# GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS OF ETHER EXTRACTS FROM TISSUE CULTURES OF CURCUMA ZEDOARIA

ANALISIS SPEKTROMETRI MASSA - KHROMATOGRAFI GAS SARI ETER DARI KULTUR JARINGAN *CURCUMA ZEDOARIA*

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**ABSTRAK**

*Untuk meneliti pengaruh derajat deferensiasi sel terhadap kandungan terpenoid pada tanaman* Curcuma zedoaria, *maka dilakukan analisis GC-MS pada ekstrak eter dari beberapa jenis kultur jaringan tanaman tersebut.*

*Hasil analisis menunjukkan bahwa derajat deferensiasi dari sel dapat mempengaruhi kandungan senyawa terpenoidnya. Kultur organ (kultur pucuk, akar dan planlet) dapat memproduksi senyawa terpenoid lebih banyak bila dibandingkan dengan kultur kalusnya.*

*Sebagian besar senyawa terpenoid yang berhasil teridentifikasi dari kultur in vitro tanaman ini berada dalam bentuk teroksigenasi.*

**ABSTRACT**

*In order to study the effect of cell defferentiation on its terpenoids content in Curcuma zedoaria plant, GC-MS analysis of the ether extracts from its tissue cultures were performed.*

*The results of the analysis showed that the degree of cell differentiation could influence their terpenoids content. The organ cultures (shoot, roots and plantlet cultures) could produce more terpenoids compared to their unorganized callus cultures.*

*Most of the identified terpenoids in these in vitro cultures were in oxygenated forms.*

**Key words**: *Curcuma zedoaria, tissue, callus and organ cultures, terpenoids, GC-MS analysis*

## INTRODUCTION

*Curcuma zedoaria* Roscoe (Zingiberaceae) is widely used as traditional medicine in Indonesia, China and Japan (Salim, 1985; Shiobara *et al.*, 1985). It can

be used as drugs of the digestive systems such as stomachics and cholagogues. Many mono and sesquiterpenoids have been isolated and identified from its rhizome (Hikino *et al.*, 1968; Matthes *et al.*, 1980 Shiobara *et al.*, 1985). Shiobara *et al.* (1985) reported that each parts of *Curcuma zedoaria* plant has a characteristical terpenoid constituents.

Yasuda *et al.* (1988) reported that ether extract of callus cultures of *Curcuma zedoaria* contained no cineole, however it was detected in the ether extract of its plantlet cultures and mother plants. The GC-profile of the ether extract of plantlet cultures was identical with the mother plants. Charlwood *et al.* (1988) described that in root cultures of *Zingiber officinale* (Zingiberaceae) α-pinene and champhene could be detected, but these compounds were not found in the shoot cultures.

The present paper describes the analysis of the ether extracts from several tissue cultures systems (callus, shoot, root and plantlet cultures) of *Curcuma zedoaria* using gas chromatography- mass spectrometry (GC-MS). The analysis is intended to study the effect of cell differentiation in the different tissue cultures systems on its terpenoid constituents.

## METODOLOGY

### Materials and Methods

***Tissue cultures of* Curcuma zedoaria** : The in vitro cultures were initiated from young shoots of *Curcuma zedoaria* Roscoe collected at Purwodadi Botanical Garden Lawang East Java Indonesia, using Murashige-Skoog's (MS) medium (Murashige and Skoog, 1962) modified with the addition of 2,4-D (2,4-dichlorophenoxyacetic acid) 1 $mgl^{-1}$, kinetin 0.1 $mgl^{-1}$ (callus cultures); BAP (benzylaminopurine) 5 $mgl^{-1}$, NAA (naphthaleneacetic acid) 0.1 $mgl^{-1}$ (shoot cultures); NAA 5 $mgl^{-1}$, BAP 0.1 $mgl^{-1}$ (root cultures), sucrose 30 $gl^{-1}$ and solidified with agar 7 $gl^{-1}$. For these experiments, the in vitro cultures were cultivated on the same medium as described above and incubated in continuous light (Phillips TL54/40W; ca. 700 lux) at $25 \pm 1^{o}C$ for 8 weeks (callus and shoot cultures); 18 weeks (root cultures). When the shoot cultures were incubated more than 8 weeks, it became plantlet cultures (in vitro cultures of plants with shoots, leaves and roots). The plantlet cultures were harvested after 16 weeks of incubation.

