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Research Article

PHYTOCHEMICAL PROFILING BY LC-ESI-MS/MS AND DETERMINATION OF ANTIOXIDANT, ANTI-ALZHEIMER AND ANTITYROSINASE ACTIVITIES OF TWO ASPARAGACEAE SPECIES: *Scilla hyacinthoides* L. AND *Scilla ingridiae* SPETA

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Abstract: *In this study, in vitro antioxidant, antialzheimer and antityrosinase activity, total phenolic and flavonoid components, phytochemical profiling, identified by Liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS/MS) analysis and also chemometric analysis, of two different parts of Scilla species (Scilla hyacinthoides L. and Scilla ingridiae Speta) were determined in detailed perspective. The highest contents of total phenolics (TPC) and total flavonoids (TFC) were determined in S1TU (S. hyacinthoides aerial parts) extract (11.72±0.00 µg GAE/mg) and S2TU (S. ingridiae aerial parts) extract (31.53±0.21 µg QE/mg). The highest ABTS and CUPRAC activities were found in S1TU and S2TU extracts. The inhibitory activities of the extracts on the enzyme acetylcholinesterase were investigated. S1TU and S2TU extracts again showed the highest activity. Although the antityrosinase enzyme inhibitory activities of the extracts were generally similar and high, the S2TA extract (S. ingridiae corm parts) showed the highest activity. LC-ESI-MS/MS was used to determine the content of phenolic components of the extracts. Fourteen different bioactive components were determined in the analyzes and their amounts were measured. The data obtained were analyzed chemometrically such as principal component analysis (PCA), hierarchical component analysis (HCA) and their relationships to each other were supported visually and numerically with Pearson correlation graphs, heat maps, etc. The research results have shown that the various components of the plant have a good effect on various biological activities, so that it can be used for various purposes in the future (especially with a good degree of antityrosinase activity), taking into account the results. The biological activities demonstrated here can rationalize the use of the plant in traditional medicine.*

Key words: *Antioxidant activity, enzyme inhibition, multivariate analysis, Pearson correlation, phytochemical profiling*

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1. Introduction

The concept of herbal medicine is defined as "botany or phytotherapy" and refers to the use of biological material containing parts of a plant, such as seeds, fruits, leaves, bulbs, tubers, roots, bark or flowers, for medicinal purposes, where the different parts of a plant are used individually or in combination and sold as various shape (tablets, capsules, powders, teas, extracts, and fresh or dried plants). People use conventional herbal products to maintain or develop their health [1]. Products made from herbal substances that are used to protect or promote health may be referred to as herbal products, or herbal medicines. Herbal treatments can be used as personal care medicine in cases such as sleep, flu and psychological disorders, digestive disorders, flu infections and stress. Traditional herbal medicines are also used to treat irritable bowel syndrome, eczema, premenstrual syndrome,

migraines, menopausal symptoms, rheumatoid arthritis, chronic fatigue syndrome, asthma, and cancer. Herbal complements are best taken under the advice of a trained healthcare provider. A study mentions that 90% of arthritis patients are treated with alternative treatments such as herbal medicines [2]. In the medical world, medicinal plants are now recognized as a complementary treatment method. This shift in opinion has not only increased the use of herbal medicines, but has also contributed to an increase in pharmacological and toxicological research on herbal medicines to prove their efficacy [3].

Throughout their lives, all plants synthesize various chemical compounds as an indispensable part of their normal metabolic activities. Primary metabolites consist of macromolecules such as proteins, fats, sugars and carbohydrates, which are found in all plant organisms. Primary metabolites play an important role in vital functions such as metabolism, reproduction and growth. SM*s* (secondary metabolites) are a group of biosynthetically produced primary metabolites that occur in a small number of plants and fulfill a more vital function, mainly phenolic compounds, alkaloids, terpenes, steroids and derivatives of sulfur compounds. Secondary metabolites play a more important role in protecting and maintaining health, such as toxins to deter predators and pheromones that attract insects for pollination [4-6]. Some types of secondary compounds that are particularly important in pharmacy today are thought to have hypolipidemic, antioxidant, hypoglycemic, and anti-inflammatory effects. In addition, some secondary compounds are also said to protect against thrombosis and cancer. Due to these properties, it is assumed that phytochemicals can protect against some diseases such as obesity, diabetes, metabolic syndrome, dyslipidemia, cardiovascular diseases and cancer [6].

The genus *Scilla* is exemplifyed by 14 taxa in Türkiye [7-9]. The pharmacopoeias of many countries list the uses of the corms; for example, the corms of this genus (*Scillae* corms) are used for their diuretic and cardiotonic features [10, 11]. The literature search revealed that only a few studies have been conducted on the species growing in Türkiye. The literature search revealed that few studies have been conducted on the species growing in Türkiye. Tanış [12] investigated the antioxidant capacity of *Scilla bifolia* L., Aktepe et al. [13] investigated the biochemical constituents, enzyme inhibition properties, antioxidant and antimicrobial properties of endemic *Scilla mesopotamica* Speta. Coşkun [14] conducted studies on the reproductive biology of *Scilla autumnalis* L. and obtained valuable results. Considering the prepotency of medicinal plants and the rare chemical and biological investigation of these species, this study was essential.

The sole aim of this work was to explore the phytochemical constituents of the species *S. hyacinthoides* and *S. ingridiae*, to show their biological capabilities, to explain them with chemometric approaches, to model them with correlation methods and to present them to scientists working in different disciplines. On the other hand, this study is also significant in many ways with respect to the species and parameters studied.

2. Materials and Methods

2.1. Plant Materials

S. hyacinthoides was collected in an oak area along the 40. km Hani road in Diyarbakır (26.05.2023). *S. ingridiae* was collected in a mountainous area in Sason district in Batman (06.05.2023). The plants was assigned by Dr. Alevcan Kaplan and provided with voucher specimens Batman 2023/27 and Batman 2023/28, which are kept at Batman University.

