

2 **Chemistry of Phorbol Ester Toxicity in *Jatropha***

3 ***curcas* Seed – A Review**

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13 **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 **improving the applications** of the seed meal.

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.

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16 *Keywords: phorbol ester, toxicity, detoxification, Jatropha curcas*

17 **1. INTRODUCTION**

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22 *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 *J. curcas* seed cake equals that of soya bean, and better than sunflower and cotton seed meal, making it an ideal stock feed, if detoxified.

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28 Scientists have focused their attention on the detoxification of the seed cake and a number of methods have been
29 **studied**. Although some physical and microbiological detoxification methods have been forwarded, they are generally not
30 cost-effective. It is believed that a chemical detoxification technique can be much faster and cheaper. Other work has
31 been done on the chemical nature and toxicity of phorbol esters. However, the **relationship between chemical structure**
32 **and toxicity of phorbol esters is not well understood. Detoxification mechanisms have also not been studied.**

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2. JATROPHA CURCAS PLANT

Family: Euphorbiaceae

Genus: *Jatropha*

Species: *Jatropha curcas* Linnaeus

J. curcas is a genus of approximately 175 succulent plants, shrubs and trees in the Euphorbiaceae family (Wirawan, 2009). It has good adaptation capacity to a large variety of soil conditions. Cultivation on dry, stony and very shallow soils is frequent (Makkar and Becker, 2009b). It can be established on marginal land, paddocks, contour ridges, hilly slopes and gullies (Tigere *et al.*, 2006).

The origin of *J. curcas* is believed to be 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). *J. curcas* now grows pantropically, from Brazil to the islands of Fiji (Achten *et al.*, 2010). Two genotypes of *J. curcas* exist: the toxic and the non-toxic varieties. The latter genotype is found in Mexico only (Makkar and Becker, 2009b). Another non-toxic species, also found in Mexico, is *Jatropha platyphylla* Müell (Makkar *et al.*, 2010b).

Seeds of *J. 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 *J. curcas* 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). *J. curcas* seed 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 *et al.*, 1998a). Research done by Chivandi *et al.* (2005) indicated higher crude protein, ash, calcium and phosphorus in *Jatropha* seed meal than in soya bean meal.

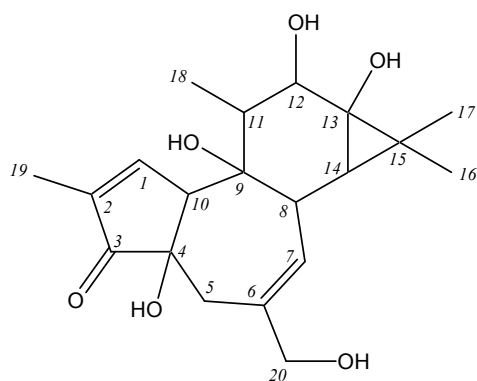
J. curcas is famous for being a potential source of raw material for biodiesel. Its popularity stems from the widespread general knowledge that it is a non-edible, oil-yielding tree, well adapted to marginal areas with poor soil and low rainfall, where it grows without competing with annual food crops (Soerawidjaja, 2010).

There are many other uses of *J. curcas*. 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 *J. curcas* (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). *J. 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 (Dowlatabad *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 *J. curcas* to mollusks has been investigated because of its relevance in schistosomiasis control. Extracts from *J. curcas* were found to be toxic against snails (Goel *et al.*, 2007). Ethanolic extracts of *J. curcas* 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, 2010a).

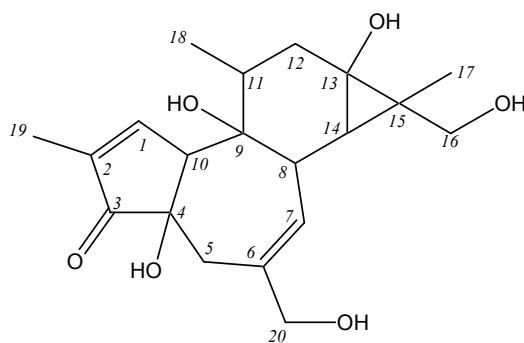
The International *Jatropha* Organization has claimed that in 2017 there will be approximately 33 million hectares of *J. curcas* cultivated land worldwide, producing 160 million tons of seeds and 95% of its total production will be in Asia (Devappa *et al.*, 2010a).