The growth rate of the tissue cultures was expressed as growth index (GI) defined as the ratio of the final fresh weight and initial fresh weight.

### Gas Chromatography Mass Spectrometry Analysis

***Sample preparation*** : To extract the terpenoids constituents, the sun air dried tissue cultures biomass (1.0 g) was extracted 3 times using a vortex- mixer (Thermolyne type 16700) with 10 ml ether of each according to Yasuda et al. (1988) and Charlwood et al. (1988). All ether extracts were combined and evaporated to dryness under $N_2$. The residue was dissolved in 100.0 μl of chloroform and 5.0 μl of

this solution was injected into the GC-MS (split ratio 1/50) system. All solvents used for extraction and dissolution were p.a. grade ( E. Merck, Germany).

***GC-MS Analysis of the extracts*** : The ether extracts were analyzed using a Jeol JMS DX 303 Gas Chromatograph Mass Spectrometer equipped with 50 m x 0.32 mm id. HP-1 (Hewlett Packard) fused capillary column (0.17 $\mu$m film thickness). Injector and separator temperatures were maintained at 300$^{o}$C. Oven temperature was programmed from 100 to 300$^{o}$C, 8$^{o}$C min$^{-1}$. The carrier gas was Helium at a flow rate of 1 ml min$^{-1}$; Mass spectra (EI) were taken at 70 eV, 300 $\mu$A with chamber temperature was 250$^{o}$C. The identification of the individual GC peaks of the total ion chromatograms were made by comparison searching in the GC-MS-library (Jeol JMA DA 5000, NBS/NIH/EPA/MSDC Library data), matching their MS data to the MS of the authentic standards (Sigma, USA) and MS data of references (Shiobara *et al.*, 1985; Setijadi, 1985).

## RESULTS AND DISCUSSIONS

Due to its complex terpenoids content in *Curcuma zedoaria* as reported by previous investigators (Shiobara *et al.*, 1985; Matthes *et al.*, 1980), a GC-MS method of analysis was selected in this work. Analysis of complex terpenoids content by GC-MS in plants or tissue cultures system were also reported by Charlwood *et al.* (1988), Setijadi (1988) and Cardenas (1993).

TIC (total ion chromatogram) in Figure 1 showed that callus cultures of *Curcuma zedoaria* did not contain monoterpene, but an unknown peak (S1) with MW (mole weight) 236 was detected. The absence of monoterpene in callus cultures of *Curcuma zedoaria* was also reported by Yasuda et al. (1988).. Sakui *et al.* (1992) had found that callus cultures of *Curcuma zedoaria* could do some biotransformation-reactions of a 10-membered sesquiterpene (germacrone) which was given as substrate into guaiane-type sesquiterpenoids, however they did not report the endogenous (sesqui)terpenoids content of their callus cultures.

Oxygenated monoterpenoids (camphor and borneol), sesquiterpenoids (elemene and *trans* caryophyllene) and oxygenated sesquiterpene (germacrone) were detected in ether extracts of the shoot cultures (see Figure 2). In addition 3 unknown peaks (MW 218-220) were also detected in this extract.

However in leaves of the plantlet cultures, some oxygenated sesquiterpenoids (germacrone, curcumenone, and furanodienone) and several unknown constituents (MW 218-220) were detected (Figure 3).

