CHAPTER ONE

LITERATURE REVIEW

1.1 Introduction

The demand for fossil-based resources such as gasoline, petro-diesel and natural gas has intensified as a consequence of economic growth worldwide. The substantial use of energy for multiple purposes and consequently, the depletion of natural resources are depleted with time and the impact of environmental pollution piling up due to exhaust emissions has become a major issue. A time will come when these natural resources will be extinct. Furthermore, the exploration of natural petroleum will be more towards deep sea oil exploration which incurs higher costs of production. In the event where cost of drilling and production per litre of crude oil in deep sea exploration is higher than the market price per litre, economically the production of resources is considered as extinction. In short, it is no longer feasible to invest in fossil-based resources. For that reason, it has triggered an interest in researchers to explore alternatives to fossil fuels; primarily biodiesel which are produced from renewable resources. Nowadays, biodiesel from both edible and non-edible vegetable oils has shown a promising solution for supplementing petroleum fuels (Murugesan *et al*., 2009). Biodiesel is well known chemically as the monoalkyl ester of long chain fatty acids synthesised by transesterification process of vegetable oils, animal fats or waste cooking oil with short chain mono-hydric alcohol in the presence of a catalyst. Biodiesel is regarded as an environment-friendly fuel due to its properties such as eco-friendly, renewable, harmless and have least pollutant emissions, particularly carbon monoxide (CO), hydrocarbon (HC) and SOx (Vyas *et al*., 2009; Worapun *et al*., 2012).

Edible vegetable oils like soybean, canola, rapeseed, olive, sunflower and corn oils have been used for biodiesel production. However, the use of such edible oils as petroleum substitutes may not be viable in Malaysia due to high demand of such oils for food and therefore are considerably expensive to be used as biofuels. In view of this, the biodiesel produced from non-edible oils such as *Jatropha (Jatropha curcas), Karanja (Pongamia pinnata), Castor (Ricinus communis),* animal fat, waste oil and tallow can be used since its production does not has an adverse effect on overall food supply. Furthermore, the cost of production of biodiesel from non-edible oils is lower than that from edible oils. Edible oils are much more valuable as cooking oil rather than converting it to non-food applications (Prasad *et al*., 2012; Wang *et al*., 2011). One of the non-edible oils as mentioned earlier, physic nut or *Barbados* nut, *Jatropha curcas* (Linnaeus) plant has great potential as a source for biodiesel. The oil content in *Jatropha curcas* seed is reported to be ranging from 30 to 50% by weight of the seed and 45 to 60% by weight of the kernel (Pramanik, 2003). The advantages of *Jatropha curcas* as compared with the other non-edible plants include rapid growth, higher seed productivity, low plantation costs, minimal input or management and well adapted to marginal areas with poor quality soils or degraded agricultural land, e. g. sandy, gravelly and saline soils (Rietzler and Brandt, 2007; Ramesh *et al*., 2006; Heller, 1996).

1.2 Biological and Chemical Properties of *Jatropha curcas*

Jatropha curcas is one of the non edible oil crops which has a potential to be exploited as a biodiesel source. Over the past decade, *Jatropha curcas* is planted in many areas worldwide for its advantage as a non-food application as compared to edible oils and its ability to grow in dry soil and on degraded land.

1.2.1 *Jatropha curcas* L. Plant

Jatropha is a genus comprising of 160-175 known species of shrubs, rhizomatous shrubs, herbs and small trees, belonging to Joannesieae tribe in the *Euphorbiaceae* family (Puri *et al*., 2007). Central America is said to be the native of the plant but it is commonly found and utilised across most of the tropical and subtropical regions of the world. Its yield is four and ten times higher than soybean and corn, respectively (Nobrega and Sinha, 2007). The physic nut *Jatropha curcas* L. was found by Linnaeus (1753) in "Species Plantarum" and the name is still applicable at present. The name *Jatropha* originated from jatr'os (doctor) and troph'e (food), both are Greek words which connote medicinal use of the plant (Kumar and Sharma, 2008). The physic nut, by definition, is a dicotyledonous deciduous small perennial tree or shrub, which can grow to a height of 8 or 10m. The *Jatropha* tree is a drought-resistant with estimated lifespan of 50 years (Sirisomboon *et al*., 2007). The *Jatropha curcas* plant, fruits with its cross-section and seeds are illustrated in Figure 1.1.

Figure 1.1(A) : *Jatropha curcas* plant

Figure 1.1(B) : Jatropha fruits

Figure 1.1(C) : Cross-section of fruits

Figure 1.1(D) : Jatropha seeds

The plant is monoecious (having unisexual reproductive organs or flowers borne on a single plant) with greenish white flowers, occasionally hermaphroditic flowers take place. Flowering happens during wet season and two flowering peaks are often observed, *i.e*. during summer (May to August) and autumn (September to November). In continuingly humid regions, flowering occurs throughout the year. The hermaphroditic flowers can be self-pollinating or by insects especially honey bees. The complex inflorescences are formed terminally or axillary with the first branching is recemose (borne in a raceme) and subsequent branching is cymose (branch stems that each ends with flower) (Heller, 1996; Kumar and Sharma, 2008). Each inflorescence yields a bunch of approximately ten or more ovoid fruits. The fleshy exocarp of the fruits dried after the seeds mature and three bivalved cocci are developed. The period of the seeds to mature are estimated to be three to four months after flowering season. Normally, five roots are formed from seedlings, one central and four peripheral. A tap root is not usually formed by vegetative propagated plants. The leaves are five to seven lobed, hypostomatic and their stomata are of paracytic (rubiaceous) type (Kumar and Sharma, 2008).

1.2.2 *Jatropha curcas* L. Seeds and Kernels: Physical Properties

Makkar *et al.* (1998) has described the physical characteristic of *Jatropha curcas* fruit and seeds in detail. The fruit has green exocarp which can be subdivided into three parts containing one seed in each. The seed dark brown in colour and has a kidney-shaped structure, with an approximate length of two to three centimetres. It is made up of a dark brown shell and a large yellowish white kernel which can be easily crushed. The hard shell is mainly composed of fibre (more than 83% neutral detergent fibre and more than 74% acid detergent fibre), lignin (more than 45%) with very little crude protein (approximated up to six percent).

The kernel is composed mainly of lipid (53.9 - 58.5%) and crude protein (22.2 - 27.2) with small amount of moisture and ash (estimated up to five percent). Sirisomboon *et al.* (2007) reported the moisture content of different parts of the *Jatropha* fruit. The hull of the fruit contained very high moisture content, 88.95± 0.54% compared to nut shell, 51.87± 1.10% and kernel, 34.09± 0.95%. The moisture contents of hull, nut shell and kernel were determined based on the method of moisture measurement of peanut (ASAE, 1998) with triplicate samples of 200 g each. In the study carried out by Salimon and Ahmed (2012), the physical properties of *Jatropha curcas* seeds from Malaysia, Indonesia and India were discussed and compared. In their work, the oil extracted was in the range of 32.71 – 33.73% at room temperature. The percentage of water was 4.1%, 3.3% and 4.2% for Malaysia *Jatropha curcas* seed oil, Indonesia *Jatropha curcas* seed oil and Indian *Jatropha curcas* seed oil respectively. Other than percentage of water, the refractive index at 25°C and viscosity at 28°C were reported to be in the range of 1.463 to 1.472 and 38±1 to 63±1 respectively.

1.2.3 *Jatropha* oil: Chemical Properties

Jatropha oil content is in the range of 40-60 g of oil per 100 g of kernels (Shah *et al*., 2004). The oil contains approximately 24.60% of crude protein, 47.25% of crude fat and 5.54% of moisture content (Kumar and Sharma, 2008). Triacylglycerol (TAG) is the most abundant (%) glyceridic compound in *Jatropha* oil which encompasses of trimesters of high aliphatic acids or fatty acids, while monoacylglycerol (MAG) (%) and diacylglycerol (DAG) (%) are the minor glyceridic compounds (Naudet, 1996). The chemical structures of partial acylglycerols (MAG and DAG) and TAG were shown in Figure 1.2.