3. PHORBOL ESTERS

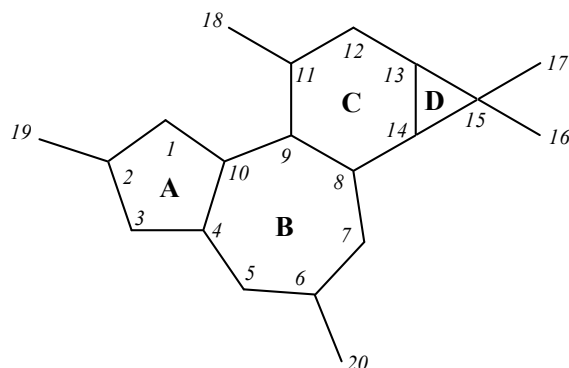
The term 'phorbol esters' is used today to describe a naturally occurring group of compounds mainly distributed in plant species of the Euphorbiaceae family. Phorbol esters are esters of phorbol, a tetracyclic diterpenoid with a tigliane skeletal structure (Haas and Mittelbach, 2000). Terpenoids are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



phorbol



12-deoxy-16-hydroxyphorbol

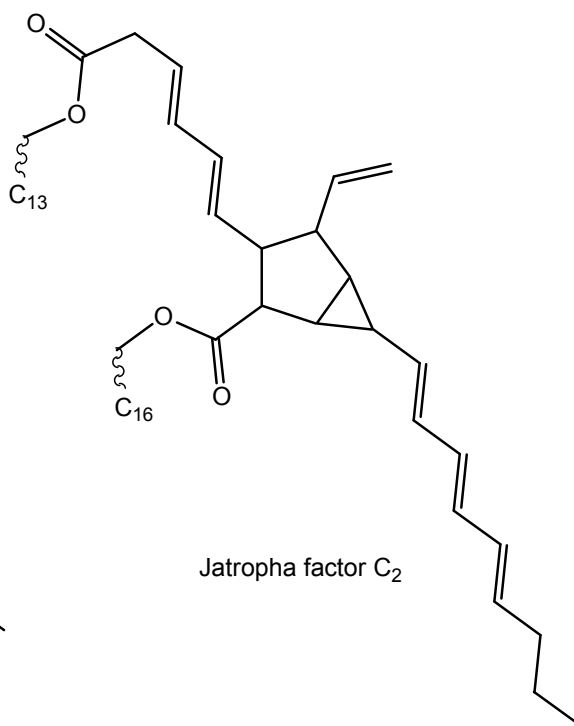
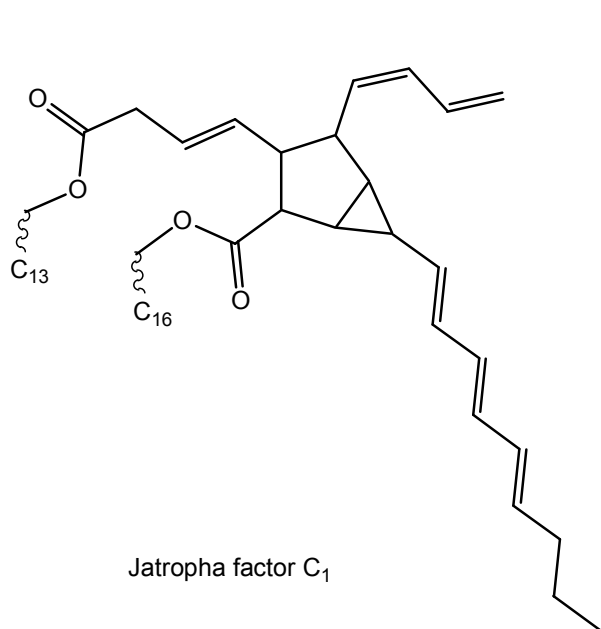
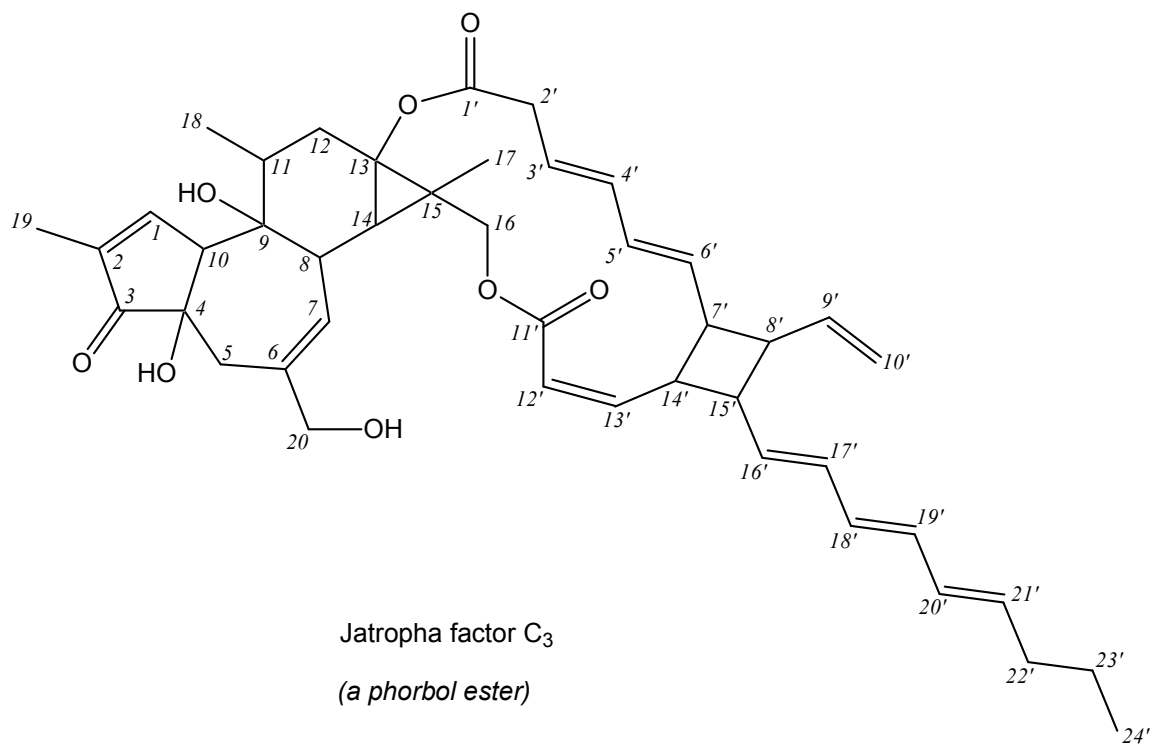


