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# *Sijil Penyertaan*

*Dengan sukacitanya sijil ini dianugerahkan*

**kepada**

**NOVIZA NAZIR**

*kerana telah menghadiri dengan jayanya*

**SEMINAR MINYAK DAN LEMAK KEBANGSAAN 2008  
(SMILE 2008)**

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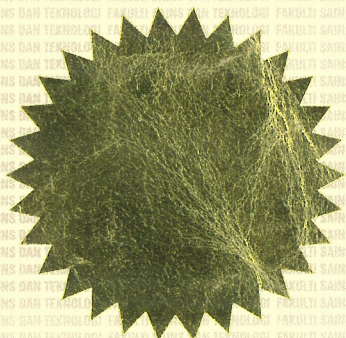
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# DETOXIFICATION OF *JATROPHA CURCAS*. L SEED OIL BY ACID-ACTIVATED BENTONITE

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## ABSTRACT

The seed oil of *Jatropha* contains several toxic or antinutritional compounds of phorbol esters, a family of compound known to cause a large number of biological effects such as tumor promotion and inflammation. Acid-activated Bentonite were used as adsorbent for detoxification of *Jatropha curcas* seed oil by adsorption method at atmospheric condition. Acid-activated Bentonite were prepared by aqueous impregnation technique. 5.3 M HCl and 40% by mass of H<sub>2</sub>SO<sub>4</sub> were supported on bentonite by aqueous impregnation, washing with deionized water till no Cl<sup>-1</sup> and SO<sub>4</sub><sup>-2</sup> ions were detected, drying overnight and calcinated at 500 °C for three hours. Six methods of bentonite-adsorption followed by NaOH neutralization for the detoxification step were compared: (A) adsorption with bentonite; (B) adsorption with HCl-activated bentonite; (C) adsorption with HCl-activated bentonite and calcinated at 500 °C (D) adsorption with H<sub>2</sub>SO<sub>4</sub>-activated bentonite; (E) adsorption with H<sub>2</sub>SO<sub>4</sub>-activated bentonite and calcinated at 500°C and (F). Without bentonite-adsorption. Color, free fatty acid (FFA), phorbol esters content, detoxification performance of detoxified *Jatropha* oil were determined.

**Keywords:** *Jatropha curcas* oil, phorbol esters, adsorption, detoxification performance, acid-activated bentonite

## INTRODUCTION

There are several oil seed species could be utilized as source for oil production. Among these, *J. curcas* which grows in tropical and sub-tropical climates accross developing world is a multipurpose species with many attributes and potentials (Openshaw 2000 and Tapanes *et al* 2008). Nutritional utilizations, however, the use of the seed oil for cooking purposes are not possible due to the content of toxic compounds. This property of *J. curcas* is the subject of many publications (Gubitza *et al.*, 1998). The observations indicates that acceptance of *Jatropha* seeds as food or feed is affected by the content of phorbol esters. The higher the phorbol esters, the lower the acceptance of *Jatropha* seeds (Makkar *et al.* 1998). The term 'phorbol esters' is used today to describe a naturally occurring family of compounds widely distributed in plant species of the families Euphorbiaceae and Thymelaeaceae. Haas *et al* (2002) reported six phorbol esters from *J. curcas* seed oil, where all compounds possess the same diterpene moiety, namely, 12-deoxy-16-hydroxyphorbol (Figure1). The analysis in the work of Haas and Mittelbach (2000) used an isocratic mixture of 80% acetonitrile and 20% water determined the retention time of the phorbol esters by to 6–11 min. Therefore the total sum of the phorbol ester peaks (retention time: 6–11 min.) was used for quantification (Fig. 2).

The biological effects of these compounds in addition to tumour promotion, bring about a wide range of biochemical and cellular effects, alter cell morphology, serve as lymphocyte mitogens and induce platelet aggregation (Blumberg, 1980 and 1981). The toxic



fraction isolated from jatropha oil not only had an irritant effect after topical application but also caused diarrhoea and mortality in the animals, indicating that there is substantial percutaneous absorption of the toxic components of the oil (Gandhi et al 1995).

