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Evaluation of Toxins, Antinutrients and Nutrients of Indian Cultivating Varieties of *Jatropha curcas*

CHAND PASHA*, KANNOJU BALAKRISHNA, NUNAVATH HANUMALAL, BANOTH SRINIVAS and BANOTH CHANDRASEKHAR

Department of Microbiology, University College of Science, Osmania University, Hyderabad-500 007, India

*Corresponding author: E-mail: cpasha21@yahoo.com

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Jatropha curcas a member of the euphorbiaceae family is a multipurpose shrub of significant economic importance because of its several biofuel and medicinal uses. The seeds contain high oil, which can be used as fuel directly or as a substitute to diesel in the transesterified form. The seed cake can be a good protein source for humans as well as for livestock, but highly toxic to a number of animal species due to the presence of toxins and antinutrient components. There is a variation in toxic, antinutrient and nutrient components in toxic, non toxic, wild and cultivable varieties. Seed, seed cake, leaves, fruits, oil and latex samples of 15 cultivating *J. curcas* varieties were collected across the India for toxins, antinutrient and nutrient characterization. The average content of principal toxin phorbol esters is 7.70 mg/g in seed, 4.24 mg/g in seed cake, 2.9 mg/g in oil and 0.73 mg/g in latex. phorbol esters were not found in young leaves and concentration was found to be increasing in leaves and fruits as age increases till a month. Curcin, phytate, trypsin inhibitor, saponin and total phenols are less than the non toxic Mexican varieties. Total protein in seed is 21 to 30 %, whereas in screw pressed seed cake it is 25 to 34 %. With increase in protein content decrease in oil content was observed.

Key Words: *Jatropha curcas*, Toxicity, Phorbol esters, Curcin, Phytate, Seed cake.

INTRODUCTION

Oil demand for energy is increasing day by day for the human needs. Biodiesel are plant derived monoalkyl esters of long chain fatty acids obtained by transesterification. Among non edible oil sources *Jatropha curcas* L is a wild oil seed plant of the tropics and/or it is now being credited as a most promising biofuel crop, ideally suited for growing in the waste lands[1]. *J. curcas* is oil bearing shrub containing 40-60 % oil in the kernel with a fatty acid composition similar to that of oils used for human nutrition[2]. The seed is rich source of oil and protein but can't be utilized for nutrient purposes due to its toxic nature. Jatropha also provides a meal (cake) as byproduct which is rich in crude protein. However the *J. curcas* seed and seed cake contains toxins and antinutrition factors were found to be toxic. Phorbol esters are the major toxic constituents present in the aerial parts of the plant especially in seeds, which make the plant unpalatable and toxic to some vertebrates, insects and snails[1]. Few studies[3-5] are available on toxic and antinutrient characterization of *J. curcas* available in different regions of India and Mexico. But the information on toxic, nutrient and antinutrient components in seed, seed cake, fruits, leaves and latex of many varieties being cultivated in India are lacking. There is a large variability in different accessions of *J. curcas* from diverse agro climatic regions[5-8]. This paper is a step towards filling the knowledge gap. An attempt was made to collect 15 cultivating varieties of *J. curcas* seed, seed cake, oil and latex each from different regions of India having different soil and climatic conditions and analyzed for toxins, antinutrients and nutrients.

EXPERIMENTAL

Fifteen cultivating varieties of *J. curcas* seeds, seed cakes, crude oils and latex were collected in India from different regions (Table-1) between June to August 2010. Different aged (5 to 30 days) leaves and fruits of N4 variety of *J. curcas* were also collected to evaluate the concentration of phorbol esters.

Mature and dried seeds were collected, dried in oven for moisture free and used after making powder with mortar and pistil. Screw pressed seed cake obtained, dried to moisture free in oven and used for experiments. Crude oil collected after screw pressing and used for experiment. Latex collected from plants after cutting branches and used for experiments. Leaves and fruits of different ages were collected and directly used for phorbol esters estimation.