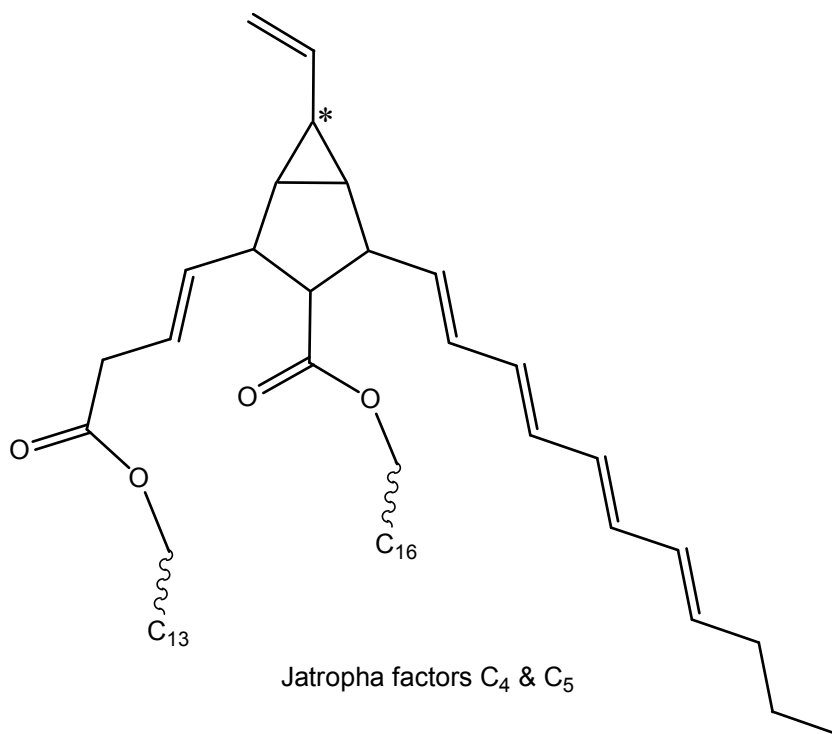
tiglane

Phorbol itself was isolated as a non-toxic crystalline solid from the plant, *Croton tiglium* L. (Crombie *et al.*, 1968), but its ester, TPA (12-O-tetradecanoylphorbol-13-acetate), present in croton oil, is the most toxic and most studied phorbol ester, known to be a co-carcinogen (Olivier *et al.*, 1992; Devappa *et al.*, 2011). However, phorbol esters from *J. curcas* are derivatives of 12-deoxy-16-hydroxyphorbol (Haas *et al.*, 2002). Phorbol esters with the same diterpenoid moiety have also been extracted from the latex of *Euphorbia cooperi* (Gschwendt and Hecker, 1970).

