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Research Article

# NUTRITIONAL, ELEMENTAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF GARDEN CRESS (*LEPIDIUM SATIVUM* L.) SEEDS

**PRAMOD J KASABE, PRANIT N PATIL, DAYANAND D KAMBLE, PADMA B DANDGE***

**Department of Biochemistry, Shivaji University, Kolhapur 416 004, (MS) India. padmadandge@gmail.com, kasabe.pramod@gmail.com**

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**ABSTRACT**

Most of the plants possess nutritional as well as medicinal, activities. And varieties of plants are used for the same purposes. The present study is to uncover the nutritional, medicinal potencies and also the free radicals scavenging properties of garden cress. Total Carbohydrates, Proteins and Iron are quantified for nutritional measurement as 60 mg, 1-2 mg and 1 mg respectively from a gram of seed sample. Atomic absorption spectroscopy shows 200-375 ppm of Iron and Flame photometry shows 0.1% of calcium concentration. The seeds posses good Ferric Reducing Antioxidant Power (FRAP) and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity with $IC_{50}$ 176.18 µg/ml. The antioxidant activity is also correlated to the total phenolic content of the seeds. The results reveal that the seeds of Garden cress possess nutritional, medicinal as well as antioxidant activities.

**Keywords:** Nutritional, Medicinal, Antioxidant, Phenolic, $IC_{50}$

## INTRODUCTION

Garden cress (*Lepidium sativum* L.) is an annual herb that belongs to the Brassicaceae family, which is rich in health promoting phytochemical constituents. The total phenolic compounds, which are known to be one of the most important groups of natural antioxidants that accumulate in minimally processed vegetables tissues, are responsible for a high antioxidant capacity. Seeds of *L. sativum* are claimed to possess varied medicinal properties like galactogogue, aperient, diuretic, alterative, tonic, demulcent, aphrodisiac, carminative and emmenagogue[1]. Seeds were largely used for the treatment of hypertension and renal diseases[2].

Garden-cress seeds are used as a medicine in India in the system of "Ayurveda". Also it has health promoting properties which can be used as a functional food. It is also reported that these seeds contain Glucosinolates, sulphur-containing glycosides found in brassicaceous plants that can be hydrolysed enzymatically by plant myrosinase or non-enzymatically to form primarily isothiocyanates and/or simple nitriles. From a human health perspective, isothiocyanates are quite important because they are major inducers of carcinogen-detoxifying enzymes the most potent inducers are benzyl isothiocyanate (BITC) present in garden cress (*Lepidium sativum L.*)[3].

The seed coat of germinating seeds contains much mucilage, which has an allelopathic substance, lepidimoide. The effects of the germinating seeds were studied to determine the potential for slowing down the hydrolysis of starch to glucose in diabetic persons. The seeds significantly lowered the glycaemic response to a test meal[4].

## MATERIALS AND METHODS

### Collection of Plant Material

Seeds of *Lepidium sativum L.* (Garden cress) were collected from local market of Dist. Sangli of Maharashtra, India. The seeds & plants were identified with the help of recent floral description. Present study is to evaluate its potential by quantifying its bioactive compounds. Along with that total phenolic content and the free radical scavenging activity of these seeds was also tested here.

Samples were prepared in three forms as aqueous extract, methanol extract and 50 % ethanol extract at concentration 100 mg/ml.

### A. Nutritional Analysis

- **Estimation of total carbohydrate by Anthrone method**

Estimation is done by Anthrone method with some modifications. 100 mg of seed powder was weighed and taken into a test tube, hydrolysed by keeping it in a boiling water bath for 3 hrs with 5 ml of 2.5 N HCL & cooled to room temperature. It was neutralised with solid sodium carbonate until the effervescence ceases. 100 ml volume was made and centrifuged at 5000 rpm for 30 min. The supernatant was collected & used for analysis. The same procedure is followed for methanol & ethanol extracts. The standards were prepared by taking various aliquots of glucose as a working standard. The volume was made 1 ml by distilled water. 4 ml of Anthrone reagent was added & heated for 8 min in water bath, cooled it. The reading was noted at 630 nm[5].

- **Estimation of Protein by Lowry's Method**

The total protein content was determined spectrophotometrically by Lowry's method using bovine serum albumin (BSA) as a standard protein. Concentrations of proteins in unknown samples are determined by plotting absorbance at 660 nm *v/s* mg protein[6].

