Evaluation of Underutilized Cereal Crop Coix Lacryma Jobi (Jobs's Tear) for Nutritiveand Nutraceutical Values

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Abstract

Coix lacryma jobi (Job's tear) is a lesser known but ancient crop. This is a minor cereal belonging to Gramineae family and the dehusked grain is consumed as staple food like rice. Very little information is available about the crop which is known to be resilient; have ability to withstand biotic stress and drought and can be grown without much inputs like irrigation, chemical fertilizers and other agrochemicals.

Nutritive values were evaluated in terms of crude protein, total carbohydrate, lipid, crude fibre and total mineral in the form of ash content apart from calorific value. Crude protein exhibited variation in the range of 13.9% to 18.5%. Likewise, lipid content varied from 5.3% to 8.2%. These values are much higher than major food crops like rice, wheat, maize etc. Carbohydrate contents were comparable to that of rice. Total mineral in the form of ash content varied from 1.35% to 3.34% which are little higher than that of major food crop. Phenolics contents varied from 0.208 mg GAE/g dm to 0.580 mg GAE/g dm. in vitro antioxidant activity in terms of DPPH – RSA varied in the range of 40.2% to 87.6% .IC_{50} values varied from 2.66 mg/ml to 12.84 mg/ml.By implication the grains of Coix lacryma jobi have impressive antioxidant value apart from rich nutritive values.

The present study shows that contrary to general perception non-conventional food crop like Coix lacryma jobi is superior to major crops like rice, maize, wheat etc. in terms of nutritive and nutraceutical values. Moreover, the crop exhibit considerable genetic diversity which can be exploited for productive and sustainable use.

Keywords — *Coix lacryma jobi*,Job's tear,nutritive value, nutraceutical value, antioxidant activity.

I. INTRODUCTION

Non-conventional, underutilized food plants have remained neglected and lesser known since they were localised to certain areas and communities for centuries. However, with changing situation, particularly climate change, gloomy food security and nutritional security scenario, such lesser known food plants are attracting attention from scientific community because of their remarkable nutritive values (Handique, 2003) and adaptability to increasingly hostile environment (Sthapit *et al.*,2010).

Since time immemorial agriculture and related vocations were and are backbone of global economy and driving force of human civilization. Since 19th century due to phenomenal technological advancement economic activities has diversified into hundred vocations but agro economy remain the mother of all economy. However, with changing time and situation particularly in the post green revolution period i.e. 1990 agriculture is under increasing stress. One emerging concept is to scientifically scrutinise the thousands of underutilized, neglected food plants which are part of ethnic food culture but with limited scientific literature. These are mostly collected from the wild or from backyard garden for domestic consumption. Often they are sold in local market at a nominal cost (Handique, 2003). In the absence of adequate scientific data there is a misconception that their food value is inferior and often termed as "poor man's food".

Job's-tears or *Coix lacryma jobi* L. is one of the cereal plants native to South-East Asia. Although classified as a minor cereal, it has a long history of cultivation in Asia. In India its cultivation is prominent in hilly terrain of Nagaland with remarkablediversity of germplasm and local tribal refer it as "Paddy substitute" (Handique *et al.*, 1986). The most promising aspect of *Coix lacryma jobi* is that it can be grown successfully in such areas where other crops are difficult to grow (Hore and Rathi, 2007). It can be grown in dryland with little moisture and also require little or no maintenance care (Handique *et al.*,1986).

Any conservation programme should precede evaluation to make conservation meaningful and productive. Keeping this in view the present study was undertaken with the objective of evaluation for basic nutritional parameters viz. protein, carbohydrate, lipid, mineral in the form of ash and crude fibre and nutraceutical parameters viz. dietary antioxidants and *in vitro* antioxidant activity.

II. MATERIALS AND METHODS

A. Collection of land races

The seeds were collected from farmers' field or household through visit to different villages in Nagaland soon after harvest season December/January. Basic information like cultivation practice, utility, traditional knowledge and beliefs associated were documented based on interaction with farmers and village elders. As per the standard practice land races were demarcated on the basis of seed morphology, seed coloration and such seed characteristics. The land races were marked as Ks-1, Ks-2...etc. Total 15 land races were collected andanalysed for the present study. In Angami Naga language *Coix* is called 'Kesi'; based on this, the land races were marked as 'Ks' followed by a numerical number.