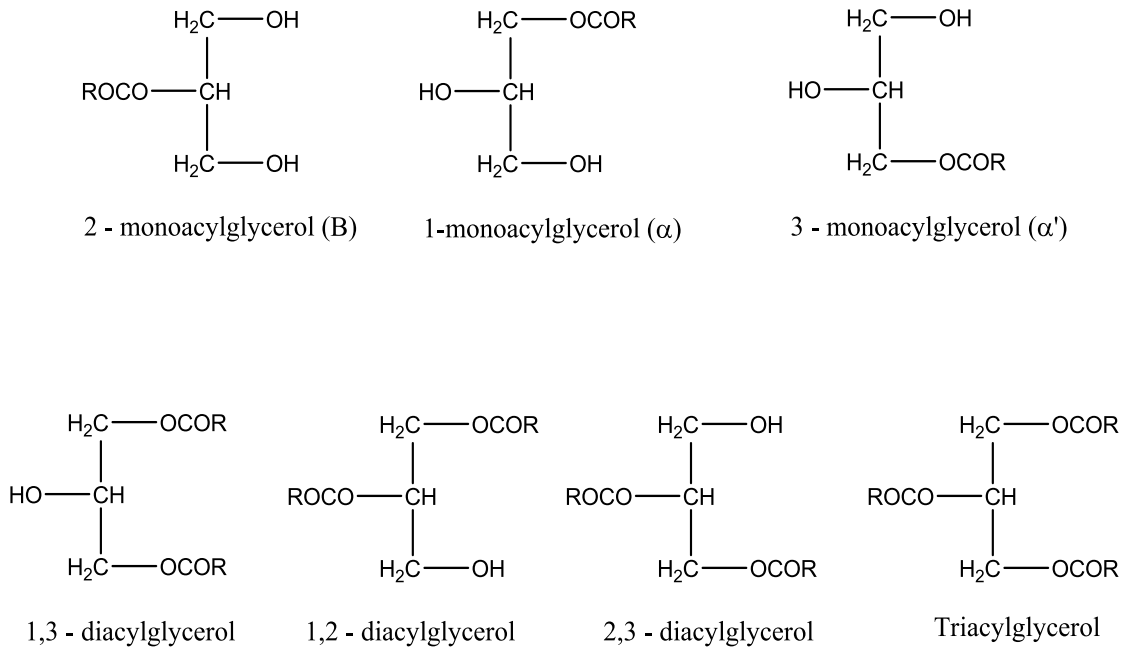


Figure 1.2 : Partial acylglycerols and TAG molecules structures

A review article by Kumar and Sharma (2008) discussed and compared the chemical properties (saponification number and iodine value) of *Jatropha curcas* seeds oil with other types of seed oil namely *Pongamia pinnata* (Pongam tree), *Madhuca indica* (Butter tree), *Euphorbia helioscopia* (Sun spurge or Madwoman's milk) and *Mesua ferrea* (Ceylon ironwood) as presented in Table 1.1.

Table 1.1 : Saponification number (SN) and iodine value (IV) of some selected seed oils

Sources	SN	IV
Jatropha curcas	202.6	93.0
Pongamia pinnata	196.7	80.9
Madhuca indica	202.1	74.2
Euphorbia helioscopia	206.7	170.9
Mesua ferrea	201.0	81.3

Source : Adapted from Kumar and Sharma (2008)

From Table 1.1, the IV of *Jatropha curcas* seed oil was higher than 90 and comparable with other seed oils. IV is a measurement of the unsaturation of fats and oils. According to Knothe (2002), higher IV values indicate higher unsaturation of fats and oils in view of the fact that standard IV for biodiesel is 120 for Europe's EN 14214. Similarly for the comparison of SN, *Jatropha curcas* seed oil has higher SN as compared to *Pongamia pinnata*, *Madhuca indica* and *Mesua ferrea* with no significant difference with *Euphorbia helioscopia.* Salimon and Ahmed (2012) reported the chemical properties of *Jatropha curcas* oil from three different countries, Malaysia, Indonesia and India which include iodine value (IV), acid value, free fatty acid (FFA), saponification value (SV) and unsaponifiable matter. The comparisons of the chemical properties for these three different countries are presented in Table 1.2.

Table 1.2 : Chemical properties of tropical *Jatropha curcas* seeds oil

Characteristic	*Jatropha curcas* seeds oil from Malaysia	*Jatropha curcas* seeds oil from Indonesia	*Jatropha curcas* seeds oil from India
IV, (wijs method)	103.2±0.6	99.8±0.5	97.9±0.7
Acid value, (mg NaOH /g)	2.4±0.2	9.9±0.3	7.6±0.1
FFA as oleic, (%)	1.68±0.02	6.99±0.03	5.35±0.05
SV, (mg/g)	197.8±0.1	183.2±0.2	156.2±0.2
Unsaponifiable matter, (%)	1.99±0.15	2.15±0.06	2.35±0.08

Source : Adopted from Salimon and Ahmed (2012)

In their findings, the chemical properties of selected *Jatropha curcas* samples from Malaysia, Indonesia and India were not significantly different ($p<0.05$) although there were slight differences in their specific parameters values. The iodine value was ranging from 97.9-103 signified that the quality of oil is as fine as commercial vegetable oils in terms of unsaturation level.

Saponification value was comparable (156.5 mg/g-197.8 mg/g) to the typical commercial seeds oil such as corn oil (187 mg/g-193 mg/g) and cottonseed oil (189 mg/g-198 mg/g) (Gunstone, 2002). Unsaponification value was comparable (1.99-2.35%) to Gunstone's analysis (2002) of corn seeds oil (1-3%) and higher than cotton seeds oil (0.5-0.7%) and soybean seed oil (1.6%). Acid value of Malaysia's *Jatropha curcas* seeds oil showed low number of 2.4 compared to India's *Jatropha curcas* seeds oil and Indonesia's *Jatropha curcas* seeds oil which are 7.6 and 9.9, respectively.

1.2.3.1 Fatty Acid Composition of *Jatropha* Oil

Fatty acid composition (FAC) of *Jatropha* oil lies within a very specific range of 14 to 22 carbon numbers. It was reported that the major fatty acids are oleic and linoleic acids, followed by palmitic, stearic and palmitoleic acids (Salimon and Ahmed, 2012). Therefore, *Jatropha* oil can be categorized as oleic - linoleic oil. Table 1.3 depicts the FAC of *Jatropha* oil.

Table 1.3 : Fatty acid composition of *Jatropha* oil (% methyl ester)

Composition (as % methyl ester)	
Myristic (C14:0)	0 – 0.1
Palmitic (C16:0)	14.0 – 15.3
Palmitoleic (C16:1)	0 – 1.3
Margaric (C17:0)	0 - 0.1
Stearic (C18:0)	3.7 – 9.8
Oleic (C18:1)	34.3 – 45.8
Linoleic (C18:2)	29.0 – 44.2
Linolenic (C18:3)	0 – 0.3
Arachidic (C20:0)	0 – 0.3
Behenic (C22:0)	0 – 0.2

Source: Adapted from Salimon and Ahmed (2012), Akbar *et al.* (2009) and Gubitz *et al.* (1999)

1.2.3.2 The Chemical Behaviour of Ester Group in Oil Molecules

Acylglycerols are composed of esters that appended to the backbone of glycerol. In natural oils and fats, 90 to 96% of the total molar mass of triacylglycerol (TAG) consist of ester groups (Naudet, 1996). The ester groups in TAG exhibit significant roles in the chemical and physical properties of the oil. As for saturated TAG, the straight structures or chains of fatty acids do not specify any distinctive chemical functional group. Carboxylic group in TAG molecules act as the functional group for chemical reactions. The carbonyl or ester group of the TAG can actively be involved in many chemical reactions by inducing a particular reactivity at the α-carbon (Ucciani and Debal, 1996). Figure 1.3 shows the graphical behaviour of nucleophilic carbonyl carbon with the acidic hydrogen at the α-carbon (Rousseau and Marangoni, 2002).

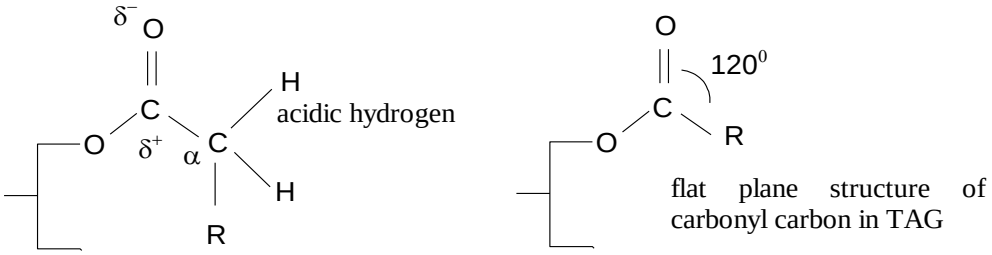


Figure 1.3 : The chemical function of acyl group in TAG

The electronegative oxygen withdraws an electron pair from the carbonyl carbon that led to partial positive charge on the carbon. This partial positively charged carbon can be attacked by nucleophiles. In addition, the sp2 orbital of the carbonyl carbon with flat plane structure may allow easier access of nucleophiles to the carbonyl carbon. The electronegativity of oxygen that is attached to the carbonyl carbon may also increase the acidity of the hydrogen attached to the α-carbon (Rousseau and Marangoni, 2002). These ester groups in TAGs are responsible for several chemical reactions during modification of oils and fats, such as alcoholysis, transesterification, interesterification, reduction (hydrogenolysis), hydrolysis and saponification (Ucciani and Debal, 1996).

1.2.4 Chemical Composition and Inedible Aspect of *Jatropha curcas*

Comprehensive information about the presence of minor chemical constituents including toxins in different parts of *Jatropha curcas* plant are important so as to fully utilise the potential of the plant. In the review paper by Kumar and Sharma (2008), they have summarised the compounds isolated from various parts of *Jatropha curcas* plant as depicted in Table 1.4.

