Fractionation of Citronella Oil and Identification of Compounds by Gas Chromatography-Mass Spectrometry

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ABSTRACT
Citronella oil is one of the most important essential oils and is widely used in the pharmaceutical, cosmetic and food industries. However, the selling price of citronella oil is still low, so efforts are needed to increase its added value by isolating the active components such as citronellal, citronellol and geraniol. This study aims to isolate the active ingredients of citronella oil that have higher economic added value. Citronella oil was obtained by the process of distillation of Cymbopogon nardus (L.) Rendle leaves. The essential oil was then fractionated by a vacuum fractionation process. The essential oil fraction was identified by Gas Chromatography - Mass Spectrometry (GC-MS). Fractions obtained from the fractionation process were identified, and the results were: F1 (D-Limonene: 72.89%), F2 (Citronellal: 50.13%), F3 (Citronellal: 74.89%), F4 (Citronellal: 88.56%), F5 (Citronellal: 84.89%), F7 (Citronellol: 57.42%), F8 (Citronellol: 44.73%), F9-1 (Geraniol: 65.56%), F9-2 (Geraniol: 64.41%) and residual (Geraniol: 32.04%). Based on these results, several active compounds from citronella oil can be obtained using the vacuum distillation fractionation method.

Keywords: citronella oil; fractionation; gas chromatography-mass spectrometry

INTRODUCTION
Indonesia is a tropical country that is rich in natural resources, especially medicinal plants. Until now, Indonesia still plays an important role in the world spices trade, including essential oils and their derivatives. Citronella oil is one of the most important essential oils and is widely used in the pharmaceutical, cosmetic and food industries. However, the value of citronella oil is still low, so efforts are needed to increase its added value by isolating the active components such as citronellal, citronellol and geraniol.

Citronella oil is an essential oil obtained from the steam distillation of Cymbopogon nardus (L.) Rendle leaves. Harianingsih et al (2017) identified essential oil from citronella oil using Gas Chromatography – Mass Spectrometry (GC-MS) and obtained 36.11% citronellal, 20.07% geraniol, and 10.82% citronellol. Fractionation of citronella oil has also been carried out with GC-MS analysis, and 80.65% citronellol, 76.63% geraniol, 95.10% citronellal and 75.95% p-menthane-3,8-diol were obtained.

The active compounds found in citronella oil include citral, citronellol, α-pinene, kampfen, sabinen, misren, β-felandren, psimen, limonene, cis-osome, terpinol, citronellal, borneol, terpinen-4-ol, α-terpineol, geraniol, farnesol, methyl heptenone, n-decaldehyde, dipenten, methyl heptenone, bornylacetate, geranylformate, terpinyl acetate, citronellyl acetate, geranyl acetate, β-element, β-cariophyline, β-bergamotene, trans-methylisoeugenol, elemol, and cariophylene oxide (Timung et al., 2016).

Research on the identification of essential oil components using GC-MS has been carried out (Madivoli et al., 2012). Gas chromatography was used...
to identify a compound found in the gas mixture and also to determine the concentration of a compound in the sample. Mass spectrometry is a method for obtaining molecular weight. Alloys of both can produce accurate data in identifying compounds that are covered by their molecular structure. This study aims to identify the compounds of fractionation of citronella oil with vacuum distillation method using GC-MS.

METHODS

Citronella oil was obtained from leaves of *Cymbopogon nardus* (L.) Rendle which was distilled in the experimental garden, Manoko – Lembang, Bandung. Citronella oil has the characteristics as shown in Table 1. From an analysis using GC-MS, the major compounds of citronella oil were characterized, as shown in Table 2. (Anwar et al., 2019a). Citronella oil (1000 mL) was then fractionated in Laboratory of Chemistry at LIPI by vacuum fractionation with packed column and reflux ratio of 10:5 and 5:10, at 0 mbar pressure as shown in Figure 1. The process of fractionation of essential oils was carried out based on the major compounds data and area (%) of the essential oils that had been obtained from GC-MS analysis at 0 mbar pressure (Table 3) (Anwar et al., 2019a). Afterwards, citronella oil fraction was identified by GC-MS.