The present research was designed to study the effects of various bentonite-adsorption treatments in detoxification of jatropha oil.

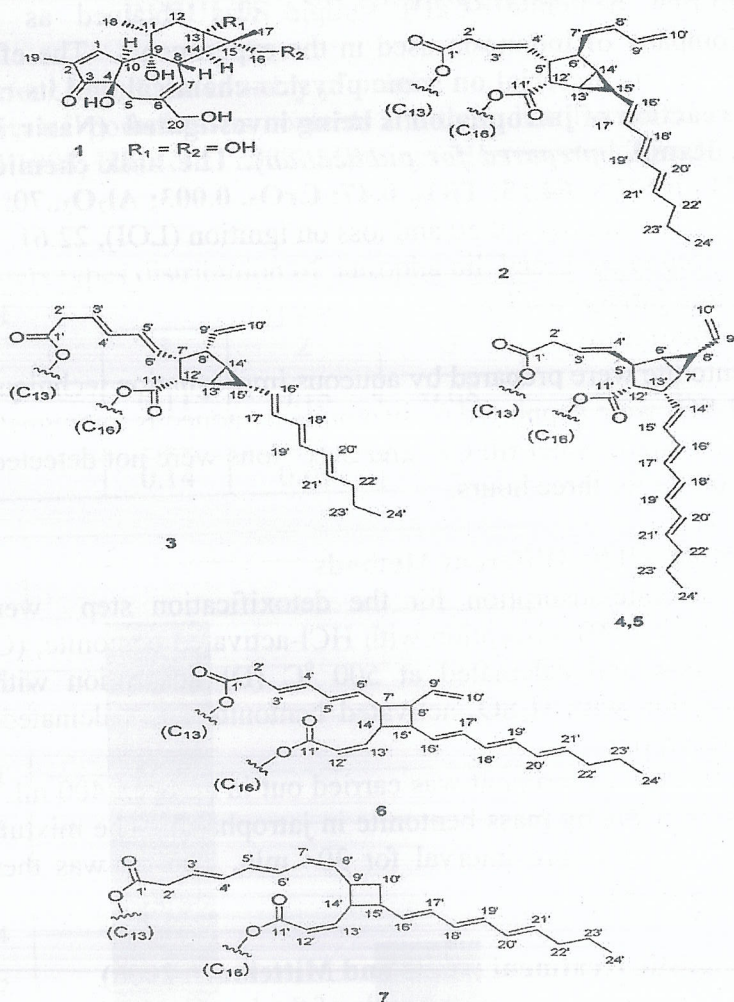


Figure 1. Phorbol esters type from jatropha oil (Haas et al 2002)

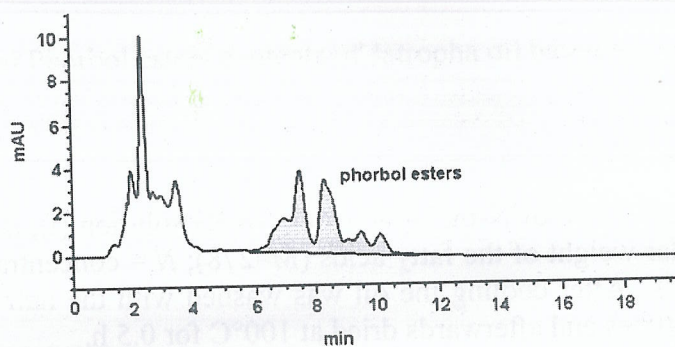


Figure 2. HPLC chromatogram of the methanol extract of untreated *Jatropha curcas* seed oil (Haas and Mittelbach, 2000)



## MATERIAL AND METHOD

### Materials

*Jatropha curcas* seeds come from Indonesia, India and Malaysia. Anhydrous methanol (MeOH), 99.8%, Sodium hydroxide (NaOH), sulfuric acid (H<sub>2</sub>SO<sub>4</sub>), and Hydrochloric acid (HCl), 37-38% pure were purchased from ChemAR®.

