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PHYTOCHEMICAL STUDY OF *JATROPHA CURCAS* CELL CULTURE

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ABSTRACT

Jatropha curcas belongs to the Euphorbiaceae family which has a high economic value as a source of biofuel.. This plant has been reported containing toxic compounds such as curcin and phorbol ester and its derivatives which may become a problem if *J. curcas* is processed to biofuel. In order to investigate the chemical constituents of this plant, a research on phytochemical and initiation of cell and organ culture has been carried out. *Jatropha curcas* collected from different regions in Indonesia showed containing relatively the same profile of chemical contents. Dominant compounds that were detected by GCMS are hydrocarbon such as 2-heptenal, decadienal, hexsadecane, pentadecane, cyclooctane etc, fatty acid such as octadecanoate acid, ethyl linoleate, ethyl stearate, hexadecanoate acid and steroid such as stigmasterol, fucosterol, sitosterol. No phorbol ester and its derivatives have been detected yet by the GCMS method. Callus and suspension cultures of *J. curcas* have been established using Murashige and Skoog medium supplemented with 4 % (w/w) sucrose, solidified with 0.9% agar and growth hormon NAA 2 mg/L : BAP 0,5 mg/L.

Key words: *Jatropha curcas*, stigmasterol, fucosterol, sitosterol, phorbol ester, fatty acid

INTRODUCTION

Jatropha curcas belongs to the family Euporbiaceae which has a multipurpose use such as medicinal plant and source of biofuel. As an alternative source of biofuel, *J. curcas* has a high economic value. The development of this plant as source of alternative energy has been supported by researches and government regulations. Since this plant is very potential, it has been cultivated in many regions in Indonesia. Several aspects have to be considered to develop this plant not only from the view point of production capacity but also the social condition of the population. Aside from a potential source of biofuel, *J. curcas* contains secondary metabolite compounds which have pharmacological and also toxicity effects. Several compounds isolated from *J. curcas* have been reported to be toxic, they are toxalbumin, cursin and phorbol ester. Toxic effects of this plant caused skin irritation, vomiting, diarhea, etc. These

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toxicities are closely related to its chemical contents. Several other secondary metabolites from *J. curcas* have been reported such as terpenoid, coumarine, flavonoid, etc. The level of these chemical contents of *J. curcas* cause the different toxicity levels of several strains of *J. curcas* (Gadir *et al.* 2003, Rug M & Ruppel 2000). High phorbol ester content has been proved to be responsible for the toxicity of *J. curcas*. This toxicity effect could not be eliminated using high temperature, since phorbol ester is very stable in high temperature. Chemical reactions can reduce phorbol ester content of *J. curcas* (Aregheore *et al.* 2003). These toxic compounds can be a problem in processing *J. curcas* to biofuel. Attempts to detoxificate toxic compounds of *J. curcas* have been done in order to reduce the toxic effects (Haas *et al.* 2000). In addition to physical and chemical detoxification, biotechnological approaches could be applied.

The toxic compounds may also be eliminated or reduced by regulation of their biosynthetic pathway. Several genes which are responsible for synthesis of toxic compounds have been cloned, furthermore the expression and properties of the enzyme have been studied (Qin *et al.* 2005). Identification of genes which are responsible for cursin production give the challenges to regulate their inhibition activities. Several tools to study the biosynthesis of toxic compounds of *J. curcas* are needed. Cell, tissue and organ culture are tools that can be used to regulate the production of secondary metabolites from plants. Furthermore, these tools can be used for study at genetic level. To understand the secondary metabolites in relation to its toxicity and side effects, a research concerning phytochemical study of *J. curcas* collected from wild types and cultivated regions have been done. In addition, cell, organ and supension cultures have been initiated. The aims of this study were to investigate the phytochemical contents of *J. curcas* and to establish the cell culture of *J. curcas* which can be used for further study.

MATERIALS AND METHOD

Plant materials were collected from different regions in Indonesia such as Padang, Lampung, Garut, Ciamis, Tasikmalaya, Bantul and Gunung Kidul. Plant organs ie. leaves, fruits and stems were separated All materials were dried at 50^0C and powdered.