B. Sample preparation

The collectedseed grains were shadedried and then dried in a hot air oven at 50 ± 2^{o}C till constant weight was recorded. The seeds were then manually dehusked and the kernels were grounded to fine powder. All the biochemical analysis was carried out on dry weight basis.

C. Determination of nutritive values

Nutritive values were determined for basic nutritional parameters. Crude protein content was determined by estimating the nitrogen content as outlined in method 2001.11, (AOAC, 2000),Lipid content was determined by ether extraction method(Thiex *et al.*, 2003; AOAC, 2000), Crude fibre was determined by AOAC method 962.09 (AOAC, 2000) and minerals in the form of ash was determined as outlined in method 942.05 (AOAC, 2000). For determination of total carbohydrate content,the dry matter was digested with 2.5N HCl for 30 minutes in a hot waterbath and then estimated by anthrone method as outlined by Clegg (1956).Calorific value was computed using the formula mentioned by Sherman (1952).

D. Determination of total phenolics content

1g of finely grinded dry matter was extracted with about 20 ml 80% methanol under continuous agitation for 8 hours; extract was separated by centrifugation and used for further analysis. For estimation of phenolics content an aliquot of the extract was taken and methanol was removed under reduced pressure in a rotary evaporator and then re-extracted with distilled water. Total phenolics contents were determined by Folin–Ciocalteu reagent method as outlined by Chang*et al.*2001). 100µl extract was diluted with 2900 µl water and allowed to react with 0.5 ml Folin–Ciocalteu reagent for 3 minutes. After that 2 ml 20% sodium carbonate was added and incubated in a waterbath set at 70^{o}C for 1 minute to develop blue colour. The absorbance of the coloured complex was recorded at 650 nm (Aquamate plus, Thermo Scientific). Phenolics contentwere quantified from standard curve prepared with gallic acid and expressed as mg gallic acid equivalent per gram of dry matter (mg GAE/g dm).

E. Determination of in vitro antioxidant activity by DPPH-RSA

1, 1- diphenyl-2-picryl hydrazyl (DPPH) radical scavenging assay (RSA) was carried out as per the method of Brand-Williams (1995) as modified by Abe *et al.*1998). 300 µl of methanol extract was taken, to which 1.7 ml methanol was added and followed by addition of 2 ml DPPH solution (0.1 mM DPPH prepared with methanol). The mixture was then incubated in dark for 30 minutes at room temperature. The reduction of DPPH was measured by change in intensity of the purple coloration at 517 nm using a UV-VIS spectrophotometer (Aquamate plus, Thermo Scientific). The percent RSA was calculated using the following equation:

$$\%RSA = \frac{A_{NC} - A_s}{A_{NC}} \times 100$$

Where, A_{NC} is absorbance of unreduced DPPH as negative control and A_S is absorbance of sample.

For determination of IC_{50} value a series of linearly increasing concentration of extracts was taken and DPPH reduction was carried out in similar manner. The reduction of DPPH radicals at different concentration was shown by decrease in absorbance maxima within wavelength range of 400 to 650 nm in an UV-VIS spectrophotometer (Aquamate plus; Thermo scientific) using the software Visionlite Scan (Thermo scientific). From the profile of gradually decreasing absorbance values, the concentration at which 50% reduction in colour intensity was worked out.

Fig 1 (A): Seed grains of *Coix lacryma jobi* land racesused in the present study(a) Ks – 1, (b) Ks – 2, (c) Ks – 3, (d) Ks – 4, (e) Ks – 5, (f) Ks – 6, (g) Ks – 7, (h) Ks – 8.

