Antioxidative and free radical scavenging activities of aqueous and methanolic bulbs extracts of *Allium hirtifolium*

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**Key words:** *Allium hirtifolium*, antioxidant, pro-oxidant, polyphenols, antiradicals

**Introduction**

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**Abstract**

The aim of the present study was to investigate the antioxidant activity of aqueous and methanolic bulbs extracts of *Allium hirtifolium* by employing various established in vitro systems including DPPH, FRAP, ABTS free-radical scavenging, superoxide and hydroxyl radicals scavenging. Pro-oxidant effect was determined by Bleomycin-dependent DNA damage method, and the Iron II chelating activity was also evaluated. Total phenolic, flavonoid, and oligomeric proanthocyanidin contents were also determined by a colorimetric method. polyphenolic, flavonoid, and proanthocyanidin contents of methanolic extract were more than that of the aqueous one and, in addition, methanolic extract had a more powerful antioxidant and antiradical effect than the aqueous extract. In Iron II chelating assay, the effect of aqueous extract was highly more than the methanolic one. It was also observed that extracts have a lower pro-oxidant effect than vitamin C and, in this regard, methanolic extract had a lower effect than the aqueous one. Generally comparing the results, in spite of making lower dry weight, methanolic extract has a more powerful antioxidant effects.

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Introduction

Oxygen metabolism continuously generates small amounts of reactive oxygen species (ROS). ROS is normally produced during physiologic processes such as cellular respiration and inflammatory defense mechanisms (Crimi et al., 2006). It has numerous harmful and undesired effects as well as a critical role in diseases such as Alzheimer (Markesbery, 1997), cancer (Dreher and Junod, 1996), ischemic diseases (Mecord, 1985), aging (Harman, 1992), Parkinson (Olanow, 1992) and digestive tract diseases (Parks et al., 1983). Antioxidants have a major role in health (Young and Woodside, 2001). By various mechanisms, antioxidants control the free radicals and put a stop to their poisonous effects.

Allium hirtifolium Boiss is belonged to liliaceae family, native to Iran and endemic to the Zagross Mountains. Bulb of A. hirtifolium is the storage tissue, which, usually, consist of a single main bulb or, rarely, two bulbs and the weight of each bulb is 8-15 times of garlic clove (Ghahremani-Majd et al., 2012). Its bulbs well known in Iranian folk medicine and widely used for treating rheumatic and inflammatory disorders (Jafarian et al., 2003). A. hirtifolium contains some useful biological secondary metabolites, which include alliin, alliinase, allicin, S-allyl-cysteine, diallyldisulphide, diallyltrisulphide, and methylallyltrisuphide (Azadi et al., 2009), furostanol and spirostanolsaponins (Barile et al., 2005) and flavonoids (Seyfi et al., 2010). In previous works, pharmacological properties such as antioxidants, antitrichomonas, antiproliferative, immunomodulatory, anti-angiogenesis, hypolipidemic, hypoglycemic, and Hepatoprotective and antimicrobial effects have been reported (Asgari et al., 2012; Azadi, Riazi, et al, 2009; Hosseini et al., 2012; Jafarian, Ghannadi, et al, 2003; Seyfi, Mostafaie, et al, 2010).

Pro-oxidants are chemicals that induce oxidative stress, either by generating reactive oxygen species or by inhibiting antioxidant systems (Puglia and Powell, 1984). Some substances can serve as either antioxidants or pro-oxidants, depending on conditions. Some of the conditions that are important include the concentration of the chemical (Herbert, 1996). Damage of DNA in the presence of a bleomycin-Fe complex has been adopted as a sensitive and specific method to examine potential pro-oxidant agents (Gouda et al., 2013).

Methanolic extracts were in previous studies investigated for antioxidant activities (Siahpoosh and Sohangir, 2013). Because of using aqueous extracts in folk medicine, this study was aimed to have an in vitro evaluation and comparison of antioxidant activities of aqueous extract of A. hirtifolium with methanolic extracts collected from Iran. The aim of the present study was to investigate the antioxidant activity of aqueous and methanolic bulbs extracts of Allium hirtifolium by employing various established in vitro systems including DPPH, FRAP, ABTS free radical scavenging, superoxide and hydroxyl radicals scavenging.

