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ANTIOXIDANT ACTIVITY OF RAW AND DIFFERENTIALLY PROCESSED UNDER-UTILIZED TROPICAL LEGUME *CANAVALIA ENSIFORMIS* L. DC SEEDS, SOUTH INDIA

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ABSTRACT

In the present study, the antioxidant activity of extracts of raw and differentially processed seed materials of Canavalia ensiformis L.DC (Jack bean) collected from South India was investigated. The raw seeds were found to contain higher levels of phenolic compounds (238.92 mg/100 g seed flour) and flavonoids (68.12 mg/100 g seed flour). By virtue of their hydrogen-donating ability, all the tested extracts were found to exhibit excellent reducing power, with the highest values being recorded in Kellanadukalli accession. Similarly, when compared with synthetic oxidants (Ascorbic acid), all the presently studied jack bean seed extracts were found to be more potent in free radical scavenging activity against α, α - diphenyl - β - picrylhydrazyl (DPPH) radicals. Among the various common processing methods employed in the present study, the cooking treatment was found to improve the antioxidant property of jack bean seed. Hence, such viable and suitable processing method could be recommended for the utilization of such under-utilized legume seeds as a source of natural antioxidants, in addition to their nutrient values.

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KEY WORDS

Jack bean; antioxidant activity; DPPH; phenols; flavonoids

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[I] INTRODUCTION

Most of the free radicals have been implicated in causation of various human ailments such as cancer, rheumatoid arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis and AIDS [1]. Free radicals due to environmental pollutants, radiation, chemicals, toxins, deep fried and spicy foods as well as physical stress, cause depletion of immune system antioxidants, change in gene expression and induce abnormal proteins [2]. The oxidant radical can be transformed into more reactive forms such as superoxide, hydrogen peroxide, singlet oxygen and hydroxyl radicals, which are collectively known as reactive oxygen species (ROS).

Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions [3]. The Food and Nutrition Board of the National Academy of Science defined a dietary antioxidant as a substance in foods that significantly decreases the adverse effects of reactive oxygen species, reactive nitrogen species, or both on normal physiological function in humans. In order to prolong the storage of foods, several synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are used currently, but these substances may be inappropriate for chronic human consumption, as recent publications have mentioned their possible toxic properties for human health and the environment [4]. Phenolic compounds and antioxidant activities in legume seeds were reported by several earlier communications, although legumes constitute one of the most abundant and least expensive sources of protein in human/animal diet [5]. Phenolic constituents, such as flavonoids, phenolic acids, diterpenes and tannins are especially worthy of notice due to their high antioxidative activity [6]. Natural phenolic antioxidants can scavenge reactive oxygen and nitrogen species (RONS) thereby preventing the onset of oxidative diseases in the body. A positive correlation between the consumption of phenolic-rich foods and a decrease of several chronic diseases has been shown to exist from epidemiological studies [7].

Recently the under-utilized legume seeds have received more attention of the researchers as an alternative protein source all around the world [8]. Sincere and concerted attempts are being made to evaluate and utilize the under-utilized legume seeds as an alternative/additional protein source in the diets of both human beings and animals, particularly developing countries [9]. Among the various under-utilized pulses, the jack bean [Canavalia ensiformis L.DC.] seeds merits a wide use in South Asian countries and other parts of the tropics as food legume. In this connection, the seeds of Canavalia ensiformis L.DC. (Jack bean), an under-utilized food legume becomes more important. C.ensiformis is distributed throughout the tropics and randomly cultivated in South India. The mature seeds of C.ensiformis are being consumed by the Indian tribal sects such as Kurumba,

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Malayali, Irula and other Dravidian groups after cooking [9]. Jack beans are found to contains higher levels of proteins (19.9 – 35.0%), lipids (0.8 – 9.9%) and other nutrients and merits a wide use in South and Southeast Asian countries and other parts of the tropical food legumes [9]. The protein qualities of this wild legume seeds seem to be similar to that of most edible legumes and hence, they are advocated to be a good source of extending protein sources. Several studies revealed that jack bean seeds contain potential of antioxidant activity [10]. Although, few reports are available on the nutritional and antinutritional properties jack bean seeds, the information regarding their antioxidant property was found to be meager. Hence, the present study was carried out with a view to analyze the antioxidant properties of raw and differentially processed seeds samples of jack bean collected from South India. The analysis included 1, 1-diphenyl-picryl-hydrazyl (DPPH), Reducing power assay, quantification of flavonoids and total phenolic compounds (TPC).

