GAS CHROMATOGRAPHIC-MASS SPECTROMETRIC
ANALYSIS OF THE MAIN COMPONENT OF VOLATILE OIL
ISOLATED FROM CURCUMA ZEDOARIA ROSC.

ANALISIS KROMATOGRAFI GAS-SPEKTROMETRI MASA DARI KOMPONEN UTAMA
MINYAK MENGGAP YANG ZEDOARI DARI CURCUMA ZEDOARIA ROSC.

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ABSTRACT
Steam distillation of Curcuma zedoaria Rosc. rhizome (one of the kunir putih) produced a colorless volatile oil. Gas chromatographic analysis of the oil showed 13 different components peaks. The 12th peak, the highest on intensity, had retention time of 22.262 seconds with the height ratio of 8.216. Mass spectrometric analysis of this peak resulted in a molecular ion of m/z 213. The fragment ions of the molecular ion were m/z 194, 167, and a base peak of m/z 105. This molecular ion is the dehydro derivative of ar-turmerone.

Key-words: C. zedoaria, kunir putih, volatile oil, ar-turmerone derivative.

ABSTRAK
Distilasi uap rizom Curcuma zedoaria Rosc. (dulang kunir putih) menghasilkan minyak menguap yang tidak berwarna. Analisis kromatografik gas terhadap minyak ini menunjukkan 13 puncak wujud retensi yang berkembang. Puncak ke-12 merupakan retensi tertinggi, dengan waktu retensi 22.262 detik serta rasio area/picak sebesar 8.216. Analisis spektrometri massa terhadap puncak ke-12 ini menunjukkan ion mollekul m/z 213. Ion fragment dari ion mollekul tersebut adalah m/z 194, 167, dan puncak dasar m/z 105. Ion mollekul tersebut adalah turunan dehydro dari ar-turmeron.

Kata-kunci: C. zedoaria, kunir putih, minyak menguap, as-turmeron.

INTRODUCTION
Curcuma zedoaria Rosc. has been clinically used for traditional treatment of cervical cancer (Wan et al., 1998). In Yogyakarta and its vicinity this rhizome is one of those that are called kunir putih. Kunir putih has been used for anti-tumor in this area.
Mitogenic activity was shown by the protein fraction of *C. zedoaria* on the both on human peripheral blood lymphocytes and on mouse cells (Takahata and Kawanishi, 1992). The essential oil of *C. zedoaria* has been found to exhibit antimicrobial activity against *Staphylococcus aureus*, *Vibrio comma*, and *Escherichia coli* (Rao and Nigam, 1970). The water extract of *C. zedoaria* demonstrated antimutagenic activity against benzo[a]pyrene-induced mutations in the microbial system of *Salmonella*. (Lee and Lin, 1988).

Carcinogenics, which were extracted in diethyl acetate from *C. zedoaria* and consisted of curcumin, demethoxy-curcumin, and bisdemethoxy-curcumin (Figure 1), were found to have a cytotoxic effect against OVCAR-1 (human ovarian cancer cells) (Wan et al., 1998).
additional compounds to the curcuminoids were isolated from C. zedoaria. These compounds were lipophilic and having chemical structures of 3,7-dimethyl-4,5,6-trisubstituted-2H-pyrone-4-carboxylic acid, curcuminoid, and galatoisol (Figure 2) (Wan et al., 1998).

(+)-ar-Turmerone (Figure 3), which was isolated from the root of C. longa, C. zedoaria, and C. zedoaria, was found to have cytotoxic activity on various cancer cell lines (Ali et al., 1997; Mudra, et al., 1989). It was reported that the α,β-unsaturated carbonyl moiety of ar-turmerone is responsible for the antitumor activity (Ali et al., 1997). Other anti-nitriteonone derivatives, such as turmerone, curcumen, turmeranoid A and B, have been isolated from the rhizome of C. longa (Keltkar and Jon, op cit Majum, 1999).

![Chemical Structures](image)

Figure 3. The chemical structures of Turmerone, ar-Turmerone, and Dehydro-ar-Turmerone

This research is to GC-MS analyze the main content of volatile oil isolated from C. zedoaria.

**MATERIALS AND METHODS**

Materials: C. zedoaria rhizome was bought from Yogakarta market on August 1998 and then identified by Lab. of Pharmaceutical Biology, Fac. of Pharmacy GUM.

Instrument: Gas chromatographic-mass spectrometer (GC-MS) QP 5000 (Shimadzu). The operational condition of the GC-MS for the volatile oil analysis was as follows: The GC-column was 30 meter of DB-5 using temperatures of 40°C for 5 minutes and 280°C for 10 minutes, and 19 Kpa Helium as the carrier. The temperatures of the injector and the detector were 280°C, while the ionizing chamber of the MS was using Electron Impact (EI) of 70 eV.

