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Recovery of Phorbol from Oil of an Egyptian *Jatropha*

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Abstract: Biofuels have been getting considerable attention because of global emphasis on reducing greenhouse gases, considering the environment and energy security. The use of biodiesel to (partially) replace fossil diesel has a significant potential for reducing pollution and creating economic benefits for farmers. *Jatropha curcas* is a non-edible crop and an important source for producing clean energy from its oil. Seeds and other parts of *Jatropha* can be processed to produce oil containing substances called phorbol esters. These esters can be recovered as valuable co-products to be used for various agricultural, medical and pharmaceutical applications. On the other hand, clays (bentonite) are usually used in fixing the organic compounds. In the present study we investigated the adsorption and desorption of phorbol esters on clays. We found that the optimum conditions for adsorption of phorbol esters were using 15% (w/v) of bentonite stirred at 100 rpm and at room temperature. Bentonite characterization was done using infrared spectroscopy and X-rays diffraction. Desorption of phorbol esters has been evaluated and achieved co-product recovery of 82-100% from *Jatropha* oil.

Key words: Adsorption, clays, phorbol esters, recovery, bio-oil

INTRODUCTION

Biofuels have been getting considerable attention because of global emphasis on reducing greenhouse gases, considering the environment and energy security. The use of biodiesel to (partially) replace fossil diesel has a significant potential for reducing pollution and creating economic benefits for farmers. Therefore, the focus now is moving towards the use of non-edible crops such as *Jatropha* and algae for biodiesel production. Oil, used for cooking oil, low-quality animal fats and side-streams from oil refining (Devappa *et al.*, 2010). *Jatropha curcas*, belongs to the Euphorbiaceae family, is a vigorous, drought, pest-resistant plant and can grow under a wide range of rainfall regimes ranging from 200 mm to over 1,500 mm per annum. It survives also on barren, eroded lands and under harsh climatic conditions (Makkar *et al.*, 2009) and has a productive life up to 50 years.

In view of the current debate of oil for food versus oil for fuel, making this toxicity a potential advantage for *Jatropha*. *Jatropha* oil can be seen as "Technical oil" and therefore does not compete directly with the food markets. At the same time this can also be a disadvantage as the toxicity of the plant oil required special precautions to be exercised during the processing of *Jatropha curcas* seed and oils (Acda, 2009). By-products of the vegetable oil pre-treatment and biodiesel production process, such

as fatty acid distillate, gums and glycerin have several applications in food and animal fodder industries.

Generally, *Jatropha* has been used for its oil while its other parts and derivatives are used for a number of agriculture/medicinal/pharmaceutical purposes and for soap production (Devappa *et al.*, 2010). Seeds of *Jatropha* contain 22-25% oil which is a rich source of phorbol esters and extraction of these esters as a co-product (Table 1) can increase the revenues for biodiesel industries. However, the presence of phorbol esters could render it unfit for nutritional purposes. Phorbol esters are diterpenes with a tigliane skeleton (Fig. 1). They are hydrophobic, heat and oil soluble (Hass and Mittelbach, 2000). Six different types of phorbol esters have been characterized from *Jatropha curcas* oil (Ahmed and Salimon, 2009). Phorbol esters are potent inducers of a range of biological effects, including co

Table 1: Phorbol esters in different parts of the toxic *Jatropha curcas* plant

Parts	Phorbol esters (mg g ⁻¹ dry matter)*
Kernel	2.00- 6.00
Leaves	1.82-2.75
Stems	0.78-0.99
Flower	1.39-1.83
Buds	1.18-2.10
Roots	0.55
Bark (outer brown skin)	0.39
Bark (inner green skin)	3.08
Wood	0.09

*As phorbol-12-myristate 13-acetate equivalent (Laila *et al.*, 2008)

