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Original Article

Evaluation of Antioxidant Potential and Free Radical Scavenging Activity of Methanol Extract from Scrophularia striata

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ABSTRACT

Objectives: The effectiveness of Scrophularia striata in controlling infections and promoting wound healing has been reported. This study aimed to investigate the antioxidant properties of the methanol extract from Scrophularia striata.

Methods: Scrophularia striata, a perennial wild plant found in various temperate and tropical areas of Iran, underwent a methanol extraction process to obtain its active compounds. The antioxidant property of the methanol extract of Scrophularia striata was evaluated by quantifying the total antioxidant level, determining the total phenol content, and conducting DPPH radical scavenging assays.

Results: As the extraction concentrations of Scrophularia striata increase, both the total antioxidant level and total phenol content rise dramatically. With the progression of time and increase in plant extract concentrations, the efficacy of DPPH radical scavenging also shows a corresponding enhancement. Moreover, the IC50% value of Scrophularia striata for DPPH radical scavenging consistently decreases over the observation period.

Conclusion: The data suggest that Scrophularia striata possesses antioxidant properties. The presence of flavonoids and phenolic compounds in Scrophularia striata highlights its potential to alleviate various disorders by modulating oxidative stress levels.

Keywords: Scrophularia Striata, Antioxidant, Oxidative Stress, Total Phenol

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Introduction

Traditional medicine, also known as herbal medicine, herbalism, or phytotherapy, is a practice that involves the use of plants and plant extracts for medicinal purposes (1). This practice has been a part of human history for millennia, with various cultures worldwide promoting health and well-being (2). Today, it is estimated that about one-third of all medicinal products originate from plants or have been modified from plants (3). The annual supply and use statistics for plants are increasing every year. The World Health Organization (WHO) stated that a large part of human society believes in and uses herbal medicines to ensure their health and well-being (4). The study of pharmacy and related sciences by most researchers in universities and prestigious research institutes around the world has

been directed towards investigating and researching the fields of identifying substances with pharmacological properties, therapeutic use, and making medicinal forms from medicinal plants (2, 5).

Reactive oxygen species (ROS) and reactive nitrogen species (RNS), by producing free radicals, can cause disorders by damaging biomolecules such as DNA, proteins, enzymes, or membrane lipids (6). The imbalance between antioxidant and oxidant systems can cause the destructive effects of radicals and induce oxidative stress (OS) (7). In the long term, damage to DNA, proteins, and other molecules leads to the development and exacerbation of cardiovascular diseases, cancer, aging, cataracts, liver bleeding, and AIDS (8). Free radicals are created by several mechanisms or can be formed by losing an electron or by taking a hydrogen atom from chemical compounds (8, 9). In addition, free radicals can be produced as a result of the activation of inflammatory cells such as macrophages and neutrophils. These cells use ROS-type free radicals to destroy foreign microorganisms (10). ROS and RNS can also induce cancer. This action of free radicals does not only take place through direct effects on the structure of DNA and its destruction but it can also affect the cell through disruption of proliferation and death messages (11, 12). Since oxidant compounds and free radicals are necessary for many physiological and metabolic processes, the body must create a balance between oxidation and reduction by using antioxidant enzymes and an antioxidant compound (13). These factors include antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, paraoxonase, and antioxidant vitamins such as vitamin E, vitamin C, and β-carotene (14). In people who have certain diseases, the body's antioxidants need external assistance which is provided through antioxidants in food and herbal medicine (15). The beneficial effects of consuming herbal medicine are partly attributed to the presence of natural phenolic and polyphenolic antioxidants (16). Today, many efforts have been made to find natural antioxidants from plant sources.

Scrophularia striata belongs to the Scrophulariaceae family (Kingdom: Plantae, Phylum: Angiosperms, Class: Eudicots, Order: Lamiales, Family: Scropulariacea, Genus: Scrophularia, Species: Boise Stripa Scropularia) (17). The Scrophulariaceae family, commonly known as the figwort family, includes over 200 genera and 4,000 species of mostly herbaceous plants (18). These plants are often herbaceous or woody, and rarely trees. Some of them are in a semi-parasitic state, despite being green, and suck the plant juice from the host plants. Some lack chlorophyll and are fully parasitic (18, 19). These plants are scattered in most areas of the Earth, especially in cold and temperate areas. Some of them are important medicinal types due to their effective materials such as glucose or essential oils. Some have a laxative or softening effect (20, 21).

