South African Journal of Botany 143 (2021) 282–290

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Sophora alopecuroides var. *alopecuroides*: Phytochemical composition, antioxidant and enzyme inhibitory activity of the methanolic extract of aerial parts, flowers, leaves, roots, and stems

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ARTICLE INFO

Article History:
Received 31 August 2020
Revised 8 October 2020
Accepted 10 October 2020
Available online 28 October 2020

Edited by KRR Rengasamy

Keywords:
Sophora alopecuroides var. *alopecuroides*
Biological activity
Chemical composition

ABSTRACT

Sophora alopecuroides L. are commonly used in traditional Chinese medicine for the treatment of various ailments such as refractory tinea, eczema, dermatitis, colpitis, bacillary dysentery, and other gastrointestinal diseases. The aim of this study was to investigate the chemical composition, antioxidant, and enzyme inhibitory activity of the aerial part, flower, leaf, root, and stems extracts of *S. alopecuroides* var. *alopecuroides*. Major compounds of the extracts were found to be ferulic acid, luteolin, hesperidin and pinoresinol. In many of the antioxidant activity assays, the root extract exhibited high activity. In CUPRAC reducing, phosphomolybdenum, ABTS, and DPPH tests, the activity of the root extract was 1.42, 2.45, 1.25, and 8.41 mg/mL, respectively. Root extract also exhibited significant activity in enzyme inhibitory assays. Tyrosinase, α-amylase, α-glucosidase, and acetyl cholinesterase (AChE) inhibitory activities of the extract were determined as 1.22, 2.97, 1.03, and 0.97 mg/mL, respectively. Chromatographic analysis showed that the root extract contained 462.25 μg/g pinoresinol and 256.23 μg/g vanillic acid as the major compounds. Pearson correlation analysis showed that besides flavonoids/phenolics, these phytochemicals also contributed to the activity of root extract. Therefore, it was concluded that these phytochemicals may be responsible for the activity of the root extract. The root extract of *S. alopecuroides* var. *alopecuroides* was thought to be an effective antioxidant and enzyme inhibitory agent.

1. Introduction

The members of the *Sophora* genus spread in both hemispheres of the earth in the tropical and subtropical regions of the world. It is known to have more than 70 species ranging from small trees to shrubs (Wang et al., 2020). *S. alopecuroides* grows widely in Western and Central Asian countries. Mongolia (Kwon et al., 2015), Iran (Jaktaji and Mohammadi, 2018), Pakistan (Choudhary et al., 2000), and Japan (Iinuma et al., 1995) are the leading countries where this species distributed. Both aerial parts and seeds of *S. alopecuroides* have been used in China since ancient times to treat fever and gastrointestinal disorders. It is also one of the plants used in traditional Chinese medicine as anti-rheumatic, antibacterial and analgesic agents (Wang et al., 2018; Zhang et al., 2017; Zhao et al., 2011). In studies conducted to scientifically confirm the ethno-pharmacological potential, *S. alopecuroides* has been reported to show antiviral (Zhang et al., 2017), antitumor (Li et al., 2016; Ling et al., 2018), neuroprotective (Wang et al., 2013; Zhao et al., 2018), antibacterial (Wan et al., 2015), antioxidant (Li et al., 2017), analgesic (Yang et al., 2015a, 2015b), cardio-protective (Guo et al., 2018), anti-inflammatory (Guo et al., 2016) and antiarrhythmic activity (Zhang and Li, 1999).

S. alopecuroides was determined to contain many compounds from the groups of polysaccharides, steroids, alkaloids, and flavonoids. Detailed studies have been conducted on quinolizidine alkaloids, and these compounds have been reported to be of great medical importance (Wang et al., 2020). *S. alopecuroides* has a highly developed root system. Therefore, it has a high tolerance against drought and alkaline substances. It is also highly resistant to sandstorms. The plant has also been proven to perfectly tolerate herbicides and pesticides (Zhao et al., 2010).

There are two varieties of *S. alopecuroides* in the Flora of Turkey named as var. *alopecuroides* and var. *tomentosa* (Boiss.) Chamberlain, of which var. *alopecuroides* is most widespread. The roots, stems, seeds, and whole plants are commonly used in traditional medicine for the treatment of various ailments such as refractory tinea, eczema, dermatitis, colitis, bacillary dysentery, and other gastrointestinal diseases (Huang et al., 2016).

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https://doi.org/10.1016/j.sajb.2020.10.009
0254-6299/

The aim of this study was to provide data on characterization of phenolic profile and to evaluate the antioxidant and enzyme inhibitory potential of the various parts of *S. alopecuroides* var. *alopecuroides*. Further, this study also aimed to clarify the relationship among the phytochemicals and their bioactivities using correlation analysis.

2. Materials and methods

2.1. Plant material

Plant species: *S. alopecuroides* var. *alopecuroides*
Locality: Sucati village, Gurun, Sivas-Turkey
Collection date: 23 June 2015
GPS coordinates: 1351 m, 38° 43′ 15.06″N and 37° 21′ 43.22″E
Taxonomist identifying the plant species: Dr. Olcay Ceylan
Herbarium number: AD-1518
All the parts of the plant material were air-dried in the shade for several weeks, and then ground using a laboratory mill.

2.2. Preparation of the methanolic extracts

The methanol extracts from different parts of *S. alopecuroides* var. *alopecuroides* were prepared by maceration for 24 h. The extracts were evaporated under reduced pressure to remove the solvent used for the extraction. They were then stored at refrigerator temperature. Data on extract yields can be found in Table 1.

2.3. Liquid chromatography–electrospray tandem mass spectrometry (LC–ESI–MS/MS) analysis

Details of the spectrophotometric and chromatographic methods performed were given in the supplementary file (Sarikurkcu et al., 2015; Tlili et al., 2019).

