Physico-chemical properties of clove oil from three forest clove accession groups in Maluku

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Abstract. Forest cloves (Syzygium aromaticum (L.) Merr. & L. M. Perry.) are classified as wild and endemic clove to Maluku islands. So far, the studies on utilization of clove oil as an essential oil are still limited. This study aims to provide information on the physicochemical properties of clove oil from three clove accession groups in Maluku. Clove oil is obtained through the distillation of parts of the plant such as flowers bud, flower stalks, and leaves. The physicochemical components of clove oil in this study were compared to those of the standard type of cultivated clove oil, for there is no standard for forest clove oil. The results of the characterization of the physicochemical properties of clove oil revealed that the oils of the three clove accession groups met the standard of clove oil in several parameters, namely color (yellow to dark brown), specific gravity (0.9559-1.101 g ml⁻¹), refractive index (1.5075-1.5467), β-caryophyllene (0.74-11.79%). However, they did not meet the standard of clove oil solubility in ethanol 70% (1:10) and total eugenol (20-28%). The clove accession groups had high oil content (in the distillation) from the flower bud section (1.33-3.00%), followed by the flower stalks (0.40-1.0%), and leaves (0.44-0.93%).

Keywords: SNI clove oil, eugenol, essential oil, Syzygium aromaticum, wild cloves

1. Introduction

Forest cloves belong to the species of wild cloves, endemic to Maluku islands [1]. In Maluku, cloves are often found on Ambon Island (Hitulama and Hitumesing Village) and Seram Island (Latu and Hualoi Villages) [2] [25]. They are classified as aromatic plants [1] from the Myrtaceae family [3–5]. Fragrant plants can generally produce essential oils through extraction from parts of plant organs [4], [6]. The Forest clove plants have a larger morphological size compared to the cultivated ones. Mahulette et al. (2019) divided forest cloves (in Maluku) into 3 accession groups, namely accessions with large, medium, and small morphological measurements of flowers, fruits, and seeds. The agromorphological differences between the forest clove accession groups can cause the variations of the composition in their volatile oils. The volatile oils are plant products having aromatic, volatile, and strong aesthetic properties, and plants are produced through secondary metabolic pathways [7].
essential oils in plants are generally used as antibacterial, antivirus, antifungus, insecticides, defense against herbivores, as well as attractants for pollinators and repellent for pests [6].

The demand for essential clove oil distilled from flowers bud (bud oil), flower stalks (stem oil), leaves (leaf oil), are very high on the market [3], [8], as it is used in fields pharmacology, agriculture, food industry, cosmetics and various other industries [3], [9]. The production of essential clove oil in Indonesia is only limited to cultivated cloves, for it is considered to be of good quality with high eugenol level, about 70-90% [7], [10], [11]. Meanwhile, the use of forest clove oil, such as an essential oil producing plant has not been carried out. So far, the utilization of wild cloves as an essential oil is still limited because they are considered as wild with low eugenol levels [1], [2]. The commercial use of wild cloves is limited to dried clove buds, not clove oil. The limited utilization of wild cloves as an essential oil producing plant led to a lack of data about the physicochemical properties. The quality of clove oil is solely showed based on the character of its physicochemical properties. The physicochemical properties of forest clove oil from accession groups in Maluku are essential because they relate to the trade standards of clove oil. The standard physicochemical properties of clove oil have been based on physicochemical parameters of cultivated clove oil, and not wild cloves. The physicochemical properties of clove oil based on the standard of clove oil include color, specific gravity, refractive index, optical rotation, solubility in ethanol, eugenol, and β-caryophyllene.

Data on the agro-morphological and the physicochemical components of forest clove oil is important in determining and informing the character of the plant and the quality standards of the oil produced. This information can be used as a reference in making forest clove oil standards as well as developing forest clove commodities towards the clove oil agro-industry.

