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Research Article

α-AMYLASE, *α*-GLUCOSIDASE AND LIPASE INHIBITORY PROPERTIES AND PHYTOCHEMICAL ANALYSIS OF ENDEMIC PLANT *Jurinea brevicaulis* Boiss.

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Abstract: Obesity, defined as New World Syndrome, causes global health problems and big economic losses. Natural products have gained increasing importance because of their antiobesity potency. The genus *Jurinea* Cass. with approximately 200 described species worldwide has been traditionally used as a therapeutic agent for colic, fever, gout and rheumatism. The aim of this study was to analyze the volatile components, to determine phenolic compounds and to evaluate *α*-amylase, *α*-glucosidase, and lipase inhibitory activities of the endemic plant species *Jurinea brevicaulis* Boiss. The widely used solid-phase microextraction technique (SPME) was employed for Gas Chromatography-Mass Spectrometry (GC/MS) analysis of the volatile components. Quantitative analysis of phenolic compounds was performed using Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC). A total of 19 volatile components were specified and o-cymene (10.60 %), *β*-bisabolene (9.30 %), and sesquicineole (57.5 %) for different terpenes were described as major components. According to the RP-HPLC analysis, sinapic acid, *p*-coumaric acid and quercetin were determined for the species. IC_{50} values of the species were determined as 36.59 ± 2.37 µg/mL and 42.56 ± 2.83 µg/mL for *α*-amylase and *α*-glucosidase assays, respectively. IC_{50} value was found as 50.31 ± 3.75 µg/mL with the lipase inhibition analysis. In conclusion, it has been determined that *J. brevicaulis* included diverse volatile components, three phenolic compounds with antiobesity effect potential, which highlights *J. brevicaulis* as the up-and-coming candidate of natural product source to be used against obesity.

Özet: Yeni dünya sendromu olarak tanımlanan obezite, küresel sağlık sorunlarına ve büyük ekonomik kayıplara neden olmaktadır. Antiobezite potansiyelleri nedeniyle doğal ürünler, giderek artan bir önem kazanmaktadır. *Jurinea* Cass. cinsi dünya çapında yaklaşık 200 tür içermektedir ve geleneksel olarak kolik, ateş, gut ve romatizma için terapötik bir ajan olarak kullanılmaktadır. Bu araştırma ile *Jurinea brevicaulis* Boiss'in uçucu bileşenlerinin analiz edilmesi, fenolik bileşiklerinin belirlenmesi ve *α*-amilaz, *α*-glukozidaz ve lipaz inhibitör etkilerinin değerlendirilmesi amaçlanmıştır. *Jurinae brevicaulis*'in uçucu bileşenlerinin Gaz Kromatografisi-Kütle Spektrometresi (GC/MS) analizi için katı-faz mikro ekstraksiyon tekniği (SPME) kullanılmıştır. *Jurinae brevicaulis*'in fenolik bileşiklerinin kantitatif analizi, Yüksek performanslı ters faz sıvı kromatografisi (RP-HPLC) kullanılarak yapılmıştır. Toplam 19 uçucu bileşen belirlenmiş ve farklı terpenler için *o*-simen (%10.60), *β*-bisabolen (%9,30) ve seskisinol (%57,5) ana bileşenler olarak tanımlanmıştır. Tür için RP-HPLC analizine göre sinapik asit, *p*-kumarik asit ve kuersetin belirlenmiştir. Türün IC_{50} değerleri *α*-amilaz ve *α*-glukozidaz deneyleri için sırasıyla 36,59 ± 2,37 µg/mL ve 42,56 ± 2,83 µg/mL olarak bulunmuştur. Lipaz inhibisyon IC_{50} değeri 50,31 ± 3,75 µg/mL bulunmuştur. Sonuç olarak, *J. brevicaulis*'in antiobezite etki potansiyeline sahip üç fenolik bileşik içerdiği, bunun da *J. brevicaulis*'i obeziteye karşı kullanılacak doğal ürün kaynağı olarak gelecek vadeden bir aday olarak öne çıkardığı belirlenmiştir.

Introduction

Obesity characterized by excess body weight causes significant deterioration in the fields of health and economy, globally (Ghouse *et al.* 2016). The prevalence rate of obesity increase is in an increasing trend as a response to the developing conditions worldwide. According to 2017 nutrition reports, 2 billion adults and 41 million children were estimated as overweight or obese worldwide (Endalifer & Diress 2020). The evaluation of obesity as a global health problem is related to the increasing risk it poses on emergence of metabolic, cardiovascular and musculoskeletal diseases, and cancer cases (Jack *et al.* 2017, Blüher 2019).

