Screening of Total Phenol and Flavonoid Contents, Antioxidant and Antibacterial Activities of the Methanolic Extract of *Allium Ampeloprasum* L. (Alliaceae) from Iran

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Abstract

The methanol extracts of leaves and bulbs of *Allium ampeloprasum* L. (Alliaceae) were analyzed for their phenolic profiles and screened for their antioxidant and antibacterial activities. Two biochemical assays, namely DPPH free radical scavenging and *β*-caroten/linoleic acid activity systems, were used to evaluate antioxidant activity. Total phenol and flavonoid contents of the species were determined by Folin-Ciocalteu and $AlCl_3$ assays, respectively. Results indicated that the extracts are different in total phenolic content. Results from antioxidant activity showed that the extracts studied in DPPH radical scavenging assay are lower active than ascorbic acid and BHT as synthetic antioxidants. Antibacterial assay showed that the bulbs extract is more effective in inactivation of *Haemophilus influenzae* and *Bacillus cereus* and the leaves extract is more effective in inactivation of *Bacillus cereus* at 100 mg ml^{-1} than others. Then, the extracts of *Allium ampeloprasum* possess strong antioxidant activity and may be used as new drug supplements in the future.

Introduction

During the last decade, the use of traditional medicine has expanded globally and is gaining popularity. It has continued to be used not only for primary health care of the poor in developing countries, but also in countries where conventional medicine is predominant in the national health care system [4], [18]. According to WHO, herbal medicines serve the health needs of about 80% of the world's population, especially for

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millions of people in the vast rural areas of developing countries. Therefore, the search on new drugs must be continued and natural products from plants, microorganisms, fungi and animals can be the source of innovative and powerful therapeutic agents for newer, safer and affordable medicines [9], [20]. On the other hand the screening of plants as a possible source of antiviral drugs has led to the discovery of potent inhibitors of *in vitro* viral growth [13], [16], [19], [24]-[26], [38]-[40]. Free radical formation is associated with the normal natural metabolism of aerobic cells. The oxygen consumption inherent in cell growth leads to the generation of reactive oxygen species (ROS). The interaction of ROS with molecules with lipid nature produces new radicals such as hydroperoxides and different peroxides [3], [30], [34]. This group of radicals (superoxide, hydroxyl and lipoid peroxides) may interact with biological systems in a clearly cytotoxic manner. In this respect, flavonoids and phenols have been shown to possess important antioxidant activity toward these radicals, which is principally based on the redox properties of their phenolic hydroxyl groups and the structural relationships between different parts of their chemical structure [7], [36], [37]. Free radicals and their uncontrolled production, in fact, are responsible for several pathological processes, such as certain tumors (prostate and colon cancers) [15] and coronary heart disease [21].

Allium ampeloprasum L. belongs to the genus *Allium* (Alliaceae) and includes about 800 species, of which 93 are known from Iran [11]. *Allium ampeloprasum* is extremely variable. As a wild plant, it is widely distributed across the Mediterranean basin (sometimes as weedy plants) through the Middle East into western and southern former U.S.S.R [14]. Minor crops of *Allium ampeloprasum* are locally cultivated from Asia Minor to Iran and the Caucasus, and sporadically in California and other regions of America and Europe [12]. *Allium* vegetables have been used in the traditional medicine for centuries [32]. Recent scientific investigations have shown that *Allium* vegetables and their constituents reduce the risk of cardiovascular disease and diabetes, stimulate immune system, protect against infections, and have anti-aging as well as anti-cancer

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effects [1], [23], [31], [32]. The aim of this research is to study total phenol and flavonoid contents and assessment of antioxidant and antibacterial activities of the methanolic leaves and bulbs extracts of *Allium ampeloprasum*.

Materials and methods

Chemicals

1, 1-Diphenyl-2-picryl hydrazyl (DPPH), quercetin, linoleic acid and *β*-carotene were purchased from Sigma (St. Louis, MO, USA). Gallic acid, Folin-Ciocalteu reagent, Tween 40, chloroform, sodium bicarbonate, aluminum chloride, potassium acetate, butylated hydroxytoluene (BHT), ascorbic acid, Muller-Hinton agar, dimethyl sulfoxide (DMSO), ethanol and methanol were purchased from Merck (Darmstadt, Germany).

