV_3	128,0	102,3	108,3	114,7	123,0	110,0
AD_1	229,0	229,3	216,0	178,3	275,3	274,0
AD_2	241,7	252,0	276,0	267,7	269,0	255,3
AD_3	264,7	283,0	260,0	267,0	267,3	271,3

NC: Normal control
LV1: Positive control of lavender essential oil at 0.1 mL concentration,
LV2: Positive control of lavender essential oil at 0.3 mL concentration
LV3: Positive control of lavender essential oil at 0.5 mL concentration,
AD1: Test group of *andaliman* essential oil at 0.1 mL concentration
AD2: Test group of *andaliman* essential oil at 0.3 mL concentration
AD3: Test group of *andaliman* essential oil at 0.5 mL concentration

Table 4: The Average of Mice Wheels Rotation in each 90 minutes of Treatment

Treatment	Average of Wheels Rotation (rata-rata ± SD)	Effects on Activity (%)
KN	258,67 ± 8,47	0
LV_1	210,50 ± 5,85	- 18,62
LV_2	163,72 ± 6,95	- 36,71
LV_3	114,38 ± 9,61	- 55,78
AD_1	233,65 ± 36,82	- 9,67
AD_2	260,28 ± 12,78	+ 0,62
AD_3	268,88 ± 7,84	+ 3,95

* = Value of effects on activity compared to normal control (0%)

(-) = Decreasing of locomotor activity, (+) = Increasing of locomotor activity

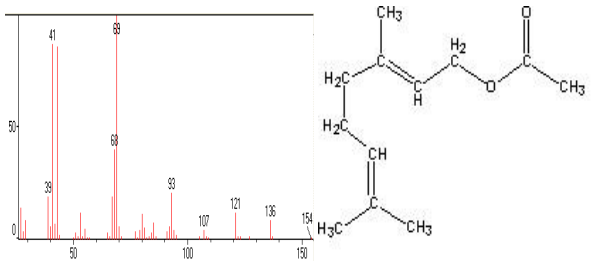

Fig. 1: Spectrum and chemical structure of geranyl acetate

However, this study was used a different method of isolation of essential oils, steam distillation, may affect the resulting compositions. In addition, the geographic and seasonal factors may be important in determining the chemical composition [17].

Locomotor Activity Test

The effect of andaliman fruit essential oil was tested towards locomotor activity of mice using Wheel cage method. The resulted data was analysed using Analysis Variance statistical method with Randomized Complete Block (RCB) design experiment model. Normal control, positive control, and test group were used as treatment. Time interval of 15 minutes was used as block. The results of mice's locomotor activity from each treatment group are shown in Table 3.

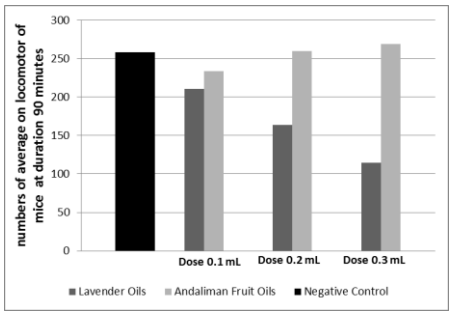

Fig. 2: Bar group of the average numbers of locomotors of mice after inhalation of essential oils within 90 minutes.

Analysis of Variance with Randomized Complete Block design at 95% confidence suggested that different concentration of essential oil resulted in different effects on the amount of wheels rotation.

Andaliman essential oil at concentration of 0.1 mL decreased the mice's locomotor activity until the 60th minute. Furthermore, *andaliman* essential oil increased the mice's locomotor activity started from 60th to 90th minute. Moreover, *andaliman* essential oil at concentration of 0.3 and 0.5 mL have significantly increased the locomotor activity of mice at 0th until 90th minute, hence it is concluded as the effective concentration. The resulted different pharmacological activity of essential oils towards mice has indicated that *andaliman* essential oils have both ability to increase and to decrease the mice's locomotor activity.

At mice group which had given 0.1 mL of *andaliman* essential oil, the percentage of wheel rotations were decreased at 9.67 %. Moreover, *andaliman* essential oils at concentration of 0.3 and 0.5 mL increased the wheels rotation for 0.62 % and 3.95 %, respectively. Newman Keuls range test indicated that there is a significant number of wheels rotation amongst group which given inhalation of essential oils at 0.1 mL, 0.3 mL, and 0.5 mL. The study on locomotor activity of *andaliman* fruit essential oils has not been reported, thus we could not compare this results. However, we found that the *andaliman* fruit have the inhibitory effects of against inflammatory biomarkers by conducting cell culture experiments in vitro [18].

CONCLUSIONS

Analysis of essential oils using GC-MS resulted in 29 chemical components with geranyl acetate as its major component (23.18 %). The locomotor activity test using modified Wheel cage method showed that inhalation of essential *andaliman* fruits oils affected the locomotor activity of mice, where at dose of 0.1 mL decreased the locomotor activity at 60th minute up to 9.67 %. The essential oils of *andaliman* fruits at dose of 0.3 mL and 0.5 mL increased the locomotor activity up to 0.62 % and 3.95 %, respectively.

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