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Research Article

Phenolic Composition, *In-vitro* Antioxidant and Enzyme Inhibition Activities of *Cardaria draba* Different Parts

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Abstract

The plant *Cardaria draba* (L.) Desv. subsp. *draba* (Brassicaceae), is traditionally used for various ailments in different cultures. We investigated different solvent extracts such as methanol, ethanol, and water extracts obtained from flowers, leaves, stems, and roots of *C. draba* for their chemical composition, as well as antioxidant and enzyme inhibition activities of acetylcholinesterase (AChE), butyrylcholinesterase (BChE), α-glucosidase, α-amylase, and tyrosinase). RP-HPLC technique was used to determine the phenolic profile of each extracts. The antioxidant activities of the extracts were determined by using DPPH and ABTS method, total phenol and flavonoid content analysis, and iron chelating assay. Enzyme inhibitory potential was evaluated by 96-microplate method. According to the results, aqueous extract of flower demonstrated the highest total phenolic content (64.32 µgGAE/ mg), while leaf ethanol extract showed the highest total flavonoid content (141.47 µgQE/ mg) among all extracts. The methanol, and water extracts of flowers were the most effective DPPH scavenger while aqueous extract of flower was the most active on ABTS scavenging. It is shown that the extracts possess promising activity against α-glucosidase than α-amylase enzyme. The methanolic extract of flower showed anti-acetylcholinesterase activity, and the ethanolic extract of the stem exhibited the best anti-butyrylcholinesterase activity among the 12 extracts. As for the anti-tyrosinase activity, higher kojic acid equivalent values were found for the aqueous extracts of roots and leaves. In conclusion, the data obtained from this study may serve as the basis for the bioassay-guided isolation of active compounds and the development of novel drugs from *Cardaria draba*.

Keywords: *Cardaria draba*, Antioxidant activity, Enzyme inhibition, HPLC

Cardaria draba'nın Farklı Kısımlarının Fenolik Bileşimi, *İn-Vitro* Antioksidan ve Enzim İnhibisyon Aktiviteleri

Öz

Cardaria draba (L.) Desv. subsp. *draba* (Brassicaceae) bitkisi geleneksel olarak farklı kültürlerde çeşitli rahatsızlıklar için kullanılmaktadır. *C. draba*'nın çiçek, yaprak, gövde ve köklerinden elde edilen metanol, etanol ve su gibi farklı solvent ekstrelerinin kimyasal bileşimleri ile antioksidan aktivitesi ve asetilkolinesteraz (AChE), butirilkolinesterazın (BChE), α-glukosidaz, α-amilaz ve tirozinaz enzimleri üzerindeki inhibisyon aktivitelerini araştırdık. Her ekstrenin fenolik profilini belirlemek için RP-HPLC tekniği kullanıldı. Ekstrelerin antioksidan aktiviteleri ise DPPH ve ABTS yöntemi, toplam fenol ve flavonoid içerik analizi ve demir şelatlama deneyi kullanılarak belirlendi. Enzim inhibitör potansiyeli 96-mikroplaka yöntemi ile değerlendirildi. Sonuçlara göre, çiçek ekstresi en yüksek toplam fenolik içeriği (64.32 µgGAE/ mg) gösterirken, yaprak etanol ekstresi ise tüm özütler arasında en yüksek toplam flavonoid içeriğine (141.47 µgQE/ mg) sahip olduğu tespit edilmiştir. Çiçeklerin metanol ve su ekstraktları en etkili DPPH

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süpürücü iken, çiçeklerin sulu ekstraktı ABTS süpürmede en aktifti. Ekstraktların α-glukosidaza karşı α-amilaz enziminden daha umut verici aktiviteye sahip olduğu gösterilmiştir. Çiçeğin metanolik özütü, anti-asetilkolinesteraz aktivitesi gösterdi ve gövdenin etanolik özü, 12 özüt arasında en iyi anti-bütirilkolinesteraz aktivitesi göstermiştir. Anti-tirozinaz aktivitesine gelince, kök ve yaprakların sulu ekstraktları için daha yüksek kojik asite eşdeğer değerleri bulundu. Sonuç olarak, bu çalışmadan elde edilen veriler, aktif bileşiklerin biyo-aktivite rehberli izolasyonu ve *Cardaria draba*'dan yeni ilaçların geliştirilmesi için temel niteliği taşımaktadır.

