South African Journal of Botany 000 (2021) 1–7

Contents lists available at ScienceDirect

South African Journal of Botany

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

Draba cemileae (Karaer): Phytochemical composition, antioxidant and enzyme inhibitory activity

Mustafa Cuce[a,*], Ersin Demir[b], Arzuhan Sihoglu Tepe[c], Cengiz Sarikurkcu[b,*]

[a] Department of Food Technology, Giresun University, Sebinkarahisar School of Applied Sciences, Giresun TR-28400, Turkey
[b] Faculty of Pharmacy, Afyonkarahisar Health Sciences University, Afyonkarahisar TR-03100, Turkey
[c] Department of Pharmacy Services, Kilis 7 Aralik University, Vocational School of Health Sciences, Kilis TR-79000, Turkey

ARTICLE INFO

Article History:
Received 1 May 2021
Revised 23 June 2021
Accepted 16 July 2021
Available online xxx

Edited by A. Ndhlala

Keywords:
Draba cemileae
Chemical composition
Antioxidant activity
Enzyme inhibitory activity

ABSTRACT

Plant polyphenols have always attracted the attention of researchers with their excellent biological activity potentials. In this study, roots, leaves and seeds of *Draba cemileae* (Karaer) were evaluated for their chemical compositions and biological activities. Spectrophotometric analysis showed that phenolics and flavonoids were present in high quantities in leaf and seed extracts (22.84 mg GAEs/g and 23.32 mg REs/g, respectively). Chromatographic analysis showed that the *p*-hydroxybenzoic acid and chlorogenic acid contents of the extracts were significantly high. The seed extract also contained 550 mg/g of rosmarinic acid. While the leaf extract showed high activity in phosphomolybdenum, CUPRAC, FRAP and ferrous ion chelating activity tests (0.88, 2.23, 1.73 and 3.68 mg/ml, respectively), DPPH and ABTS scavenging activity tests resulted in the superiority of the seed extract (3.75 and 2.53 mg/ml, respectively). The leaf extract also showed the highest activity in α-amylase and tyrosinase inhibitory activity tests (1.78 and 4.99 mg/ml, respectively). On the other hand, the seed extract exhibited higher activity than the others in α-glucosidase, AChE and BChE inhibitory activity tests (7.14, 1.13 and 5.11 mg/ml, respectively). Correlation coefficients between the composition and the biological activities were over 0.9. It was concluded that *D. cemileae* could be a new and effective source of antioxidant and enzyme inhibitory phytochemicals in medicine, food and cosmetics industries.

1. Introduction

Plant polyphenols are compounds containing hydroxyl groups attached to aromatic rings in their structure (Zhou et al., 2019). It is estimated that the number of plant polyphenols identified so far, including those that can be consumed, is several thousand (Stagos, 2020). Plants have been the richest source of exogenous antioxidant compounds for many years. Authorities claim that almost two-thirds of the plant species distributed on the world have various biological/pharmacological activities and almost all of them contain strong antioxidant compounds (Krishnaiah et al., 2011). After the discovery of ascorbic acid, the first exogenous antioxidant compound, plants were relied upon for the treatment of many diseases caused by increased oxidative stress (Boo, 2019; Burgos-Morón et al., 2019; Pawlowska et al., 2019). Many researchers agree that polyphenols can be used as antioxidants in the treatment of diseases caused by oxidative stress (Jin et al., 2018). Therefore, plant-derived antioxidants are a promising source of reference to combat the problems caused by oxidative stress (Jin et al., 2018; Kasote et al., 2015).

Diabetes is a chronic disease that occurs due to inadequate carbohydrate metabolism or impairment of cell surface receptors. The high level of glucose in the blood causes many health problems (Mathers and Loncar, 2006; Shaw et al., 2010). The enzymes responsible for metabolizing polysaccharides or disaccharides in the body are α-amylase and α-glucosidase. Inhibiting these enzymes to slow down carbohydrate metabolism is one of the important strategies in the treatment of diabetes (Azad et al., 2017; Kim et al., 2000; Ramasubbu et al., 1996; Zhen et al., 2017). Today, some synthetic enzyme inhibitors are used to reduce the blood glucose level. However, these substances cause some side effects such as edema, hypoglycemia, excessive weight gain, anemia, gastrointestinal disorders and lactic acidosis (Magaji et al., 2020). Therefore, researchers also focused on plants to discover new and alternative α-amylase and α-glucosidase inhibitors to treat diabetes.

In addition to the therapeutic properties mentioned above, phytochemicals also exhibit promising activities for the treatment of disorders in the cholinergic system. It has been determined that the metabolic activity of cholinesterases (ChEs) is closely related to the pathology of various neurological diseases, especially Alzheimer's disease (AD). Especially in AD, high ChE activity contributes to the increase of amyloid plaque aggregation as well as slowing down neural conduction. Symptoms such as memory loss, irreversible

* Corresponding authors.
E-mail addresses: mustafa.cuce@giresun.edu.tr (M. Cuce), sarikurkcu@gmail.com (C. Sarikurkcu).

https://doi.org/10.1016/j.sajb.2021.07.028
0254-6299/

neurological tissue degeneration, inability to perform daily vital activities are frequently seen in AD (Choubdar et al., 2019; Vickers, 2017). Today, the most effective approach in the treatment of AD is the use of cholinesterase inhibitors (donepezil, galantamine, rivastigmine) (Ahmad et al., 2019; Yiannopoulou and Papageorgiou, 2020). Despite being clinically effective, these compounds have been reported to have common side effects such as diarrhea, nausea, and vomiting. Therefore, researchers have focused on the development or isolation of new phytochemicals (Cheenpracha et al., 2016; García et al., 2015; Kiełczewska et al., 2021; Liu et al., 2017; Mohebbi et al., 2018; Richmond et al., 2013).

Tyrosinase is a critical enzyme that converts monophenols to diphenols and then to melanin through *o*-quinone oxidation (Tian et al., 2019; Yu et al., 2019). Melanin is a pigment found in the skin of organisms that acts as a filter against UV rays. However, if it is synthesized excessively, freckles and age spots occur on the skin. Overexpression of melanin synthesis has also been reported to be associated with melanoma (Chang, 2009). Polyphenol oxidases, which are structurally similar to tyrosinase, cause browning in fruits and vegetables. Browning leads to deterioration of quality and taste in these foods (Brotzman et al., 2019; Chang, 2009). Inhibition of tyrosinase is one of the most rational solutions in the treatment of skin diseases due to abnormal melanin synthesis in medicine, in the preparation of skin whitening preparations in the cosmetics industry and in the prevention of browning in the food industry. Today, there are some synthetic substances used as tyrosinase inhibitors. However, natural tyrosinase inhibitors are more preferred on the grounds that their biocompatibility capacity is higher and they are sustainable. For this reason, researchers are investigating plant species for the discovery of new and effective tyrosinase inhibitors (Wang et al., 2020; Zolghadri et al., 2019).

