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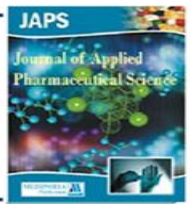

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Antioxidant potentials in sprouts vs. seeds of *Vigna radiata* and *Macrotyloma uniflorum*

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ABSTRACT

Antioxidant activity of methanol extracts of two common legumes, *Vigna radiata* (Green gram) and *Macrotyloma uniflorum* (Horse gram) for their seeds and sprouts was investigated by adopting various *in vitro* models such as reducing power assay, DPPH assay, total phenolic assay and total antioxidant assays. The results showed higher antioxidant abilities in the sprouts than their seeds for the various antioxidant tests performed. Sprouts described above are being used in traditional diet as a beneficial source of food with very high nutritional value and support the concept of functional foods and the results are discussed.

Key words: Antioxidant activity, seeds, sprouts.

INTRODUCTION

Highly reactive free radicals, especially oxygen-derived radicals, which are formed by exogenous chemicals or endogenous metabolic processes in the human body, are capable of oxidizing cellular bio-molecules such as nucleic acids, enzymes, proteins, lipids and carbohydrates, and consequently may adversely affect immune functions resulting in cell death and tissue damage (Nilsson et al, 2004). Oxidative damage plays a significant pathological role in human diseases. Cancer, emphysema, cirrhosis, atherosclerosis and arthritis have all been correlated with oxidative damage (Halliwell et al, 1984). Almost all organisms are well protected against free radical damage by enzymes such as superoxide dismutase and catalase or compounds such as ascorbic acid, tocopherol and glutathione (Niki et al, 1994). When the mechanism of antioxidant protection becomes unbalanced by factors such as ageing, deterioration of physiological functions may occur resulting in diseases and accelerating ageing. Synthetic antioxidants are widely used to reduce oxidative damage because they are effective and cheaper than natural types. However, their safety and toxicity have been of major concerns (Imaida et al, 1983). Much attention has been focused on the use of antioxidants especially natural antioxidants to protect the human body from the oxidative damage by free radicals. Dietary antioxidants protect against free radicals such as reactive oxygen species in the human body. Modern research is now directed towards natural antioxidants originated from plants due to safe therapeutics.

The importance and health benefits of grain consumption in the prevention of chronic diseases such as cancers and heart disease have been documented (Smigel et al, 1992; Thompson et al, 1994). Grains contain unique phytochemicals that complement those in fruits and vegetables

when consumed together. Phenolic compounds present in grains have antioxidant properties associated with the health benefits of grains and grain products. Pulses are the dried edible seeds of cultivated legumes. Pulses contain more protein than any other plant. The nutritive properties of pulses resemble in many respects those of the whole cereal grains. The practice of utilizing germinated seed or sprouting or young seedlings of pulses as a fresh vegetable is widespread in the Orient. There is an amazing increase in nutrients in sprouted pulses when compared to their dried embryo. In the process of sprouting, the vitamins, minerals and protein increase substantially with corresponding decrease in calories and carbohydrate content (Chavan and Kadam, 1989). Further, sprouting of the pulses not only improves nutritive value but also digestibility.

Consumption of seeds and sprouts has become increasingly popular among people interested in improving and maintaining their health status by changing dietary habits. The seeds and sprouts are excellent examples of 'functional food', lowering the risk of various diseases and exerting health promoting effects in addition to its nutritive value (Paweł Pas´ko et al, 2009). The present investigation is aimed to evaluate the antioxidant benefits of seeds and their sprouts utilizing two common legumes.

The green gram, *Vigna radiata* (Fabaceae) is one of the important pulse crops in India. It is a protein rich staple food. It contains about 25 percent protein. It supplies protein requirement of vegetarian population of the country. This is consumed in the form of split pulse as wells as whole pulse, which is an essential supplement of cereal based diet. In addition to being an important source of human food and animal feed, Green gram also plays an important role in substaining soil fertility by improving soil physical properties and fixing atmospheric nitrogen.

The horse gram, *Macrotyloma uniflorum* (Fabaceae) is normally used to feed horses, though it is also commonly used in dishes. In traditional ayurvedic cuisine, horse gram is considered a food with medicinal qualities. It is prescribed for persons suffering from jaundice or water retention and as part of a weight loss diet. It is useful in iron deficiencies and is considered helpful for maintain body temperature in the winter season.

