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ORIGINAL ARTICLE

Assessment of the Antioxidant Potential of Methanolic Extracts from *Leontodon lanata* (L.) Fisch, *Convolvulus arvensis* L., and *Ziziphora capitata* L.

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ABSTRACT: Synthetic antioxidants are widely used today to mitigate oxidative stress and counter the harmful effects of free radicals. However, due to the potential side effects associated with synthetic antioxidants, there is growing interest in natural alternatives. The antioxidant properties of medicinal plants offer a compelling rationale for their application in the food, pharmaceutical, and personal care industries. Since plant-based foods, particularly fruits and vegetables, are the richest sources of antioxidants, this study aimed to investigate the antioxidant properties of methanolic extracts from *Leontodon lanata* (L.) Fisch, *Convolvulus arvensis* L., and *Ziziphora capitata* L. *L. lanata* was harvested from the Ilam region, while C. arvensis and Z. capitata were collected from the Andimeshk to Dehloran area. The total antioxidant capacity of these plants was assessed using the ferric reducing antioxidant power (FRAP) assay. The results revealed that *L. lanata* exhibited the highest antioxidant activity with a capacity of 3.87 mmol Fe^{2+} L^{-1}. In comparison, the antioxidant capacities of *C. arvensis* and *Z. capitata* were measured at 1.29 mmol Fe^{2+} $L^{-1}$and 1.69 mmol Fe^{2+} L^{-1}, respectively. Among the plants tested, *L. lanata* demonstrated the most potent antioxidant effect. These findings suggest that methanolic extracts *of L. lanata, C. arvensis, and Z. capitata*, particularly *L. lanata*, could be valuable as natural supplements in the food and pharmaceutical industries, supporting health and therapeutic applications.

INTRODUCTION

Free radicals are generated through natural reactions during cellular respiration in aerobic organisms, especially in animals [1]. Free radicals, such as reactive oxygen species (ROS), are naturally produced in different metabolic pathways in cells and play a key role in signaling process of cells. The body may generate free radicals in response to ultraviolet rays, tobacco smoke, and alcoholic drinks [2]. High amounts of free radicals are dangerous for cells and may cause damage to cell components like proteins, DNA, phospholipids, and cell membranes, leading to tissue destruction and different diseases, such as inflammation, cancer, etc [3]. Low level of antioxidants in the body leads to early aging, damaged or mutated cells, damaged tissue, and activation of faulty genes in DNA; and it causes an increased pressure on immune system function [4].

Foods containing antioxidants and food antioxidants are an important part of the food industry. In the past,

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antioxidants were primarily used to control oxidation and mitigate damage, but today many are used for their health benefits [3]. In the cosmetic industry, antioxidants are used as ingredients that help protect the skin against oxidative damage and free radicals. These substances aim to improve the appearance and quality of the skin, reduce the signs of aging and maintain the health of the skin. Antioxidants can be obtained from various sources such as vitamins (such as vitamin C and E), minerals, plant extracts and other nutritional values [2-4].

Antioxidants that must be obtained from external sources include vitamins A, C, and E, along with beta-carotene, lycopene, lutein, selenium, manganese, and zeaxanthin. The richest sources of these antioxidants are plant-based foods, particularly fruits and vegetables. Additionally, plant foods contain other antioxidant compounds such as flavonoids, flavones, catechins, polyphenols, and phytoestrogens, which also serve as important phytonutrients. Foods abundant in antioxidants are often referred to as "superfoods" or "functional foods" due to their health-promoting properties [2-4]. The antioxidant activity of plant extracts has been studied for many years; however, most of the studies have been conducted over the past 30 years. During this period, research studies have been conducted on medicinal plants with traditional usage [5]. Antioxidants are important compounds capable of protecting cells against oxidation and are effective in preventing and treating the oxidative damage [6]. The important antioxidants contained in tocopherol include folic acid, ascorbic acid, carotenoid pigments, phenylacrylic acids, and polyphenols, namely flavonoids and anthocyanins that constitute the largest group of natural phenols [7]. Some antioxidants are made by the body itself, and others are provided through feeding and eating foods containing antioxidants, which may also possess anti-inflammatory properties [8]. The most important sources of antioxidants are medicinal plants, fruits, and vegetables. Antioxidants are found in different compounds like carotenoids (e.g., beta-carotene), lycopene, and vitamin C. These compounds are some examples of antioxidants that inhibit the oxidation process or cell reaction to oxygen, peroxide, or free radicals [8].

Considering the unwanted side effects of artificial antioxidants, this study aims to investigate the effect of medicinal plants such as *Leontodon lanata* (L.) Fisch, *Convolvulus arvensis* L and *Ziziphora capitata*, which are useful and anti-inflammatory medicinal plants in traditional medicine and ethnobotanical knowledge of western Iran. They are oxidants and were studied to prove their antioxidant effect.