In this study, roots, leaves and seeds of *Draba cemileae* (Karaer) were evaluated for their chemical compositions and biological activities. In addition to spectrophotometric and chromatographic analysis, total antioxidant activities of the extracts based on their chlorogenic acid equivalents (CAEs) of total phenolic and flavonoid contents were determined by performing square wave stripping voltammetry (SWSV) on multi-walled carbon nanotube paste electrode (MWCNTPE).

2. Materials and methods

2.1. Plant material and extract preparation

D. cemileae was collected from Tamzara village, Sebinkarahisar, Giresun-Turkey on 14 June 2020 (1348 m, 40° 20' 47.67"N 38° 26' 32.62"E), authenticated by Dr. Mustafa Cuce, and deposited (Herbarium number: KTUB, CUCE & GULTEPE 796) at the Department of Biology, Karadeniz Technical University (Trabzon-Turkey). The plant was firstly divided into different parts (roots, leaves, and seeds), air-dried in the shade for several weeks, and then ground using a laboratory mill.

Air-dried samples (2 g) were individually extracted with 40 ml of methanol for 30 min in a sonication bath (30 °C). The extracts were filtered and then concentrated. They were stored at +4 °C.

2.2. Determination of the phenolic compositions of the extracts

Details of the spectrophotometric (Zengin et al., 2015a) and chromatographic (Movahhedin et al., 2016) methods were given in supplementary file. All details regarding the SWSV analysis (Demir, 2019) applied for the determination of the total antioxidant activity of the extracts in terms of chlorogenic acid equivalent were also specified in the supplementary file.

2.3. Biological activity

Details of the biological activity tests (antioxidant capacity and enzyme inhibitory activities) can be found in supplementary file (Apak et al., 2006; Kocak et al., 2016; Ozer et al., 2018; Tepe et al., 2011; Zengin et al., 2015a, 2015b).

2.4. Statistical analysis

Details of the statistical analysis applied to the data obtained from the biological activity tests were given in the supplementary file.

3. Results and discussion

3.1. Chemical composition

The yields of the MeOH extracts obtained from the roots, leaves and seeds of *D. cemileae* were given in Table 1. According to the data in the table, the highest yield belonged to root extract (11.9%). It was followed by the seed and leaf extracts, respectively.

Data obtained from the spectrophotometric analysis were also presented in Table 1. While leaves were the richest plant parts in terms of phenolics (22.84 mg GAEs/g), the seed extract was found to contain higher amount of flavonoids than the others (23.32 mg REs/g). Root extract was the poorest in terms of both phenolics and flavonoids. Statistical analyzes showed that both phenolic and flavonoid profiles of root, leaf and seed extracts were significantly different from each other.

Chromatographic analyzes were also performed to determine the amount of certain phytochemicals in the extracts. According to the data presented in Table 2, the compound with the highest amount in the extracts was chlorogenic acid. While the amount of this phytochemical was too close to each other in root and seed extracts (1350 and 1300 μg/g, respectively), it was determined that the leaf extract contained 450 μg/g chlorogenic acid. Another compound found in significant amounts in the extracts was *p*-hydroxybenzoic acid. It was determined that there was 550 μg/g rosmarinic acid in the seed extract.

D. cemileae is a new species introduced to literature by Dr. Fergan Karaer in 2012 (Karaer, 2012). Therefore, chemical composition of this species have not previously been reported. Furthermore, there is no data in the literature regarding the chemical composition of other *Draba* species too. In this respect, the current study could be assumed as the first report.

3.2. Antioxidant activity

The data obtained from the antioxidant activity assays were subjected to an analysis called relative antioxidant capacity index (RACI) and the index values of each extract were calculated. According to the findings given in Fig. 1, the most effective part of *D. cemileae* in terms of antioxidant activity was leaves (RACI index: 0.62). It was followed by extracts of seeds and roots, respectively (RACI indexes 0.17 and -0.79, respectively). The results of the statistical analyzes performed to determine the correlation between the RACI indexes of the extracts and their antioxidant activities were given in Fig. 2. According to the data in the figure in question, all antioxidant activity data

Table 1
Extract yields, total flavonoid and phenolic contents of the samples.

Assays	Roots	Leaves	Seeds
Yield (%)	11.9	1.74	1.84
Total flavonoids (mg REs/g extracts)	0.87 ± 0.01^{c}	18.61 ± 0.05^{b}	23.32 ± 0.14^{a}
Total phenolics (mg GAEs/g extracts)	10.97 ± 0.21^{c}	22.84 ± 0.52^{a}	19.53 ± 0.07^{b}

REs: Rutin equivalent
GAEs: Gallic acid equivalent
Data expressed with different superscripts in the same row are different from each other.

Table 2
Results of chromatographic analysis

No.	Phenolics	Concentration (μg/g extract)		
		Roots	Leaves	Seeds
1	Gallic acid	nd	nd	nd
2	Protocatechuic acid	nd	nd	nd
3	(+)-Catechin	nd	nd	nd
4	*p*-Hydroxybenzoic acid	700 ± 15^b	900 ± 15^a	450 ± 15c
5	Chlorogenic acid	1350 ± 50^a	450 ± 5^b	1300 ± 50^a
6	Caffeic acid	250 ± 10^b	400 ± 10^a	300 ± 10^b
7	(-)-Epicatechin	nd	nd	nd
8	Syringic acid	nd	nd	nd
9	Vanillin	nd	nd	150 ± 3
10	*p*-Coumaric acid	50 ± 2^b	50 ± 2^b	150 ± 2^a
11	Ferulic acid	150 ± 1c	200 ± 1^b	250 ± 1^a
12	Sinapic acid	nd	nd	nd
13	Benzoic acid	nd	nd	nd
14	*o*-Coumaric acid	nd	nd	nd
15	Rutin	nd	nd	nd
16	Hesperidin	nd	nd	nd
17	Rosmarinic acid	nd	nd	550±50
18	Eriodictyol	nd	nd	nd
19	*trans*-Cinnamic acid	nd	nd	nd
20	Quercetin	nd	nd	nd
21	Luteolin	nd	nd	nd
22	Kaempferol	nd	nd	nd
23	Apigenin	nd	nd	nd

Data expressed with different superscripts in the same row are different from each other.

