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Methods of detoxification of *Jatropha curcas* L. seed cake for its use as protein supplement in animal feed-An overview

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Seed cake, left as residue after extraction of oil from seeds of *Jatropha curcas* L. is rich in crude proteins and has the potential to be used as an animal feed, but the presence of toxic component and antinutrients limits its current use as organic manure. In this article, the toxic components and antinutrients present in *J. curcas* seed cake, various detoxification methods adopted for their removal and techniques used to estimate have been reviewed and discussed in details.

Keywords: Animal feed, Antinutrients, Detoxification, *Jatropha curcas* L., Seed cake, Phorbol esters.

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Introduction

Jatropha curcas L., a tropical plant belonging to the Euphorbiaceae family is a small tree or shrub of immense economic importance because of its several industrial and medicinal uses. In India, *J. curcas* is commonly known as Ratanjyot, Jamalgota, Chandrajyot, etc. *J. curcas* grows as a small tree or shrub of 3-5 m but can attain upto 8-10 m under favourable condition. It is a seed bearing plant containing 40-60 % (w/w) oil in the seed kernel. The oil has a fatty acid composition similar to that of common edible oils, but was found toxic in nature and for that reason its application for human and animal consumption is not possible[1]. However, the oil can be used for making biodiesel, a substitute for diesel fuel. Also, the oil has many medicinal uses in curing disease like dysentery, haemorrhoids, gonorrhoea, infertility, smallpox, skin infection, etc[2-4].

A considerable quantity of seed cake is left as a by-product after oil extraction from the *J. curcas* seed. One ton of seed yields approximately 615 kg of kernels with a potential oil recovery of approximately 51 % using solvent extraction method**.** The residual seed cake of about 264 kg is found to be rich in crude proteins (50-58 % depending on the residual oil)[5,6]. The types of essential amino acids, present in seed cake are comparable with those of Food and Agriculture Organization reference protein for a growing child of 2-5 years of age except lysine[5]. Further, a comparison of amino acid composition of the seed cake with soybean cake revealed an almost similar pattern for all essential amino acids, except lysine and sulphur amino acids. Lysine is lower whereas sulfur amino acids are higher in *J. curcas* seed cake. Thus, the seed cake has the potential to be used as a protein supplement for animals[6], but cannot be used as such owing to the presence of toxic and antinutritional components[7 -10]. Several cases of *J. curcas* seed poisoning in humans have been reported on accidental consumption of seeds exhibiting symptoms such as giddiness, vomiting and diarrhoea[11]. The major toxic components reported in the seed cake are phorbol esters (PEs) besides antinutrients such as trypsin inhibitor, lectin, saponin and phytic acid[12-16].

Thus, the use of *J. curcas* seed cake as a protein supplement for livestock feed would only be viable if detoxified by a suitable low cost detoxification process. Once the seed cake is freed from PEs and antinutrients, it has the potential to be a competitor to soybean meal in the animal feed market. This article gives an overview of the toxic and antinutrient components reported in the *J. curcas* seed cake, methods available for their estimation and the outcomes of various detoxification techniques adopted for its removal.

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Toxic & antinutrient components and methods of estimation

Different studies revealed that PEs ranged from 0.21 to 0.47 mg/g of seed cake whereas lectin activity varied from 51.3 to 102 using haemagglutination assay. Similarly wide variations were observed for saponin (1.8 to 3.4 % diosgenin equivalent), trypsin inhibitor (18.4 to 27.5 mg of trypsin inhibited per g sample) and phytate (6.2 to 10.1 % phytic acid equivalent) in the seed cake. The acceptable limits for making the seed cake non toxic is PEs (0.11 mg/g), lectin activity (51 using haemagglutination assay), saponins (3.4 % diosgenin equivalent), trypsin inhibitor activity (26.5 mg trypsin inhibited per g sample) and phytates (8.9 % phytic acid equivalent)[6, 17-19]. Table 1 summarizes the details of the methods available for their estimation.

