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 MENGUAP YANG DIISOLASI 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 this oil showed 13 different time-retention peaks. The 12th peak, the highest in intensity, had retention time of 22.262 seconds with area/height ratio of 8.316. Mass spectroscopic analysis of this peak resulted a molecular ion of *m/z* 212. The fragment ions of this molecular ion were *m/z* 194, 167, and a base peak of *m/z* 105. This molecular ion is the detetrahydro derivative of ar-turmerone.

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

ABSTRAK

Distilasi-uap rizom *Curcuma zedoaria* Rosc. (salah satu dari kunir putih) menghasilkan minyak menguap yang tidak berwarna. Analisis kromatografi gas terhadap minyak ini menunjukkan 13 puncak waktu retensi yang berbeda. Puncak ke-12 mempunyai intensitas tertinggi, dengan waktu retensi 22,262 detik serta rasio area/tinggi pucak sebesar 8,316. Analisis spektroskopi-massa terhadap puncak ke-12 ini memberikan ion molekul *m/z* 212. Ion fragmen dari ion molekul tersebut adalah *m/z* 194, 167, dan suatu puncak-dasar *m/z* 105. Ion molekul tersebut adalah turunan detetrahidro dari ar-turmeron.

Kata-kunci: *C. zedoaria*, kunir putih, minyak menguap, turunan ar-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 three that are so-called *kunir putih*. *Kunir putih* has been used for anti-tumor in this area.

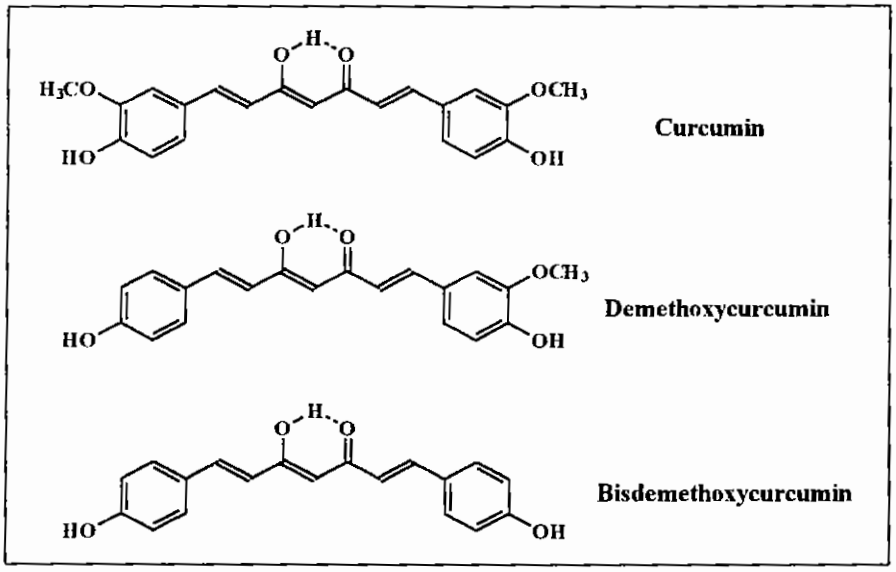

Figure 1.: The chemical structures of Curcumin, Demethoxycurcumin, and Bisdemethoxy-curcumin

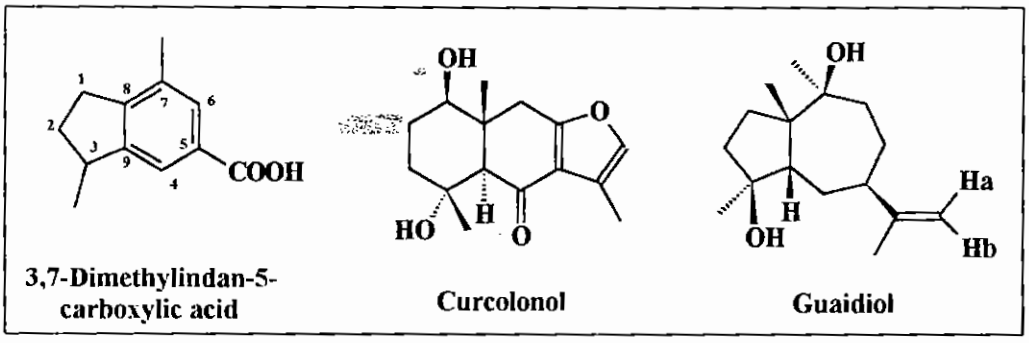

Figure 2. The chemical structures of 3,7 Dimethylindan-5-carboxylic acid, Curcolonol, and Guaidiol.

Mitogenic activity was shown by the protein fraction of *C. zedoaria* on the both on human peripheral blood lymphocytes and on mouse cells (Tachibana 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[α]pyrene-induced mutations in the microsomal system of *Salmonella* (Lee and Lin, 1988).

