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ANTIOXIDANT POTENTIALS OF RAW AND SPROUTED MOTH BEAN *VIGNA ACONITIFOLIA* (JACQ.) MARECHAL SEEDS : AN UNDERUTILIZED FOOD LEGUME

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Abstract:

The aim of study was to evaluate the antioxidant potentials of methanolic extract of raw and sprouted moth bean seeds Vigna aconitifolia (Jacq.) Marechal by in vitro assays such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, reducing power assay, total Phenolic assay and total antioxidant assays. The results showed high antioxidant potentials in the sprouts than raw seeds of moth bean for the various antioxidant aasays performed. Sprouted and raw seeds of mothbean are being used in traditional diet as nutritional recipies to support the concept of functional foods. We concluded through our studies, sprouting of moth beans increases the health benefits by increasing its free radical scavenging abilities higher than raw seeds.

Key words: *Moth bean; Sprouting; High antioxidant potentials; functional foods*

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INTRODUCTION:

Food antioxidants such as amino acids, peptides, proteins, tocopherols, carotenoids, flavonoids and other phenolic compounds play a significant role as physiological and dietary antioxidants, thereby augmenting the body's natural resistance to oxidative damage [1,2]. Epidemiological evidences indicate that intake of food-derived antioxidants can offer advantageous effects to consumers health such as lowering the incidence of cardiovascular disease, cancers and age-related degenerative processes [3].

Legumes constitute the main staple foods in many developing countries by providing significant quantities of energy, protein, fibre, and selected micronutrients to the animal and human diet. Significant levels of antioxidants have been detected in legumes and legume-based products. It has been suggested that antioxidants may contribute to the health benefits of legume-based foods by reducing the incidence of aging-related chronic diseases including heart diseases and some types of cancer [4]. Recently, there is a need to examine the underutilized legumes to meet out the protein energy malnutrition. The utilization of these underutilized legumes is limited due to the presence of certain antinutritional compounds. Several processing techniques such as germination, soaking and cooking and dry heat treatment have been used to overcome the antinutrients. During sprouting, a large portion of the original nutritional value of the legume seeds is retained, and the amounts of some active substances increase significantly.

The moth bean *Vigna aconitifolia* (Jacq) Marechal seeds is one of the most important underutilized short-season, summer-cultivated legumes. It is grown widely throughout the tropics and subtropics [5,6]. The seeds and sprouts are excellent sources of antioxidants in China, India, Bangladesh, and Southeast Asia [7]. However, information with particular reference to the antioxidant properties of raw and sprouted seeds is scare. Hence, our study aimed to evaluate the antioxidant properties of raw and sprouted moth beans.

MATERIALS AND METHODS:

Collection of plant materials

The moth bean seeds were purchased from a local market of Coimbatore,Tamil Nadu. Then seeds were thoroughly cleaned and screened to remove broken and cracked grains, dust and other foreign materials, dried and stored in airtight containers. A part of beans were allowed to sprout for 12 h and dried under sunlight for 24 h and stored in airtight containers until further use.

Extraction

The dry seeds of moth beans were milled to flour using flour miller. Exactly 500 g of flour was weighed and extracted with 500 ml of methanol for 7 days in dark under room temperature with intermittent shaking. After 7 days, the whole extracts are filtered using muslin cloth at first and then through filter paper. The filtrate is maintained in dark. To the residue, 100 ml of fresh solvent was added and refluxed for 1 h followed by filtration and finally both the filtrate were mixed together and concentrated. The yield of crude extracts were noted and stored in desiccators for maximum of 3 days; later preserved in a deep freezer (-20 °C) for further use. Similar procedure was also followed for sprouts of moth bean. The dried extract thus obtained was used for the assessment of antioxidant activity by *in vitro* assays.

Qualitative phytochemical analysis

The preliminary qualitative phytochemical studies were carry out for testing the different chemical groups present in methanol extracts of moth bean raw seeds and their sprouts as per method of Trease and Evans (1978) [8].

