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Research Article

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ANTIOXIDANT ACTIVITY OF GARLIC (*ALLIUM SATIVUM* L.) CROPS GROWN IN NORTH WESTERN INDIAN MOUNTAINS

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Abstract

Allium sativum L. (Garlic), most commonly used as a favorite additive in Indian food possesses antimicrobial, antimutagenic, antiplatelet and antihyperlipidemic properties. Very little scientific information is available on the antioxidant properties of garlic crops produced locally in Kullu Valley of Himachal Pradesh, India. The present study investigates the total phenolics and flavonoids contents in methanol extracts of garlic bulbs and their antioxidant activities using *in-vitro* assays such as 1, 1- diphenyl-2- picrylhydrazyl (DPPH) radical, 2, 2'-azinobis-3-ethylbenzothiazoline-6-disulphonic acid (ABTS) and ferric reducing antioxidant power (FRAP).. The results showed that garlic bulbs had significant amounts of total phenolics and flavonoids, and also possess potential antioxidant activities. The test parameters varied significantly with altitudes ($p<0.05$). Pearson's relationship study revealed that soil properties had significant influence on the antioxidant activities of *A. sativum.* This study concludes that local agricultural practices and micro-climatic conditions of Kullu Valley potentially affect antioxidant potential of *A. sativum* crops. This study also suggests that locally produced garlic crops can be consumed by mountain peoples for their health benefits.

Keywords: *Allium sativum*; Altitudes; Antioxidant activities; Soil properties; Kullu Valley

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Introduction

Farming systems have strong influence on soil properties, such as organic matter, pH and major nutrients such as Na, K and Ca. The uptake and accumulation of trace metals such as Cd, Zn, Cu, etc. in vegetable crops also varied with seasons and agriculture fields (Sharma *et al.*, 2007). The climate changes influence the antioxidant properties *of Labisia pumila* Blume was reported by Ibrahim and Jaafar (2011). Garlic (*Allium sativum* L.) has been widely used as both a folk medicine and as a spice for thousands of years. Tocopherols, flavonoids, carotenoids and amino acids have been recognized as potent natural antioxidants and the antioxidative activity of spices has also been reported (Kim et al. 1997). Organosulfur compounds of garlic, promotes lipid lowering, antithrombotic, anti-blood coagulation, anti-hypertension, anticancer, antioxidant and antibacterial effects (Rahman et al. 2012). Lanzotti (2006) demonstrated that total phenolics content in red onion (*A. cepa* L. var. *ascalonicum* Backer) was higher than garlic (*A. sativum*). Environment factors such as altitude, temperature and physiological properties of the soil influenced antioxidant activities of medicinally and economically important plants (Sharma et al., 2012; 2013).

Very little scientific information is available on antioxidant properties of garlic, one of the main cash crops produced locally in Kullu Valley of Himachal Pradesh, India. Therefore, the present study investigated the total phenolics and flavonoids contents in methanol extracts of garlic bulbs and their antioxidant activities using *in-vitro* assays such as 1, 1- diphenyl-

2- picrylhydrazyl (DPPH) radical, 2, 2'-azinobis-3-ethylbenzothiazoline-6-disulphonic acid (ABTS) and ferric reducing antioxidant power (FRAP). A correlation study was also performed to assess the effects of altitudes and soil properties on antioxidant activities of garlic crops.

MATERIAL AND METHODS:-

The samples of edible parts of garlic crops (bulbs) were collected from agriculture fields of Pahnala, Tarigran and Kamandh, located at 1500m, 1800m and 2100m amsl, respectively in Mohal-Khad watershed of Kullu Valley of Himachal Pradesh, India at the age of maturity during May-June 2014. Soil samples were also collected in triplicates along garlic samples. Both soils and cloves of garlic were transported to laboratory. Soils were kept as such in a room at normal temperature for air dry till a constant weight was obtained. The samples of cloves of garlic were washed under running tap water to remove the adhered soil particles and other unwanted plant materials. The bulbs were separated from whole plants and kept in a refrigerator at 4 ^{0}C till antioxidant extraction.

