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ORIGINAL ARTICLE

Isolation of the Major Compounds and Determination of Biological Activities in the Underground Parts of *Trachystemon orientalis* D.Don

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ABSTRACT

Objectives: *Trachystemon orientalis* D.Don, an edible plant, is widely used in folk medicine. This study aimed to investigate the antioxidant and lipase inhibitory activities of the extracts and isolated compounds from the underground parts of *T. orientalis* (TOU).

Materials and Methods: Isolation studies were carried out on the subextracts (the chloroform, ethyl acetate and remaining aqueous) prepared from the methanol extract of TOU using various chromatographic methods, and the structures of the purified compounds were determined by 1D-NMR, 2D-NMR, and mass spectroscopy techniques. To determine antioxidant activity, ferric-reducing antioxidant power (FRAP) and Cu(II) ion-reducing antioxidant capacity (CUPRAC) assays were performed. Lipase inhibitory activity was determined using an *in vitro* spectrophotometric method.

Results: In the isolation studies, rosmarinic acid (**1**) was isolated from the ethyl acetate subextract and danshensu (**2**), globoidnan B (**3**), and rabdosiin (**4**) were isolated from the remaining aqueous subextract. These compounds were isolated from TOU for the first time. The ethyl acetate subextract had higher activity than the other extracts in the FRAP and CUPRAC assays (794.818 ± 8.999, 583.06 ± 5.882 µM Trolox equivalents (TE)/g), respectively] and rosmarinic acid exhibited the highest activity [1260.273 ± 4.499, 608.250 ± 1.195 µM TE/g, respectively). Lipase enzyme inhibitory studies showed that the remaining aqueous and ethyl acetate subextracts had significant inhibitory activity [half maximal inhibitory concentration $(IC)_{50}$ = 38.131 ± 0.720, 38.841 ± 1.359 µg/mL respectively). All isolated compounds inhibited lipase, and rosmarinic acid was the most effective (IC_{50} = 49.421 ± 1.448 µg/mL).

Conclusion: According to the results of this study, *T. orientalis* and its isolated compounds may be a promising natural therapeutic agent for the treatment of obesity *via* its high antioxidant capacity and lipase inhibitory activity.

Keywords: Antioxidant, isolation, lipase inhibition, obesity, *Trachystemon orientalis*

INTRODUCTION

Overweight and obesity are defined as excessive fat accumulation caused by an imbalance in lipid metabolism.[1] It has been reported that there were two billion overweight adults in 2016, and 650 million were affected by obesity.[2] Obesity and hyperlipidemia are associated with oxidative stress and are risk factors for many metabolic disorders, such as atherosclerosis, diabetes, hypertension, and cardiovascular diseases.[3,4] Inhibition of lipid digestion and absorption in the gastrointestinal

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tract is an important option for the treatment or prevention of obesity. In this context, inhibition of the pancreatic lipase enzyme, the primary lipase that breaks down triacylglycerols into monoglycerides and fatty acids, is targeted.[4,5] Orlistat, the most widely used drug for treating obesity approved by the European Medicines Agency and Food and Drug Administration, inhibits pancreatic lipase.[6] However, due to side effects such as steatorrhea, diarrhea, abdominal pain, acute kidney injury, and increased risk of osteoporosis, the search for more effective compounds with fewer side-effect profiles continues.[5,6] Natural products are under investigation for the discovery of safer and more effective pancreatic lipase inhibitors.[7]

Trachystemon orientalis D. Don is the only species of *Trachystemon* D. Don genus of the Boraginaceae family.[8] It is a perennial and herbaceous plant with a black color and tuberous rhizome, reaching 30-40 cm in height.[9] The plant, known as "Hodan, Ispıt, Kalirin, Kaldırayak, Tamara and Acı Hodan" in Türkiye, grows in the Black Sea Region, Caucasus, and Bulgaria.[10] The aerial parts of the plant are used as vegetables, and pickles are made from the petioles and roots.[11,12] In addition to its use as food, it is also used as a folk medicine in Türkiye.

It is used as a diuretic, antipyretic, sudorific, and antidepressant and for sore throats.[13] Its roots are used for anti-inflammatory, wound healing, and rheumatism, breast cancer, stomach pain, and swelling.[14-16] It has been shown to contain flavonoids, phenolic compounds, anthocyanins, tannins, essential oils, mucilage, saponin, resin, and fatty acids.[10,17,18] Studies have shown that it has antioxidant, allelopathic, herbicidal, antiviral, antifungal, antimutagenic, antidiabetic, and butyrylcholinesterase inhibitory activities.[10,12,14,18-20] In an *in vitro* study, it has been shown that rhizomes have anticancer effects on endometrial cancer cells.[21]

To the best of our knowledge, no study has been conducted on the isolation of major compounds from the underground parts (rhizomes and roots) of (TOU). This study aimed to investigate the antioxidant and lipase inhibitory activities of the extracts and isolated compounds from the underground parts of *T. orientalis* (TOU).