Evaluation of *in vitro* antioxidant activity

Reducing power assay

The reducing power of the extracts was evaluated according to method of Oyaizu (1986). From the stock solution, different concentrations of the samples were added to 2.5 ml of phosphate buffer and 2.5 ml of 1% Potassium ferricyanide solution. This mixture was kept at 50ºC in water bath for 20 min. After cooling, 2.5 ml of 10% Trichloro acetic acid was added and centrifuged at 3000 rpm for 10 min. 2.5 ml of supernatant was mixed with 2.5 ml of distilled water and 0.5ml of 0.1% ferric chloride and kept for 10 min. The absorbance of resulting solution was measured at 700nm. Increase in absorbance of the reaction mixture indicates increased reducing power. The experiment was conducted in triplicates and values are expressed as equivalents of ascorbic acid in µg / mg of extract.

2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

DPPH free radical scavenging assay was measured using the method of Wong et al. (2006). DPPH solution was added to 3 ml of methanol and the absorbance was noted at 516 nm. Different volume levels of test sample (0.5, 1, 1.5, 2 and 2.5ml) was made up to 3 ml with methanol. 3ml of DPPH solution was added to each of the above test tubes. All the test tubes were incubated at room temperature in dark for 30 minutes. Absorbance was taken at 517 nm in UV-visible spectrophotometer after 15 min and Percentage of inhibition was calculated by using the formula,

$$\%\text{Inhibition} = \frac{(\text{ODofcontrol} - \text{OD of sample})}{\text{ODofcontrol}} \times 100$$

Results have also been reported as IC50, which is the amount of antioxidant necessary to decrease the initial DPPH• concentration by 50%. All the tests were performed in triplicates and for IC50 values the graph was plotted .

Total phenolics assay

Different concentrations (5, 10, 15, 20 and 25 µg) of pyrocatechol were taken and made up to 1ml with distilled water. To the above prepared solutions 1 ml of the FC (Folin-Ceocalteau) reagent is added; shaken well for 3min. To each test tube 3ml of sodium carbonate is added to provide the alkaline condition. All the test tubes are incubated for 2 h at room temperature. The resulting solution is blue in colour and its absorbance is measured at 760 nm. The experiment was conducted in triplicates and values are expressed as equivalents of pyrocatechol in µg / mg of extract. Similarly 50 µg of both the extract samples were taken in two test tubes and the total phenolics assay is carried out as above to get the blue coloured resulting solution and its OD value is measured.

Total antioxidant assay

The total antioxidant capacity was measured by spectrophotometeric method of Prieto et al. (1999). Different concentrations of extracts are taken in different test tubes. The volume of each test tube is made up to 1 ml with distilled water. 1ml of acid reagent consisting of 0.6M H_2SO_4 + 28 mM Na_2HPO_4 + 4 mM Ammonium molybdate is added to each of the test tube. The above solution was incubated for 90 min at 95 °C. The absorbance of the resulting solution was measured at 695 nm. The experiment was conducted in triplicates and values were expressed as equivalents of ascorbic acid in µg / mg of extract.

RESULTS AND DISCUSSION:

Qualitative phytochemical analysis

The methanolic extracts of the sprouts and raw seeds of *Vigna aconitifolia* (Jacq.) Marechal seeds showed the presence of carbohydrates, proteins, amino acids, saponins, steroids, triterpenoids, glycosides, flavonoids, polyphenols and tannins , alkaloids (Table 1).

