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ORIGINAL ARTICLE

LC–ESI-MS/MS-BASED PHYTOCHEMICAL PROFILE, ANTIOXIDANT AND ENZYME INHIBITION ACTIVITIES OF EXTRACTS FROM *COUSINIA AINTABESIS* BOISS. & HAUSSKN.

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Abstract

This study aimed to evaluate the antioxidant activity, the inhibitory effect on digestive enzymes linked to diabetes and the phytochemical composition of an active sub-extract obtained from *Cousinia aintabensis* (*Asteraceae*). The antioxidant effect of extracts was investigated with DPPH$^{\bullet}$, ABTS$^{\bullet+}$ and FRAP tests and the inhibition effect was tested on α-amylase and α-glucosidase. The quantitative and qualitative determination of the compounds within the active extract was carried out by LC-MS/MS. The highest total phenolic (185.816 ± 0.003 mg GAE/g extract) and total flavonoid content (147.423 ± 0.003 mg CA/g extract) was found in ethyl acetate sub-extract of *C. aintabensis* (CAE). It also showed the highest antioxidant activity on DPPH$^{\bullet}$ (IC50 = 0.473 ± 0.056 mg/mL), ABTS$^{\bullet+}$ (at a conc. of 0.5 mg/mL equivalent to 0.543 ± 0.005 µM Trolox) and FRAP (at a conc. of 1 mg/mL equivalent to 2586 ± 6.421 mmol Fe^{2+}) tests. Furthermore, the highest α-amylase and α-glucosidase activity were also found in CAE (IC50 = 0.361 ± 0.010 µg/mL for α-glucosidase and 1.010 ± 0.010 for α-amylase). According to LC-MS/MS analysis, the main compounds of CAE were cynarin and isorhamnetin 3-*O*-rutinoside.

Rezumat

Acest studiu și-a propus evaluarea activității antioxidante, a efectului inhibitor asupra enzimelor digestive implicate în diabet precum și a compoziției fitochimice a unui subextract activ obținut din *Cousinia aintabensis* (*Asteraceae*). Efectul antioxidant a fost investigat cu ajutorul testelor DPPH$^{\bullet}$, ABTS$^{\bullet+}$ și FRAP. Efectul inhibitor a fost testat asupra α-amilazei și α-glucozidazei. Determinarea cantitativă și calitativă a compușilor a fost efectuată prin LC-MS/MS. Cel mai mare conținut total de fenoli (185,816 ± 0,003 mg GAE/g extract) și de flavonoide (147,423 ± 0,003 mg CA/g extract) a fost găsit în subextractul de acetat de etil (CAE). De asemenea, acesta a arătat cea mai mare activitate antioxidantă asupra DPPH$^{\bullet}$ (IC50 = 0,473 ± 0,056 mg/mL), ABTS$^{\bullet+}$ (la o concentrație de 0,5 mg/mL echivalent cu 0,543 ± 0,005 µM Trolox) și FRAP (la o concentrație de 1 mg/mL echivalent cu 2586 ± 6,421 mmol Fe^{2+}). CAE a inhibat activitatea α-amilazei și α-glucozidazei (IC50 = 0,361 ± 0,010 µg/mL pentru α-glucozidază și 1,010 ± 0,010 pentru α-amilază). Conform analizei LC-MS/MS, principalii compuși ai subextractului au fost cinarina și izoramnetina 3-O-rutinozida.

Keywords: *Cousinia*, *Asteraceae*, diabetes, antioxidant, LC-MS/MS

Introduction

Diabetes mellitus (DM) is a chronic disease that occurs with a deficiency in insulin production or its action. One of the primary mechanisms for attempting to treat DM (Type II) is the inhibition of certain enzymes such as alpha-amylase and alpha-glycosidase [1]. The hyperglycaemia increases the generation of reactive oxygen species (ROS) that bind to carbohydrates, proteins, lipids or DNA, thereby enable cellular and tissue damages, leading to oxidative stress in T2D [2]. Thus, oxidative stress is predicted as the primary causal factor of T2D associated complications. Moreover, antioxidants are molecules that can inhibit or stop the initiation or propagation of oxidative chain reactions and can therefore prevent or repair the damage caused by ROS [3]. For centuries, medicinal plants were used to treat diabetes due to their availability, the fewer side effects and low cost.