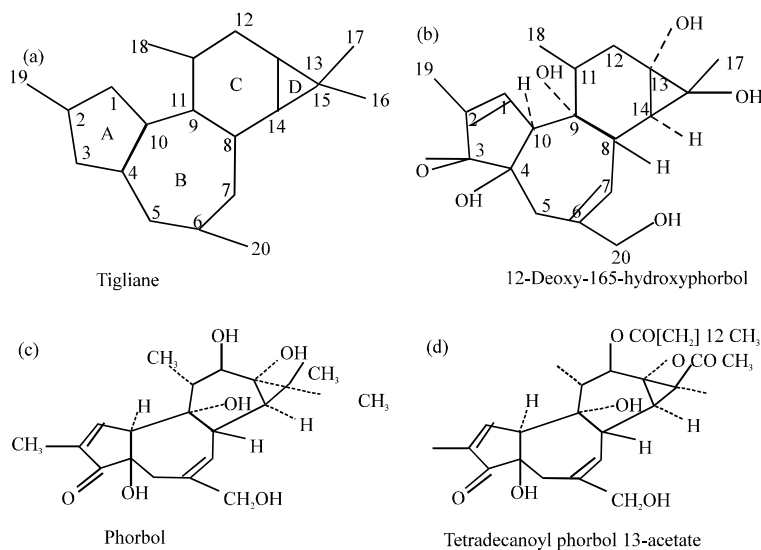


Fig. 1(a-d): Phorbol esters diterpenes with a tiglane skeleton

carcinogenicity and tumor growth (King *et al.*, 2009). Such toxic and co-carcinogenic effects are potential constraints to the widespread of *Jatropha*. Phorbol esters are used as a pharmaceutical tool for the investigation of biochemical processes such as carcinogenesis and also in many agricultural applications such as pesticides, molluscicides, insecticides, bacteriocides and fungicides (Iehilaslii *et al.*, 2010). Isolated phorbol esters could have a high commercial retail value (ca USD 2000/g). Kumar and Sharma (2008) stated that complete removal of the toxins is therefore necessary before *Jatropha* oil can be used in industrial applications or in human medicine. The extraction of phorbol esters from oil, in addition to providing an invaluable product, will also make the process of biodiesel production friendly to the work and environment (Makkar and Becker, 2009).

The oil of the *Jatropha* plant can serve as biofuel in adapted diesel engines especially in remote and difficult to access regions, the use of plant oil might be recommended. Reddy and Ramesh (2006) used neat *Jatropha* oil in a direct-injection diesel engine which showed lower emissions of hydrocarbons and oxides of nitrogen compared to those from mineral diesel. Pramanik (2003) established that 40-50% of *Jatropha* oil can substitute for diesel without any engine modification and preheating of the blends. From the energetic point of view it can be an advantage not to esterify the oil but to use it directly. The energy content of the *Jatropha* oil has been found to range from below 37 to 39.5 MJ kg⁻¹.

Clays play an important role in adsorption of polar organic compounds, mainly due to the high surface areas associated to their small particle size. The sorption of

phorbol esters on clays has been studied to test the influence of adsorption at different solid/oil ratio and different stirring time (Russell and Fraser, 1994).

The objective of this study was to assess the effect of clays (bentonite) on adsorption of phorbol esters from *Jatropha* oil and then recovery of these esters by desorption technique. Clay's characterization was done using infrared spectroscopy and x-ray diffraction.

MATERIALS AND METHODS

Materials: *Jatropha curcas* seed oil was obtained from Department of Chemical Engineering, National Research Centre, Egypt. The experimental adsorbent (Bentonite) was obtained from Alexandria City, Egypt. Phorbol 12-myristate-13-acetate (PMA; GAS number 16561-29-8) was obtained from Sigma (St. Louis, USA). All other chemicals/solvents used were of analytical grade. Methanol used was of HPLC grade.

Methods

Activation of clay (bentonite): The activation process was carried out by adding 100 g clay to 100 mL of 2M H₂SO₄ (The mass ratio of clay to acid was 1:1) and then the mixture was dried in vacuum dryer at 90°C for 1 h. Grinded samples were sieved through 106 µm opening. The Bentonite characterization was done using IR-Spectroscopy and X-ray diffraction.

