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Exploring the Antioxidant Properties of Methanolic Extracts from *Capsicum annuum*, *Hypericum helianthemoides*, and *Dianthus orientalis*

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A B S T R A C T

Objective: Antioxidants, whether produced naturally within the body or obtained from external sources, are crucial in safeguarding health by defending cells from the damage caused by oxidative stress. Phytochemicals, naturally occurring in medicinal plants, possess significant antioxidant properties and are gaining widespread attention for their health benefits. Numerous studies have explored the antioxidant capacities of various plant species. In this study, we investigate the antioxidant activity of methanolic extracts from *Capsicum annuum*, *Hypericum helianthemoides*, and *Dianthus orientalis* Adams.

Materials & Methods: The aerial parts of the plants were first dried and finely ground. Methanolic extracts were prepared by combining the plant material with methanol. The total antioxidant capacity of the extracts was then determined using the Ferric Reducing Antioxidant Power [FRAP] assay. The results were measured at a wavelength of 570 nm using an ELISA reader.

Results: Our findings revealed that the methanolic extracts of *Hypericum helianthemoides*, *Capsicum annuum*, and *Dianthus orientalis* Adams exhibited maximum total antioxidant capacities of 2.79, 2.48, and 1.96 mmol Fe^{2+}/L, respectively.

Conclusion: The findings from this study highlight the significant antioxidant capacities of methanolic extracts derived from *Capsicum annuum*, *Hypericum helianthemoides*, and *Dianthus orientalis* Adams. These plants demonstrated strong potential in reducing oxidative stress, a key factor in the prevention of various chronic diseases, including cardiovascular disorders, neurodegenerative diseases, and cancer. Given their substantial antioxidant activity, these plants could be further explored for development into natural therapeutic agents or incorporated into functional foods and nutraceuticals. Additionally, their incorporation into pharmaceutical formulations could provide a natural alternative to synthetic antioxidants, offering fewer side effects and promoting better consumer health. Future research should focus on identifying the specific bioactive compounds responsible for these antioxidant properties and investigating their mechanisms of action in clinical settings.

Keywords: Antioxidants, Medicinal plants, Methanolic extracts, *Capsicum annuum*, *Hypericum helianthemoides*, *Dianthus orientalis* Adams

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Introduction

The global medicinal market is dominated by medicinal plant materials and the herbal remedies they produce. Throughout the history of mankind, herbal remedies and medicines have played a vital role in the treatment of disease [1]. The impact of medicinal plants on world health has made them an important topic worldwide. The health care system of a large part of the world's population has been sustained by herbal medicine, which has played a crucial role. The design and development of novel pharmaceuticals has been aided by the use of medicinal herbs, which are fundamental components of alternative medicine [2, 3]. There are several factors contributing to the increased use of medicinal plants for disease treatment. Plants and their derivatives are widely believed to be safe and effective therapeutic agents. In addition, they have no adverse effects and are affordable [2, 3]. Over time, the use of medicinal plants has experienced significant growth. The utilization of these plants has been prevalent since ancient times and is now recognized as a source of modern medicine [4]. In spite of the advancements in medicine, medicinal plants continue to play a crucial role in Iran as curatives for various health issues [6]. In Iran, which has a variety of climates and geographical zones, at least 2300 species with aromatic and medicinal properties can be found [7].

Antioxidants play a vital role in public health by neutralizing free radicals, thus preventing cellular damage and reducing the risk of chronic diseases such as heart disease, cancer, and diabetes [4]. In the food industry, they act as natural preservatives that prevent the oxidation of products, while in the pharmaceutical and health sectors, they contribute to skin health and immune system support. Utilizing antioxidants enhances quality of life and promotes overall public health [5-7].