Plant materials

Allium ampeloprasum was collected from Hamedan province, West Iran in April to May 2011. Voucher specimens were deposited in the herbarium of Bu-Ali Sina University (BASU), Hamedan, Iran. Plant materials were dried at room temperature and ground in a mortar. For preparation of methanolic extract, 20 g of plant powder was extracted in 250 ml of methanol by Soxhlet. The solvent was removed under the vacuum at temperature below 70°C and the yielding a waxy material. Finally, the extracts were lyophilized and kept in the dark at 4°C until tested.

Determination of total phenol content

Total phenol content was estimated through the Folin-Ciocalteu method [29]. Then 0.5 ml of plant extracts or gallic acid (standard phenolic compound) was mixed with Folin-Ciocalteu reagent (5 ml) and aqueous Na_2CO_3 (4 ml, 1M). The mixtures were allowed to stand for 15 min and the total phenols were determined by spectrophotometrically (Perkin Elmer, USA) at 765 nm. Gallic acid was used as a

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standard for calibration curve. Total phenol values were expressed in terms of mg equal gallic acid in 1 g powder dry plant.

Determination of total flavonoid content

The flavonoids content was determined by aluminium trichloride method using quercetin as a reference compound [8]. This method is based on the formation of a complex flavonoid-aluminum having the maximum absorptivity at 415 nm, after remained react at room temperature for 30 min. Briefly, 0.5 ml of each extract (1:10 g ml^{-1}) in methanol was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. The calibration curve was prepared by preparing quercetin solutions at different concentrations from 12.5 to 100 g ml^{-1} in methanol.

DPPH free radical scavenging activity

The hydrogen atom or electron donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of the purple-colored methanol solution of DPPH. The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the extracts. In order to determine the radical scavenging ability, the method reported by Mensor et al. [22] was used. Briefly, methanolic solution of 0.5 ml of DPPH (0.3 mM) was added to 2.5 ml of the different concentrations of plant extracts (0.2, 0.4, 0.6, 0.8, and 1 mg ml^{-1}). The samples were first kept in the dark and allowed to react at room temperature and then their absorbance was read at 517 nm after 30 min. The antiradical activity (I) was determined using the following formula:

$I\% = 1- (A_s – A_b)/A_c \times 100$

Blank samples (A_b) contained 1 ml methanol and 2.5 ml of various concentrations of extract; control sample (A_c) containing 1 ml of 0.3 mM DPPH and 2.5 ml methanol. The optical density of the samples, the control and the empty samples were measured in comparison with methanol. The discoloration was plotted against the sample

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concentration in order to calculate the IC_{50} value, which is the amount of sample necessary to decrease the absorbance of DPPH by 50% [17].

Antioxidant activity in *β*-carotene/linoleic acid model system

In this assay antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation [5]. A stock solution of β-carotene/linoleic acid was prepared as follows: first, 0.5 mg of β-carotene was dissolved in 1 ml of chloroform, then 25 µl of linoleic acid and 200 mg of Tween 40 were added. The chloroform was subsequently evaporated using a vacuum evaporator. Then 100 ml of distilled water saturated with oxygen (30 min at 100 ml min^{-1}) were added with vigorous shaking. Aliquots (2.5 ml) of this reaction mixture were transferred to test tubes, and 350 µl portions of the extracts (2 g $^{l-1}$ in ethanol) were added before incubating for 48 h at room temperature. The same procedure was repeated with BHT at the same concentration and a blank containing only 350 µl of ethanol. After incubation period, absorbance of the mixtures was measured at 490 nm. Antioxidant capacities of the samples were compared with those of BHT and the blank.

Antibacterial activity assay

Extracts were dissolved in DMSO to a final concentration of 1 mg ml-1. Antibacterial activities of the methanolic extracts were evaluated against 4 gram positive and negative bacteria, namely *Haemophilus influenzae* PTCC 1623, *Pseudomonas aeruginosa* PTCC 1430, *Bacillus cereus* PTCC 1247 and *Staphylococcus aureus*, by disc diffusion method [6] at 4 different concentrations. The extracts were dissolved in DMSO to make a 100 mg ml^{-1} solution and other concentrations make from this concentration, and then apply on the blank paper discs. Dried discs were placed onto Muller-Hinton agar medium that previously inoculated with a bacterial suspension (1.5×108 of bacteria in ml). The cultures were incubated at 37°C for 24 h. The antibacterial activity against each test organism was quantified by determining mean zone of inhibition. Gentamicin, penicillin, tetracycline, ampicillin and neomycin were also used as positive controls.