A calcium-rich bentonite (CaB) sample was obtained as powder from PT. Superintending Company of Indonesia used in the experiments. The effects of calcinating and acid activation of this material on some physico-chemical and its catalytic properties on esterification reaction of jatropha oil is being investigated (Nazir, N. M.A. Yarmo, J. Salimon, and N. Ramli, *prepared for publication*). The bulk chemical analysis of the bentonite (mass %) is SiO<sub>2</sub>, 64.15; TiO<sub>2</sub>, 0.47; CrO<sub>3</sub>, 0.003; Al<sub>2</sub>O<sub>3</sub>, 0.70; Fe<sub>2</sub>O<sub>3</sub>, 0.10; MgO, 0.70; CaO, 0.03; Na<sub>2</sub>O, 0.20; K<sub>2</sub>O, 0.50 and loss on ignition (LOI), 22.61.

### Preparation of crude jatropha oil

#### Acid activation of Bentonite

Acid-activated Bentonite were prepared by aqueous impregnation technique. 5.3 M HCl and 40% by mass of H<sub>2</sub>SO<sub>4</sub> were supported on bentonite by aqueous impregnation (at 80 °C and 4 h), washing with deionized water till Cl<sup>-1</sup> and SO<sub>4</sub><sup>-2</sup> ions were not detected, drying overnight and calcinated at 500 °C for three hours.

#### Adsorption of Jatropha oil by Different Methods

Six methods of bentonite-adsorption for the detoxification step were compared: (A) adsorption with bentonite; (B) adsorption with HCl-activated bentonite; (C) adsorption with HCl-activated bentonite and calcinated at 500 °C (D) adsorption with H<sub>2</sub>SO<sub>4</sub>-activated bentonite; (E) adsorption with H<sub>2</sub>SO<sub>4</sub>-activated bentonite and calcinated at 500 ° and (F) without bentonite-adsorption

Each detoxification experiment was carried out in an open 400 mL beaker containing a stirred suspension of a 2% by mass bentonite in jatropha oil. The mixture was then heated to 80°C, kept at this temperature interval for 30 min. The oil was then filtered through Whatman No. 41 filter paper.

#### Neutralization by caustic treatment (Haas and Mittelbach 2000)

After determining the content of free fatty acids of the bentonite-adsorbed-oil, 20 g of the oil were heated to 70°C under constant stirring at 800 rpm in a beaker. 2.5 M aqueous NaOH was added to the oil, corresponding to an excess of 20 % needed to neutralize the free fatty acids. The mixture was stirred for 20 min. The appropriate amount of alkaline solution NaOH to neutralize the free fatty acids was calculated by the following equation (Bokisch, 1993):

$$L = \frac{d \cdot \text{FFA} \cdot 10000}{M \cdot N}$$

where  $L$  = appropriate volume of  $N$ -molar aqueous NaOH solution (l);  $d$  = density of the oil  $M$  = average molecular weight of the fatty acids ( $M=278$ );  $N$  = concentration of the aqueous NaOH solution (mol:l). After cooling the oil was washed with the half amount of distilled water (w:w) for three times and afterwards dried at 100°C for 0.5 h.



### Analysis of oils

The FFA content of jatropha oil was determined by titration with standard 0.01 N NaOH solution. The color index of biodiesel was determined by using a *Lovibond Automatic Tintometer Model F* (The Lovibond Limited, UK). Phorbol esters composition was analysed using *HPLC Ultimate 3000*.