2.2. Preparation of Plant Extracts for Biological Activity and Phytochemical Profiling

The species collected in the field were separated into aerial parts and corms and dried in an airy room protected from sunlight. The corms were cut in half and dried to prevent mold growth. The dried plants were then cut into small pieces, weighed and coded according to the plant parts. The aerial parts

(flowers, stems, and leaves) of the species *S. hyacinthoides* were labeled with S1TU, the corms with S1T2, the aerial parts of the species *S. ingridiae* with S2TU and the corms with S2TA. Then the plant parts were weighed, briefly, S1TU-63 g, S1TA-87.1 g and S2TU-25 g, S2TA 23.5 g were weighed and extraction procedures were performed with ethanol, the solvents were evaporated until the plants were completely dried under the fume hood to ensure that there was no solvent left in the plants. Finally, the tares of the dried extracts were taken and the % (percentage) yield was calculated. The extracts were stored in closed containers until analysis.

2.3. Phytochemical Profiling via LC-ESI-MS/MS Methods

The LC-ESI-MS/MS method was used to analyze the phytochemical constituents of the plants. A previously ingrained and developed LC-ESI-MS/MS method was used to identify the phenolic content of *Scilla* species in the study [15].

2.4. Evaluation of TPC and TFC Contents

The TPC of plants was expressed as pyrocatechol and TFC content or quercetin equivalent [16-18]. The following equations were used to calculate the TPC and TFC of the extracts:

PE*s*. pyrocatechol equivalents (Absorbance = 0.032x + 0.0445 pyrocatechol (μg) (R^2 = 0.9947)

QEs. quercetin equivalents (Absorbance = 0.0288x + 0.0352 quercetin (μg) (R^2 = 0.9951)

2.5. Antioxidant Activity

To appraise the antioxidant activity of the extracts, the methods of the radical scavenger DPPH and the ABTS cation radical scavenger as well as the reducing antioxidant capacity of copper (CUPRAC) were used [19-22].

2.6. Anticholinesterase Inhibiton Activity

A spectrophotometric method improved by Ellman et al. [23] was used to determine the acetyl and butyryl cholinesterase inhibitory activities.

2.7. Tyrosinase Inhibiton Activity

The tyrosinase inhibition experiments were conducted according to the protocol of Hearing and Jiménez [24].

2.8. Chemometric and Statistical Analysis

Chemometrics is essentially the statistical analysis of data obtained through chemical processes. Multivariate data analyzes are processed and interpreted using chemometric algorithms [25]. To perform chemometric analyzes, the results obtained from the experiments were transferred to programs such as OriginPro 2024b Academic edition, Graphad Prism 5, and Xlstat 2024 after their calculation and the data were analyzed. The results of TPC and TFC, antioxidant, tyrosinase inhibitory and anticholinesterase activities were expressed as mean values ± S.E (standard error). The outcomes were analyzed using an unpaired *t*-test and one-way analysis of variance ANOVA. Differences were considered statistically significant at $p < 0.05$.

3. Results and Discussion

For a thousand of years, nature has been the source of medicines, and an astonishing number of modern treatments have been defined from natural sources. Most of plants have long been used as herbal folk remedies to treat different conditions, and their diverse NP*s* have inspired the improvement of new medicines. With the discovery of new molecular targets (MT*s*) based on proteins, the need for new structural and chemical diversification in screening is growing. NP*s* will play a critical role in meeting this need through the continued detection of the world's biodiversity, much of which remains undiscovered. While drug development from medicinal plants (MP*s*) remains an significant source of new therapeutics, there are several obstacles, including defining and conducting appropriate HTS (high-throughput screening) experiments, increasing the supply of biologically active molecules and sourcing plant material. Exploration of these natural resources requires multidisciplinary, nationwide and international partnerships in many process (synthesis, discovery, and design drug) improvement practices [26]. Such studies are innovative and intriguing given the strategies to incorporate the therapeutic agents use of plant NP*s* worldwide to support future drug exploration from plant sources [26].

In this regard, the phytochemical components and biological abilities of the species with the common names "dağ soğanı" (*S. hyacinthoides*) and "ala sümbül" (*S. ingridiae*) in Türkiye were studied and evaluated. First, the efficacy of solvent on extraction yield was investigated. The effect of solvent on the percentage yield is shown in Table 1, Figure 1, and Figure 2. The highest yield was found to be 4.77 % for S2TA. The other tree extracts showed almost similar percentage yields. Extraction is the most important step to obtain and isolate bioactive phytochemicals from different plant materials. The extraction adequacy is affected by the chemical nature of the phytochemicals, the extraction technique used, the particle size of the sample, the solvent used and the presence of solvent-insoluble substances. The extraction efficiency depends on the polarity of the solvent, pressure, extraction method, T °C (temperature), E_t (extraction time), pH, and combination of the sample [27-29]. Aydın et al. [30] documented that the yield of methanol extracts from tuber, leaf and flower of *Scilla siberica* subsp. *armena* (Grossh.) Mordak species were 1.27 g, 1.80 g and 1.04 g, respectively. Mulholland et al. [31] extracted the species *Scilla zebrina* Baker with dicholoromethane and reported an extraction yield of 7.28 g. Yaman et al. [29] assumed that the variations in extract yield were due to differences in species, location, harvest time and extraction methods.