Phorbol esters

PEs are the major impediment to the commercial use of *J. curcas* seed cake as a feedstock[25-29]. A variety from Mexico, which was non-toxic in feeding trials also contained saponins and lectins similar to those in toxic varieties, but was almost free from PEs. Thus, it was concluded that PEs contributes predominantly to the toxicity of *J. curcas* seed, seed cake and oil[30]. PEs are esters of tigliane diterpenes[31]. At least six types of PEs are found in *J. curcas* seeds[32-34]. The most studied PE is Phorbol – 12- myristate - 13- acetate (PMA), synonym: 12-O-tertradeconoyl phorbol - 13- acetate (TPA). The PEs are reported to mimic the action of diacylglycerol, an activator of protein kinase C which regulates different signal transduction pathways. Interference with the activity of protein kinase C affect a number of processes including phospholipid and protein synthesis, enzyme activities, DNA synthesis, phosphorylation of proteins, cell differentiation and gene expression[35]. The PEs even at very low concentration show toxicological manifestations in animal feed diet[36-37]. During the mechanical extraction of oil from seeds, 70-75 % of PEs comes with the oil but the rest are still retained in the seed cake, thus making both the cake and oil non-edible[38].

Lectins

Lectins are glycoproteins having a high degree of specificity toward the sugar component[39]. Lectins inhibit growth of animals. They are also called phytohemagglutins because they agglutinate red blood cells. During intestinal transit, about 60 % of the lectin bind to the intestinal epithelium where it causes disruption of the brush border and atrophy of the microvilli and reduces the viability of the epithelial cells. As a consequence of the interaction of lectin with epithelial surface of the small intestine, there is an increase in the weight of the small intestine. Other physiological effects are lowering of the insulin levels in the blood, inhibition of the disaccharides & proteases in the intestine, degenerative changes in the liver & kidneys, increased protein catabolism, breakdown of stored fat & glycogen, disturbance in mineral metabolism and interference with absorption of iron and lipid from the diet[20]. Lectin activity has been expressed as inverse of minimum amount of sample in mg/mL of the assay which produced agglutination.

Saponins

Saponins comprise a family of structurally related compounds containing a steroid or triterpenoid aglycone linked to one or more oligosaccharide moieties by glycosidic linkage. The carbohydrate moiety consists of pentoses, hexoses or uronic acids. The presence of both polar (sugar) and nonpolar (steroid or triterpene) groups provide saponins with strong surface active properties that are responsible for many of its adverse and beneficial effects. The aglycone (sugar free) portions of the saponins are termed sapogenins[20].

Saponins are reported to affect the permeability of the small intestinal mucosal cells and thus have effect on active nutrient transport. Saponins have also been shown to inhibit various digestive enzymes, including trypsin and chymotrypsin, and are also known to inhibit protein degradation by forming saponin-protein complexes[40].

Trypsin inhibitors

Trypsin inhibitors are chemicals that reduce the availability of biologically active trypsin, an enzyme essential for nutrition in many animals, including humans[39]. Trypsin inhibitors interfere with the physiological process of digestion through interference with the normal functioning of pancreatic proteolytic enzymes in non-ruminants leading to severe growth depression[20].

The trypsin inhibitor activity is expressed either in terms of number of trypsin units inhibited per mg sample or as mg of pure trypsin inhibited per g of sample. One trypsin unit is an arbitrary unit defined as an increase in 0.01 absorbance at 410 nm per 10 mL of reaction mixture under the prescribed conditions.

Table 1—Methods of estimation of toxic and antinutrients content of *J. Curcas* L. seed cake