In the *Asteraceae* family *Cousinia* Cass. is one of the most diverse genus. It consists of 600 - 700 species distributed in Central and South-West Asia and represents 38 species and 6 sections in Turkey. *Cousinia aintabesis* Boiss. & Hausskn. is a perennial herb from Cynaroidae Bunge. section. This is a biennial plant with spiny-dentate leaves [4].

Despite the considerable investigation of taxonomy, systematics and phylogeny of *Cousinia* species, their biological and phytochemical research is limited. A study investigated the cytotoxic activity of ethanolic extracts of seven *Cousinia* species against fibrosarcoma WEHI 164 cancer cell line, and *C. verbascifolia* was found with the highest activity (IC_{50} = 18.4 ± 0.59 µg/mL). In the same study, these species reported MMP inhibitory and antibacterial activities. On the

other hand, *C. verbascifolia* fractions were active against ovarian cancer (OVCAR-3), colorectal cancer (HT-29) cell lines and apigenin, caffeic acid was obtained from bioactive fraction [5, 6]. Investigating *C. aitchisonii* led to the isolation of sesquiterpene compounds, namely desoxygenerin and raserolide. These compounds were found cytotoxic against breast cancer (MCF-7) cell line (IC_{50} = 4.5 µg/mL and 4.6 µg/mL, respectively) [7]. In another study, four *Cousinia* species were investigated for cytotoxic activity against A549 and Colo205 cell lines. According to the results, fraction-3 from *C. stenocephala* was found more active than others (IC_{50} = 49.2 ± 0.51 µg/mL) [8]. In a study, the cytotoxic activity of *C. ermenekensis* methanolic extract was determined against A549, Colo205, HepG2, and Beas-2b cell lines. As a result, it was more active against Colo 205 cell line (IC_{50} = 69 µg/mL) [9]. In another study, four *Cousinia* species were investigated for cytotoxic activity against the HepG2 cell line. As a result, *C. davisiana* methanolic extract and ethyl acetate sub-extract were more cytotoxic (IC_{50} = 150 and 89 µg/mL, respectively) [10].

In the literature, systematic and taxonomic studies are generally performed on the *Cousinia* genus. On the other hand, phytochemical and bioactivity studies are very few. Therefore, we aimed to investigate the anti-oxidant effect of the species, as well as the inhibitory activity on α-amylase and α-glucosidase. In this study, the antioxidant capacity, measured by radical scavenging activity (using the DPPH, ABTS and FRAP methods) and the inhibitory activity on digestive enzymes were assessed by measuring the decrease of reducing sugars released by either α-amylase or α-glucosidase on specific substrates. Then the phytochemical profile of extract and quantitative analyses of phenolic compounds were determined by LC-MS/MS.

Materials and Methods

Plant material

The flowering aerial parts of *C. aintabensis* (CA) were harvested from Mardin in July 2017 and identified by Prof. Dr. Osman Tugay. Herbarium specimen was stored at the Herbarium of Selçuk University, Turkey (Voucher No., KNYA 15.332).

Preparation of extracts

Air-dried aerial parts of *C. aintabensis* (500 g) were powdered and extracted three times with methanol by maceration at room temperature. Then, the combined macerates were filtered and evaporated to dryness under reduced pressure at 37°C. Then, the methanolic (CAM, yield %10) extract was dispersed with water (CAW, yield %20) and partitioned with *n*-hexane (CAH, yield %30), ethyl acetate (CAE, yield %15) and *n*-butanol (CAB, yield %25) respectively. Four sub-extracts were obtained from CAM extract and stored in the dark at -20°C.

Evaluation of total phenolic (TPC) and total flavonoid (TFC) contents

The TPC was performed by the method of Saeed *et al*. and expressed as gallic acid equivalents (GAE)/g extract [11]. The (TFC) of extract and sub-extracts was estimated as milligrams of catechin equivalent through the method of Marinova *et al*. [12].