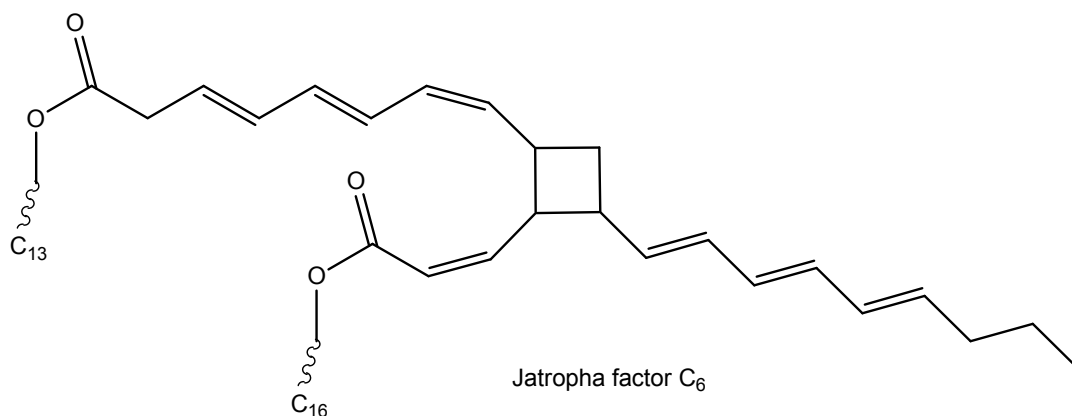
Adolf *et al.* (1984) were the first to identify phorbol esters in *J. curcas*. Although they concluded that the irritant principles of the plant represented 12-deoxy-16-hydroxyphorbol-13-acylates with highly unsaturated acid moieties, they could not elucidate their exact chemical structures. Hirota *et al.* (1988) identified 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 *et al.* (2002) isolated and did a thorough structural determination on all phorbol esters found in *J. curcas*.

Six phorbol esters have been characterized from *J. 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 *J. 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 *J. curcas* seed oil from Malaysia (0.23%), Indonesia (1.58%) and India (0.58%). Saetae and Suntornsuk (2010b) demonstrated a variation in phorbol ester content in *J. curcas* plants from different provinces of Thailand. However, Makkar *et al.* (1998a) 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 *J. curcas* plant (Makkar and Becker, 2009b), they are concentrated in the seed kernel and their levels in seed oil are approximately four times higher than those in the seed cake (Hiral *et al.*, 2009).

4. 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). According to Demissie and Lele (2010), toxicity to sheep, goats, calves, chicks and fish by consumption of *Jatropha* seed or seed meal has been reported. The seed also has a high level of 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).

A summary of the effects of phorbol esters on different animals is given in Table 1 below.

Table 1. Toxicity of phorbol esters to different animals

Animal	Application	Symptoms
Humans	Ingestion	Burning and pain in mouth and throat, vomiting, delirium, muscle shock, high pulse, death
Ruminants	Ingestion	Diarrhoea, gastrointestinal inflammation
Rabbits and rodents	Topical	Erythema, oedema, necrosis, diarrhea, scaling and thickening of skin, tumor formation
	Ingestion	Lung and kidney damage, loss of weight, death
Goats and sheep	Ingestion	Lack of appetite, reduced water intake, diarrhea, dehydration and haemorrhagic effects in different organs
Guinea pigs	Topical	Inflammation, inhibition of milk secretion
Fish	Ingestion	Lower metabolic rate, increase in fecal mucus production, rejection of feed

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 PKC 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 phorbolmyristate acetate (PMA), also known as PTA, in mouse skin showed that its metabolite, phorbololmyristate 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). While phorbol-13-acetate was reported as effective, the closely related 12-deoxyphorbol-13-acetate exhibited inhibitory or antitumor properties (Carroll 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 PKC, 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 PKC (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 nuclear magnetic resonance (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)

5. DETOXIFICATION

The *J. 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.*, 1998b). 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 ruminants, like cattle, are as prone to phorbol ester toxicity as monogastric animals (Makkar and Becker, 2010a).

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

Although Aregheore *et al.* (2003) had suggested that the use of 4% NaOH, followed by washing with water could effectively reduce phorbol esters, studies by Sumiati *et al.* (2010) showed that addition of 4% NaOH and heating in an autoclave at 121°C for 30 minutes, only partially reduced toxicity of *J. curcas* seed cake.

