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Research Article

EFFECT OF SPROUTING ON *IN VITRO* ANTIOXIDANT POTENTIAL OF SOME VARIETIES OF CHICKPEA SEEDS (*CICER ARIETINUM* LINN.)

JASNEET KAUR, SATNAM SINGH*

Department of Pharmacognosy, Amar Shaheed Baba Ajit Singh Jujhar Singh Memorial College of Pharmacy, Bela - 140 111, Punjab, India. Email: drssb13pu@gmail.com

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ABSTRACT

Objective: The aim of the present study is to access the effect of sprouting on *in vitro* antioxidant potential of some varieties of *Cicer arietinum* Linn. (Chickpea seeds).

Methods: The unsprouted and sprouted seeds of its newly developed varieties *viz*. PBG-1, GPF-2, PBG-5 were powdered and then was extracted with methanol by direct maceration method and their antioxidant activity was evaluated using four different methods (reducing power, free radical scavenging activity (2,2-diphenyl-1-picrylhydrazyl), total antioxidant activity, and ferric reducing antioxidant power [FRAP] activity).

Results: Sprouting clearly increased the flavonoid content of seeds to 104%, 117%, 341%. There was visible increase in total antioxidant activity of methanol extracts of sprouted seeds which were evident by increased activity from 0.298 to 0.397 at concentration 60 µg/ml in var. PBG-5 and increased FRAP from 0.332 to 0.387 at concentration 40 µg/ml in var. PBG-1.

Conclusion: Thus, it may be concluded from the study that sprouting visibly increased the *in vitro* antioxidant activity of selected chickpea seeds varieties.

Keywords: *Cicer arietinum* Linn., *In vitro* antioxidant activity, Sprouting, Total flavonoid content, Total antioxidant activity, Ferric reducing antioxidant potential assay.

INTRODUCTION

Pulses belong to the family of leguminous plant are an important source of macronutrients (such as proteins, carbohydrates, and dietary fiber), micronutrients, vitamins, carotenoids, and phenolic compounds. Pulses are carriers of phenolic compounds and have increased antioxidant potential. Cereal grains are a good source of antioxidants. Most of the antioxidants in cereal grains are contained in bran and germ which can be used as free radical scavengers. The antioxidant potential is attributed to polyphenolic compounds and bioflavonoids, ascorbic acid, tocopherols and carotenoids. It is widely recognized that free radical-induced oxidative stress is the main cause for many human diseases and cereal bran can reduce this oxidative stress due to their polyphenolic content. Naturally occurring antioxidant supplements from plants are vital to counter the oxidative damage in cells. These provide a very good therapeutic potential in the prevention of cancer, cardiovascular diseases, and complications of diabetes due to their antioxidative effect. The main advantage of using cereal bran as antioxidants is that it can be taken easily in our daily food requirements and protects our body from various diseases induced by oxidative stress. Antioxidants act as protective factors against oxidative damage in the human body and prevent the development of chronic diseases such as cancer, heart disease, stroke, and cataracts [1].

Chickpea (*Cicer arietinum* Linn.) is one of the oldest and most widely consumed legumes in the world. It is a staple food crop in some tropical and subtropical countries. Chickpea seeds are a cheap source of high quality protein in the diet of millions in the developing countries.

Traditionally it is used as food item but in addition, they are also a good source of carbohydrates, minerals and trace elements and also used as antifungal, antibacterial, antipyretic, antidiarrheal, inflammation, cold coughs, etc. [2].

Isoflavones are the main bioactive components of sprouted chickpea seeds with biological activities, including antioxidative, estrogenic, insecticidal, antifungal, and antimicrobial properties. Polyphenols are the major plant compounds with antioxidant activity having biological properties such as anticarcinogenicity, antimutagenicity, antiallergenicity, and antiaging activity have been reported for natural antioxidants [3,4].

Recent studies have shown that chickpea seeds have antioxidant, antidiarrheal, and antipyretic activities of the hydroalcoholic extract [5], anti-inflammatory activity of methanolic and ethanolic extracts [6], antibacterial activity, antihyperglycemic activity, antidiabetic plant [7].

