CHAPTER II

THEORY AND LITERATURE REVIEW

2.1 *Jatropha curcas* L.

Jatropha curcas is the native plant of Central and South American countries. This plant crop has wide variations in the morphological characteristics in its stems, leaves, flowers, fruits, and seeds. *J. curcas* is a drought-resistant species which is widely cultivated in the tropics as a living fence. This plant has been used for many purposes; such as medicine, pesticide, soap production, diesel fuel, etc. (Heller, 1996). It contains high amounts of oil in the seed. Its oil can be used directly or indirectly in engines. Besides from the oil, kernel meal is one of interest since it contains high nutrition. Chivandi et al. (2005) reveals that *J. curcas* meals contain crude protein even higher than soybean. Therefore, to develop technique that is able to eliminate phorbol esters from seed meal would be a promising solution since the meal will be value-added for further production i.e. feedstock.

2.1.1 Oil content

J. curcas becomes more interest as an alternative fuel because its seed contains high amounts of oil. Furthermore, it is non-edible plant, so there is not competitive with being food purpose. Previous studies reported the oil content in *J. curcas* seeds and in *J. curcas* kernels (Table 2.1). Koedklai (2007) revealed that four kilograms of seeds can produce 1 liter of oil. The major component most found in *J. curcas* oil is in triglyceride form as shown in Figure 2.1. The major free fatty acids in the *J. curcas* oil are unsaturated consisting of oleic acid and linoleic acid (Banerji et al., 1985; Kandpal and Madan, 1994; Foidl et al., 1996; Akintayo, 2004; Berchmans and Hirata, 2008; Chhetri et al, 2008). The compositions of free fatty acid exist in *J. curcas* oil is shown in Table 2.2.

Table 2.1 Oil proportion in *Jatropha curcas* seeds (%wt)

Whole seed	Kernel	Reference
-	48.5	Banerji et al., 1985
37.4	46-48.6	Kandpal and Madan, 1994
-	58-60	Aderibigbe et al., 1997
-	43-59	Makkar et al., 1997
-	53.9-58.5	Makkar et al., 1998
30-50	45-60	Pramanik, 2003
47.25	-	Akintayo, 2004
-	44	Shah et al., 2005

Table 2.2 Free fatty acid in *Jatropha curcas* oil*

Fatty acid		Formula	Systemic name	%
Myristic	14:0	$C_{14}H_{28}O_2$	Tetradecanoic	0.1
Palmitic	16:0	$C_{16}H_{32}O_2$	Hexadecanoic	13.38-19.5
Palmitoleic	16:1	$C_{16}H_{30}O_2$	cis-9-Hexadecanoic	0.88-0.9
Stearic	18:0	$C_{18}H_{36}O_2$	Octadecanoic	2.3-7.4
Oleic	18:1	$C_{18}H_{34}O_2$	cis-9-Octadecanoic	34.3-49.0
Linoleic	18:2	$C_{18}H_{32}O_2$	cis-9,cis-12-Octadecanoic	29.7-43.2
Linolenic	18:3	$C_{18}H_{30}O_2$	cis-6,cis-9,cis-12-Octadecanoic	0.2
Arachidic	20:0	$C_{20}H_{40}O_2$	Eicosanoic	0.2-0.3
Behenic	22:0	$C_{22}H_{44}O_2$	Docosanoic	0.2
Total saturated				20.8-26.3
Total unsaturated				72.7-78.7

Note: * Adapted from Banerji et al., 1985; Kandpal and Madan, 1994; Foidl et al., 1996; Akintayo, 2004; Berchmans and Hirata, 2008; Chhetri et al, 2008

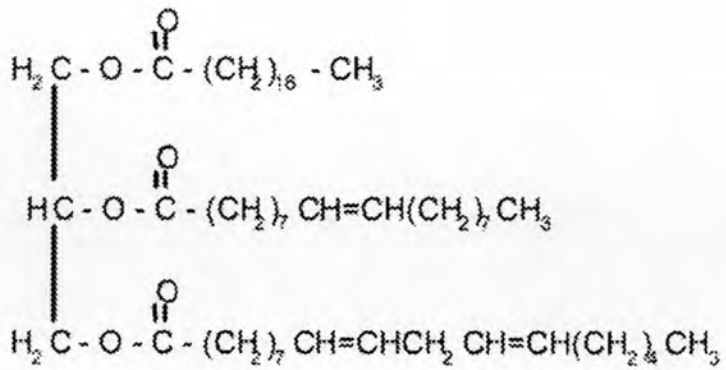

Figure 2.1 Formula structure of *J. curcas* oil (Jongschaap et al., 2007)