Component	Method of estimation [20-24]
Phorbol esters	Makkar et al[20] described a method which is based on extraction of cake with methanol. About 0.5 g of the test sample was extracted four times with methanol. The quantitative estimation was done by loading a suitable aliquot of the extract into a HPLC fixed with a reverse phase C18 column. The separation was performed at room temperature (23 ^{o}C) and the flow rate was 1.3 mL/min using a gradient elution. The four phorbol esters peak appearing between 25.5 and 30.5 min were detected at 280 nm. Phorbol-12-myristate 13- acetate (PMA) was used as an external standard whose peak appeared between retention time 31 and 32 min. The area of the four phorbol ester peaks was then summed and results were expressed equivalent to PMA.
Trypsin inhibitor	Makkar et al[20] described a method in which the trypsin inhibitor activity (TIA) is measured indirectly by inhibiting the activity of trypsin. A synthetic substrate, benzyl-DL-arginine - para-nitroanilide (BAPNA) is subjected to hydrolysis by trypsin to produce yellow colored p-nitroanilide. The degree of inhibition by the plant extract to produce yellow color is a measure of TIA and is measured at 410 nm using a spectrophotometer. Smith et al[21] described a method involving extraction of the inhibitors from the sample in 0.01 M NaOH at pH 9.5 and mixing the unfiltered suspension with bovine trypsin. The activity of the remaining trypsin is then measured by offering it BAPNA under standard condition. The p-nitroaniline released is measured using spectrophotometer at 410 nm. The TIA was expressed in terms of mg pure trypsin inhibited per g of sample.
Lectin	Makkar et al[20] described two methods based on agglutination of red blood cells**.** First one is latex agglutination assay and another is haemagglutination assay. A series of serial twofold dilutions are made in both methods, which are added to a solution of red blood cells. Both assays can be used to detect lectin activity in seed cake, but the haemagglutination assay is better due to its higher sensitivity and shorter time duration for agglutination to appear. The haemagglutination activity was defined as the inverse of the amount of material in mg/mL in the last dilution giving positive agglutination. Aregheore et al[22] described a method in which lectin activity was investigated using latex agglutination and haemagglutination assays. The cake extract prepared using 0.9 % (w/v) NaCl solution was mixed with an equal volume of latex bead in a round bottomed well of microtitre plate. The plates were gently shaken at room temperature for 3-4 h. For latex agglutination assay, agglutination of the bead was seen as a uniform circular clump at the bottom of the well, while a negative pattern (indicating no agglutination) was a suspended form similar to that of blank. For haemagglutination assay, plates were left at room temperature for 1-2 h and read. A positive pattern which indicated agglutination was a uniform coating of the bottom of the well by erythrocytes while a negative pattern (indicating no agglutination) was a circular clump of erythrocytes surrounded by a concentric clear zone of equal size to the blank. For both the assay, lectin activity is expressed as reciprocal of the minimum quantity (in mg) of *J. curcas* L. seed cake/mL of the assay which produced agglutination.
Saponin	Makkar et al[20] described four methods and its quantitative evaluation is based on the separation of saponins on thin layer chromatography (TLC) plate. The saponin spots are located by their violet blue color produced by spraying the vanillin-perchloric acid or sulfuric acid reagent. The principle that saponins have hemolytic activity is also used for detecting hemolytic saponins. The developed TLC plates are sprayed with 6 % cattle erythrocyte solution. The clear zone against the red blood erythrocyte area indicates the presence of saponins. The haemolytic activity is expressed as the inverse of the minimum amount of saponin extract/mL assay medium in the highest dilution that started producing the hemolysis. Hiai et al[23] described a spectrophotometric method to determine total saponin (triterpenoid and steroidal) content. To 0.5 g ground samples taken in a centrifuge tube 10 mL of 80 % aqueous methanol was added. The contents were stirred overnight using magnetic stirrer. The tubes were centrifuged at 3000 g for 10 mins and supernatants were collected in volumetric flasks. The final volume was made to 25 mL with 80 % aqueous methanol. Aliquot samples from the flask were used for saponin determination. The results are expressed as disogenin equivalent from a standard curve of different concentration of diosgenin in 80 % aqueous methanol.
Phytates	Makkar et al[20] described two methods for determination of phytic acid (phytate). In the first method, phytate is extracted with trichloroacetic acid and precipitated as ferric salt. The iron content of the precipitate is determined spectrophotometrically and the phytate phosphorus content is calculated from this value, assuming a constant 4 Fe/6 P molecular ratio in the precipitate. In the second method, phytate is extracted with 3.5 % (w/v) HCl and further purified through an AG1-X8 chloride anion exchange column. The pink color of the wade reagent is due to the reaction between ferric ion and sulfosalicylic acid with an absorbance maximum at 500 nm. In the presence of phytate, the iron becomes bound to the phosphate ester and is unavailable to react with sulfosalicylic acid, resulting in a decrease in pink color intensity. Results are expressed as mg/100 g of sample. Vaintraub and Lapteva[24] described a colorimetric method which is based on decoloration of Fe^{3+} sulfosalicylate complex (wade reagent) by phytate. One mL of wade reagent was added to 3 mL of the assayed solution of phytate (extracted using 3.5 HCl and diluted with distilled water) and the mixture was centrifuged. The absorbance at 500 nm was measured using spectrophotometer. The phytate concentration was calculated from the difference between the absorbance of the control (3 mL H_2O + 1 mL of wade reagent) and that of the assayed sample.