Anahtar Kelimeler: *Cardaria draba*, Antioksidan aktivite, Enzim inhibisyon, YBSK

1. Introduction

The plant *Cardaria draba* L. (Synonym: *Lepidium draba* L.) belongs to Brassicaceae family, commonly known as hoary cress and traditionally used for various ailments in different cultures (Roughani et al., 2018). The name of genus comes from heart shaped fruits in Greeks (Halimi, 2014). Previous studies of *C. draba* have been reported its secondary metabolites such as flavonoids, phenolics, glucosinolates and alkaloids (Fréchard et al., 2002; Senatore et al., 2003; Mahomoodally etl al., 2018). Different biological activities of *C. draba* extracts were also reported (Sharifi-Rad *et al., 2015;* Ouissem et al., 2018; Seebaluck-sandoram et al., 2019; Bicha et al., 2016; Naser et al., 2019; Kaya et al., 2015).

Alzheimer's disease is the most common neurodegenerative disorder among elder people. In the pathology of this disease, cholinergic theory is accepted that the decrease in the amount of acetylcholine, an important neurotransmitter, has been observed in patients. Acetylcholinesterase (AChE) inhibitors have been one of the therapeutic strategies in the treatment of Alzheimer's disease (Askin et al., 2017). To date, the inhibitory effects of many plant extracts on cholinesterase have been investigated (Yildiztekina et al., 2015).

Tyrosinase is a copper containing enzyme involved in melanin biosynthesis. Its inhibitors have been extensively studied from natural resources due to relationship with Parkinson's disease and skin whitening effect (Neagu et al., 2015). The tyrosinase inhibitors can be used in medicine, cosmetic and food industry for developing new products. As a result, the search on determination of natural sources including the compounds responsible for the tyrosinase inhibitory activity is increasing day by day (Namjoyan et al., 2016).

Medicinal plants have been used as a source of therapeutic agents throughout human history (Boutemak et al., 2015; Benahmed-Bouhafsoun et al., 2015). Today they become a potential source for identification of biological active compounds for a variety of human ailments. In this study, the different solvent extracts (methanol, ethanol and aqueous) from different parts (flowers, leaves, stems and roots) of *C. draba* from Turkey were investigated in terms of their chemical composition, antioxidant, and enzyme inhibition activities for the first time.

2. Material and Method

2.1. Plant materials

The plant material was collected from Konya, located in inner Anatolia of Turkey in June 2018 (C4 Konya: Between Konya and Sarayönü, Dokuz village location, 1050-1100m, 18.06.2018) and authenticated by the botanist Prof. Dr. Yavuz Bagcı of Selcuk University. The voucher specimen (Y. Bağcı 4180) was maintained in the KNYA herbarium of the Selçuk University.

2.2. Preparation of extracts

The plant material was dried at the shade for about two weeks and powdered by laboratory type mill. The different parts (10g) of *Cardaria draba* L. (flower, stem, root and leaf) were macerated separately with methanol, ethanol and water at room temperature for 24 h. After filtration, the plant residue was extracted twice with the same solvent. After the filtrate were combined, the solvent was evaporated under low pressure at 40 ºC. The yield of the extracts was given in Table 3. All the extracts stored at -20°C until further experiments.

2.3. Chemical composition

The phenolic compounds in different extracts of C. draba were qualitatively and quantitatively analyzed by HPLC-DAD. An Agilent 1200 liquid chromatography (LC) system with diode array detector (DAD) (Agilent Technologies, Wilmington, DE, USA) was employed for this purpose. The separation of phenolic compounds in different extracts was performed by an ACE-C18 (4.6 mm × 250 mm; 5 µm) column. The mobile phase comprised of solvent mixtures were (A) ultra-pure water with 0.1% acetic acid, (B) ultra-pure water with 0.1% methanol and (C) ultra-pure water with 0.1% acetonitrile, respectively. The injection volume was 10 µL and detection wavelength was set at 280nm. The flow rate was 0.8 ml. min^{-1}. The column temperature was maintained at 40 ºC. The gradient elusion program was as follows: 0-8 min 80:12:8 A: B: C. 75:15:10 at 8-10min, 70:18:12 at 10-24 min, 65:20:15 at 24-32 min, 50:35:15 at 32-40 min, 25:60:15 at 40-45 min and then back to 80:12:8 to recondition the column for 5 min.