2.1.2 Nutrient in seed

The *J. curcas* seed meal is nutrient rich as it contains crude protein at about 26.0% (Makkar et al., 1997). This amount of crude protein in *J. curcas* kernel is higher than that of soybean (Chivandi, Kachigunda and Fushai, 2005). Several studies revealed the amount of crude protein found in each parts of *J. curcas* seeds as described in Table 2.3. The essential amino acids of meals almost meet the FAO reference protein, except lysine (Makkar et al., 1998). Even thought, its kernels contain a lot of protein. The kernels are not suitable for animal feeds since they contain some toxin.

Table 2.3 Crude protein content in *Jatropha curcas* seeds by percent weight

Seed	Kernel	Meal*	Reference
26.75	-	-	Liberalino et al.,1988
-	19-31	-	Makkar et al., 1997
-	22.2-27.7	57.3-64.4	Makkar et al., 1998
24.60	-	-	Akintayo, 2004
-	-	57.7	Chivandi et al., 2005

Note: * Meal is defatted kernel

2.1.3 Phorbol esters

Phorbol esters are known as significant toxic compounds found in *J. curcas* seed (Aregheore et al., 2003). According to the Division of Occupational Health and Safety, phorbol esters are toxic and suspected carcinogens. They are easily absorbed into the body by the dermal route and the ingestion. The possible effects of contact with phorbol esters are the severe irritation of tissues (the skin, eyes, mucous membrane, and lungs) and induced sensitivity. They are a derivative of the tigliane family of a tetracyclic diterpene, the general structure of phorbol esters group are shown in Figure 2.2a. Many studies have reported the concentrations of phorbol esters in *J. curcas* from several sources (Table 2.4). Since Phorbol esters are organic compound, they can be miscible and/or partition in oil phase, thus the results show that phorbol esters can be found both in the extracted oil and also in the residual meal or the cake. Phorbol esters are thermal resistant, so they are not easily destroyed by only heat (Aregheore et al., 2003).

Table 2.4 Phorbol esters in *Jatropha curcas*

Oil (mg/g*)	Kernel (mg/g*)	Reference
-	2.17-2.70	Makkar et al., 1998
-	0.11**	Makkar et al., 1998
3.1	-	Haas et al., 2000
-	1.78	Aregheore et al., 2003
-	6.5	Chivandi et al., 2005
3.8540	-	Nokkaew et al., 2008a
3-6	1-2	Nokkaew et al., 2008b

Note: * mg/g as TPA equivalent

** Non- toxic Mexico variety

There are many different compounds of phorbol esters group found in *J. curcas* seeds; such as the derivative of 12-deoxy-16-hydroxyphobol (Figure 2.2c) -- 12-deoxy-16-hydroxyphorbol-13-acylate (Adolf et al., 1984), and 12-deoxy-16-

hydroxyphorbol-4'-[12',14'-butadienyl]-6'-[16',18',20'-nonatrienyl]-bi-cyclo[3.1.0] hexane-(13-o)-2'-[carboxylate]-(16-o)-3'-[8'-butenoic-10']ate (DHPB, Figure 2.2d) (Hirota et al., 1998). However, the determination of the concentration of phorbol esters by HPLC generally uses 12-o-tetradecanoyl-phorbol-13-acetate (TPA) as the external standard (Makkar et al., 1997; Hass and Mittelbach, 2000). The molecular weight of TPA is 616.92 g/mol and the formula is $C_{36}H_{56}O_8$. The structure of TPA is shown in Figure 2.2b.