In the basal-stems of the plantlet cultures, germacrone and 2-*cis*-6-*trans* farnesal (oxygenated sesquiterpenoids) could be identified, an unknown peak (M) with MW 136 and some unidentified constituents (MW 204, 218 and 220) were also detected (Figure 4).

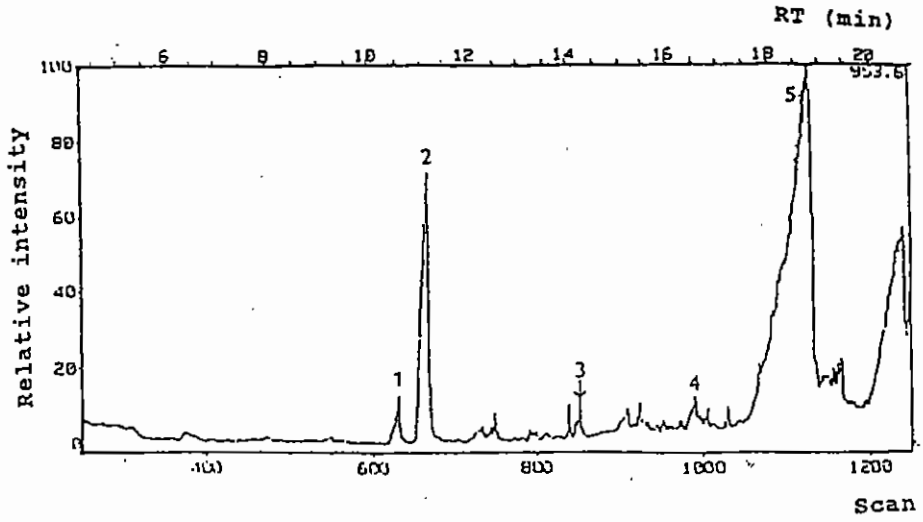


Figure 1. TIC of extract from callus culture of *C.zedoaria.* Unknown(S1) = 1 MW 236 : 2 = DTBPC; 3 = myristic acid; 4 = lauric acid; 5 = palmitic acid