- **Estimation of Iron**

Here iron concentration is determined by using spectrophotometer as described by Achar B.N. and Bellappa S. with some modification. Aliquots of Std. Ferric Ammonium Sulphate (FAS) were taken in test tubes and the final volume is adjusted to 1 ml, 0.2 ml conc. HCl and 0.1 ml $H_2O_2$ .is added followed by 0.5 ml ammonium thiocyanate in each test tube. Samples were analyzed by using same protocol. Absorbance is taken at 470 nm[7].

### B. Elemental analysis

- **Determination of iron by atomic absorption spectroscopy:**

1 gm powder of Garden cress was digested in $HNO_3$:$HClO_4$ (3:1) for 30 min & volume was made 100 ml. Readings were taken on atomic absorption spectroscope. For standard data 7.0213 g Ammonium ferrous sulphate hexahydrate is dissolved in 400 ml double distilled water, 15 ml conc. $H_2SO_4$ was added & volume was made 1 L by double distilled water. From this stock 10 fold diluted solution is prepared. Aliquots of 2, 4, 6, 8 &10 ml were taken by adjusting volume to 100 ml the readings were recorded. The experiment was carried out in triplicate for accuracy of result data.

- **Determination of calcium by flame photometer:**

1 gm powder of Garden cress was digested in $HNO_3$:$HClO_4$ (3:1) for 30 min & volume was made 100 ml. Readings were taken on flame photometer. For standard 2.4973 g calcium carbonate was dissolved 25 ml 1:1 HCl & the volume was made 1 L to get 1000 ppm solution of calcium. From that 100 ppm solution was prepared. By taking 1, 2, 3, 4, 5 ml aliquots volume was made to 100 ml. Readings are noted on flame photometer. The experiment was carried out in triplicate for accuracy of result data.

**C. Free Radical Scavenging Activity:**

The extract is prepared in methanol by using soxhlet apparatus and analysed for its antioxidant properties.

- **Ferric reducing antioxidant assay:**

For the measurement of reductive ability i.e. ferric reducing antioxidant power we investigated $Fe^{3+}$-$Fe^{2+}$ transformation in the presence of the extract using the method described by Srinivas Leela *et al.* with some modifications[8]. Various concentrations of extracts (10 µg, 50 µg &100 µg) were mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) & 2.5 ml of 1 % potassium ferricyanide. The mixture was incubated at $50^0$C for 20 min, 2.5 ml of 10 % trichloroacetic acid (w/v) was added. 5 ml of above solution was mixed with 5 ml of distilled water & 1 ml of 0.1 % of ferric chloride. The absorbance was measure spectrophotometrically at 700 nm. Butylated Hydroxy Anisole (BHA) was used as standard antioxidant.

- **Free radical scavenging activity by DPPH Method :**

1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay is carried out with some modifications[9]. Different concentration of methanolic extracts & BHA were taken in different test tubes. The volume was adjusted to 100 µl, by adding methanol, 3 ml of a 0.1 mM methanolic solution of a DPPH was added to these tubes & shaken vigorously. The tubes were allowed to stand in dark at room temperature for 30 min. The control was reported as above without any extract. DPPH radical scavenging activity is measured by reduction in intensity of purple colour and quantified by decrease in absorbance at wavelength 517 nm. Radical scavenging activity was calculated using the following formula:

$$\%\,\text{radical scavenging activity} = \frac{\text{Control O.D.} - \text{sample O.D.}}{\text{Control O.D.}} \times 100$$

- **Determination of total phenolic compounds**

Total phenolic content was estimated by using Folin-Ciocalteau method with some modifications. 125 µl methanolic extract of seeds of plant were mixed separately with 0.5 ml distilled water followed by 125 µl Folin-Ciocalteau reagent. After 5 min, 1.25 ml of a 7% aqueous sodium carbonate solution was added. Total volume is adjusted to 3 ml by distilled water and samples were allowed to stand for 90 min. Absorbance was recorded at 760 nm and concentrations of total phenolic compounds in the samples were determined as microgram of Gallic acid equivalent[10].

**RESULTS AND DISCUSSIONS**

**A. Spectrophotometric Nutritional Analysis**

- **Determination of total carbohydrates**

Garden cress is the rich source of carbohydrate. Three samples were prepared and estimation is carried out in triplicate. Results are analysed by using Microsoft Office Excel and expressed in mg/g as Mean ± S.D. form in Table 1.

