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Evaluation of in Vitro Antioxidant & Antidiabetic Effects of Garden Cress Seed (Lepidium Sativum)

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Abstract

Foods rich in antioxidants have gained much attention due to their health promoting activities. These foods are also referred as a neutraceuticals as they prevent many of degenerative diseases like diabetes mellitus, cancer, blood pressure and heart diseases. Garden cress seed have been used in India since ancient time. It possesses numerous health benefits such as antianaemic effect, Antimicrobial Activity, Antidiabetic and Anti-oxidant effect. In the present study, Garden cress seed was evaluated for its in vitro antioxidant and antidiabetic activities. Antioxidants activities of Garden cress seed was studies by DPPH and ABTS method. Whole seed showed 22.63% DPPH radical inhibition and 13.78% ABTS radical inhibition. Antidiabetic activity was studies by using Non enzymatic Glycosylation of hemoglobin (NEGH) and α amylase inhibition activity. Non enzymatic Glycosylation of hemoglobin inhibition was 71.42% and α amylase inhibition was 66.53%. Results suggest potential health benefiting activities of Garden cress seed. These benefits must be utilized by incorporating seed in daily diet.

Keywords: Antioxidants; Antidiabetic; Degenerative diseases; Inhibition.

INTRODUCTION

There is growing scientific evidence associating diets rich in antioxidant compounds which occur particularly in plant foods with a lower risk of developing cardiovascular disease, certain kinds of cancer and age related degenerative processes.[8] Particular attention has been paid to their role as "free radical scavengers" and has provoked numerous studies into phenolic compounds in many plants such as fruits, vegetables, nuts and plant seed. Fruits, vegetables and nuts are popular for their health beneficial effects. Among the lists of functional foods which possess various neutraceuticals properties, Garden cress seed is one of the plant seed with high nutritional value and functional properties.[13]

Garden cress seed belongs to Brassicaceae family and its scientific name is Lepidium sativum. Common names of Garden cress seed includes Common Cress (English), Halim (Bengali), Aseliyo (Gujrati), Chansur (Hindi), Allibija, Kapila (Kannada), Alian (Kashmiri) Asali (Malayalam), Ahaliva, Haliv (Marathi), Allivirai (Tamil) and Adityalu, Aadalu (Telugu).[3] In spite of great medicinal value, seed has not received much attention. Only few studies are available which describe chemical composition of seed.[13,4] In traditional medicinal system, Garden cress seed have been widely used in treating various health issues such as hypertension, diabetes and kidney diseases and in prevention of cancer, cardiovascular

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diseases and mild glycemia in diabetic patients.[7]

Research revealed that Garden cress seed possesses strong anticancer effect on breast cancer cell.[11] Traditionally Garden cress seed are used in fracture healing long back. A study conducted on 6 adult rabbits reveled that compared to control group there was significant increase in fracture healing among experiment group who were fed Garden cress seed mixed food.[1] An animal study conducted on rats proven its effect on reducing blood cholesterol.[2] The results of this study suggest that Garden cress seed can also be used in treating hypercholesterolemia. Garden cress seed have positive effect on bronchial asthma also. A study conducted on 30 males and female reveled that feeding of one gram of ground seed powder for 4 weeks significantly improve different pulmonary functions without causing any adverse effect.[9] As Garden cress seed possess numerous health promoting compounds, it must be further studied and can be added in various food products.

Objectives

- To investigate the in vitro Antioxidant activity of Garden cress seed (Lepidium sativum).
- To investigate the in vitro Antidiabetic activity of Garden cress seed (Lepidium sativum).

METHODOLOGY

For the study Garden cress seed procured from local market and cleaned manually. Cleaned seed than ground in to fine powder with mortal pastel. Powdered sample stored in refrigerator till further analysis. Seed was cleaned manually, finely grind with mortal pastel and stored in refrigerator till further analysis.