Adsorption experiment: Adsorption was carried out following the batch technique using various mass adsorbent (clay:oil) (w/v) ratio. *Jatropha* oil was mixed

with activated bentonite with various mass ratio (zero, 10, 20, 25, 30, 35 and 40%) and then these mixtures were stirred at room temperature for 5, 15 or 25 min at 100 rpm. Thereafter, the mixtures were filtered and were subjected to separation of residual phorbol esters by extraction with methanol using separating funnels. Separation was repeated 3 times and each time the mixture was left for gravity settling for 15 min to get upper methanolic layer and lower oily layer. Both layers were separated and HPLC analysis for phorbol esters was done.

Desorption of esters: Clay samples were soaked after adsorption process in methanol for overnight with methanol to clay ratio of 100:1. The mixtures (clay and methanol) were shaken for 48 h and allowed to settle for removing out the methanol before HPLC analyses. The desorption was performed to recover the phorbol esters.

The high performance liquid chromatography (HPLC) analysis: The adsorptive concentrations were determined by HPLC following a couple of methods as the following:

- **Method A:** reversed phase chromatogram column (125×4 mm) with particle size of 5 μm , thermally controlled at 25°C and a mixture of acetonitrile HPLC grade to water in ratio of 80:20 (v/v) were used at a flow rate 1 mL min⁻¹ and detector wavelength 280 nm. A total of 10 μL sample solution were injected
- **Method B:** HPLC on a Waters Symmetry 300™ column (C₁₈ 5 μm , 4.6×150 mm, 1 mL min⁻¹, 25°C) with Waters 1525 HPLC binary pump and Waters 2996 photodiode array detector equipped with millennium

software. The degassed mobile phase consisted of phosphoric acid 85% (A) and acetonitrile (B). Eluting with the gradient programmed as follows; 60% solution A and 40% solution B at 0 min, 40-50 B at 10 min, 50-75% B at 30 min and 75-100% B at 45 min. After each run the column was washed extensively with tetrahydrofuran. The flow rate was 1 mL min⁻¹ and UV detection was performed at 280 nm. Just before injection onto the HPLC column, an internal standard containing phorbol 12-myristate 13-acetate) was added

Characterization of clay: The clay was analysed for its vibrational spectra with the aid of Fourier transform infrared spectroscopy instrument (Perkin Elmer 2000 model) in the range 450-4000 cm⁻¹ as potassium bromide pellet.

Patterns X-ray diffraction of clay were obtained on a powder X-ray diffractometer Model Philips with CuK α radiation having a scanning speed of 0.04°/sec. The clay was subjected to X-ray diffraction to determine its chemical composition.

RESULTS AND DISCUSSION

Characteristics of clays used in this study are presented in Table 2. Results indicated that montmorillonite and Kaolinite represented the major fractions of clays which have been confirmed by the infrared spectra illustrated in Fig. 2. The Kaolin spectrum revealed an intense band at 3430 cm⁻¹ corresponding to stretching vibration of OH groups coordinated to Al-Al