Estimation of selected toxins

Phorbol ester extraction and estimation: Phorbol esters extraction and estimation is carried out by modified method of Gaudani *et al.*,[9]. Same method was used for seed, seed cake, oil and latex. For extraction 8 g of sample was suspended in 20 mL methanol and sonicated four times each of 10 min on

ultrasonic bath. Methanol was pooled to about 80 mL and evaporated completely on water bath and 40 mL double distilled water was added to remaining primary extract. Further, phorbol esters were extracted thrice with each of 20 mL of ethyl acetate. For better formation of two layers 1 g sodium chloride was added during ethyl acetate extraction. About 200 mg sodium sulphate was added to collected ethyl acetate extract to remove the moisture. Ethyl acetate phase was transferred into other flask and concentrated by heating on a water bath till no residual ethyl acetate was left. Resultant extract was dissolved in 2 mL of HPLC grade methanol.

HPLC analysis: Phorbol ester of *J. curcas* seed, oil, cake and latex were determined by using high performance liquid chromatography (SHIMADZU HPCL system) equipped with a reverse phase C_{18} column (Phenomenex, size: 150 × 4.60 mm, 5 µm) SPD M20A photodiode array detector, LC 20AD PUMP-B, LC-20AD PUMP-A, DGU-20A3 degasser and LC solutions software. The column temperature was controlled at 25 ºC and the flow rate was 1.3 mL min^{-1}. The solvents used were 1.75 mL orthophosphoric acid (85 %) in 1 L distilled water (A) and acetonitrile (B). All solvents were filtered and degassed by inline degasser. The gradient used was as follows: 0-10 min, 40 % B; 10-40 min, 50 % B; 40-55 min, 75 % B; 55-60 min, 100 % B. The column was equilibrated with 40 % B. The phorbol esters peaks appeared between 28 and 32 min. The peaks were integrated at 280 nm and results were expressed as equivalent of phorbol 12-myristate 13-acetate (Sigma Chemicals), whose peak appeared at 34 min. Each analysis was conducted in triplicates.

Curcin extraction and heamagglutination activity: 100 g of ground solid samples and 100 g of liquid samples were directly used for curcin extraction by suspending in 500 mL of 50 mM sodium phosphate buffer for over night. Supernatant was separated and ammonium sulphate was added to bring 60 % saturation. Precipitated protein was collected by centrifugation at 10,000 rpm, 4 ºC for 0.5 h. Precipitated protein was buffer exchanged against 50 mM sodium phosphate buffer for 24 h at 4 ºC in a dialysis bag. Crude protein obtained was loaded on Sephadox-G100 column and eluted with 100 mM Tris HCL 8.8 pH. Total protein eluted was estimated by Lowry's method.

Haemagglutination activity of extracted curcin and samples was carried out. Purified curcin and *J. curcas* samples were taken in the range of 1 to 200 mg and suspended in 1 mL of 50 mM phosphate buffer pH 7. 1 mL of trypsinized cattle blood was centrifuged and supernatant was discarded. To the washed pellet containing reticulocyte, samples suspended and centrifuged 1 mL buffer containing curcin was added. The mixture was applied on slides and after 1hr haemagglutination was observed under microscope. Minimum amount of samples showing haemagglutination was recorded.

Estimation of selected antinutrients

Phytic acid extraction and estimation: Phytic acid from the sample of *J. curcas* was extracted by Latta and Eskin method[10] and estimated using Wade and Morgan method[11].

Saponins: Total saponin content was determined using a spectrophotometric method, described by Hiai *et al.*[12]. The concentration of saponins was read off from a standard curve of diosgenin in 80 % aqueous methanol and results were expressed as diosgenin equivalents.

Trypsin inhibitor activity: Standard trypsin assay was conducted by taking 0.01 to1 mg/mL sigma trypsin in potassium phosphate buffer pH 7. To 1 mL of phosphate buffer pH 7 containing 86 µg/mL BAEE (*N*-α-benzoyl-L-arginine ethyl ester), 1 mL enzyme was added and incubated for 5 mins at 37 ºC. After incubation absorbance at 253 nm was measured. 1 g of sample was suspended in 100 mL of 100 mM phosphate buffer pH 7. After vortex and centrifugation 10 µL of supernatant was added to 1 mg trypsin assay. Absorbence was measured at 253 nm against suitable blank. Less differential activity in sample compared to the standard is inferred as trypsin inhibited.