Inhibition of food digestion and absorption aimed to reduce energy intake are significant obesity treatment strategies (Marrelli *et al.* 2013). Pancreatic lipase (PL) secreted by the pancreas plays an important role in fat metabolism and is responsible for converting triglycerides to glycerol and fatty acids by hydrolysis (George *et al.* 2020, Liu *et al.* 2020). Carbohydrates are absorbed in the small intestine after being broken down into monosaccharides by *α*-amylase and *α*-glucosidase as the two major enzymes responsible for their hydrolysis. *α*-amylase secreted by the pancreas breaks carbohydrates into oligosaccharides and *α*-glucosidase completes the breakdown into monosaccharide units (Mahmood 2016). PL, *α*-amylase and *α*-glucosidase inhibitors are therapeutic agents for preventing obesity because of their roles in decelerating triglyceride digestion and retarding the absorption of glucose (Tucci 2010).

Natural compounds have recently gained attention as an alternative strategy to develop more effective and safe agents to be used againts obesity (Marrelli *et al.* 2013, Mohamed *et al.* 2014). Natural phenolic compounds recognized as safe and effective agents to treat or prevent obesity are increasingly reported by researchers and became popular as potential antiobesity agents (Sergent *et al.* 2012). Similarly, volatile compounds from natural sources are well-known to possess antiobesity properties by various underlying mechanisms (Rashed *et al.* 2017).

The genus *Jurinea* Cass. (Asteraceae) is represented with approximately 200 species worldwide (Susanna *et al.* 2006). The genus have long been used traditionally, with the roots of the plants used to obtain gum, aromatic oils for treatment of diarrhea, eye infection, gout, stomachache and rheumatism. Some members have also been used for folk medicine in treatment of colic and puerperal fever in the form of oral decoction (Shah *et al.* 2014, Singh *et al.* 2016). Previous studies showed that members of *Jurinea* contains sesquiterpene lactones and triterpenes as major constituents which possess antileishmanial, anti lipid peroxidation, DNA protection, antibacterial, antifungal, anticholinesterase and antioxidant effects (Singh *et al.* 2016). *Jurinea brevicaulis* Boiss is an endemic species for Türkiye, and was demonstrated to have cytotoxic effects in cancerous cells and high antioxidant activity (Abudayyak *et al.* 2020).

Triterpenes, a large structurally diverse group of natural compounds, display various biological activities including antitumor, antibacterial, antiviral, anti-inflammatory and anti-diabetic (Zhang *et al.* 2017). Bioactive molecules including saponins, phenols, terpenes, glycosides, alkaloids, carotenoids and polysaccharides from natural resources have been known to exhibit lipase inhibitory activity (Singh *et al.* 2015). For instance, lipase inhibitory activity was reported for the chloroform extract and fractions of *Jurinea tzar-ferdinandii* Davidov, and the fractions were demonstrated to contain triterpenes, flavonoids and sesquiterpene lactones (Trendafilova *et al.* 2018).

The antidiabetic and antilipase activities of *Jurinea* members are known to be related with the phytochemical contents of the plants in question (Singh *et al.* 2015, Singh *et al.* 2016, Trendafilova *et al.* 2018). In the present study, we aimed to investigate the α-amylase, α-glucosidase and lipase inhibitory activities of *J. brevicaulis* to reveal its antiobesity potential and to determine the volatile and phenolic contents of the plant by using Solid-phase microextraction Gas Chromatography-Mass Spectrometry (SPME-GC/MS) and RP-HPLC techniques, respectively.

Materials and Methods

Plant material and extraction

The aerial parts of *J. brevicaulis* were collected from Erzincan, Türkiye in July 2017. The voucher specimens are deposited at the Herbarium of Erzincan University, Faculty of Science with voucher number “10958”. The identification of the specimens were performed by Prof. Dr. Ali KANDEMIR.

The collected specimens were air-dried and powdered. The powdered samples were extracted with methanol (2 L×8 h, three times) at room temperature and filtrated. The filtrate was evaporated using a rotary evaporator at 40°C to acquire dry crude residue.