Table 1.4 : Compounds isolated from different parts of *Jatropha curcas* plant

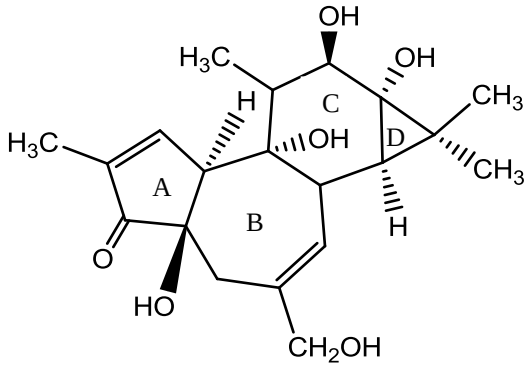
Various part	Chemical composition
Aerial	Organic acids (*o*- and *p*-coumaric acid), *p*-OH benzoic acid, protocatechuic acid, resorsilic acid, saponins and tannins)
Stembark	β-Amyrin, β-sitosterol and taraxerol
Leaf	Cyclic triterpenes stigmasterol, stigmast-5-en-3β, 7 β-diol, stigmast-5-en-3β, 7 α-diol, cholest-5-en-3β, 7 β-diol, cholest-5-en-3fl, 7α-diol, campesterol, β-sitosterol, 7-keto- β-sitosterol as well as the β-D-glucoside of β-sitosterol. Flavonoids apigenin, vitexin, isovitexin. Leaf also contain the dimer of a triterpene alcohol ($C_{63}H_{117}O_9$) and two flavonoidal glycosides
Latex	Curcacycline A, a cyclic octapeptide Curcain (a protease)
Seed	Curcin (a lectin) Phorbol esters Esterase and Lipase
Kernel	Phytates, saponins and a trypsine inhibitor
Root	β-sitosterol and its β-D-glucoside, marmesin, propacin, the curculathyranes A and B and the curcusones A-D. diterpenoids jatrophol and jatropholone A and B, the coumarin tomentin, the coumarin-lignan jatrophin as well as taraxerol.

Source : Adopted from Kumar and Sharma (2008)

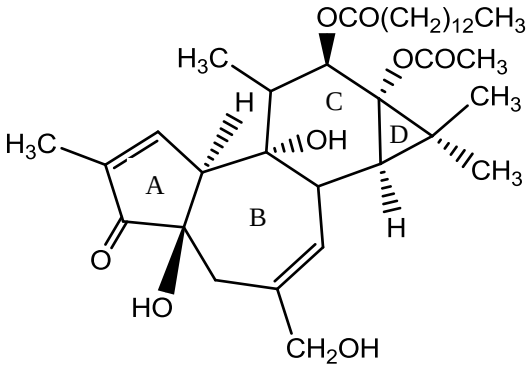
Although seeds of physic nut are a good source of triacylglycerol, there are toxic and anti-nutritive compounds in the seeds of *Jatropha curcas* due to the presence of curcin (a toxic lectin) and phorbol esters. The presence of phorbol esters in *Jatropha* seed and kernel has been identified as the main toxic agent accountable for *Jatropha* toxicity (Adolf *et al*., 1984; Makkar *et al*., 1997). Therefore, either *Jatropha* seeds or *Jatropha* oil are most unlikely to be used in food application for fear of contamination. Its usage in the non-food application particularly, biodiesel production fits well.

1.2.4.1 Phorbol Esters

The name 'phorbol' is used to represent the family of naturally occuring compounds that can be referred to as tigliane diterpenes (Evans, 1986). Phorbol esters are defined as "polycyclic compounds in which two hydroxyl groups on neighbouring carbon atoms are esterified to fatty acids" (Goel *et al*., 2007). The active phorbol esters, TPA (4β-12-O-tetradecanoylphorbol-13-acetate), are the tetracyclic diterpenoids commonly acknowledged for their tumor promoting activity. Several other plants, namely, *Sapium indicum, S. japonicum, Euphorbia frankiana, E. cocrulescence, E. ticulli, Croton spareiflorus, C. tigilium, C. ciliatoglandulifer, Excoecaria agallocha and Homalanthus nutans*, are also reported to contain toxic phorbols (Beutler *et al*., 1989). Phorbol esters have the basic skeleton of tetracyclic diterpene carbon framework known as tigliane. Phorbol is considered as a basic alcohol moiety in the phorbol esters as illustrated in Figure 1.4.



Phorbol

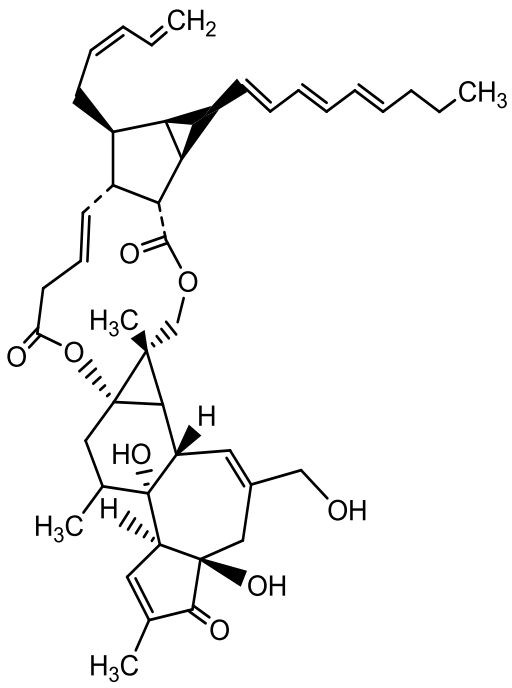


Tetradecanoyl Phorbol-13-Acetate

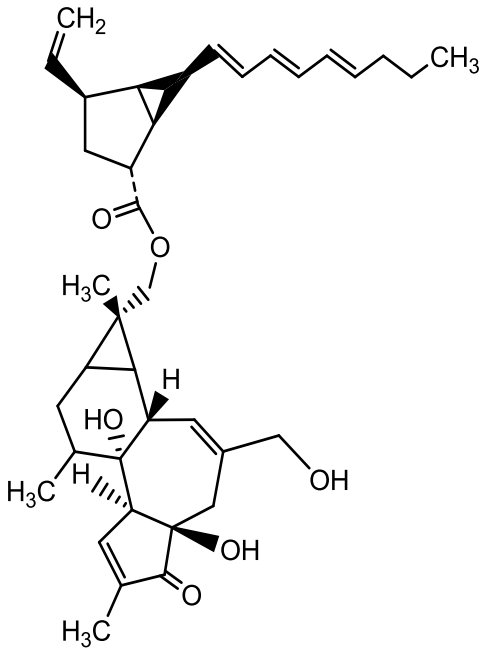
Figure 1.4 : Structures of Phorbol and Tetradecanoyl Phorbol-13-Acetate

Phorbol, which is the parent diterpene of phorbol esters, contains five hydroxyl groups with distinctive reactivity towards acylation (Goel, *et al*., 2007; Hecker and Schmidt, 1974). Ring A is attached to the seven membered ring B. Ring C, a cyclohexane, is cis-linked to Ring D. Since there are possibility of different position of OH group in Ring C, the phorbols can be divided into two groups, α and β. The stereochemistry of the OH group in the phorbols structure makes the phorbols either an active (β) or inactive (α) form.

The dormant α-phorbol esters have analogous lipophilicity and physicochemical properties as the active β phorbol, but are incapable to stimulate the interaction between phorbol esters and protein kinase C (which regulates assorted signal transduction pathways and other cellular metabolic activities) due to conformational shifts (Silinsky and Searl, 2003). The active phorbols, TPA and PDBu (4β-phorbol-12, 13-dibutyrate) vary only by their substitutions at positions 12 and 13 in Ring C (Goel, *et al*., 2007). In the study done by Haas *et al.* (2002), six types of phorbol esters were identified, namely, *Jatropha* factors C1 to C6 as shown in Figure 1.5.