Table 1. Qualitative phytochemical analysis in methanolic extract of raw and sprouted *Vigna aconitifolia* (Moth bean) seeds

Phytochemical composition	Vigna aconitifolia	
	Raw	Sprouted
Carbohydrates	+	+
Proteins	+	+
Amino acids	+	+
Alkaloids	+	+
Triterpenoids	+	+
Steroids	+	+
Flavonoids	+	+
Glycosides	+	+
Saponins	+	+
Polyphenols	+	+
Tannins	+	+

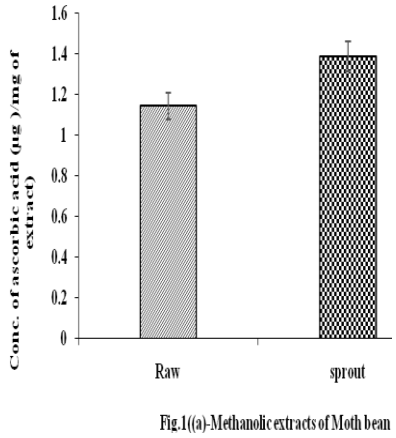

Fig.1((a)-Methanolic extracts of Moth bean

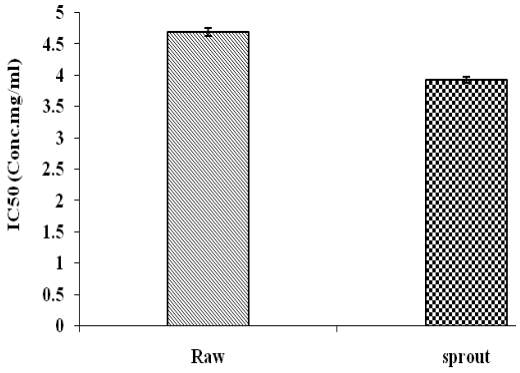

Fig.1(b)-Methanolic extract of Moth bean

Fig. 1(a). Reducing Power assay (Equivalents of ascorbic acid) and Fig.1(b). DPPH radical scavenging activity (IC_{50}) of methanolic extracts of sprouts and raw seeds of Moth bean.

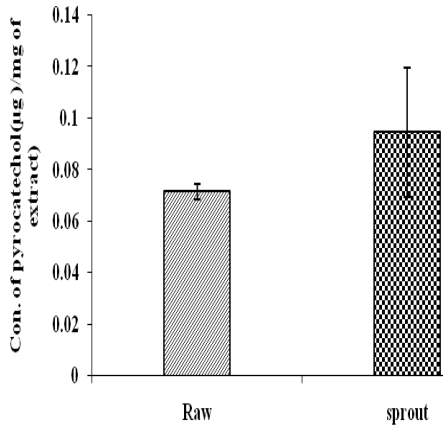

Fig.2(a)-Methanolic extracts of Moth bean

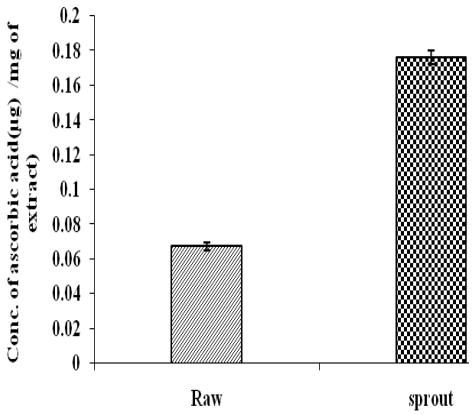

Fig.2(b)-Methanolic extracts of Moth bean

Fig. 2(a). Total phenolics assay (Equivalents of pyrocatechol) and Fig.2.(b).Total antioxidant assay (Equivalents of ascorbic acid) of methanolic extracts of sprouted and raw seeds of moth bean

Reducing power assay

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reductive ability was measured in terms of Fe^{3+} to Fe^{2+} transformation in the presence of different concentrations of the extract. The methanolic extracts of sprouts and seeds of moth bean showed potent reducing ability. The results were presented as their ascorbic acid equivalents shown in the **Fig. 1(a)**. The sprouts and seeds of moth bean showed 1.391 and 1.144 µg of ascorbic acid / mg of extract respectively. The results revealed the dose dependent reducing ability for our methanolic mothbean extracts. From the results it was revealed that the sprouts exhibited greater reducing power ability compared to seeds in terms of ascorbic acid equivalents.