2.4. *In-vitro* antioxidant activity

The antioxidant activity of the different extract was assayed by DPPH and ABTS methods, total phenol and flavonoid content, and iron chelating assay.

2.4.1. DPPH radical scavenging activity

The DPPH radical scavenging activity of extracts obtained from *C. draba* different parts was investigated by colorimetric method (Clarke et al., 2013). Briefly, 180 µL of 0.1mM DPPH solution prepared in methanol was added to 40 µL of sample solutions at different concentrations (0, 50, 100, 250, 500, and 1000 µg/mL). After 30 min, the absorbance was read at 517 nm. The DPPH free radical scavenging potential was calculated using the following equation:

Inhibition %= (Acontrol-Asample)/Acontrol ×100.

2.4.2. ABTS radical scavenging activity

Firstly, the $ABTS^{\bullet+}$ stock solution was produced by reacting 7 mM ABTS in H_2O with 2.45 mM potassium persulfate in the dark for 16 h. The working solution was prepared freshly from

the stock by diluting with methanol to get an absorbance of 0.70 ± 0.02 at 734 nm. Then 180 µL of $ABTS^{\bullet+}$ solution was added to 20 µL of sample solution at different concentrations. After 10 min, the absorbance of the mixture was read at 734 nm (Re et al., 1999).

2.4.3. Total phenol content

The concentrations of phenolic contents of the extract were estimated with Folin-Ciocalteu method and expressed as gallic acid equivalents (mg GAE/g) as dry weight basis and the values were presented as means of triplicate analyses[2] (Elizabeth, 2007).

2.4.4. Total flavonoid content

The concentrations of flavonoid contents in the extract were determined using aluminum chloride colorimetric method and expressed as gallic acid equivalents (mg GAE/g) as dry weight basis (Bag et al., 2015).

2.4.5. Iron chelating assay

The ferrous ion chelating activity of the methanol extracts was evaluated by monitoring the decrease in absorbance at 562 nm of the iron (II)-ferrozine complex. Briefly, 50 µL of 0.1mM $FeSO_4$ was added to 50 µL sample or positive control- EDTA at different concentrations, followed by 100 µL of 0.2 mM ferrozine. The mixture was allowed to equilibrate for 10 min before measuring the absorbance at 562 nm. The ability of the sample to chelate ferrous ion was calculated relative to the control (Chai et al., 2014).

2.5. Enzyme inhibition activity

2.5.1. Acetylcholinesterase and butyrylcholinesterase inhibitory activity

Estimation of anticholinesterase inhibitory activity of the extracts was performed by *in vitro* assays described previously (Ellman et al., 1961; Eruygur and Uçar, 2018). All samples were prepared their stock solution by dissolving in methanol at 5000 µg/mL concentration. Aliquots of 140 µL of 0.1 M phosphate buffer (pH 8.0), 20 µL sample solution at different concentration, and 20 µL of AChE/BChE solution were mixed and incubated for 10 min at room temperature and 10 µL of 0.5Mm DTNB was added. The reaction was then initiated by the addition of 10 µL of 0.71 Mm acetylthiocholine iodide (or 0.22 Mm butyrylthiocholine iodide) as substrate. After incubation for 10 min, the hydrolysis of the substrate was monitored using a Multiscango (Thermo scientific) Elisa reader at 412nm. Galanthamin was used as positive control.