2. Materials and Methods

2.1. Plants material

Forest clove plant was collected from Wanath Hamlet, Hitumessing Village, Central Maluku Regency, Ambon Island (03°36'21.7" E - 128°11'19.9" S, at an altitude of 186 m), in March 2018. The plant used in this study had previously been identified based on herbarium specimens at Herbarium Bogoriense, Bogor-Indonesia LIPI-Cibinong Research Center (BO 1726070) as the *Syzygium aromaticum* (L.) Merr. & L.M. Perry.) [2]. The population of wild cloves can be distinguished mainly based on the size of the morphology of the leaves so that the sampling of forest clove accession groups in this study referred to the grouping of wild cloves according to Mahulette et al. (2019), namely the accession group with large leaf morphology (first group), moderate (second group), and small (third group). The criteria for accession in group I, group with a leaf length of 20-22 cm and leaf width of 9-10 cm; group II with the accession group with leaf length of 17-19 cm and leaf width of 8-9 cm; group III the accession group with a leaf length of 14-16 cm and a leaf width of 6-7 cm. The leaves taken were the fourth leaf from shoots [12]. Plants from each accession group consisted of 10 plants, so there were a total of 30 forest cloves. The plants were selected as healthy and aged > 15 years.

2.2. Forest clove oil distillation

Parts of the plant distilled from each accession group were 2 kg of flowers bud, 3 kg of flower stalks, and 6 kg of leaves. The flower buds and flower stalks to be distilled were dried in the sun for 3-4 days, while the leaves were only reduced by 10-15% using ventilated drying ovens at 30 °C before distillation. The clove leaves that have been swayed were immediately distilled, while the dried flower buds and flower stalks were ground first. The distillation process was carried out at 100 °C for 6-10 hours. The produced oil was then put into a measuring cup to determine the oil content. Next, the oil was added with anhydrous sodium sulfate (Na₂SO₄) as much as 2% of the oil volume. The oil was then filtered using filter paper to obtain pure clove oil, which was then stored in a dark bottle and labeled for analysis.
2.3. Analysis of physicochemical properties
The analysis of physicochemical properties was carried out at the Spice and Medicinal Crops Research Institute (BALITTRO), Bogor. The work procedure for analyzing the physicochemical properties of clove oil refers to the Indonesian National Standard (SNI) 06-2387-2006 [13]. The standard color determination was done visually and then compared to the 2015 RHS color chart standard. The determination of the density using the gravimetric method used pignometer at 20 °C; the refractive index used the refractometry method at 20 °C; and the solubility in 70% ethanol used volumetric method with turbidity comparison solution, namely silver nitrate 0.1 N. the total eugenol was determined using cassia flask with 4% Potassium Hydroxide (KOH) solution, while β-caryophyllene used gas chromatography (GC) technique Agilent Technologies 6890N with mobile phase in the form of nitrogen with velocity flowrate 30 ml minute⁻¹, flame ionization detector (FID) was done at 250 ° C, hydrogen flowed at a velocity of 30 ml minute⁻¹, and gas flow rate was 300 ml minute⁻¹. The temperature of the tools used was: injector temperature of 200°C, temperature column of 80°C, the final temperature of the column of 200°C with a rise in temperature of 5°C minute⁻¹.

2.4. Data analysis
Data from the results of the physicochemical analysis of forest clove oil were compared to the standard of clove oil. The Indonesian National Standard (SNI) for clove oil used were: SNI: 06-4267-1996 (bud oil), SNI: 06-4374-1996 (stem oil), and SNI: 06-2387-2006 (leaf oil) [13]–[15]. The international standards used were ISO 3141: 1997 (E) and Food Chemical Codex Edition IV.

3. Results
3.1. Forest clove oil content
Forest clove oil from the three forest clove accession groups on Ambon Island had varying oil content (Figure 1). The oil distillation results revealed that the highest levels of forest clove oil were obtained in group III (3.00%), followed by group II (1.67%) and the group I (1.33%). The highest oil content from the distillation of the flower stalk was obtained from the group I (1.00%), followed by group III (0.77%), and group II (0.40%). The highest leaf oil content was found in group III (0.93%), followed by the group I (0.44%) and group II (0.34%).

