Essential oil components of forest clove variants from Ambon Island, Maluku

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Abstract. Forest clove is native to Maluku from the non-aromatic class. Forest cloves have several variants, including the accession group with a small morphological size in the population. So far, the complete information on essential components contained in this accession group is not studied. The research aims to determine the forest clove accession group's total essential oils with small morphological sizes originating from its distribution area on Ambon Island, Maluku. The analyses were performed on flowers, flower stalks, and leaves using Gas Chromatography-Mass Spectrometry (GC-MS). The analysis results identified 11 components in the flower section, 14 components on the flower stalk, and 13 components on the leaf. The main components analysis of the heat map profile revealed that the small morphological-sized forest clove accession groups' main components were germacrene-D, α-cubebene, eugenol, δ-cadinene, α-copaene, methyl eugenol.

1. Introduction

Forest cloves are classified as non-aromatic and endemic cloves from the Maluku Islands [1]–[5]. A previous study conducted by [3] identified three forest clove accessions in Maluku's distribution area. The forest clove variants found in the last research consisted of 3 accessions, namely forest clove accessions with large, medium, small leaf, flower, fruit, and seed morphological sizes [4], [3]. Characterization of the essential components of forest clove accession groups with large morphological sizes has been carried out [1]; however, the accessions groups with medium and small morphological sizes have not been informed. The study of clove essential components is important, considering that essential oil is currently very commercial in the trading market. It is widely used in the biopharmaceutical sector as an antioxidant, antibacterial, antifungal, antivirulence, and antiseptic [6], [7].

Forest clove with small morphological size on Ambon Island is found in Hitulama and Hitumesing villages, Central Maluku Regency [3], [8]. This accession grows together with other forest clove accession in one population. Its existence can be distinguished based on the appearance of plant morphology, which is somewhat different from the two other forest clove accession groups [3], [4]. So
Physico-chemical studies of the essential oils of the forest clove accession group of small morphological sizes have not been reported. However, complete information on the essential components of this accession group is not yet available. The forest clove accession group with small morphological size was reported in the previous study to have low oil content, namely 3% on flowers, 0.77% on flower stalks, and 0.93% on leaves [2]. Also, it was reported that this accession group had not met the standard of oil trade because it had low eugenol levels [2].

Further research is needed on the potential utilization of other essential components. This study aims to inform the small morphological-sized forest clove accession group's complete essential components using Gas Chromatography-Mass Spectrometry (GC-MS). This information is vital to inform the potential of the essential components in this forest clove accession group to help develop these commodities towards biopharmaceuticals.

2. Methods

Samples for oil distillation of the forest clove accession group of small morphological sizes were taken from forest cloves more than 15 years old on Ambon Island (03°36'17.3'' E - 128°11'15.3'' S, at an altitude of 179 masl). Samples were taken from 10 clove trees from the forest clove accession group with small morphological sizes, and then they were composited. Samples for distillation consisted of 3 kg of dried clove flowers with 14% moisture content, 5 kg of dried flower stalks, and 6 kg of withered leaves using a ventilated drying oven at 30 °C. The handling of material samples to be distilled refers to the procedure [6]. Oil distillation was carried out at The Spice and Medicinal Crops Research Institute (BALITTRO), Bogor, Indonesia, using the steam-hydro distillation method for 8 hours. The distilled oil is then analyzed for its essential components using Gas Chromatography-Mass Spectrometry (GC-MS) at the Regional Health Laboratory of DKI Jakarta Province. Gas Chromatography-Mass Spectrometry (GC-MS) analysis using the Agilent Technologies 7890 Gas Chromatograph. The specifications for the tools used consist of columns in the form of HP Ultra; column length 30 x 0.25 (mm) I.D x 0.25 (μm) film thickness; mobile phase rate 1.2 μ/min; pressure 12 kPa; injector split ratio 8: 1; injector temperature 250 °C. The GC-MS chromatogram peak was then compared with the mass spectra of the NIST 2005 v.2.0 and Wiley 7 library 2003 [9], [10] to determine the component names.

The GC-MS results were presented qualitatively, including component name, component class, retention time (RT), quality (Q), and concentration (%). The main components are displayed in a heat map profile using the R Stat 3.1.0 software with the metabolomics package.