The aim of the present investigation is to study the effect of sprouting on antioxidant activity on the chickpea varieties procured from Punjab Agricultural University, Ludhiana, Punjab, India.

METHODS

Plant material

The plant consists of dried seeds of *C. arietinum* Linn. varieties (PBG-1, GPF-2, PBG-5) belonging to family Fabaceae.

Collection and authentication

The seed part of the plant *C. arietinum* Linn. varieties (PBG-1, GPF-2, and PBG-5) and family Fabaceae was collected from "Punjab Agricultural University, Ludhiana, Punjab, India" in the month of October and seeds were stored in air tight container. It was authenticated and identified as *C. arietinum* Linn. varieties (PBG-1, GPF-2, and PBG-5) by Dr. M.S. Gill., Head of Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India. The voucher of a specimen of the sample was deposited in the herbarium of Pharmacognosy Department, Amar Shaheed Baba Ajit Singh Jujhar Singh Memorial College of Pharmacy, Bela, Ropar, Punjab, India.

Reagents

Folin–Ciocalteu reagent, sodium carbonate, aluminum chloride, sodium acetate, quercetin, sulfuric acid, sodium phosphate,

ammonium molybdate, ascorbic acid, ferrous chloride, ammonium thiocyanate, potassium ferricyanide, trichloroacetic acid, ferric chloride and methanol, gallic acid, 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ). All chemicals of analytical grade were purchased from companies like Loba Chemie (Mumbai, Maharashtra, India), Merck Specialities Private Limited and Himedia Laboratories Private Limited.

Preparation of extracts

C. arietinum Linn. seed extract was prepared according to a modified method. Seed powder (8 g) of each was extracted trice with 48 ml of methanol for 3 hrs in an electrical shaker or in sonicator at 40°C. The extracts were filtered through Whatman No. 2 filter paper and evaporated under vaccum using rotary evaporated. The residual crude methanolic powdered extract was weighed [8].

In the case of sprouting, seeds of the three varieties (PBG-1, GPF-2, PBG-5) were taken and washed with clean water. A cotton bed was prepared to spread and germinate seeds. 500 g of seeds were spread on wet cotton bed at room temperature with ample light and air. Seeds were covered with a similar type of cotton covering. Water was sprinkled as and when required to keep bedding wet. Sprouts with 1.5-3.0 cm germinate length were picked up for analysis. It took about 72 hrs to germinate up to this length. Fine powder of raw sprouts was prepared for extraction. Powders were soaked in 85% methanol for 3 days at room temperature. Supernatant was vacuum filtered, concentrated to 1/3 volume under reduced pressure in a rotary evaporator (50±1°C) and lyophilized to dry. Extracts were refrigerated until analysis [9].

Total phenolic content

The total phenolic content of seed extracts was determined, using the Folin–Ciocalteu reagent. The reaction mixture contained 20 µl of seed extracts was mixed with 100 µl of the freshly prepared Folin–Ciocalteu reagent and a further 1.58 ml of distilled water. The mixture was shaken vigorously, and 300 µl of sodium carbonate (20% w/v) were added and the mixture was again shaken for 2 minutes. After the mixture was left to stand for 2 hrs at room temperature, the absorbance at 765 nm was measured by using a spectrophotometer. Gallic acid was used as a standard, and results were calculated as gallic acid equivalents (mg/g of bran) [10].

Total flavonoid content

Reagents like $AlCl_3$ (0.1 g/ml) and CH_3COONa (1 M) were prepared, then prepared dilutions for quercetin (standard) of 5, 10, 15, 20, and 25 µg/ml. For sample preparation, 1 mg/ml solution of extract was prepared in methanol. 0.5 ml of solution was taken and added 1.5 ml ethanol. To this added 0.1 ml of $AlCl_3$ and 0.1 ml of CH_3COONa reagent and added 2.8 ml distilled water and kept for 30 minutes. Absorbance was taken at 415 nm. Then, 0.5 ml of standard dilutions of quercetin was taken and added 1.5 ml ethanol. To this added 0.1 ml of $AlCl_3$ and 0.1 ml of CH_3COONa reagents followed by 2.8 ml distilled water and kept for 30 minutes after that absorbance was taken at 415 nm. For the preparation of the blank solution, 2 ml of ethanol + 0.1 ml of $AlCl_3$ + 0.1 ml of CH_3COONa reagents and then added 2.8 ml distilled water. After taking the absorbance of standard dilutions, a calibration curve was plotted. Flavonoid content in the drug was calculated by using standard calibration curve [11].