Extraction, isolation and identification of secondary metabolites

To isolate secondary metabolites from *J. curcas*, each plant material from different organs and collection regions was extracted using soxhlet apparatus and solvent with increasing polarity such as n-hexane, ethyl acetate and methanol. Extracts were checked by Thin Layer Chromatography (TLC). Chromatogram profile was used as the first indication to choose the best system for further isolation. n-Hexane extract of leaves, stems and fruits were further fractionated using vacuum liquid chromatography using silica gel 60 H as adsorbents, the eluents were the mixtures of n-hexane and dichloromethane. The compositions of the eluents consisted of n-hexane 100 %, n-hexane dichloromethane (9:1) till 1:9, and dichloromethane 100%, yielded 12 fractions (fraction 1-12). The fractions were checked by TLC. Several

fractions contained the dominant compounds. Identification of isolated compounds and selected fractions were conducted by GCMS. Spectrums were analyzed and compared with available library.

Callus, cell, and suspension culture of *J.curcas*

The explants used in this experiment were leaves and seeds. The sterile leaves or seeds were cut into slices and callus induction was obtained using media with different compositions. These media were modifications of the Murashige and Skoog (Murashige & Skoog 1962) or the Gamborg's B5 medium (Gamborg *et al.* 1968). Medium contained the combination of macro and micro-nutrient, carbon sources and other nutritions as well as growth hormones is needed. All media are supplemented with 4 % (w/w) sucrose and solidified with 0.9% agar. The callus cultures were grown under an L/D regime (16/8 h: 3000 lux) at 26°C. The compositions of growth hormones were as follows : 1) NAA 2 mg/L : BAP 0.5 mg/L, 2) NAA 2.22 mg/L : BAP 0.5 mg/L, 3) NAA 2.5 mg/L : BAP 0.5 mg/L, 4) NAA 2.22 mg/L:BAP 0.4 mg/L. Cell suspension cultures were initiated by transferring callus clumps into 100 mL sterile conical flasks with 50 mL of liquid medium of the same composition as described above but without agar. Cultures were incubated on a rotary shaker (100 rpm) at 26°C under an L/D regime (16/8 h: 3,000 lux, daylight L 36W/10, OSRAM, Germany). After 1 month, 50 mL of cell suspension cultures were transferred into a 500 mL conical flask, with fresh medium yielding a total volume of 300 mL. Subcultures were prepared every 3 weeks by adding 100 mL of a full-grown cell suspension culture to 200 mL of fresh medium.

RESULTS AND DISCUSSION

Phytochemical screening showed that *J. curcas* contains alkaloid, flavonoid, tanin chatecate, saponin and steroid/triterpenoid (Table 1). Screening results were summarized in Table 1. Samples from other collection regions gave the same result as shown in the Table. Based on the screening results, the extraction process was used as previously mentioned in Materials and Method section. TLC analysis resulted that chromatogram profile of n-hexane, ethyl acetate and methanol extracts from *J. curcas* collected from different places showed relatively the same, respectively. From GCMS data of fraction and isolated compounds, several spectrums showed containing hydrocarbon, fatty acid and steroid compounds. However, phorbol ester and its derivatives reported having toxic effects, could not be detected. It was probably due to low content of phorbol ester. To confirm whether these compounds did not exist in *J. curcas* from selected regions, another GCMS measurement and or using different columns and conditions should be done. From these confirmations, no phorbol esters contents were detected. Based on GCMS, compound 1 has molecular weight of 412 with m/z: 83, 159, 255, and 300. These fragmentation patterns were similar to β-stigmasterol (Fig. 1). Compound 2 has molecular weight of 284 with m/z 73, 129 and 241 which were similar to octadecanoic acid (Fig. 2). Compound 3 has molecular weight of 152 with m/z 81 which were similar to 2,4 decadienal (Fig. 3). Dominant

Table. 1. Phytochemical screening of *Jatropha curcas* collected from Ciamis, Garut and Tasik

Crude sample	Alkaloid	Flavonoid	Tancin		Quinon	Saponin	Steroid/ terpenoid
			Chatecate	Gallat			
Leaves							
Ciamis	+	+	+	-	-	+	+
Garut	+	+	+	-	-	+	+
Tasik	+	+	+	-	-	+	+
Stem							
Ciamis	+	+	+	-	-	+	+
Garut	+	+	+	-	-	+	+
Tasik	+	+	+	-	-	+	+