Soil pH and electrical conductivity (EC) was measured in suspension of soil to water in a ratio of 1:5 (w/v) using a glass electrode attached to a multi-parameter system (Eutech, PC 700). Organic carbon (OC) in soil was determined using a method of Walkley and Black's rapid titration (Allison, 1986) and organic matter (OM) was calculated using a multiplying factor i.e. 1.72. Total nitrogen content in soil was determined following micro – KJEldaHAL technique through the Gerhardt Automatic Analyzer (KES 12L, KEL PLUS, India). Soil (1g) was digested with 15 ml of HNO_3, H_2SO_4 and $HClO_4$ in the ratio of 5:1:1 at 80^{0}C till a transparent solution was obtained (Allen *et al*., 1986) and used for determination of total P using method of Olsen and Sommus (1982). Total K, Na and Ca were determined in digested sample using a flame photometer (MJ 272, Rescholar, India). Available N of the soil was determined in the solution of TISAB and samples in a ratio of 2:1 using ion analyzer equipped with nitrate electrode (pH/Ion 340i, WTW, Germany). Available P was also estimated in soil samples using the method of (Olsen *et al*., 1954).

Bulbs (1 g approx, fresh weight) were crushed in 10ml of 80% (v/v) methanol using a mortar and pestle and kept at 4 ^{0}C in a refrigerator for overnight. The extracts were centrifuged at 5000×g and suspension was maintained to 10ml using 80% (v/v) methanol and stored in a refrigerator at 4 ^{0}C for the further analysis. The amounts of total phenolics (TPC) and flavonoids (TFC) in methanol extracts of garlic bulbs expressed as mg gallic acid equivalent (GAE)/100g fresh weight and mg quercetin equivalents (QE) /100g fresh leaf, respectively were quantified using Folin-Ciocalteu reagent and $AlCl_3$ (prepared in absolute alcohol) reagents as per methods described by Wolfe *et al.* (2003) and Ordon-Ez *et al. (*2006), respectively. Antioxidant capacity of the extracts expressed as mM ascorbic acid equivalents (AAE)/100g fresh weight was further assessed using three *in-vitro* assays namely, 1, 1- diphenyl-2- picrylhydrazyl (DPPH) radical, 2, 2'-azinobis-3-ethylbenzothiazoline-6-disulphonic acid (ABTS) and ferric reducing antioxidant power (FRAP) using the methods of Liyana- Pathirama *et al.* (2005), Re *et al*. (1999) and Benzjie and Strain (1996), respectively.

Generated data were presented as mean ± SE of three independent measurements. Treatment means were separated using Duncan's Multiple Range Test and were considered significant at 5%. Pearson's correlation was also developed between the antioxidant properties of garlic and soil properties. All the analysis were also performed by using SPSS software

RESULTS AND DISCUSSION:-

The physicochemical properties of growing media i.e. soils are influencing the growth and development of plants by altering their production capacities of enzymatic and non-enzymatic antioxidants (Sharma *et al.,* 2010; 2013). The physicochemical properties of soil collected from different study sites were given in Table 1. Soil pH was found slightly alkaline, varied significantly between the sites and ranged between 7.63-7.76 (Table 1).

Table 1: Altitudinal changes in physicochemical properties of soil in Mohal-Khad watershed, Kullu, Himachal Pradesh

Soil properties	Altitudes (m amsl)			P - levels
	1500	1800	2100	
pH (water)	7.76 ± 0.46	7.71 ± 0.22	7.63 ± 0.08	0.953
EC (mS^{-1})	246.33 ± 45.3	286.67 ± 10.4	296.67 ± 20.8	0.899
OC (%)	2.28 ± 0.03	2.82 ± 0.10	1.92 ± 0.07	0.000
OM (%)	3.93 ± 0.06	4.86 ± 0.18	3.31 ± 0.12	0.000
Total N (%)	0.49 ± 0.01	0.82 ± 0.01	1.03 ± 0.03	0.000
Total P(µg/g dw)	36.02 ± 0.12	35.09 ± 1.05	33.23 ± 0.33	0.055
Total K (g/kg dw)	2.75 ± 0.10	3.48 ± 0.19	4.28 ± 0.05	0.000
Total Na (g/kg dw)	0.36 ± 0.03	0.45 ± 0.01	0.43 ± 0.02	0.040
Total Ca(g/kg dw)	0.38 ± 0.02	0.36 ± 0.02	0.46 ± 0.01	0.005
Available N (g/kg dw)	0.56 ± 0.03	0.33 ± 0.02	0.22 ± 0.01	0.000
Available P (µg/g dw)	0.50 ± 0.02	0.78 ± 0.02	0.43 ± 0.02	0.000

Values are mean ± SE of three replicates.

Values followed by the different letter in a row are significantly different from each other at p<0.05.

