

Research Article

Evaluation of climatic and geographical effects on bioactive compounds and minerals profile of two selected samples of white turnip

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Abstract

With the rise of various disorders in human beings related with free radicals perceived to recompense the scrutiny on synthetic and natural antioxidants to control the numerous perilous ailments and other carcinogenic hazardous diseases. Vegetables and fruits are the major source of natural antioxidants because these are majorly composed of natural antioxidants. Therefore, current study was carried out to focus on the significance and assessment of antioxidants in the ethanol and methanol extracts of turnip peels. Turnip samples were collected from two different regions of Pakistan i.e. Soon Valley and Sargodha region. Further two different solvent systems i.e. Methanol and Ethanol were used for each variety of turnip. Evaluated the antioxidant activity through DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity, total flavonoid contents, determined the total phenolic contents via Folin-Ciocalteu method and reducing power assay. Estimation of mineral profile was done by wet digestion method. In the samples of turnip percentage yield of methanolic extracts exhibited maximum antioxidant activity as compared to ethanolic extracts. Analysis of results revealed that methanolic extract of Sargodha region turnip possessed maximum concentration of TPC (Total phenolic content) as compared to the 80% ethanolic extract of soon valley region. In 100% methanolic extract radical scavenging activity exhibited maximum % inhibition as compared to ethanolic extract. In the extracts of turnip peels 100% methanol exhibited greater reducing potential. Mineral composition and levels vary across two different samples of turnip indicated the maximum concentration of minerals in Sargodha region turnip. As a consequence of this study solvents that were used possessed significant results ($P < 0.05$) for the antioxidant compounds extraction from turnip samples.

Keywords: Turnip; Antioxidant potential; Reducing powers; Moisture content; Minerals profile

Introduction

For human life fruits and vegetables are the vital permutation of nutrients because they

contain different kind of vitamins, water, antioxidants, proteins, minerals, lipids, antioxidants, flavonoids and phenolics etc.

Crucial diseases such as cardiovascular and cancer are generally restrained by daily utilization of fruits and vegetables. In our society increased rate of deaths occurs due to these diseases. Possibility of various diseases like Alzheimer disease, cancer and other ailments that are linked with human health can be declined by the intake of vegetables and fruits [1]. Phenolic antioxidants terminate free radicals and often act as metal chelators [2].

Compounds that inhibit the effect of free radicals generated by various metabolic mechanisms of body are called antioxidants. With the passage of time and progression of technologies, studies disclosed the significance of antioxidants [3]. Fruits and vegetables made up of substantial amount of dietary antioxidants which prevent the cells from aging and encourage the new cells growth.

Qualitative food is a vital demand of all human beings that dispense them different nutrients like vitamins, carbohydrates, minerals and protein in their daily uptake. For the scarcity of these nutrients fruits and vegetables appraised as extensive way. Scarcity of nutrients becomes the reason of numerous ailments as linked with human health [4]. In our body liquids and tissues inorganic components like minerals are responsible for the suitable working of many functions of biological system. Chemically minerals are employed by various sources in human body and considered as essential elements [5]. From different sources intake of minerals manifested the nutritional advantage in various physiological functions and lack of minerals causes significant health issues. Minerals insufficiency is very menacing to human health.

Vitamin C protects us from cancer, diabetes, high blood pressure and obesity by arresting the process of oxidation and enormously found in turnip which is a *brassica* vegetable. Cruciferous root vegetable

Brassica rapa is circular in shape with purple crown and in white colour, holding green leaves at top. Annually *Brassica rapa* is grown-up in European countries but normally it is found in winter season of year from October to March. Vitamin C and antioxidants contributed by turnip terminate the oxidation processes that produce oxidative stress. Valuable substances are present in *Brassica rapa* that lessen the possibility of numerous diseases [6].

Functional constituents in natural sources are affected by varieties, cultivation, Climatic and Geographical influences. Thus, the nature of extracting solvents plays an important role in the yield of extracts, their antioxidant activities and other important properties. In this scenario, we were motivated to evaluate bioactive attributes, biological activities, minerals profile and other antioxidant compounds found in white turnip extracted by different solvents system, to optimize the extraction process and to check the geographical effects on cultivars.

