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Analysis of minerals profile, phenolic compounds and potential of Garlic (*Allium sativum*) as antioxidant scavenging the free radicals

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Abstract

Allium sativum is the most cultivated vegetable and use for flavouring in country cuisines. This species is believed to have medicinal properties and act as remedy of oxidative related disorder. In this study, it was recorded that garlic has high profile of total phenolic (40.80±2.91 mg GAE/100 g), flavonoid content (4.59±1.28 mg RE/100 g) and potential candidate in amelioration of oxidative stress. To really check this ability three free radicals namely FRAP (The ferric reducing ability of plasma), DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis 3-ethylbenzthiazoline-6-sulphonic acid) were used to evaluate the antioxidant capacity of the garlic. Garlic showed significantly different results in the case of every free radical having the values of 35.22±6.63, 28.82±11.61 and 231.64±25.02 mg vitamin C equivalent per 100 g for FRAP, DPPH and ABTS respectively. Similarly in the case of copper chelating, Iron chelating, Superoxide radical scavenging and Hydroxyl radical scavenging the values were 21.44 ±1.19, 0.69± 0.09, 4.87± 0.95 and 9.09± 1.71 mg TE equivalent/g. The garlic samples also showed significant variations in crude protein (13.83±3.26%), crude fat (0.51±0.086%), ash content (4.40±0.19%) and crude fibres (2.17±0.58%).The mineral analysis of garlic showed higher concentration of potassium (48.75±3.69) followed by calcium (24.79±2.78). The other minerals were Na, Fe, P, Zn, Cu, Mn and Mg with concentration of 4.06±0.32 mg/100g, 3.93±0.21, and 9.86±0.55, 0.53±0.01, 0.010±0.00, 0.010±0.00 and 2.63±0.25 mg/100g, respectively. It is concluded, garlic has high profile of minerals and phenolic compounds which increase the potential as strong antioxidant in the scavenging the free radicals.

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Introduction

The interest of using plant material as home remedy of various health disorders has been increasing in the developing and developed countries because of nutraceutical importance (Khalid *et al.*, 2011). Traditional plants like garlic and cinnamon are using extensively as a raw material in the pharmaceutical industry. About 70% of population around the world use traditional medicine derived from plant materials for the treatment and cure of their diseases (Sharma & Goyal, 2010). Therefore great emphasis was given to analyze the minerals, phenolic and nutritional profile of these plants.

Allium is the most cultivated vegetable species among the Alliaceae family (Mnayer *et al.*, 2014) with 700 species around the world (Tepe *et al.*, 2005). *Allium sativum* is the most commonly used in India and Pakistan as popular cuisines and medicinal properties (Khalid *et al.*, 2014; Nicastro *et al.*, 2015). It not only provides carbohydrates content but also minerals, antioxidant, vitamins, polyphenols and carotenoids (Liu *et al.*, 2014). It is also the richest source of sulfur containing compounds (ajoene, allicin, alliin, allyl sulfides, allyl disulfides, allyl trisulfides, cysteine, cycloalliin, cysteine sulfoxides, cystine, diallyl sulfides, dimethyl sulfides, glutathione, disulfides, methionine, methyl sulfides, sulfanes, pseudoscordinine, thiosulfinates, scordinine, trisulfides and tetrathiol) (Choudhary, 2008; Butt *et al.*, 2009) and minerals like phosphorus, calcium, iron, selenium and germanium increasing its potential as antioxidant, therapeutic, antibacterial and anti-carcinogen agent (Touloupakis and Ghanotakis, 2010; Nicastro *et al.*, 2015). Different toxic materials are the source of oxidative stress by generating reactive oxygen species (ROS) in the cell. In human body the ROS include, hydrogen peroxide, peroxy radical, hydroxyl radicals, superoxide anion radical hypochlorous acid, nitric oxide radical and singlet oxygen (Rajendran *et al.*, 2014). Over production of ROS may cause easily damage to cell and tissue by lipid peroxidation damage to protein and DNA (Doreswamy *et al.*, 2004; Cadet and Wagner, 2013). Garlic compounds showed modulating effects on oxidative stress by scavenging the ROS and increases the cellular antioxidant enzymes and glutathione in the cell (Borek, 2001; Tsubura *et al.*, 2011). It can be possibly utilized in various health disorders against colon tumor development (Jikihara *et al.*, 2015), non-alcoholic steatohepatitis (Wu *et al.*, 2015), murine colitis (Fasolino *et al.*, 2015), induces apoptosis in human gastric carcinoma cell line (Zhang *et al.*, 2015), cell cycle arrest in cancer cell line (Bagul *et al.*, 2015), reduce blood pressure (Wang *et al.*, 2015) and as anticancer (Nicastro *et al.*, 2015). Garlic attains the property of antioxidant due to the presences of the phenolic compounds and most importantly the flavonoids contents (Chen *et al.*, 2013; Queiroz *et al.*, 2014). In the present study, minerals profile, total phenolic compounds, flavonoids and aitnoxidantive potential of garlic species was evaluated.

