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Research Article

Polyphenolic composition and Antioxidant Effect of Aerial Parts and Roots Extracts from *Scorzonera veratrifolia*

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Abstract: Antioxidant activities of the different extracts (*n*-heptane, chloroform, methanol) from the roots and aerial parts of *Scorzonera veratrifolia* by maceration method, as well as total phenolic and flavonoid content were examined first time in this study. The findings revealed that the methanol extract from *S. veratrifolia* aerial parts exhibited greater DPPH radical scavenging (IC_{50}: 0.62±0.60 mg/mL) and iron (III) reduction capacity (1.56±0.03 mM Fe^{2+}/mg extract). Furthermore, aerial parts methanol extract has the highest concentration of total phenolic (46.3±1.1 mgGAE/g extract) and flavonoid (0.013±0.002 mg QE/mg extract) compounds. Based on these findings, the main phenolic content of aerial parts methanol extract was analyzed by LC-ESI-QTOF/MS, as this extract was found to contain the strongest antioxidant as well as the highest amount of phenolics and flavonoids as compared to the others. Quinic acid, chlorogenic acid, rutin, liquiritin, quercetin hexoside, luteolin-7-*O*-rutinoside, and di-*O*-caffeoylquinic acid compounds were identified as major compounds in methanol extract. The findings showed that aerial parts of *S. veratrifolia*, rather than its roots, could be used as a source of antioxidants.

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1. INTRODUCTION

The accumulation of reactive species formed under oxidative stress conditions defined as the disruption of antioxidant and pro-oxidant balance in the organism causes irreparable damage to biological macromolecules in living cells. As a result, oxidative stress causes various diseases such as cancer, coronary heart disease, diabetes, hypertension, cellular deterioration, mutations, and immune system disorders (Chedea *et al*., 2010). Internal enzymatic defenses against oxidative damage are not entirely effective, and a series of internal and external free radical-scavenging antioxidants act as the second defense system. Antioxidants are compounds that prevent or delay the oxidation of that compound at a lower concentration than that of the oxidizable compound (Akyüz *et al*., 2013). Antioxidants are either a group of enzymes that

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strengthen the antioxidant capacity of the cell, or molecules that inactivate free radicals or prevent chemical reactions initiated by free radicals by participating in physiological, biochemical, or cellular processes (Halliwell, 2008; Papetti *et al*., 2006). Phenolic compounds are secondary metabolites found in large amounts in plants. The antioxidant effects of herbal products are due to the phenolic compounds in their ingredients (Turumtay *et al*., 2014). Phenolic compounds exert antioxidant effects by destroying free radicals and chelating with metal ions that can form lipid peroxidation (Huyut *et al*., 2017).

Scorzonera species (Asteraceae) are found in traditional medicine as analgesic, antirheumatic, anthelmintic, automatic, diuretic and wound-healing, hypertension, pulmonary edema, kidney disorders, diabetes, and diarrhea (Acıkara *et al*., 2013; Sarı *et al*., 2009). *Scorzonera* species have been found to contain dihydroisocoumarin, bibenzyl derivatives, flavonoids, lignans, stilbene derivatives, quinic and caffeic acid derivatives, sesquiterpene, sesquiterpene lactones and triterpene compounds (Sarı, 2010). It has been determined by literature search that *Scorzonera* species show antioxidant, analgesic, anti-inflammatory, and wound-healing activities (Tsevegsuren *et al*., 2007; Wang *et al*., 2009). *Scorzonera veratrifolia* Fenzl plant is a perennial herbaceous plant. This plant spreads in Eastern Anatolia and grows on dry rocky slopes. The roots and latex of this plant are used as wound healing. Benzylfithalide, scorzoveratrin, scovoveratroside, chlorogenic acid, chlorogenic acid methyl ester, cryptochlorogenic acid, 4,5-dicaffeoylquinic acid and 3,5-dicaffeoylquinic acid were isolated from only the root of *Scorzonera veratrifolia*, and their antimicrobial activity was examined (Sarı *et al*., 2009; Sarı, 2010).

No studies on the antioxidant activity of *Scorzonera veratrifolia* (*S. veratrifolia*) and the analysis of phenolic compounds have been found in the literature searches. The first aim of this study was to examine the antioxidant activities of *n*-heptane, chloroform and methanol extracts obtained from aerial parts and roots of this plant. The last aim of our study is to analyze the phenolic contents of the methanol extract from the above-ground parts of the plant, which shows the strongest activity with LC-MS/MS system.

2. MATERIAL and METHODS

2.1. Identification and Collection of Plant Samples

The aerial parts and roots of *S. veratrifolia* were collected from Tunceli in Turkey by Dr. Ahmet Dogan. The taxonomic description of the plant samples was made by Dr. Ahmet Dogan from Marmara University, Pharmacy Faculty (MARE:13917).

2.2. Preparation of Different Extracts and Extract Yield

The plant samples were dried at room temperature. Using the maceration procedure with *n*-heptane (4x1000 mL), chloroform (3x700 mL) and methanol (4x1000 mL) solvents, extracts were produced from the aerial parts of the plant (200 g). Also, extracts were obtained from the roots of the plant (50 g) using *n*-heptane (4x300 mL), chloroform (3x400 mL) and methanol (4x500 mL) solvents, respectively. The solvents were then filtered via filter paper and evaporated under low pressure in a rotary evaporator, with the raw extracts preserved in the refrigerator. Table 1 shows the yield percentages and extract quantities of the various extracts from the plant. The aerial components of the methanol extract (10.12 g) were found to be more abundant than the other extracts. Furthermore, when the yield percentages of the extracts were compared, it was discovered that the methanol extract (11.96%) made from the plant roots had the highest yield percentage.

Table 1. Amount and percentage yield (%) of *S. veratrifolia* in different extracts.

Extracts	Amount (g)	Yield (%)
Aerial parts *n*-heptane	7.55	3.78
Aerial parts chloroform	3.64	1.82
Aerial parts methanol	10.12	5.06
Root methanol	5.98	11.96
Root chloroform	2.45	2.05
Root heptane	1.12	1.02

2.3. Determination of Total Amount of Phenolic Substances

Distilled water was added to the extracts prepared at concentrations of 1-5 mg/mL and taken into tubes of 0.1 mL each, and their volume was 4.6 mL. 0.1 ml Folin-Ciocalteu reagent and 0.3 mL of 2% sodium carbonate solution added to the absorbance of the color that occurs after being kept in a shaking water bath for 2 hours under room conditions. It was measured at 760 nm in comparison to a standard (Samatha *et al*., 2012).

Preparation of the calibration curve of gallic acid: total phenolic substance was determined by using Folin-Ciocalteu reagent to the gallic acid solutions prepared at concentrations of 0.05-0.40 mg/mL. The calibration curve was prepared by plotting the concentrations against the measured absorbances and the correct equation was obtained [(Abs=75.63 [GA]x-0.044 (R^2 = 0.9963)]. From the obtained equation, the total phenolic amounts of the samples were calculated as the equivalent of mg gallic acid (mg GAE/g extract).

