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Antioxidant Properties of Methanolic Extract of *Sigesbeckia orientalis* L. from Rize

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ABSTRACT

Sigesbeckia orientalis Linnaeus is a traditional herbal medicine that has been used for many years to treat inflammatory diseases. The genus Sigesbeckia comprises three species: *Sigesbeckia pubescens* Markino (SP), *Sigesbeckia glabrescens* Makino (SG), and *Sigesbeckia orientalis* L. (SO). *S. orientalis* has been used in Eastern medicine to alleviate rheumatic disorders, improve joint mobility, and provide protection against toxicity. This plant is naturally found in countries such as China, the Philippines, and Vietnam. In Türkiye, particularly in the Black Sea region, *S. orientalis* is known as Sarıteçan. In this study, the antioxidant capacity of the methanolic extract of *S. orientalis* was evaluated by determining the total phenolic content (TPMC) and total flavonoid content (TFC). The antioxidant activity was assessed using Ferric Reducing Antioxidant Power (FRAP), Cu (II) Ion Reducing Antioxidant Capacity (CUPRAC), and 2,2-Diphenyl-1-picrylhydrazyl Free Radical Scavenging Capacity (DPPH) assays. The total phenolic content (TPMC) of *S. orientalis* extract was determined to be 18 ± 0.57 mgGAE/g dry weight, and the total flavonoid content (TFC) was 8.55 ± 0.21 mg QE/g dry weight. The DPPH (SC50) was found to be 380 ± 12 µg/mL, while the CUPRAC and FRAP activities were 74 ± 1.99 and 19 ± 1.38 mg TE/g dry weight, respectively. These results demonstrate that *S. orientalis* grown in the Rize İkizdere region possesses strong antioxidant properties. Therefore, *S. orientalis* may have significant potential not only in traditional medicine but also in modern complementary medicine.

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Rize Yöresinde Yetişen *Sigesbeckia orientalis* L. Metanollü Ekstresinin Antioksidan Özellikleri

ÖZET

Sigesbeckiae orientalis Linnaeus, uzun yıllardır enflamatuvar hastalıkların tedavisinde kullanılan geleneksel bir bitkisel ilaçtır. Sigesbeckiae cinsine ait üç türü bulunmaktadır: *Sigesbeckia. pubescens* Markino (SP), *Sigesbeckia glabrescens* Makino (SG) ve *Sigesbeckia orientalis* L. (SO). *S. orientalis* doğu tıbbında romatizmal rahatsızlıkları hafifletmek, eklemlerin hareketliliğini iyileştirmek ve toksisiteye karşı koruma sağlamak için kullanılmıştır. Bu bitki Çin, Filipinler ve Vietnam gibi Asya ülkelerinde doğal olarak yetişmektedir. Türkiye'de özellikle Karadeniz Bölgesinde yetişen *S. orientalis* Sarıteçan olarak adlandırılmaktadır. Bu çalışmada, *S. orientalis*'in metanollü ekstresinin antioksidan kapasitesi toplam fenolik madde miktarı (TPMA) ve toplam flavonoid miktarı (TFA) belirlenerek, antioksidan aktivitesi Ferrik İndirgeyici Antioksidan Güç (FRAP), Cu (II) İyonu İndirgeyici Antioksidan Kapasite (CUPRAC) ve 2,2-difenil-1-pikrilhidrazil Serbest Radikalleri Temizleme Kapasitesi (DPPH) analizleri yapılarak değerlendirildi. *S. orientalis* ekstresinin toplam fenolik madde miktarı (TPMA) 18 ± 0.57 mgGAE/g kuru ağırlık olarak belirlenirken toplam flavonoid miktarı (TFA) ise 8.55±0.21 mg QE/g kuru ağırlık olarak belirlendi. DPPH (SC_{50}) 380±12 µg/mL bulunurken CUPRAC ve FRAP aktiviteleri

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sırasıyla 74 ± 1.99 ve 19 ±1.38 mg TE/g kuru ağırlık bulundu. Bu sonuçlar, Rize İkizdere bölgesinde yetişen *S. orientalis* bitkisinin güçlü antioksidan özelliklere sahip olduğunu ortaya koymaktadır. Bu nedenle, *S. orientalis* bitkisi, geleneksel tıbbın ötesinde modern tamamlayıcı tıpta da araştırılmaya değer bir potansiyele sahip olabilir.