Materials and methods

Sample preparation and extraction

Earlier than extraction, selected varieties of turnip from two different areas i.e. Sargodha and Soon valley were physically cleansed and swabbed with deionized distilled water. Separate out the peels from pulp of turnip samples and cut them into small segments, after that dehydrated the samples of turnip at room temperature in shadow. To resolve the content of moisture in an electric oven these samples were placed for 2 hours at 100°C before exposure to air. After the step of drying, samples of turnip were minced into powder of fine particles then moved all the powder of fine particles across the 1mm strainer. The moisture of fresh samples was calculated by using formula. All chemicals that were employed in different experiments of this study were analytical grade and

purchased from Sigma-Aldrich Chemical Corporation, Germany.

Sample extraction

In the extraction procedure all the fine powder material was specifically 10g measured and accomplished the extraction in 100 mL (1:10) of two distinct solvents i.e. methanol and ethanol. The extraction was done through (OS- 752) shaker at 170 rpm for 6 hours [7]. The extraction was repeated thrice in order to complete extraction of phenolic compounds and minerals. After that these extracts were filtered and for the evaporation of solvents passed these samples below the reduced pressure of 45°C through rotary evaporator. After the solvents evaporation remnants are obtained and labelled the obtained residues for the perseverance of yield %.

Determination of antioxidant activity

Determination of TPC (Total phenolic content)

Assess the total contents of phenolic substances in dissimilar samples of *Brassica rapa* via Folin-Ciocalteu using spectrophotometer. Mixture of reaction was made by mixing + 0.5N Folin reagent + 1mL sample extract (0.1 mL) of turnip. Then leave it at room temperature for 15 minutes. After that added 2.5 ml of 7% saturated Na₂CO₃ and left it in dark for 30 minutes. Through spectrophotometer absorbance at 760 nm was noted with a 1 cm cell. Manipulated the Gallic acid as calibration standard and calculating the results in mg/100g. The assay was performed three times and average results were obtained [8].

Determination of TFC (Total flavonoid content)

50mg of *Brassica rapa* samples extracts were added in 1mL of distilled water then added 4 mL of distilled water in order to make the volume up to 5 mL. Further added 0.3 mL of 5 % NaNO₃ and left this mixture for 5 minutes. Add 3mL of 10% AlCl₃ to the

reaction mixture. Then add 2ml NaOH 1M after 6 minutes. Through UV spectrophotometer absorbance was measured at 510 nm [9]. Values of TFC were showed in mg/100g. Catechin was manipulated as a calibration standard. The analysis was performed three times and average results were obtained.

DPPH scavenging activity assay

Evaluation of DPPH examination was executed for the analysis of free radicals in the sample solution. Analysis of antioxidant activity of turnip samples from two different areas was also determined by conducting the DPPH scavenging assessment through spectrophotometer. For this reason, reaction mixture was prepared by dissolving 10 mg of sample in 1mL distilled water. Six varied concentration of standard (0.25, 0.5, 0.75, 1, 1.5 and 2) mg/mL were prepared. After that added distilled water in order to make the volume up to 400 µL. In each test tube added 2mL of DPPH reagent (0.1mM). 0.1mM DPPH reagent solution was made by dissolving 10mg DPPH in 50ml of methanol. After adding DPPH reagent solution all the sample solutions were left for 30 minutes in dark. By using a standard Butyl hydroxyl anisole (BHA) absorbance was measured at 517 nm [10].

$$\% \text{Inhibition} = \frac{\text{Absorbance (Blank)} - \text{Absorbance (sample)}}{\text{Absorbance (Blank)}} \times 100$$

Reducing power assay

Reducing power ability of the *Brassica rapa* samples from two different areas was determined by reducing power assay. In this method 50 mg of samples extracts of turnip were dissolved in to 5 mL of Sodium phosphate buffer NaH₂PO₄ acid=29.94g, NaHPO₄ base=7.146g, pH=6.6). Then potassium ferricyanide (1.0%, 2mL) was added into reaction mixture and kept this reaction mixture at 50°C for 20 minutes. Added 2mL of 10% trichloroacetic acid and kept it in a centrifuge for 10 minutes (220v-CHINA) at 980 rpm. After the centrifugation of reaction mixture upper

most film of solution (2.5 mL) was diluted with 2.5mL distilled water and ferric chloride (0.5mL, 0.1%) was added in to the reaction mixture. The absorbance at 700 nm was recorded by using spectrophotometer. The experiment was performed three times and average results were obtained [11].

