



Evaluation of Enzyme Inhibitory Effects and Phytochemical Analysis by LC-Q-ToF-MS of Some *Polygonum* L. Species Growing in Türkiye

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ABSTRACT

Polygonum species have strong biological activity due to their important bioactive components. *Polygonum* species have been studied for enzyme inhibition activity, wound healing, anti-inflammatory, antibacterial, antiviral, antioxidant effects, immunomodulatory activity, anti-aging potential, estrogenic activity and are used abroad as a source of resveratrol. For this purpose, biological activity studies, and phytochemical analysis were carried out in *P. aviculare* L., *P. cognatum* Meissn., *P. patulum* Bieb. subsp. *patulum*, and *P. setosum* Jacq. subsp. *setosum* growing in Turkish flora. *Polygonum* ethanol extracts were tested for their cholinesterase, elastase, collagenase, and tyrosinase enzyme

inhibitory activities with spectrophotometry. *P. aviculare* root extract had the highest acetylcholinesterase inhibitory activity ($IC_{50} = 55.94 \pm 2.63$ μ g/mL), while the best butyrylcholinesterase inhibitory activity was observed for *P. aviculare* ($IC_{50} = 17.02 \pm 3.42$ μ g/mL) and *P. patulum* ($IC_{50} = 18.36 \pm 0.15$). μ g/mL root extracts. All *Polygonum* root extracts showed moderate inhibitory activity against tyrosinase. In this study indicated that *P. aviculare* root ($IC_{50} = 169.33 \pm 3.58$ μ g/mL) had the highest anti-elastase activity. *P. cognatum* roots ($IC_{50} = 200.30 \pm 2.26$ μ g/mL) have potent inhibition against collagenase. LC-Q-ToF-MS analysis in *Polygonum* extracts indicated that resveratrol was not present in the extracts.

Keywords: *Polygonum*, Enzyme inhibition, LC-Q-ToF-MS

1. Introduction

The most typical cause of early dementia in older people is Alzheimer's disease (AD). The characteristics of AD are progressive memory loss, a deterioration in language abilities, and other cognitive impairments. Although the actual mechanism of AD is unknown, several variables including the cholinergic deficit, excessive transition metals, amyloid peptide, tau protein aggregation, and oxidative stress have been related to it, (Uriarte-Pueyo et al. 2011; Ustun et al. 2012). The most prescribed medications for treating the symptoms of AD at the moment is acetylcholinesterase/butyrylcholinesterase (AChE/BChE) inhibitors, which enhance acetylcholine (ACh) levels at cerebral cortex synapses via blocking AChE/BChE that breaks down ACh. Numerous natural compounds, primarily alkaloids, have been identified as AChE inhibitors (Uriarte-Pueyo et al. 2011). Researchers continually search for natural cholinesterase inhibitors that have fewer side effects than the currently used cholinesterase inhibitory drugs such as tacrine, donepezil, and galanthamine. Wrinkles are caused by the degradation of elastin and collagen, the main proteins of connective tissue that give the skin its elasticity and resistance. Aging-related elastase and collagenase are triggered by the release of free oxygen radicals (Oxlund et al. 1988). Tyrosinase (TYR) is a copper-containing melanogenic enzyme commonly found in microorganisms, plants, and animals. TYR causes enzymatic browning in fruits and mushrooms and melanogenesis in humans. The search for safe and effective TYR inhibitors for hyperpigmentation treatment, skin whitening, and spot removal has recently become necessary (Chang 2009; Seo et al. 2003). One of the main objectives of the beauty industry has been to find new anti-wrinkle and skin-lightening chemicals or extracts and scientifically prove their effectiveness by inhibiting these enzymes, which include elastase, collagenase, and TYR (Deniz et al. 2020; Hong et al. 2014). The genus *Polygonum* L. (Polygonaceae) is represented by 43 taxa, 8 of which are endemic to Türkiye. Numerous medicinal species, including *P. multiflorum*, *P. cuspidatum*, *P. bistorta*, *P. aviculare*, and *P. tinctorium*, are found in the *Polygonum* genus (Demirpolat 2022). Different parts of the plants have been used in Traditional Chinese Medicine and as folk remedies. In addition,

diverse chemical components, including terpenes, glycolipids, stilbenes, anthraquinones, flavonoids, and stilbene derivatives, have been found in this genus (Dong et al. 2014). At the same time, *Polygonum* species have numerous ethnobotanical uses, such as relieving excessive menstrual bleeding, decreasing kidney stones, and wound healing in various countries (Gürdal et al. 2013; Sagirolu et al. 2017). This study aimed to analyze the presence of bioactive compounds in four *Polygonum* species growing in Türkiye and to investigate the new biological activities of these compounds.

2. Material and Methods

2.1. Plant material and extraction

The collection sites and dates of the herbal materials used in this experimental studies are given in Table 1. The collected plant materials were pressed and dried according to standard herbarium techniques. The voucher specimens were kept in KNYA herbarium, Selcuk University. The plant materials were identified by Osman TUGAY. The collected *Polygonum* species were divided into aerial parts and roots. They were dried in the shade and powdered. Then, each sample was macerated once with 96% ethanol at room temperature for seven days. Filter paper was used to filter the resulting extract. The residual filtrate was dried off in a rotavapor (Büchi, Switzerland) at 40 °C with a low vacuum (Kekilli et al. 2021; Ozer et al. 2021). The extracts were stored at +4°C to test further for enzyme inhibition assays and LC-Q-ToF-MS analysis.