## RESULTS AND DISCUSSIONS

### Phorbol esters content based on country of origin

There were variability of phorbol esters contents and their distribution based on countries of origin (Makkar et al 1998, Haas et al 2002, Martı́nez-Herrera et al 2006)(Table 1 and Figure 3 and 4)

Table 1. Phorbol esters types distribution of jatropha oil based on countries of origin

No	Country of origin	Type of phorbol esters distribution				
		1	2	3	4	5
1	India	0.11	0.15	0.08	-	-
2	Indonesia	0.22	0.44	0.33	0.24	0.35
3	Malaysia	0.14	0.09	-	-	-

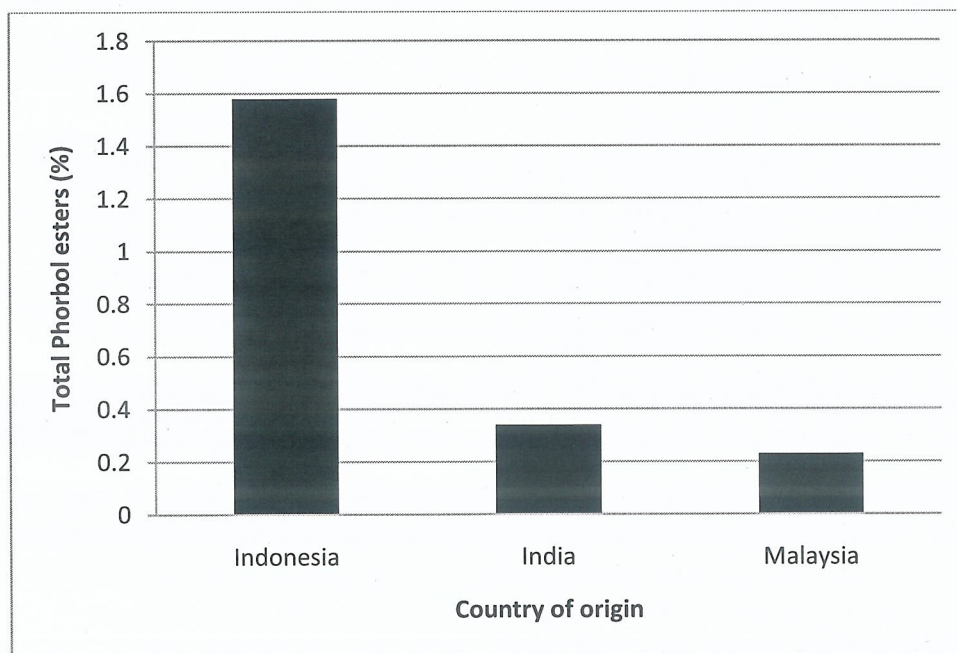


Figure 3. Total Phorbol esters contents of Jatropha oil based on country of origin