Solvent extraction is among the most successful means of detoxifying *J. curcas* seed cake. Chivandi *et al.* (2004) reported that a hexane/ethanol solvent system reduced phorbol ester content in the seed meal by 70%. Sequential extraction involving non-polar solvent followed by polar solvent has been recommended by Gaur *et al.* (2011). Approximately 80% of phorbol esters present in *Jatropha* oil were extracted using methanol (Devappa *et al.*, 2010b). Nevertheless, Saetae and Suntornsuck (2010a) claimed that 90% ethanol was just as effective as 99.5% methanol for complete phorbol ester

extraction from *J. curcas* seed cake. They preferred ethanol since it is safer 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.* (2010c) 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 *J. 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 (2010a) studied the effect of *Aspergillus niger* and *Penicillium* sp. treated *J. curcas* kernel cake, as stock feed for West African dwarf goat. They concluded that fungi-treated seed meal can partially replace soybean cake for growing goat by as much as 50%. Other work by Belewu *et al.* (2010b) 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 another study by Belewu *et al.* (2010c), it was concluded that growing goats can be fed a diet consisting of 50% soybean cake plus 50% *Rhizopus oligosporus* treated *J. curcas* kernel cake. Sumiati *et al.* (2009) used *Rhizopus oryzae* to detoxify *J. 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). Rosa *et al.* (2010) have demonstrated that *Aspergillus niger* is not only a detoxifier but a nutritional enrichment additive. Joshi *et al.* (2011) have claimed that phorbol esters can be completely degraded in 9 days by *Pseudomonas aeruginosa* under optimized solid-state fermentation of deoiled *J. 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.

6. FUTURE RESEARCH

The role of chemists in finding the most appropriate detoxification method for the *J. 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.

6.1 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.

6.2 Detoxification Mechanisms

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

6.3 Chemical Detoxification Methods

Use of chemicals to detoxify the *J. curcas* seed can be quite fast and economically 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.

7. CONCLUSION

The toxicity of *J. 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 detoxifying methods is necessary.

REFERENCES

1. Abdel-Hafez AA-M, Nakamura N, Hattori M. Biotransformation of phorbol by human intestinal bacteria. Chemical & Pharmaceutical Bulletin. 2002; 50(2): 160-164.
2. Abou-Arab AA, Abu-Salem FM. Nutritional quality of *Jatropha curcas* seeds and effect of some physical and chemical treatments on their anti-nutritional factors. African Journal of Food Science. 2010; 4(3): 93-103.
3. Achten WMJ, Nielsen LR, Aerts R, Lengkeek AG, Kjaer ED, Trabucco A, Hansen JK, Maes WH, Graudal L, Akinnifesi FK, Muys B. Towards domestication of *Jatropha curcas*. Biofuels. 2010; 1(1): 91-107.
4. Adebawale KO, Adedire CO. Chemical composition and insecticidal properties of underutilized *Jatropha curcas* seed oil. African Journal of Biotechnology. 2006; 5(10): 901-906.
5. Adolf W, Opferkuch HJ, Hecker E. Irritant phorbol derivatives from four *Jatropha* species. Phytochemistry. 1984; 23: 129-132.
6. Ahmed WA, Salimon J. Phorbol ester as toxic constituents of tropical *Jatropha curcas* seed oil. European Journal of Scientific Research. 2009; 31(3): 429-436.
7. Aregheore EM, Becker K, Makkar HPS. Detoxification of a toxic variety of *Jatropha curcas* using heat and chemical treatments, and preliminary nutritional evaluation with rats. The South Pacific Journal of Natural and Applied Sciences. 2003; 21: 50-56.
8. Becker K, Makkar HPS. Effects of phorbol esters in Carp (*Cyprinus carpio* L). Veterinary and Human Toxicology. 1998; 40(2): 82-86.
9. Belewu MA, Akande BA. Biological upgrading of the nutritional quality of *Jatropha curcas* kernel cake: effect on performance characteristics of goat. International Research Journal of Biotechnology. 2010a; 1(2): 19-22.
10. Belewu MA, Belewu KY, Ogunsola FO. Nutritive value of dietary fungi treated *Jatropha curcas* kernel cake: Voluntary intake, growth and digestibility coefficient of goat. Agriculture and Biology Journal of North America. 2010b; 1(2): 135-138.
11. Belewu MA, Eniolorunda OO, Ilori GI. Response of goat to fungi (*Rhizopus oligosporus*, *Rhizopus nigrican*) treated *Jatropha curcas* kernel cake. Archives of Applied Science Research. 2010c; 2(4): 255-261.
12. Carroll GL, Little DR. Inter- and intramolecular reductive coupling reactions: An approach to the phorbol skeleton. Organic Letters. 2000; 2(18): 2873-2876.
13. Chivandi E, Mtimuni JP, Read JS, Makuza SM. Effect of processing method on phorbol esters concentration, total phenolics, trypsin inhibitor activity and the proximate composition of the Zimbabwean *Jatropha curcas* provenance: A potential livestock feed. Pakistan Journal of Biological Sciences. 2004; 7(6): 1001-1005.
14. Chivandi E, Kachigunda B, Fushai F. A comparison of the nutrient and antinutrient composition of industrially processed Zimbabwean *Jatropha curcas* and *Glycine max* meals. Pakistan Journal of Biological Sciences. 2005; 8(1): 49-53.

