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Determination of total antioxidant content of methanolic extracts of *Cynara scolymus*, *Echinacea purpurea* and *Portulaca oleracea*

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Abstract

Background: 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 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 methanolic extracts of *Cynara scolymus*, *Echinacea purpurea* and *Portulaca oleracea*. Aerial parts of *C. scolymus*, *E. purpurea* and *P. oleracea* were dried and ground.

Methods: 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.

Results: The results revealed that the total antioxidant capacity was found as 3.45, 1.16 and 1.68 mmol Fe^{2+}/L for *C. scolymus*, *E. purpurea* and *P. oleracea*, respectively. Based on our results, *C. scolymus*, *E. purpurea* and *P. oleracea* showed a potent antioxidant activity.

Conclusion: It is recommended that utilization of *C. scolymus*, *E. purpurea* and *P. oleracea* in food and pharmaceutical industries could possibly possess beneficial health effects.

Introduction

Antioxidants have attracted the attention of scientists for their numerous benefits [1, 2]. Lack of antioxidants can induce oxidative stress and develop various diseases including neoplasms, cardiovascular diseases, neurodegenerative diseases and inflammatory disorders [3-5]. Oxidative stress refers to conditions in which the production of free radicals particularly the generation of reactive oxygen species (ROS) overcomes their neutralization by antioxidants [6-8]. ROS are naturally produced in low amounts in the body, but their overproduction can induce mitochondrial dysfunction, endoplasmic reticulum stress and eventually damage macromolecules and tissue injury [5-9]. To solve this problem, the use of antioxidants, especially oral antioxidants with natural origin is a useful solution [10]. It has been reported that the dietary intake of natural plant antioxidants has an inverse relationship with the incidence of diseases [11,12]. Medicinal plants have exerted healing effects due to their ability to the production of a wide range of antioxidant compounds such as polyphenols, terpenoids and vitamins [13,14]. The use of medicinal plants to treat various diseases has a long history [15,16]. This has led to use medicinal plants and the plant derived antioxidant compounds in finding treatment strategies to successfully treatment of human diseases [17,18].

Cynara scolymus is one of the medicinal plants with high antioxidant properties which grow in Mediterranean area. It is a perennial plant belongs to Asteraceae family which commonly known as artichoke. In addition to its edible use as a vegetable, *C. scolymus* has a long history in the treatment of diseases. Antioxidant constituents including phenolic acids and flavonoids are abundant in *C. scolymus*. Apigenin and luteolin are the most important bioactive compound which found in artichoke. Numerous healing properties, both in traditional medicine and in new research, such as antimicrobial, anticancer, hypoglycemic and hepatoprotective properties have been listed for *C. scolymus* [19]. *Echinacea purpurea* is a perennial plant belonging to Asteraceae family. *E. purpurea* could exert several biological and pharmacological effects including antioxidant, antimicrobial, anti-inflammatory, anti-tumoral and immunomodulatory properties. Researchers have indicated that the presence of bioactive compounds such as caffeic acid, caftaric acid, cichoric acid, echinacoside, alkylamide, α-pinene and limonene are associated with the healing effects of *E. purpurea* [20]. *Portulaca oleracea* is an annual plant belongs to the Portulacaceae family. It could grow in the tropical and subtropical regions including United States and Mediterranean countries. *P. oleracea* is used as a folk remedy via exerting antioxidant, antibacterial and anti-inflammatory effects. Flavonoids, alkaloids and terpenoids are the most well-known bioactive compound extracted from *P. oleracea* [20]. Hence, the aim of this study was to evaluate the antioxidant properties of *C. scolymus*, *E, purpurea* and *P. oleracea*.

Methods

Collection of Plant Materials

The aerial parts of *C. scolymus*, *E. purpurea* and *P. oleracea* were collected from Dehloran county 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 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.

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 [21].

Determination of Antioxidant Activity

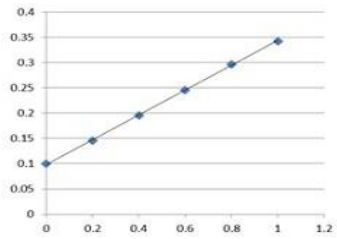
The total antioxidant capacity of the plants was assessed by ferric iron reducing antioxidant power (FRAP) assay [21].

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, the R2 solution was mixed in a ratio of 1: 1 and after vortexing, 5 times its volume was added to R1 solution. The resulting solution is the stock solution of an antioxidant kit [21].

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
R2=0.9997

Figure 1: Linear equation obtained from different concentrations of standard solution.

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 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 [22].

Results

As shown in Table-1, the results revealed that the total antioxidant capacity was found as 3.45, 1.16 and 1.68 mmol Fe^{2+}/L for *Cynara scolymus*, *Echinacea purpurea* and *Portulaca oleracea*, respectively.