Table 1. Effect of solvent types on extract yield of two *Scilla* species

Plant samples	Extraction yield (%)
S1TU	3.28±0.3
S1TA	3.22±0.09
S2TU	3.06±0.12
S2TA	4.77±0.29

[†]It indicates the mean value and standard deviation

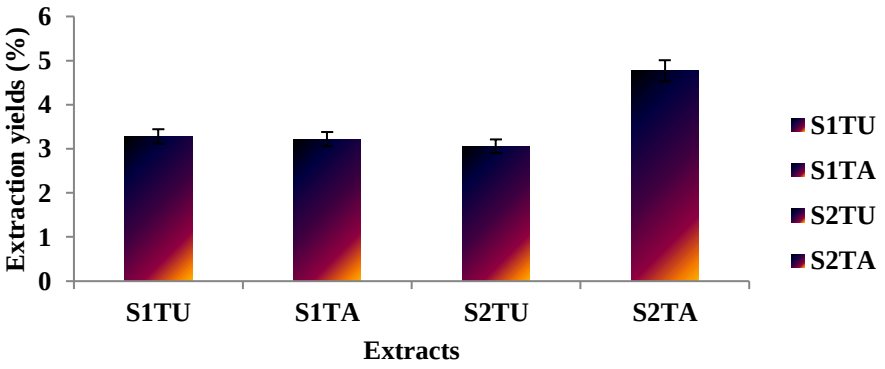

Figure 1. Extraction yields of two *Scilla* species

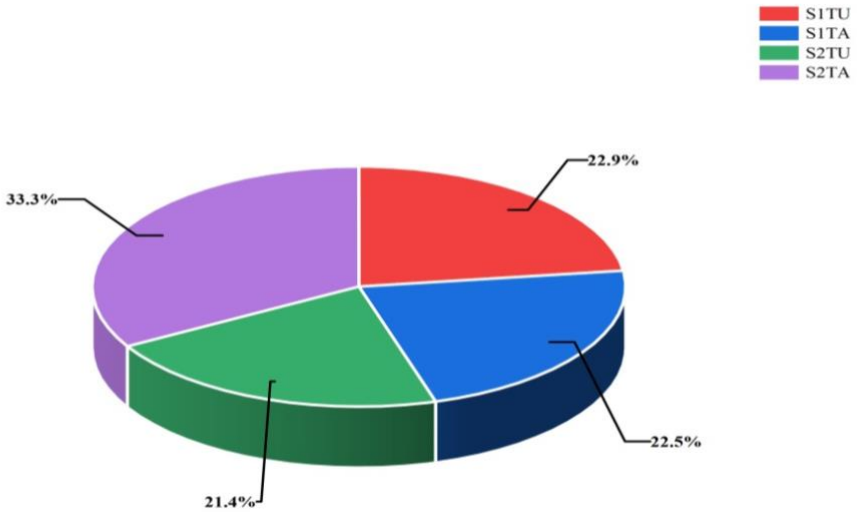

Figure 2. Visualization of % yields of extracts of two different *Scilla* species with pie chart

For the identification, pathological characteristics analysis and remedy of numerous diseases, sensitive and specific methods are required for the determination and characterization of endogenous level of these steroids in body tissues or, fluids as well as for their quantification and content in phytochemical analyses. More recently, LC-ESI-MS/MS has gained acceptance for these objectives due to its specificity and sophistication [32]. The chromatograms of the phytochemical constituents are shown in Figure 3. The content and amounts of these phytochemicals are summarized in Table 2. The correlations of the phytochemicals with each other are shown in Figure 4, and the correlations of the extracts with each other according to their content are depicted in Figure 5. As can be clearly shown in Table 2, a total of 14 phytochemicals were identified. The number of ingredients was determined as follows: S1TU (*n*=13), S2TA (*n*=12), S1TA = S2TU (*n*=11), from most to least. It can be said that the most important chemical compound is vanillic acid (VA). However, the S1TU extract contains shikimic acid, which is not present in the other extracts and not in this quantity. VA (4-hydroxy-3-methoxybenzoic acid) is a derivative of hydroxybenzoic acid, which is mainly used as a flavouring agent. It is also formed as an intermediate in the production of vanillin from ferulic acid [33,34]. In addition to its use as a food additive, it is thought to have anticoagulant, antioxidant, antimicrobial, antioxidant, anti-inflammatory, anti-venous, cardioprotective, hepatoprotective and neuroprotective effects, as well as anti-inflammatory effects by lowering the pain threshold [34-37]. In addition, VA has also been used in wound healing by suppressing the expression of MITF (microphthalmia-associated transcription factor) and melanogenic enzyme sinB16F10 in cells and decreasing melanin

