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# Chemical Composition of the Essential Oils in *Eugenia caryophyllata*, Thunb from Amboina Island

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**Abstract:** The essential oils in *Eugenia caryophyllata*, Thunb from Amboina Island on the bud, leaf and stem by steam distillation were isolated. Result of isolation was analyzed by GC and GC-MS. Five component were identified in the bud, leaf and stem oil. Main chemical component in the bud oil are eugenol (81.13-84.44 %), eugenyl acetate (11.60-15.02 %) and  $\beta$ -caryophyllene (3.45-4.60 %) respectively and eugenol (81.06-86.04 %),  $\beta$ -caryophyllene (11.95-16.16%), eugenyl acetate (2.02-3.05%) in leaf oil, while in stem oil eugenol (97.20-98.83%).

**Keywords:** *Eugenia caryophyllata*, Essential Oils, Chemical Composition, Amboina Island

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## 1. Introduction

The clove tree, *Eugenia caryophyllata*, Thunb (= *Syzigium aromaticum*, L.), (Myrtaceae), grown naturally in Moluccas Islands, East Indonesia, and were cultivated in Tanzania, Madagascar, Sri Langka, India, Malaysia, Brazil, Jamaica and Guinea [1]. Local name of these trees from Moluccas Islands is *cengkeh*; the trunk of tree can grow as height as 8-12 meters, on altitudes of 10.0-1400.0 m above the sea level in Amboina Island. Clove can be used in cooking, either whole or in ground form. The spice is used in Europa and Asia, and in Indonesia its use for cigarettes, that is well known as "kreteks" [2]. Furthermore, the essential oil of clove has been widely use as spice and is well known for its medicinal properties. It is active against oral bacteria associated and fungi [3]. Previous studies also have reported antifungal [4-6], antioxidant [7-9], antibacterial [10,13], and anti-inflammatory [1, 13] properties of clove oil. Main component of clove oil, eugenol, has been used as an anti-cancer [14-15], and as starting material for synthesis analog L- $\alpha$ -metil DOPA [16]. Research studies [2, 18-20] of clove essential oil from different parts of the world showed that the major component of the clove oil is usually eugenol,  $\beta$ -caryophyllene,  $\alpha$ -humulene, caryophyllene oxide and eugenyl acetate respectively, although different in concentration. A comparison of the published results reveals a great variability in chemical compositions of the clove essential oils. Eighteen components were identified in essential oil from Turkey

clove bud, where major components are eugenol (87%), eugenyl acetate (8.01%) and  $\beta$ -caryophyllene (3.56%), [2]. There are ten components in clove essential oil, with the main components are eugenol (77.81%), eugenyl acetate (21.30%) and  $\beta$ -caryophyllene (8.44%) [17]. Thirty-eight components were identified in the clove leaf oil from Bangladesh, and the main components are eugenol (74.30%), eucalyptol (5.80%),  $\alpha$ -cadinol (2.43%). Thirty one components in bud oil were identified and the main components are eugenol (47.70%),  $\beta$ -caryophyllene (18.90%), benzene-1-ethyl-3-nitro (11.10%) and benzoic acid 3-(1-methylethyl) (8.90%) [18]. Other work that was carried out on the bud and leaf essential oil of clove from India and Madagascar were found 28 and 35 components respectively from the bud oils and the leaf oil from Madagascar is 22 components. Major components in the bud oil from India are eugenol (70 %) followed by  $\beta$ -caryophyllene (19.5%) and eugenyl acetate (2.1%). A comparison components of bud and leaf oils from Madagascar is 82.6% : 82.0% for eugenol, 7.2% : 13.0% for  $\beta$ -caryophyllene, and 6.0% : 0.4% for eugenyl acetate [19]. The research studies to compare of Madagascar, Indonesia and Zanzibar bud, leaf and stem essential oils have found 10 component. Major component in the bud essential oil from Madagascar and Indonesia are eugenol (72.08 -80.31% and 77.32 - 82.36%),  $\beta$ -caryophyllene (2.36 - 6.38% and 5.34 - 8.64%) and eugenyl acetate (11.68 -21.32% and 8.61 - 10.55%). Major components in the leaf essential oil from Madagascar and Indonesia are eugenol (80.87 - 83.35% vs

75.04 - 77.54%),  $\beta$ -caryophyllene (11.65 - 15.02% vs 17.04 - 19.53%) and eugenyl acetate (0.29 - 1.45% vs 0 - 0.06%). Major component oil of Madagascar, Indonesia and Zanzibar stem essential oils are eugenol (91.81 - 96.65%, 88.76 - 89.28% and 87.52 - 89.47%),  $\beta$ -caryophyllene (1.66 - 4.48%, 7.40 - 7.75% and 7.19 - 9.70%), [20]. This paper reports results of chemical composition of clove bud, leaf and stem oils obtained from original trees grown in Amboina Island. So far, no study has been done on the chemical composition of clove in Amboina Island.