In vitro antioxidant activity and hypoglycaemic properties

Assay of DPPH radical scavenging capacity

DPPH radical scavenging activity of extract and sub-extracts was estimated according to Gyamfi and Aniya [13]. In this assay, the different concentrations of samples were obtained after dilution of the stock solution (4 mg/mL). The concentrations were 0.025, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 and 2.0 mg/mL. In this study, butylated hydroxyanisole (BHA) was used as a positive control, and the absorbance measurement was carried out at 517 nm.

Assay of ABTS radical scavenging capacity

The ABTS assay was performed according to Thaipong *et al*. and results were expressed as Trolox equivalents [14]. The absorbance was read at 734 nm.

Ferric reducing ability power assay (FRAP)

The antioxidant capacity of extract and sub-extracts was also established with the FRAP assay and estimated as mmol Fe^{2+} *per* g extract, according to Guo *et al.* [15].

In vitro hypoglycaemic assay

In this study, the method of Paşayeva L. was used for the *in vitro* evaluation of hypoglycaemic activity [16]. The used concentrations of extracts and sub-extracts ranged between 4 - 2000 µg/mL. After the reaction stopped, the absorbance of samples was read at 540 nm. In this study, the positive control was acarbose. Therefore, the α-amylase inhibitory activity was calculated according to equation (1):

$$(\%) = [1 - (A_{sample}/A_{control})] \times 100 \quad (1).$$

To determine the α-glucosidase inhibitory activity, the concentration of extracts and sub-extracts were prepared between 37 - 1200 µg/mL. According to the method, the increasing absorbance at the end of the reaction due to the released p-nitrophenol was measured at 400 nm. The α-glucosidase inhibitory activity was calculated according to equation (1).

LC-MS/MS assay

LC-ESI-MS/MS (Shimadzu LCMS-8040) was used to conduct the phytochemical analyses in the active sub-extract. The stock solution of the active sub-extract was prepared at 10 µg/mL. The Restek C-18 (150 mm × 4.6 mm, 3 µm) column was used. The mobile phase was acetonitrile (A) and water: acetic acid (99:1, v/v) (B) as 60% solvent A and 40% solvent B at a flow rate of 0.3 mL/min.

Statistical analysis

GraphPad Prism Software Version 8.0 (La Jolla, CA, USA) was used for the statistical analysis. The results are expressed as mean ± standard deviation (S.D.). In

addition, statistically significant values were compared using two-way ANOVA with Tukey Multiple Comparison Test and p-values less than 0.05 were considered statistically significant.

Results and Discussion

In vitro α-glucosidase and α-amylase inhibition of extract and sub-extracts

The inhibitory effect of *C. aintabensis* methanolic extract and sub-extracts on α-glucosidase and α-amylase activity are shown in Table I. A significant inhibitory effect was considered for crude extracts if the IC_{50} value was below 50 µg/mL. Based on this, CAM and CAE sub-extracts decreased α-glucosidase activity with 0.483 ± 0.013 and 0.361 ± 0.010 µg/mL, as well as α-amylase activity with 1.363 ± 0.015 and 1.010 ± 0.010 µg/mL IC_{50} values. The positive control was acarbose (IC_{50} = 0.054 ± 0.0012 µg/mL for α-glucosidase and IC_{50} = 0.117 ± 0.015 µg/mL for α-amylase). None of the other sub-extracts inhibited α-glucosidase and α-amylase. As a result, the CAE sub-extract showed appreciable α-glucosidase and α-amylase inhibitory activity compared to acarbose ($p < 0.001$). Previously, α-glucosidase and α-amylase activity has not been reported for this *Cousinia* species.

