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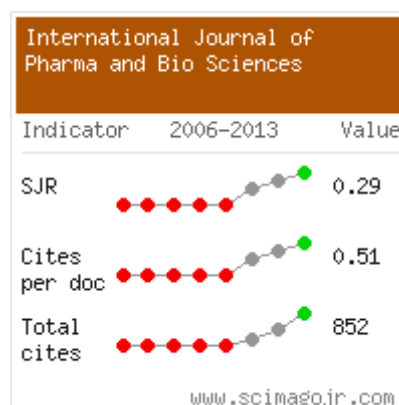
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## ESSENTIAL OIL OF RED GALANGAL (*ALPINIA GALANGA* (L) WILLD) RHIZOMES AS SLIMMING AROMATHERAPY

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### ABSTRACT

Red galangal (*Alpinia galanga* (L) Willd) is an aromatic plant which has potential as aromatherapy. The aim of this research was to evaluate and identify the red galangal essential oil as slimming aromatherapy through *in vivo* observation in adult male *Sprague Dawley* rats. The oil was separated by distillation and fractionated by silica gel column chromatography resulting five fractions. Crude essential oil, fractions 1 and 2 were further analyzed by gas chromatography-mass spectrometry and performed *in vivo* assay. The major compounds in the crude oil were  $\beta$ -bisabolene (11.78%) and trans-caryophyllene (9.10%); in fraction 1 was bicyclo-2-heptene (12.08%); and pentadecane (11.09%) in fraction 2. After five weeks of inhalation with 0.1% concentration, body weight showed significant difference ( $P < 0.05$ ) and animals inhaling the crude oil had the lowest body weight compared to fraction 1 but not different compared to control and fraction 2 treated animals. In conclusion, red galangal's essential oil compound  $\beta$ -bisabolene and trans-caryophyllene were suggested as the responsible compounds that has slimming aromatherapy effect.

**KEY WORDS:** aromatherapy, slimming, red galangal, essential oil



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## INTRODUCTION

Obesity is a very serious problem which is a result of a long term imbalance between calories consumed and calories expended<sup>1-2</sup>. People who have excess weight tend to have higher total plasma cholesterol (TPC) and triglyceride (TG) in their blood and lower HDL (high density lipoprotein)<sup>3</sup>. These conditions lead to the risk of various diseases such as high blood pressure and coronary heart disease<sup>4</sup>. Lean *et al.* also stated that obesity is a causative factor of cancer and metabolic syndrome<sup>5</sup>. According to National Health and Nutrition Examination Survey in USA, 65% adults (approximately 127 million people) do not have ideal weight and their obesity caused more than 300,000 deaths every year<sup>1</sup>. Many efforts have been done to lose weight such as consuming synthetic medicine as well as natural products which commonly taken orally. Currently, there are two types of medicine in the market. First, medicine that act centrally by suppressing appetite and increasing satiety. Second, medicine that works in the intestine by inhibiting the absorption of fat<sup>6</sup>. Generally, obese patient prefer to consume natural (traditional) therapy rather than the synthetic one. They believed it is safer and cheaper. Aromatherapy herbal plant based medicine is a new approached and recently more explored as slimming alternative medicine. The plant compound that has potential as the slimming aromatherapy is the essential oils<sup>7-8</sup>. Essential oils have been reported to stimulate the release of endorphins, relieving physical and emotional discomfort as well as promoting a feeling of joy and well-being<sup>9</sup>. One of the plants that contain these oils are red galangal rhizomes and the essential oil content between 0.3% - 0.5%<sup>10</sup>. Red galangal rhizome has been reported to have several medicinal properties such as anti-inflammatory, analgesic, anti-hyperglycemic, anti-allergy, antimicrobial, antifungal, anticancer, antibacterial and antioxidant<sup>10</sup>. Study of the active compound in red galangal as slimming aromatherapy has never been reported. Therefore, the aim of this study was to identify the active compound from the crude essential oil as well as the fractions of red galangal that has potential as slimming aromatherapy in adult male *Sprague Dawley* rat.