Estimation of mineral profile

For the analysis of minerals in the samples of *Brassica rapa* from two different areas, the labelled samples were used for evaluation of minerals as SV (Soon valley turnip), SS (Sargodha turnip).

Wet digestion method

In this method of wet digestion, measured 2g each of the dried samples of *Brassica rapa* and put them in to four isolated beakers. One beaker was labelled as blank. Then 20 mL of nitric acid was added in to the samples of turnip and kept at the temperature of 70-90°C on an electric hot plate for 2 hours in a fume hood chamber. After 10 minutes burning of sample with nitric acid, 3-5mL H₂O₂ (30%) was added and gently added nitric acid in to each beaker. In an empty beaker nitric acid was also added and labelled as blank. The hot plate temperature was elevated up to 120-130°C after 2 hours and settled the sample with constant addition of hydrogen peroxide and nitric acid for 1 hour. All solutions amount was conserved from 25 to 30mL. Process of digestion was continued till the solutions turned in to transparent. Then solutions were sanctioned to cool and percolated the cool solutions with filter paper of Whatmann (0.4µm). After the filtration process distilled water was added to make the final volume up to 30mL [12].

Mineral analysis

Assessment of mineral profile from the turnip samples was carried out by making different standard solutions of Ni²⁺, Cr³⁺, Co³⁺, Cu²⁺ and Cd²⁺. Prepare all these standards by the stock solution of 1000 mg/L. For the analysis of each element six

concentrations of standard were prepared (5ppm, 10ppm, 15ppm, 20ppm, 25ppm and 30ppm).

Standards were prepared by using formula

$$C_1V_1 = C_2V_2$$

Absorbance of different concentration of standards was determined by atomic absorption spectrophotometer (AAS). Plotted the absorbance versus concentration and the development of continuous curve resolved the accurate concentration of the sample. Since the calibration of varied concentration of elements reference reading was also taken. Results were assembled for statistical analysis [13].

Statistical analysis

All the preliminary efforts accomplished in the laboratory were replicated three times and statistical study of data was described as Mean ± standard deviation [14].

Results and discussion

Yield of crude extracts

Brassica vegetables are considered as potential source of antioxidants, intensely nutritional value and have substantial proposed health effects on human beings. Materials obtained from plant species can be extracted in particular solvents and greatly depend upon nature, concentration of the solvent utilization, and their mixing up performance [15]. For the ultimate extraction of antioxidant compounds appropriate approaches are required. In dissimilar solvents ethanol (80%, 100%) and methanol (80%, 100%) % yield of turnip samples from two regions Sargodha and Soon valley was determined. In the samples of *Brassica rapa* % yield of methanolic extracts possessed higher antioxidant activity as correlated with ethanolic extracts.

Moisture content

Current investigation was conducted to estimate the biochemical configuration of two varieties of *Brassica rapa* peels to determine the content of moisture between Sargodha and Soon valley samples.

Proximate investigation of two dissimilar turnip samples content of moisture was analysed as shown in Figure 1. Different moisture content values were recognized in each gram of sample with the help of analysis of variance. Statistical study revealed significant variations ($P > 0.05$)

among 5g sample from both regions [16, 17]. The error bar on the data indicates the error value.

Content of moisture in Sargodha region sample of turnip revealed maximum % in contrast to Soon valley region sample.