Scrophularia striata, a monkey flower, is native to the Zagros mountainous areas. Its local name in Ilam is Thirsty. Its height is between 10 and 50 cm. In the western part of Iran, it is used in traditional and localized brewing plants to treat deep and superficial internal infections (22).

The use of traditional medicine due to fewer side effects has increased in recent years. Accordingly, this study aims to investigate the antioxidant effects of Scrophularia striata ethanolic extract using different methods. The results of this study can provide new insights into the potential application of this plant in the treatment of diseases related to oxidative stress.

Methods and Materials

Collection of Plant

The Scrophularia striata plant considered in this research was collected from the southwestern heights of Ilam in the Cham Gardlan village in early April. It was then dried in the shade and transferred to the laboratory for testing. The dried plant was powdered using a grinder and prepared for extraction.

Preparation of Alcoholic Extract

In this study, methanol was selected as the solvent for the extraction of Scrophularia striata (23). 100 grams of dry powder were dissolved in 500 cc of methanol and kept in the dark for three days. During this period, the solutions were stirred every few hours. The solvent was then removed using a distillation apparatus, resulting in 500 mg of dried methanolic extract.

FRAP Method

This method is based on the ability of the compound of interest to reduce ferric (Fe 3) ions to ferrous (Fe 2) ions in the presence of a TPTZ (Tripyridyl-s-Triazine) solution at low pH. The Fe 2+ TPTZ complex is a violet-colored complex with maximum absorption at a wavelength of 593 nm (24). A FRAP reagent was prepared by mixing acetate buffer (300 mM), iron chloride (20 mM), and TPTZ (10 mM) in a ratio of 10:1:1. Concentrations of 100 µL, 70 µL, 50 µL, and 25 µL from 2 mg/L solutions were prepared and mixed with 2 ml of FRAP solution for 10 minutes, and the absorbance of each was measured at 37 °C at a wavelength of 593 nm. Copper sulfate in different concentrations was used to draw the standard curve.

DPPH Method

This method is based on the ability of the compound of interest to donate an electron or hydrogen to the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical,

thereby neutralizing the DPPH free radical. DPPH is an unstable free radical that can accept an electron or hydrogen radical to become stable. DPPH has the highest absorption at a wavelength of 517 nm (purple dye) (25, 26).

200 mg of dried methanolic extract was dissolved in 1 ml of DMSO solvent, and then different concentrations were prepared (0.1 mg/ml, 0.3 mg/ml, and 0.5 mg/ml). Different concentrations of control and standard (Quercetin) were added to 2 ml of DPPH solution, and the absorbance was read at 517 nm after 10, 15, and 20 minutes.

The percentage of inhibition or reduction of DPPH by the antioxidant compound can be calculated from the following relationship: DPPH inhibition percentage = (A DPPH - A sample/A DPPH) *100 (A DPPH: absorbance of DPPH in the absence of a sample, A Sample: DPPH absorption in the presence of the sample).

Indeed, the IC50% of various plant extractions was calculated at 10, 15, and 20 times.

Total Phenolic

To obtain the standard curve, the Terbium (Tb) sensitized fluorescence method at room temperature was employed. 2 ml of aqueous solution + 0.01 M Tb + 0.25 ml Tris buffer 0.05 M with pH = 7, and standard solutions of quercetin were used. The fluorescence intensity of the solutions was read after 5 minutes at 545 nm with excitation at 310 nm against the blank.

The previously prepared Scrophularia striata extracts (100 µL, 70 µL, 50 µL, and 25 µL concentrations of 2 mg/L plant extracts) were added to the Tb+ solution and Tris buffer, and their total phenol content was measured using the standard increase method.