Materials and methods

Physiochemical analysis of garlic species

The local species *A. sativum* was cultivated in the plots and irrigated on weekly bases. The pH of the soil was 7.6 with standard mineral profile. After maturation of the bulb, the samples were taken from the middle row of plots. The taxonomic status was confirmed from department of Botany GC University Faisalabad. About 200 g of raw garlic was crushed with electrical juicer, disinfected with 70% ethanol and dried in the laminar flow (ESCO EN® Class H13 HEP). This crushed material was then mashed through fine ferbric mesh with mesh size of 100-120 µm and disinfected with ethanol and dried in the laminar flow hood. At this moment, the garlic extract was expected to 100% pure and then diluted with mili-q water for desired dilution.

Moisture content was determined with method no 44-19 in AACC (2000). 2 g of raw garlic was placed in the preheated and weighed metallic dish and dried the garlic extract at 130°C for 2 h in the hot air oven. The moisture content of garlic was calculated according to the formula.

$$\% \text{ Moisture Content} = \frac{W1 - W2}{Wighted\ of\ sample} x100$$

Where W_1 is weight of garlic + dish just before heating W_2 in the weight of garlic extract + dish just after heating.

Total ash content was determined according to the method 08-01 in AACC (2000). 3 g of garlic extracts was placed in pre-weighed crucible and ignited at 500 °C in the muffle furnace (model EHRET TK/L 4105) for 2 h. Remaining material was cooled, weighed and total ash content was calculated according to the formula

$$\text{Ash (\%)} = \frac{\text{Residue weight}}{Weighted\ of\ sample} x100$$

Where W_1 is weight of garlic + dish before heating
W_2 in the weight of garlic extract + dish after heating
AACC (2000) method no. 984-13 and Kjeltech apparatus (Kjeltec™ 8000) was used for determination of per cent nitrogen in the garlic sample. The sample was digested by using the digestion mixture of K_2SO_4. $FeSO_4$, $CuSO_4$ in the ratio of 100:5:10 respectively until green colour appeared. Distil water was used for dilution and making the sample up to 250 ml in the volumetric flask. 10 ml of each digested samples and 10ml of 40% NaOH was taken in the distillation apparatus and liberated ammonia was collected in 4% boric acid solution beaker. The methyl red was used as titration indicator. The resultant ammonium borate was used for calculation of nitrogen in the samples using the 0.1 N H_2SO_4 solution as titrating agent until light golden colour appeared. The crude protein was calculated by estimating the percentage of nitrogen in the samples with following formula.

$$\text{\% Nitrogen} = \frac{\text{Volume of H2SO4 used } x\ 0.0014\ x\ volume\ of\ dilution\ (250ml)}{Volume\ of\ distillate\ x\ Weighted\ of\ sample} x100$$

% Crude protein = Nitrogen X factor (6.25).