Electrical conductivity in soil ranged between 246.33 mS^{-1} - 296.67 mS^{-1}. Organic carbon, organic matter, total N, P, Na, K, Ca in soil ranged between 1.92%-2.82%, 3.31%- 4.86%, 0.49%-1.03%, 33.32µg g^{-1}dw-36.02µg g^{-1} dw, 0.36 g/kg dw-0.45g/kg dw, 2.75g/kg dw-4.28g/kg dw and 0.36g/kg dw-0.46g/kg dw, respectively (Table 1). Available N and P in test soils also ranged from minimum to maximum as 0.22g/kg dw to 0.56g/kg dw and 0.43µg g^{-1} dw to 0.78µg g^{-1} dw, respectively. The highest soil pH, available N and total P were found at low altitude i.e. 1500m amsl, whereas EC and content of total N, K and Ca in soil were found at highest altitude i.e. 2100m amsl (Table 1). The highest content of available P, Organic carbon and organic matter in the soil was found at 1800m amsl. These variations in soil properties were ascribed to local agricultural practices of at different altitudes.

Table 2: Altitudinal changes in biochemical yields of edible part of *A. sativum* grown in Mohal-Khad watershed, Kullu, Himachal Pradesh

Biochemical yields	Altitudes (m amsl)			P- levels
	1500	1800	2100	
Total Phenolics (mgGAE/ 100g fw)	60.49 ± 0.38	41.43 ± 0.37	67.37 ± 0.62	0.000
Total Flavonoids (mgQE/100g fw)	109.43 ± 3.21	118.02 ± 2.51	164.90 ± 3.55	0.000
DPPH (mM AAE/100g fw)	24.57 ± 2.03	42.03 ± 2.34	48.71 ± 1.85	0.000
ABTS (mM AAE/100g fw)	9.88 ± 0.02	9.59 ± 0.02	9.71 ± 0.01	0.000
FRAP (mM AAE/100g fw)	10.62 ± 0.09	12.67 ± 0.31	16.31 ± 0.47	0.000

Values are mean ± SE of three replicates.
Values followed by the different letter in a row are significantly different from each other at p<0.05.

Numbers of studies have shown that high total phenolics contents in plants are associated with greater antioxidant activity (Zheng *et al.,* 2001; Iwalokun *et al.,* 2006; Sharma *et al.,* 2012, 2013). In the present study, the total phenolics contents showed significant variations between the sites (Table 2). The contents of total phenolics in the bulbs of *A. sativum* (mg GAE/100g) among different study sites varied between 41.4 - 67.3 with their minimum and maximum contents at 1800m amsl and 2100m amsl, respectively (Table 2). Significant variations in the total phenolics contents in fruits of a wild edible plant *Myrica esculenta* have also been reported by Rawat *et al*. (2011). The total flavonoids contents in bulbs of *A. sativum* expressed as mg QE/100g dw ranged between 109.43 to 164.90 (Table 2). Like total phenolics contents, minimum and maximum total flavonoids contents were also found at 1500m amsl and 2100m amsl, respectively (Table 2). Earlier reports regarding three varieties of garlic demonstrated that the flavonoid and steroid contents in garlic extract had a contribution in an antioxidant capacity (Narendhirakaman, 2010). Variations in total phenolics and total flavonoids contents in different parts of *A. sativum* may be due to local climatic conditions and soil physico-chemical parameters. The results of the present study clearly indicate that the medicinal potential of *A. sativum* plants is controlled by multiple environmental factors and highest total phenolics and flavonoids contents are found at an altitude of 2100m amsl. A similar finding was also reported by Rawat *et al*. (2011).

Antioxidant capacities of bulbs extract of *A. sativum* evaluated in terms of DPPH, ABTS and FRAP assays were given in Table 2. DPPH assay has been used by most of the scientists for the determination of primary antioxidant activity of economically important plants (Sharma *et al.,* 2012, Carene *et al.,* 2014). Antioxidant compounds in a plants extract have potential to donate hydrogen which react with DPPH radical and consequently decrease its content in the reaction mixture (Kumaran and Kavan, 2007). DPPH, ABTS and FRAP activities in the methanol extracts of garlic bulbs (mM AAE/100g fw) varied between 24.57-48.71, 9.59-9.88 and 10.62-16.31, respectively (Table 2). Minimum and maximum DPPH and FRAP activities were found at 1500m amsl and 2100m amsl in bulbs, respectively, whereas ABTS activity was found at 1800m amsl and 1500m amsl. Study of Najja *et al*., (2011) showed that trends of DPPH activity in *A. roseum* were similar with ABTS activity in the present study. The significant variations in antioxidant properties of methanol extracts of bulbs ($P< 0.05$) are described to local agricultural practices and altitudinal variations. In the present study, DPPH and FRAP activities in bulbs of *A. sativum* were maximum at altitude of 2100m amsl. Rawat *et al*. (2011) also reported significant variations in antioxidant activities of fruits of *M. esculenta* populations growing at different altitudes ranging between 1775m-2100m amsl. Moure *et al.,* (2000) pointed out that the antioxidant activity of phenolic compounds may depend on factors such as growing conditions, quality and origin i.e. geographical locations of plants as well as the extraction and purification methods used to determine the antioxidant activity.

