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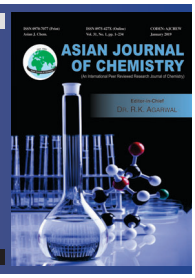

Analysis of Antinutritional Components of Oil and Meal in Different Provenances of *Jatropha curcas* from India

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The present work describes the estimation of antinutritional components such as phorbol esters in oil, trypsin inhibitors and phytates in the defatted meal of *Jatropha curcas* seeds collected from different geographical locations of India. The phorbol ester, trypsin inhibitor and phytate content in the defatted seed meal were extracted and estimated. Phorbol esters, the major toxic components vary from 0.9 to 3.2 mg/g in oil and 0.2 to 1.6 mg/g in the defatted meal. Similarly, a wide variation was observed for trypsin inhibitor (5.72-23.09 mg/g of defatted seed meal) and phytate (7.9-10.1 %) content in the meal of seeds collected from different geographical locations of India. The study also examined the variation of concentration of antinutritional components with the effect of meteorological parameters mainly climatological temperature and rainfall over stations. The phorbol esters content in the seed and meal are increased/decreased from region to region with the subsequent variation of temperature and rainfall. A similar trend is also observed in variation of trypsin inhibitors in seed cake whereas the variation of phytate concentration is not correlated with rainfall and temperature.

Keywords: *Jatropha curcas*, Antinutritional components, Phorbol ester, Trypsin inhibitor, Phytate.

INTRODUCTION

The search for alternative fuels has brought biodiesel into light which is easily-biodegradable, renewable and has low environmental problems. In India, non-edible oils from various plants like jatropha (*Jatropha curcas*), karanja (*Pongamia pinnata*), mahua (*Madhuca indica*), neem (*Azadirachta indica*), Simarouba (*Simarouba glauca*), *etc*. are used for bio-diesel production [1]. Among these, *Jatropha curcas* is being credited as a promising biofuel crop [2]. *Jatropha curcas* L. (Euphorbiaceae) bears 40-60 % oil in its kernel [3] with a fatty acid composition [4] similar to that of other oils used for human nutrition. However, due to its toxic nature, the oil can't be used for nutrition. The seed cake obtained after oil extraction may serve as highly nutritious protein (50-58 % depending on the residual oil) supplement in animal feed but because of the toxins present in it, presently being used only as a fertilizer [5,6]. Phorbol esters are the major toxic constituents present especially in seeds, which make the plant unpalatable and toxic to some vertebrates, insects and snails [3]. The seed cake is toxic to rats, mice and ruminants and therefore can't be used as an animal feed [7]. The cake has high trypsin inhibitor and lectin activities, which can be inactivated by heat treatment [8]. In addition, high concentration of the antimetabolic, metal-chelating and heat-stable factor named phytic acid has been reported in *Jatropha curcas* meal [9]. Saponin, another antinutritional component, is also known to be present (2.1-2.9 %, w/w) in seed meal [7].

The adoptions of biodiesel, as a fuel would necessitate its production at a large scale, which will produce the by-products, *e.g*. seed cake in very large amounts. The prime objective of this study is an estimation of antinutritional components such as, phorbol esters in oil and phorbol esters, phytates and trypsin inhibitors present in the defatted kernel (meal) obtained from *Jatropha curcas* seeds which in turn were obtained from different locations of India. The effect of agro-climatic conditions such as temperature and rainfall on concentration of antinutritional components was studied.

EXPERIMENTAL

Jatropha curcas seeds were collected from 10 different locations such as Bhubaneswar (Odisha state), Khandwa (Madhya pradesh state), Coimbatore (Tamil Nadu state), Rajkot (Gujarat state), Dehradun (Uttaranchal state), Raipur (Chattisgarh state), Faridabad, Gurgoan, Sohana (all situated in Haryana state) and Udaipur (Rajasthan state) of India. *N*-benzoyl-L-arginine *p*-nitroanilide (BAPNA), sodium phytate and phorbol-12-myristate 13-acetate were purchased from Sigma Chemical Co., St. Louis, USA. All other reagents were of analytical grade.

