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Chemical Profiling of Clove Bud Oil (*Syzygium aromaticum*) from Toli-Toli and Bali by GC-MS Analysis

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Abstract. Indonesia is the largest clove producer in the world. In 2012, total world clove production is 113,215 tons where nearly 71 % (79,250 tons) comes from Indonesia. Although Indonesia is a major producer of clove in the world, research and publications about cloves in this country are scarce and hence knowledge about characteristics of difference varieties of cloves is very limited. The present study was aimed to compare major and minor constituents in clove oil responsible for their flavor based on origin which are cloves from Toli-Toli and Bali. The clove bud oil was isolated from clove bud (*Syzygium aromaticum*) using steam distillation. The compounds of clove bud oil was analyzed using GC-MS. The major compounds of clove oil were eugenol, caryophyllene, α-humulene and eugenyl acetate with composition 66.37 %, 15.38 %, 1.97 % and 12.99 %, respectively (Toli-Toli) and clove from Bali were 72.34 %, 12.51 %, 2.34 % and 5.33 %, respectively. The unique minor compounds of clove oil from Toli-Toli were (+)-δ-cadinene (0.13 %) and β-caryophylladienol (0.19 %) while in clove oil from Bali were valencene (0.17 %), δ-selinene (0.22 %) and alloaromadendrene (0.24 %). A total of 36 compounds were identified from the clove bud oil Toli-Toli and 38 compounds from the clove bud oil Bali.

Keywords: *Syzygium aromaticum*, steam distillation, chemical composition.

INTRODUCTION

Essential oils plant and other products of secondary metabolites have good uses in medicines, food flavorings, perfumes and pharmaceutical industry [1]. The main producer of clove in the World are Madagascar, India, Sri Lanka, and Indonesia [2-4]. In 2012 production of clove in Indonesian is around 79,250 thousand tons, while the production of cloves in the world in the same year reached 113,215 thousand tons, contributing 70.99 % of the total world production [5]. Most cloves are planted in Indonesia, Madagascar and Tanzania with the percentages of 71 %, 13 % and 7 % [5].

The clove tree is an annual plant that can grows the height of 10-20 m, has oval leaves that blooms in its tip of leaves and crimson flowers in numerous groups of terminal clusters [1]. Clove oil is an essential oil was produced by steam distillation of clove (*Syzygium aromaticum* or *Eugenia caryophyllata* or *Eugenia aromaticum*), belongs to the family *Myrtaceae*, is an important role in providing a spicy aroma [4].

Clove oil has biological activities such as antiseptic, aromatherapy [1], antiviral [3], antifungal, insecticide, antioxidant [4], antimicrobial to against pathogens [6], and aphrodisiac [7]. Clove oil also can be used in various ways such as treating bronchitis, cough, fever and sore throat [6]. It has been reported that *Syzygium aromaticum* can be used as a cure asthma and other allergic diseases with oral use [1]. The high content of eugenol in clove bud oil was contribute to antimicrobial activity. These phenolic compounds can damage proteins and react with the cell

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membrane phospholipids changing its permeability [4]. In the food and beverage, cloves are used in toothpastes, soaps, cosmetics, perfumes, and cigarettes [8].

Essential oils can be obtained through water distillation, steam distillation and soxhlet extraction. This process is not expensive but can lead to thermal degradation, hydrolysis and some water-soluble compounds [9, 10].

Major compound in clove bud oil and clove leaf oil were eugenol and β-caryophyllene [11, 12]. Alma also reported that clove oil contains eugenol, eugenol acetate and β-caryophyllene.

Although Indonesia is the world's largest producer of cloves, research and publications on clove in this country are still scarce and therefore knowledge of the different characteristics of both major and minor chemical compounds in clove oil Bali and Toli-Toli is conducted.

MATERIALS AND METHODS

The main equipment used in this study was a set of distillation equipment and heating mantle. Supporting equipment used include an analytical balance, stone boiling, thermometer as a temperature controller, separating funnel, glassware (Pyrex) as container, Gas Chromatography-Mass Spectroscopy (GC-MS) Agilent 5975C for analysis. The materials used in this study were dried cut cloves that obtained from PT H.M. Sampoerna, distilled water as a solvent and $MgSO_4$ anhydrous (Merck).

Sample Collected

Samples were dried clove buds from Toli-Toli and Bali obtained from PT H.M. Sampoerna, Sukerejo, East Java, Indonesia. The moisture content of dried clove buds was 15 %. Samples used in this study was dried cutted clove buds.

Extraction of Clove Oil

Dried cut cloves (100 g) from Toli-Toli and Bali were put in a 3000 mL round bottom flask. The ratio of samples and water was 1:30. Steam distillation was carried out for 6 hours. The distillate was collected until no more drop oil. Distillate containing the oil was separated from water. The distillate was dried with anhydrous magnesium sulfate. The oil samples were stored at 4 °C. Clove oil were analyzed using GC-MS [8, 12].