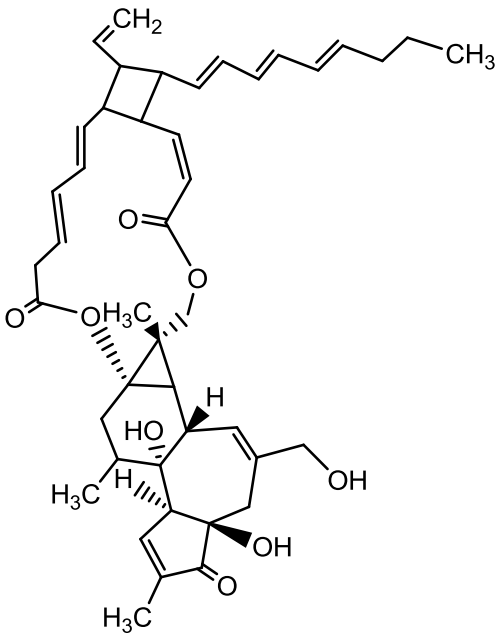


Jatropha Factor C_1

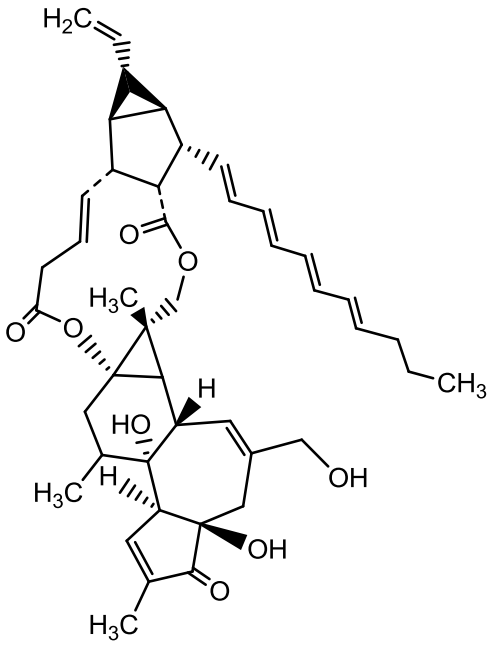


Jatropha Factor C_2

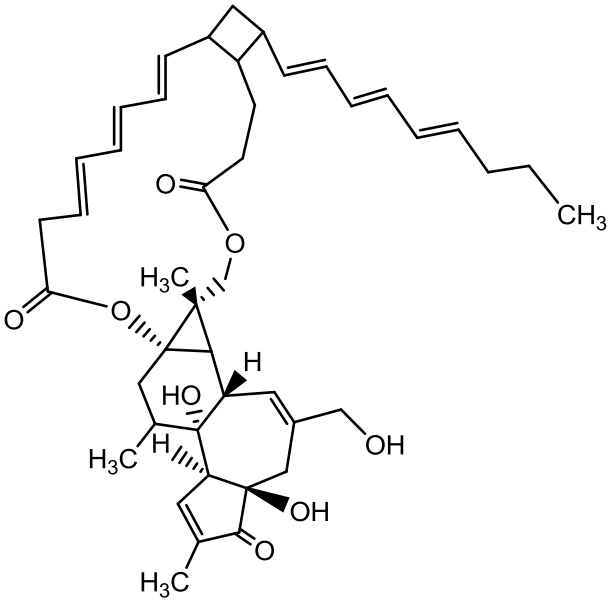
Figure 1.5 : Phorbol esters identified in *Jatropha curcas* seeds and oil.



Jatropha Factor C_3



Jatropha Factor C_4 and C_5



Jatropha Factor C_6

‘**Figure 1.5**, continued’

1.2.4.2 Toxicity of *Jatropha* Oil towards Human and Animals

It was reported that in 1854, in Birmingham, more than thirty children were intoxicated by *Jatropha curcas* seeds (Gubitz *et al*., 1999). In recent years, in South America, South Africa and Asia, numerous cases of *Jatropha curcas* poisoning in humans have been reported after accidental ingestion of the seeds (Gubitz *et al*., 1999). Symptoms such as vomiting, diarrhoea and giddiness and in extreme condition, death have been recorded (Becker and Makkar, 1998). In most cases, ingestion of three to five seeds causes marked nausea, gastro-intestinal irritation, abdominal pain, vomiting and sometimes diarrhoea. In severe cases, clinically dehydrated patients nevertheless made rapid recovery after intravevenous fluid replacement (Joubert *et al*., 1984; Abdu *et al*., 1986; Mampane *et al*., 1987).

Even though all the patients fully recovered, we should not underrate the toxic components of this oil crop as Shah and Sanmukhani (2010) reported that the inception of *Jatropha curcas* poisoning in human is very fast and immediate. The phorbol esters affect human and animals biologically by causing tumor promotion, lymphocyte mitogenesis, blood platelet activation, cell proliferation, inflammation (erythema of the skin), prostaglandin production and stimulation of degranulation in neutrophils (Aitken, 1986; Haas and Mittelbach, 2000). A recent finding by Raghunath *et al.* (2012) testified on the new symptom by an eight years old child following the consumption of the *Jatropha curcas* nuts. Besides mild gastroenteritis and hypovolemic shock, purpuric spots were developed over whole of the body as exhibited in Figure 1.6. The purpuric spots were noticeable all over the knees and healed after three weeks.

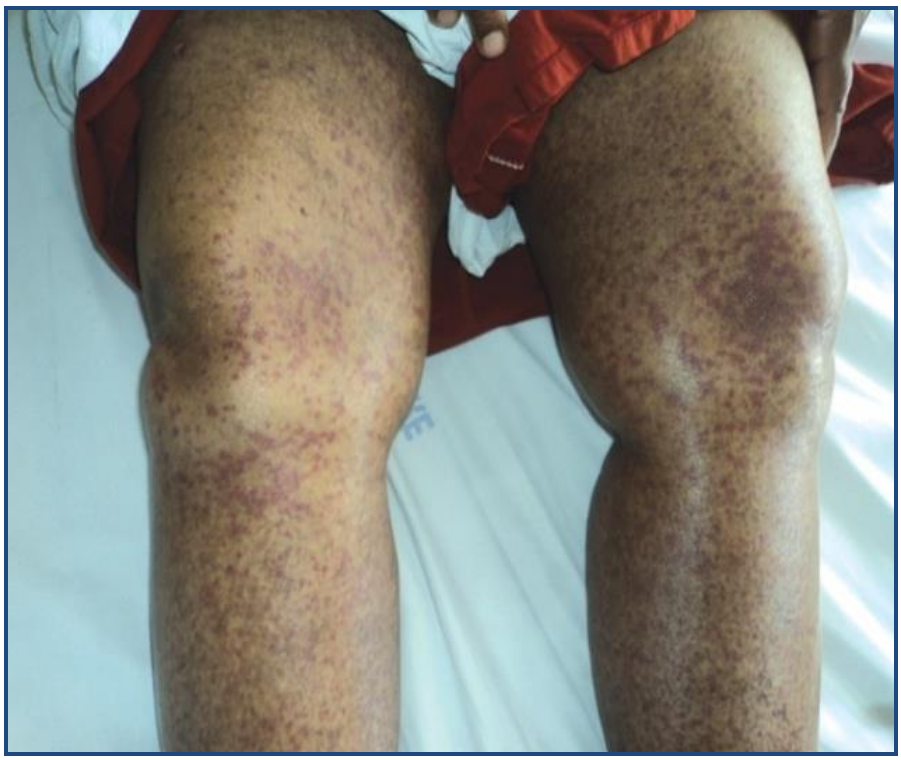


Figure 1.6 : Multiple purpuric spots over the lower limbs.

The toxicity studies have also been reported for human, rodents and livestock (Adam, 1974; Adam and Magzoub, 1975; Ahmed and Adam, 1979; Joubert *et al*., 1984). Several authors have reported on intoxication in rats after being fed with *Jatropha* oil from an Indian diversity and the symptom were severe diarrhoea, dehydration and inflammation of intestines (Gandhi *et al*., 1995). In a large number of the studies, the animals were force-fed either raw *Jatropha curcas* seeds or defatted seed meals, leaves or assorted organic solvents or aqueous extracts. Article by Gubitz *et al*. (1999) on the exploitation of *Jatropha curcas*, described the minimal lethal dose of *Jatropha* seeds for three different animal species as tabulated in Table 1.5.

Table 1.5 : Minimal lethal dose of *Jatropha curcas* seeds in animals

Animal	Amount of seeds fed		Death (day)
	Ratio of mass of seeds fed per mass of animal, (g/kg)	Total mass of seed fed, (g)	
Sheep	7.4	67	9
Goat	1.5	8	12
Calf	3.0	36	12

Source : Adapted from Gubitz *et al*. (1999)

1.2.4.3 Extraction and Detection of Phorbol Esters in *Jatropha* Oil

Haas and Mittelbach (2000) suggested the usage of high performance liquid chromatography (HPLC) to determine the phorbol esters content. Briefly, the extraction of phorbol esters was carried out repeatedly by using technical grade methanol and the combined extracts were centrifuged and transferred into a 100 mL volumetric flask, which was topped up with methanol. 4β,9α,12β,13α,20-pentahydroxytiglia-1,6-dien-3-on-12β-myristate-13-acetate in methanol was used as an external standard for quantification.

Ahmed and Salimon (2009) reported on phorbol ester content of three different countries of tropical *Jatropha curcas* seed from Malaysia, Indonesia and India by using HPLC method as suggested by Haas and Mittelbach (2000). A comparison of the phorbol ester peaks with an external standard phorbol ester (4α-phorbol-12,13-didecanoate) and soybean oil peak was carried out in order to calculate the total content of phorbol esters. In their finding, it was depicted that the phorbol ester level was low in Malaysian seed oil (0.23%), whereas the level of phorbol esters on Indonesian and Indian seed oil were 1.58% and 0.58% respectively.

In 2010, Saetae and Suntornsuk detected toxic compounds and anti-nutritional factors in *Jatropha curcas* seed cake and its protein isolated by ethanol extraction and analysed by HPLC according to the modified method of Haas and Mittelbach (2000). The phorbol 12-myristate 13-acetate (PMA) (Sigma Chemical) was used as an external standard. In their study, high levels of phorbol esters (0.73±0.06 mg/g; calculated in equivalent to PMA) and lectin contents (13.15±0.45 Hemagglutinating units (HU)/mg protein) were identified in *Jatropha curcas* seed cake whereas they were not detected in the detoxified *Jatropha curcas* seed cake and its protein isolated from the detoxified seed cake. The observation suggests that ethanol extraction could be effective method to completely remove the phorbol esters and lectin. *Jatropha* meal can be fully utilised for various industrial applications including edible product and by-product if the toxic and anti-nutritive factors are effectively removed.