Table 1- Collection Sites and Dates of *Polygonum* Species

<i>Samples</i>	<i>Collection sites</i>	<i>Collection date</i>
<i>P. aviculare</i>	C4 Konya; Çumra-Bozkir, step, 1060 m	May, 2020
<i>P. cognatum</i>	C4 Konya; Hadim-Calca, 1600 m	May, 2020
<i>P. patulum</i>	C4 Konya; Cumra-Bozkir, step, 1060 m	May, 2020
<i>P. setosum</i>	C4 Konya; Seydisehir, 1570 m	June, 2020

2.2. Analysis of compounds in extracts by LC-Q-ToF-MS

Analyzes were performed on the Agilent 1260 series HPLC system coupled with Agilent 6550 iFunnel High-Resolution Mass Spectrometer. Agilent Jet Stream Electrospray ionization technique was used in negative mode. MS operating mode 2 GHz Extended Dynamic Range. Thermo Hypersil Gold C-18 (4.6 mm x 150 mm x 3 µm) column was used for chromatographic separation. Agilent MassHunter Software B06.00 and Metlin Metabolit database were used for analysis and data evaluation. HPLC and MS parameters are given in Table 2 in detail.

Table 2- HPLC and MS Parameters

Analysis time	60 min.
Mobile Phase A	Ammonium acetate (5 mM)
Mobile Phase B	Acetonitrile
Flow	0.6 mL/min
Streaming program	0 min- 5 % B 0.5 min- 5 % B 20 min- 20 % B 30 min-40 % B 38 min- 70 % B 42 min- 90 % B 50 min- 90 % B 50.1 min- 5 % B
Ionization mode	Negative
Dryer gas temperature	250 °C
Dryer gas flow, N2	14 L/min
Nebulizer	20 psi
Sheath gas temperature	375 °C
Sheath gas flow, N2	12 L/min
Capillary Voltage	4500 V
Nozzle Voltage	2000 V
Mass reading range	100-1700 amu
Reference ions	966.725

The same conditions were applied to the extracts for analyzing resveratrol. Identification and quantifications were made by injecting standard reference substances for catechin, quercetin 3-glycoside, and quercetin 3-galactoside compounds, whose presence was detected in *Polygonum* extracts.

2.3. Enzyme inhibition assays

2.3.1. AChE-BChE inhibition

AChE and BChE inhibitory activity of the extracts was assessed using a modified Ellman spectrophotometric technique (Ellman et al. 1961). The environment parameters and other compounds were similar to those described in previous research (Orhan et al. 2007). Galanthamine hydrobromide was used as the reference drug.

2.3.2. Elastase inhibition

Elastase inhibition was tested using the spectrophotometric method of Kraunsoe et al., modified by Lee et al. (Kraunsoe et al. 1996; Lee et al. 2009). Previous study provides a thorough explanation of the enzyme inhibition experiments (Deniz et al. 2020). N-(Methoxysuccinyl)-Ala-Ala-Pro-Val-chloromethyl ketone (Sigma) was used as the reference.

2.3.3. Collagenase inhibition

Wart and Steinbrink's modified spectrophotometric technique was used to evaluate collagenase inhibition. (Barrantes et al. 2003; Van Wart and Steinbrink, 1981). Previous study provides a thorough explanation of the enzyme inhibition experiments (Deniz et al. 2020). 1,10-Phenanthroline was used as the reference.

2.3.4. TYR inhibition

L-DOPA (Sigma, USA), developed by Masamoto et al. (1980), was used in a spectrophotometric approach to evaluate TYR inhibition (Deniz et al. 2020; Lee et al. 2009). Kojic acid was used positive control.

The mean \pm standard deviation of the percentage of inhibitions from four experiments is used to present the results. The following formula was used to determine the samples' percentage of enzyme inhibition. $\text{Inhibition \%} = 100 - [(A1 / A2) \times 100]$

A1= Sample solutions' average absorbance

A2= Average absorbance of negative controls

3. Results

3.1. Phytochemical analysis of extracts by LC-Q-ToF-MS

Resveratrol was not detected by LC-Q-ToF-MS in *Polygonum* species. The identified compounds of *Polygonum* extracts as a result of LC-Q-ToF-MS analysis according to retention time (Rt), formula, and precursor ions are given in Table 3.

Table 3- Chemical Composition of *Polygonum* spp. According to LC-Q-ToF-MS Analysis

<i>Chemical name</i>	<i>R_t (min)</i>	<i>Formula</i>	<i>M-H ion</i>
Quinic acid	3.46	C ₇ H ₁₂ O ₆	191.0560
Galloilfructose/Glucose	3.69	C ₁₃ H ₁₆ O ₁₀	331.0630
Galloilfructose/Glucose	4.6	C ₁₃ H ₁₆ O ₁₀	331.0595
Gallic Acid	5.69	C ₇ H ₆ O ₅	169.0141
1-O-Galloylfructose	7.72	C ₁₃ H ₁₆ O ₁₀	331.0631
Gallocatechin/Epigallocatechin	8.66	C ₁₅ H ₁₄ O ₇	305.0597
Procyanidin B1	12.2	C ₃₀ H ₂₆ O ₁₂	577.1165
Catechin	13.82	C ₁₅ H ₁₄ O ₆	289.0662
Epicatechin	17.34	C ₁₅ H ₁₄ O ₆	289.0657
3-Galloylprocyanidin B1/B2	18.19	C ₃₇ H ₃₀ O ₁₆	729.1206
Catechin/Epicatechin gallate	23.62	C ₂₂ H ₁₈ O ₁₀	441.0698
Quercetin 3-glucoside	23.86	C ₂₁ H ₂₀ O ₁₂	463.0792
Rutin	23.89	C ₃₀ H ₂₆ O ₁₄	609.1327
Quercetin 3-galactoside	24.79	C ₂₁ H ₂₀ O ₁₂	463.0798
Quercetin	31.55	C ₁₅ H ₁₀ O ₇	301.0316

Total ion (TIC) and MS chromatograms of the compounds obtained from *Polygonum* extracts are given in Figure 1-8.