Total phenols: Total phenols were determined using a spectrophotometric method described by Makkar *et al.*[13]. Total phenolics were quantified by the Folin-Ciocalteu reagent and results were expressed as tannic acid equivalents.

Estimation of selected nutrients

Crude proteins: Crude protein was determined in accordance with the standard methods of AOAC[14].

Carbohydrates: Soluble sugars were estimated by DNS method[15], insoluble sugars were estimated by anthrone method[16]. Soluble and insoluble sugars are collectively taken to give total sugar content.

Oil content estimation: The oil content of seeds was determined in accordance with the standard methods of AOAC[14].

Statistical analysis: Values represented were the means and standard deviations for three replicates carried out in three times separately (n = 9).

RESULTS AND DISCUSSION

J. curcas seeds are rich in oil, proteins and minerals[13]. Similar to that of oils used for human nutrition, the seed is rich source of oil, protein and minerals[17] but cannot be utilized for nutrition purposes due to high content of toxins and antinutrient factors[18]. Chemical composition proved that *J. curcas* seed and seed cake are good source of protein. The seed and seed cake are also rich in macro elements like K, Ca, Na, Mg and P and micro elements like Mn, Se, Zn[19]. *J. curcas* oil is looked upon as one of the most appropriate renewable alternative source for biodiesel production. But biodiesel production from *J. curcas* oil is not economical due to less yield and high cost[2]. Research is being carried out to add value to byproducts or wastes of *J. curcas*[20]. *J. curcas* seeds are highly toxic to number of animal species due to presence of toxins like phorbol esters and curcin, antinutrient factors such as phytic acid, trypsin inhibitors, saponins and phenols at high concentration. Hence there is a need to screen the *J. curcas* to select less or non toxic varieties for utilization of seed cake for animal feed. Makkar and Becker[21] reported that *J. curcas* nut is causing poisoning in humans after consumption of the seeds. Trypsin inhibitors, lectin activity and phytic acid which are antinutrient factors has been reported in Mexico non-toxic varieties also.

Phorbol esters were found in all the samples tested *i.e.* seed, seed cake, oil, latex, leaves and fruits. Tender leaves below 10 days age are not having phorbol esters but as the age of

TABLE-1
J. curcas CULTIVATING VARIETIES COLLECTED FROM DIFFERENT AREAS OF INDIA. PLANT VARIETY ITS GEOGRAPHICAL POSITION AND OIL CONTENT OF PLANT VARIETIES IS MENTIONED

S. No	Plant variety	Area/GPS NO.	Oil (%)
1	N-1	Zaheerabad. A.P. and 17.6823, 77.6271	34
2	N-2	Nandan Biomatrics, A.P. and 17.6761, 77.6225	30
3	V-1	Vikarabad, A.P. and 17.3335, 77.9043	37
4	S-1	Petroleum and Energy Studies Department, Dehradun and 30.3364, 77.9610	32
5	RK	Delhi and 28.5470, 77.1899	30
6	DOD	Petroleum University, Dehradun and 30.2671, 78.0770	25
7	I-1	Gulburga and 17.3067, 76.8747	27
8	CJC-19	Tamilnadu Biofuel Department (T.A.U) and 13.0921, 80.2245	26
9	N-4	Nandan biomatrics, A.P and 17.6761, 77.6225	26
10	K-3	Kolkatha, West Bengal and 22.6013, 88.3630	22
11	Jam	Jammu and Kashmir and 32.6948, 75.0146	25
12	Than	Thane, Mumbai and 19.1870, 72.9608	28
13	W-1	Warangal and 18.0694, 79.5252	30
14	T.A.U	Chennai, Tamilnadu and 13.0714, 80.2489	28
15	KTDM	Kothagudem, A.P. and 17.5466, 80.6468	32