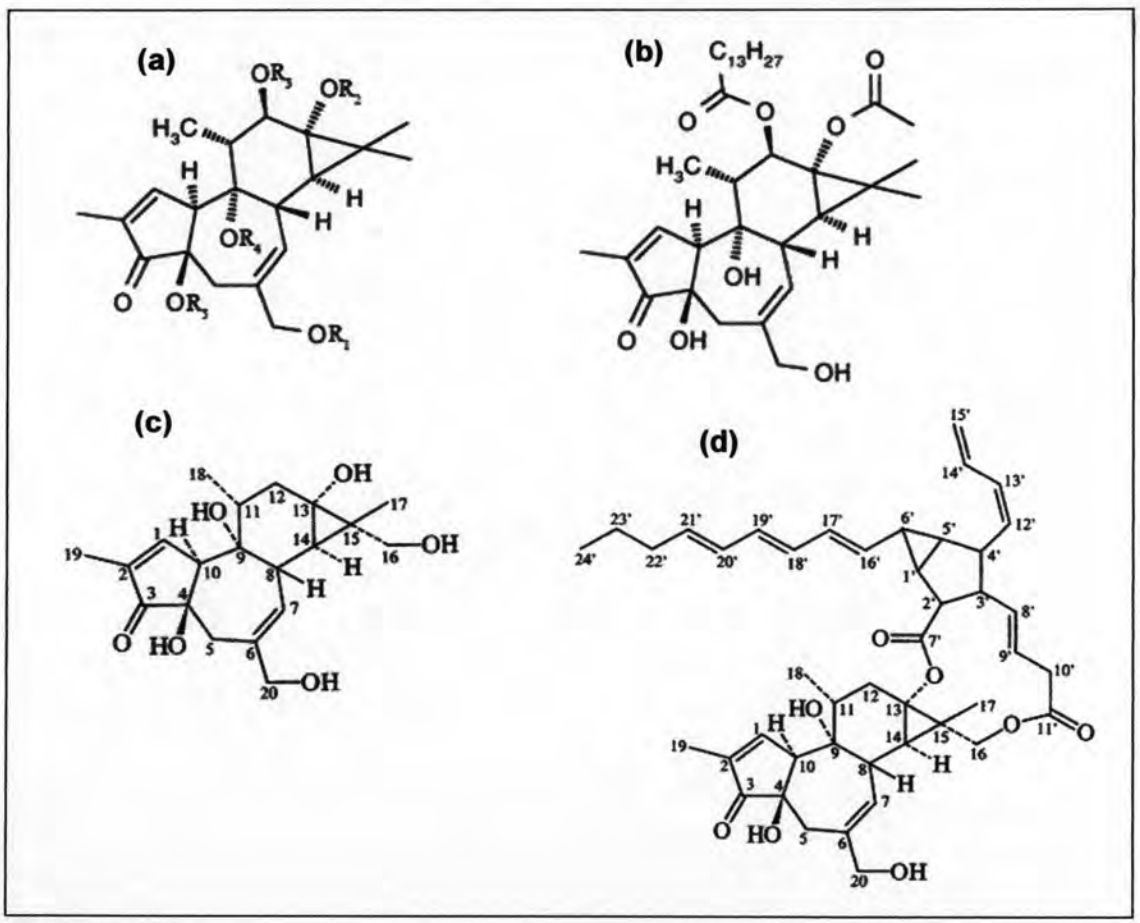

Figure 2.2 The structure of phorbol esters: (a) general structure, (b) 12-o-tetradecanoyl -phorbol-13-acetate (TPA), (c) 12-deoxy-16-hydroxyphorbol, and (d)12-deoxy-16-hydroxyphorbol-4'-[12',14'-butadienyl]-6'-[16',18',20'-nonatrienyl]-bi-cyclo [3.1.0]hexane-(13-o)-2'-[carboxylate]-(16-o)-3'-[8'-butenoic-10']ate (DHPB)

The toxicity studies of these compounds were done in many animal models such as carps, mice, rats, and snail. The test animals were force for feeding. The symptoms like water intake reduction, diarrhea and other hemorrhagic effects in different organs (Goel et al., 2007). The result on effect from toxicity studies are shown in Table 2.5.

Table 2.5 The toxicity studies of phorbol esters

Animals test	Phorbol ester level	Effects	Reference
Carp *Cyprinus carpio* L.	15 ppm feed < 31 ppm feed	- The threshold level - Lower average metabolic growth rate - Fecal mucus production - Rejection of feed	Becker and Makkar, 1998
Carp *Cyprinus carpio* L. and Rats	Use non-toxic variety from Veracruz, Mexico	No effect to both animals	Makkar and Becker, 1999
Snails -*Biomphalaria glabrata* -*Bulinus truncates* and *B. natalensis*	 50 ppm* 25 ppm* 0.2 ppm* 1 ppm*	 LC50 LC100 LC50 LC100	Rug and Rupple, 2000

Note: *Using methanol extraction from *J. curcas* oil

2.2 Oil Extraction Method

The general method to extract oil from the *J. curcas* seeds is pressing technology (Division of Occupational Health and Safety, 2006). The pressing technology is used by small and medium scale that have some different in the machine characters; nevertheless, this method cannot extract all the oil from the seed. Therefore, the large scale extraction or the industrial extractions use organic solvent to rise up the extractable oil (Jongschaap et al., 2007). The efficiency of oil extraction in different technologies is shown in Table 2.6.