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INTRODUCTION

Plants have been essential to human survival since antiquity, fulfilling various roles including nourishment, shelter, protection, warmth, and medicinal uses (Tohma et al., 2016; Yılmaz et al., 2023; Karagecili et al., 2023). Throughout history, people have relied on plants for nutrition and for finding remedies for various health problems (Kendir & Güvenç, 2010). The prevalence of synthetic pharmaceuticals, characterized by a singular active ingredient and frequent adverse effects, has prompted a transition towards natural medicines in contemporary healthcare. The utilization of plants, referred to by different terms like alternative medicine and phytotherapy, in healthcare services has recently gained prominence (Dave, 2019). The genus Sigesbeckia comprises three species: *Sigesbeckia pubescens* Markino (SP), *Sigesbeckia glabrescens* Makino (SG), and *Sigesbeckia orientalis* Linnaeus (SO). These species are part of the Asteraceae family of plants. The aerial components of these species are utilized in the treatment of various ailments, particularly inflammatory conditions, referred to as Xi-Xian in traditional Chinese medicine. Siegesbeckia is a genus of annual plants indigenous to tropical, subtropical, and temperate climates globally (Chen et al., 2022; Tao et al., 2021). *S. glabrescens* is native to countries in East Asia, including China, Japan, and Korea. *S. pubescens* is found in parts of Korea, China, and around Kocaeli, Türkiye. *S. orientalis* is primarily found in the eastern Black Sea region of Türkiye, including Rize, Artvin, and Trabzon. This herbaceous plant, referred to by locals as the Sarıteçan, blooms naturally between April and July, with peak flowering occurring during this period. (Ejder, 2020).

Plants can synthesize a wide variety of organic chemicals, with secondary metabolites including polyphenols, volatile compounds, and alkaloids being crucial for their adaptability and defense mechanisms (Güven et al., 2024; Celik et al., 2024; Bayrak et al., 2023). Siegesbeckia is acknowledged for its variety of bioactive substances that exhibit health-promoting qualities, including phenolics (flavonoids), carotenoids, capsaicinoids, fatty acids, vitamin C, and vitamin E. There is an increasing emphasis on investigating the functions and applications of many physiological bioactive chemicals, particularly kirenol, recognized as one of the most important bioactive ingredients (Ren et al., 2020; Sukanya et al., 2022). Plants possess antioxidant, antibacterial, anti-inflammatory, and antiviral capabilities owing to the bioactive compounds they contain. Phenolic acids and flavonoids are the predominant categories of polyphenols (Zagoskina et al, 2023). Phenolics and flavonoids, present in nearly all plants, including fruits and vegetables, function as natural antioxidants (Apak et al., 2007). Free radicals exhibit high reactivity and instability owing to the presence of unpaired electrons within their structure. These radicals tend to react with proteins, lipids, nucleotides, DNA, and coenzymes, causing harmful effects. These include diseases of the cardiovascular system, cancer, nervous system disorders like diabetes, Parkinson's, and Alzheimer's, and rheumatoid arthritis. The antioxidant systems within the body act to prevent the damage caused to cells by free radicals, which can lead to the development of various diseases (Topal & Gulcin, 2022; Karagecili et al., 2023; Gulcin, 2020). Antioxidant substances react with free radicals, playing a crucial role in preventing the damage caused by these radicals (Alpkent & Demir, 2006; Öğretmen, 2022). When the antioxidant defense system is inadequate, it is necessary to consume natural antioxidants externally to reduce the effects of free radicals. Plants possess natural antioxidant properties attributed to flavonoids and phenolic acids, which are secondary metabolites produced as part of their metabolism (Bayrak et al., 2023).

S. orientalis grows spontaneously in many areas, from temperate to tropical regions, and grows best where the temperature is in the range of 10-27°C during the growing season. *S. orientalis* can bloom year-round in warm climates, but flowering is at its highest during the rainy season. Propagation of flowers can occur under moist conditions. Phytochemical analysis of *S. orientalis* leaves indicates that they contain high amounts of saponins, alkaloids, flavonoids, oxalates, tannins, phytates, and phenolics. Additionally, the leaves of *S. orientalis* contain significant amounts of vitamins A and C, which act as antioxidants, reducing the harmful effects of free radicals

by donating electrons to various anti-oxidative enzymatic and non-enzymatic reactions (Sukanya et al., 2022). It is recognized that the active metabolite content of *S. orientalis* varies depending on several factors such as altitudinal differences, different parts of the plant, environmental conditions, growing conditions, plant development stages, genotypes, etc. (Pradhan et al.2018).