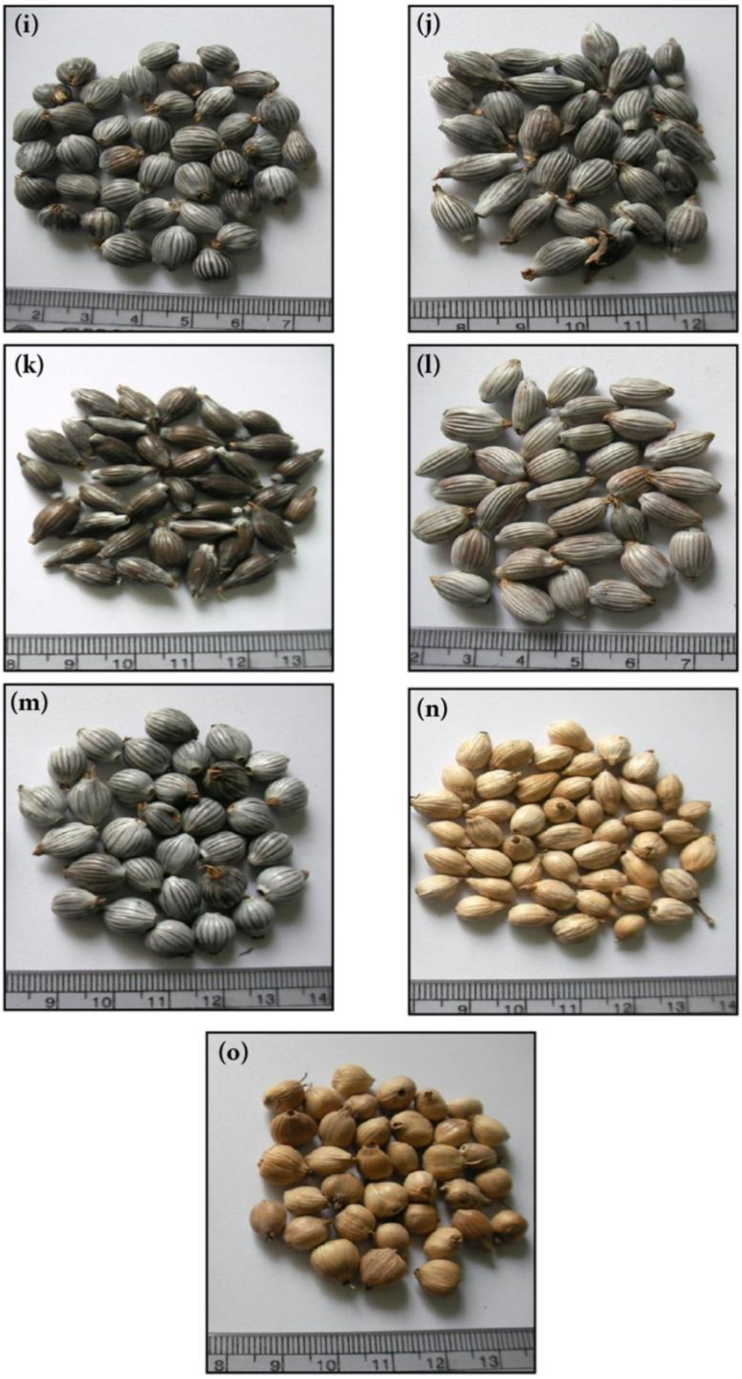

Fig 1 (B): Seed grains of *Coix lacryma jobi* land racesused in the present study(i) Ks – 9, (j) Ks – 10, (k) Ks – 11, (l) Ks – 12, (m) Ks – 13, (n) Ks – 14, (o) Ks – 15.

F. Statistical analysis

All the biochemical analysis were carried out in triplicates. The standard error of mean and analysis of variance (one-way ANOVA) was computed using SPSS statistics v 22.0. The critical difference (CD) was calculatedat 5% and 1% probability level. Using the same software coefficient ofcorrelation between total phenolics content and *in vitro*antioxidant activity was worked out and pearson's correlation coefficient was calculated at 0.001 and 0.05 level of significance.

III.RESULTS

The nutritional value viz. protein, lipid, crude fibre, mineral in the form of ash and carbohydrate content exhibited significant variation among the land races. The nutraceutical values were evaluated in terms of total phenolics content and *in vitro* antioxidant activity were worked out with respect to DPPH-RSA%. IC_{50} values were worked out based on DPPH reduction exhibited by gradually increasing concentration of methanolic extract of grain.