2.4. Determination of the Total Amount of Flavonoid Substances

Total flavonoid amounts of plant extracts were determined by aluminum chloride color measurement method equivalent to quercetin (Samatha *et al*., 2012). Briefly, 0.5 mL of each of the different extracts was taken and mixed with distilled water of 2 mL. Then, 0.15 mL of 5.0% (w/v) $NaNO_2$ solution was added, and this mixture was left for 6 minutes. Then, 0.15 mL of 10% $AlCl_3$ solution was added to the mixture, and after 6 minutes of incubation, 2 mL of 4.0% (w/v) NaOH solution was added to the mixture. Finally, the total volume of the mixture was completed with 5 mL of distilled water, and the absorbance values against the reference solution were measured at 510 nm, in which the pink colored flavonoid-aluminum complex in the alkaline medium gave maximum absorbance after 15 minutes.

2.5. DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was used to test the extracts' free radical scavenging activity (Wei *et al*., 2010). 3.9 mL of 0.1 mM DPPH solution was added to 0.1 mL of extracts prepared at known concentration and standard solution (ascorbic acid). After mixing the mixture with vortex, it was left at room temperature and in the dark for 30 minutes. UV-vis spectroscopy was used to determine the activity of the extracts, with at least three duplicate measurements taken at 517 nm. The control was prepared under the same conditions using 0.1 mL methanol instead of sample and standard substance. The absorbance of the control was measured daily. Before the IC_{50} value was calculated, the % DPPH radical scavenging activity was calculated with the formula given below:

$$\text{DPPH radical removal capacity} = [(A_0 - A_1)/A_0] \times 100]$$

A_0: Absorbance of the control solution,

A_1: Plant extracts and absorbance of standard solutions

IC_{50} is the concentration of extract or standard material that causes a 50% reduction in initial DPPH concentration. This value was calculated using the correct equation obtained by placing

the % free radical removal activity values against the studied concentrations and the results were given as IC_{50} = mg/mL.

2.6. Iron (III) Reduction/Antioxidant Power (FRAP) Method

Acetate buffer (pH 3.6) 25 mL of 300 mM, 2.5 mL of TPTZ solution (solution of 10 mM TPTZ in 40 mM HCl), and 2.5 mL of 20 mM $FeCI_3.6H_2O$ were mixed and kept at 37 ºC for 30 minutes. In the fourth minute, an increase in absorbance was detected at 593 nm. against the reference prepared by mixing 3.8 mL of the FRAP reagent with 0.2 mL of extract and by adding distilled water instead of the extract. The absorbance values of the extracts at 593 nm were compared with the values of the calibration plot [Abs=0.8226 [$FeSO_4$]x-0.0338 (R^2=0.9940)] prepared with $FeSO_4.7H_2O$, and the FRAP value was expressed as mM $FeSO_4$/mg extract (Benzie & Strain, 1996).

2.7. LC-ESI-QTOF/MS Analysis

The phenolic compounds of methanol extract from aerial parts of plant were determined by LC-ESI-QTOF/MS technique. An Agilent 6530 was used for the separation and analysis of phytochemicals. The chromatographic separation was performed on reverse phase Agilent Poroshell C18 (3.0 x 150 mm, 2.7 µm) analytical column. The column temperature was set to 30 °C. The separation was carried with a gradient binary mixture of solvent A (0.1% aqueous formic acid) and solvent B (acetonitrile) at a flow rate of 0.4 mL/min:0-2 min 10% B; 2-6 min 10-50%B; 6-8 min 50%B; 8-12 min 10-90% B; 12-14 min 10-90% B; 14-14.01 min 90-10% B and stop time is 20.00 min. The electrospray ionization mass spectrometer (ESI/MSn) in negative ion mode was used to create the MS spectra of the most abundant ion. Helium was employed as the collision gas. Nitrogen was employed as a nebulizing gas, a 10-arbitrary-unit auxiliary gas flow, and a 35-arbitrary-unit sheath gas flow. The spray voltage was set to 5.00 kV, while the capillary temperature and voltage were set to 300 degrees Celsius and 35.00 volts, respectively. 10 mg of the extract was dissolved in 3 mL of methanol-water solution (2:1 v/v). The filtrate was then filtered into the vial to 1.5 mL using a filter and syringe and 10 µL sample was injected to LC.

2.8. Statistical Analysis

All the results were performed in triplicate and illustrated in terms of mean±SD. One-way analysis of variance was performed following ANOVA procedures, and significant differences between means were determined by Tukey Multiple Comparison test. $p< 0.05$ was considered statistically significant.

3. RESULTS / FINDINGS

3.1. Determination of Total Flavonoid and Phenolic Content

Total phenolic and flavonoid amounts of different extracts of plant were determined in this study. According to the results, 46.3±1.1 gallic acid equivalents per gram extract was detected as the number of total phenolics of the methanol extract from plant's aerial parts. Besides, it was determined that the methanol extract (0.013±0.020 quercetin equivalents per milligram extract) from plants aerial parts had the highest amount of flavonoid compounds. In this study, the phenolic compounds content of the aerial parts and root extracts of the plant was compared, and it was found that all extracts from the aerial parts contained a higher amount of phenolic and flavonoid compounds than the root extracts (Table 2).

The total flavonoid and phenolic contents of the different extracts (water, ethyl acetate, chloroform, ether, *n*-butanol) of the aerial parts and roots of the *S. paradoxa* was determined. According to the results, it was determined that the *n*-butanol extracts from the aerial parts contain higher amounts of phenolic (11.86±0.10 mg tannic acid equivalents/g extract) and

flavonoid (6.42±0.04 mg rutin equivalents/g extract) compounds than the other extracts (Nasseri et al., 2015). In our study, it was observed that methanol extract of *S. veratrifolia* aerial parts contains higher amounts of phenolic (46.3±1.1gallic acid equivalents per gram extract) and flavonoid (0.013±0.020 quercetin equivalents per milligram extract) compounds than other extracts.

Table 2. Total phenolic and flavonoid contents of different extracts from various parts of *S. veratrifolia*.

Extracts	Total phenolic (mgGAE/g extract)	Total flavonoid (mg QE/mg extract)
Aerial parts methanol	46.3 ± 1.1[a]	0.013±0.002[a]
Aerial parts chloroform	16.5 ±1.5[b]	0.0055±0.011[b]
Aerial parts *n*-heptane	8.1±2.3[c]	0.002±0.003[c]
Root methanol	10.0 ±1.6[d]	0.004±0.011[d]
Root chloroform	4.1±2.5[e]	0.001±0.025[e]
Root *n*-heptane	1.2 ±1.9[f]	0.001±0.032[f]

The mean of three independent determinations (n=3) is used to calculate the standard deviation. GAE–Gallic acid equivalents; QE-Quercetin equivalents. a-f Means in a row without a common superscript letter differ ($p < 0.05$), as analyzed by one-way ANOVA.