Curcuminoids, which were extracted in ethyl-acetate from *C. zedoaria* and consisted of curcumin, demethoxycurcumin and bisdemethoxycurcumin (Figure 1), were found to have a cytotoxic effect against OVCAR-3 (human ovarian cancer cells) (Wan *et al.*, 1998). Three

additional compounds to the curcuminoids were isolated from *C. zedoaria*. These compounds were nonbioactive and having chemical structures of 3,7-dimethylindan-5-carboxylic acid, curcolonol, and guaiadiol (Figure 2) (Wan *et al.*, 1998).

(+)-ar-Turmerone (Figure 3), which was isolated from the root of *C. longa, C. xanthorhiza,* and *C. zedoaria*, was found to have cytotoxic activity on various cancer cell lines (Ahn *et al.*, 1997; Mathes, *et al.*, 1980). It was reported that the α,β-unsaturated carbonyl moiety of ar-turmerone is responsible for the antitumor activity (Ahn *et al.*, 1997). Other ar-turmerone derivatives, such as turmerone, curlone, turmeronol A and B, have been isolated from the rhizome of *C. longa* (Kelkar and Rao, *op cit* Majeed, 1995).

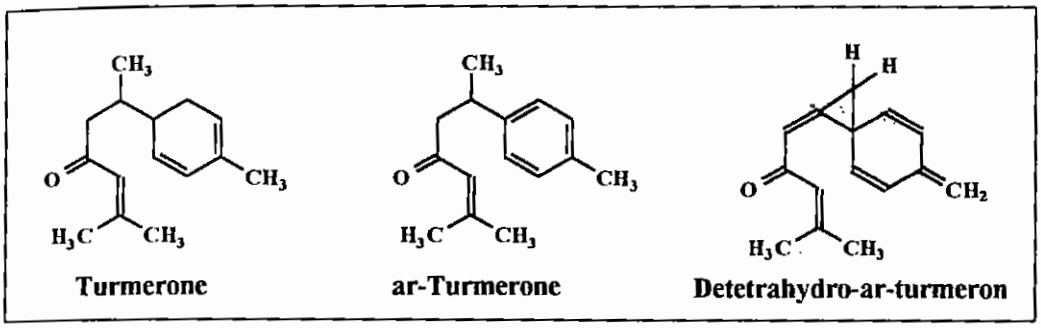

Figure 3. The chemical structures of Turmerone, ar-Turmerone, and Detetrahydro-Turmerone

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

MATERIALS AND METHODS

Materials: *C. zedoaria* rhizomes was bought from Yogyakarta market on August 1998 and then identified by Lab. of Pharmaceutical Biology, Fac. of Pharmacy GMU.

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 DBI using temperatures of 40°C for 5 minutes and 280°C for 10 minutes, and 10 Kpa Helium as the carrier. The temperature 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-distillated, 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.

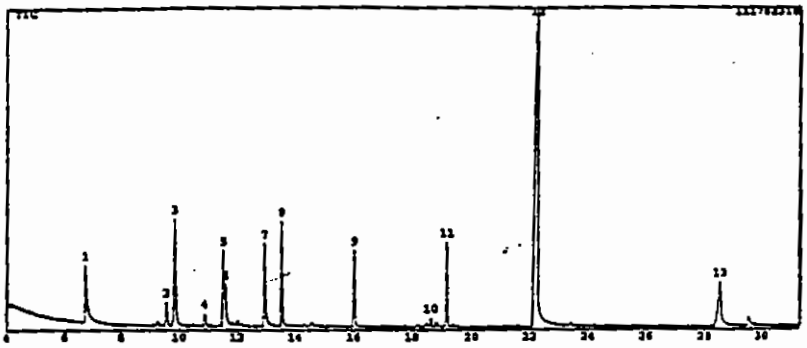

Figure 4. The gas chromatographic peaks of volatile oil components isolated from *C. zedoaria*.

In the gas chromatographic spectrogram, 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 spectroscopic analysis of the 12th peak-compound resulted 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 in figure 6.

It was known that rhizome/root of the Curcuma species (such as *C. longa* and *C. xanthoriza*) contains cytotoxic compounds, *i.e.*, ar-turmerone and turmerone (Ahn *et al.*, 1997). The ar-turmerone is a sesquiterpene derivative having α,β-unsaturated carbonyl moiety and aromatic ring system, and has a molecular weight of 216. Other compound, turmerone, is a dihydro derivative of the ar-turmerone and shows a molecular weight of 218 (Majeed *et al.*, 1995; Ahn *et al.*, 1997).