- **Estimation of Protein**

Protein estimation is carried out by using Lowry's Method. It is carried out by using dry and soaked seed samples. The experiment is carried out in triplicate manner and results are expressed in Mean ± S.D. form in Table 1.

- **Estimation of Iron**

Here iron estimation is carried out by using spectrophotometer. It is carried out by using dry and soaked seed samples. The experiment is carried out in triplicate manner and results are expressed in Mean ± S.D. form in Table 1. The dry samples show higher concentration of iron as compared to soaked one.

**B. Elemental Analysis**

- **Determination of iron**

Determination of iron is also done by using atomic absorption spectroscope. The experiment is carried out by using dry and soaked seed samples for various time periods. The experiments are done in triplicate manner and results are expressed as Mean ± S.D. form in Table 2.

- **Determination of calcium**

$Ca^{2+}$ was determined by the flame photometry. The experiment is carried out by using dry and soaked seed samples. Soaking is done for various time periods. The experiments were carried out in triplicate manner and results are expressed as Mean ± S.D. form in Table 3. The result data shows prolonged soaking decreases the $Ca^{2+}$ concentration in the samples.

**C. Free Radical Scavenging Activity**

- **Ferric reducing antioxidant assay**

The results are shown in the Fig.1. The reducing power increased as the extract concentration increased from 10 µg to 100 µg, indicating some compounds in Garden cress seeds could react with free radicals to convert them into more stable products and to terminate radical chain reaction.

- **Free radical scavenging activity by DPPH Method**

In the DPPH radical scavenging assay, the Garden cress seeds show better inhibition of DPPH at concentrations 100, 150 and 200 µg of methanolic extracts. Results are compared with standard free radical scavenger BHA at concentration 10, 50 and 100 µg and shown in the Figure 2. The estimated $IC_{50}$ concentration is shown in Table 4.

- **Determination of total phenolic compounds**

In the methanolic extract of Garden cress seeds, the total phenolic compounds were determined and expressed as Gallic acid equivalents Garden cress extract. The Garden cress seeds are good source for phenolic compounds. Results are expressed in Table 5.

**Table 1: Spectrophotometric analysis of nutritional components in Garden cress seed**

| Nutritional compounds | | Concentration (mg/g) |
|---|---|---|
| Carbohydrates | | 60 ± 2 |
| Proteins | Dry seed | 1.64±0.050 |
| | 24 hours soaked seed | 1.92±0.056 |
| Iron | Dry seed | 1.05 ± 0.02 |
| | 24 hours soaked seed | 0.275 ± 0.02 |

**Table 2: Concentration of iron checked by atomic absorption spectroscope**

| Micronutrients (Iron) | Garden cress seed ppm |
|---|---|
| Dry powder | 279.68 ± 0.02 |
| Sample 1 (8 hr Soaked) | 374.04 ± 0.02 |
| Sample 2 (16hr Soaked0 | 365.04 ± 0.02 |
| Sample 3 (24 hr Soaked) | 210.35 ± 0.02 |

**Table 3: Concentration of calcium checked by flame photometer**

| Nutrients (calcium) | Garden cress seeds (%) |
|---|---|
| Dry powder | 0.102 ± 0.002 |
| Sample 1 (8 hr Soaked) | 0.096 ± 0.002 |
| Sample 2 (16hr Soaked0 | 0.091 ± 0.002 |
| Sample 3 (24 hr Soaked) | 0.080 ± 0.002 |

**Table 4: DPPH radical scavenging activity of methanolic extract**

| Source | $IC_{50}$ (µg/ml) |
|---|---|
| Garden cress seeds | 176.18 |

**Table 5: Concentration of total phenolic compounds**

| Source | Total Phenolic Compounds (mg/mg GAE) |
|---|---|
| Garden cress seeds | 0.021± 0.002 |

