

Chemistry of Phorbol Ester Toxicity in *Jatropha curcas* Seed – A Review

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ABSTRACT

The toxicity of *Jatropha curcas* seeds emanates mainly from phorbol esters. It is known that the seeds are a valuable source of oil, which can be used to produce biodiesel. The cake generated as a by-product, after oil pressing is a protein-rich potential stock feed. Researchers, the world over, have studied these phorbol esters and investigated ways of making the seed meal more useful than the *status quo*.

This review paper outlines the previous research done on the *Jatropha curcas* plant, the toxicity of phorbol esters and the efforts made to detoxify the seed. It also highlights the knowledge gaps concerning the chemistry of phorbol ester toxicity and the probable areas of future research.

Key words: phorbol ester, toxicity, detoxification, *Jatropha curcas*

INTRODUCTION

Jatropha curcas has been touted as a world-wide wonder plant. It is drought resistant, making it suitable for low-rainfall areas. The seeds contain greater than 30% oil that can be processed to produce good-quality biodiesel and soap. Unfortunately, the remaining high-protein seed cake cannot be used as stock feed because it contains toxic phorbol esters. Studies have shown that the nutritional value of *Jatropha* seed cake equals that of soya bean, and better than sunflower and cotton seed meal, making it an ideal stock feed, if detoxified.

Scientists have focused their attention on the detoxification of the seed cake and a number of methods have been suggested. Although some physical and microbiological detoxification methods have been forwarded, they are generally not cost-effective. It is believed that a chemical detoxification technique can be much faster and cheaper. Other work has been done on the chemical nature and toxicity of phorbol esters. However, the chemistry of phorbol ester toxicity is still not well understood.

JATROPHA CURCAS PLANT

Family:	Euphorbiaceae
Genus:	<i>Jatropha</i>
Species:	<i>Jatropha curcas</i> Linnaeus

Common name: Physic nut

Swedish naturalist, Carolus Linnaeus (1753) was the first to name physic nut, *Jatropha L.* (Kumar and Sharma, 2008). The name “jatropha” is derived from the Greek words, *iatrós* (physician) and *trophé* (nutrition), hence the common name, *physic nut*. *Jatropha* is a genus of approximately 175 succulent plants, shrubs and trees in the Euphorbiaceae family (Wirawan, 2009). Physic nut, by definition, is a small deciduous tree or large shrub, which can reach a height of 3 – 5 metres, but is capable of attaining a height of 8 – 10 metres under favourable conditions (Kumar and Sharma, 2008). It bears fruits from the second year of its establishment and the economic yield stabilizes from the fourth or fifth year, onwards (Jimu *et al.*, 2009). The average seed-bearing life span of the plant has been recorded as 50 years (Karavina *et al.*, 2011).

Physic nut has excellent adaptation capacity to a large variety of soil conditions. Cultivation on dry, stony and very shallow soils is frequent (Makkar and Becker, 2009). It can be established on marginal land, paddocks, contour ridges, hilly slopes and gullies (Tigere *et al.*, 2006). However, *Jatropha curcas* does not tolerate sticky, impermeable and waterlogged soils. The plant also requires sufficient sunshine and cannot grow well under shade (Ye *et al.*, 2009).

The delineation of the original area of species distribution of *Jatropha* has been the subject of a long debate but, currently, there is a growing agreement that the *Jatropha* centre of origin is Mexico and continental Central America. Portuguese people learned

about *Jatropha*'s medicinal properties in the 16th Century, and later established commercial plantations for soap and lamp oil production on the Cape Verdean Islands and Guinea Bissau. Later, *Jatropha* genotypes adopted in West Africa were spread across other Portuguese colonies in Africa (Mozambique, Angola) and into Asia (India, China and Indonesia). *Jatropha* now grows pantropically, from Brazil to the islands of Fiji (Achten *et al.*, 2010). Two genotypes of *Jatropha curcas* exist: the toxic and the non-toxic varieties. The latter genotype is found in Mexico only (Makkar and Becker, 2009). Another non-toxic species, also found in Mexico, is *Jatropha platyphylla* Müell (Makkar *et al.*, 2010).

Seeds of *Jatropha curcas* plant are rich in oil and protein. Significant quantities of macrominerals (Na, K, Mg, Ca, P) and microminerals (Mn, Fe, Zn) have also been found in the seeds (Abou-Arab and Abu-Salem, 2010). The seeds contain about 300 – 350g/kg oil, which can be used as a fuel directly or in its transesterified form, as a substitute for diesel. The protein quality of the meal obtained from shelled *Jatropha* seeds is high. Levels of essential amino acids (except lysine) are higher in *Jatropha* seed cake than in the FAO reference protein for a growing child of 3 – 5 years (Makkar *et al.*, 2008). *Jatropha curcas* meals contain high true protein, high energy and low fibre. The nutritional value of *jatropha* meal compares favourably with those from conventional seed meals, such as soyabean (Makkar, Aderibigbe and Becker, 1998). Research done by Chivandi and others (2005) indicated higher crude protein, ash, calcium and phosphorus in *Jatropha* seed meal than in soyabean meal.

Jatropha curcas L. is probably the most highly promoted oilseed crop at present in the world. It is famous for being a potential source of raw material for biodiesel. The popularity of *Jatropha curcas* stems from the widespread general knowledge that it is a non-edible, oil-yielding tree, well adapted to marginal areas with poor soil and low rainfall, where it grows without competing with annual food crops (Soerawidjaja, 2010). Physico-chemical and performance characteristics of *Jatropha* biodiesel are comparable to those of fossil diesel and conform to the American and European standards (Parawira, 2010). Current raw materials for biodiesel are soybean oil (USA), rapeseed oil (Europe), palm and coconut oils (South East Asia) and tallow and lard (Australia and Newzealand), all of which are edible (Soerawidjaja, 2010). In fact, biodiesel was found to have a number of advantages over mineral fuels, including being safe, non-polluting and renewable (Karavina *et al.*, 2011).