MATERIALS AND METHODS

Plant materials

Two plant materials of *Vigna radiata* and *Macrotyloma uniflorum* were selected. The seeds were purchased fresh from a local store of Shivamogga, Karnataka.

Extraction

The dry seeds *viz. Vigna radiata* (DSVR) and *Macrotyloma uniflorum* (DSMU) were collected and are powdered in a mill separately. Exactly 500g was weighed and extracted in 500ml 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 marc, 100 ml fresh solvent was added and refluxed for 1hour 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^{o}C) for further use. Similar procedure was also followed for 24hrs sprouts of *Vigna radiata* (SPVR) and *Macrotyloma uniflorum* (SPMU). The dried extract thus obtained was used for the assessment of antioxidant activity through four *in vitro* models.

Qualitative phytochemical analysis

The preliminary qualitative phytochemical studies were performed for testing the different chemical groups present in methanol extracts of two different seeds and their sprouts (Trease and Evans, 1978).

Evaluation of *in vitro* antioxidant activity

Reducing power assay

The reducing power of the extracts was evaluated according to Oyaizu, 1986 (Oyaizu, 1986). From the stock solution, different concentrations of the samples are added to 2.5ml of phosphate buffer. 2.5ml of 1% Potassium ferricyanide solution is added. 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 methanol which serves as blank and the absorbance was noted immediately at 516 nm. Different volume levels of test sample (0.5, 1, 1.5, 2 and 2.5ml) was made up to 3ml 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 (SL159, Elico India P. Ltd.) after 15 min using methanol as a blank. The absorbance was also noted for the blank. Percentage of inhibition was calculated by using the formula,

$$\% \text{ Inhibition concentration} = \frac{\text{O.D. of Control} - \text{O.D. of Sample}}{\text{O.D. of Control}} \times 100$$

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

Total phenolics assay

Different concentrations (5, 10, 15, 20 and 25µg) of pyrocatechol were taken and made up to 1ml with distilled water. To the above prepared solutions 1ml 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 2h at room temperature. The resulting solution is blue in colour and its absorbance is measured at 760nm. 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 O.D. 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 1ml with distilled water. 1ml of acid reagent consisting of 0.6M H_2SO_4 + 28mM Na_2HPO_4 + 4mM Ammonium molybdate is added to each of the test tube. The above solution is incubated for 90 min at 95^oC. The absorbance of the resulting solution is measured at 695nm. The experiment was conducted in triplicates and values are expressed as equivalents of ascorbic acid in µg / mg of extract.

RESULTS AND DISCUSSION

Qualitative phytochemical analysis

The methanolic extracts of the sprouts and seeds of *Vigna radiata* and *Macrotyloma uniflorum* showed the presence of carbohydrates, proteins, amino acids, steroids, triterpenoids, glycosides, flavonoids, polyphenols and tannins alkaloids. Analysis also revealed that none of the extracts under study gave positive results for saponins (Khandelwal, 2006).

Table 1. Qualitative phytochemical analysis in methanol extracts of sprouts and seeds of *Vigna radiata* and *Macrotyloma uniflorum.*

	Vigna radiate		*Macrotyloma uniflorum*	
	sprouts	**seeds**	**sprouts**	**seeds**
Carbohydrates	+	+	+	+
Proteins	+	+	+	+
Amino acids	+	+	+	+
Steroids	+	+	+	+
Triterpenoids	+	+	+	+
Glycosides	+	+	+	+
Saponins	-	-	-	-
Flavonoids	+	+	+	+
Alkaloids	+	+	+	+
Polyphenols and tannins	+	+	+	+

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 *Vigna radiata* and *Macrotyloma uniflorum* were showed potent reducing ability. The results were presented as their ascorbic acid equivalents shown in the Fig 1. The sprouts and seeds of *Vigna radiata* showed 0.206 and 0.091 µg of ascorbic acid / mg of extract respectively. Similarly the methanolic extracts of sprouts and seeds of *Macrotyloma uniflorum* showed 0.643 and 0.153 µg of ascorbic acid / mg of extract respectively. The results revealed the dose dependent reducing ability for all the extracts. From the results it was revealed that the sprouts exhibited greater reducing power ability compared to seeds in terms of ascorbic acid equivalents. On the basis of the results of reducing power assay, the methanolic extracts of both the plants was placed in the following order. Sprouted *Macrotyloma uniflorum* > Sprouted *Vigna radiata* > seed *Macrotyloma uniflorum* > seed *Vigna radiata.*