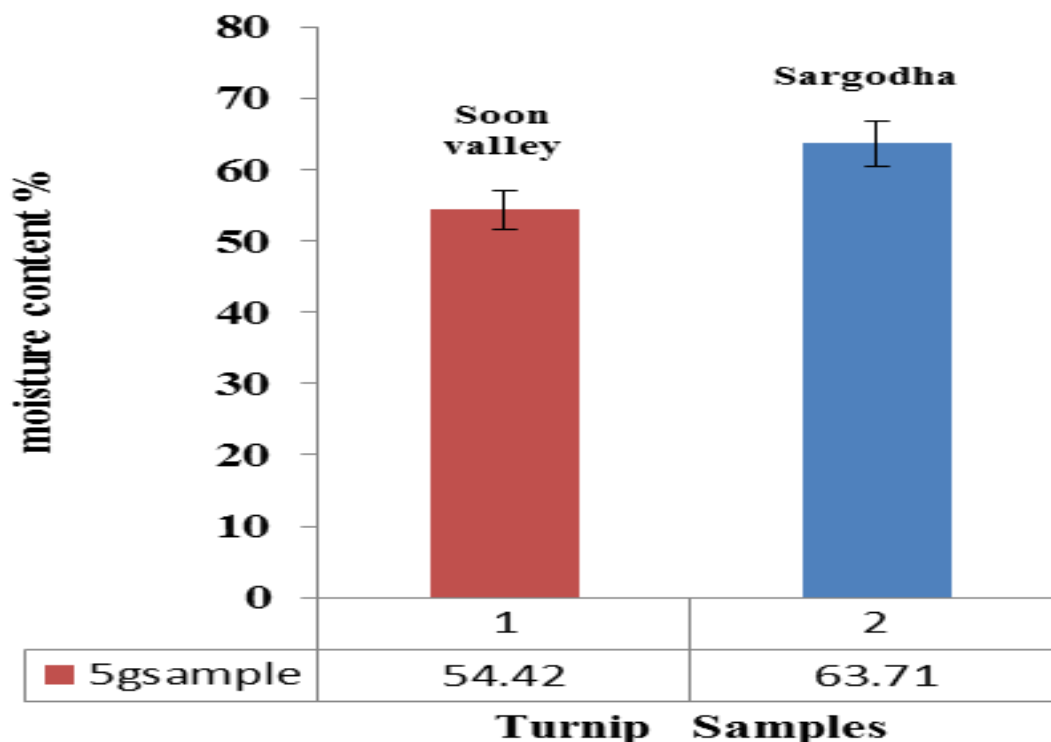


Figure 1. Moisture content

Total phenolic contents

Secondary metabolites that are derived by plant products such as fruits and vegetables are known as phenolic contents [18]. Total phenolic contents of Sargodha and Soon valley samples of turnip were illustrated. Analysis showed difference in 80% and 100% (methanolic, ethanolic) solvents extracts that were assessed by employing Folin-Ciocalteu method and using Gallic acid as standard for its amplified potency,

lesser intervention as well as expeditious analysis of phenolics [19]. Analysis of soon valley and Sargodha region turnip peels possessed higher quantity of total phenolic contents in 100% methanolic extracts as correlated with 80% ethanolic extracts as shown in Figure 2. Analysis of variance revealed significant effects ($P < 0.05$). These both solvents demonstrated to be very efficacious in TPC.