1.2.5 Application of *Jatropha curcas*

Jatropha plant has been used for different purposes in different societies throughout the world. It is used as ornamental plants in South and Central America, Africa and Asia. Typically, *Jatropha* plant is grown as a live hedge to protect agricultural fields from damage by animals such as cattle or goats as it can be easily propagated by seeds and branch cuttings (Gubitz *et al.*, 1999). Besides improving yield of seeds, the hedge also act as inexpensive bio-fence as compared to wire fence (Kumar and Sharma, 2008). The first commercial application of *Jatropha curcas* was reported from Lisbon, where the oil imported from Cape Verde, was used for soap production and as potential domestic fuel for cooking and lightings. (Gubitz *et al*., 1999; Islam *et al*., 2011).

All parts of *Jatropha curcas* have high prospective to be used in conventional medication and for veterinary purposes due to its purgative properties, to be applied externally, in treating skin infection and rheumatism. Decoction of the leaves have been used as treatment for coughs or as antiseptics after birth while the branches can be used as chewing sticks (Heller, 1996 and Gubitz *et al.,* 1999). The latex of *Jatropha curcas* contains an alkaloid known as "Jatrophine" is believed to possess anti-cancerous properties (Nabi *et al*., 2007). The average of 30-35% of linoleic acid (C18:2) content in *Jatropha* kernel has great possible interest in skincare production (Kumar and Sharma, 2008). Table 1.6 presents the medicinal uses of various parts of *Jatropha*.

Table 1.6 : Medicinal uses of the various parts of *Jatropha curcas*

Part of the plant	Medicinal use
Seeds[a]	To treat arthritis, gout and jaundice
Tender twig / stem	Toothache, gum inflammation, gum bleeding, pyorrhoea
Plant sap	Dermatomucosal diseases
Plant extracts	Allergies, burns, cuts and wounds, inflammation, leprosy, leucoderma, scabies and small pox
Water extracts of branches	HIV and tumor
Roots	Antidotes for snakebite

[a]External application

Source : Adapted from Kumar and Sharma (2008); Islam *et al.* (2011)

The bark, leaf and tender stems of *Jatropha curcas* yields a dark blue to blackish brown dye which is reported to be used for colouring cloth, fish nets and lines. The plants and fruit hulls are used for firewood. *Jatropha* wood is very light wood and is not popular as fuel wood source because it burns rapidly. Seed cake results in very high-quality charcoal that has the potential to be used in high valued markets. But pressed cake is much more valuable to use as an organic manure to increase crop production in marginal land (Kumar and Sharma, 2008).

In the last few years, the application of *Jatropha* oil as a fuel in diesel engines has been reported by many researchers (Gubitz *et al*., 1999; Mohibbe *et al*., 2005; Kumar and Sharma, 2008; Achten *et al*., 2008; Koh and Ghazi, 2011). This non-edible oil is a source for biodiesel production without compromising the food industry (Tapanes *et al*., 2008; Divakara *et al.*, 2010). *Jatropha* oil can be used as fuel in diesel engines directly and by blending it with methanol (Gubitz *et al.*, 1999). *Jatropha curcas* has been acknowledged to be applied for biodiesel production since it has desirable physicochemical and performance characteristics as diesel fuel (Jain and Sharma, 2010). The following Table 1.7 presents the comparison of *Jatropha* oil and *Jatropha* oil methyl ester specifications with biodiesel standards, such as European Standard (EN 14214) and American Standard (ASTM D6751).

Table 1.7 : The comparison of *Jatropha* oil and *Jatropha* oil methyl ester specifications with biodiesel standards

Properties	*Jatropha* oil	*Jatropha* oil methyl ester	Biodiesel standards[e]	
			EN 14214	ASTM D6751
Density at 15°C (kg m^{-3})	918.6[b]	880[b]	860-900	-
Viscosity at 40°C (mm^2 s^{-1})	49.93[b]	2.35-2.47[c]	3.5-5.0	1.9-6.0
Cetane index	40-45[b]	50[b]	51<	47<
Flash point (°C)	210-240[c]	170[b]	120<	130<
Pour point (°C)	-3[c]	-6 to 2[c]		-15 to 10[d]
Carbon residue (%)	64[b]	0.5	0.3>	0.50>

[b]Kumar and Sharma (2008)
[c]Koh and Ghazi (2011)
[d]ASTM D6751-02
[e]ASTM D6751 and EN 14214

Source : Adapted from Kumar and Sharma (2008); Koh and Ghazi (2011) and ASTM Standards (2003)

Based on the Table 1.7, *Jatropha* oil and *Jatropha* oil methyl ester met with the standard requirements of American and European countries. In addition to that, it is significant to point out that the oil of *Jatropha curcas* is a viable alternative to diesel fuel.

1.3 Oil Extraction Process

Oil extraction is conducted in many different ways depending on several factors such as the morphology and size of fruit or oil seed, cost and oil quality. Various oil extraction methods available are, namely, mechanical extraction, solvent extraction, aqueous extraction and enzymatic assisted aqueous oil extraction.

1.3.1 Mechanical Extraction

The mechanical oil extraction is considered a conventional method and widely used to obtain the *Jatropha* oil due to its simplicity, reliable and reasonably inexpensive. It can be executed by batch processes, mainly hydraulic pressing, and by continuous processes either mechanically or working presses. Both batch and continuous processes of mechanical extraction, apply pressure on the oil seeds to facilitate oil release.

Salimon and Ahmed (2012) had extracted dried *Jatropha* seeds by using cool mechanical extraction (homemade screwed-press extruder). The percentage of oil extracted was found to be in the range of 30.32 to 33.73%. This result was in agreement with previous work by Chhetri *et al.* (2008) which reported of low oil yield (27.8%) of *Jatropha* oil extracted by using cold pressing method. Both findings were compared with oil yield obtained by solvent extraction, 60% (Salimon and Rozaini, 2008).

The cold press extraction produced about 75% of the total oil extracted by the solvent given that the organic solvent used has a greater ability to extract most of the oil available in the oil seed (Gunstone *et al*. 2007).

1.3.2 Solvent Extraction

In general, solvent extraction of oil can be defined as a process of hauling out the oil molecules that are partly bound to the protein and carbohydrate complexes of oil seeds by diffusing the oil fraction from the oil seeds into a non-polar solvent such as hexane. Generally, solvent extraction is dependent on the nature of the solvent and oil, reaction time between solvent and seeds, temperature of the process, particle size of the seed and the ratio of solvent to the meal (Sayyar *et al*., 2009).

Hexane has been commercially used in the oil extraction of edible and non-edible oilseeds due to its low vaporization temperature (boiling point 63° - 69°C), low corrosiveness, low greasy residual effect, and pleasant aroma and flavour for the milled products (Becker, 1978; Johnson and Lucas, 1983). The oil recovery from solvent extraction that can be achieved is up to 99% as compared to mechanical extraction which is limited to 90-95% of the oil present in the seeds (Beerens, 2010). Although solvent extraction produces high oil recovery in a large scale of oil extraction, unfortunately in long term production, the usage of hydrocarbon solvent such as hexane or petroleum ether can affect human health and the environment as more volatile products are liberated into the atmosphere (Rosenthal *et al*., 1996). The disadvantages of solvent extraction method have also been reported by Bargale (1997). The overall process of solvent extraction is hazardous to human health. Some traces of solvents are constantly present in the meal even though desolventisation has eliminated a large amount of the solvents used in the oil extraction.

As a result, toxic meals are produced. As a consequence of these limitations of solvent extraction, a number of new combination approaches such as two-phase solvent extraction (Rubin *et al*., 1984; Liu *et al.*, 1994) and enzyme assisted solvent extraction (Sosulski *et al.*, 1988; Dominguez *et al.*, 1995; Tano and Ohta, 1995) have been utilised in order to improve solvent extraction technology.

1.3.3 Aqueous Oil Extraction

Aqueous extraction has received much interest in recent years as it is deemed to be an environmentally cleaner method as compared to conventional methods of oil extraction. In addition, since it eliminates solvent consumption, aqueous extraction has advantages in terms of cost savings, environment and safety concerns as well as nutrition issues (Rosenthal *et al*., 1996).

Unfortunately, the oil yield obtained using this method alone produced low oil yield and required longer duration time of extraction and relatively high oil content in the residue and in some cases in the protein isolate. This problem has discouraged the commercial application of aqueous oil extraction method (Rosenthal *et al*., 1996). Soya bean (Yoon *et al*., 1991), avocado (Buenrostro and Lopez, 1986), sunflower (Lanzani *et al.*, 1975) and coconut oils (Barrios *et al.*, 1990) are examples of edible oils which had been investigated using this method at laboratory scale.

1.3.4 Enzymatic Assisted Aqueous Oil Extraction

Enzymatic assisted aqueous oil extraction (EAAOE) is one of the alternative environment-friendly oil extraction process based on isolation of oil and protein from the oil seeds by suspending fine powder seeds in water and removing out the oil layer after centrifugation process (Sharma *et al.*, 2002[b]).

This method can eliminate the possibility of hazardous detonation since the whole process is in water medium (Rosenthal *et al.*, 1996). This relatively new technique has been investigated using various oil seeds such as peanut (Rosenthal *et al*., 1996), sunflower (Lanzani *et al*., 1975), rice bran (Sharma *et al*., 2001), white pitaya (Rui *et al*., 2009) and sesame (Latif and Anwar, 2010).

