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Free Radical Scavenging and Antioxidant Activity of Underutilized Processed Jack Bean (*Canavalia ensiformis*) and Barnyard Millet *(Echinochloa frumentacae)* Flour Extracts

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ABSTRACT

In the present study, the antioxidant activity of extracts of raw and differentially processed *Canavalia ensiformis* (Jack bean) and *Echinochloa frumentacae* (Barnyard millet) was investigated. These indigenous underutilized legume and millet exhibit high nutritive values as well as antioxidant potential. *In-vitro* antioxidant activity of aqueous processed (soaking, cooking, autoclaving and germination) flour extracts were determined by DPPH free radical scavenging assay, total phenolic and flavonoids content using reference standard ascorbic acid, gallic acid and quercetin respectively. All the analysis was made with the use of UV-Visible Spectrophotometer. The DPPH radical scavenging activity of the extract was increased with the increasing concentration. The highest radical scavenging effect was observed in germinated Jack bean and Barnyard millet flour extract with IC_{50}, 5 and 7 µg/ml respectively. The highest total phenolic content (mgGAE/100g) was found to be 82.0±0.4 and 21.3±0.1 in germinated and total flavonoids content (mgQE/100g) was 61.8±0.3 and 43.5±0.6 of aqueous extracts of both germinated flours.Among the various processing methods employed in the study, the germinated treatment was found to improve the antioxidant property followed by autoclaved, soaked and cooked flours. The findings suggest that germination process is viable and suitable processing method which could be recommended for the utilization of these underutilized legume and millet as a source of natural antioxidants.

1. INTRODUCTION

Antioxidants are radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neuro-degeneration, Parkinson's diseases, mongolism, ageing process and perhaps dementias[1]. Oxygen radicals induce oxidative stress that is believed to be a primary factor in various diseases as well as normal process of ageing. Plants are an important source of organic antioxidant chemicals, which are widely used as ingredients in dietary supplements. The relationship between the intake of food rich in antioxidant components and the alleviation of illnesses caused by oxidative damage has become an important research topic in the food and medicine arena. Based on their mechanism of activity, the antioxidants are classified as chain breaker (or free radical inhibitor), peroxide decomposer, metal inactivator, or oxygen scavenger[2]**.**

Plants are an important source of organic antioxidant chemicals which are widely used as ingredients in dietary supplement. Legumes, which play a crucial role in many diets worldwide, are also utilized for health purposes in the prevention of pathologies such as cancer, heart disease, diabetes, and neurodegenerative processes. Seeds from several species of legumes are major sources of dietary protein for millions of people in developing countries. Although conventional legumes fulfill food and feed stuff needs in most of the countries, their production is not enough to meet requirements of the increasing population and animal feed industries. Hence, there is a need for exploitation of neglected underutilized legumes[3]**.**

Jack bean (*Canavalia ensiformis*) is one of the under exploited tropical dry beans. It is, however, fairly widely distributed, being cultivated in Africa, Asia, West Indies, America and India[4]. The jack bean can be grown in marginal soils and arid to semi arid regions. It has therefore, great potential in most tropical and subtropical parts of the world. It ranks among the underutilized legumes that could improve protein deficiency in human nutrition, particularly in developing countries. The role of *Canavalia* in medicine and pest control has also been projected in several research works which have also been made on possible future lines of action to exploit it[5]. Information with respect to the antioxidant and free radical scavenging activity of raw as well as processed seeds of Jack bean is scanty.

Millets are rich in B vitamins, especially niacin, pyridoxine and folic acid, as well as the minerals such as calcium, iron, potassium, magnesium, zinc and contain no gluten, so they are not suitable for raised bread, but they are good for people who are gluten-intolerant. Barnyard millet *(Echinochloa frumentacaea)* is called by several other names, *ooda, oadalu, sawan, sanwa, and sanwank.* It has an excellent rejuvenating capacity compared to other cereal crops[6]**.** Nutritionally, Barnyard millet is an important crop which contains good amount of digestible protein, and both soluble and insoluble fractions of dietary fiber[7]. Polyphenolic compounds such as flavonoids, phenolic acids, and DPPH (radical scavenging activity) which are of great interest for their radical-scavenging activity are expected to be effective in the prevention of many diseases and morbid states. However, knowledge of antioxidant compounds in millets, including Japanese barnyard millet, is still superficial.