Soil properties are playing an important role in uptake and accumulation of mineral nutrients and consequently affecting growth and development of the plants by enhancing their free radical scavenging potential (Sharma *et al.,* 2010a, b; 2013). Therefore, in the present study the relationships between physico-chemical properties of soil with total phenolics and flavonoids contents in methanol extracts of *A. sativum* and their antioxidant activities was developed and the results were given in Table 3.

Table 3: Correlation between soil and extract properties of edible part of *A. sativum* grown in Mohal-Khad watershed, Kullu, Himachal Pradesh

Soil properties	Extract properties				
	TPC	TFC	DPPH	ABTS	FRAP
pH	-0.03 NS	-0.18 NS	+0.02 NS	+0.05 NS	-0.17 NS
EC	-0.03 NS	+0.29 NS	+0.20 NS	-0.33 NS	+0.04 NS
OC	-0.95**	-0.67 NS	-0.18 NS	-0.48 NS	-0.51 NS
OM	-0.95**	-0.67 NS	-0.18 NS	-0.48 NS	-0.51 NS
Total N	+0.14 NS	+0.84**	+0.94**	-0.64 NS	+0.95**
Total P	-0.36 NS	-0.71*	-0.73*	+0.24 NS	-0.80**
Total K	+0.26 NS	+0.91**	+0.86**	-0.57 NS	+0.92**
Total Na	-0.40 NS	+0.40 NS	+0.65 NS	-0.83**	+0.47 NS
Total Ca	+0.75*	+0.88**	+0.46 NS	+0.05 NS	+0.72*
Available N	-0.05 NS	-0.82**	-0.91**	+0.72*	-0.81**
Available P	-0.99**	-0.52 NS	+0.02 NS	-0.67 NS	-0.32 NS

Levels of significance: *=p<0.01, **=p <0.001; NS: not significant

Results showed that significant and both positive and negative relationships between the soil properties and total phenolic and flavonoid contents in the methanol extracts of *A. sativum.* Organic carbon and organic matter had negative and significant relationship with TPC (R = 0.95, P<0.001; R = 0.95, P<0.001). Total N content of soil showed significant and positive relationship with TFC (R =0.84, P<0.001), DPPH (R =0.94, P<0.001) and FRAP (R =0.95, P<0.001) activity. Total P had significant and negative relationship with TFC (R =0.71, P<0.01), DPPH (R =0.73, P<0.01) and FRAP (R =0.80, P<0.001) activity. K had significant and positive relationship with TFC (R =0.91, P<0.001), DPPH (R =0.86, P<0.001) and FRAP (R =0.92, P<0.001) activity. Ca further showed significant and negative relationship with TPC, TFC and FRAP (R =0.75, P<0.01; R =0.88, P<0.001 and R =0.72, P<0.01, respectively). Na showed significant and negative relationship with ABTS (R =0.83, P<0.001) activity. Available N showed significant and positive relationship with ABTS (R =0.72, P<0.01) activity and negative relationship with TFC (R =0.82, P<0.001), DPPH (R =0.91, P<0.001) and FRAP activity (R =0.81, P<0.001) in the bulb of *A. sativum.* Available P in soil showed significantly and negative relationship with TPC (R =0.99, P<0.001). The present results clearly indicate that the TPC, TFC in extracts of *A. sativum* and their antioxidant properties are significantly affected by physico-chemical properties of the soil (Table 3). However, soil pH and EC did not show any relationship with TPC, TFC and DPPH, ABTS and FRAP activities of *A. sativum.*

Conclusions

In the present study, total phenolics and flavonoid contents in methanol extracts of *A. sativum* collected from three different altitudes of Kullu Valley and their antioxidant properties were evaluated. The study revealed that edible parts i.e. bulbs of *A. sativum* are a rich source of nutritional total phenolics and possess potent antioxidant activities. It was observed that these properties of *A. sativum* were further influenced by altitudinal changes as well as soil properties significantly (p<0.05). Thus, management of soil quality could be mean to improve the antioxidant properties of *A. sativum*. The present study suggests that locally produced garlics can be consumed by mountain peoples for their health benefits.

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