2.5.2. α-glucosidase inhibitory activity

The α-glucosidase (Saccharomyces cerevisiae, EC3.2.1.20, Sigma) inhibitory activity was evaluated according to a previously described method (Yang et al., 2015) with minor modifications. 100 µL of 0.2 U/ml α-glucosidase solution in 0.1M phosphate buffer (pH 6.8) was incubated with 50 µL of extract or acarbose at different concentrations at 37ºC for 15 min. Then, 50 µL of 5mM pNPG (*P*-nitrophenyl-α-D-glucopyranoside) was added as substrate and the absorbance change was recorded at 405 nm after 30 min of incubation. Acarbose was used as positive control and phosphate buffer was used as control instead of sample.

2.5.3. α-amylase inhibitory activity

α-amylase inhibitory activity was assayed according to the procedure described by Özek et al. with as minor modification (Özek, 2018). Amylase activity was determined using soluble starch (1%) as a substrate in 20 mM potassium phosphate buffer (pH 6.9). Briefly, 25 µL of extract or acarbose at different concentrations, 50 µL of α-amylase solution (0.8 U/ml) was mixed with 50 µL of the potassium phosphate buffer. After incubation at 37 ºC for 10 min, 50 µL of the starch solution (1%) was added and the mixture re-incubated at 37 ºC for 20 min. The reaction was terminated by adding 25 µL of HCI and then by adding of 100 µL of I_2/KI solution and absorbance of the extracts was measured at 630 nm.

2.5.4. Tyrosinase inhibitory activity

Tyrosinase inhibitory activity of extracts was determined spectrophotometrically using mushroom tyrosinase (Kim et al., 2005) with slight modification. L-tyrosine was used as substrate and kojic acid was used as standard inhibitors of tyrosinase. The percent inhibition of tyrosinase and IC_{50} values were calculated using a calibration curve developed from the tyrosinase inhibitory activity as percentages (inhibition %) against sample concentrations (µg/mL).

2.6. Statistical analysis

All data on the biological activity tests were the average of triplicate analyses (mean ± SD). All antioxidant and enzyme inhibitory activity tests were carried out for six concentrations and the results are expressed as IC_{50}. The significant differences ($p < 0.05$) between the tested samples was evaluated with One-way ANOVA followed by Tukey's multiple range.

3. Results and Discussion

3.1. Chemical composition of phenolic

The detection and quantitative analysis of phenolic substances are extremely important for plants to excipient in food supplements or pharmaceutical preparations. HPLC method is the most widely used in qualitative and quantitative analysis of plant extracts, drugs, pesticides, and biological samples. The phenolic profiling of *C. draba* extract by HPLC-DAD, was reported for the first time. As shown in Figure 1, 18 mixed standards were successfully separated under the optimized gradient elusion program. A representative chromatogram of the identified phenolic compounds in *C. draba* extracts was also presented in Figure 2. In the current study, sinapic acid was the most dominant phenolic compounds detected in all the *C. draba* extracts except root aqueous extract which included more abundantly caffeic acid. High antioxidant activity is attributed to high amount of total phenolic content (Ceylan et al., 2015). The leading phenolic compound found in *C. draba* flower extract was sinapic acid, ranging from 8009.17-14331.25 µg/g dry weight, followed by root methanol extract (1638.21 µg/g dw) and leaf ethanol extract (1120.23 µg/g dw). Caffeic acid was also found to be as major phenolic compound in flower aqueous extract (7511.94 µg/g dw).

Table 1. The content of phenolic compounds in the methanol, ethanol and water extracts of different part of Cardaria draba (µg/g, n=3)