2.4. Determination of the activity potentials of the samples

Antioxidant, and enzyme inhibitory activities of the extracts were performed according to the literature given (Apak et al., 2006; Kocak et al., 2016; Odabas Kose et al., 2010; Sarikurkcu et al., 2018; Zengin et al., 2015). Details of the experimental procedures can be found in supplementary file.

2.5. Statistical analysis

The data obtained from the activity tests were presented as mean ± standard deviation. All tests were performed in triplicate. One-way ANOVA by Tukey's test was used for statistical evaluation of the data (significance level is 5%). Correlations between variables were determined by using Pearson's correlation test. Statistical analysis was carried out using the SPSS v22.0 software.

3. Results and discussion

Quantities of flavonoids, phenolics and selected phytochemicals in MeOH extracts were given in Tables 1 and 2, respectively. The RACI (relative antioxidant capacity index) values of the extracts can be seen in Fig. 1. Table 3 and Fig. 2 contain antioxidant activity data. Correlation of antioxidant activity data with RACI values was presented in Fig. 3. While data from enzyme inhibitory activity tests were presented in Table 4 and Fig. 4, the correlations between phytochemicals and assays were also given in Table 5.

3.1. Comparison of the phytochemical compositions of plant parts

As stated in Section 2.2, the yields of the extracts were given in Table 1. Yields of aerial part, flower, leaf, and root extracts were between 13 and 14%. However, the yield of the stem extract was lower than the others. Except for the root extract, the amount of phenolics in all other extracts was higher than the flavonoids. The total flavonoid contents of the aerial part, flower, leaf, and stem extracts were between 5.73–7.94 mg QEs/g. However, the flavonoid content of the root extract was two times higher than the others. The amount of phenolics in aerial part and stem extracts were almost equal to each other. On the other hand, the phenolic contents of flower, leaf and root extracts were found to be between 9.01–10.61 mg GAEs/g.

Ferulic acid, luteolin, hesperidin and pinoresinol were the main components (Table 2). Additionally, it was also determined that apigenin and vanillic acid were in high amounts in the extracts. Ferulic acid, a carboxylic acid, was the main component in all extracts except the extract obtained from root. Pinoresinol, a lignan, was the major compound in aerial part, root, and stem extracts. While luteolin, which is a flavonoid with a yellow crystalline appearance, was the main component of aerial part and flower extracts, another flavonoid, apigenin, was high in aerial parts and leaves. Vanillic acid was also found to be in high quantities in root and stem extracts. In addition, it was found that hesperidin was the main component in flowers, *p*-coumaric acid in leaves, and protocatechuic acid, syringic acid, and vanillin in stem.

Essential oil of *S. alopecuroides* has been previously analyzed by many researchers. In these reports, flavonoids and flavonoid glycosides (Bian et al., 2014; Iinuma et al., 1995; Kwon et al., 2015; Ni et al., 2014; Wan et al., 2015; Yang et al., 2013; Ye et al., 2009), steroid compounds (Bian et al., 2014; Ma and Zhang, 2003), polysaccharides (Cao et al., 2014; Wu et al., 2018) and volatile oil components (Chen and Yang, 2006) were documented. But limited data was available on the chemical composition of var. *alopecuroides* in the literature. In these studies, the researchers have focused especially on alkaloids isolated from the seeds of the variety in question (Kianbakht and Dabaghian, 2016; Kianbakht et al., 2017; Kucukboyaci et al., 2011). Therefore, the chemical composition data presented in the current study was the first record on this variety.

Table 1
Extract yields and chemical compositions of the samples[1].

Samples	Extraction yield (%)	Total flavonoid (mg QEs/g extract)	Total phenolic (mg GAEs/g extract)
Aerial parts	13.12	6.86 ± 0.05^{bc}	8.61 ± 0.68^{a}
Flowers	13.14	7.61 ± 0.16^{b}	9.01 ± 0.68^{a}
Leaves	13.36	7.94 ± 0.21^{b}	10.61 ± 0.01^{a}
Roots	14.76	16.82 ± 0.69^{a}	10.05 ± 1.25^{a}
Stems	6.88	5.73 ± 0.05^{c}	8.33 ± 0.85^{a}

[1] Data with the same superscripts in the same column were statistically similar ($p < 0.05$). GAEs: Gallic acid equivalent, QEs: Quercetin equivalent.

Table 2
Amounts (μg/g extract) of phytochemicals in the samples[1].

