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FREE RADICAL SCAVENGING ACTIVITY OF *ALLIUM ASCALONICUM*

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ABSTRACT
50g of *Allium ascalonicum* L was homogenized in 100 mL of methanol and the extract was used for the determination of radical scavenging activity, chain-breaking activity and reducing capacity. Time dependent free radical scavenging activity was studied for 20, 40 and 60 mg of extract of *A.ascalonicum*. It was observed that 20mg extract *A. ascalonicum* scavenges 43 % after 5 hr and 60% after 24 hr. Ascorbic acid (control) had showed 84% of NO scavenging activity while 60 mg of *A.ascalonicum* extract showed nearly 60% of NO scavenging activity which is highly significant. The free radical scavenging effect of *A.ascalonicum* could be due to high amounts of quercetin present in it. The other phytochemicals present in the extract of *A.ascalonicum* could have the synergetic effect. These findings are correlated with its phytochemical analysis.

INTRODUCTION

Free radicals are unstable molecules that include the hydrogen atom, nitric oxide (NO) and reactive oxygen species. They naturally occur in the body as a result of chemical reactions during normal cellular processes and can also be formed in response to excess pollution, UV rays etc. The increasing acceptance of free radicals in common place and important biochemical intermediates, they have been implicated in a large number of human diseases.

Production of free radicals causes depletion of antioxidants. Low levels of essential antioxidants in the circulation have been found to be associated with an increased risk of cancer (Skryzdlewska *et al*., 2001). Currently available synthetic antioxidants like BHT, BHA, tertiary butylated hydroquinone and gallic acid esters have been known to cause negative health effects.

Phytochemicals and antioxidant constituents in plant material have raised interest among scientists for their roles in maintenance of human health. Frequent consumption of fruits and vegetables is associated with a lowered risk of cancer, heart disease, hypertension and stroke (Vinson *et al.,* 2001 and Wolfe and Liu, 2003).

Polyphenols are common constituents of food of plant origin and major antioxidants of our diet (Ranilla *et al.,* 2007). Flavonoids, a large family of polyphenolic compounds that are ubiquitous in nature and are categorized, according to chemical structure, into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones (Stewart *et al.,* 2000). Many of these phytochemicals may help to protect cells against oxidative damage caused by free radicals (Wada and Ou, 2002).

Our previous study showed that methanolic extract of *A.ascalonicum* possessed high amounts of total phenols and flavonoids (52.54mg/100g FW, 681mg /100g FW) and quercetin was the major flavonoid present (by HPLC analysis) (Vanitha *et al.,* 2009).

Many recent studies showed that *Allium* have antioxidant effect what could be of the great importance in preventing and treating different diseases and may contribute to its therapeutic potential. Although there is abundant literature about medicinal properties of *Allium ascalonicum* there is scanty work on its free radical scavenging effect and antioxidant potential. It was therefore planned to study the antioxidant capacity and scavenging activity, chain breaking activity to assess their antioxidant potential. These studies may through light on its use as a therapy for free radical induced cancer.

MATERIALS AND METHODS

Red onions (*Allium ascalonicum.L*) were obtained from the local vendor without any external defects. The external peel was removed and bulbs which are uniform were selected for analysis. Analysis was carried out on the edible portion and the results were expressed in terms of fresh weight (FW).

Extraction

50g of *Allium ascalonicum* tissue was homogenized in 100

mL of methanol using a Waring blender at 2000 rpm for 1 min at 4ºC. The extract was stirred for 10 min at 4ºC and filtered through four layer of cheesecloth and the residue was re-extracted under the same condition with 100 mL of methanol. The combined filtrate was concentrated under vacuum at 65ºC to dryness and the dry residue was dissolved in methanol. These methanolic extracts were used for the studies.