Total antioxidant activity by ammonium molybdate reduction method

Total antioxidant activity was measured in different concentrations. Extracts were mixed with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate), after 90 minutes incubation at 95°C, sample were cooled to room temperature. Then, the absorbance of the solution was measured at 695 nm using spectrophotometer. Methanol (0.3 ml) was used as blank. The antioxidant activity was expressed as the number of equivalents of ascorbic acid [12].

Determination of reducing power

Extracts (10, 25, 50 mg/ml) were mixed with phosphate buffer (2.5 ml, 2.0 M, pH 6.6). The diluted sample was then mixed with 2.5 ml of 1% potassium ferricyanide and incubated at 50°C for 20 minutes. About 2.5 ml of 10% trichloroacetic acid were added, and the mixture was centrifuged for 10 minutes. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and 1% ferric chloride (0.5 ml), and absorbance was measured at 700 nm. The standard curve was prepared using various concentration of ascorbic acid [13].

Ferric reducing antioxidant power (FRAP) assay

FRAP assay is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of TPTZ, forming an intense blue Fe^{2+} TPTZ complex with an absorption maximum at 593 nm. This reaction is pH-dependent (optimum pH 3.6). The absorbance decrease is proportional to the antioxidant content. 0.2 ml of the extract is added to 3.8 ml of FRAP reagent (10 parts of 300 mM sodium acetate at pH 3.6, 1 part of 10.0 mM TPTZ solution and 1 part of 20.0 mM $FeCl_3$. $6H_2O$ solution), and the reaction mixture is incubated at 37°C for 30 minutes and the increase in absorbance at 593 nm is measured. $FeSO_4$ is used for calibration. The antioxidant capacity based on the ability to reduce ferric ions of the sample is calculated from the linear calibration curve and expressed as Mmol $FeSO_4$ equivalents per gram of sample. Butyl hydroxytoluene, butylated hydroxyanisole, ascorbic acid, quercetin, catechin, or trolox can be used as a positive control [14].

RESULT AND DISCUSSION

Total phenolic content

The total phenolic content of the methanol extract of three varieties of *C. arietinum* L. seed (PBG-1, GPF-2, PBG-5) was estimated using Folin–Ciocalteu method. The greater amount signifies the presence of different constituents having a phenolic moiety in their structures. The phenolic content for non-sprouted seed varieties was found to be 118.5, 118, and 110 (mg gallic acid equivalents/g of extract) for methanolic extracts of the chickpea seeds in varieties PBG-1, GPF-2, and PBG-5, respectively. The phenolic contents were found to be maximum in variety PBG-1. The standard plot was shown in Fig. 1.

The phenolic content for sprouted seed varieties was found to be 119, 123, and 111 (mg gallic acid equivalents/g of extract) for methanolic extracts of the chickpea seeds in varieties PBG-1, GPF-2, and PBG-5, respectively.

Total flavonoids content

The total flavonoids content of the methanol extract of three varieties of *C. arietinum* L. seed (PBG-1, GPF-2, PBG-5) was estimated taking quercetin as standard. The standard curve of quercetin was plotted, and all the findings were made in comparison to quercetin. The greater amount signifies the presence of more flavonoids moieties in the constituents. The flavonoid content for non-sprouted seed varieties was found to be 22.1, 12.9, and 6.84 (mg quercetin equivalent/g of extract) for methanolic extracts of the chickpea seeds of varieties PBG-1, GPF-2, and PBG-5, respectively. The flavonoid content was found to be