SPME-GC/MS analysis

Solid-phase microextraction (SPME)

To reveal the volatile components, the contained fiber (polydimethylsiloxane/divinyl-benzene (65 µm-blue hub plain) was preconditioned at 250°C in the injection port of a GC for 10 min, then plant extracted for 30 min with the fiber attached in a SPME apparatus. After collecting the sample on the fiber, the SPME device was placed in the injector of the GC and GC-MS devices run for a 62 min GC analysis period using the RTX-5M column. The fiber found in a manual fiber SPME device contained to reveal volatile components was polydimethylsiloxane/divinyl-benzene (65 µm-blue hub plain) (a precondition of the fiber at 250°C in the injection port of a GC for 10 min) for 30 min. After sampling, the SPME device was located into the injector of the GC and the GC-MS instruments through GC

analysis time of 62 min using the RTX-5M column. The SPME fibers were prepared in the GC injector for 30 min at 250°C. Extractions were achieved at 50°C at 30 min for incubation and 10 min for extraction.

Gas chromatography-mass spectrometry/flame ionization detector (SPME-GC/MS-FID)

The SPME procedure included ~1.00 g of plant material placed in a 10 mL vial. Fibers with extracted aroma compounds were then inserted into the GC injector in split mode at a split ratio of 1:10. Thermal desorption was performed at 250°C for 4 min.

A Restek Rxi-5MS capillary column (60 m length, 0.25 mm i.d. and a 0.25 µm phase thickness in split mode) was performed to assist with the separation processes. The baseline oven temperature was 60°C for 2 min, which was subsequently increased by 3°C per min to 240°C and finally held at 250°C for another 4 min. Helium was performed as the carrier gas with a constant flow-rate of 1 mL min^{-1}. Electronic impact mode was benefited to detect once the ionization voltage had been stabilized at 70 eV. The mass acquisition was performed in scan mode (40-450 *m/z*). All volatile components were compared to their respective RI (relative to C7-C30 alkane standards) for the specifications. Mass spectral data were subjected to comparisons against those held in the FFNSC1.2 and Wiley and NIST library. Also, all findings were checked in previous studies (Bicchi *et al.* 2008, Kanbolat *et al.* 2018).

RP-HPLC analysis

Preparing sample solutions

The crude methanolic extracts were redissolved in HPLC grade methanol and filtered through 0.45µm membranes.

HPLC conditions

HPLC analysis of *J. brevicaulis* was conducted with a previously validated HPLC method (Korkmaz *et al.* 2019). The quantitative analysis of *J. brevicaulis* in terms of *p*-hydroxybenzoic acid, vanillic acid, syringaldehyde, *p*-coumaric acid, sinapic acid, benzoic acid, and quercetin was performed using a reverse-phase column (150×4.6 mm i.d, 5 µm) (Waters Spherisorb, Milfort, MA, USA) on an HPLC system (Shimadzu Corporation, LC 20 AT, Kyoto, Japan). The mobile phase consists of two solvents system - A: 100% methanol and B: 2% acetic acid in water (pH 2.8) - and the separations were carried out using gradient mode, at a flow rate of 1.5 mL/min, injection volume of 20 µL. The signals were detected at 232, 270, 280, 290, and 320 nm by a diode array detector (DAD). The compounds in the plant material were identified by comparing retention times and spectral data with pure standards. Calibration curves of the standards were used for quantitative analysis. The HPLC analyzes were repeated three times. Seven standards were performed with the same HPLC conditions to control the repeatability of the methods before quantitative analysis.

In vitro biological activity studies

α-amylase inhibition

The inhibition of α-amylase was determined using the modified iodine/potassium iodide method (Yang *et al.* 2012). *α*-amylase was dissolved in phosphate buffer (0.01 M, pH 6.9/0.006 M NaCl) to prepare 2 Units/mL. Concentration ranges of 12.5-100 µg/mL of the extract and acarbose used as positive control were prepared in phosphate buffer. Briefly, 100 µL sample solutions or distilled water (negative control) and 900 µL *α*-amylase solutions were mixed in test tubes. 100 µL extract solutions (sample blank) or distilled water (negative control blank) and 900 µL phosphate buffer were combined in different test tubes without *α*-amylase solutions. After preincubation at 37°C for 10 min, 500 µL starch solutions were added to each test tube. Incubation at 37°C for 10 min was carried out for reaction mixtures. The reactions were ended using HCl solutions. Finally, 300 µL of iodide solution and 10 mL distilled water were added to each test tube and the absorbances were measured at 620 nm (SpetrostarNano BMG LABTECH). The percent inhibition of α-amylase was specified with the below equation (Eq. 1):

$$\text{Inhibition (\%)} = \left[1 - \frac{A_S - A_{SB}}{A_N - A_{NB}}\right] \times 100 \qquad \text{Eq. 1}$$

A_S: Absorbency of the sample
A_{SB}: Absorbency of the sample blank
A_N: Absorbency of the negative control
A_{NB}: Absorbency of the negative control blank