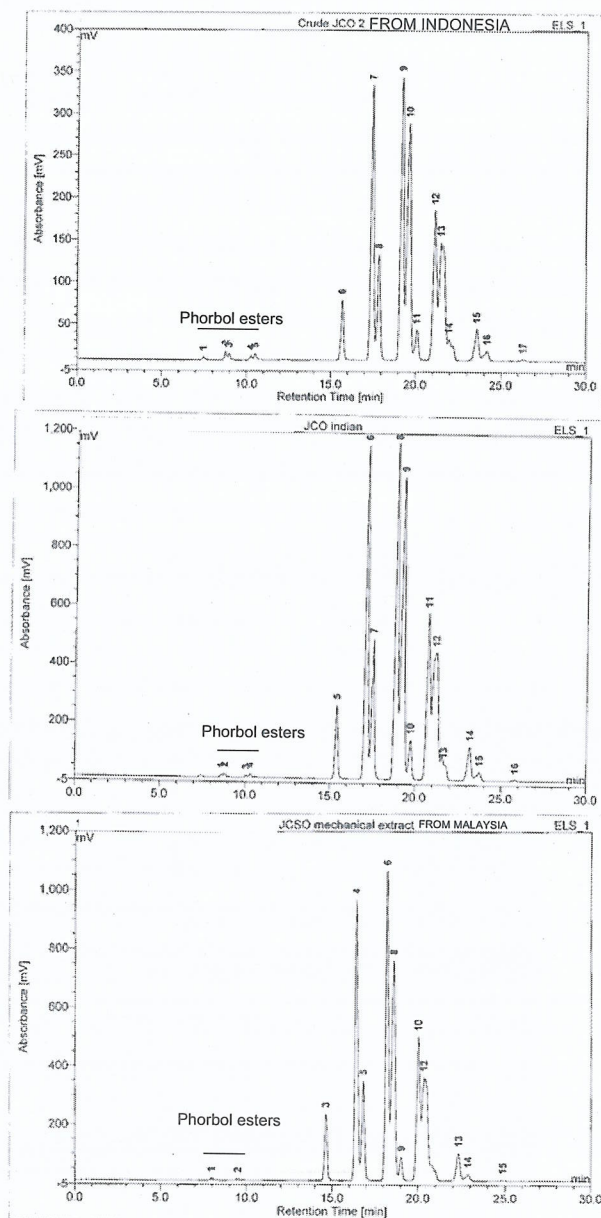


Figure 4. HPLC Chromatograph of phorbol esters types Distribution of *J. curcas* oil from Indonesia (a), India (b) and Malaysia (c)

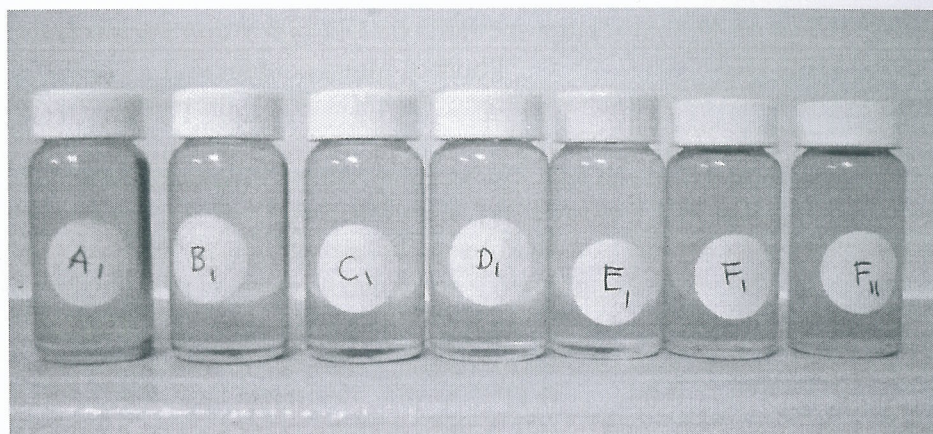
**Effect of Bentonite adsorption on Color**

Significant effect on color scale was achieved with adsorbents treatments, although there was no significant effect on color scale was achieved among adsorbents treatments (Figure 5).

**Effect of Detoxification on FFA Content of Jatropha oil**

No significant reduction on FFA was achieved with adsorbents treatments. But, after neutralization the FFA content become zero (Figure 6).





Treatment	A	B	C	D	E	F
Lovibond color index	10 Yellow, 1.3 Red	10 Yellow, 1.1 Red	10 Yellow, 1.1 Red	10 Yellow, 1.1 Red	10 Yellow, 1.1 Red	20 Yellow, 2 Red