349 15. Crombie L, Games ML, Pointer DJ. Chemistry and structure of phorbol, the diterpene parent of co-carcinogens of
350 Croton oil. Journal of the Chemical Society. 1968; C: 1347-1362.
351

352 16. Demissie AG, Lele SS. Bioassay-assisted identification of phorbol ester from *Jatropha curcas* (Linn.) tissue culture.
353 International Journal of Pharma and Bio Sciences. 2010; 1(3): 1-7.
354

355 17. Devappa RK, Makkar HPS, Becker K. Optimization of conditions for the extraction of phorbol esters from *Jatropha* oil.
356 Biomass and Bioenergy. 2010a; 34(8): 1125-1133.
357

358 18. Devappa RK, Maes J, Makkar HPS, De Greyt W, Becker K. Quality of Biodiesel prepared from phorbol ester extracted
359 *Jatropha curcas* oil. Journal of the American Oil Chemists' Society. 2010b; 87(6): 697-704.
360

361 19. Devappa RK, Makkar HPS, Becker K. Biodegradation of *Jatropha curcas* phorbol esters in soil. Journal of Science of
362 Food and Agriculture. 2010c; 90: 2090-2097.
363

364 20. Devappa RK, Makkar HPS, Becker K. *Jatropha* diterpenes: A review. Journal of the American Oil Chemists' Society.
365 2011; 88(3): 301-322.
366

367 21. Dowlathabad MR, Sreeyapureddy A, Adhikari A, Bezawada K, Nayakanti D. Pharmaceutical investigation and
368 biopesticidal activity of *Jatropha curcas* L. seed oil on digestive enzymic profiles of *Cnaphalocrocis medinalis* (Rice leaf
369 folder) and *Helicoverpa armigera* (Cotton boll worm). International Research Journal of Pharmacy. 2010; 1(1): 194-200.
370

371 22. Dunphy WG, Delclos KB, Blumberg PM. Characterization of specific binding of [³H] phorbol 12, 13-dibutyrate and [³H]
372 phorbol 12-myristate 13-acetate to mouse brain. Cancer Research. 1980; 40: 3635-3641.
373

374 23. Gaur S, Viswanathan B, Seemamahannop R, Kapila S, Book NL. Development and evaluation of an efficient process
375 for the detoxification of *Jatropha* oil and meal. Nature Precedings. 2011; doi 10.1038/npre.2011.6118.1.
376

377 24. Goel G, Makkar HPS, Francis G, Becker K. Phorbol esters: Structure, biological activity and toxicity in animals.
378 International Journal of Toxicology. 2007; 26: 279-288.
379

380 25. Gschwendt M, Hecker E. Tumor-promoting compounds from *Euphorbia cooperi*: Di- and triesters of 16-hydroxy-12-
381 deoxy-phorbol. Tetrahedron Letters. 1970; 8: 567-570.
382

383 26. Haas W, Mittelbach M. Detoxification experiments with the seed oil from *Jatropha curcas* L. Industrial Crops and
384 Products. 2000; 12: 111-118.
385

386 27. Haas W, Sterk H, Mittelbach M. Novel 12-deoxy-16-hydroxyphorbol diesters isolated from the seed oil of *Jatropha*
387 *curcas*. Journal of Natural Products. 2002; 65(10): 1434-1440.
388

389 28. Hecker E. Three stage carcinogenesis in mouse skin – Recent results and present status of advanced model system
390 of chemical carcinogenesis. Toxicologic Pathology. 1987; 15(2): 245-258.
391

392 29. Hipal G, Mayank G, Nikhil G, Soham T, Prasad P, Girish G, Vamsi KK, Reddy MP, Sethiya BD, Rathod MR. Isolation
393 and characterization of phorbol esters (toxin) from the *Jatropha curcas* L. International Journal of Microbiology Research.
394 2009; 1(1): 1-7.
395

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

31. Jacobs S, Sahyoun NE, Saltiel AR, Cuatrecasas P. Phorbol esters stimulate the phosphorylation of receptors for insulin and somatomedin C. *Biochemistry*. 1983; 80: 6211-6213.

