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Evaluation of Climatic and Topographical Effects on Bioactive Compounds and Mineral Profiles of Two Selected Samples of Cauliflower

Tahir Mehmood¹*, Lubna Nawaz¹, Mudassir Iqbal^{2,3}, Shoaib Ahmad Malik⁴, Aftab Safdar Khan¹, Faiza Siddique¹ and Qudsia Tabassam²

Abstract

The principal endeavor for this study was to appraise the phenolic content, total flavonoid content, antioxidant activity, antimicrobial activity and mineral profiling in Cauliflower (*Brassica oleracea botrytis*) extracts from two different regions, which are Soon valley and Sargodha vicinity, having diverse environmental and ground water impact. The extracts, taken out by 100%, 80% methanol and distilled water separately, showed crude yield in the range of 3.93 - 21.3 g/100g with no significant difference between the two areas. TPC (Total phenolic content) and TFC (total flavonoid content) were between 10.15 - 11.76 GAE mg/100mg and 15.72-33.50 mg/g respectively. Although TPC and TFC also differ in values depending upon the solvent system used but the extracts of Soon valley exhibit higher values, especially individual phenolic acids profile was significantly better for the extracts of Soon valley. Similarly, the reducing potential and DPPH radical scavenging assay showed the values of 214.28-1196.46 mg/mL and 0.55-3.16 mg/mL respectively. Moreover, the extracts demonstrated minerals contents in the range of 0.049-1.9650 mg/kg, with significant higher quantities of Pb and lower quantities of Co in the samples of Sargodha region. The extracts exhibited significant antimicrobial activity against *E. coli* (11.37- 21.81 mm/DW), *S. aureus* (8.22-18.27mm/DW), *B. substilis* (9.5-19. 56 mm/DW), and *S. typhae* (8.49-13.5 mm/DW), but the samples from Sargodha region were more potent. Study indicates that Cauliflower from Soon valley has more antioxidant activity, fewer amounts of heavy metals and lesser antimicrobial activity as compared to Sargodha region.

Keywords: Phenolics, Anitoxidants, Biological activity, Mineral profile, Cauliflower

Full length article Received: 29 Jan, 2016 Revised: Feb, 9, 2016 Accepted: Feb. 09, 2016 Available online: Feb.15, 2016 **Affiliations of authors**: ¹Department of Chemistry, University of Sargodha, 40100 Sargodha, Pakistan

²Department of Chemistry, School of Natural Sciences, National University of Sciences and Technology (NUST), H-12, Islamabad, 44000, Pakistan.

³Department of Chemistry, University of Fribourg, Chemin du Musee 9, Fribourg 1700, Switzerland.

⁴Department of Biochemistry, Sargodha Medical College, University of Sargodha, 40100 Sargodha, Pakistan.

*Corresponding Author: tahiruosbiochem@yahoo.com, tahirmehmood@uos.edu.pk

1. Introduction

The most important, safer and compatible sources of natural antioxidants are plants, vegetables and fruits. Substantial evidence is reported in the literature that the intake of natural products have been associated with lower incidences of chronic diseases such as cancer and heart disease. In addition to vitamins and minerals, phytochemicals such as flavonoids and phenolics may exert preventive and/or therapeutic effects for human diseases. Many of these natural products have antioxidant activity and may help to protect the cells against the oxidative damage caused by free radicals (Dimitios *et al.*, 2006). In this scenario, functional foods have a potentially positive effect on health beyond basic nutrition. (Sánchez-Salcedo *et al.*, 2015; Venkatesh *et al.*, 2008).

In living organisms oxidation reactions produce free radicals which cause the oxidation of bio-molecules like lipids, DNA, RNA and proteins etc. (Niwa *et al.*, 2001; Bergman *et al.*, 2001). Extra affinity of Lipids to lose electrons make them target for peroxidation and ultimately for the causation of diseases. Whereas the food issues concerned, the shelf life of fresh and processed foods

decreases by oxidative reactions. Lipids oxidation effects the color, taste and consistency of food (Siddiq *et al.*, 2005; Anwar *et al.*, 2007) resulting in economic loss (Akram *et al.*, 2009). The human health is also affected due to toxicity of oxidized oil and other preserved food stuff (Anwar *et al.*, 2006). Damage by free radicals in biological systems is retarded by antioxidants. Antioxidants have the ability to capture or quench those radicals that are involved in causing damage to human body and food. Inhibition of lipid per-oxidation and other radical mediated processes are also done by antioxidants (Anwar *et al.*, 2009). They are also very important in increasing the shelf life of food which is affected by oxidative reactions. Synthetic antioxidants are mostly used in food processing and pharmaceuticals industries, but they have many side effects such as Liver damage and carcinogenic effects (Anwar *et al.*, 2008). So, there is need to explore and isolate natural antioxidants, which have very fewer or no side effects and can be used in medicines in place of synthetic antioxidants (Karoui *et al.*, 2013).