3.2. Scavenging Activity of DPPH Radical Assay

The DPPH test is a widely used spectrophotometric method to determine the antioxidant capacity of plants or foods (Bursal *et al*., 2020). The IC_{50} values of the extracts and standards for DPPH radical scavenging were found as; ascorbic acid (0.004±0.003)> aerial parts methanol (0.62±0.60)>roots chloroform (1.14±0.01)>roots heptane (1.37±0.21)>roots methanol (2.76±0.28)>aerial parts chloroform (2.82±0.04)>aerial parts *n*-heptane (4.15±0.02). According to the findings, the methanol extract of aerial parts had much higher DPPH radical scavenging activity than the other extracts. (Table 3).

3.3. Ferric Ions (Fe^{3+}) Reduction Abilities of Different Extracts

The reduction potentials of different extracts obtained from the plants aerial parts and roots were determined by reduction systems including FRAP capability. Fe^{3+} reducing powers of the extracts and standards were decreased as; BHT (1.2±0.21)>aerial parts methanol (1.56±0.03)>roots methanol (1.02±0.05)>aerial parts chloroform (0.980±0.002)>roots chloroform (0.97±0.47)> roots *n*-heptane (0.82±0.51)> aerial parts *n*-heptane (0.22±0.06). In this study, it was found that methanol extracts from the aerial parts and roots of the plant had potent iron reduction capacity from other extracts, while they had a lower capacity than the standard compound (Table 3).

Table 3. Free radical scavenging activity and FRAP of different extracts from various parts of *S. veratrifolia.*

Extracts/standards	DPPH· (IC_{50}: mg/mL)	FRAP assay (mM Fe^{2+}/mg extract)
Aerial parts methanol	0.62±0.60[a]	1.56± 0.03[a]
Aerial parts chloroform	2.82±0.04[b]	0.98± 0.002[b]
Aerial parts *n*-heptane	4.15±0.02[c]	0.22± 0.06[c]
Root methanol	2.76±0.28[d]	1.02± 0.05[d]
Root chloroform	1.14±0.01[e]	0.97± 0.47[e]
Root *n*-heptane	1.37±0.21[f]	0.82± 0.51[f]
Ascorbic acid	0.004±0.003[g]	
BHT		1.2± 0.21[g]

Values are mean of triplicate determination (n=3) ± standard deviation; a-g Means in a row without a common superscript letter differ ($p< 0.05$), as analyzed by one-way ANOVA.

3.4. Identification of phytochemical compounds by LC-ESI-QTOF/MS

In the present study, the antioxidant activities of the extracts obtained from different parts of the plant using different solvents, as well as the total phenolic and flavonoid substance amounts were compared among themselves. The methanol extract from the plants aerial parts was shown to have the highest antioxidant activity as well as total phenolic and flavonoid contents. In line with this result, the LC-QTOF/MS device was used to qualitatively analyze the main phenolic molecules in methanol extract that might be responsible for the activity (Figure 1). The presence of quinic acid, chlorogenic acid, rutin, liquiritin, quercetin hexoside, luteolin-7-*O*-rutinoside and di-*O*-caffeoylquinic acid (Table 4) in the methanol extract from aerial parts was determined in this investigation.

Figure 1. MS base peak chromatogram (BPC) of methanol extract from *S. veratrifolia*.

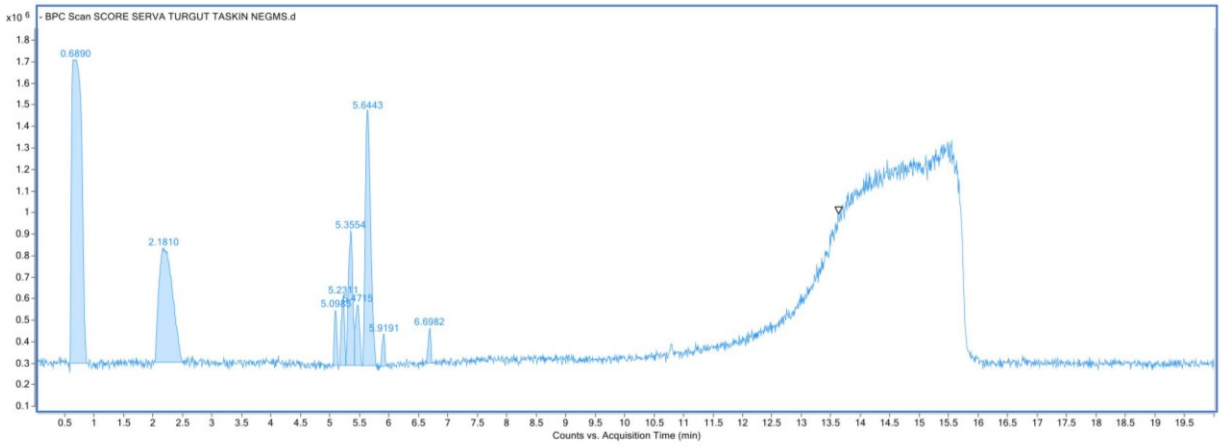

Table 4. Identification of polyphenols in *S. veratrifolia* by LC-ESI-TANDEM MS data

Rt (Min)	$[M-H]^-$	Other MS-MS ions (M/Z)	Identification	Reference
0.7173	191.0569	171,155,127,93,85	quinic acid	*
2.0564	353.0907	191	chlorogenic acid	*
2.1073	707.1843	353,191	chlorogenic acid dimer	*
5.0809	609.1528	300,271,151	rutin	*
5.2578	417.1193	255,169	liquiritin	(Simirgiotis *et al.,* 2015).
5.2829	463.0923	300,271,151,112,69	quercetin hexoside	Hofmann *et al.,* 2016
5.3589	593.1568	285,255,151	luteolin-7-*O*-rutinoside	Simirgiotis *et al.,* 2015
5.4431	515.1266	353, 179,135	di-*O*-caffeoylquinic acid	Schütz *et al.,* 2005

*Compounds identified by comparing retention times and MS data with those of reference

As shown in Supporting Information, quinic acid gave a molecular ion at m/z 191. This compounds MS/MS fragmentation pattern revealed a distinct m/z at 171, 155 with water loss and then 93 fragments of mass with the loss of carbon dioxide. Chlorogenic acid produced deprotonated molecules at m/z 353. The MS/MS fragmentation pattern obtained from chlorogenic acid revealed a characteristic m/z at 191 and 161 by separating the caffeoyl portion and separating the quinic and one molecule of water, respectively. The precursor ion at m/z 707 was identified as chlorogenic acid dimer, and the fragment ion at m/z 353 was a fragment of chlorogenic acid (m/z 354) that lost a hydrogen ion fragment when mass spectrometry was used in negative mode. The fragment of quinine (m/z 192), which lost a hydrogen ion fragment, was another ion at m/z 191. Rutin produced deprotonated molecules at m/z 609 and the sugar portion was separated and gave a fragment of quercetin aglycone at a molecular weight of 300 g/mol. The peak with retention time at 5.26 was liquiritin and gave m/z 255, the molar mass of the