![Figure 1. The oil content of Forest clove accession groups](image-url)
3.2. The color of forest clove oil
The distillate forest clove oil showed normal characteristics in terms of physical oil color (Figure 2). The results revealed that the overall physical color of clove oil was visually following the standard of clove oil, which is yellow to dark brown. The physical color of the forest clove oil was compared to the 2015 RHS color chart standard (Table 1). Based on the results obtained, the flower oils in groups I and II belonged to the yellow group, namely vivid yellow (9A) and brilliant yellow (8B), while group III was in the green, yellow group, namely light greenish yellow (1C). The overall clove flower oil in the wild belonged to the grayed orange color group, which is dark reddish orange (175B), brownish orange (N167B) and brilliant greenish yellow (1A), respectively. The leaf oil of the entire clove accession group was included in the yellow group color, i.e., each of the greenish yellow (3A), light greenish yellow (1C), and brilliant greenish yellow (4A).

![Figure 2. Physical color characteristics of forest clove oil. (A) bud oil; (B) stem oil; (C) leaf oil). (I) Forest clove accession group I; (II) Forest clove accession group II; (III) Forest clove accession group III.](image)

3.3. Physico-chemical properties
The forest clove physicochemical properties described in this study were specific gravity, refractive index, solubility in ethanol, total eugenol, and β-caryophyllene (Table 2). The quality was compared to the standards of flower, flower stalks, and cultivated clove leaf oils. The Indonesian National Standard (SNI) was used as follows: 06-4267-1996 (bud oil), SNI: 06-4374-1996 (stem oil), and SNI: 06-2387-2006 (leaf), International Standard (ISO) 3141: 1997 (E) and Food Chemical Codex Edition IV. The overall standard showed an almost equal range in all clove oil. The physicochemical results of forest clove oil showed that the whole accession groups of forest clove had a specific gravity between 0.9559-1.101 g ml⁻¹. Likewise, the index of refraction at a temperature of 20°C had also a specific value where the overall clove oil in the forest clove accession group was in the range of 1.5075-1.5467.

In contrast to the solubility in 70% ethanol and total eugenol, where the clove oil in the accession groups was insoluble in ethanol 70% (1:10) with low total eugenol levels (20-28%). The levels of β-caryophyllene obtained by the clove oil in accession groups were between 0.74-11.79% and not exceeding 17%, as in the overall standard of clove oil.
Table 1. The color standard of forest clove oil based on the 2015 RHS color chart.

| Forest clove accession groups | Bud oil | Stem oil | Leaf oil |
|------------------------------|---------|----------|---------|
| Code                        | Color   | Color groups | Code | Color | Color groups | Code | Color | Color groups |
| I 9A                        | Vivid yellow | Yellow group | 175B | Dark orange | reddish orange group | 3A | Brilliant greenish yellow | Yellow group |
| II 8B                      | Brilliant yellow | Yellow group | N167B | Brownish orange | Graded orange group | 1C | Light greenish yellow | Yellow group |
| III 1C                     | Light greenish yellow | Green, yellow group | 1A | Brilliant greenish yellow | Yellow group | 4A | Brilliant greenish yellow | Yellow group |

Notes: 1 Bud oil = oil distilled from the flowers, 2 stem oil = flower stalk parts, 3 leaf oil = leaf parts. Data obtained from the results of the distillation of clove took place in March 2018.

Table 2. Physico-chemical of clove oil from three accession groups (Syzygium aromaticum (L.) Merr. & L. M. Perry.) in Maluku.

| No. | Test                          | Forest clove accession group. I | Forest clove accession group. II | Forest clove accession group. III |
|-----|-------------------------------|--------------------------------|--------------------------------|----------------------------------|
|     |                               | Bud oil | Stem oil | Leaf oil | Bud oil | Stem oil | Leaf oil | Bud oil | Stem oil | Leaf oil |
| 1   | Specific gravity (20°) (g ml⁻¹) | 0.9987  | 1.0108  | 1.1091  | 1.0221  | 1.0033  | 1.0068  | 0.9559  | 1.0043  | 0.9768  |
| 2   | Bias index (20°)               | 1.5173  | 1.5281  | 1.5467  | 1.5283  | 1.5233  | 1.5236  | 1.5075  | 1.5216  | 1.5133  |
| 3   | 70% ethanol solubility         | 1 : 10  | 1 : 10  | 1 : 10  | 1 : 10  | 1 : 10  | 1 : 10  | 1 : 10  | 1 : 10  | 1 : 10  |
|     |                               | (unsoluble) | (unsoluble) | (unsoluble) | (unsoluble) | (unsoluble) | (unsoluble) | (unsoluble) | (unsoluble) | (unsoluble) |
| 4   | Total Eugenol (%)             | 20      | 20      | 21      | 26      | 22      | 26      | 23      | 28      | 24      |
| 5   | β-caryophyllene (%)           | 5.30    | 1.70    | 0.74    | 11.79   | 3.32    | 6.16    | 6.40    | 3.29    | 5.88    |