Several researchers had attempted to extract *Jatropha* oil by EAAOE. In most EAAOE reported, the enzymes used were usually made up of varieties such as protease, cellulase, pectinase, xylanase and amylase (Shah *et al*., 2005). Winkler *et al*. (1997) reported the EAAOE using several cell wall degrading enzymes for extracting *Jatropha* oil. The best result, 86% oil recovery or 47.3% oil yield (based on oil content) was obtained using an alkaline protease. However, the oil yield reported by Shah *et al.*(2005) was lower than oil yield reported by Winkler *et al.* (1997), 64% oil recovery or 28.2% (based on oil content) by using alkaline protease component. The differences in oil yield obtained were due to the inherent nature of seed material used (Shah *et al*., 2005). The selection of protease and cellulase in the EAAOE were parallel with the morphology and composition of the kernel. Oil in the kernel of *Jatropha* was dispersed in matrices of protein and cellulosic cells. In order to release the oil, both protein and cellulosic complexes must be broken down. In this respect, protease was stipulated to be involved in the hydrolysis of protein in the cell membranes, lipid body membranes and in the cytoplasm of the oilseeds while cellulase will break the structure of cotyledon cell walls present in the kernel. In the review paper by Rosenthal *et al.* (1996), the proteolytic enzymes interrupt the cytoplasmic network which is mainly composed of proteins, therefore making the internal structure less strictly bound and dense thus enabling easier elimination of protein and lipid in the cell. Figure 1.7 illustrates the effect of enzyme treatment on a ground oilseed.

It can be observed from Figure 1.7 that enzyme action makes the composition of ground oilseed more permeable; the extent depends on particle size (Rosenthal *et al*., 1996).

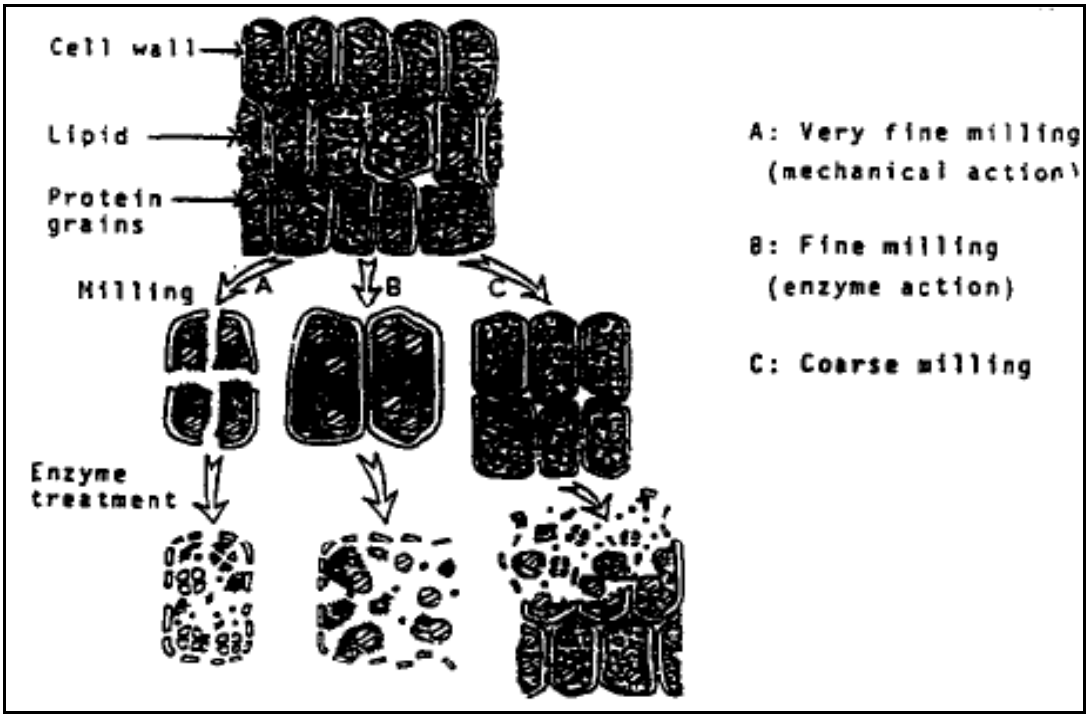


Figure 1.7 : Effect of milling and and enzymatic treatment on oilseed cell structure

Source : Adopted from Rosenthal *et al.* (1996)

1.4 Biodiesel

Biodiesel is an alternative diesel substitute which is comprised of mono-alkyl esters of long chain fatty acids. Biodiesel is commonly referred to as fatty acid methyl esters (FAME) which can be derived from edible and non-edible oils (*e.g.* palm, peanut, rapeseed, canola, olive, soybean, sunflower, safflower, coconut) or animal fats (*e.g.* tallow) as well as recycled waste oils (*e.g.* waste cooking oils). The most frequent method used for mass production of biodiesel is by transesterification of triacylglycerols (TAGs). The preference of feedstock depends mostly on the inherent properties of the oil and the quality of final product may vary and modification to the production may be necessary (Knothe *et al.*, 2005). Currently, the most common feedstock for biodiesel production is from edible oils.

Nevertheless, this approach has raised objections from several associations, claiming biodiesel is competing for resources with the food industry (Koh and Ghazi, 2011; Prasad *et al*., 2012). Qian *et al.* (2010) reported that the usage of such edible oil for biodiesel in China is not feasible, as they are far too expensive to be used at present due to lack of supply. For this reason, the usage of non-edible oil as the feedstock for biodiesel is highly recommended. *Jatropha curcas* has been found to be more suitable for biodiesel production as it has advantages as non-edible oil as well as its characteristics and productivity as discussed in Section 1.2. Besides its advantages such as biodegradable and renewable, *Jatropha* oil methyl esters has a number of other well established advantages such as high flash point (190-195°C); which lead to safer handling and storage, and excellent lubricity (Gubitz, 1999; Knothe *et al*., 2005; Berchmans and Hirata, 2008). There are a few methods that have been introduced to reduce the high viscosity of vegetables to facilitate their uses in universal diesel engines without problem, namely, blending with petrodiesel, pyrolysis, microemulsification (cosolvent blending), esterification and transesterification (Schwab *et al.*, 1987 and Knothe *et al.*, 2005).

1.4.1 Esterification Process

Esterification is the chemical method for ester preparation. In general, a free fatty acid of degraded oil (*e. g*. waste cooking oils), react with an alcohol in a presence of heat to produce an alkyl ester and water. The process varies from the transesterification process in that the reaction happens directly between the alcohol and the fatty acid molecule. There are no intermediate steps of cleaving the fatty acid chains from the glycerol backbone. Therefore, no glycerol is produced during esterification reaction (Altic, 2010). The esterification reaction of a fatty acid molecule with alcohol to form a methyl ester and water molecule as a side product was shown in Figure 1.8.

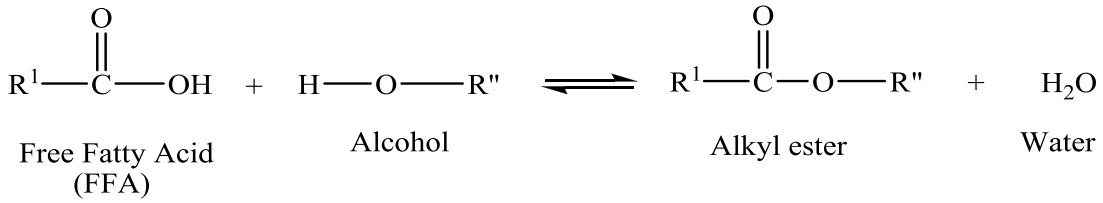


Figure 1.8 : Basic esterification reaction

In the esterification reaction, the stoichiometry of reaction is a 1:1 molar ratio of alcohol to FFA, to produce one mole of biodiesel and one mole of water, where R^1 represents long chain of fatty acids. Short chain alcohols, for instance methanol and ethanol react in the esterification process rapidly because they are relatively small molecules and contain no carbon atom side chains that would hinder their reaction (Bhatt and Patel, 2012).

1.4.2 Transesterification

Transesterification is considerably the most standard method that leads to the products typically recognised as biodiesel. Transesterification process is also known as alcoholysis which involves the reaction of triacylglycerols with alcohols (*e.g.* methanol, ethanol, propanol or butanol) in the presence of a catalyst to produce alkyl esters of oils and fats and glycerol as a by-product. Figure 1.9 depicts the transesterification reaction.

