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THE POTENCY OF CLOVE (*SYZYGIUM AROMATICUM*) ESSENTIAL OIL AS SLIMMING AROMATHERAPY BY *IN VIVO* ASSAY

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ABSTRACT

Clove leaves (*Syzygium aromaticum*) contain essential oils which consists of eugenol, β-caryophyllene and other compounds commonly used as perfume. The objective of this research was to analyze the potency of clove leave oil, eugenol, nonpolar extract, β-caryophyllene and isoeugenol as slimming aromatherapy by *in vivo* assay. Clove oil was obtained by distillation and the oil was extracted using an alkaline solution until eugenol and nonpolar extract were obtained. β-Caryophyllene was isolated from nonpolar extract using preparative thin layer chromatography. The crude essential oil and isolation-producted compounds were identified using gas chromatography-mass spectrometry. The results of *in vivo* assays showed that inhalation of isoeugenol had a potential for slimming by decreasing body fat tissues of rats. Lipid profile tests showed a decrease of triglyceride concentration in the clove oil group and the eugenol group compared to our negative control. In addition, there was a trend of decreasing body weight and total cholesterol concentration for the clove oil treatment.

KEYWORDS: **Clove oil, Essential oil, Isoeugenol, Slimming aromatherapy and *Syzygium aromaticum***

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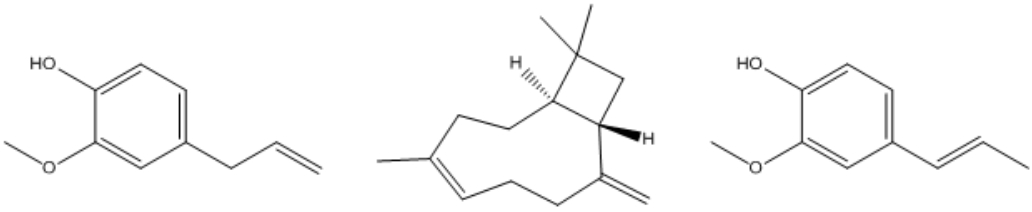
INTRODUCTION

One of Indonesia's herbal plants containing essential oil is the clove (*Syzygium aromaticum*). The clove has been reported having several biological activities including antifungal [1], anticancer [2], antibacterial [3] and antioxidant [4]. The clove oil contains major components: eugenol and β-caryophyllene [5]. Eugenol is the main component for synthesis of isoeugenol. Isoeugenol has the potency as an antioxidant [6] and an antiarthritis [7]. Additionally, isoeugenol is also a natural compound used as a raw material in perfume. Akunna et al. [8] reported the aroma of perfume can cause weight loss. Meanwhile, β-caryophyllene has the potency as an anti-inflammatory agent [9] and an antibacterial agent [10]. However, the potency of clove oil and its components in slimming aromatherapy has not been reported yet. Aromatherapy can be defined as an alternative method using volatile materials derived from plants, such as essential oils and other aromatic compounds [11]. Studies on the essential oil potency from several plants in slimming aromatherapy has been previously investigated [12]. Grapefruit essential oil was reported to stimulate the sympathetic nerve and control the white and brown adipose tissue [12,13]. In addition, grapefruit oil can inhibit the parasympathetic gastric nerve, increase both lipolysis and body temperature and suppress the appetite of test animals. So, it causes the decrease of body weight [13,14]. The opposite result is achieved for lavender oil [15] and *Zingiber zerumbet* oil [16] which increases the body weight of test animals by suppressed the activity of the sympathetic nerve and lipolysis. Aromatherapy can be a solution to replace the synthetic drugs used to overcome obesity. Obesity needs to be overcome because it can cause degenerative diseases, such as hypercholesterolemia, constriction of blood vessels, diabetes, high blood pressure and coronary heart disease [17]. Synthetic drugs, such as sibutarmin [18] and orlistat [19] are two types of slimming drugs commonly used to treat obesity. However, both types of those drugs have been reported to have harmful side effects. It is reported that sibutarmin can increase the risk of cardiovascular disease [20]. Meanwhile, orlistat also can cause diarhea, dyspepsia, flatulence and abdominal bloating [21]. So, the objective of this research is to analyse the potency of clove leaf oil, eugenol, nonpolar extract, β-caryophyllene and isoeugenol (Fig. 1) in the form of slimming aromatherapy by *in vivo* assay using rats.