[II] MATERIALS AND METHODS

2.1. Collection of the seed samples

Five different accessions of jack bean seed materials were collected from different agroclimatic regions of Western Ghtas, South India from the natural stands [Table–1]. Soon after collection, the immature and damaged seeds were removed and the mature seeds were dried in the sun light for 24 h and stored in plastic containers in refrigerator (50C), until further use.

Table: 1. Collection details of various accessions of *Canavalia sp*.

Location	Seed coat colour	District	State	Date of collection
Bannari	white	Erode	Tamilnadu	22.05.2009
Kuppanatham	Red	Madurai	Tamilnadu	22.05.2009
Ayodhyapattinam	white	Salem	Tamilnadu	29.05.2009
Arachalur	white	Erode	Tamilnadu	29.05.2009
Keelanadukalli	white	The Nilgiris	Tamilnadu	10.06.2009

2.2. Processing methods

Five separate batches of whole seeds of jack beans were taken and the first batch was soaked in distilled water for 24 h at room temperature (30 ± 20 C) in the bean to water ratio of (1:10) w/v. The second batch of seeds was cooked at 90 - 950 C for 1 h in the bean to water ratio of (1:10) w/v. The third batch of seeds were roasted for 30 min at 100 – 110 0 C in an iron pot along with clean fine sand to prevent the burning of the seed coat and to ensure the uniform distribution of heat. The fourth batch of seeds as soaked in distilled water for overnight were germinated in sterile Petri dishes lined with a wet filter paper for 24 h at room temperature (32 ± 20C) in the dark. After each treatment, the treated seeds were rinsed with distilled water, separately, and then dried at 55 0 C for 6 h in a hot air oven. The fifth batch of raw seeds was stored as such without any treatment.

2.3. Preparation of extracts

Seed samples were weighed (10 g each) and blended with Waring blender and soaked with methanol [in ratio seed flour: methanol (1: 10)] for seven days and filtered using Whatman No. 1 paper. The methanol was completely removed by vacuum evaporator at 50oC to give viscous mass. The crude extracts were weighed and stored at 0 – 4oC before analysis.

2.4. DPPH radical scavenging activity

DPPH scavenging activity was carried out by the method of Blois [11]. Two different concentrations (500 & 250 µg/ml) of *Canavalia ensiformis* seed extracts (Methanol) were dissolved in DMSO (dimethyl sulfoxide) and taken in test tubes in triplicates. Then 5 ml of 0.1mM ethanol solution of DPPH (1, 1, Diphenyl-2- Picrylhydrazl) was added to each of the test tubes and were shaken vigorously. They were then allowed to stand at 37oC for 20 minutes. The control was prepared without any extracts. Methanol was used for base line corrections in absorbance (OD) of sample and measured at 517nm. A radical scavenging activity was expressed as 1% scavenging activity and was calculated by the following formula.

$$\text{Radical scavenging activity (\%)} = \frac{\text{OD Control} - \text{OD Sample} \times 100}{\text{OD control}}$$

2.5. Reducing power assay

Reducing activity was carried out by using the method of Oyaizu [12]. Two different concentrations (500 & 250 µg/ml) of Canavalia ensiformis seed extracts (Methanol) were dissolved in DMSO (dimethyl sulfoxide) and taken in test tubes in triplicates. To the test tubes, 2.5 ml of sodium phosphate buffer and 2.5 ml of 1% Potassium ferric cyanide solution was added. These contents were mixed well and were incubated at 50oC for 20 minutes. After incubation 2.5ml of 10% TCA was added and were kept for centrifugation at 3000rpm for 10 minutes. After centrifugation 5ml of supernatant were taken and to this 5ml of distilled water was added. To this about 1ml of 1% ferric chloride was added and was incubated at 35o C for 20 minutes. The O.D (absorbance) was taken at 700nm and the blank was prepared by adding every other solution but without extract and ferric chloride (0.1%) and the control was prepared by adding all other solution but without extract.

2.6. Total phenols

Total phenolic contents were determined by McDonald et al. [13] method. A dilute extract of each seed extract (0.5 ml of 1:10g ml –l) or garlic acid (standard phenolic compound) was mixed with 5 ml of Folin Ciocalteu reagent (1:10 diluted with distilled water) and 4 ml of aqueous sodium carbonate (1 M). The mixtures were allowed to stand for 15 min and the total phenols were determined by measuring absorbance at 765

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nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg/ml solutions of gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg g-l of dry mass), which is a common reference compound.

