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Phytochemical Analysis of *Solidago Virgaurea*: Quantitative Analysis of Bioactive Compounds By LC-MS/MS, Ascertaining the Antioxidant Activity and Phenolic Profile by Potentiometric Sensors

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ABSTRACT

Solidago virgaurea L. subsp. virgaurea (SVSV) has long been used in traditional medicine for treating urinary tract disorders, nephrolithiasis, and inflammatory-related conditions. This study aimed to evaluate the antioxidant capacities and phenolic profiles of *SVSV* extracts. Antioxidant activities were assessed using the FRAP and DPPH assays. Moreover, total phenolic content was determined by Folin-Ciocalteu reagent (FCR). The advanced technique, a potentiometric sensor, was utilized for antioxidant assays and FCR. Phenolic composition in plant extract was determined by LC–MS/MS. *Solidago virgaurea* methanolic extract exhibited strong antioxidant potential with a FRAP value of 19.07 µmol TE/mg extract and a DPPH radical scavenging activity of 78.8% at 128 ppm. The total phenolic content was found to be 413.82 mg GA/mg extract. Compared to standards, *SVSV* methanolic extract showed higher DPPH activity (78.80%) than BHT (68.25%), BHA (49.06%), and Trolox (65.25%), and was the same as with gallic acid (78.55%). LC-MS/MS analysis revealed that the major phenolic compounds were trans-cinnamic acid (126.19 µg/g), chlorogenic acid (58.83 µg/g), and vanillin (26.17 µg/g), along with rutin (11.36 µg/g), gentisic acid (11.03 µg/g), and quercetin (2.47 µg/g). These findings demonstrate that *SVSV* possesses remarkable antioxidant capacity and a diverse phenolic profile, supporting its potential use as a functional food ingredient and natural source of antioxidants.

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Introduction

Due to their bioactive compound content, plants have been utilized in traditional medicine and have played a significant role in the development of new drugs (Demirtas et al., 2013; Elmastas et al., 2004; Topçu et al., 1999). In addition, Aromatic and medicinal plants have been reported to reveal a broad spectrum of biological activities (Dede et al., 2019; Yildiz et al., 2017).

Oxidative stress occurs in living organisms when free radical species and active oxygen are produced in excess of the required amount. (Erenler et al., 2025; Erenler et al., 2014). This process leads to oxidative damage to cellular components (lipids, proteins, and DNA), leading to the development of many chronic diseases such as cancer, diabetes, cardiovascular diseases, and neurodegenerative diseases (Başar et al., 2025; Elmastaş et al., 2015). For this reason, the discovery and evaluation of natural antioxidants are among the primary research areas in both pharmaceutical research and plant and food science today (Erenler et al., 2015; Yildiz et al., 2024).

Natural antioxidants of plant origin generally consist of phenolic compounds, flavonoids, phenolic acids, and tannins (Erenler et al., 2016; Yaman et al., 2024). These compounds act by directly quenching free radicals, chelating metal ions, or activating antioxidant enzyme systems. In particular, the antioxidant power of phenolic compounds emerges thanks to the interaction of hydroxyl groups in their structure with radicals (Djermane et al., 2025; Kamah et al., 2025; Merzouki et al., 2024).

SVSV is a perennial herbaceous plant belonging to the Asteraceae family and is native to North America, South America, and Eurasia, and includes about 139 species. It is also found in the flora of Turkey with different subspecies and is known as goldenrod grass. This plant, which is used as a diuretic, kidney stone, and anti-inflammatory agent in traditional medicine, has attracted great attention in recent

years for its phytochemical components and biological activities. Like many plants used in phytotherapy, *SVSV* has a rich profile in phenolic compounds, flavonoids, and other antioxidant metabolites (Fursenco et al., 2020).