A. *Nutritive value of Coix lacryma jobi*

Crude protein content in *Coix* is found to vary significantly (P < 0.05) in the range of 13.8% in Ks – 13to 18.5% in Ks – 8.As many as 9 land races exhibited protein content above 15% which is remarkable. The mean value for the 15 land races has been found to be 15.66% which is considerably more than any other major cereal. Lipid content also exhibited remarkably high values compared to any other major cereal. Lipid content varied in the range of 5.3% in Ks – 12 to 8.2% in Ks – 4. The mean value for the 15 land races has been found to be 6.61% and the variation among the land races has been found to be highly significant. Among the major components crude fibre occurred in lowest proportion. Crude fibre content varied from 0.4% in Ks – 8 and Ks – 11. Highest crude fibre content was observed in Ks – 3 and Ks – 5 both with 0.85%. The overall mean for the land races has been found to be 0.637%. However, the intraspecific variation was statistically significant. Like protein and lipid content ash content has been found to be very impressive compared to other major cereal. Ash content varied from 1.35% in Ks – 15 to 3.34 in Ks – 6. The overall mean for the land races has been found to be 2.17% with significant intraspecific variation. Carbohydrate constituted the major fraction of the nutritional component in terms of relative proportion. Carbohydrate content varied from 64.7% in Ks – 8 to 77% in Ks – 15. As many as 9 land races exhibited carbohydrate content above 70%. The overall mean has been found to be 70.77% which is comparable to that of other cereal crop.

The variation in nutritional component is reflected in calorific value which exhibited considerable variation. Calorific value varied from 390.8 kcal/100g in Ks – 9 to 430.2 kcal/100g in Ks – 15. The overall mean for the cultivars has been found to be 405.1 kcal/100g.

TABLE I

Nutritional parameters of 15 land races of *Coix lacryma jobi*. The values represent % dry matter

Land races	Protein content (%) ± SEm	Lipid content (%) ± SEm	Crude fibre (%) ± SEm	Ash content (%) ± SEm	Carbohydrate content (%) ± SEm	Calorific value (kcal/100g)
Ks-1	16.0 ± 0.216	6.0 ± 0.342	0.76 ± 0.028	2.00 ± 0.236	70.0 ± 0.540	398.0
Ks-2	14.8 ± 0.170	7.0 ± 0.413	0.70 ± 0.024	1.70 ± 0.401	75.0 ± 0.540	422.2
Ks-3	15.0 ± 0.237	6.5 ± 0.434	0.85 ± 0.028	1.95 ± 0.378	72.0 ± 0.406	406.5
Ks-4	15.3 ± 0.119	8.2 ± 0.319	0.55 ± 0.036	1.55 ± 0.309	70.0 ± 0.539	415.0
Ks-5	14.1 ± 0.294	6.2 ± 0.413	0.85 ± 0.028	2.30 ± 0.236	73.1 ± 0.473	404.6
Ks-6	15.4 ± 0.196	6.0 ± 0.392	0.55 ± 0.038	3.34 ± 0.331	69.0 ± 0.505	391.6
Ks-7	16.8 ± 0.356	7.0 ± 0.365	0.54 ± 0.035	2.00 ± 0.309	67.0 ± 0.438	398.2
Ks-8	18.5 ± 0.196	5.8 ± 0.266	0.40 ± 0.040	2.30 ± 0.331	64.7 ± 0.473	389.0
Ks-9	17.0 ± 0.237	6.0 ± 0.478	0.60 ± 0.033	1.90 ± 0.309	67.2 ± 0.471	390.8
Ks-10	14.8 ± 0.242	7.0 ± 0.289	0.45 ± 0.035	2.25 ± 0.356	72.0 ± 0.406	410.2
Ks-11	18.2 ± 0.276	6.0 ± 0.448	0.40 ± 0.033	2.40 ± 0.307	66.1 ± 0.371	391.2
Ks-12	16.5 ± 0.170	5.3 ± 0.418	0.70 ± 0.036	3.20 ± 0.378	69.5 ± 0.442	389.0
Ks-13	13.8 ± 0.125	7.4 ± 0.392	0.80 ± 0.033	2.60 ± 0.330	69.5 ± 0.442	425.8
Ks-14	14.8 ± 0.152	7.0 ± 0.289	0.65 ± 0.026	1.65 ± 0.309	73.0 ± 0.478	414.2
Ks-15	13.9 ± 0.125	7.8 ± 0.418	0.75 ± 0.031	1.35 ± 0.378	77.0 ± 0.508	430.2
Mean	15.66	6.61	0.637	2.17	70.77	405.1
CD at 5%	0.770	1.356	0.115	1.167	1.692	
CD at 1%	1.037	1.827	0.155	1.571	2.278	

B. *Nutraceutical value of Coix lacryma jobi*

Like nutritional components phenolics content also exhibited significant interspecific variation. Phenolics content varied in the range of 0.208 mg GAE/g dm in Ks-12 to 0.580 mg GAE/g dm in Ks-19 and 0.469 mg GAE/g dm in Ks-26. Statistically the variation has been found to be highly significant (P < 0.05).

