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# *In vitro* antioxidant effects of different extracts obtained from the leaves and seeds of *Allium ampeloprasum* subsp. *persicum*

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**ABSTRACT**

**Introduction:** This study evaluated antioxidant effects of hexane, chloroform, chloroform-methanol, aqueous and butanol extracts of leaves and seeds of *Allium ampeloprasum* subsp. *persicum*.
**Methods:** Various extracts were evaluated for total phenolic content, ferric reducing antioxidant power (FRAP), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and $H_2O_2$ scavenging activities.
**Results:** Total phenolic content varied from 2.46 to 8.12 mg gallic acid equivalents (GAE) per gram for various extracts with the highest level for butanol leave extract. Butanol leave extract showed the lowest $IC_{50}$ of DPPH scavenging. FRAP assay showed stronger antioxidant capacity for leaves than seeds' extracts and butanol extract was comparable to ascorbic acid at the concentration 50 µg/mL. In $H_2O_2$ scavenging activity assay, butanol and chloroform-methanol leave extracts showed the least $IC_{50}$ value.
**Conclusion:** Our results revealed moderately low amount of phenolic compounds and weak DPPH scavenging activity for all extracts of *A. ampeloprasum* subsp. *persicum*. However, leaves extracts showed good total antioxidant capacity and $H_2O_2$ scavenging activity.

*Implication for health policy/practice/research/medical education:*
The results of this study revealed better antioxidant activity for leaves extracts of *A. ampeloprasum* subsp. *persicum* than its seeds extracts through ferric reducing and scavenging of hydrogen peroxide. Therefore the leaves may be considered as the valuable plant part for medicinal and nutritional applications.

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## Introduction

Oxidative stress has an important role in the pathogenesis of various diseases such as cancer, diabetes, aging, hypertension, atherosclerosis and neurodegenerative disorders (1). Antioxidants are substances which are able to prevent and protect against diseases related to oxidative stress through preventing the free radical formation, scavenging and neutralizing reactive oxygen species, and inhibiting oxidative reactions (2). Therefore, widespread interest has been recently concerned on the evaluation of antioxidant plants and phytochemicals for reducing the risk of various diseases and improving the quality of life (3,4). *Allium* with around 800 species is an important genus of Amaryllidaceae family which widely spread in the northern hemisphere. The members of *Allium* genus are rich in various bioactive constituents including flavonoids, sulphuric compounds and saponins with a variety of biological activities including antimicrobial, antihypertensive, antihyperlipidemic, antidiabetic, anti-atherosclerotic and anticarcinogenic effects (5). *Allium ampeloprasum* subsp. *persicum* is an endemic Iranian plant which is known as Persian Leek and widely cultivated all over the country (6). This edible vegetable is used as food or traditional herbal medicine. It has been used for the treatment of headache, hemoptysis, asthma, obesity, constipation, hemorrhoids and goat. It is also reported as an aphrodisiac, emmenagogue and diuretic agent in folk medicine (6). The seeds of *A. ampeloprasum* subsp. *persicum* have also been traditionally used as an appetizer and aphrodisiac, and for the treatment of neuralgia, vitiligo,

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chronic diarrhea, freckle and hemorrhoids (7). Since there is limited information about the antioxidant activities of different polarity extracts of leaves and seeds of this subspecies, the present study was aimed to investigate the antioxidant properties of hexane, chloroform, chloroform-methanol, aqueous and butanol extracts obtained from the leaves and seeds of *A. ampeloprasum* subsp. *persicum in vitro*.

## Materials and Methods

### Chemicals

The commercial kits for assessment of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, ferric reducing antioxidant power (FRAP) and hydrogen peroxide ($H_2O_2$) scavenging activity were purchased from Hakiman Shargh Research Co. (Isfahan, Iran).

### Plant material and preparation of extracts

The leaves and seeds of *A. ampeloprasum* subsp. *persicum* were purchased from a local market in Isfahan in August 2017. After identification of the plant, a voucher specimen (No. 2751) was deposited at the Herbarium of the School of Pharmacy and Pharmaceutical Sciences, Isfahan, Iran. Hexane, chloroform, chloroform-methanol, butanol and aqueous extracts were evaluated in this study. For preparation of each extract, the seeds or air-dried leaves were finely powdered and respectively extracted with hexane, chloroform, chloroform-methanol (9:1) or methanol using maceration method for 24 hours at room temperature. Methanol fraction was suspended in water for making aqueous extract and then extracted with butanol. After filtration and concentration under vacuum by rotary evaporator, each extract was freeze-dried and kept in the refrigerator till used for the assays.