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Statistical analysis

The data were recorded as means ± SE. Analysis of variance was performed by Excel and SAA (VER, 9.2) procedures. Statistical analysis was performed using *P* value< 0.05 was regarded as significant.

Results and discussion

Total phenol and flavonoid contents

Phenolic compounds are a class of antioxidant agents, which act as free radical terminators and their bioactivities may be related to their abilities to chelate metals, inhibit lipoxygenase and scavenge free radicals [7]. The content of total phenols was measured by Folin-Ciocalteu reagent in terms of Gallic acid equivalent (standard curve equation: $y = 0.0041 x – 0.022$, $R^2 = 0.9982$). Phenol content in the bulbs extract (8.64 ± 0.26 mg g^{-1} DW) was higher than that of the leaves (6.25 ± 0.5 mg g^{-1} DW). The flavonoid content in terms of quercetin equivalent (the standard curve equation: $y = 0.0067 x + 0.0132$, $R^2 = 0.999$) in the bulbs and leaves extracts are not significantly different (Table 1). The compounds such as flavonoids, which contain hydroxyls, are responsible for the radical scavenging effect [10]. It is well-known that phenolic compounds contribute to quality and nutritional value in terms of modifying color, taste, aroma, and flavor and also in providing health beneficial effects. They also serve in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to survive and prevent molecular damage and damage by microorganisms, insects, and herbivores [39].

Table1. Total phenol and flavonoid contents of the leaves and bulbs extracts of *Allium ampeloprasum*

Extract	Total phenols (mg g^{-1} DW^a)	Total flavonoids (mg g^{-1} DW^a)
Leaves	6.25^b ± 0.5	4.19^c ± 0.07
Bulbs	8.64^a ± 0.26	3.25^c ± 0.03

DW^a, dry weight. Experiment was performed in triplicate and expressed as mean ± SE. Values in each column with different superscripts are significantly different ($P < 0.05$).

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DPPH free radical scavenging activity

Antioxidant properties, especially radical scavenging activities, are very important due to the deleterious role of free radicals in foods and biological systems [35]. Methanolic extracts were subjected to screening for their possible antioxidant activity by DPPH scavenging assay free radical-scavenging capacities of the corresponding methanolic extracts were measured by DPPH assay and the results are shown in Table 2. Leaves and bulbs extracts of *Allium ampeloprasum* showed a lower potency than ascorbic acid in scavenging of DPPH free radical. The higher antioxidant activity is reflected in a lower IC_{50}. The IC_{50} value of ascorbic acid as the standard compound was 0.125, while that of bulbs extract was 0.46 represents a lower antioxidant activity as the same as leaves extract (0.47) (Table 3). DPPH radical was used as a stable free radical to determined antioxidant activity of natural compounds [28]. Antioxidant properties, especially radical scavenging activities, are very important due to the deleterious role of free radicals in foods and biological systems [35]. The effects of phenolic compounds on DPPH radical scavenging are thought to be due to their hydrogen donating ability [27]. It is reported that the decrease in the absorbance of DPPH radical caused by phenolic compounds is due to the reaction between antioxidant molecules and radicals, resulting in the scavenging of the radical by hydrogen donation and is visualized as a discoloration from purple to yellow [33].

Table2. DPPH radical scavenging activity of the leaves and bulbs extracts of *Allium ampeloprasum* and ascorbic acid

Extract	DPPH concentration (mg ml^{-1})					Average
	0.2	0.4	0.6	0.8	1	
Leaves	$34.5^{e} \pm 1.5$	$47.77^{d} \pm 4.65$	$63.44^{c} \pm 1.03$	$72.44^{b} \pm 2.47$	$83.7^{a} \pm 0.63$	60.37
Bulbs	$32.35^{e} \pm 1.92$	$42.52^{d} \pm 1.81$	$64.72^{c} \pm 7.47$	$66.6^{c} \pm 1.62$	$77.46^{b} \pm 0.84$	56.73
Ascorbic acid	$80^{a} \pm 0.8$	$80.84^{a} \pm 1.7$	$81.2^{a} \pm 1.4$	$80.84^{a} \pm 2.3$	$80.16^{a} \pm 1.9$	80.61