TABLE-2
TOXINS (PHORBOL ESTERS AND CURCIN) CONTENT OF DIFFERENT VERITIES OF *J. curcas* SAMPLES

Variety	Phorbol esters (mg/g)				Curcin (mg/100 g)		
	Seed	Seed cake	Oil	Latex	Seed	Seed cake	Latex
N-1	8.8 ± 0.1	5.28 ± 0.2	3.0 ± 0.3	0.9 ± 0.5	12 ± 0.1	48 ± 0.2	27 ± 0.2
N-2	6.08 ± 0.2	4.19 ± 0.3	2.1 ± 0.2	0.5 ± 0.2	10 ± 0.1	46 ± 0.1	32 ± 0.2
V-1	9.29 ± 0.1	5.94 ± 0.2	2.2 ± 0.2	0.2 ± 0.2	20 ± 0.3	43 ± 0.3	33 ± 0.1
S-1	7.2 ± 0.1	2.52 ± 0.2	3.5 ± 0.2	1.2 ± 0.1	15 ± 0.8	52 ± 0.3	33 ± 0.2
RK	5.1 ± 0.3	1.75 ± 0.3	3.3 ± 0.3	1.1 ± 0.4	16 ± 0.4	49 ± 0.2	32 ± 0.3
DOD	7.9 ± 0.9	4.75 ± 0.6	3.1 ± 0.2	1 ± 0.5	17 ± 0.2	62 ± 0.2	30 ± 0.2
I-1	7.3 ± 0.2	4.3 ± 0.2	3.2 ± 0.4	0.7 ± 0.3	14 ± 0.3	42 ± 0.3	34 ± 0.2
CJC-19	5.6 ± 0.5	1.88 ± 0.3	3.4 ± 0.3	0.6 ± 0.2	17 ± 0.2	45 ± 0.4	28 ± 0.2
N-4	13.5 ± 0.2	7.1 ± 0.4	3.5 ± 0.2	0.4 ± 0.4	13 ± 0.2	45 ± 0.3	32 ± 0.3
K-3	9.9 ± 0.1	5.1 ± 0.3	4.3 ± 0.1	0.2 ± 0.3	11 ± 0.3	46 ± 0.2	31 ± 0.3
Jam	7.4 ± 0.1	2.8 ± 0.3	3.1 ± 0.2	0.8 ± 0.2	16 ± 0.2	46 ± 0.3	28 ± 0.5
Than	8.7 ± 0.3	5.4 ± 0.1	2.5 ± 0.2	1.1 ± 0.1	13 ± 0.1	45 ± 0.2	32 ± 0.5
W-1	5.33 ± 0.4	4.39 ± 0.6	0.4 ± 0.2	0.9 ± 0.3	15 ± 0.2	52 ± 0.1	27 ± 0.2
T.A.U	5 ± 0.3	4.13 ± 0.1	1.7 ± 0.1	0.6 ± 0.2	18 ± 0.2	47 ± 0.1	32 ± 0.1
KTDM	8.5 ± 0.4	4.1 ± 0.2	4.3 ± 0.3	0.8 ± 0.1	18 ± 0.2	52 ± 0.1	34 ± 0.1
Average	7.70 ± 0.2	4.24 ± 0.28	2.90 ± 0.22	0.73 ± 0.26	15 ± 0.25	48 ± 0.22	31 ± 0.24

leaves increased, concentration of phorbol esters also increased. Phorbol esters were found in all ages of fruits and also showed an increase by age till one month. Phorbol esters in 3 to 25 days old fruits was in the a range of 0.4 to 1.5 mg/g. Phorbol ester content in 1 month old leaves was 0.5 mg/g. When average phorbol esters content of these samples compared, whole seed contains 7.70 mg/g, seed cake 4.24 mg/g, oil 2.90 mg/g and latex 0.73 mg/g (Table-2). Herrera *et al.*[3] also reported that phorbol esters are present at high levels in the kernels which are the main toxic agents responsible for toxicity. Phorbol esters were reported in high concentration in the kernel meal of Mexican 4.05 mg/g[5], Medagascar-6.16 and Uganda-6.6 mg/g toxic genotypes[6]. Similar observation is made in the present study also as the average phorbol content is 4.24 mg/g in seed cake. Some of the phorbol content in seed cake and oil is almost equal to seed phorbol content, indicating no possible degradation during screw pressing. In present study, phorbol esters concentration in seeds is maximum (N4 veraiety-13.50 mg/g) compared to seed cake, oil and latex samples. Herrera *et al.*[3] reported phorbol concentration of 0.35-1.44 mg/mL in the Mexican nontoxic samples. In this study phorbol esters range was 1.75 mg/g to 7.1 mg/g in seed cakes. Young leaves (brown coloured) are not having any phorbol esters and its content is increased as age increased. Fruits of all ages are having phorbol esters but young fruits are having less phorbol esters. All 15 cultivating varieties of *J. curcas* studied were commercial varieties used in India with high oil content (Table-1), but phorbol esters content is also high.