4',7-dihydroxyflavanon (Simirgiotis *et al*., 2015). At m/z 463, quercetin hexoside formed a deprotonated molecule, and a fragment of the aglycon ion peak provided a quercetin monosaccharide at m/z 300.0303 ([M-H-162]$^-$ loss of hexose fragments) (Hofmann *et al*., 2016). There is a parent ion at m/z 593 and fragment ions corresponding to the luteolin aglycon at m/z 285, 255 and 151. This compound has been proven in the relevant literature to be luteolin-7-*O*-rutinoside. (Simirgiotis *et al*., 2015). The peak with retention time at 5.44 was tentatively deduced as di-*O*-caffeoylquinic acid (Schütz *et al*., 2005), which gave fragment ion at m/z 353 M-H-caffeoyl as follows; m/z 173 fragments was formed by the separation of M-H-caffeoyl-quinic molecule ions.

4. DISCUSSION and CONCLUSION

According to the literature research, there are just a few research studies on this species. It was also found that the water extract (3.36±0.28 mg tannic acid equivalents/g extract) from the roots contains high amounts of phenolics, while the *n*-butanol extract (0.15±0.01 mg rutin equivalents/g extract) contains high amounts of flavonoids (Nasseri *et al*., 2015). Unlike this study, the methanol extract of *S. veratrifolia* roots was found to possess greater levels of phenolic (10.00±0.01 gallic acid equivalents per gram extract) and flavonoid (0.004±0.011 quercetin equivalents per milligram extract) compounds than other extracts. In this study, the antioxidant potential of root and aerial parts extracts of the *S. veratrifolia* for the first time was examined in comparison with the standard.

The antioxidant capacities of *Scorzonera* species have been investigated in the studies. It was stated that water extract from the aerial parts of *S. suberosa* (IC_{50}: 42.33±1.60 mg/mL) S. laciniata (IC_{50}: 77.07±1.88) *S. latifolia* (IC_{50}: 29.36±1.46) showed median DPPH radical scavenging activity (Erden *et al*., 2013). In the present study, it was observed that the methanol extract (IC_{50}: 0.62±0.60 mg/mL) from *S. veratrifolia* aerial parts has higher DPPH radical scavenging activity than the water extract from aerial parts of *S. suberosa, S. laciniata* and *S. latifolia*. The DPPH radical scavenging potential of the methanol:water (80:20 v/v) extract from the aerial parts and roots of the *S. latifolia* was determined. It has been determined that the aerial parts (IC_{50}:1.036 mg/mL) have an effective radical scavenging effect compared to the root extract (IC_{50}:4.102 mg/mL) (Açıkara *et al*., 2017). In the present study, the DPPH radical scavenging activity of the aerial parts and root parts of *S. veratrifolia* was examined in parallel with the literature. When the results were examined, it was discovered that methanol extracts from *S. veratrifolia* aerial parts (IC_{50}: 0.62±0.60 mg/mL) and roots (IC_{50}: 2.76±0.28 mg/mL) had higher radical scavenging activity than methanol: water (80:20 v/v) extracts from *S. latifolia* aerial parts and roots.

A previous study found that the methanol extract (10.8%, 50 µg/mL) from the aerial parts of *S. tomentosa* L showed lower radical scavenging activity compared to ascorbic acid (96.78 %, 100 µg/mL) (Karagöz *et al*., 2015). In our present study, it was determined that all extracts of *S. veratrifolia* showed lower activity than ascorbic acid in parallel with the literature.

It was stated that the methanol extract (IC_{50}: 18.81 mg/mL) from the *S. paradoxa* aerial parts showed more effective DPPH radical scavenging activity than the root extract (IC_{50}: 88.9 mg/mL) (Nasseri et al., 2015). According to our findings, it was determined that the methanol extract of *S. veratrifolia* aerial parts (IC_{50}: 0.62±0.60 mg/mL) has more effective DPPH radical scavenging activity than the root (IC_{50}: 2.76±0.28 mg/mL) extract and has a stronger radical scavenging effect than the methanol extracts from *S. paradoxa* aerial parts and roots.

In the literature search, benzylfithalide, scorzoveratrin, scovoveratroside, chlorogenic acid, chlorogenic acid methyl ester, cryptochlorogenic acid, 4,5-dicaffeoylquinic acid and 3,5-dicaffeoylquinic acid compounds were isolated from the root of the *S. veratrifolia*, but no study was found about the analysis of phenolic compounds both in the aerial parts by LC-ESI-

QTOF/MS system (Sarı *et al*., 2009; Sarı, 2010). The phenolic compounds analysed in methanol extract of aerial parts, which possesses strong antioxidant activity, were investigated for the first time in this work. It was analyzed by HPLC system that the methanol: water (80:20 v/v) extract from aerial parts of the *S. latifolia* contained high amounts of chlorogenic acid and hyperoside compounds (Açıkara *et al*., 2017). In our current study, it was determined by HPLC system that the methanol extract from *S. veratrifolia* aerial parts contains chlorogenic acid like *S. latifolia* in parallel with the literature.

This study provided information about antioxidant activity and phytochemical composition of *S. veratrifolia*. The antioxidant activity of different extracts from the root and aerial parts of the plant was examined and the aerial parts methanol extract was determined to have the highest antioxidant activity. Furthermore, when compared to other extracts, the methanol extract contained the highest amount of phenolics and flavonoids. It was analyzed by LC-ESI-QTOF/MS that the methanol extract from aerial parts substantially contained quinic acid, chlorogenic acid, rutin, liquiritin, quercetin hexoside, di-*O*-caffeoylquinic acid, and luteolin-7-*O*-rutinoside compounds. These analysed compounds show antioxidant properties because they contain many hydroxyl groups attached to their aromatic rings. Hence, this extract can be used as a natural medicinal and nutritional source in the future after detailed analysis tests.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the author(s).

Authorship Contribution Statement

Authors are expected to present author contributions statement to their manuscript such as; **Duygu Taskin**: Investigation, Writing-original draft, Supervision. **Mert Gecim**: Investigation. **Ahmet Dogan**: Plant collection **Ayfer Beceren**: Supervision.