Table I

α-amylase and α-glucosidase IC_{50} values of *C. aintabensis* extracts and sub-extracts

Extracts	α-glucosidase inhibition IC_{50} (mg/mL)	α-amylase inhibition IC_{50} (mg/mL)
CAM	0.483 ± 0.013***	1.363 ± 0.015*
CAH	n.d	n.d
CAE	0.361 ± 0.010***	1.010 ± 0.010**
CAB	n.d	n.d
CAW	n.d	n.d
Acarbose	0.054 ± 0.0012**	0.117 ± 0.015*

n.d: Not detected. Values are shown as mean ± standard deviation (n = 3). Different letters for the same column indicate significant differences at *p < 0 .05, **p < 0.001 and ***p < 0.0001. CAM: C. aintabensis methanol extract, CAH: C. aintabensis hexane sub-extract, CAE: C. aintabensis ethyl acetate sub-extract, CAB: C. aintabensis n-butanol sub-extract, CAW: C. aintabensis water sub-extract. IC_{50}: half maximal inhibitory concentration

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The TPC and TFC of CAM extract were 48.411 ± 0.005 mg GAE/g extract and 30.127 ± 0.003 mg CA/g extract, respectively (Table II). Moreover, it was found that the CAE sub-extract contains higher total contents of phenolic compounds (185.816 ± 0.003 mg GAE/g extract) as well as total flavonoids (147.423 ± 0.003 mg CA/g extract) than the other extracts. Furthermore, lower total phenolic and flavonoid content results were detected in water extract (39.737 ± 0.902 mg GAE/g extract and 12.503 ± 1.250 mg CA /g extract).

Table II

Total bioactive compounds and antioxidant capacity results of *C. aintabensis*

Extracts	TPC (mg GAE/g extract)	TFC (mg CA/g extract)	DPPH (IC_{50}) (mg/mL)	FRAP (mmol Fe^{2+}/g extract/ mmol Fe^{2+}/g trolox)	ABTSa (µM Trolox/g extract/ µM Trolox/g BHA)
CAM	48.411 ± 0.005	30.127 ± 0.003	0.694 ± 0.048*	350 ± 17.736*	0.247 ± 0.002***
CAH	14.602 ± 0.002	6.178 ± 0.070	0.945 ± 0.040*	168 ± 5.445*	0.051 ± 0.004**
CAE	185.816 ± 0.003	147.423 ± 0.003	0.473 ± 0.056**	2586 ± 6.421**	0.543 ± 0.005***
CAB	42.531 ± 0.004	16.503 ± 0.005	0.836 ± 0.006	390 ± 11.090*	0.113 ± 0.003***
CAW	16.015 ± 0.007	7.756 ± 0.078	1.061 ± 0.006	183 ± 14.201	0.059 ± 0.008**
Trolox	-	-	-	2616 ± 10.223	-
BHA	-	-	0.056 ± 0.004	-	0.611 ± 0.004**

Values are mean ± SD, n = 3. Different letters for the same column indicate significant differences at *p < 0.05, **p < 0.001 and ***p < 0.0001. [a]concentration at 0.5 mg/mL. CAM: *C. aintabensis* methanol extract, CAH: *C. aintabensis* hexane sub-extract, CAE: *C. aintabensis* ethyl acetate sub-extract, CAB: *C. aintabensis n*-butanol sub-extract, CAW: *C. aintabensis* water sub-extract. IC_{50}: half maximal inhibitory concentration

Antioxidant Activity

1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\bullet+}$) Scavenging Activity

According to our results, CAM extract showed moderate antioxidant activity with 0.694 ± 0.048 mg/mL IC_{50} values (Table II). In this study, the highest DPPH radical scavenging activity was determined for CAE sub-extract (IC_{50} = 0.473 ± 0.056 mg/mL) and was followed by CAB, CAH and CAW extracts (IC_{50} = 0.836 ± 0.006 mg/mL, 0.945 ± 0.040 mg/mL and 1.061 ± 0.006 mg/mL, respectively). In this study, none of the extracts showed higher activity than BHA (IC_{50} = 0.056 ± 0.004 mg/mL).

2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{+\bullet}$) Scavenging Activity

The ABTS radical scavenging activity of extract, sub-extracts and BHA were determined at 0.50 mg/mL. The methanolic extract and CAE sub-extract revealed the highest activity than the others (0.247 ± 0.002 and 0.543 ± 0.005 µM Trolox/g extract). The CAE

sub-extract was found to be more active than the methanolic extract. The activity of the CAE was found to be higher than the activity of BHA ($p < 0.0001$) (0.611 ± 0.004 µM Trolox/g BHA).