RESULTS AND DISCUSSION

The fractionation process was carried out using vacuum fractionation at 0 mBar pressure and reflux ratio of 10:5 and 5:10. The results of fractionation in these conditions can be seen in Table 4.

The fractionation process of citronella oil is done by varying the amount of distillate volume. This distillate volume refers to the results of identification by GC-MS of citronella oil. Fraction 1 (F1) has major compound D-Limonene (72.89%), Fraction 2 (F2) has major compound Citronellal (50.13%), Fraction 3 (F3) has major compound Citronellal (74.89%), Fraction 4 (F4) has major compound Citronellal (88.56%), Fraction 5 (F5) has major compound Citronellal (84.89%), Fraction 6 (F6) has major compound Citronellal (55.38%), Fraction 7 (F7) has major compound Geraniol (65.56%), Fraction 8 (F8) has major compound Geraniol (64.41%) and residue has major compound Geraniol (32.04%). The best results of citronellal fraction are obtained by F4 (88.56%). From the five fractions (F2-F6), the fraction F4 has high citronellal area and purity (95), the fraction F5 has high citronellal area and same purity (98), fraction F9-1 has high geraniol area and purity (94).
1. Stirring motor  
2. Magnetic stir bar  
3. Heating jacket  
4. Feeding pump  
5. Feed thermocouple  
6. Distillation column  
7. Column heating jacket  
8. Cooling  
9. Pressure difference measuring device  
10. Thermocouple peak  
11. Reflux unit  
12. Manometer  
13. Condenser  
14. Vacuum sensor  
15. Vacuum hose  
16. Distillate coolant  
17. Container bottle  
18. Fraction container  
19. Fraction divider  
20. Motor dividers  
21. Control panel  
22. Trap tube  
23. Vacuum controller faucet  
24. Vacuum pump  
25. Aluminium frame

**Figure 1. Bench scale fractionation distillation unit** (Agustian E, Sulaswatty. A, 2005)

**Figure 2. Fraction of Cymbopogon nardus (L.) rendle with the highest compounds**

Figure 2 shows the fraction with the highest component. The difference in yield obtained is influenced by fractionation time and reflux ratio (whether 10:5 or 5:10). According to Egi et al., one method to improve the efficiency of the separation process is to use a reflux technique, i.e., some products are returned to the system to move from the liquid phase to the vapor phase. (Siwi & Rusli, 2013). The optimum reflux ratio to obtain high D-Limonene, citronellal, citronelol and geraniol purity in this study is 10:5.

**CONCLUSION**

D-Limonene, citronellal, citronelol and geraniol are the main compounds of citronella oil which can be separated using vacuum fractionation distillation. The difference in the volume of distillate, reflux ratio and the time of fractionation distillation greatly influences the purity of each major compound.
Table 3. The results of fractionation of the essential oil of *Cymbopogon nardus* (L.) rendle (Anwar et al, 2019a)