### Total phenolic assay

Total phenolic content of various extracts of leaves and seeds of *A. ampeloprasum* subsp. *persicum* was measured using Folin-Ciocalteu reagent (8). Briefly, the diluted reagent and $Na_2Co_3$ solution (20%) were mixed with plant samples. Then, UV absorbance was detected at 765 nm using a UV-visible spectrophotometer. Total phenolics were estimated using a standard curve obtained from different concentrations of gallic acid. The total phenolic content was presented as milligram of gallic acid equivalents (GAE) per gram of dried plant extract.

### DPPH radical scavenging activity assay

The scavenging effect of different extracts on DPPH free radical was evaluated based on previous studies (9). Briefly, methanol solution of DPPH was added to different concentrations of plant extracts (10-1000 µg/mL). The mixture was gently homogenized and after 30 minutes incubation in dark condition at room temperature, the absorbance was read at 517 nm using a microplate reader/spectrophotometer. The ability of plant extracts for scavenging DPPH was estimated using the formula $[(A_0\text{-}A_1)/A_0] \times 100$, where $A_0$ is the absorbance of the control, and $A_1$ is the absorbance of the sample. Ascorbic acid was used as the standard reference. The half maximal inhibitory concentration ($IC_{50}$) values were calculated using their calibration curve.

### Ferric reducing antioxidant power assay

The total antioxidant capacity of different plant extracts was evaluated by FRAP method. In this colorimetric assay, the reduction of ferric-tripyridyltriazine complex to ferrous form is expressed as antioxidant capacity. Briefly, the FRAP reagent was added to different concentrations of plant extracts (10-1000 µg/mL). Absorbance was read at 570 nm using a microplate reader/spectrophotometer. The FRAP value of samples was calculated using a standard curve of $FeSO_4 \times 7H_2O$ and expressed as micromole of FeII equivalents per liter (10).

### Hydrogen peroxide scavenging activity assay

The ability of different plant extracts to scavenge $H_2O_2$ was measured based on the ferrous ion oxidation by xylenol orange reagent (FOX1) Method. A solution of $H_2O_2$ (10 µL, 2 mM) was mixed with 10 µL of plant extracts (10-1000 µg/mL). Then, FOX1 reagent was added (190 µL) and the absorbance of solution was measured at 540 nm using a microplate reader/spectrophotometer. The percentage of $H_2O_2$ scavenging was estimated using the formula $[(A_0\text{-}A_1)/A_0] \times 100$, where $A_0$ is the absorbance of the control, and $A_1$ is the absorbance of the sample. $IC_{50}$ values were calculated for plant and standard samples (11).

### Statistical analysis

Data were expressed as mean ± standard error of mean (SEM). For statistically evaluation, a one-way analysis of variance (ANOVA) followed by Tukey post hoc test was used (SPSS software version 16.0). $P$ values <0.05 were considered as significant.

## Results

### Total phenolic assay

For both seeds and leaves samples, butanol extract showed the highest and hexane extract showed the lowest level of phenolics and the total phenolic content in the leaves extract was higher than that of the seeds (Table 1).

### DPPH radical scavenging activity assay

$IC_{50}$ of ascorbic acid was 32 µg/mL. DPPH scavenging activity was in the following order for various seeds extracts: chloroform-methanol > chloroform > butanol > hexane > aqueous extract, and for leaves extracts: butanol > aqueous > chloroform-methanol > hexane > chloroform extract (Table 1).

**Table 1.** Total polyphenolic content as mg gallic acid equivalents (GAE)/g and half maximal inhibitory concentration ($IC_{50}$) for 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity of various extracts obtained from seeds and leaves of *A. ampeloprasum* subsp. *persicum*

| | Total polyphenolic content (mg GAE/g) | | DPPH scavenging activity ($IC_{50}$, µg/mL) | |
|---|---|---|---|---|
| Extract | Seeds | Leaves | Seeds | Leaves |
| Butanol | 5.33 ± 0.38[a] | 8.12 ± 0.41[a] | 668[a] | 315[a] |
| Aqueous | 4.81 ± 0.42[a] | 7.34 ± 0.35[a] | 792[a] | 413[b] |
| Chloroform-methanol | 5.15 ± 0.52[a] | 7.45 ± 0.51[a] | 457[b] | 439[b] |
| Chloroform | 3.86 ± 0.38[b] | 3.23 ± 0.64[b] | 556[c] | 585[c] |
| Hexane | 2.95 ± 0.28[c] | 2.46 ± 0.55[c] | 720[a] | 554[c] |

Values in each column with different superscripts are significantly different ($P < 0.05$).