There are many other uses of *Jatropha curcas* L. Traditionally, the plant has been used as a hedge and living fence, not edible by livestock. Growing *Jatropha* reclaims eroded and waste land. The seed oil can be used as a fuel for lighting and is a raw material for making high quality soap. Residue from seed pressing is good organic fertilizer (Yan, 2008). All parts of *Jatropha* (seeds, leaves and bark) have been used in traditional medicine and for veterinary purposes for a long time (Kumar and Sharma, 2008). This is typical of most members of the Euphorbiaceae family, whose medicinal properties may be due to stress factors such as drought and extreme temperatures that induce synthesis of survival and defence chemicals (Mwine and Van Damme, 2011). *Jatropha curcas* seed oil has insecticidal properties, especially against *Callosobruchus maculatus* – a seed

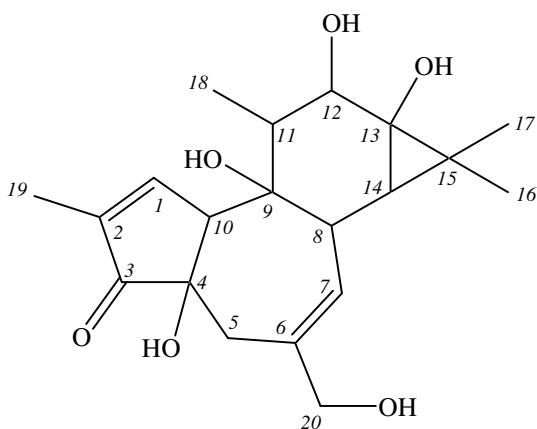
beetle in cowpeas (Adebawale and Adedire, 2006), *Cnaphalocrocis medinalis* – rice leaf folder and *Helicoverpa armigera* – cotton boll worm (Dowlathabad *et al.*, 2010). Alcohol extract from *Jatropha* leaf was found to have antibiotic effect on *Escherichia coli* and *Staphylococcus aureus* (Ye *et al.*, 2009). The toxicity of *Jatropha curcas* to mollusks has been investigated because of its relevance in schistosomiasis control. Extracts from *J. curcas* L. were found to be toxic against snails (Goel *et al.*, 2007). Ethanolic extracts of JCL seed cake also showed antifungal activities against important fungal phytopathogens: *Fusarium oxysporum*, *Pythium aphanidermatum*, *Lasiodiplodia theobromae*, *Curvularia lunata*, *Fusarium semitectum*, *Colletotrichum capsici* and *Colletotrichum gloeosporioides* (Saetae and Suntornsuck, 2010).

The International *Jatropha* Organization has claimed that in 2017 there will be around 33 million hectares of *Jatropha curcas* cultivated land worldwide, producing 160 million tons of seeds and 95% of its total production will be in Asia (Devappa *et al.*, 2010).

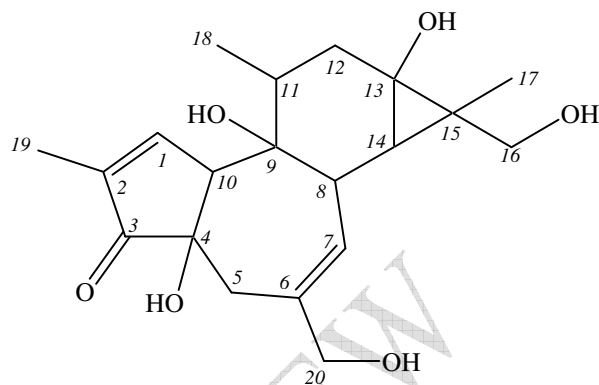
PHORBOL ESTERS

The term ‘phorbol esters’ is used today to describe a naturally occurring group of compounds mainly distributed in plant species of the Euphorbiaceae family. Phorbol esters are esters of phorbol, a tetracyclic diterpenoid with a tigliane skeletal structure (Haas and Mittelbach, 2000). {Terpenoids are classified according to the number of carbon atoms they carry: monoterpenoids have 10 carbons, diterpenoids have 20, and so on}

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phorbol

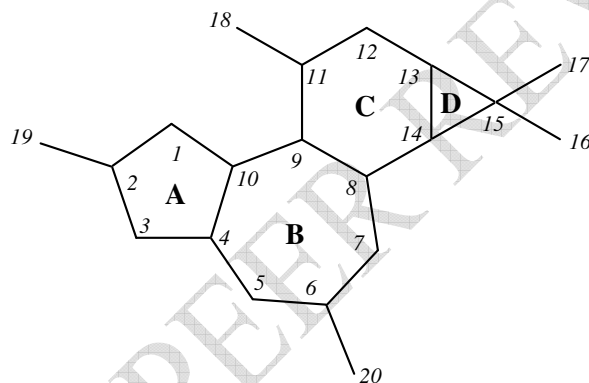


12-deoxy-16-hydroxyphorbol

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tiglane

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145 Phorbol itself was isolated as a non-toxic crystalline solid from the plant, *Croton tiglium*

146 *L.* (Crombie *et al.*, 1968), but its ester, TPA (12-*O*-tetradecanoylphorbol-13-acetate),

147 present in croton oil, is the most toxic and most studied phorbol ester, known to be a co-

148 carcinogen (Devappa *et al.*, 2010; Olivier *et al.*, 1992). However, phorbol esters from

149 *Jatropha curcas* are derivatives of 12-deoxy-16-hydroxyphorbol (Haas *et al.*, 2002).