Method no 30-20 mentioned in the AACC (2000) was used for crude fat calculation. About 5 grams of garlic powder was put in the cellulose thimbles fitted extractor. The crude fat extracted out with hexane in the fat cups. The cups were weighed and of fat of the sample calculated according to the formula.

$$\text{Crude Fat (\%)} = \frac{\text{Weight of extracte fat}}{Weighted\ \ sample} x100$$

For crude fibre, about 1 g of garlic sample was taken in the beaker and added 100 ml of 1.25% H_2SO_4. Then it was reflux for 30 min and filtered through sintered glass crucible in vacuum, wash with distil water to neutralize the sample. The washed material was again refluxed for 30 min in the beaker with 100 ml 1.25% NaOH. The material was again washed with distil water till neutralized. The washed material was dried in oven at 130 °C for 1 h cooled in desiccator then weighed. Residue was ignited for 6 h weighed in pre-weighed crucible. The crude fibre was then calculated according to the formula [method no 926-09 in AOAC (2006)].

$$\text{\% Crude Fiber} = \frac{\text{A} - \text{B}}{Weighted\ of\ sample} x100$$

A= crucible and residue weight
B= crucible and ash weight

The carbohydrates content was determined by subtracting the sum up of percentage of moisture, lipid, protein, fiber and finally ash content from 100 (Otitoju, 2009).

For mineral content in the garlic sample, 1 g of garlic sample was taken in the crucible of muffle furnace and ignited at 550 °C for 6 h. The ash was dissolved in 10% 10 ml HNO_3, heated for 20 min at low heat, filtered and filtrate obtained was used for mineral content determination. Ca, Mg, Fe, P and Zn were determined through atomic absorption (Aurora TRACE AI1200 Atomic Absorption Spectrometer) while Na, K was determined through flame photometer (Jenway PFP7).

The data was represented as mg/g sample which was then multiply with 100 to represent as mg/100g sample (AOAC, 2006). The calibrated pH (InoLab 720) meter was used to record the pH according to procedure in AOAC (2006). The total acidity of garlic extract was calculated after titrating it against 0.1 N NaOH solutions till persistent pink colour.

Total phenolic, Flavonoids content and antioxidant activity

100 ml of samples were inserted into the Soxlet apparatus with 70% ethanol as extraction solvent in the round flask. After the assemblance, the soxlet apparatus was left running at 60 °C for 12 h. All the extraction solvent was removed in the rotary evaporation in the round flask (Büchi ® rotary evaporator Model R-200). The extract was subjected to freezing drying for final removal of water content. Finally the extract was refrigerated at 4 °C for further use.

Total phenolic content was determined by Folin-ciocalteau micro method. About 60µl garlic extract was diluted with deionized milli-Q water to make the volume up to 4.8 ml. 300 µl of Folin-ciocalteau reagent was added and allows for 10 min. Then 900 µl of 20% Na_2CO_3 solution was added to this mixture and kept it for 80 min at 40 °C. Gallic acid (50 µg) was used as standard reference because of having wide spectrum of phenolic compounds. The absorbance was recorded at 765nm reported as mg gallic acid of per gram fresh weight.

Total phenolic compound was calculated by the following formula.

$$\text{Values of TPC}\left(\text{mg}\frac{\text{GAE}}{\text{g}}\right) = \frac{\left[\frac{\text{SA}-\text{CA}}{\text{Slope}}\right]\left(\frac{10}{\text{U}}\right)}{(2)(1000)}$$

SA= Sample absorbance for TPC
CA= control absorbance with no extracts
Slope= slope of standard curve
(10/U)= total volume of extract/used volume of extract
1000= µg to mg converting factor.

The result was multiply with 100 for calculation of mg/100g sample. The methodology of Shen *et al.* (2009) was followed for estimation of flavonoids content.

FRAP assay was performed according to the protocol Benzie and Strain (1999). The results were represented as mg vitamin C equivalent. For ABTS radical cation decolorization assay, the Re *et al.* (1999) method of ABTS was used with slight modification. The results were represented as mg vitamin C equivalent.