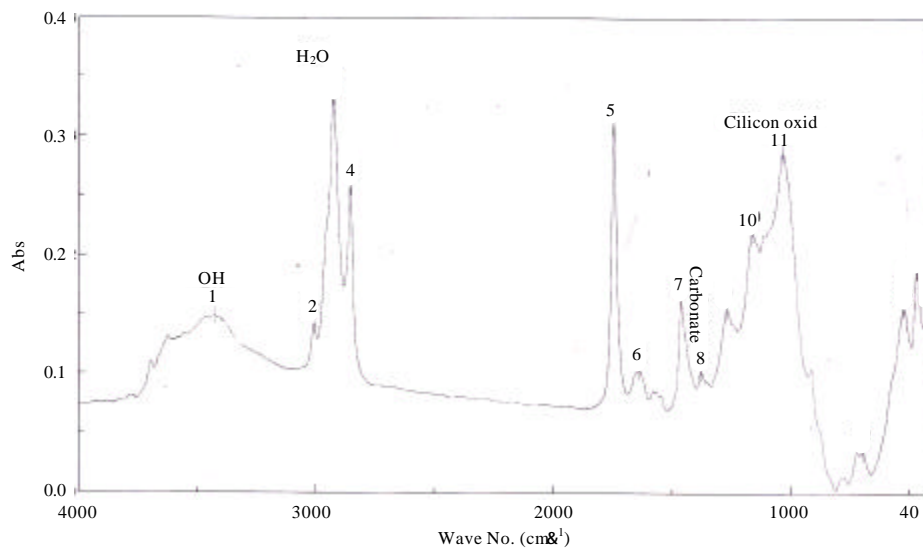


Fig. 2: Bentonite characterization interpretation infrared chart

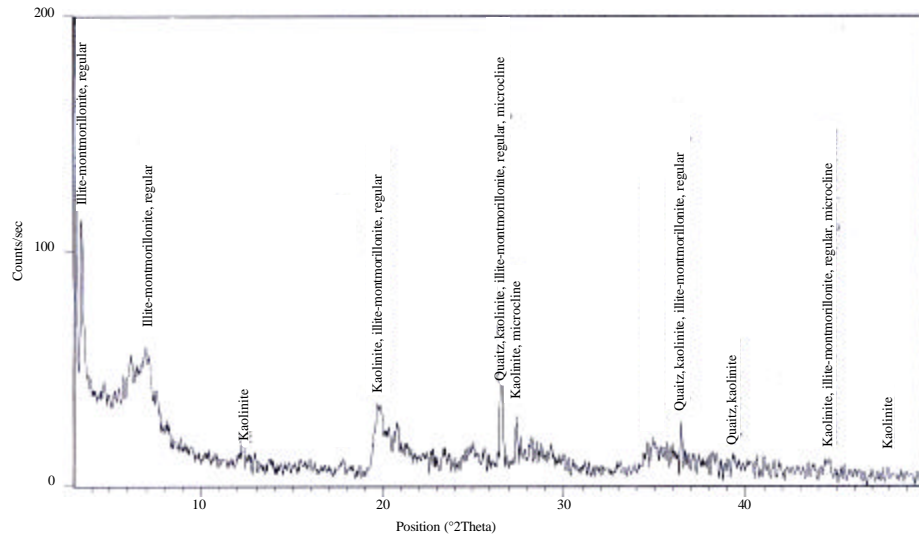


Fig. 3: Clay X-ray diffraction for composition of mineralogical phase

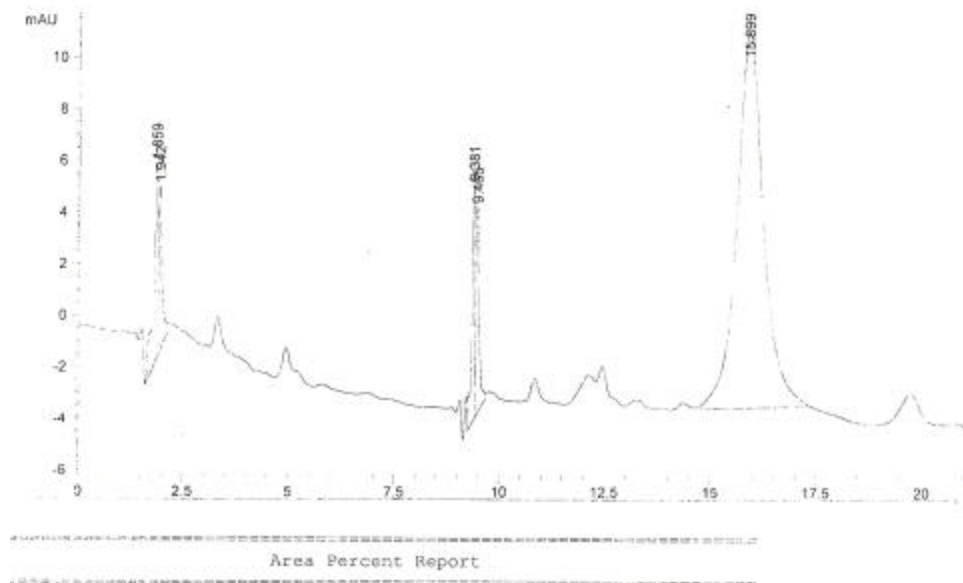


Fig. 4: Chromatogram of standard phorbol ester 12-myristate 13-acetate (method A)