Figure 1
Structures of eugenol, β-caryophyllene and isoeugenol



eugenol β-caryophyllene isoeugenol

MATERIALS AND METHODS

(i) Plant material

Syzygium aromaticum leaves used in this experiment were collected in the morning from the Conservation and Cultivation Unit of Biopharmaca Research Center, Bogor Agricultural University, Bogor, Indonesia in November 2013. The sample was identified by Herbarium Bogoriense, Cibinong, Indonesia.

(ii) Isolation of essential oil from clove leaves

About 750 g of chopped clove leaves were distilled for 3 hours with water (ratio 1:2) at 95-105°C. The distillate obtained was stood for 24 hours and the oil separated using a separation funnel. The yield of clove leaf essential oil was 0.16% (w/w) based on the dry weight.

(iii) Isolation of eugenol

The optimum conditions for isolating eugenol were based on the research by Fitria and Kawira [22]. To the clove oil distillate are added 4% NaOH solution. The mixture was separated with a separation funnel to obtain the eugenol phase, which is soluble in NaOH, and a nonpolar extract. Furthermore, the eugenol phase was put into a separation funnel and extracted with technical n-hexane 3x. The eugenol phase which was not soluble in n-hexane was separated, and 3% HCl added to give a ratio of 1:2. Then the mixture was allowed to stand for 24 hours. The eugenol phase was separated with a separation funnel and washed with distilled water. We then added anhydrate Na_2SO_4 and filtered the mixture.

(iv) Isolation of β-caryophyllene with preparative thin-layer chromatography (PTLC)

β-Caryophyllene, which was presumed to exist in the nonpolar extract, was separated by PTLC. The best eluent was used which was n-hexane and ethyl acetate (19:1). The spot with same R_f value as the standard R_f of β-caryophyllenewas dredged and dissolved in n-hexane [23]. This mixture was then centrifuged. The solvent containing β-caryophyllene was then re-concentrated.

(v) Gas chromatography-mass spectrometry (GC-MS) analysis

The crude essential oil, eugenol, the nonpolar extract and β-caryophyllene were analysed by GC-MS (Shimadzu-QP-5050A). Column: HP-5MS, 60 m x 250 µm ID x 0.25 µm film thickness. Temperature program: from 70°C to 290°C (40 minute) at 15°C.minute^{-1}. Injection temperature: 290°C. The injection port

temperature was 290°C and that of the detector was 250°C. Injection mode: split (50:1). Inlet pressure: 18.03 psi. The carrier gas was helium with a flow rate of 1 mL.minute^{-1}. The mass spectrometer conditions were as follows: ionization voltage 70 eV. MS source temperature at 250°C; MS quadruple temperature at 150°C; interface temperature at 290°C; electron ionization mass spectra was acquired over the mass range 40-800 m/z.

(vi) In vivo Analysis

Thirty-six 2-month-old male Sprague-Dawley rats weighing 250-260 g were used in this study. Before treatment, the rats had been adapted for 2 weeks. For the first week of the adaptation period, rats were given standard feed, whereas at the second week of the adaptation period, rats were given a high-fat diet (HFD). The rats were grouped into 6 groups, each group consisting of 6 rats. During the treatment period, all groups of rats were given a high fat diet at a dose of 25 g/animal/day [24] and given mineral water *ad libitum*, but each group was given different treatments. Group 1 was used as a negative control without any inhalation treatment. Treatment groups 2, 3, 4, 5 and 6 received a high fat diet and inhaled clove oil, eugenol, nonpolar extract, β-caryophyllene and isoeugenol are diluted 100 × in water. During 5-weeks of treatment, body weight was measured every week and the residue of feed consumed was weighed each day. Blood samples were collected after the rats had fasted for 12 hours and had been sedated by injecting ketamine and xylazine with doses of 80 mg.kg^{-1} and 10 mg.kg^{-1} based on body weight. At the end of the adaptation period, blood samples were taken through the vein of the rat's tail. Meanwhile, at the end of each treatment period, blood samples were taken exsanguinous. The lipid profile of the resultant blood was analyzed (triglycerides and total cholesterol) using the standard routine kit of Biolabo SA (Maizy, France). Furthermore, body fat tissue from the abdominal cavity and perineal area were removed and weighed. All procedures using animals have been approved by the Animal Ethics Committee of Bogor Agricultural University (No. 04.2013 IPB).