| Name of Fraction | Major Compound       | R TIME | % AREA | SI | Volume (ml) |
|------------------|----------------------|--------|--------|----|-------------|
| F1               | Beta Myrcene         | 8.063  | 1.26   | 96 | 25          |
|                  | dl-Limonene          | 9.387  | 55.90  | 99 |             |
|                  | dl-Limonene          | 9.649  | 2.96   | 98 |             |
|                  | Citronelal           | 22.282 | 36.38  | 98 |             |
|                  | L-linalool           | 25.863 | 1.51   | 91 |             |
|                  | Isopulegol           | 26.863 | 1.38   | 99 |             |
| F2               | D L limonene         | 9.339  | 17.17  | 99 | 137.96      |
|                  | Citronelal           | 22.349 | 76.00  | 98 |             |
|                  | Linalool             | 25.686 | 2.81   | 97 |             |
|                  | Isopulegol           | 26.530 | 1.22   | 99 |             |
|                  | Isopulegol           | 26.878 | 2.81   | 99 |             |
| F3               | dl-Limonene          | 9.330  | 1.46   | 99 | 12.38       |
|                  | Citronellal          | 22.378 | 91.12  | 98 |             |
|                  | Linalool L           | 25.868 | 2.78   | 97 |             |
|                  | Neo-isopulegol       | 26.530 | 1.37   | 99 |             |
|                  | Isopulegol           | 26.873 | 3.26   | 99 |             |
| F4               | dl-Limonene          | 9.335  | 1.03   | 98 | 348.27      |
|                  | Citronellal          | 22.359 | 90.10  | 98 |             |
|                  | Linalool L           | 25.868 | 1.96   | 91 |             |
|                  | Neo-isopulegol       | 26.530 | 1.85   | 99 |             |
|                  | Isopulegol           | 26.878 | 5.06   | 99 |             |
| F5               | Citronellal          | 22.282 | 44.77  | 98 | 3.41        |
|                  | Alpha-terpinolene    | 25.882 | 1.06   | 95 |             |
|                  | Neo-isopulegol       | 26.535 | 1.89   | 99 |             |
|                  | Isopulegol           | 26.882 | 5.65   | 99 |             |
|                  | Beta-elemene         | 28.697 | 1.87   | 99 |             |
|                  | Citronellol Acetate  | 32.268 | 4.33   | 94 |             |
|                  | Geranyl Acetate      | 37.244 | 1.75   | 91 |             |
|                  | Citronellol          | 37.625 | 25.37  | 98 |             |
|                  | Geraniol             | 41.716 | 13.32  | 97 |             |
| F6               | Citronelal           | 22.206 | 4.27   | 98 | 147.12      |
|                  | Isopulegol           | 26.873 | 1.40   | 99 |             |
|                  | Beta-elemene         | 28.735 | 8.71   | 99 |             |
|                  | Caryophyllene        | 29.087 | 10.92  | 94 |             |
|                  | Citronellol Acetate  | 32.287 | 11.79  | 94 |             |
|                  | Citronellol          | 37.692 | 58.22  | 98 |             |
|                  | Geraniol             | 41.692 | 4.69   | 97 |             |
| F7               | Citronellol Acetate  | 32.302 | 18.98  | 94 | 25          |
|                  | Geranyl Acetate      | 37.264 | 1.29   | 91 |             |
|                  | Citronellol          | 37.692 | 64.84  | 98 |             |
|                  | Geraniol             | 41.716 | 13.77  | 97 |             |
Table 3. continued

| Name of Fraction | Major Compound       | R TIME | % AREA | SI | Volume (ml) |
|------------------|----------------------|--------|--------|----|-------------|
|                  | Citronellol Acetate  | 32.28  | 11.82  | 94 | 125.68      |
|                  | Geranyl Acetate      | 37.26  | 6.03   | 91 |             |
|                  | Citronellol          | 37.65  | 37.42  | 98 |             |
|                  | Geraniol             | 41.77  | 44.72  | 96 |             |
| F9               | Geraniol             | 41.75  | 41.37  | 96 | 198.4       |
|                  | Geranyl Acetate      | 37.24  | 14.83  | 91 |             |

Table 4. The results of fractionation of the essential oil of *Cymbopogon nardus* (L.) rendle