Analyte	Retention time (min)	Samples											
		CDSM	CDSE	CDSA	CDFM	CDFE	CDFA	CDRM	CDRE	CDRA	CDLM	CDLE	CDLA
Gallic acid	4.69	-	298.02	-	58.12	275.17	442.87	66.43	57.90	221.20	73.44	619.79	73.984
3,4-dihydroxy benzoic acid	6.98	124.19	369.96	192.29	236.41	243.88	318.19	94.02	90.65	150.40	-	241.24	182.46
Catechin	7.97	208.19	136.42	114.72	138.81	282.53	365.23	130.50	119.12	128.94	-	342.15	-
Chlorogenic acid	8.79	110.72	92.11	-	133.88	125.40	92.27	106.47	61.08	-	-	-	-
4-hydroxy benzoic acid	10.65	79.63	144.29	-	870.19	376.00	941.29	91.18	-	871.27	-	244.61	-
1,2-dihydroxy benzene	11.09	3.46	120.07	174.50	286.79	262.37	432.26	175.85	115.98	281.73	83.019	167.74	223.54
Epicatechin	11.40	128.69	133.92	109.87	105.58	152.82	-	106.30	-	-	-	-	166.61
Vanilic acid	11.80	10.927	9.957	13.035	-	-	83.0295	-	-	15.736	-	10.231	26.934
Caffeic acid	12.18	1006.51	1086.94	370.25	543.795	851.36	7511.94	407.49	451.10	332.4	346.81	1626.86	351.10
Vanilin	17.63	-	26.23	24.282	29.298	-	22.845	-	-	-	-	-	-
p-Cumaric acid	18.27	-	11.031	13.556	226.09	7.334	23.126	-	-	-	-	7.965	9.561
Sinapic acid	19.17	515.86	987.23	190.56	14331.25	18497	8009.17	1638.21	175.59	-	120.75	1120.23	119.43
Trans-Ferulic acid	20.07	33.734	36.149	27.967	710.65	1573.41	753.68	107.32	-	-	-	46.674	34..42
Elagic acid	21.17	300.59	35.918	-	28.042	-	41.086	102.41	-	-	14.672	24.755	-
Rutin	22.40	100.59	104.24	101.99	148.22	174.78	102.82	204.13	-	-	-	108.82	-
Salicylic acid	32.88	178.48	476.05	263.07	201.59	639.66	1415.39	-	-	-	-	612.18	356.48
Quercetin	36.26	-	97.79	74.722	322.17	128.78	72.06	37.649	-	86.89	38.022	-	-
Campherol	39.97	-	99.43	94.95	137.60	148.07	82.86	145.79	-	89.00	-	179.48	-

Not: CDSM: methanol extract of stems; CDSE: ethanol extract of stems; CDSA: aqueous extract of stems; CDFM: methanol extract of flowers; CDFE ethanol extract of flowers; CDFA: aqueous extract of stems; CDRM: methanol extract of roots; CDRE: ethanol extract of roots; CDRA: aqueous extract of roots; CDLM: methanol extract of leaves; CDLE: ethanol extract of leaves; CDLA: aqueous extract of leaves

Table 2. Statistical Analysis for the Calibration Curve of Phenolic Compounds

Analyte	Equation of the line	Correlation coefficient (R^2)	Linearity Range (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)
Gallic acid	$27.969x + 29.661$	0.9985	1 - 111	2.318	7.024
3,4-dihydroxy benzoic acid	$18.243x - 5.419$	0.9997	1 - 111	1.051	3.186
Catechin	$15.880x - 10.151$	0.9986	1 - 111	2.245	6.804
Chlorogenic acid	$27.902x - 5.483$	0.9993	1 - 111	1.603	4.857
4-hydroxy benzoic acid	$20.520x + 0.406$	0.9992	1 - 111	1.701	5.155
1,2-dihydroxy benzene	$19.153x - 1.697$	0.9993	1 - 111	1.621	4.912
Epicatechin	$14.514x - 0.147$	0.9972	1 - 111	3.189	9.662
Vanilic acid	$46.875x + 22.624$	0.9979	1 - 111	2.778	8.420
Caffeic acid	$5.434x - 9.184$	0.9915	1 - 111	5.636	17.079
Vanilin	$51.466x + 6.730$	0.9995	1 - 111	1.298	3.933
p-Cumaric acid	$102.581x + 16.824$	0.9996	1 - 111	1.193	3.616
Sinapic acid	$10.607x + 4.067$	0.9993	1 - 111	1.568	4.753
Trans-Ferulic acid	$41.345x + 1.066$	0.9997	1 - 111	0.991	3.004

Elagic acid	8.613x +31.529	0.9964	1 - 111	3.644	11.042
Rutin	33.475x -44.748	0.9983	1 - 111	2.485	7.529
Salicylic acid	5.872x +8.639	0.9949	1 - 111	4.342	13.157
Quercetin	36.193x +0.810	0.9998	1 - 111	0.883	2.674
Campherol	22.258x -6.550	0.9994	1 - 111	1.528	4.630