Phytic acid

Phytic acid/phytates, a cyclic compound (1,2,3,4, 5,6-hexatris dihydrogen phosphate myoinositol) is a storage form of phosphorus bound to inositol in the fiber of legumes, nuts and seeds. Phytates are known to decrease the absorption of minerals particularly calcium, zinc and iron[41,24]. Phytic acid, as a result of possessing negative charge at a wide range of pH values has strong affinity to bind metal ions such as calcium, zinc and iron in foods making the minerals unavailable for absorption. In addition, phytic acid is known to form complex with proteins and starch, resulting in reduced digestibility of these nutrients[42].

Detoxification techniques

It was observed that trypsin inhibitors, lectins and phytates present in *J. curcas* seed cake are not responsible for permanent acute toxicity[43]. Trypsin inhibitors and lectins are heat labile and can be destroyed by moist heating[44-46]. But PEs and phytates are found to be insensitive to heat. The adverse effect of phytates could be mitigated by addition of phytates in diet[42]. The PEs are found to withstand roasting temperature as high as 160 ^{o}C for 30 mins[47-49]. Hence, the heat treatment is not at all effective to remove PEs from toxic genotype. Thus, the main issue is to establish a method for removal of PEs from the seed cake.

Due to the polar nature of PEs, high polarity solvents like methanol, ethanol and isopropyl alcohol have been used to selectively remove them from the seed cake. The use of ethanol has the advantage of being relatively non-toxic compared to methanol[50-51].

Different techniques such as chemical, thermal, biological and radiation treatment have been adopted in the past to remove antinutrients and toxic components from seed cake. Various methods adopted for detoxification along with their effect on the antinutrients are summarized in Table 2.

Out of the different methods used so far, chemical treatment method is considered environment unfriendly whereas biological method is time consuming and cumbersome. Radiation processing seems to be effective, less expensive and environment

Table 2—Detoxification techniques used for *J. Curcas* L. seed cake to remove phorbol esters and antinutrients

Methods	Outcome
Thermal treatment	
Moist heating at 121 ^{o}C for 30 mins[5, 52]	Total inactivation of trypsin inhibitor and lectin. No effect on phorbol esters and phytates.
Heating at 160 ^{o}C for 30 mins[47-49]	Phorbol esters and phytate remains unaffected
Chemical treatment	
Extraction with ethanol (80 %) or methanol (92 %)[53]	Saponins and phorbol ester reduced by 95 % after four extraction
Extraction with 0.1 M NaOH in 90% methanol and 85 % ethanol[51]	Phorbol ester reduces by 100 %
Ethanol extraction followed by treatment with 0.07 % $NaHCO_3$[54]	Phorbol ester content decreases by 99 %
Double solvent extraction with ethanol followed by moist heat treatment[55]	Phorbol ester reduces by 70.7 %
Petroleum ether extraction[55]	Phorbol ester reduces by 67.7 %
$NaHCO_3$ moistting combined with heat treatment at 90 ^{o}C for 30 mins and water wash [18]	Remove 76.5 % phorbol esters
Biological treatment	
Solid state fermentation of seed cake with white rot fungi *Bjerkandera adusta* or *Phlebia rufa* for 30 days at 28 ^{o}C[56]	Phorbol ester reduced by 91 and 97 %, respectively
Solid state fermentation of seed cake with Pseudomonas aeruginosa PseA strain within 9 days at 30 ^{o}C, pH=7 and 65 % relative humidity[57]	Phorbol ester reduces to undectable level
Fermentation of seed cake with *Cunninghamella echinulata* CJS- 90 for 6–12 days[58]	Phorbol esters reduces by 75-100 %, phytate content decreases by 65-96 %, Saponin content reduces by 55-99 %, trypsin inhibitor and lectin was removed completely
Radiation treatment	
Gamma irradiation of cake (50 kGy for 30 mins)[18]	Phorbol ester reduces by 71.3%
Gamma irradiation of cake (30 kGy in presence of sensitizer) at a rate of 2.5 kGy/hr[59]	Phorbol ester reduces by 75.8 %
$NaHCO_3$ moistting combined with 3 min ozone treatment at ozone dose of 50 mg/L[18]	Phorbol ester reduces by 75.2 %