Oil content determination in defatted seed meal: The seeds were cleaned to remove all foreign materials. The cleaned seeds were dried under sun and then in hot air oven. The seeds were decorticated manually to obtain kernel. The oil content in the kernel was determined by solvent extraction. About 50 g of kernel of each kind of sample were grinded to fine powder, sieved through a 2 mm sieve. For oil content determination the powdered samples of each location were extracted separately in soxhlet apparatus using petroleum ether (60-80 ºC) as solvent. The extract was concentrated in rotary evaporator; the residual oil was cooled and weighed [10]. The dehaulled cake/meal was dried, stored in freeze and used for further experiments.

Extraction of phorbol esters fraction from oil and defatted kernel: Extraction of phorbol esters from oil was carried out by the solvent-solvent extraction method [11]. The oil was extracted in methanol-water (9:1, v/v) mixture using separating funnel. The constituents soluble in the methanol-water mixture were extracted in *n*-hexane by solvent-solvent extraction. The methanol-water layer was concentrated in a rotary evaporator and a viscous oily fraction was separated from the aqueous solution after concentration and again extracted with diethyl ether. The combined ether layer was washed with water, the lower water layer was discarded and the ether layer was evaporated. A brown viscous mass was obtained and dissolved in tetrahydrofuran for determination of phorbol esters by HPLC. Similarly, the meal was extracted in methanol-water (9:1, v/v) mixture in an orbital shaker for 8 h at 180 rpm at 25 ºC and the rest procedure as above was followed for extraction of phorbol esters.

Identification and quantification of phorbol esters: The phorbol esters content of Jatropha oil and meal was quantified by HPLC [3]. The phorbol esters fraction was analyzed using Waters 600 HPLC system equipped with a reverse phase C18 column (Waters Spherisorb, 5 µm, 250 mm × 4 mm i.d.) and a photodiode array detector. The column temperature was kept at 25 ºC and the flow rate was kept 1.3 mL/min. The solvents used were 1.75 mL orthophosphoric acid (85% v/v) in 1 L distilled water (A) and acetonitrile (B). All the solvents were filtered and degassed by Waters inline degasser. The gradient used was as follows: 0-10 min, 60 % A and 40 % B; 10-40 min, 50 % A and 50 % B; 40-55 min, 25 % A and 75 % B; 55-60 min, 100 % B. The peaks were integrated at 280 nm and the results were expressed as equivalent of phorbol-12-myristate 13-acetate, whose peak appeared at 50 min. The phorbol esters fractions obtained from oil and meal of jatropha seed sourced from different locations were analyzed as above and each analysis was conducted in triplicate.

Extraction of phytate from jatropha kernel meal: Phytates from meal was extracted by following a modified procedure of Harland and Oberleas [12]. The initial extraction was carried out with 0.6 N HCl as this extraction is more efficient in extracting total phytate.

Estimation of phytate content: Phytate content was determined by a colorimetric procedure described by Latta and Eskin [13]. The meal extract (3 mL, appropriately diluted) containing phytate was taken in test tubes. Wade′s reagent (1 mL; 0.03 % $FeCl_3 \cdot 6H_2O$ and 0.3 % sulfosalicylic acid in distilled water) was added to it and then vortexed for 5 s. The solution was then centrifuged at 5000 × g at 25 ºC for 15 min and the supernatant was read at 500 nm. The amount of total phytate in the meal sample was calculated from the standard curve obtained with pure sodium phytate.

Extraction of trypsin inhibitory activity: Jatropha meal (10 g) was extracted at room temperature with 0.25 N sulphuric acid (40 mL) for 1 h. The extract was then clarified by centrifugation (10000 × g; 4 ºC). Solid ammonium sulphate was added to the clear supernatant to give 70 % w/v saturation. After standing overnight, the precipitate was centrifuged at 10000 × g, 4 ºC for 15 min, dissolved in minimum volume of 50 mM acetate buffer, pH 6.0 containing 250 mM NaCl and then dialyzed against the same buffer. The trypsin inhibitor activity was estimated in the extract [14].