Materials and methods

Chemicals

The 6-hydroxy-2, 5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-azinobis(3-ethylbenzothiazoeline-6-sulfonic acid) diammonium salt (ABTS), and 2,4,6-tripyridyls-triazine (TPTZ), 1,1-Diphenyl-2-picolryldrazyl (DPPH), 2-deoxy-D-ribose, xanthine, xanthine oxidase (XOD), thiobarbituric acid (TBA), ferric chloride and L-ascorbic acid, nitroblue tetrazolium (NBT) and Folin-Ciocalteu reagent were purchased from Sigma; FeSO₄·7H₂O, FeCl₃ anhydrous were purchased from Fluka Co. All other chemicals used were of analytical grade supplied by Merck.

Plant material

Fresh and ripe bulbs were used in the experiments. The Allium hirtifolium, natively known as Mooseer or Persian shallot, was collected from a region of Iran named Aligudarz. The herbal product extract was prepared according to Iranian herbal pharmacopeia (Ghasemi et al., 2003) that explained in previous work (Siahpoosh and Javedani, 2012). Bulbs were washed thoroughly in water, cut into small pieces
using a kitchen mixer, and extracted with water or methanol for 48h at room temperature. The extract was centrifuged at 3500 rpm for 20 min to obtain the supernatant and the rest was also re-extracted under the same conditions. The combined extracts were filtered through 0.45 μm of filter membrane, concentrated in a rotoevaporator and dried with Freeze Dryer.

**Total phenolic content determination**

Total phenolic compound amount in extracts was determined by the Folin–Ciocalteu method: 0.5 ml of each extract and 2.5 ml of a 1/10 aqueous dilutions of Folin-Ciocalteu reagent were mixed. 2 ml of Na₂CO₃ 7.5% was, after 5 minutes, added and incubated at room temperature for 120 minutes. Absorption at 765 nm was measured by using a spectrophotometer. The total phenolic content was expressed as Tannic acid (Singleton and Rossi, 1965).

**Total flavonoid content determination**

The flavonoid content was estimated by the AlCl₃ method: 1 ml of methanolic extract solution was added to 1 ml of 2% methanic AlCl₃, 6H₂O. The absorbance was measured 10 min later at 430 nm (Xma 3000, Human, Korea). Results were expressed in mg rutin/100 mg dry weight of extract matter by a comparison with standard rutin (Quettier-Deleu C, 2000).

**Determination oligomeric proanthocyanidin content**

This method was described by Quettier-Deleu et al. 0.5 ml extract solution, 6 ml of n-butanol:HCl (95:5;v:v) and 0.2 ml of 2% (w:v) solution of NH₄Fe(SO₄)₂, 12H₂O in 2 M HCl were mixed and heated during 40min at 95±2°C in a water bath. Absorption of cooling extract were measured at 550 nm. The proanthocyanidin content was expressed in mg of cyanidin chloride/ 100 mg dry weight of extract (Quettier-Deleu et al., 2000).

**DPPH free radicals scavenging activity assay**

To 3.9 ml of DPPH solution (0.025 g/l), 0.1 ml of sample solution was added and the absorbance was measured at 515 nm. The tubes were then incubated at room temperature for 30 minutes under dark conditions and the absorbance was measured at 517 nm. Inhibition of DPPH radical was calculated using the equation I (%) = 100 × (A₀ – Aₛ)/A₀, where A₀ is the absorbance of the control (containing all reagents except the test compound), and Aₛ is the absorbance of the tested sample; The IC₅₀ value represents the concentration of the sample that caused a 50% inhibition.(Brand-Williams et al., 1995).

**Ferric-reducing antioxidant power (FRAP) assay**

The FRAP reagent contained 2.5 ml of a 10 mM tripydyltriazine (TPTZ) solution in 40 mM HCl plus 2.5 ml of 20 mM FeCl₃ · 6H₂O and 25 ml of 0.3 M acetate buffer at pH 3.6. Freshly prepared FRAP reagent (3.0 ml) were mixed with 30 μl of sample and 10 μl of distilled water; the reaction mixtures were later incubated at 37° C. Absorbance at 593 nm was read with reference to reagent blank containing distilled water, which was also incubated at 37°C. Aqueous solutions of known Fe(II) concentrations in the range of 100–2000 μM (FeSO₄·7H₂O) were used for calibration (Biglari et al., 2008). The parameter equivalent concentration (EC₁) was defined as the concentration of antioxidant having a ferric-TPTZ reducing ability equivalent to that of 1 mmol/L FeSO₄·7H₂O. EC₁ was calculated as the concentration of antioxidant giving an absorbance increase in the FRAP assay equivalent to the theoretical absorbance value of a 1 mmol/L concentration of Fe(II) solution, determined using the corresponding regression equation. Tannic acid was used as standard.