Table 2.6 The Efficiency of oil extraction (Jongschaap et al., 2007)

Method	Efficiency (%)
Pressing technology	
- Hand powered small scale pressing	60
- Mechanized pressing equipment	75
- Commercially available pressing system	90
Industrial extraction with organic solvent (mainly hexane)	nearly 100

2.3 Surfactant

"A surfactant (a contraction of the term surface-active agent) is a substance that, when present at low concentration in s system, has the property of adsorbing onto the surfaces or interfaces of the system and of altering to a marked degree the surface or interfacial free energies of those surfaces (or interfaces). The term interface indicates a boundary between any two immiscible phases; the term surface denotes an interface where one phase is a gas, usually air" (Rosen, 2004).

Surfactant has the amphipathic structure; in the other word, one surfactant consists of both hydrophobic group and hydrophilic group. Hydrophobic group is the structure that can dissolve in less polar solvent, whereas hydrophilic group. Surfactants are classified in 4 types: anionic, cationic, zwitterionic, and nonionic.

2.3.1 Interfacial Tension Reduction Phenomena

In liquid phase, the energy to bring one molecule at interior phase is smaller than to bring it at surface or another phase. The minimum energy that used to bring molecule from interior to surface is called surface tension or interfacial tension. Interfacial tension is surface free energy per unit area.

The two bulk phases have potential energy between phases greater than the same bulk phase or interior phase. The interfacial tension of two different phases is the combination of the surface free energies per unit area of each phase minus with the interaction per unit area across the interface as expressed equation 2.1.

$$\gamma_I = \gamma_a + \gamma_b - 2\gamma_{ab} \qquad [2.1]$$

The surface free energies per unit area of two phases are γ_a and γ_b, and γ_{ab} is the interaction energy per unit area across between two phases.

Surfactants can reduce the interfacial tension between immiscible phases; i.e. water/oil, because the amphipathic structure. The hydrophilic part is able to adsorb with the polar phase and the hydrophobic part is able to adsorb with the non-polar phase. Thus, surfactants are in the between phases and due to the reduction of tension across the interface (Rosen, 2004). Moreover, the interfacial tension decreased when increasing the concentration of surfactant, is constant until release the cmc or critical micelle concentration (Tadros, 2005).

2.4 Ultrasonic Extraction

Ultrasonic extraction or sonication is one method for extraction substances from the solid phase. Sonicator produces the ultrasonic energy wave and this wave facilitate the solvent contacts with the sample well. The advantages of ultrasonic extraction are faster and less solvent consumption than the conventional extraction method (Soxhlet) due to more energy is added to the system and promote coalescence between molecules. However, there are some disadvantages may be generated. For instance, the extraction efficiency of some substances may not be high as other method. Mitra (2003) mentioned that some organophosphorus compounds can be

destroyed by the ultrasonic vibration. In addition, more energy may be needed as compared to conventional shaking.

Following the guideline from EPA method 3550C, ultrasonic extraction method is more suitable for semivolatile and nonvolatile organic compound extraction from solid phase. At lower concentration than 20 mg/kg of organic analyzes, the samples are required more than one time extraction; whereas, at medium/high concentration more than 20 mg/kg, only one time extraction is enough by 2 g of sample and 20 ml of solvent. The ultrasonic disrupter is required to have a minimum power voltage of 300 watts.

2.5 Related work

Because the fossil fuel are depleting, many alternative fuels have been developed in order to be substitution energy. As mentioned earlier, *Jatropha curcas* seed is one of alternative fuels has been proposed in many researches as a source of energy. In 1985, Banerji et al. studied about 4 plant-seed in *Jatropha* genus; *J. curcas, J. glandulifera, J. gossypifolia* and *J. multifida* and found that the oil content (extracted with petroleum ether in a Soxhlet apparatus) in all species is 27.2% – 48.5%. Among the 4 plant seeds, the one yields the highest oil is *J. curcas* seed. In addition, they found that almost fatty acid in of all plant seeds are unsaturated, 78.7% of *J. curcas* oil are unsaturated oil. Oleic acid, linoleic acid and palmitic acid in *J. curcas* oil are 49.0%, 29.7% and 18.5%, respectively. The energy value of *J. curcas* oil is 41.77 kJ/g; however, the highest heat value was found in the oil obtained from *J. multifida* seeds. Ten years later, Kandpal and Madan (1995) reported their study that *J. curcas* contain oil about 37.4% from whole seeds and 46.0 – 48.6% from kernels of seeds (extracted by petroleum ether). The saturated and unsaturated fatty acids of oil are 20.1% and 79.9%, respectively. They compared between *J. curcas* oil and diesel oils and found that the calorific value of *J. curcas* oil are a little lower; however, the sulphur content in *J. curcas* oil is much lower than diesel.

