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Determination and Evaluating the Antioxidant Properties of *Ziziphus nummularia* (Burm.f.) Wight & Arn., *Crataegus pontica* K.Koch and *Scrophularia striata* Boiss

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RESEARCHERS have shown an increasing attention to the use of medicinal plants because of their beneficial effect on human health. The antioxidant properties of medicinal plants is a strong reason to use them in the food and pharmaceutical industries. Hence, the aim of this study was to evaluate the antioxidant properties of *Ziziphus numnularia*, *Crataegus pontica* and *Scrophularia striata*. Aerial parts of *Ziziphus numnularia*, *Crataegus pontica* and *Scrophularia striata* were dried and ground. Then, plant samples were prepared using homogenizing plant powders in methanol solution. Finally, the total antioxidant capacity of the plants was assessed by ferric iron reducing antioxidant power (FRAP) assay. The results indicated that the total antioxidant capacity was found as 3.16, 2.95 and 1.14 mmol Fe²⁺/L for *Ziziphus numnularia*, *Crataegus pontica and Scrophularia striata* showed a potent antioxidant activity. It is recommended that utilization of *Ziziphus numnularia*, *Crataegus pontica* and *Scrophularia striata* in food and pharmaceutical industries could possibly possess beneficial health effects.

Keywords: Antioxidant Activity, Medicinal Plants, Ziziphus nummularia, Crataegus pontica, Scrophularia striata.

Introduction

Concerns about the side effects of chemical drugs, including drug resistance, have led to increased tendencies to use compounds with natural origin, such as natural compounds extracted from medicinal plants [1]. Humanity has historically been highly dependent on medicinal plants for the treatment of variety of diseases and disorders [2-6]. Today, many populations in developing countries need to use medicinal plants to meet their basic medical needs. Despite the widespread use of chemical drugs, medicinal plants still play an important role in the production of drugs in the pharmaceutical industry [7]. The presence of bioactive compounds in medicinal plants causes the numerous biological effects such as antioxidant properties [8, 9]. Natural antioxidants derived from medicinal plants are composed of polyphenols (including phenolic acids and flavonoids), carotenoids and vitamins [10]. These natural antioxidants are recognized for their ability to scavenge reactive oxygen species (ROS) [11-13]. Oxidative stress is a harmful event that is responsible for development of a wide range

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of disease [14]. In fact, ROS induce tissue injury through DNA damage and oxidation of lipid and proteins [15-17].

The increment of consumption of plant derived natural antioxidants can be a compensatory way to reduce the harmful effects of ROS [18-20]. Ziziphus nummularia or Ramilak is an important plant in the Rhamnaceae family. The growing location of Z. nummularia is in South and West Asia and some African countries including Zimbabwe, Mauritania, Nigeria and Uganda. Traditional medicine has enumerated countless properties for this plant in the treatment of diabetes, cutaneous diseases, cold and pain. Studies have revealed the correlation between the presence of phytoconstituents including tannins, flavonoids and alkaloids and biological effects of Ziziphus nummularia such as anti-inflammatory, antibacterial and particularly antioxidant properties [21]. Crataegus pontica is another interesting plant which commonly known as Hawthorn. It is a small tree which belongs to the Rosaceae family. C. pontica is widely found in Asia, Europe and particularly in Iran. Almost all parts of C. pontica have been possessed various pharmacological effects such as antioxidant, anti-inflammatory and antimicrobial properties. It has been reported that C. pontica contains various active constituents including polyphenols, flavonoids ad triterpenoids [22]. Scrophularia striata is an annual medicinal plant from Scrophulariaceae family which is widely consumed in Iranian traditional medicine. Researchers have confirmed the presence of different active constitutes including alkaloids, flavonoids in various parts of S. striata. Antioxidant, anti-tumor, anti-microbial and anti-inflammatory effects are some of the numerous pharmacological properties of S. striata [23]. Hence, the aim of this study was to evaluate the antioxidant properties of S. striata, C. pontica and S. striata.

Materials and Methods

Collection of Plant Materials

The aerial parts of *Z. nummularia, C. pontica* and *S. striata* were collected from Dehloran Countiy of Ilam Province, Southwest of Iran, in March 2022. The plants were identified according to the morphological features of Ilam Province Plant Flora at the

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Biotechnology and Medicinal Plants Research Center, Ilam University of Medical Sciences, Ilam, Iran. Collected plants were air dried in the shade and then were ground and used for antioxidant evaluation. The characteristics of the mentioned medicinal plants were shown in Table 1.

Plant Sample Preparation

After drying the plant, 1 g of the 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

The total antioxidant capacity of the plants was assessed by ferric iron reducing antioxidant power (FRAP) assay [24].

Stock Solution Preparation

A volume of 2.2 mL of R2b solution was added to the parent bottle R2a and vortexed until complete dissolution and R2 solution was obtained. Then, the R2 solution was mixed in a ratio of 1: 1 and after vortex, 5 times its volume was added to R1 solution. The resulting solution is the stock solution of an antioxidant kit [24].

Standard Solution Preparation

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

Y=0.2447x + 0.0988

 $R^2 = 0.9997$

Procedure

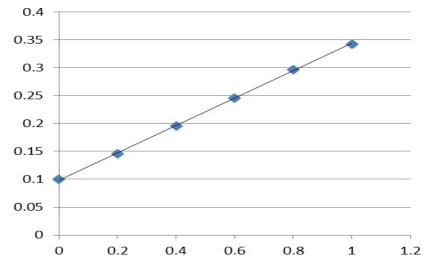
First, 5 μ L of the prepared plant solution was added to each well and then 250 μ L of the prepared working solution was added to each well containing the plant solution. The microplate was then incubated at 35-50 °C for 30 minutes and finally read at 570 nm with the ELISA reader [24].