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6. APPENDIX

Supporting Information

Figure S1. MS/MS spectra and fragmentation patterns of quinic acid in *S. veratrifolia.*

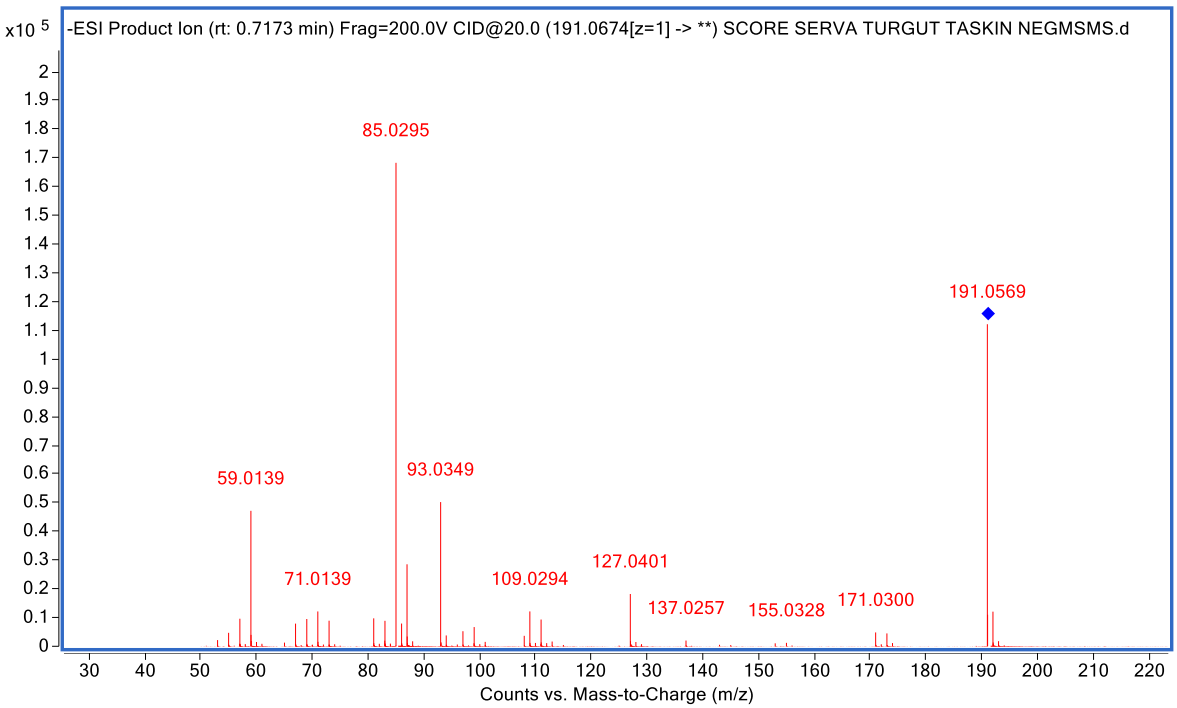

Figure S2. MS/MS spectra and fragmentation patterns of chlorogenic acid in *S. veratrifolia.*

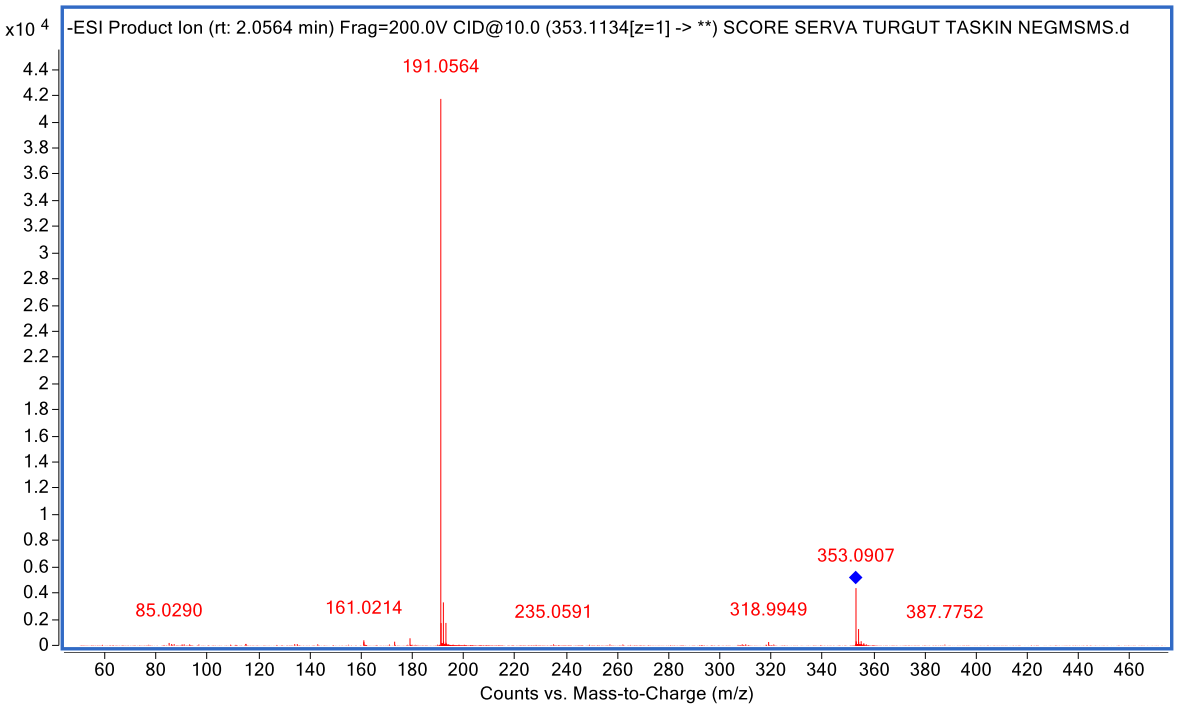

Figure S3. MS/MS spectra and fragmentation patterns of chlorogenic acid dimer in *S. veratrifolia.*