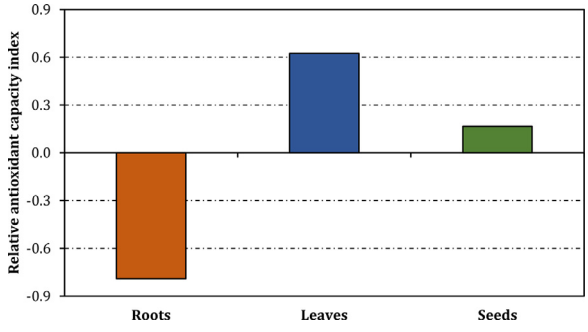

Fig. 1. Relative antioxidant capacity index of samples extracts.

except the ferrous ion chelating activity test were highly correlated with RACI indices.

The data obtained regarding the antioxidant activity potentials of the extracts were given in Table 3. According to the data in the table, the leaf extract showed the highest antioxidant activity in phosphomolybdenum, reducing power (CUPRAC and FRAP) and ferrous ion chelating activity tests (0.88, 2.23, 1.73 and 3.68 mg/ml, respectively). In phosphomolybdenum and reducing power (CUPRAC and FRAP) assays, the activity of the leaf extract was followed by the extract obtained from the seed, while the root extract was the second in ferrous ion chelating activity test. On the other hand, the seed extract showed the highest activity in radical scavenging assay. DPPH and ABTS radical scavenging capacities of the seed extract were determined to be 3.75 and 2.53 mg/ml, respectively. However, radical scavenging activity of leaf extract was too close to the root extract. As with other antioxidant activity tests, the root extract exhibited the weakest activity in radical scavenging assays. However, none of the extracts exhibited as potent activity as trolox or EDTA. According to the results of statistical analysis, the activities of the leaf and seed extracts were similar in phosphomolybdenum, CUPRAC, DPPH and ABTS assays. While root and leaf extracts showed similar activity in ferrous ion chelating activity test, the activities of all extracts were statistically different from each other in FRAP assay.

In the present study, in addition to the test systems given above, the antioxidant activities of the extracts were also analyzed in terms of CAEs by electrochemical method. 0.6 ml, 0.3 ml and 0.2 ml of plant root, leaf and seed samples were added to the electrochemical cell, respectively, and then SWS voltammograms were obtained (Fig. 3). As seen in the figure, approximately +0.4 V anodic peak was observed at different concentrations of plant samples. Since this peak potential has almost the same potential as the anodic peak of chlorogenic acid, it is possible to calculate the total antioxidant value in plant samples in terms of CAEs. As a result, total antioxidant levels of root, leaf and seed extracts were found as 0.79, 2.43 and 5.73 mg / g CAEs, respectively (Fig. 4).

As mentioned above, there is no report in the literature regarding the antioxidant activity of *D. cemileae*. However, in order to better understand the antioxidant activity of root and leaf extracts, it is useful to examine some literature data on the contribution of chlorogenic acid and *p*-hydroxybenzoic acid, which were found to be relatively high in the extracts. In addition, the literature data on the contribution of rosmarinic acid to the activity was also discussed below.

In a study conducted to determine the contribution of some phenolic acids to antioxidant activity in *Aegilops cylindrica*, it was reported that chlorogenic acid, along with some other phenolic acids and flavonoids, significantly contributed to DPPH radical scavenging activity and is one of the defense mechanisms of cells against oxidative stress (Kiani et al., 2021). In another study investigating the free radical scavenging activity of *Miscanthus sacchariflorus*, radical scavenging activities of the samples on DPPH and ABTS were reported as 28.85−99.25 and 25.65−83.62 μg/ml, respectively, and as a result of chemical composition analysis, it was found that the major compounds of the extracts were *p*-hydroxybenzoic acid and chlorogenic acid (Ghimire et al., 2021). As there are numerous reports in the literature that rosmarinic acid contributes significantly to the antioxidant activities of extracts (Righi et al., 2021; Song et al., 2021; Zeljkovic et al., 2021), there are also reports showing that this compound itself has direct antioxidant activity (Hyatt et al., 2021; Phromnoi et al., 2021; Wang et al., 2021).

3.3. Enzyme inhibitory activity

Data on the enzyme inhibitory activities of the extracts were given in Table 4. The extracts exhibited different activity profiles in digestive enzyme inhibition tests in which anti-diabetic activity was investigated. While leaf extract exhibited the highest activity in the α-amylase inhibitory activity assay, it was determined that the efficiency of the seed extract was higher in α-glucosidase inhibitory activity test. Leaf extract showing high activity in the α-amylase inhibitory activity test took the last place in α-glucosidase assay. The extracts exhibited higher inhibitory activity on α-amylase than α-glucosidase. The α-amylase/α-glucosidase inhibitory activities of the extracts were statistically significantly different from each other.

To the best of our knowledge, neither α-amylase nor α-glucosidase inhibitory activities of *D. cemileae* have been previously studied. However, there are some reports that the main compounds given in Table 2 may contribute to the inhibitory activity on these enzymes. In a study by Chung et al. (2019), the extract of *Tupistra nutans* roots was rich in phenolic acids (including *p*-hydroxybenzoic acid) and there was a correlation between composition and α-glucosidase inhibitory activity. The fact that the phytochemical in question exhibits α-glucosidase inhibitory activity has also been confirmed by Choi et al. (2012) in the study on yeast α-glucosidase. In a study carried out by Chen et al. (2020) on HepG2 cells, it was reported that chlorogenic acid showed moderate α-glucosidase inhibitory activity. According to Tolmie et al. (2021), who investigated *in silico* α-amylase and α-glucosidase inhibitory activities of rosmarinic acid, the