MATERIALS AND METHODS

Chemicals and instrumentation

Ethyl acetate and chloroform were purchased from Sigma-Aldrich (St. Louis, USA), methanol from Riedel-de Haën (France), and *n*-hexane from Isolab (Eschau, Germany). For antioxidant activity studies, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and 2,4,6-tripyridyl-s-triazine (TPTZ) were obtained from Fluka Chemie GmbH (Buchs, Switzerland); for lipase inhibition studies, *p*-nitrophenyl butyrate (*p*-NPB) and Tris-HCl were obtained from Sigma-Aldrich. For column chromatography (CC), Sephadex LH-20 (Sigma-Aldrich) and silica gel 60 [normal phase silica gel (Merck 9385, Merck 7734), and reverse-phase silica gel (Merck 9303)] were used. For thin layer chromatography (TLC), silica gel 60 F_{254} 20 x 20 cm (Merck 5554) was used. TLC points were determined by sputtering 1% Vanillin/H_2SO_4 using an ultraviolet lamp (Mineralight UVGL-58). Nuclear magnetic resonance (NMR) spectra were obtained using a Bruker Ascend™ 400 MHz/54 mm ultra-low height. Mass spectroscopy (MS) analyses were performed using Thermo TSQ Quantum Access Max. In addition, a shaker (Heidolph Unimax 1010) was used for extraction, and a rotary evaporator (Heidolph Hei-VAP Precision) was used for solvent evaporation. All absorbance measurements were carried out using a BMG Labtech Spectrostar Nano spectrophotometer. A Starter 3000 OHARUS pH meter was used for all pH measurements.

Plant material

T. orientalis was collected from Sümer Village (Fındıklı district, May 2019, Rize province, Türkiye) and authenticated by Prof. Dr. Ufuk ÖZGEN, one of the authors. The voucher specimen (KATO 15486) was deposited at the KATO Herbarium (in the Faculty of Forestry, Karadeniz Technical University), Trabzon, Türkiye.

Extraction and isolation

Air-dried plant materials were cleaned and powdered, and then the powder (250 g) was extracted with methanol (two times, 1.5 L) at room temperature (25 °C). The combined and filtered extracts were evaporated at 40 °C. The methanolic extract (TOU-M, 6.3 g) was suspended in a mixture of water: methanol (9:1). The obtained suspension was partitioned with chloroform, and after evaporation, a chloroform subextract (TOU-C, 0.6 g) was acquired. To obtain an ethyl acetate subextract (TOU-E, 0.25 g), a water: methanol mixture (9:1) was partitioned with ethyl acetate, and the solvent was evaporated. After the remaining aqueous phase had evaporated to dryness, the remaining aqueous subextract (TOU-A, 5.5 g) was obtained.

TOU-E (0.2 g) was chromatographed over Sephadex LH-20 CC with MeOH as the eluent to yield 21 fractions. Fractions 13-17 were combined, and compound 1 (150 mg) was obtained.

TOU-A (5.3 g) was subjected to vacuum liquid chromatography followed by elution with water: methanol mixture gradiently (100:0 → 0:100). The 111 fractions (A) were gathered. After fractions (A) 5-6 (38 mg) were combined, they were applied to Sephadex LH-20 CC using MeOH to provide 30 fractions and fractions 22-26 gave compound 2 (13.3 mg). Fractions (A) 10-12 (25.2 mg) were combined and chromatographed over Sephadex LH-20 CC using MeOH as the eluent to yield 13 fractions, and fractions 5-8 were combined to yield compound 3 (10 mg). Fraction (A) 41 gave compound 4 (15 mg).

Each collected fraction was subjected to TLC to determine the compounds (mobile phase: EtOAc: MeOH: H_2O 7:2:1, reagent: 1% Vanillin: H_2SO_4). The fractions were combined according to their R_f values on a TLC plate and used for further analysis.

Structure identification

The structure of the isolated compounds was identified with the help of 1D-NMR, 2D-NMR, and MS.

Ferric reducing antioxidant power (FRAP) assay

The basis of the FRAP assay was to determine the ability of the samples to reduce Fe^{+3} to Fe^{+2}.[22] Ethanol solutions of five different concentrations (62.5-1000 μM) of Trolox were used

for calibration. Samples of TOU-M and subextracts (10 mg/mL) were prepared. Samples' solvents were used as blanks. FRAP reagent (1.5 mL) was added to the sample solutions (50 µL). The tubes were incubated (at 25 ˚C, 20 min) after cortexin. Next, the absorbances of the samples were determined at 595 nm with the help of a spectrophotometer. The FRAP values of the samples were compared with those of Trolox (standard) and expressed as µM Trolox equivalent antioxidant capacity (TEAC) per g sample.

Cu(II) ion reducing antioxidant capacity (CUPRAC) assay

The principle of the CUPRAC assay is to measure the copper reduction capacity of the samples.[23] Methanol solutions with five different concentrations (62.5-1000 µM) of Trolox were used for calibration. Samples of TOU-M and subextracts (10 mg/mL) were prepared. Samples' own solvents were used as blanks. Five hundred µL of each sample solution was taken 1000 µL of $NH_4CH_3COO^-$ and 1000 µL of $CuCl_2$. $2H_2O$ was added. Then, 1000 µL of Nc reagent was pipetted into the test solutions, while 1000 µL of reagent solvent (methanol) for the sample blank was pipetted at 20 sec intervals. After cortexin the tubes, they were retained in the dark (at 25 ˚C, 30 min). The absorbance was then measured at 450 nm using a spectrophotometer. The activity of the samples was expressed as TEAC (µM) by comparing it with Trolox (standard).