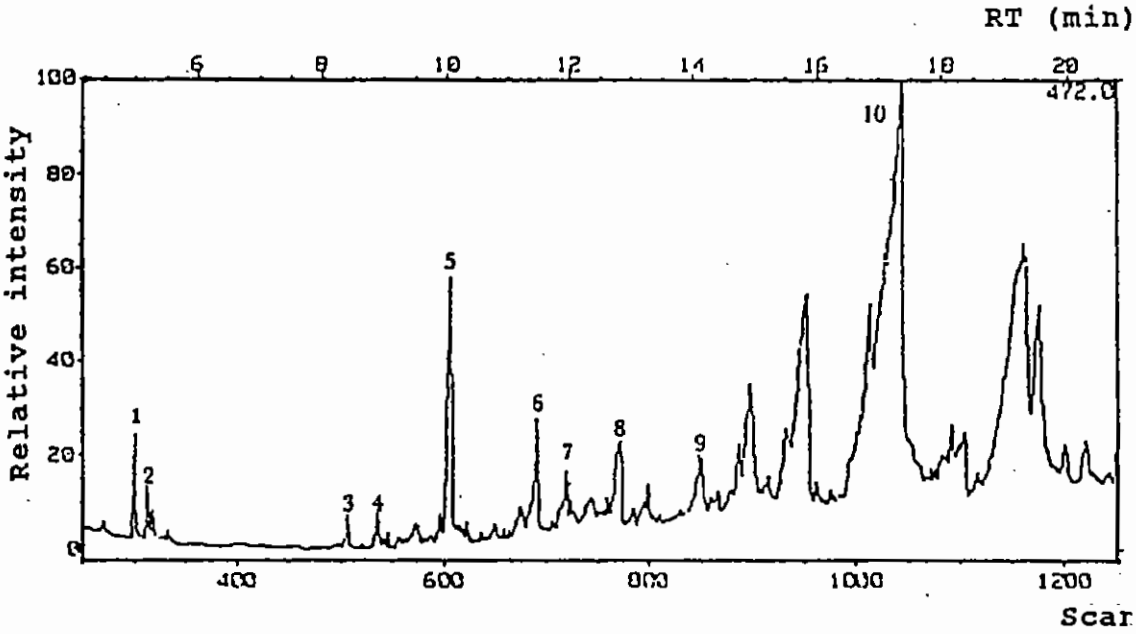


Figure 2. TIC of ether extract from shoot culture of *C. zedoaria.*
1 = camphore; 2 = borneol; 3 = elemen; 4 trans caryophyllen; 5 = DTBPC; 6 = gemacrone; 7 = unknown (S2), MW = 220; 8 = Unknown (S3), MW = 218; 9 = Unknown (S4), MW = 220; 10 = palmitic acid.

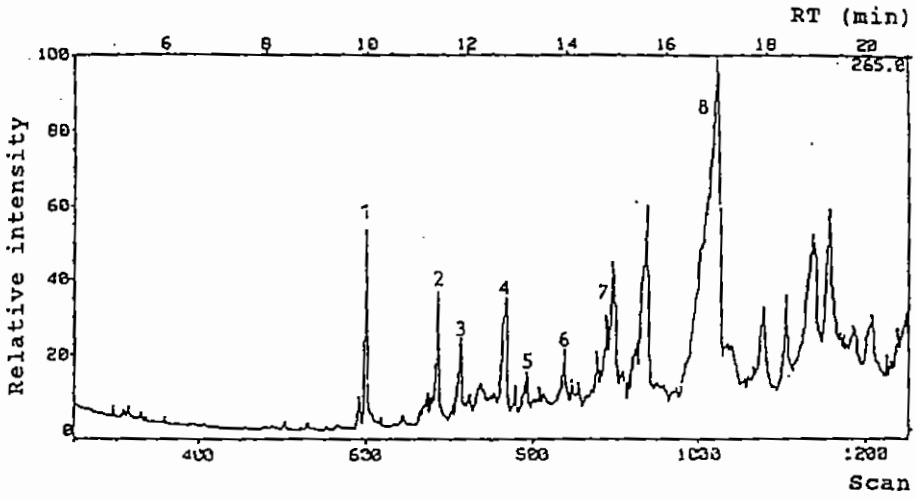


Figure 3. TIC of ether extract from leaves of plantlet cultures of *C.zedoaria*. 1 = DTBPC; 2 = germacron; 2 = unknown (S5) MW 220; 4 = unknown (S3) MW 218; 5 = unknown (S6) MW 220; 6 = furanodienone; 7 = curcumenon; 8 = palmitic acid.