levels and tyrosinase activity with or without MSH (melanocyte-stimulating hormone) stimulation [38,39]. It also inhibited inflammatory pain by activating nuclear factor Kappa B (NF-κB) and inhibiting cytokine production, oxidative stress, and neutrophil recruitment [39,40]. Vanillic acid also showed anti-cancer effects by inhibiting tumor growth. It treated acute myocardial injury by hypoxia/reoxygenation by reducing oxidative stress of H9c2 cells [39, 41]. It also controlled lipopolysaccharide-induced neurotoxicity in mice by regulating c-Jun N-terminal kinase in the brain [36, 39]. SA (shikimic acid) is properly distributed in many herbal species and is known to exhibit various bioactivities, including antioxidant, analgesic, anti-inflammatory, and antithrombotic effects as well as activities against biofilms [42, 43]. The richness of the species in such valuable secondary metabolites confirms the parameters of the study. To look at the data obtained in the study from a different angle and to civilize the data, the heat map was created using the Pearson correlation matrix and shown in Figure 3. There is a strong positive correlation between protocatechuic acid and hydroxybenzaldehyde, vanillic acid, caffeic acid, syringic acid, protocatechuic acid ethyl ester and naringenin. With the exception of chlorogenic acid and shikimic and trans-cinnamic acid, moderate correlations were generally observed. there is a strong positive correlation between hydroxybenzaldehyde and protocatechuic acid, vanillic acid, caffeic acid, syringic acid, protocatechuic acid ethyl ester and naringenin. there is a strong positive correlation between vanillic acid and protocatechuic acid, hydroxybenzaldehyde, caffeic acid, syringic acid, protocatechuic acid ethyl ester and naringenin. there is a strong positive correlation between *p*-coumaric acid and vanillin and salicylic acid. there is a strong positive correlation between salicylic acid and *p*-coumaric acid, vanillin. Scutellarin showed a close to strong to moderate correlation with chlorogenic acid. there is a strong positive correlation between protocatechuic acid ethyl ester and protocatechuic acid, hydroxybenzaldehyde and protocatechuic acid, vanillic acid, caffeic acid, syringic acid and naringenin. a moderate to strong correlation exists only between *trans*-cinnamic acid and vanillin. A strong correlation was found between naringenin and protocatechuic acid, protocatechuic acid, vanillic acid, caffeic acid, syringic acid and protocatechuic acid ethyl ester. Surprisingly, protocatechuic acid, hydroxybenzaldehyde, vanillic acid, protocatechuic acid ethyl ester and naringenin always showed a high correlation with each other. This correlation suggests that they may play a common role in the biological activities of the plant. Aktepe et al. [13] detected chicory (0.564 µg/mL), transferulic acid (0.501 µg/mL), caffeic acid (0.165 µg/mL) and chlorogenic acid in the chloroform extract as a result of LC-MS/MS analysis of *S. mesopotamica*. They found that the levels (0.158 µg/mL) were again quite high compared to other extracts. The same researcher found from the LC-MS/MS results that the amounts of caffeic acid, *p*-coumaric acid, transferferulic acid, kaempferol and gentisic acid in the ethyl acetate extract were quite high (1.933 µg/mL, 0.301 µg/mL, 0.295 µg/mL, 0.229 µg/mL, and 0.162 µg/mL, respectively). However, the LC-MS/MS results of the methanol extract of *S. mesopotamica* Speta showed high levels of chichoric acid, vanillic acid, chlorogenic acid and citric acid (0.559 µg/mL, 0.228 µg/mL, 0.159 µg/mL and 0.111 µg/mL), ascorbic acid, 4-hydroxybenzoic acid, epicatechin, transferulic acid, fumaric acid and catechin were lower (0.08 µg/mL, 0.052 µg/mL, 0.047 µg/mL, 0.047 µg/mL, 0.041 µg/mL, 0.037 µg/mL, and 0.036 µg/mL, respectively) [13]. These results show that plants, even if they belong to the same genus, can synthesize many different bioactive secondary metabolites. Figure 5 shows the evaluation of the extracts in terms of their content. In this respect, a strong correlation was found between S2TU and S1TA, between S1TA and S1TU and a moderately positive correlation between S2TA and S2TU. This can also be seen as an indicator of the parallelism between the ingredients and the extract results.

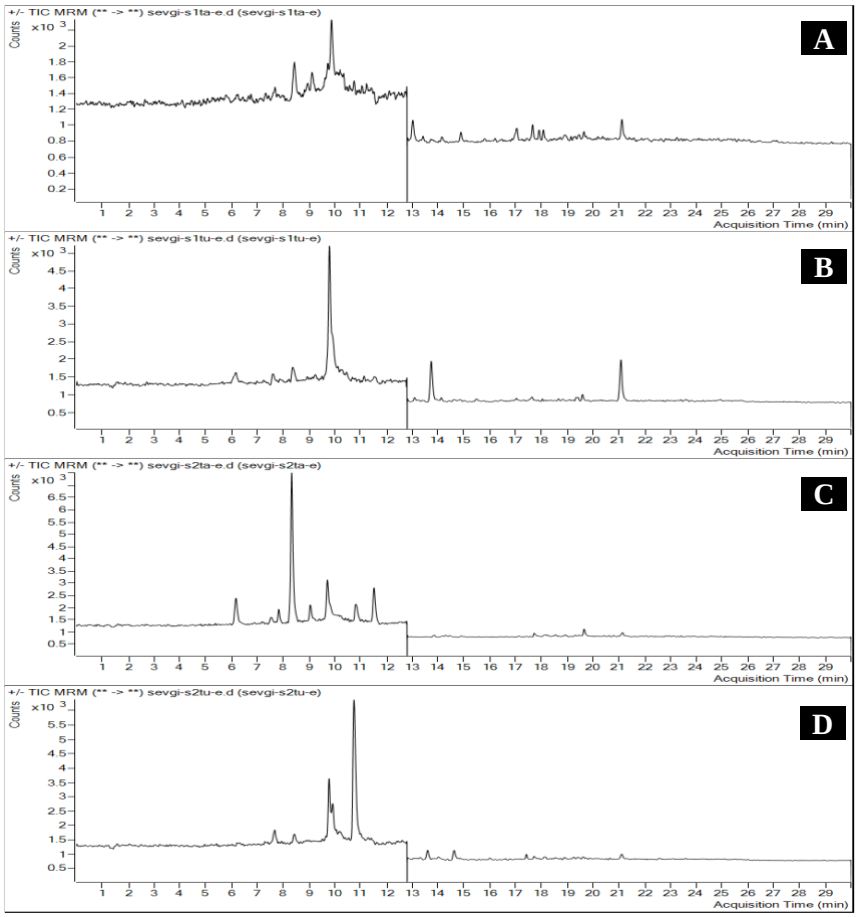

Figure 3. Overlapped two *Scilla* species extract samples chromatogram from LC-ESI-MS/MS analysis, **A**-S1TA, **B**-S1TU, **C**-S2TA, **D**-S2TU

Table 2. LC- ESI-MS/MS profiling of two different parts of two different *Scilla* species

Analytes	RT (min.)	R^2	Quantitative results (ng analyte/mL extract)			
			S1TA	S1TU	S2TA	S2TU
Shikimic acid	1.53	0.99	n.d	**841.9**	n.d	n.d
Protocatechuic acid	6.30	0.99	7.1	40.2	141.7	9.5
Chlorogenic acid	7.93	0.99	7.0	9.1	13.2	13.9
Hydroxybenzaldeyde	8.40	0.99	13.0	13.7	32.7	16.4
Vanillic acid	7.95	0.99	**325.1**	**285.5**	**3709.4**	**205.3**
Caffeic Acid	8.45	0.99	29.8	18.4	473.9	12.4
Syringic acid	9.12	0.99	18.8	12.4	30.3	14.2
Vanillin	9.72	0.99	10.8	104.6	45.7	54.2
p-coumaric acid	9.89	0.99	136.8	161.7	151.3	162.3
Salicylic acid	10.21	0.99	61.4	148.6	126.2	127.7
Scutellarin	10.68	0.99	0.4	0.5	16.7	114.8
Protocatehuic ethyl ester	11.43	0.99	6.1	8.9	81.2	9.0
Trans-cinnamic acid	14.04	0.99	n.d	106.9	n.d	n.d
Naringenin	15.09	0.99	n.d	n.d	27.8	n.d