Statistical Analysis

To minimize error, each sample was tested in three test tubes, and the average of the three readings was calculated. All data are represented as means ± SD. Differences between groups were analyzed using one-way ANOVA/Kruskal-Wallis tests, followed by post-hoc Tukey and Mann-Whitney tests. Statistical analysis was performed with SPSS software version 26 (SPSS Inc., Chicago, IL), and graphs were prepared using GraphPad Prism software (8.4.3).

P-values < 0.05 were considered statistically significant.

Results

Total Antioxidant Levels

Figure 1 presents the results of a FRAP test conducted to determine the overall antioxidant power of Scrophularia striata extract. As shown in Figure 1, the total antioxidant levels increased significantly in 70 µl ($P = 0.01$) and 100 µl ($P < 0.001$) of Scrophularia striata compared to 25 µl of 2 mg/L plant extracts.

DPPH Free Radical Scavenging

Figure 2 illustrates the DPPH radical scavenging by various concentrations (0.5, 0.3, and 0.1 mg/dl) of Scrophularia striata extract at 10, 15, and 20 times. Table 1 shows the IC50% of various concentrations of Scrophularia striata extract. Based on the results obtained 10, 15, and 20 minutes after the test, the DPPH inhibition percentage increased significantly for concentrations of 0.3 mg/dl and 0.5 mg/dl compared to 0.1 mg/dl (P < 0.001). The IC50% for different concentrations (10, 15, and 20 IC50%) are 30, 26, and 24 mg/dl, respectively.

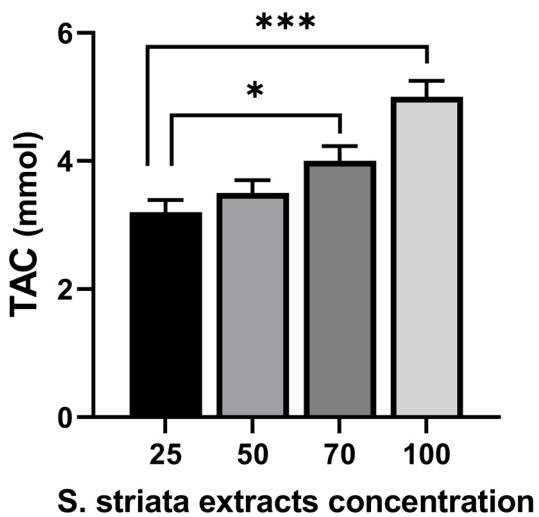

Figure 1: Total antioxidant levels in Scrophularia striata excretion concentrations (25 **µl**, 50 **µl**, 70 **µl**, 100 **µl** of 2 mg/L plant extracts). The data are presented as mean ± SD, and One-way ANOVA/Kruskal-Wallis tests with post-hoc Tukey and Mann-Whitney were used to analyze the data. The significance levels are: * $p < 0.05$, *** $P < 0.001$.

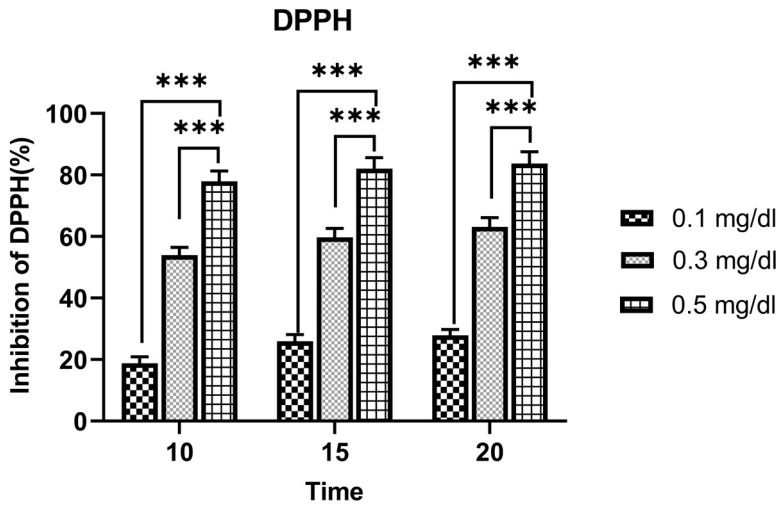

Figure 2: DPPH radical scavenging by 0.1, 0.3, and 0.5 mg/dl of Scrophularia striata extract at 10, 15, and 20 times. The data are presented as mean ± SD, and One-way ANOVA/Kruskal-Wallis tests with post-hoc Tukey and Mann-Whitney were used to analyze the data. The significance levels are: *** $P < 0.001$.