Ferric-reducing ability power (FRAP)

Table II shows FRAP values obtained from CA extract and sub-extracts. According to these results, ethyl acetate sub-extract from CA extract was the most active (FRAP: 2586 ± 6.421 mmol Fe^{2+}/g extract) and was followed by CAB, CAM, CAW and CAH (FRAP: 390 ± 11.090, 350 ± 17.736, 183 ± 14.201 and 168 ± 5.445 mmol Fe^{2+}/g extract, respectively). Trolox was used as a standard in this test.

LC-MS/MS Analyses of Bioactive Compounds

The mass spectra revealed the presence of apigenin [17], caffeic acid-3-glucoside, caffeic acid derivative-I, [18], chlorogenic acid, isorhamnetin 3-*O*-rutinoside [17], kaempferol-3-*O*-glucoside [19], malic acid [20], quinic acid [21], rutin [22], cynarin, isoquercitrin [18] and others. The total ion chromatogram and mass spectra of the sub-extract are shown in Figures 1 and 2. In addition, molecular ion, retention time (RT) and MS/MS data of identified compounds are given in Table III. The substances were identified using the NIST (National Institute of Standards and Technology) mass spectral database (version 2.3, USA) and literature data.

Table III

Mass spectral characteristics of phytochemicals in CAE sub-extract

Pik No	RT (min)	$[M-H]^-$ (*m/z*)	MS/MS (*m/z*)	Compounds	References
1	3.70	455	189, 207, 248, 456	Betulinic acid	[42]
2	4.51	377	341, 215, 179, 161,119	Caffeic acid derivative-I	[18]
3	5.15	191	191, 93, 85	Quinic acid	[21]
4	5.16	353	191	Chlorogenic acid	[17]
5	5.21	341	179,135	Caffeic acid-3-glucoside	[18]
6	5.61	179	135, 87	Caffeic acid	[43]
7	6.12	515	353, 191, 179	Cynarin	[18]
8	6.82	477	477, 315, 287, 271, 187	Nepetin 7-glucoside	[44]
9	7.23	133	115	Malic acid	[20]
10	7.61	463	300, 271	Isoquercitrin	[40]
11	8.10	609	301	Rutin	[22]
12	10.22	623	315	Isorhamnetin 3-*O*-rutinoside	[17]
13	11.83	537	284, 537	Hinokiflavone	[45]
14	12.0	447	273, 285, 257, 151	Kaempferol-3-*O*-glucoside	[46]
15	13.45	225	181,165	Myristoleic acid	[42]
16	13.61	269	-	Apigenin	[47]