Notes : + = contain secondary metabolites
- = did not contain secondary metabolites

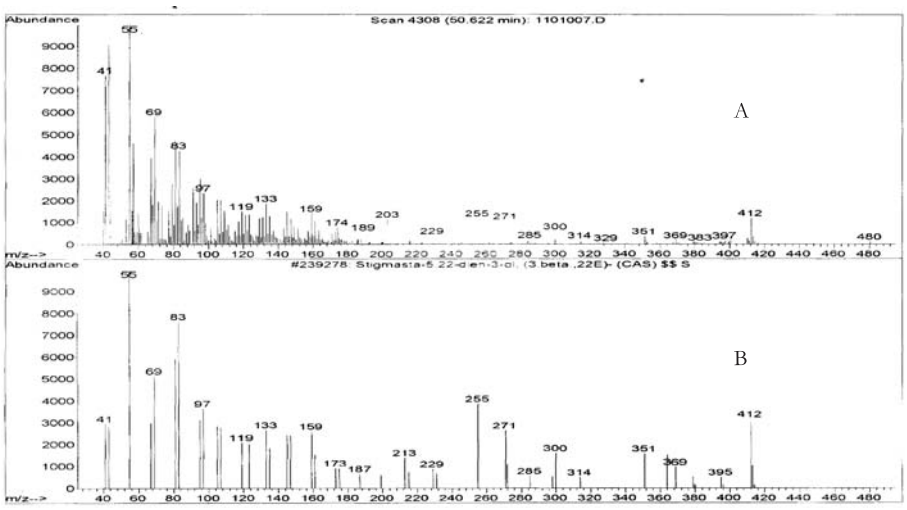

Figure 1. GCMS spectrum and fragmentation pattern of A) isolated compound of *Jatropha curcas* and B). B-stigmasterol

compounds detected by GCMS were hidrocarbon such as 2-heptenal, decadienal, hexsadecane, pentadecane, cyclooctane etc, fatty acid such as octadecanoate acid, ethyl linoleate, ethyl stearat, hexadecanoate acid etc and steroid such as stigmasterol, fucosterol, sitosterol.

Using leaf as the explant, callus can grow within 22 days, callus growth started with the folding of the green leaves, then callus slowly proliferated. Callus was soft, compact and yellow green. The optimum callus formation was found at 82 days after inoculation. At day 90, callus started to be brownies and then died for more than 100 days. Then callus were subcultured every month (Fig. 4). While using seeds as the explant, callus can grow in a week after inoculation. Callus was easily proliferated. Formed callus was soft, compact, pale green. The optimum callus formations of seeds were found at 63 days after inoculation (Fig. 5). Using similar medium without agarose, cell suspension culture were grown, yielded cell suspension culture of seed

