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Antioxidant effects of methanolic extracts of *Anthemis Susiana* Nabelek, *Alyssum campestre*, and *Gundelia tournefortii*

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ABSTRACT

One of the prominent effects of free radical activity in the body is damage to biological and vital molecules such as nucleic acids, proteins and lipoprotes. So, free radicals, especially reactive oxygen species, play an important role in medical issues. Using compounds that neutralize free radicals, i.e. antioxidants, is a suitable choice to minimize the damage caused by their production. Herbs are recognized as rich sources of antioxidants and consumption of these plants can be effective for human health. The present study was conducted to evaluate the antioxidant property of native medicinal plants including Babouneh shoshi, *Anthemis Susiana* Nabelek, Ghodoumeh dokhtari, *Alyssum campestre*, and Kangar, *Gundelia tournefortii* in Ilam Province, Iran. Total antioxidant capacity was measured using at 570 nm with the enzyme-linked immunosorbent assay (ELISA). The results showed that the total antioxidant capacities of Babouneh shoshi, Ghodoumeh dokhtari, and Kangar are 4.29, 1.01 and 1.25 mmol Fe^{2+} L^{-1}, respectively. Generally, there are several natural antioxidant compounds in Iranian medicinal herbs which made them as reliable sources for therapeutic purposes. The essential oils of these plants are the source of herbal secondary metabolites with antioxidant and antimicrobial effects that are recommended for their use in the food, pharmaceutical, and health industries.

Keywords: Antioxidant, Plants, *Anthemis Susiana*, *Alyssum campestre*, *Gundelia tournefortii*.
Article type: Research Article.

INTRODUCTION

Medicinal plants are favoured by people in traditional Iranian medicine to treat diseases. There are about 7500-8500 plant species in Iran, of those, 200 more species exhibit medicinal and economic value. Nowadays, medicinal plants have displayed an interesting effect in the treatment of diseases. Therefore, exploring the biological properties of these plants and understanding the mechanism of their therapeutic effects has attracted a lot of attention. Phenolic constituents are recognized as a main class of natural herbal metabolites comprising flavonoids, anthocyanins and tannins that are regularly presented in various parts of vegetables, fruits and nuts such as leaves, seeds and roots. Herbal chemical compounds play a significant role in the production of food and medications because of their wide spectrum of desirable biological properties, such as antioxidant effects. Antioxidants are constituents that protect body against free radicals as important factors in the occurrence of oxidative stress in connection with the pathogenesis of various important disorders. It is now well established that oxidative degradation due to the activity of this development causes several diseases and atherosclerosis, vascular, chronic such as heart disease, aging, cataract, and inflammation (Valko *et al*. 2006). Research has shown that

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some plants with properties such as controlling the oxidation of lipids can show antioxidant effects (Wong *et al.* 2020). Moreover, the presence of bioactive compounds in plants has made them a good source to replace the synthetic ones (Annegowda *et al*. 2012). When using medicinal plants, it is necessary to be aware of the existence of the antioxidant compounds and their biological properties (El-Ghorab *et al*. 2007). In Iran, using these plants has been traditional and native for a long time. *Alyssum campestre* is one of the native medicinal plants in Iran and belongs to the Cruciferae / Brassicaceae family, which is very valuable medicinally and in traditional medicine. Phenolic compounds include a variety of metabolites such as many cyclic compounds such as phenols, flavonoids, flavonoids, tannins, and lignin and are one of the largest groups in all plants that exert their antioxidant activity by preventing the formation of primary precursors and scavenging the radicals produced (Akhtarian *et al*. 2015). *Gundelia tournefortii* is another native plant in Asian regions, including Iran, whose flowers, leaves, seeds, and roots are used for food and medicine (Ertuğ 2000). In Iran, the fresh flower buds of this plant are sold in local markets in spring (AL-Qadhi *et al*. 2020). This plant is known as a medicinal plant in the some Middle East countries with temperate, mountainous and semi-desert regions, especially Egypt, Iran, Iraq, Turkey, Syria, Jordan, Azerbaijan, Armenia and Turkmenistan (Karis *et al*. 2001). This plant is found as a wildling in early autumn and late winter in the hills of the western and southern regions of Iran (Jamshidzadeh *et al*. 2020). The genus *Chamomile* or *Anthemis* (Babouneh) belongs to the Asteraceae family, of those, about 130 species are distributed in the world. This genus has 39 species of annual and perennial herbaceous plants in Iran, of those, 15 species are exclusive to Iran and other species, in addition to Iran in Iraq, Afghanistan, Pakistan, Anatolia, Central Asia, Libya, Cyprus, Sinai, Syria, Palestine, Transcaucasia, Turkmenistan, Lebanon and Caucasus (Karis *et al*. 2001). In recent years, due to the adverse side effects of synthetic medicinal compounds, the tendency to use herbal plants has increased. Due to the increasing tendency of human beings to cure diseases using plant-derived natural constituents, this study aimed to investigate the antioxidant effect of native medicinal plants of *Anthemis Susiana* (Babouneh Shoshi), *Alyssum campestre* Nabelek (Ghodoumeh dokhtari), and *Gundelia tournefortii* (Kangar) in Ilam Province, Iran.