**ABTS⁺ free-radical scavenging activity**

The ABTS⁺ radical was generated by chemical reaction with potassium persulfate (K₂S₂O₈). 25 ml of ABTS (7 mM) was, for this purpose, spiked with 440 μl of K₂S₂O₈ (140 mM) and permitted to position in darkness at the room temperature for 12–16 h that is the time required for formation of the radical. Taking a volume of the previous solution and diluting it in ethanol, the working solution was prepared until its absorbance at λ = 734 nm was 0.70 ± 0.02. The reaction took place directly in the measuring cuvette.
100 μl of the sample or the standard (Trolox) were added to 2 ml of the ABTS·+ radical, The absorbance was measured 6 minutes after mixing the reagent. Trolox was used as standard and IC₅₀ values were calculated using linear regression analysis (Ak and Gülçin, 2008; Miser-Salihoglu et al., 2013; Re et al., 1999).

Iron chelation
The chelation of iron (II) ions by the Allium hirtifolium extracts were studied, as described by Kanatt et al (2007). An aliquot of the extract (1 ml) was added to 100 μl of 1 mM FeCl₂ and 3.7 ml of distilled water. The reaction was initiated by adding 200 μl of 5 mM ferrozine. After 20 min incubation at room temperature, the absorbance at 562 nm was recorded. EDTA was used as a positive control. The control contained all the reaction reagents except the extract or positive control. The Fe²⁺-chelating activity was calculated using the equation: Chelation activity (%) = [(A₀ - Aₛ)/A₀] × 100 where, A₀ is the absorbance of control and Aₛ the absorbance of extract (Kanatt et al., 2007).

Non-site-specific hydroxyl radical scavenging activity
Total Hydroxyl radicals scavenging activity assay of extracts was determined by the deoxyribose method: The reaction mixture contained 100 μl of extract, 500 μl of 5.6 mM 2-deoxy-d-ribose in KH₂PO₄–NaOH buffer (50 mM, pH 7.4), 200 μl of premixed 100 μM FeCl₃, 104 mM EDTA (1:1 v/v) solution, 100 μl of 1.0 mM H₂O₂, and 100 μl of 1.0 mM aqueous ascorbic acid were dissolved in water. Tubes were incubated at 50°C for 30 minutes. Thereafter, 1 ml of 2.8% TCA and 1 ml of 1.0% TBA were added to each tube. The samples were heated in a water bath at 50°C for 30 minutes. The extent of oxidation was estimated from the absorbance rate of the solution at 532 nm. The inhibition percentage values were calculated from the absorbance of the control (Ac) and of the sample (As) using the following equation: Inhibition (%) = ((Ac-As)/Ac) × 100, where the controls contained all the reaction reagents except the extract or positive control substance (Halliwell et al., 1987).

Superoxide anion scavenging activity assay
Superoxide anion scavenging activity of Allium hirtifolium was measured using the xanthine/xanthine oxidase method. A 0.5 ml of samples was added to a 1.0 ml mixture of 0.4 mM xanthine and 0.24 mM nitroblue tetrazolium chloride (NBT) in 0.1 M phosphate buffer (pH 8.0). A 1.0 ml solution of xanthine oxidase (0.049 units/ml), diluted in 0.1 M phosphate buffer (pH 8.0), was added and the resulting mixture incubated in a water bath at 37 °C for 40 min. The reaction was terminated by adding 2.0 ml of 69 mM sodium dodecyl sulphate (SDS) and the absorbance of NBT was measured at 560 nm (Que et al., 2006).