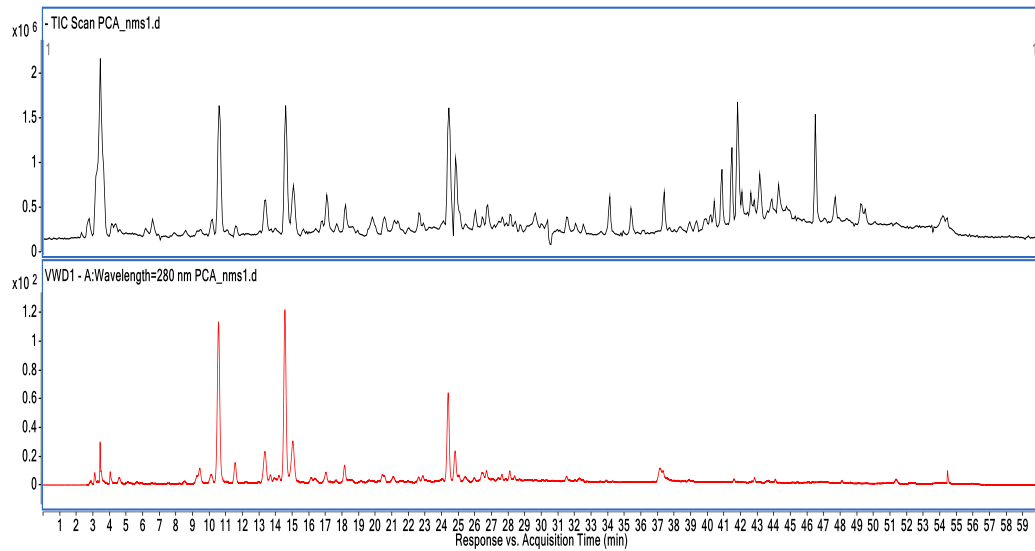


Figure 1- TIC and MS Chromatogram of *P. cognatum* Aerial Part Extract

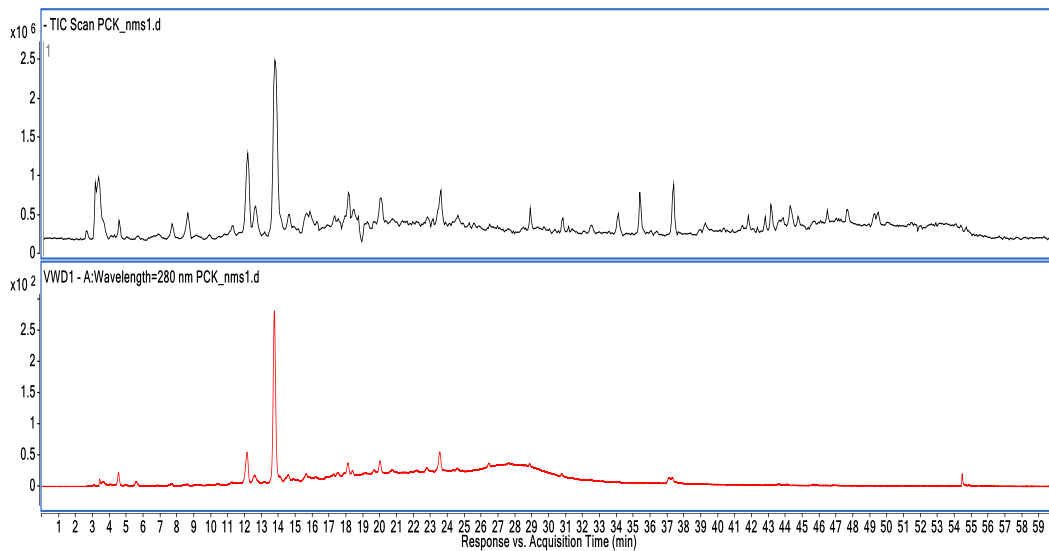


Figure 2- TIC and MS Chromatogram of *P. cognatum* Root Extract

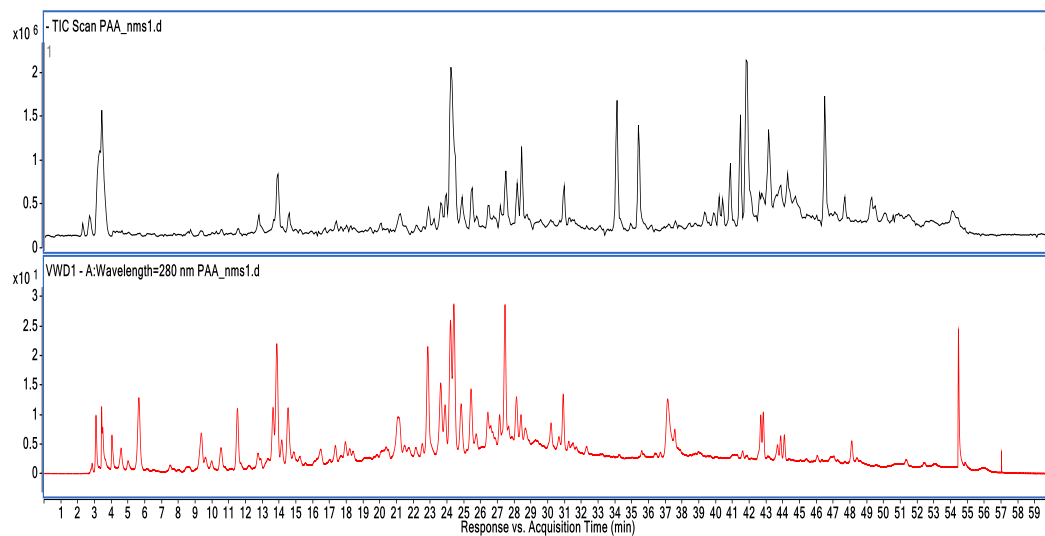


Figure 3- TIC and MS Chromatogram of *P. aviculare* Aerial Part Extract

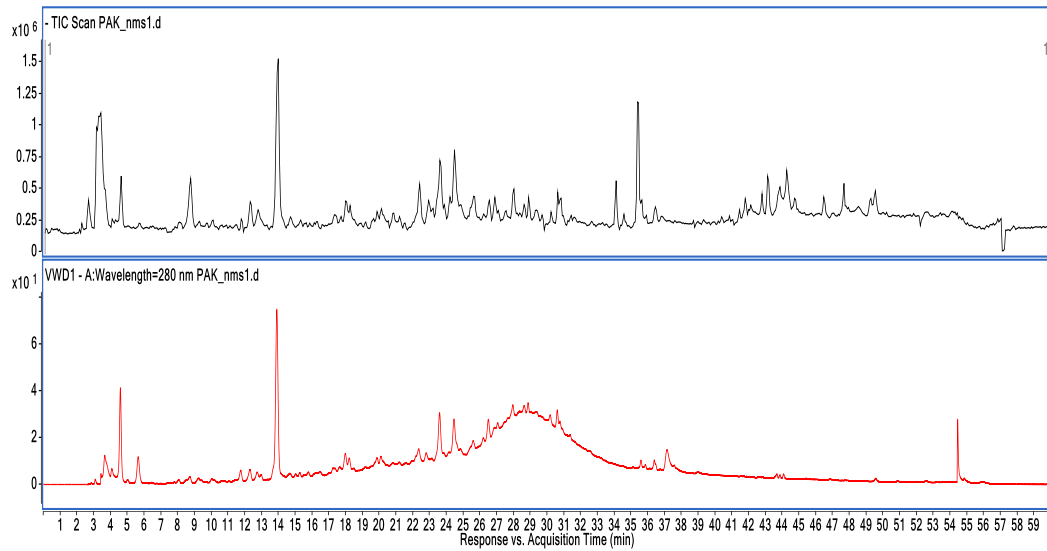