MATERIALS AND METHODS

Plant preparation

In April 2022, *Oliveria decumbens* Vent. and the *Caparis spinosa* L. were prepared from Dehloran City in Ilam Province (Western Iran). The morphological keys of the book of plant flora of Ilam Province were used for identification of the plant. The plant was approved by the BMPRC affiliated to the Ilam University of Medical Sciences. Collected herbs were cleaned and air-dried in the shade. Then, the dried plant was ground by a mixer and used for evaluation of the antioxidant effect. The details of collected medicinal plant are shown in Table 1.

Table 1. Medicinal plant details of the present study.

Plant name	scientific name	Plant family	Collection area	Geographical coordinates
Babouneh shoshi	*Anthemis Susiana*	Compositae	Dehloran	32° 41' 28" North, 47° 15' 58" East
Ghodoumeh dokhtari	*Alyssum campestre*	Brassicaceae	Ilam	32° 41' 28" North, 47° 15' 58" East
Kangar	*Gundelia tournefortii*	Asteraceae	Dehloran	32° 41' 28" North, 47° 15' 58" East

Determination of antioxidant activity

Preparation of plant samples

At first, we dried the fresh plant, then 1 g plant powder was shaken with 100 mL methanol for 6 h. The present solution was passed through a strainer and centrifuged in a Falcon tube for 10 min at 6000 rpm. The obtained solution was the final plant one for testing.

Preparation of Stock Solution

An aliquot of 2.2 mL Reaction 2b (R2b) solution was mixed to the Reaction 2a (R2a) solution and was vortexed until complete dissolution, hence Reaction 2 (R2) solution was prepared. The latter was then mixed in a ratio of 1:1 and after vortexing for 5 times, its volume was added to the Reaction 1 (R1) solution as the stock solution of an antioxidant kit (According to the kit instruction's manual).

Standard solution preparation

In this step, the standard solution was prepared with doses of 0, 0.2, 0.4, 0.6, 0.8 and 1 µL to obtain the linear equation based on it. The linear equation resulting from different concentrations of the standard solution is shown in Fig. 1.

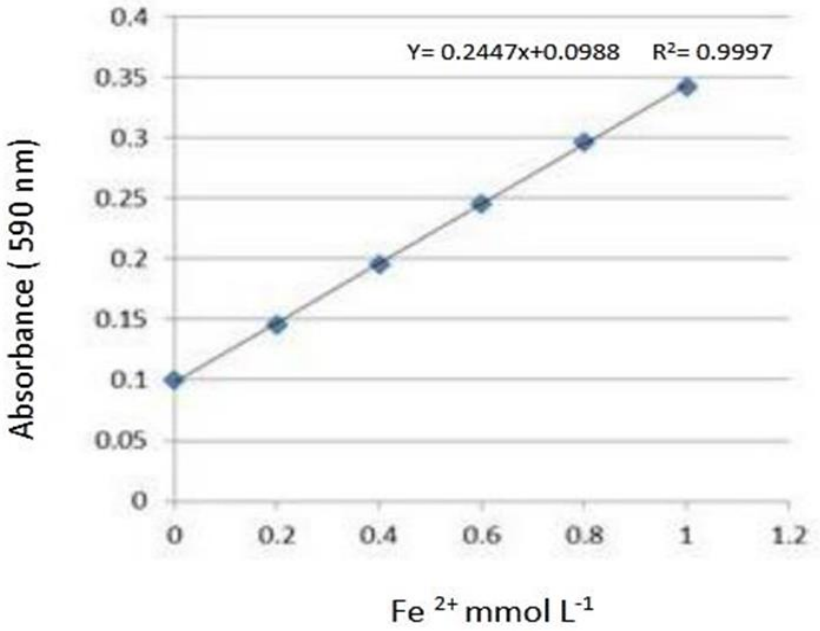

Fig. 1. The standard curve of different concentrations of standard solution.

Procedure

At first, 250 µL of the prepared working solution was added to each well, then 5 µL of the plant sample solution was added to each well. The microplate was incubated for 30 min at 35-50 °C. Finally, the antioxidant potential of each plant was read at a wavelength of 570 nm with an ELISA reader.

RESULTS

The results showed that the total antioxidant capacity of Babouneh shoshi, Ghodoumeh dokhtari, and Kangar are 4.29, 1.01, and 1.25 mmol Fe^{2+} L^{-1}, respectively (Table 2).

Table 2. Total antioxidant capacity of native medicinal plants.

Common name	Scientific name	Total antioxidant capacity
Babouneh shoshi	*Anthemis Susiana*	4.29 mmol Fe^{2+} L^{-1}
Ghodoumeh dokhtari	*Alyssum campestre*	1.01 mmol Fe^{2+} L^{-1}
Kangar	*Gundelia tournefortii*	1.25 mmol Fe^{2+} L^{-1}

The results of this study, as in other studies, have shown that plants with higher phenolic compounds exhibit high anti-radical activity (Arumugam *et al*. 2010; Bai *et al*. 2010).