Determination of free radical scavenging activity

Free radical scavenging activity was determined by the method of Koleva *et al.,* (2002). Different concentrations of plant extract were added, to methanolic solution of DPPH 1M. After incubation for 15 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated for three times. BHT was used as standard control. Free radical scavenging activity toward DPPH was estimated from the following equation:

$$\% \text{ inhibition} = (A_{control} - A_{smple}/A_{control}) \times 100$$

Determination of nitric oxide scavenging activity

Nitric oxide scavenging activity was measured spectrophotometrically (Govindarajan *et al.,* 2003). Sodium nitroprusside (5mmol) in phosphate buffered saline was added to different concentrations of the extract dissolved in methanol and incubated at 25ºC for 30 min. A control without the test compound but with an equivalent amount of methanol was taken. After 30 min of incubation 1.5 mL of the incubation solution was removed and diluted with 1.5 mL of Griess reagent (1%sulphanilamide, 2% phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with naphthyl ethylene diamine dihydrochloride was measured at 546 nm.

Determination of chain breaking activity

The chain-breaking activity was assessed by the method of Manzocco *et al.* (1998). A volume of 3 mL of 6.1×10^{-5} M DPPH in methanol was added to different concentrations of extract. After 60 min incubation at room temperature, the absorbance was read at 515 nm. The chain-breaking activity was expressed by the reaction rate *k* and calculated by the following equation:

$$1/A^3 - 1/A_0^3 = -3kt$$

Where A_0 is initial optical density, *A* is optical density at increasing time, *t*. The reaction rate was expressed as *k* mL^{-1} ($-OD^{-3}$ min^{-1} mL^{-1}).

Assessment of reducing activity

The reducing capacity (*RP*) of the extract was assessed by the method of Oyaizu (1986). 2 mL of extract was added to potassium ferricyanide (2.5mL, 10g/L^{-1}) and the mixture incubated at 50ºC for 20 min. Trichloroacetic acid (2.5mL, 100g/L^{-1}) was added to the mixture, which was then centrifuged at 650 × *g* for 10 min. The supernatant (2.5 mL) was mixed with distilled water (2.5mL) and ferric chloride (0.5 mL, 1g/L^{-1}). The absorbance was read at 700 nm. Higher absorbance indicated greater reducing capacity which is calculated as follow:

$$RP = [A_m / A_b - 1] \times 100$$

A_m = absorbance of reaction mixture
A_b = absorbance of blank mixture (distilled water instead of extract).

Statistical analysis

All determinations were conducted in triplicate, and experiment was run for six different preparations. The values are expressed as mean ± SD. Statistical analysis was done by students't' test and " p " value was arrived at to assess the statistical significance of changes observed. P value less than 0.02 was considered significant.

RESULTS AND DISCUSSION

Time dependent free radical scavenging activity of *A.ascalonicum* has been shown in Table 1. It was observed from the Table 1 that 20 mg of methanolic extract of *A.ascalonicum* produced nearly 43% scavenging and 60 mg showed 63% scavenging after 5hr. Increase in concentration of the methanolic extract showed proportional increase in the % of scavenging as shown in Table 1. After 24 hr incubation of DPPH with 60 mg of methanolic extract of *A.ascalonicum* there was nearly 77% scavenging (Fig. 1). Table 2 shows Pearson's correlation between phenolic content and free radical scavenging activity of *A.ascalonicum*. It is observed from the table that there is a positive correlation between total phenolic contents and radical scavenging activity of *A.ascalonicum*. (r = 0.675). It is observed from the Table 2 that the correlation between the flavonoid contents and free radical scavenging activity of *A.ascalonicum* is high (r = 0.7537) when compared to the total phenolic contents and radical scavenging activity. Thus it is clearly evident that flavonoids play a major role in scavenging the free radicals. Fig. 2 shows the nitric oxide scavenging activity of the methanolic extract of *A.ascalonicum*. It is observed from the figure that there is a dose dependent NO scavenging activity. Ascorbic acid (control) had showed 84% of NO scavenging activity. 60 mg of *A.ascalonicum* extract showed nearly 60% of NO scavenging activity which is highly significant. Table 3 shows the comparison of scavenging activity of methanolic extract of *A.ascalonicum* on ROS and RNS. It is observed from the table that 20mg of extract could efficiently scavenge nitrogen free radicals than oxygen free radicals significantly ($p < 0.001$).Table

