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PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT POTENTIAL OF GARLIC (ALLIUM SATIVUM L.) EXTRACTS IN DIFFERENT SOLVENTS

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ABSTRACT The selection of appropriate solvent for extraction of phytoconstituents is an important aspect to achieve the maximum concentration of desired phytoconstituents in plant extracts. Hence, the present study was undertaken to study the effect of different solvents (acetone, ethanol and distilled water) on extraction of total phenols, flavonoids, ascorbic acid and sugars from garlic (*Allium sativum* L.). The antioxidant activity of different extracts of garlic was evaluated by two different methods *viz.* 2,2′-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity and β-carotene bleaching method. The results revealed that water extract contained the highest total phenols (2.33 mg GAE/g fwb), flavonoids (33.22 mg CE/100g fwb), ascorbic acid (34.49 mg/100g fwb), total sugars (1.93 g/100g fwb), reducing sugars (0.63 g/100g fwb) and non-reducing sugars (1.30 g/100g fwb) contents as well as exhibited the highest DPPH free radical scavenging activity (47.79%) and antioxidant activity (29.33%) by β-carotene bleaching method.

Keywords: *Allium sativum*, solvents, total phenols, flavonoids, ascorbic acid, sugars, antioxidant activity

INTRODUCTION

Spices and herbs have been effectively used in the indigenous systems of medicine and food in India. They contain bioactive compounds imparting antioxidant, preservative and antimicrobial properties to the food. Human body is constantly exposed to a variety of oxidizing agents and the body is equally inbuilt with antioxidants. Antioxidants are often reducing agents such as ascorbic acid and polyphenols which terminate the chain reactions by removing free radical intermediates. Extraction of antioxidants from a plant material can be carried out using different techniques and solvents because of diversity of chemical nature of these compounds and often unique distribution of these compounds in the plant matrix[1-2]. Various solvents are being frequently used for extraction of plant antioxidant compounds. However, the extract yields and antioxidant efficacy of the resulting extracts is strongly affected by polarity of the solvent as well as the chemical nature of the extracted compounds.

Allium sativum, commonly known as garlic, is a species in the onion genus, *allium*. It is a member of Liliaceae family. Garlic is an herb and is best known as a flavouring agent for food. Garlic produces a chemical called allicin which is responsible for its smell. Allicin, diallyl disulphide and diallyl trisulphide appeared to be the main antioxidant compounds in the garlic volatiles[3]. *Allium sativum* oil protects stomach against alcohol damage for its antioxidant qualities. The activity of destroying reactive oxygen species and high phenolic content of *Allium sativum* aquatic extract depends on the presence of allicin. Allin (+ -S- allyl –L-cysteine sulphoxide) is a precursor of allicin and it does not have antioxidant activity in a linoleic acid oxidation system[4]. When garlic cloves are cut, crushed or chopped, the cysteine sulfoxides which are odorless, are very rapidly converted to a new class of compounds "the thiosulfinates" which are responsible for the odor of freshly chopped garlic. Survey of the literature reveals that no systematic work has been done on the phytochemical analysis and antioxidant potential of garlic (*Allium sativum* L.) extracts in different solvents. Thus, the objective of the present study was to evaluate the efficacy of different solvents towards the extraction of total phenols, flavonoids, ascorbic acid and sugars as well as the antioxidant activity of the extracts produced from garlic.

EXPERIMENTAL

Plant material and extraction

Fresh and fully mature garlic (*Allium sativum* L.) were procured from the Research Farm of Department of Vegetable Crops of CCS Haryana Agricultural University, Hisar. One variety of garlic i.e. variety HS-17 was taken for investigation. Garlic buds were peeled off and cut into pieces and homogenized in Waring blender to make pulp. 15 gram of homogenized samples were extracted with 70 mL of solvents (acetone, ethanol and water) in conical flasks by shaking on a mechanical shaker for 1hr 30min. Extracts were filtered and residues were again extracted twice (each shaking time 1 hr) with 50 and 30 mL respective solvents. Filtrates of each solvent from three extraction steps were pooled and their volumes were noted. Extracts were then used for estimation of total phenolics, flavonoids, ascorbic acid and sugars contents and for evaluation of antioxidant activity.

Chemicals

The commercially available chemicals from Sigma-Aldrich, Qualigens, Merck and Hi-Media of highest purity, were used for various experimental procedures.

Estimation of moisture content

Moisture content was estimated by the standard procedure of AOAC[5]. The data of moisture content was used to calculate the amount of various phytochemicals on dry weight basis (dwb).