Method: The pre-washed rhizomes were chopped and steam-distilled, and the colorless volatile oil produced was collected. This volatile oil was run on the GC-MS to analyze its main components.

**RESULTS AND DISCUSSION**

The steam distillation of C. zedoaria rhizome resulted colorless volatile oil. The gas chromatographic analysis of this volatile oil gave 13 peaks of retention time (Figure 4) with the area-height ratio as shown in Table 1.

![Table 1](image)
In the gas chromatographic spectrum, the volatile oil sample showed 13 peaks of retention time, in which the twelfth peak has the highest intensity (Figure 4). The retention time of the 12th peak is 22.262 seconds with area relative to height (A/H) 8.316 (Table 1). Therefore, the 12th peak represents a major component within the sample.

Mass spectrometric analysis of the 12th peak compound reported a molecular ion at m/z 212, and fragment ions at m/z 194, 167, 105, 91, and 77 (Figure 5). The ion at m/z 105 is the base peak. The mass spectrometric fragmentation analysis of the molecular ion of m/z 212 is shown in Figure 6.

It was known that the components of the Curcuma species such as C. longa and C. aromatica contain cytotoxic compounds, i.e., a-quatercumene and turmerone (Alas et al., 1997). The a-quatercumene is a sesquiterpene derivative having α-l Shamanarat carbonyl moiety and aromatic ring system, and has a molecular weight of 216. Other compound, turmerone, is a diterpenoid derivative of the a-quatercumene and shows a molecular weight of 118 (Majid et al., 1995; Alas et al., 1997).

Table 1: The gas chromatographic-peak retention time and area-height ratio (A/H) of volatile oil components isolated from C. aromatica.

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Retention Time (seconds)</th>
<th>Peak Area (A)</th>
<th>Peak Height (B)</th>
<th>A/H Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.736</td>
<td>17947595</td>
<td>19497738</td>
<td>4.100</td>
</tr>
<tr>
<td>2</td>
<td>9.543</td>
<td>27659796</td>
<td>8051904</td>
<td>2.413</td>
</tr>
<tr>
<td>3</td>
<td>9.848</td>
<td>12303651</td>
<td>26514288</td>
<td>3.674</td>
</tr>
<tr>
<td>4</td>
<td>10.881</td>
<td>16459501</td>
<td>4446345</td>
<td>3.702</td>
</tr>
<tr>
<td>5</td>
<td>11.518</td>
<td>79004891</td>
<td>25882115</td>
<td>3.057</td>
</tr>
<tr>
<td>6</td>
<td>11.582</td>
<td>55156873</td>
<td>17852163</td>
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</tr>
<tr>
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<td>93827076</td>
<td>28337543</td>
<td>3.240</td>
</tr>
<tr>
<td>8</td>
<td>12.528</td>
<td>11170539</td>
<td>36513464</td>
<td>3.079</td>
</tr>
<tr>
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<td>77960378</td>
<td>26202057</td>
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</tr>
<tr>
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<td>40980768</td>
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<td>22.539</td>
<td>98234156</td>
<td>14251554</td>
<td>0.959</td>
</tr>
</tbody>
</table>
Figure 5: The mass spectrogram of the GC-12th peak compound. This GC-compound was having the highest area/height ratio in the gas chromatogram.

Figure 6: The mass spectrometric fragmentation analysis of the 12th peak compound.

The 12th peak which has molecular ion of 212, seems to be a sesquiterpene derivative. Loss of a neutral molecule, most probable water, from this molecular ion gave fragment ion at m/z 194. Retaining of neutral radical from the fragment ion (m/z 197) resulted a fragment ion of m/z 167, which was then losing a neutral fragment to give fragment ion at m/z 105. The fragment ion at m/z 105, forms a base peak in this mass spectrum. It is assumed that the base peak was due to formation of tropolium ion, a common characteristic for bezilium derivative (Figure 6)(Silverstein et al., 1991). It was concluded therefore that the main content of the C. zedoaria...
was the dehydro-derivative of ar-tumerone as shown in figure 1. This derivative compound did not come from ar-tumerone which might release its free hydrogen due to the high temperature (207°C) in the GC-fragment. This fact was confirmed by the GC-MS analysis result of ar-tumerone using the same temperature (unpublished data). Comparing to ar-tumerone this compound is much less stable due to its cyclohexene innercore which means that this compound is much more reactive than ar-tumerone. The compound still has the 4-hydroxylated caryophyllene moiety which is responsible for the antimicrobial activity. Based on these reasons, it can be assumed that the dehydro-derivative ar-tumerone may have a stronger antimicrobial active than ar-tumerone.

CONCLUSION

It is concluded that the main constituent of the volatile oil of C. zizanioides is dehydro-derivative of ar-tumerone.

REFERENCES