Traditional treatments such as soaking and germination have been used to improve nutritional as well as antioxidant property of the legume and millet. With constantly increasing awareness on good nutrition and healthy living, the consumption of millet and legumes are rising by developing food products based on them to enhance its utilization. Underutilized jack bean and barnyard millet have less popularity and lack of awareness about its antioxidant potential. Therefore, the aim of present study is to evaluate the free radical scavenging and antioxidant activity of underutilized processed *Canavalia ensiformis* and *Echinochloa frumentacae* flour.

2. MATERIALS AND METHODS

2.1. Collection of the seed samples

Seeds of *Canavalia ensiformis* and *Echinochloa frumentacae* were collected from a local producer (Jaipur seed house). After collection, the immature and damaged seeds were removed and the mature seeds were dried in the sunlight and stored in plastic containers.

2.2. Development of processed flours

Preparation of the seed flour after removing the immature and damaged seeds, the mature seeds of jack bean and barnyard millet were dried in the open sunlight for 2 days and 50 g of each air dried seeds were powdered in a grinding mill to obtain 60 mesh size powder. Care was taken to clean the grinding mill thoroughly after powdering each sample and before starting to powder a

new sample to avoid mixing up of samples. The fine seed powdered samples were stored in screw-cap bottles until further use.

2.3. Processing methods

Four separate batches of whole seeds of jack bean and barnyard millet were taken and the first batch was soaked in distilled water for 24 h at room temperature to water ratio of (1:10) w/v. The second batch of seeds was cooked at 80-100^{0}C for 20 min in the bean and millet to water ratio of (1:10) w/v. The third batch of seeds were autoclaved for 30 min at 15 psi at 121°C in an autoclaving instrument 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 steeped in distilled water for overnight were germinated in sterile Petri dishes lined with a wet filter paper for 36 h at room temperature (37°C) in the dark. After each treatment, the treated seeds were rinsed with distilled water, separately, and then dried at 55°C for 6 h in a hot air oven. The fifth batch of raw seed flour was stored as such without any treatment.

2.4. Preparation of extracts

100 g of each plant powder was extracted in 800 ml of water by maceration (48 h).The precipitate was removed under the vacuum at temperature below 50ºC and the extracts were freeze dried at 0-4ºC before analysis.

2.5. Chemicals

2, 2-diphenyl-1-picrylhydrazyl (DPPH), Quercitin, Gallic acid, Sodium Carbonate. All other reagents used were of analytical grade.

2.6. DPPH radical scavenging activity

The ability of the methanolic extracts to scavenge free radicals was determined against a very stable free radical DPPH (2, 2-diphenyl-1-picrylhydrazyl) determined spectrometrically[8]. Aliquots of the sample extract at different concentrations 0.02, 0.05, 0.1, 0.15, 0.25 mg/ml were added to 1 mM methanolic solutions of DPPH. Each mixture was vortexed vigorously and left for 30 min at room temperature in the dark. The absorbance was measured at 517 nm and activity was expressed as percentage DPPH scavenging relative to control using the following equation:

DPPH scavenging activity (%) = (Absorbance of control − Absorbance of sample)/ Absorbance of control × 100

IC_{50} Value was also calculated.

2.7. Determination of total phenolic content

Total phenols were determined by Folin Ciocalteu reagent[9]. A dilute extract of each seed (0.5 ml of 1:10 g ml^{-1}) or gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and 4 ml of aqueous sodium carbonate (1 M). The mixtures were kept at dark ambient condition for 15 min and the total phenols were determined by spectrophotometry at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg/ L solutions of Gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of Gallic acid equivalent (mg/g of dry mass), which is a common reference compound.