Figure 4- TIC and MS Chromatogram of *P. aviculare* Root Extract

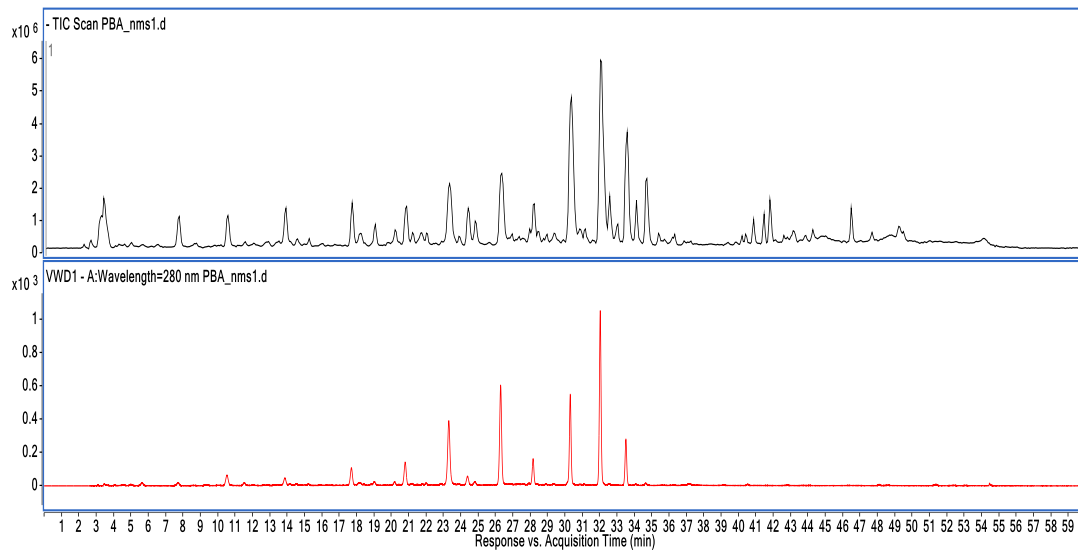


Figure 5- TIC and MS Chromatogram of *P. patulum* Aerial Part Extract

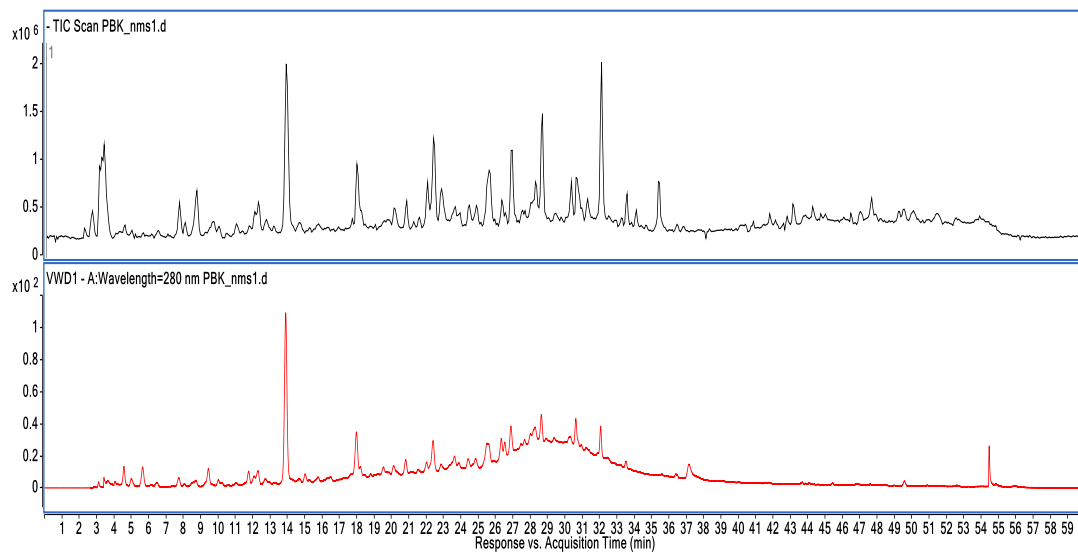


Figure 6- TIC and MS Chromatogram of *P. patulum* Root Extract

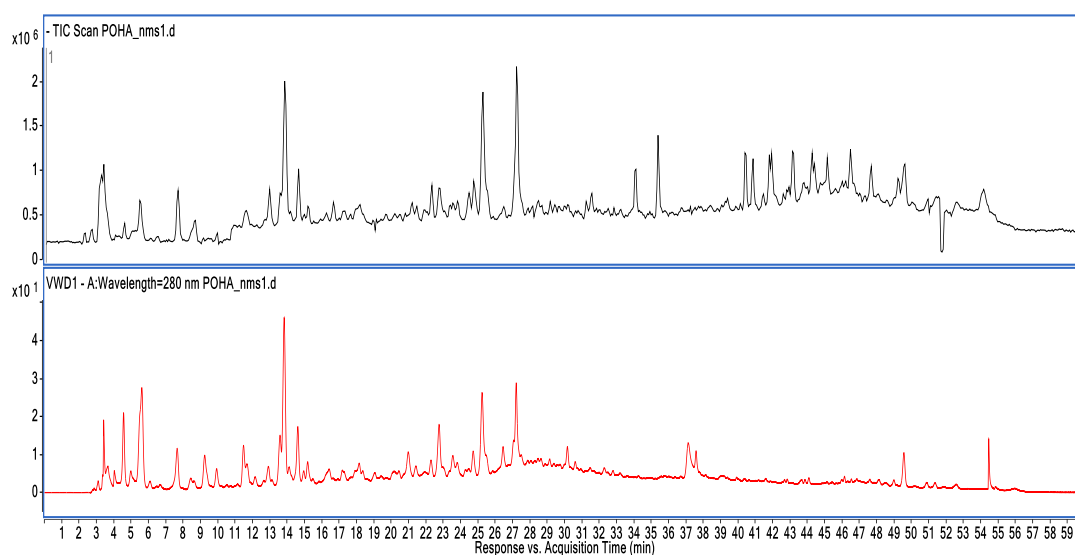


Figure 7- TIC and MS Chromatogram of *P. setosum* Aerial Part Extract