RT: Retention time

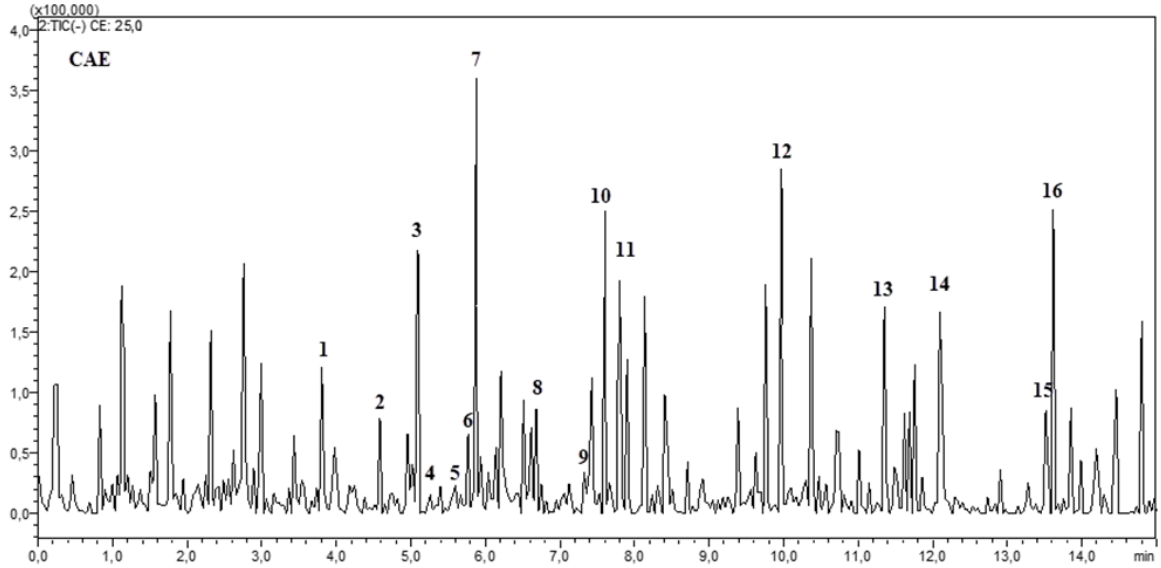

Figure 1.
TIC profile of CAE sub-extract

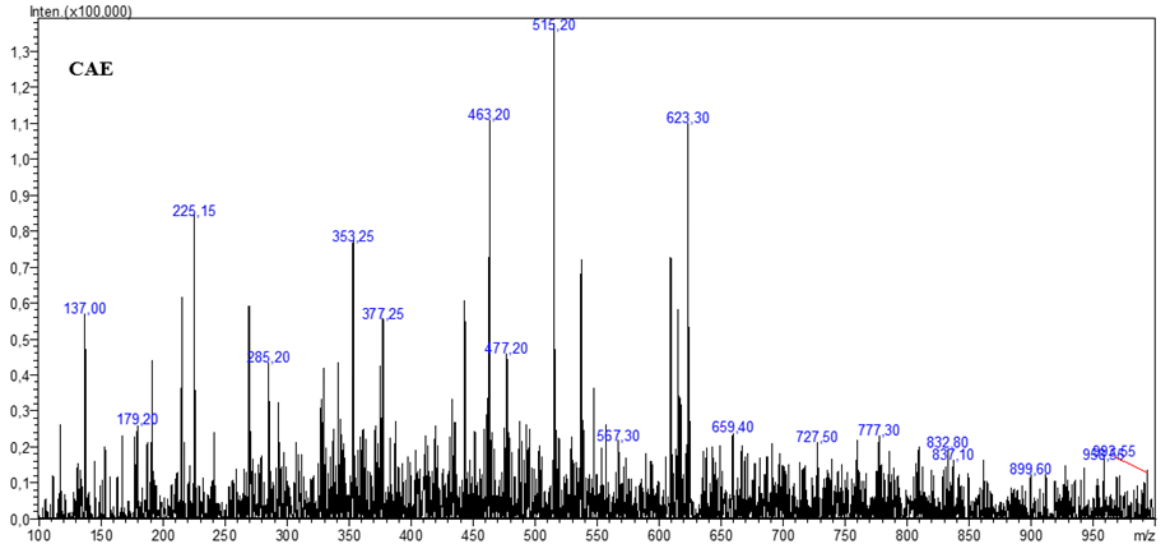

Figure 2.
Mass spectra of CAE sub-extract

Quantitative Analysis of Compounds

Compounds were analysed in Q1Scan (Product Ion Scan) mode and then optimized by an automatic MRM optimization function.

As a result of LC-MS/MS analysis for malic acid, the loss of water $[M-H-H_2O]^-$ provided an ion at m/z 115 and with the loss of CO_2, an intense ion at m/z 71 [23]. The peak was identified as a chlorogenic acid (m/z 353), produced to the loss of one of the caffeoyl moieties $[M^-H^-caffeoyl]^-$, and subsequent fragmentation of ion yielded the fragments at m/z 191 (deprotonated quinic acid), 179 [caffeic acid-H]$^-$, 135 and the peak of the ion at m/z 173 (the absence of a C4 substituent) [24]. Fragmentation of $[M-H]^-$ ion of rutin (m/z 609) gives two major ions at m/z 300 and 301, showing the rhamnose–glucose unit loss. Fragmentation of isorhamnetin 3-O-rutinoside $[M^-H]^-$ ion (m/z 623) resulted ions m/z 285, 300 and 315. Isorhamnetin represents specific fragmentation with the loss of CH_3 radical from the deprotonated aglycone, thus giving m/z 315 → m/z 300 and the m/z 285 patterns as a result of fragmentation in the C-ring [25]. The peak at m/z 515 was observed as cynarin (1,3- dicaffeoylquinic acid) and at MS^2 produced m/z 353 as a 3-caffeoylquinic, to the loss of one of the caffeoyl moieties [M-H-caffeoyl]$^-$ yielded the fragments at m/z 191 (deprotonated quinic acid) and 179 [caffeic acid$^-$H]$^-$ [26]. The predominant signal detected in the first quadrupole (Q1) was $[M^- H]^-$ at m/z 463 and identified as an isoquercitrin, while the product ions were observed at m/z 300, m/z 271. The most sensitive product ion spectra for this analyte showed a loss of m/z 163 from the deprotonated molecular ion ($[M^- H]^-$, m/z 463), producing a molecular fragment with a mass of 300 [27].