pairs and another band located at 2924 cm^{-1} corresponding to H_2O -stretching vibration which in accordance with the results obtained by Taylor (1999). However, according to Madjeva and Komadel (2001), broad band situated at 1377 cm^{-1} attributed to the presence of carbonate solid phase and these results were confirmed by X-ray diffraction. Spectra show a large band around cm^{-1} equivalent to Si-O-stretching (Hass *et al.*, 2002). The X-ray powder different pattern of clays has been given in Fig. 3. The principal equidistance of the

most intense lines as well as the hkl plans relative to these rays. The charts illustrate composition of mineralogical phase; montorillonite, illite, quartz, microcline and anatase.

The obtained chromatograms of both Standard phorbol ester and phorbol esters obtained from *Jatropha curcas* oil are shown in Fig. 4 and 5. Figure 4 shows chromatogram of standard phorbol-12-myristate-13-acetate at retention time of 15 min (Method A). While Fig. 5 shows chromatogram of standard phorbol-12-myristate-13-acetate at retention time of 55 min

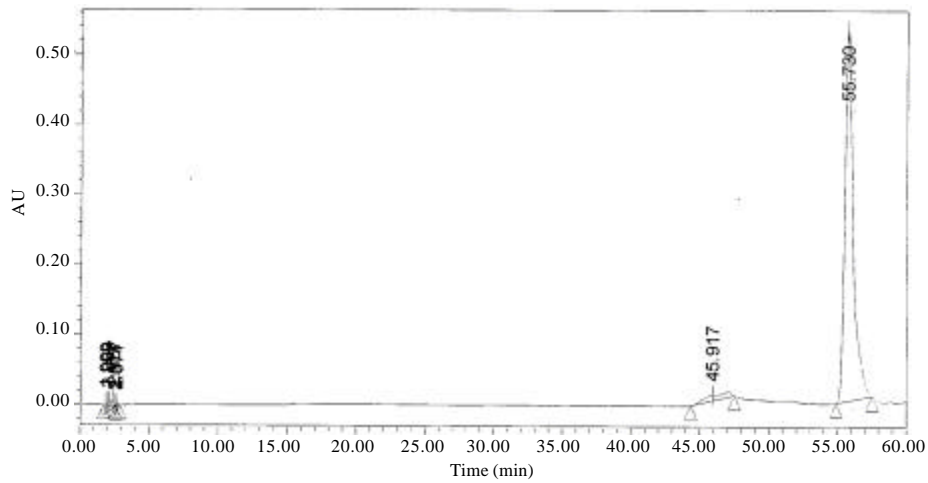


Fig. 5: HPLC Chromatogram of standard phorbol ester 12-myristate 13-acetate (method B)