DPPH radical scavenging activity

DPPH was used to determine the proton scavenging activity of the methanolic extracts of seeds and sprouts of moth bean and their IC_{50} values in **Fig. 1(b)** .The IC_{50} value, a measure of the extract concentration which is required for 50% scavenging of the free radical DPPH, was determined. The IC_{50} values for sprouts and seeds of moth bean showed 3.93 and 4.69 mg/ml respectively. While, the similar activity was 0.011 mg/ml of ascorbic acid for standard. The results revealed that the IC_{50} values for methanolic extracts of moth bean were found to be at lower concentration in sprouts and higher concentration in raw seeds.

Total phenolics assay

The total phenolics of the methanolic extracts of sprouts and seeds of moth bean were studied. The results were presented as their pyrocatechol equivalents in the **Fig. 2.** The methanolic extracts of sprouts and seeds of moth bean showed 0.09 and 0.072µg of pyrocatechol / mg of extract respectively. From the results it was revealed that sprouts possesed greater total phenolics compared to seed extracts in terms of pyrocatechol equivalents.

Total antioxidants assay

Total antioxidant assay is determined depending on the reduction of molybdenum present in the phosphomolybdic acid. The molybdenum which is present in Mo (IV) will be converted into Mo (V) by the antioxidant activity of the phytochemicals present. This will subsequently form the phosphate/Mo (V) complex at acidic pH which is green in colour and whose absorbance is measured. The methanolic extracts of sprouts and seeds of moth bean showed potent total antioxidant capacity. The results were presented as their ascorbic acid equivalents shown in the **Fig. 2.** The methanolic extracts of sprouts and seeds of moth bean showed 0.18 and 0.067 µg of ascorbic acid / mg of extract respectively. From the results, it was revealed that sprouts exhibited higher total antioxidant capacity compared to seed extracts in terms of ascorbic acid equivalents. According to Chavan and Kadam (1989) "the metabolic activity of resting seeds increases as soon as they are hydrated during soaking. Complex biochemical changes occur during hydration and subsequent sprouting. The reserve chemical constituents, such as protein, starch and lipids, are broken down by enzymes into simple compounds that are used to make new compounds. During sprouting of

mothbean the metabolic activity of seeds increases by hydrating. Most of the biochemical changes occur during hydration and during sprouting . Further, sprouting treatment generally improves their vitamin value, especially the Vitamins A, E, C & B complex [9]. These changes are brought about by enzymes which become active during germination [10].

CONCLUSION:

Epidemiological studies have strongly suggested that diets play a crucial role in the prevention of chronic diseases such as cancer and heart disease, diabetes, and Alzheimers's disease. Consumption of grains, as well as fruits and vegetables, has been associated with reduced risk of chronic diseases [11]. This has been hypothesized to be because they contain phytochemicals that combat oxidative stress in the body by helping to maintain a balance between oxidants and antioxidants. An imbalance caused by overproduction of oxidants leads to oxidative stress, resulting in damage to large biomolecules such as lipids, DNA, and proteins. Oxidative damage increases the risk of degenerative diseases such as cancer and cardiovascular diseases. Antioxidants reduce oxidative damage of biomolecules by modulating the effects of reactive oxidants [12]. The results of our investigation have shown that sprouts have higher antioxidant activity than raw seeds, which may be a result of difference in the content of polyphenols and other compounds. Methanolic extracts of sprouted mothbean had higher antioxidant capacity than raw seeds methanolic extracts . In sprouted seeds antioxidant potential increases due to their generation of several antioxidant phytochemicals, vitamins. Moth bean seeds and sprouts are being used in traditional diet as a beneficial source of food with very high nutritional value and hence now support the concept of functional foods.

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