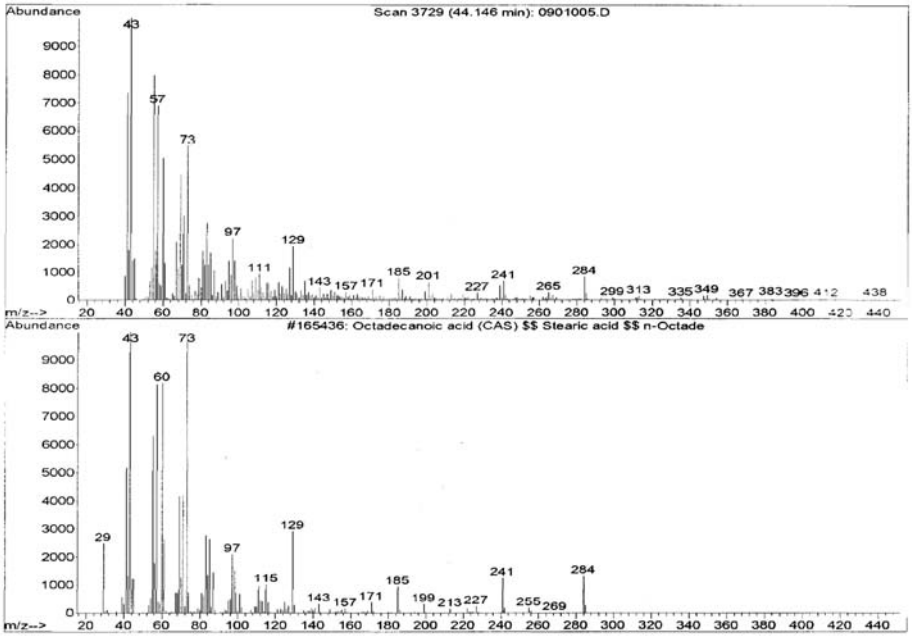

Figure 2. GCMS spectrum and fragmentation pattern of A) isolated compound of *Jatropha curcas* and B). octadecanoic acid

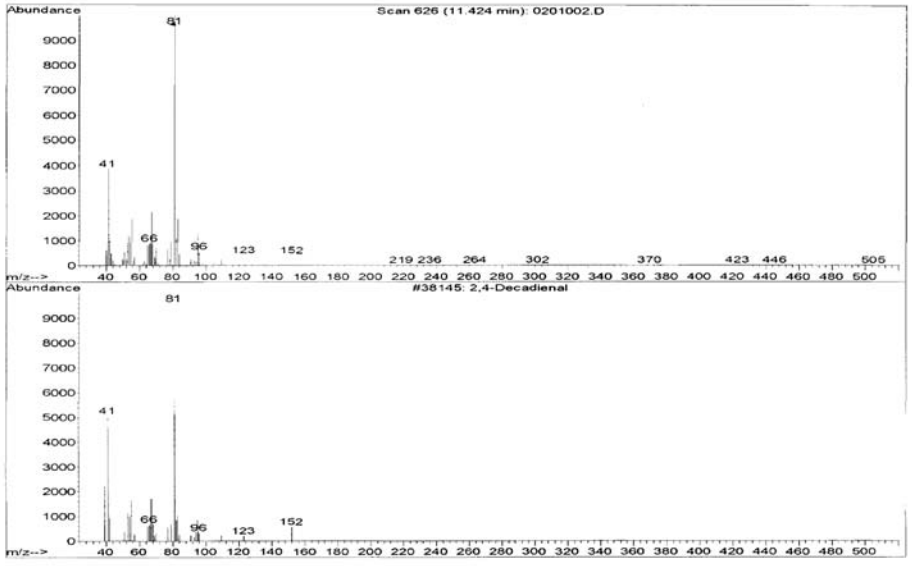

Figure 3. GCMS spectrum and fragmentation pattern of A) isolated compound of *Jatropha curcas* and B). 2,4-decadienal

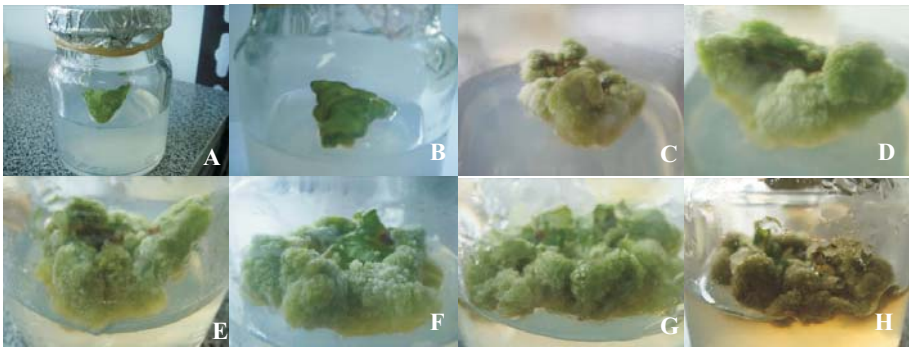

Figure 4. Callus culture of *J. curcas* leaf in growth phase (A) 22 days, (B) 29 days, (C) 45 days, (D) 52 days, (E) 59 days, (F) 65 days, (G) 82 days and (H) 90 days, incubation in MS medium added with NAA 2 mg/L : BAP 0.5 2mg/mL.