## 2. Materials and Methods

### 2.1. Material

Bud, leaf and stem plant of clove were collected from different locations in northern, southern, eastern and western parts of Amboina Island, Moluccas. Bud (4 samples), Leaf (4 samples) and Stem (12 samples) were deposited in the Organic Chemistry Laboratory, Pattimura University, Ambon. Raw material was dried on the sun for five until seven days.

### 2.2. Isolation of Essential Oil from Bud, Leaf and Stem of *Eugenia Caryophyllata*

The dry plant materials (2.0 kg) of bud, leaf (1.6 kg.) and stem (1.2 kg) of *Eugenia caryophyllata* (Figure 1) were obtained by steam-distillation method by conventional steam distiller, for 6 hours. The essential oil samples were dried by anhydrous sodium sulfate ( $\text{Na}_2\text{SO}_4$ ) and stored in cool and brown bottle until analysis conducted.

### 2.3. Gas Chromatographs (GC)

GC analysis of clove essential oil was performed on a Shimadzu QP-2010, equipped with a FID and Rtx-5 using a fused silica capillary column (30 m x 0.25 mm ID, film thickness 1.0  $\mu\text{m}$ ). Oven temperature was from 70  $^\circ\text{C}$  for 5 minutes and programmed heating from 70 to 220  $^\circ\text{C}$  at a rate of 10 $^\circ\text{C}$  for 6 minutes, and from 280  $^\circ\text{C}$  for 5 minutes, injector temperature 270  $^\circ\text{C}$ ; detector temperature 280  $^\circ\text{C}$ ; pressure of carrier nitrogen gas at inlet 7 psi, split 100 and volume was 0.2  $\mu\text{L}$ .

### 2.4. Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analyses of clove essential oil were performed using Shimadzu QP-2010 Plus at 70 eV and 320  $^\circ\text{C}$  with auto sampler, system equipped with Rtx-5 fused silica capillary column (30 m x 0.25 mm ID, film thickness 1.0  $\mu\text{m}$ ). Oven temperature was from 70  $^\circ\text{C}$  for 5 minutes, than programmed heating from 70 to 220  $^\circ\text{C}$  at a rate of 10  $^\circ\text{C}$  for 6 minutes, and from 280  $^\circ\text{C}$  for 5 min, injector temperature 270  $^\circ\text{C}$ . 100% pure helium gas was used as a carrier gas at the constant flow rate 2.30 mL/min, split ratio 100, ion source temperature 225  $^\circ\text{C}$ . The spectrum of the unknown component was compared with the spectrum of known components stored in the Wiley 7 library. Name, molecular

weight and structure of the component of the test materials were ascertained.

## 3. Results and Discussion

### 3.1. Performance of Bud, Leaf and Stem Essential Oil of Clove

The essential oils of bud, leaf and stem were distilled from dry plant material on equipment of the steam distillation (Figure 2). Average yields of bud, leaf and stem essential oils were calculated toward dry material. The results of clove oil from steam-distillation revealed that from bud have yield (7.05% w/w), leaf (3.21%, w/w) and stem (3.58 %, w/w).



Figure 1. *Eugenia caryophyllata*, Thunb.



Figure 2. Conventional steam-distillation.

### 3.2. Chemical Composition of Bud, Leaf and Stem Clove Essential Oils from Amboina Island

The clove of bud essential oil (4 samples), leaf essential oil (4 samples) and stem essential oil (12 samples) of *Eugenia caryophyllata*, Thunb were analyzed by GC and GC-MS. Five components were identified and quantified, as shown in Table 1, according to their elution order on the Rtx-5 column. Components were presented from the lowest to the highest concentration. The major components of bud, leaf and stem oil are eugenol (81.13 - 84.44%, 81.06 - 86.04% and 97.20 - 98.83 %),  $\beta$ -caryophyllene (3.45 - 4.60%, 11.95 - 16.16%) and 0.49 - 1.73 %) and eugenyl acetate (11.60 - 15.02%, 2.02 - 3.05% and 0.68 - 0.73 %). Three major components were observed by GC-MS analysis for ion chromatogram in bud, leaf and stem clove oils. In comparison to standard library data, the peak at  $m/z$  164 ( $M^+$ ),  $C_{10}H_{12}O_2$ , was identified as eugenol (Figure 3 A), the peak at  $m/z$  204 ( $M^+$ ),  $C_{15}H_{24}$ , was identified as  $\beta$ -caryophyllene (Figure 3 B) and the peak at  $m/z$  206 ( $M^+$ ),  $C_{12}H_{14}O_3$ , was identified as eugenyl acetate (Figure 3 C). The are two minor components in the mass