GC-MS Analysis

The essential oils from clove bud were analyzed by using GC-MS electron impact ionization (EI) method on GC-Agilent 7890A gas chromatograph coupled to a GC-MS Agilent 5975C mass spectrometer using HP-5 MS as a capillary column (30 m x 0.25 mm; 0.25 µm film thickness). The GC oven temperature was kept at 40 to 200 °C at a rate of 6 °C/min, 200 to 250 °C at a rate of 30 °C/min and then kept at 280 °C. The injector temperature was 250 °C. The amount of injection was 1 µL. The carrier gas was helium with flow rate 1 mL/min. MS spectra were taken at EI ion source of 70 eV.

Identification of the Compounds

Compound identification was conducted by comparing the Wiley library data. Percentage composition was computed from GC peak areas on HP-5 MS column.

RESULTS AND DISCUSSION

Clove oil from Toli-Toli contained 4 major compounds they are eugenol (66.37 %), β-caryophyllene (15.38 %), α-humulene (1.97 %), and eugenol acetate (12.99 %) with the total percentage was 96.71 % of clove oil. In addition, clove oil also contains some minor compounds such as chavicol (0.18 %), α-copaene (0.46 %), (+)-δ-cadinene (0.13 %), cadina-1,4-diene (0.15 %), caryophyllene oxide (0.47 %), and caryophylla-3,8(13)-dien-5β-ol (0.17 %) (Table 1).

Bali Clove oil also contained 4 major compounds: eugenol (72.34 %), β-caryophyllene (12.51 %), α-humulene (2.34 %), and eugenol acetate (5.33 %) with the total percentage was 92.52 % of clove oil. In addition, clove oil also contains some minor compounds that chavicol (0.25 %), α-copaene (0.52 %), valencene (0.17 %), δ-selinene (0.22 %), cadina-1, 4-diene (0.25 %) and caryophyllene oxide (0.18 %) (Table 1).

TABLE 1. Chemical compositions of clove buds oil from Toli-Toli and Bali

No.	RT (min)	Name of compound	Percentage	
			Bali	Toli-Toli
1.	11.084	(E)-4,8-Dimethyl-1,3,7-Nonatriene	0.01	0.01
2.	12.262	Benzyl Acetate	0.04	0.02
3.	13.017	Methyl Salicylate	0.07	0.04
4.	14.431	Chavicol	0.25	0.18
5.	16.582	α-cubebene	0.03	0.03
6.	16.962	Eugenol	72.34	66.37
7.	17.210	α –copaene	0.52	0.46
8.	17.543	β-elemene	n.d	0.02
9.	18.189	Caryophyllene	12.51	15.38
10.	18.340	(+)-(E)-β-Caryophyllene	0.23	0.03
11.	18.787	Valencene	0.17	n.d
12.	18.884	α-humulene	2.34	1.97
13.	19.029	Aromadendrene	0.04	0.03
14.	19.216	(-)-δ-selinene	0.22	n.d
15.	19.271	(+)-δ-cadinene	n.d	0.13
16.	19.397	α-muurolene	0.08	0.01
17.	19.555	β-selinene	0.08	0.03
18.	19.730	α-selinene	0.07	0.06
19.	19.802	α-muurolene	0.18	0.02
20.	19.875	E,E-α-farnesene	0.25	0.07
21.	19.947	δ-cadinene	0.03	n.d
22.	19.990	Unknown	0.05	0.03
23.	20.098	α-amorphene	0.07	0.04
24.	20.279	Aceteugenol	5.34	12.99
25.	20.461	Cadina-1,4-Diene	0.25	0.15
26.	20.557	α-cadinene	0.03	0.01
27.	20.678	α-calacorene	0.04	0.02
28.	20.884	Unknown	0.11	0.08
29.	20.998	Unknown	0.12	0.01
30.	21.246	Unknown	0.16	0.05
31.	21.512	Caryophyllene Oxide	0.18	0.47
32.	21.681	Unknown	2.35	n.d
33.	21.850	γ-gurjunene	n.d	0.06
34.	22.013	Unknown	n.d	0.05
35.	22.177	Unknown	n.d	0.09
36.	22.279	Alloaromadendrene	0.24	n.d
37.	22.346	Cadina-1,4-Diene	n.d	0.08
38.	22.449	Unknown	n.d	0.07
39.	22.515	Unknown	0.14	n.d
40.	22.521	β-caryophylladienol	n.d	0.19
41.	22.896	Cycloheptane, 4-Methylene-1-Methyl-2-(2-Methyl-1-Propen-1-Yl)-1-Vinyl-	0.13	0.20
42.	23.156	Caryophylla-3,8(13)-Dien-5β-Ol	0.08	0.17
43.	23.470	2,3,4-Trimethoxyacetophenone	0.56	0.26
44.	24.805	Benzyl Benzoate	0.03	0.06

RT = retention time, nd = not detected

Figure 1 was investigated about comparison major constituents in clove bud oil from Bali and Toli-Toli. The highest eugenol and α-humulene comes from clove Bali with the percentage of composition was 72.34 % and 2.34 %, respectively. While the highest β-caryophyllene and aceteugenol comes from clove Toli-Toli with the percentage of composition was 15.38 % and 12.99 %, respectively.