Previous pharmacological research on *SVSV* has revealed that the herb exerts antioxidant, anti-inflammatory, antimicrobial, and diuretic effects (Wojnicz et al., 2021). *Solidago virgaurea* showed antioxidant capacity and DPPH radical scavenging activity (IC_{50} = 74.66 µg/mL) and exhibited antimicrobial action against *Staphylococcus aureus, E. faecalis, E. coli,* and *B. cereus* at the level of MIC = 50 µg/mL (Ferrante et al., 2020). In another anticancer study, the part isolated with Sephadex G-100 showed an in vitro cytotoxic effect in various tumor cell lines such as PC3 (prostate), MDA435 (breast), C8161 (melanoma) and H520 (small cell lung cancer); SCID at a dose of 5 mg/kg suppressed tumour growth in the mouse model and no apparent toxicity was noted (Gross et al., 2002). . The limitation of studies on *SVSV* plant and the newness of the method used in the analysis make the study unique. The limited studies on *SVSV* and the newness of the method used in the analysis make the study original. The increase in sensor studies offers more sensitive, fast, and reliable methods for evaluating the antioxidant activity of plant extracts.

In this study, antioxidant activity and total phenolic compounds were determined by potentiometric sensors, and the quantitative analysis of phenolic compounds was evaluated by LC-MS/MS. Thus, the aim is to create a scientific basis for the potential use of the plant in food and pharmaceutical applications. The antioxidant capacities of *SVSV* extracts were determined using a voltage-based, color-change-unaffected potentiometric sensor developed as an alternative to methods commonly employed in literature. This sensor offers a wide range of measurements, eliminating the need for many chemicals typically used in tests.

Figure 1. *Solidago virgaurea* plant

The potentiometric sensor tests were applied in DPPH (2,2-diphenyl-1-picrylhydrazyl radical) scavenging, FRAP (Ferric Reducing Antioxidant Power), and FCR (Folin-Ciocalteu Reagent) test in addition, the phenolic compound profile of the extract was revealed by LC-MS analysis. The findings obtained in this context provide essential data to evaluate the potential of the plant as a functional food component.

Materials and Methods

Plant Material and Extract Preparation

Solidago virgaurea L. subsp. virgaurea was collected from Artvin province and was identified by Ozgur Eminagaoglu, Ph.D. A specimen was deposited at the Herbarium (No: 7755). The plant was dried in the shade, ground into powder, and extracted with methanol for 48 hours. The extract solution was subjected to a rotary evaporator to yield the dry extract.

Chemicals

The reagents and solvents used in this study were Folin-Ciocalteu reagent (FCR), DPPH (1,1-diphenyl-2-picrylhydrasyl), BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), Trolox, ascorbic acid (vitamin C) and gallic acid acetonitrile (ACN) (≥% 99.9%), methanol (≥% 99.9%), ethanol (≥% 99.9%,. All chemicals are sourced from Merck (St. Louis, USA).

Antioxidant Activity Analysis

Evaluation of Antioxidant Biosensors

The antioxidant potential was analyzed using the potentiometric sensor designed as an alternative to commonly applied spectrophotometric techniques (Erenler et al., 2024).. The evaluation covered three widely used assays: DPPH radical scavenging, Folin–Ciocalteu reagent (FCR) total phenolic determination, and the Ferric Reducing Antioxidant Power (FRAP) test. In contrast to conventional UV–Vis spectrometry, this approach relies on the detection of potential differences. The method can be employed for a wide range of sample types—including transparent, turbid, or colored matrices—without the limitations of optical measurements. For this purpose, three separate electrodes were specifically developed and applied, each corresponding to one of the assays.