Lipase inhibition

Lipase inhibition was evaluated using the method[24] and the substrate was *p*-nitrophenyl butyrate (*p*-NPB). The prepared extracts and orlistat (standard) were diluted with a buffer solution (0.1 M Tris-HCl buffer, pH 8.0) at different concentrations (12.5-400 and 6.25-100 µg/mL, respectively). The experimental microplate wells were prepared as follows: enzyme solution (ES) (90 µL, 200 units/mL), substrate solution (SS) (5 µL, 10 mM), buffer solution (BS) (5 µL); B: ES (90 µL, 200 units/mL), BS (10 µL); C: ES (90 µL, 200 units/mL), sample solution (5 µL), SS (5 µL, 10 mM); D: ES (90 µL, 200 units/mL), sample solution (5 µL), BS (5 µL). Microplates were incubated for 15 min at 37 ˚C before and after substrate addition. Microplates were read at 405 nm using a microplate reader. The equation given below was used to determine the percentage of pancreatic lipase enzyme inhibition. All samples were run in 3 parallels.

$$\%\textbf{Pancreatic Lipase Inhibition} = \frac{(\mathbf{A}-\mathbf{B})-(\mathbf{C}-\mathbf{D})}{(\mathbf{A}-\mathbf{B})} \times \mathbf{100}$$

The half maximal inhibitory concentration $(IC)_{50}$ values for enzymatic inhibition of the samples were determined from the equation of the graph obtained using the percentage enzyme inhibition values and the logarithm of the corresponding concentration.

Statistical analysis

In the activity studies, each sample was studied in triplicate. The results were obtained from graphs plotted using Microsoft Excel. Experimental results were presented as means ± standard deviation (SD).

RESULTS

Isolation of major compounds

According to the results of the isolation studies, four known compounds (two phenolic acids, and two arylnaphtalene lignans) from TOU were purified. Rosmarinic acid (1) (phenolic acid) from TOU-E, danshensu (2) (phenolic acid), globoidnan B (3), and rabdosiin (4) (arylnaphtalene lignans) from TOU-A were isolated. The structures of the purified compounds are presented in Figure 1.

Compound 1: ESI-MS (m/e) 361.67 $[M+H]^-$, ($C_{18}H_{16}O_8$); Proton Nuclear Magnetic Resonance (^{1}H-NMR) (400 MHz, CD_3OD): δ 7.57 (*d*, *J*=15.9 Hz, 1H, H-7), 7.07 (*d*, *J*=2.1 Hz, 1H, H-2), 6.97 (*dd*, *J*=8.2 Hz, *J*=2.1 Hz, 1H, H-6), 6.80 (*d*, *J*=8.2 Hz, 1H, H-5), 6.78 (*d*, *J*=2.0 Hz, 1H, H-2'), 6.72 (*d*, *J*=8.0 Hz, 1H, H-5'), 6.64 (*dd*, *J*=8.1 Hz, *J*=2.1 Hz, 1H, H-6'), 6.29 (*d*, *J*=15.9 Hz, 1H, H-8), 5.21 (*dd*, *J*=8.3 Hz, *J*=4.3 Hz, 1H, H-8'), 3.12 (*dd*, *J*=14.3 Hz, *J*=4.4 Hz, 1H, H-7'a), 3.03 (*dd*, *J*=14.3 Hz, *J*=8.3 Hz, 1H, H-7'b); Carbon Nuclear Magnetic Resonance (^{13}C-NMR) (100 MHz, CD_3OD): δ 173.7 (C-9'), 168.6 (C-9), 149.8 (C-4), 147.9 (C-3), 146.9 (C-7), 146.3 (C-3'), 145.4 (C-4'), 129.4 (C-1'), 127.8 (C-1), 123.3 (C-6), 121.9 (C-6'), 117.7 (C-2'), 116.6 (C-5), 116.4 (C-5'), 115.4 (C-2), 114.5 (C-8), 74.8 (C-8'), 38.0 (C-7'). ^{1}H-NMR and ^{13}C-NMR data are in agreement with the previously published data for Rosmarinic acid.[25,26] NMR and MS spectra of Rosmarinic acid are presented in Figures S1-S3.

Compound 2: ESI-MS (m/e) 199.91 $[M+H]^-$, ($C_9H_{10}O_5$); ^{1}H-NMR (400 MHz, CD_3OD): δ 6.63 (*d*, *J*=1.5 Hz, 1H, H-2), 6.58 (*d*, *J*=8.0 Hz, 1H, H-5), 6.49 (*dd*, *J*=8.0 Hz, *J*=2.0 Hz, 1H, H-6), 4.14 (*dd*, *J*=7.6 Hz, *J*=4.1 Hz, 1H, H-2'), 2.86 (*dd*, *J*=14.0 Hz, *J*=3.8 Hz, 1H, H-3'a), 2.65 (*dd*, *J*=13.9 Hz, *J*=7.9 Hz, 1H, H-3'b); ^{13}C NMR (100 MHz, CD_3OD): δ 178.3 (C-1'), 146.1 (C-3), 145.1 (C-4), 130.7 (C-1), 122.1 (C-6), 117.8 (C-2), 116.3 (C-5), 73.5 (C-2'), 41.3 (C-3'). ^{1}H-NMR, ^{13}C-NMR, Correlation Spectroscopy, Heteronuclear Single Quantum Coherence (HSQC), Heteronuclear Multiple Bond Correlation (HMBC), and MS data are agreement with the data given in the literature for Danshensu.[27] NMR and MS spectra of Danshensu are presented in Figures S4-S9.