friendly method for removing PEs from *J. curcas* seed Cake[18]. Siddhuraju et al (2002) stated that irradiation is one possible alternative and additional processing technique for reducing both heat stable and heat labile antinutrients[60]. Radiation treated material does not create any toxicological or other harmful effects. This has been confirmed by a long term laboratory study in which animals were fed with dry milk protein irradiated at 45 kGy[18]. No mutagenic effects were noted, no tumors were formed and no toxic effects were apparent in the animals over nine successive generations[18].

Conclusion

J. curcas seed cake is a potential source of protein supplement in animal feed. Presence of various toxic components and antinutrients make it non-edible for animal consumption. Lectins and trypsin inhibitors are heat labile and can be deactivated by moist heating. Phytic acid can be compensated by adding phytates in the diet, but PEs are found to be quite stable to heat even at 160 °C. Thus the main issue was to remove PEs from the seed cake. Chemical method is environment unfriendly and it is expensive to produce *J. curcas* seed meal by this method. Biological method is time consuming and cumbersome. Radiation treatment method can be a possible green alternative. So, taking into account the above factors, radiation treatment which is an emerging and environment friendly technique can be considered as an effective process for detoxification of *J. curcas* seed cake for its use as protein supplement in animal feed.

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Reference

1 Makkar H, Maes J, Greyt W D and Becker K, Removal and degradation of phorbol esters during pre-treatment and transesterification of *Jatropha curcas* oil, *J Am Oil Chem Soc*, 2009, **86**, 173-181.

2 Ahmed W A and Salimon J, Phorbol ester as toxic constituents of tropical *Jatropha curcas* seed oil, *Eur J Scientific Res*, 2009, **31**(3), 429-436.

3 Raja S A, Smart D S R and Lee C L R, Biodiesel production from *Jatropha* oil and its characterization, *Res J Chem Sci*, April 2011, **1**(1), 81-87.

4 Pradhan S, Sahoo N K, Naik S N and Sahoo P K, Estimation of toxic phorbol ester in *Jatropha curcas* oil, cake and biodiesel, *Asian J Chem*, 2010, **22**(5), 4069-4074.

5 Martinez- Herrera J, Siddhuraju P, Francis G, Davila-Ortiz G and 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 Chem*, 2006, **96**(1), 80-89.

6 Makkar H P S and Becker K, *Jatropha curcas* a promising crop for the generation of biodiesel and value added coproducts, *Eur J Lipid Sci Technol*, 2009, **111**(8), 773-787.

7 Srivastava M, Kumar A and Pal M, Phytochemical investigation on *Jatropha curcas* seed cake, *Int J Pharm Life Sci*, 2010, **1**(6), 357-362.

8 Saetae D and Suntornsuk W, Toxic compound, anti nutritional factors and functional properties of protein isolated from detoxified *Jatropha curcas* seed cake, *Int J Mol Sci*, 2011, **12**(1), 66-77.

9 Apiwatanapiwat W, Vaithanomsat P, Somkhang P and Malapant T, Optimization of protein hydrolysate production process from *Jatropha curcas* cake, *World Acad Sci Engineering Technol*, 2009, **3**(5), 103-106.

10 Devappa R K, Harinder M P S and Becker K, Biodegradable of *Jatropha curcas* phorbol ester in soil, *J Sci Food Agric*, 2010, **90**(12), 2090-2097.