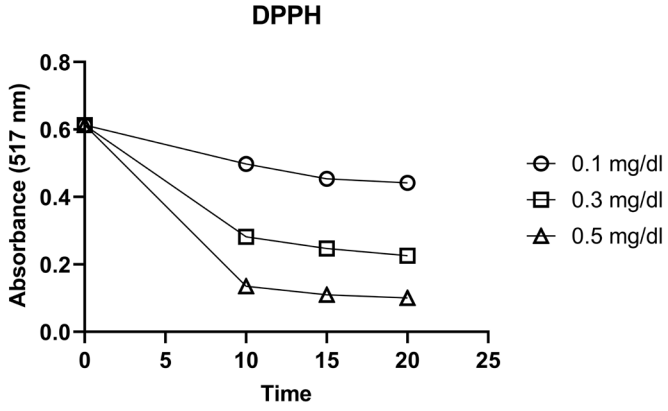

Figure 3: DPPH absorption in 0.1, 0.3, and 0.5 mg/dl of plant extract at 10, 15, and 20 times.

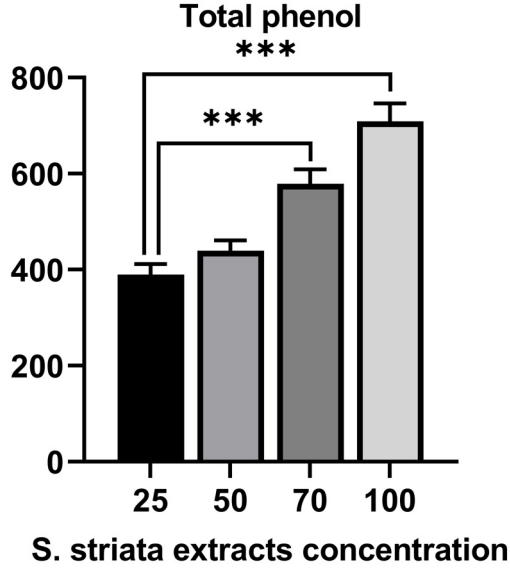

Figure 4: Total phenol content of the Scrophularia striata extract concentrations (25 **µl**, 50 **µl**, 70 **µl**, 100 **µl** of 2 mg/L plant extracts). The data are presented as mean ± SD, and One-way ANOVA/Kruskal-Wallis tests with post-hoc Tukey and Mann-Whitney were used to analyze the data. The significance levels are: *** $P < 0.001$.

Table 1: DPPH radical scavenging and IC50% by 0.1, 0.3, and 0.5 mg/dl of S. striata extract at 10, 15, and 20 times.

Concentration / Time (minute)	0.1 (mg/mL)	0.3 (mg/mL)	0.5 (mg/mL)	IC50 (mg/mL)
10	18.76	53.99	77.97	0.30
15	25.93	59.7	82.05	0.26
20	27.89	63.13	83.68	0.24

The data indicate that as time passes, the concentration of the sample required to inhibit 50% of DPPH free radicals decreases. Indeed, with various doses of Scrophularia striata extract, DPPH absorption reduced over time as shown in Figure 3.

Total Phenolic Content

Figure 4 presents the total phenol content of various concentrations of Scrophularia striata extract. The total phenol content increased significantly for concentrations of 70 µl ($P < 0.001$) and 100 µl ($P < 0.001$) compared to 25 µl of 2 mg/L plant extracts. This indicates that with an increase in plant extraction concentration, the total phenol content enhances.

Discussion

The findings of this study indicate that as the concentration of Scrophularia striata methanol extract increases, the total amounts of antioxidants and the total level of phenol content also increase. Additionally, DPPH radical scavenging improves as time progresses and Scrophularia striata concentrations rise. The IC50% of Scrophularia striata for DPPH radical scavenging declines over time.