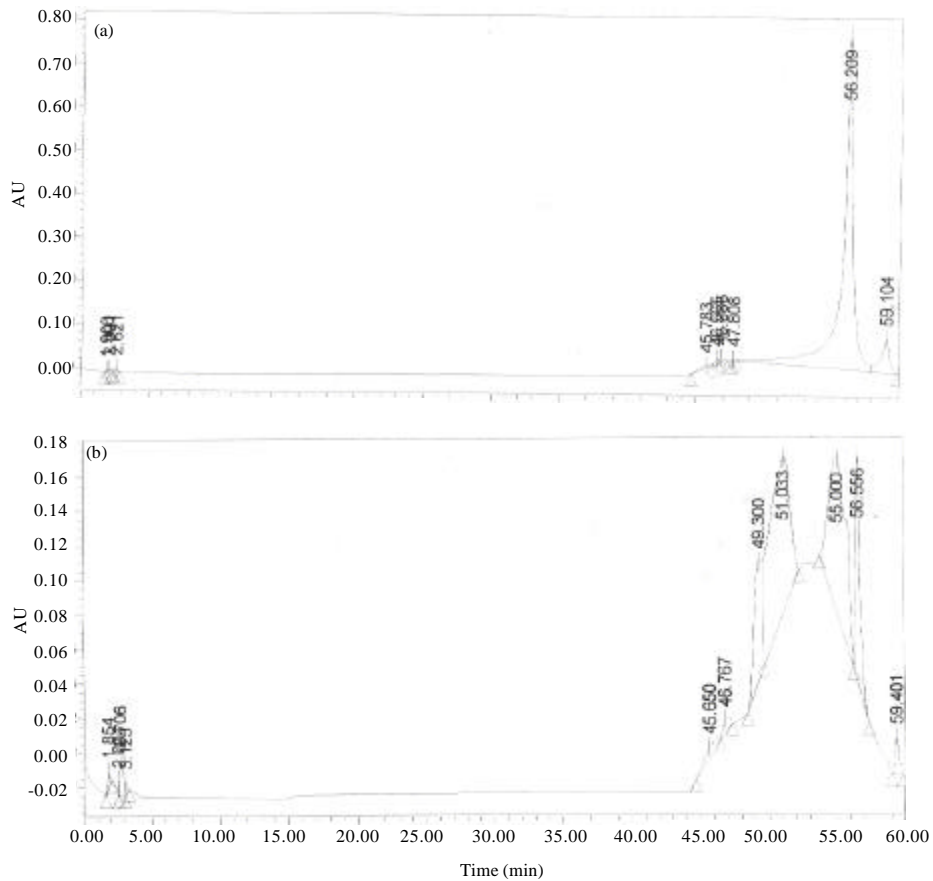


Fig. 6(a-b): HPLC Chromatogram showing Bentonite efficiency for phorbol adsorption using method B. The four peaks of phorbol esters are enlarged as shown in the lower Figure

(Method B). Figure 6 demonstrated a group of four peaks during retention time at 45-60 min indicating the presence

of phorbol ester in *Jatropha* seed oil. Hass *et al.* (2002) illustrated that phorbol esters in *jatropha* seed oil might

Table 2: Chemical composition of original clays (bentonite)

Main minerals	Least minerals
Kaolinite, $Al_2SiO_5(OH)$	Quartz, low- SiO_2
Illite-Montmorillonite	Microlite
$[Na \times (Al, Mg)_2SiO_{10}(OH)_2 \cdot H_2O]$	Anatase, $sys-TiO_2$
Illite	

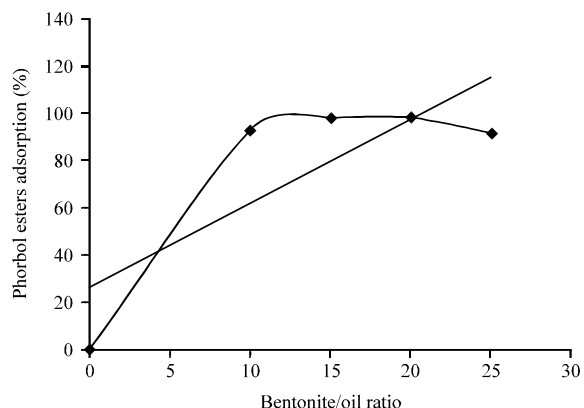


Fig. 7: Effect of Bentonite solid/oil ratio on phorbol esters adsorption (%) at 5 min stirring

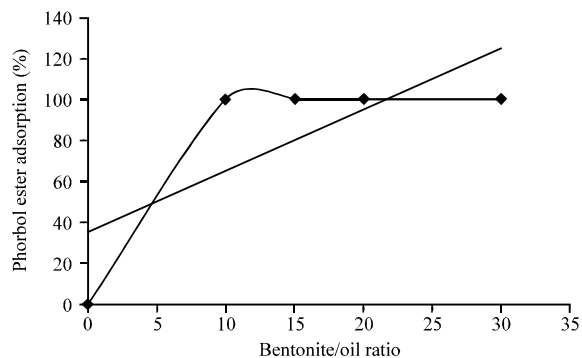


Fig. 8: Effect of Bentonite solid/oil ratio on Phorbol esters adsorption (%) at 15 min stirring

be in different forms of phorbol esters types which can explain the difference between forms of the peaks appeared at 1-5 min and the form of standard phorbol 12-myristate-13-acetate.