S. orientalis is reported to possess pharmacological properties with anti-inflammatory, antiallergic, antithrombotic, anti-atherosclerotic, microcirculation-enhancing, wound-healing, and anticancer effects. Research on the plant is primarily focused on cancer and inflammation. While the phenolic content of the plant has been analyzed, there is limited research on its antioxidant content and capacity (Tao et al.,2021).

These findings may enhance our comprehension of the health benefits linked to *S. orientalis*, offering significant insights for the pharmaceutical and food industries. These discoveries could facilitate the development of functional foods, dietary supplements, or medications. This study aims to examine the antioxidant content and activity of the methanolic extract of the *S. orientalis* (Sarıteçan) collected from the İkizdere area of Rize.

MATERIALS and METHODS

Preparation of Extracts:

The plant was collected on April 15, 2017, from a location in the İkizdere district of Rize, specifically in the area between Ovit Mountain and its surrounding region, at an altitude of approximately 1800-2000 meters (Figure 1). The plant's species identification was carried out by Prof. Dr. Serdar Makbul, a faculty member from the Biology Department, Faculty of Arts and Sciences, Recep Tayyip Erdogan University.

The aerial components of *S. orientalis* samples were detached, weighed (15-20 g), and pulverized using a mortar and pestle. The plant material was subsequently treated with 100 mL of methanol (MeOH) and maintained in a magnetic stirrer for 48 hours. Due to its elevated polarity, methanol facilitates the effective dissolution of polar phytochemicals, including phenolic and flavonoid molecules. Therefore, methanol was favored as a solvent during the extraction process. The extracts were filtered using filter paper, and the solvents were evaporated in an evaporator at 40°C. The extracted samples were prepared in DMSO and preserved in the freezer for experimental usage (Ejder, 2020).

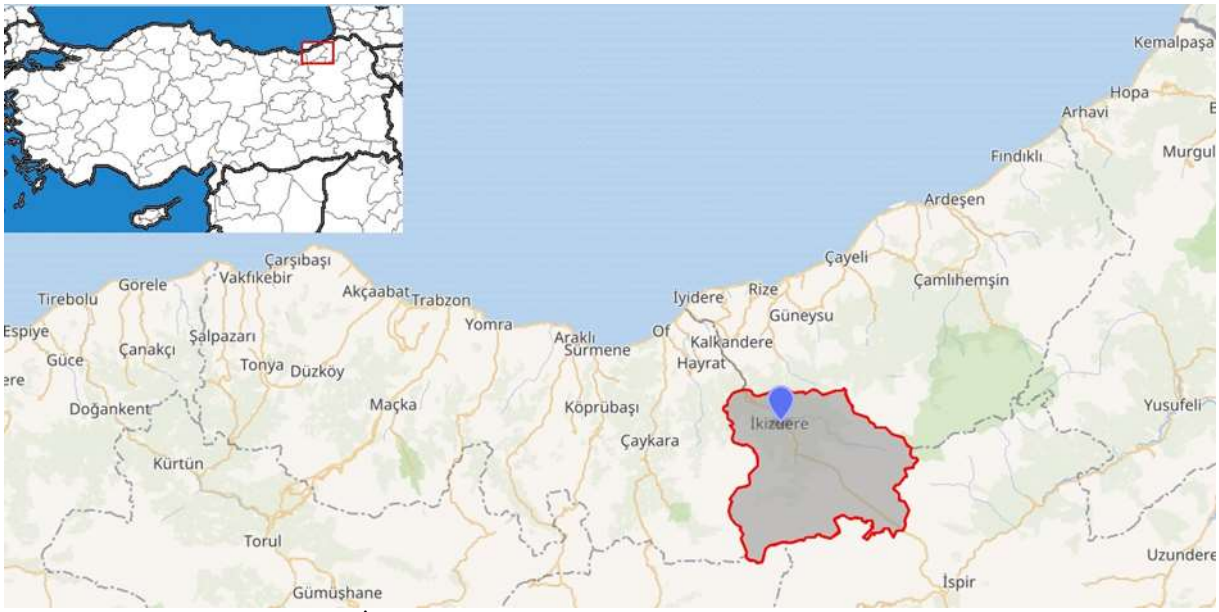

Figure 1. The location of the İkizdere district of Rize province on the map of Türkiye.
Resim 1. Rize ili İkizdere ilçesinin Türkiye haritası üzerindeki konumu.