Compound 3: ESI-MS (m/e) 536.65 $[M-H]^-$ ($C_{27}H_{22}O_{12}$); ^{1}H-NMR (400 MHz, CD_3OD): δ 7.49 (*s*, 1H, H-4), 6.71 (*s*, 1H, H-5), 6.66

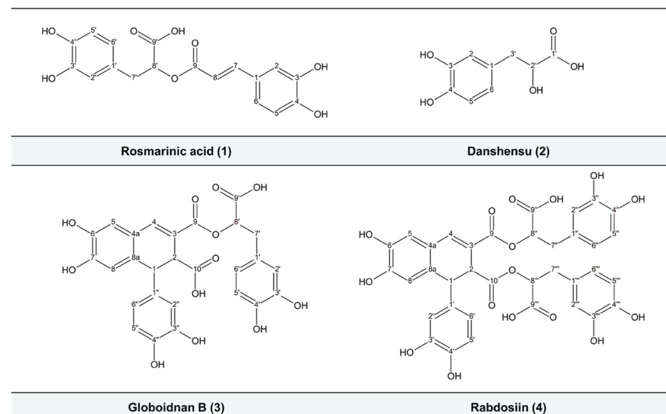

Figure 1. Chemical structures of the isolated compounds

(*d*, *J*=1.5 Hz, 1H, H-2′), 6.57-6.48 (*m*, 3H, H-8, H-5′, H-5″), 6.45 (*s*, 1H, H-6′), 6.39 (*d*, *J*=1.8 Hz, 1H, H-2″), 6.31 (*dd*, *J*=8.2 Hz, *J*=1.9 Hz, 1H, H-6″), 4.94 (*s*, 1H, H-8′), 4.32 (*s*, 1H, H-1), 3.74 (*s*, 1H, H-2), 2.97-2.84 (*m*, 2H, H-7′); ^{13}C-NMR (100 MHz, CD_3OD): δ 177.5 (C-10), 177.3 (C-9′), 169.2 (C-9), 147.5 (C-6), 144.9 (C-3′), 144.9 (C-3″), 144.2 (C-4″), 143.8 (C-7), 143.4 (C-4′), 138.9 (C-4), 138.1 (C-1″), 132.2 (C-8a), 131.2 (C-1′), 125.6 (C-4a), 125.3 (C-3), 122.2 (C-6′), 119.9 (C-6″), 118.0 (C-2′), 117.3 (C-8), 117.2 (C-5), 116.5 (C-5′), 116.5 (C-5″), 115.9 (C-2″), 77.5 (C-8′), 51.0 (C-2), 47.1 (C-1), 38.2 (C-7′). ^{1}H-NMR, ^{13}C-NMR, HSQC, and HMBC data are consistent with the published data for Globoidnan B.[28,29] NMR and MS spectra of Globoidnan B are presented in Figures S10-S15.

Compound 4: ESI-MS (m/e) 717.16 $[M-H]^-$, ($C_{36}H_{30}O_{16}$); ^{1}H-NMR (400 MHz, CD_3OD): δ 7.52 (*s*, 1H, H-4), 6.71 (*s*, 1H, H-5), 6.63-6.59 (*m*, 3H, H-5″, H-2‴, H-5‴), 6.57 (*d*, *J*=8.1 Hz, 1H, H-5′), 6.52 (*s*, 1H, H-2″), 6.50 (*s*, 1H, H-2′), 6.45 (*d*, *J*=8.0 Hz, 2H, H-6″, H-6‴), 6.28 (*t*, *J*=8.6 Hz, 2H, H-8, H-6′), 4.98 (*t*, *J*=5.8 Hz, 1H, H-8″), 4.04 (*s*, 1H, H-1), 3.84 (*d*, *J*=2.0 Hz, 1H, H-2), 2.92-2.74 (*m*, 4H, H-7″, H-7‴), H-8‴ (overlapped the solvent peak); ^{13}C-NMR (100 MHz, CD_3OD): δ 173.7 (C-9‴), 173.6 (C-9″), 173.6 (C-10), 168.1 (C-9), 149.2 (C-7), 146.3 (C-3″), 146.2 (C-3‴), 146.0 (C-4′), 145.6 (C-4‴), 145.3 (C-3′), 145.2 (C-6), 145.0 (C-4″), 141.3 (C-4), 136.7 (C-1′), 131.5 (C-1‴), 129.6 (C-1″), 129.4 (C-8a), 124.9 (C-4a), 122.2 (C-3), 122.2 (C-6‴), 121.7 (C-6″), 120.1 (C-6′), 117.9 (C-5), 117.6 (C-8), 117.5 (C-5‴), 117.4 (C-2″), 117.4 (C-2′), 116.6 (C-2‴), 116.4 (C-5″), 115.9 (C-5′), 75.8 (C-8″), 75.4 (C-8‴), 50.0 (C-2), 46.7 (C-1), 38.0 (C-7″), 38.0 (C-7‴). ^{1}H-NMR, ^{13}C-NMR, HSQC, and HMBC data are consistent with the previous data for Rabdosiin.[29,30] NMR and MS spectra of Rabdosiin are presented in Figures S16-S21.