2.8. Determination of total flavonoids content

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

3. RESULTS AND DISCUSSION

Table 1: Antioxidant Activities (Free Radical Scavenging) of Germinated and Autoclaved Jack Bean Flour and Barnyard Millet Flour

Concentration mg/ml	Ascorbic acid	UB	UJ	GB	GJ	AB	AJ
0.02	47.4	26.6	37.2	36.8	35.2	30.2	38.5
0.05	59.7	32.8	42.1	45.9	49.8	37.1	45.1
0.1	61.9	41.9	47.4	57.7	59.4	49.3	51.8
0.15	73.8	49.7	51.4	59.8	68.3	57.9	59.2
0.25	**85.2**	**55.8**	**61.1**	**68.6**	**72.6**	**60.1**	**69.9**

Value expressed as percent

UB -Unprocessed barnyard millet extract UJ -Unprocessed jack bean extract

GB -Germinated barnyard millet extract GJ -Germinated jack bean extract

AB -Autoclaved barnyard millet extract AJ -Autoclaved jack bean extract

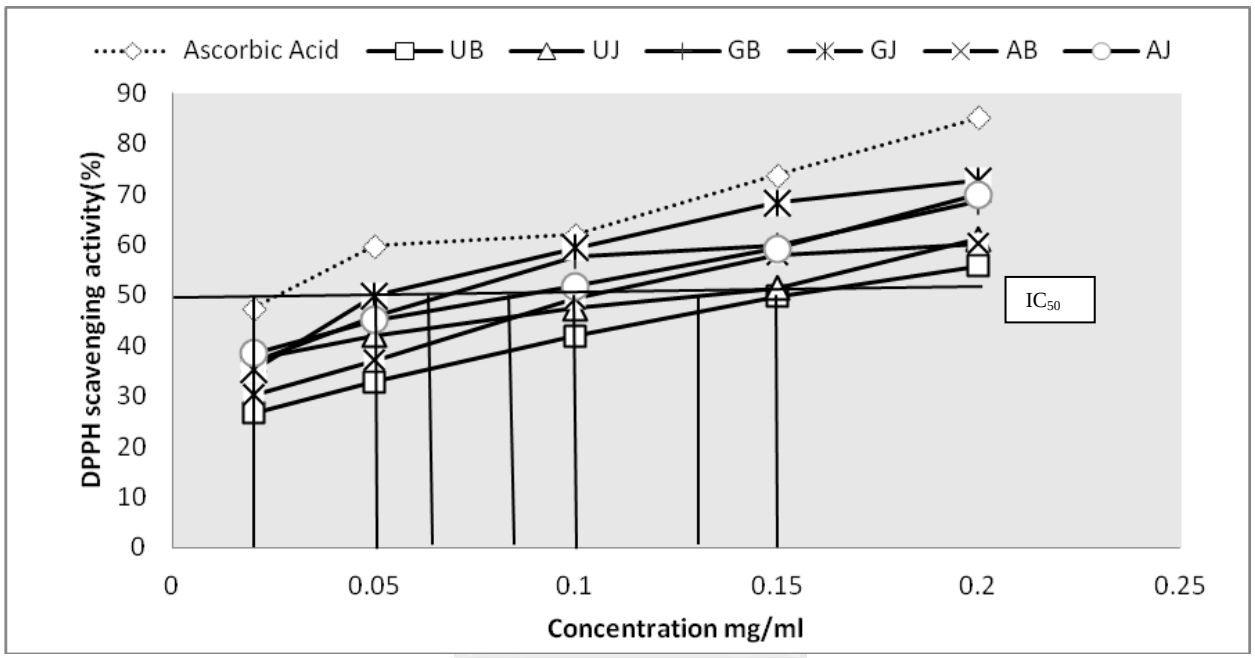

Figure 1: Antioxidant activity (DPPH) of germinated and autoclaved Jack Bean Flour and Barnyard Millet Flour