α-glucosidase inhibition

The inhibition of *α*-glucosidase was determined using the modified version of the method of Palanisamy *et al.* (2011). *α*-glucosidase was dissolved in phosphate buffer (0.1M, pH 6.8/2% BSA) to prepare 0.4 Unit/mL. Concentration ranges of 12.5-100 µg/mL of the extract and acarbose used as positive control were prepared in phosphate buffer (0.01 M, pH 6.9/0.006 M NaCl). Briefly, 20µL sample solutions or distilled water (negative control), 20µL glutathione and 20 µL *α*-glucosidase solutions were mixed in test tubes. 20 µL extract solutions (sample blank) or distilled water (negative control blank), 20µL glutathione solutions, and 20 µL phosphate buffer were combined in different test tubes without *α*-glucosidase solutions. 20µL of *p*-Nitrophenyl *β-D*-glucuronide solutions were added to each test tube. Incubation at 37°C for 15 min was carried out for reaction mixtures. Finally, the reactions were ended using 800µL of Na_2CO_3 solutions. The absorbances were measured at 400nm (SpetrostarNano BMG LABTECH). The percent inhibition of *α*-glucosidase was specified with Eq 1.

Lipase inhibition

Dry methanolic extract of *J. brevicaulis* was tested for lipase inhibition activity by the modified method using *p*-nitrophenyl butyrate (*p*-NPB) (CAS: 2635-84-9) as substrate (Bustanji *et al.* 2011). Firstly, the extract was dissolved in 0.1 M Tris-HCl buffer (pH 8.0) in different concentration ranges of 12.5-100 µg/mL. Orlistat, used as

the positive control to detect lipase inhibitory effect, was prepared in the concentration range of 12.5-100 µg/mL. The test procedure was planned by assigning 4 wells of a plate as A, B, C and D. A included 90 µL enzyme solution [(Crude porcine PL type II (Sigma, EC 3.1.1.3)-(200 units/mL)], 5 µL substrate solution (10 mM *p*-NPB in acetonitrile); 5 µL buffer solution (0.1 M Tris-HCl buffer, pH 8.0), B included 90 µL enzyme solution, 10 µL buffer solution, C included 90 µL enzyme solution, 5 µL substrate solution, 5 µL sample solution and D included 90 µL enzyme solution, 5 µL buffer solution and 5 µL sample solution. Then the plate was incubated at 37°C for 15 min. Following the incubation, a substrate solution (10 mM *p*-NPB in acetonitrile) was mixed into the corresponding well. The plate was once more incubated at the 37°C for 15 min. The absorbances of the reaction mixtures were measured at 405 nm using a spectrophotometer (SpetrostarNano BMG LABTECH). Each sample was run three times to control the repeatability of the assay. The percentage of lipase inhibitory effect was estimated with the following equation (Eq. 2):

$$\text{Inhibition (\%)} = \left[\frac{(A-B)-(C-D)}{(A-B)}\right] \times 100 \qquad \text{Eq. 2}$$

A: Absorbency of negative control
B: Absorbency of negative control blank
C: Absorbency of sample
D: Absorbency of a sample blank

Statistical analyses

All analysis of with the extract and positive controls were performed in triplicate. The linear graphs were created by plotting logarithm of concentrations (Log [C]) (x axis) against the enyzme percentage inhibitions of the samples and positive controls (y ordinate). The regression coefficients were detected for all graphs ($R^2 \geq 0.99$). IC_{50} values for three enzymes were determined using equations of the graphs. The standard deviation of IC_{50} values were calculated. Differences of inhibition percentages between the extracts and positive controls were evaluated by one-way analysis of variance (ANOVA). The differences were considered as significant when the *p*-value was less than 0.05.

Results

Results of SPME-GC/MS analysis

Volatile components of *J. brevicaulis* were investigated by SPME-GC/MS analysis and the results are presented in Table 1. The methanol extract of *J. brevicaulis* extract was found to have monoterpene (6.1%), sesquiterpene (21.7%) and sesquiterpene derivatives (64.5%). The most abundant volatile component found in *J. brevicaulis* was sesquicineole (57.5%).

A total of 19 volatile compounds were identified as hexanal, 2-hexenal, hexanol, myrcene, *α*-phellandrene, *o*-cymene, *α*-cubebene, bergamotene, trans-*β*-caryophyllene, *α*-trans-bergamotene, *β*-farnesene, *α*-curcumene, cubebene, *β*-bisabolene, sesquicineole, *γ*-cadinene, spatulenol, *β*-bisabolol and *α*-bisabolol by using the SPME-GC/MS method (Table 1).