[a]R.T.:Retention time, R^2: Correlation coefficient, n.d: not determined, S1TA: *Scilla hyacinthoides* corms ethanol extract, S1TU: *Scilla hyacinthoides* aerial parts ethanol extract, S2TA: *Scilla ingridae* corms ethanol extract, S2TU: *Scilla ingridae* aerial parts ethanol extract, n.d: not detected. Phytochemical classes of the studied compounds; **Phenolic aldehydes:** vanillin, **Phenolic acids:** Protocatechuic acid, chlorogenic acid, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, salicylic acid, *Trans*-cinnamic acid, Shikimic acid (3,4,5-trihydroxy-1-cyclohexene-1-carboxylic acid), Protocatehuic ethyl ester, **Flavonoids:** Naringenin, Scutellarin, **Hydroxybenzaldehydes**: Hydroxybenzaldeyde

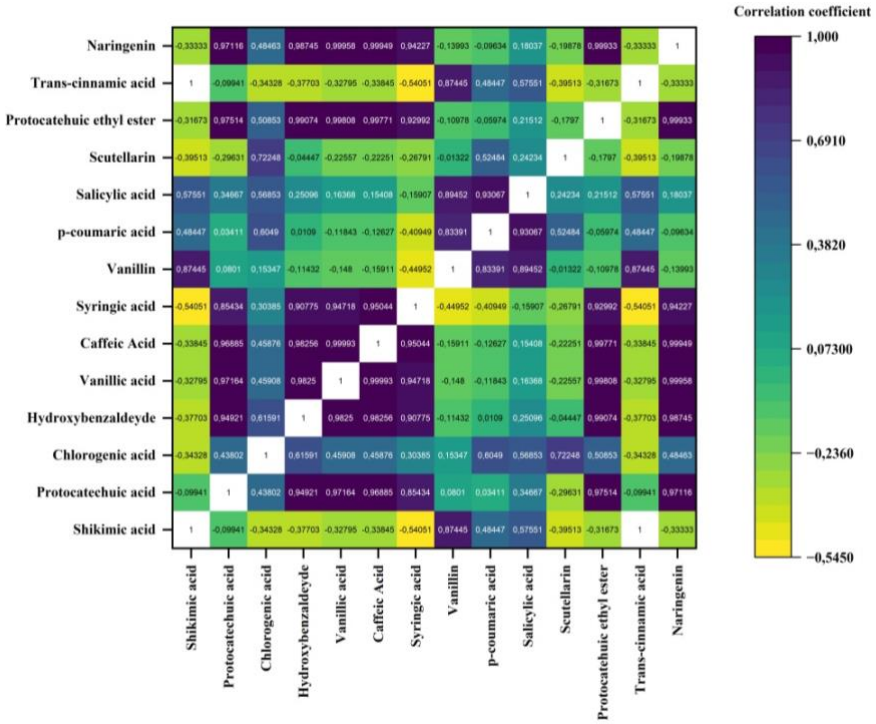

Figure 4. Heatmap with Pearson correlation matrix of two different *Scilla* species phytochemical component

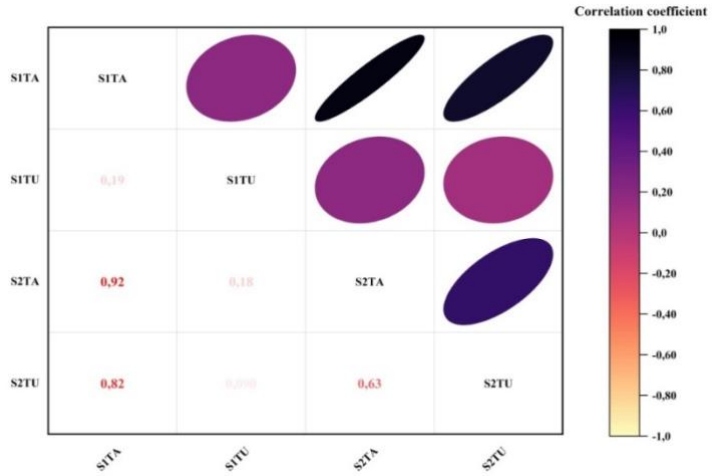

Figure 5. Correlation plot analysis of different parts extracts of two *Scilla* species

In the present study, the TPC and TFC as well as the antioxidant features of the extracts acquired from the plant were investigated and negotiated. S1TU (11.72±0.00 µg PE*s*/mg extract), S1TA (7.03±0.00 µg PE*s*/mg extract)=S2TA(7.03±2.21µg PE*s*/mg extract), S2TU (5.99±0.90 µg PE*s*/mg extract) demonstrated the highest total phenolic content, while S2TU (31. 53±0.21 µg QE*s*/mg extract), S1TU (22.04±0.22 µg *QEs*/mg extract), S2TA (6.41±0.20 µg QE*s*/mg extract), S1TA (5.41±0.35 µg QE*s*/mg extract). Interestingly, the extracts were not active in DPPH activity in the antioxidant activity method. On the other hand, S1TU (IC_{50}: 425.66±4.13 µg/mL) proved to be the most active extract in the ABTS method, while S2TU (IC_{50}: 185.88±0.30 µg/mL) showed the highest activity in the CUPRAC method (Table 3). Phenolic compounds that contribute to antioxidant capacity