The protocol of Brand-Williams *et al.* (1995) with modification was used for DPPH radical scavenging. 0.8 mM DPPH radical solution was prepared with 95% ethanol. 100-1000 µl of garlic extract was diluted with 95% ethanol and deionized water in ratio of 1:1 up to 5.4 ml. 0.6 ml of DPPH solution was mixed and decrease in absorbance was recorded after 1 min, compared with standard curve of 0-40 µg ascorbic acid and results were reported as mg Vit-C equivalent. The values of total FRAP, ABTS, DPPH were calculated by according to formula.

$$\text{Values of FRAP, ABTS, DPPH}\left(\text{mg}\frac{\text{VCE}}{\text{g}}\right) = \frac{\left[\frac{\text{SA}-\text{CA}}{\text{Slope}}\right]\left(\frac{10}{\text{U}}\right)}{(2)(1000)}$$

The results were multiply with 100 for calculation of mg per 100g sample.

The protocol of Sánchez-Vioque *et al.* (2012) was followed for garlic copper chelating activity using pyrocatechol. The chelating activity was expressed as mg/g sample.

Iron chelating potential of garlic was measured using the methodology of Decker and Welch (1990) with some modification. About 10 ml of sample was mixed with 20 ml of solution of $FeCl_2$ then 20 µl of 2.4 mM ferrozine was added to this mixture and incubated for 10 min at room temperature. After 10 min, absorbance was measured at 562 nm using ETDA as standard. The garlic chelating activity was expressed in the term of mg/g EDTA. For superoxide scavenging, Su *et al.* (2009) was followed. The superoxide scavenging was expressed in the term of mg TE equivalent/g. Hydroxyl radical scavenging assay was done using the methodology of Zhang *et al.* (2011). The results were expressed as mg TE equivalent/g.

Results and discussion

Garlic has the long history of about 5000 years as remedy and health promoter. The plant usually contains complex chemical profile but investigators showed all plants contain phenolic compounds but in different concentrations. The phenolic compound is generic term and refers to the compounds that have at least one aromatic ring and one or more attached hydroxyl group.

Chemical composition analysis plays a vital role to assess the nutritional quality and quantity of plant materials constituting the human diet. Carbohydrates act as energy reservoirs where the protein and fat have development and repair role. The micro minerals develop the immune systems. The dietary fibre improves the functionality of digestive tract, increases the growth of intestinal inhabiting beneficial microflora and lowers the cholesterol level.

The phytochemical analysis of garlic was done presents proximate analysis of raw garlic (*A. sativum*). The results show variation of moisture content among the raw and powder garlic. The raw garlic contained 64.20±3.90% moisture and 34.91±3.26% dry content. However the powder garlic showed much less moisture content (Table 1).

Table 1. The physiochemical analysis of powdered garlic.

Serial No.	Parameters	Values (%)
1	Moisture content	4.75 ± 1.37^{C}
2	Ash content	4.40 ± 0.19^{CD}
3	Crude protein	13.83 ± 3.26^{B}
4	Crude fat	0.51 ± 0.086^{D}
5	Carbohydrate	71.01 ± 4.66^{A}
6	Crude fiber	2.17 ± 0.58^{CD}

Values are Means ±SD of five replicates

Values sharing the same letter in the same column are not significantly different at 5% probability level.

The pH recorded was acidic (5.76±0.51) having 0.50±0.089 total acidity. Table 1 also represents the garlic samples are rich in the carbohydrates (72.01±4.66%), crude protein (13.83±3.26%), crude fat (0.51±0.086%) and crude fibres (2.17±0.58%).

The mineral analysis of *A. sativum* showed higher concentration of potassium (48.75±3.69) followed by calcium (24.79±2.78). The other minerals were Na, Fe, P, Zn, Cu, Mn and Mg with concentration of 4.06±0.32 mg/100g, 3.93±0.21, and 9.86±0.55, 0.53±0.01, 0.010±0.00, 0.010±0.00 and 2.63±0.25 mg/100g, respectively (Table 3).