Total phenolic matter analysis (TPMA):

The Folin-Ciocalteu assay (Folin & Ciocalteau, 1927) was employed to assess the total phenolic content (TPC) of the samples. This method involved the formation of colored complexes with Folin reagents in an alkaline environment, leading to the development of a characteristic purple-violet complex. Gallic acid was employed as the reference standard. The results are expressed as mg GAE/g dry.

Total flavonoid (TFA) analysis:

The total flavonoid content in the extracts was determined spectrophotometrically utilizing the aluminum

chloride/potassium acetate method (Christ & Müller, 1960). Quercetin (QE) served as the reference standard. The results are reported as mg QE/g dry sample.

Methods for Determination of Antioxidant Activity Based on Electron Transfer

Copper ion-reducing antioxidant capacity (CUPRAC) activity:

The cupric reducing antioxidant capacity (CUPRAC) method works by creating a Cu(I)-neocuproine chelate, which can only happen when an antioxidant substance is present. This chelate is produced from the Cu(II)-neocuproin complex generated by Cu (II) of 2,9-dimethyl-1,10-phenanthroline, displaying a peak absorbance at 450 nm (Apak et al., 2008). Trolox served as the reference standard. The results were expressed as grams of Trolox equivalent per gram of dry extract (TECA/g dry sample).

Ferric Reducing Antioxidant Power (FRAP) activity:

The samples' total antioxidant capacity was assessed using the ferric reducing antioxidant power (FRAP) assay (Benzie & Strain, 1996). This approach entails the reduction of the Fe (III)-TPTZ (2,4,6-tris(2-pyridyl)-S-triazine) molecule to its blue Fe (II)-TPTZ form in the presence of antioxidants, with absorbance measured at 595 nm. A standard curve was established utilizing different concentrations of Trolox, spanning from 1000 to 31.25 µmol/mL. The results are presented as mg TEFA/g dry extract.

Free Radical Scavenging (DPPH) Activity:

The DPPH radical (2,2-diphenyl-1-picrylhydrazyl) employed in this work was obtained from a commercial supplier. The antioxidant activity of the samples was evaluated using the modified Coundent method (Blois, 1958). The result was denoted as SC_{50}, with lower SC_{50} values indicating greater radical scavenging activity.

RESULT and DISCUSSION

In this study, TPMA and TFA levels were determined in *S. orientalis* plant. In addition, antioxidant activity was investigated by CUPRAC, FRAP, and DPPH measurements in methanolic extracts.

The TPMA results were investigated, and it was found that the *S. orientalis* plant extract contained 18 ± 0.57 mg GAE/g dry weight. The TFA content was found to be 8.55 ± 0.21 mg QE/g dry weight. Researchers used CUPRAC and FRAP tests to find out how antioxidant-rich the extract was. The CUPRAC activity was 74 ± 1.99 mg TE/g dry weight, and the FRAP activity was 19 ± 1.38 mg TE/g dry weight. The DPPH activity (SC_{50}) was found to be 380 ± 12 µg/mL. The antioxidant capacity and antioxidant activity results of *S. orientalis* plant extract have been presented in Table 1.

Table 1. Antioxidant capacity and activity results of S. orientalis plant extract.
Tablo1. *S. orientalis* bitki ekstresinin antioksidan kapasite ve aktivite sonuçları.

S. orientalis Antioxidant Capacity and Activity	
TPMA (mg GAE/g dw)	18 ± 0.57
TFA (mg QE/g dw)	8.55 ± 0.21
CUPRAC (mg TE/g dw)	74 ± 1.99
FRAP (mg TE/g dw)	19 ± 1.38
DPPH (SC_{50}, µg/mL)	380 ± 12