The *in vitro* antioxidant activity based on DPPH reduction was found to be reflective of phenolics content of respective land races. *in vitro* antioxidant activity were expressed as percent radical scavenging activity (RSA%) with the generalization that higher RSA% imply higher antioxidant activity and vice versa. Highest antioxidant activity was found in Ks-19 with RSA 87.6%; the same land race also exhibited highest phenolics content. Low level of antioxidant activity in the range of 32.8% to 40% were observed in Ks-29, Ks-23 and Ks-22 which exhibited relatively lower phenolics content. Analysis antioxidant activity in terms of IC_{50} was found in case of KS-6 with IC_{50} value 2.66 mg/ml followed by KS-12 with 3.00 mg/ml. KS-15 also exhibited appreciable IC_{50} value of 3.38 mg/ml. On the other hand, least antioxidant activity was recorded in KS-11 with 12.84 mg/ml and KS-4 with 11.34 mg/ml. It is noteworthy that a low IC_{50} value imply high antioxidant potency and vice versa. It appears that land races with higher phenolics content exhibit high antioxidant activity, while those with lower phenolics content exhibited comparatively lesser antioxidant activity. This signifies an apparent positive

TABLE II

Nutraceutical parameters of 15 land races of *Coix lacryma jobi.*

Land races	Total phenolics content (mg GAE/g dm) ± SEm	*In vitro* antioxidant activity	
		DPPH-RSA (%) ± SEm	DPPH-IC_{50} (mg/ml) ± SEm
Ks-1	0.100 ± 0.017	65.83 ± 0.669	10.86 ± 0.170
Ks-2	0.266 ± 0.014	69.71 ± 0.756	6.010 ± 0.166
Ks-3	0.268 ± 0.021	70.49 ± 0.471	6.180 ± 0.260
Ks-4	0.208 ± 0.017	67.77 ± 0.669	11.34 ± 0.237
Ks-5	0.334 ± 0.005	70.68 ± 0.754	4.540 ± 0.196
Ks-6	0.580 ± 0.014	87.57 ± 0.802	2.660 ± 0.260
Ks-7	0.300 ± 0.019	74.56 ± 0.568	5.060 ± 0.047
Ks-8	0.313 ± 0.018	40.00 ± 0.662	4.180 ± 0.233
Ks-9	0.279 ± 0.026	31.26 ± 0.802	8.120 ± 0.144
Ks-10	0.264 ± 0.012	76.50 ± 0.712	7.870 ± 0.233
Ks-11	0.221 ± 0.009	66.80 ± 0.566	12.84 ± 0.178
Ks-12	0.469 ± 0.015	66.99 ± 0.613	3.000 ± 0.262
Ks-13	0.147 ± 0.019	61.55 ± 0.568	9.070 ± 0.260
Ks-14	0.228 ± 0.005	36.31 ± 0.471	9.260 ± 0.191
Ks-15	0.389 ± 0.021	32.82 ± 0.754	3.380 ± 0.223
Mean	0.291	61.26	6.958
CD at 5% probability level	0.059	2.350	0.748
CD at 1% probability level	0.079	3.165	1.007

Coefficient of correlation between phenolics and DPPH-RSA is 0.176 (NS, $P = 0.531$, $P > 0.05$) and for phenolics and DPPH-IC_{50} at $P < 0.001$ is (-) 0.817.

for IC_{50} based on DPPH reduction by gradient solution with increasing proportion of methanolic extract showed considerable diversity. Best correlation between phenolics content and antioxidant activity.