This study shows garlic contains higher amount of total phenolic compounds (40.80±2.91 mg GAE/100g). Flavonoid which is one of the most important phenolic compounds with known antioxidant properties. It's more important role is free radical scavenging. The present study shows flavonoid in garlic was 4.59±1.28 RE/100 g recorded (Table-2). The percentage yield of oil was found to be 22.10±5.70 %. The color of oil was light yellow and the specific gravity was 0.92±0.05 cm^3. The oil had pungent odor and was liquid at room temperature indicating the presence of oleic acid and linoleic acid and other unsaturated fatty acids.

The FRAP, DPPH and ABTS inhibition were 35.22±6.63, 28.82±11.61 and 231.64±25.02 mg vitamin C equivalent per 100 g, respectively (Table 4). The maximum inhibition was done in the case of ABTS free radicals (231.64±25.02 mg/100g) where minimum was found in the case of DDPH free radical. The FRAP radical scavenging was not much different. The inhibition of these free radicals confirmed the

role as antioxidant and hence utilized in the amelioration of oxidative stress.

Currently, the garlic was analysed for physiochemical, mineral, total phenolic and its antioxidant potential analysis. This study recorded that the *A. sativum* has 64.20±3.90% moisture corresponding to the studies of Khalid *et al.* (2014), dry matter 34.91±3.26%. The dry content was slightly different from the findings of Khalid *et al.* (2014) who estimated the 32.73±0.84% dry content. The difference is probably due to the environmental conditions and different storage methods. The pH values were similar with the pH values presented by Ahmed and Shivhare (2001) and Khalid *et al.* (2014). Further, the results represents the garlic samples were rich in the carbohydrates (72.01±4.66%). These results show analogy with carbohydrates content values observed by Sultan *et al.* (2014) and Al-Numair *et al.* (2007).

Table 2. Total phenolic and antioxidant activity of Garlic against FRAP, DPPH, ABTS radicals.

Serial No.	Parameters	Values
1	Total phenolic mg GAE/100 g	40.80 ± 2.91^{A}
2	Flavonoids mg RE/100 g	4.59 ± 1.28^{B}

Values are Means ±SD of three replicates

Values sharing the same letter in the same column are not significantly different at 5% probability level.

The sample also showed significant variations in crude protein (13.83±3.26%), crude fat (0.51±0.086%), ash content (4.40±0.19%) and crude fibres (2.17±0.58%). These results are similar with the studies of Kumar *et al.* (2010) and Khalid *et al.* (2014). Moreover these findings are also in agreement with limits defined by WHO (World health organization).

Extensive research was carried out on the minerals analysis of medically important herbs and plants. The *A. sativum* showed higher concentration of potassium (48.75±3.69) followed by calcium (24.79±2.78). These findings about the minerals are comparable with Khalid *et al.* (2014). They reported the high concentration of K (48.06+0.32 mg/100g) among all the minerals in the *A. sativum* followed by Ca (24.33±1.95 mg/100g). The other minerals were Na, Fe, P, Zn, Cu, Mn and Mg with concentration of 4.06±0.32 mg/100g, 3.93±0.21, and 9.86±0.55, 0.53±0.01, 0.010±0.00, 0.010±0.00 and 2.63±0.25 mg/100, respectively (Table 3).

Table 3. Mineral content of Garlic powder (mg 100 g^{-1}).

Serial No.	Elements	Values (mg/100g)
1	Na	4.54 ± 0.61^{CD}
2	Ca	24.79 ± 2.78^{B}
3	Fe	3.79 ± 0.80^{CD}
4	P	8.23 ± 2.03^{C}
5	K	48.75 ± 3.69^{A}
6	Zn	0.47 ± 0.07^{D}
7	Cu	0.014 ± 0.006^{D}
8	Mn	0.016 ± 0.02^{D}
9	Mg	2.69 ± 0.47^{CD}

Values are mean ±SD of five replicates

Values sharing the same letter in the same column are not significantly different at 5% probability level.