Purified curcin at 5 mg concentration was found to cause complete heamagglutination of 1 mL leucocytes. In assays, substrates were used from 1 to 200 mg to evaluate the heamagglutination. Based on substrate required for heamagglutination and minimum curcin required for 1 mL reticulocytes heamagglutination (5 mg), the curcin content of samples was estimated. On average, curcin content is 15 mg/g in seed (in 198 mg seed sample required for heamagglutination), 48 mg/g in seed cake (in 104 mg seed cake) and 31 mg/g in latex (in 161 mg latex) (Table-2). Heamagglutination studies with oil were not appropriate, as oil is disturbing the heamagglutination. No curcin was detected in leaves and fruits of below 10 days old and subsequent increase with age was noted (data not shown). Lectin related component curcin is another toxic factor in

TABLE-3
ANTINUTRIENT FACTORS (PHYTIC ACID, SAPONIN, TOTAL PHENOL AND TRYPSIN INHIBITORS) CONTENT OF DIFFERENT VARIETIES OF *J. curcas* SAMPLES

	Phytic acid (%)				Saponin (%)				Total phenols (%)				Trypsin inhibitors (mg of trypsin inhibited per g of sample		
Variety	Seed	Seed cake	Oil	Latex	Seed	Seed cake	Oil	Latex	Seed	Seed cake	Oil	Latex	Seed	Seed cake	Latex
N-1	8 ± 0.2	5.5 ± 0.2	0.1 ± 0.06	1.2 ± 0.03	2.1 ± 0.01	2.6 ± 0.02	0.7 ± 0.21	3.1 ± 0.31	0.7 ± 0.08	2 ± 0.06	0.4 ± 0.02	10 ± 0.01	26.0 ± 2.1	21.0 ± 3.9	26.8 ± 2.1
N-2	9.8 ± 0.12	3.9 ± 0.21	0.1 ± 0.08	1.8 ± 0.04	2.4 ± 0.09	2.0 ± 0.03	0.6 ± 0.05	4.6 ± 0.21	0.9 ± 0.03	3 ± 0.05	0.2 ± 0.06	11 ± 0.03	32.0 ± 2.3	23.0 ± 2.2	29.0 ± 1.43
V-1	8 ± 0.6	3.8 ± 0.2	0.8 ± 0.05	1 ± 0.05	2.0 ± 0.05	4.8 ± 0.06	0.4 ± 0.36	4.3 ± 0.02	0.5 ± 0.07	2 ± 0.03	0.1 ± 0.02	9 ± 0.40	26.0 ± 1.9	26.0 ± 4.3	22.5 ± 2.5
S-1	7.6 ± 0.5	3.3 ± 0.7	0.6 ± 0.05	1.2 ± 0.01	3.0 ± 0.04	5.0 ± 0.04	1.4 ± 0.09	5.8 ± 0.32	1.0 ± 0.02	5 ± 0.01	0.1 ± 0.04	8 ± 0.37	21.0 ± 3.1	21.0 ± 3.3	28.3 ± 1.8