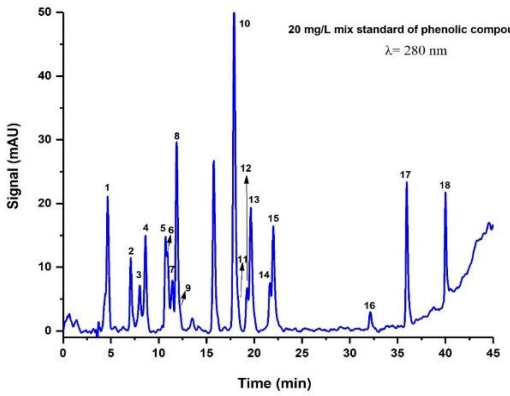

Figure 1. HPLC Chromatogram of mixed standard phenolic compounds (peaks 1: gallic acid; 2: 3,4-dihydroxy benzoic acid; 3: Catechin; 4: Chlorogenic acid; 5: 4-hydroxy benzoic acid; 6: 1,2-dihydroxy benzene; 7: Epicatechin; 8: Vanilic acid; 9: Caffeic acid; 10: Vanilin; 11: p-Cumaric acid; 12: Sinapic acid; 13: Trans-Ferulic acid; 14: Elagic acid; 15: Rutin; 16: Salicylic acid; 17: Quercetin; 18: Campherol

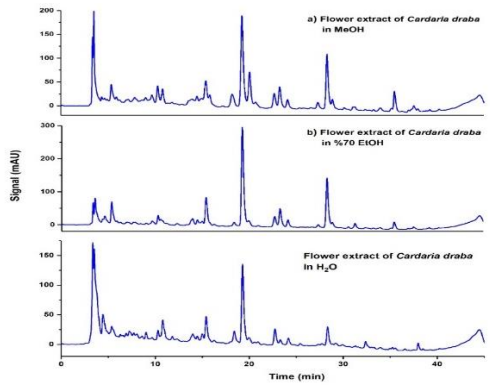

Figure 2. Representative HPLC chromatograms of phenolic compounds of flower extract of *Cardaria draba* in MeOH (a), in %70 EtOH (b), in H_2O (c)

3.2. Antioxidant activity

The free radical scavenging activity of the extract of *C. draba* was tested by two discoloration methods, namely DPPH and ABTS assays. In these methods, the radical scavenging potential was calculated as the percentage of decrease in the initial concentration of the violet-colored DPPH and green colored $ABTS^{\bullet+}$ solution and expressed as IC_{50} values. The aqueous and methanol extract showed highest DPPH radical scavenging activity with least IC_{50} values, 1.27± 1.69 and 1.57± 0.71 mg/mL respectively. In terms of $ABTS^{\bullet+}$ radical scavenging, the methanol extract of the roots exhibited highest scavenging effect with least IC_{50} value of 0.07± 2.18 mg/mL.

The measured TPC levels of the extracts expressed as gallic acid equivalents (GAE), TFC level expressed as quercetin equivalent (QE). According to the results, the TPC and TFC were affected by the extracting solvents as shown in Table 3. There were significant differences in the amount of flavonoids and phenolic compounds in methanol, ethanol and water extracts. Among the extract, the flower aqueous extract demonstrated the highest TPC (64.32 mg GAE/ g), followed by the flower methanol extract (40.04 mg GAE/ g) and stem ethanol extract (12.86 mg GAE/ g) had least activity. However, the leaf ethanol extract showed the highest TFC (141.47 mg QE/ g) followed by leaf methanol extract (103.73 mg QE/ g) while the root methanol extract displayed the least TFC (1.21 mg QE/ g). Mahomoodally et al[3]. found that the TPC was highest in acetone extract (31.67 mg GAE/g extract), and the TFC was highest in aqueous extract (26.98 mg RE/g extract). Compared to our results, TFC and TPC quantities were similar with the literature. In the current study, in order to estimate the effect of extraction solvent on phenolic composition and mostly populated plant parts, we have studied different parts of the *C. draba*, including flower, leaf, stem and root in different extract with different polarity by methanol ethanol and water as solvent.