Table 1. Antioxidant activities of the extracts and isolated compounds

Samples	CUPRAC[a]	FRAP[b]
TOU-M	442.972 ± 7.378	677.545 ± 1.285
TOU-C	65.472 ± 4.317	52.394 ± 5.143
TOU-E	583.06 ± 5.882	794.818 ± 8.999
TOU-A	354.083 ± 6.191	770.273 ± 3.857
Rosmarinic acid	608.250 ± 1.195	1260.273 ± 4.499
Danshensu	221.306 ± 0.851	919.364 ± 7.071
Globoidnan B	478.389 ± 1.264	813.909 ± 2.571
Rabdosiin	483.667 ± 1.534	1041.182 ± 8.357

[a]The CUPRAC value is the copper reducing antioxidant power (μM Trolox equivalent/gram), [b]the FRAP value indicates the iron reducing antioxidant power (μM Trolox equivalent/gram).
TOU-M: The methanol extract of the underground parts of *Trachystemon orientalis*, TOU-C: The chloroform subextract of the underground parts of *T. orientalis*, TOU-E: The ethyl acetate subextract of the underground parts of *T. orientalis*, TOU-A: The remaining aqueous subextract of the underground parts of *T. Orientalis*, CUPRAC[a]: Cu(II) ion reducing antioxidant capacity, FRAP: Ferric reducing antioxidant power

Antioxidant activity

To define the antioxidant activities of the extracts and isolated compounds of TOU, FRAP, and CUPRAC assays were performed, and the results are presented in Table 1. It was determined that the isolated compounds had better antioxidant activity than the extracts. Rosmarinic acid showed the highest activity (FRAP: 1260.273 ± 4.499, CUPRAC: 608.250 ± 1.195 μM TE/g, respectively). TOU-E exhibited higher activity than the other extracts in both tests (FRAP: 794.818 ± 8.999, CUPRAC: 583.06 ± 5.882 μM TE/g, respectively).

Lipase inhibition

The IC_{50} values for lipase inhibitory activities of the extracts and isolated compounds are presented in Table 2. It was observed that the extracts and isolated compounds had weaker lipase inhibitory activities than the standard (orlistat). In addition, TOU-A and TOU-E exhibited higher activity (IC_{50} = 38.131 ± 0.720, 38.841 ± 1.359 μg/mL, respectively) than the other extracts and isolated compounds. Rosmarinic acid demonstrated the highest activity (IC_{50} = 49.421 ± 1.448 μg/mL) among the compounds.

DISCUSSION

Obesity is a serious public health problem, which is defined as the epidemic of the 21st century by the World Health Organization and affects both developed and developing countries.[5,6] Inhibiting the digestion and absorption of nutrients is one of the most important treatment strategies for preventing obesity. Therefore, inhibition of pancreatic lipase, which plays a key role in the digestion of triglycerides, is an interesting therapeutic approach.[31] Herbal products are under investigation for the discovery of effective and safe new pancreatic lipase inhibitor compounds. Natural products, such as saponins, polyphenols, flavonoids, and terpenes, obtained from plants have been reported to be effective.[32]

Table 2. Lipase inhibitory activities of the extracts and isolated compounds

Samples	IC_{50} (μg/mL) ± SD[a]
TOU-M	54.370 ± 0.937
TOU-C	ND[b]
TOU-E	38.841 ± 1.359
TOU-A	38.131 ± 0.720
Rosmarinic acid	49.421 ± 1.448
Danshensu	65.160 ± 4.443
Globoidnan B	79.881 ± 3.435
Rabdosiin	56.801 ± 2.052
Orlistat	17.581 ± 0.714

[a]Standard deviation, [b]ND: Not determined, TOU-M: The methanol extract of the underground parts of *Trachystemon orientalis*, TOU-C: The chloroform subextract of the underground parts of *T. orientalis*, TOU-E: The ethyl acetate subextract of the underground parts of *T. orientalis*, TOU-A: The remaining aqueous subextract of the underground parts of *T. orientalis*, IC_{50}: Half maximal inhibitory concentration, SD: Standard deviation

In this study, isolation studies were performed on subextracts prepared from TOU-M. As a result of the isolation studies, rosmarinic acid was isolated from TOU-E, danshensu, globoidnan B, and rabdosiin were isolated from TOU-A. These compounds were purified from the underground parts of the plant for the first time. According to the literature, no previous isolation studies on *T. orientalis* have been reported. Rosmarinic acid was determined in the roots of the plants using high-performance liquid chromatography.[33]