In the present study, the seed materials of two under-utilized legume, Jack bean and barnyard millet were collected from Jaipur seeds house. After collection, the antioxidant DPPH scavenging properties and the levels of phenols and flavonoids were analyzed and the results are expressed in the Table 1-3. The IC_{50} value (µg/ml) is the concentration of antioxidant at which 50% inhibition of free radical activity is observed (Scavenging Effect=Inhibitory Concentration or IC)[11]**.** Radical scavenging activity employing DPPH has been extensively used in the field of processing for screening the antioxidant capacity of agricultural products[12]. The assay was carried out in the aqueous extract of processed jack bean and barnyard millet with different concentrations such as 0.02, 0.05, 0.1, 0.15, 0.25 mg/ml. As shown in Table 1 and Figure 1, germinated flour extract showed the highest antioxidant activity 72.6% with $IC_{50;}$ 5 µg/ml for Jack Bean and 68.6 % with $IC_{50;}$ 7 µg/ml in barnyard millet when compared to standard ascorbic

acid 85.2% with $IC_{50;}$ 2.8 µg/ml. The 1, 1-diphenyl-β-picryl hydrazyl (DPPH) a stable nitrogen centered free radical has been used to evaluate the antioxidant activity of natural products by measuring the radical scavenging capacity in a relatively short period of time required for 50% inhibition. Antioxidants due to their scavenging activity are used in the management of many diseases such as cardiovascular disease, obesity, diabetes and hypertension[13]. All presently studied flours extracts (differentially processed) were found to exhibit more effective free radical scavenging activity against DPPH. The free radical inhibitory activity of processed flours ranged between 35-72% which is in agreement with that of the previous reported on various legumes such as *Phaseolus vulgaris*[14]**,** *Vigna aconitifolia*[15], *Mucuna pruriens*[16]**,** and IC_{50} was ranged between 1.9 -31 µg/ml for sorghum, foxtail and proso millet when compared with standard ascorbic acid, BHA and BHT respectively[17]**.** When considering the effect of various processing methods on the free radical inhibition activity of flour extract, all the processed samples showed moderate to higher levels of free radical scavenging activity than those of unprocessed flour. The study also reported that the highest radical scavenging activity was showed by legume, *Canavalia gladiata* with IC_{50}; 59.23 µg/ml which is higher than that of *Canavalia ensiformis* ($IC_{50;}$ 34.35 µg /ml) in concentration of 500 and 250 µg/ml and also showed that the extract of *C. gladiata*, which contains highest amount of flavonoids and phenolic compounds, exhibited the greatest antioxidant activity[18].

Table 2: Quantitative Assessment of Total Phenolic Content in Unprocessed and Differentially Processed Seeds of Jack bean and Barnyard millet

Flours	Total Phenols (mg GAE/100g)				
	Unprocessed	**Soaked**	**Cooked**	**Autoclaved**	**Germinated**
Jack bean	90.5±0.9	57.6±0.2* (36.35↓)	41.1±0.4* (54.5↓)	65 .0±0.1* (28.1↓)	82.0±0.4* (08.9↓)
Barnyard	26.7±0.6	15.6±0.1* (41.5↓)	17.5±0.8* (34.4↓)	16.5±1.0* (38.2↓	21.3±0.1* (20.2↓)

Values are mean of three replicates ±SD and expressed on g/100g sample JBF; jack bean flour, BMF; barnyard millet flour, *Indicates significant difference at P 0.05 level, NS-Non Significant Figures in parenthesis indicate percent decrease ↓ & Increase ↑