Due to very high oil content in seeds, *J. curcas* oil has attracted many researchers to investigate its potential to develop *J. curcas* as an alternative fuel. Foidl et al. (1996) studied the properties of biodiesel from *J. curcas* oil by methyl and

ethyl transesterification. Both methyl and ethyl esterification reduce the viscosity at 30°C from 52 cSt of crude oil to 4.84 and 5.54 cSt, respectively. The ester fuel can be used in engines directly. The both ester fuel have good quality and meet the existing standard for vegetable-oil-derived fuel. For the application of biodiesel from *J. curcas* oil, Singh et al. (2008) revealed that performance of de-gummed and de-wax oil, and methyl transertered oil could be used as pure biodiesel in CI engine without any problem.

In comparison, an oil extraction method by petroleum-based solvent, i.e. ether or hexane is considered as a potential source of air pollution while a pressing method seems to be cleaner technology even though this technique is much lower efficiency. To compromise advantage and disadvantage of these two techniques, several approaches have been developed to obtain high efficiency of and also environmental friendly technique. Shah et al. (2005) extracted *J. curcas* oil from kernels by combination of ultrasonication and aqueous enzymatic oil extraction. They found that the highest oil yield is obtained by 5 min ultrasonic extraction after using an alkaline protease at pH 9.0.

Naksuk (2006) studied the oil extraction from the palm kernels and the soybeans by using the surfactant-based solution. The researcher conducted the mix-surfactant solution consisted of 3% Comperlan KD as nonionic surfactant, 0.1% Alfoterra 145-5PO and Alfoterra 145-8PO as anionic surfactants, and with NaCl scan. The results show that this method gives the high efficiency extraction up to 85% of the method using hexane Soxhlet extraction. The quality of both palm and soybean oils obtained from different extraction methods--hexane and surfactant-based solution--are similar. Therefore, the researcher concludes that surfactant aqueous-based system is promising as an alternative oil extraction method.

As generally being recognized, *J. curcas* seeds not only contain oil with high nutrient but also contain several types of toxin. Adolf et al. (1984) reports that 12-deoxy-16-hydroxyphorbol extracted from *J. curcas* oil by methanol irritate to the ear of rat. In 1988, Liberalino et al. found that the seeds contain the high protein with a good ratio of essential amino acid; however, the toxicity is very high. All rats were fed with raw seeds died within 2 – 3 days, with cooked seed with in 6 – 8 days, and with roasted seeds within 14 – 16 days. Makkar et al. (1997) extensively studied on

the nutrient and toxin of different provenances of *J. curcas*. Eighteen *J. curcas* in different planting areas from the West and East Africa, North and Central America, and Asia were used in this experiment. They found that there are the diversity in the amount of nutrient and toxin in variety of seed stains. The kernels contain crude protein 19 – 31%, lipid 43 – 59%, neutral detergent fiber 3.5 – 6.1%, and ash 3.4 – 5.0%. The toxins in defatted kernels or meals are trypsin inhibitor activity 18.4 – 27.5 mg of trypsin inhibited/g, saponins 1.8 – 3.4% as diosgenin equivalent, and phytate 6.2 – 10.1% as phytic acid equivalent. Moreover, phorbol esters remain in 17 provenances around 0.87 – 3.32 mg/g of kernel but cannot be detected in the seeds from Papantla, Mexico. Moreover, Makkar et al. (1998) continued their research and found that about 90% of crude protein in *J. curcas* meals is true protein and the levels of essential amino acid meet the FAO reference protein, except lysine.

