COMPARISON OF FREE RADICAL SCAVENGING ACTIVITY OF TWO MAIN VARIETIES OF *CICER ARIETINUM* SPROUTS

Beenu Tom^{1*}, Thiruselvi M²

¹M. Sc. Biochemistry, Bharathiar University, RVS College of Arts and Science, Department of Biochemistry, Coimbatore, Tamilnadu, India

²Assistant professor, Bharathiar University, RVS College of Arts and Science, Department of Biochemistry, Coimbatore, Tamilnadu, India

*Corresponding Author Email: beenuelstom@gmail.com

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ABSTRACT

The purpose of the study was to evaluate the extent of free radical scavenging properties and antioxidant effects of crude extracts of sprouted *Cicer arietinum* (Chick pea/Chana/Bengal gram) seeds. Two main varieties of *Cicer arietinum* seeds viz. Kabuli-chana (cream seed-coat) and Bengal gram (brown seed-coat) were examined and compared for their free radical scavenging properties and antioxidant effects. A crude methanol extract of the sprouted seeds were prepared. Free radical scavenging properties were evaluated against stable 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) and hydrogen peroxide radical and the extent of antioxidant effect was assessed by lipid peroxidation induced by ferrous sulphate on the lipid present in the liver homogenate. The results showed that the two *Cicer arietinum* extracts differed in their capacities to quench or inhibit DPPH, hydrogen peroxide and lipid peroxide. Brown colored *Cicer arietinum* sprouts showed the greatest activity against DPPH radicals, hydrogen peroxide radicals and lipid peroxide compared to the cream variety. This study is clearly indicating that brown colored *Cicer arietinum* is effective in scavenging free radicals and has the potential to be a powerful antioxidant.

Keywords: *Cicer arietinum*, free radical, antioxidant, DPPH, hydrogen peroxide, lipid peroxide.

INTRODUCTION

Legume seed sprouts are popular foods globally. It is also reported that seed priming improve the antioxidant enzymes activity which decrease the adverse effects of Reactive Oxygen Species (ROS)¹. Free radicals are chemical elements, which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability². Production of free radicals in the biological system results is due to the imbalance in the level of pro-oxidant and antioxidant known as oxidative stress³. Antioxidants are chemical compounds that can bind to free oxygen radicals thus preventing these radicals from damaging healthy cells which could lead to cancer⁴. Many antioxidants can be obtained from food sources such as sprouted grains, fruits and vegetables. Consumption of high amounts of antioxidant substances may have a positive impact on human health, particularly the prevention of cancer and inflammatory diseases⁵. Seeds of *Cicer arietinum* are one of the ancient and widely consumed legumes in tropical and subtropical countries. Chickpeas are a rich source of zinc, folate and protein. They are also very high in dietary fiber and hence a healthy source of carbohydrates for persons with insulin sensitivity. They can assist in lowering of cholesterol in the bloodstream and are effective in conditions like anemia, digestive system disorder, painful menstruation, skin and hair disease and sexual dysfunction. In the present study an attempt has been made to evaluate the scavenging effect of *Cicer arietinum* sprouted seeds on selected free radicals and oxidants and to test the effect of sprouted seed extract on biomolecular damage induced *in vitro*.

MATERIALS AND METHODS

Collection of plant species

Two types of seeds were used in the present study, namely brown coat colored *Cicer arietinum* seeds and cream coat

colored *Cicer arietinum* seeds. The seeds were collected from the local markets of Coimbatore city, India and maintained sterile.

Seed germination

Healthy grains were selected and washed in clean water. Then soak the seeds in water and drain off the water after 12 hours. A cotton bed was prepared to spread and germinate grains. The grains were spread on wet-cotton bed at room temperature with ample light and air. Grains were covered with similar type of cotton covering. Water was sprinkled as and when required to keep bedding wet.

Preparation of crude extract

The fourth day sprouts were taken and extracts were prepared using methanol as solvent. 1g of the samples were taken and homogenized with 1ml of the respective solvents; Then centrifuged at 4000rpm for 20mins. The supernatant containing the sprouted seed extract was then transferred to a pre-weighed beaker and the extracts were concentrated by evaporating the solvent at 60°C. The crude extract was weighed and dissolved in a known volume of dimethyl sulphoxide, to obtain a final concentration of 20mg/5µl. In order to identify the chemical nature of the active component present in the plants, a preliminary phytochemical screening was done.

DPPH scavenging assay

The free radical scavenging activity of sprouted seed extracts were determined by the DPPH which is a stable free radical which when acted upon by an antioxidant undergoes a change in color that can be quantified spectrophotometrically⁶. 3ml 1mM DPPH-Methanol solution was added to 2.5µl and 5µl of the plant sample. DPPH solution with methanol was used as a positive control and methanol alone act as blank. When DPPH reacts with antioxidant, it is reduced and the color

change from deep violet to light yellow measured at 518nm. The present inhibition is calculated by the following Formula

$$\text{Scavenging capacity \%} = 100 - (A_1 - A_0) / A_0 \times 100$$

A_0 – Absorbance of control

A_1 – Absorbance in the presence of sample of plant extract and standard.