RK	9.4 ± 0.8	5.9 ± 0.6	0.3 ± 0.03	1.5 ± 0.05	1.8 ± 0.05	4.2 ± 0.07	0.8 ± 0.32	7.3 ± 0.14	1.3 ± 0.06	2 ± 0.01	0.3 ± 0.04	4 ± 0.17	23.0 ± 3.5	26.0 ± 4.9	29.9 ± 4.2
DOD	7.2 ± 0.6	5.6 ± 0.29	0.8 ± 0.0 7	1 ± 0.07	2.1 ± 0.06	3.2 ± 0.04	0.4 ± 0.01	3.2 ± 0.27	1.9 ± 0.05	4 ± 0.05	0.2 ± 0.02	5 ± 0.26	26.0 ± 2.2	19.0 ± 2.1	28.9 ± 2.9
I-1	7.3 ± 0.3	3.2 ± 0.34	0.5 ± 0.07	1.6 ± 0.04	2.0 ± 0.01	4.2 ± 0.09	0.5 ± 0.04	8.6 ± 0.69	0.8 ± 0.02	2 ± 0.08	0.3 ± 0.03	5 ± 0.25	30.0 ± 3.3	18.0 ± 3.9	27.0 ± 6.3
CJC-19	7.0 ± 0.2	4.4 ± 0.13	0.7 ± 0.04	1.7 ± 0.06	2.6 ± 0.03	2.8 ± 0.09	0.8 ± 0.01	9.1 ± 0.34	1.6 ± 0.010	3 ± 0.01	0.5 ± 0.02	4 ± 0.13	23.0 ± 5.2	26.0 ± ± 6.2	29.2 ± 4.3
N-4	8.8 ± 0.3	5.2 ± 0.24	0.9 ± 0.06	1.8 ± 0.03	3.2 ± 0.01	4.2 ± 0.09	1.0 ± 0.07	5.6 ± 0.05	2.0 ± 0.05	2 ± 0.07	0.2 ± 0.072	7 ± 0.61	25.0 ± 3.3	26.0 ± 3.8	24.9 ± 2.9
K-3	8.9 ± 0.5	4.8 ± 0.62	0.3 ± 0.03	1.3 ± 0.05	2.8 ± 0.07	3.6 ± 0.08	0.9 ± 0.06	9.9 ± 0.31	1.9 ± 0.06	4 ± 0.03	0.1 ± 0.03	12 ± 0.33	26.0 ± 2.8	20.0 ± 2.7	23.0 ± 3.7
Jam	9.1 ± 0.6	4.9 ± 0.31	1 ± 0.01	1.1 ± 0.03	2.6 ± 0.06	3.1 ± 0.04	0.8 ± 0.02	6.3 ± 0.51	0.9 ± 0.05	3 ± 0.12	0.2 ± 0.03	11 ± 0.85	28. 0 ± 5.6	20.0 ± 2.6	27.0 ± 3.1
Than	7.6 ± 0.5	5.3 ± 0.11	0.3 ± 0.025	1.9 ± 0.09	2.8 ± 0.05	3.8 ± 0.07	0.6 ± 0.032	10.0 ± 0.22	0.7 ± 0.09	2 ± 0.08	0.2 ± 0.04	6 ± 0.1	28.0 ± 3.8	20.0 ± 1.8	20.0 ± 3.8
W-1	7.2 ± 0.3	4.8 ± 0.51	0.9 ± 0.33	2 ± 0.02	1.5 ± 0.04	2.6 ± 0.09	0.7 ± 0.02	5.5 ± 0.38	0.5 ± 0.04	3 ± 0.016	0.4 ± 0.01	10 ± 0.4	29.0 ± 1.9	24.0 ± 3.2	25.3 ± 2.5
T.A.U	8.9 ± 0.5	3.9 ± 0.18	0.2 ± 0.23	1.4 ± 0.01	2.2 ± 0.24	3.1 ± 0.01	1.0 ± 0.06	7.8 ± 0.41	2.0 ± 0.06	4 ± 0.05	0.1 ± 0.03	11 ± 0.5	26.0 ± 3.9	21.0 ± 3.5	27.0 ± 3.7
KTDM	7.5 ± 0.8	5.1 ± 0.31	0.8 ± 0.42	1.7 ± 0.09	2.3 ± 0.04	3.5 ± 0.05	0.8 ± 0.07	9.5 ± 0.24	1.8 ± 0.07	3 ± 0.02	0.2 ± 0.04	8 ± 0.6	27.0 ± 1.9	25.0 ± 1.8	23.9 ± 1.3
Average	8.1+ 0.45	4.6 ± 2.37	0.5 ± 0.10	1.4 ± 0.04	2.3 ± 0.04	3.5 ± 0.05	0.7 ± 0.09	6.7 ± 0.29	1.2 ± 0.05	2.9 ± 0.04	0.2 ± 0.03	8.0 ± 0.33	26.4 ± 3.12	22.4 ± 3.30	26.1 ± 3.10