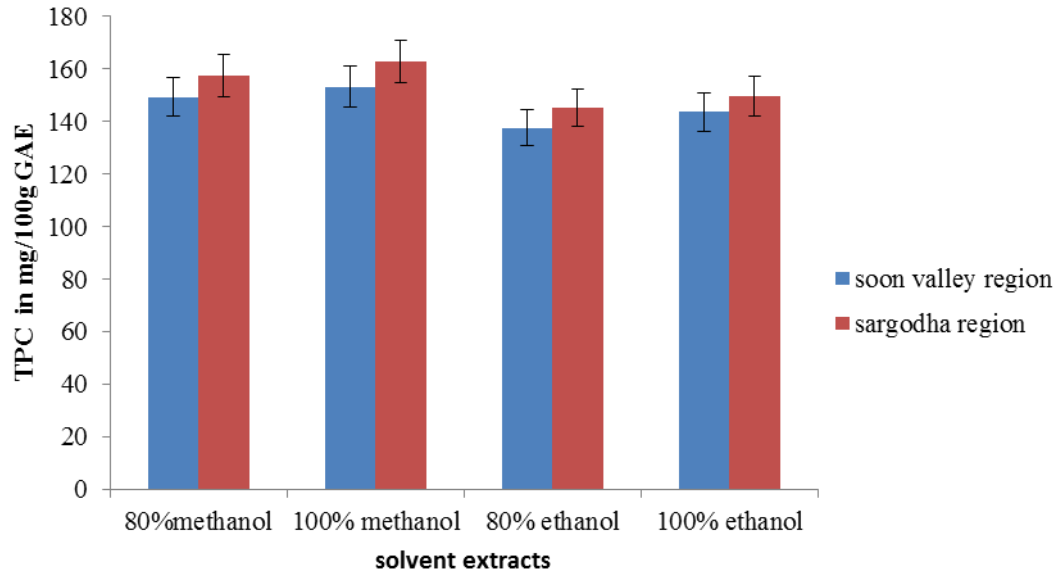


Figure 2. Total phenolic content of turnip sample

Total flavonoid content

Plants appraised as affluent source of flavonoids contributing the potent antioxidant activity, granted as the most significant phenolics in plants preventing the oxidation of lipoproteins [20]. Total flavonoid contents (mg/100g) of turnip peel samples from Sargodha and Soon valley in both ethanol (80%, 100%) and methanol

(80%, 100%) solvents were determined as shown in Figure 3. 100% methanolic extracts of both regions revealed highest amount of TFC. Methanolic extract of Sargodha region possessed highest TFC values. Analysis of variance showed significant results ($p < 0.05$) in different extracts [21].

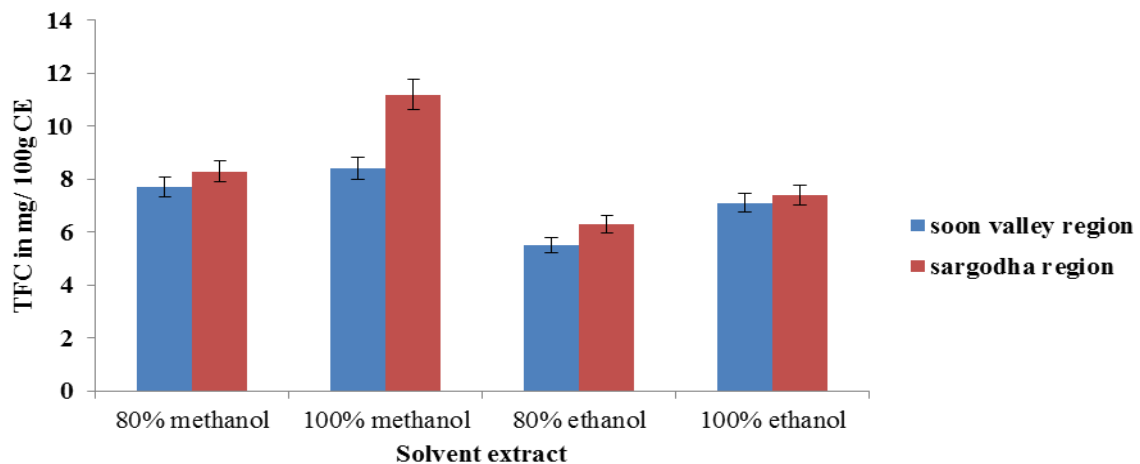


Figure 3. Total flavonoid content of turnip sample

Percentage inhibition of radical DPPH

Radical scavenging activity grants a fundamental function for human beings because free radicals are very dangerous to

our health because they are elaborated in various diseases such as cataracts, aging, cancer, brain dysfunction besides cardiovascular disorders. In alcoholic

solution, by stabilizing free radical, DPPH provides as a perfect substrate. To determine the antioxidant prospective of various fruits and vegetables free radical was employed [22]. DPPH is known for stabilizing free radical because it attains hydrogen and electron, thus stabilizing the free radicals that are responsible for many ailments in human body.

Radical scavenging activity is assessed by its reducing absorbance as long as DPPH