Vegetables, fruits, cereals and some other plants contain phenolic compounds as a major antioxidant group. The phenolic compound retains this ability due to its redox potential acting as reducing agent and quenches the singlet oxygen (Atoui *et al.*, 2005). The normal physiological functions depend upon the adequate status of body antioxidants which can be maintained by increasing the intake of dietary antioxidant. These antioxidants prevent the several generative diseases by lowering the oxidative stress (Tepe *et al.*, 2005). Some synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are also being utilized but they have toxic and carcinogenic effects on animal models (Liu *et al.*, 2014). So consumers are more amenable towards the natural antioxidant than synthetic one. This study shows garlic contains higher amount of total phenolic compounds (40.80±2.91 mg GAE/100g).

Table 4. Radicals scavenging ability of garlic (Vit. C equivalent mg 100 g^{-1})

Serial No.	Radicals	Values
1	FRAP (mg/100g)	35.22 ± 6.63^{B}
2	DPPH (mg/100g)	28.82 ± 11.61^{B}
3	ABTS (mg/100g)	231.64 ± 25.02^{A}
4	Copper chelating activity (mg/g)	21.44 ± 1.19^{C}
5	Iron chelating activity (mg/g)	0.69 ± 0.09^{F}
6	Superoxide scavenging (mg/g)	4.87 ± 0.95^{E}
7	Hydroxyl scavenging (mg/g)	9.09 ± 1.71^{D}

Values are Means ±SD of three replicates

Values sharing the same letter in the same column are not significantly different at 5% probability level.

These findings are similar to results of Che Othman *et al.* (2011) (37.60±2.31 mg GAE/100g). The phenolic and flavonoids compounds have the ability of free radical scavenging and act as antioxidant. In this study, the antioxidant capacity of *A. sativum* was determined by FRAP, DPPH and ABTS assay. FRAP assay measures the sample ability to reduce the Fe^{3+}-TPT (ferric- tripyridyltriazine) to Fe^{2+}-TPT (Leong and Shui, 2002). DPPH and ABTS assay is the reduction of DPPH and ABTS radicals (Re *et al.*, 1999). The values of 35.22±6.63, 28.82±11.61 and 231.64±25.02 mg vitamin C equivalent per 100g for FRAP, DPPH and ABTS, respectively was significantly different for *A. sativum* (**Error! Reference source not found.**). Al-Numair *et al.* (2007) and Settharaksa *et al.* (2012) estimated slightly different values for *A. sativum* again possibly due to extraction solvent and analytical methods. The garlic showed significant ABTS radicals scavenging at pH similar to human body. Both phenolic and sulfhydryl compounds probably involved in the antioxidant activity against ABTS free radicals. Even cooking doesn't alter or slightly affected the antioxidant profile (Liu *et al.*, 2014). The scavenging of DPPH is accompanied due to electron transport. The estimated results usually showed lower antioxidant activity than real one due to influence of pH and extraction solvents. The difference in the antioxidant assay methods may also give different results (Nicastro *et al.*, 2015). The FRAP assay may determine the electron transfer ability of test compound in the acidic environment (Wojdyło *et al.*, 2007). The active compound donates the electron reducing the Fe^{3+} to Fe^{2+}.

The antioxidant activity of test extracts could not be explain on the basis of their phenolic compounds estimation only but required proper characterization of each compound present (Ghasemi *et al.*, 2009). It is because the antioxidant activity largely depends upon the compound structure. Up till now, only flavonoid has been studied and its role as proton donating and radical scavenging has been determined

(Wojdyło *et al.*, 2007). Further, the extracts are mixture of very complex and distinct compounds.

Conclusion

It is concluded from the present study that garlic is rich in minerals and phenolic compounds. It is powerful antioxidant and could be used in the remedy of various oxidative stress related health disorders. The present work also suggests the characterization of other phenolic compounds in different species.

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