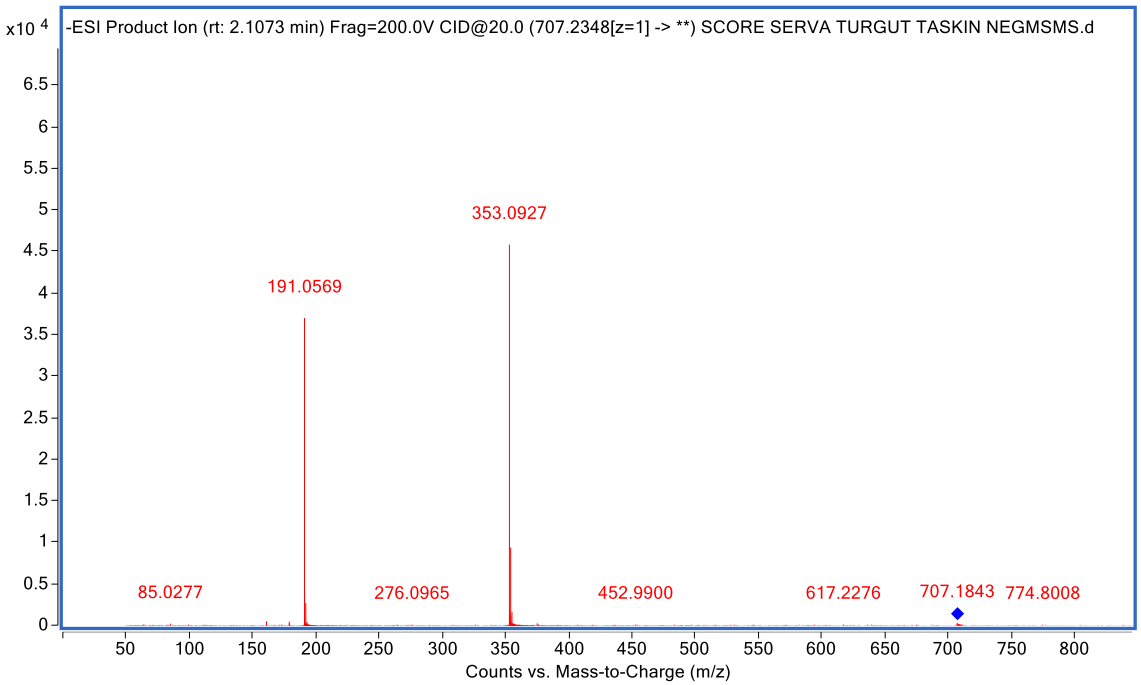

Figure S4. MS/MS spectra and fragmentation patterns of rutin in *S. veratrifolia.*

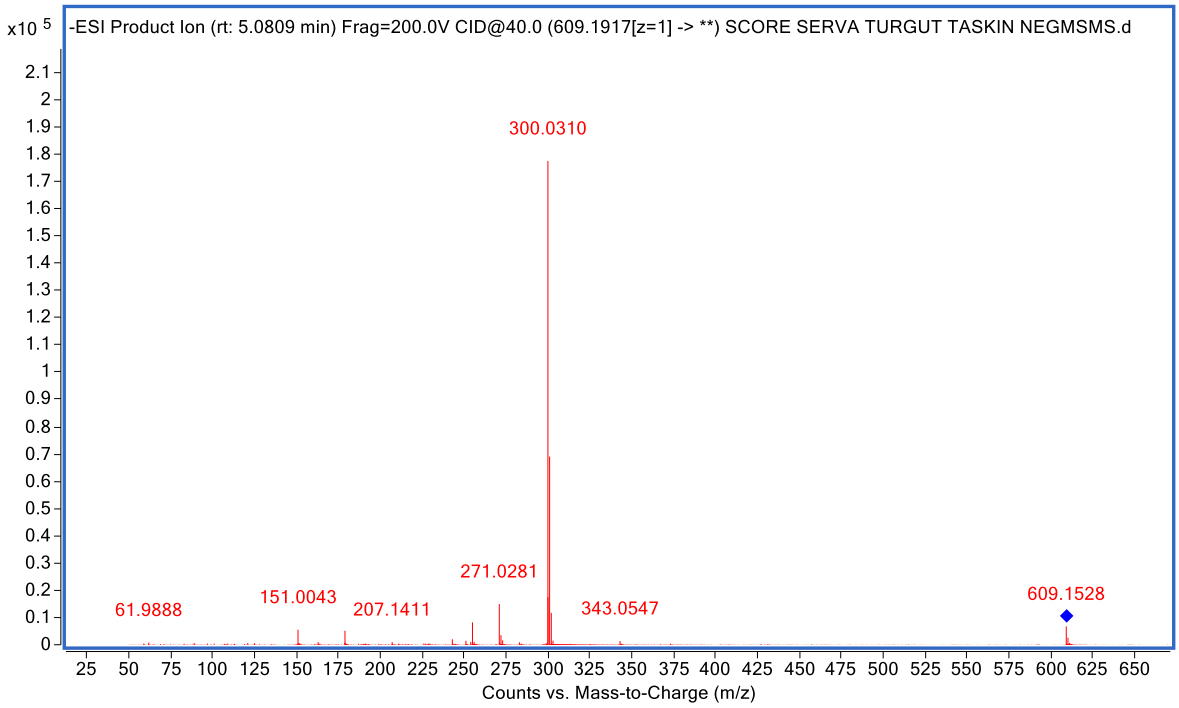

Figure S5. MS/MS spectra and fragmentation patterns of liquiritin in *S. veratrifolia.*

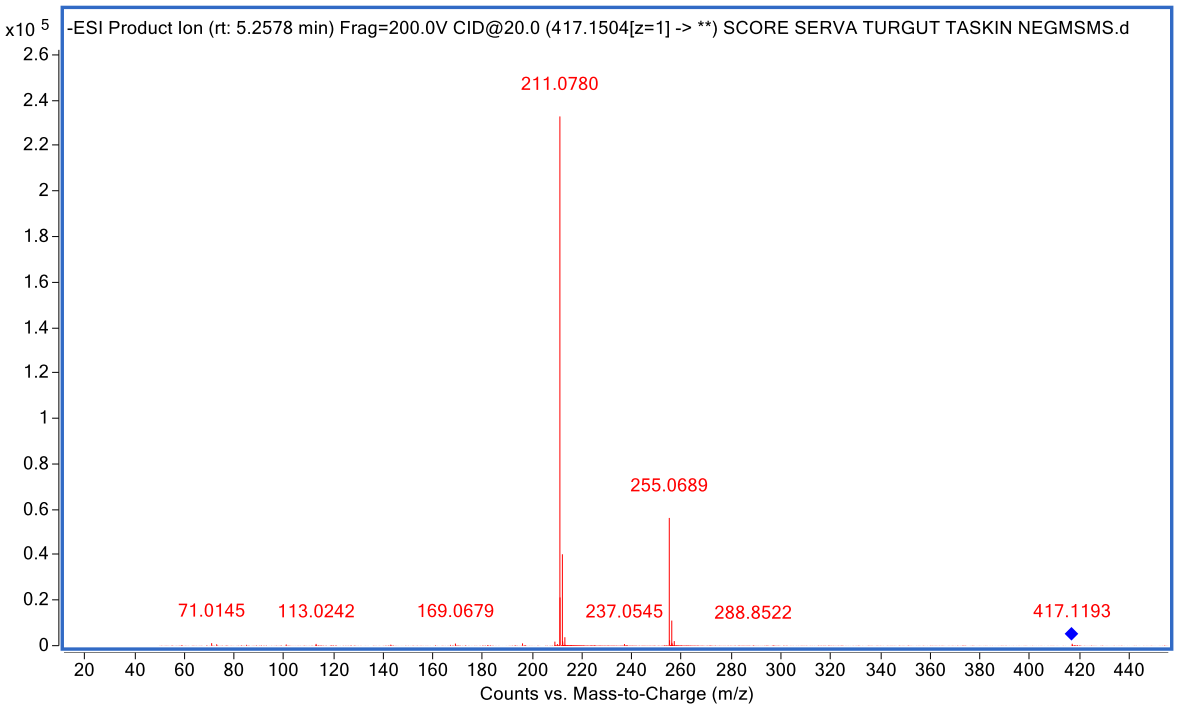

Figure S6. MS/MS spectra and fragmentation patterns of quercetin hexoside in *S. veratrifolia.*