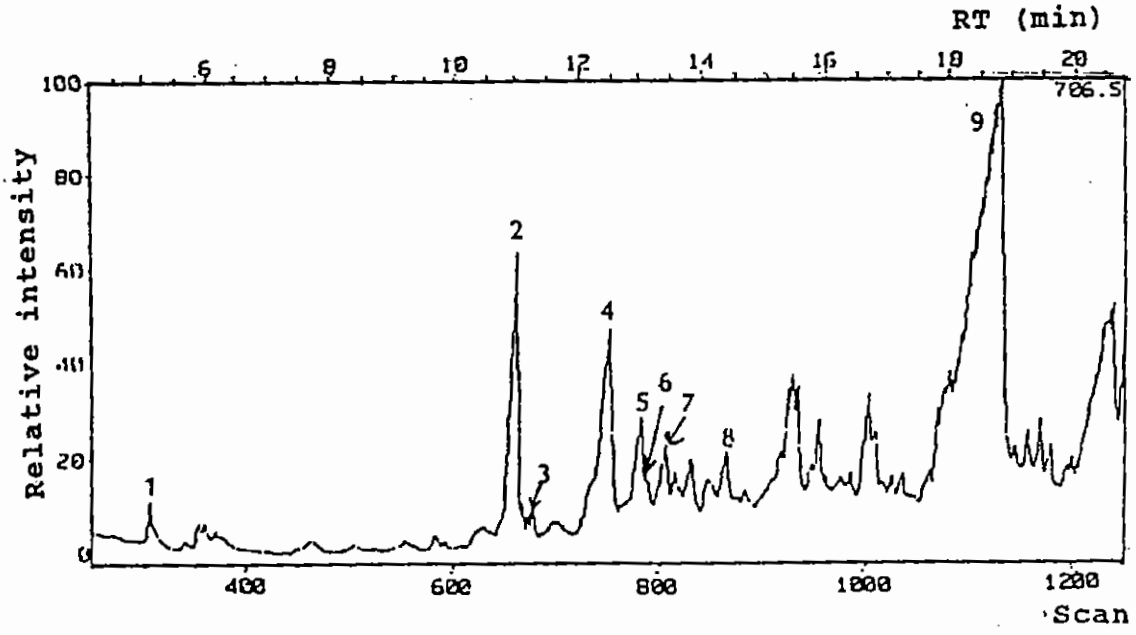


Figure 4.TIC of extract ether from basal-stemes of culture of *C. zeodaria*. 1 = unknown (M1) MW 136; 2 = DTBPC; 3 unknown (S7) MW 204; 4 = germa crone; 5 = unknown (S5) MW 220; 6 = unknown (S8) MW 218; 7 = unknown (S9) MW 220; 8 = 2-cis-6-trans farnesal ; 9 = palmitic acid.

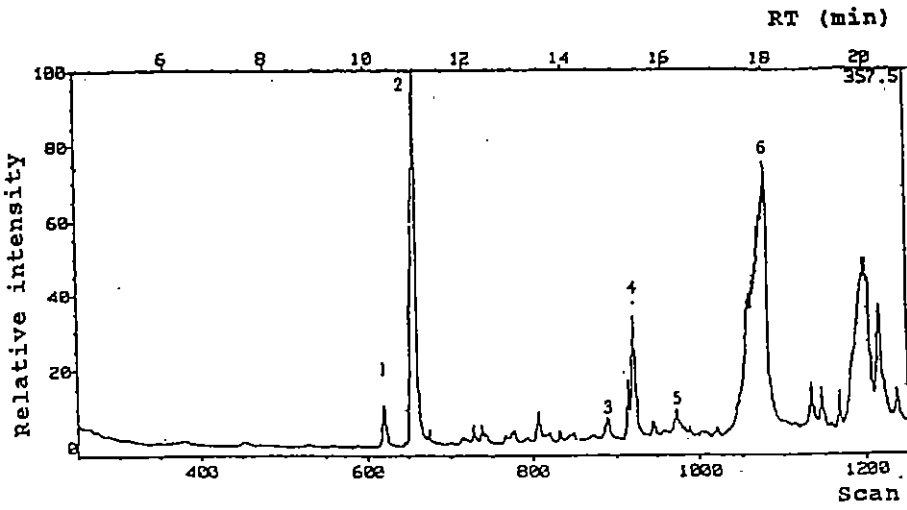


Figure 5. TIC of ether extract from root cultures of *C.zedoaria*, 1 = Unknown (S1), MW 236 ; 2 = DTBPC;3 = myristic acid; 4 = unknown (S10), MW 234; 5 = lauric acid; 6 = palmitic acid.