Estimation of trypsin inhibitory activity: The trypsin inhibitory activity was determined by measuring the hydrolytic activity towards the substrate BAPNA [15]. Following stock solutions were prepared: (a) 50 mM Tris-HCl, pH 8.2 containing 20 mM $CaCl_2$ (assay buffer); (b) 50 mM BAPNA in assay buffer; (c) Trypsin in assay buffer (0.2 mg/mL). Reaction mixture contained different amount of trypsin inhibitor appropriately diluted in assay buffer (0.55 mL), BAPNA (3 mL, 41 mM final concentration) and trypsin (0.05 mL, 2 µg). Incubation was carried out for 10 min at 25 ºC. The reaction was terminated with 30 % acetic acid (0.6 mL). Absorbance was read at 410 nm.

Variation of aninutritional components with agro climatic conditions: The agro climatic conditions such as temperature, rainfall, altitude and longitude of the 10 different locations from where seeds were collected are shown in Table-1. The variation of antinutritional components was plotted against average temperature and rainfall of respective stations.

RESULTS AND DISCUSSION

Identification and quantification of phorbol esters: Phorbol esters are the major toxic constituent present in oil and meal of Jatropha seed. The phorbol esters (four peaks) appeared between 41 and 48 min in the LC analysis. The phorbol esters content of Jatropha oil and meal collected from different locations of India is listed in Table-2. It is observed that phorbol esters content of oil (PECO) varies from 0.9 to 3.2 mg/g of oil. Phorbol esters content varies in oils because the oils are extracted from seeds, which in turn collected from different geographical locations of India. There is a wide variation in phorbol esters content of the oil of different locations of India. The oil extracted from Rajkot (Gujarat) seed has highest phorbol esters content (3.2 mg/g) and that of oil from Sohana (Haryana) seed has lowest phorbol ester content. Hass and Mittelbach [16] reported 0.31 %

TABLE-1
ORIGIN OF SEEDS AND AGRO CLIMATIC CONDITIONS OF COLLECTING SITES

Stations name	Latitude (°)	Longitude (°)	Climatological mean rainfall (cm)	Climatological mean temperature (°C)
Bhubaneswar (Odisha)	20.15	85.50	14.43	16.7
Khandwa (Madhya Pradesh)	21.50	76.22	9.15	13.9
Coimbatore (Tamil Nadu)	11.00	76.58	20.92	19.8
Dehradun (Uttaranchal)	30.19	78.02	6.21	11.4
Rajkot (Gujarat)	22.18	70.42	6.31	14.3
Raipur (Chattisgarh)	21.14	81.39	11.01	13.8
Faridabad (Haryana))	28.14	77.28	7.12	10.0
Gurgoan (Haryana)	28.25	77.00	5.11	9.2
Sohana (Haryana)	28.15	77.05	7.12	10.0
Udaipur (Rajasthan)	24.35	73.42	6.21	11.4

TABLE-2
AMOUNT OF PHORBOL ESTERS PRESENT IN JATROPHA OIL AND CAKE OF DIFFERENT LOCATIONS OF INDIA

Area of seed collection	Phorbol ester content of oil (g/kg of oil)	Phorbol ester content of cake (g/kg of cake)
Bhubaneswar (Odisha)	2.2 ± 0.01	0.7 ± 0.02
Khandwa (Madhya Pradesh)	1.8 ± 0.01	0.6 ± 0.01
Coimbatore (Tamil Nadu)	2.5 ± 0.03	1.3 ± 0.05
Dehradun (Uttaranchal)	2.0 ± 0.05	0.9 ± 0.05
Rajkot (Gujarat)	3.2 ± 0.04	1.6 ± 0.01
Raipur (Chattisgarh)	2.1 ± 0.02	0.6 ± 0.01
Faridabad (Haryana)	1.4 ± 0.04	0.4 ± 0.002
Gurgoan (Haryana)	1.1 ± 0.03	0.5 ± 0.02
Sohana (Haryana)	0.9 ± 0.02	0.2 ± 0.01
Udaipur (Rajasthan)	1.0 ± 0.002	0.4 ± 0.01

(3.10 mg/g of oil) of phorbol ester in *Jatropha curcas* oil collected from Nicaragua. It is also reported that 3.77 mg/g phorbol ester present in cold pressed oil [17].