The Capsicum genus, which includes hot peppers, stands as one of the oldest and most widely used vegetables, condiments, and spices worldwide [8]. Chili peppers, commonly known as *Capsicum annuum*, have been utilized in both traditional medicine and as a vegetable since ancient times [9]. Spicy foods have been shown to have a positive impact on promoting a healthy lifestyle through epidemiological research [8, 10]. Anti-inflammatory, analgesic, and blood glucose-regulating agents that act as nutraceuticals can be found in peppers. The compounds that often contribute to these positive properties include alkaloids, flavonoids, vitamin C and E, carotenoids, and capsaicin [8]. *Hypericum*, a significant genus in the Hypericaceae family, encompasses more than 400 species of herbaceous plants [11]. *Hypericum* is a genus that encompasses multiple species that were traditionally valued as medicinal plants [12]. Trauma, gastroenteritis, hysteria, rheumatism, neuralgia, depression, ulcers, and bedwetting are among the conditions treated with these plants. For their sedative, antibacterial, antioxidant, and anti-inflammatory effects, *Hypericum* species, such as *Hypericum helianthemoides*, have been employed for a long time. The key constituents found in *Hypericum* species include hypericin, phenolic acids, tannins, flavonoids, rutin, quercitrin, isoquercitrin, and chlorogenic acid [11]. The *Dianthus* genus, which is part of the Caryophyllaceae family, encompasses over 300 species. These plants species including *Dianthus orientalis* Adams have a rich history in traditional medicine, where they were employed to address a variety of ailments, such as stomach aches, throat and gum infections, toothache, wounds, and cardiovascular conditions. Polyphenols, alkaloids, saponins, flavonoids, and vitamins are all components present in *Dianthus orientalis* Adams that contribute to its pharmacological properties [13-15].

Secondary metabolites, produced by medicinal plants, are small bioactive molecules [16, 17]. Various diseases can be managed through the use of these compounds, which include antioxidant, anticancer, antimicrobial, and anti-inflammatory activities [18, 19]. Hence, the present paper aims to investigate the antioxidant activity of methanolic extracts of *Capsicum annuum*, *Hypericum helianthemoides* and *Dianthus orientalis* Adams.

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Materials and Methods

Plant Preparation

In July 2023, researchers collected the above-ground portions of *Capsicum annuum*, *Hypericum helianthemoides*, and *Dianthus orientalis* Adams from Khuzestan province in southwest Iran. The Biotechnology and Medicinal Plants Research Center at Ilam University of Medical Sciences identified and validated the plants using morphological criteria from the book on the plant flora of Ilam province. After being harvested, the plants were cleaned and dried under darkness [20]. The attributes of the medicinal plants employed in this research are delineated in Table 1

Table 1. The attributes of the medicinal plants employed in this study.

Scientific name	English Name	Family	Main Chemical Compounds	Plant Type	Traditional Uses
Capsicum annuum	Bell Pepper, Chili Pepper	Solanaceae	Capsaicinoids [Capsaicin], Carotenoids [Capsanthin, Capsorubin], Flavonoids, Vitamin C	Annual	Used for treating digestive issues, stimulating circulation, and relieving pain. Also used for improving appetite and reducing inflammation in traditional medicine.
Hypericum helianthemoides	St. John's Wort [Helianthemoides]	Hypericaceae	Hypericin, Hyperforin, Flavonoids, Phenolic Acids, Essential Oils	Perennial	Used for treating depression, anxiety, and wound healing in traditional medicine. Known for its antibacterial and antiviral properties.
Dianthus orientalis Adams	Eastern Pink	Caryophyllaceae	Flavonoids, Anthocyanins, Phenolic Compounds, Essential Oils	Perennial	Used traditionally for treating fever, sore throat, and skin conditions. Known for its antiseptic and anti-inflammatory properties.

Extract Preparation

Following the plant drying process, 1 gram of the desiccated plant powder was homogenized in a 100 ml methanol solution. For 6 hours, the mixture was subjected to agitation in the same solution. Subsequently, the resulting solution was transferred into a tube and subjected to

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centrifugation at 6000 rpm for 10 minutes. The sample was made up of the centrifuged solution.

Determination of the Antioxidant Activity

The Ferric Iron Reducing Antioxidant Power [FRAP] assay of the Naxifer kit was used to assess the total antioxidant capacity of the plants. First, 2.2 mL of Reagent 2b [R2b] solution [Naxifer, Iran] was mixed with the original R2a solution and thoroughly vortexed until complete dissolution, resulting in the formation of the Reagent 2 [R2] solution. After being mixed in a 1:1 ratio and vortexed five times, it was combined with Reagent 1 [R1] solution, resulting in the formation of the working solution for the antioxidant kit. The kit's standard solutions, containing volumes of 0, 0.2, 0.4, 0.6, 0.8, and 1 µL, were made and added to microplate wells 1 through 6. The linear equation resulting from the concentrations of 0, 0.2, 0.4, 0.6, 0.8, and 1 ML of the standard solution is shown in Figure-1. It should be noted that well number 1 had no standard or zero concentration. For 30 minutes, the microplate was incubated at 35°C, and then it was observed to have an absorbance of 570 nm using the ELISA reader [21].