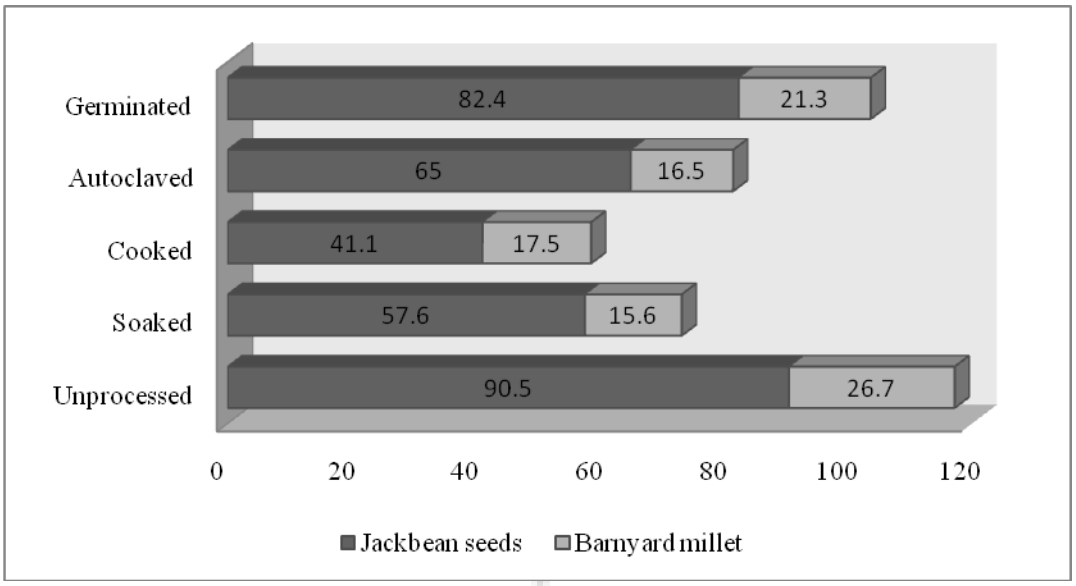

Figure 2: Effect of Different Processing on Total Phenolic Content of Jack Bean Flour and Barnyard Millet Flour

Phenolic compounds are a class of antioxidant agents which acts as free radical terminaters[19].The total phenolic content were 90.5±0.9 and 26.7±0.6 mg GAE/100g in unprocessed jack bean and barnyard millet flour extract as shown in Table 2 among the various processing such as soaking, cooking, autoclaving and germination, the total phenolic content were 57.6±0.2, 41.1±0.4, 65.0±0.1 and 82.0±0.4 mg GAE/100g in jack bean and 15.6±0.1, 17.5±0.8, 16.5±1.0 and 21.3±0.1 mg GAE/100g in barnyard respectively and minimum reduction was established in germinated flour were 8.9% in jack bean and 20.2% in barnyard millet flours. The level of elimination of phenolic compounds in the presently studied materials was found to be higher in soaking and cooking, since these compounds are water soluble in nature and mostly located in the seed coat, the decrease on the level of phenol compounds during this processing treatment might be due to leaching out into the soaking medium. The total phenols content was decreased significantly at $P<0.05$ level when compared to unprocessed flours.

Table 3: Quantitative Assessment of Total Flavonoids Content in Unprocessed and Differentially Processed Seeds of Jackbean and Barnyard millet

Flours	Flavonoids content (mg QE/100g)				
	Unprocessed	Soaked	Cooked	Autoclaved	Germinated
Jackbean	73.1±0.2	34.5±0.7* (52.8↓)	46.3±0.2* (47.1↓)	54.6±0.5NS (25.3↓)	61.8±0.3* (15.4↓)
Barnyard	56.9±0.6	33.9±0.4* (40.4↓)	44.8±0.3* (21.2↓)	30.4±0.4* (46.5↓)	43.5±0.6* (23.5↓)

Values are mean of three replicates ±SD and expressed on g/100g sample JBF; jack bean flour, BMF; barnyard millet flour, *Indicates significant difference at P 0.05 level, NS-Non Significant Figures in parenthesis indicate percent decrease ↓ & Increase ↑