Compounds	Aerial parts	Flowers	Leaves	Roots	Stems
Verbascoside	nd[2]	nd	nd	2.56 ± 0.10	nd
Vanillin	36.96 ± 0.03^{c}	21.34 ± 0.42^{d}	17.68 ± 0.05^{e}	62.23 ± 0.93^{b}	103.58 ± 1.54^{a}
Vanillic acid	142.22 ± 1.73^{c}	160.38 ± 0.92^{c}	87.84 ± 3.80^{d}	256.23 ± 8.75^{b}	302.52 ± 11.08^{a}
Taxifolin	nd	nd	nd	nd	nd
Syringic acid	51.87 ± 1.72^{c}	23.11 ± 0.13^{d}	14.13 ± 0.42^{e}	62.75 ± 2.99^{b}	109.93 ± 0.21^{a}
Sinapic acid	11.71 ± 0.05^{b}	13.47 ± 0.26^{b}	6.25 ± 0.92^{c}	2.26 ± 0.27^{d}	18.60 ± 1.54^{a}
Rosmarinic acid	2.48 ± 0.27^{b}	3.44 ± 0.07^{a}	2.35 ± 0.05^{bc}	1.90 ± 0.09^{c}	3.23 ± 0.07^{a}
Quercetin	2.56 ± 0.01^{d}	6.60 ± 0.17^{a}	2.16 ± 0.01^{d}	3.60 ± 0.16^{c}	5.26 ± 0.17^{b}
Pyrocatechol	nd	nd	nd	nd	nd
Protocatechuic acid	67.58 ± 1.68^{c}	150.08 ± 2.41^{a}	50.23 ± 0.19^{d}	39.68 ± 0.31^{e}	116.84 ± 0.39^{b}
Pinoresinol	334.08 ± 15.65^{b}	202.48 ± 7.59^{c}	65.13 ± 0.12^{d}	462.25 ± 25.98^{a}	423.44 ± 11.59^{a}
p-Coumaric acid	74.43 ± 0.84^{c}	125.64 ± 1.22^{b}	145.04 ± 3.73^{a}	14.71 ± 0.29^{e}	33.46 ± 0.30^{d}
Luteolin 7-glucoside	1.89 ± 0.12^{c}	4.20 ± 0.16^{b}	2.46 ± 0.05^{c}	14.75 ± 0.08^{a}	2.46 ± 0.41^{c}
Luteolin	290.80 ± 7.70^{b}	1750.67 ± 80.61^{a}	88.36 ± 1.74^{c}	12.86 ± 0.50^{c}	88.17 ± 9.83^{c}
Kaempferol	nd	nd	nd	4.03 ± 0.07	nd
Hyperoside	49.68 ± 1.16^{b}	270.99 ± 2.29^{a}	40.21 ± 0.16^{c}	1.95 ± 0.17^{e}	25.34 ± 0.84^{d}
Hesperidin	11.78 ± 0.26^{b}	2170.62 ± 167.51^{a}	12.95 ± 1.35^{b}	10.87 ± 0.10^{b}	16.51 ± 1.48^{b}
Gallic acid	16.68 ± 0.05^{b}	26.49 ± 0.83^{a}	10.84 ± 0.18^{c}	3.11 ± 0.25^{d}	24.34 ± 1.54^{a}
Ferulic acid	454.93 ± 3.46^{c}	1019.15 ± 13.14^{a}	622.93 ± 36.39^{b}	90.76 ± 2.02^{e}	216.82 ± 2.35^{d}
Eriodictyol	3.95 ± 0.12^{d}	14.20 ± 0.02^{a}	1.43 ± 0.08^{e}	5.20 ± 0.25^{c}	6.10 ± 0.03^{b}
Chlorogenic acid	1.87 ± 0.01^{b}	2.22 ± 0.45^{b}	6.47 ± 0.61^{a}	1.24 ± 0.06^{b}	2.52 ± 0.11^{b}
Caffeic acid	27.68 ± 3.96^{b}	51.53 ± 0.29^{a}	28.50 ± 4.31^{b}	14.54 ± 0.17^{c}	22.79 ± 0.52^{bc}
Apigenin 7-glucoside	nd	nd	nd	nd	nd
Apigenin	207.33 ± 7.21^{c}	433.12 ± 0.52^{a}	333.50 ± 1.80^{b}	17.24 ± 1.36^{e}	58.11 ± 0.06^{d}
4-Hydroxybenzoic acid	27.04 ± 0.36^{d}	69.04 ± 0.01^{a}	32.47 ± 0.53^{c}	63.30 ± 1.00^{b}	28.00 ± 1.10^{d}
3-Hydroxybenzoic acid	nd	6.03 ± 0.51	nd	nd	nd
3,4-Dihydroxyphenylacetic acid	5.42 ± 1.13^{b}	6.00 ± 0.10^{b}	4.27 ± 0.89^{b}	9.49 ± 0.87^{a}	11.70 ± 0.07^{a}
2-Hydroxycinnamic acid	nd	nd	1.49 ± 0.02	nd	nd
2,5-Dihydroxybenzoic acid	38.18 ± 0.58^{b}	115.89 ± 17.05^{a}	36.46 ± 0.39^{bc}	6.02 ± 0.42^{c}	28.61 ± 2.94^{bc}
(+)-Catechin	nd	nd	nd	nd	nd
(-)-Epicatechin	nd	nd	nd	nd	nd

[1] Data with the same superscripts in the same row were statistically similar ($p < 0.05$).
[2] nd: not detected.

3.2. Antioxidant activity

RACI values were given in Fig. 1. It has been determined that stem extract clearly showed stronger antioxidant activity compared to others. It was followed by aerial part, flower and leaf extracts, respectively. IC_{50} values (mg/mL) and positive control equivalents of antioxidant activity data were also given in Table 3 and Fig. 2, respectively.

CUPRAC reducing activities of extracts were higher than their FRAP reducing activities. Root extract showed higher activity in CUPRAC reducing assay than others. The activity potential of flower, leaf and aerial part extracts were too close to each other. On the other hand, stem extract showed the lowest activity. In FRAP assay, all the extracts showed almost equal activity. The aerial part extract showed the highest reducing power potential. Root, leaf, stem, and flower extracts followed the activity of the aerial part extract, respectively. The activities of the samples were highly correlated with RACI values (Fig. 3). While there was a high correlation between flavonoid content of extracts and their CUPRAC reducing activities, no significant correlation was determined between the results of CUPRAC and FRAP assays and the major compounds (Table 5).