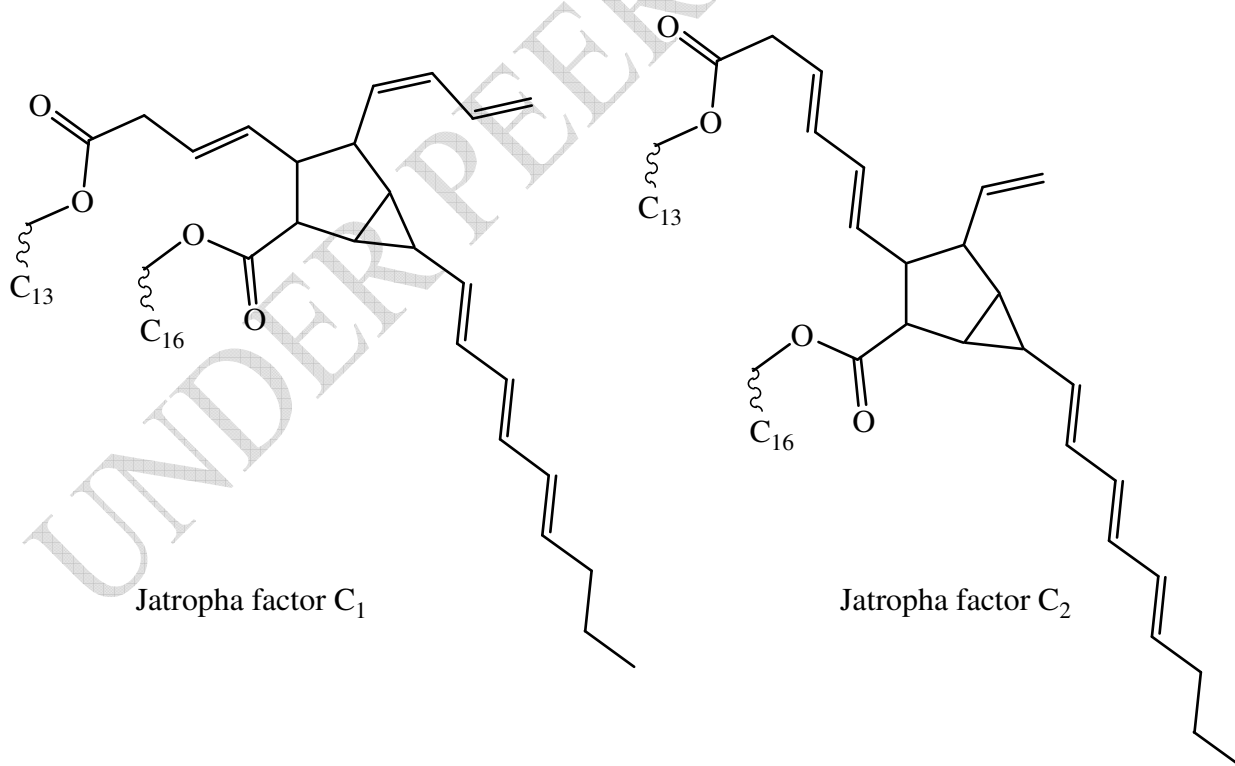
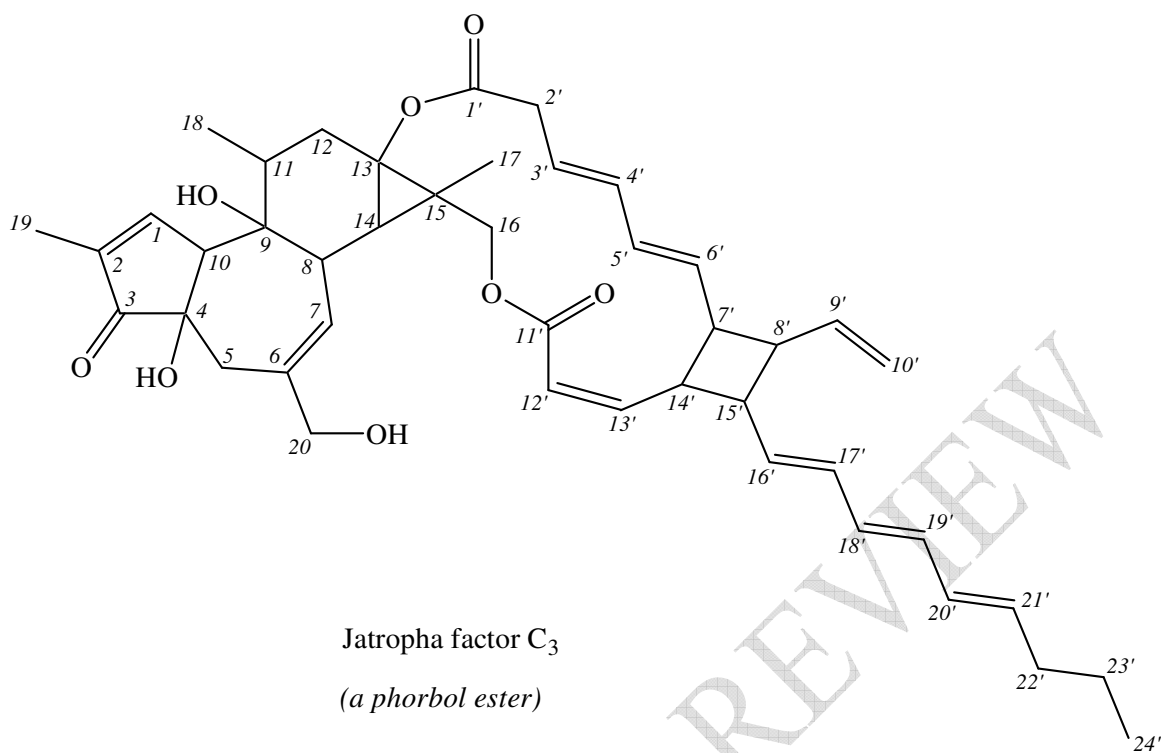
150 Phorbol esters with the same diterpenoid moiety have also been extracted from the latex

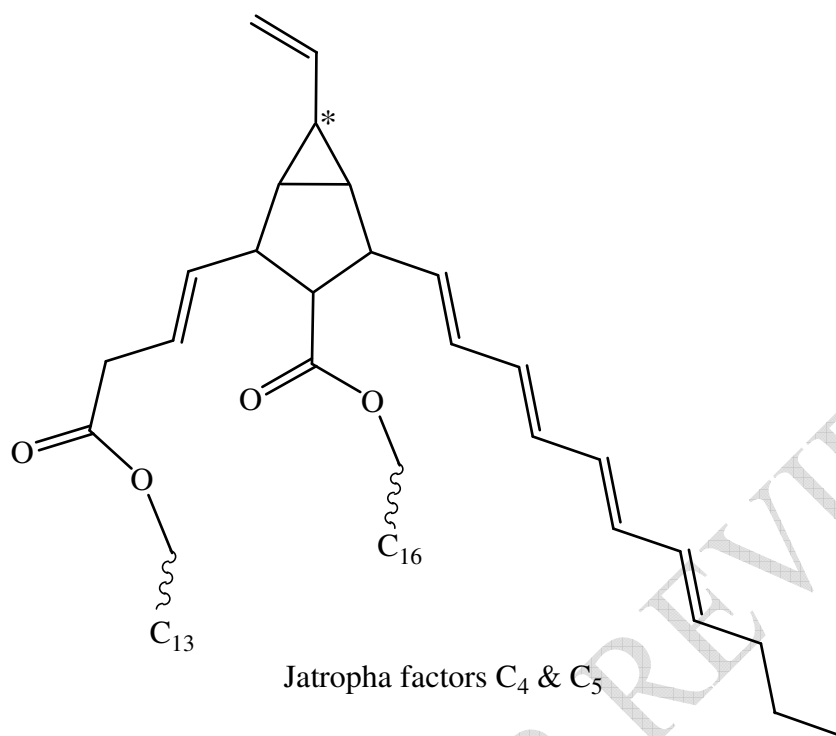
151 of *Euphorbia cooperi* (Gschwendt and Hecker, 1970).

