THE CONTENTS OF VOLATILE OIL ISOLATED FROM Kaempferia galanga RHIZOMES. MASS SPECTROSCOPIC APPROACH

KUNDANGAN MINYAK ATSIRI YANG DIISOLASI DARI UMBI Kaempferia galanga PENDEKATAN SECARA SPEKTROMETRIK MASSA

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Abstract
Gas chromatographic-mass-spectroscopic (GC-MS) analysis was carried out to volatile oil isolated from Kaempferia galanga rhizomes. It was found that eight components comprised the oil, in which five of them (compounds 3, 4, 6, 7, and 8 peaks) were the main component. The mass-spectroscopic analysis resulted that the four (compounds 3, 4, 6, and 8 peaks) of the five compounds were δ-phenylacetonitrile, α-terpinene, ethyl cinnamate and shikimol δ-kaempferolide and respectively, while compound 7 was probably isookadicetone ketone or 4-benzyl methyl. Compounds 1 and 2 were assumed as β-phenylacetonitrile derivatives, while compound 3 needs to be structurally elucidated using NMR-spectroscopic method in further.

Keywords: GC-MS, Kaempferia galanga, δ-phenylacetonitrile, α-terpinene, ethyl cinnamate, dihydro-β- sesquinaptholide

Abstrak
Dilakukan analisis kandungan minyak atsiri dari umbi Kaempferia galanga secara kromatografis gas spektroskopik massa (K-G-SM). Didapatkan delapan komponen pentanun minyak atsiri umbi, lima diantaranya (senyawa dengan puncak 3, 4, 6, 7, dan 9) merupakan komponen pokok. Dari hasil analisis spektroskopik massa dinyatakan bahwa empat senyawa dengan puncak 3, 4, 6, dan 8 dari lima senyawa tersebut berturut-turut adalah: δ-phenylacetonitrile, α-terpinene, ethyl cinnamate dan shikimol δ-kaempferolide dan, sedangkan senyawa 7 dibagai isookadicetone keton, sementara 4-benzyl methyl. Senyawa 1 dan 2 diduga derivat β-phenylacetonitrile sedangkan asetil senyawa 5 perlu dieksplorasi lebih lanjut dengan metoda NMR spektroskopik.

Kata kunci: K-G-SM, Kaempferia galanga, δ-phenylacetonitrile, α-terpinene, ethyl cinnamate, dihydro-β-sesquinaptholide.

INTRODUCTION
In the course of finding anticancer agents, some Yogyakarta local rhizomes known as kurut path group had been investigated. Several species are grouped into the kurut path, such as: Curcuma zedoaria, C. mangga, Kaempferia rotunda and K. galanga. The comparison of the cytotoxic activity on the cancer lines, the secondary metabolites contents, protein contents and volatile oil contents of those species have been carried out by the group of kurut path, in EUC-biotechnology-GMU, Yogyakarta. The analysis of the volatile oil contents using Gas Chromatographic-Mass Spectroscopic method (GC-MS) of K. galanga will be described in this paper.

METHODS
Material. K. galanga rhizomes were collected from Yogyakarta market.
Instrument. Gas Chromatographic-Mass Spectrometer (GC-MS) QP 5000 (Shimadzu).
Method. The isolation of volatile oil. Pre-washed rhizomes of K. galanga were chopped and steam-distilled. The volatile oil obtained was collected. This volatile oil was run on the GC-MS to analyze its main

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RESULTS AND DISCUSSION

The gas chromatogram of the volatile oil isolated from *Karsospiza galanga* showed 8 peaks of retention times as shown in figure 1 and table 1. These peaks showed that there were 8 compounds (compounds 1 to 8), contained in the volatile oil, in which compounds 3, 4, 6, 7, and 8 were the main components, while the rest (compounds 1, 2, and 5) were the small components.

Figure 1: The gas chromatogram of volatile oil isolated from *Karsospiza galanga* rhizomes.

Table I. The gas chromatogram-peak, retention times, molecular ions and fragment ions of 8 compounds contained in the volatile oil of *Karsospiza galanga*.