In 2005, Chivandi et al. compared the nutrient and antinutrient between industrially processed Zimbabwean *J. curcas* and *Glycine max* (soybean) meals. The soybean meals were derived by hexane extraction and the Jatropha meals were derived by hexane-ethanol extraction. They found that Jatropha meals contain crude protein 577.00 g/kg dry mass that has a significant ($p<0.05$) higher than 470.80 g/kg dry mass of soybean. However, phorbol esters were also found in the Jatropha meals about 0.8 mg/g while it cannot be observed this compound in soybean meals. Further result shows that the hexane-ethanol extraction can reduce phorbol esters from 6.5 mg/g of raw kernels to 0.8 mg/g, 87.69% phorbol esters reduction.

To fully utilize *J. curcas* seeds, detoxification methods are needed. Hass and Mittelbach (2000) studied the phorbol esters detoxication in the *J. curcas* seed oil. They investigated the phorbol esters reduction in the traditional oil refining process and found that the oil by pressing extraction contain phorbol esters at about 0.31%. When the oil pass the degumming and deodorization process, there are almost no effect with the amount of phorbol esters. While the deacidification and bleaching can reduce the amount of phorbol esters up to 55%.

Aderibigbe et al. (1997) studied about chemical component, effect of heat to organic matter- and nitrogen-degradability, and some antinutritent of 2 varieties *J. curcas* meal. They found that moist heat treatment (67% moisture at 100°C for 60

min and 80% moisture at 130°C for 30 min can increase in vitro rumen nitrogen degradability (IVDN) and decrease trypsin inhibitor.

Aregheore et al. (2003) investigated detoxification of a toxic variety of *J. curcas* by using heat and chemical treatment. They found that only heat treatment in an autoclave at 121°C for 30 min cannot destroy phorbol esters but can inactivate lectin. Combination between heat and chemical can reduce phorbol esters from meals. NaOH, NaOCl, and methanol were used in this experiment. 4.0% NaOH (w/w) mixed with 10% NaOCl (v/w) can reduce phorbol ester down to 0.13 mg/g. The best method that can reduce phorbol esters from 1.78 mg/g to 0.09 mg/g is heat-treated and washed 4 times with 92% methanol. This method is better to detoxifying *J. curcas* meal but may be unsuitable in economic term. Due to the high protein content in *J. curcas* meal, the researchers carried out the preliminary study on nutritional evaluation with rats by using detoxified meal from the different detoxify method. They found that rats ignored the meal from NaOH-treated because of the strong smell of NaOH while they intaked the diet meal from heat treatment and 4x methanol washing and had gain weight.

Besides heat and chemical treatment to detoxicify phorbol esters in *J. curcas* oil, Nokkaew et al. (2008b) proposed detoxification of phorbol esters in the oil by using several adsorbents. Their results showed that bentonite 200 perform the best on phorbol esters adsorption from *J. curcas* oil. The optimal condition in adsorption process was 3.2% (w/v) of bentonite 200 with 100 rpm stirring rate for 15 min at room temperature which provided 96 – 98% removal. Moreover, they also studied the phorbol esters removal from the press cake or meal. They found that phorbol esters was removed lower as the non-toxic meal when washing meal with 2 – 3% (w/w) of potassium hydroxide for 45 min at room temperature then followed with 95% ethanol for one night.

Although phorbol esters are toxic compound, they are considered as a useful compound. Rug and Ruppel (2000) found that the methanol extract from *J. curcas* oil for enrich phorbol esters, the aqueous extract and *J. curcas* crude oil toxify to intermediate snail hosts and larvae of schistosomes. Among these three liquid, the methanol extract has the highest toxicity to the snails at LC_{50} = 5 ppm and LC_{100} = 25 ppm while Bayluscide® which is a commercial pesticide for snail killing can kill all

snails at 1 ppm. Therefore, to separate phorbol esters from Jatropha oil and its meal will be another target of the research because all composition, oil, meal, and phorbol esters can be fully utilized and this approach can be considered high efficiency on resources utilization.

Thus, in this present study introduced surfactant aqueous-based solution to extract phorbol esters from Jatropha seeds instead of destruction by oxidizing agent. The study emphasized on the removal of phorbol esters from the meals as well as other quality of the meal surfactant solution was evaluated. From the literature review and previous studies (Makkar et al., 1998; Naksuk, 2006; Nokkaew et al., 2008b), the mixing surfactant between nonionic and anionic surfactant were selected for use to extract the phorbol esters from *J. curcas* meals in this present work. Moreover, the sample preparation method before determination by HPLC was investigated to determine the most efficiency extraction procedure in order to obtain more precise and reliable experimental result.