DPPH• Scavenging Activity

Determination of DPPH• scavenging capacity (DPPH-RSA) was carried out by monitoring the amount of unreacted DPPH• remaining after its interaction with antioxidants in the extract. In the assay, 10 mL of plant extract (128 ppm) was mixed with 10 mL of DPPH• solution (100 µg mL^{-1}). The mixture was kept for 30 minutes to ensure complete reaction, and the potential values were recorded by immersing the DPPH-SPMB sensor directly in the sample. The radical scavenging capacity was expressed as a percentage based on the following formula, with results summarized in Table 2...

$$\%RSA = \frac{[(E1-E0)-(E2-E0)]}{E1-E0} x100 \quad (1)$$

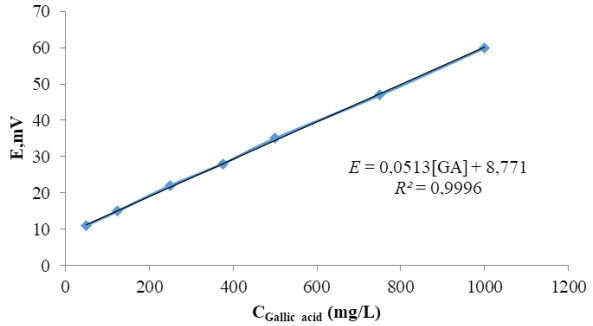

Figure 2. FCR – GA calibration graph

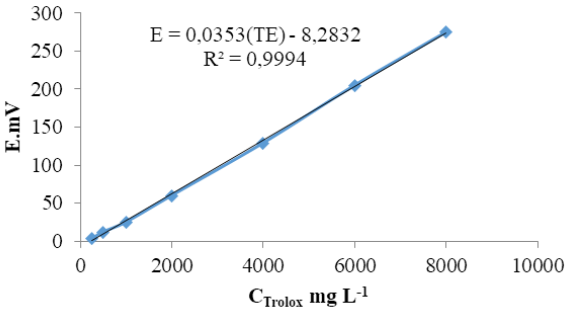

Figure 3. Calibration graph of FRAP-TE quantity

Table 1 LC-MS/MS Gradient program

Time	A (Water)	B (Acetonitrile)
5.00 Min	75.0 %	25.0 %
15.00 Min	25.0 %	75.0 %
16.00 Min	0.0 %	100.0 %
20.00 Min	0.0 %	100.0 %
22.00 Min	85.0 %	15.0 %
40.00 Min	85.0 %	15.0 %

Here, E_0 refers to the potential of the plant extract, E_1 to the potential of the standard DPPH$^{\bullet}$ solution, and E_2 to the potential value measured after the 30-minute reaction period (Isildak et al., 2022).

Total Phenolic Compound

The evaluation of total phenolic content in the extracts was achieved using FCR-selective PVC membrane biosensors (FCR-SPMB) (Işıldak & Yıldız 2024). This approach estimates antioxidant capacity by measuring phenolic compounds, which are expressed as gallic acid equivalents (GAE). A calibration curve of gallic acid with FCR was generated to calculate the equivalents corresponding to the amount of FCR reduced by antioxidant molecules present in the extracts.

SVSV extracts were prepared at 0.5 mg mL^{-1} in 20 mL tubes. For the analysis, 2.0 mL of extract was diluted with 16.0 mL of deionized water and combined with 2.0 mL of 0.5 mmol L^{-1} FCR reagent, followed by vortex mixing. After 30 minutes, the samples were analyzed potentiometrically. The total phenolic content was determined using the calibration equation (E = 0.0513 [GA] + 8.771, R^2 = 0.9996), and expressed as gallic acid equivalents (FCR-GAE). The calculated values are presented in Table 2.

The results were calculated as the total phenolic amount in the plant extract, expressed as mg/g extract. FCR-GAE test results obtained by potentiometric methods are given comparatively in Table 2.