## MATERIALS AND METHODS

### Materials

Materials in this study were dried red galangal from Takengon-Aceh Province (Indonesia), distilled water, ethyl acetate, chloroform, methanol, *n*-hexane, silica gel, silica gel aluminum plate G<sub>60</sub>F<sub>254</sub> from Merck, *propylthiouracil* (PTU) and kits for TPC and TG (BIOLABO). The laboratory animals were adult male *Sprague Dawley* rat obtained from the Biopharmaca Research Center - Bogor Agricultural University. The diets were standard diet and high fat diet added egg yolk and coconut oil.

### Instrumentation

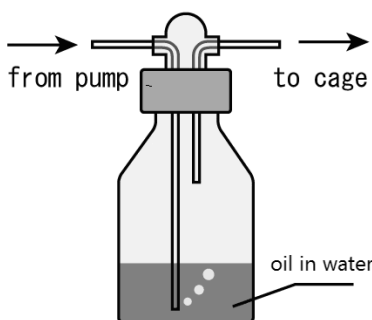
The instruments used in this study were oven, distiller, chromatography vessel, capillary pipe, *Gas Chromatography-Mass Spectrometry* (GC-MS) (Shimadzu-Qp-505), as well as animal cages equipped with inhalator tube.

### Procedure

This research was performed in two stages. The first stage was the collection of samples, determination of moisture and ash content, and isolation of essential oil by using Dean-Stark distillation. Further fractionation of essential oil by using silica gel open chromatography column with *n*-hexane and ethyl acetate as eluting solvent and final fraction identification with GC-MS. Thin layer chromatography (TLC) analysis for each group was performed by silica gel and *n*-hexane:ethyl acetic (9:1) as eluting solvent. The second stage was *in vivo* assay to evaluate the essential oil of red galangal and the two selected fractions as slimming aromatherapy in 24 adult male rats. After two weeks of adaptation period, all animals were fed high fat diet with cholesterol of 25 g/day/animal added PTU 0.01% and water given *ad libitum*. Animals were divided into four treatment groups (n= 6 animals). The treatment groups were animals a) as control group not given inhalation (Group I); b) given red galangal crude essential oil (Group II); c) treated with inhalation of fraction 1 (Group III); and d) treated with inhalation of fraction 2 (Group IV). The concentration of all aromatherapy was

0.1% given for 5 weeks. The inhalation of oils was performed like shown in Figure 1. The oil gas from the bottle was distributed to all cages

in the treatment process. The amount of diets given to the animals were recorded daily and body weight were recorded every week.



**Figure 1**  
**Apparatus for oils inhalation to the animal cages.**

Fasting blood samples of 1 ml in ethylenediaminetetraacetic acid (EDTA) were collected from the tail vein after animals sedated with ketamine HCl and xylazine intraperitoneally (80mg/kg-weight and 10mg/kg-weight, respectively) at pre-treatment and at the end of experiment taken intracardially. The blood was to analyzed TPC and TG. Animals were euthanized by exsanguination. After confirming death, body fat tissues from the abdominal cavity and perineal area was collected and measured for its total weight. All procedures performed on these animals have been approved by the animal ethic committee of Bogor Agricultural University (No. 04.2013 IPB). Data from all parameters (body weight, fat tissues weight, plasma lipid, and diet consumption) were analyzed using complete randomized group method and ANOVA (*Analysis of Variance*) with confidence level of 95% ( $\alpha$  level=0.05). Data that was significantly different ( $p < 0.05$ ) further tested by Duncan's post hoc test.

## RESULTS AND DISCUSSION

### **Determination of Red Galangal and Extraction of Its Essential oil**

Plant determination of red galangal from Takengon was performed by the Herbarium Bogorienses Laboratory of Indonesia Institute of Sciences Cibinong Bogor, Indonesia. It was

confirmed that it belongs to the subfamily of Alpinioideae and species *Alpinia galanga*<sup>12</sup>. The moisture and ash content were determined 6.02% and 4.78%, respectively. Moisture content less than 10% has longer storage time and more resistance to microorganism contamination. The maximum ash content requirement for medicinal plant in Indonesia was 5%<sup>13</sup>. Therefore, this red galangal can be further used as medicinal plant. Isolation of essential oil from 500 g dried red galangal rhizome results was 0.3% v/w of yellowish oil. This result was similar to Bermawie *et al.* which reported that essential oil content of red galangal from Banten, Yogyakarta and Central Java, Indonesia was between 0.3-0.5%<sup>10</sup>.