Table 3. Antioxidant activity of different part extracts of Cardaria draba

Plant parts	Extracts	Extract yield (%)	$DPPH^{\bullet}$ assay IC_{50} (mg/mL)	$ABTS^{\bullet+}$ assay IC_{50} (mg/mL)	Total phenol content (mg GAE/g)	Total flavonoid content (mg QE/g)	Iron ion chelating activity IC_{50} (mg/mL)
Flower	MeOH	26.78	1.57± 0.71	0.51± 3.36	40.04±3.36	61.66± 1.14	4.21± 1.72
	EtOH	45.12	2.02± 1.34	0.45± 1.28	34.16±3.09	75.40± 2.85	1.05± 2.43
	H_2O	29.79	1.27± 1.69	0.30± 1.70	64.32±2.41	75.37± 2.41	1.04± 3.77
Stem	MeOH	11.51	6.51± 0.80	0.62± 2.23	13.82±3.86	3.11± 2.68	18.08± 2.14
	EtOH	18.01	9.04± 0.94	0.68± 2.66	12.86±2.02	6.52± 1.99	2.12± 2.54
	H_2O	14.85	11.14±1.36	0.74± 3.36	20.36±1.46	25.05± 5.43	2.60± 1.69

Root	MeOH	8.89	8.30± 0.46	0.07± 2.18	17.99±1.91	1.21± 0.65	6.97± 2.14
	EtOH	14.22	6.14± 1.15	0.56± 1.69	20.67±3.11	1.36± 0.16	1.05± 2.29
	H_2O	9.05	2.39± 2.04	0.43± 2.14	29.84±2.22	25.73± 6.54	1.34± 3.77
Leaf	MeOH	19.66	3.11 ± 0.79	0.48± 2.53	21.89±3.11	103.73± 6.86	2.20± 0.94
	EtOH	34.69	3.12± 8.27	0.87± 2.27	24.92±2.44	141.47± 2.04	0.68± 2.11
	H_2O	24.54	5.27± 2.49	0.44± 1.47	21.42±1.67	12.13± 0.74	1.25± 1.46
Quercetin			0.11± 0.68				
BHT				0.032±0.92			
EDTA							0.52±1.00

3.2. Enzyme inhibitory activity

3.2.1. Acetylcholinesterase and butyrylcholinesterase inhibitory activity

In the present study, methanol, ethanol and aqueous extract from flowers, stems, leaves and roots of C. draba were evaluated for their inhibitory effect on Alzheimer's disease related key enzymes: acetylcholinesterase and butyrylcholinesterase by in-vitro method. The stem ethanol extract exhibited the higher AChE inhibitory effect with 75.39 mg Galanthamine equivalent/g (mg GALAE/g) dry weight extract, it was followed by flower ethanol extract with 48.88 mg GALAE/g dry extract. In terms of BChE inhibitory activity, the flower methanol extract was exhibited highest butyrylcholinesterase inhibitory activity (36.48 mg GALAE/g dw). Sarikurkcu et al. (2017) was reported the C. draba extract showed AChE and BChE inhibitory effects with 5.71 and 24.38 mg GALAE/g, which were lower than our results

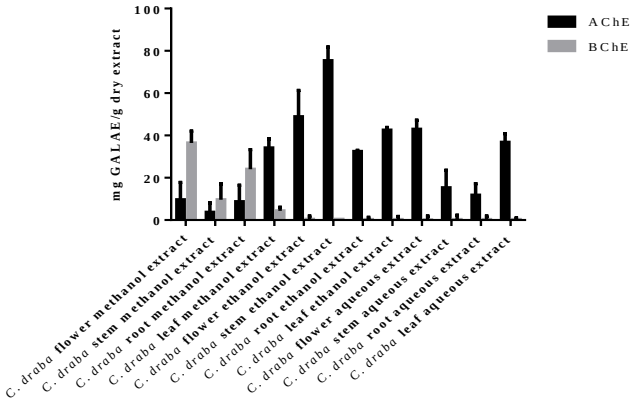

Figure 3. Acetylcholinesterase and butyrylcholinesterase inhibitory activities of different *C. draba* extracts