Desert ivy (*Convolvulus arvensis*) is a perennial herbaceous plant with branches up to 100 mm long, broad branches and branched stems, herbaceous leaves, ovate petioles, thread-like peduncles, membranous sepals, ovate ovary and white or pink calyx with Dark red streaks. *Convolvulus arvensis* is an herbaceous perennial plant in the Convolvulaceae family that is native to Europe and Asia. This plant is laxative, anti-spasmodic, effective in wound treatment, and has anti-hemorrhoid, antioxidant, anti-chromium and anti-tumor properties [9]. *Z. capitata* is an annual herb in the family Lamiaceae. This plant has a stem of 5-25 cm, standing, hard and brittle, leaves 4-35 mm long, leaves next to the flowers with various shapes. This plant is an anti-flatulence, disinfectant, wound healer, sedative, expectorant, stomach tonic, gastric problems remover, and a body lice killer with external use [10].

Hairy horsehair herb (*Leontodon lanata* (L.)) without hair or spider hair and cabbage green with a stem height of up to 30 cm, standing, deeply branched. The leaves are fresh fleshy and have tabular and purple flowers. *L. lanata* (L.) Fisch is in the Compositae family. This plant is an edible vegetable with antimicrobial and antioxidant effects [11]. The present study aims to evaluate the antioxidant effect of methabolic extract of medicinal plants *L. lanata* (L.) Fisch, *C. arvensis* L, and *Z. capitata*.

MATERIALS AND METHODS

Plant preparation

Medicinal plants *L. lanata* (L.) Fisch, *C. arvensis* L, and *Z. capitata* was collected in June, 2023. This plant is native to Iran and the provinces of Ilam and Khuzestan. The plant *L. lanata* was collected from Ilam and two medicinal plants *C. arvensis* L. and *Z. capitata* L. were collected from Andimeshk to Dehlaran (60 km from Dehlran). Using the morphological keys from the book of plant flora by the botanist of the research center for biotechnology and medicinal plants and natural resources of Ilam city to identify the medicinal plants. The collected plants were cleaned and shade-dried with full air circulation. The dried plant was powdered using a mixer grinder and used for an antioxidant activity test. Table 1 shows the specifications of the above-mentioned medicinal plants used in this study.

Table 1. Specifications of the studied medicinal plants.

Plant Scientific name	Herbal family	Collection *region*	Herbarium code	The geographical location
***Leontodon lanata* (L.) Fisch**	Asteraceae	Ilam, Ilam	187	33.638531°N 46.422649°E
***Convolvulus arvensis* L.**	Convolvulaceae	Khuzestan, Andimeshk	265	32°24′52″N 47°49′24″E
***Ziziphora capitata* L.**	Lamiaceae	Khuzestan, Andimeshk	598	32°24′52″N 47°49′24″E

Determination of Antioxidant Activity of Methanolic

Extracts

Preparation of Plant Sample

To prepare the plant sample, 1 gram of the homogenized dry plant powder was shaken for 6 hours with 100 mL of methanol. The resulting solution was transferred to a plastic falcon tube and centrifuged at 6000 rpm for 10 minutes. The supernatant obtained after centrifugation was used as the sample [12].

Preparation of Working Solution

For the working solution, 2.2 mL of reactive substance 2b (R2b) was added to the parent solution of reactive substance 2a (R2a) and vortexed until fully dissolved, forming the reactive solution 2 (R2). This solution was then mixed with 5 times its volume of R1 solution after vortexing. The resulting mixture served as the working solution for the antioxidant assay kit [12].

Preparation of Standard Solution

A standard solution was prepared with concentrations of 0, 0.2, 0.4, 0.6, 0.8, and 1 mL. The linear equation was derived based on the absorbance of Fe^{2+} at varying concentrations [12]. The total antioxidant capacity was assessed through the ferric reducing antioxidant power (FRAP) method, which measures the ability of the sample to reduce ferric ions, following a single electron transfer mechanism.

Method

In this assay, 5 microliters of the prepared plant extract solution were added to each well of the microplate, followed by the addition of 250 µL of the working solution to each well. The plate was then incubated for 30 minutes at temperatures ranging between 35 and 50°C. The antioxidant activity was determined by measuring absorbance at 570 nm using an ELISA reader [12].

RESULTS

The results of the antioxidant activity of the plants' extract showed the standard antioxidant activity to be 1 mmol Fe^{2+} L^{-1} [12]. As shown in Table 2, all medicinal plants studied in this research had antioxidant effects.

Table 2. Total antioxidant capacity of methanolic extract of medicinal plants analyzed in this study

Methanolic extract	Total antioxidant capacity
***Leontodon lanata* (L.) Fisch**	3.87 mmol Fe^{2+} L^{-1}
***Convolvulus arvensis* L.**	1.29 mmol Fe^{2+} L^{-1}
***Ziziphora capitat* L.**	1.69 mmol Fe^{2+} L^{-1}

According to the obtained results, the methanolic extract of *L. lanata* (L.) Fisch with an antioxidant capacity of 3.87 mmol Fe^{2+} L^{-1} had the strongest antioxidant activity amongst medicinal plants studied in this research.