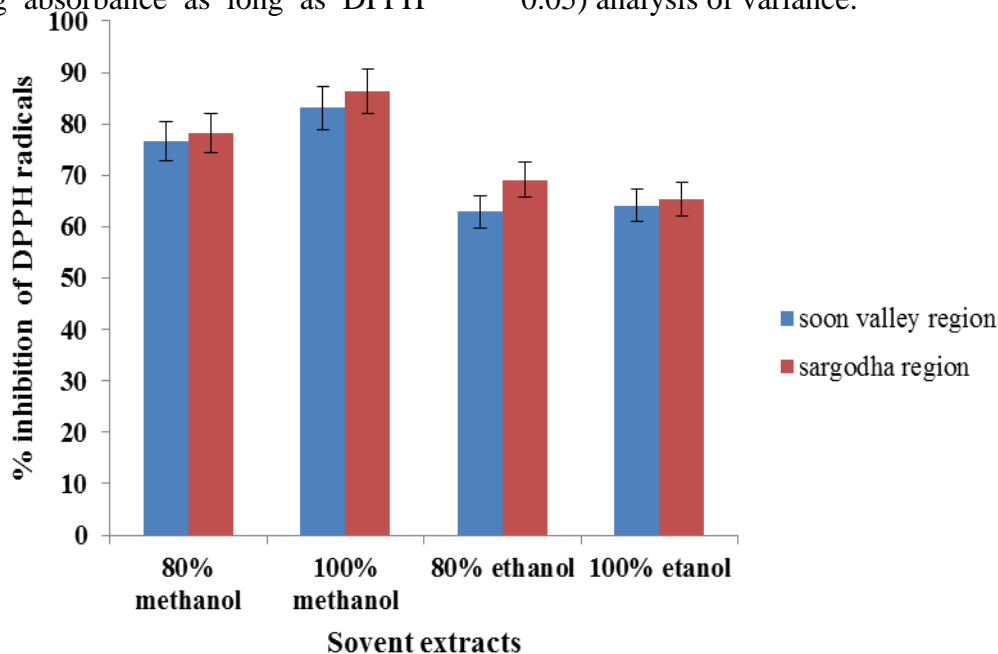


Figure 4. DPPH radical scavenging activity of turnip sample

FRAP assay

FRAP (ferric reducing antioxidant power assay) is a kind of added component for the determination of antioxidant activity swiftly, reproducibly, as well as quietly. Allocated comparable results of authenticity with distilled water and dissimilar solvents. In this practice according to the principle with the change of colour iron is reduced to another form. Reducing activity of the substances confined on the certainty of colour stability, maximal colour acknowledged as greater antioxidant activity [23].

reaction that is executed at 517nm [6]. Both samples of *Brassica rapa* peels revealed considerable amount of radical scavenging activity as shown in Figure 4. This scavenging activity showed variation with the previous scrutinized activity. In the prevailing investigation the activity of 100 % methanol from the samples of *Brassica rapa* exhibited maximum % inhibition of radical DPPH and showed significant ($P < 0.05$) analysis of variance.

Table 1 illustrated the reducing potential of varied extracts of *Brassica rapa* in dissimilar solvents. Concentration was deliberated in mg/mL, with the rise in concentration of the samples of turnip, reducing activity was lifted. Correlation of *Brassica rapa* samples from both regions Sargodha and Soon valley showed that 100% methanol possessed highest antioxidant activity as related to other sample extracts. Statistical study interpreted significant ($P < 0.05$) discrepancy between two assortments of Soon valley and Sargodha region.

Table 1. Effect of reducing power assay on turnips from two different origins

Solvents	Conc.(mg/mL)	Soon valley (S.V)	Sargodha (S.S)
80% Methanol	0.25	1.94± 0.06	1.98 ± 0.05
	0.5	2.05 ± 0.08	2.27 ± 0.06
	0.75	2.43± 0.04	2.69 ± 0.07
	1.0	2.87± 0.04	2.96 ± 0.08
	1.5	3.21± 0.05	3.45± 0.09
100% Methanol	0.25	1.98± 0.01	2.12 ± 0.04
	0.5	2.18± 0.03	2.38± 0.04
	0.75	2.53± 0.05	2.76± 0.07
	1.0	2.83± 0.06	3.55± 0.08
	1.5	3.64± 0.03	3.81± 0.08
80% Ethanol	0.25	1.82 ± 0.02	1.93 ± 0.07
	0.5	1.98 ± 0.05	2.14 ± 0.06
	0.75	2.16 ± 0.04	2.39± 0.07
	1.0	2.48± 0.06	2.59± 0.05
	1.5	2.61± 0.05	2.69 ± 0.06
100% ethanol	0.25	1.91 ± 0.04	2.27± 0.06
	0.5	2.44± 0.02	2.57± 0.05
	0.75	2.59± 0.06	2.64± 0.06
	1.0	2.71± 0.06	2.95± 0.07
	1.5	3.13± 0.08	3.67± 0.08

Values are mean ± SD (n=3) of three separate experiments

Estimation of mineral profile and few heavy metals

Minerals are contemplated inorganic substances that are generally requisite in lesser amounts on regular basis. Mineral contents intake in plants, human beings and animals significantly differ in various ratios. Calcium, phosphorous and iron concentrations differ in various purposes of body, for example Ca^{2+} is essential in the actual bone maintenance and also for the proper functioning of nerves as well as muscles [24]. Chief element of cytochrome, Fe^{3+} , carries out cellular respiration. Mg^{2+} and Zn^{2+} serve as a cofactor in many enzymes and moreover in different biological paths.