spectrum, the peak at  $m/z$  204 ( $M^+$ ),  $C_{15}H_{24}$ , was identified as humulene and the peak at  $m/z$  177 ( $M^+$  -  $CH_3CO$ .) was identified as caryophyllene oxide.

Based on this result, it can be inferred that oil from stem contain higher eugenol content than bud and leaf oil, but lower  $\beta$ -caryophyllene and eugenyl acetate content than on bud and leaf oil from Amboina Island. Moreover oil from leaf contains higher  $\beta$ -caryophyllene content than bud and stem, and oil from bud contain higher in eugenyl acetate content than oil from leaf and stem.

A typical gas chromatogram of Amboina Island clove oil is shown in Figure 4 (oil from bud), Figure 5 (oil from leaf) and Figure 6 (oil from stem).

This study reveals that the clove essential oils compounds from Amboina Island (bud, leaf and stem) have differences when compared to the earlier reports [1-2, 17-20]. The highest concentration of eugenol on stem oil makes it potentially use as medicines because it exhibit antibacterial, antifungal, anti-inflammatory activity, anticancer and antioxidant properties [1, 3-15].

Table 1. Chemical composition of bud, leaf and stem clove essential oils from Amboina Island.

Compound Name	M.W	Formula	Bud (%)	Leaf (%)	Stem (%)
Eugenol	164	$C_{10}H_{12}O_2$	81.13 - 84.44	81.06 - 86.04	97.20 - 98.83
$\beta$ -caryophyllene	204	$C_{15}H_{24}$	3.45 - 4.60	11.95 - 16.16	0.49 - 1.73
Humulene	204	$C_{15}H_{24}$	0.38 - 0.50	1.22 - 1.80	0.00 - 0.30
Eugenyl acetate	206	$C_{12}H_{14}O_3$	11.60 - 15.02	2.02 - 3.05	0.68 - 0.73
Caryophyllene oxide	220	$C_{15}H_{24}O$	0.05 - 0.24	0.00 - 0.37	0.00 - 0.89