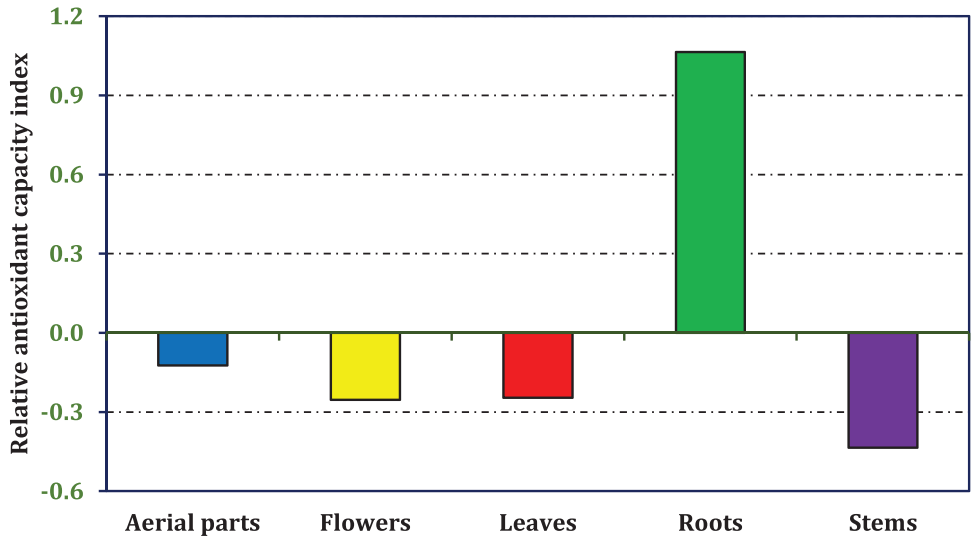

Fig. 1. RACI values of the extracts.

Table 3
Antioxidant activity (IC_{50}: mg/ml) of the samples[1].

Samples	CUPRAC reducing	FRAP reducing	Phosphomolybdenum	ABTS radical	DPPH radical	Ferrous ion chelating
Aerial parts	3.95 ± 0.06^{c}	2.67 ± 0.08^{b}	3.82 ± 0.37^{c}	2.81 ± 0.05^{c}	12.88 ± 1.74^{c}	1.02 ± 0.01^{b}
Flowers	$3.61 \pm 0.66b^{c}$	3.34 ± 0.25^{c}	2.92 ± 0.18^{b}	3.03 ± 0.10^{c}	13.61 ± 3.68^{c}	1.02 ± 0.01^{b}
Leaves	3.91 ± 0.25^{c}	2.99 ± 0.31^{bc}	3.59 ± 0.55^{c}	3.01 ± 0.22^{c}	14.82 ± 1.67^{c}	1.03 ± 0.01^{b}
Roots	1.42 ± 0.07^{ab}	2.94 ± 0.03^{bc}	2.45 ± 0.01^{b}	1.25 ± 0.01^{b}	8.41 ± 0.58^{b}	1.03 ± 0.01^{b}
Stems	4.42 ± 0.12^{c}	3.17 ± 0.11^{c}	2.85 ± 0.09^{b}	3.34 ± 0.01^{d}	15.57 ± 2.94^{b}	1.02 ± 0.01^{b}
Trolox	0.31 ± 0.02^{a}	0.11 ± 0.01^{a}	1.15 ± 0.01^{a}	0.26 ± 0.01^{a}	0.25 ± 0.01^{a}	–
EDTA	–	–	–	–	–	0.03 ± 0.003^{a}

[1] Data with the same superscripts in the same column were statistically similar ($p < 0.05$). EDTA: Ethylenediaminetetraacetic acid (disodium salt).

In phosphomolybdenum assay, root extract showed the highest activity. The total antioxidant activity of the root extract was 2.45 mg/mL (471.84 mg TEs/g). IC_{50} values of stem and flower extracts were also below 3.0 mg/mL, while the activities of leaf and aerial part extracts were 3.59 and 3.82 mg/mL, respectively. Activity data were highly correlated with RACI values (Fig. 3). As with the