Phorbol esters content in the cake (PECC) was found to vary from 0.2-1.6 mg/g of the cake. Devappa and Swamylingappa [18] have reported 1.35 mg/g of phorbol ester in dehaulled meal and 0.72 mg/g of phorbol ester in ghani pressed meal of jatropha seeds collected from Karnataka state of India. The cake (meal) of seed collected from Rajkot (Gujarat) has highest phorbol esters content (1.6 ± 0.01mg/g) but the cake of seed collected from Sohana (Haryana) has lowest phorbol ester content (0.2 ± 0.01 mg/g) among other loaction seeds. The phorbol esters content varies as these are of different geographical locations. The variation of phorbol esters content in oil and cake was plotted against temperature and rainfall, shown in Fig. 1. Temperature and rainfall has positive influence on phorbol esters contents

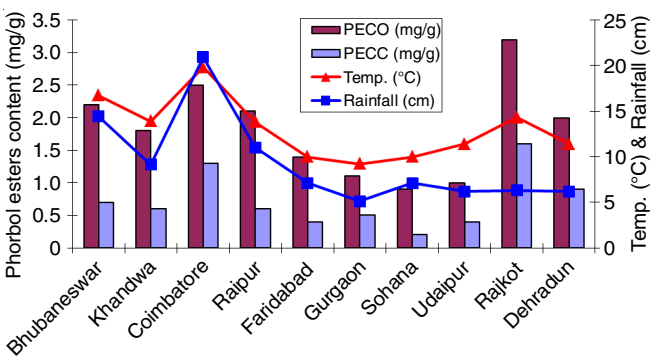

Fig. 1. Variation of phorbol esters in oil and cake of Jatropha seed with temperature (°C) and rainfall (cm)

of oil and cake of the seed collected from different locations of India. As temperature of the region increases phorbol esters concentration increases and phorbol esters concentration decreases with decrease in temperature. A similar trend is also observed for effect of rainfall on phorbol esters content of oil and meal of the seed collected from different locations of India.

Estimation of phytate content: The contents of phytic acid are reported in Table-3 and varied from 7.9 to 10.1 g /100 g of meal. The meal of Uttaranchal kernel has highest (10.1 %) phytic acid content and the meal of Gurgoan (Haryana) kernel has lowest phytic acid content (7.9%). The phytic acid content has been found to vary from 6.2 to 10.1 % in different provenances of defatted jatropha kernels [3]. Martinez-Herrera *et al.* [7] reported 8.54-9.27 % of phytic acid present in defatted kernel meal collected from four different regons of Mexico. The results obtained for the kernel meal of different locations of India is comparable with the result of other researchers. The phytic acid contents of the cake of seed collected from different domains was plotted against temperature and rainfall of the respective region (Fig. 2). It is observed that no such regular co-relation is found between phytate content of the seed, temperature and rainfall of the region of seed collection. The phytate content of the seed may be influenced by other parameters like quality and composition of the soil. The phytate content in Jatropha meals is very high (7.9-10.1 %) compared to the phytate content of soyabean meal (1.5 %) and peanut presscake (1.4 %) [8]. Phytates decrease protein digestibility by forming complexes and also by interacting with enzymes such as trypsin and pepsin [19]. The high level of phytate in Jatropha meal would also decrease the bioavailability of minerals, especially Ca^{2+} and Fe^{2+} [7].