2.7. Flavonoids

Aluminum chloride colorimetric method was used for flavonoids determination [14]. Each seed extracts (0.5ml of 1:10 g/ml) in methanol were separately mixed with 1.5 ml of methanol, 0.1ml of 10% aluminum chloride, 0.1ml of 1M potassium acetate and 2.8ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415nm with a double beam Perkin Elmer UV/Visible spectrophotometer (USA). The calibration curve was prepared by preparing quercetin solution at concentrations 12.5 to 100g ml -1 in methanol.

[III] RESULTS

In the present study, the seed materials of five different accessions of an under-utilized legume, Jack bean were collected from different agro-ecological regions of South India. After collection, the antioxidant (DPPH and reducing power assay) properties and the levels of poly phenols and flavonoids were analyzed and the results were expressed in the **Tables 1-7**.

3.1. DPPH assay

Radical-scavenging activity employing DPPH has been extensively used in the field of food processing for screening the antioxidant capacity of agricultural produce [15] (Sanchez-Moreno, 2002). The assay was carried out in the methanol extracts with two different concentrations like 500 and 250µg/ml. The antioxidant activity of raw seeds of five different jack bean accessions was found to be ranged from 38.04 – 68.51%. Highest antioxidant activity was recorded by Arachalur accession **[Tables– 2 and 3]**.

3.2. Reducing power assay

The reducing power assay of five different accessions of raw jack bean seeds were given in table 4 and 5, which was found to be ranged from 0.202-0.722 of the seed flour. Among the five different accessions, the Ayodhyapattinam germplasm have exhibited the highest reducing activity.

3.2. Total phenols and flavonoids

The total phenols content of five different accessions of raw jack bean seed flour was found between 135.47 and 238.92 mg/100g of seed flour (Table 6). Among the five different accessions, the Keelanadukalli germplasm showed the highest levels of poly phenols. The flavonoids content of five different accessions of raw jack bean seed flour was found between 52.05 - 68.12 mg/100g of seed flour (Table 7). Bannari germplasm has registered the highest level of flavonoids.

Table: 2. Antioxidant activity (DPPH assay) of raw and moist heat treated jack bean (*Canavalia ensiformis*)*

Accessions	Percentage of Free Radical Scavenging Activity					
	Raw Seeds (µg/ml)		Soaking (µg/ml)		Cooking (µg/ml)	
	250	500	250	500	250	500
Bannari	45.61 ± 0.603	56.42 ± 0.419	30.91 ± 0.135	53.79 ± 0.123	28.72 ± 0.190	72.45 ± 0.045
Ayodhyapattinam	38.04 ± 0.047	63.6 ± 0.230	28.47 ± 0.095	50.86 ± 0.112	34.79 ± 0.202	73.47 ± 0.170
Arachalur	42.61 ± 0.213	68.51 ± 0.215	22.28 ± 0.05	52.63 ± 0.088	40.45 ± 0.220	75.17 ± 0.090
Kuppanatham	45.42 ± 0.14	57.6 ± 0.075	28.24 ± 0.210	48.54 ± 0.205	39.24 ± 0.100	74.47 ± 0.182
Keelanadukalli	42.42 ± 0.120	66.35 ± 0.183	29.51 ± 0.181	50.13 ± 0.280	34.85 ± 0.150	75.88 ± 0.040

Table: 3. Antioxidant activity (DPPH assay) of raw, germination and dry heat treated jack bean (*Canavalia ensiformis*)*

Accessions	Percentage of Free Radical Scavenging Activity					
	Raw Seeds (µg/ml)		Roasting (µg/ml)		Germination (µg/ml)	
	250	500	250	500	250	500
Bannari	45.61 ± 0.603	56.42 ± 0.419	33.25 ± 0.060	66.14 ± 0.083	42.39 ± 0.240	70.83 ± 0.005
Ayodhyapattinam	38.04 ± 0.047	63.6 ± 0.230	36.60 ± 0.133	65.81 ± 0.046	39.61 ± 0.035	74.76 ± 0.057
Arachalur	42.61 ± 0.213	68.51 ± 0.215	35.13 ± 0.066	69.52 ± 0.080	36.48 ± 0.036	74.39 ± 0.147
Kuppanatham	45.42 ± 0.14	57.6 ± 0.075	29.33 ± 0.068	66.57 ± 0.109	32.91 ± 0.062	72.8 ± 0.1
Keelanadukalli	42.42 ± 0.120	66.35 ± 0.183	28.43 ± 0.085	68.27 ± 0.280	32.42 ± 0.098	70.66 ± 0.149