Table 1: Time dependent free radical scavenging activity of *allium ascalonicum* extract

Concentration of extract(mg/ ml)	% of inhibition with time (hours) 1/2	1	1 1/2	2	2 1/2	3	3 1/2	4	4 1/2	5
20	9.2 ± 0.3	12.4 ± 0.7	17 ± 1.0	21.0 ± 1.5	25.1 ± 1.8	28.3 ± 2.1	31.2 ± 2.5	35.1 ± 2.9	38.1 ± 3.2	42.5 ± 3.5
40	16.86 ± 0.9	20.1 ± 1.2	25.3 ± 1.7	29.8 ± 2.1	33.4 ± 2.5	38.3 ± 2.9	42.2 ± 3.4	45.5 ± 4.0	49.1 ± 4.2	53.1 ± 4.7
60	27.69 ± 1.3	32.73 ± 1.9	35.9 ± 2.3	40.9 ± 2.9	44.7 ± 3.3	48.8 ± 3.9	52.1 ± 4.3	55.8 ± 4.8	60.6 ± 5.2	63.1 ± 5.8

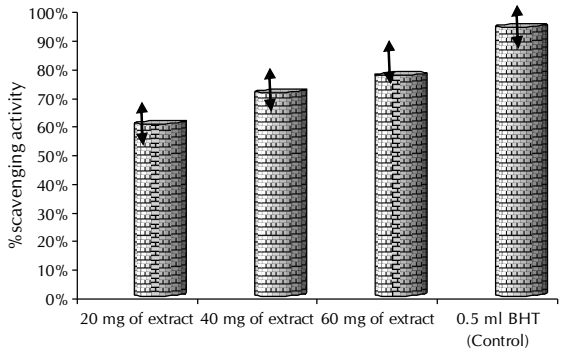

Figure 1:Free radical scavenging activity of *Allium ascalonicum* in 24hr. Values are mean ± S.D for 6 different preparations

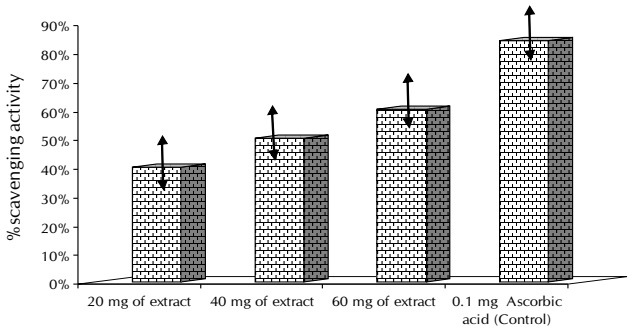

Figure 2:Nitric Oxide scavenging activity of *Allium ascalonicum;* Values are mean ±S.D. for 6 different preparations