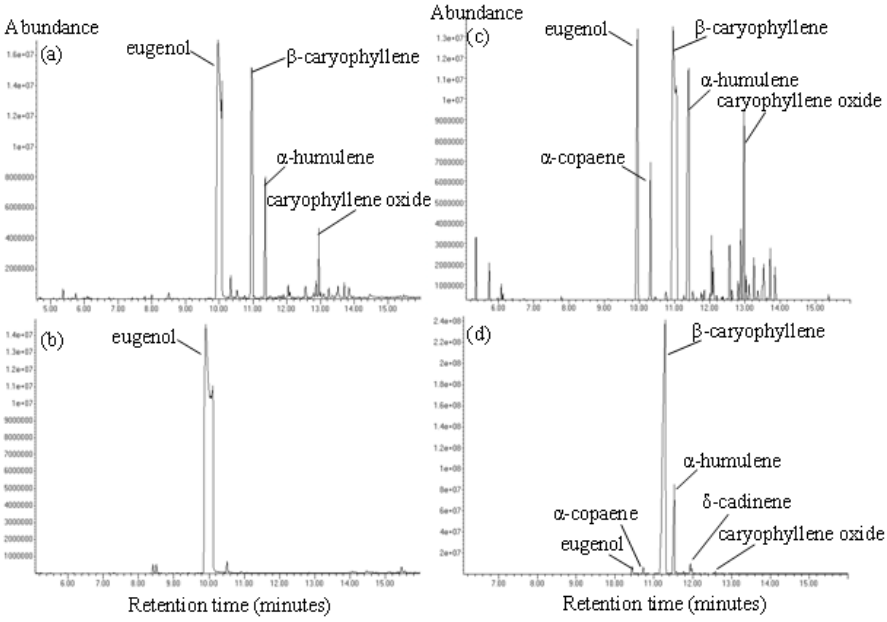
(vii) Statistical analysis

The data for body weight, body fat tissues weight and lipid profiles of the test animals were analysed using a completely randomized block design and with an analysis of variance (ANOVA) at a confidence level of 95% (α level of 0.05) using SPPS 20 software. The Duncan's multiple range test was also used [16].

RESULTS AND DISCUSSION

The result of GC-MS analysis showed that the major compounds in the essential oil of clove leaves were eugenol (58.6%), β-caryophyllene (21.4%), α-humulene (5.6%) and caryophyllene oxide (2.9%) (Fig. 2a). Jirovetz et al. [5] indicated similiar components in the essential oil of clove leaves from their isolation, including eugenol (76.8%), β-caryophyllene (17.4%) and α-humulene (2.1%). Eugenol in clove oil was dissolved in 4% NaOH, creating salts of Na-eugenolate, while nonpolar components was not dissolved. Therefore, the nonpolar extract, which contains β-caryophyllene, can be separated. Eugenol was obtained by adding 3% HCl into the Na-eugenolate solution. The yield of eugenol and nonpolar extract were 57.0% and 15.0%, respectively. A chromatogram of eugenol isolate is shown in Figure 2b. This showed there was a significant change indicated by an increasing of eugenol concentration from 58.6% to 84.8%. In addition, this chromatogram showed there was no β-caryophyllene, α-humulene and caryophyllene oxide which was identified in clove oil previously. Meanwhile, the chromatogram of nonpolar extract (Fig. 2c) showed there has been an increase in concentration of some nonpolar compounds,

Figure 2
Total ion chromatogram of clove oil (a), isolated eugenol (b), nonpolar extract (c) and isolated β-caryophyllene (d)



such as β-caryophyllene (35.1%), α-humulene (11.8%), caryophyllene oxide (6.7%) and α-copaene (4.6%), while the concentration of polar components (eugenol) decreased to 12.2%. Isolation of β-caryophyllene using preparative TLC resulted in 6 separated-spots. The spot at R_f 0.95 was collected because it has same R_f value as standard β-caryophyllene. This result (Fig. 2d)

Table 1
Constituents of clove oil, eugenol, nonpolar extract and β-caryophyllene

Group name	Compound name	Percentage (%)			
		Clove oil	Isolated eugenol	Nonpolar extract	Isolated β-caryophyllene
Phenyl propanoid	Eugenol	58.56	84.82	12.20	0.98
Sesquiterpene	α-Copaene	-	-	4.57	0.85
	β-Caryophyllene	21.37	-	35.10	82.56
	α-Humulene	5.57	-	11.79	12.12
	δ-Cadinene	-	-	-	1.05
Sesquiterpene alcohol	Caryophyllene oxide	2.94	-	6.66	0.31