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Retention Time (sec)</th>
<th>Mass (%)</th>
<th>Molecular Ion (m/z)</th>
<th>Fragment ions (m/z)</th>
<th>Substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.505</td>
<td>2.09</td>
<td>136</td>
<td>121, 107, 93 (bp), 79, 67, 53, 41.</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>12.945</td>
<td>2.91</td>
<td>139</td>
<td>136, 121, 110, 95 (bp), 67, 53, 41.</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>17.431</td>
<td>21.76</td>
<td>176</td>
<td>158, 148, 131 (bp), 103, 91, 77, 51.</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>18.355</td>
<td>6.27</td>
<td>212</td>
<td>184, 177, 113, 99, 85, 71, 57 (bp).</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>21.017</td>
<td>30.17</td>
<td>206</td>
<td>177, 161 (bp), 147, 134, 114, 103, 89, 77, 53, 51.</td>
<td></td>
</tr>
</tbody>
</table>

Compound 3, which had molecular ion m/z 136 and fragment ions as shown in Table 1, most probably was β-phellandrene (Figure 2). This molecular ion under an α-cleavage produced a fragment ion m/z 121, which...
then underwent a 1,2-H shift rearrangement resulting a tropolium-like ion (McLafferty, 1980; Silverstein et al., 1991; Williams and Fleming, 1995; De-Hoffmann et al., 1996).

Figure 2A: Mass-spectrum of compound 3 containing in K. glutona volatile oil. B: The fragmentation pattern of compound 3.

By releasing a neutral molecule of ethylene from the tropolium-like ion resulted a fragment ion of cyclopentene carbocation (m/z 93) which was the base peak. This ket ion then again could release neutral molecules: methane or methylecyclopropane. By releasing the methane the cyclopentene carbocation would produce fragment ion of cyclopentadiene carbocation (m/z 77), while the releasing methylecyclopropane would produce fragment ion of cyclopropane carbocation (m/z 41).

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Figure 3. A. Mass-spectrogram of compound 4 contained in K. palung volatile oil. B. The fragmentation pattern of compound 4.
The trypsofilm-like ion could also release either a methyl or a methylene carbene to give a fragment ion of m/z 107. It is known that trypsofilm easily breaks up and releases acetylactone (De Hoffmann et al., 1996). This case occurred to the trypsofilm-like ion by releasing acetylactone carbene or producing cyclopentadienone carbonyl with m/z 106. Based on the pattern of fragmentation it was confirmed that the molecular ion (m/z 156) was β, γ-phenylthio (Figure 2). Similar fragmentation pattern of β, γ-phenylthio was indicated in the mass-spectrum of Cremors using volatile oil (Sobhio, 2001).

Compound 4, which had molecular ion m/z 734 and fragment ions as shown in Table 1, was assigned a α-satrapinol (Figure 3). The pattern of the fragmentation was found mostly to follow the Kashi Mandubtun Kyla (KMR) (McLaury, 1962; Williams and Findlay, 1995) α-Cleavage of the molecular ion (m/z 154) resulted in an odd fragment ion of m/z 129. This odd fragment ion, following the KMR, then released a methylene carbene to produce another even fragment ion of m/z 125.

**Figure 4A**

**Figure 4B**

B. The fragmentation pattern of compound 6.

The last fragment ion men undergo twice single bond breaking released a radical hydroxyl and resulted an odd fragment ion m/z 108. After breaking a radical methyl, this ion yielded even fragment ion m/z 95. Beside that, the even fragment ion of m/z 125 again following the KMR released a methylene carbene to produce another even fragment ion of m/z 111. This last even ion again undergoes twice KMR resulted after even fragments ions of subsequently m/z 97 and 71 by releasing methylene carbene and acetylactone respectively. The even fragment ion m/z 111 could also release a water molecule and yielded fragment ions m/z 93. The fragment ion m/z 97 lost a radical hydrogen and yielded a fragment ion of m/z 96 that in further released respectively methylene carbene and radical hydrogen, and resulted subsequently fragment ions m/z 82 (not observed) and 81.

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The fragment ions of m/z 59, 55 and 43 (the base peak) were respectively isopropenyl, isobutene, and isopropene carboxyls that could be derived from the breaking of the molecular ion. Base on the analysis of the fragmentation pattern, it was concluded that the molecular ion m/z 154 was α-terpinene (Figure 3).