Rosmarinic acid is a polyphenolic compound formed by the esterification of caffeic acid and danshensu.[34] It is commonly found in plants of the Boraginaceae family.[35] In the Boraginaceae family, globoidnan B, and rabdosiin were isolated from the roots of *Symphytum officinale* L. and danshensu from leaves of *Cordia americana* (L.) Gottschling & J.S.Mill.[36,37] Danshensu, globoidnan B, and rabdosiin have been found in Boraginaceae plants (the aerial parts and the roots of *S. officinale*; the leaves and the roots of *S. ibericum* Steven).[38,39]

In this study, the pancreatic lipase inhibitory and antioxidant activities of the extracts and isolated compounds of TOU were evaluated. According to the results of the pancreatic lipase inhibition experiment, the extracts and compounds were less active than orlistat, and TOU-A showed the highest lipase inhibitory activity. TOU-E had an IC_{50} value similar to that of TOU-A. It was observed that the activity of rosmarinic acid was similar to that of the extracts. It is thought that the activity of the extracts is higher due to the synergistic effects of the isolated phenolic compounds. In an *in vitro* study evaluating the pancreatic lipase inhibition of *Rosmarinus officinalis* L. extract and its phenolic compounds, including rosmarinic acid, the IC_{50} value of the extract was found to be 13.8 µg/mL, whereas that of rosmarinic acid was 125.2 µg/mL. It has been suggested that the effect of the extract may be due to the synergistic effect of rosmarinic acid and other phenolic acids.[7] In another study, it was determined that rosmarinic acid showed high lipase inhibitory activity (IC_{50} = 62.8 ± 2.7 µM) and this result supports our findings.[40] Only a few studies are showing that rosmarinic acid plays a role in different obesity-related mechanisms apart from pancreatic lipase inhibition. A previous study found that it suppresses adipogenesis, lipolysis, and inflammation.[41] In another study, its effects on adipogenesis and lipid metabolism were investigated, and it was reported that it inhibits inflammation and excessive lipid accumulation in human adipocytes.[42]

Obesity causes a decrease in antioxidant capacity by increasing oxidative stress and decreasing the activity of antioxidant enzymes.[1] In this study, according to the antioxidant activity results, TOU-E showed the highest activity among the extracts. In terms of antioxidant activity, the activities of the isolated compounds were better than those of the extracts. It was observed that the activity of rosmarinic acid was the best among the isolated compounds. The isolated compounds are believed to be responsible for the activity of the extracts. In a study, the authors attributed the high antioxidant activity of the extracts prepared from the aerial parts and roots of *T. orientalis* to rosmarinic acid in its content.[33] It has been shown that rosmarinic acid has free radical-scavenging properties and is effective against oxidative reactive oxygen species.[43] In this study, TOU-A had an antioxidant activity similar to that of TOU-E. Compounds from this subextract may be responsible for the activity. In a study, it was shown that danshensu has higher scavenging activity for free hydroxyl radicals, superoxide anion radicals, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radicals compared with vitamin C.[44] It has been reported that the root extracts of *S. officinale* rich in compounds such as rosmarinic acid, globoidnan B, and rabdosiin, as well as extracts prepared from the aerial parts of *S. anatolicum*, show high antioxidant activity owing to their phenolic acids.[39,45] It has been determined that the antioxidant activity (using the DPPH and ABTS assays) of rabdosiin is higher than that of globoidnan B and rosmarinic acid.[37] Phenolic compounds show radical scavenging, metal chelation, and hydrogen donor properties.[32] *T. orientalis* exhibits high antioxidant and antilipase activity *via* its phenolic compounds, and therefore, it may be a promising therapeutic agent for the treatment of obesity.

Study limitations

Further *in vivo*, clinical studies and toxicological analyses are needed to comprehensively reveal the effect of *T. orientalis* on obesity.

CONCLUSION

This study revealed that TOU-E and TOU-A prepared from TOU and the isolated compounds from these extracts have high antioxidant and pancreatic lipase inhibitory activities. The current study is the first to evaluate the effects of *T. orientalis* on obesity *via* lipase inhibition. It is thought that the compounds (1-4) isolated from the plant for the first time and responsible for high antioxidant and antilipase activities. From the perspective of these results, *T. orientalis* is an important natural source that can be evaluated for the treatment of obesity.

Ethics

Ethics Committee Approval: Not required.

Informed Consent: Not required.

Authorship Contributions

Surgical and Medical Practices: A.N.K., T.S., M.B., Ş.K., Concept: A.N.K., M.B., Ş.K., U.Ö., S.Ö.Ş., Design: A.N.K., T.S., U.Ö., Data Collection or Processing: A.N.K., T.S., M.B., Ş.K., U.Ö., Analysis or Interpretation: A.N.K., T.S., M.B., Ş.K., U.Ö., A.D., İ.Ç., Literature Search: A.N.K., T.S., M.B., Ş.K., Writing: A.N.K., T.S., M.B., Ş.K.

Conflict of Interest: The authors have no conflicts of interest to declare.