*The data are means of triplicate determinations and ± standard errors

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Table: 4. Antioxidant activity (reducing power assay) of raw and moist heat treated jack bean (*Canavalia ensiformis*)*

Accessions	Raw Seeds (µg/ml)		Soaking (µg/ml)		Cooking (µg/ml)	
	250	500	250	500	250	500
Bannari	0.336 ± 0.002	0.686 ± 0.001	0.340± 0.002	0.549 ± 0.001	0.347 ± 0.00	0.763 ± 0.001
Ayodhyapattinam	0.327 ± 0.002	0.722 ± 0.001	0.227 ±0.001	0.610 ± 0.001	0.333 ± 0.001	0.790 ± 0.001
Arachalur	0.221 ± 0.002	0.488 ± 0.002	0.243 ± 0.001	0.523 ± 0.002	0.318 ± 0.003	0.791 ± 0.009
Kuppanatham	0.247 ± 0.001	0.685 ± 0.003	0.331 ± 0.001	0.563 ± 0.002	0.297 ± 0.00	0.828 ± 0.002
Keelanadukalli	0.202 ± 0.002	0.430 ± 0.00	0.333 ± 0.001	0.578 ± 0.002	0.302 ± 0.001	0.840 ± 0.001

*The data are means of triplicate determinations and ± standard errors

Table: 5. Antioxidant activity (reducing power assay) of raw, germination and dry heat treated jack bean (*Canavalia ensiformis*)*

Accessions	Raw Seeds (µg/ml)		Roasting (µg/ml)		Germination (µg/ml)	
	250	500	250	500	250	500
Bannari	0.336 ± 0.002	0.686 ± 0.001	0.395 ± 0.001	0.720 ± 0.001	0.295 ± 0.002	0.720 ± 0.00
Ayodhyapattinam	0.327 ± 0.002	0.722 ± 0.001	0.296 ± 0.001	0.759 ± 0.001	0.353 ± 0.003	0.793 ± 0.001
Arachalur	0.221 ± 0.002	0.488 ± 0.002	0.263 ± 0.002	0.682 ± 0.010	0.301 ± 0.001	0.795 ± 0.001
Kuppanatham	0.247 ± 0.001	0.685 ± 0.003	0.455 ± 0.003	0.751 ± 0.003	0.408 ± 0.001	0.824 ± 0.00
Keelanadukalli	0.202 ± 0.002	0.430 ± 0.00	0.351 ± 0.009	0.422 ± 0.002	0.421 ± 0.001	0.750 ± 0.002

*The data are means of triplicate determinations and ± standard errors

Table: 6. Quantitative estimation of Poly phenols in raw and differentially processed seed samples of *Canavalia ensiformis**

Accessions	Poly phenols content (mg/100 g seed flour)				
	Raw Seeds	Soaking	Cooking	Roasting	Germination
Bannari	146.58 ± 8.22	113 ± 0.25	121 ± 0.05	130.4 ± 0.02	142 ± 0.24
Ayodhyapattinam	152.45 ± 0.04	118.1 ± 0.24	128 ± 0.18	132.7 ± 0.35	137 ± 0.25
Arachalur	135.47 ± 2.16	108 ± 0.04	106.2 ± 0.06	125.5 ± 0.12	127.28 ± 0.06
Kuppanatham	221.29 ± 0.03	118.39 ± 0.05	113.84 ± 0.05	138.69 ± 0.23	184 ± 0.08
Keelanadukalli	238.92 ± 0.67	179.2 ± 0.18	132.66 ± 0.05	124.24 ± 0.14	121.81 ± 0.13

*The data are means of triplicate determinations and ± standard errors

Table: 7. Quantitative estimation of Flavonoids in raw and differentially processed seed samples of *Canavalia ensiformis*