was also supported by qualitative analysis using KLT and anisaldehyde-H_2SO_4 reagent. β-Caryophyllene (the standard and isolation product) showed violet-colored spot appropriating with literature [23]. The yield obtained was 40.8% and contained 82.6% in the highly puritied sample (Table 1). According to our GC-MS chromatogram (Fig. 2d), the purity of β-caryophyllene which previously was 35.1%, increased substantially, but α-humulene was also present (12.1%). Both compounds are isomer components which have similar polarities. This means that β-caryophyllene cannot be separated from α-humulene in our preparative TLC process. The effects of the inhalation to the test animals was by observing the initial and final weight, fat deposits and lipid profile. The results of analysis of variance (ANOVA) showed that the body weight of each group was not significantly different at the end of the adaptation period ($P > 0.05$), whereas at the end of inhalation, there was significantly different ($P < 0.05$). Post hoc Duncan analysis showed that groups which inhaled clove oil had the lower average of body weight (238 g) compared to groups which inhaled β-caryophyllene but the result was not significantly different compared with the negative control ($P > 0.05$). Data in Table 2 showed that the group which inhaled clove oil had lowest level of feed consumption compared with the other groups during 5 weeks of

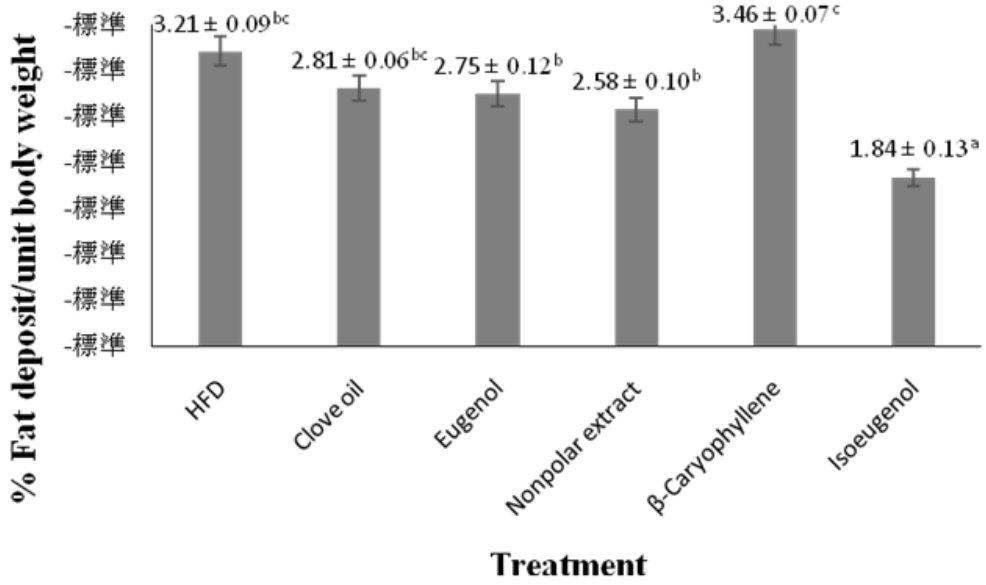
Table 2
Average body wight and feed consumption in all groups

Treatment Groups	Body weight (g)		Feed Consumption (g/Animal/day)	
	Before treatment	After treatment	Adaptation period	Treatment period
HFD (High Fat Diet)	282 ± 14[a]	252 ± 25[ab]	25 ± 0	16 ± 6
HFD + clove oil	275 ± 18[a]	238 ± 13[a]	25 ± 0	14 ± 5
HFD + eugenol	282 ± 16[a]	258 ± 24[ab]	25 ± 0	15 ± 5
HFD + nonpolar extract	279 ± 18[a]	246 ± 24[ab]	25 ± 1	14 ± 5
HFD + β-caryophyllene	283 ± 18[a]	260 ± 36[b]	25 ± 0	16 ± 5
HFD + isoeugenol	282 ± 13[a]	252 ± 29[ab]	25 ± 0	16 ± 5

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

treatment. This was one factor that caused rats which inhaled clove oil to have the lowest average body weight compared with other groups. The potency of slimming aromatherapy also can be seen from the percentage of body fat tissues per unit body weight of rats after the inhalation period. Figure 3 showed that the groups which inhaled clove oil had a lower percentage of body fat tissues than the negative control. Meanwhile, inhalation of β-caryophyllene revealed a higher percentage of body fat tissues than the negative control. This result correlates with body weight of the three groups, but these results were