Minerals are involved in fighting the diverse ailments that are extremely hazardous to health. Heavy metals in food cause dangerous health effects [25]. Estimation of

minerals by wet ashing method is used to determine different types of minerals. Strong acids and oxidizing agents are used and heated in anticipation of the organic matter is digested. The main advantage of this method is less loss of mineral contents and it is more frequent than dry method because it takes less temperature. Current study analysed the heavy metal contents and mineral profile from wet ashing method in turnip samples from both regions Sargodha and Soon valley. Heavy metal determination from peels of *Brassica rapa* revealed that concentration of Zn^{2+} in turnip was highest i.e. 26.73mg/kg in Soon valley and 34.21 mg/kg in Sargodha region [26]. Study was executed to assess the antioxidant potential, content of moisture and minerals profile of samples of *Brassica rapa* from Sargodha and Soon valley. The result of minerals is shown in Figure 5.

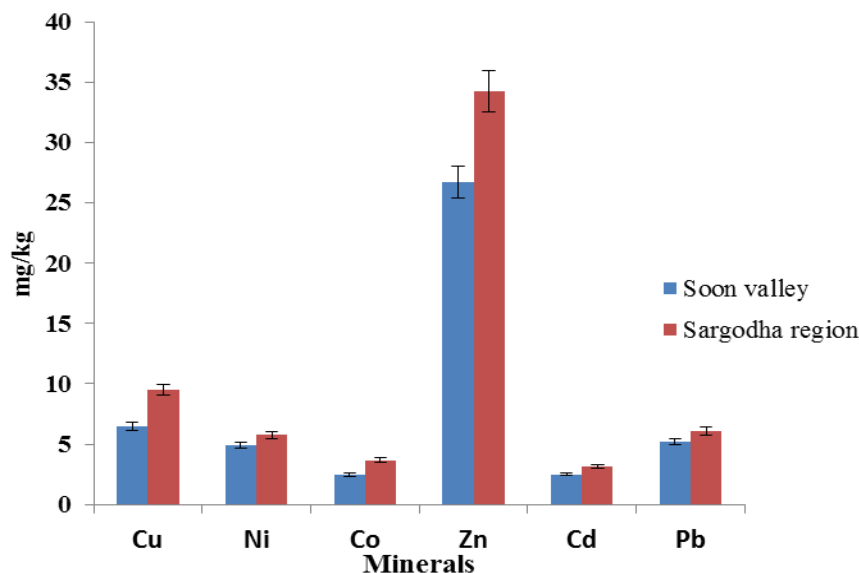


Figure 5. Mineral profile of turnip of two selected regions

Conclusion

The two selected samples of turnip revealed excellent antioxidant potential. By juxtaposing current study results with earlier employed sources of antioxidants, it is culminated that turnip is a powerful source of antioxidants. Results among two different varieties of turnip showed that antioxidant activity was superior in Sargodha region sample as compared to Soon valley sample. Among two different solvents i.e. methanol and ethanol (80%, 100%), methanol showed privileged yield as compared to ethanol. Higher antioxidant activity was observed in methanol solvent. Estimation of minerals through wet ash method from the peels of *Brassica rapa* revealed that Zn^{2+} concentration in turnip was highest 34.21mg in Sargodha region as compared 26.73mg in Soon valley. So, present results concluded that Sargodha region turnip showed maximum antioxidant activity and mineral profile as compared to Soon valley region turnip.

Authors' contributions

Conceived and designed the experiments: T Mehmood & M Iqbal, Performed the experiments: S Bashir & F Siddique,

Analyzed the data: T Mehmood, Z Jabeen & H Arshad, Contributed reagents/ materials/ analysis tools: T Mehmood & M Iqbal Wrote the paper: T Mehmood, F Siddique & H Arshad.

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