Table 1. Major volatile components of *J. brevicaulis* based on SPME-GC/FID-MS analysis.

Retention Time	% Area	Name	Retention Index	Class
8.653	1.9085	Hexanal	815	Aldehyde
10.248	3.4967	2-Hexenal	861	Aldehyde
10.700	1.6240	Hexanol	874	Alcohol
15.264	1.0069	Myrcene	991	Monoterpene
15.967	2.1571	*α*-Phellandrene	1007	Monoterpene
16.788	2.9726	*o*-cymene	1026	Monoterpene
31.697	0.9117	*α*-Cubebene	1380	Sesquiterpenoid
32.089	1.3849	Bergamotene	1390	Sesquiterpene
33.510	3.8766	Trans-*β*-caryophyllene	1426	Sesquiterpene
33.911	1.2653	*α*-trans-Bergamotene	1437	Sesquiterpene
34.530	1.3262	*β* -Farnesene	1453	Sesquiterpene
35.662	1.9741	*α*-Curcumene	1482	Sesquiterpene
35.848	0.8875	Cubebene	1487	Sesquiterpene
36.637	9.2959	*β*-Bisabolene	1508	Sesquiterpene
36.911	57.4778	Sesquicineole	1516	Sesquiterpenoid
37.324	1.6839	*γ*-Cadinene	1527	Sesquiterpene
39.453	1.1653	Spatulenol	1586	Sesquiterpenoid
42.538	1.5262	*β*-bisabolol	1674	Sesquiterpenoid
42.964	1.4318	*α*-Bisabolol	1686	Sesquiterpenoid
Total	97.373			

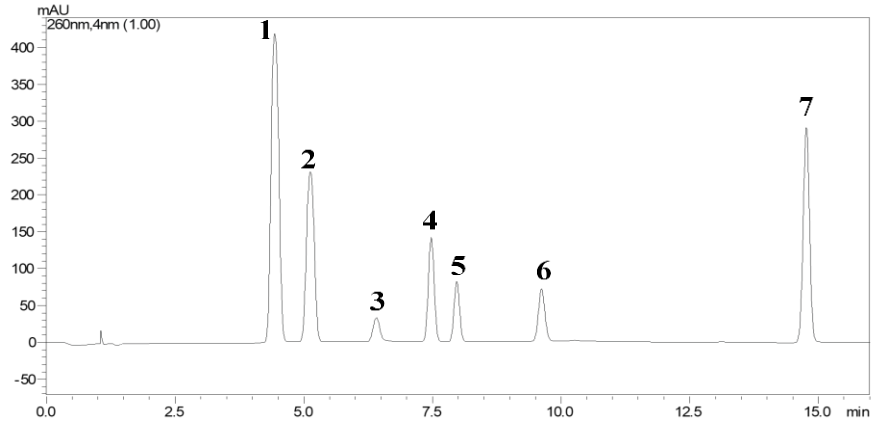

Fig. 1. HPLC chromatogram of the standards. Peak identification: 1. *p*-hydroxybenzoic acid, 2. vanillic acid, 3. syringaldehyde, 4. *p*-coumaric acid, 5. sinapic acid, 6. benzoic acid, 7. Quercetin.

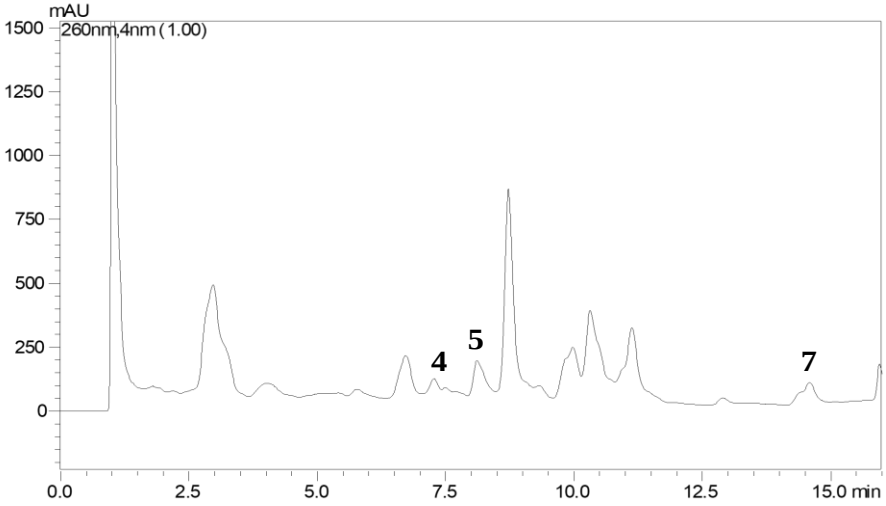

Fig. 2. HPLC chromatogram of the methanol extract of *J. brevicaulis*. Peak identification: 4. *p*-coumaric acid, 5. sinapic acid, 7. Quercetin.