| Name of fraction | Mayor Compound | Area (%) | SI | T head | T flash | Vol.theoretical (Vol. Real) (ml) | Reflux |
|------------------|----------------|----------|----|--------|--------|---------------------------------|--------|
| F1               | Beta-myrcene   | 1.87     | 96 | 67.0   | 97.0   | 10.285 (9)                      | 10 : 5 |
|                  | D-limonene     | 72.89    | 99 |        |        |                                 |        |
|                  | Citronellal    | 19.45    | 93 |        |        |                                 |        |
|                  | Isopulegol     | 1.09     | 94 |        |        |                                 |        |
| F2               | D-limonene     | 42.46    | 99 | 80.4   | 100.3  | 42.79 (45)                      | 10 : 5 |
|                  | Citronellal    | 50.13    | 90 |        |        |                                 |        |
|                  | Linalool       | 2.57     | 97 |        |        |                                 |        |
|                  | Isopulegol     | 1.06     | 95 |        |        |                                 |        |
|                  | Isopulegol     | 2.41     | 97 |        |        |                                 |        |
| F3               | D-limonene     | 16.62    | 99 | 81.3   | 99.2   | 11.455 (13)                     | 10 : 5 |
|                  | Citronellal    | 74.89    | 94 |        |        |                                 |        |
|                  | Linalool       | 2.97     | 96 |        |        |                                 |        |
|                  | dl-Isopulegol  | 1.41     | 96 |        |        |                                 |        |
|                  | Isopulegol     | 3.22     | 99 |        |        |                                 |        |
| F4               | D-limonene     | 1.20     | 99 | 80.0   | 106.6  | 441.99 (301)                    | 10 : 5 |
|                  | Citronellal    | 88.56    | 94 |        |        |                                 |        |
|                  | Linalool       | 2.69     | 96 |        |        |                                 |        |
|                  | Isopulegol     | 1.62     | 93 |        |        |                                 |        |
|                  | Isopulegol     | 3.83     | 99 |        |        |                                 |        |
| F5               | Citronellal    | 84.89    | 95 | 84.8 - 85.7 | 116.7 | 441.99 (143)                  | 5 : 10 |
|                  | Linalool       | 2.07     | 96 |        |        |                                 |        |
|                  | Isopulegol     | 7.07     | 99 |        |        |                                 |        |
| F6               | Citronellal    | 55.38    | 95 | 87.5 - 89.7 | 115.5 - 116.7 | 2.63 (2.9)   | 10 : 5 |
|                  | Citronellal    | 2.56     | 58 |        |        |                                 |        |
|                  | Isopulegol     | 2.29     | 96 |        |        |                                 |        |
|                  | L-alpha-terpineol| 1.18 | 95 |        |        |                                 |        |
|                  | Citronellol    | 7.59     | 98 |        |        |                                 |        |
| F7               | B-Elemene      | 5.41     | 91 | 89.8 - 105.2 | 89.8 - 125.5 | 206.19 (206)   | 5 : 10 |
|                  | Caryophyllene  | 5.52     | 99 |        |        |                                 |        |
|                  | Citronellol    | 57.42    | 98 |        |        |                                 |        |
### Table 4. continued

| Name of fraction | Mayor Compounds          | Area (%) | SI | T head     | T flash    | Vol. theoretical (Vol. Real) (ml) | Reflux |
|------------------|-------------------------|----------|----|------------|------------|-----------------------------------|--------|
| F8               | Geraniol                | 9.10     | 94 | 105.5 - 130.7 | 105.5 - 125.5 | 3.225 (4)                        | 10 : 5 |
|                  | Citral                  | 2.19     | 97 |            |            |                                   |        |
|                  | Geranyl isobutyrate     | 4.13     | 90 |            |            |                                   |        |
|                  | Citronellol             | 44.73    | 98 |            |            |                                   |        |
|                  | Geraniol                | 30.37    | 94 |            |            |                                   |        |
| F9.1             | Germacrene              | 1.46     | 99 | 130.7 - 150.5 | 125.5 - 165.9 | 192 (192)                        | 5 : 10 |
|                  | Geranyl Acetate         | 12.21    | 91 |            |            |                                   |        |
|                  | Citronellol             | 14.40    | 98 |            |            |                                   |        |
|                  | Geraniol                | 65.56    | 94 |            |            |                                   |        |
| F9.2             | Citral                  | 1.00     | 94 | 101 - 101.6 | 193 - 326  | 88 (3)                            | 5 : 10 |
|                  | Geranyl Acetate         | 15.79    | 91 |            |            |                                   |        |
|                  | Citronellol             | 10.41    | 98 |            |            |                                   |        |
|                  | Geraniol                | 64.41    | 94 |            |            |                                   |        |
|                  | Geranyl Acetate         | 12.53    | 91 |            |            |                                   |        |
|                  | Geraniol                | 32.04    | 94 |            |            |                                   |        |
|                  | Eugenol                 | 1.67     | 98 |            |            |                                   |        |

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