4 shows the reducing capacity and chain breaking activities of methanolic extract of *A.ascalonicum*. 60mg of *A.ascalonicum* extract showed higher reducing capacity and chain breaking activity. DPPH (1,1 diphenyl picryl hydroxyl) is a stable (free) radical. In the presence of an antioxidant this DPPH radical obtains one or more electrons and their absorbance decreases as reported by Koleva *et al*., (2002). It has been recognized that flavonoids show antioxidant activity. It has been reported by Kessler *et al*., (2003) that flavonoids act through scavenging or chelating processes where as phenolic compounds act as free radical terminators. The high rate of scavenging activity observed in the methanolic extract of *A.ascalonicum* may be due to high concentration of flavonoids and phenols already observed earlier (Vanitha *et al.,* 2009). In fruits and vegetables many researchers have also reported a statistically significant relationship between total phenolics and antioxidant activity (Connor *et al.,* 2002; Kaur and Kapoor, 2002 and Moyer *et al.,* 2002). It has been reported by Miller *et al*. (2000) that free radical scavenging activity depends upon phenolic and sulphur compounds of *A.cepa*. It has also been reported by Miller *et al*. (2000) that quercetin showed highest scavenging activity. As quercetin is the major component present in *A.cepa* the significant free radical scavenging activity noted in our study may be due to their high content. A direct relationship between antioxidant activity and phenolic content of plant extracts has been reported by Landbo and Meyer (2001). It has been reported by Heinonen (2002) that onions contain large amounts of flavonoids and hence shows increased antioxidant activity. It has been reported by Gennaro *et al.,* (2002) and Marotti and Piccaglia (2002) that red onions contain high levels of flavonoids and there was a predominance of quercetin compounds such as quercetin-4- glucosides, quercetin- 3, 4- diglucosides. It has been reported by Griffiths *et al.,* (2002) that red onions contains both anthocyanins and flavonoids in higher amounts and hence have higher antioxidant activity. NO is a free radical and is a highly reactive molecule within the biological system reacting with other free radicals. NO can react rapidly in the intracellular environment to form nitrate, nitrite and S-nitrosothiols. These metabolites play a key role in mediating many xenotoxic effects such as DNA damage. NO can cause DNA damage via peroxynitrite formation. It is observed from present study that methanolic extract of *A.ascalonicum* inhibits the formation of NO to about 60%. This antioxidant activity of the extract can be justified by the presence of high concentrations of phenols, flavonoids and tannins. Quercetin prevents free radical induced damage by several ways. One way is the direct scavenging of free radicals. Since it was observed that there was high amount of quercetin present in the extract when compared to other flavonoids, the higher nitrogen radical scavenging activity may be due to this. Recent studies have indicated that quercetin is not only scavenges free radicals but also interact with intracellular signaling pathways and thereby reduce the oxidative damage. Quercetin significantly inhibited TNF- α (Tumor Necrosis Factor) production and gene expression in a dose dependent manner as reported by Aggarwal (2000), Aggarwal *et al.,* (2001) and Wajant *et al*. (2001). This indicates that quercetin can modulate the immune response and has anti-inflammatory activity also. The reducing activity of *Allium ascalonicum* extracts observed (Table 4) may due to the high antioxidant activity. It has been reported by Amagase *et al*. (2001) that high antioxidant activity of *A.cepa* is due to the presence of organo sulphur compounds. The reducing capacity and chain breaking

Table 2: Karl pearson's correlation co-efficient between total phenolic content and free radical scavenging activity of *Allium ascalonicum*

Variables	r	P
Total phenols and free radical scavenging activity	0.675	0.001
Total flavonoids and free radical scavenging activity	0.7537	0.001

Table 3: Comparison of ROS and RNS scavenging activity of methanolic extract of *Allium ascalonicum*

Parameter	20mg methanolic extract	40mg methanolic extract	60mg methanolic extract
ROS scavenging activity	9.20± 0.3	16.86±0.9	27.69±1.3
RNS scavenging activity	a)****36.21±1.5	b)****48.11±2.4	c)****60±4

Values are mean ± S.D for 6 different preparations.; **** $p < 0.001$; a) Comparison between ROS and RNS activity at 20mg methanolic extract of *Allium ascalonicum*; b) Comparison between ROS and RNS activity at 40mg methanolic extract of *Allium ascalonicum;* c) Comparison between ROS and RNS activity at 60mg methanolic extract of *Allium ascalonicum*

Table 4: Reducing capacity and chain breaking activity of *Allium ascalonicum* extract

Extract concentration (mg/mL)	Reducing capacity (%)	Chain breaking activity (O.D $^{-3}$ min^{-1} mL $^{-1}$)
20	52.94 ± 1.5	0.174 ± 0.07
40	64.71 ± 2.7	0.294 ± 0.01
60	105.88 ± 6.0	0.374 ± 0.02

Value are mean ±S. D. for 6 different preparations

activity may be due to the presence of high concentration of phenols and flavonoids. This preliminary study highlights the free radical scavenging potential of *A.ascalonicum*. The high reducing and antioxidant activity observed in the *A.ascalonicum* extract may be due to high contents of flavonoids and phenols. However more studies are needed to be carried out to evaluate the impact of *A.ascalonicum* phytochemicals on cancer cell lines to show its antiproliferative effect.

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