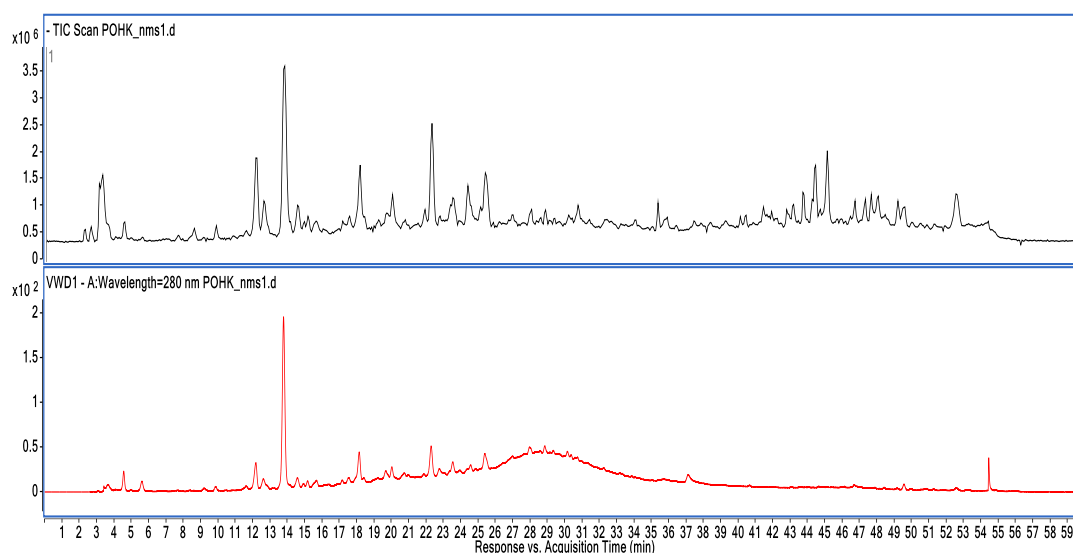


Figure 8- TIC and MS Chromatogram of *P. setosum* Root Extract

Catechin and quercetin glucosides were determined as major components by LC-Q-ToF-MS analysis on the extracts. The quantity of catechin and quercetin 3-glycoside/3-galactoside was calculated by external calibration using a standard mixture of the compounds. Results are given in Table 4-5.