3. Results and discussion

3.1. The morphology of the small forest clove accession

The small morphological size of the forest clove accession had morphological differences with the other two forest clove accession groups in Ambon Island's distribution population. Forest cloves of small morphological size have smaller flower, fruit, and seed sizes in their population (Figure 1). The results of previous studies on forest clove accessions conducted by [1], [2], [4] indicated that small morphological size forest cloves had the smallest morphological sizes of leaves, flowers, fruits, and seeds compared to other forest clove accessions. According to [4], forest clove accessions of small morphological size have leaves length and width of ± 15.17 cm and 6.99 cm, respectively. The leaf morphology size is different from other forest clove accessions, which can reach ± 20.36 cm in length and 9.86 cm in width [1], [2], [4]. Apart from leaf size, it was also reported that there were differences in the morphology of flowers, fruits, and seeds. Small morphological groups of forest cloves were reported to have flower bud lengths and flower buds diameter of ± 2.35 cm and ± 0.57 cm, respectively, length and diameter of ripe fruit ± 2.75 cm and 1.40 cm, respectively, length and diameter of seed ± 1.97 cm and ± 0.90 cm, respectively [4].

3.2. Essential oils of small morphological size forest clove accession

GC-MS analysis identified 11 components in flower, 14 components on the flower stalk, and 13 components in leaves. Most of the components consisted of sesquiterpenoid fractions (Table 1). The forest clove accession group with a small morphological size had the highest concentration of volatile
components in flowers, namely α-cubebene (27.83%), then eugenol (12.90%), germacrene-D (11.26%), δ-cadinene (8.58%), 2'-3', 4' trimethoxycacetophenone (8.44%); on flower stalks in the form of eugenol (24.26%), δ-cadinene (12.60%), methyleugenol (8.91%), 2'-3', 4' trimethoxycacetophenone (8.79%); and the leaves are eugenol (22.83%), then followed by α-cubebene (17.62%), α-copaene (13.00), δ-cadinene (7.03%), caryophyllene (6.90%), methyleugenol (6.35%).

The number of components contained in the small morphological size of the forest clove accession group of the flower, flower stalks, and leaf parts obtained in the study showed similarities to the number of components in the large morphological size forest clove accession group obtained in the previous study on Ambon Island by [1]. Previous studies on the large morphological-sized forest clove accession group consisted of 13 components in flowers, 11 components on flower stalks, and six components on leaves. This difference can be seen in the amount and concentration of content in part being analyzed. According to [11], [12], variations in the number of components can occur in the same species depending on environmental factors, plant genetics, and the part being analyzed. According to [1], [2], [13], forest cloves are classified as non-aromatic cloves, which contain up to 14 essential components, which is different from aromatic cloves, which only have six essential components. Even though there were more essential components, the eugenol content in the forest clove accession group of small morphological sizes in this study was at a low concentration (<25%). The low level of eugenol is in line with the results obtained in previous studies on the large morphological size forest clove accession group in its distribution area in Maluku [1], [2]. According to [2], [14], forest cloves are classified as non-aromatic wild type cloves which have low eugenol content in all parts of the plant.

Eugenol is a phenylpropanoid which is pale yellow (pale yellow), where the fraction will give the cloves a spicy aroma [15], [16]. The low levels of eugenol contained in forest cloves showed a difference with the aromatic clove group, which had the highest eugenol content in all parts of the plant, namely around 70-90% [13], [17], [18]. The difference in eugenol levels is due to forest cloves belonging to the wild type of non-aromatic cloves, which differ morphologically and metabolically from aromatic cloves[1], [3], [13], [19]. Although low eugenol levels were found, the small morphological size of forest cloves showed various variations in components and concentrations in the flower, flower stalks, and leaves.
3.3. The essential oil of forest clove accession groups with small morphology