FRAP Ferric (III) Reducing Antioxidant Power Test

The ferric ion (Fe^{3+}) reducing activity of the samples was measured with FRAP-selective PVC membrane biosensors (FRAP-SPMB) (Isildak et al., 2023). Antioxidant compounds present in the extracts reduced Fe^{3+} to Fe^{2+}, and the remaining Fe^{3+} concentration was measured to assess ferric reducing power. A calibration plot was established using Trolox as a standard, allowing the Fe^{3+} reducing power of plant extracts to be quantified potentiometrically. For the test, extracts were prepared at a concentration of 12.8 mg mL^{-1} in 20 mL volumes. From each, 2.0 mL was combined with 18.0 mL deionized water and 2.0 mL of 8.0 mg mL^{-1} Fe^{3+} solution. After vortexing, the mixtures were incubated in the dark for 30 minutes before potential readings were recorded. The reducing power was expressed as Trolox equivalents using the regression line (E = 0.0353 [TE] − 8.2832, R^2 = 0.9994; Figure 3). Results are listed in Table 2.

LC-MS Analysis

LC-MS/MS analyzes were performed with an Agilent (6460 Triple Quad) device. The column in the device was a 50 mm × 4.6 mm, 2.7 µm Poroshell 120 EC-C18. 120 g of the sample to be analyzed was taken and placed in a 2 mL Eppendorf and placed in the device. 1 mL of a mixture of equal proportions of acetonitrile, alcohol, and water was added and mixed. Vortex and sonication were performed to form a homogeneous mixture. 0.8 mL of hexane was added to the resulting homogeneous mixture and mixed. Then, centrifugation at 7000 rpm for 5 minutes resulted in a solid phase. The solid phase formed after centrifugation was filtered (0.25 µm) and placed in the device. The appropriate program was selected from the device. The selected program was set to a flow rate of 0.400 mL/min, an injection volume of 4.00 µL, a column temperature of 30 °C, and an analysis time of 40 minutes. Phase A contained 5 mM ammonium formate and 1% formic acid. Phase B consisted of 0.1% formic acid (Yaman et al., 2022).

Results

Antioxidant Activity Results

The antioxidant activity of *SVSV* extract was determined using FRAP, FCR, and DPPH methods. The results obtained were compared with standard antioxidants (BHT, BHA, Trolox, ascorbic acid and gallic acid). The measurement results are given in Table 2.

Antioxidant Activity

In the DPPH assay, *SVSV* extract (78.8%) exhibited a much higher activity than Trolox (65.25%), BHT (68.25%), and BHA (49.06%). These results demonstrate that *SVSV* extract possesses a remarkably strong antioxidant capacity. Especially in the DPPH test, *SVSV* demonstrated radical scavenging activity at a similar level to gallic acid, indicating that the plant has a structure rich in phenolic compounds. Hence, *SVSV* has the potential to be used in the functional food and pharmaceutical fields.

In the FRAP (Ferric Reduction Antioxidant Power) test, the value of *SVSV* (19.07 µmol TE/mg extract) is higher than BHT (16.81 µmol TE/mg extract), but about the same level as BHA (19.22 µmol TE/mg extract) and lower than Trolox (20.73 µmol TE/mg extract), vitamin C (24.23 µmol TE/mg extract) and Gallic acid (27.80 µmol TE/mg extract). In the FCR (Folin-Ciocalteu Reagent) test, *Solidago virgaurea* (413.82 mg GA/mg extract) exhibits a value close to Trolox (404.33 mg GA/mg extract), but is stronger than standards such as BHT (289.77 mg GA/mg extract) and BHA (279.26 mg GA/mg extract); however, it lags behind vitamin C (492.88 mg GA/mg extract) and especially Gallic acid (796.73 mg GA/mg extract), which has the highest value. Overall, RAN-1 exhibited higher antioxidant potential in all three activity tests compared to BHT and BHA from the tested standards.

LC-MS Analysis Results

The phenolic compound profile of *SVSV* extract was determined by LC-MS/MS method. The results obtained are given in Table 2.