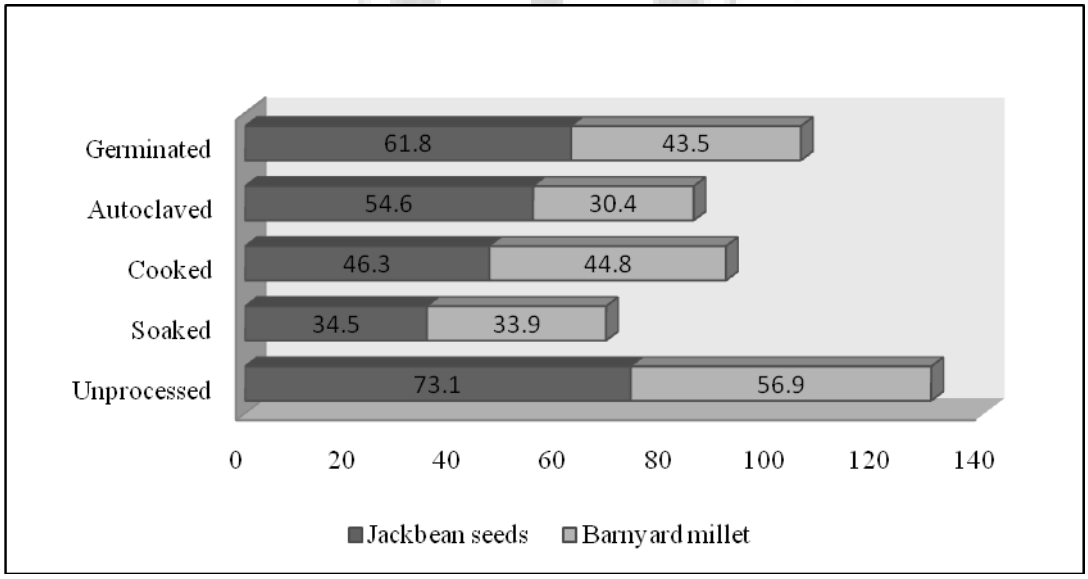

Figure 3: Effect of Different Processing on Total Flavonoids Content of Jack Bean Flour and Barnyard Millet Flour

It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging and chelating processes [20]. The flavonoids content of processed and unprocessed flour extract of jack bean and barnyard millet were presented in Table and Figure 3. There was a

significant decrease in flavonoids content during autoclaving (54.6±0.5 mg QE/100g) and germination (61.8±0.3 mg QE/100g) flour by 25% and 15% in jack bean when compared to the unprocessed flour (73.1±0.2 mg QE/100g) at 0.05 level. Similarly, the flavonoids content also significantly decreased in autoclaved (46%) and germinated (23%) in barnyard flour extract. The results were in agreement with Pradeep and Guha that total phenols and flavonoids content was decreased by 5-20% for the *Panicum sumatrense*.

4. CONCLUSION

The results of the present study demonstrated that the processed jack bean and barnyard millet flour constitute a rich source of antioxidants and other secondary metabolites in addition to appreciable levels. All the bioactive compounds in 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 both seed flours, the germination and autoclaving appear to be more effective. Hence such a viable processing method could be recommended for the versatile utilization of underutilized flours as source of natural antioxidants. Free radicals are often generated as by-products of biological reactions or from exogenous factors. The involvements of free radicals in the pathogenesis of a large number of diseases are well documented. A potent scavenger of free radicals may serve as a possible preventative intervention for the diseases. The results from *in vitro* experiments demonstrate that the raw and processed flours of both jack bean and barnyard millet have significant antioxidant activities. They are natural and economic sources of dietary antioxidant, which may put off diseases caused by free radical. The difference in the antioxidant activity of the processed flour extracts may be attributed to the changes in the chemistry and in the level of total phenol contents as influenced by the processing methods. It has enhanced the scope of its utilization by using different processing methods which will definitely expands its utility for human consumption. Further research is needed to be carried out on human trial to authenticate its medicinal uses by the countryside communities.

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