Minerals, available in trace amount in living systems, are the major part of different enzymes that regulate the biological processes (Dashti *et al.*, 2004). There are 25 elements or minerals that are found in human body and among them 20 are most important (Crews, 1998). Lack of a particular mineral may lead to a specific disease in the living beings, like deficiency of iron may result in anemia and deficiency of iodine may cause goiter. Therefore, all these minerals and nutrients must be in proper range and concentration in body fluids, as higher amount also disturb the normal physiologic and metabolic functions of body or living beings. Fruits and vegetables have been a good source of all these required minerals (Connor, 1995) and thus their use is helpful in preventing diseases, maintaining body and blood pH and retaining proper physiologic functions (Underwood, 1971).

Brassica family (broccoli, Cauliflower, cabbage and Brussels sprouts) are the vegetables which are excessively used in the world and have enough amounts of antioxidants and other valuable phyto-chemicals in them (Gulcin *et al.*, 2004; Sharma *et al.*, 2005). Cauliflower plants can grow in a wide range of climatic, topographical and soil conditions, which may affect its chemical composition and nutritional status of the plants. Cauliflower may also contain different concentration of several minerals depending upon the area, atmospheric conditions, underground water and different types of fertilizers which are used to assist its growth during cultivation.

In this scenario, it is of interest to evaluate antioxidant attributes, biological activities, phenolic acids composition, and minerals profile of cauliflower, thereby to assess the effect of climatic, topographical and soil conditions on phenolics and flavonoids composition, antioxidant activity, and mineral profile. For this purpose depending upon the diverse environmental and ground water impact, two different samples of Cauliflower from two different areas, such as Sargodha region (S.R) and Soon Valley (S.V) of Pakistan, were collected and their above mentioned parameters were assessed by using organic solvent systems.

2. Material and Methods

2.1. Sample collection and preparation

Brassica olereacea samples were collected from Soon valley and Sargodha vicinity. The samples were thoroughly washed to remove dust and other impurities and were dried in open air. Controlled grinding is performed so that powder particles can pass through 80 mesh sieve. Ground powder of samples was placed in refrigerator for further analysis. All chemicals which were used in research analysis, were German made and of analytical grade, bought from Sigma Aldrich corporation.

2.2. Preparation of extracts

Extraction of samples was done by different solvents e.g. 100%, 80% methanol and distilled water. Ratio of sample and solvent was kept as 1/10 (w/v). Extraction was done for 6 hours thrice by using orbital shakers. Extract was separated by the process of filtration by keeping residue left behind. These extracts were concentrated by using rotary evaporator at reduced pressure. After that extract was kept in refrigerator at a temperature of 4 °C and then used for further analysis (Chatterjee *et al.*, 2010).

2.3. Total phenolic contents (TPC) determination

Folin Ciocalteu is used to assess the amount of TPC. 1 mL of distilled water is added in 100 mg crude extract of samples. 20 μ L of this prepared sample (2 mg/mL) was taken in test tube and marked the volume up to 0.5 mL by adding 480 μ L of distilled water. Then 0.1 mL (100 μ L) of Folin Ciocalteu (0.5 N) was added and keep the mixture for 15 minutes. Then 2.5mL of 7% Na2CO3 (w/v) solution was added to make the total volume 3.1mL. Incubation was performed for 30 minutes on room temperature. TPC amounts were calculated by using Gallic acid calibration curve within the range of (10-300) ppm (R2=0.9991) Figure. 1A. Results were expressed as Gallic acid equivalents GAE mg/100 g of dry plant matter.

2.4. Determination of Total Flavonoid Contents (TFC)

The contents of total flavonoid were determined by the procedure followed by (Zhishen*et al.* 1999). The results were expressed as catchin equivalent (10-500 ppm) (R2=0.918) CE mg/g of dry weight. Assay was performed in triplicate. Amounts of TFC were calculated by using calibration curve of catechin in the range of (10-500) ppm having (R2=0.991) Figure 1B and results were calculated as Catechin Equivalent (CE) per dry matter.