Table 2. Antioxidant activity results of *SVSV* extract and standards

ppm		FRAP*	DPPH**	FCR***
128	*SVSV*	19.07+0.79	78.80+0.45	413.82+0.44
10	BHT	16.81+1.07	68.25+0.25	289.77+1.20
10	BHA	19.22+0.85	49.06+0.62	279.26+0.90
10	Trolox	20.73+0.87	65.25+0.80	404.33+0.80
10	Vitamine C	24.23+0.89	89.250.44	492.88+0.47
5	Gallic Acid	27.80+0.86	78.55+0.85	796.73+0.84

*µmol TE/mg extract, **% RSA, ***mg GA/mg extract

Table 3. LC-MS/MS phenolic compound analysis of *Solidago virgaurea* extract (µg/g extract)

Compounds	Rt (min)	Concentration (mg/gr extract)
Chlorogenic Acid	2.38	58.83
Gentisic Acid	2.66	11.03
Caffeic Acid	3.35	0.12
Rutin	3.71	11.36
P-Coumaric Acid	4.87	1.21
Vanillin	5.29	26.17
Salicylic Acid	7.66	9.52
Trans-Cinnamic Acid	11.03	126.19
Morin	10.43	2.25
Quercetin	10.43	2.47

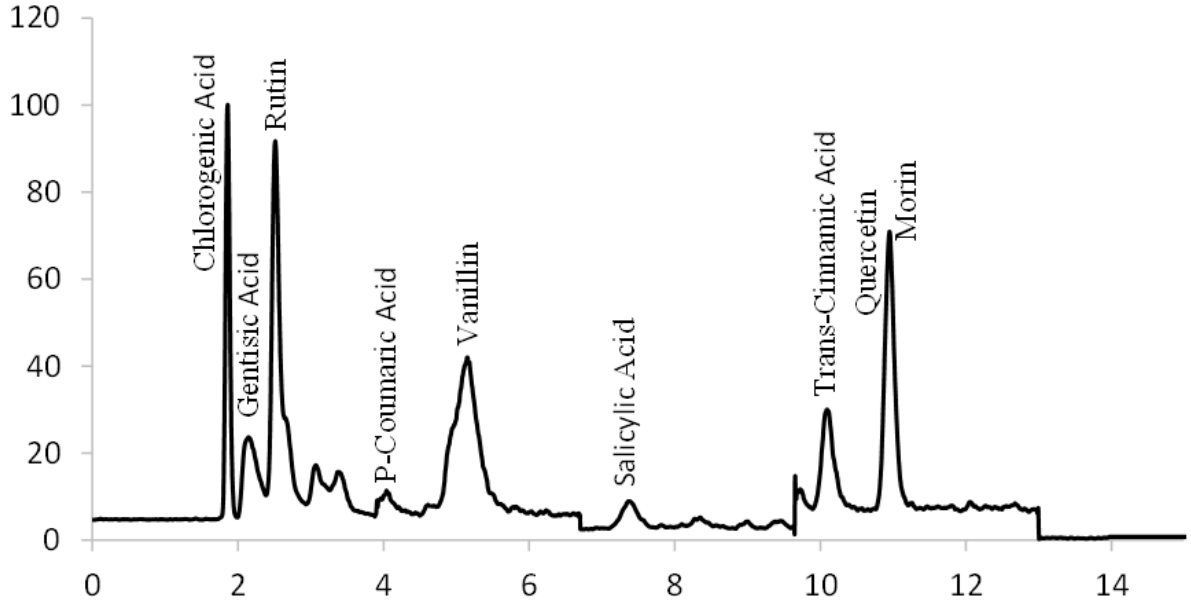

Figure 4. *SVSV* methanolic extract LC-MS/MS chromatogram

According to the results, the most concentrated compound in the extract was trans-cinnamic acid (126.19 µg/g). This was followed by chlorogenic acid (58.83 µg/g) and vanillin (26.17 µg/g), respectively. In addition, rutin, gentisic acid, and salicylic acid were also found in significant amounts in the extract.