### **Fractionation Essential Oil of Red Galangal**

Red galangal essential oil (about 2 g) was fractionated with *n*-hexane and ethyl acetate by using step gradient method resulted in 5 fractions. Table 1 showed that the highest yield was fraction 1 (63.5%) which has 6 spots followed by fraction 2 which has 4 spots. The TLC spots of fraction 1 and 2 were different which means the component in fraction 1 and 2 should be different. Therefore, the crude essential oil, fraction 1 and fraction 2 were further analyzed by GC-MS and used for *in vivo* analysis.

**Table 1**  
**Fractions of essential oil from red galangal rhizome**

Fraction	Total spot in TLC analysis	Yield (%)
1	6	63.5
2	4	8.8
3	3	3.6
4	4	7.1
5	3	1.3

**Composition of Crude Essential Oil, Fraction 1 and Fraction 2**

Composition analysis of crude essential oil, fraction 1 and 2 using GC-MS showed different compound composition (Table 2). The dominant component in the crude essential oils was  $\beta$ -bisabolene (11.78%) and trans-caryophyllene (9.10%). Fraction 1 has 3

main major compounds such as bicyclo-2-heptene (12.08%),  $\beta$ -bisabolene (11.61%) and eremophila (10.28%). On the other hand, main compound in fraction 2 was pentadecane (11.09%) and trans- $\alpha$ -bergamotene (9.02%). The structure of major compounds were shown in Figure 2.

**Table 2**  
**Concentration of major compounds of the crude essential oil, fraction 1 and fraction 2**

Group	Compound	Concentration of major compound (%) in		
		Crude essential oil	Fraction 1	Fraction 2
Monoterpene	$\alpha$ -Pinene	0.69	0.13	-
	$\beta$ -Pinene	0.49	0.13	-
	$\beta$ -Elemene	6.36	3.97	0.84
	Limonene	-	0.15	-
Monoterpene alcohol	1.8-Cineol	3.94	-	1.29
	Linalool	-	0.04	-
	$\alpha$ -Bisabolol	1.86	-	-
	Bergamotol	1.94	-	-
	Granil acetate	0.89	-	-
	Cavikol	0.32	-	-
	$\beta$ -Fencyl alcohol	0.04	-	-
	$\alpha$ -Terpineol	0.86	-	-
Sesquiterpene	Trans-caryophyllene	9.10	-	3.59
	$\beta$ -Sesquiphellandrene	5.42	1.72	1.35
	Bicyclo-2-heptene	-	12.08	6.30
	$\beta$ -Bisabolene	11.78	11.60	2.68
	Trans- $\alpha$ -bergamotene	2.39	-	9.02
	Eremophila	-	10.28	-
	Pentadecane	-	-	11.69