DISCUSSION

Antioxidants are essential molecules that protect the body from free radicals, which can contribute to the development of diseases such as diabetes and cancer. Studies have revealed that medicinal plants like *Ferula assa-foetida* (both bitter and sweet varieties) and *Bunium persicum* exhibited antioxidant capacities of 1.18, 1.09, and 1.75 mmol Fe^{2+} L^{-1}, respectively [12]. Another investigation showed that methanolic extracts of *Ziziphus spina-christi*, *Salvia rosmarinus*, and *Satureja khuzistanica* displayed antioxidant potentials of 7 mmol Fe^{2+} L^{-1}, 1.93 mmol Fe^{2+} L^{-1}, and 12.99 mmol Fe^{2+} L^{-1}, respectively [13]. Similarly, total antioxidant capacities for *Anthemis susiana*, *Alyssum campestre*, and *Gundelia tournefortii* were recorded as 4.29, 1.01, and 1.25 mmol Fe^{2+} L^{-1}, respectively [14]. Research on the antioxidant activity of *Allium ampeloprasum* demonstrated a total antioxidant capacity of 3.06 mmol Fe^{2+} L^{-1}, confirming its significant antioxidant properties [15]. Using the FRAP assay, another study measured total antioxidant capacities of 2.21, 0.78, and 7 mmol Fe^{2+} $L^{-1}$for *Mentha longifolia*, *Pistacia khinjuk*, and *Eucalyptus globulus*, respectively [16]. An investigation into Nasturtium officinale revealed its total antioxidant capacity to be 2.83 mmol Fe^{2+} L^{-1} [17]. Further analysis found antioxidant capacities of 4.1, 2.35, and 0.46 mmol Fe^{2+}/L for *Quercus brantii*, *Thymbra spicata*, and *Citrullus colocynthis*, respectively [18]. Additionally, *Falcaria vulgaris* displayed a total antioxidant capacity of 2.86 mmol Fe^{2+} L^{-1} [19]. Finally, the methanolic extracts of *Cynara scolymus*, *Echinacea purpurea*, and *Portulaca oleracea* demonstrated antioxidant capacities of 3.45, 1.16, and 1.68 mmol Fe^{2+} L^{-1}, respectively [20]. This study found the medicinal plant extracts with antioxidant properties to have high levels of phenolic compounds [21]. Plant extracts contain different antioxidant compounds including polyphenols. Previous studies have shown the abundant biological activities of these compounds, especially flavonoids. According to these studies, most of the properties of plant species extracts can be assigned to these compounds [22, 23]. Convolvulus arvensis contains main compounds, including steroids, flavonoids, phenols, lipids, coumarins, campesterol, stigma-sterol, and beta-sitosterol as the most abundant steroid compound [24]. The results of a study showed that *Z. capitata* contained chemical compounds, including pulegone, limonene, 1,8-cineole, p-mentha-3-en-8-ol, neomenthol, bornyl acetate, and piperitone [25]. Sesquiterpene lactone and its glycosides are among the main components of *L. lanata* [26]. The results of phytochemical studies showed that medicinal plants of the *Leontodon genus* contained phenolic compounds [27, 28]. As medicinal plants containing phenolic and polyphenolic compounds have antioxidant effects, studied medicinal plants also contained phenolic and polyphenolic compounds, they have antioxidant properties, too. These compounds have hydroxyl groups in their chemical structure, which act as antioxidant agents [29]. Polyphenols have high reducing power. They can react with free radicals of oxygen and nitrogen and reduce oxidative stress, and finally, decrease the damage to the body cells and tissues [30]. These antioxidants mostly are safe and can prevent or treat diseases such as diabetes mellitus [31, 32]. Hence, the daily consumption of plants with high levels of antioxidant activity might be useful in these patients. Antioxidants act as absorbers of free radicals and thus protect the body from damage caused by the presence of free radicals [30-32]. Antioxidants may also boost the immune system and lower the risk of various diseases and infections [33-35]. These antioxidant compounds, through mechanisms such as binding to oxidant molecules or neutralizing free radicals, help diminish the harmful effects of oxidants that contribute to chronic conditions like diabetes, cardiovascular diseases, and kidney disorders, while mitigating their destructive potential [36-43]. Since free radicals are crucial contributors to the development of numerous illnesses, antioxidant agents can play a significant role in both the prevention and treatment of such health issues.

CONCLUSIONS

Today, natural antioxidants are healthier and safer than synthetic ones and are not restricted to terrestrial sources and seaweeds are a rich source of antioxidant compounds. As the medicinal plants studied in this research have natural antioxidants, metabolic extract of the above-mentioned medicinal plants has antioxidant properties and can be used as food or therapeutic supplements if preclinical and clinical studies are approved.

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Conflict of interests

The authors declare no competing interest.

Consent for publication

Not applicable.

Availability of data and materials

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