11 Chikpah S K and Demuyakor B, Evaluation of physicochemical properties of *Jatropha curcas* seeds from four different agro-climatic areas of Ghana, *Int J Curr Res*, 2012, **4**(12), 446-450.

12 Malviya S N, Malakar R, Yadav M, Mishra A and Tiwari A, Estimation and characterization of protein present in seed extract of *Jatropha curcas*, *Advance Res Pharmaceut Biolog,* 2011, **1**(1), 35-44.

13 Harinder M P S, Francis G and Becker K, Preparation of protein concentrate form *Jatropha curcas* screw pressed seed cake and toxic and antinutritional factors in protein concentrate, *J Sci Food Agric*, 2008, **88**(9)**,** 1542-1548.

14 Makkar H P S, Becker K and Sohmook B, Edible provenance of *Jatropha curcas* from Quintana Roo state of Mexico and effect of roasting on antinutrients and toxic factors in seed, *Plant Foods Human Nutrition,* 1998, **52**(1), 31-36.

15 Trabi M, Gubitz G M, Steiner W and Foidi N, Toxicity of *Jatropha curcas* seeds in Biofuels and industrial product from *J. curcas,* GM Gubitz, M Mittelbach and M Trabi, Eds, Dbv-Verlag für die Technische Universität Graz, 1997, 173-178.

16 Makkar H P and Becker K, Are *Jatropha curcas* phorbol esters degraded by rumen microbes, *J Sci Food Agric*, 2010, **90**(9), 1562-1565.

17 Saetae D and Suntornsuk W, Antifungal activities of ethanolic extract from *Jatropha curcas* seed cake, *J Microbiol Biotechnol*, 2010, **20**(2), 319-324.

18 Diwani G I El, Rafei Sh A El and Hawash S I, Ozone for phorbol esters removal from Egyptian *Jatropha* oil seed cake, *Adv Appl Sci Res*, 2011, **2**(4), 221-232.

19 Makkar H P S, Becker K, Sporer F and Wink M, Studies on nutritive potential and toxic constituents of different provenance of *Jatropha curcas*, *J Agric Food Chem*, 1997, **45**(8), 3152-3157.

20 Makkar H P S, Siddhuraju P and Becker K, *Plant Secondary Metabolites* in Methods in Molecular Biology*,* E John and M Walker, Eds, Humana Press Inc, Toyota, New Jersey, 2007, 1-105.

21 Smith C, Van M W, Twaalfhoven L and Hitchcock C, The determination of trypsin inhibitor levels in foodstuff, *J Sci Forest Agric,* 1980, **31**(4), 341-350.

22 Aregheore E M, Makkar H P S and Becker K, Assessment of lectin activity in a toxic and a non-toxic variety of *Jatropha curcas* using latex agglutination and haemagglutination methods and inactivation of lectin by heat treatments, *J Sci Food Agric*, 1998**,** **77**(3), 349-352.

23 Hiai S, Oura H and Nakajima T, Color reaction of some sapogenins and saponins with vanillin and sulfuric acid, *Planta Med,* 1976, **29**(2), 116-122.

24 Vaintraub I A and Lapteva N A, Colorimetric determination of phytate in unpurified extracts of seeds and the products of their processing, *Anal Biochem*, 1988, **175**(1), 227-230.

25 Punsuvon V, Nokkaew R and Karnasuta S, Determination of toxic phorbol esters in biofertilizer produced with *Jatropha curcas* seed cake, *Sci Asia*, 2012, **38,** 223-225.

26 Kapila S, Viswanathan B, Lorbert S and Seemamahannop R, Characterization and quantification of phorbol esters by Tandem ESI-FT-ICR-MS, *Curr Trends Mass Spectrometry*, Special issue, May 2012, (www.spectroscopyonline.com).

27 Nafisa A, Kurchania A K and Swati B, Biomethanisation of *Jatropha curcas* defatted waste, *J Engineer Technol Res*, 2010, **2**(3), 038-043.