Bleomycin-dependent DNA damage
0.5 ml DNA (1 mg/ml) was mixed together with 0.05 ml bleomycin sulphate (1.5 units/ml, approximately 0.6 mM), 0.1 ml MgCl₂ (0.05 M), 0.05 ml Tris buffer (1.0 M, pH 7.4), 0.1 ml FeCl₃ (0.5 mM) or 0.1 ml buffer (for the reaction blank tubes), and the reaction was started by addition of 0.1 ml of sample dissolved in ethanol. Tubes were incubated at 37° for 10 min to develop the MDA-TBA chromogen which was read at 532 nm after cooling (Nd et al., 2000). The extent of DNA damage was measured by increase in absorbance at 532 nm (Liu and Ng, 2000).

Statistical analysis
The data determined was expressed as the mean of three replicate determinations and presented as mean ± SD (standard deviation). The IC₅₀ values were estimated by linear/non-linear regression.

Results and discussion
Total extracts from the aqueous and methanolic extracts were 39.44% and 1.57%, respectively, indicating that Mooseer polar compounds soluble in water are more than those dissolved in methanol. Allium species has important compounds such as
organosulphuric, polyphenolic, saponin, and other compounds, which have high structural varieties, thus they are dissolved in solvents with different polarities (Liu and Yeh, 2001; Mikail, 2010). Typically many biological effects of Allium spp. are related to the thiosulfinates and volatile sulfur compounds. However, these compounds are not stable and give rise to product transformation. For this reason, some studies have focused on polar compounds such as polyphenols, which are more stable in cooking and storage (Ghahremani-Majd, Dashti, et al, 2012).

### Table 1. Total phenolic, flavonoids and Oligomeric proanthocyanidins compounds in methanolic and aqueous extracts of Allium hirtifolium. Values are expressed as a mean±standard deviation (n=3).

<table>
<thead>
<tr>
<th>Extract Type</th>
<th>Total phenolic content</th>
<th>Flavonoid content</th>
<th>Olig. proanthocyanidins content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous extract</td>
<td>1.1 ± 0.04</td>
<td>0.05 ± 0.005</td>
<td>10.55 ± 0.03</td>
</tr>
<tr>
<td>Methanolic extract</td>
<td>18.1 ± 0.9</td>
<td>1.07 ± 0.1</td>
<td>64.22 ± 0.04</td>
</tr>
</tbody>
</table>

a: Data are expressed as mg of Tannic acid equivalents per g dry extract.

b: Data are expressed as mg of Rutin equivalents per 100mg dry extract.

b: Data are expressed as µg of Cyanidin chloride equivalents per 100mg dry extract.

### Table 2. Pro-oxidant Effects of methanolic and aqueous extract on Ferric Bleomycin Induced DNA Damage.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Conc. (µg/ml)</th>
<th>Extent of DNA damage (OD 532 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>0.020 ± 0.001</td>
</tr>
<tr>
<td>Vitamin C</td>
<td></td>
<td>2.023 ± 0.500</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>2.059 ± 0.100</td>
</tr>
<tr>
<td>Methanolic extract</td>
<td>100</td>
<td>0.050 ± 0.005</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.090 ± 0.006</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>100</td>
<td>0.080 ± 0.009</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.150 ± 0.010</td>
</tr>
</tbody>
</table>

The values are mean±SD (n=3).

Flavonoid content was estimated using the AlCl₃ method. The principle of aluminum chloride colorimetric method is that aluminum chloride forms stable acid complexes with a C-4 keto group and C-3 or C-5 hydroxyl group, therefore this method was proved to be specific only for flavones and flavonols (Chang et al, 2002). Results showed that methanolic extract have higher flavonoid contents than aqueous extract (Table 1). Cheung et al (2003) reported that the amount of phenolic compounds in organic extracts was higher than in water extracts (Cheng and Crisoto, 1995).

Oligomeric proanthocyanidins strictly refer to dimer and trimer polymerizations of catechins. They are found in most plants and thus are a part of the human diet (Santos-Buelga and Scalbert, 2000). Besides the free radical scavenging and antioxidant activity,
proanthocyanidins exhibit vasodilatory, anticarcinogenic, anti-allergic, anti-inflammatory, antibacterial, cardioprotective, immune-stimulating, anti-viral and estrogenic activities, as well as being inhibitors of the enzymes phospholipase A2, cyclooxygenase and lipoxygenase (Bagchi et al., 2000)

The content of proanthocyanidins was determined after n-butanol/HCl hydrolysis using a colorimetric assay. In this assay heat-stable anthocyanidins are liberated from proanthocyanidins and they can be determined colorimetrically at a wavelength of 550 nm (Amarowicz and Pegg, 2006).