Table 4- Major Compounds of *Polygonum* sp. Quantified Using LC-Q-ToF-MS Analysis

<i>Plant parts</i>	<i>Catechin (mg/g)</i>	<i>Quercetin 3-glucoside/3-galactoside (mg/g)</i>
<i>P. aviculare</i> aerial parts	2.78	0.93
<i>P. aviculare</i> roots	4.09	1.36
<i>P. cognatum</i> aerial parts	1.38	0.46
<i>P. cognatum</i> roots	4.62	1.54
<i>P. patulum</i> aerial parts	5.86	1.95
<i>P. patulum</i> roots	8.18	2.72
<i>P. setosum</i> aerial parts	5.06	1.69
<i>P. setosum</i> roots	10.46	3.48

Table 5- Regression Equations and LOD/LOQ of the Major Compounds

Compounds	Equation	r^2	LOD/LOQ ($\mu\text{g/ml}$)
Catechin	$y = 5.723x + 2.850$	0.9997	0.61/1.98
Quercetin 3-glucoside/3-galactoside	$y = 14.367x + 11.022$	0.9957	0.81/2.67

3.2. Enzyme inhibition results

Among the *Polygonum* extracts, *P. aviculare* root extract ($\text{IC}_{50} = 55.94 \pm 2.63 \mu\text{g/mL}$) showed the highest inhibition activity against AChE. All extracts were found to have significant inhibitory activity against AChE, and their IC_{50} values were determined. When the enzyme inhibitory activity of *Polygonum* extracts against BChE was evaluated, *P. aviculare* ($\text{IC}_{50} = 17.02 \pm 3.42 \mu\text{g/mL}$) and *P. patulum* ($\text{IC}_{50} = 18.36 \pm 0.15 \mu\text{g/mL}$) root extract exhibited the highest inhibition. Considering the BChE inhibition activity of galanthamine hydrobromide ($\text{IC}_{50} = 37.94 \pm 3.17 \mu\text{g/mL}$), the reference, roots of *P. aviculare* ($\text{IC}_{50} = 17.02 \pm 3.42 \mu\text{g/mL}$), *P. patulum* ($\text{IC}_{50} = 18.36 \pm 0.15 \mu\text{g/mL}$), *P. cognatum* ($\text{IC}_{50} = 31.01 \pm 1.98 \mu\text{g/mL}$), and *P. setosum* ($\text{IC}_{50} = 33.26 \pm 3.21 \mu\text{g/mL}$) displayed very potent inhibitory activity. All root extracts were found to be more active than the aerial parts extracts for both AChE and BChE (Table 6).

Table 6- Cholinesterases Enzyme Inhibition Activity of *Polygonum* sp.

Samples	Parts	Inhibition (Inhibition% \pm S.D. ^a) 100 $\mu\text{g/mL}$ ^b	
		AChE	BChE
<i>P. aviculare</i>	Aerial part	37.67 ± 4.56	56.74 ± 2.52 ($\text{IC}_{50} = 104.33 \pm 2.16 \mu\text{g/mL}$)
	Root	63.93 ± 1.75 ($\text{IC}_{50} = 55.94 \pm 2.63 \mu\text{g/mL}$)	88.07 ± 1.10 ($\text{IC}_{50} = 17.02 \pm 3.42 \mu\text{g/mL}$)
<i>P. patulum</i>	Aerial part	42.01 ± 1.80	54.41 ± 1.21 ($\text{IC}_{50} = 95.68 \pm 2.11 \mu\text{g/mL}$)
	Root	57.51 ± 2.89 ($\text{IC}_{50} = 74.65 \pm 1.15 \mu\text{g/mL}$)	86.78 ± 1.09 ($\text{IC}_{50} = 18.36 \pm 0.15 \mu\text{g/mL}$)
<i>P. cognatum</i>	Aerial part	- ^c	22.25 ± 1.50
	Root	51.60 ± 2.99 ($\text{IC}_{50} = 74.10 \pm 4.22 \mu\text{g/mL}$)	84.55 ± 1.83 ($\text{IC}_{50} = 31.01 \pm 1.98 \mu\text{g/mL}$)
<i>P. setosum</i>	Aerial part	41.55 ± 1.01	58.05 ± 1.74 ($\text{IC}_{50} = 46.75 \pm 0.70 \mu\text{g/mL}$)
	Root	55.50 ± 2.21 ($\text{IC}_{50} = 81.21 \pm 1.49 \mu\text{g/mL}$)	86.39 ± 1.10 ($\text{IC}_{50} = 33.26 \pm 3.21 \mu\text{g/mL}$)
Galanthamine hydrobromide ^d		90.47 ± 0.76 ($\text{IC}_{50} = 0.91 \pm 0.03 \mu\text{g/mL}$)	70.95 ± 1.51 ($\text{IC}_{50} = 37.94 \pm 3.17 \mu\text{g/mL}$)

^a Standard deviation, ^b Final concentration, ^c No activity, ^d Final concentration is 100 $\mu\text{g/mL}$

All *Polygonum* root extracts exhibited moderate inhibitory activity against TYR. *P. cognatum* (48.64%) and *P. setosum* (45.99%) root extracts had the highest inhibition (Table 6). Root extracts of *P. aviculare* ($\text{IC}_{50} = 169.33 \pm 3.58 \mu\text{g/mL}$), *P. patulum* ($\text{IC}_{50} = 326.63 \pm 2.84 \mu\text{g/mL}$), and *P. setosum* ($\text{IC}_{50} = 247.50 \pm 5.02 \mu\text{g/mL}$) displayed remarkable inhibitory activity against elastase (Table 6). While root of *P. aviculare* ($\text{IC}_{50} = 220.50 \pm 1.13 \mu\text{g/mL}$) and *P. patulum* ($\text{IC}_{50} = 297.70 \pm 2.40 \mu\text{g/mL}$), aerial parts ($\text{IC}_{50} = 220.35 \pm 3.46 \mu\text{g/mL}$) and root ($\text{IC}_{50} = 200.30 \pm 2.26 \mu\text{g/mL}$) of *P. cognatum* extracts showed high inhibition against collagenase, the inhibition values of these extracts were found to be similar to each other. The *P. cognatum* ($\text{IC}_{50} = 200.30 \pm 2.26 \mu\text{g/mL}$) root extract has the highest anti-collagenase activity among the extracts. In general, all extracts showed intense anti-collagenase activity (Table 7).