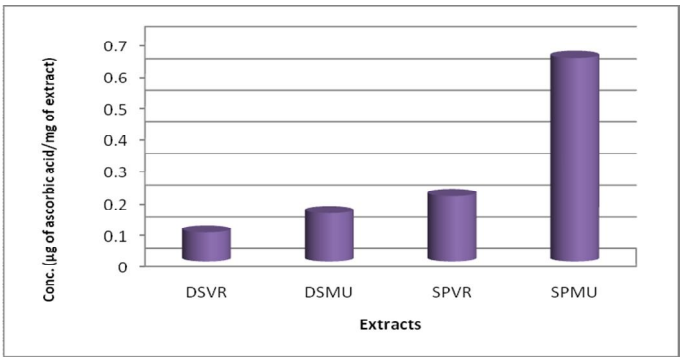

Fig 1. Reducing power assay in methanol extracts of sprouts and seeds *Vigna radiata* and *Macrotyloma uniflorum* (Equivalents of ascorbic acid).

DSVR – Dry seeds *Vigna radiata*
DSMU – Dry seeds *Macrotyloma uniflorum*
SPVR – Sprouted *Vigna radiata*
SPMU – Sprouted *Macrotyloma uniflorum*

DPPH radical scavenging activity

In this study, DPPH was used to determine the proton scavenging activity of the methanolic extracts of seeds and sprouts of two legumes and their IC_{50} values (**Fig 2).**

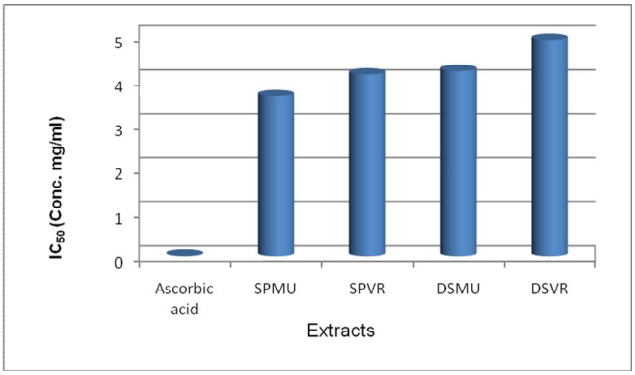

Fig 2. DPPH radical scavenging activity (IC_{50}) of methanol extracts of sprouts and seeds *Vigna radiata* and *Macrotyloma uniflorum.*

The IC_{50} value, a measure of the extract concentration which is required for 50% inhibition of the free radical DPPH, was determined. The IC_{50} values for sprouts and seeds of *Vigna radiata* showed 4.148 and 4.93 mg/ml respectively. Similarly the IC_{50} values for sprouts and seeds of *Macrotyloma uniflorum* 3.66 and

4.225 mg/ml respectively while, the similar activity was 0.011 mg/ml for standard. The results revealed that the IC_{50} values for methanol extracts of both the plants were found to be highest in sprouts of *Macrotyloma uniflorum* and lowest in seeds of *Vigna radiata.* On the basis of the results of DPPH radical scavenging activity for IC_{50}, the plants were placed in the following order. Sprouted *Macrotyloma uniflorum* > sprouted *Vigna radiata* > seed *Macrotyloma uniflorum* >seed *Vigna radiata.*

Total phenolics assay

The total phenolics of the methanolic extracts of sprouts and seeds of *Vigna radiata* and *Macrotyloma uniflorum* were studied. The results were presented as their pyrocatechol equivalents shown in the **Fig 3.**

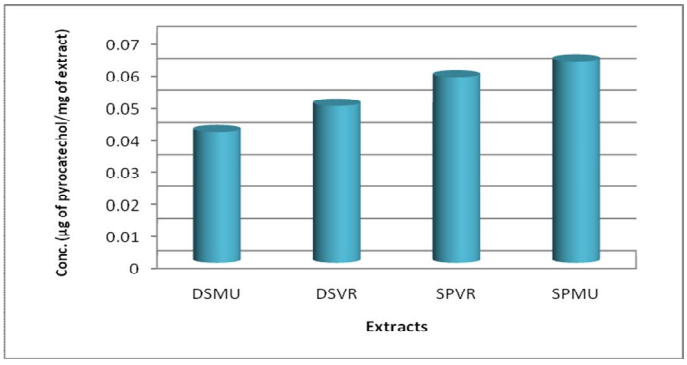

Fig 3. Total phenolics assay in methanol extracts of sprouts and seeds *Vigna radiata* and *Macrotyloma uniflorum* (Equivalents of pyrocatechol).