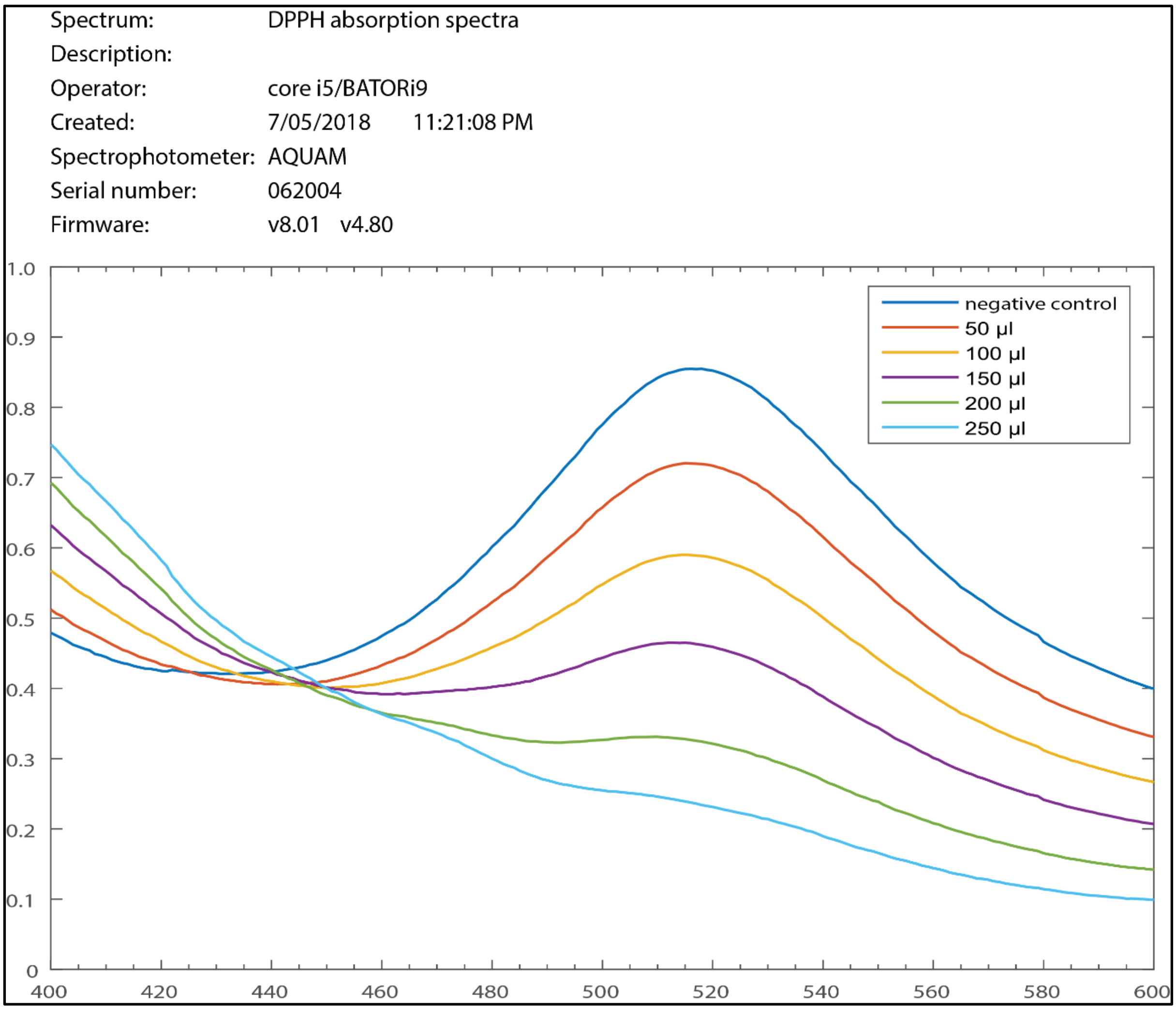

Fig 2: Absorption spectra for methanolic extract of grain of Ks – 12 with gradient solutions of increasing concentration to estimate IC_{50} value.