Sample extraction for analyzing antioxidant activity: Methanol: Distilled water (80:20) was used as a solvent for the extraction of Garden cress seed. 300mg of ground powder was taken in 50ml conical flask. Then 5ml of solvent was added. The mixture then was shaken for 30 minutes using a mechanical shaker (NOVA) at 30rpm. After shaking, the content of flask was centrifuged (REMI) at 3000 rpm for 10 minutes and supernants were collected in sugar tubes. Again 5 ml of the same solvent was added to flask and process was repeated. Both supernants were combined; filtered and volume was adjust to 16.3 ml. the obtained extract was stored at –20°C and used to analyzed for their total antioxidant capacity two methods i.e. ABTS and DPPH.

Sample extraction for analyzing antidiabetic activity:

One gram of seed powder soaked in 10ml of methanol for 48 hours at 37°C with occasional shaking. The extract was filtered through a cotton plug followed by Whatman No. 1 filter paper. The filtrate was then evaporated up to 5 ml and stored at 4°C until use.

Evaluation of antioxidant and antidiabetic effect

DPPH Radical Scavenging Activity

For analyzing DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging capacity of Garden cress seed extract 0.2 ml of cereal extract was taken and volume was made up to 1 ml with methanol. Then 3 ml of DPPH reagent (1 mM in methanol) were added. The content was mixed properly. It was incubated at 37°C for 20 minutes. After incubation the absorbance was measured 517 nm in a UV visible double beam spectrophotometer (Hitachi 22OS, Japan). For control, 3 ml of DPPH was added to 1.0 ml of methanol. For standard, known concentration of trolox (10-40 µg) was taken and volume was made up to 1 ml with methanol. Pure methanol was used as a blank and percent inhibition was calculated using the following formula: % inhibition = (Abs of control – Abs of sample)/Abs of control x 100.[5]

Total Phenolic Compounds Estimation

For estimating total phenolic compounds, 0.05 ml aliquote from each extraction was taken in a test tube and volume was made up to 1 ml with distilled water. To this, 1 ml each of folin ciocalteau reagent diluted with water (1:2) and 35 % Na_2CO_3 were added. The contents were incubated for 30 min at room temperature. 2 ml of distilled water was added and intensity of blue colour was recorded at 620 nm in UV visible double beam spectrophotometer (Hitachi 220S, Japan). Gallic acid of known concentration (5-20 mg) was used as standard.

TEAC Measurement by ABTS Method

The TEAC (Trolox equivalent antioxidant capacity) of cereal extract was measured using the modified 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid diammonium salt (ABTS) radical decolonization assay. This method was given by *Re et al (1999)*. 7mmol/L ABTS stock solution was reacted with 2.45mmol/L potassium persulphate

to prepare ABTS radical cation (ABTS+) and incubated in the dark at room temperature for 12–16 h. The ABTS+ solution was diluted with 5mM PBS (Potassium phosphate buffered saline, pH 7.4) to an absorbance of 0.7 at 734 nm before use. For assay, 20 µl of cereal extract was taken, volume was made up to 1 ml with ethanol and 3 ml of ABTS was added to it. The contents were vortexed for 10 seconds. The discoloration caused by reduction of the cation by antioxidant from the sample measured at 734 nm in a UV visible spectrophotometer (Hitachi 200S, Japan). 1.0 ml of ethanol was added to 3 ml of ABTS and used as a control. For Standard, known concentration of trolox (5-20 µg) was taken and the volume was made up to 3 ml with ethanol and thereafter all test tubes were treated in the same way as sample. Percent inhibition was calculated using the following formula: % inhibition = (Abs of control – Abs of sample)/Abs of control x 100[10]

In-Vitro antidiabetic activity

Non–enzymatic glycosylation of hemoglobin assay

Antidiabetic activity of Garden cress seed were investigated by estimating degree of Non–enzymatic glycosylation of hemoglobin and was measured in colorimetrically at 520nm. For the assay, 2% glucose, 0.06% hemoglobin and 0.02% sodium azid solutions were prepared in 0.01 M phosphate buffer (pH 7.4). 1ml of each of the above mentioned solution was mixed and in that 1ml of extracted sample was added to above mixture. This mixture was incubated in dark at room temperature for 72 hours. The degree of glycosylation of hemoglobin was measured colorimetrically at 520nm. Trolox was used as a standard. All the tests were performed in triplicate.[12]