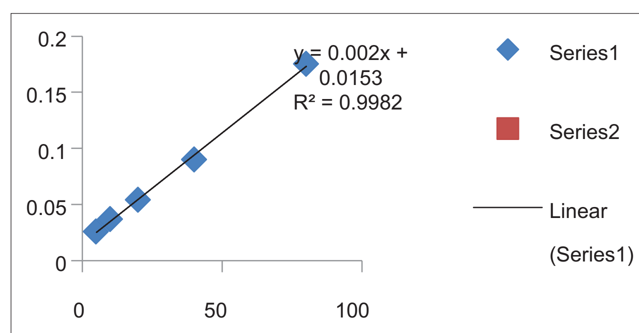

Fig. 1: Standard plot for gallic acid

the highest in variety PBG-1 among all the varieties. The standard plot for quercetin is shown in Fig. 2.

The flavonoid content for sprouted seed varieties was found to be 45.6, 28, and 30 (mg quercetin equivalent/g of extract) for methanolic extracts of the chickpea seeds of varieties PBG-1, GPF-2, and PBG-5, respectively. Sprouting clearly increased the flavonoid content of seeds to 104%, 117%, 341%.

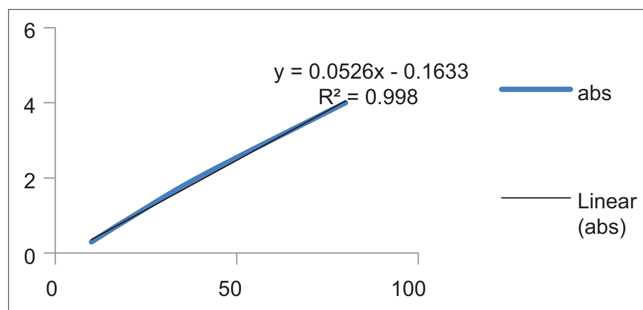

Fig. 2: Standard plot for quercetin

Reducing power of various extract of C. arietinum Linn.

In an overall reducing power analysis the test can be arranged as: BHT> CAME (S2)> CAME (S1)> CAME (S3)

There was concentration dependent increase of reducing power in both cases, i.e., non-sprouted and sprouted seeds. There was increase in reducing power of methanol extracts of sprouted seeds which were evident by increased activity from 0.384 to 0.398 at concentration 100 µg/ml in var. GPF-2 as shown in Fig. 3.

Total antioxidant activity of various extracts of C. arietinum Linn.

As in earlier study, there was concentration dependent increase of total antioxidant activity of methanol extracts in both cases, i.e., non-sprouted and sprouted seeds. There was a visible increase in total antioxidant activity of methanol extracts of sprouted seeds which were evident by increased antioxidant activity from 0.298 to 0.397 at concentration 60 µg/ml in var. PBG-5 as shown in Fig. 4.

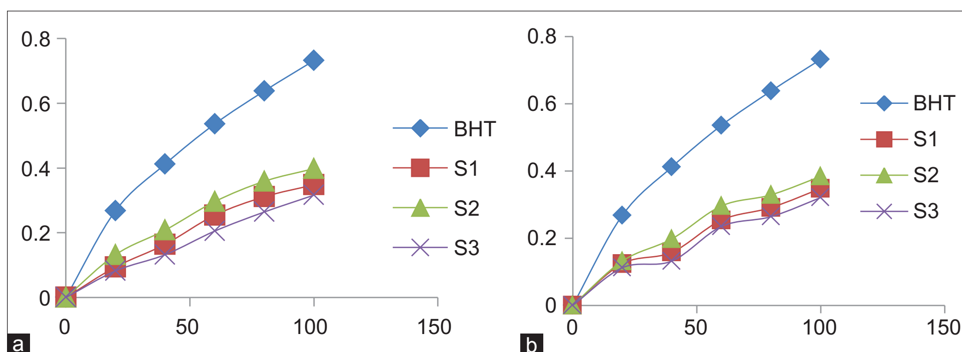

Fig. 3: Reducing power of methanol extracts of non-sprouted and sprouted seed varieties CAME (S1=PBG-1), CAME (S2=GPF-2), and CAME (S3=PBG-5) (where CAME: *Cicer arietinum* methanol extract) (a) Non-sprouted seed varieties. (b) Sprouted seed varieties