32. Jayaraman P, NesaPriya S, Parameshwari S, Priya SS, Jawahar N, Babu HS. Occurrence of storage fungi in *Jatropha* (*Jatropha curcas* L.) seeds. *African Journal of Microbiology Research*. 2011; 5(5): 475-480.

33. Joshi C, Mathur P, Khare SK. Degradation of phorbol esters by *Pseudomonas aeruginosa* PseA during solid-state fermentation of deoiled *Jatropha curcas* seed cake. *Bioresource Technology*. 2011; 102: 4815-4819.

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

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

36. Li C-Y, Devappa RK, Liu J-X, Lv J-M, Makkar HPS, Becker K. Toxicity of *Jatropha curcas* phorbol esters in mice. *Food and Chemical Toxicology*. 2010; 48: 620-625.

37. Makkar HPS, Aderibigbe AO, Becker K. Comparative evaluation of non-toxic and toxic varieties of *Jatropha curcas* for chemical composition, digestibility, protein degradability and toxic factors. *Food Chemistry*. 1998a; 62(2): 207-215.

38. Makkar HPS, Becker K, Schmook B. 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*. 1998b; 52: 31-36.

39. Makkar HPS, Francis G, Becker K. 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*. 2008; 88: 1542-1548.

40. Makkar HPS, Maes J, De Greyt W, Becker K. Removal and degradation of phorbol esters during pre-treatment and transesterification of *Jatropha curcas* oil. *Journal of the American Oil Chemists' Society*. 2009a; 86: 173-181.

41. Makkar HPS, Becker K. *Jatropha curcas*, a promising crop for the generation of biodiesel and value-added coproducts. *European Journal of Lipid Science and Technology*. 2009b; 111: 773-787.

42. Makkar HPS, Becker K. Are *Jatropha curcas* phorbol esters degraded by rumen microbes? *Journal of the Science of Food and Agriculture*. 2010a; 90: 1562-1565.

43. Makkar HPS, Kumar V, Oyeleye OO, Akinleye A, Angulo-Escalante MA, Becker K. Traditional wisdom confirmed by scientific research: *Jatropha* species from Mexico is non-toxic. *Nature Precedings*. 2010b; hdl 10101/npre.2010.4155.1.

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

443 45. Mwine JT, Van Damme P. Why do Euphorbiaceae tick as medicinal plants? A review of Euphorbiaceae family and its
444 medicinal features. Journal of Medicinal Plants Research. 2011; 5(5): 652-662.
445

446 46. Olivier GWJ, Rowan MG, Branch SK, Mahon MF, Molloy KC. Two esters of Synadenol, a new lathyrane diterpenoid,
447 from the latex of *Synadenium compactum* (Euphorbiaceae): A crystal structure analysis. Journal of the Chemical Society,
448 Perkin Transactions. 1992; 1: 1831-1835.
449

450 47. Rosa TS, Castro AM, Torres AG, Freire DMG. Analysis of nutritional composition and detoxification of *Jatropha curcas*
451 cake after solid-state fermentation. The 32nd Symposium on Biotechnology for Fuels and Chemicals. April 19-22, 2010.
452

453 48. Saetae D, Suntornsuk W. Antifungal activities of ethanolic extract from *Jatropha curcas* seed cake. Journal of
454 Microbiology and Biotechnology. 2010a; 20(2): 319-324.
455

456 49. Saetae D, Suntornsuk W. Variation of phorbol ester contents in *Jatropha curcas* from different provinces in Thailand
457 and the application of its seed cake for starter broiler diets. American-Euroasian Journal of Agriculture and Environmental
458 Sciences. 2010b; 8(5): 497-501.
459

460 50. Saetae D, Suntornsuk W. Toxic compound, anti-nutritional factors and functional properties of protein isolated from
461 detoxified *Jatropha curcas* seed cake. International Journal of Molecular Sciences. 2011; 12: 66-77.
462