The methanolic extracts of sprouts and seeds of *Vigna radiata* showed 0.058 and 0.049 µg of pyrocatechol / mg of extract respectively while, sprouts and seeds of *Macrotyloma uniflorum* showed 0.063 and 0.041 µg pyrocatechol / mg of extract respectively. From the results it was revealed that sprouts exhibited greater total phenolics compared to seed extracts in terms of pyrocatechol equivalents. On the basis of the results of total phenolics, the methanolic extracts of both the plants was placed in the following order. Sprouted *Macrotyloma uniflorum* > sprouted *Vigna radiata* > seed *Vigna radiata* > seed *Macrotyloma uniflorum*.

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 *Vigna radiata* and *Macrotyloma uniflorum* were showed potent total antioxidant capacity. The results were presented as their ascorbic acid equivalents shown in the **Fig 4.** The methanolic extracts of sprouts and seeds of *Vigna radiata* showed 0.148 and 0.043 µg of ascorbic acid / mg of extract respectively. Similarly sprouts and

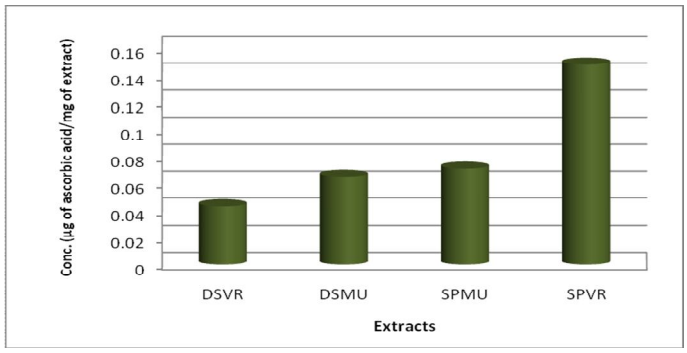

Fig 4. Total antioxidant capacity in methanol extracts of sprouts and seeds *Vigna radiata* and *Macrotyloma uniflorum* (Equivalents of ascorbic acid).

seeds of *Macrotyloma uniflorum* showed 0.071 and 0.065 µg of ascorbic acid / mg of extract respectively. The results revealed the dose dependent total antioxidant capacity for all the extracts. From the results it was revealed that sprouts exhibited higher total antioxidant capacity compared to seed extracts in terms of ascorbic acid equivalents. On the basis of the results of total antioxidant assay, the methanolic extracts of both the plants was placed in the following order. Sprouted *Vigna radiata* > sprouted *Macrotyloma uniflorum* > seed *Macrotyloma uniflorum* > seed *Vigna radiata*.

Ranking of the methanolic extracts of sprouts and seeds of *Vigna radiata* and *Macrotyloma uniflorum* based on the results of four assays *viz*, reducing power, DPPH, total phenolics and total antioxidant assay can be placed in the following order. Sprouted *Macrotyloma uniflorum* > Sprouted *Vigna radiata* > seed *Macrotyloma uniflorum* > seed *Vigna radiata* .

Epidemiological studies have strongly suggested that diets play a crucial role in the prevention of chronic diseases such as cancer and heart disease, cancer, diabetes, and Alzheimers's disease (Willett, 1994). Consumption of grains, as well as fruits and vegetables, has been associated with reduced risk of chronic diseases (Willet et al, 1995). 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 (Wagner et al, 1992). Antioxidants reduce oxidative damage to biomolecules by modulating the effects of reactive oxidants (Fraga et al, 1991). The results of our investigation have shown that sprouts have higher antioxidant activity than seeds, which may be a result of difference in the content of polyphenols and other compounds. 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. Further, sprouting treatment generally improves their vitamin value, especially the Vitamins A, E, C & B complex (Cuddeford, 1989; Shipard, 2005). These changes are brought about by enzymes which become active during germination

(Chavan and Kadam, 1989). Therefore it is apparent that the antioxidant potentials of sprouts could be due to their generation of several antioxidant phytochemicals, vitamins etc. Alternative crops and sprouts described above are being used in traditional diet as a beneficial source of food with very high nutritional value and support the concept of functional foods.

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