### Ferric reducing antioxidant power assay

The results indicated an increasing trend in total antioxidant capacity with increasing the extracts concentrations (Figure 1). There was a significant difference in FRAP value between different seeds extracts and ascorbic acid. The antioxidant activity of seeds extracts was in the following order at the concentration 100 µg/mL: chloroform-methanol > hexane > chloroform = butanol > aqueous extract. For the leaves extracts, the results showed stronger antioxidant capacity than the seeds' extracts and the butanol extract was comparable to ascorbic acid at the concentration 50 µg/mL. The FRAP values were in the following order for leaves extracs: butanol > chloroform-methanol > aqueous > hexane > chloroform extract (Figure 2).

### Hydrogen peroxide scavenging activity assay

There was increasing trend in $H_2O_2$ scavenging activity with increase in the sample concentration. $IC_{50}$ value of ascorbic acid was 41 µg/mL. The scavenging activity of seeds extract with $H_2O_2$ was in the following order: chloroform-methanol = chloroform ($IC_{50}$= 235 µg/mL) > butanol ($IC_{50}$= 471 µg/mL) > hexane = aqueous extract ($IC_{50}$= 937 µg/mL) (Figure 3). For the leaves extracts, the results were in the following order: butanol ($IC_{50}$= 100 µg/mL) > chloroform-methanol ($IC_{50}$= 145 µg/mL) > aqueous ($IC_{50}$= 227 µg/mL) > hexane ($IC_{50}$= 687 µg/mL) > chloroform extract ($IC_{50}$= 810 µg/mL) (Figure 4).

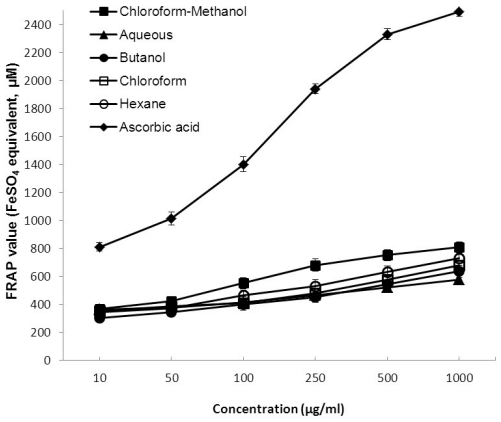


**Figure 1.** FRAP values of various extracts obtained from seeds of *A. ampeloprasum* subsp. *persicum* (25-1000 µg/mL) determined as ferrous sulfate equivalents. Values are means ± SEM from three independent experiments.

## Discussion

*Allium ampeloprasum* L. is an *Allium* species with wide traditional and dietary uses. Despite the potentially valuable medicinal and nutritional profile of this species, there was no research regarding antioxidant activity of various polarity extracts of leaves and seeds of *A. ampeloprasum* subsp. *persicum*. In the present study, total phenolic contents varied from 2.46 to 8.12 mg GAE/g for various extracts of *A. ampeloprasum* subsp. *persicum,* and there were higher levels in the leaves extract than that of the seeds. Our data are in agreement with the values

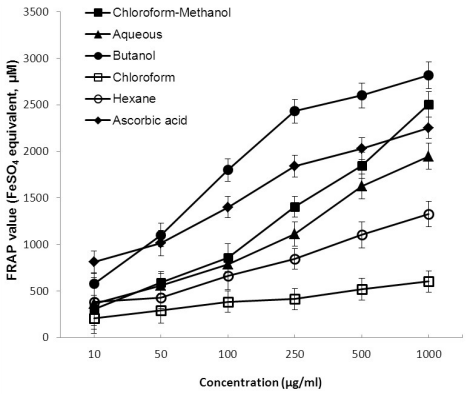


**Figure 2.** FRAP values of various extracts obtained from leaves of *A. ampeloprasum* subsp. *persicum* (25-1000 µg/mL) determined as ferrous sulfate equivalents. Values are means + SEM from three independent experiments.

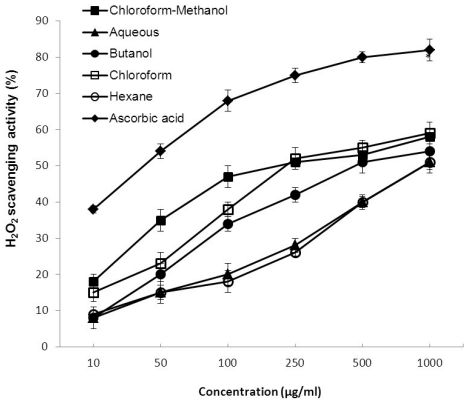


**Figure 3.** H2O2 scavenging activity of various extracts obtained from seeds of *A. ampeloprasum* subsp. *persicum* (25-1000 µg/mL) tested by FOX1 Method. Values are means + SEM from three independent experiments.