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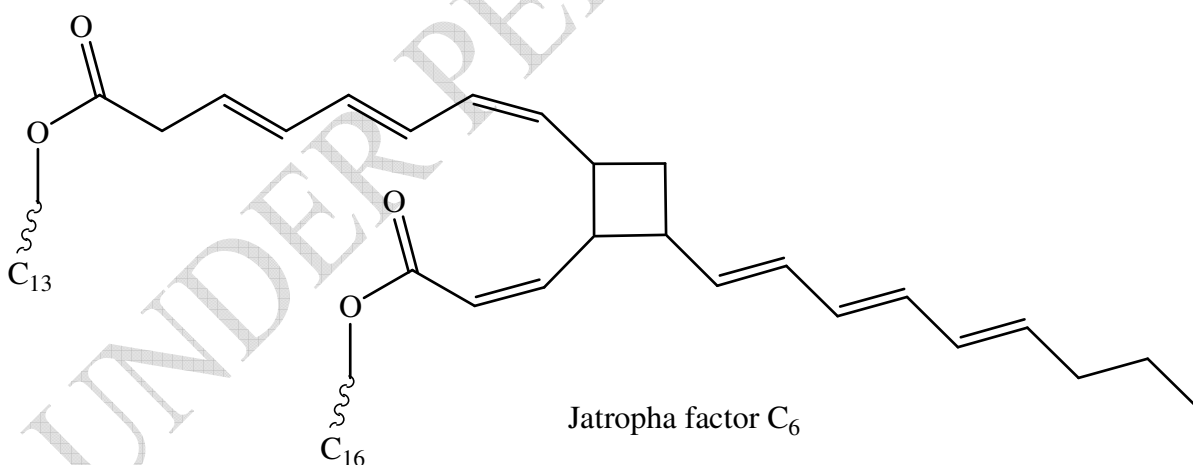
Adolf and co-workers (1984) were the first to identify phorbol esters in *Jatropha curcas* L. Although they concluded that the irritant principles of the plant represented 12-deoxy-16-hydroxyphorbol-13-acylates with highly unsaturated acid moieties, they could not elucidate their exact chemical structures. Hirota and fellow researchers (1988) managed to identify the macrocyclic, dicarboxylic acid, diester structure of one of the phorbol esters and showed that it is a tumor promoter with weaker activity than TPA. Later, Haas and others (2002) isolated and did a thorough structural determination on all phorbol esters found in *Jatropha curcas*.

Six phorbol esters have been characterized from *Jatropha curcas* seed oil and designated as Jatropha factors C₁, C₂, C₃, epimers C₄ & C₅ and C₆, with the molecular formula C₄₄H₅₄O₈. All of them are intra-molecular diesters of the same diterpenoid. Jatropha factor C₆ was found to be the least stable. It has been assumed that the diester group biosynthetically arises from two originally separated monoacids (Haas *et al.*, 2002). The full structure of jatropha factor C₃ is shown below; the other esters are given without the 12-deoxy-16-hydroxyphorbol portion.





(They are isomeric at C*; C₄ has S and C₅ has R configuration)



The content of phorbol esters in *Jatropha curcas* can vary according to the region in which the plant grows. Ahmed and Salimon (2009) have shown a variation in the phorbol ester content of the *Jatropha curcas* seed oil from Malaysia (0.23%), Indonesia (1.58%)

and India (0.58%). Saetae and Suntornsuk (2010) demonstrated a variation in phorbol ester content in *Jatropha curcas* plants from different provinces of Thailand. However, Makkar, Aderibigbe and Becker (1998) had previously found similar levels of phorbol esters in plants from Cape Verde (0.27%), Nicaragua (0.22%) and Nigeria (0.23%).

Although the phorbol esters are distributed in different parts of the *Jatropha curcas* plant (Makkar and Becker, 2009), they are concentrated in the seed kernel and their levels in seed oil are higher than those in the seed cake (Hipal *et al.*, 2009).

PHORBOL ESTER TOXICITY

Phorbol esters are the most potent tumor promoters known. They exhibit a remarkable ability to amplify the effect of a carcinogen but are themselves not carcinogenic (Wender *et al.*, 1998). The seeds from *J. curcas* have been reported to be toxic to humans, rodents and livestock and phorbol esters have been identified as the main toxic agent (Becker and Makkar, 1998). Symptoms of intoxication in humans are: burning and pain in mouth and throat, vomiting, delirium, muscle shock, decrease in visual capacity and high pulse. In Nigeria and Burkina Faso, seeds and seed oil are added to Strophanthus arrow poison. In Gabon seeds are grated with palm oil to kill rats. The Shamba of Usambara region of Tanzania used *Jatropha* seeds as an ordeal poison (Wink *et al.*, 1997). Toxicity to sheep, goats, calves, chicks and fish by consumption of *Jatropha* seed or seed meal has been reported. The seed has also the highest known molluscicidal activity (Demissie and Lele, 2010). Common symptoms of topical application to rabbits, mice and rats were erythema,

oedema, necrosis, diarrhea, scaling and thickening of skins. Ingestion of phorbol esters cause lung and kidney damage, resulting in fatality (Li *et al.*, 2010).

The toxicity of phorbol esters is mainly due to their action on biological cell membranes. The phorbol esters are amphiphilic molecules and have a tendency to bind to phospholipid membrane receptors. The receptors are usually the primary targets of the phorbol esters. The most investigated activity of the phorbol esters is their binding and activation of protein kinase C (PKC), an enzyme that plays a critical role in signal transduction pathways and also regulates cell growth and differentiation. It has been proposed that the phorbol esters convert protein kinase C into a constitutive active form that is irreversibly inserted into the membrane. During normal signal transduction, the enzyme is activated by diacylglycerol, which is then rapidly hydrolyzed. Upon activation, PKC enzymes are translocated to the plasma membrane to conduct various other signal transduction pathways. The phorbol esters act as analogues for diacylglycerol and are stronger PKC activators that are hardly metabolized by the cell. They hyperactivate PKC and trigger cell proliferation, thus amplifying the efficacy of carcinogens. (Goel *et al.*, 2007)

Phorbol esters also affect insulin binding by stimulating PKC-induced phosphorylation of insulin receptors. It is believed that protein kinase C regulates insulin (Jacobs *et al.*, 1983).

The relationship between the chemical structure and toxicity of phorbol esters has received considerable attention from scientists, without conclusive results. For instance, the lack of toxicity of phorbol, as contrasted to its esters, both *in vivo* and *in vitro* had strongly indicated that adequate hydrophobicity was one of the requirements for activity (Sharkey and Blumberg, 1985). Studies on effects of phorbol myristate acetate (PMA), also known as PTA, in mouse skin showed that its metabolite, phorbolol myristate acetate (PHMA) was nearly as effective a tumor promoter as PMA (Segal *et al.*, 1975) – here, the carbonyl group in PMA is converted to a hydroxyl group to give PHMA. Other esters found to bind specifically to PKC are phorbol-12,13-didecanoate, phorbol-12,13-dibutyrate, phorbol-12,13-dibenzoate, phorbol-12,13-diacetate, phorbol-12,13,20-triacetate, phorbol-13-acetate (Dunphy *et al.*, 1980) and phorbol-12-tetradecanoate (Kikkawa *et al.*, 1983). However, phorbol-13,20-diacetate and 4-*O*-tetradecanoylphorbol-13-acetate were ineffective; thus, were declared as non-promoters (Yuspa *et al.*, 1976). Interestingly, while phorbol-13-acetate was reported as effective, the closely related 12-deoxyphorbol-13-acetate exhibited inhibitory or antitumor properties (Carrol and Little, 2000).