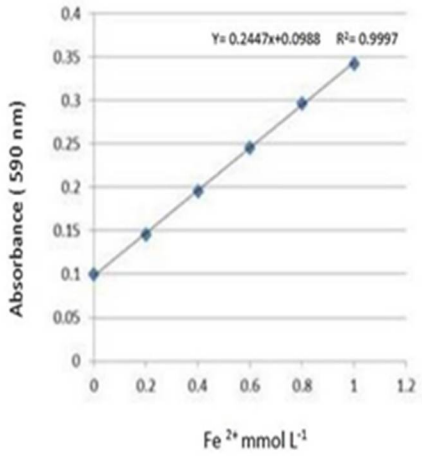

Figure1. Linear equation resulting from different concentrations of the standard solution

Results

The total antioxidant capacity was determined to be 2.79 mmol Fe^{2+}/L for *Hypericum helianthemoides*, 2.48 mmol Fe^{2+}/L for *Capsicum annuum*, and 1.96 mmol Fe^{2+}/L for *Dianthus orientalis* Adams. Furthermore, the results exhibited that the value for the total antioxidant capacity of standard was found as 1 mmol Fe^{2+}/L. As can be seen from the table 2, complete antioxidant effects are present in all medicinal plants in this study.

Table 2. Total antioxidant capacity of Capsicum annuum, Hypericum helianthemoides and Dianthus orientalis Adams

Common Name	Scientific name	Total antioxidant capacity [mmol Fe^{2+}/L]
Chili pepper	*Capsicum annuum*	2.48 mmol Fe^{2+}/L
Zagrossi St John's wort	*Hypericum helianthemoides*	2.79 mmol Fe^{2+}/L
Georgian pink	*Dianthus orientalis* Adams	1.96 mmol Fe^{2+}/L

C. annuum is rich in capsaicin and carotenoids, with capsaicin being particularly renowned for its pain-relieving and anti-inflammatory properties. *H. helianthemoides* contains hypericin and hyperforin, which are associated with antidepressant and anti-inflammatory activities. *D. orientalis* includes flavonoids and anthocyanins, contributing to its antioxidant and anti-inflammatory effects. The presence of these compounds indicates that each plant has a unique mechanism of action and therapeutic potential, especially concerning antioxidant activities. *C. annuum* is recognized as an annual plant, meaning it completes its life cycle within a year and is typically cultivated for immediate use. In contrast, both *H. helianthemoides* and *D. orientalis* are perennial plants, suggesting they can live for several years, which may offer advantages for

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sustainable harvesting and continuous use in traditional medicine. The antioxidant, anti-inflammatory, and antibacterial properties of these plants highlight their potential for synergistic effects when used in combination or within herbal formulations. This synergy could enhance therapeutic efficacy, making them suitable candidates for research in herbal medicine and dietary supplements. *C. annuum* has soft, fleshy tissues, particularly in its fruits. These tissues are well-suited for retaining water and nutrients, and since its fruits are edible, they are typically tender and easily digestible. *H. helianthemoides* features woody, resilient tissues that provide strength. Its leaves are usually firm with specific textures that may help protect against environmental factors. *D. orientalis* possesses soft and somewhat fibrous tissues. Its flowers often exhibit delicate, colorful textures that enhance the plant's aesthetic appeal.

Discussion

Antioxidants, which are bioactive compounds, protect cell integrity by preventing the negative effects of free radicals [22]. The emergence of free radicals from metabolic processes can result in oxidative stress and cellular damage. It is noteworthy that antioxidants have shown their effectiveness in hindering or preventing the oxidation of various molecules in the biological environment [23]. The protection against diseases associated with free radicals is crucial thanks to phytochemicals, which are chemical components found in medicinal plants [24]. Antioxidant compounds, which are primarily produced as secondary metabolites within plants, provide a variety of health benefits and aid in disease prevention [22]. This study set out with the aim of assessing the antioxidant activity of methanolic extracts of *Capsicum annuum*, *Hypericum helianthemoides* and *Dianthus orientalis* Adams.