The quantitative results of compounds are given in Table IV. Accordingly, cynarin (161.638 ± 0.203 µg/mg extract), isorhamnetin 3-O-rutinoside (147.975 ± 1.943 µg/mg extract), isoquercitrin (136.014 ± 0.190 µg/mg extract), rutin (64.985 ± 0.291 µg/mg extract) and quinic acid (53.486 ± 0.032 µg/mg extract) were the main constituents of CAE sub-extract.

Table IV

Quantification of main compounds in *C. aintabensis* extract and sub-extracts

Constituent	Rt (min)	Content (µg/mg extract)				
		CAM	CAH	CAE	CAB	CAW
Chlorogenic acid	5.1	12.028 ± 0.136	4.216 ± 0.423	2.753 ± 0.490	n.d.	33.982 ± 0.303
Quinic acid	5.2	37.715 ± 0.044	35.558 ± 0.045	53.486 ± 0.032	n.d.	17.466 ± 0.047
Caffeic acid	5.6	25.328 ± 0.933	n.d.	n.d.	8.094 ± 0.099	10.292 ± 0.169
Cynarin	6.1	11.213 ± 0.153	97.319 ± 0.088	161.638 ± 0.203	n.d.	n.d.
Isoquercitrin	6,9	7.700 ± 0.516	1.166 ± 0.057	136.014 ± 0.190	1.245 ± 0.129	n.d.
Malic acid	7.2	7.961 ± 0.038	n.d.	3.547 ± 1.032	0.281 ± 0.062	103.246 ± 3.291
Rutin	8	11.620 ± 0.073	1.640 ± 0.060	64.985 ± 0.291	8.623 ± 0.167	1.654 ± 0.014
Isorhamnetin 3-*O*-rutinoside	10.2	87.273 ± 0.914	0.254 ± 0.094	147.975 ± 1.943	59.669 ± 0.043	0.109 ± 0.058

We investigated the enzyme inhibition, the antioxidant capacity and the phytochemical composition of *C. aintabensis* extract and sub-extracts. According to our results regarding the enzyme inhibitory activity, the CAE sub-extract showed the highest α-amylase and α-glycosidase inhibition effect. There is no previous report on the α-amylase and α-glycosidase inhibition effect of *C. aintabensis* species. However, there are

many studies on the literature's correlation between phenolic content, antioxidant activity, and α-amylase and α-glycosidase inhibition effect [28]. We can say that the higher effect of the CAE sub-extract may be related to the high amount of phenolic and flavonoid content. Previous studies investigated the antioxidant effect and enzyme inhibition activity of *C. iconica*. As a result, the ethyl acetate sub-extract and methanolic extracts were more active on α-amylase and in the antioxidant assay. So, the highest antioxidant activity on DPPH$^{\bullet}$ (IC_{50} = 90.397 ± 0.575 µg/mL) was shown for *C. iconica* methanolic (CIM) extract, and the highest $ABTS^{+\bullet}$ and iron-chelating activities were found in CIE (IC_{50} = 70.70 ± 0.38, and 615.10 ± 2.47 µg/mL, respectively). CIE also showed high activity on α-amylase with 864.57 ± 0.68 µg/mL IC_{50} values. In the same study, rutin and cynarin were determined as the main constituents of CIM and CIE [18]. These findings supported our results.