It has been observed that shorter chain derivatives of phorbol are irritant but not promoting, these being two biological effects that could be considered as independent (Szallasi *et al.*, 1993). The presence of a C₂₀ hydroxyl group has been seen to be important for the irritant and tumor promoting activities of phorbol esters. Introduction of an acetyl group in the 20-position gives rise to lower irritancy (Wu *et al.*, 2009). Therefore, it may be postulated that the spatial position of this function, as determined by

their particular carbon skeleton and relative to specifically binding cellular structures, may play a delicate role in the hydrophilic interaction of the esters with membranes and receptors (Hecker, 1987). One way to weaken or abolish tumor promoting efficacy of a phorbol ester without impairing its mitogenic and irritant activities is to introduce double bonds into the long chain fatty acid residue at position 12 of the phorbol moiety (Sorg *et al.*, 1982).

Although many biological activities of phorbol esters are mediated through their direct interaction and activation of protein kinase C, there are some phorbol esters that are active without being tumor promoters, such as ostodin and prostratin. It has also been reported that 12-*O*-acetylphorbol-13-decanoate potently inhibits the cytopathic effects of human immunodeficiency virus (HIV) type 1 without appreciable activation of protein kinase C (Abdel-Hafez *et al.*, 2002).

Phorbol, the parent diterpenoid of phorbol esters, contains five hydroxyl groups with different reactivities towards acylation. Ring A is on the left and trans- linked to the 7-member ring B. Ring C is 6-membered and cis- linked to the cyclopropane D ring. The two categories of phorbols, α and β differ in their OH group in ring C. The placement of OH group makes the phorbol ester an active (β) or inactive (α) type, which results in spatial arrangement of ring D and precludes activation of PKC and other structurally similar phorbol esters receptors. (Goel *et al.*, 2007)

Information about structure of PKC has been limited by its large size, which makes NMR structural analysis quite difficult. It should be emphasized that the competitive binding to PKC, exhibited by diacylglycerol and the different phorbol esters, does not require that they possess similar recognition subunits. They could be interacting differently with functionality in the receptor domain. (Wender *et al.*, 1998)

DETOXIFICATION

The *Jatropha curcas* seed cake is a by-product of oil extraction from the seed. Although the seed meal has been found to be protein-rich, its utilization as a stock feed is hampered by the presence of toxic phorbol esters and the antinutritional factors that include lectin, saponin, phytic acid, and trypsin inhibitor. Detoxification of the seed meal is, therefore, of paramount importance. Several biological, chemical and physical detoxification methods have been suggested.

Roasting of seeds inactivates trypsin inhibitor and reduces lectin activity. However, saponins, phytic acid and phorbol esters are not affected by roasting. In Mexico, Quintana Roo state, non-toxic *J. curcas* seeds are consumed by humans, after roasting. The consumption of raw seeds is known to produce cramps and uneasy feeling in the stomach (Makkar *et al.*, 1998). It is not possible to destroy phorbol esters by heat treatment because they are heat stable and can withstand roasting temperature as high as 160°C for 30 minutes (Aregheore *et al.*, 2003). Although rumen microbes can degrade lectins, phytic acid and trypsin inhibitor, they cannot degrade phorbol esters. This is why

300 ruminants, like cattle, are as prone to phorbol ester toxicity as monogastric animals
301 (Makkar and Becker, 2010).

302
303 Complete degradation of phorbol esters by stripping or deodorization of *Jatropha curcas*
304 oil at 260°C, with 3 mbar pressure and 1% steam injection has been reported by Makkar
305 *et al.* (2009). The procedure involves steam injection through the seed oil, in a closed
306 oven, at 260°C and low pressure. Vapours escaping from the oil are condensed in a water
307 cooler that is connected to a vacuum pump.

308
309 Although Aregheore *et al.* (2003) had suggested that the use of 4% NaOH, followed by
310 washing with water could effectively reduce phorbol esters, studies by Sumiati and others
311 (2010) showed that addition of 4% NaOH, then heating in an autoclave at 121°C for 30
312 minutes, did not produce satisfactory results.

313
314 Solvent extraction is among the most successful means of detoxifying *J. curcas* seed
315 cake. Chivandi and co-researchers (2004) realized that a hexane/ethanol solvent system
316 was useful in reducing phorbol ester content in the seed meal. Sequential extraction
317 involving non-polar solvent followed by polar solvent has been recommended by Gaur
318 and others (2011). Approximately 80% of phorbol esters present in *Jatropha* oil were
319 extracted using methanol (Devappa *et al.*, 2010). Nevertheless, Saetae and Suntornsuck
320 (2010) claimed that 90% ethanol was just as effective as 99.5% methanol for complete
321 phorbol ester extraction from *J. curcas* seed cake. They preferred ethanol since it is safer
322 for humans and other animals than methanol. Ethanol was also found to be efficient in

removing lectin and reducing saponin, phytic acid and trypsin inhibitor in the seed cake (Saetae and Suntornsuck, 2011). Research done by Martínez-Herrera *et al.* (2006) indicated that extraction with ethanol, followed by treatment with 0.07% NaHCO₃ decreased phorbol ester content by 98% in *Jatropha* seeds; there was also a considerable decrease in lectin activity.

Devappa *et al.* (2010) have demonstrated that *Jatropha* phorbol esters are biodegradable in the environment. The degraded products are innocuous. Degradation rates of phorbol esters in the soil are influenced by temperature and moisture levels. The occurrence of storage fungi in *J. curcas* seeds points to the use of these microorganisms in detoxifying the seed meal. Different species of *Aspergillus*, *Penicillium*, *Mucor* and *Rhizopus* were isolated from stored *Jatropha* seeds (Jayaraman *et al.*, 2011). Solid-state fermentation of *Jatropha curcas* seed cake, using various microorganisms has been tried by a number of researchers. Here, the method constitutes a fermentation process performed on a non-soluble material that acts both as physical support and source of nutrients in the absence of free flowing liquid. The same technique had been employed to eliminate or reduce antinutritional factors in other plants, such as saponin from whole soybean tempe by *Rhizopus oligosporus*, caffeine and tannins from coffee husk by *Aspergillus* sp. and gossypol from cotton seed by *Candida tropicalis* (Joshi *et al.*, 2011).

Belewu and Akande (2010) studied the effect of *Aspergillus niger* and *Penicillium* sp. treated *Jatropha curcas* kernel cake, as stock feed for West African dwarf goat. They concluded that fungi treated *J. curcas* seed meal can partially replace soybean cake for

growing goat by as much as 50%. Other work by Belewu *et al.* (2010) showed that inoculation of *J. curcas* kernel cake with *Aspergillus niger* and incubation at room temperature, for 7 days, managed to detoxify the seed meal. In yet another study by Belewu *et al.* (2010), it was concluded that growing goats can be fed a diet consisting of 50% soybean cake plus 50% *Rhizopus oligosporus* treated *Jatropha curcas* kernel cake. Sumiati and others (2009) used *Rhizopus oryzae* to detoxify *Jatropha curcas* seed meal by solid-state fermentation for 3 days. The procedure eliminated phorbol esters, reduced trypsin inhibitor by 68% and fat content by 93%. Fermentation using *Rhizopus oligosporus* was also described as an effective method of detoxifying *J. curcas* seed meal and increasing its nutritive value for poultry because there was reduction of phytic acid, an increase in protein utilization efficiency and retention of calcium and phosphorus in broilers (Sumiati *et al.*, 2010). Again, Rosa *et al.* (2010) have demonstrated that *Aspergillus niger* is not only a detoxifier but a nutritional enrichment additive. Joshi and co-workers (2011) have claimed that phorbol esters can be completely degraded in 9 days by *Pseudomonas aeruginosa* under optimized solid-state fermentation of deoiled *Jatropha curcas* seed cake. It is interesting to note that Tjakradidjaja *et al.* (2009) obtained poor results, on trials with *Aspergillus niger*, *Rhizopus oligosporus*, *Rhizopus oryzae*, *Trichoderma viridae* and *Trichoderma reesei*, in the development of mice feed. This led them to conclude that the use of fermented *J. curcas* seed meal with various moulds at 5% in ration is not effective as protein source for male mice.