J. curcas. About 0.78 to 12.5 mg/mL curcin was reported in Mexican varieties[5]. Becker and Makkar[21] found that lectin activity was similar in both non toxic and toxic genotypes. Curcin was extracted and purified from *J. curcas* seed cake and used to evaluate the minimum curcin required for heamagglutination of 1 mL reticulocytes. High curcin samples will require little substrate for heamagglutination and *vice versa*. Seed cake and latex showed almost equal curcin, where as seeds are having higher curcin. Sample required for heamagglutination in this study is more than 100 mg where as in non toxic Mexican varieties it is less than 100 mg[5], indicating less curcin in studied *J. curcas* varieties.

The average phytic acid content is 8.1 % in seed, 4.6 % in seed cake, 0.5 % in oil and 1.4 % in latex (Table-4). More phytic acid is present in seed, very little amount is passed in oil. Seed cake is also having less phytic acid than seed indicating possible degradation of phytic acid by screw press oil extraction (Table-3). Makkar and Becker[22] reported anti-metabolic, metal-chelating properties of *J. curcas* were due to phytic acid. Maximum phytic acid content in seed is 9.8 %, where as it is minimum in seed cake and oil indicating possible degradation during screw press oil expelling. The similar phytic acid content was reported in Mexican varieties[23]. However, Egyptian varieties contained high concentration of phytic acid[18]. The average saponins concentration is 2.3 % in seed, 3.5 % in seed cake, 0.7 % in oil and 6.7 % in latex (Table-3). Saponins are natural triterpene plant glycosides found in many plant species and effects physiological balance[24]. 1.9-2.3 % saponin in raw seed meal was reported in edible provenances of *J. curcas* from Mexico. Saponins concentrations were not decreased after roasting seed meal[25]. Toxic and non toxic Mexican varieties contain 1.1 to 3.7 % saponins[5]. Egyptian seed varieties contain less saponins than other varieties[19]. Saponin concentration was estimated and maximum of 10 % was found in latex in present study. Saponin contents of seed and seed cake levels from present test results showed similar to Mexican and Egyptian genotypes seeds. The average trypsin activity inhibited per gram sample is 26.4 mg with seed samples, 22.4 mg with seed cake and 26.1 mg latex. The seed cake inhibited less trypsin activity than seed samples indicating possible degradation of trypsin inhibitors during oil expelling. Trypsin inhibitors in oil were not calculated due to improper miscibility, whereas 1 g of latex inhibited average 26.1 mg trypsin (Table-3). About 30 to 35 mg/g tripsin inhibitors were reported in nontoxic Mexican variety kernel cakes[5], whereas in studied seed cakes, it is 18 to 26 mg/g. Kernel seeds have high concentration of trypsin inhibitors activity compared to shell and whole seeds of the Egyptian varieties[19]. These Egyptian concentrations are