Inhibition of salivary amylase enzyme

For this assay, 1ml of extract or standard of different concentration (2,1, 0.5 mg/mL) was taken in test tubes. In each test tube 20μL of α-amylase was added and incubated for 10 minutes at 37°C. After the incubation, 200 μL of starch solution (1%) was added in each test tube. Again this mixture was incubated for 1 hour at 37°C. After re-incubation, 200μL of iodine solution and 10mL of distilled water was added. Finally, absorbance of the mixture was read at 565nm. Sample, substrate and α-amylase blank were prepared by following above method.[6]

RESULTS

Looking to the analytical procedures, entire study can be divided into two parts viz. analysis of antioxidants and analysis of antidiabetic activities.

Antioxidant and Antidiabetic Activity of Garden cress seed

S. no.	Antioxidant Activity				Antidiabetic Activity			
	DPPH (% inh)	ABTS (% inh)	TPC (mg%)	FRAP (RP%)	HbA_1 (% inh)		α-amylase (% inh)	
					Std*1	GCS	Std*2	GCS
1	22.63	11.23	780.55	46.45	72.83	71.42	71.23	66.66
2	26.12	14.33	801.89	48.81	76.34	65.38	70.00	67.69
3	25.32	12.42	791.31	42.51	77.55	75.67	69.11	68.65
4	19.22	17.22	776.54	51.96	78.84	67.85	72.00	65.57
5	22.29	13.33	798.21	50.43	77.31	69.49	67.69	64.40
6	20.21	14.12	782.23	48.23	75.63	71.38	71.43	66.21
Mean	22.63	13.78	788.46	48.07	76.42	70.20	70.24	66.53
SEM	1.11	0.83	4.19	1.35	0.85	1.44	0.67	0.62
CV	12.02	14.81	1.30	6.89	2.71	5.02	2.32	2.27

% inh = % inhibition

RP% = Reduction Power

HbA_1 = Glycosylated by Non Enzymatic Glycosylation of Hemoglobin

Std*1 = Standard 1 i.e. Trolox

Std*2 = Standard 2 i.e. Metformin

In vitro Antioxidant activity

ABTS & DPPH radical scavenging activity

Although the DPPH• free radical is ubiquitously used to estimate the potential free radical-scavenging activity of natural products, the ABTS•+ free radical is commonly used when issues of solubility or interference arise and the use of DPPH• based assays becomes inappropriate. The percent of inhibition of GC extracts was 13.78±0.83 and 22.63±1.11 for ABTS and DPPH radical respectively.

Total Phenolic Compounds

The role of phenolics as natural antioxidants has attracted considerable interest due to their pharmacological functions. The mean value of total phenolic content of seed extract was 788.46±4.19 mg%.

In vitro Antidiabetic activity

To study Antidiabetic effect of Garden cress seed % inhibition of Non Enzymatic Glycosylation of Hemoglobin and α-amylase these two tests were performed. Mean value of Non Enzymatic Glycosylation of Hemoglobin is 70.20 ± 1.44 (% inhibition) where as standard shown 76.42±0.85 (% inhibition). In case of % inhibition of α-amylase Garden cress seed shown 66.53± 0.62 and standard shown 70.24± 0.67.

All experiments were carried out in triplicate (n = 3) for each analysis and their means ± Standard deviation were reported. Differences between variable were tested for significance by using a one way analysis of variance procedure, Ducan, using level of significance $P \leq 0.05$ using SPSS.

DISCUSSION

Garden cress seed possess many neutraceutical properties and to prove it few researches have been carried out in vitro as well as in vivo. Based on present study also it can be concluded that garden cress seed possess antidiabetic and antioxidant activities. Most of the previous studies including this, have proven that garden cress seed can be utilized in treating diabetitic, hyperglycemia, fracture healing and to get various phytochemicals. Though only few in vivo studies have been carried out, looking to the health benefits further researches should be carried out to prove its positive effect on human.

CONCLUSION

The results of present study concluded that Garden cress seed possess significant antioxidant activity. The potential pharmacological activity of seed might be due to the presence of phytochemicals and soluble fiber.

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