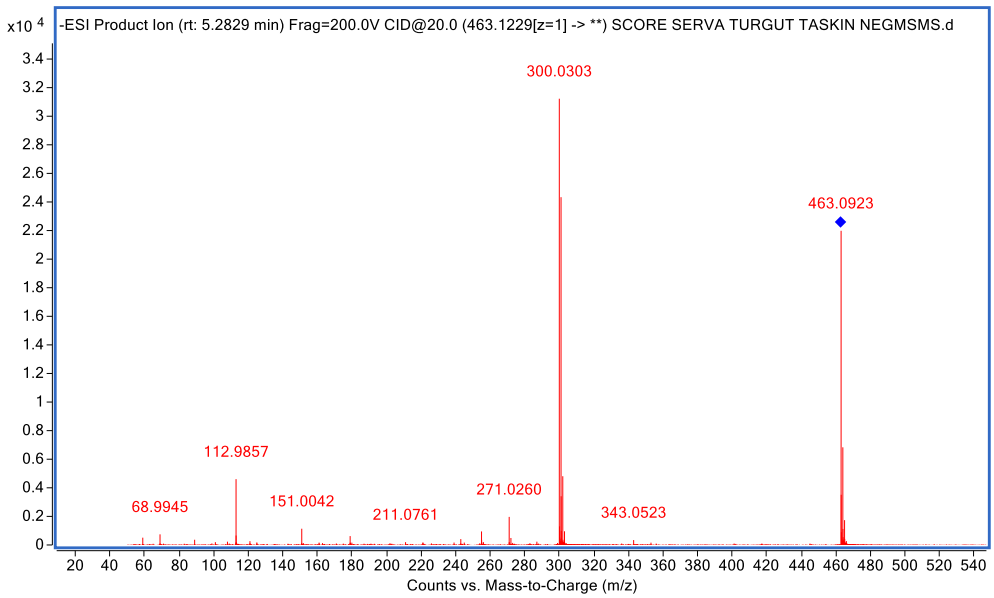

Figure S7. MS/MS spectra and fragmentation patterns of luteolin-7-O-rutinoside in *S. veratrifolia.*

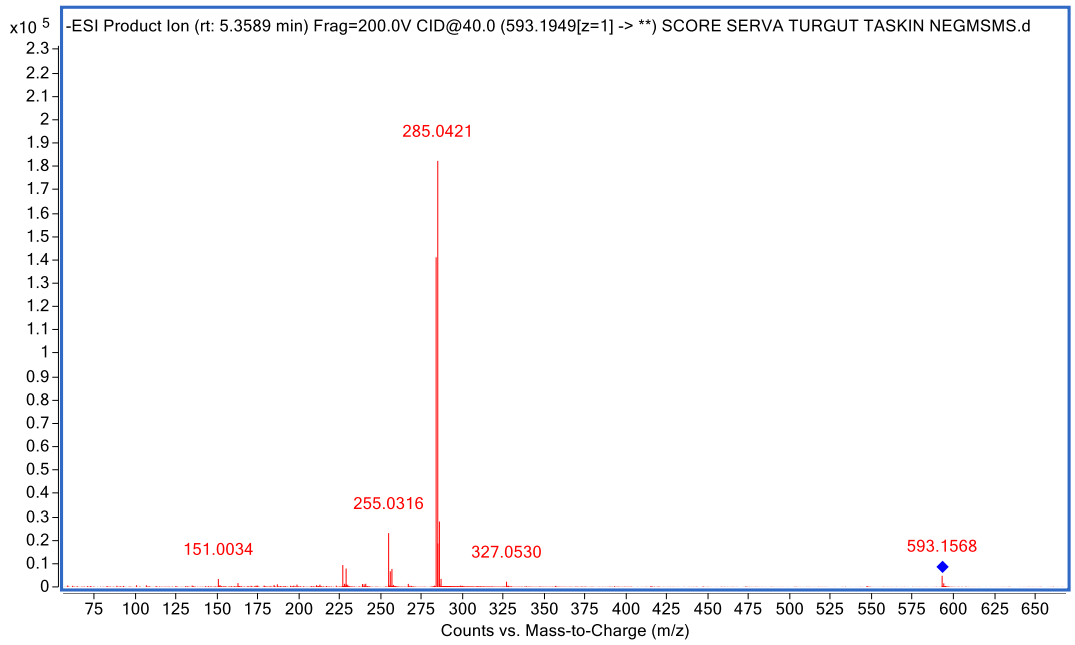

Figure S8. MS/MS spectra and fragmentation patterns of di-O-caffeoylquinic acid in *S. veratrifolia.*