Figure 5. Color variability of bentonite-adsorbed oil

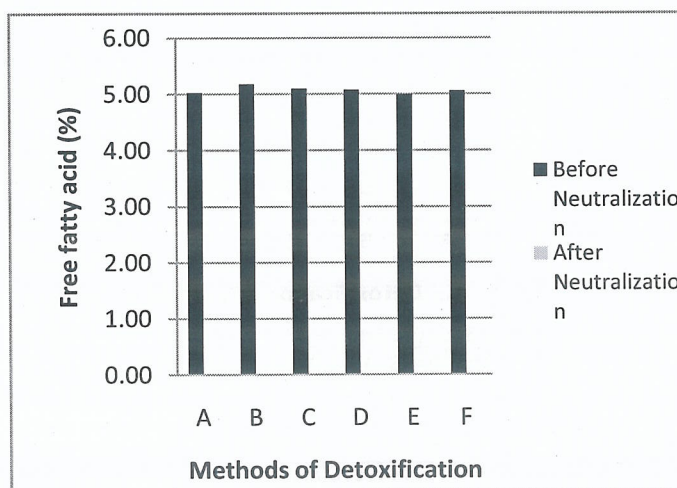


Figure 6. Effect of Bentonite adsorption on FFA Content

#### Effect of Detoxification methods on Detoxification Performance of Jatropha oil

As starting material for experiments on bentonite-adsorption, 20 g of neutralized *J. curcas* seed oil with a phorbol esters content of 1.58% was used. A series of adsorbents at 2% concentration were tested with stirring for 30 min at 80°C. The highest reduction of the phorbol ester content was reached by using H<sub>2</sub>SO<sub>4</sub>-activated bentonite followed by neutralization (0.14% or 8.9% phorbol esters to the phorbol esters content in untreated oil (control) (Table 2, Fig. 7). For the the best result of detoxification for nutritional utilization, the use of seed oil for cooking purposes, the increasing amount of adsorbents should be used. It is because that increasing amount of adsorbents leads to a higher reduction of the phorbol ester content (Haas and Mittelbach, 2000). However, before it can be recommended for human consumption, long term toxicological studies by feeding diets containing oil from this non-toxic jatropha need to be conducted on rats or other laboratory animals ( Makkar et al. 1998).



Table 2. Effect of Detoxification methods on Detoxification Performance of Jatropha oil

TREATMENTS	Phorbol esters content (%)					TOTAL	Detoxification Performance
	TYPE1	TYPE2	TYPE3	TYPE4	TYPE5		
Control	0.22	0.44	0.33	0.24	0.35	1,58	
A	0,06	0,08	0,04	0,06		0,24	85
B	0,07	0,09		0,08	0,09	0,33	79
C	0,08	0,10			0,07	0,25	84
D		0,08	0,06			0,14	91
E	0,10	0,12		0,09		0,31	80
F	0,09	0,11		0,08		0,28	81

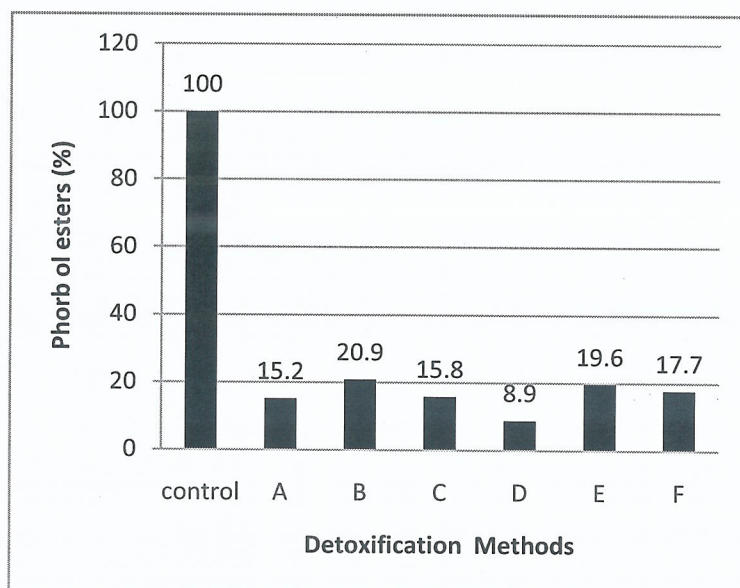


Figure 7. Effect of Detoxification methods on phorbol esters of jatropha oil in relation to the phorbol esters content in untreated oil (control)

### CONCLUSION

Based on the objectives of this study we can conclude as follow:

- There were variation of phorbol esters content and types based on the countries of origin of jatropha oil, where jatropha oil from Indonesia has the highest phorbol esters content and jatropha oil from Malaysia has the lowest phorbol esters content.
- Bentonite has an ability of physically and chemically to adsorb toxic compounds from jatropha oil.
- Detoxification of jatropha oil with  $H_2SO_4$ -activated bentonite followed by neutralization treatment using NaOH was the best detoxification method with detoxification performance of 91% for 2% by mass bentonite adsorbent loaded in jatropha oil.

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