FUTURE RESEARCH

The role of chemists in finding the most appropriate detoxification method for the *Jatropha curcas* seed cannot be overlooked. Research on structure-toxicity relationships and detoxification mechanisms of phorbol esters is important. Investigations on possible chemical detoxification methods are also useful.

Structure-Toxicity Relationship

The active sites that are responsible for phorbol ester toxicity are still unknown. More work is required to determine the appropriate structure or particular groups that give rise to toxic properties of phorbol esters.

Detoxification Mechanisms

Some researchers have claimed success in the detoxification of *Jatropha curcas* seed. Their work should be used to determine the mechanism of degradation of phorbol esters.

Chemical Detoxification Methods

Use of chemicals to detoxify the *Jatropha curcas* seed can be quite fast and efficient. The major challenge, however, is the generation of secondary toxins. Another option is to identify and isolate an enzyme that degrades the phorbol esters; this protein can then be produced in bulk.

CONCLUSION

The toxicity of *Jatropha curcas* seeds emanates mainly from phorbol esters. Their structure-toxicity relationships have not been well studied. Biological, physical and some chemical detoxification methods of *Jatropha* seed have been proposed, but further research on detoxification mechanisms and chemical methods is necessary.

REFERENCES

Abdel-Hafez A.A.-M., Nakamura N. & Hattori M. (2002); "Biotransformation of phorbol by human intestinal bacteria"; Chem. Pharm. Bull.; 50(2): 160 – 164.

Abou-Arab A.A. & Abu-Salem F.M. (2010); "Nutritional quality of *Jatropha curcas* seeds and effect of some physical and chemical treatments on their anti-nutritional factors"; African Journal of Food Science; 4(3): 93 – 103.

Achten W.M.J., Nielsen L.R., Aerts R., Lengkeek A.G., Kjaer E.D., Trabucco A., Hansen J.K., Maes W.H., Graudal L., Akinnifesi F.K. & Muys B. (2010); "Towards domestication of *Jatropha curcas*"; Biofuels; 1(1): 91 – 107.

Adebowale K.O. & Adedire C.O. (2006); "Chemical composition and insecticidal properties of underutilized *Jatropha curcas* seed oil"; African Journal of Biotechnology; 5(10): 901 – 906.

414

415 Adolf W., Opferkuch H.J. & Hecker E. (1984); "Irritant phorbol derivatives from four
416 *Jatropha* species"; *Phytochemistry*; 23: 129 – 132.

417

418 Ahmed W.A. & Salimon J. (2009); "Phorbol ester as toxic constituents of tropical
419 *Jatropha curcas* seed oil"; *European Journal of Scientific Research*; 31(3): 429 – 436.

420

421 Aregheore E.M., Becker K. & Makkar H.P.S. (2003); "Detoxification of a toxic variety
422 of *Jatropha curcas* using heat and chemical treatments, and preliminary nutritional
423 evaluation with rats"; *S. Pac. J. Nat. Sci.*; 21: 50 – 56.

424

425 Becker K. & Makkar H.P.S. (1998); "Effects of phorbol esters in Carp (*Cyprinus carpio*
426 *L.*"); *Vet. Human Toxicol*; 40(2): 82 – 86.

427

428 Belewu M.A. & Akande B.A. (2010); "Biological upgrading of the nutritional quality of
429 *Jatropha curcas* kernel cake: effect on performance characteristics of goat"; *International*
430 *Research Journal of Biotechnology*; 1(2): 19 – 22.

431

432 Belewu M.A., Belewu K.Y. & Ogunsola F.O. (2010); "Nutritive value of dietary fungi
433 treated *Jatropha curcas* kernel cake: Voluntary intake, growth and digestibility coefficient
434 of goat"; *Agriculture and Biology Journal of North America*; 1(2): 135 – 138.

435

436 Belewu M.A., Eniolorunda O.O. & Ilori G.I. (2010); “Response of goat to fungi
437 (*Rhizopus oligosporus*, *Rhizopus nigricans*) treated *Jatropha curcas* kernel cake”; Archives
438 of Applied Science Research; 2(4): 255 – 261.

439

440 Carroll G.L. & Little D.R. (2000); “Inter- and intramolecular reductive coupling
441 reactions: An approach to the phorbol skeleton”; Organic Letters; 2(18): 2873 – 2876.

442

443 Chivandi E., Mtimuni J.P., Read J.S. & Makuza S.M. (2004); “Effect of processing
444 method on phorbol esters concentration, total phenolics, trypsin inhibitor activity and the
445 proximate composition of the Zimbabwean *Jatropha curcas* provenance: A potential
446 livestock feed”; Pakistan Journal of Biological Sciences; 7(6): 1001 – 1005.

447

448 Chivandi E., Kachigunda B. & Fushai F. (2005); “A comparison of the nutrient and
449 antinutrient composition of industrially processed Zimbabwean *Jatropha curcas* and
450 *Glycine max* meals”; Pakistan Journal of Biological Sciences; 8(1): 49 – 53.

451

452 Crombie L., Games M.L. & Pointer D.J. (1968); “Chemistry and structure of phorbol, the
453 diterpene parent of co-carcinogens of Croton oil”; J. Chem. Soc.; C: 1347 – 1362.

454

Demissie A.G. & Lele S.S.(2010); “Bioassay-assisted identification of phorbol ester from *Jatropha curcas* (Linn.) tissue culture”; International Journal of Pharma and Bio Sciences; 1(3): 1 – 7.

Devappa R.K., Makkar H.P.S. & Becker K. (2010); “Optimization of conditions for the extraction of phorbol esters from *Jatropha* oil”; Biomass and Bioenergy; doi10.1016/j.biombioe.2010.03.001.

Devappa R.K., Maes J., Makkar H.P.S., De Greyt W. & Becker K. (2010); “Quality of Biodiesel prepared from phorbol ester extracted *Jatropha curcas* oil”; J. Am. Oil Chem. Soc.; doi 10.1007/s11746-010-1547-4.