H₂O₂ scavenging assay

The H₂O₂ scavenging ability of leaf extracts was determined according to the method of Ruch *et al*⁷. A solution of H₂O₂

(40mM) was prepared in phosphate buffer (p^H 7.4). Plant extract at the concentration of 10mg/10μl was added to a H₂O₂ solution (0.6ml, 40mM). The total volume was made up to 3ml. The absorbance of the reaction mixture was recorded at 230nm. The blank solution contained phosphate buffer without H₂O₂. The percentage of H₂O₂ scavenging of sprouted seed extract and standard compound was calculated as:

$$\text{Scavenging activity \%} = (A_0 - A_1) / A_0 \times 100$$

Table I: DPPH Radical Scavenging Activity

Sample concentration	Percent radical scavenging activity (%)	
	Brown <i>Cicer arietinum</i>	Cream <i>Cicer arietinum</i>
50	35.56	26.42
100	48.32	44.42
150	66.48	58.83
200	80.14	64.78
250	89.48	75.80

Table II: Hydrogen Peroxide Radical Scavenging

Sample concentration	Percent radical scavenging activity (%)	
	Brown <i>Cicer arietinum</i>	Cream <i>Cicer arietinum</i>
50	16.42	11.2
100	25.40	19.01
150	36.78	27.32
200	51.15	38.41
250	67.58	50.79

Table III: Extent of Inhibition of *in vitro* Lipid Peroxidation in Goat Liver Homogenate

Sample concentration	Percent radical scavenging activity (%)	
	Brown <i>Cicer arietinum</i>	Cream <i>Cicer arietinum</i>
50	15.26	11.35
100	21.05	18.32
150	30.56	24.62
200	39.25	32.34
250	43.45	38.89

Figure I: DPPH radical scavenging activity

Figure II: Hydrogen peroxide radical scavenging activity

Figure III: Lipid peroxidation activity

Inhibition of *in vitro* lipid peroxidation in liver homogenate

An *in vitro* model of goat liver homogenate was used for the induction of lipid peroxidation with ferrous ions as prooxidants⁸. Application of the relevant sprouted seed extract in the medium was tried with an objective of assessing the extent of inhibition of *in vitro* lipid peroxidation by the measurement of thiobarbituric acid reactive substances in the experimental mixture and TBARS was measured using

spectrophotometer at 535nm. A 5% liver homogenate was prepared in cold TBS. 50μl of it was used in the assay. Fresh sample (0.5g) was weighed accurately and homogenized in 1ml of cold TBS. Aliquots of 50μl of it was used in the assay. Ferrous sulphate at a final concentration of 10μmoles was added in the assay medium to induce oxidation. The final volume in the test tubes were made up to 500μl with cold TBS. Controls were prepared for each sample containing the respective sprouted seed extract (50μl), liver homogenate

(500µl) and TBS to make up the final volume to 500µl. Pro-oxidant was not added to the control tubes. A blank containing no sprouted seed extract, no liver homogenate, but only FeSO₄ and TBS to make a final volume of 500µl was also prepared. An assay medium corresponding to 100% oxidant was prepared by adding all the other constituents except the relevant plant extract and the volume was made up to 500µl with the TBS. The experimental medium corresponding to auto-oxidation contained only the liver homogenate and TBS to make the final volume to 500µl. All the tubes were incubated at 37°C for one hour. Following the incubation period, 500µl of 70% alcohol was added to all the tubes to stop the reaction. 1ml of 1% TBA was added to all the, followed by boiling in a hot water bath for 20 minutes. After cooling to room temperature, the tubes were centrifuged. To the clean supernatant collected into tubes, added 500µl of acetone and measured the TBARS at 535nm in a spectrophotometer.

RESULTS

The effects of methanolic extracts of *Cicer arietinum* sprouted seeds were analyzed on a battery of free radicals namely DPPH and non-radical oxidant hydrogen peroxide. The DPPH scavenging activity of the extracts of *Cicer arietinum* sprouted seeds was analyzed using a photometric assay for several concentrations ranging from 50-250µg/ml. The per cent scavenging activity of the different extracts are listed in Table I and Figure I. It was observed that the extract of brown colored *Cicer arietinum* sprouted seeds showed more effective result than cream colored *Cicer arietinum* sprouted seeds. The brown coat colored sprouted seeds showed a maximum radical scavenging activity of 89.48% at 250µg concentrations. The hydrogen peroxide scavenging activity of the extracts of *Cicer arietinum* was analyzed and the results are listed in Table II and Figure II. Both the extracts showed considerable hydrogen peroxide scavenging activity. The extracts of brown coat colored *Cicer arietinum* sprouted seeds were found to be more effective than the cream coat colored *Cicer arietinum* sprouted seeds as they showed a maximum radical scavenging activity of 67.58% at 250µg concentration. The effect of *Cicer arietinum* sprouted seed extracts on the extent of peroxidation induced by ferrous sulphate on the lipid present in the liver homogenate was assessed and the results are presented in Table III and figure III. Ferrous sulphate resulted in a marked increase in the level of LPO. The presence of *Cicer arietinum* sprouted seed extracts in the reaction medium caused a noticeable decline in this increase. The brown *Cicer arietinum* sprouted seed extracts were found to be more effective.

DISCUSSION

In this study the brown coat colored *Cicer arietinum* was found to be more effective against free radicals. Chick pea Protein Hydrolysate showed a high rate of DPPH radical scavenging activity and antioxidant activity in a study⁹. The DPPH radical scavenging activities was well proved and correlated with the ferric reducing antioxidant capacity of the Cow pea seed extracts¹⁰. H₂O₂ is highly important because of its ability to penetrate biological membranes. H₂O₂ itself is not very reactive, but it can sometimes be toxic to cell because of it may give rise to hydroxyl radical in the cells. The results showed that extracts and fractions of *Cicer arietinum* pod wall had an effective H₂O₂ scavenging activity¹¹.

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