Among the flavonoids, quercetin (2.47 µg/g) and morin (2.25 µg/g) were particularly detected. These compounds have been widely reported in the literature to have antioxidant, anti-inflammatory, and anticancer properties. Low amounts of caffeic acid and p-coumaric acid have also been detected. In LC-MS/MS analysis, it was observed that certain phenolic compounds, including gallic acid, epigallocatechin, catechin, luteolin, kaempferol, and resveratrol, were undetectable in the extract. This suggests a chemical difference specific to the phenolic profile of *Solidago virgaurea*.

Discussion

In this study, the antioxidant activities of *SVSV* extracts were determined by two different methods (FRAP, DPPH), and total phenol content was indicated by FCR. These analyses were executed using the potentiometric sensors. Moreover, the phenolic compound profile was revealed by LC-MS analysis. The findings suggest that the plant has a high antioxidant capacity and offers chemical content rich in phenolic compounds. The antioxidant activity was attributed to the high content of bioactive compounds in the plant extract. Moreover, the synergic effect of these compounds may cause to the antioxidant effect.

It is known that the FRAP and DPPH assays used in antioxidant capacity assessment are based on different mechanisms. FRAP measures the reducing power, and DPPH measures the free radical scavenging capacity. Therefore, the fact that the plant extract gives similarly high values in different methods indicates a versatile antioxidant potential. It was found to be superior to synthetic antioxidants such as BHT and BHA with an extract value of 19.07 µmol TE/mg in FRAP analysis, and gave similar results to Trolox. With 413.82 mg of GA/mg extract in terms of total phenolic content, it is understood that the plant is rich in phenolic compounds. Notably, it demonstrated 78.8% activity in the DPPH test, indicating that it has a free radical scavenging capacity almost identical to gallic acid.

These findings are in line with previous studies in the literature on the antioxidant properties of *Solidago* species. For example, (likanov et al.2021) *SVSV* extract has high phenolic content and antioxidant capacity. The data obtained in our study support these results and confirm the potential of the plant as a functional food or phototherapeutic agent.

LC-MS analysis revealed the diversity of phenolic compounds in *Solidago virgaurea* extract. The highest concentration was trans-cinnamic acid. Cinnamic acid and its derivatives are reported to possess antimicrobial, anti-inflammatory, and antioxidant properties. Therefore, it can be considered that cinnamic acid is one of the primary culprits for the extract's high antioxidant power. In addition, phenolic compounds such as chlorogenic acid (58.83 µg/g) and vanillin (26.17 µg/g) were also found in high amounts in the extract. Chlorogenic acid is a powerful antioxidant commonly found in coffee and certain vegetables, and is known for its lipid peroxidation-suppressing effects. Vanillin, on the other hand, is widely used as a flavoring agent in foods, and it also attracts attention due to its free radical scavenging effect. Rutin (11.36 µg/g) and quercetin (2.47 µg/g) are significant flavonoids that reveal considerable biological properties. Quercetin has anti-inflammatory, antihypertensive, and anticancer properties, as well as potent antioxidant effects.

The presence of morin (2.25 µg/g) also enhances the extract's antioxidant profile. The presence of these flavonoids may have contributed to the extract's high DPPH activity values. In the literature, studies on *SVSV* also report similar phenolic compounds. In *Solidago* species, rutin has been identified as a derivative of chlorogenic acid and quercetin. Cinnamic acid derivatives are among the dominant phenolics in *Solidago* plants. The results obtained in our study are in agreement with these findings, confirming that the extract contains characteristic phenolic compounds.

Conclusion

The phytochemistry with antioxidant activity of *SVSV* was presented. The bioactive compounds, including trans-cinnamic acid, chlorogenic acid, vanillin, rutin, gentisic acid, and quercetin, were determined as major natural compounds in this plant. Hence, *SVSV* could be a valuable source of corresponding bioactive compounds for purification. Since this plant displayed considerable antioxidant effects, it can be utilized as a natural antioxidant agent. Furthermore, this study may inspire new research, such as in vivo studies, investigations of other biological effects, and isolation of bioactive compounds.

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