Table 2 discussed about study of major constituents in clove bud oil from the different area. It has been reported by Alma that major constituents of clove bud oil using steam-dsitillation produced eugenol, β-caryophyllene, α-humulene and aceteugenol with the percentage of composition was 87 %; 3.56 %; 0.40 % and 8.01 %, respectively. While Safrudin was reported the same major constituent in clove bud oil with the different composition was 72.40 %; 12.61 %; 1.56 % and 9.59 %, resepectively using hydro-distillation process. The different composition in clove bud oil due to several things such as sample of clove bud, distillation process, geographic condition and etc.

The different composition in clove bud oil from clove Bali and Toli-Toli can be seen in Fig. 2. For example benzyl acetate, methyl salicylate and chavicol was found in clove Bali, while caryophyllene oxide and caryophylla-3,8(13)-dien-5β-ol was found in clove Toli-Toli with the high intensity.

Clove oil can be extracted from clove buds, clove leaves and clove stems. The main components in clove oil were eugenol, eugenol acetate, and caryophyllene. Eugenol was a phenyl group propanoid like anetol, estragol and cinnamaldehide formed through shikimate acid pathway and were often found in essential oils [2].

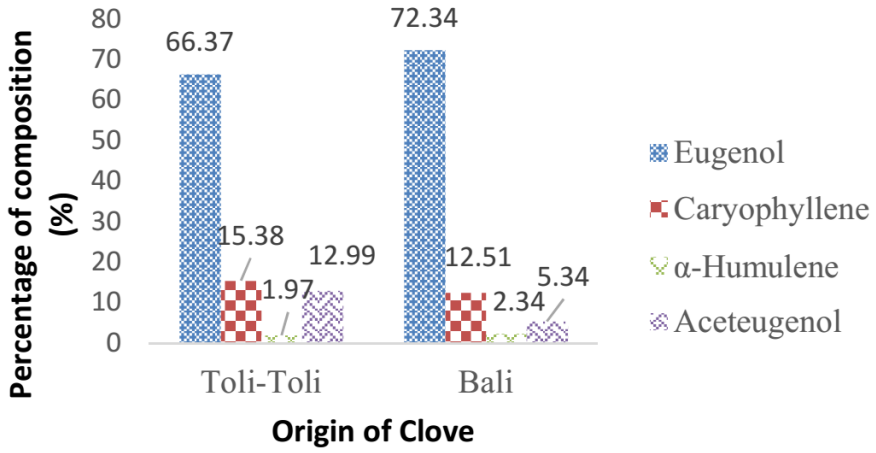

FIGURE 1. Comparison major constituents in Clove Oil

TABLE 2. Chemical composition in major compounds of clove bud oil

No.	Author/Reference	Compound (%)			
		Eugenol	β-Caryophyllene	α-Humulene	Eugenyl acetate
1.	Alma et al/[1]	87[a]	3.56[a]	0.40[a]	8.01[a]
2.	Bhuiyan et al/[4]	49.71[b]	18.94[b]	-	-
3.	Safrudin/[8]	72.40[b]	12.61[b]	1.56[b]	9.59[b]
4.	Memmou/[10]	47.6[b]	35.4[b]	-	13.4[b]
5.	Lee et al/[13]	89.2	-	-	8.6
6.	Kapoor et al/[14]	27.1	8.7	1.1	-
7.	Jirovetz et al/[15]	76.8	17.4	2.1	1.2
8.	Sohilait/[16]	81.13[a]	3.45[a]	0.38[a]	11.60[a]
9.	Our results (Toli-Toli)	66.37[a]	15.38[a]	1.97[a]	12.99[a]
10.	Our results (Bali)	72.34[a]	12.51[a]	2.34[a]	5.34[a]