The most remarkable result to emerge from the data is that complete antioxidant effects are present in all medicinal plants in this study. The current study found that the maximum total antioxidant capacity of the methanolic extract of *Hypericum helianthemoides* was quantified at 2.79 mmol Fe^{2+}/L, respectively. The significance of *Hypericum* species in both traditional and modern medicine exhibits a progressive upward trend. Our experiments are consistent with previous results provided by Akdeniz and colleagues. They showed that *Hypericum helianthemoides* is rich in monoterpene compounds, including α-pinene, which cause it to have potent antioxidant properties [12]. Our finding is in agreement with Ghasemi Pirbalouti's *et al.* findings which demonstrated significant antioxidant properties of *Hypericum helianthemoides*. The authors revealed that the antioxidant activity of *Hypericum helianthemoides* was found as 253.4±7.7 using DPPH assay. Ghasemi Pirbalouti and colleagues showed that α-pinene, β-ocimene, β-pinene and β-caryophyllene were the main constituents in *Hypericum helianthemoides* [11]. The findings of the current study are consistent with those of Javidnia *et al.* who reported potent antioxidant capacity of *Hypericum helianthemoides* [25]. *Hypericum helianthemoides* has antioxidant effects that may account for the presence of bioactive compounds [11].

This study highlighted that the antioxidant activity of methanolic extract of *Capsicum annuum* was 2.48 mmol Fe^{2+}/L. Consistent with our results, numerous scholars have extensively written about the antioxidant activity of *Capsicum annuum* [26, 27]. The claim that *Capsicum annuum* has a high antioxidant capacity has been supported by empirical evidence [27, 28]. The increase in phenols and capsaicinoids in fresh pepper [*Capsicum annuum*] has been demonstrated by Loizzo et al., leading to greater antioxidant activity. The ferric reducing antioxidant power of pepper samples [86.3±2.8] was higher than that of the positive control [63.2±4.3], as determined by the authors [28]. The study conducted by Choi and colleagues showed that *Capsicum annuum*, especially yellow pepper, had the highest antioxidant capacity based on high polyphenol and carotenoid content and free radical scavenging activity [27]. Based on our findings, it is believed that the methanolic extract of *Dianthus orientalis* Adams has a significant antioxidant capacity of 1.96 mmol Fe^{2+}/L.

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Data from several studies have identified the potent antioxidant activity of *Dianthus* species especially *Dianthus orientalis* Adams [29, 30]. Findings in the present study are consistent with the findings of Saboora *et al*. The DPPH assay performed by Saboora and colleagues revealed that *Dianthus orientalis* has a potent ability to scavene free radicals, 6 times more than the standard. *Dianthus orientalis* is rich in effective compounds that play an essential role in the antioxidant richness of this plant. [31]. There are several pieces of evidence about the presence of various bioactive compounds in the methanolic extract of *Dianthus orientalis* Adams. The most abundant compounds in *Dianthus orientalis* Adams are phenolic compounds, which include cinnamic acid, vanillic acid, coumaric acid, and oleuropein, alongside flavonoids and alkaloids. According to previous evidence, the presence of these compounds in this plant could be linked to *Dianthus orientalis* Adams' antioxidant capacity [32].

The plant *Capsicum annuum* plays a vital role in combating oxidative stress through various mechanisms. It helps improve the body's antioxidant status by reducing free radicals and boosting the activity of antioxidant enzymes such as superoxide dismutase [SOD], catalase [CAT], and glutathione peroxidase [GPx]. Additionally, *Capsicum annuum* has anti-inflammatory effects, modulates the immune system, and inhibits lipid peroxidation, contributing to overall health [33]. *Hypericum helianthemoides* is recognized as a potent antioxidant by neutralizing free radicals and protecting cells from oxidative stress. Other mechanisms of this plant include antidepressant, antiviral, and antibacterial effects, highlighting its broad use in both traditional and modern medicine [34]. *Dianthus orientalis Adams* acts as an effective antioxidant by increasing glutathione levels and reducing lipid peroxidation. This plant also holds a special place in medicinal research due to its anti-tumor properties, cardiovascular health benefits, and anti-inflammatory effects [35]. Each of these plants possesses multiple antioxidant and non-antioxidant mechanisms, which lead to their extensive applications in medicine and disease treatment [36-38].

Conclusion

According to our research, *Capsicum annuum*, *Hypericum helianthemoides*, and *Dianthus orientalis* Adams possess strong antioxidant effects. The study has gone some way towards enhancing our understanding about the role of medicinal plants in the medical, pharmaceutical, and health care fields, these botanicals as dietary supplements or pharmaceutical agents. In general, the investigation of traditional Iranian edible plants reveals their significance as important reservoirs of natural antioxidant compounds. More research is necessary to determine the effectiveness of these plants in clinical conditions.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare no competing interest.

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