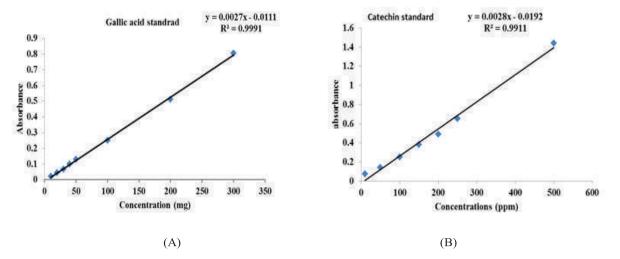


Figure 1. A. Gallic acid standard curve used for the calculation of Gallic acid equivalents for tested samples. B. Catechin standard curve used for the calculation of Catechin equivalents for tested samples

2.5. Assessment of antioxidant activity

The antioxidant activity of samples was assessed by 1, 1 diphenyl-2-picrylhydrazyl radical (DPPH) scavenging assay described by (Zhuang *et al.*, 2012). BHA that is synthetic antioxidant was used as a positive control. Calculation was made at IC50, concentration of extract that is scavenged/ neutralized 50% of DPPH free radicals.

2.6. Phenolics profile calculation

Phenolics profile was assessed by HPLC individually. Separation was carried out on HPLC ODS reversed phase column (Petropoulos et~al., 2002). Acidified acetonitrile (99.5%) was mobile phase at a constant rate of 1 mL/min. 20 μ L sample was injected in column every time and detection wavelength was 280 nm. Phenolic compounds were detected by comparing their retention time with pure standards of targeted compounds.

2.7. Reducing power determination

Cauliflower extracts reducing power was determined according to the procedure described by (Sahito *et al.* 2002) with a slight modification. Different concentrations of each sample (1-3 mg/mL) were made in phosphate buffer (pH 6.6) and total volume of solution was marked to 3.5 mL. Then 2 mL of 1% potassium ferricyanide was added. Place the mixture for 20 minutes in oven at 50 °C. Then 1 mL of 10% trichloroacetate was added and centrifuged for 10 minutes at 650 rpm. 2.5 mL of supernatant was separated and then 2.5 mL of distilled water was introduced and absorbance was taken at 700 nm. Reducing power was calculated by linear line equation.

2.8. Mineral Profiling

Wet digestion of the samples (both from Soon valley and Sargodha region) was performed to make the samples transparent and free from any organic matter. One gram of both the samples was weighed and treated with 5 mL of conc. HNO₃ in a beaker separately. The samples were heated at 70-80 °C for ten minutes followed by increase of temperature to 150 °C. Solvent was evaporated and residue was kept overnight. 5mL of conc. H₂SO₄ and 5 mL of H₂O₂ was added drop wise followed by 10 mL of deionized water. Then the solution was filtered and total volume of filtrate was made to 15 mL with deionized water (Adeyeye *et al.* 1999; Akpanyung 2005).

2.9. Preparation of Standard solutions for analysis

For determination of mineral profiling, standard solutions of seven metals were prepared from their parental salts. Standard solutions for Cd, Co, Cu, Ni, Cr, Pb were prepared from their sulphate salts and Zn solution was prepared from ZnCl₂ for determination of concerned elements by atomic absorption spectrophotometer. Absorption of series of standard solutions of known concentrations of each metal was determined and concentration of cauliflower samples was determined from graph.

2.10. Antibacterial activity

The Antibacterial activity of the extracts was determined by measuring minimum inhibitory concentration method (MIC). Nutrient agar (Oxoid) was prepared and then autoclaved. Before transferring this medium in sterilized Petri plates, 100 µL inoculum was added in medium while it was liquid and quite cool, mixed and then poured into petri plates. After that 6 mm Wicks paper discs were laid flat on growth medium and 100 ul was put on each disc. The Petri plates were then incubated at 37 °C for 24 h, for the growth of bacteria. The extracts having antibacterial activity, inhibited the bacterial growth and clear zone of inhibition was formed. The zone of inhibition was measured in millimeters (Sarpeleh *et al.*, 2009). The bacterial strains that used were *Bacilus Substilis*, *Streptococcus aureus*, *Salmonella typahe* and *E-coli*. Ciprofloxacin was used as standard.

2.11. Statistical analysis

Statistical Analysis Data was expressed as mean values at 95% confidence interval. Analysis of variance was performed using ANOVA procedures. Significance difference for values with respect to means of samples and solvents were determined. P> 0.05 means no significance difference. Analysis was done by using SPPS 16.0 software.