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

Peak No.	Retention Time **(seconds)**	Peak area (A)	Peak height **(H)**	A/H **Ratio**
1	6.736	19947359	19497768	4.100
2	9.563	27650706	8051904	3.434
3	9.848	133232651	36531408	3.647
4	10.881	16459601	4446345	3.702
5	11.518	78904891	25812015	3.057
6	11.595	55154871	13953013	3.953
7	12.931	91827676	28337543	3.240
8	13.528	111790538	36301864	3.079
9	16.021	77790538	26220357	2.967
10	18.635	10293325	3287351	3.131
11	19.157	79210841	29268040	2.706
12	22.262	913874739	109890868	8.316
13	28.539	98234156	14321554	6.859

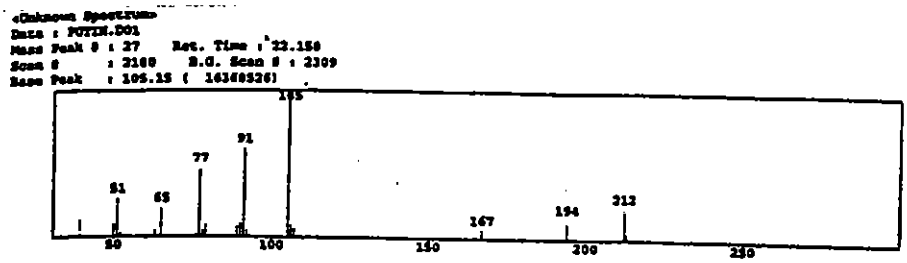

Figure 5.: The mass spectrogram of the GC-12th peak compound. This GC- compound was having the highest area/height ratio in the gas chromatogram.

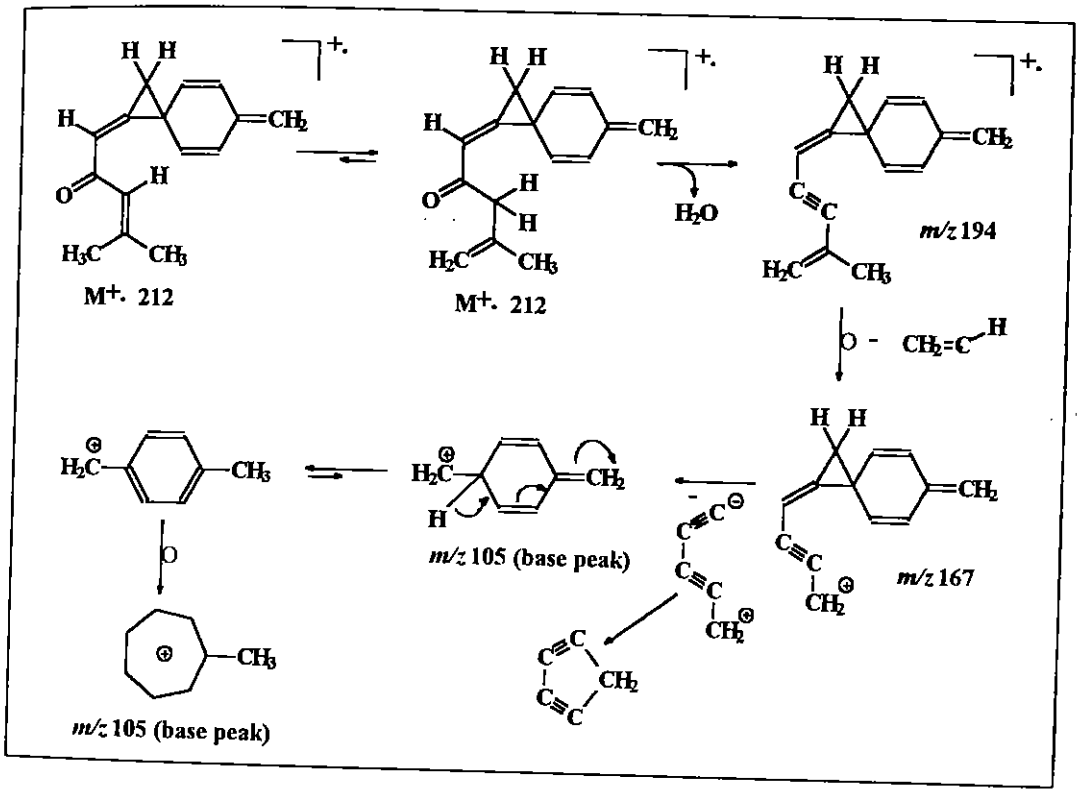

Figure 6.: The mass spectrometric fragmentation analysis of the 12th peak compound.

The 12th peak which has molecular ion of 212, seems to be a sesquiterpene derivative. Lost of a neutral molecule, most probable water, from this molecular ion gave fragment ion at *m/z* 194. Releasing of a 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 tropylium ion, a common characteristic for benzilium derivative (Figure 6)(Silverstein *et al.*, 1991). It was concluded therefore that the main content of the *C. zedoaria*

was the detetrahydro-derivative of ar-turmerone as shown in figure 3. This derivative compound did not come from ar-turmerone which might release its four hydrogens due to the high temperature (280°C) in the GC-column. This fact was confirmed by the GC-MS analysis result of ar-turmerone using the same temperature (unpublished data). Comparing to ar-turmerone this compound is much less stable due to its cyclopropylen structure; which means that this compound is much more reactive than ar-turmerone. The compound still has the α,β-unsaturated carbonyl moiety which is responsible to the antitumor activity. Based on these reasons, it can be assumed that the detetrahydro derivative ar-turmerone may have a stronger antitumor activity than ar-turmerone.

CONCLUSION

It is concluded that the main content of the volatile oil of *C. zedoaria* is detetrahydro-derivative of ar-turmerone.

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