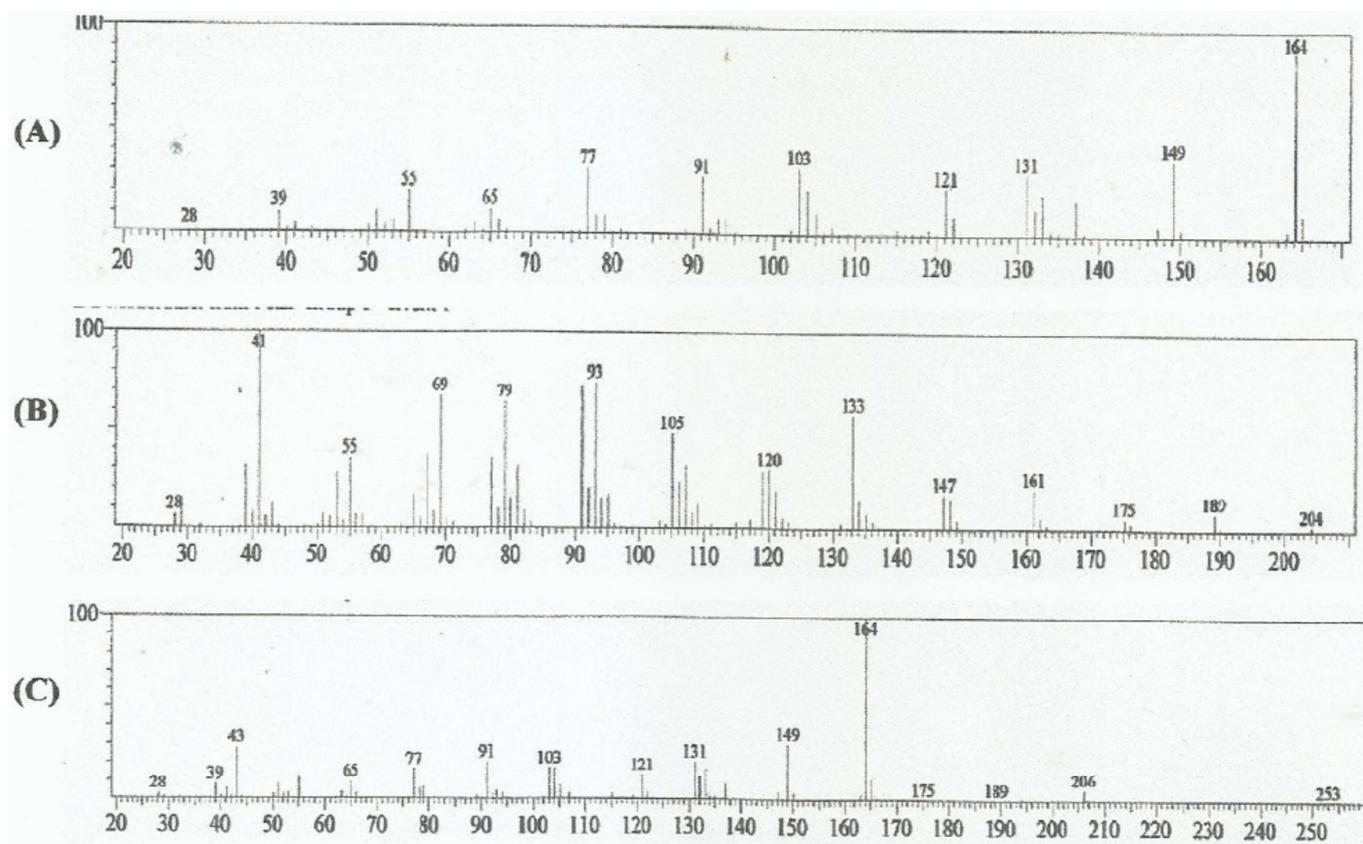


Figure 3. Mass spectrum of three major component in clove oil, eugenol (A),  $\beta$ -caryophyllene (B), eugenyl acetate (C).

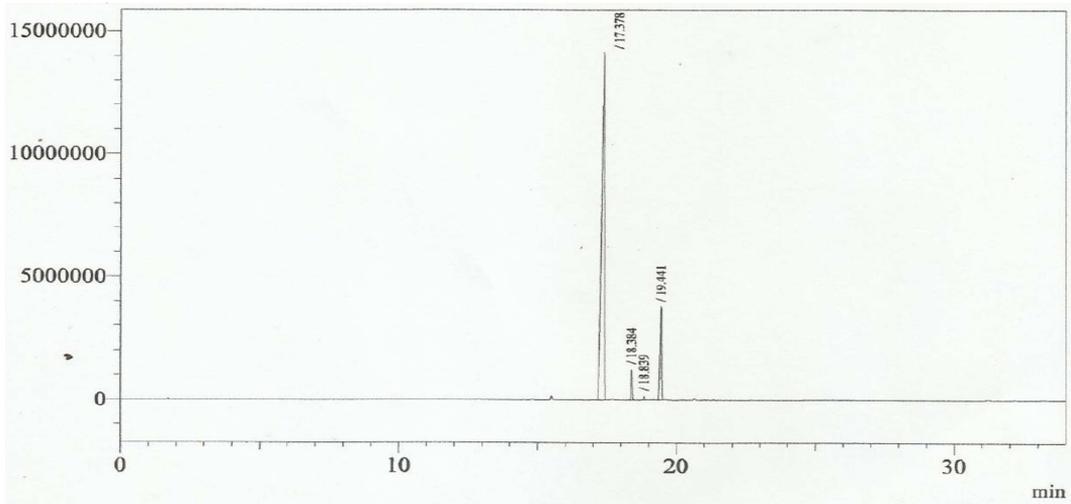


Figure 4. A typical gas chromatogram of the essential oil from Amboina Island bud oils.