3. Results and Discussion

3.1. Extraction yields

The extraction of different compounds from plant material is dependent upon quantity, composition and nature as well as time duration consumed in extraction (Hsu *et al.*, 2006). Therefore optimal conditions should be availed to get excellent extraction yield. Data for the percentage yield of cauliflower samples extracted in pure methanol, 80% methanol and distilled water for Soon Valley and Sargodha regions is given in table 1.

Percent yield of dried extract of cauliflower in 80% methanol, 100 % methanol and in distilled water was observed to be 21.30%, 18% and 3.93% for Soon valley region, and 17.52%, 17.65% and 4.96% for Sargodha region respectively. It became evident from the results that generally high extraction yield was observed in cauliflower samples from Soon valley as compared to samples from Sargodha region. Statistical analysis (P>0.05) showed insignificant variation in percent yield of both extracts of cauliflower.

Table 1. Extraction yield (g/100g of dry weight) of cauliflower extracts using different solvents

Solvent system used for extraction	Soon valley cauliflower % Extraction yield	Sargodha region cauliflower
	70 Extraction yield	% Extraction yield

80% methanol	21.3 ± 0.397	17.52 ± 0.320
100% methanol	18.0 ± 0.246	17.65 ± 0.316
Distilled water	3.93 ± 0.203	4.93 ± 0.203

3.2. Total Phenlic content

The Folin ciocalteu method was used to access the total phenolic contents from cauliflower samples because this method has less interference and rapid in finding phenolic from sample (Sultana *et al.* 2007). The total phenolic contents of the dried extracts of cauliflower in 80% methanol, 100% methanol and in distilled water were observed to be 11.76, 11.45 and 11.17 (GAE) mg/100 g for soon valley region and 10.86, 10.59 and 10.15 (GAE) mg/100 g for Sargodha region respectively. It was clear from the results that TPC was in high concentration in the samples of soon valley as compared to the samples of Sargodha region. The values showed a slight difference between the TPC of the two regions. The maximum concentration was observed in samples extracted in 80% methanol. Furthermore, a minute effect of solvent composition on extracts of both regions was seen there. (Table 2)

Sample region	Solvent type	TPC(GAE) mg/100g
	80% methanol	11.76 ± 0.40
Soon valley	100% methanol	11.45 ± 0.30
	Distilled water	11.17 ± 0.09
	S.R 80 % methanol	10.86 ± 0.22
Sargodha region	S.R100% methanol	10.59 ± 0.08
	Distilled water	10.15 ± 0.30

Table 2. Total Phenolic content in two different samples of cauliflower

3.3. HPLC analysis for determination of individual phenolic acids

Three types of phenolic acids (gallic acid, protocatechuic acid and p-coumaric acid were present in cauliflower sample of both regions given in Table 3. Gallic acid was present dominantly in both regions. In Soon valley sample, Gallic acid was present in 100% methanol (10.52 mg/L) and in distilled water extract (24.14 mg/L) but was absent in 80% methanol. Similarly p-coumaric acid was (1.18 mg/L) in 80% methanol and (14.68 mg/l) in 100 % methanol, while the amount of protocatechuic acid was observed as (25.07 mg/l) in only 80% methanol. In Sargodha region sample, Gallic acid was present in 100% methanol as (16.81 mg/L) and in 80% methanol as (4.08 mg/L). The amount of protocatechuic acid was present in only distilled water extract as (36.3 mg/kg). While p-coumaric acid was absent in all three sample extracts of Sargodha region. It became evident from the results that the concentration of gallic acid is high in Sargodha region than in soon valley in same solvent (100% methanol). Furthermore the amount of protocatechuic acid was higher in distilled water extract than in 80% methanol.

Table 3. HPLC analysis for determination of phenolic acids

Sample region	Solvent type	Phenolic acids in mg/kg		
		Protocatechuic acid	1 *	Gallic acid
			acid	

Soon valley	80% methanol 100% methanol Distilled water	25.07± 0.061 - -	1.18± 0.085 14.68± 0.060	- 10.52± 0.260 24.14± 0.523
Sargodha region	80 %methanol 100%methanol Distilled Water	- 36.3± 0.176	- - -	4.08± 0.09 16.81± 0.136

3.4. Total Flavonoid content

TF contents were determined following a reported procedure (Dewanto *et al.* 2002). The total flavonoid content of dried extract of cauliflower in 80% methanol, 100% methanol and in distilled water was observed to be 15.72, 32.28 and 33.4 mg/g from soon valley region and 24.07, 22.50 and 33.50 mg/g from sample of Sargodha region respectively. High content of flavonoids was observed in extract of distilled water for both regions (Table 4).