DISCUSSION

Nowadays, special attention is paid to the protective effects of antioxidants of natural origin. Using herbs to treat a wide range of diseases is rapidly increasing. Iran has many facilities for the production and export of medicinal plants (Zarabi *et al.* 2017; Falahi *et al*. 2019). There are several pieces of evidence about the presence of phytochemical constituents in aromatic and medicinal plants. These chemical compounds exhibit various health-related benefits including antioxidant and antimicrobial properties which can affect human health through removing both oxidative stress and pathogens (Ceylan *et al*. 2019). Positive correlation is found between the measurement of antioxidant activity and phenol content in Qadoomeh plant as water-soluble antioxidant

compounds. Probably the high level of these compounds in methanolic extract could be a major reason for the high regenerative power at this stage (Ibrahim *et al*. 2013). Research shows that since most antioxidant compounds are polar, methanolic solvents show the highest anti-radical activity (Hayouni *et al*. 2007). Due to the complex structure of the extracts, it is not easily possible to express the correlation between the antiradical activity and the compounds in the extracts, which can be attributed to the difference in the type and amount of the compounds in them. In a study by Bahrami Kia *et al.* (2015) the antioxidant activity of methanolic extracts of *G. tournefortii* was checked. The results showed significant antioxidant activity in different active ingredients and contained significant levels of phenolic acids, flavonoids, and ascorbic acid. In general, it has a high level of antioxidant activity (Ibrahim *et al*. 2013). In different studies, plants showed high antioxidant properties containing one or more types of these antioxidants. Similar studies in different countries have evaluated the total antioxidant capability of medicinal herbs in the respective regions. For instance in Singapore, the antioxidant powers of twenty seven fruit species were reported in Copa (2003). The antioxidant effects of 45 medicinal herbs were examined in Italy during 2003, in addition to 34 vegetables and 30 fruit species (Bahrami *et al*. 2015). Similar to the present study, Bahrami *et al*. (2015) reported that the ethanolic extract of Kangar in a dose-dependent process causes the accumulation of DPPH free radicals. In addition, the amount of IC_{50} was 92.2 mg mL^{-1} for this plant. According to their results, it can be said that artichoke has antioxidant properties and can be used as a medicinal plant and food source to modify the diet (Bahrami *et al*. 2015). Fazeli Nasab *et al.* (2017) reported that IC_{50} levels of rosemary hydroalcoholic extract were 80.92 μg μL^{-1}. The anti-radicalization of rosemary is related to the presence of polar compounds such as rosmarinic acid and other phenolic acids. According to their studies, the main phenols of rosemary extract exhibiting this activity include carnosic, carnosol and rosmarinic acid (Fazeli-Nasab *et al*. 2017). Chon *et al.* (2009) reported that based on antioxidant and anti-proliferative properties of Korean mulberry, some plant parts could be recommended as preventative and therapeutic agents. Methanol extracts and fractions of mulberry branches, roots and leaves dependently increased radical scavenging activity (over 70%). Highly correlated with the radical scavenging activity was shown by the total phenolic content of the extract of this plant in experiments. In addition, the methanolic extract of fermented mulberry leaves at a concentration of 1000 mL exhibited an anti-proliferative effect on the human stomach cancer cell line (SNU-601; Chon *et al*. 2009). Plant extracts and essential oils display antioxidant effects due to their phenolic substances and antioxidant compounds (Eftakhari *et al*. 2022; Khademian Amiri *et al*. 2022; Usunobun *et al*. 2022; Shahsavari *et al*. 2022; Shahsavari *et al*. 2022; Javaid *et al*. 2023). Many chemical compounds can cause lesions in body organs, especially the occurrence of cancer (Manouchehri *et al*. 2021). So, using substances, natural antioxidants and medicinal plants may be considered as an important approach in preventing the occurrence of many diseases (Hasanvand *et al*. 2019; Altememy *et al*. 2023; Amiri *et al*. 2023). The number of hydroxyl groups in the structure of antioxidant molecules is not usually the determinant factor of its antioxidant activity. The position of phenolic hydroxyl groups, the presence of other functional groups in the molecule, such as double bonds, as well as combination of hydroxyl and ketone groups, play an important role in antioxidant activity. The antioxidant activity of natural extracts is usually attributed to their ability to donate hydrogen.

CONCLUSION

Nowadays, one of the problems of the food industry is the employment of various synthetic compounds as preservatives, and the potential dangers of each of these compounds for human health have been suggested. Therefore, research on natural sources of antioxidants to replace synthetic compounds seems necessary. According to the results, it can be said that *Anthemis susiana* Nabelek, *Alyssum campestre*, and *Gundelia tournefortii* exhibit antioxidant properties and can be used as medicinal plants and food sources to modify the diet.

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