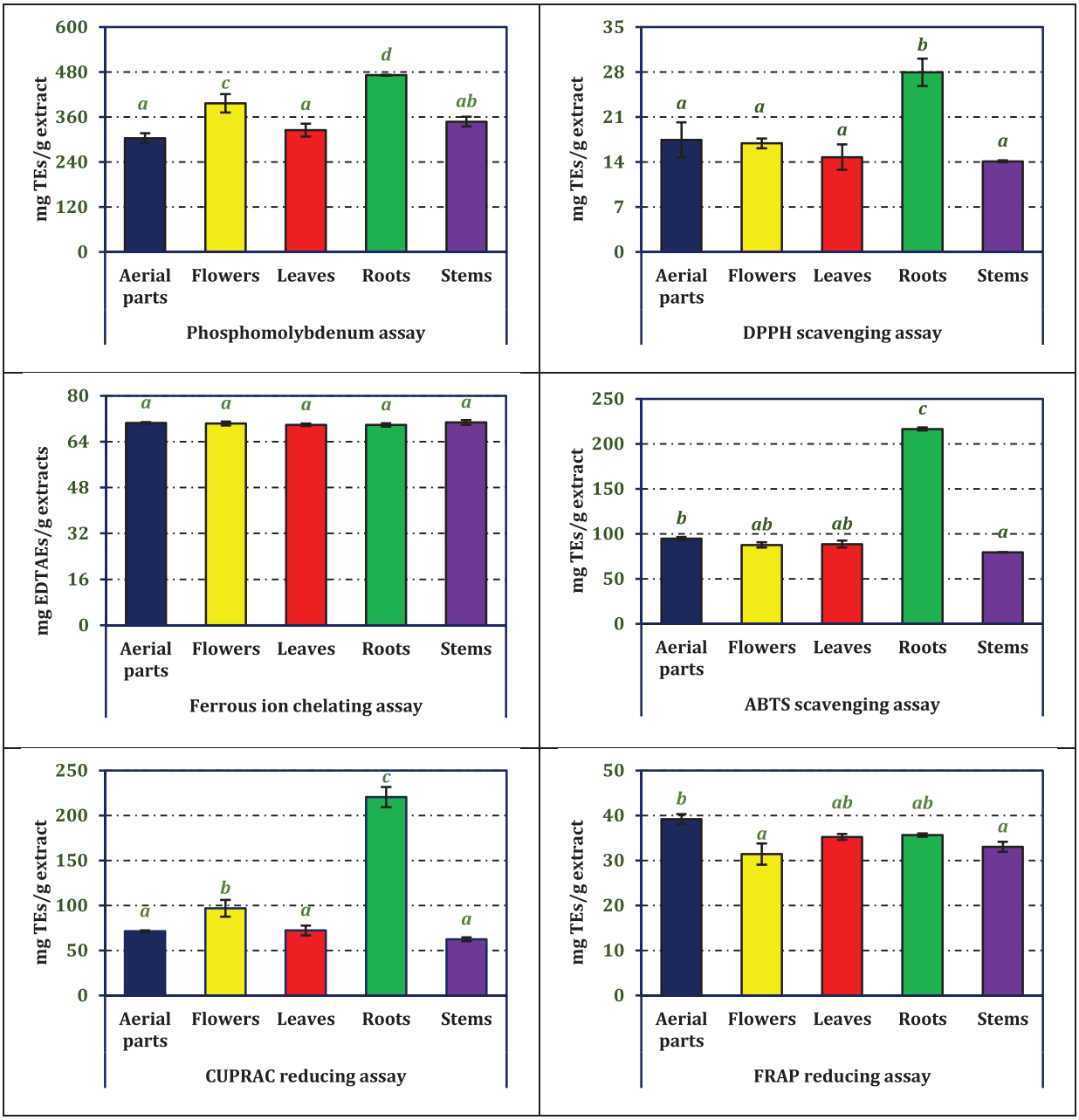

Fig. 2. Antioxidant activity of methanol extracts from different parts of *S. alopecuroides* var. *alopecuroides* TEs: trolox equivalents, EDTAEs: ethylenediaminetetraacetic acid (disodium salt) equivalents. Data with the same superscripts were statistically similar at 5% significance level.

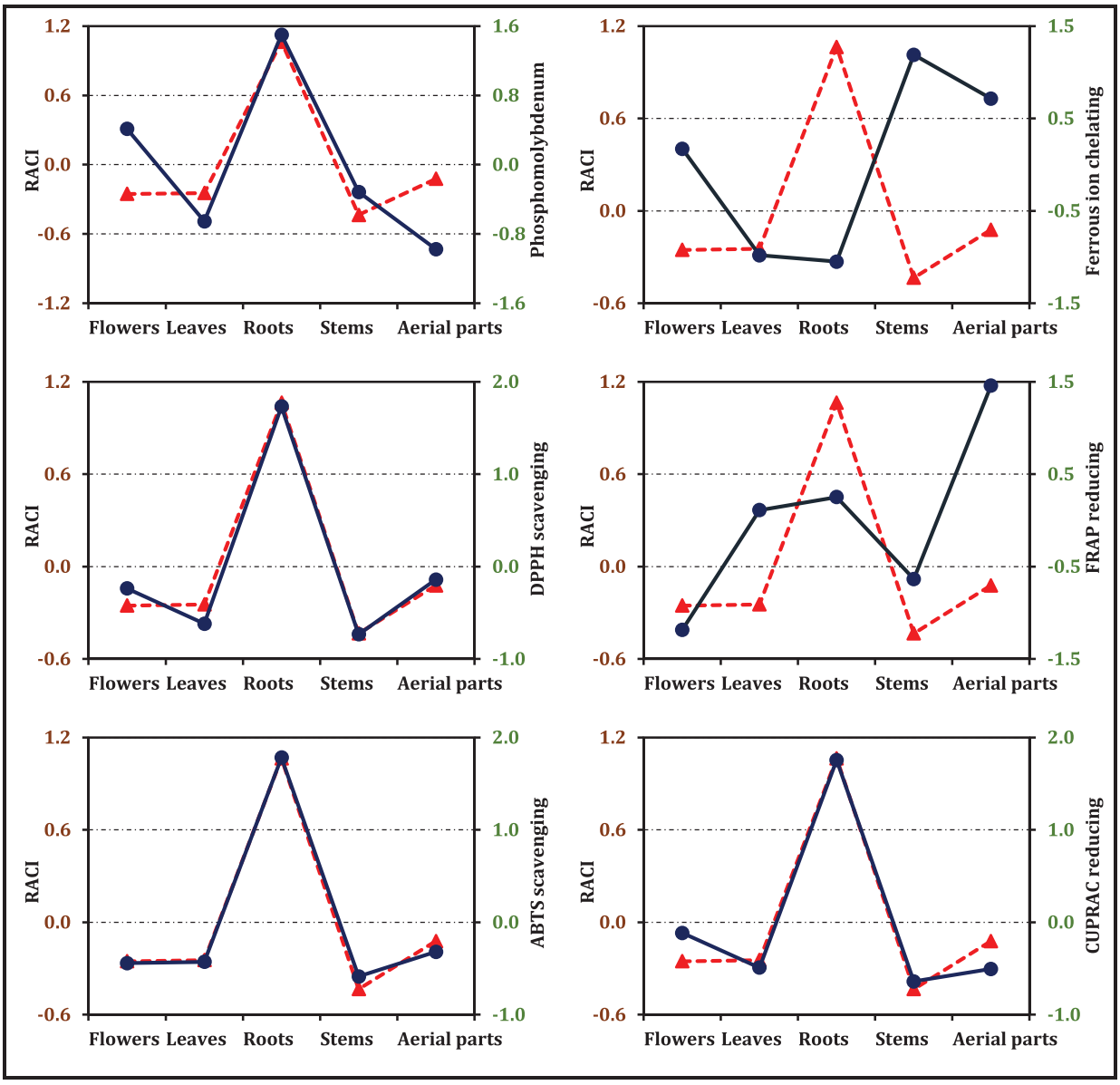

Fig. 3. Correlation between the antioxidant activity (solid dark blue line with circle) and RACI (dashed red line with triangle). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

reducing power test, correlation analysis showed that flavonoids were responsible for the activities of the extracts (Table 5). Pearson correlation analysis showed that vanillic acid contributed significantly to activity.