Sample region Solvent type TFC (mg/g) Soon valley 80% methanol 15.72 ± 0.085 100% methanol 32.28 ± 0.165 Distilled water 33.4 ± 0.216 80 % methanol 24.07 ± 0.041 Sargodha region 100% methanol 22.50 ± 0.257 Distilled water 33.50 ± 0.238

Table 4. Determination of total flavonoids content

3.5. DPPH radical scavenging assay

All cauliflower samples extracted in 100% methanol, 80% methanol and distilled water were observed to exhibit appreciable scavenging activity ranging from 65.0-73.0%, 56.0-88.0%, 23-48% for soon valley region and 52.0-92.0%, 64.0-77.0%, 54.0-73.0% for sample of Sargodha region. DPPH radical scavenging activity increased in a concentration dependent manner (table 5). IC 50 values for 100% methanol, 80%methanol and distilled water were 453, 287.82 and 341.34 mg/mL for soon valley region and 214.28, 1196.46 and 447.36 mg/mL for Sargodha region.

3.6. Reducing power

The reducing potential was measured over the concentration range of 1-3 mg/mL. Results showed general increase in activity when concentration increased. Reducing potential of 80% methanol, 100% methanol and distilled water extracts of cauliflower were in the range of 0.155-3.14, 0.991-3.022, 0.871-3.08 respectively for soon valley sample and 1.09-3.16, 0.55-2.62 and 0.96-3.01 respectively for Sargodha region sample. Significant difference in reducing potential of both regions was observed at 2 mg/mL in 100% methanol cauliflower extract. The reducing potentials of various extracts are given in Table 6.

Table 5. Determination of DPPH radical scavenging activity (IC50)

Sample region	Solvent	IC 50 (mg/mL)

	80% methanol	287.82 ± 0.05
Soon valley	100% methanol	453.00 ± 0.015
	Distilled water	341.34 ± 0.10
Course dhe masiem	80% methanol	1196.46 ± 0.07
Sargodha region	100% methanol	214.28 ± 0.065
	Distilled water	447.36 ± 0.0035

3.7. Minerals Profile

The concentrations of various metals in Cauliflower from two selected regions are given in Figure 2. There is a considerable variation in mineral contents of vegetable among the two selected regions. Mineral concentrations are higher in Cauliflower of Sargodha region as compared to Soon Valley region. The difference of concentration is attributed to different quality of water used for irrigation purpose in the two selected regions. Regarding the mineral contents of Soon valley region, highest concentration was found for Ni (0.923 mg/kg) and lowest concentration was of Cd (0.049 mg/kg). While in Sargodha region, highest concentration was found for Pb (1.965 mg/kg) and lowest concentration was of Cd (0.061 mg/kg). The magnitude of minerals of Soon Valley region were of the order Cd < Co < Cu < Cr < Pb< Zn < Ni. The magnitude of minerals of Sargodha region was found to be Cd < Co < Cu < Cr < Pb< The accumulation of minerals in vegetables collected from Sargodha region is majorly due to irrigation by contaminated water usually.

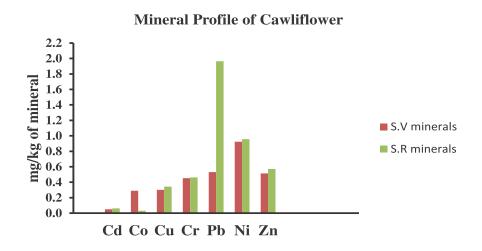


Figure 2. Minerals Profile of different metals

The Concentration of Pb was found to be (1.965 mg/kg) in Sargodha region and (0.531 mg/kg) in Soon valley region. According to Sharma *et al.* (2005) and Muchuweti *et al.* (2006) the concentration of Pb in vegetables collected from polluted waste water area exceeded the safety limits (2.5 mg/kg) and (2 mg/kg) respectively. The Concentration of Cu was found to be (0.343 mg/kg) in Sargodha region and (0.301 mg/kg) in Soon valley region. In polluted areas of Varanasi, India, the concentration of copper (2.25-5.42 mg/kg) was observed within safe limits (Tandi *et al.*, 2005). The Concentration of Cr was found to be (0.461 mg/kg) in Sargodha region and (0.452 mg/kg) in Soon valley region. And chromium concentration was within safety limit in vegetables grown in industrial area (Muchuweti *et al.*, 2006). The Concentration of Cd was found to be (0.061 mg/kg) in Sargodha region and (0.049

mg/kg) in Soon valley region. Various vegetables (cucumber, tomato, green pepper, lettuce, parsley, onion, bean, eggplant, pepper mint, pumpkin and okra) from Turkey were analyzed and higher content of Cd (0.24–0.97 mg/kg) injurious to human health was observed (Demirezen *et al.*, 2006). Our present study showed the both cauliflower samples to be the least accumulator of Cd.