Results of RP-HPLC analysis

The HPLC chromatogram of the standards is shown in Fig. 1. The phytochemical content of *J. brevicaulis* were determined by comparing retention times and spectral data with pure standards.

The quantitative HPLC analysis revealed presence of *p*-coumaric acid (4.98 mg/g crude extract), sinapic acid (28.12 mg/g crude extract) and quercetin (3.53 mg/g crude extract) (Fig. 2).

In vitro biological activity studies

α-amylase and α-glucosidase inhibition

The *α*-amylase and *α*-glucosidase inhibition percentages of *J. brevicaulis* and acarbose are given in Fig. 3 and Table 2. According to *α*-amylase inhibition tests, IC_{50} values of the extract of *J. brevicaulis* and acarbose were found 36.59±2.37 and 21.81±1.17 µg/mL, respectively. According to *α*-glucosidase inhibition tests, IC_{50} values of the acarbose and the extract of *J. brevicaulis* were detected as 28.95±1.38 µg/mL and 42.56±2.83 µg/mL, respectively.

Lipase inhibition

Inhibition percentages of lipase of the extract of *J. brevicaulis* were found 15.16±2.35, 31.76±3.58, 48.01±2.05, 68.92±2.28 µg/mL for the 12.5, 25, 50, and 100 µg/mL, respectively. The IC_{50} value of the extract was determined as 50.31±3.75 µg/mL and IC_{50} value of the positive control orlistat was 13.80±1.07 µg/mL (Fig. 3, Table 2).

Table 2. The results of *α*-amylase, *α*-glucosidase, and lipase inhibition (µg/mL).

	***α*-amylase**	***p* value**	***α*-glucosidase**	***p* value**	**Lipase**	***p* value**
Methanol extract	36.59 ± 2.37	0.0151*	42.56 ± 2.83	0.0272*	50.31 ± 3.75	0.0207*
Acarbose	21.81 ± 1.17	0.0056*	28.95 ± 1.38	0.0071*	-	
Orlistat	-		-		13.80 ± 1.07	0.0025*