Table 7- TYR, Elastase, and Collagenase of Five *Polygonum* sp.

Samples	Parts	Inhibition (Inhibition % \pm S.D. ^a) 333 μ g/mL ^b		
		TYR	Elastase	Collagenase
<i>P. aviculare</i>	Aerial part	- ^c	-	33.13 \pm 4.07 ^d
	Root	24.95 \pm 1.83	68.87 \pm 0.29 (IC ₅₀ = 169.33 \pm 3.58 μ g/mL)	69.63 \pm 2.92 (IC ₅₀ = 220.50 \pm 1.13 μ g/mL)
<i>P. patulum</i>	Aerial part	-	13.65 \pm 1.22	27.52 \pm 2.50
	Root	42.87 \pm 0.59	50.97 \pm 0.35 (IC ₅₀ = 326,63 \pm 2.84 μ g/mL)	55.54 \pm 3.52 (IC ₅₀ = 297.70 \pm 2.40 μ g/mL)
<i>P. cognatum</i>	Aerial part	-	16.47 \pm 0.94	56.87 \pm 5.66 (IC ₅₀ = 220.35 \pm 3.46 μ g/mL)
	Root	48.64 \pm 1.66	11.04 \pm 1.22	74.46 \pm 3.53 (IC ₅₀ = 200.30 \pm 2.26 μ g/mL)
<i>P. setosum</i>	Aerial part	-	6.90 \pm 1.76	36.31 \pm 2.31
	Root	45.99 \pm 2.30	62.92 \pm 0.98 (IC ₅₀ = 247.50 \pm 5.02 μ g/mL)	16.37 \pm 2.51 ^d
Reference		84.56 \pm 0.27 ^e (IC ₅₀ = 0.68 \pm 0.05 μ g/mL)	99.65 \pm 0.08 ^f (IC ₅₀ = 2.65 \pm 0.36 μ g/mL)	87.39 \pm 2.85 ^g (IC ₅₀ = 27.95 \pm 0.37 μ g/mL)

^a Standard deviation, ^b Final concentration, ^c No activity, ^d Final concentration is 166 μ g/mL, ^e Alpha-Kojic acid (133 μ g/mL) ^f N-(Methoxysuccinyl)-Ala-Ala-Pro-Val-chloromethyl ketone (66,67 μ g/mL), ^g 1,10-Phenanthroline (66,67 μ g/mL)

4. Discussion

Polygonum is a genus with a wide distribution in Türkiye and is rich in endemic species. The studies investigated the potential of different *Polygonum* species for treating neurodegenerative diseases. In a study, *P. multiflorum* showed potent inhibition against cholinesterases (IC₅₀AChE= 9.11 μ g/mL, IC₅₀BChE= 4.83 μ g/mL) (Li et al. 2017). Methanol extracts of *P. maritimum* had high inhibition towards AChE (leaves: IC₅₀= 0.27 mg/mL; roots: IC₅₀= 0.17 mg/mL) and BChE (leaves: IC₅₀= 0.62 mg/mL; roots: IC₅₀= 0.61 mg/mL) (Rodrigues et al. 2018). The aerial parts of four *Polygonum* species growing in the area of Istanbul were examined for their anti-AChE potential and other biological activities. The maximum inhibitory activity against AChE was 400 μ g/mL (> 80%) for *P. istanbulicum* and *P. patulum* subsp. *pulchellum* extracts. These were followed by the *P. aviculare* (75.59 \pm 2.24%) and *P. lapathifolium* extracts (60.55 \pm 2.76%) (Yilmaz-Ozden et al. 2021). In the cholinesterase inhibition study of *P. hydropiper* essential oil, leaf and flower essential oils of the plant were highly inhibited against AChE (IC₅₀= 120 μ g/ml and 220 μ g/ml, respectively) and BChE (IC₅₀= 130 μ g/ml and 225 μ g/ml, respectively) (Ayaz et al. 2015). In the current study, the AChE inhibition activities of the ethanol extracts of *P. aviculare* and *P. patulum* aerial parts were determined as 37.67% and 42.01%, respectively, at 100 μ g/mL concentration. In previous studies, the cholinesterase inhibitory activity of *P. aviculare* and *P. patulum* extracts was higher than our results.

Cosmetic-related enzyme inhibitory activities were found to be high in some species of *Polygonum*. Studies have reported that formulations containing *P. aviculare* and *P. minus* extracts have wrinkle-reducing effects (Haris et al. 2014; Loing et al. 2013). (+)-Taxifolin isolated from *P. hydropiper* sprout (70%) and acetone extract of *P. maritimum* showed potent anti-TYR activity (Miyazawa et al. 2007; Rodrigues et al. 2019). In addition, in a study, the anti-melanogenic effect of *P. tinctorium* flower extract was examined, and the extract decreased the amount of melanin and TYR activity. In the same study, it also strongly inhibited the expressions of TYRP1 and TYRP2. In the study, HPLC analysis of the plant extract indicated the main compounds as isoquercitrin and quercetin (Chung et al. 2018). It was found that the methanolic extract of *P. hydropiper* sprout performed a dose-dependent collagenase inhibition, while the methanolic extract of *P. lapathifolium* (IC₅₀= 0.0003 \pm 0.0002 μ g/mL) had high elastase inhibitory activity (Kawaguchi et al. 2019; Sokmen et al. 2017). The collagenase inhibition by hyperoside, the major compound in *P. hydropiper*, was high (IC₅₀= 1.9 μ g/mL) and may be responsible for the effect (Kawaguchi et al. 2019). The elastase inhibitory activities of the aqueous extract of *P. cuspidatum* and the fraction obtained from the aqueous extract were found to be 53.56% and 61.27%, respectively. Polydatin as a resveratrol derivative, which is the main component of the extract, possessed 82.53% inhibition (Xiao-jing et al. 2012).