TABLE-4
NUTRIENT COMPONENTS (PROTEIN AND CARBOHYDRATES) CONTENT OF DIFFERENT VARIETIES OF *J. curcas* SAMPLES

Variety	Total protein (%)				Total carbohydrates (%)			
	Seed	Seed cake	Oil	Latex	Seed	Seed cake	Oil	Latex
N-1	24 ± 1.1	25 ± 1.1	0.1 ± 0.01	12 ± 0.8	25 ± 1.1	40 ± 2.5	4.5 ± 0.3	13 ±0.7
N-2	22 ± 1.3	28 ± 1.1	0.3 ± 0.02	13 ± 1.0	30 ± 1.3	44 ± 2.2	1.8 ± 0.1	11 ± 1
V-1	21 ± 1.3	26 ± 1.5	0.1 ± 0.01	19 ± 1.1	33 ± 1.4	41 ± 2.1	4.5 ± 0.3	20 ± 1.1
S-1	23 ± 1.1	26 ± 1.4	0.4 ± 0.05	14 ± 0.9	33 ± 1.2	44 ± 2.2	2.2 ± 0.4	14 ± 1.2
RK	30 ± 1.2	34 ± 1.3	0.5 ± 0.03	11 ± 1.3	31 ± 1.2	44 ± 2.3	2.5 ± 0.2	16 ± 1.3
DOD	26 ± 1.2	29 ± 1.2	0.1 ± 0.04	13 ± 1.2	28 ± 1.1	48 ± 2.4	3.5 ± 0.2	17 ± 1.2
I-1	28 ± 2.2	31 ± 1.2	0.1 ± 0.07	13 ± 0.9	31 ± 1.2	46 ± 2.2	2.2 ± 0.3	14 ± 0.8
CJC-19	26 ± 1.1	28 ± 1.1	0.3 ± 0.02	12 ± 1.2	33 ± 1.2	43 ± 2.4	1.8 ± 0.4	16 ± 1.2
N-4	25 ± 1.2	27 ± 1.2	0.2 ± 0.04	17 ± 1.3	32 ± 1.0	45 ± 2.3	4.2 ± 0.3	14 ± 1.2
K-3	29 ± 1.1	31 ± 1.0	0.3 ± 0.02	15 ± 1.1	27 ± 1.5	48 ± 2.6	2.1 ± 0.2	12 ± 1.1
Jam	25 ± 1.2	27 ± 1.1	0.4 ± 0.06	15 ± 1.0	31 ± 1.3	42 ± 2.7	1.5 ± 0.3	16 ± 1
Than	24 ± 1.3	25 ± 1.2	0.5 ± 0.03	14 ± 1.3	32 ± 1.1	44 ± 2.1	2.3 ± 0.2	13 ± 1.3
W-1	23 ± 1.2	29 ± 1.3	0.1 ± 0.01	20 ± 1.4	30 ± 1.2	43 ± 2.4	1.2 ± 0.4	16 ± 1.4
T.A.U	24 ± 1.4	29 ± 1.2	0.1 ± 0.05	18 ± 1.1	24 ± 1.3	45 ± 2.5	2.3 ± 0.1	18 ± 1.3
KTDM	22 ± 1.2	25 ± 1.3	0.7 ± 0.02	13 ± 1.2	29 ± 1.2	42 ± 2.2	1.4 ± 0.3	17 ± 1.2
Average	24.8 ± 1.27	28.1 ± 1.20	0.28 ± 0.03	14.6 ± 1.12	29.9 ± 1.22	43.9 ± 2.34	2.5 ± 0.26	15.1 ± 1.13

closely similar to the concentration of trypsin inhibitors activity expressed in our test seeds, seed cakes. The average total phenol content is 1.2 % in seed, 2.9 % in seed cakes, 0.2 % in oil and 8 % in latex (Table-3). Latex showed maximum concentration of 12 % total phenols. Total phenol content is 0.5 to 2 % in seed, 2 to 5 % in seed cake, where as 0.399 % was showed non-toxic seeds[19].

The average total protein content is 24.8 % in seed, 28.1 % in seed cake, 0.28 % in oil and 14.6 % in latex (Table-4). 18-33 % of crude protein was reported in toxic Mexican varieties[5] and 32 % in seeds of Egyptian varieties[19]. 27-30 % protein was found in kernel seed in edible non toxic provenances of Mexican *J. curcas*[25]. Present results showed that seed cakes contain maximum 34 % total protein. There is more protein in seed cake than seed, but it is not proportion to the removed oil. This is due to denaturation of protein with temperature generated in screw pressing. There is more protein in only organic solvent oil extraction seed cake than screw pressed cake[26]. The average total sugars content is 29.9 % in seed, 43.9 % in seed cake, 2.5 % in crude oil and 15.1 % in latex (Table-4). Arab and Salem[19] showed 30.11 % carbohydrates content in seed. Herrera *et al*.[3] reported below 12 % carbohydrates in 4 varieties defatted kernel of different agro climatic regions of Mexico.

All the 15 verities studies were being cultivated for biofuels production and seeds contain high oil content in the range of 22-37 % (Table-1). In current study it is observed that there is a negative correlation of phorbol concentration with protein and also there is a negative correlation between protein and oil contents.

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