Figure 3
Percentage of body fat tissues per body weight of rats in each group



not significantly different (P > 0.05). Most interesting is the result produced by the group inhaling isoeugenol. This group has the lowest percentage of body fat tissues at 1.8%, and the result was significantly different compared with negative controls (P < 0.05). A compound or mixture of compounds has potency as a slimming drug if it can stimulate fat burning, block absorption of fats and reduce appetite [25]. According to our data, isoeugenol showed potency in slimming aromatherapy. Inhalation of isoeugenol can not decrease both appetite and body weight, but it can reduce the weight of fat tissues in a significant way (P < 0.05). The mechanism of slimming aromatherapy has correlation with an aroma effect in which there is influence on the activity of the sympathetic nerve. The aroma of an active compound which causes increasing sympathetic nerve activity will decrease both appetite and body weight [13]. Meanwhile, the aroma causing a decreasing of sympathetic nervous activity will increase both appetite and body weight [15,16]. High sympathetic nerve activity will be proportional with the increased lipolysis. It will increase the level of triglycerides which were divided into free fatty acids so, it will increase heat production through the activity of uncoupling protein 1 (UCP1) [26], which will then affect an increase of both body temperature and energy consumption through uncoupling oxidative phosphorylation. The total concentration of cholesterol in each inhalation group was not significantly different compared with negative control (P > 0.05). The lowest and the highest values for total concentration of cholesterol were produced by groups which inhaled eugenol and nonpolar extract, respectively.

Table 3
Total concentration of cholesterol and triglycerides after the inhalation period

Groups	Cholesterol (mg/dL)	Triglyceride (mg/dL)
HFD (High Fat Diet)	138.844 ± 45.152^{abc}	52.860 ± 7.881^{b}
HFD + clove oil	123.940 ± 23.557^{ab}	31.892 ± 11.704^{a}
HFD + eugenol	120.572 ± 16.415^{a}	32.446 ± 5.656^{a}
HFD + nonpolar extract	167.145 ± 11.909^{c}	37.408 ± 16.864^{ab}
HFD + β-caryophyllene	147.526 ± 8.520^{abc}	46.251 ± 15.124^{ab}
HFD + isoeugenol	155.257 ± 17.597^{bc}	38.477 ± 9.757^{ab}

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

Meanwhile, analysis of triglyceride concentration showed the group which inhaled clove oil had the lowest concentration and it was significantly different compared with negative control, and which resulted in the highest triglyceride concentration ($P < 0.05$) (Table 3). The total concentration of cholesterol and triglycerides produced by each group was within in the normal range [27].

CONCLUSION

Inhalation of isoeugenol at a concentration at 1% showed their potency in slimming aromatherapy by decreasing body fat tissues of rats. The aroma of isoeugenol can stimulate the oxidation of fat in the body of rats. Meanwhile ,inhalation of both clove oil and eugenol can decrease triglycerides concentration in the blood.