are among the most important compounds found in plants [44]. Flavonoids have many antioxidant properties, such as scavenging radicals and activating enzyme systems [45]. It can be said that the reason why very high antioxidant activity results were not obtained in the work is due to the relatively low amount of phenols and flavonoids. However, the S2TU extract with the highest flavonoid content demonstrated the highest CUPRAC activity, which confirms the known theories of this method. Tanış [12] calculated the TPC and TFC in the methanolic extract of *S. bifolia* as 21.56 mg GAE/g and 27.70 mg RE/g. The DPPH radical scavenging feature of the methanol extract of *S. bifolia* was recorded to be 31.91±0.27 mg TE/g. The ABTS radical scavenging feature was found to be 37.99±1.98 mg TE/g. They reported that the plant extract had a stronger scavenging effect on ABTS radicals. In the FRAP and CUPRAC tests, which are reducing power tests, 31.39 mg TE/g and 83.62 mg TE/g were detected, respectively. The copper reducing power of the plant extract is higher than the iron reducing power. Although differences in terms of the solvent used and the type of solvent were considered, the reducing power is similar to this work. It was noteworthy that the extracts showed antioxidant activity proportional to their high reducing power. Yasuda et al. [46] investigated the antioxidant activity of the MeOH (methanolic) extract of *S. scilloides* (Lindl.) Druce corms using $ABTS^{\bullet+}$ and DPPH methods. The authors of the paper stated that, the $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ clearance abilities were determined as EC_{50} of 51.2 µg/mL and 1.039 µg/mL, respectively. In another research, the total phenolic content of *S. siberica* subsp. *armena* was determined as leaf extract (53.59.59211 µg GAE/mg extract), flower extract (43.88158 µg GAE/mg extract), tuber extract (38.05263 µg GAE/mg extract) and BPE (blue pollen extract) (34.77.632 µg GAE/mg extract) [30]. As a result of the $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ trials, they found that the leaf extract demonstrated the best activity (IC_{50}=949.81 µg/mL and IC_{50}=94.07 µg/mL, respectively) compared to the standards. In their study, extracts from the aerial parts of *Scilla* species were found to be more efficient than extracts from the corm [30]. This result is consistent with presented study.

Table 3. TPC, TFC, and antioxidant activity results of two *Scilla* species

Treatments	TPC (*µg PEs*/mg extract)[a]	TFC (*µg* QE*s*/mg extract)[b]	IC_{50} values (µg/mL)[c]		$A_{0.5}$ values (µg/mL)[d]
Samples			DPPH Free Radical	ABTS Cation Radical	CUPRAC
S1TU	11.72±0.00	22.04±0.22	>1000	425.66±4.13	202.87±0.04
S1TA	7.03±0.00	5.14±0.35	>1000	713.59±5.05	352.46±3.34
S2TU	5.99±0.90	31.53±0.21	>1000	>1000	185.88±0.30
S2TA	7.03±2.21	6.41±0.20	>1000	519.09±5.24.	245.06±0.53
BHA[e]	-	-	3.22±0.08	2.74±0.03	4.14±0.17
α-TOC[e]	-	-	1.41±0.04	8.48±0.43	13.64±0.32
BHT[e]	-	-	16.71±0.80	4.44±0.30	3.93±0.24

[†]Values expressed are means ± standard deviation of three parallel measurements (p<0.05)

[a] *PEs*. pyrocatechol equivalents (y = 0.032x + 0.0445 R^2 = 0.9947)

[b] QE*s*. quercetin equivalents (y = 0.0288x + 0.0352 R^2 = 0.9951)

[c] Values were given as IC_{50} for DPPH free and ABTS cation radical scavenging activities

[d] Values were given as $A_{0.5}$ for CUPRAC activity

[e] Standart compounds

The anticholinesterase and antityrosinase abilities of the extracts are shown here (Table 4). The most active extracts in terms of AChE inhibition are S1TU (23.90±0.46 % inhibition) and S2TU (23.90±0.75% inhibition) at 200 µg/mL concentration. For BChE inhibition, S1TU (23.04±0.01 % inhibition) and S2TU (21.49±0.87 % inhibition) were identified at 200 µg/mL concentration. In the

tyrosinase inhibition assay, all extracts showed activity above 50%, with the most successful extracts identified as S2TA (57.30±1.68 % inhibition) and S1TA (54.17±1.07 % inhibition) at 200 μg/mL concentration. As a result, the aerial parts were found to be more successful in anticholinesterase activity, while the tubers were more successful in antityrosinase activity. As mentioned above, the main compound of the extracts was reported as VA. In support of the enzyme results, previous studies have reported that vanillic acid is also used in wound healing by suppressing the expression of MITF (microphthalmus-associated transcription factor) and melanogenic enzyme sinB16F10 in cells and reducing melanin levels and tyrosinase activity with or without MSH (melanocyte-stimulating hormone) stimulation [38,39]. In another study, it was found to have a neuroprotective effect [37]. Aktepe et al. [13] showed that all extracts of *S. mesopotamica* were efficient in inhibiting AChE. The authors of the paper documented that the main phenols defined in the extracts of *S. mesopotamica* act as AChE inhibitors. Not many studies were found in the literature review, with the exception of Aktepe et al. [13] on the enzyme inhibition of plants belonging to the genus *Scilla* on the acetylcholinesterase activity of *S. mesopotamica* and Aydın et al. [30] on the antidiabetic activity of *S. siberica* subsp. *armena*. In this case, the data were presented in the study. The authors of the paper have been reviewed and presented for the first time and have helped to enhance the value and originality of this study.