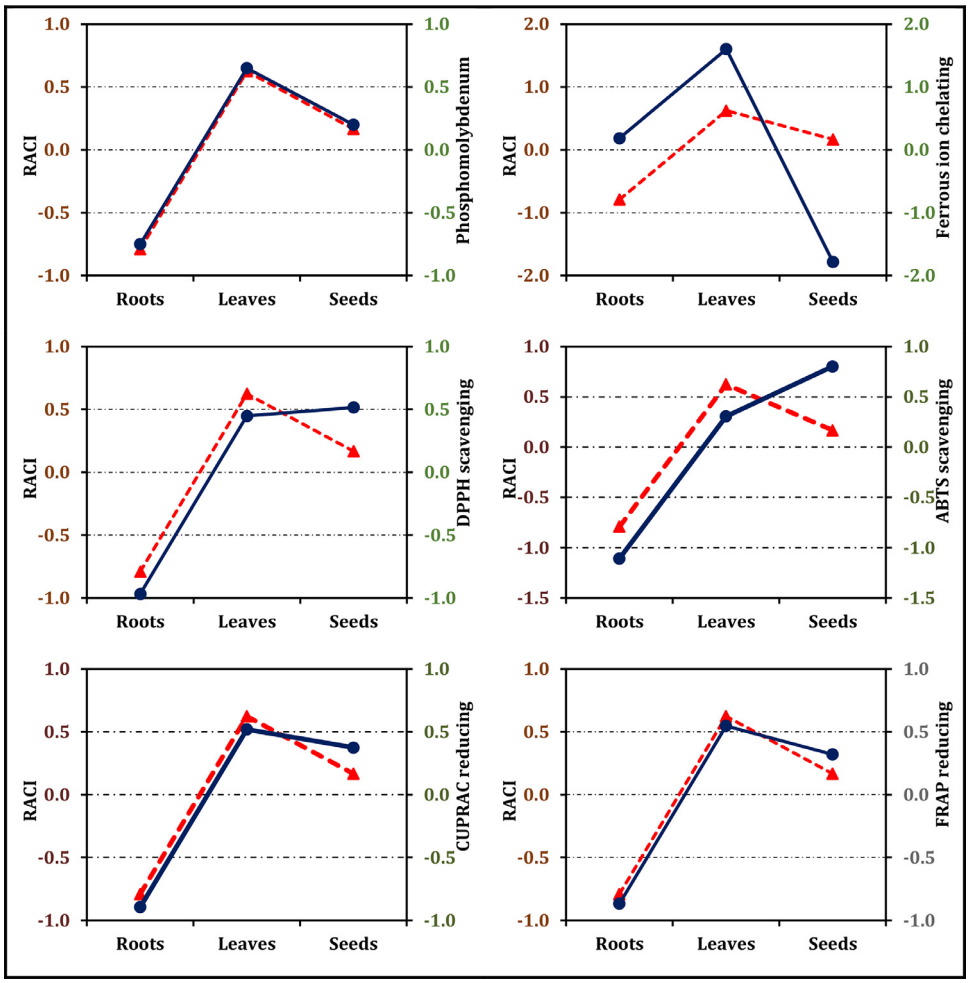

Fig. 2. Relative antioxidant capacity index (dashed red line with triangle) and antioxidant activity (solid dark blue line with circle) of samples extracts (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

Table 3
Antioxidant activities of the samples.

Antioxidant activity	Unit	Roots	Leaves	Seeds	Trolox	EDTA
Phosphomolybdenum	EC_{50}: mg/ml	1.09 ± 0.01^{c}	0.88 ± 0.02^{b}	0.94 ± 0.02^{b}	0.38 ± 0.01^{a}	-
	mg TEs/g extract	348.51 ± 4.04^{b}	430.83 ± 11.05^{a}	404.92 ± 6.74^{a}		
CUPRAC reducing	EC_{50}: mg/ml	3.27 ± 0.03^{c}	2.23 ± 0.01^{b}	2.30 ± 0.03^{b}	0.15 ± 0.01^{a}	-
	mg TEs/g extract	45.65 ± 0.39^{b}	66.94 ± 0.13^{a}	64.74 ± 0.97^{a}		
FRAP reducing	EC_{50}: mg/ml	2.61 ± 0.01^{d}	1.73 ± 0.01^{b}	1.83 ± 0.01^{c}	0.070 ± 0.001^{a}	-
	mg TEs/g extract	26.95 ± 0.01^{c}	40.63 ± 0.18^{a}	38.43 ± 0.19^{b}		
DPPH scavenging	IC_{50}: mg/ml	4.56 ± 0.09^{c}	3.78 ± 0.05^{b}	3.75 ± 0.05^{b}	0.082 ± 0.001^{a}	-
	mg TEs/g extract	18.05 ± 0.37^{b}	21.78 ± 0.27^{a}	21.96 ± 0.26^{a}		
ABTS scavenging	IC_{50}: mg/ml	3.41 ± 0.09^{c}	2.71 ± 0.03^{b}	2.53 ± 0.05^{b}	0.13 ± 0.01^{a}	-
	mg TEs/g extract	37.15 ± 0.98^{b}	46.76 ± 0.57^{a}	50.14 ± 0.91^{a}		
Ferrous ion chelating	IC_{50}: mg/ml	4.15 ± 0.23^{b}	3.68 ± 0.01^{b}	5.04 ± 0.22^{c}	-	0.039 ± 0.001^{a}
	mg EDTAEs/g extract	9.45 ± 0.52^{a}	10.64 ± 0.02^{a}	7.79 ± 0.35^{b}		

TEs: Trolox equivalent
EDTAEs: Ethylenediaminetetraacetic acid (disodium salt) equivalent
Data expressed with different superscripts in the same row are different from each other.

phytochemical in question showed inhibitory activity equivalent to acarbose.

In both cholinesterase inhibitory activity tests, the seed extract was found to be more effective than the others. The inhibitory activities of this extract on AChE and BChE were found to be 1.13 and 5.11 mg/ml, respectively. Root extract showed the weakest activity in both test systems. The inhibitory activity of the root extract on the enzymes in question was 1.31 and 15.31 mg/ml, respectively. The extracts exhibited higher inhibitory activity on AChE than BChE. While the activities of the extracts were statistically similar in AChE inhibitory activity test, they were found to be different from each other in BChE inhibitory activity assay.

Cholinesterase inhibitory activity of *D. cemileae* was first brought to the literature with this study. Therefore, there is no enough literature data to discuss the cholinesterase inhibitory activity of the plant species in question. However, considering that the seed extract showed high activity on both cholinesterase, it would be useful to discuss some literature data on the inhibitory activities of

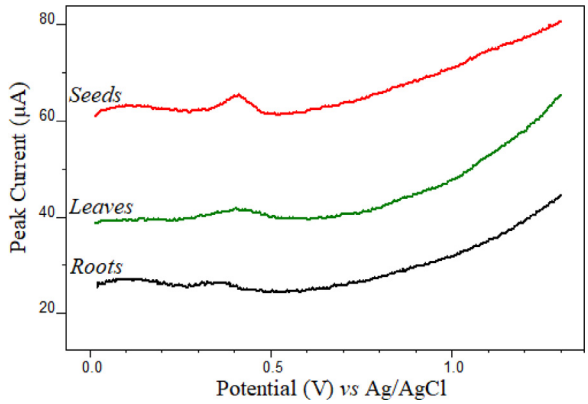

Fig. 3. SWSV pathway for the *D. cemileae* plant samples as total antioxidant capacity on MWCNTPE in pH 5.0 BR buffer solution.