Proanthocyanidins are higher in methanolic extract (64.22 µg cyanidin/100 mg dry extract) than in the aqueous extract (10.55 µg cyanidin /100 mg dry extract).

**Fig. 1.** Free radical (1,1-diphenyl-2-picrylhydrazyl (DPPH)) scavenging activity of methanolic and aqueous extracts from Allium hirtifolium at different concentrations. Each value represents means ± SD (n= 3).

*The scavenging activity for DPPH radicals*

DPPH molecule that contains a stable free radical has been widely used to evaluate the radical scavenging ability of antioxidants. The assay is based on the reduction of DPPH radicals in methanol which causes an absorbance to drop at 515 nm. The free radical scavenging activities of the two extracts were also assayed by using DPPH. As shown in Fig. 1.

Both methanolic and aqueous extracts reacted directly with and quenched DPPH radicals to different degrees with increased activities at higher concentrations. At all of the concentrations tested, methanolic extract showed significantly stronger activities than aqueous extract. At the concentration of 0.63 mg/ml, the scavenging activity of methanolic extract reached a plateau of 85%. However, at the same concentration, the scavenging effect of aqueous extract was lower than 1%. Therefore, although both methanolic and aqueous extracts showed DPPH scavenging activity, methanolic was a significantly stronger scavenger for DPPH radicals. More powerful effects of methanolic extract can be related to higher polyphenolic compounds in the methanolic extract (about 18 times).IC$_{50}$ content was 0.59 mg/ml and 3.4 mg/ml for methanolic and aqueous extracts, respectively, indicating that the methanolic extract has a more powerful effect than the aqueous one. In another study on the Mooseer of Iran’s different regions, IC$_{50}$ content was calculated to be 60.9-267.2 mg/ml, which is highly more than the IC$_{50}$ calculated here in this study (Ghahremani-Majd, Dashti, et al, 2012).

**Fig. 2.** Antioxidant activity of methanolic and aqueous extracts from Allium hirtifolium determined by FRAP assay. Each value represents means ± SD (n= 3).

*Ferric reducing activity based on FRAP assay*

Ferric reducing antioxidant power is to measure the ability of antioxidant presence in the herb extracts to reduce the ferric ion Fe$^{3+}$-TPTZ complex to blue colored ferrous ion Fe$^{2+}$-TPTZ by electron donor in acidic medium(Huang et al., 2005). The reducing power (EC$_1$ value) of herb extracts are summarized in Figure 2. Generally, the reducing properties are associated with the presence of compounds, which exert their action by breaking the free radical chain through donating a hydrogen atom (Othman et al., 2007). Figure 2 shows that methanolic extract of *A. hirtifolium* exhibited higher EC$_1$ (3.99 mmol Fe(II)/mg extract) antioxidant activity than aqueous
extract (50.23 mmol Fe(II)/mg extract). In the present study, this method also showed similar results compared to those obtained by both DPPH assay. The EC₅₀ for tannic acid was 0.99 mmol Fe(II)/mg tannic acid.

**Fig. 3.** ABTS free-radical scavenging activity of methanolic and aqueous extracts from Allium hirtifolium at different concentrations. Each value represents means ± SD (n= 3).

**ABTS' free radical scavenging activity**

ABTS radical cation decolorization assay is a rapid and reliable method widely used to assess the total amount of radicals that can be scavenged by an antioxidant (Ghahremani-Majd, Dashti, *et al.*, 2012). This assay is based on the ability of antioxidants to scavenge ABTS⁺ radicals. It can measure antioxidant capacities of lipophilic and hydrophilic components in a sample, and is a method usually used for the evaluation of antioxidant capacity (Huang, Ou, *et al.*, 2005). Rate of ABTS radical scavenging in different concentrations is indicated in Figure 3. As observed, there is a direct relationship between the rate of control and concentration. IC₅₀ was calculated to be 0.09 and 0.85 mg/ml for methanolic and aqueous extracts, respectively. Different studies indicate that using different solvents have a high effect on the rate of ABTS radical scavenging and this effect is different based on the polarity and non-polarity of compounds available plants (Huang, Ou, *et al.*, 2005; Othman, Ismail, *et al.*, 2007]). Higher effect of methanolic extract can be due to the higher content of polyphenolic, flavonoid, and proanthocyanin compounds of this extract to the aqueous one. Direct relationship between rate of ABTS radical control and content of polyphenolic compounds was observed in different studies (Manian *et al.*, 2008; Su *et al.*, 2007). IC₅₀ content for Trolox was calculated to be 10.76 µmol/ml.