[a]Steam distillation, [b]Hydrodistillation

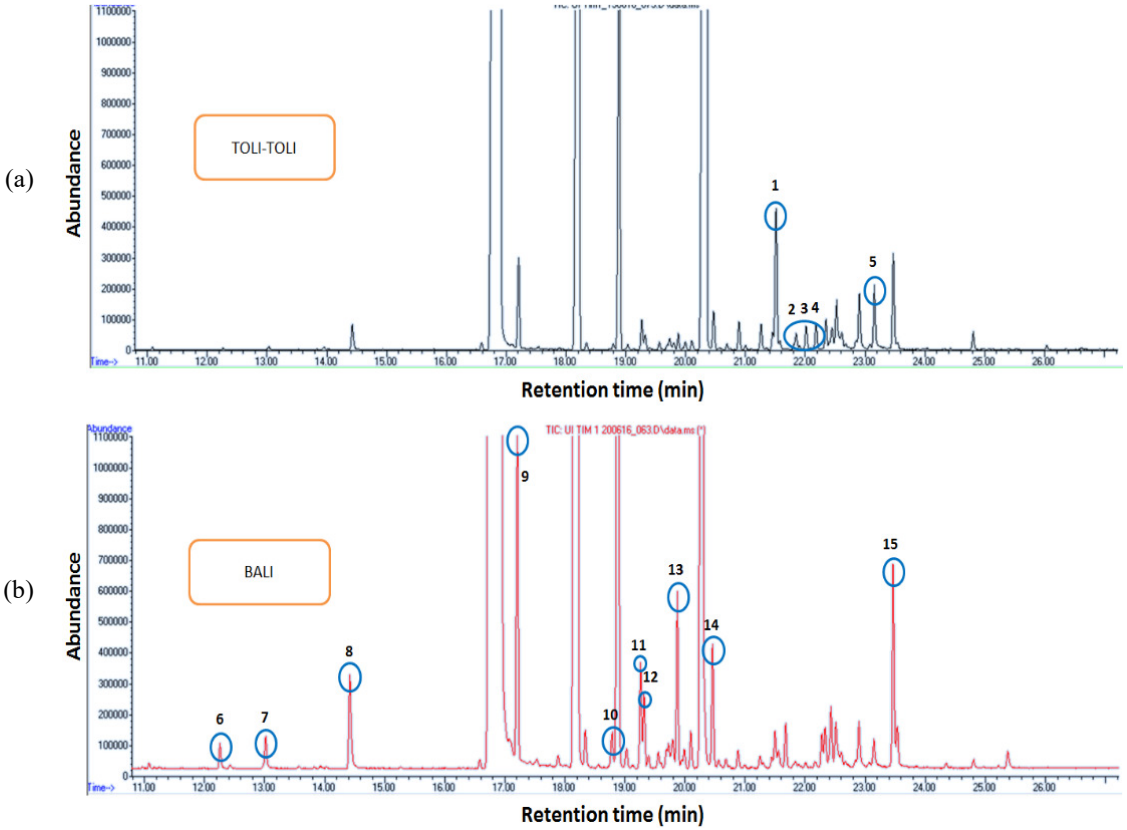

FIGURE 2. Gas chromatogram of the essential oil from clove (a) Toli-Toli and (b) Bali.
Note: 1. Caryophyllene oxide, 2. Globulol, 3. Unknown, 4. Unknown, 5. Caryophylla-3,8(13)-dien-5β-ol, 6. Benzyl acetate, 7. Methyl salicylate, 8. Chavicol, 9. α-copaene, 10. Valencene, 11. δ-selinene, 12. δ-cadinene, 13. α-farnesene, 14. Cadina-1,4-diene, and 15. 2,3,4-trimethoxyacetophenone

Differences compound of clove buds Toli-Toli and Bali due to several things: the influence of climate, soil conditions, geographic origins and the influence of fertilization[18]. Climatic influences include a high temperature and sea level, the number and nature of rain, sunshine intensity and wind. Changes in soil chemical properties and differences in solar radiation should be sought. Both factors involve the activation or inactivation of certain enzymatic groups, leading to the predominance of a particular biosynthetic pathway [17].

CONCLUSIONS

In this study, we compared major and minor constituents in clove oil from Toli-Toli and Bali using steam distillation method. Chemical composition of clove oil from Toli-Toli and Bali were analyzed using GC-MS. This study showed that clove oil from Toli-Toli contained 4 major compounds: eugenol (66.37 %), β-caryophyllene (15.38 %), α-humulene (1.97 %), and eugenol acetate (12.99 %) with the total percentage was 96.71 % of clove oil. While the Bali clove oil contains 4 major compounds were eugenol (72.34 %), β-caryophyllene (12.51 %), α-humulene (2.34 %) and eugenol acetate (5.33 %) with the total percentage was 92.52 % of clove oil. The unique minor compounds of clove oil from Toli-Toli were (+)-δ-cadinene (0.13 %) and β-caryophylladienol (0.19 %) while in clove oil from Bali were valencene (0.17 %), δ-selinene (0.22 %) and alloaromadendrene (0.24 %).

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