In biological systems, the production of free radicals is inevitable. The body neutralizes their harmful effects to some extent by designing antioxidant defense mechanisms, using antioxidant vitamins such as vitamin E, vitamin C, and β-carotene, or supplementary herbal medicine with antioxidant properties (14, 27, 28). However, if the production of free radicals increases or the antioxidant factors decrease, the damage caused by them increases, leading to oxidative stress. This imbalance between the production of free radicals and peroxide substances and a defect in the antioxidant defense system can lead to oxidative stress (29, 30).

Internal sources of oxidative stress include peroxisomes and enzymes, especially detoxifying enzymes from the P450 complex, xanthine oxidase, and nicotinamide adenine dinucleotide oxidase complexes. External sources include pollution, radiation, light rays, and chemical compounds like anti-cancer drugs, cigarette smoke, and alcohol (31, 32). In cases of mild or moderate oxidative stress, tissues often neutralize oxidative stress by increasing the antioxidant defense. However, in cases of severe oxidative stress, cells can be damaged and may lead to cell death (33).

Today, it is clear that free radicals are involved in the pathogenesis of more than one hundred types of diseases such as diabetes mellitus, rheumatoid arthritis, cardiovascular diseases, asthma, AIDS, malaria, cancers, aging, alcohol poisoning etc. They affect various tissues such as the kidney, lung, heart, skin, brain, joints, gastrointestinal tract, eyes, blood vessels, red blood cells and other tissues during oxidative stress (8, 34-37).

It is now clear that exposure to oxidative stress is a common occurrence, and in order to combat these conditions, it is necessary to increase antioxidant capacity through the consumption of antioxidant products. One effective approach in this regard is the consumption of fruits and plants with high antioxidant properties. Herbal medicine has been used worldwide, including by locals and traditional Iranians, to treat diseases and enhance antioxidant capacity (38).

Scrophularia striata, native to the Zagros mountainous areas, is used in local and traditional medicine (21, 22). People in the province of Ilam have long used this plant experimentally to treat a variety of illnesses including inflammation and infection of the eyes and ears, skin burns, infectious wounds, episiotomy, pain, gastrointestinal disorders, colds, hemorrhoids, and boils (39, 40).

This study was conducted to investigate the antioxidant properties and activity of Scrophularia striata using several methods. Vitamins A, C, and E are plant components that support antioxidant ability. Additionally, phenolic substances such as flavonoids and phenolic acids have antioxidant properties (41).

The data from this study demonstrated that total antioxidant capacity and total phenol content increased with augmented Scrophularia striata concentration. Indeed, DPPH radical scavenging increases with Scrophularia striata methanol extraction, and IC50% of Scrophularia striata is responsible for DPPH radical scavenging, which declines with time. This method showed that Scrophularia striata extract collected free radicals and increased the redox state of the system (26).

Azadmehr et al.'s study on the PC12 cell line demonstrated that Scrophularia striata decreases ROS generation and cell apoptosis and also suggests the presence of neuroprotective agents in this plant (42). Kamalipourazad et al. showed that the antioxidant

responses of Scrophularia striata were boosted through enhanced activity of an effective series of scavenging enzymes: superoxide dismutase, peroxidase, catalase, and the biosynthesis of non-enzymatic antioxidants such as phenolic acids, echinacoside, and flavonoids compounds (43). Khodamoradi reported Scrophularia striata has a dose-response relationship, and with a higher dose, this plant's antioxidant activity rises, which is similar to what our study found (44). Because it contains unique components like flavonoids and phenolic compounds, the Scrophularia striata plant can counteract hepatotoxicity by boosting the antioxidant state and enhancing liver function (23).

Conclusion

Scrophularia striata indeed holds potential for treating a variety of illnesses and disorders. However, as you rightly pointed out, there has been limited research examining the phytochemistry of this plant. Despite the promising results from this study, further research is indeed needed to isolate and identify potent chemicals with pharmacological and bioactive properties in different parts of the plant.

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Conflict of Interest

Authors state no conflict of interest.

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