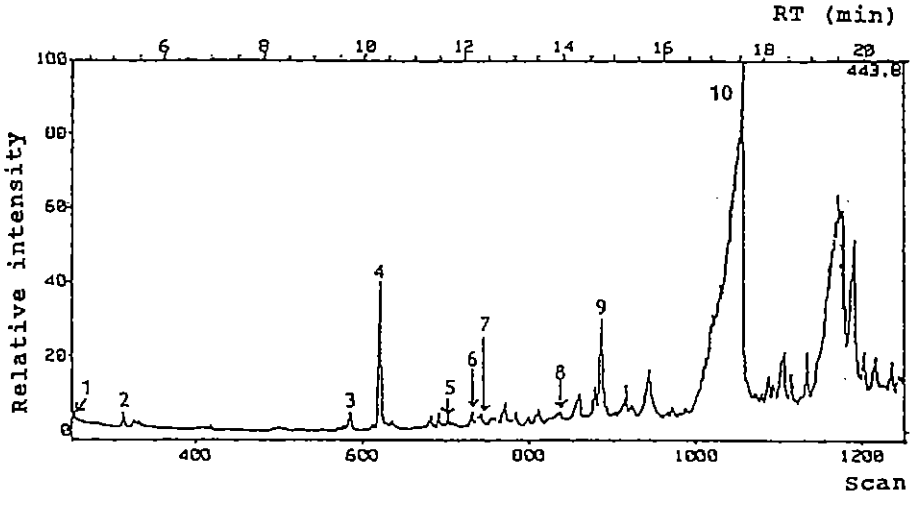


Figure 6. TIC of ether extract from roots of plantlet cultures of *C.zedoaria*. 1 = Cineole; 2 = camphor; 3 = unknown S1, MW 236; 4 = DTBPC; 5 = germacrone; 6 = unknown S2, MW 220; 7 = unknown S11, MW 218; 8 = un known S12, MW 234; 9 = unknown S13, MW 234; 10 = palmitic acid.