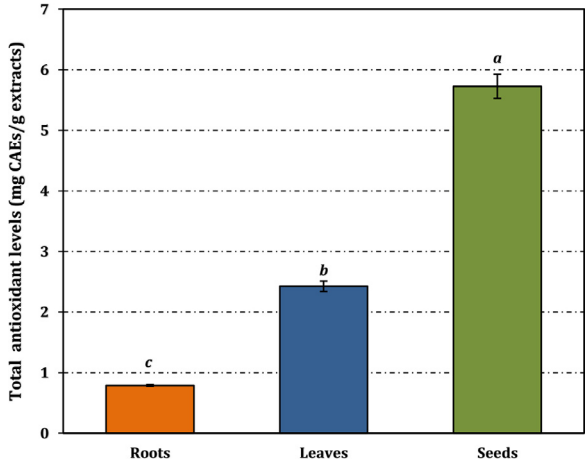

Fig. 4. Total antioxidant levels of methanolic extracts from different parts of *D. cemileae* by electrochemical method (CAEs: Chlorogenic acid equivalents). Values indicated by the same superscripts within the same row are not different from the honestly significant difference after Tukey's post hoc test at 5% significance level.

phytochemicals which were found to be in high quantities in the extracts on AChE and BChE. In a study on cholinesterase inhibitory activity of *p*-hydroxybenzoic acid isolated from *n*-butanol extracts of *Nelumbo nucifera* stamens, it was reported that the phytochemical exhibited non-competitive inhibitory activity on AChE and BChE and activity values were 20.07 and 62.29 μM, respectively (Jung et al., 2010). In a study conducted on cyclosporine induced hypertensive rats, orally administered chlorogenic acid significantly reduced the activity of both cholinesterase (Agunloye et al., 2019). According to Senol et al. (2017) rosmarinic acid exhibited significant BChE inhibitory activity.

The data obtained from the test system in which tyrosinase inhibitory activities of the extracts were investigated showed that the leaf extract was the most effective inhibitory agent among the others. The tyrosinase inhibitory activity of this extract was determined as 4.99 mg/ml (Table 4). It was followed by the root and seed extracts, respectively. The tyrosinase inhibitory activities of the extracts were statistically different from each other.

In the literature, there is no information regarding the tyrosinase inhibitory activity of *D. cemileae*. However, there are some studies on the tyrosinase inhibitory activities of the main compounds given in Table 2. According to Chen et al. (2005) and Azizuddin et al. (2011), *p*-hydroxybenzoic acid is a competitive tyrosinase inhibitor. In the tyrosinase inhibitory activity test performed with *p*-hydroxybenzoic acid isolated from *Ficus erecta* var. *sieboldii*, the compound was reported to exhibit 0.98 mM inhibitory activity (Park et al., 2012). In addition, the strong tyrosinase inhibitory activity of *p*-hydroxybenzoic acid beta-d-glucosyl ester, which is a derivative of the compound in question, was reported by Shim et al. (2020). In a study by Cheng et al. (2020), it was reported that chlorogenic acid exhibited inhibitory effect on polyphenol oxidase, which was structurally similar to tyrosinase, and prevented browning in fresh cut potatoes.

3.4. Correlation between the parameters

Table 5 shows the correlation between the chemical composition and biological activity data. Although the correlation coefficients given in the table are hypothetically calculated based on the amounts of the phytochemicals in the extracts, it is a good way to have an idea about the contribution of the compounds to the activity. According to the data presented in Table 5, phenolics/flavonoids contributed to the antioxidant activities of the extracts at a high rate. This finding is already accepted by many researchers. According to the data in the table, total phenolics and flavonoids also contributes statistically significantly to α-amylase and BChE inhibitory activity, respectively (correlation coefficients above 0.9). Among the individual components, *p*-hydroxybenzoic acid contributed to ferrous ion chelating activity, caffeic acid to α-amylase inhibitory activity, *p*-coumaric acid to α-glucosidase inhibitory activity, and ferulic acid to ABTS radical scavenging and BChE inhibitory activities. As stated above, the data in the table in question are hypothetical and should be evaluated together with the literature data.

4. Conclusions

The lack of a similar study on *D. cemileae* makes the present study important in terms of the literature. It was concluded that the extracts obtained from leaves and seeds exhibited higher antioxidant

Table 4
Enzyme inhibitory activities of the samples.

Enzyme inhibitory activity	Unit	Roots	Leaves	Seeds	Acarbose	Galantamine	Kojic acid
α-Amylase inhibition	IC_{50}: mg/ml	2.58 ± 0.04^{d}	1.78 ± 0.01^{b}	2.09 ± 0.06^{c}	0.50 ± 0.01^{a}		
	mg ACEs/g extracts	193.75 ± 3.25^{c}	280.45 ± 1.95^{a}	238.76 ± 6.63^{b}			
α-Glucosidase inhibition	IC_{50}: mg/ml	23.55 ± 0.82^{c}	43.69 ± 0.40^{d}	7.14 ± 0.09^{b}	1.38 ± 0.05^{a}		
	mg ACEs/g extract	52.83 ± 2.07^{b}	25.41 ± 0.30^{c}	189.43 ± 2.36^{a}			
AChE inhibition	IC_{50}: mg/ml	1.31 ± 0.01^{b}	1.25 ± 0.20^{b}	1.13 ± 0.04^{b}		0.0027 ± 0.0001^{a}	
	mg GALAEs/g extract	2.09 ± 0.01^{a}	2.21 ± 0.35^{a}	2.43 ± 0.08^{a}			
BChE inhibition	IC_{50}: mg/ml	15.31 ± 1.16^{d}	7.86 ± 0.10^{c}	5.11 ± 0.29^{b}		0.0057 ± 0.0001^{a}	
	mg GALAEs/g extract	0.38 ± 0.03^{c}	0.74 ± 0.01^{b}	1.14 ± 0.06^{a}			
Tyrosinase inhibition	IC_{50}: mg/ml	5.07 ± 0.07^{b}	4.99 ± 0.01^{b}	5.16 ± 0.30^{b}			0.13 ± 0.01^{a}
	mg KAEs/g extract	25.79 ± 0.35^{a}	26.16 ± 0.04^{a}	25.38 ± 1.48^{a}			

GALAEs: Galantamine equivalent
KAEs: Kojic acid equivalent
ACEs: Acarbose equivalent
Data expressed with different superscripts in the same row are different from each other.

Table 5
Pearson correlation coefficients between the parameters.