<u>Results</u>

As shown in Table 2, the results revealed that the total antioxidant capacity was found as 3.16, 2.95 and 1.14 mmol Fe²⁺/L for *Z. nummularia, C. pontica and S. striata,* respectively.

Persian Name	Family	Collection Area	Geographic Coordinate
Mive konarak	Rhamnaceae	Dehloran County	32° 41' 28" North, 47° 15' 58" East
Zalzalak	Rosaceae	Dehloran County	32° 41' 28" North, 47° 15' 58" East
Gole miemoni	Scrophulariaceae	Dehloran County	32° 41' 28" North, 47° 15' 58" East
	Name Mive konarak Zalzalak	NameFamilyMive konarakRhamnaceaeZalzalakRosaceaeGole miemoniScrophulariaceae	NameFamilyCollection AreaMive konarakRhamnaceaeDehloran CountyZalzalakRosaceaeDehloran CountyGole miemoniScrophulariaceaeDehloran County

TABLE 1. The characteristics of the used medicinal plant.



Fig, 1. Linear equation obtained from different concentrations of standard solution

TABLE 2. Total antioxidant capacity of Ziziphus nummularia, Crataegus pontica and Scrophularia striata.

Plant	Total Antioxidant Capacity	
Ziziphus nummularia	3.16 mmol Fe ²⁺ /L	
Crataegus pontica	2.95 mmol Fe ²⁺ /L	
Scrophularia striata	1.14 mmol Fe ²⁺ /L	

Discussion

In recent times, the tendency to use medicinal plants has continued to grow, because of their antioxidant properties and health-related effects [25]. Healing properties of medicinal plants are related to the presence of vitamins, minerals and various phenolic compounds in plants. In the group of phenolic compounds, special attention should be paid to phenolic acids, which can fight coronary heart disease, inflammation, diabetes and cancer. The healing properties of the medicinal plants depends the presence of vitamins, minerals and different phenolic compounds. Phenolic compounds are one of the most important antioxidants in medicinal plants that researchers have focused on their benefits [26]. Oxidative stress plays a central role in the development of many diseases such as cancer, diabetes, coronary heart disease and inflammation. Since, prescribing antioxidants is a beneficial way to prevent the progression of such diseases [8]. Hence, the aim of present study was to evaluate total antioxidant capacity of *Z. nummularia*, *C. pontica* and *S. striata* using FRAP assay.

The findings of our study revealed that the total antioxidant capacity of *Z. nummularia* was obtained as $3.16 \text{ Fe}^{2+}/\text{L}$. *Z. nummularia* is known as a medicinal plant with potent antioxidant property which has a great free radical scavenging potential. In a study, Bahmani and the colleagues

investigated the phytochemical composition of Z. nummularia. Limonene, β -Myrcene, α - Pinene, Tetradecane and Hexadecane were identified as the main bioactive compounds in essential oil of Z. nummularia which could exert high antioxidant effect [21]. In another similar study, Gupta and the colleagues evaluated the antioxidant activity of Z. nummularia fruits using FRAP assay. They showed that Z. nummularia has a potent free radical scavenging property which is related to its high phenolic content [27]. In a similar study, Dureja and Dhiman understood that methanolic extract of Z. nummularia fruits exhibited a significant DPPH radical scavenging activity [28]. The difference in the values of the antioxidant activity of Z. nummularia could be due to the difference in the preparation of plant samples and modifications in the method of measuring the antioxidant activity [21]. The results of another part of our study indicated that the total antioxidant capacity of C. pontica was obtained as 2.95 Fe^{2+/L}. The antioxidant capacity of various parts of C. pontica has been reported previously. H. Bardakci and the colleagues reported that C. pontica is a rich source of antioxidant molecules especially phenolic compounds which is caused exhibiting antioxidant activity. They also found the antioxidant property of C. pontica through the value of FRAP assay as 0.5 mM FeSO4 eq. in 1 g sample [29].

The differences in the phytochemical constituents and subsequently the antioxidant capacity of C. pontica could be due to the growth of this plant in different geographical areas and ecological conditions [21]. In a study, Dolatkhani and Jameie approved the high antioxidant activity of Crataegus pontica through the remarkable free radical scavenging potential [30]. The results of our study also showed that the total antioxidant capacity of S. striata was found as 1.14 Fe²+/L. S. striata is known for its numerous biological and pharmacological properties, especially its antioxidant effect. The antioxidant property has been associated with the presence of bioactive compounds especially phenolic compounds and flavonoids [23]. F Yousefbeyk et al. demonstrated the antioxidant activity of S. striata using FRAP assay. The results of their investigation indicated that the methanol extract of S. striata was found as 664.4 mmol FeII/g dry extract which this value exerts the considerable antioxidant capacity of S. striata [31]. Various factors such as geographical area, plant growth and ecological conditions and type of plant sample preparation are the most

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important factors involved in the difference in the level of the antioxidant activity [21].The antioxidant properties of medicinal plants have been studied in various studie [32-38]. The reason for their antioxidant properties is the presence of secondary phenolic compounds, flavonoids and other compounds [39-44].

Conclusion

In conclusion, the findings of the present study exhibited the considerable antioxidant activity of Z. nummularia, C. pontica and S. striata. According to the results, the above mentioned medicinal plants could exert potent antioxidant capacity through scavenging free radicals. With the above mentioned findings, it can be said that Z. nummularia, C. pontica and S. striata showed noticeable antioxidant potential and this high antioxidant potential could play role in their medicinal effects.

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Conflict of Interest

The authors declared no competing interests.

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