ABTS scavenging activities of the extracts were higher than DPPH. Root extract exhibited the strongest scavenging activity on both radicals (1.25 and 8.41 mg/mL, respectively). Aerial part, leaf, and flower extracts followed the root extract, respectively. Stem extract showed the weakest scavenging activity on both radicals (3.34 and 15.57 mg/mL, respectively). Scavenging activities of extracts on radicals were highly correlated with their RACI values (Fig. 3). Statistically, there was a correlation between radical scavenging activity and

Table 4
Enzyme inhibitory activities (IC_{50}: mg/ml) of the samples[1].

Samples	Tyrosinase inhibition	α-Amylase inhibition	α-Glucosidase inhibition	AChE inhibition	BChE inhibition
Aerial parts	1.29 ± 0.02^{bc}	3.80 ± 0.12^{d}	2.86 ± 0.09^{b}	1.07 ± 0.01^{d}	1.17 ± 0.01^{b}
Flowers	1.44 ± 0.08^{c}	4.49 ± 0.04^{e}	13.06 ± 0.09^{d}	1.15 ± 0.01^{e}	1.41 ± 0.01^{c}
Leaves	1.31 ± 0.01^{bc}	3.08 ± 0.03^{b}	4.16 ± 0.23^{c}	1.02 ± 0.01^{c}	1.17 ± 0.02^{b}
Roots	1.22 ± 0.02^{b}	2.97 ± 0.01^{b}	1.03 ± 0.01^{a}	0.97 ± 0.01^{b}	1.34 ± 0.03^{c}
Stems	1.35 ± 0.05^{bc}	3.50 ± 0.05^{c}	4.51 ± 0.61^{c}	1.15 ± 0.02^{e}	1.50 ± 0.03^{d}
Kojic acid	0.37 ± 0.01^{a}	–	–	–	–
Acarbose	–	1.21 ± 0.07^{a}	1.77 ± 0.04^{a}	–	–
Galanthamine	–	–	–	0.003 ± 0.0002^{a}	0.006 ± 0.001^{a}

[1] Data with the same superscripts in the same column were statistically similar at $p < 0.05$.

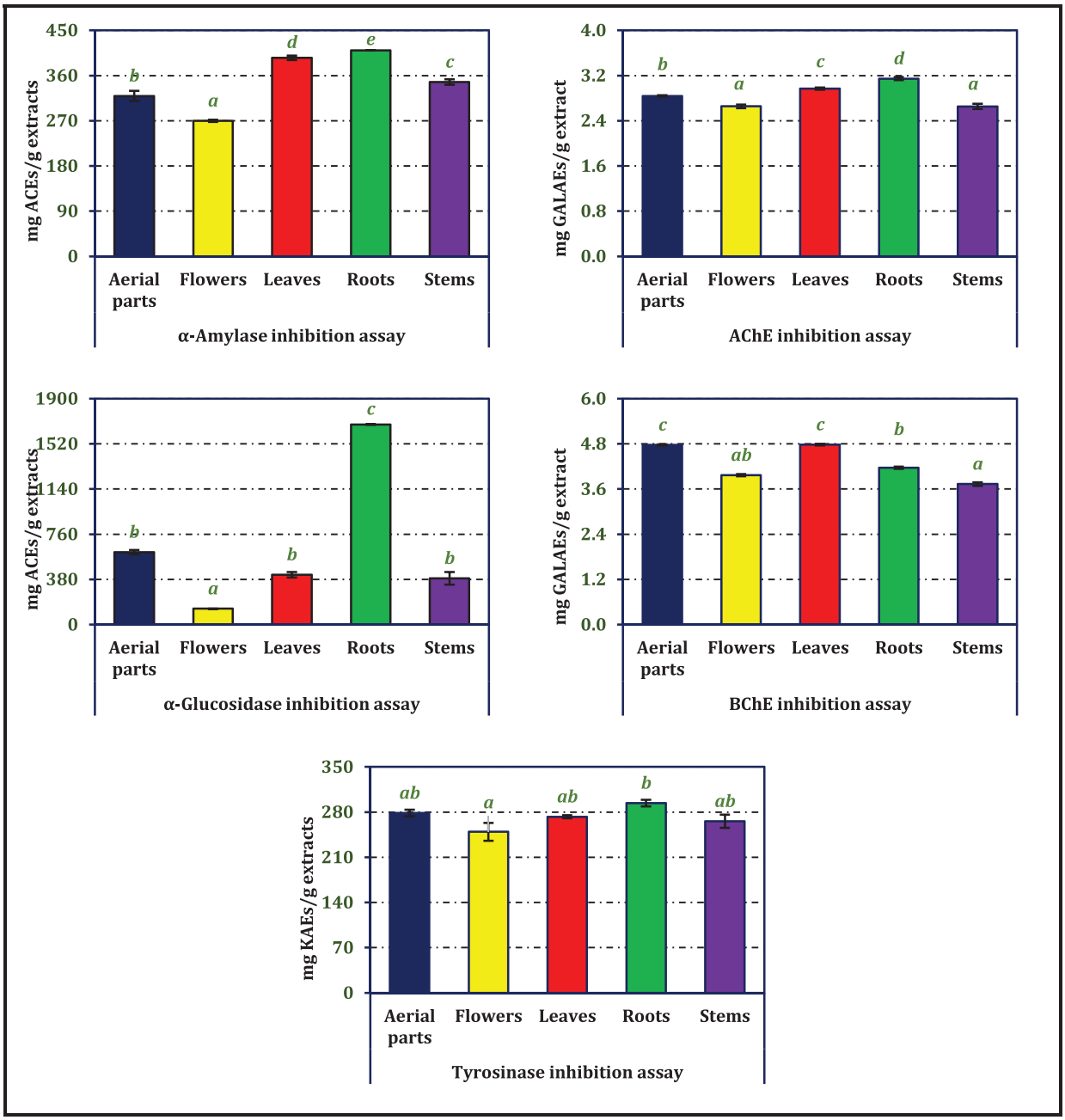

Fig. 4. Enzyme inhibition activity of methanol extracts from different parts of *S. alopecuroides* var. *alopecuroides*. ACEs: acarbose equivalents, GALAEs: galanthamine equivalents, KAEs: kojic acid equivalents. Data with the same superscripts were statistically similar at 5% significance level.

flavonoids. Also, vanillic acid and pinoresinol were other compounds contributing to this activity (Table 5).