463 51. Segal A, Van Duuren BL, Maté U. The identification of phorbolol myristate acetate as a new metabolite of phorbol
464 myristate acetate in mouse skin. Cancer Research. 1975; 35: 2154-2159.
465

466 52. Sharkey NA, Blumberg PM. Highly lipophilic phorbol esters as inhibitors of specific [³H] phorbol 12,13-dibutyrate
467 binding. Cancer Research. 1985; 45: 19-24.
468

469 53. Soerawidjaja TH. Potential of *Jatropha curcas* L. as a non-edible feedstock of biodiesel fuel. 7th Biomass-Asia
470 Workshop; Jakarta, Indonesia. 29 Nov.-1 Dec., 2010.
471

472 54. Sorg B, Fürstenberger G, Berry DL, Hecker E, Marks F. Preparation of retinoic acid esters of phorbol derivatives.
473 Journal of Lipid Research. 1982; 23: 443-447.
474

475 55. Sumiati Y, Yusriani Y, Astuti DA, Suharti S. Feeding fermented *Jatropha curcas* L. meal supplemented with cellulose
476 and phytase to kampung chicken. The 1st International Seminar on Animal Industry; Bogor, Indonesia. 2009; 191-197.
477

478 56. Sumiati Y, Sudarman A, Nurhikmawati I, Nurbaeti. Detoxification of *Jatropha curcas* meal as poultry feed. The 2nd
479 International Symposium on Food Security, Agricultural Development & Environmental Conservation in Southeast and
480 East Asia; Bogor, Indonesia. 2010; 61-66.
481

482 57. Szallasi Z, Krsmanovic L, Blumberg PM. Nonpromoting 12-deoxyphorbol 13-esters inhibit phorbol 12-myristate 13-
483 acetate induced tumor promotion in CD-1 mouse skin. Cancer Research. 1993; 53: 2507-2512.
484

485 58. Tigere TA, Gatsi TS, Mudita II, Chikuvire TJ, Thamangani S, Mavunganidze Z. Potential of *Jatropha curcas* in
486 improving smallholder farmers' livelihoods in Zimbabwe: An exploratory study of Makosa Ward, Mutoko district. Journal of
487 Sustainable Development in Africa. 2006; 8(3): 1-9.
488

489 59. Tjakradidjaja AS, Suryahadi, Mahajati R. Effectivity of *Jatropha curcas* seed meal fermented with various moulds as
490 protein source for male mice (*Mus musculus*). The 1st International Seminar on Animal Industry; Bogor, Indonesia. 2009;
491 157-164.

- 493 60. Wender PA, Martin-Cantalejo Y, Carpenter AJ, Chiu A, DeBrabander J, Harran PG, Jimenez J-M, Koehler MFT, Lippa
494 B, Morrison JA, Müller SG, Müller SN, Park C-M, Shiozaki M, Siedenbiedel C, Skalitzky DJ, Tanaka M, Irie K. The
495 chemistry-medicine continuum: Synthetic computer, spectroscopic and biological studies on new chemotherapeutic leads.
496 Pure and Applied Chemistry. 1998; 70(3): 539-546.
497
- 498 61. Wink M, Koschmieder C, Sauerwein M, Sporer F. Phorbol esters of *J. curcas* – Biological activities and potential
499 applications; Biofuel and Industrial Products from *Jatropha curcas*. G.M. Gübitz, M. Mittelbach & M. Trabi: Graz, Austria;
500 1997; 160-166.
501
- 502 62. Wirawan SS. Potential of *Jatropha curcas* L. Joint Task 40 / ERIA Workshop; 28 Oct., 2009; Tsukuba, Japan.
503
- 504 63. Wu Q-C, Tang Y-P, Ding A-W, You F-Q, Zhang L, Duan J-A. ¹³C-NMR data of three important diterpenes isolated
505 from Euphorbia species. Molecules. 2009; 14: 4454-4475.
506
- 507 64. Yan H. Is *Jatropha curcas* a solution to sustainable energy supply? Jatropha International Congress; Singapore.
508 December 17-18, 2008.
509
- 510 65. Ye M, Li C, Francis G, Makkar HPS. Current situation and prospects of *Jatropha curcas* as a multipurpose tree in
511 China. Agroforestry Systems. 2009; 76: 487-497.
512
- 513 66. Yuspa SH, Lichti U, Ben T, Patterson E, Hennings H, Slaga TJ, Colburn N, Kelsey W. Phorbol esters stimulate DNA
514 synthesis and ornithine decarboxylase activity in mouse epidermal cell cultures. Nature. 1976; 262: 402-404.
515