Table 4. Enzyme inhibition activities results of two *Scilla* species

Treatments	**Inhibition % (μg/mL)**		
Samples	**AChE**	**BChE**	**Tyrosinase**
S1TU	23.90±0.46	23.04±0.01	50.83±0.31
S1TA	12.30±0.76	8.64±0.02	54.17±1.07
S2TU	23.90±0.75	21.49±0.87	51.47±1.08
S2TA	18.77±0.76	4.56±0.01	57.30±1.68
Galanthamin[a]	91.01 ±0.22	80.46±0.18	-
Kojic acid[b]	-	-	95.05±0.37

[†]Values are means of three parallel measurment ± standard deviation (*n*= 3)
[a]Standard compound for AChE and BChE
[b]Standard compound for tyrosinase

At this point in the study, the data obtained in the biological activity experiments were analyzed chemometrically and subjected to correlation tests, and an attempt was made to uncover the correlations of the results by testing their accuracy. The diagrams of PCA and HCA are depicted in Figure 6 and Figure 7, respectively. Figure 8 shows a heat map of the triangle with the Pearson correlation matrix. As can be seen in Figure 6, PC1 explains 52.90% of the data and PC2 explains 30.81%. To elucidate the PC2 components, ABTS, CUPRAC, and TFC activities contribute in the negative squares and comprise the S1TU and S1TA extracts of the plant. DPPH remained in the center as no extract showed activity. It also contributed to the elucidation of PC1 components in the positive quadrature comprising the S2TU and S2TA extracts of the plant and with TPC, AChE, BChE, and anti-tyrosinase activities. However, as can be seen in Figure 7, the dendrogram is divided into two branches with two separate groups, one containing S2TU and the other S1TA and S2TA branching from the same node to which S2TA is connected. A correlation analysis was performed to obtain further knowledge on the relationship between chemical combination and biological activities. Figure 8 shows the triangle heat map with Pearson correlation matrix diagram of the phytochemical compositions and biological activities contained in the extracts to illustrate their relationship. The closer the values are to +1, the higher the correlation is, but the lower this value is, the lower the

correlation relationship is, which is illustrated by the coloring of the heat map (the more the color goes towards black, the stronger the correlation, while the more the color goes towards yellow, the lower the correlation). TPC showed a strong positive correlation with transcinnamic acid, vanillin and shikimic acid. TFC showed a high correlation with naringenin, scutellarin and *p*-coumaric acid. The antioxidant ABTS activity showed a strong positive correlation with the CUPRAC method. However, other phytochemicals showed weak or negative correlations at different levels. Öztürk et al. [47] found in the multivariate analysis of *Alcea fasciculiflora* Zohary species that the secondary metabolites that showed high positive correlation with the phytochemical content of the species and the antioxidant activity methods (ABTS, DPPH, MCA, PPBD), *p*-coumaroylhexaric acid isomer, luteolin, apigenin, *p*-coumaroylhexaric acid, *p*-coumaroylhexaric acid isomer, *p*-coumaroylhexaric acid isomer, feruloylhexaric acid, feruloylhexaric acid isomer, feruloylhexaric acid isomer, feruloylhexaric acid isomer. Intriguingly, AChE activity showed the strongest positive correlation with protocatechuic acid ethyl ester (*n*=1), while hydroxybenzaldehyde (*n*=0.98), caffeic acid (*n*=0.99), syringic acid (*n*=0.89), vanillic acid (*n*=0.99), protocatechuic acid (*n*=0.99) and anti-tyrosinase activity (*n*=0.8), which belong to biological activities, showed strong positive correlations. BChE activity is protocatechuic acid ethyl ester (*n*=0.99), hydroxybenzaldehyde (*n*=0.99), caffeic acid (*n*=0.97), syringic acid (*n*=0.86), vanillic acid (*n*=0.98), protocatechuic acid (*n*=0.98).) showed strong positive correlations with biological activities and anti-tyrosinase activity (*n*=0.76). The tyrosinase inhibition activity of protocatechuic acid ethyl ester (*n*=0.85), hydroxybenzaldehyde (n=0.82), caffeic acid (*n*=0.88), syringic acid (*n*=0.98), vanillic acid (*n*=0.88) and protocatechuic acid (*n*=0.76) showed strong positive correlations. The reason why there are no clear or high correlations in the antioxidant activity results suggests that the amounts, types and combinations of total phenolic and flavonoid substances have little influence as shown and expressed in Table 1. Öztürk et al. [47] showed that AChE activity was determined by the secondary metabolites of *A. fascuculifolia* species such as luteolin, tiliroside, kaempferol-O-p-coumaroyl-O hexoside, apigenin, kaempferol, N-feruloyltyramine. Authors of the paper documented that it was significantly positive with presented study. In this section, which contains the most striking part of the study, it should be emphasized and underlined that the secondary metabolites responsible for enzyme inhibition are protocatechuic acid ethyl ester, hydroxybenzaldehyde, caffeic acid, syringic acid, vanillic acid and protocatechuic acid.

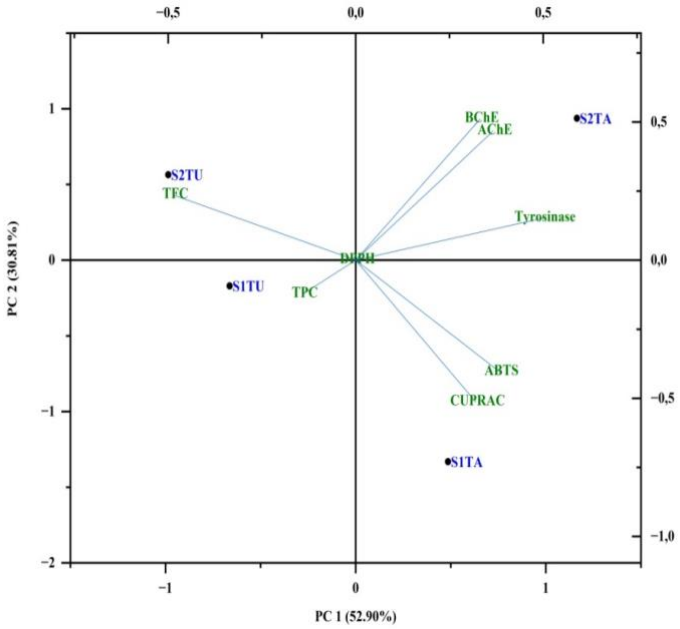

Figure 6. Principal component analysis of biological activity profiles of two *Scilla* species extracts in Table 3, and Table 4