Plant	Total Antioxidant Capacity
Cynara scolymus	3.45 mmol Fe^{2+}/L
Portulaca oleracea	1.68 mmol Fe^{2+}/L
Echinacea purpurea	1.16 mmol Fe^{2+}/L

Table 1: Total antioxidant capacity of *Cynara scolymus, Echinacea purpurea* and *Portulaca oleracea.*

Discussion

Oxidation reactions not only are a harmful process in the human body but also for many food products. Excess in generation of ROS is called oxidative stress which could induce development of variousdamaging conditions such as incidence of cancer, diabetes, inflammatory diseases and coronary heart diseases[23]. Meanwhile, antioxidants can protect the body against damage caused by oxidative stress as a shield.Antioxidants play their protective role by neutralizing free radicals by donating electrons to them and stopping the auto-oxidative chain reaction caused by ROS [24].One way to supply exogenous antioxidants is by consuming rich sources of antioxidants. Medicinal plants have always been considered by researchers due to having rich sources of bioactive compounds with abundant biological and pharmacological properties particularly antioxidant effect[25].Therefore, the present study was designed to evaluate total antioxidant capacity of *C. scolymus*, *E, purpurea* and *P. oleracea*using FRAP assay.

The results of our study indicated that the total antioxidant activity of *C. scolymus*was obtained as 3.45 Fe^{2+}/L.The presence of bioactive constituents such as phenolic compounds especially phenolic acids and flavonoids play role in increasing total antioxidant activity of medicinal plants[26].*C. scolymus*is recognized as a plant containing potent antioxidants. *C. scolymus*has a high rank in terms of containing antioxidant compounds among different medicinal plants.

In this regard, M. Ben Salem and the colleagues reported considerable antioxidant potential of *C. scolymus*. The results of their study showed that the value oftotal antioxidant activity of ethanolic extract of *C. scolymus* leavesusing FRAP assay was obtained as 527.79 mol Fe (II)/g DW. They concluded that *C. scolymus* possessed significant antioxidant capacity.Although there was a difference in the values obtained, two studies confirmed the antioxidant effects of this plant. There were differences due to the differences in thegeographical area, plant growth and ecological conditions and type of plant sample preparation [27].

In another study, Emanuel and the colleagues confirmed the ability of *C. scolymus* to scavenge free radicals. They showed that the presence of high quantities of phenols and flavonoids are related to capable antioxidant activity of *C. scolymus* [28]. In our study, the findings demonstrated that the total antioxidant capacity of *E. purpurea* was found as 1.16 Fe^{2+}/L.*E. purpurea*exerts a remarkable antioxidant effect due to the presence of bioactive compounds including cichoric acid.

In a study, K.O. Mohamed Sharifand the colleagues investigated biological effects of extracts of *E. purpurea*. They found that the aqueous extract of *E. purpurea*leaves exhibited strong antioxidant activity. In this study, the value of total antioxidant activity of aqueous extract of *E. purpurea*leaves using FRAP assay was found as 196mg TE/g which represents a high antioxidant capacity [29].

In another study, A. Wojdyło and the colleagues investigated antioxidant activity of a series of selected herbs including *E. purpurea*. In their study, the value of total antioxidant activity of aqueous extract of *E. purpurea* leaves using FRAP assay was found as 94.6 lMtrolox/100 g dw [30]. The results of another part of our study indicated that the total antioxidant capacity of *P. oleracea* was found as 1.68 Fe^{2+}/L. *P. oleracea* is known by its high nutritive value and healing properties. The antioxidant effect of *P. oleracea* could be related to the high total phenolic and flavonoid content.

Free radicals are toxic products of oxygen metabolism that contain at least one unpaired electron. Excessive production of free radicals during a process called oxidative stress causes damage to different parts of the cell such as proteins, DNA and cell membrane [31-37]. These irreversible damages lead to changes in cell function or cell death. Oxidative damage to vital molecules ultimately leads to chronic diseases such as heart disease, cancer, diabetes, Alzheimer's, Parkinson's, arthritis and infertility [38-43].

The human body needs both types of oxidants (free radicals) and antioxidants for normal metabolism, signal transmission, and regulation of cellular activities [44-45]. Medicinal plants and herbal antioxidants can contain compounds that can neutralize many diseases [46-62].

In a similar study, Md. Kamal Uddin and the colleagues evaluated the antioxidant properties of *Portulaca*

oleracea. They showed that the value of total antioxidant activity of extract of *Portulaca oleracea* using FRAP assay was found as 4.3 mg GAE/g. They concluded that this antioxidant property could relate to its high phenolic and flavonoid content [63]. In another study, it has been shown that *Portulaca oleracea* particularly its ornamental type could exert considerable antioxidant property. They revealed that the value of total antioxidant activity of extract of *Portulaca oleracea* using FRAP assay ranged from 7.39±0.08 to 104.2±6.34 μ mol TE/gDW [64]. Taking together, we concluded that the three chosen medicinal plants including *C. scolymus*, *E. purpurea* and *P. oleracea* shows a potent antioxidant property and this could be a reason for their use in the food and pharmaceutical industries. Based on our findings, it is recommended that utilization of *C. scolymus*, *E. purpurea* and *P. oleracea* could possibly possess beneficial health effects.

Competing Interest

The authors declare that there is no conflict of interest.

Author Contributions

Samira Shokri: Data analysis
Kourosh Saki: Monitoring of research
Yeganeh mazaheri and Samira Shokri: Drafting
Gholamreza Jahed khaniki: Manuscript scanning
Samira Shokri: Data evaluation

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