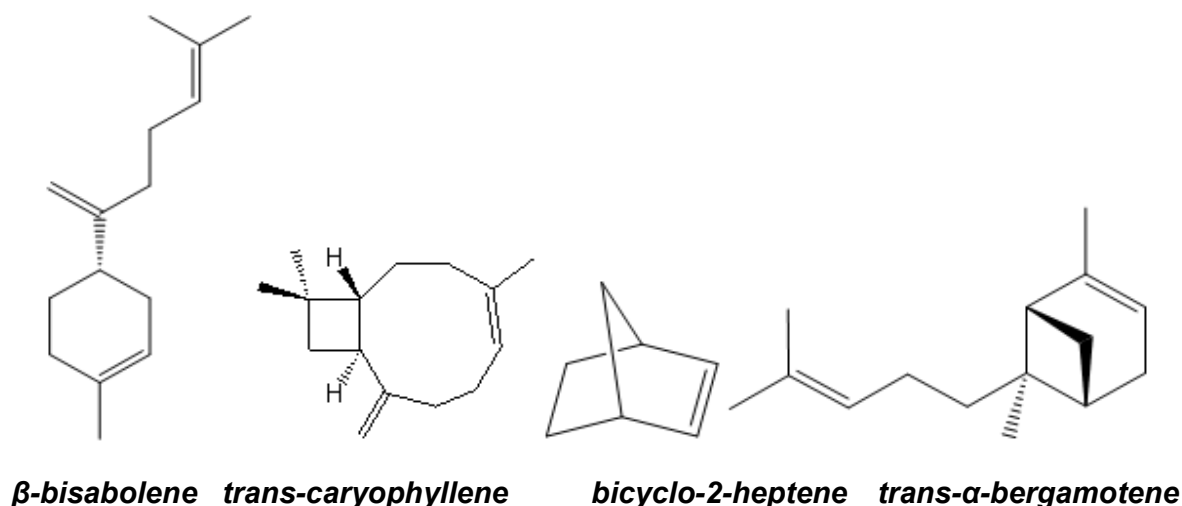


Figure 2

**Structure of *β*-bisabolene, trans-caryophyllene, bicyclo-2-heptene and trans- $\alpha$ -bergamotene**

### **Aromatherapy In Vivo Study**

Inhalation response to the tested animal was by observing the initial and final weight, fat deposits, and changes in lipid profile (TPC and TG). Total daily diet consumption in all four groups were not significantly different through all treatment period ( $P > 0.05$ , data not shown). For their body weight, at pre-treatment there was no difference among all groups ( $P > 0.05$ ), whereas at the end of treatment there was significant difference ( $P < 0.05$ ). Post hoc Duncan analysis showed that animals treated with crude essential oil showed lower body weight compared to fraction 1 treated animals but not different compared to the other two groups (Table 3). Unexpected finding, there were an increase of body weight response shown in animals treated with fraction 2 which was similar to the effect of inhalation by *Zingiber zerumbet*<sup>14</sup>. The highest percent

reduction of body weight was shown in animals that inhaled crude essential oil (13.67%). The body weight reduction mechanism with aromatherapy method was reported by stimulating through the olfactory system. The inhaled active compound affected the autonomic nervous system related to lipolysis mechanisms and heat production in adipose cells, as well as decreased in appetite that lead to weight reduction<sup>15</sup>. Shen *et al.* stated that the aroma of grapefruit oil with a dominant content of limonene affect the autonomic nervous system. This increased of lipolysis through histaminergic and reduced in appetite leads to weight loss<sup>16</sup>. However, Shen *et al.* also reported that lavender aroma and its active compound linalool influenced the autonomic nervous system, lipolysis through histaminergic response and increase of appetite<sup>17</sup>.

**Table 3**  
**Average body weight male rat at pretreatment and post treatment**

Treatment groups	Pretreatment body weight (g) ( $P > 0.05$ )	Post treatment body weight (g) ( $P < 0.05$ )
(I) Control	282.3 $\pm$ 13.6 <sup>a</sup>	251.8 $\pm$ 25.3 <sup>ab</sup>
(II) Crude Essential Oil	283.2 $\pm$ 22.0 <sup>a</sup>	244.8 $\pm$ 25.8 <sup>a</sup>
(III) Fraction 1	287.7 $\pm$ 14.8 <sup>a</sup>	266.8 $\pm$ 16.3 <sup>b</sup>
(IV) Fraction 2	289.0 $\pm$ 6.2 <sup>a</sup>	258.9 $\pm$ 16.1 <sup>ab</sup>