Estimation of total phenols content

Total phenols content of extracts was determined using Folin-Ciocalteu method[6]. Aliquots of 0.2 ml of extracts were mixed with 1 ml of 1 mol/L Folin-Ciocalteu reagent. After that, 2.0 ml of 20% (w/v) sodium carbonate solution was added. The solutions were mixed and volume was made up to 10.0 ml with distilled water. The absorbance was measured at 730 nm using UV-VIS double beam Spectrophotometer Model 2203 (Systronics Co.). A calibration curve was prepared using gallic acid as standard. Results were expressed as mg GAE/g on fresh weight as well as dry weight basis.

Estimation of flavonoids content

Flavonoids content of extracts was estimated according to the colorimetric assay[7]. In 1.0 ml of extract, 4.0 ml of double distilled water and 0.3 ml of 5% (w/v) $NaNO_2$ were added. After 5 min., 0.3 ml of 10% (w/v) $AlCl_3$ was added. Immediately, 2.0 ml of 1 M NaOH was added and the volume was made up to 10.0 ml with double distilled water. The solution was mixed thoroughly and the absorbance was measured at 510 nm using UV-VIS double beam Spectrophotometer Model 2203 (Systronics Co.). A calibration curve was prepared using catechin as standard. Results were expressed as mg CE/100g on fresh weight as well as dry weight basis.

Estimation of ascorbic acid content

Ascorbic acid was estimated by titrimetric method[8]. 1.0 ml of each extract was taken and 5.0 ml of 2% (w/v) meta-phosphoric acid in glacial acetic acid was added. Total volume of this mixture was made up to 10.0 ml with distilled water. It was titrated against 2,6-dichlorophenol indophenol dye of 2 ppm until a pink end point was obtained. Ascorbic acid content was calculated from the standard curve of ascorbic acid. Results were expressed as mg/ 100g on fresh weight as well as dry weight basis.

Estimation of total, reducing and non-reducing sugars content

Sugars were estimated by the method of Hulme and Narain[9].

Reducing sugars

One ml of extract was diluted to 15 times with distilled water. Five ml of potassium ferricyanide solution was added to five ml of diluted extract in a test tube. The tubes were covered with aluminium foil and kept for 15 minutes in boiling water bath. The tubes were then cooled under tap water and 5 ml of iodine-zinc solution and 3 ml of acetic acid solution were added to it. The liberated iodine was titrated with sodium thiosulphate ($NaHSO_4$) (0.01N) using starch as an indicator. The end point was the disappearance of blue colour and appearance of milky white colour. A blank with 5 ml of distilled water was also run simultaneously. The results were calculated by the following formula and expressed in g of sugar per 100 g.

$$\text{Sugar (\%)} = \frac{[\text{NaHSO}_4 \text{ used in blank - NaHSO}_4 \text{ used in sample}] \times 0.338 \times \text{DF} \times \text{V} \times 100}{\text{volume of aliquot} \times \text{weight of sample} \times 1000}$$

Where, DF = dilution factor

V = volume of stock solution

Total sugars

To 5 ml of sugar extract, 0.8 ml of concentrated hydrochloric acid was added and kept for 15 minutes at 68 °C in boiling water bath. The acidity was neutralized by adding a little anhydrous sodium carbonate till the effervescence stopped. After this, the volume was made to 20 ml. One ml of extract was diluted to 15 times with distilled water. Five ml of this diluted solution was used for estimation of total sugars as described in the above method of reducing sugars.

Non-reducing sugars

Non-reducing sugars = Total sugars – Reducing sugars

Antioxidant Activity

DPPH free radical scavenging activity

The antioxidant activity of the extracts was evaluated by 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method[10]. For this, in 0.2 mL of ethanol extract, 3.0 mL of 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH; 0.1 mM in 100 % ethanol) was added and mixed thoroughly for 5 min. For antioxidant activity in acetone and water extracts, DPPH stock solutions were prepared in acetone and 50%

(v/v) ethanol : water, respectively and remaining procedure was same. A control was also made containing 0.2 mL of each solvent instead of extract. The absorbance of the sample as well as control was measured at 517 nm after 30 min. of incubation in dark at room temperature using the UV-VIS double beam Spectrophotometer Model 2203 (Systronics Co.) against a blank containing respective solvent. For each sample, three replications were carried out. The percentage of DPPH scavenged (% $DPPH^*_{sc}$) was calculated using:

$$\% \text{ DPPH}^*_{sc} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where, $A_{control}$ is the absorbance of control and A_{sample} is the absorbance of the sample.