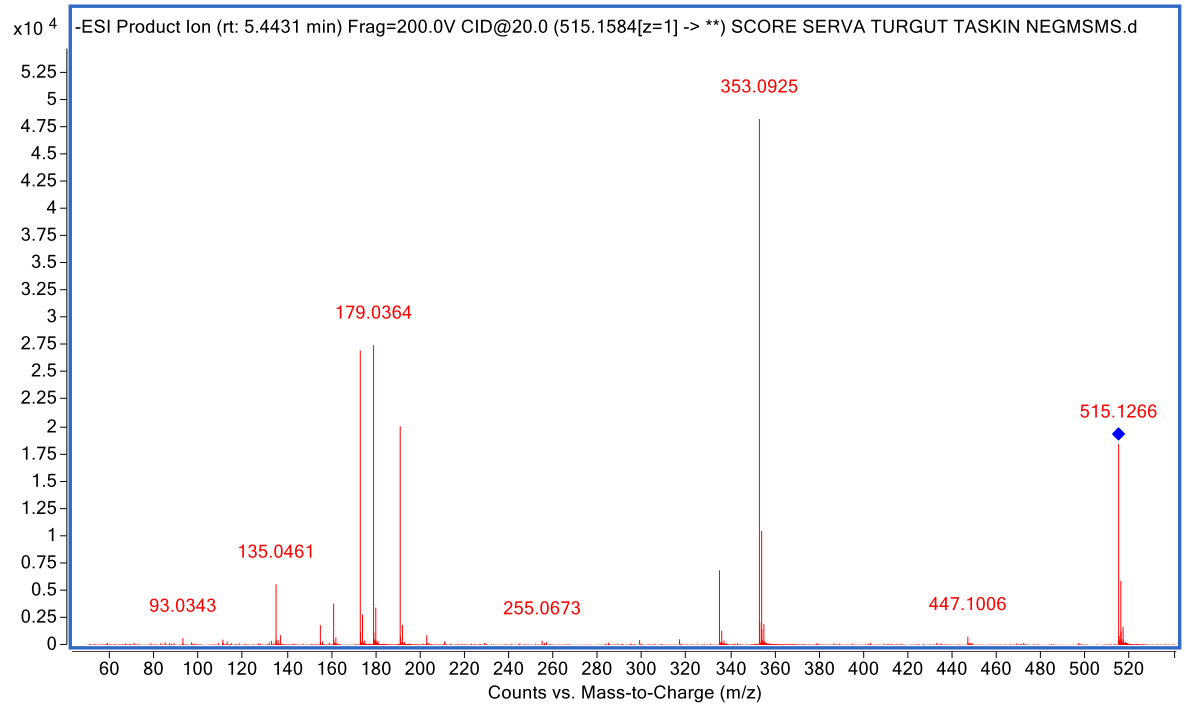

Figure S9. An unknown compounds MS/MS spectra and fragmentation patterns at 5.63 retention time.

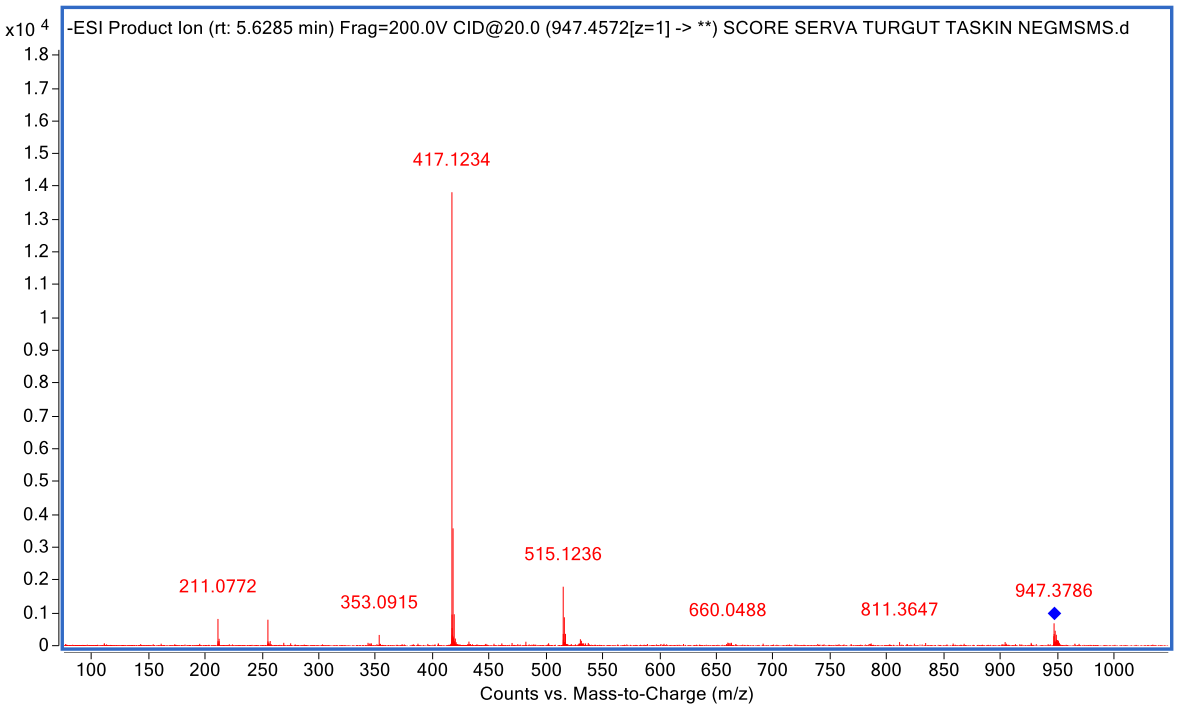

Figure S10. An unknown compounds MS/MS spectra and fragmentation patterns at 5.91 retention time.

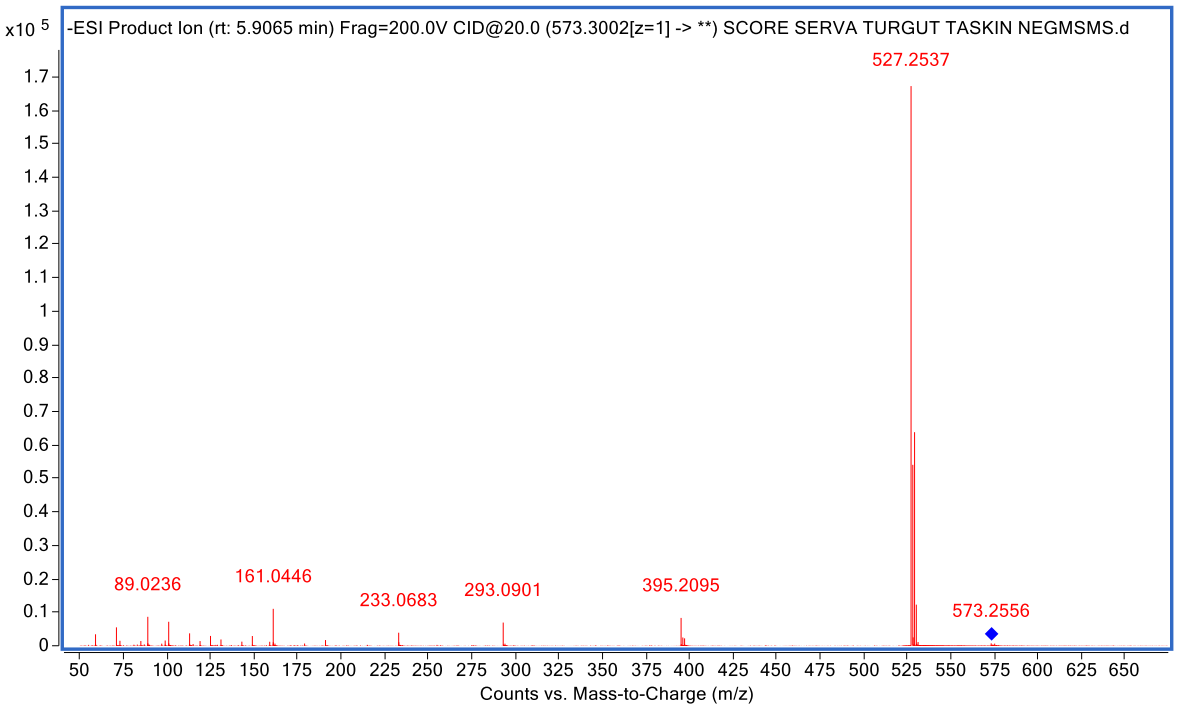

Figure S11. An unknown compounds MS/MS spectra and fragmentation patterns at 6.67 retention time.