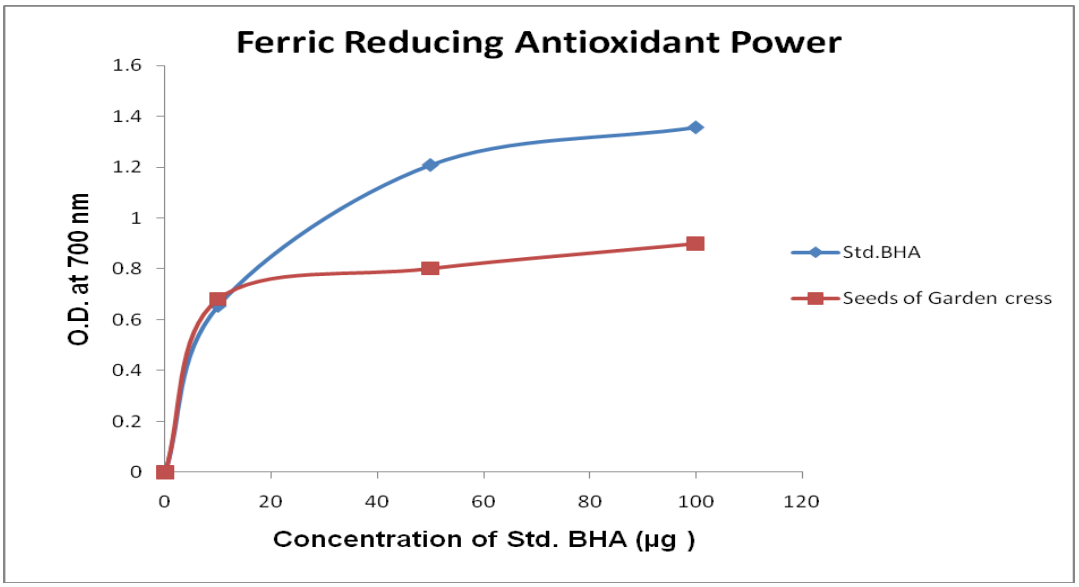


**Fig. 1: Ferric reducing antioxidant assay**

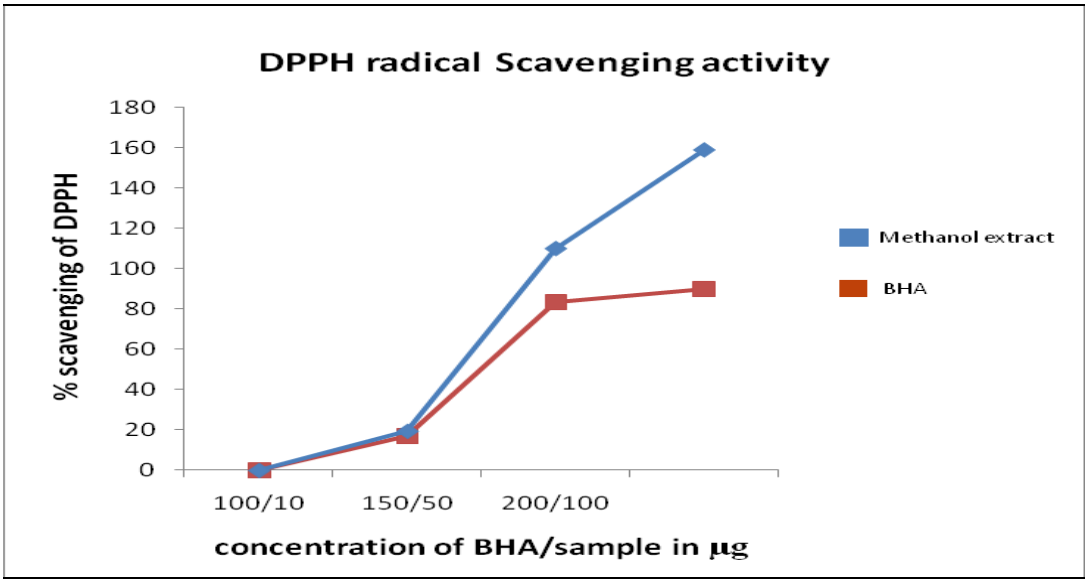


**Fig. 2: DPPH Radical scavenging Activity**

## CONCLUSION

The present study shows that the seeds of Garden cress could be used as food supplement in human diet as it contains considerable amount of iron and calcium. Presence of high carbohydrates, macro and micro elements and antioxidant properties would increase its utilization. The very low anti nutritional factors in the tubers may not hamper its nutritional value.

Garden cress seeds evaluated in present study may be use to help the human body to reduce oxidative damage when the natural mechanism of antioxidant protection becomes unbalanced by factors such as ageing, deterioration of physiological functions may occur resulting in diseases like cancer, cirrhosis, various inflammatory diseases etc. and accelerating ageing.

Due to high free radical scavenging potential leads to consumption of mixed or balanced diet may show rich nutritional as well as medicinal value of the Garden cress.

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