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REFERENCES

1. Vangoori Y, Dakshinamoorthi A, Kavimani S. Prominent pancreatic lipase ınhibition and free radical scavenging activity of a myristica fragrans ethanolic extract *in vitro*. Potential Role in Obesity Treatment. Maedica (Bucur). 2019;14:254-259.
2. Rakhra V, Galappaththy SL, Bulchandani S, Cabandugama PK. Obesity and the Western diet: How we got here? Mo Med. 2020;117:536-538.
3. Huang R, Zhang Y, Shen S, Zhi Z, Cheng H, Chen S, Ye X. Antioxidant and pancreatic lipase inhibitory effects of flavonoids from different citrus peel extracts: An *in vitro* study. Food Chem. 2020;326:126785.
4. Liu TT, Liu XT, Chen QX, Shi Y. Lipase Inhibitors for obesity: a review. Biomed Pharmacother. 2020;128:110314.
5. Hou XD, Qin XY, Hou J, Tang H, Ge GB. The potential of natural sources for pancreatic lipase inhibitors: a solution to the obesity crisis? Expert Opin Drug Discov. 2022;17:1295-1298.
6. de la Garza AL, Milagro FI, Boque N, Campión J, Martínez JA. Natural inhibitors of pancreatic lipase as new players in obesity treatment. Planta Med. 2011;77:773-785.
7. Bustanji Y, Issa A, Mohammad M, Hudaib M. Inhibition of hormone-sensitive lipase and pancreatic lipase by *Rosmarinus officinalis* extract and selected phenolic constituents. J Med Plant Res. 2010;4:2235-2242.
8. Yıldırımlı S. The chorology of the Turkish species of Boraginaceae family. The Herb Journal of Systematic Botany. 2000;7:257-272.
9. Baytop T. Hodan (Herba Boraginis). In: Türkiye'de bitkiler ile tedavi (geçmişte ve bugün) (1st ed). İstanbul: Istanbul University Press, No. 3255, 1984:256.
10. Sacan O. Antioxidant activity, total phenol, and total flavonoid contents of *Trachystemon orientalis* (L.) G. Don. Eur J Biol. 2018;77:70-75.
11. Koca AD, Yıldırımlı S. Ethnobotanical properties of Akçakoca district in Düzce (Türkiye), HJBC. 2010;38:63-69.
12. Yılar M, Onaran A, Yanar Y, Belguzar S, Kadıoglu I. Herbicidal and antifungal potential of *Trachystemon orientalis* (L.) G. Don (Kaldırık). Iğdır Univ J Inst Sci. 2014;4:19-27.
13. Gul V, Seckin Dinler B. Some medical and aromatic plants growing naturally in the Kumru region (Ordu). ISUBÜ ZFD. 2016;11:146-156.
14. Yeşilada E, Sezik E, Honda G, Takaishi Y, Takeda Y, Tanaka T. Traditional medicine in Türkiye IX: folk medicine in north-west Anatolia. J Ethnopharmacol. 1999;64:195-210.
15. Uzun E, Sariyar G, Adsersen A, Akkol EK, Aslan S, Yılmaz F, Kocabas M. Traditional medicine in Sakarya province (Türkiye) and antimicrobial activities of selected species. J Ethnopharmacol. 2004;95:287-296.
16. Sagiroglu M, Arslanturk A, Akdemir Z, Turna M. An ethnobotanical survey from Hayrat (Trabzon) and Kalkandere (Rize/Türkiye). Biodicon. 2012;5:31-42.
17. Dimcheva V, Kaloyanov N, Karsheva M. The polyphenol composition of *Cistus incanus* L., *Trachystemon orientalis* L. and *Melissa officinalis* L. infusions by HPLC-DAD method. Open J Anal Bioanal Chem. 2019;3:31-38.
18. Demir E. Profile of fatty acids, vitamins, phytosterols and phenolic acids in *Trachystemon orientalis* plant and evaluation of its antioxidant activity. EJOSAT. 2022;33:112-118.
19. Ozen T. Antioxidant activity of wild edible plants in the Black Sea Region of Türkiye. Grasas Y Aceites. 2010;61:86-94.
20. Boğa M, Hacıbekiroğlu I, Kolak U. Antioxidant and anticholinesterase activities of eleven edible plants. Pharm Biol. 2011;49:290-295.
21. Erguven M, Ozsoy N, Aktas E, Bitis L, Taskın T, Bilir A. *Trachystemon orientalis* has anticancer activity which is highly induced by rhizomes on endometrial cancer *in vitro*. Anti-Cancer Drugs. 2015;26:e12.
22. Benzie IF, Strain JJ. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol. 1999;299:15-27.
23. Apak R, Guclu K, Demirata B, Ozyurek M, Celik SE, Bektaşoğlu B. Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. Molecules. 2007;12:1496-1547.
24. Bustanji Y, Al-Masri IM, Mohammad M, Hudaib M, Taha M, Najib N. Pancreatic lipase inhibition activity of trilactone terpenes of Ginkgo biloba. J Enzyme Inhib Med Chem. 2011;26:453-459.
25. Ferlinahayati F, Gultom RPJ, Herlina H, Eliza E. Steroid compounds from *Gynura pseudochina* (Lour) DC. Molekul. 2017;12:8-13.
26. Badem M, Sener SO, Kanbolat S, Kucuk T, Uludağ A, Yılmaz S, Arslan M. Evaluation of biological activities of *Barbarea integrifolia* and isolation of a new glucosinolate derivated compound. Z Naturforsch C J Biosci. 2021;76:375-382.
27. Sidoryk K, Filip K, Cmoch P, Laszcz M, Cybulski M. Efficient synthesis and physicochemical characterization of natural danshensu, its S isomer and intermediates thereof. J Mol Struct. 2018;1153:135-148.
28. Basli A, Delaunay JC, Pedrot E, Riche C, Nicolas L, Gueguen Y, Toutain B, Boudier A. New cyclolignans from *Origanum glandulosum* active against β-amyloid aggregation. Rec Nat Prod. 2014;8:208-216.
29. D'Urso G, Masullo M, Seigner J, Rezzonico E, Dal Toso R, Brighenti V, Bresciani A, Delcarlo M, Pezzoni D, Bonati M, Montalto G. LC-ESI-FT-MS^n metabolite profiling of *Symphytum officinale* L. roots leads to isolation of comfreyn a, an unusual arylnaphthalene lignan. Int J Mol Sci. 2020;21:4671.
30. Flegkas A, Milosević Ifantis T, Barda C, Samara P, Tsitsilonis O, Skaltsa H. Antiproliferative activity of (-)-rabdosiin isolated from *Ocimum sanctum* L. medicines (Basel). 2019;6:37.
31. Buchholz T, Melzig MF. Polyphenolic compounds as pancreatic lipase inhibitors. Planta Med. 2015;81:771-783.
32. Hadrich F, Cher S, Gargouri YT, Adel S. Antioxidant and lipase inhibitory activities and essential oil composition of pomegranate peel extracts. J Oleo Sci. 2014;63:515-525.
33. Bıyık B, Yılmaz Sarıaltın S, Gökbulut A, Çoban T, Çoşkun M. *Trachystemon orientalis* (L.) G. Don as a valuable source of rosmarinic acid: biological activities and HPLC profiles. Turk J Pharm Sci. 2023;20:141-148.
34. Habtemariam S. Molecular pharmacology of rosmarinic and salvianolic acids: potential seeds for Alzheimer's and vascular dementia drugs. Int J Mol Sci. 2018;19:458.
35. Petersen M, Simmonds MS. Rosmarinic acid. Phytochemistry. 2003;62:121-125.
36. Geller F, Schmidt C, Göttert M, Schröder J, Schüßler R, Losch P, Schunk L, Weber T. Identification of rosmarinic acid as the major active constituent in *Cordia americana*. J Ethnopharmacol. 2010;128:561-566.
37. Trifan A, Skalicka-Woźniak K, Granica S, Trifan G, Sinan KI, Zengin G, Tomczyk M, Góra J, Kwiatkowska B. *Symphytum officinale* L.: Liquid-liquid chromatography isolation of caffeic acid oligomers and evaluation