**Fig. 4.** Fe(II) chelating activity of methanolic and aqueous extracts from Allium hirtifolium at different concentrations. Each value represents means ± SD (n= 3).

**Iron chelation**

Foods are often contaminated with transition metal ions which may be introduced by processing methods. Bivalent transition metal ions play an important role as catalysts of oxidative processes, leading to the formation of hydroxyl radicals and hydro peroxide decomposition reactions via Fenton chemistry. These processes can be delayed by iron chelation and deactivation(Hinneburg *et al.*, 2006). As observed in Figure 4, methanolic and aqueous extracts have an Iron II chelating activity, but the aqueous extract has a higher chelating activity. EC₅₀ content for Iron II chelating for aqueous extract, methanolic extract, and EDTA as positive control is 0.14 mg/ml, 0.78 mg/ml, and 5.34 µg/ml, respectively. Results of various
references indicate that polyphenolic content in organic extracts is more than in aqueous extracts (Alonso Borbalán et al., 2003; Cheng and Crisoto, 1995). Higher content of polyphenolic compounds is not a reason of a more powerful chelating activity of heavy metals. The ability for phenolic compounds to chelate metal ions depend on their backbone structures and the variation and kind of functional groups present in the chemical structure. A sample high in polyphenols might not chelate metal if the polyphenols present did not have suitable groups that could chelate the cations. Bidentate ligands are more powerful scavengers of metal cations than monodentate ligands, for example, catechol binds ferric ions tightly whereas phenol does not (Wong et al., 2006).

Lower chelating effect of methanolic extract to the aqueous one, in spite of the existence of higher amounts of polyphenolic, flavonoid, and proanthocyanidin compounds in the methanolic extract, can be related to the structure of polyphenolic compounds (existence of more hydroxyl groups) (Andjelković et al., 2006; Hider et al., 2001) and other compounds in the aqueous extract with nonphenolic structure such as organic acids, amino acids, and sugars (Wang et al., 2009; Wong, Leong, et al., 2006). A similar result was reported by Zhao et al (2008) and wang et al (2009), that stating poor correlation between the ferrous ion-chelating ability with both polyphenolic content and antioxidant activities (various assays e.g. DPPH and ABTS cation radical scavenging activity and reducing power) (Wang, Jónsdóttir, et al, 2009; Zhao et al., 2008).

**Non-site-specific hydroxyl radical scavenging activity**

The interaction of iron ions with hydrogen peroxide in biological systems can lead to formation of a highly-reactive tissue-damaging species that is thought to be the hydroxyl radical. Hydroxyl radical capable of modifying almost every molecule in the living cells (Winterbourn, 1995). This radical has the capacity to cause strand damages in DNA leading to carcinogenesis, mutagenesis, and cytotoxicity. Moreover, hydroxyl radicals are capable of the quick initiation of lipid peroxidation process as by abstracting hydrogen atoms from unsaturated fatty acids (Cooke et al., 2003). The scavenging activity of hydroxyl radical of extracts was measured and the percentage inhibition was calculated (Figure 5). The methanolic and aqueous extracts demonstrated a concentration-dependent effect and methanolic extracts showed superior hydroxyl radical scavenging activity.

![Fig. 5. Hydroxyl radical scavenging activity of methanolic and aqueous extracts from Allium hirtifolium. Each value represents the mean ± SD (n=3)](image)

IC$_{50}$ content of methanolic extract, aqueous extract, and mannitol was calculated to be 0.13 mg/ml, 0.86 mg/ml, and 0.12 mg/ml, respectively. In this assay, hydroxyl radical is produced of the reaction between Iron II and H$_2$O$_2$. Hydroxyl radical attacks deoxyribose it degrades into fragments that react with TBA on heating at low pH to form a pink color. Thus, compounds able of chelating Iron II or scavenging the hydroxyl radical can make positive responses in this reaction(Ardestani and Yazdanparast, 2007). Given that the rate of aqueous extract’s Iron II chelation is more than the methanolic extract, but the results in this assay is highly lower than the methanolic extract, it can be concluded that that higher ability of methanolic extract in this assay is related to the
ability of scavenging the hydroxyl radical created in the reaction.