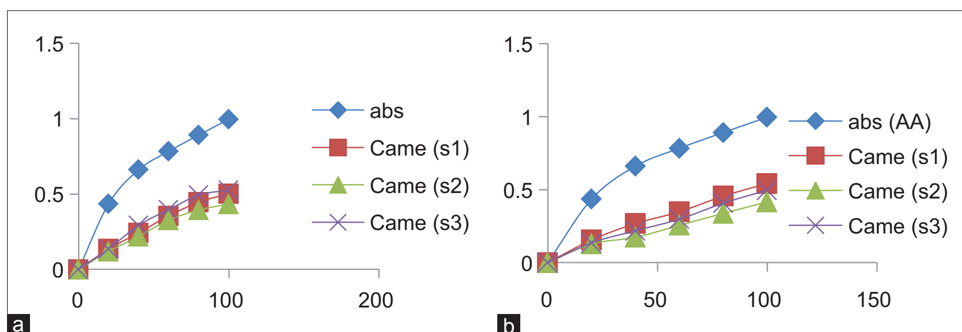

Fig. 4: Total antioxidant activity of methanol extracts of non-sprouted and sprouted seed varieties *Cicer arietinum* methanol extract (CAME) (S1=PBG-1), CAME (S2=GPF-2), and CAME (S3=PBG-5). (a) Sprouted seed varieties. (b) Non-sprouted seed varieties

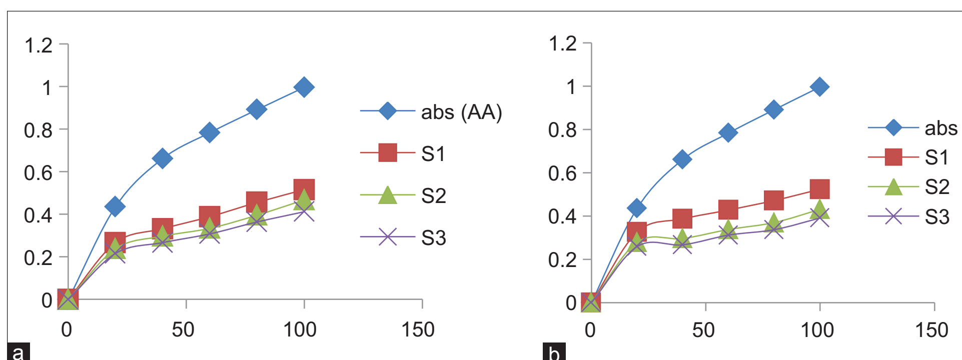

Fig. 5: Ferric reducing antioxidant power of methanol extracts of non-sprouted and sprouted seed varieties *Cicer arietinum* methanol extract (CAME) (S1=PBG-1), CAME (S2=GPF-2), and (PBG-5). (a) Non-sprouted seed varieties. (b) Sprouted seed varieties

Ferric reducing antioxidant power of various extracts of C. arietinum Linn.

In an overall FRAP analysis the test can be arranged as:
Ascorbic acid> CAME (S1)> CAME (S2)> CAME (S3)

In this study, again there was concentration dependent increase of FRAP in both cases, i.e., non-sprouted and sprouted seeds. However, there was a visible increase in FRAP of methanol extracts of sprouted seeds which was evident by increased activity from 0.332 to 0.387 at concentration 40 µg/ml in var. PBG-1 as shown in Fig. 5.

CONCLUSIONS

In conclusion, the results validate that seed extract has antioxidant activity. This is the first study which investigates the antioxidant activity of methanol extracts of *C. arietinum* L. seed varieties in comparison to its sprouted seeds. The results revealed the potentiation of total flavonoid content and antioxidant potential of varieties PBG-1, GPF-2, and PBG-5. The results were found to be encouraging for further assessment and to elucidate both mechanisms and to identify the bioactive compounds.

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