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Determination of Antioxidant Capacity of Methanolic Extract of *Allium ampeloprasum* subsp *Iranicum*

Somayeh Shahsavari[1], Sudip Kumar Mandal[2*], Dhiraj Kumar[3]

[1]Biotechnology and Medicinal Plants Research Center, Ilam University of Medical Sciences, Ilam, Iran
[2]Department of Pharmaceutical Chemistry, Dr. B. C. Roy College of Pharmacy and Allied Health Sciences, Durgapur, West Bengal, India
[3]Department of Pharmacy, Institute of Technology and Management, GIDA, Gorakhpur, Uttar Pradesh, India

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*Corresponding author:
E-mail: somayeh.shahsavari@gmail.com

ABSTRACT

Introduction: The unique properties of medicinal plants particularly their effects on human health have attracted the attention of many researchers. The antioxidant properties of medicinal plants are a strong reason to use them in the food and pharmaceutical industries. The aim of this study was to evaluate the antioxidant properties of *Allium ampeloprasum* subsp Iranicum.

Methods: Aerial parts of *A. ampeloprasum* were dried and ground. Plant samples were prepared using homogenizing plant powders in a methanol solution. The total antioxidant capacity of the plants was assessed by ferric iron-reducing antioxidant power (FRAP) assay.

Results: The results of the evaluation of the antioxidant activity of *A. ampeloprasum* exhibited that the total antioxidant capacity was obtained as 3.06 mmol Fe2+/L.

Conclusion: *A. ampeloprasum* showed potent antioxidant activity. It is recommended that the utilization of *A. ampeloprasum* in food and pharmaceutical industries could possibly possess beneficial health effects.

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Intorduction

Human reliance on the use of medicinal plants for treatment of various diseases has a long history. Although the use of chemical and synthetic drugs became extremely popular in the last half century, their side effects on health quickly caused people to turn towards medicinal plants again (Jamshidi-Kia et al, 2018; Regassa et al., 2022). It has been shown that medicinal plants have beneficial effects on human health due to their effective substances, medicinal and antioxidant compounds along with their potential therapeutic effects on various body organs and various diseases (Yu et al., 2021; Bagheri et al., 2019). Medicinal plants, as rich sources of antioxidants, play important role in the pharmaceutical industry and the treatment of diseases (Bagheri et all., 2021; Gulcin et al., 2020). Antioxidants are molecules or compounds that act as neutralizer of free radicals. The free radicals cause molecules to be damaged and lose their function. Antioxidants are responsible for the primary defense against these oxidative damages (Holst et al., 2008; Minh et al., 2016). Antioxidants are compounds that

significantly reduce the speed of oxidative reactions with different mechanisms at a low concentration compared to the substrate (Shahrajabian et al., 2021). Since plants produce a significant amount of phytochemicals with several biological activities including antioxidant effect, these phytochemicals have particular importance (García-Herrera et al., 2014). Phytochemicals are potential sources of compounds with antioxidant activity. Phytochemicals including alkaloids, terpenes and phenolic compounds have recognized as the most important compounds with antioxidant activity (Polito et al., 2022).

Allium ampeloprasum (Family Amaryllidaceae), which is commonly known as wild leek, is a plant with food and medicinal uses. Mediterranean region including southern Europe, northern Africa and western Asia are recognized as the main habitats of *A. ampeloprasum*. Although the growth of *A. ampeloprasum* has been observed in other parts of the world including America (northern and southern), Australia and India (Dokhani et al., 2022). Review of literature has showed use of this plant in traditional medicine to treat inflammatory diseases, cough, spasm and sore throat. *A. ampeloprasum* has been identified as a magical herb with amazing biological properties. Antioxidant, antimicrobial, anticancer, hypocholesterolemic, anti-inflammatory, anti-toxic and immunostimulating effects have been explained for various parts of *A. ampeloprasum* as therapeutic properties (Pelkonen et al., 2014). There are several types of phytochemicals which are related to biological properties of this plant including sulphur containing constituents, saponins, flavonoids and vitamins (Rahimi-Madiseh et al., 2017). Hence, the aim of the present study was to determine the antioxidant activity of methanolic extract of *A. ampeloprasum* subsp *Iranicum*.

Materials and Methods

Plant Materials

Collection of *Allium ampeloprasum* subsp *Iranicum* (aerial parts) was carried out from Ilam County of Ilam Province, Southwest of Iran, in May 2022. *A. ampeloprasum* was identified according to the morphological features of Ilam Province Plant Flora at the Biotechnology and Medicinal Plants Research Center, Ilam University of Medical Sciences, Ilam, Iran. *A. ampeloprasum* was air dried in shade, then ground and finally used for antioxidant assessment. The characteristics of *A. ampeloprasum* have been shown in Table 1.

Table 1. Characteristics of *Allium ampeloprasum* subsp Iranicum.

Scientific name	*Allium ampeloprasum* subsp *Iranicum*
Persian name	Tareh kouhi
Family	Amaryllidaceae
Collection area	Ilam county
Geographic coordinate	33° 37' 60" North, 46° 25' 60" East

Plant Sample Preparation

After drying the plant, 1 g of dry powder of the studied plants was homogenized using 100 mL of methanol solution and was shaken in the same solution for 6 hours. The resulting solution was then poured into a plastic falcon and centrifuged at 6000 rpm for 10 minutes. The resulting solution was used as a sample.

Determination of Antioxidant Activity

Total antioxidant capacity of the plants was assessed by ferric iron reducing antioxidant power (FRAP) assay.

Stock Solution Preparation

2.2 mL of R2b solution was added to the parent bottle R2a and vortexed until complete dissolution and R2 solution was obtained. Then, R2 solution was mixed in a ratio of 1: 1 and after vortexing 5 times of its volume was added to R1 solution. The resulting solution is stock solution of an antioxidant kit.