28 Saetae D and Suntornsuk W, Variation of phorbol ester contents in *Jatropha curcas* from different provinces in Thailand and the application of its seed cake for starter broiler diets, *Am-Eurasian J Agric Environ Sci*, 2010, **8** (5), 497-501.

29 Prasad L, Pradhan S, Das L M and Naik S N, Experimental assessment of toxic phorbol ester in oil, biodiesel and seed cake of *Jatropha curcas* and use of biodiesel in diesel engine, *Appl Energy*, 2012, **93**, 245-250.

30 Wink M, Koschmieder C, Sauerwein M and Sporer F, Phorbol esters of *J. curcas*-Biological activities and potential applications in Biofuels and Industrial Product from *J. curcas,* G M Gubitz, M Mittelbach and M Trabi, eds, Dbv-Verlag für die Technische Universität Graz, 1997, 160-166.

31 Devappa R K, Isolation characterization and potential agro-pharmaceutical application of phorbol esters from *Jatropha curcas* oil, Thesis, Faculty of Agricultural Science, University of Hohenheim, Stuttgart, Germany, 2012.

32 Devappa R K, Angulo-Escalante M A, Makkar H P S and Becker K, Potential of using phorbol esters as an insecticide against *Spodoptera frugiperda*, *Indian Crop Prod*, 2012, **38**, 50-53.

33 Roach J S, Devappa R K, Makkar H P S and Becker K, Isolation, stability and bioactivity of *Jatropha curcas* phorbol esters, *Fitoterapia*, 2012, **83**(3), 586-592.

34 Devappa R K, Maes J, Makkar H P S, Greyt W D and Becker K, Quality of biodiesel prepared from phorbol ester extracted *Jatropha curcas* oil, *J Am Oil Chem Soc*, 2010, **87**(6), 697-704.

35 Goel G, Makkar H P, Francis G and Becker K, Phorbol esters: structure, biological activity and toxicity in animals, *Int J Toxicol*, 2007, **26**(4), 279-288.

36 Lin J, Zhou X, Wang J, Jiang P and Tang K, Purification and characterization of curcin, a toxic lectin from the seed of *Jatropha curcas*, *Prep Biochem Biotechnol*, 2010, **40**(2), 107-118.

37 Oskoueian E, Abdullah N, Ahmad S, Saad W Z, Omar A R and Ho Y W, Bioactive compounds and Biological activities of *Jatropha curcas* L. kernel meal extract, *Int J Mol Sci*, 2011, **12**(9), 5955 - 5970.

38 Devappa R K, Rajesh S K, Kumar V, Makkar H P S and Becker K, Activities of *Jatropha curcas* phorbol esters in various bioassays, *Ecotox Environ Safe*, April 2012, **78**, 57-62.

39 Devappa R K, Makkar H P S and Becker K, Nutritional, biochemical and pharmaceutical potential of proteins and peptides from *Jatropha*: Review, *J Agric Food Chem*, 2010, **58** (11), 6543-6555.

40 Gangwal A, Extraction, estimation and thin layer chromatography of saponins: A review, *Int J Pharm Chem Sci*, 2013, **2**(3), 1366-1368.

41 Hallberg L, Rossander L and Skanberg A B, Phytate and the inhibitory effect of bran on iron absorption in man, *Am J Clin Nutr*, 1987, **45** (5), 988-96.

42 Makkar H P S and Becker K, Challenges and opportunities for using byproducts from the production of biodiesel from *jatropha* oil as livestock feed. In Proceedings of Animal Nutrition Association World conference, New Delhi, 14-17 Feb 2009, 168-170.

43 Makkar H and Becker K, Detoxification of *Jatropha curcas* meal for feeding to farm animal species and fish, US Pat 2011/0311710 A1 to Jatrosolutions GMBH, Stuttgart-Hohenheim, 22 Dec, 2011.

44 Azzaz N A E, El-Nisr N A, Elsharkawry E E and Elmotleb E A, Chemical and Pathological evaluation of *Jatropha curcas* seed meal toxicity with or without heat and chemical treatment, *Aust J Basic Appl Sci*, 2011, **5**(12), 49-59.