Table 6. Determination of reducing power

Sample Type	Concentration (mg/mL)	Absorbance (nm)	Reducing Power (mg/mL)
	1	0.444	1.034 ± 0.002
S.V 80%	1.5	0.471	0.155 ± 0.003
Methanol	2	0.491	1.934 ± 0.004
	2.5	0.512	0.233 ± 0.005
	3	0.554	3.140 ± 0.003
	1	1.082	0.991 ± 0.004
S.V 100%	1.5	1.252	1.434 ± 0.003
methanol	2	1.542	2.18 ± 0.001
	2.5	1.616	2.382 ± 0.003
	3	1.862	3.022 ± 0.005
	1	1.006	0.871 ± 0.003
	1.5	1.164	1.79 ± 0.001
S.V Distilled	2	1.182	1.880 ± 0.002
water	2.5	1.264	2.360 ± 0.002
	3	1.39	3.080 ± 0.002
	1	0.649	1.090 ± 0.002
	1.5	1.692	1.480 ± 0.003
S.R 80%	2	0.742	1.920 ± 0.003
	2.5	0.791	2.36 ± 0.003
	3	0.88	3.160 ± 0.003
	1	0.498	0.790 ± 0.002
S.R 100%	1.5	0.556	0.550 ± 0.005
	2	0.582	$1.69\ 0\pm0.003$
	2.5	0.616	2.090 ± 0.003
	3	0.629	2.620 ± 0.004
	1	0.401	0.95 ± 0.003
	1.5	0.442	1.67 ± 0.002
S.R Distilled	2	0.451	1.83 ± 0.002
water	2.5	0.492	2.550 ± 0.004
	3	0.512	3.010 ± 0.004

Table 7. Antibacterial activity of different extracts of Cauliflower

	Antibacter	Antibacterial activity (mm) of different thyme extracts against four			
Solvents	bacterial species				
	Bacillus subtilus	Staphylococcus aureus	Salmonella typhi	Escherichia coli	
	Soon Valley Cauliflower				

100% methanol	13.56 ± 0.49^{a}	12.26 ±1.76 ^a	8.166±0.15 ^a	18.23± 0. 20 ^a
80% methanol	11.23 ± 0.20^{b}	8.22 ± 0.25^{b}	11.26±0.25 ^b	15.46 ± 0.20^{b}
Distilled water	$9.5 \pm 0.25^{\circ}$	$7.30 \pm 0.\ 10^{c}$	8.59±0. 10°	$11.37 \pm 0.43^{\circ}$
100%	Sargodha Region Cauliflower			
methanol	19. 56 ± 0.41^a	18.27 ± 0.15^{a}	13.5 ± 0.47^{a}	21.81 ± 0.27^{a}
80% methanol	15.66 ± 0.41^{b}	14.36 ± 0.15^{b}	11.96 ± 0.20^{b}	17.47 ± 0.25^{b}
Distilled water	10.91 ± 0.15^{c}	$8.5 \pm 0.13^{\circ}$	8.49±0.15°	14.88 ±0.30°
Ciprofloxacin	15.2 ± 0.2^{d}	10.21 ± 0.32 d	11.63±0.15 ^d	12.37 ±0.25 ^d

The Concentration of Zn was found to be (0.572 mg/kg) in Sargodha region and (0.513 mg/kg) in Soon valley region. When various vegetables (cucumber, tomato, green pepper, lettuce, parsley, onion, pepper mint, pumpkin and okra) were analyzed it was observed that the Zn concentration (3.56–4.592 mg/kg) was within the recommended international standards (Demirezen *et al.*, 2006). The Concentration of Ni was found to be (0.957 mg/kg) in Sargodha region and (0.923 mg/kg) in Soon valley region. The Concentration of Co was found to be (0.312 mg/kg) in Sargodha region and (0.289mg/kg) in Soon valley region. According to survey report of different fruits and vegetables in Egypt Pb, Co, Cu and Ni were observed with high values (Radwan *et al.*, 2006).