Devappa R.K., Makkar H.P.S. & Becker K. (2010); “Biodegradation of *Jatropha curcas* phorbol esters in soil”; J. Sci. Food Agric.; 90: 2090 – 2097.

Devappa R.K., Makkar H.P.S. & Becker K. (2010); “*Jatropha* diterpenes: A review”; J. Am. Oil Chem. Soc.; doi 10.1007/s11746-010-1720-9.

Dowlathabad M.R., Sreeyapureddy A., Adhikari A., Bezawada K. & Nayakanti D. (2010); “Pharmaceutical investigation and biopesticidal activity of *Jatropha curcas* L. seed oil on digestive enzymic profiles of *Cnaphalocrocis medinalis* (Rice leaf folder) and

476 *Helicoverpa armigera* (Cotton boll worm)”; International Research Journal of Pharmacy;
477 1(1): 194 – 200.

478
479 Dunphy W.G., Delclos K.B. & Bumberg P.M. (1980); “Characterization of specific
480 binding of [³H] phorbol 12, 13-dibutyrate and [³H] phorbol 12-myristate 13-acetate to
481 mouse brain”; Cancer Research; 40: 3635 – 3641.

482
483 Gaur S., Viswanathan B., Seemamahannop R., Kapila S. & Book N.L. (2011);
484 “Development and evaluation of an efficient process for the detoxification of *Jatropha* oil
485 and meal”; Nature Precedings; doi 10.1038/npre.2011.6118.1.

486
487 Goel G., Makkar H.P.S., Francis G. & Becker K. (2007); “Phorbol esters: Structure,
488 biological activity and toxicity in animals”; International Journal of Toxicology; 26: 279 -
489 288.

490
491 Gschwendt M. & Hecker E. (1970); “Tumor-promoting compounds from *Euphorbia*
492 *cooperi*: Di- and triesters of 16-hydroxy-12-deoxy-phorbol”; Tetrahedron Letters; 8: 567
493 -570.

494
495 Haas W. & Mittelbach M. (2000); “Detoxification experiments with the seed oil from
496 *Jatropha curcas* L.”; Industrial Crops and Products; 12: 111 -118.

Haas W., Sterk H. & Mittelbach M. (2002); “Novel 12-deoxy-16-hydroxyphorbol diesters isolated from the seed oil of *Jatropha curcas*”; *Journal of Natural Products*; 65(10): 1434 – 1440.

Hecker E. (1987); “Three stage carcinogenesis in mouse skin – Recent results and present status of advanced model system of chemical carcinogenesis”; *Toxicologic Pathology*; 15(2): 245 – 258.

Hipal G., Mayank G., Nikhil G., Soham T., Prasad P., Girish G., Vamsi K.K., Reddy M.P., Sethiya B.D. & Rathod M.R. (2009); “Isolation and characterization of phorbol esters (toxin) from the *Jatropha curcas* L.”; *International Journal of Microbiology Research*; 1(1): 1 – 7.

Hirota M., Suttajit M., Suguri H., Endo Y., Shudo K., Wongchai V., Hecker E., Fujiki H. (1988); “A new tumor promoter from the seed oil of *Jatropha curcas* L., an intramolecular diester of 12-deoxy-16-hydroxyphorbol”; *Cancer Research*; 48: 5800 – 5804.

Jacobs S., Sahyoun N.E., Saltiel A.R. & Cuatrecasas P. (1983); “Phorbol esters stimulate the phosphorylation of receptors for insulin and somatomedin C”; *Biochemistry*; 80: 6211 – 6213.

Jayaraman P., NesaPriya S., Parameshwari S., Priya S.S., Jawahar N. & Babu H.S. (2011); "Occurrence of storage fungi in jatropha (*Jatropha curcas* L.) seeds"; African Journal of Microbiology Research; 5(5): 475 – 480.

Jimu L., Nyakudya I.W. & Katsvanga C.A.T. (2009); "Establishment and early field performance of *Jatropha curcas* L. at Bindura University farm, Zimbabwe"; Journal of Sustainable Development in Africa; 10(4): 445 – 469.

Joshi C., Mathur P. & Khare S.K. (2011); "Degradation of phorbol esters by *Pseudomonas aeruginosa* PseA during solid-state fermentation of deoiled *Jatropha curcas* seed cake"; Bioresource Technology; 102: 4815 – 4819.

Karavina C., Zivenge E., Mandumbu R. & Parwada C. (2011); "*Jatropha curcas* production in Zimbabwe: Uses, challenges and way forward"; Modern Applied Sciences; 5(2): 239 – 243.

Kikkawa U., Takai Y., Tanaka Y., Miyake R. & Nishizuka Y. (1983); "Protein kinase C as a possible receptor protein of tumor-promoting phorbol esters"; The Journal of Biological Chemistry; 258(19): 11442 – 11445.

Kumar A. & Sharma S. (2008); "An evaluation of multipurpose oil seed crop for industrial uses (*Jatropha curcas* L.): A review"; *Industrial Crops and Products*; 28(1): 1 – 10.

Li C.-Y., Devappa R.K., Liu J.-X., Lv J.-M., Makkar H.P.S. & Becker K. (2010); "Toxicity of *Jatropha curcas* phorbol esters in mice"; *Food and Chemical Toxicology*; 48: 620 – 625.

Makkar H.P.S., Aderibigbe A.O. & Becker K. (1998); "Comparative evaluation of non-toxic and toxic varieties of *Jatropha curcas* for chemical composition, digestibility, protein degradability and toxic factors"; *Food Chemistry*; 62(2): 207 – 215.

Makkar H.P.S., Becker K. & Schmook B. (1998); "Edible provenances of *Jatropha curcas* from Quintana Roo State of Mexico and effect of roasting on antinutrient and toxic factors in seeds"; *Plant Foods for Human Nutrition*; 52: 31 – 36.

Makkar H.P.S., Francis G. & Becker K. (2008); "Protein concentrate from *Jatropha curcas* screw-pressed seed cake and toxic and antinutritional factors in protein concentrate"; *Journal of the Science of Food and Agriculture*; 88: 1542 – 1548.

Makkar H.P.S., Maes J., De Greyt W. & Becker K. (2009); "Removal and degradation of phorbol esters during pre-treatment and transesterification of *Jatropha curcas* oil"; J. Am. Oil Chem. Soc.; 86: 173 -181.

Makkar H.P.S. & Becker K. (2009); "*Jatropha curcas*, a promising crop for the generation of biodiesel and value-added coproducts"; Eur. J. Lipid Sci. Technol.; 111: 773 – 787.

Makkar H.P.S. & Becker K. (2010); "Are *Jatropha curcas* phorbol esters degraded by rumen microbes?"; Journal of the Science of Food and Agriculture; 90: 1562 – 1565.