In the case of ferrous ion chelating activity, activity potential of the extracts were almost equal. Chelating activities of aerial part, flower, and stem extracts were 1.02 mg/mL (70.52, 70.32, and 70.70 mg EDTAEs/g, respectively), and IC_{50} values of leaf and root extracts were determined as 1.03 mg/mL (69.88 and 69.85 mg EDTAEs/g). The Tukey test confirmed that the extracts were statistically similar. The chelating activities of the samples did not correlate with their RACI values (Fig. 3). Pearson correlation analysis showed that phenolics contributed more to the chelating activity than the activities given above. The statistical analysis in question showed that vanillic acid and pinoresinol partially contributed to the activity, as in the radical scavenging test (Table 5).

According to the literature records, the antioxidant activity of *S. alopecuroides* var. *alopecuroides* has not been studied. However, antioxidant activity of the plant species evaluated in the current study was studied only by Li et al. (2017). According to this report, the IC_{50} of alkaloid-rich extract of *S. alopecuroides* was reported as 0.40 mg/mL in the DPPH radical scavenging test. This value was better than the radical scavenging capacity of the species given here. This may be due to the contribution of alkaloids to scavenging activity.

As can be seen from the data given above, root extract was more active in most of the antioxidant activity tests than the others. According to the results of chromatographic analyzes, pinoresinol and vanillic acid were the major compounds of the root extract. Although these compounds partially contributed to antioxidant activity, it would be reasonable to discuss the literature data on their antioxidant activities. In a study carried out by Deveci et al. (2019), MeOH extract of *Porodaedalea pini* was reported to exhibit high antioxidant activity. Chemical structures of main components were

Table 5
Correlations among phenolic compounds and assays[1].

	CUPRAC	FRAP	TAP	ABTS	DPPH	FICA	TIA	AAIA	AGIA	AChEIA	BChEIA
FRAP	0.049										
TAP	0.915[2]	−0.349									
ABTS	0.981[3]	0.208	0.831								
DPPH	0.978[3]	0.227	0.833	0.983[3]							
FICA	−0.624	−0.072	−0.491	−0.611	−0.555						
RACI	0.975[3]	0.264	0.804	0.996[3]	0.990[3]	−0.629					
TIA	0.633	0.698	0.297	0.768	0.710	−0.463					
AAIA	0.482	0.317	0.263	0.590	0.445	−0.640	0.799				
AGIA	0.901[2]	0.394	0.681	0.967[3]	0.933[2]	−0.531	0.899[2]	0.691			
AChEIA	0.728	0.506	0.435	0.806	0.747	−0.830	0.877	0.824	0.845		
BChEIA	−0.162	0.769	−0.494	−0.061	−0.053	−0.380	0.371	0.253	0.045	0.471	
TFC	0.986[3]	0.138	0.854	0.987[3]	0.967[3]	−0.718	0.715	0.599	0.928[2]	0.830	−0.029
TPC	0.431	0.074	0.296	0.435	0.351	−0.968[3]	0.402	0.684	0.385	0.770	0.461
Vanillic acid	0.348	−0.287	0.478	0.355	0.312	0.357	0.181	0.144	0.378	−0.114	−0.808
p-Coumaric acid	−0.527	−0.185	−0.459	−0.599	−0.572	−0.206	−0.584	−0.338	−0.687	−0.236	0.451
Ferulic acid	−0.435	−0.404	−0.247	−0.569	−0.475	0.020	−0.801	−0.704	−0.731	−0.491	0.114
Hesperidin	−0.066	−0.663	0.230	−0.249	−0.134	0.092	−0.775	−0.770	−0.477	−0.524	−0.374
Hyperoside	−0.204	−0.592	0.073	−0.376	−0.254	0.153	−0.824	−0.830	−0.585	−0.583	−0.268
Pinoresinol	0.481	0.155	0.442	0.533	0.541	0.327	0.469	0.148	0.603	0.104	−0.501
Luteolin	−0.145	−0.574	0.127	−0.318	−0.188	0.167	−0.788	−0.839	−0.528	−0.567	−0.299
Apigenin	−0.461	−0.292	−0.330	−0.572	−0.496	−0.089	−0.712	−0.579	−0.710	−0.374	0.285

[1] In this table, Pearson Correlation Coefficients among the parameters were given. TAP: total antioxidant activity by phosphomolybdenum method. AAIA: α-amylase inhibitory activity, AChEIA: acetyl cholinesterase inhibitory activity, BChEIA: butyryl cholinesterase inhibitory activity, AGIA: α-glucosidase inhibitory activity, TIA: tyrosinase inhibitory activity, ABTS: ABTS radical scavenging activity, DPPH: DPPH radical scavenging activity, CUPRAC: CUPRAC reducing power potential, FRAP: FRAP reducing power potential, TFC: total flavonoid content, TPC: total phenolic content, FICA: rerrous ion chelating activity. [2] Significant at $p < 0.05$ [3] Significant at $p < 0.01$.

analyzed by IR, 1D-NMR, and 2D-NMR techniques and pinoresinol was found in the extract in high quantity. Additionally, Khummanee et al. (2019) reported the antioxidant activity of pinoresinol-α-D-glucoside. According to the literature search, vanillic acid was another compound having antioxidant activity. Rodboon et al. (2020), reported that protocatechuic and vanillic acids were significant antioxidant and tyrosinase inhibitory agents used by riceberry during germination. Salau et al. (2020) reported that vanillin and vanillic acid can improve metabolic complications related to Fe^{2+} induced brain tissue damage by modulating the antioxidant defense system. Literature data on pinoresinol and vanillic acid were thought to support the findings presented in the current study.