3.8. Determination of Biological Activity

The values of antibacterial activity of soon valley Cauliflower and Sargodha valley Cauliflower against *Bacillus subtilus*, *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* ranged from 9.1-29.46 mm of dry weight (Table 7). The maximum value was observed for 100% methanol (21.81 mm) Sargodha region Cauliflower extract against *E. coli* while minimum was observed for distilled water (9.1 mm) of Soon valley Cauliflower against *Staphylococcus aureus*. The antibacterial activity values for soon valley Cauliflower were in range of: 8.16-18.23 for 100% methanol, 8.22-15.46 for 80% Methanol and 7.30-11.37 for distilled water and for Sargodha region cauliflower were in range of: 13.5-21.81 for 100% methanol, 11.96-17.47 for 80% methanol and 8.5-14.88 for distilled water. Among the extraction solvents, overall the order of solvents for antibacterial activity against selected microorganisms was: 100% methanol > 80% methanol > distilled water showing significant (p < 0.05). From above presented results, it is concluded that Sargodha Region Cauliflower showed greater antibacterial activity as compared to Soon Valley Cauliflower against selected bacterial strains. These results are also comparable to those reported by (Khalid *et al.*, 2011) that Cauliflower has antimicrobial activity.

Conclusion

The study showed that the climatic and topographical changes, and variation in the composition of ground water and soil conditions affect the antioxidant attributes, phenolic acids composition, biological activities and minerals profiling of the cultivated crop, which here in the study is cauliflower. Although the extract quality greatly depends upon the solvent extraction system used, but the values of TPC (Total phenolic content), TFC (total flavonoid content), individual profiling of phenolic acids, reducing power, DPPH (2,2-diphenylpicrylhydrazyl) radical scavenging and mineral profiling assay greatly differ depending upon the area of the plant cultivation, which ultimately is linked to diverse climatic and topographical variations. Study indicates that Cauliflower from Soon valley has more antioxidant activity and fewer amounts of heavy metals as compared to Sargodha region. Moreover, the antimicrobial activity was found to be greater in Sargodha region Cauliflower. Cauliflower from Sargodha region might have higher antimicrobial activity due to greater minerals content of heavy metals, probably due to relatively polluted environment.

References

Adeyeye, E. and M. K. Otokili. 1999. Proximate composition and some nutritionally valuable minerals of two varieties of Capsicum annum. Discov. Innov.11, 75-81.

Akpanyung, E.O. 2005. Proximate and mineral composition of bouillon cubes produced in Nigeria. Pak. J. Nutri. 4:327-329.

Akram, N., F. Anwar and S. A. Raza. 2009. Effects of sunlight exposure on the quality and oxidative stability of sunflower and soybean oils. Asian J. Chem. 21: 2789-2798.

Anwar, F., M. Ali, A. I. Hussain and M. Shahid. 2009. Antioxidants and antimicrobial activities of essential oil and extracts of fennel (*Foeniculumvulgare* Mill.) seeds from Pakistan, Flavour Frag J. 24: 170-176.

Anwar, F., A. I. Hussain, S. Iqbal and M. I. Bhnanger. 2007. Enhancement of the oxidative stability of some vegetables oils by blending with *Moringaoleifera* oil. Food Chem. 103: 1181-1191.

Anwar, F., A. Jamil, I. Iqbal and M. A. Sheikh. 2006. Antioxidant activity of various plant extracts under ambient and accelerated storage of sunflower oil. Grasas Y Aceites. 57: 189-197.

Anwar, F., R. Naseer, M. I. Bhanger, S. Ashraf, F. N. Talpor and F. A. Aladedunye. 2008. Physico-chemical characteristics of Citrus seeds and seed oils from Pakistan, J. Am. Oil Chem. Soc. 85: 321-330.

Berggan, M., L. Varshavsky, H. H. Gottlieb and S. Grossman. 2001. The antioxidant activity of aqueous spinach extract: chemical identification of active fraction. Phytochem. 58: 143-152.

Chatterjee, S., P. S. Variyar and A. Sharma. 2010. Bioactive lipid constituents of fenugreek. Food Chem. 119: 349-353.

Connor, B.B. 1995. Vernacular health care responses to HIV and AIDS. Alternative Therapies 1(5):35-52/wood RJ (2000). Assessment of marginal zinc status in humans. J.Nutr. 130: 1350-1354.

Crews, H.M. 1998. Speciation of trace elements in foods, with special references to cadmium and selenium: is it necessary? SpectrochemicalActa.Part B. 53: 213-219.

Dashti, B., F. Al-Awadi, R. Alkandari, A. Ali and J. Al-Otaibi. 2004. Macro- and microelements contents of 32 Kuwaiti composite dishes. Food Chem. 85: 331-337.