	TAP	DPPH	ABTS	CUPRAC	FRAP	FICA	AChEIA	BChEIA	TIA	AAIA	AGIA
DPPH	0.920										
ABTS	0.825	0.966									
CUPRAC	0.967	0.986	0.935								
FRAP	0.975	0.973	0.913	0.997							
FICA	0.209	-0.120	-0.352	-0.002	0.056						
RACI	0.987	0.930	0.836	0.974	0.986	0.217					
AChEIA	0.461	0.510	0.602	0.492	0.460	-0.504					
BChEIA	0.629	0.860	0.937	0.790	0.759	-0.571	0.656				
TIA	0.180	0.003	-0.054	0.060	0.042	0.306	-0.078	-0.333			
AAIA	0.973	0.847	0.728	0.916	0.935	0.370	0.275	0.480	0.279		
AGIA	0.052	0.390	0.578	0.269	0.215	-0.941	0.606	0.794	-0.441	-0.134	
Total flavonoid	0.860	0.981	0.993	0.956	0.939	-0.284	0.604	0.933	-0.100	0.761	0.537
Total phenolic	0.981	0.947	0.859	0.982	0.991	0.177	0.356	0.673	0.111	0.968	0.092
Chlorogenic acid	-0.760	-0.500	-0.301	-0.613	-0.659	-0.784	0.099	-0.027	-0.299	-0.864	0.587
p-Hydroxybenzoic acid	0.247	-0.110	-0.318	0.029	0.087	0.987	-0.455	-0.570	0.404	0.416	-0.951
Caffeic acid	0.889	0.720	0.574	0.805	0.832	0.532	0.057	0.283	0.384	0.970	-0.336
p-Coumaric acid	0.204	0.530	0.701	0.416	0.364	-0.895	0.638	0.874	-0.376	0.024	0.987
Ferulic acid	0.660	0.880	0.960	0.815	0.781	-0.570	0.672	0.993	-0.226	0.518	0.777

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, FICA: Ferrous ion chelating activity

and enzyme inhibitory activity than the root extract. Although not all of the phytochemicals in the extracts have been documented, *p*-hydroxybenzoic acid, chlorogenic acid and especially rosmarinic acid in the seed extract are thought to contribute significantly to the activities. It was concluded that the *D. cemileae* could be considered as one of the new and alternative phytochemical sources in medicine, food and cosmetics industries.

Declaration of Competing Interest

The authors confirm that there are no known conflicts of interest.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.sajb.2021.07.028.

References

Agunloye, O.M., Oboh, G., Ademiluyi, A.O., Ademosun, A.O., Akindahunsi, A.A., Oyagbemi, A.A., Omobowale, T.O., Ajibade, T.O., Adedapo, A.A., 2019. Cardio-protective and antioxidant properties of caffeic acid and chlorogenic acid: mechanistic role of angiotensin converting enzyme, cholinesterase and arginase activities in cyclosporine induced hypertensive rats. Biomed. Pharmacother. 109, 450–458.
Ahmad, G., Rasool, N., Rizwan, K., Imran, I., Zahoor, A.F., Zubair, M., Sadiq, A., Rashid, U., 2019. Synthesis, in-vitro cholinesterase inhibition, in-vivo anticonvulsant activity and in-silico exploration of N-(4-methylpyridin-2-yl) thiophene-2-carboxamide analogs. Bioorg. Chem. 92, 103216.
Apak, R., Güçlü, K., Özyürek, M., Esin Karademir, S., Erçağ, E., 2006. The cupric ion reducing antioxidant capacity and polyphenolic content of some herbal teas. Int. J. Food Sci. Nutr. 57, 292–304.
Azad, S.B., Ansari, P., Azam, S., Hossain, S.M., Shahid, M.I.B., Hasan, M., Hannan, J., 2017. Anti-hyperglycaemic activity of Moringa oleifera is partly mediated by carbohydrase inhibition and glucose-fibre binding. Biosci. Rep. 37, BSR20170059.
Azizuddin, Khan, A.M., Choudhary, M.I., 2011. Tyrosinase inhibitory potential of natural products isolated from various medicinal plants. Nat. Prod. Res. 25, 750–753.
Boo, Y.C., 2019. Can plant phenolic compounds protect the skin from airborne particulate matter? Antioxidants 8, 379.
Brotzman, N., Xu, Y., Graybill, A., Cocolas, A., Ressler, A., Seeram, N.P., Ma, H., Henry, G.E., 2019. Synthesis and tyrosinase inhibitory activities of 4-oxobutanoate derivatives of carvacrol and thymol. Bioorg. Med. Chem. Lett. 29, 56–58.
Burgos-Morón, E., Abad-Jiménez, Z., Martinez de Maranon, A., Iannantuoni, F., Escribano-López, I., López-Domènech, S., Salom, C., Jover, A., Mora, V., Roldan, I., 2019. Relationship between oxidative stress, ER stress, and inflammation in type 2 diabetes: the battle continues. J. Clin. Med. 8, 1385.
Chang, T.S., 2009. An updated review of tyrosinase inhibitors. Int. J. Mol. Sci. 10, 2440–2475.
Cheenpracha, S., Jitonnom, J., Komek, M., Ritthiwigrom, T., Laphookhieo, S., 2016. Acetylcholinesterase inhibitory activity and molecular docking study of steroidal alkaloids from Holarrhena pubescens barks. Steroids 108, 92–98.
Chen, Q.X., Song, K.K., Qiu, L., Liu, X.D., Huang, H., Guo, H.Y., 2005. Inhibitory effects on mushroom tyrosinase by p-alkoxybenzoic acids. Food Chem. 91, 269–274.
Chen, Y.S., Geng, S., Liu, B.G., 2020. Three common caffeoylquinic acids as potential hypoglycemic nutraceuticals: Evaluation of alpha-glucosidase inhibitory activity and glucose consumption in HepG2 cells. J. Food Biochem. 44 (9), e13361. https://doi.org/10.1111/jfbc.13361 In this issue.
Cheng, D., Wang, G.L., Tang, J.L., Yao, C.Y., Li, P.F., Song, Q., Wang, C.L., 2020. Inhibitory effect of chlorogenic acid on polyphenol oxidase and browning of fresh-cut potatoes. Postharvest Biol. Technol. 168. https://doi.org/10.1016/j.postharvbio.2020.111282.
Choi, S.J., Kim, J.K., Jang, J.M., Shin, K.H., Lim, S.S., 2012. Rapid identification of the alpha-glucosidase inhibitory compounds from Thunberg's geranium (Geranium thunbergii Sieb. et Zucc.). Food Sci. Biotechnol. 21, 987–996.
Choubdar, N., Golshani, M., Jalili-Baleh, L., Nadri, H., Küçükkilinç, T.T., Ayazgök, B., Moradi, A., Moghadam, F.H., Abdolahi, Z., Ameri, A., 2019. New classes of carbazoles as potential multi-functional anti-Alzheimer's agents. Bioorg. Chem. 91, 103164.
Chung, I.M., Chelliah, R., Oh, D.H., Kim, S.H., Yu, C.Y., Ghimire, B.K., 2019. Tupistra nutans Wall. root extract, rich in phenolics, inhibits microbial growth and alpha-glucosidase activity, while demonstrating strong antioxidant potential. Rev. Bras. Bot. 42, 383–397.
Demir, E., 2019. Sensitive and selective pathway of total antioxidant capacity in commercially lemon, watermelon and mango-pineapple cold teas by square wave adsorptive stripping voltammetry. Gazi Univ. J. Sci. 32, 1123–1136.
García, M.E., Borioni, J.L., Cavallaro, V., Puiatti, M., Pierini, A.B., Murray, A.P., Peñéñory, A.B., 2015. Solanocapsine derivatives as potential inhibitors of acetylcholinesterase: synthesis, molecular docking and biological studies. Steroids 104, 95–110.
Ghimire, B.K., Sacks, E.J., Kim, S.H., Yu, C.Y., Chung, I.M., 2021. Profiling of phenolic compounds composition, morphological traits, and antioxidant activity of Miscanthus sacchariflorus L. accessions. Agronomy 11, 31 -Basel.
Hyatt, J.R., Zhang, S.Y., Akoh, C.C., 2021. Comparison of antioxidant activities of selected phenolic compounds in O/W emulsions and bulk oil. Food Chem. 349,, 129037. https://doi.org/10.1016/j.foodchem.2021.129037 In this issue.
Jin, T., Song, Z., Weng, J., Fantus, I.G., 2018. Curcumin and other dietary polyphenols: potential mechanisms of metabolic actions and therapy for diabetes and obesity. Am. J. Physiol. Endocrinol. Metab. 314, E201–E205.
Jung, H.A., Jung, Y.J., Hyun, S.K., Min, B.S., Kim, D.W., Jung, J.H., Choi, J.S., 2010. Selective cholinesterase inhibitory activities of a new monoterpene diglycoside and other constituents from Nelumbo nucifera Stamens. Biol. Pharm. Bull. 33, 267–272.
Karaer, F., 2012. Draba cemileae (Brassicaceae), a new species from NE Anatolia, Turkey, Annales Botanici Fennici. BioOne 1-2, 111–116.
Kasote, D.M., Katyare, S.S., Hegde, M.V., Bae, H., 2015. Significance of antioxidant potential of plants and its relevance to therapeutic applications. Int. J. Biol. Sci. 11, 982–991.
Kiani, R., Arzani, A., Maibody, S., 2021. Polyphenols, flavonoids, and antioxidant activity involved in salt tolerance in wheat, Aegilops cylindrica and their amphidiploids. Front. Plant Sci. 12, 13.
Kiełczewska, U., Jorda, R., Gonzalez, G., Morzycki, J.W., Ajani, H., Svrčková, K., Štěpánková, Š., Wojtkielewicz, A., 2021. The synthesis and cholinesterase