3.3. Enzyme inhibitory activity

The data obtained on the inhibitory activities of the extracts on tyrosinase, α-amylase, α-glucosidase, AChE and BChE were given in Table 4 in IC_{50} (mg/mL) and in Fig. 4 in the positive control equivalent. The enzyme inhibitory activity of *S. alopecuroides* var. *alopecuroides* has not previously been studied.

In tyrosinase inhibition assay, the activity was in the range of 1.22–1.44 mg/mL. The root extract was the most active one. Pearson correlation analysis also confirmed that flavonoids contributed more to the activity than phenolics (Table 5). It was also understood that pinoresinol partially contributed to the activity.

Literature data confirmed the partial contribution of pinoresinol to the tyrosinase inhibitory potential of the samples. Azhar ul et al. (2006) reported that pinoresinol available in the MeOH extract of *Vitex negundo* showed moderate tyrosinase inhibitory activity. On the other hand, Rodboon et al. (2020) reported that vanillic acid was among the phytochemicals that germinating riceberry used to suppress the process of melanogenesis.

As understood from the data in both Table 4 and Fig. 4, some extracts showed stronger inhibitory activity on α-amylase than α-glucosidase. As with the tyrosinase inhibition assay, root extract exhibited the highest α-amylase inhibition. The IC_{50} value of this extract was 2.97 mg/mL (410.31 mg ACEs/g). The IC_{50} values of leaf, stem, and aerial part extracts were between 3.0–4.0 mg/mL, while flower extract exhibited an inhibitory activity higher than 4.0 mg/mL. The activity potentials of leaf and root extracts were statistically similar. However, the activities of other extracts were different. According to the correlation analysis, flavonoids and phenolics contributed almost equally to the activity (Table 5). Pinoresinol was also found to contribute to the activity. In α-glucosidase inhibitory activity test, the root extract showed again a high activity. The extract obtained from the aerial part followed the root extract. While the IC_{50} values of leaf and stem extracts were found to be close to each other (around 4.0–4.5 mg/mL), the activity of the flower extract was quite low. As can be seen from the data given above, except for aerial part, flower and root extracts, the inhibitory activities of leaf and stem extracts were statistically similar. Flavonoids had a high contribution to the activity (Table 5). Pinoresinol and vanillic acid also contributed to the activity.

Rasouli et al. (2017) analyzed the inhibitory activity of 26 polyphenols on α-amylase/α-glucosidase through molecular docking and virtual screening studies. Among these molecules, they reported that pinoresinol significantly inhibited α-glucosidase. Similar findings have also been reported by Kwon et al. (2014). There were also some reports in the literature regarding the inhibitory activity of vanillic acid on α-amylase/α-glucosidase. In a study carried out on the butanol fraction of *Dacryodes edulis* by Erukainure et al. (2020), the extract rich in vanillic acid has been reported to effectively inhibit α-glucosidase. In addition, vanillic acid isolated from the leaves of *Rubus suavissimus* using chromatographic methods exhibited moderate activity on α-glucosidase (Liu et al., 2020).

The inhibitory activities of the extracts on AChE were higher than that of BChE. The extracts showed inhibitory activity on AChE in the range of 0.97–1.15 mg/mL. As with other enzyme inhibition tests, the root extract showed the strongest activity (IC_{50}: 0.97 mg/mL, 3.15 mg GALAEs/g). AChE inhibitory activities of leaf, aerial part, flower, and stem extracts were almost equal. According to statistical analysis, activities of flower and stem extracts were similar. It was found that both flavonoids and phenolics significantly contributed to the AChE inhibitory activities of the extracts (Table 5). In BChE inhibitory activity assay, a different activity profile was observed. Aerial part and leaf extracts exhibited equal inhibitory activity on BChE (IC_{50}: 1.17 mg/mL). In addition, the activities of these extracts in terms of positive control equivalent were almost equal (4.77 and

4.78 mg GALAEs/g, respectively). The inhibitory activities of root, flower, and stem extracts were between 1.34–1.50 mg/mL. BChE inhibitory activities of aerial part-leaf and flower-root extract pairs were not statistically different, but the inhibitory activity of the stem extract was different from the others (Table 5).

In a study reported by Tang et al. (2016), pinoresinol isolated from *Catalpa bungei* showed inhibitory activity on BChE while it was not effective on AChE. In order to clarify the difference between the literature data and the data presented here, it was thought that the cholinesterase inhibitory activity of pinoresinol should be elucidated by a further study. Since there were no reports on the inhibitory activity of vanillic acid on cholinesterases, it was thought that a study should also be planned to make a judgment on the cholinesterase inhibitory activity of this molecule.

4. Conclusion

It has been concluded that the activity potential of the root extract may be due to the high amount of pinoresinol and vanillic acid. Indeed, literature data supported this idea. The root extract of *S. alopecuroides* var. *alopecuroides* was thought to be an effective antioxidant and enzyme inhibitory agent. However, more detailed techniques need to be applied to identify the components responsible for the activity.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

Dr. Olcay Ceylan carried out the taxonomic identification of the plant material. In addition, Vesile Sarikurkcu assisted the process of biological activity tests. We would like to thank them for their contributions.

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