S. orientalis is an herbal medicine that has been traditionally used to treat rheumatism in China for many years, since at least the Tang dynasty (Linghu et al., 2020). It has been reported that extracts collected from the whole plant *S. orientalis* have anti-inflammatory and antirheumatic properties and strong antioxidative activity (Pradhan et al., 2018; Chen et al., 2022). A phytochemical study of *S. orientalis* revealed hundreds of compounds, which have been classified into three main groups: sesquiterpenes, diterpenes, and phenolics (Tao et al., 2021). The HPLC analysis of extracts of *S. orientalis* plant revealed the presence of rutin, quercitrin, chlorogenic acid, caffeic acid, 3-caffeoylquinic acid, 4-caffeoylquinic acid, 3,4-dicaffeoylquinic acid, and 3,5-dicaffeoylquinic acid. The plant contains a multitude of phenolic compounds, including kaempferol-3-O-rhamnoside and kaempferol-3-O-rutinoside. (Nguyen et al., 2017; Tran et al., 2023). Research has demonstrated that the antioxidant activity of plant extracts was closely correlated with the concentration of phenolic compounds present. High levels of phenolics have been shown to exhibit strong antioxidant effects by neutralizing free radicals (Teixeira et al., 2017; Asif, 2015).

Recent studies have further expanded our understanding of *S. orientalis*' bioactive properties. A study conducted

by Zhou et al. (2024) demonstrated that diterpenoids isolated from *S. orientalis* exhibited significant antimicrobial activity, particularly against antibiotic-resistant bacteria. This finding highlights the potential of *S. orientalis* not only for its traditional use but also for its relevance in addressing modern health challenges, particularly in combating drug-resistant infections.

A recent study on *S. pubescens* highlighted the isolation of novel diterpenoids with potent anti-inflammatory effects, particularly in the context of diabetic retinopathy. This study demonstrated that these diterpenoids, especially compound 5, effectively reduced oxidative stress and inflammatory responses *in vitro* and *in vivo*. Recent research has identified bioactive compounds with significant anti-inflammatory properties, further emphasizing the potential of Sigesbeckia species in the development of natural therapeutic agents (Liu et al., 2025). Moreover, while *S. orientalis* has been used for centuries in traditional medicine, its modern applications are expanding. The strong antioxidant properties of the plant have made it an attractive candidate for research into new therapies, particularly in the treatment of age-related diseases and chronic inflammation. The therapeutic value of S. orientalis could be enhanced through further exploration of its bioactive compounds and their roles in reducing oxidative damage and inflammation (Teixeira et al., 2017; Asif, 2015).

In this study, *S. orientalis* extract was prepared with methanol solvent in order to investigate the antioxidant capacities and activities of *S. orientalis* plant growing in İkizdere of Rize. We used TPMA and TFA analyses to determine the antioxidant capacity of the *S. orientalis* plant extract prepared with methanol. Also, CUPRAC, FRAP, and DPPH were analyzed for the determination of antioxidant activity.

Hung et al. (2017) purchased plant materials of the *S. orientalis* species from the Yuanshan Company in Kaohsiung City, Taiwan. They extracted the plant materials using ethanol and subsequently separated the ethanol extract using solvents of varying polarity (hexane, ethyl acetate, and methanol). In this study, it was observed that the total amount of phenolic and flavonoid substances in the ethyl acetate extract was the highest, followed by ethanol, methanol, and hexane. That study by Hung et al. (2017) measured the total amount of flavonoids (1.9±0.1 mg QE/g dry weight) and phenolic substances (15.7±0.4 mg GAE/g dry weight). This study, on the other hand, measured the total amount of flavonoids (8.55±0.21 mg QE/g dry weight) and phenolic substances (18.57±0.57 mg GAE/g dry weight) in the same solvent extract. In the same study, DPPH activity was investigated in extracts prepared with 4 different solvents. They found that DPPH scavenging activities were highest in ethyl acetate and lowest in hexane. They determined the IC_{50} value (346.6 ± 34.7 µg/mL) in methanolic extracts. In the present study, DPPH scavenging activity was found to be (SC_{50} = 380 ± 12.0 µg/mL), compatible with the study by Hung et al. (2017).