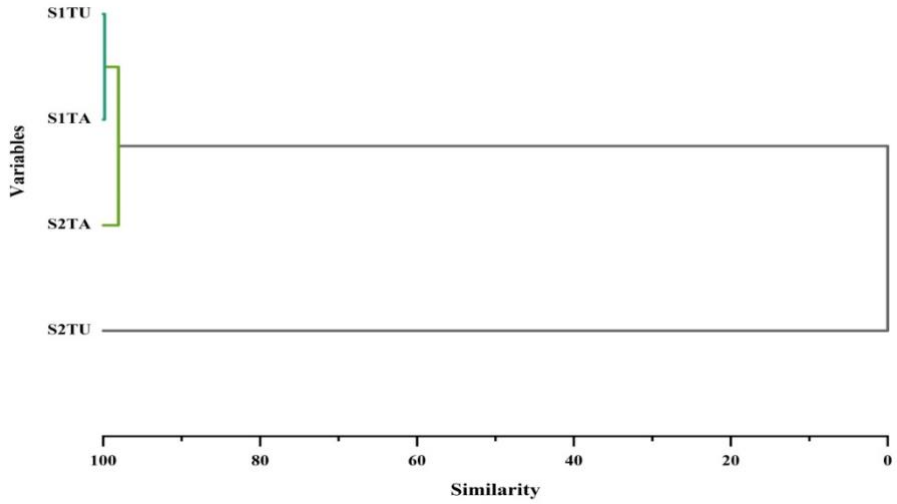

Figure 7. Hierarchial component analysis dendrogram of two *Scilla* species extracts displayed in Table 3, and Table 4

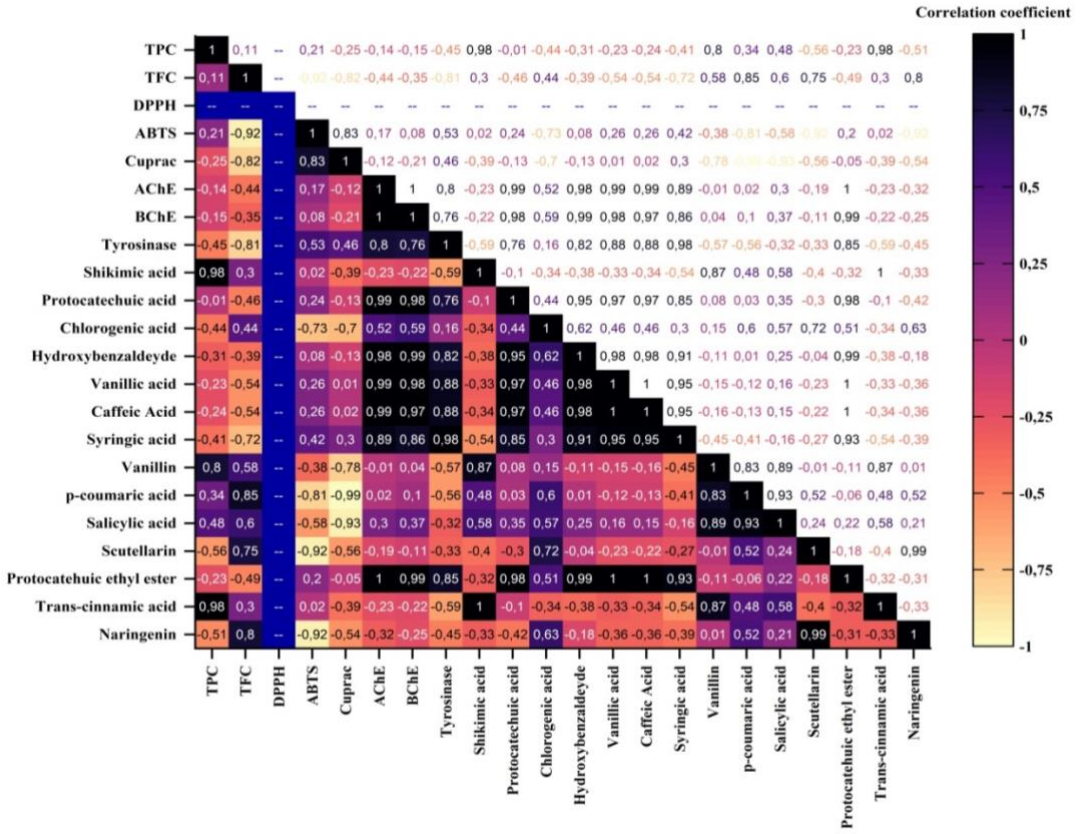

Figure 8. Triangle heat map with Pearson correlation matrix with secondary metabolites and biological activities displayed in Table 2, Table 3, and Table 4

4. Conclusion

In this work, the ethanol extracts of *S. hyacinthoides* and *S. ingridiae* species were found to contain important phytochemical compounds (the most important compound is vanillic acid), and although the total amount of phenols and flavonoids was low, they showed antioxidant activity in different amounts, and the ability to inhibit enzymes was quite successful. Remarkably, in agreement with the results of chemometric data and correlation analysis, it is underlined and emphasized that the secondary metabolites responsible for enzyme inhibition could be protocatechuic acid ethyl ester, hydroxybenzaldehyde, caffeic acid, syringic acid, vanillic acid and protocatechuic acid. These valuable and very important results of the study are supported by many researchers. It is expected to broaden its horizons and vision. The cosmetics, pharmaceutical and medical industries in particular will benefit from the results, as they are at the forefront of the sectors in which they are used. This makes the research results promising candidates for new therapeutic applications in the biomedical field.

Ethical statement

The author declares that this document does not require an ethics committee approval or any special permission. This study does not cause any harm to the environment.

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Conflict of Interest

No conflict of interest or common interest has been declared by the author.

Authors' Contributions

A.K: Conceptualization, Methodology, Formal analysis, Writing - Original draft preparation
A.K.: Conceptualization, Methodology, Resources, Investigation
A.K: Methodology, Formal analysis, Writing
A.K: Conceptualization, Methodology, Formal analysis, Writing - Original draft preparation
Author read and approved the final manuscript.

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