Compound 6, which had molecular ion m/z 176 and fragment ions as shown in Table 1, was assigned as ethyl cinnamate (Figure 4). Releasing a radical ethoxy group via α-cleavage from the molecular ion produced fragment ion m/z 131 which was the base peak. The fragment ion then released a neutral molecule of carbon monoxide to produce a fragment ion m/z 103. Twice losing of acetyl esters from the ion yielded fragment ions at m/z 77 and 51 respectively. Fragment ions m/z 158 and 148 were formed directly from the molecular ion by releasing neutral molecules of water and carbon monoxide. It was concluded then that the molecular ion of m/z 176 was ethyl cinnamate.

Compound 7, which had molecular ion m/z 212 and fragment ions as shown in Table 1, was not easy to be assigned. However the end of the fragmentation releasing mostly neutral molecules (seven times) with molecular weight of 14 that most probably was a methylene group. This indicated that the molecular ion was an

Unknown Spectrum
Data: Knauer B. DB 5
Mass Peak : 29, Ret. Time: 18.142
Scan B : 1698 B. O. Scan B : 2624
Base Peak : 72.10 [232569]

![Mass-spectrum of compound 7 contained in K' gurage volatile oil.](image)

The fragmentation pattern of compound 7.

Figure 5: A. Mass-spectrum of compound 7 contained in K' gurage volatile oil. B. The fragmentation pattern of compound 7.

After the first fragmentation, the molecular ion releasing a molecule with molecular weight of 28 and yielded a fragment ion which then fragmented and released a molecular weight of 43.

The first molecule released by the molecular ion could be a carbon monoxide, while the second molecule released was probably a propane. Base on these assumptions, the molecular ion might be an isoalkane ketone that most probably was isodecylcinnamyl ketone, or its cyclic derivative of 4-butylnaphthalene (Figure 5). To decide which one was true between these two, NMR-spectrometric analysis should be carried out to the pure substance.

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The molecular ion of compound 8 was m/z 206 that was 2 larger than that of β-sesquiphellandrene. Some of its fragment ions (Table 1) were also 2 larger than those of β-sesquiphellandrene (Suibhyo, 2000). By analyzing the fragmentation pattern and comparing it to that of β-sesquiphellandrene, the compound 8 was assigned as dihydro-β-sesquiphellandrene (Figure 6).

**Figure 6:** A. Mass-spectrogram of compound 8 contained in K. galanga volatile oil.
B. The fragmentation pattern of compound 8.

Again the KMR occurred in the fragmentation process of compound 8. The even fragment ions of m/z 191 following the KMR fragmented into another even fragment ion m/z 177 by releasing a neutral molecule methylene methane. The even ion resulted again following the rule released a methane and produced another even ion m/z 161 that was the base peak. This later ion once again followed the rule to produce even ion m/z 153.
The content of volatile oil

gave releasing acrylene. The last even ion then fragmented into ion m/z 134 and 118 after releasing a radial hydrogen and methane group respectively. Based on the fragmentation pattern, compound 6 was concluded as dihydro-β-sesquiphellandrene (Figure 6). The small compounds 1 and 2 had identical molecular ion and similar fragmentation-ion pattern to that of compound 3 (β-phellandrene, m/z 136), (Table 1). These two compounds could be the isomers of C10-

The small compounds 1 and 2 had identical molecular ion and similar fragmentation-ion pattern to that of compound 3 (β-phellandrene, m/z 136), (Table 1). These two compounds could be the isomers of C10-

phenanthrene; and their fragmentation patterns were proposed as shown in figure 7. Compound 5, that had molecular ion m/z 131, was difficult to be assigned from its mass-spectrum. Other analytical method like NMR-spectroscopy is necessary to carry out to structurally elucidate this compound.

CONCLUSION

There were five main compounds contained in volatile oil isolated from K. galanga, four of those were β-phellandrene-terpinol, ethyl cineol, dihydro-β-sesquiphellandrene respectively, whilst the other one was probably isosedoxyanethole or 4-butylsthongol.

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