Notes: 1 Group I = accession with a large morphological measure, 2 groups. II = medium, 3 groups. III = small; Bud oil = oil from the distillation of flowers, stem oil = flower stalks, leaf oil = leaf part. Data were obtained from the distillation, and physico-chemical analysis of Ambon Forest clove oil from the Island took place in March 2018.
4. Discussion

The results of the distillation of parts of flower buds, flower stalks, and leaves from the three clove accession groups showed that the levels of whole clove oil were still relatively low compared to those of the cultivated ones in the previous study. Cultivated cloves have the highest oil content in the flower buds section (10-20%), followed by flower stalks (5-10%), and leaves (<5%) [7], [16]–[18]. The difference in oil content was determined by differences in plant species [19], where cloves are classified as wild cloves characterized by low eugenol levels [1]. The results of the distillation from the morphological part of the flower buds, flower stalks, and leaves of the entire forest clove accession groups showed high oil content in the distillation of the flower buds (bud oil), following the flower stalks and the leaves. The high oil content in the flower section is supported by the results of previous studies on cultivated cloves [19], [20], where clove oil production is determined by parts of the plant organ and other factors such as the particle size of raw materials, and duration of distillation [19], [21].

The analysis of the physicochemical properties of forest clove oil obtained from the whole accession group showed normal physical characteristics such as oil color. The results showed that the overall physical color of clove oil was visually following the clove oil standard, which is yellow to dark brown. These results were following previous studies on cultivated clove oil, which showed that the normal color of clove oil is light yellow [22], [23]. The brightness of the oil color is determined by several factors, such as the quality of the raw material and the refining method [18]. The distillation method using inadequate equipment can reduce the quality of the oil color or even darken the color due to the buildup of Fe ions [18], [24].

The results of the physicochemical analysis showed that the density of the whole wild cloves in the accession group ranged from 0.9559 to 1.091 g ml⁻¹ so that it was in the range set by the Indonesian National Standard for bud oil, stem oil and leaves oil, 1.04-1.07 g ml⁻¹; 1.033-1.063 g ml⁻¹; 1.025-1.049 g ml⁻¹ [13]–[15], or based on International Standard (ISO) 3141: 1997 (E) and Food Chemical Codex Edition IV, namely 1.0355-1.0455 g ml⁻¹. The refractive index parameter also showed conformance with the clove oil standard, which is in the range 1.5075-1.5467. The refractive index value met the standards set by Indonesian National Standard for bud oil, stem oil and leaf oil, namely 1.529-1.537; 1.510-1.520; 1.528-1.535 [13]–[15], as well as the International Standard (ISO) 3141: 1997 (E) and Food Chemical Codex IV Edition 1.5260-1.5330.

The physicochemical of whole forest clove accession groups was not soluble in 70% ethanol and had low total eugenol ranging from 20-28%. The solubility requirement in the 70% ethanol based on the Indonesian National Standard for bud oil, stem oil, leaf oil and international standards for cultivated clove oil is 1:2 (clear). Likewise with the total eugenol standard, which is 80-95% for bud oil, 78-95% for stem oil, a minimum of 78% for leaf oil [13]–[15], 80-82% for International Standard (ISO) 3141: 1997 (E) and Food Chemical Codex Edition IV. The levels of β-caryophyllene obtained from the whole oil in the forest clove accession groups were between 0.74-11.79% so that they were still within the specified range, which did not exceed 17%. The physicochemical standard of clove oil obtained is determined by various factors such as plant species, plant parts, particle size, preparation methods, storage methods and storage used [18]–[21].

5. Conclusion

The physicochemical properties of the clove oil in the entire accession groups in Maluku, based on the standard of clove oil, were only met in terms of color parameters, specific gravity, refractive index, and β-caryophyllene, did not fulfill parameters such as solubility in 70% ethanol and total eugenol. The essential oils of all forest cloves had low oil content and total eugenol levels.

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