TABLE-3
AMOUNT OF PHYTATES AND TRYPSIN INHIBITORS PRESENT IN MEAL OF JATROPHA SEED FROM DIFFERENT LOCATIONS OF INDIA

Area of seed collection	Phytate (%)	Trypsin inhibitors (g/kg of cake)
Bhubaneswar (Odisha)	8.9 ± 0.2	13.01 ± 0.04
Khandwa (Madhya Pradesh)	9.5 ± 0.6	17.01 ± 0.02
Coimbatore (Tamilnadu)	8.6 ± 0.1	23.09 ± 0.04
Dehradun (Uttaranchal)	10.1 ± 0.7	17.20 ± 0.01
Rajkot (Gujarat)	9.8 ± 0.5	13.67± 0.01
Raipur (Chattisgarh)	8.5 ± 0.1	14.33 ± 0.05
Faridabad(Haryana)	9.8 ± 0.4	12.56 ± 0.01
Gurgaon (Haryana)	7.9 ± 0.1	5.72 ± 0.04
Sohana (Haryana)	9.6 ± 0.5	7.93 ± 0.04
Udaipur (Rajsthan)	8.2 ± 0.1	7.94 ± 0.03

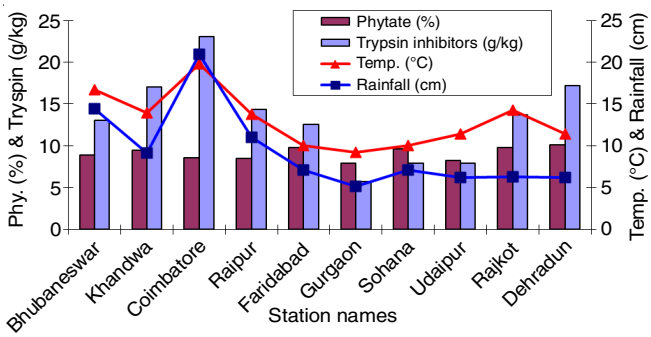

Fig. 2. Variation of phytates and trypsin inhibitors in meal of Jatropha seed with temperature (°C) and rainfall (cm)

Estimation of trypsin inhibitory activity: Trypsin inhibitory activity in the defatted kernel varies from 5.72 to 23.09 mg/g of the meal. Table-3 represents the trypsin inhibitory activity of the meal of different locations of India. The meal of Tamil nadu seed kernel has highest trypsin inhibitor activity (23.09 mg/g) and the meal of Gurgoan seed kernel has lowest trypsin inhibitor activity (5.72 mg/g) among the meal of seed kernel of different locations of India. Makkar *et al*. [3] reported that trypsin inhibitor activity varies from 18.4 to 27.5 mg of trypsin inhibited/g of dry mass in the meal of *Jatropha curcas* collected from different provenances. Smith *et al*. [20] reported trypsin inhibitor activity of 18.6 to 30 mg/g for soyabean meals. The consumption of raw soyabean meal produces adverse effect in monogastrics [21,22]. Trypsin inhibitors hinder the physiological process of digestion by interfering with the normal functioning of pancreatic proteolytic enzymes in non-ruminants, leading to severe growth inhibition. The variation in concentration of trypsin inhibitors of the meal of particular region was plotted against temperature and rainfall of the respective region (Fig. 2). Change in temperature and rainfall has positive influence on trypsin inhibitor content of the meal of the seed collected from different locations of India. Trypsin inhibitory activity of meal increases with increase in temperature and rainfall of the place of seed collection.

Conclusion

Jatropha has been identified as the energy crop for obtaining biodiesel by the Government of India. As Jatropha oil is a potential source for biodiesel preparation, large amount of Jatropha bye-product (meal and other bio-mass) is generated and handled by the rural people. Since the meal is used as fertilizers, biopesticides, animal feeding, molluscocides, mosquito reppelant and for production of biogas, it should be handled carefully due to presence of toxic constitutents. Phorbol ester are the most toxic component in the meal among other toxic components namely, trypsin inhibitor and phytates. The Indian sub-continent with a varied agro-climatic condition influences the physiology of plants and hence the chemical composition in the seeds. With differing temperature and rainfall conditions have tremendous effect on varying antinutritional components of *Jatropha curcas*. The phorbol esters of oil and cake of the seed and trypsin inhibitor content of the cake increases with increase in temperature and rainfall of the region where as phytate content of the cake is not affected by change in temperature and rainfall of the region of seed collection.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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