IV. DISCUSSION

Nutritive value or relative proportion of major nutritional component is the major criterion for assessing a food crop, particularly a non-conventional one. Among them protein content is considered as prime factor to ascertain nutritional superiority. *Coixlacryma jobi* is a lesser known but an ancient crop and considered as cereal (Hore and Rathi 2007). However, a comparison with conventional and cultivated cereal shows that *Coix* is superior to all of them. In case of rice depending on the cultivars and cultivation practice, protein content may vary from 7.03% to 8.43% (Dutta Roy *et al.*, 2010) in scented rice (Joha Dhan); 9.3% to 13.5% in case of some deep water paddy (Bao Dhan) (Baruah *et al.*, 2006; Loying *et al.*, 2010). Tiwary (2010) working with indigenous Boro or spring rice found variation in the range of 7.9% to 12.65% with a mean value of 10.43%. Similarly, another major cereal, maize has a mean value of 11.1% (Gopalan *et al.*,1989). By contrast in the present study, protein content varies from 13.9% to 18.5% implying significant variation due to genotype. However, carbohydrate content varied from 64.7 to 77.0% andthe carbohydrate content has been found to be comparable to other major cereals like paddy wheat and maize. On the other hand, lipid content and mineral content (ash content) have been found to be appreciably high. In case of rice, depending upon cultivar, lipid content may vary from 2.42% to 4.64% in deep water rice (Baruah *et al.*, 2006). For scented rice (joha dhan) the range of variation was 2.03% to 3.73% (Dutta Roy *et al.*, 2010). Tiwary(2010) working with Boro (spring rice) found lipid in the range of1.71% to 3.91% with mean of 2.41%. In the present study, lipid content varied from 5.3% to 8.2%. By comparison most varieties of wheat have mean value of about 1.5% to 1.7% while for maize the corresponding value is 0.9% to 3.6% (Gopalan *et al.*, 1989). The available information shows that *Coix* landraces are superior to all major cereals, so far as lipid content is concerned. For total mineral in the form of ash content also *Coix* land races are superior to other major cereal. Loying *et al.* (2010) working with deep water paddy reported ash content in the range of 1.13% to 2.0% which is one of highest range. Other rice researchers also reported ash content within this range or little lower. Other cereals like maize has a mean value of 0.8 % to 1.5 %; wheat in the range of 1.5 to 2.7 % (Gopalan *et al.*, 1989).In the present study ash content vary from 1.35% to 3.34% which clearly shows that most landraces of *Coix* are superior to major cultivated food crops.

In the present study the *Coix* land races exhibited considerable antioxidant activities and most land races contain impressive amount of phenolics which is a well-known dietary antioxidant. Apart from phenolics there are may be other dietary antioxidants which cumulatively exert their antioxidant activity. There are diverse report about correlation between dietary antioxidants and observed *in vitro* antioxidant activity. Many workers have reported significant positive correlation between both (Subhashree *et al.*,2009; Gulleria *et al.*, 2011, Gogoi and Rajkhowa 2015) while others did not find significant correlation. This is attributed to the phytochemical diversity of antioxidants. In the present study there was a positive correlation between phenolics and RSA%; however, statistically it was not significant. Saikia *et al.*2016) working with diversity of phenolics observed that while some phenolics have strong antioxidant property due to higher redox potential and others are poor. By implication a grain sample may have high total phenolic content and still its overall antioxidant activity may be poor. In the present study an appreciable positive correlation have been found between phenolic and antioxidant activity implying that in case of *Coix* the observed antioxidant activity is due to observed concentration of phenolics. The antioxidant potency based on IC 50 value exhibited considerable variation like phenolics and RSA%. However, the findings are impressive. It is noteworthy that unlike RSA% in case of IC_{50}, a lower value denotes higher antioxidant potency. In the present study there was negative correlation between phenolics and IC_{50} ($r = -0.817$, $P < 0.001$). Information on rice for IC_{50} can be considered for comparison with *Coix* since their food values are comparable. Gogoi and Rajkhowa (2015) working with anthocyanin rich red rice of deep water paddy reported IC_{50} in the range of 1.66 mg/ml to 10.37 mg/ml. However, Rao*et al.*2010) working with indigenous paddy cultivar of Kerala (India) known as Njavara reported IC_{50} value 30.85 mg/ml. In the present study for the 15 land races of *Coix*IC_{50} value varied from 2.66 mg/ml to 12.84 mg/ml. The findings are in agreement with earlier report for paddy and it appear that its IC_{50} value and antioxidant potency are comparable to paddy.

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