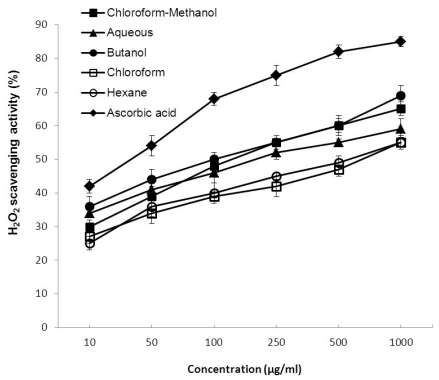


**Figure 4**. H2O2 scavenging activity of various extracts obtained from leaves of *A. ampeloprasum* subsp. *persicum* (25-1000 µg/mL) tested by FOX1 method. Values are means + SEM from three independent experiments. .

reported by some researchers (12). However, our results showed moderately low amount of phenolic compounds in different extracts of seeds and leaves of *A. ampeloprasum* subsp. *persicum*.

In the DPPH assay, the higher antioxidant activity is reflected in the lower $IC_{50}$ value. It has been proposed that samples with $IC_{50}$ lower than 50 µg/mL are very strong antioxidants, with 50-100 µg/mL are strong, with 101-150 µg/mL are moderate, and with $IC_{50}$ greater than 150 µg/mL are weak antioxidants (9). Butanol leave extract which contains more phenolic compounds such as flavonoids showed the lowest $IC_{50}$ of DPPH scavenging (315 µg/mL). However, all extracts exhibited weak antioxidant activity. Similar to our results, Karamian and Hosseini reported the $IC_{50}$ of DPPH 470 µg/mL for methanol leaves extract of *A. ampeloprasum* (13). Weak DPPH scavenging activity has also been reported for seeds of other *Allium* species including *A. cepa* (14).

Our results showed weak total antioxidant capacity for all seeds extract compared to ascorbic acid. However, chloroform-methanol extract which was rich in both polar and nonpolar compounds and the hexane extract containing nonpolar compounds exhibited more FRAP value. Several mono- and polyunsaturated fatty acids including linoleic, oleic and palmitic acid and also some phytosterols with antioxidant activities have been identified in seeds of *Allium* species (15). Phytosterols possess antioxidant property through radical scavenging and stabilizing the membranes (16,17).

Unlike seeds extract, leaves extracts showed good total antioxidant capacity comparable to that of standard in a dose-dependent manner. Butanol extract exhibited the highest FRAP value that may be due to the relatively high contents of tannins, flavonoids and saponins.

Regarding $H_2O_2$ scavenging activity, butanol leave extract showed the less $IC_{50}$ value indicating its strong antioxidant capacity. Chloroform-methanol extract also indicated moderate antioxidant capacity.

Various antioxidant activities have been reported for *Allium* species that may be attributed to different phytochemicals constituents including water-and lipid-soluble organosulfur compounds microelements, dietary fibers, saponins, polyphenols and flavonoids (18). Several known and new saponins such as spirostane and cholestane saponins have been isolated from *A. ampeloprasum* subsp. *persicum* (6). Important biological effects including effective antioxidant activities have also been established for natural saponins in various investigations (19).

## Conclusion

In conclusion, our results showed moderately low amount of phenolic compounds and weak DPPH scavenging activity for all extracts of *A. ampeloprasum* subsp. *persicum*. However, leaves extracts showed good total antioxidant capacity and $H_2O_2$ scavenging activity. The best antioxidant activity was also found for butanol leave extract which may be attributed to high contents of tannins, flavonoids and saponins in this extract.

## Authors' contributions

LS and BZ contributed to the idea and design of the study. The experimental procedures were done by SFN under the supervision of LS and BZ. The manuscript was prepared and written by LS and approved by all authors.

## Conflict of interests

The authors declare no conflicts of interest. The authors alone are responsible for the content of the paper.

## Ethical considerations

All procedures were approved by the ethics committee of Isfahan University of Medical Sciences (IR.MUI.REC.1395.1.072) and the authors observed ethical issues in accordance with the guidelines of National Committee of Ethics for Biomedical Researches.

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