β-carotene bleaching method

Antioxidant activity was measured by β-carotene bleaching method[11]. 1.0 mg of crystalline β-carotene was dissolved in 5.0 mL of $CHCl_3$ and 0.1mL of linoleic acid and 0.9 mL of tween 20 (200 mg) were added. The solvent was removed at 40ºC using a vacuum evaporator and the mixture was immediately diluted with 250 mL of double distilled water. To 0.2 mL of test sample, 4 mL of this emulsion was added. A control containing 0.2 mL of solvent and 4.0 mL of emulsion was also used. The test tubes were placed in a water bath at 50ºC after covering with aluminium foil. The absorbance at 470 nm was recorded with a UV-VIS double beam Spectrophotometer Model 2203 (Systronics Co.) at intervals of 30 min, until the colour of β-carotene disappeared from the control tubes. The above mixture without β-carotene served as blank. All determinations were carried out in triplicates. The antioxidant activity was calculated using the following equation:-

$$A_A(\%) = \frac{[(A_o)_{control} - (A_t)_{control}] - [(A_o)_{sample} - (A_t)_{sample}]}{[(A_o)_{control} - (A_t)_{control}]} \times 100$$

where, $(A_0)_{control}$ and $(A_0)_{sample}$ are the absorbance values measured at zero time of incubation for the control and sample, respectively and $(A_t)_{control}$ and $(A_t)_{sample}$ are the corresponding values at the end of the reaction time.

RESULTS AND DISCUSSION

The results of estimation of total phenols, flavonoids, ascorbic acid and sugars in different extracts of garlic buds and evaluation of their antioxidant activity by using

two testing methods: 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity and β-carotene bleaching method have been presented under. The moisture content in garlic was found to be 35.23%.

Total phenols content

Total phenols content in various extracts of garlic varied widely. On fresh weight basis, water extract of garlic contained the highest total phenols content i.e. 2.33 mg GAE/g fwb followed by ethanol (1.75 mg GAE/g fwb) and acetone (1.50 mg GAE/g fwb) extracts (Table 1). Similar trend was observed on dry weight basis (Table 2). Our finding is in agreement with previous investigation which reported that higher content of polyphenols was obtained with an increase in the polarity of the solvent used for extraction process and reported that water extracts of *Juniperus phenicea* and *Quercus coccifera* have higher phenolic content as compared to acetone extracts[12]. Water extract of Jackfruit pulp also had highest phenolics content in comparison to methanol and acetone extracts which are of lower polarity[13].

Flavonoids content

Flavonoids content in various extracts of garlic varied widely. On fresh weight basis, water extract of garlic contained the highest flavonoids content i.e. 33.22 mg CE/100g fwb followed by ethanol (30.78 mg CE/100g fwb) and acetone (28.89 mg CE/100g fwb) extracts (Table 1). Similar trend was observed on dry weight basis (Table 2). Other research workers have also reported similar findings. Flavonoids content of *Allium sativum* extracts ranged from 41.6 to 69.9 mg quercetin eq./100g fwb[14]. Water was best solvent for extracting flavonoids compound rather than methanol, ethanol and acetone used for extraction of flavonoids[13]. Hence, the concentration of flavonoids in plant extacts depends on the polarity of solvents used in the extract preparation. In present study, it was found that highest concentration of flavonoids was obtained in water extracts which is highly polar in comparison to ethanol and acetone.

Ascorbic acid content

Ascorbic acid content in various extracts of garlic varied widely. On fresh weight basis, water extract of garlic contained the highest ascorbic acid content i.e. 34.49 mg/100g fwb followed by ethanol (31.43 mg/100g fwb) and acetone (29.04 mg/100g fwb) extracts (Table 1). Similar trend was observed on dry weight basis (Table 2).

Ascorbic acid is a water soluble antioxidant. It is one of rarer compounds containing an acidic hydroxyl group which is completely dissociated at neutral pH responsible for maximum solubility in water[15]. In present study, ascorbic acid content was found to be highest in water extract of garlic in comparison to ethanol and acetone extracts which may be due to high solubility of ascorbic acid (at neutral pH) in water in comparison to ethanol and acetone.