inhibitory activities of solasodine analogues with seven-membered F ring. J. Steroid Biochem. Mol. Biol. 205, 105776.
Kim, J.S., Kwon, C.S., Son, K.H., 2000. Inhibition of alpha-glucosidase and amylase by luteolin, a flavonoid. Biosci. Biotechnol. Biochem. 64, 2458–2461.
Kocak, M.S., Sarikurkcu, C., Cengiz, M., Kocak, S., Uren, M.C., Tepe, B., 2016. Salvia cadmica: phenolic composition and biological activity. Ind. Crops Prod. 85, 204–212.
Krishnaiah, D., Sarbatly, R., Nithyanandam, R., 2011. A review of the antioxidant potential of medicinal plant species. Food Bioprod. Process. 89, 217–233.
Liu, Y.M., Feng, Y.D., Lu, X., Nie, J.B., Li, W., Wang, L.N., Tian, L.J., Liu, Q.H., 2017. Isosteroidal alkaloids as potent dual-binding site inhibitors of both acetylcholinesterase and butyrylcholinesterase from the bulbs of Fritillaria walujewii. Eur. J. Med. Chem. 137, 280–291.
Magaji, U., Sacan, O., Yanardag, R., 2020. Alpha amylase, alpha glucosidase and glycation inhibitory activity of Moringa oleifera extracts. S. Afr. J. Bot. 128, 225–230.
Mathers, C.D., Loncar, D., 2006. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med. 3, e442.
Mohebbi, G., Nabipour, I., Vazirizadeh, A., Vatanpour, H., Farrokhnia, M., Maryamabadi, A., Bargahi, A., 2018. Acetylcholinesterase inhibitory activity of a neurosteroidal alkaloid from the upside-down jellyfish Cassiopea andromeda venom. Rev. Bras. Farmacogn. 28, 568–574.
Movahhedin, N., Zengin, G., Bahadori, M.B., Sarikurkcu, C., Bahadori, S., Dinparast, L., 2016. Ajuga chamaecistus subsp. scoparia (Boiss.) Rech. f.: a new source of phytochemicals for antidiabetic, skin-care, and neuroprotective uses. Ind. Crops Prod. 94, 89–96.
Ozer, M.S., Kirkan, B., Sarikurkcu, C., Cengiz, M., Ceylan, O., Atilgan, N., Tepe, B., 2018. Onosma heterophyllum: phenolic composition, enzyme inhibitory and antioxidant activities. Ind. Crops Prod. 111, 179–184.
Park, S.H., Oh, T.H., Kim, S.S., Kim, J.E., Lee, S.J., Lee, N.H., 2012. Constituents with tyrosinase inhibitory activities from branches of Ficus erecta var. sieboldii King. J. Enzym. Inhib. Med. Chem. 27, 390–394.
Pawlowska, E., Szczepanska, J., Koskela, A., Kaarniranta, K., Blasiak, J., 2019. Dietary polyphenols in age-related macular degeneration: protection against oxidative stress and beyond. Oxidative Med. Cell Longev. 2019, 9682318.
Phromnoi, K., Suttajit, M., Saenjum, C., Limtrakul, P., 2021. Inhibitory effect of a rosmarinic acid-enriched fraction prepared from Nga-mon (Perilla frutescens) seed meal on osteoclastogenesis through the RANK signaling pathway. Antioxidants 10 (2), 307. https://doi.org/10.3390/antiox10020307.
Ramasubbu, N., Paloth, V., Luo, Y., Brayer, G.D., Levine, M.J., 1996. Structure of human salivary α-amylase at 1.6 Å resolution: implications for its role in the oral cavity. Acta Crystallogr. Sect. D. Biol. Crystallogr. 52, 435–446.
Richmond, V., Murray, A.P., Maier, M.S., 2013. Synthesis and acetylcholinesterase inhibitory activity of polyhydroxylated sulfated steroids: Structure/activity studies. Steroids 78, 1141–1147.
Righi, N., Boumerfeg, S., Deghima, A., Fernandes, P.A.R., Coelho, E., Baali, F., Cardoso, S.M., Coimbra, M.A., Baghiani, A., 2021. Phenolic profile, safety assessment, and anti-inflammatory activity of Salvia verbenaca L. J. Ethnopharmacol. 272, 113940. https://doi.org/10.1016/j.jep.2021.113940.
Senol, F.S., Slusarczyk, S., Matkowski, A., Perez-Garrido, A., Giron-Rodriguez, F., Ceron-Carrasco, J.P., den-Haan, H., Pena-Garcia, J., Perez-Sanchez, H., Domaradzki, K., Orhan, I.E., 2017. Selective *in vitro* and in silico butyrylcholinesterase inhibitory activity of diterpenes and rosmarinic acid isolated from Perovskia atriplicifolia Benth. and Salvia glutinosa L. Phytochemistry 133, 33–44.
Shaw, J.E., Sicree, R.A., Zimmet, P.Z., 2010. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res. Clin. Pract. 87, 4–14.