Recent studies have focused on using polyphenolic and flavonoid compounds in formulating nutritional and medicinal supplements to treat free radical damage and oxidative stress-related diseases. Since the last decade, the high antioxidant activity of the identified compounds in our study, the α-amylase and α-glycosidase inhibition properties, received much attention [29]. It was shown that the inhibitory capacity of flavonoids was correlated with the number of OH groups on the B ring, the 2,3-double bond and hydroxylation at the 5th position [30]. With the ability to strongly bind to proteins resulting in insoluble and indigestible complexes, phenolic compounds are considered enzyme inhibitors [31]. Some of the identified substances in active sub-extract are known for their α-amylase and α-glycosidase inhibition properties. Numerous studies in the literature have reported α-amylase and α-glycosidase inhibition properties of the major compounds cynarin, isorhamnetin 3-O-glucoside, rutin, quinic acid, isoquercitrin [32-34]. Specifically, cynarin (1,3 dicaffeoylquinic acid), the CAE sub-extract's primary content, was reported as a potent inhibitor on key digestive enzymes linked to DM Type II [35]. According to these results, we can consider that the potent α-amylase and α-glycosidase inhibition activity of CAE may be explained by the presence of these phenolic and flavonoid compounds.

Many studies have shown that natural antioxidants can prevent oxidative stress-related diseases such as cancer, rheumatoid arthritis, cardiovascular diseases, and neurodegenerative diseases. In this direction, flavonoids and phenolic acids are known as potential natural antioxidant compounds related to the capacity to scavenge free radicals and reduce Fe^{3+} to Fe^{2+} [36, 37]. In our study, the assessment of antioxidant properties showed that CAE sub-extract was found to be more active than the others, which was coherent with the result of TPC and TFC that developed a higher amount of phenolic and flavonoid content in this sub-extract. To the best of our knowledge, the higher antioxidant capacity of CAE sub-extract may be due to the higher amount of phenolic compounds and flavonoids quantified and the highest content of cynarin, isorhamnetin 3-O-glucoside, rutin, quinic acid, isoquercitrin, which are among the most efficient free radical scavengers. According to Rice-Evans *et al*., the antioxidant activity of phenolic acids and their esters is related to the number of -OH groups in the molecule and the electron-withdrawing properties of the carboxylate group [38]. It is well known that the antioxidant effect of flavonoid compounds is related to the structural conformation of these compounds. The antioxidant activity of flavonoids is linked to -OH groups, 2,3-double bond in conjunction with the 4th carbonyl group in ring C, allowing for delocalization of the phenoxyl radical electron to the flavonoid nucleus [39]. The antioxidant effect of these compounds has previously been reported [40].

LC-MS/MS analysis also confirmed the presence of higher phenolic and flavonoid compounds. Hereby, 10 flavonoids and flavonoid glycosides, 12 phenolic acids and derivatives, 1 pentacyclic triterpene and 1 hydroquinone glycoside were determined in CAE sub-extract and major antioxidant compounds, as cynarin, isorhamnetine3-O glucoside, rutin, quinic acid, isoquercitrin were quantified in this sub-extract. As a result, cynarin was found to have the highest amount in this extract and was followed by isorhamnetin-3-O glucoside, rutin, quinic acid and isoquercitrin.

As a result, it was shown that the ethyl acetate sub-extract, which is rich in phenolic and flavonoid compounds, besides more efficient antioxidant activity, also presented α-amylase and α-glucosidase inhibitory activity. Other researchers also described the positive relationships between the antioxidant effect, the enzyme inhibitory activity and the high phenolic and flavonoid content [41].

Conclusions

In this study, the biological activity and phytochemical composition of *C. aintabensis* were investigated. According to our results, the ethyl acetate sub-extract of this species developed the highest antioxidant activity, as well as α-amylase and α-glycosidase inhibitory effects. There are limited studies on *C. aintabensis*. In this regard, our study brings new scientific approaches and enable further research.

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Conflict of interest

The authors declare no conflict of interest.

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