Table 1: Total phenols, flavonoids and ascorbic acid contents in garlic on fresh weight basis (fwb)

Solvent	Total phenols content (mg GAE/g fwb)	Flavonoids content (mg CE/100g fwb)	Ascorbic acid content (mg/100g fwb)
Acetone	1.50 ± 0.03	28.89 ± 0.50	29.04 ± 0.01
Ethanol	1.75 ± 0.03	30.78 ± 0.59	31.43 ± 0.16
Water	2.33 ± 0.03	33.22 ± 0.35	34.49 ± 0.16

Table 2: Total phenols, flavonoids and ascorbic acid contents in garlic on dry weight basis (dwb)

Solvent	Total phenols content (mg GAE/g dwb)	Flavonoids content (mg CE/100g dwb)	Ascorbic acid content (mg/100g dwb)
Acetone	2.31 ± 0.04	45.13 ± 0.73	45.38 ± 0.03
Ethanol	2.69 ± 0.04	48.09 ± 0.92	49.12 ± 0.26
Water	3.58 ± 0.04	51.90 ± 0.56	53.90 ± 0.25

Total sugars, Reducing sugars and Non-reducing sugars contents

Contents of total sugars, reducing sugars and non-reducing sugars in various extracts of garlic varied widely (Table 3). On fresh weight basis, water extract of garlic contained the highest (1.93, 0.63 and 1.30 g/100g fwb, respectively) total sugars, reducing sugars and non-reducing sugars contents followed by ethanol (1.24, 0.43 and 0.81 g/100g fwb, respectively) and acetone (0.85, 0.27 and 0.59 g/100g fwb, respectively) extracts. Similar trend was observed on dry weight basis (Table 4).

Table 3: Total sugars, reducing sugars and non-reducing sugars contents in garlic on fresh weight basis (fwb)

Solvent	Total sugars content (g/100g fwb)	Reducing sugars content (g/100g fwb)	Non-reducing sugars content (g/100g fwb)
Acetone	0.85 ± 0.05	0.27 ± 0.05	0.59 ± 0.11
Ethanol	1.24 ± 0.05	0.43 ± 0.05	0.81 ± 0.10
Water	1.93 ± 0.09	0.63 ± 0.01	1.30 ± 0.10

Table 4: Total sugars, reducing sugars and non-reducing sugars contents in garlic on dry weight basis (dwb)

Solvent	Total sugars content (g/100g dwb)	Reducing sugars content (g/100g dwb)	Non-reducing sugars content (g/100g dwb)
Acetone	1.33 ± 0.08	0.42 ± 0.08	0.92 ± 0.17
Ethanol	1.93 ± 0.09	0.67 ± 0.08	1.26 ± 0.15
Water	3.02 ± 0.14	1.05 ± 0.01	1.97 ± 0.17

Antioxidant Activity

DPPH free radical scavenging activity

2,2'-diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical (purple colour) and it transforms to non radical form (yellow colour) by abstracting one electron and hence, it is widely used as a measure for the electron donation capacity of antioxidants under assay conditions. DPPH free radical scavenging activity (%) of the acetone, ethanol and water extracts of garlic varied greatly (Table 5). The aqueous extract of garlic showed highest (47.79%) DPPH free radical scavenging activity followed by ethanol extract (38.44%) and acetone extract (20.43%). Ollanketo *et al.*[16] found that using water for extraction was most effective for antioxidant activity as compared to 70% ethanol and methanol and Settharaksa *et al.*[17] also reported DPPH free radical scavenging activity obtaining from water extraction was high when compared with ethanol and methanol extraction. Fresh garlic had 21.69% DPPH scavenging activity which was generally associated with the total phenol content. As the total polyphenolic content of extract increases, antioxidant activity also increases[18]. Our results of antioxidant activity by DPPH method in acetone, ethanol and water extracts of garlic are in agreement with other research workers.

β-carotene bleaching method

Antioxidant activity was measured by β-carotene bleaching method that is based on the ability of an antioxidant to inhibit lipid peroxidation. Antioxidant activity (%) of the acetone, ethanol and water extracts of garlic by β-carotene bleaching method varied greatly (Table 5). The aqueous extract of garlic showed highest (29.33 %) antioxidant activity followed by ethanol extract (26.45%) and acetone (18.36%) extracts. The antioxidant activity of two types of garlic i.e. fresh *Allium sativum* and three-month old *Allium sativum* by β-carotene bleaching method was studied and found to be 35.36% and 10.2%, respectively[19].

Table 5: Antioxidant activity of different extracts of garlic

Solvent	DPPH free radical scavenging activity (%)	β-carotene bleaching method (%)
Acetone	20.43 ± 0.49	18.36 ± 0.16
Ethanol	38.44 ± 0.02	26.45 ± 0.07
Water	47.79 ± 0.33	29.33 ± 0.13

CONCLUSION

Results of present study showed that garlic is a rich source of total phenols, flavonoids, ascorbic acid and sugars & solvent play a vital role in the extraction of these phytoconstituents. Water extract contained highest total phenols, flavonoids, ascorbic acid and sugars contents as well as exhibited highest antioxidant activity by both methods.

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