Experiment was performed in triplicate and expressed as mean ± SE. Values in each row with different superscripts are significantly different ($P < 0.05$)

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Table3. IC_{50} values of DPPH scavenging activity of the leaves and bulbs extracts of *Allium ampeloprasum* and ascorbic acid

Extract	IC_{50}
Leaves	0.47
Bulbs	0.46
Ascorbic acid	0.125

Antioxidant activity in *β*-carotene/linoleic acid model system

In the *β*-carotene/linoleic acid system, oxidation of linoleic acid was inhibited by the leaves (40.2%) and bulbs (37.3%) extracts (Table 4). Results from present study showed that the leaves and bulbs extracts of *Allium ampeloprasum* have significantly different in inhibition of linoleic acid oxidation.

Table4. Inhibition percentages of the linoleic acid oxidation by the leaves and bulbs extracts of *Allium ampeloprasum* and BHT.

Extract	Inhibition (%)
Leaves	$40.2^{b} \pm 0.95$
Bulbs	$37.3^{c} \pm 0.26$
BHT	$95.66^{a} \pm 2.3$
Control^{-}	$26^{d} \pm 1$

Experiment was performed in triplicate and expressed as mean ± SE. Values in column with different superscripts are significantly different ($P < 0.05$).

Antibacterial activity

Antibacterial activities of the leaves and bulbs extracts of *Allium ampeloprasum* were evaluated here against 4 gram negative and positive bacteria, namely *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Staphylococcus aureus* by disc diffusion method in 4 different concentrations (100, 50, 25 and 12.5 mg ml^{-1} of extracts). Results indicated that bulbs extract has a strong antibacterial activity against gram negative and positive bacteria. *Bacillus cereus and Haemophilus influenzae* were the most sensitive bacteria to the bulbs extract in most of concentrations. In addition, the leaves extract could only inhibit *Bacillus cereus* at 100 mg ml^{-1}, but had no effect on *Haemophilus influenzae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Results from antibacterial activities of all extracts are presented in Tables 5 & 6. It seems that

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the extracts are great sources of phenolic compounds and represent the highest antibacterial activity against gram-positive bacteria.

Table5. Average inhibition zone of evaluated bacterial strains against the leaves extract of *Allium ampeloprasum*.

Bacteria	Inhibition zone				
	Concentration (mg ml^{-1})				
	100	50	25	12.5	DMSO (Solution)
Haemophilus influenzae	0	0	0	0	NA
Pseudomonas aeruginosa	0	0	0	0	NA
Bacillus cereus	4 ± 6.92	0	0	0	NA
Staphylococcus aureus	0	0	0	0	NA

NA, No active. Experiment was performed in triplicate and expressed as mean ± SE.

Table6. Average inhibition zone of evaluated strains against the bulbs extract of *Allium ampeloprasum*.

Bacteria	Inhibition zone				
	Concentration (mg ml^{-1})				
	100	50	25	12.5	DMSO (Solution)
Haemophilus influenzae	5.33^{b} ± 4.72	9.33^{a} ± 1.53	10^{a} ± 0	12^{a} ± 0	NA
Pseudomonas aeruginosa	0	0	0	0	NA
Bacillus cereus	3^{bc} ± 5.2	0	9.33^{a} ± 1.15	10.33^{a} ± 0.58	NA
Staphylococcus aureus	0	0	0	0	NA

NA, No active. Experiment was performed in triplicate and expressed as mean ± SE. Values in each row with different superscripts are significantly different ($P < 0.05$).

Conclusion

The results obtained from present study showed that the leaves and bulbs extracts of *Allium ampeloprasum*, which contain similar amounts of flavonoids and phenol compounds, represent similar antioxidant activity. The basis of β-carotene linoleate assay is discoloration of β-carotene in reaction with linoleic acid free radical. Radical is formed at elevated temperatures upon removal of hydrogen atom located between two double bonds of linoleic acid [2]. Our data indicate that the leaves and bulbs of *Allium ampeloprasum* are potential sources of secondary metabolites and their methanolic extracts possess good antioxidant activity. However, further studies are needed to evaluate the *in vivo* potential of these extracts in animal models and also isolation and characterization of the active antioxidant compounds. Determination of the antioxidant

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compounds of plant extracts and essential oils will help to develop new drug supplement for antioxidant therapy. From this point of view, the results presented here could be considered as the first information on the antioxidant activity of the plant species studied.

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