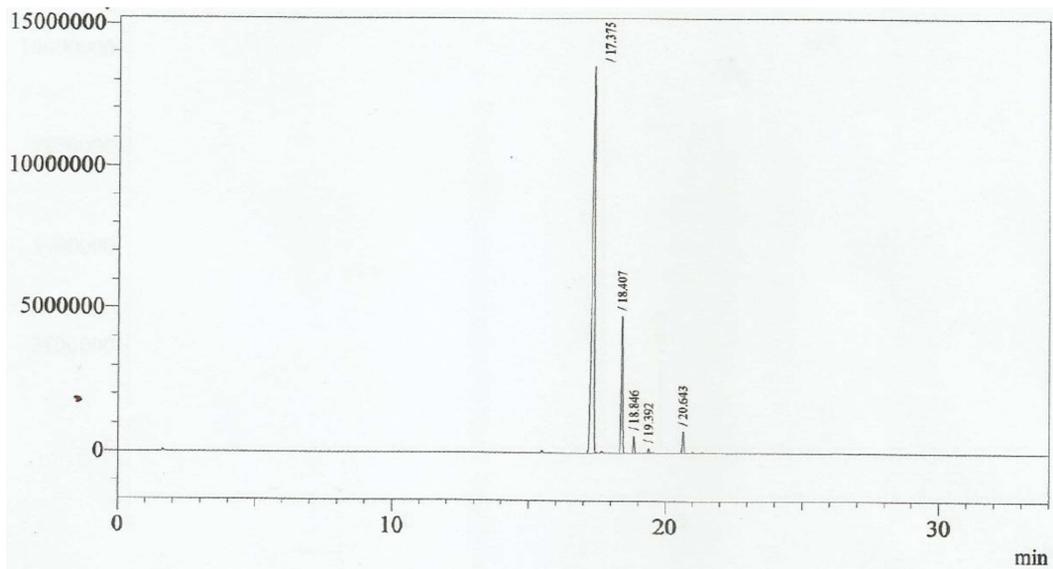


Figure 5. A typical gas chromatogram of the essential oil from Amboina Island leaf oils.

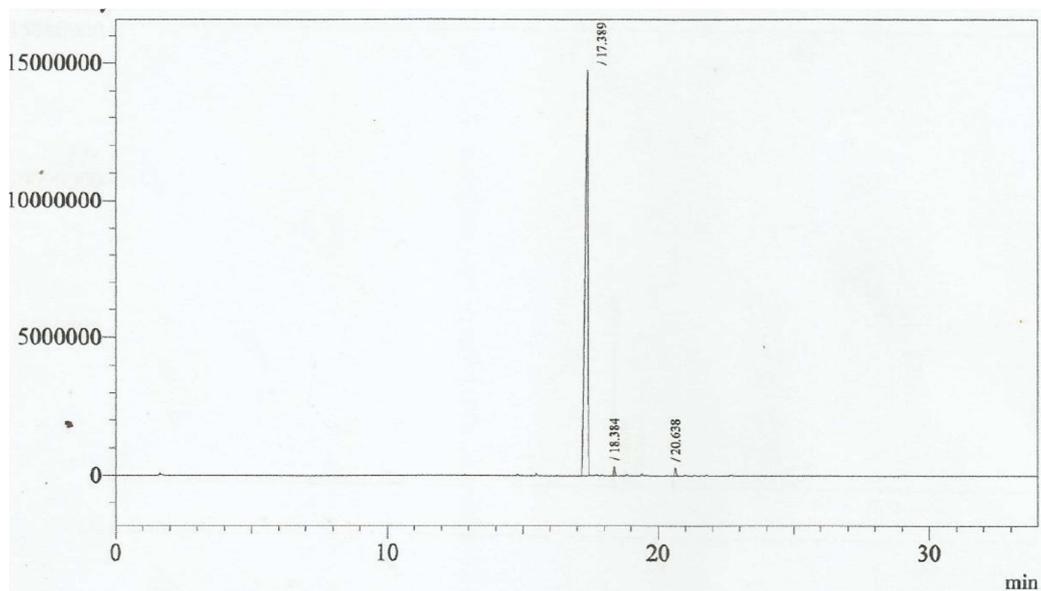


Figure 6. A typical gas chromatogram of the essential oil from Amboina Island stem oils.

## 4. Conclusions

The chemical composition of essential oils from clove (*Eugenia caryophyllata*) provided from Amboina Island was investigated. Essential oil from bud, leaf and stem clove oil, was obtained from steam distillation method, and its chemical composition was determined by GC and GC-MS. The result indicated that the essential oils mainly had about 81.13 - 84.44 % eugenol, 11.60 - 15.02 % eugenyl acetate and 3.45 - 4.60 %  $\beta$ -caryophyllene in the oil from bud, 81.06 - 86.04 % eugenol, 11.95 - 16.16 %  $\beta$ -caryophyllene and 2.02 - 3.05% eugenyl acetate in oil from leaf, 97.20 - 98.83% eugenol in oil from stem, thus only one main component.

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