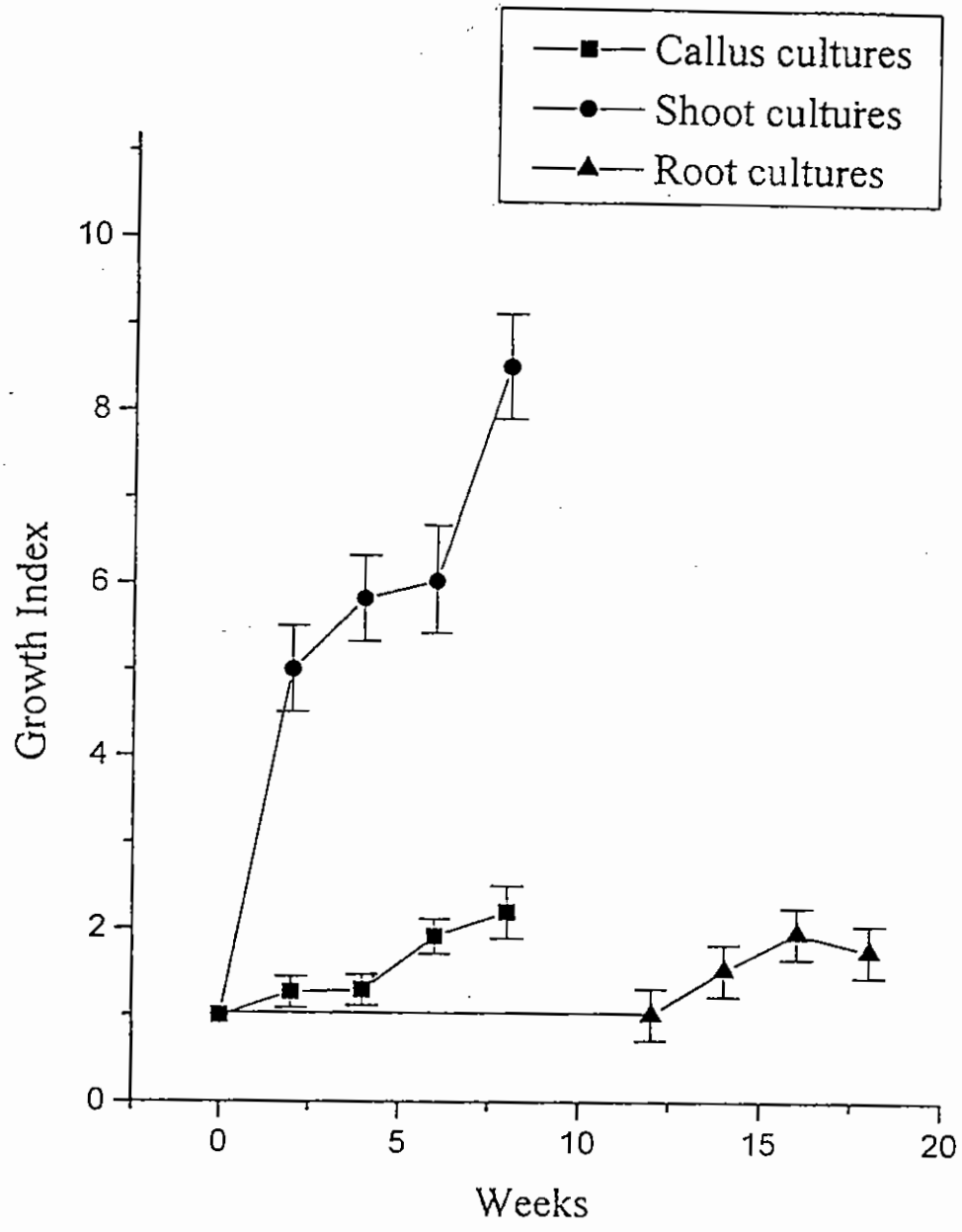


Figure 7. Growth rate of callus, shoot and root cultures of *Curcuma zedoaria*. Values represent mean ± SE.

In the root cultures of *Curcuma zedoaria*, mono and sesquiterpene were not detected but 2 unknown peaks (MW 234, 236) were detected (Figure 5). Cineole and camphor (oxygenated monoterpenoids), germacrone (oxygenated sesquiterpene) and several unidentified components were detected in the extract of roots from the plantlet cultures (Figure 6).

These results showed that the degree of cell differentiation of these tissue cultures could influence their terpenoids content. It suggests that monoterpenoids can not be produced by unorganized cells of *Curcuma zedoaria*. The organ cultures (shoot, root and plantlet cultures) of *Curcuma zedoaria* have the capacity for producing of more terpenoids. This results supported the statement of Shiobara (1988) which stated that each organs of the plant had a characteristical terpenoid contents. The capacity of organ cultures to produce more terpenoids compared to its callus cultures was also reported by Cardenas (1992). Most of the terpenoids which could be identified in these cultures of *Curcuma Zedoaria* were in the oxygenated forms.

Some fatty acids palmitic, lauric and myristic acid were also identified in the ether extracts of these tissue cultures due to their solubility in the ether. In addition, an anti oxidant 2,6- di-*tert*-butyl-p-cresol with MW 220 (DTBPC) was identified in all extracts, it might be come from the ether as a stabilizer.

Figure 7 showed that callus and roots cultures of *Curcuma zedoaria* grew relatively slower compared to its shoot cultures, thus the shoot cultures could produce more biomass. Therefore the shoot cultures of *Curcuma zedoaria* is recommended for studying factors which may influence the biosynthesis of terpenoids in *Curcuma zedoaria* plant cells. This work also showed that the shoot cultures could produce relatively more terpenoids.

From their MW, the EI-MS fragmentation and RT (retention time), the unknown peaks (S1-13, M) are deduced as sesquiterpenoid (S) or monoterpene (M). To elucidate their structure, other spectroscopy methods of analysis (UV, IR, CD, $^{1}$H-NMR and $^{13}$C-NMR) from the individuals substances are required.

## CONCLUSIONS

This work showed that the organ cultures of *Curcuma zedoaria* could produce relatively more terpenoids compared to the unorganized cells (callus cultures). Cell differentiation could induce the biosynthesis of terpenoids in this cell cultures.

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