3.2.2. α-amylase and α-glucosidase inhibitory activity

The different extracts obtained from C. draba were evaluated against the antidiabetic activity enzymes: α-glucosidase and α-amylase. According to the results, the α-amylase was more sensitive to different extracts of this plant. When compared with α-glucosidase activity, all the tested extracts have shown better α-amylase inhibitory activity, ranging from 3.21 to 162.42 mg ACAE / gr dry weight. Among the extracts, the best α-amylase inhibitory effects were observed for leaf aqueous extract (162. 41 mg ACAE /g dw), followed by root and leaf ethanol extracts (120.36 and 107.39 mg ACAE /g dw, respectively).

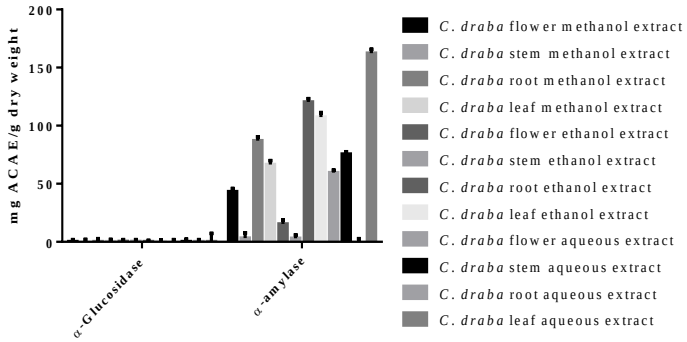

Figure 4. α-amylase and α-glucosidase inhibitory activity of different *C. draba* extracts

3.2.3. Tyrosinase inhibition activity

Results from the tyrosinase inhibitory activity of *C. draba* extracts were shown in Figure 5. In the present study, all the tested extracts exhibited inhibitory effects on tyrosinase. The inhibitory effects on tyrosinase of the extracts decreased in the order: root aqueous extract (581.87 mg KOJE/g dw extract) > leaf aqueous extract (543.64 mg KOJE/g dw) > root methanol and ethanol extract (342.31 and 289.42 mg KOJE/g dw, respectively) > stem methanol extract (283.31 mg KOJE/g dw).

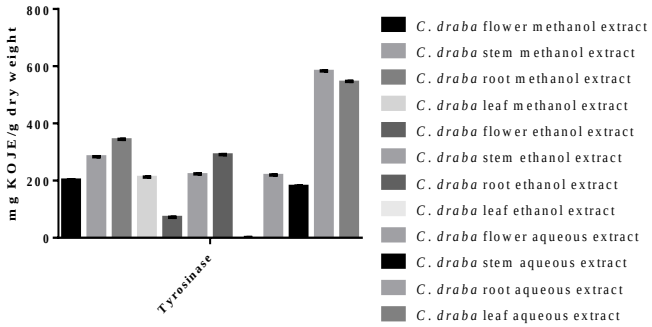

Figure 5. Tyrosinase inhibitory activity of different *C. draba* extracts

4. Conclusions and Recommendations

The current study was successfully developed by HPLC-DAD method to identify and quantify phenolic compounds in different part of *C. draba* extracts. The *in vitro* antioxidant activity was determined by DPPH, ABTS, iron chelating, TPC and TFC methods. Inhibition effects of all extracts on AChE, BChE, α-glucosidase, α-amylase and tyrosinase, are important enzymes for the treatment of AD, DM, and Parkinson's diseases, were investigated. A total of 18 phenolic compounds were identified and quantified in *C. draba* extracts, which was reported for the first time. The observed highest sinapic acid amount in flowers is important for isolation of this compound as an alternative resource. Our results will provide a preliminary data for investigations to exploit new natural antioxidant and enzyme inhibitor substances present in the extracts of this plant species studied. It is important to consider that the phenolic compounds, antioxidant, and enzyme inhibitory activity are not correlated with each other, so it is suggesting that those extracts characterized by lower phenolic content and antioxidant activity, were also potential enzyme inhibitors. Further studies are needed to identify the compounds responsible for AChE, α-amylase and tyrosinase inhibition activity.

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