Demirezen, D and A. Ahmet. 2006. Heavy metal levels in vegetables in turkey are within safe limits for Cu, Zn, Ni and exceeded for Cd and Pb. J. Food Qual. 29: 252-265.

Dewanto, V., X. Wu, K. K. Adom and R. H. Liu. 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J. Agri. Food Chem. 50: 3010-3014.

Eva, M., S. Salcedo, M. Pedro, C. García-Viguera, F. Hernández and J. J. Martínez. 2015. (Poly)phenolic compounds and antioxidant activity of white (*Morus alba*) and black (*Morusnigra*) mulberry leaves: their potential for new products rich in phytochemicals. J. functional foods.

Gulcin, I., G. I. Sat, S. Beydemir, M. Elmastas and O. I. Kufrevioglu. 2004. Comparison of antioxidant activity of clove (*Eugenia caryophylata*Thunb) buds and lavender (*Lavandulastoechasl*.) Food Chem. 87: 393-400.

Hsu, J. L., C. Y. Su and J. W. Lin. 2006.Resection of a granular cell tumor of the larynx followed by midializationlaryngoplasty with bipedicledsternohyoid muscle transposition. Otolaryngology. 135: 983-985.

Karoui, J.I. and B. Marzouk. 2013. Characterization of bioactive compounds in Tunisian bitter orange (*Citrus aurantium* L.,) peel and juice and determination of their antioxidant activities. BioMed. Res. Int. 1-12.

Muchuweti, M., J.W. Birkett, E. Chinyanga, R. Zvauya, M.D. Scrimshaw and J.N. Lester. 2006. Heavy metal content of vegetables irrigated with mixtures of wastewater and sewage sludge in Zimbabwe: Implications for human health. Agric. Ecosys. Environ. 112: 41-48.

Niwa, T., U. Doi, Y. Kato and T.Osawa, T. 2001. Antioxidant properties of phenolic antioxidants isolated from corn steep liquor. J. Agric Food Chem. 49: 177-182.

Petropoulos, G.A. 2002. Fenugreek - The genus Trigonella. Taylor and Francis, London and New York, 255.

Radwan, M.A. and K. A. Salama. 2006. Market basket survey for some heavy metals in egyptian fruits and vegetables. Food chem. Tox. 44: 1273-1278.

Sahito, A., T. G. Khazi, M. A. Jakhrani, G. H, Kazi, G. Q. Shar, M. A. Ana M.A.Memon. 2002. Investigation of *Momordicacharantia*Linn;and*Syziginmjambolana*Linn; using atomic Elemental absorption apectrophotometer. The Nucleus. 39: 49-45.

Sarpeleh, A., K. Sharifi and A.Sonbolkar, A. 2009: Journal of Plant Diseases and Protection. 5, 208.

Sharma, S.R., P. K. Singh, V. Chable and S. K. Tripathi. 2005. A review of hybrid cauliflower development. J. New Seeds. 6:151-193.

Siddiq, A., F. Anwar, M. Manzoor and A. Fatima, A. 2005. Antioxidant activity of different solvent extracts of *Moringaoleifera* leaves under accelerated storage of sunflower oil. Asian J. Plant Sci. 4: 630-635.

Sultana, B., F. Anwar and R.Przybylski. 2007. Antioxidant potential of corncob extracts for stabilization of corn oil subjected to microwave heating. Food Chemistry. 104: 997-1005.

Tandi, N.K., J. Nyamangara and C. Bangira, C. 2005. Environmental and potential health effects of growing leafy vegetables on soil irrigated using sewage sludge and effluent: A case of Zn and Cu. J. Environ. Sci. Health. 39, 461-471.

Underwood, E.J. 1971. Trace Elements in Humans and Animal Nutrition, 3rd Edition, Academic press, New York p.116./;darby WJ (1976). Trace elements in human health and disease, Prasad As and oberleas D.Eds (Academic Press, New York, San Francisco, London) 1:17.

Venkatesh, K.P and S. Chauhan. 2008. Mulberry Life enhancer. J. Med Plants Res. 2: 271-278.

Zhishen, J., T. Mengchemg and W. Jianming. 1999. The determination of falvonoid contents in mulberry and their scavenging effects on superoxide radicals, Food Chem. 64: 555-559.

Zhuang, Y., L. Chen, L. Sun and J. Cao. 2012. Bioactive characteristics and antioxidant activities of nine peppers. J. Functional Foods. 4: 331-338.