Makkar H.P.S., Kumar V., Oyeleye O.O., Akinleye A., Angulo-Escalante M.A. & Becker K. (2010); "Traditional wisdom confirmed by scientific research: *Jatropha* species from Mexico is non-toxic"; Nature Precedings; hdl 10101/npre.2010.4155.1.

Martínez-Herrera J., Siddhuraju P., Francis G., Dávila-Ortíz G. & Becker K. (2006); "Chemical composition, toxic/antimetabolic constituents, and effects of different treatments on their levels, in four provenances of *Jatropha curcas* L. from Mexico"; Food Chemistry; 96: 80 -89.

Mwine J.T. & Van Damme P. (2011); "Why do Euphorbiaceae tick as medicinal plants? A review of Euphorbiaceae family and its medicinal features"; Journal of Medicinal Plants Research; 5(5): 652 – 662.

581

582 Olivier G.W.J., Rowan M.G., Branch S.K., Mahon M.F. & Molloy K.C. (1992); “Two
583 esters of Synadenol, a new lathyrane diterpenoid, from the latex of *Synadenium*
584 *compactum* (Euphorbiaceae): A crystal structure analysis”; *Journal of the Chemical*
585 *Society, Perkin Transactions*; 1: 1831 – 1835.

586

587 Parawira W. (2010); “Biodiesel production from *Jatropha curcas*: A review”; *Scientific*
588 *Research and Essays*; 5(14): 1796 – 1808.

589

590 Rosa T.S., Castro A.M., Torres A.G. & Freire D.M.G. (2010); “Analysis of nutritional
591 composition and detoxification of *Jatropha curcas* cake after solid-state fermentation”;
592 The 32nd Symposium on Biotechnology for Fuels and Chemicals; April 19 – 22, 2010.

593

594 Saetae D. & Suntornsuk W. (2010); “Antifungal activities of ethanolic extract from
595 *Jatropha curcas* seed cake”; *J. Microbiol. Biotechnol.*; 20(2): 319 – 324.

596

597 Saetae D. & Suntornsuk W. (2010); “Variation of phorbol ester contents in *Jatropha*
598 *curcas* from different provinces in Thailand and the application of its seed cake for starter
599 broiler diets”; *American-Euroasian J. Agric. & Environ. Sci.*; 8(5): 497 – 501.

600

Saetae D. & Suntornsuk W. (2011); "Toxic compound, anti-nutritional factors and functional properties of protein isolated from detoxified *Jatropha curcas* seed cake"; International Journal of Molecular Sciences; 12: 66 - 77.

Segal A., Van Duuren B.L. & Maté U. (1975); "The identification of phorbolol myristate acetate as a new metabolite of phorbol myristate acetate in mouse skin"; Cancer Research; 35: 2154 – 2159.

Sharkey N.A. & Blumberg P.M. (1985); "Highly lipophilic phorbol esters as inhibitors of specific [³H] phorbol 12,13-dibutyrate binding"; Cancer Research; 45: 19 – 24.

Soerawidjaja T.H. (2010); "Potential of *Jatropha curcas* L. as a non-edible feedstock of biodiesel fuel"; 7th Biomass-Asia Workshop; 29 Nov. – 1 Dec., 2010; Jakarta, Indonesia.

Sorg B., Fürstenberger G., Berry D.L., Hecker E. & Marks F. (1982); "Preparation of retinoic acid esters of phorbol derivatives"; Journal of Lipid Research; 23: 443 – 447.

Sumiati Y., Yusriani Y., Astuti D.A. & Suharti S. (2009); "Feeding fermented *Jatropha curcas* L. meal supplemented with cellulose and phytase to kampong chicken"; The 1st International Seminar on Animal Industry 2009; Bogor, Indonesia; 191 -197.

Sumiati Y., Sudarman A., Nurhikmawati I., & Nurbaeti (2010); “Detoxification of
Jatropha curcas meal as poultry feed”; The 2nd International Symposium on Food
Security, Agricultural Development & Environmental Conservation in Southeast and East
Asia; Bogor, Indonesia; 61 – 66.

Szallasi Z., Krsmanovic L. & Blumberg P.M. (1993); “Nonpromoting 12-deoxyphorbol
13-esters inhibit phorbol 12-myristate 13-acetate induced tumor promotion in CD-1
mouse skin”; Cancer Research; 53: 2507 – 2512.

Tigere T.A., Gatsi T.S., Mudita I.I., Chikuvire T.J., Thamangani S. & Mavunganidze Z.
(2006); “Potential of Jatropha curcas in improving smallholder farmers’ livelihoods in
Zimbabwe: An exploratory study of Makosa Ward, Mutoko district”; Journal of
Sustainable Development in Africa; 8(3): 1 – 9.

Tjakradidjaja A.S., Suryahadi & Mahajati R. (2009); “Effectivity of Jatropha curcas seed
meal fermented with various moulds as protein source for male mice (Mus musculus)”;
The 1st International Seminar on Animal Industry 2009; Bogor, Indonesia; 157 – 164.

Wender P.A., Martin-Cantalejo Y., Carpenter A.J., Chiu A., DeBrabander J., Harran
P.G., Jimenez J.-M., Koehler M.F.T., Lippa B., Morrison J.A., Müller S.G., Müller S.N.,
Park C.-M. Shiozaki M., Siedenbiedel C., Skalitzky D.J., Tanaka M. & Irie K. (1998);

“The chemistry-medicine continuum: Synthetic computer, spectroscopic and biological studies on new chemotherapeutic leads”; Pure and Applied Chemistry; 70(3): 539 – 546.

Wink M., Koschmieder C., Sauerwein M. & Sporer F. (1997); “Phorbol esters of *J. curcas* – Biological activities and potential applications”; Biofuel and Industrial Products from *Jatropha curcas*; G.M. Gübitz, M. Mittelbach & M. Trabi; Graz, Austria; 160 – 166.

Wirawan S.S. (2009); “Potential of *Jatropha curcas* L.”; Joint Task 40 / ERIA Workshop; 28 Oct., 2009; Tsukuba, Japan.

Wu Q.-C., Tang Y.-P., Ding A.-W., You F.-Q., Zhang L. & Duan J.-A. (2009); “¹³C-NMR data of three important diterpenes isolated from *Euphorbia* species”; Molecules; 14: 4454 – 4475.

Yan H. (2008); “Is *Jatropha curcas* a solution to sustainable energy supply?”; *Jatropha* International Congress; December 17 – 18, 2008; Singapore.

Ye M., Li C., Francis G. & Makkar H.P.S. (2009); “Current situation and prospects of *Jatropha curcas* as a multipurpose tree in China”; Agroforest Syst; 76: 487 – 497.

Yuspa S.H., Lichti U., Ben T., Patterson E., Hennings H., Slaga T.J., Colburn N. &
Kelsey W. (1976); "Phorbol esters stimulate DNA synthesis and ornithine decarboxylase
activity in mouse epidermal cell cultures"; Nature; 262: 402 – 404.

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