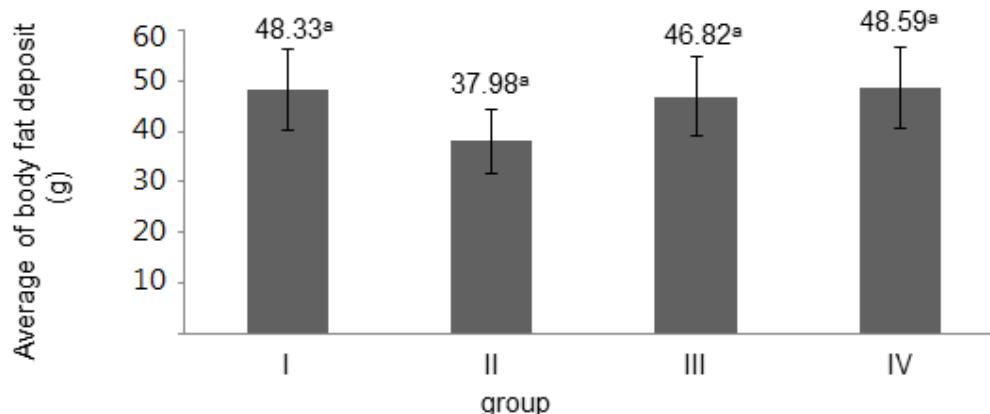
Number that following by the same superscripts were not significantly different ( $P > 0.05$ ) (Duncan's multiple range test).

According to WHO, obesity is defined as an excess in accumulation of fat that can be harmful to health<sup>18</sup>. The total weight of body fat

tissue deposit shown in Figure 3. The average body fat deposit from each group was not significantly different ( $P > 0.05$ ). However,

Group II showed a trend of lower average body fat deposit compared to the other groups. This trend showed that the crude essential oil tend to respond in a reduction of fat deposit which

leads to weight reduction. The reason of this insignificant result can be due to the possibility of relatively short treatment time and the limited number of animals in each group.



**Figure 3**

**Average of body fat deposit after treatment. Group (I) control, (II) red galangal crude essential oil, (III) fraction 1 and (IV) fraction 2**

Average concentration of TG after treatment showed no significant difference ( $p > 0.05$ ) (Table 4). Farouk *et al.*<sup>15</sup> reported that male rat albino normal TPC concentration was around 115 mg/dl. Also reported the concentration of TPC in white rat (*Rattus*

*norvegicus*) was between 97.44-102.40 mg/dl<sup>19</sup>. Sukcow *et al.* and Fahri *et al.* reported that the concentration of TG in white rat was around 25-145 mg/dl<sup>19-20</sup>. These previous results illustrated that the concentration of TPC and TG in this study was in the normal range.

**Table 4**

**Total plasma cholesterol and triglycerides concentration after 5 weeks treatment**

Treatment groups	Total plasma cholesterol (mg/dl) ( $P > 0.05$ )	Triglycerides (mg/dl) ( $P > 0.05$ )
(I) Control	107.3 ± 34.9 <sup>a</sup>	47.69 ± 6.99 <sup>a</sup>
(II) Crude Essential Oil	106.6 ± 45.7 <sup>a</sup>	47.97 ± 10.30 <sup>a</sup>
(III) Fraction 1	79.9 ± 63.9 <sup>a</sup>	37.83 ± 8.48 <sup>a</sup>
(IV) Fraction 2	83.5 ± 10.7 <sup>a</sup>	43.51 ± 13.09 <sup>a</sup>

Number that following by the same superscripts was not significantly different ( $P > 0.05$ ) (Duncan's multiple range test)

## CONCLUSION

Fractionation of essential oils using polarity improvement methods result in five fractions with the selected fractions were fraction 1 and 2. GC-MS results showed that the dominant compounds in crude essential oil of red galangal were  $\beta$ -bisabolene (11.78%) and trans-caryophyllene (9.10%). Fraction 1 contains 4 main compounds which were bicyclo-2-heptene (12.08%),  $\beta$ -bisabolene (11.61%),  $\beta$ -farnesena (10.28%) and eremophila (10.28%), whereas the main

compound in fraction 2 were pentadecane (11.09%) and trans- $\alpha$ -bergamotene (9.02%). The result of 5 weeks of inhalation in the adult male rats showed that the crude essential oil with concentration of 0.1% has potential as slimming aromatherapy.

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