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REFERENCES

1. Pinto E., Silva L.V., Cavaleiro C., Salgueiro L. Antifungal activity of the clove essential oil from *Syzygium aromaticum* on *Candida*, *Aspergillus* and *dermatophyte* species. J Med Microbiol, 58 (11): 1454-1462, (2009).
2. Aisha A.F., Abu-Salah K.M., Alrokayan S.A., Siddiqui M.J., Ismail Z., Majid A.M. *Syzygium aromaticum* extracts as good source of betulinic acid and potential anti-breast cancer. Braz J Pharmacogn, 22 (2): 335-343, (2012).
3. Kuntamalla S., Akula S. Clove oil for the control of flacherie disease and its effect on economic traits of silkworm, *Bombyxmoril*, *lepidoptera*; (*mbicidae*). Int J Pharm Bio Sci. 6 (1): 1311-1323, (2015).
4. Ivanovica J., Dimitrijevic-Brankovic S., Misic D., Ristic M., Zizovic I. Evaluation and improvement of antioxidant and antibacterial activities of supercritical extracts from clove buds. J Funct Foods, 5 (1): 416-423, (2013).
5. Jirovetz L., Buchbauer G., Stoilova I., Stoyanova A., Krastanov A., Schmidt E. Chemical composition and antioxidant properties of clove leaf essential oil. J Agric Food Chem, 54 (17): 6303-6307, (2006).
6. Findik E., Ceylan M., Elmasta M. Isoeugenol-based novel potent antioxidants: synthesis and reactivity. Eur J Med Chem, 46 (9): 4618-4624, (2011).
7. Kaur G., Sultana S. Evaluation of antiarthritic activity of isoeugenol in adjuvant induced arthritis in murine model. Food Chem Toxicol, 50(8): 2689-2695, (2012).
8. Akunna G.G., Saalu L.C., Ogunmodede O.S., Ogunlade B., Adefolaju G.A., Bello A.J. The effects of two Nigerian made parfumes on the liver of adult Wistar rat. J Med Sci, 11 (5), 220-225, (2011).
9. Fernandes E.S., Passos G.F., Medeiros R., da Cunha F.M., Ferreira J., Campos M.M., Pianowski L.F., Calixto J.B. Anti-inflammatory effects of compounds alpha-humulene and (−)-trans-caryophyllene isolated from the essential oil of *Cordia verbenacea*. Eur J Pharmacol, 569 (3): 228-236, (2007).
10. Huang M., Sanchez-Moreiras A.M., Abel C., Sohrabi R., Lee S., Gershenzon J., Tholl D. The major volatile organic compound emitted from *Arabidopsis thaliana* flowers, the sesquiterpene (E)-β-caryophyllene, is a defense against a bacterial pathogen. New Phytol, 193 (4): 997-1008, (2011).
11. Damayanti R., Batubara I., Suparto I.H. Essential oil of red galangal (*Alpinia galanga* (L) Willd) rhizomes as slimming aromatherapy. Int J Pharm Bio Sci. 6 (1): 283-289, (2015).
12. Niijima A., Nagai K. Effect of olfactory stimulation with flavor of grapefruit oil and lemon oil on the activity of sympathetic branch in the white adipose tissue of the epididymis. Exp Biol Med, 228 (10): 1190-1192, (2003).
13. Shen J., Niijima A., Tanida M., Horii Y., Maeda K., Nagai K. Olfactory stimulation with scent of grapefruit oil affects autonomic nerves, lipolysis and appetite in rats. Neurosci Lett, 380 (3): 289-294, (2005).
14. Farouk H., El-Sayeh B.A., Mahmoud S.S., Sharaf O.A. Effect of olfactory stimulation with grapefruit oil and sibutramine in obese rats. J Pak Med Stud, 2 (1): 3-10, (2012).
15. Shen J., Niijima A., Tanida M., Horii Y., Maeda K., Nagai K. Olfactory stimulation with scent of lavender oil affects autonomic nerves, lipolysis

and appetite in rats. Neurosci Lett, 383 (1-2): 188-193, (2005).
16. Batubara I., Suparto I.H., Sadiah S., Matsuoka R., Mitsunaga T. Effect of *Zingiber zerumbet* essential oils and zerumbone inhalation on body weight of Sprague Dawley rat. Pak J Biol Sci, 16 (19): 1028-1033, (2013).
17. J. Giannessi, B. Alviarand and A. Agusta, Variously substituted derivatives of guanidine, and their use as medicines with anti-diabetes and/or anti-obesity activity, U.S. Patent US 7368605, 2008.
18. Li M.F., Cheung B.M.Y. Pharmacotherapy for obesity. Br J Clin Pharmacol, 68 (6): 804-810, (2009).
19. Lewis D.R., Liu D.J. Direct measurement of lipase inhibition by orlistat using a dissolution linked *in vitro* assay. Clinic Pharmacol Biopharm, 1 (3): 1-3, (2012).
20. Li M.F., Cheung B.M.Y. Rise and fall of anti-obesity drugs. World J Diabetes, 2 (2): 19-23, (2011).
21. Snow V., Barry P., Fitterman N., Qaseem A., Weiss K. Pharmacologic and surgical management of obesity in primary care: a clinical practice guideline from the american college of physicians. Ann Intern Med, 142 (7): 525-531, (2005).
22. Fitri N., Kawira J.A. 2006. Comparison of variables in the isolation and purification of eugenol from clove oil. Media Litbang Kesehatan, 16 (2): 16-21, (2006).
23. Egon Stahl, Ed. Thin Layer Chromatography, 2nd Edn, Springer-verlag: 856-857, (1969).
24. Jann Hau and Gerald L Van Hoosier, Ed. Handbook of Laboratory Animal Science, 2nd Edn, Vol 1, CRC Press: 282-284, (2003).
25. Birari R.B., Bhutani K.K. Pancreatic lipase inhibitors from natural sources: Unexplored potential. Drug Discov Today, 12 (19-20): 879-889, (2007).
26. Argyropoulos G., Harperk M.E. Uncoupling proteins and thermoregulation. J Appl Physiol, 92 (5): 2187-2198, (2002).
27. [NCEP] National Cholesterol Education Program. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. JAMA. 285 (19): 2486-2497, (2001).