In the phytochemical analysis study, *Polygonum* extracts were found to be rich in phenolic compounds. In previous studies, it was determined that *Polygonum* species are rich in flavonoids, which is one of the chemotaxonomic indicators of the genus, and that they have a wide content of triterpenoids, anthraquinones, coumarins, phenylpropanoids, lignans, sesquiterpenoids, stilbenoids and tannins, in addition to flavonoids (Narasimhulu et al. 2014). This study determined that the major components in *P. setosum*, *P. cognatum*, *P. aviculare*, *P. patulum* species were catechins, phenolic acids, quercetin, and quercetin glycosides. In one study, quercetin (IC₅₀-AChE = 34.46 μ g/mL, IC₅₀-BChE = 99.25 μ g/mL) had high anticholinesterase activity potential, while catechin and epicatechin had no or low inhibitory activity on AChE and BChE (IC₅₀ > 1000 μ g/mL) (Li et al. 2023). In a study, catechin showed low inhibitory activity against AChE (6.45%) and BChE (18.26%) (Okello and Mather, 2020). In another study where catechin was tested against cholinesterase, it was found that it did not cause any inhibition against either AChE or

BChE (Orhan et al. 2019). Another study found that quercetin had significantly high inhibitory abilities of AChE ($IC_{50} = 0.181$ mM) and BChE ($IC_{50} = 0.203$ mM) (Ademosun et al. 2016). It can be said that the presence of quercetin and its glycosides in *Polygonum* extracts contributes more to the cholinesterase inhibitory activity than catechin.

In this study on catechin, a major component in *Polygonum* extracts, the inhibition of collagenase by green tea polyphenols (catechin and epigallocatechin gallate) was evaluated. Catechin (70%) and epigallocatechin gallate (88%) highly inhibited the collagenolytic activity of collagenase versus collagen in a dose-dependent manner (Madhan et al. 2007). It was found that catechin isolated from *Callistemon lanceolatus* Sm. showed high inhibition ($IC_{50}=20.2$ μ g/mL) against elastase enzyme (Kim et al. 2009). In the study examining the anti-TYR effect of polyphenolic compounds found in green tea; catechin, epicatechin and epigallocatechin inhibitory activities were examined. All three compounds showed moderate TYR inhibitory activity in the study (No et al. 2009). In another study investigating the elastase, collagenase, and TYR inhibitions of *Cotinus coggygia* Scop. leaves and pedicels, the active fractions obtained from pedicel extract as a result of activity-guided fractionation were methyl gallate, quercetin 3-glucoside, quercetin 3-galactoside (Deniz et al. 2020). In the study investigating the human neutrophil elastase inhibitory effect of *Drosera madagascariensis* DC., it was determined that the compounds responsible for the enzyme inhibition were quercetin, quercetin 3-galactoside (hyperoside), and quercetin 3-glucoside (isoquercetin) (Melzig et al. 2001). In another study, quercetin 3-O-rhamnoside, quercetin 3-O-galactoside, quercetin 3-O-arabinoside were found to have 32.61%; 38.05%; 30.47% anti-collagenase; 39.46%; 50.84%; 41.25% anti-elastase activity, respectively (Ozbilgin et al. 2018). When compared with all of studies, it is predicted that the cosmetic-related enzyme inhibition activities of *Polygonum* species may be due to both catechin and quercetin/quercetin glycosides. In this study, it was determined that the roots of *Polygonum* species were much more active than the aerial parts. At the same time, based on LC-Q-ToF-MS analyses, the catechin and quercetin glycosides were found at a higher rate in the root extracts than the aerial part extracts of *Polygonum* species. The components mentioned above are found in *Polygonum* root extracts at a higher rate than in aerial parts such as catechin and quercetin glycoside, which explains their more powerful cosmetic related enzyme inhibition.

In literature studies, the name *P. cuspidatum* is now included in the records as a synonym of *Reynoutria japonica* Houtt.. Likewise, *P. ciliinerve* (*Reynoutria ciliinervis* (Nakai) Moldenke) and *P. multiflorum* (*Reynoutria multiflora* (Thunb.) Moldenke), which were found to contain resveratrol, were also found to be transferred to the *Reynoutria* genus by conducting phylogenetic studies. *R. japonica* was recorded for the first time in Türkiye in 2020 (Karaer et al. 2020).

5. Conclusions

It has been determined that the *Polygonum* species studied do not contain resveratrol. On the other hand TYR, collagenase, elastase, and cholinesterase are all inhibited by them. Glycosides of quercetin and catechin, which are more abundant in plant root extracts, may be the components causing these effects. Other compounds that may be responsible for the studied activities can be detected by performing activity-guided fractionation in active extracts. In light of all these data, the *Polygonum* species studied should be examined with further research related to the development of herbal cosmetic products due to their activity and bioactive compounds. In this study, some of the *Polygonum* species grown in Türkiye were screened for the first time against cosmetic and inflammation-related enzymes and investigated in terms of their phytochemical contents.

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Conflict of interest

The authors confirm that there are no conflicts of interest associated with this publication.

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