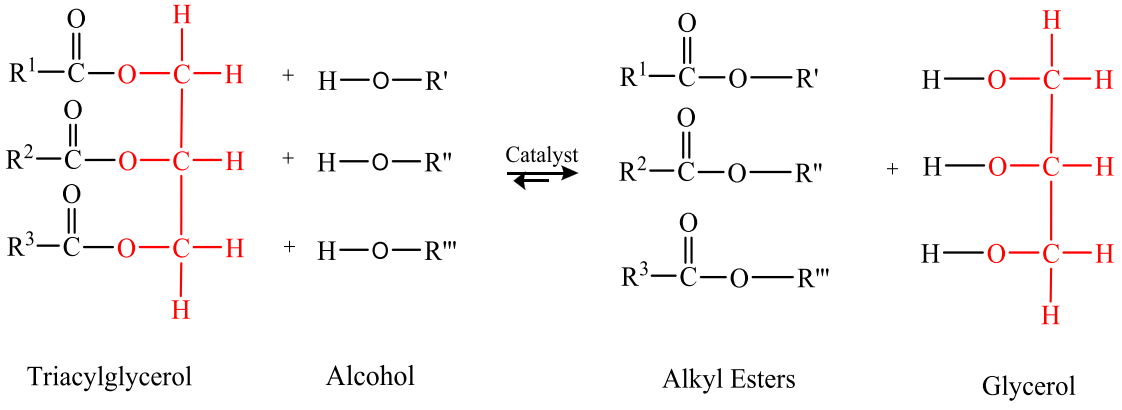


Figure 1.9 : General transesterification reaction

In the transesterification reaction, di- and monoacylglycerols are formed as intermediates (Knothe, 2005). The stoichiometry of the reaction is a 3:1 molar ratio of alcohol to oil, to produce three moles of biodiesel and one mole of glycerol, where R^1, R^2, and R^3 represent long chain of fatty acid of carbon number 12 to 18. In theory, since transesterification is a reversible reaction, the presence of excess alcohol and catalyst (a strong acid or base) are essential to accelerate the conversion and to shift the equilibrium towards the formation of alkyl esters (the right side of the transesterification reaction) (Ma and Hanna, 1999; Knothe *et al*., 2005). For this reason, the reverse reaction of transesterification does not occur or is mostly negligible in the production of vegetable oil alkyl esters (Knothe *et al*., 2005).

Methanol is preferable to be used in the process due to its low cost as compared with other alcohols even though other alcohols such as ethanol, isopropanol, butanol or amyl alcohol produce biodiesel with enhanced fuel properties (*e.g.* better cold flow properties) (Choo *et al*., 2005; Ramadhas *et al.*, 2005; Knothe *et al.*, 2005). In regards to biodiesel fuel with enhanced fuel properties, the comparison of fuel characteristics of alkyl esters of crude palm oil (CPO) was reported by Choo *et al.* (2005) as in Table 1.8.

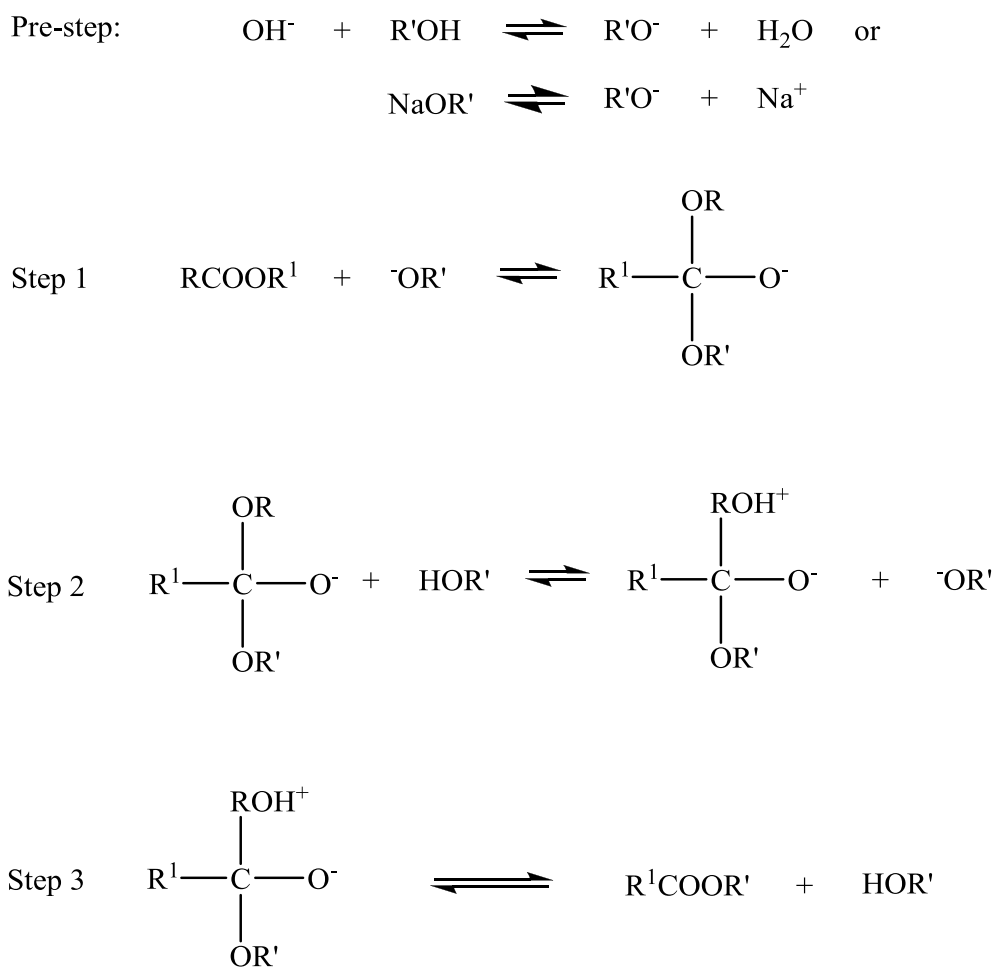
Table 1.8 : The comparison of fuel characteristics of alkyl esters of crude palm oil (CPO)

Test	Methyl esters	Ethyl esters	Isopropyl esters
Density at 40°C, (kg/L)	0.855	0.857	0.854
Viscosity at 40°C, ($\times 10^{-6}$ m^2s^{-1})	4.4	4.7	5.2
Pour Point, (°C)	15	12	6
Cloud point, (°C)	16	16	7

Source : Adapted from Choo *et al.* (2005)

In their findings, ethyl and isopropyl esters possessed good fuel properties such as lower pour point (6 to 12°C) and higher viscosity (4.7 to 5.2 × 10^{-6} m^2s^{-1}) as compared with methyl esters. Nevertheless, methyl ester exhibited comparable fuel properties and may be the favoured selection in terms of availability and cost. Moreover, methanol reacts with triacylglycerol (TAG) rapidly because of its properties, for instance; polar character and short chain alcohol (Sanli and Canakci, 2008; Demirbas, 2005; Knothe *et al*., 2005). Therefore, the duration of transesterification is shorter. Meneghetti *et al.* (2006) reported that the production of biodiesel from castor oil was faster using methanol than ethanol. The optimum yield of esters was obtained after one hour of reaction time with methanol or five hours with ethanol. In spite of that, there are exceptions in some countries. In Brazil, for example, where ethanol is relatively inexpensive and readily available, ethyl esters are used as fuel (Knothe *et al*., 2005). Overall, transesterification reaction can be preceded either by base catalysts (*e.g.* sodium hydroxide or potassium hydroxides or sodium methoxide) or acid catalysts (*e.g.* sulphuric acid or hydrochloric acid) to enhance the reaction rate and ester yield.

The catalyst was mixed alcohol and alkoxide group was formed (Sridharan and Mathai, 1974). The reaction mechanism for base-catalysed transesterification was formulated in three major steps (Eckey, 1956; Sridharan and Mathai, 1974; Demirbas, 2008). The first step of the mechanism was an attack on the carbonyl carbon atom of the triacylglycerols molecule by the anion of the alcohol (methoxide ion) to form a tetrahedral intermediate. Subsequently, in the second step, the tetrahedral intermediate reacted with an alcohol (methanol) to regenerate the anion of the alcohol (methoxide ion). In the final step, rearrangement of the tetrahedral intermediate resulted in the formation of a fatty acid ester and a diacylglycerol. Figure 1.10 summarises the mechanism of base-catalysed transesterification.



Notes: where R-OH: diacylglycerols, R^1: long chain alkyl group, and R': short alkyl group

Figure 1.10 : The mechanism of base-catalysed transesterification of triacylglycerols with alcohol (Eckey, 1956)

In the review article by Mathiyazhagan and Ganapathi (2011), transesterification process containing high free fatty acid (FFA) oil samples (more than 1%), a base catalyst is unfavourable. The presence of high FFA would lead the reaction to saponification reaction as the reaction consumes large amount of base catalyst to neutralise the FFA. Figure 1.11 illustrates hydrolysis reaction of alkyl esters and saponification reaction of FFA alkyl ester with KOH catalyst.

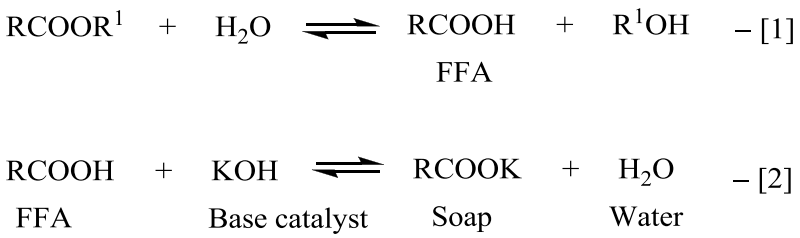


Figure 1.11 : Hydrolysis of alkyl esters and saponification reaction of FFA with KOH catalyst

Water is formed during saponification reaction (the base catalysts will react with the FFA to form soap and water) (Equation 1, Figure 1.11), impedes the transesterification through the hydrolysis reaction of alkyl esters (Equation 2, Figure 1.11) (Komintarachat and Chuepeng, 2010; Encinar *et al.*, 2007). This reaction is undesirable as soap formation lowers ester yield and causes difficulty in the separation of alkyl esters from the glycerol (Komintarachat and Chuepeng, 2010; Gerpen, 2005). Thus, acid catalyst will be more efficient to catalyse transesterification of oil with higher FFA content and the reaction demands more alcohol than base catalyst. As such, waste cooking oil requires higher ratio of alcohol *i.e.* 15:1 when subjected to acid catalysed reaction (Mathiyazhagan and Ganapathi, 2011). Besides type of catalyst (either base or acid), transesterification reaction is also dependent on other reaction parameters which include molar ratio of alcohol to oil, temperature, reaction time, catalyst concentration, agitation speed and degree of refinement. Inaccurate assessment of these parameters in transesterification reaction would affect the biodiesel production and difficulty to achieve optimal product yield (Mathiyazhagan and Ganapathi, 2011).