Shim, S.Y., Lee, Y.E., Song, H.Y., Lee, M.N., 2020. p-Hydroxybenzoic acid beta-d-glucosyl ester and cimidahurinine with antimelanogenesis and antioxidant effects from Pyracantha angustifolia via bioactivity-guided fractionation. Antioxidants 9, 13.
Song, E.S., Choi, J.Y., Gwon, H.E., Lee, K.Y., Choi, S.G., Islam, M.A., Chun, J.Y., Hwang, J.N., 2021. Phytochemical profile and antioxidant activity of Dracocephalum moldavica L. seed extracts using different extraction methods. Food Chem. 350, 128531. https://doi.org/10.1016/j.foodchem.2020.128531.
Stagos, D., 2020. Antioxidant activity of polyphenolic plant extracts. Antioxidants 9, 19.
Tepe, B., Sarikurkcu, C., Berk, S., Alim, A., Akpulat, H.A., 2011. Chemical composition, radical scavenging and antimicrobial activity of the essential oils of Thymus boveii and Thymus hyemalis. Rec. Nat. Prod. 5, 208–220.
Tian, J.L., Liu, T.L., Xue, J.J., Hong, W., Zhang, Y., Zhang, D.X., Cui, C.C., Liu, M.C., Niu, S.L., 2019. Flavanoids derivatives from the root bark of Broussonetia papyrifera as a tyrosinase inhibitor. Ind. Crops Prod. 138, 111445.
Tolmie, M., Bester, M.J., Apostolides, Z., 2021. Inhibition of alpha-glucosidase and alpha-amylase by herbal compounds for the treatment of type 2 diabetes: a validation of in silico reverse docking with *in vitro* enzyme assays. J. Diabetes. https://doi.org/10.1111/1753-0407.13163 In press.
Vickers, N.J., 2017. Animal communication: when i'm calling you, will you answer too? Curr. Biol. 27, R713–R715.
Wang, J.J., Wang, S.Q., Guo, H.Y., Li, Y., Jiang, Z.H., Gu, T., Su, B.X., Hou, W.G., Zhong, H.X., Cheng, D.D., Zhang, X.J., Fang, Z.P., 2021. Rosmarinic acid protects rats against post-stroke depression after transient focal cerebral ischemic injury through enhancing antioxidant response. Brain Res. 1757, 147336. https://doi.org/10.1016/j.brainres.2021.147336.
Wang, R., Wang, G., Sui, W., Zhou, C., Li, S., Ji, Y., Si, C., 2020. Tyrosinase inhibitory performance of hydrolysate from post-washing liquor of steam exploded corn stalk and its fractionation enhancement. Ind. Crops Prod. 154, 112652.
Yiannopoulou, K.G., Papageorgiou, S.G., 2020. Current and future treatments in alzheimer disease: an update. J. Cent. Nerv. Syst. Dis. 12, 1179573520907397.
Yu, Q., Fan, L., Duan, Z., 2019. Five individual polyphenols as tyrosinase inhibitors: inhibitory activity, synergistic effect, action mechanism, and molecular docking. Food Chem. 297, 124910.
Zeljkovic, S.C., Siskova, J., Komzakova, K., De Diego, N., Kaffkova, K., Tarkowski, P., 2021. Phenolic compounds and biological activity of selected mentha species. Plants 10 (3), 550. https://doi.org/10.3390/plants10030550 -Basel.
Zengin, G., Sarikurkcu, C., Gunes, E., Uysal, A., Ceylan, R., Uysal, S., Gungor, H., Aktumsek, A., 2015a. Two Ganoderma species: Profiling of phenolic compounds by HPLC-DAD, antioxidant, antimicrobial and inhibitory activities on key enzymes linked to diabetes mellitus, Alzheimer's disease and skin disorders. Food Function 6, 2794–2802.
Zengin, G., Sarikurkcu, C., Uyar, P., Aktumsek, A., Uysal, S., Kocak, M.S., Ceylan, R., 2015b. Crepis foetida L. subsp rhoeadifolia (Bleb.) Celak. as a source of multifunctional agents: cytotoxic and phytochemical evaluation. J. Funct. Foods 17, 698–708 .
Zhen, J., Dai, Y., Villani, T., Giurleo, D., Simon, J.E., Wu, Q., 2017. Synthesis of novel flavonoid alkaloids as α-glucosidase inhibitors. Biorg. Med. Chem. 25, 5355–5364.
Zhou, Y.P., Jiang, Z.L., Lu, H.Y., Xu, Z.Y., Tong, R.S., Shi, J.Y., Jia, G.Q., 2019. Recent advances of natural polyphenols activators for Keap1-Nrf2 signaling pathway. Chem. Biodivers. 16, e1900400.
Zolghadri, S., Bahrami, A., Hassan Khan, M.T., Munoz-Munoz, J., Garcia-Molina, F., Garcia-Canovas, F., Saboury, A.A., 2019. A comprehensive review on tyrosinase inhibitors. J. Enzym. Inhib. Med. Chem. 34, 279–309.