* Statistically significant, $p < 0.05$

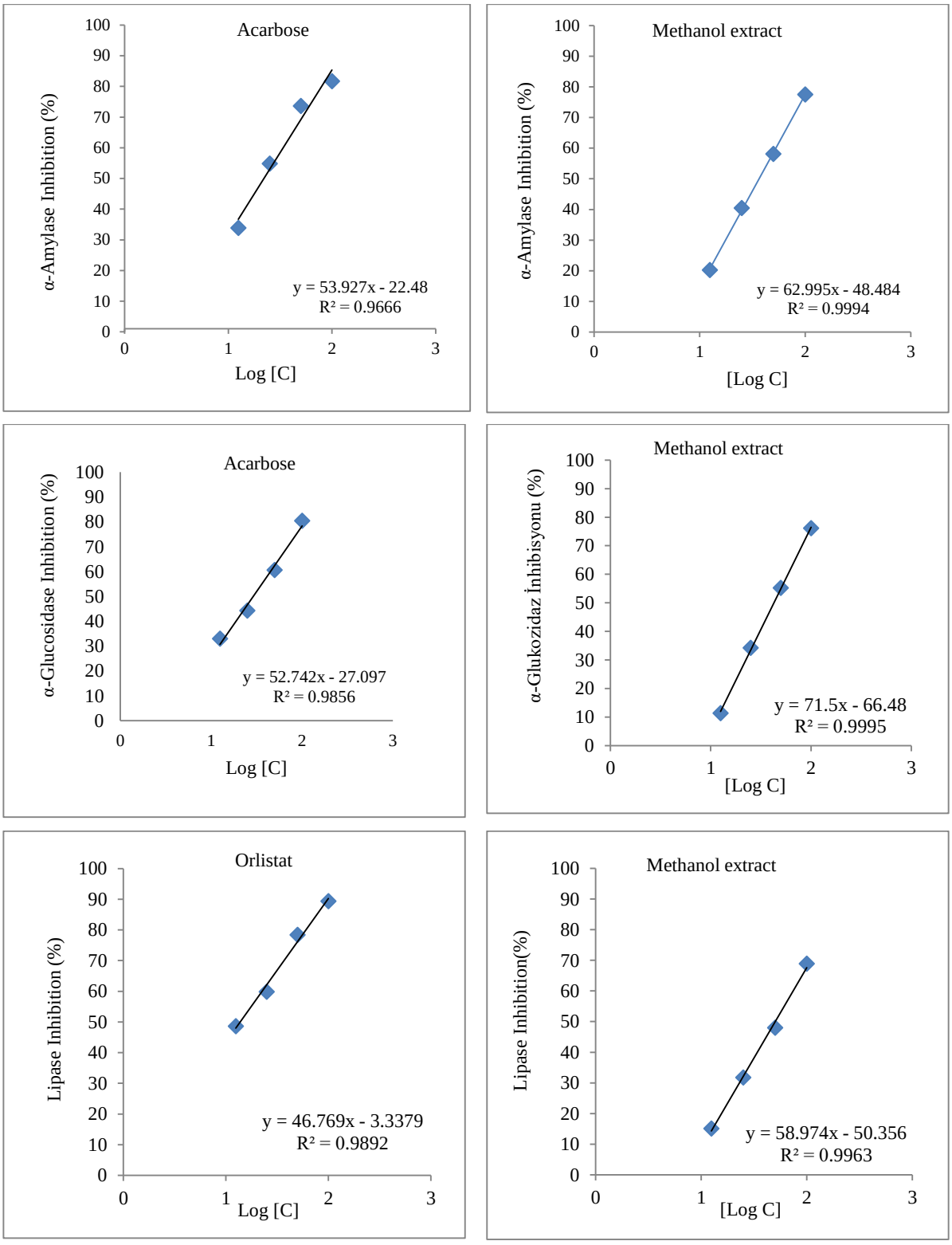

Fig. 3. The graphs of enzyme inhibition analysis.

Discussion

Obesity described as "New World Syndrome" is a provocative risk factor for metabolic, cardiovascular and musculoskeletal diseases and various cancers types. Natural products have recently been preferable to combat with obesity and related metabolic diseases. The major treatment approach in natural product use is the deceleration of food digestion and absorption. A number of natural products like valoneaic acid, acarbose, α-amylase inhibitors, α-glucosidase inhibitors, lipase inhibitors from natural sources have been clinically used for obesity and related diseases. Therefore, natural products obtained from plants have played important roles as antiobesity agents (Sukhev & Singh 2013, Jawed *et al.* 2019).

Natural products provide beneficial contributions in the process of new drug development studies. They can lead to generate new drug candidates for many disorders due to their effective ingredients with limited side effects. Thus, researches have recently focused on identification and application of natural resources in health related cases (Calixto 2019, Bhardwaj *et al.* 2021). Members of the genus *Jurinea* has been used in folk medicine for colic, fever, gout and rheumatism and are known to posses antileishmanial, anti-lipid peroxidation, DNA protection, antibacterial, antifungal, anticholinesterase and antioxidant effects (Singh *et al.* 2016). The antiobesity potencial of *J. brevicaulis*, an endemic species for Türkiye, was evaluated with the present study for the first time by means of determining its retardation effect on food digestion and absorption by investigating the inhibitor effects of extract from the plant on α-amylase, α-glucosidase inhibitors and lipase. The results revealed that the extract of *J. brevicaulis* have significant inhibitory effect on α-amylase (IC_{50} = 36.59±2.37 µg/mL), α-glucosidase (IC_{50} = 42.56 ± 2.83 µg/mL) and lipase (IC_{50} = 50.31±3.75 µg/mL).

The volatile contents of natural resources have been an important issue in development of antiobesity agents (Rashed *et al*. 2017). According to limited GC/MS analysis studies on *Jurinea*, 6-*n*-butyl- 2,3,4,5-tetrahydropyridine, cyclohexene,3,5,5-trimethyl, 1,3-(D2)-Menth-2-ene were determined as major components of *J. leptoloba* DC. (Rustaiyan & Taherkhnai 2013).