Standard Solution Preparation

Standard solution at 0, 0.2, 0.4, 0.6, 0.8 and 1 µL was also prepared. The linear equation obtained from the different concentrations of the standard solution is illustrated in Figure 1.

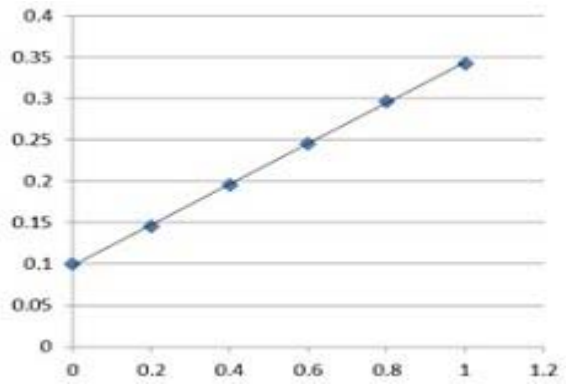

Figure 1. Linear equation obtained from different concentrations of standard solution.
Y= 0.2447X + 0.0988
R2=0.9997

Procedure

First, 5 µL of the prepared plant solution was added to each well and then 250 µL of the prepared working solution was added to each well containing plant solution. The micro-plate was then incubated at 35-50 °C for 30 minutes and finally read at 570 nm with ELISA reader (Dokhani et al., 2022).

Results

The results of evaluation of antioxidant activity of *A. ampeloprasum* exhibited total antioxidant capacity as 3.06 mmol Fe^{2+}/L.

In the mentioned method, number of 1 mmol Fe^{2+}/L is standard number of antioxidant capacity. According to the obtained results, antioxidant capacity of *A. ampeloprasum* plant is strong and has more than 3 times of standard limit of antioxidant effect.

Discussion

Medicinal plants are valuable natural resources that are used as raw materials to produce safe medicines for many diseases (Bose et al., 2020; Mandal et al.m 2020). The goal of current investigation was to assess the antioxidant capacity of methanolic extract of *A. ampeloprasum*. Today, due to the fact that herbal medicines have good effects in the treatment of various diseases, a new approach to the use of herbal medicines has been created (Benkeblia et al., 2005). *A. ampeloprasum* is a medicinal herb with a wide range of application in pharmaceutical and dietary industries. The presence of different phytochemicals with various biological properties including antioxidant effect has been reported in *A. ampeloprasum* (Dokhani et al., 2022). In our study, the findings of estimation of antioxidant activity of *A. ampeloprasum* exhibited that the total antioxidant capacity through FRAP assay was obtained as 3.06 mmol Fe^{2+}/L. In a similar study of Feghhi-Najafabadi and her colleagues, revealed that various parts of *A. ampeloprasum* exert an antioxidant property. The authors concluded that various extracts of leaves of *A. ampeloprasum* had better antioxidant capacity compare to other parts of this plant. Furthermore, results of the mentioned study exhibited that extracts of leaves, especially chloroform-methanol at a concentration of 1000 µg/mL, had a strong antioxidant capacity found through FRAP assay (FRAP value=2500 $FeSo_4$ equivalent, µM) supporting the results of our study. They also reported that leaf-extracts showed a potent H_2O_2 scavenging activity (Ashraf et al., 2013). Findings of the above study displayed that presence of tannins, flavonoids and saponins is related to high FRAP value in chloroform-methanol extract of the leaves. Results of the investigation confirmed antioxidant effects of this plant in line with our results. However, differences in FRAP value can be attributed to different methods for extract preparation (Nehdi et al., 2020). Several antioxidant properties have been considered for *A. ampeloprasum* that can be related to a wide spectrum of active ingredients such as sulfur constituents, polyphenols, flavonoids, saponins and dietary fibers. For instance, various recognized saponins including spirostane and cholestane saponins have been identified from *A. ampeloprasum*. In a study conducted by Pelkonen et al., various biological properties of *A. ampeloprasum* including antioxidant and antimicrobial properties were evaluated. The results of this study indicated that ethanolic extract of onion bulbs of *Allium ampeloprasum* had a potent antioxidant capacity through DPPH assay. In this study, authors concluded that strong antioxidant capacity of *A. ampeloprasum* may be attributed to the abundant presence of phytochemicals constituents such as flavonoids, alkaloids, saponins, and steroids (Pelkonen et al., 2014). Our findings agreed with results of above mentioned study. However, difference in type of extracts and used part of the plant made some differences in results, both studies confirmed strong antioxidant effect of this plant. The antioxidant activity of seed oil of *A. ampeloprasum* has also been evaluated in a study conducted by Nehdi et al. This study revealed that seed oil of *A. ampeloprasum* had a significant antioxidant capacity through ABTS assay (136.30 µM TEAC/g oil). The authors found that potent antioxidant activity may be attributed to high total flavonoid phenolic contents of this plant (Nehdi et al., 2020).

Conclusions

In sum, we concluded that the extract of *A. ampeloprasum* had a potent antioxidant property and this could be a reason for their use in food and pharmaceutical industries. Based on our findings, it is recommended that utilization of *A. ampeloprasum* can be benefitted for health.

Conflict of interest

There is no conflict of interest.

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Consent for publications

The authors approved the manuscript for publication.

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None.

Authors' contributions

SS conceived the research idea and SKM designed the work. SSH carried out the experiment, SS and DK wrote the first draft of the manuscript, DK carried out the literature search, SS carried out the statistical analysis, and SKM supervised the study. All authors read and approved the final manuscript for publication.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the author.

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