| Components          | class | RT   | Q   | Cons. (%) | Components          | class | RT   | Q   | Cons. (%) | Components          | class | RT   | Q   | Cons. (%) |
|---------------------|-------|------|-----|-----------|---------------------|-------|------|-----|-----------|---------------------|-------|------|-----|-----------|
| α-cubebene          | S     | 21.187 | 99 | 27.83     | Eugenol             | PP    | 50.064 | 98 | 24.26     | Eugenol             | PP    | 50.059 | 98 | 22.83     |
| Eugenol             | PP    | 50.059 | 98 | 12.90     | δ-cadinene          | S     | 36.859 | 99 | 12.60     | α-cubebene          | S     | 21.149 | 99 | 17.62     |
| Germacrene-D        | S     | 34.097 | 98 | 11.26     | Methylguaiacol      | PP    | 47.773 | 98 | 8.91      | α-copaene           | S     | 23.006 | 99 | 13.00     |
| δ-cadinene          | S     | 36.816 | 98 | 8.58      | δ-cadinene          | S     | 36.857 | 87 | 8.79      | δ-cadinene          | S     | 36.854 | 99 | 7.03      |
| 2'-3',4'Trimethocyacetophenone | S   | 56.573 | 90 | 8.44      | Caryophyllene       | S     | 21.125 | 99 | 6.74      | Caryophyllene       | S     | 28.202 | 99 | 6.90      |
| Caryophyllene       | S     | 28.225 | 99 | 5.87      | Methyleugenol       | PP    | 47.768 | 98 | 6.35      | Methyleugenol       | PP    | 47.768 | 98 | 6.35      |
| α-copaene           | S     | 23.044 | 99 | 17.49     | α-copaene           | S     | 22.982 | 99 | 6.28      | Methylguaiacol      | PP    | 47.768 | 98 | 6.35      |
| Germanol            | S     | 40.473 | 87 | 1.40      | Germanol-D          | S     | 34.063 | 98 | 5.79      | Germanol-D          | S     | 34.054 | 99 | 5.71      |
| Cadina-1,4-diene    | S     | 38.068 | 97 | 1.36      | Naphtalene,1,2,3,4,4a,7,hexahydro-1,6-dimethyl-4-(1-methyllethyl)α-cadinol | A     | 31.740 | 89 | 5.41      | Naphtalene,1,2,3,4,4a,7,hexahydro-1,6-dimethyl-4-(1-methyllethyl)α-cadinol | A     | 31.740 | 89 | 5.41      |
| γ-Muurolene         | S     | 33.216 | 99 | 1.28      | Methylguaiacol      | PP    | 47.764 | 98 | 1.08      | Methylguaiacol      | PP    | 47.764 | 98 | 1.08      |
| Methylguaiacol      | PP    | 47.764 | 98 | 1.08      | Methylguaiacol      | PP    | 47.764 | 98 | 1.08      | Methylguaiacol      | PP    | 47.764 | 98 | 1.08      |
| Note: Data uses the 15-year-old clove accession group during the harvest season in March 2018; retention time (RT); quality (Q); sesquiterpenoid (S), phenylpropanoid (P), aliphatic (A), monoterpenoid (M)
3.4. The main components of the forest clove accession group of small morphological size

The heat map profile of the essential components of the forest clove accession group of small morphological sizes in this study showed six main components: germacrene-D, δ-cadinene, eugenol, α-cubebene, α-copaene, methyleugenol (Figure 2). These components are found in almost all parts of the flower, flower stalk, and leaf. The results showed similarities with the main component in the large morphological-sized forest clove accession group previously formulated by [1]. The similarity between the main components in the forest clove accession group obtained in the previous study was due to the similarity of plant species. According to [20], [21], the same species will show the similarity of the components contained due to genetic proximity.

The results obtained in the forest clove accession group of small morphological size and previous studies on the large morphological size of forest clove accessions showed that forest cloves were classified as wild non-aromatic type cloves with almost the same main volatile components. These results differ from the aromatic group of clove's main components, where the main components are eugenol, caryophyllene, and eugenol acetate with high concentrations [13], [17]. The difference in main components between forest cloves and aromatic cloves is more due to differences in plant species [1], [3].

Main components are depicted on the peak of the GC-MS chromatogram (Figure 3), where germacrene-D has a peak at m/z 204.0 (M+), δ-cadinene (CAS) at m/z 204.0 (M+), eugenol at m/z namely 164 (M+), α-cubebene at m/z 204.0 (M+), α-copaene at m/z 204.0 (M+), methyleugenol 178.0 (M+).
4. Conclusion
The forest clove accession group of small morphological size had the same main component as the previous study in the forest clove accession group's large morphological size. However, the differences were found in the concentrations contained in the flower, flower stalks, and leaves. The main components obtained in the small morphological size forest clove accession group consisted of 6 components: germacrene-D, δ-cadinene, eugenol, α-cubebene, α-copaene, and methyleugenol.

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