As stated previously, plant volatile compounds own the potency to be used as antiobesity agents. The essential oil of *Pinus koraiensis* Siebold & Zucc. exhibited suppressing effect fat accumulation and triglyceride levels in rats fed a high-fat diet, and this antiobesity effect was affiliated with the volatile compounds α-pinene, β-pinene, β-phellandrene, bicyclohept-3-ene, borneol, camphene, (+)-limonene, 3-carene, fencyl and 4-carene (Rashed *et al.* 2017). In another similar study, the essential oil of *Prangos gaubae* (Bornm.) Hernst. & Heyn was proved to exert α-amylase and α-glucosidase inhibitory effect, and caryophyllene oxide, caryophyllene, germacrene D, and spathulenol were determined as major volatile components (Bahadori *et al.* 2017). Essential oils of *Curcuma longa* L. rhizome was demonstrated to show antiobesity effect in obese diabetic rats with α-amylase and α-glucosidase inhibitory effects. Essential oils of *C. zanthorrhiza* Roxb. also exhibited antiobesity effect in obese rats. According to another study, turmerone, α-phyllandrene, curlone, 1,8-cineole, ar-turmerone, β-turmerone, and β-caryophyllene were detected as major volatile components for *C. longa*, and α-curcumene and camphor for *C. zanthorrhiza* (Dosoky & Setzer 2018). The essential oil of *Rhynchanthus beesianus* W.W.Sm. displayed a moderate α-glucosidase inhibition effect. While major volatile components of the essential oil were found as bornyl formate, borneol, cineole and methyleugenol, other volatile contents including sesquicineole, α-phellandrene, α-curcumene, β-bisabolene, γ-cadinene were also determined (Zhao *et al.* 2020). *Jurinea brevicaulis* has some similar volatile components compared to aforementioned antiobesity effective plants, thus, the proposed antiobesity potency of *J. brevicaulis* may be associated with its volatile components.

Phenolic compounds from natural sources possess the potency to treat obesity owing to mechanisms like reduction of the levels of α-amylase, α-glucosidase, lipase, inhibition of the adipogenesis process, reduction of lipid accumulation, and suppression of appetite (Dirar *et al.* 2019, Yen *et al.* 2020). Previous studies showed that the genus *Jurinea* has a rich content in terms of phenolic compounds. Hispidulin and quercetin-3-*O*-rutinoside were isolated from *J. mongolica* Maxim. (Dumaa *et al.* 2018) and chlorogenic acid, pectolineragenin, hispidulin, catechin, caffeic acid and rutin from *J. macrocephala* DC., *J. tzar-ferdinandii* Davidov and, *J. dolomiaea* Boiss. (Shah *et al.* 2014, Trendafilova *et al.* 2018, Kumar & Agnihotri 2020). Based on the fact that phenolic compounds have antiobesity potential, phenolic compounds of *J. brevicaulis* were investigated by HPLC technique and *p*-coumaric acid, sinapic acid, quercetin among phenolic compounds were detected. These phenolic compounds were described from the genus for the first time. Former studies revealed that *p*-coumaric acid decreased the body weight of rats fed a high-fat diet and prevented obesity by activating thermogenesis (Ragab *et al.* 2015, Alam *et al.* 2016, Han *et al.* 2020, Xiong *et al.* 2020). Also, *p*-coumaric acid inhibited the adipogenesis process in 3 T3-L1 adipocytes and induced inhibitory effects of α-amylase, α-glucosidase and lipase (Ragab *et al.* 2015, Alam *et al.* 2016, Han *et al.* 2020, Xiong *et al.* 2020). Similarly, quercetin enhanced the antiobesity effect via decreasing effect on body weight, inhibitor effect on adipogenesis and inhibitory effect on α-amylase (IC_{50} = 0.5 mM), α-glucosidase (IC_{50} = 7 mM) and lipase (IC_{50} = 53.05 µM) (Ragab *et al.* 2015, Tadera *et al.* 2006, Park *et al.* 2019, Yen *et al.* 2020). Sinapic acid was determined to exhibit strong α-amylase and α-glucosidase inhibitory effect but moderate lipase

inhibitory effects (Karamać & Amarowicz 1996, Jeong *et al.* 2012, Alaofi 2020). The antiobesity potency of *J. brevicaulis* can be related to *p*-coumaric acid, quercetin and sinapic acid detected in its methanolic extract.

Conclusion

In the present study, the phenolic components of *J. brevicaulis* was investigated and *p*-Coumaric acid, sinapic acid and quercetin were determined and quantified as the phenolic compounds. Nineteen volatile components were detected and quantified. The results also showed that *J. brevicaulis* showed strong *α*-amylase, *α*-glucosidase and lipase inhibitory effects. Thus, *J. brevicaulis* can be regarded as a promising candidate of natural product for New World Syndrome, obesity.

Ethics Committee Approval: Since the article does not contain any studies with human or animal subject, its approval to the ethics committee was not required.

Data Sharing Statement: All data are available within the study.

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