Figure 7 illustrates the efficiency of clays (Bentonite) for phorbol esters adsorption. The adsorption efficiency is due to the strong acidity of activated clays resulting in hydrolysis reaction simultaneously with the adsorption of phorbol esters.

The experimental optimum conditions for phorbol esters adsorption on bentonite using various times of adsorption and various concentrations of clay: oil mixtures are shown in Fig. 7-9. Figure 7 shows that the percentage of phorbol esters adsorption did not exceed 80%. As shown in Fig. 8, the continuous increase in adsorption (%) of phorbol esters from *Jatropha* seed oil

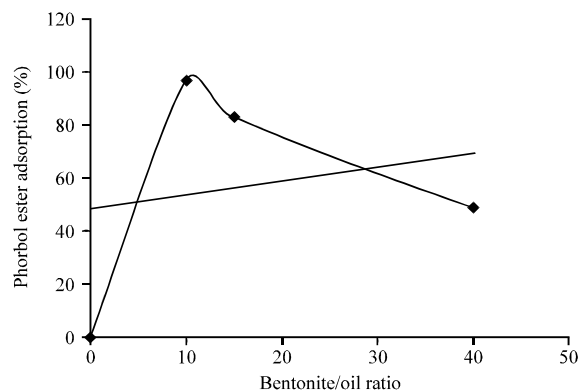


Fig. 9: Effect of Bentonite solid/oil ratio on phorbol esters adsorption (%) at 25 min stirring

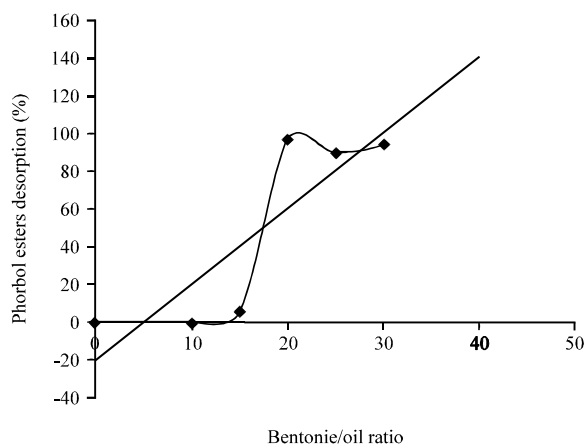


Fig. 10: Effect of Bentonite solid/oil ratio on phorbol esters desorption using methanol

with bentonite were appeared at optimum condition as depicted in the figure. The equilibrium point from adsorption experiments were 15 min of adsorption time, 15% (w/v) of bentonite, room temperature and 100 rpm of stirring rate that illustrated high phorbol esters adsorption efficiency of 82-100%. However, Fig. 9 shows that the adsorption (%) decreased up to 60% along with increasing the stirring time to 25 min. According to Devappa *et al.* (2010), increasing stirring time changed the color of the oil to deeper color and decreased the content of free fatty acids which might explain the decrease in the adsorption percentage. The methanol was used in our study as a suitable and cheap solvent than using the mixed solvent which ultimately would increase the cost of extraction. Although the mixed solvent systems may be effective in reducing the phorbol esters content in the oil but they are expensive for industrial use (Devappa *et al.*, 2010).

On the other hand, desorption of phorbol esters from Bentonite was studied. As shown in Fig. 10, continuous

increase in desorption of phorbol esters was observed with increasing of stirring time which are in accordance with the results obtained of the optimum conditions for bentonite adsorption (15 min stirring time, 100 ppm, room temperature).

CONCLUSION

Phorbol esters can be recovered from *Jatropha* oil after adsorption on bentonite using methanol as solvent as phorbol esters can be easily dissolved in water and alcohol. The rich fractions containing phorbol esters can be collected and concentrated from methanol to be used for various agricultural, medicinal and pharmaceutical applications. The extraction of phorbol esters will make the process of biodiesel preparation friendly for both workforce and environment. The main advantage of using Bentonite is the simple release of phorbol esters without need to separate oil from solvent by physical methods.

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