1.4.3 Standards for Biodiesel

Technical standards are essential for quality control and assurance. It is necessary to give guarantee to the biodiesel quality and therefore it is necessary to assess the properties of biodiesel produced such as its fuel properties, hazards, safety and environmental concerns. Hence, an approved biodiesel standard is significant to gain wider acceptance from producers, suppliers, retailers and end users as the standards will limit the amount of contaminants in biodiesel fuel (Knothe, 2005; Prankl and Worgetter, 1999).

In 1991, the primary quality standard, ON C 1990 was introduced by Austria, together with other European countries (Körbitz, 1997). Eventually, the European Union (EU) established the biodiesel standard EN 14214 in 2003, which superseded individual country standards (DIN, 2003). Likewise, the US passed American Society for Testing and Materials (ASTM) D 6751 in 2001 which standardise 14 fuel properties including kinematic viscosity, water content, cetane number, flash point, phosphorus content, carbon residue and cloud point (ASTM, 2003).

Nicolas and Repussard (1994) have mentioned that a standard is a written document approved by a recognised body in accordance with the International Standard Organisation (ISO). Other than that, a standard is also characterised as a written document available to the public and drawn up by the consensus parties concerned and is to the benefit of all. It is intended for repeated or continuous application and normally not mandatory, except for being explicitly referred to in regulations (Prankl and Worgetter, 1999). The specifications of the two most important international biodiesel standards used to analyse biodiesel, ASTM D6751 and EN14214 are presented in Table 1.9 and Table 1.10 respectively.

Table 1.9 : The standard specifications of American standard for biodiesel fuels (ASTM D6751)

Property	Limits		Test Method
	Minimum	Maximum	
Flash point, °C	130.0	-	D93
Kinematic viscosity at 40°C, mm^2/s	1.9	6.0	D445
Cetane number	47	-	D613
Sulphated ash content, %(m/m)	-	0.020	D874
Copper strip corrosion	-	No. 3	D130
Acid value, mg KOH/g	-	0.80	D664
Free glycerol, %(m/m)	-	0.20	D6584
Total glycerol, %(m/m)	-	0.240	D6584
Phosphorous content, %(m/m)	-	0.001	D4951
Carbon residue (100% sample), %(m/m)	-	0.050	D4530
Cloud point, °C	Report customer		D2500
Distillation T90 AET, °C	-	360	D1160
Sulfur (S 15 Grade), ppm	-	0.0015	D5453
Sulfur (S 500 Grade), ppm	-	0.05	D5453
Water and sediment, %vol.	-	0.050	D2709

Source : Adopted from ASTM D6751

Table 1.10 : The standard specifications of European standard for biodiesel fuels (EN 14214)

Property	Limits		Test Method
	Minimum	Maximum	
Flash point, °C	101.0	-	ISO CD3679e
Kinematic viscosity at 40°C, mm^2/s	3.5	5.0	EN ISO 3104
Cetane number	51.0	-	EN ISO 5165
Sulphated ash content, %(m/m)	-	0.02	ISO 3987
Copper strip corrosion (3 h at 50°C), rating	Class 1	-	EN ISO 2160
Acid value, mg KOH/g	-	0.5	pr EN 14104
Free glycerol, %(m/m)	-	0.02	pr EN 14105m pr EN 14106
Total glycerol, %(m/m)	-	0.25	pr EN 14105m
Phosphorous content, %(m/m)	-	0.01	pr EN 141101
Carbon residue (10% bottoms), %(m/m)	-	0.3	EN ISO 10370
Density at 15°C, kg/m^3	860	900	EN ISO 3675 EN ISO 12185
Sulfur content, mg/kg	-	10	-
Water content, mg/kg	-	500	EN ISO 12937
Total contamination, mg/kg	-	24	EN 12662
Oxidation stability at 110°C, h	6	-	pr EN 14112
Iodine value	-	120	pr EN 14111
Linolenic acid methyl ester, %(m/m)	-	12	pr EN 14103d
Polyunsaturated (≥4 double bonds) methyl esters, %(m/m)	-	1	pr EN 14103
Ester content, %(m/m)	96.5	-	pr EN 14103d
Methanol content, %(m/m)	-	0.2	pr EN 141101
Monoglyceride content, %(m/m)	-	0.8	pr EN 14105m
Diglyceride content, %(m/m)	-	0.2	pr EN 14105m
Triglyceride content, %(m/m)	-	0.2	pr EN 14105m
Akaline metals (Na + K), mg/kg	-	5	pr EN 14108 pr EN14109

Source : Adopted from EN14214

A comparison of the parameters specified between the European Biodiesel Standard (EN 14214) and the US Biodiesel Standard (ASTM D6751) from the Table 1.9 and Table 1.10 showed that the limits in both standards are the same or almost similar. The main differences between these standards are their proposed applications and the specified test methods. The EN14214 emerged to be more comprehensive and specific than ASTM D6751. It has more stringent specification on viscosity, cetane number and copper strip corrosion; and included additional parameters such as ash content, total contamination, oxidative stability, density, iodine value, contents of ester, linolenic acid methyl ester, polyunsaturated methyl ester, mono-, di- and triacylglycerols.

1.4.4 Benefits of Biodiesel

There are many distinct benefits of using biodiesel as compared with diesel fuel (Cao, 2008). Biodiesel is considered to be environmental friendly, biodegradable and renewable fuels as compare with diesel fuel. It is derived from renewable natural resources, thus reducing dependency on petroleum diesel. It can be domestically produced, offering the possibility of reducing petroleum imports. Reductions of exhaust emissions in relative to conventional diesel fuel, by generating lower emissions of hydrocarbons, particulates and carbon monoxide. Biodiesel has a relatively higher flash point (> 150°C), leading to safer handling and storage. Biodiesel provides greater lubricity than petroleum diesel, thus reducing engine wear and tear. In fact, biodiesel can be used as a lubricity enhancer for low sulphur petroleum diesel formulations (Cao, 2008).

1.5 Objectives of Present Study

The present study is aimed at investigating the singular and combinatory effect of enzyme used, namely, Alcalase® 2.4L and Celluclast® 1.5L, in combination and step-wise addition of enzymatic assisted aqueous oil extraction (EAAOE); and the role of each parameter such as particle sizes of the seeds, pH, ratio of distilled water to mass of *Jatropha* seeds, enzyme concentration, duration and temperature of incubation time during the oil extraction stage. The present study also looked into an improved oil recovery from the oil-water mixture after extraction and to further improve the oil yield. The effect of ultrasonication process on enzymatic extraction was explored and monitored under the observation of polarising microscope. The understanding of each parameter involved in the extraction stage will enable the establishment of an optimum extraction process.

The improved oil recovery from the oil-water mixture after extraction has further improved the oil yield. *Jatropha* oil was evaluated for its physicochemical properties such as density, free fatty acid content or acidity, moisture content, iodine value, peroxide value and saponification value; the results attained were compared with the data of the *Jatropha* oil extraction using solvent extraction by means of Soxhlet method. The determination of fatty acid composition of *Jatropha* oil was done by using gas chromatography - flame ionisation detector (GC-FID) and gas chromatography – mass spectrometry (GCMS). In addition to that, the detection of toxic compounds in *Jatropha* oil, phorbol esters was investigated by using high performance liquid chromatography (HPLC) and liquid chromatography – mass spectrometry (LCMS). Besides oil extraction process, the transesterification process of *Jatropha curcas* was investigated. The transesterification process was concentrated on high free fatty acid content of *Jatropha* oil.

Two types of experiments were designated which include double-stage transesterification and pre-treatment of high free fatty acid content by acid catalysed esterification prior to transesterification process. The percentage of ester content of the methyl ester was calculated and determined by using gas chromatography - flame ionisation detector (GC-FID). The *Jatropha* oil methyl ester obtained was evaluated accordingly.

To summarise, objectives of present study are as below:

i. To investigate and optimise the parameters involved in the enzymatic assisted aqueous extraction process of *Jatropha curcas* oil.

ii. To investigate the transesterification process of *Jatropha curcas* oil which has high free fatty acid content.

iii. To analyse the properties of *Jatropha* oil and *Jatropha* oil methyl esters.

iv. To quantify toxic compounds (phorbol esters) in *Jatropha curcas* using high performance liquid chromatography (HPLC) and liquid chromatography mass spectrometry (LCMS).