45 Oskoueian E, Abdullah N, Saad W Z, Omar A R, Puteh M B and Ho Y W, Anti-Nutritional Metabolites and Effect of Treated *Jatropha curcas* kernel meal on rumen fermentation in vitro, *J Anim Vet Adv*, 2011, **10**(2), 214-220.

46 Makkar H P S and Becker K, Potential of *J.curcas* seed meal as a protein supplement to livestock feed, constraints to its utilization and possible strategies to overcome constrants in Biofuels and Industrial Product from *J. curcas*, G M Gubitz, M Mittelbach and M. Trabi, Eds, Dbv-Verlag für die Technische Universität Graz, 1997, 190-205.

47 Belewu M A and Sam R, Solid state fermentation of *Jatropha curcas* kernel cakes : proximate composition and antinutritional components, *J Yeast Fungus Res*, 2010, **1**(3), 44-46.

48 Kumar A and Sharma S, An evaluation of multipurpose oil seed crop for industrial uses *(Jatropha curcas* L.) : A Review, *Indian Crop Prod*, 2008, **28**(1), 1-10.

49 Chang Chin-Feng, Weng, Jen-Hsien, Lin Kao-Yung, Liu Li-Yun and Yang Shang-Shyng, Phorbol Esters Degradation and Enzyme Production by Bacillus using *Jatropha* Seed Cake as Substrate, *Int J Environ Pollut Remediat*, 2014, **2**, 30-36.

50 Devappa R K, Makkar H P S and Becker K, Localization of antinutrients and quantitative identification of toxic components on *Jatropha curcas* seed, *J Sci Food Agric*, 2012, **92**, 1519-1525.

51 Punsuvon V and Nokkaew R, Comparison of detoxification methods on phorbol esters in deoiled *Jatropha curcas* meal for animal feeds, *J Chem Chem Eng*, 2013, **7**, 533-538.

52 Gogoi R, Niyogi U K and Tyagi A K, Reduction in trypsin inhibitor activity in *Jatropha* cake by chemical, thermal and radiation treatment, *Res J Chem Sci*, 2014, **14**(1), 1-6.

53 Makkar H P S and Becker K , *Jatropha curcas* toxicity: identification of toxic principles, *Toxic plants and other natural toxicants,* T Garland and A C Barr, Eds, Cab International, New York, 1998, 554-558.

54 Makkar H P S, Kumar V and Becker K, Use of detoxified *Jatropha* kernel meal and protein isolate in diets of farm animals in Biofuel co-products as livestock feed - opportunities and challenges, Harinder P S Makkar, Ed, Food and Agriculture Organization, United Nations, Rome, 2012, 351-378.

55 Chivandi E, Mtimuni J P, Read J S and Makuza S M, 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, *Pak J Biol Sci*, 2004, **7**(6), 1001-1005.

56 de Barros C R M, Ferreira L M M, Nunes F M, Bezerra R M F and Dias A A, Guedes C V, Cone J W, Marques G S M and Rodrigues A M, The potential of white-rot fungi to degrade phorbol esters of *Jatropha curcas* L. seed cake*, Eng Life Sci,* 2011, **11** (1), 107-110.

57 Joshi C, Mathur P and Khare S K, Degradation of phorbol esters by *Pseudomonas aeruginosa* PseA during solid-state fermentation of deoiled *Jatropha curcas* seed cake. *Bioresour Technol*, 2011, **102** (7), 4815-4819.

58 Somashekar D, Development of biological method for degradation of toxins like phorbol esters and reduction of antinutrients in *Jatropha curcas* seed cake by solid state fermentation, 4th International conference and exhibition on Analytical and bionalytical techniques, Hampton Inn Tropicana, Las Vegas, Nevada, United States, 15-17 Oct, 2013.

59 Gogoi R, Niyogi U K and Tyagi A K, Reduction of phorbol ester content in *Jatropha* cake using high energy gamma radiation, *J Radiat Res Appl Sci*, 2014, **7**(3), 305-309.

60 Siddhuraju P, Makkar H P S and Becker K, The effect of ionizing radiation on antinutritional factors and the nutritional value of plant materials with reference to human and animal food, *Food Chem*, 2002, **78**(2), 187-205.