Accessions	Flavonoids content (mg/100 g seed flour)				
	Raw Seeds	Soaking	Cooking	Roasting	Germination
Bannari	68.12 ± 0.25	28.93 ± 0.07	43 ± 0.01	32.25 ± 0.74	32.80 ± 0.02
Ayodhyapattinam	54.25 ± 0.24	30.77 ± 1.02	30.21 ± 1.71	35.65 ± 2.05	40.14 ± 0.31
Arachalur	52.05 ± 0.46	37.7 ± 0.14	31.62 ± 0.42	28.52 ± 1.33	37.46 ± 0.18
Kuppanatham	59.92 ± 0.11	38.80 ± 0.32	34.60 ± 1.59	36.14 ± 0.29	42.1 ± 0.31
Keelanadukalli	61.28 ± 0.24	42.45 ± 0.31	39 ± 0.28	27.08 ± 4.21	46.4 ± 0.05

*The data are means of triplicate determinations and ± standard errors

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[IV] DISCUSSION

The α, α,diphenyl-β-picrylhydrazyl (DPPH) a stable nitrogen centered free radical, has been used to evaluate the antioxidant activity of natural products by measuring the radical quenching capacity in a relatively short period of time. All the presently studied seed extracts (both raw and differentially processed seeds) were found to exhibit more effective free radical inhibition activity against DPPH. The free radical inhibition activity of raw seed materials of jack bean was ranged between 38.04 and 68.51%, which is in agreement with that of the previous reports on *Phaseolus vulgaris* [16]; *Vigna aconitifolia* [17]; *Mucuna pruriens var.utilis* [18]. Among the five different accessions, the arachalur germplasm showed the highest free radical scavenging activity.

When considering the effect of various common processing methods on the free radical inhibition activity of seed extract in the presently studied wild legume grain, all the processed samples showed moderate to higher levels of free radical inhibition activity than those of raw seeds **[Tables–2 and –3]**. The free radical inhibition activity of seed extracts of cooked samples were found to be higher (72.45, 73.47, 75.17, 74.47 and 75.88%) than that of other processed seed samples of the present study and previous reports of free radical inhibition activity of processed samples of Arachis hypogea (23.69%) [2].

Reducing power assay is often to evaluate the ability of natural antioxidant to donate electron or hydrogen [19]. Samples with high reducing power were reported to have a better ability to electrons. It has been widely accepted that the higher level of absorbance at 700 nm indicates greater reducing power of the test samples [20]. Two different concentrations (500 & 250µg/ml) of the seed extracts of raw jack bean seeds were found to When considereing the effect of various common processing methods on the reducing power of jack bean seeds of the present study, all the processed samples showed higher level of reducing power than those of raw seeds **[Tables–6 and –7]**. However, among the differentially processed samples, the extracts of cooked samples registered the maximum level of reducing power in similar with earlier report in Arachis hypogeal [2].

Phenolic and other phytochemical antioxidants found in fruits, vegetables and legumes are bioactive compounds capable of neutralizing free radicals and may play a role in the prevention of certain diseases. Also, dietary supplements and food fortification may be an alternative route to the consumption of minor plant components that may have health effects. A significant level of polyphenols and flavonoids was reduced during soaking treatment in jack bean seeds **[Table–7]**.The level of elimination of phenolic compounds in the presently studied seed materials was found to be higher when compared to a previous report on cowpea and pigeonpea [21]. Since the phenolic compounds are water soluble in nature and mostly located in the seed coat, the decrease on the level of phenolic compounds during soaking treatment might be due to leaching out into the soaking medium.

Effect of various simple and cost-effective processing methods on the levels of antioxidant properties of five different accessions of jack bean seeds were given in tables 2-5. Among the various post-harvest treatments employed, cooking was showed the highest antioxidant activity (72.45 – 75.88%). The effect of various simple and cost-effective processing methods on the levels of phenolic constituents of five different accessions of jack bean seed was presented in **tables –6 and –7**. Among the different processing methods employed in the present study cooking was showed the higher levels of flavonoids and poly phenols (43 – 46.4 mg/100g of seed flour & 132 - 184 mg/100g of seed flour).

[V] CONCLUSION

The results of the present study demonstrated that the seed materials of jack bean constitute a rich source of antioxidants and other secondary metabolites in addition to appreciable levels of various bioactive compounds. All the bioactive compounds in jack bean grains were found to exhibit potential antioxidant activity through in vitro model. When considering the effect of various common processing methods on the antioxidant property of jack bean seeds, the cooking appear to be more effective. Hence such a viable processing method could be recommended for the versatile utilization of jack bean seed as source of natural antioxidants, in addition to protein and other nutrients.

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