of their influence on pro-inflammatory cytokine release in LPS-stimulated neutrophils. J Ethnopharmacol. 2020;262:113169.

38. Trifan A, Zengin G, Sinan KI, Wolfram E, Skalicka-Woźniak K, Luca SV. LC-HRMS/MS phytochemical profiling of *Symphytum officinale* L. and *Anchusa ochroleuca* M. Bieb. (Boraginaceae): Unveiling their multi-biological potential *via* an integrated approach. J Pharm Biomed Anal. 2021;204:114283.

39. Trifan A, Zengin G, Sinan KI, Skalicka-Woźniak K, Minceva M, Luca SV. *Symphytum ibericum* Steven: LC-HRMS/MS-based phytochemical profile, *in vitro* antioxidant and enzyme inhibitory potential. Chem Biol Technol Agric. 2022;42:9

40. Lee IC, Bae JS, Kim T, Kwon OJ, Kim TH. Polyphenolic constituents from the aerial parts of *Thymus quinquecostatus* var. *japonica* collected on ulleung island. J Korean Soc Appl Biol Chem. 2011;54:811-816.

41. Rui Y, Tong L, Cheng J, Wang G, Qin L, Wan Z. Rosmarinic acid suppresses adipogenesis, lipolysis in 3T3-L1 adipocytes, lipopolysaccharide-stimulated tumor necrosis factor-α secretion in macrophages, and inflammatory mediators in 3T3-L1 adipocytes. Food Nutr Res. 2017;61:1330096.

42. Vasileva LV, Savova MS, Tews D, Wabitsch M, Georgiev MI. Rosmarinic acid attenuates obesity and obesity-related inflammation in human adipocytes. Food Chem Toxicol. 2021;149:112002.

43. Alagawany M, Abd El-Hack ME, Farag MR, Taha AE, Elnesr SS, Saadeldin IM, Swelum AA, Abdel-Latif MA, El-Saadony MT. Rosmarinic acid: modes of action, medicinal values and health benefits. Anim Health Res Rev. 2017;18:167-176.

44. Zhao GR, Zhang HM, Ye TX, Liu H, Liu M, Zhang Y. Characterization of the radical scavenging and antioxidant activities of danshensu and salvianolic acid B. Food Chem Toxicol. 2008;46:73-81.

45. Trifan A, Czerwińska ME, Zengin G, Sinan KI, Wolfram E, Skalicka-Woźniak K, Luca SV. Influence of pyrrolizidine alkaloids depletion upon the biological activity of *Symphytum officinale* L. extracts. J Ethnopharmacol. 2023;303:116010.

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