![Graph showing scavenging activity of methanolic and aqueous extracts from Allium hirtifolium.](image)

**Fig. 6.** Superoxide scavenging activity of methanolic and aqueous extracts from Allium hirtifolium. Each value represents the mean ± SD (n=3).

**Superoxide anion scavenging activity assay**
Superoxide anion radical is one of the strongest ROS among the free radicals and get converted to other harmful reactive oxygen species such as hydrogen peroxide and hydroxyl radical, damaging biomolecules which results in chronic diseases (Al-Mamun et al., 2007). Xanthine oxidase is a flavoprotein that catalyses the oxidation of hypoxanthine to xanthine and generates superoxide and uric acid then Superoxide radicals were assayed by the reduction of NBT (Sahgal et al., 2009). Fig. 6 illustrates the superoxide radical-scavenging ability of extracts. Methanolic and aqueous extracts showed a concentration-dependent scavenging of superoxide radicals and had an IC50 value of 3.59 µg/ml for methanolic extract and 34.88 µg/ml for aqueous extract. Extracts from other plant sources, such as tea, potato peel and garlic, have also been reported to have good superoxide radical-scavenging activity (Kanatt, Chawla, et al, 2007).

**Bleomycin-dependent DNA damage**
Bleomycin, an antitumor antibiotic, binds to DNA by using its bithiazole and terminal amine residues, and it also complexes with metals. Bleomycin binds iron ions and the bleomycin–iron complex will degrade DNA in the presence of O2 and a reducing agent such as ascorbic acid. DNA degradation is accompanied by the formation of a product similar to malondialdehyde (MDA), which combines with TBA to form a pink (TBA)2-MDA adduct (Aruoma, 2003; Gouda, Eldien, et al, 2013). Damage of DNA in the presence of a bleomycin–Fe complex has been adopted as a sensitive and specific method to examine potential prooxidant agents. (Gutteridge et al., 1981). The bleomycin–iron(III) complex by itself is inactive in inducing damage in DNA. Oxygen and a reducing agent are required for the damage of DNA to occur. If the samples to be tested are able to reduce the bleomycin–Fe3+ to bleomycin–Fe2+, DNA degradation in this system will be stimulated, resulting in a positive test for pro-oxidant activity. L-Ascorbic acid was used as a reducing agent can reduce Fe3+ to Fe2+. (Aruoma, 2003; Gouda, Eldien, et al, 2013).

Although vitamin C is a strong antioxidant, a high intake of vitamin C may lead to, in some situations, a pro-oxidant activity in the body when free transition metals are available at the same (Liu and Ng, 2000). The pro-oxidant activity of vitamin C was 20 times higher than that of A. hirtifolium extracts when tested using bleomycin-dependent DNA damage as a parameter. Methanolic extract had little pro-oxidant effect than aqueous extract (Table 2).

**Conclusions**
In this study, using various in vitro assay systems, the antioxidant potential of methanolic and aqueous extracts of Allium hirtifolium extract were evaluated based on DPPH, FRAP, ABTS free-radical, superoxide, and hydroxyl radical scavenging activities. In addition, we further evaluated pro-oxidant effect by Bleomycin-dependent DNA damage method and Iron chelating activity of extracts, as well as total phenolic, flavonoids and Oligomeric proanthocyanidins compounds were determined in extracts.
Results indicate that although the dry weight resulted from the aqueous extract is much more than the methanolic extract, but the content of polyphenolic compounds and antioxidant effects of the methanolic extract are more. To vitamin C, both extracts have much lower pro-oxidant effect, but the aqueous extract’s rate of Iron II chelating was more powerful than the methanolic extract. Additional studies are being conducted to evaluate the antioxidant capacity in vivo and in vitro environments.

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**References**


http://dx.doi.org/10.1016/j.foodchem.2009.02.041

http://dx.doi.org/10.1016/0378-4274(95)03532-X

http://dx.doi.org/10.1016/j.foodchem.2005.07.058

http://dx.doi.org/10.1136/jcp.54.3.176

http://dx.doi.org/10.1016/j.foodchem.2007.08.018