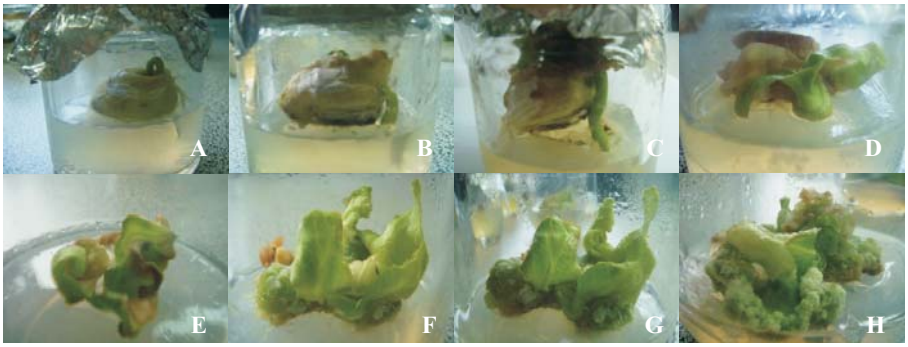

Figure 5. Callus culture of *J. curcas* seeds in growth phase (A) 7 days, (B) 14 days, (C) 17 days, (D) 21 days, (E) 28 days, (F) 35 days, (G) 42 days, (H) 63 days, incubation in MS medium added with NAA 2 mg/L : BAP 0.5 2mg/mL.

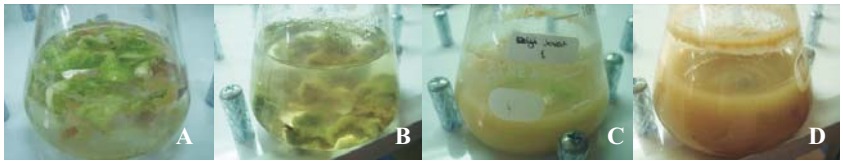

Figure 6. Cell suspension culture of *J. curcas* seeds in growth phase (A) 14 days, (B) 60 days, (C) 70 days, and (D) 80 days, , incubation in MS medium added with NAA 2 mg/L : BAP 0.5 2 mg/mL without agarose

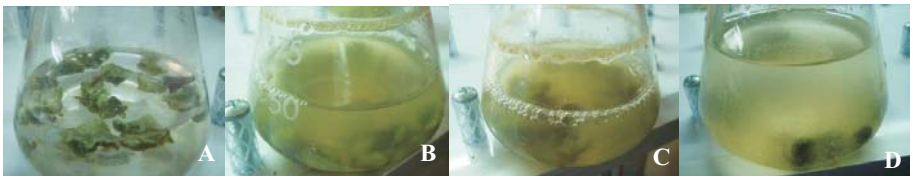

Figure 7. Cell suspension culture of *J. curcas* leaf in growth phase (A) 14 days, (B) 25 days, (C) 40 days, and (D) 60 days, , incubation in MS medium added with NAA 2 mg/L : BAP 0.5 2 mg/mL without agarose

and leaf. But the amounts of cell suspension culture were not enough for further experiment with elicitor. Scale up production of cell suspension culture is in progress (Fig. 6 and 7)

Long time is needed to optimize callus formation (approximately 2 months for the seeds and almost 3 months for the leaves). Therefore, the cell suspension cultures were not enough for the elicitor treatment. The secondary metabolites of original plant (seed, leaf) have different profiles compared to the callus and cell suspension. It could be concluded that the callus and cell suspension produced additional compounds. However, this research should be continued to determine the produced compounds of cell suspension and callus culture. Some bands of TLC chromatogram of original plants were not found in the callus and cell suspension culture.

CONCLUSIONS

Analysis of n-hexane, ethyl acetate and methanol of leaves, stems and fruits of *J. curcas* collected from different location in Indonesia (Sumatera Barat, Lampung, Jawa Barat dan Yogyakarta) showed a similar profile (qualitatively). Based on the GCMS analysis of fractions and isolates of *J. curcas* showed that the dominant compounds were hydrocarbon, fatty acid and steroid. GCMS data did not show any phorbol ester compound and its derivaties which has a toxic effect. The growth of callus and suspension cultures of leaves and seeds of *J. curcas* could be optimized in MS Medium added with NAA 2 mg/L : BAP 0,5 mg/L. Secondary metabolites content of callus and suspension culture showed different profiles. It can be concluded that the induction of callus and cell suspension can influence the production of secondary metabolites of plants.

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