A study by Pradhan et al. (2018) looked at diploid and tetraploid parts of *S. orientalis* plants and found that the parts above ground had more total phenolic matter and total flavonoid matter than the parts below ground. In this study, only the above-ground part was used. The TPMA levels were higher than those found by Pradhan et al. (9.70 ± 0.10 mg/g DW) in tetraploid leaves, but the total flavonoid content was the same (7.73 ± 0.02 mg/g DW). Across all three studies, the total flavonoid content remained lower than the total phenol content in *S. orientalis*. Additionally, Pradhan et al. (2018) reported the highest DPPH levels in the leaves of the tetraploid cytotype (16.71 ± 1.68 µg/mL). DPPH activity in this study and that of Hung et al. (2017) was lower than that of Prandhan et al. (2018). The content of bioavailable compounds in plants varies according to the region where they grow. Therefore, it was concluded that the differences between the studies may be due to both the extraction methods and the growing regions. It has been demonstrated that there was a positive correlation between the antioxidant activity (DPPH, FRAP, and CUPRAC) of a plant or fruit extract and the concentration of phenolic compounds present. High levels of phenolic compounds have been shown to exhibit strong antioxidant activity by inhibiting free radicals and donating electrons from phenol groups (Junsathian et al. 2022). The present study has identified a correlation between the phenolic and flavonoid contents of *S. orientalis* and its antioxidant activity. Previous studies have investigated the DPPH free radical scavenging activity of *S. orientalis* (Hung et al. 2017, Prandhan et al. 2018), and the results of the present study are consistent with those of these previous studies. In this study, the CUPRAC activity for *S. orientalis* plant extract was determined to be 300 µmol TE/g DW (0.30 mmol TE/g DW). As a result of the literature review, no study on the CUPRAC activity of *S. orientalis* was found. This shows that our study provides an important contribution to the literature in this field.

There is limited data on the FRAP activity of *S. orientalis*. A study looked at the antioxidant power and total phenolic content of 223 medicinal plant infusions (Li et al., 2013). For *S. orientalis*, the total phenolic content was found to be 11.61 mg GAE/g, and the FRAP activity was found to be 192.82 µmol Fe (II)/g. The total phenolic content of the *S. orientalis* extract in our study was higher (18.57 mg GAE/g DW), but the FRAP activity levels were different from those in this study (192.82 µmol Fe (II)/g). It is thought that this difference may be due to the extraction method, analysis conditions, or ecological factors in which the plant grows.

Prior research on the antioxidant properties of *S. orientalis* has predominantly utilized the DPPH technique (Yang et al., 2016). This study's thorough assessment of the methanol extract using CUPRAC, FRAP, and DPPH

techniques offers a distinctive contribution to the field. In the literature, there is no research on CUPRAC analysis, and just one study utilizing the FRAP approach exists. This work is among the first to investigate the antioxidant capabilities of *S. orientalis* from a broader perspective.

These findings emphasize the importance of further research into the bioactive compounds of *S. orientalis*, as they could play a critical role in the development of modern complementary therapies. By combining its traditional uses with modern scientific findings, *S. orientalis* has the potential to be integrated into contemporary medical treatments.

The presence of bioactive compounds in *S. orientalis* and other plants indicates that these species have potential applications in the food and pharmaceutical industries (Sacan, 2019; Choi et al., 2021). In this context, the results of our study are expected to provide a basis for further research on *S. orientalis* and comparative analysis with other species.

CONCLUSIONS

In recent years, there has been a global increase in interest in herbal products as a means of maintaining optimal health, in part due to the increasing toxicity of synthetic drugs. Furthermore, this interest has facilitated the investigation of plants that are found in their natural habitat and not cultivated. *S. orientalis* is a plant that has been known for its anti-inflammatory effect for many years and is found growing spontaneously in nature. The present study provides useful information about the antioxidant content and capacity of the *S. orientalis* plant from İkizdere, Rize. The study's findings revealed that the methanolic extracts of this plant were rich in antioxidants. This was shown by checking the levels of TFA, TPMA, DPPH, FRAP, and CUPRAC. Notably, this study is the first to evaluate the antioxidant properties of *S. orientalis* using the CUPRAC method, highlighting the contribution of this research to the existing body of knowledge. While previous studies on this plant have typically employed different solvents, such as ethanol or water, our work with methanol provides a more thorough exploration of its bioactive compounds. The bioactive compounds in *S. orientalis* have shown promising therapeutic potential, particularly for their antioxidant effects. These findings confirm that *S orientalis*, a plant native to the Rize İkizdere region, possesses significant antioxidant properties. Already utilized in traditional medicine for various ailments, these properties further supplement the plant's potential for integration into modern complementary medicine.

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Contribution of Authors

The authors state that they have contributed equally to the article.

Conflict of Interest

The authors declare no conflict of interest.

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