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Isolation of the major compounds and determination of biological activities of the underground parts of *Trachystemon orientalis*

Therapeutic potential of *Trachystemon orientalis*

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ABSTRACT
Objectives: *Trachystemon orientalis* (L.) G. Don, an edible plant is widely used in folk medicine. This study aimed to investigate antioxidant and lipase inhibitory activities of the extracts and isolated compounds of the underground parts of *T. orientalis* (TOU).
Material and Methods: The 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 MS spectral methods. To determine the antioxidant activity, ferric reducing antioxidant power (FRAP) and Cu(II) ion reducing antioxidant capacity (CUPRAC) assays were applied. Lipase inhibitory activity was determined by *in vitro* spectrophotometric method.
Results: By the isolation studies, rosmarinic acid (**1**) was isolated from the ethyl acetate subextract, and danshensu (**2**), globoidnan B (**3**), and rabdosiin (**4**) from the remaining aqueous subextract. These compounds were isolated from TOU for the first time. The ethyl acetate subextract had higher activity compared to 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 the ethyl acetate subextracts had significant inhibitory activity (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 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 as a result of imbalance in lipid metabolism.[1] It has been reported that there were two billion overweight adults in 2016 and 650 million of them 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 tract is an important option for the treatment or prevent of obesity. In this context, inhibition of 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 treatment of obesity approved by European Medicines Agency (EMA) and Food and Drug Administration (FDA), 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 (L.) G. Don is the only species concerning the *Trachystemon* G. 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, ıspıt, kaldirik, kaldırayak, tamara and acı hodan" in Türkiye, grows in the Black Sea Region, Caucasus, and Bulgaria.[10] Its aerial parts are used as vegetables, and pickles are made from its petioles and roots.[11,12] In addition to its use as food, it is used as a folk medicine in Türkiye. It is used as a diuretic, antipyretic, sudorific, antidepressant, and for sore throats.[13] Its roots are used as antiinflammatory, wound healing, and for 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 our knowledge, no study was found on the isolation of the major compounds from the underground parts (rhizomes and roots) of *T. orientalis* (TOU). This study was designed to purify the main compounds of the extracts different polarities prepared from TOU, elucidate their structures, and perform antioxidant and lipase inhibitory activity studies on the extracts and isolated compounds.

MATERIAL AND METHODS

Chemicals and Instrumentation

Ethyl acetate and chloroform were purchased from Sigma-Aldrich (St. Louis, USA), methanol from Riedel-de Haën (France), *n*-hexane from Isolab (Eschau, Germany), respectively. 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 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)), for thin layer chromatography (TLC), silica gel 60 F_{254} 20 × 20 cm (Merck 5554) were used. TLC points were determined by sputtering 1% Vanillin/H_2SO_4 and using a UV lamp (Mineralight UVGL-58). Nuclear magnetic resonance (NMR) spectra have been obtained by Bruker Ascend^{TM} 400 MHz/54 mm ULH. For Mass Spectroscopy (MS) analyses, Thermo TSQ Quantum Access Max was used. In addition, a shaker (Heidolph Unimax 1010) was used during the extraction and a rotary evaporator (Heidolph Hei-VAP Precision) was used for solvent evaporation. All absorbance measurements were carried on a BMG Labtech Spectrostar Nano spectrophotometer. Starter 3000, OHARUS pH meter was used a for all pH measurements.

Plant Material

T. orientalis was collected from xxx (Fındıklı district, May 2019, xxx province, Türkiye) and authenticated by Prof. Dr. xxx, one of authors. The voucher specimen (No. xxx 15486) was deposited at the xxx Herbarium (in Faculty of Forestry, xxx University), xxx, Türkiye.

Extraction and Isolation

Air dried the plant materials were cleaned, powdered, and then the powder (250 g) 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 mixture of water:methanol (9:1). The suspension obtained was partitioned with chloroform and after the evaporation, the chloroform subextract (TOU-C, 0.6 g) was acquired. To obtain the ethyl acetate subextract (TOU-E, 0.25 g), the water:methanol mixture (9:1) was partitioned with ethyl acetate and the solvent evaporated. After the remaining aqueous phase was evaporated to dryness, the remaining aqueous subextract (TOU-A, 5.5 g) was obtained.

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

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

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

Structure Identification

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 is 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 at 10 mg/mL concentration were prepared. Samples' own solvents were used as blanks. FRAP reagent (1.5 mL) was added to sample solutions (50 µL). The tubes were incubated (at 25 °C, 20 min) after vortexing. Next, the absorbances of the samples were determined at 595 nm with the help of spectrophotometer. The FRAP values of the samples were compared with Trolox (standard) and expressed as µM TEAC per g sample.

Cu(II) Ion Reducing Antioxidant Capacity (CUPRAC) assay

The principle of the CUPRAC assay is to measure the copper reducing capacity of the samples.[23] Methanol solutions of five different concentrations (62.5-1000 µM) of Trolox were used for calibration. Samples of TOU-M and subextracts at 10 mg/mL concentration were prepared. Samples' own solvents were used as blanks. 500 µL of each sample solution was taken and 1000 µL of $NH_4CH_3COO^-$ and 1000 µL of $CuCl_2.2H_2O$ were 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 s intervals. After vortexing the tubes, they were retained in the dark and (at 25 °C, 30 min). And then the absorbances were measured at 450 nm with 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 buffer solution (0.1 M Tris-HCl buffer, pH 8.0) at different concentrations (12.5-400 and 6.25-100 µg/mL, respectively). Experimental microplate wells were prepared as follows: A: 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). Incubation of microplates was performed for 15 min at 37°C both before and after addition of substrate. Microplates were read at 405 nm with a microplate reader. The equation given below was used to determine % Pancreatic Lipase Enzyme Inhibition values. 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 inhibitor concentration 50 (IC_{50}) values for enzyme inhibition of the samples were found from the equation of the graph obtained using the % enzyme inhibition values and the logarithm of the corresponding concentration.

RESULTS

Isolation of the Major Compounds

According to the results of 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 have been isolated. Structures of purified compounds are presented in Figure 1.

Compound 1: ESI-MS (m/e) 361.67 $[M+H]^-$, ($C_{18}H_{16}O_8$); ^{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); ^{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 agreement with the previous published data for Rosmarinic acid.[25,26]

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, COSY, HSQC, HMBC and MS data are agreement with the data given in the literature for Danshensu.[27]

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 (*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]

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]

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

DISCUSSION

Obesity is a serious public health problem, which is defined as the epidemic of the 21st century by World Health Organization, affecting both developed and developing countries.[5,6] Inhibiting the digestion and absorption of nutrients is one of the most important treatment strategies for prevention of obesity. Therefore, inhibition of pancreatic lipase, which has 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]

In this study, isolation studies were carried out on the subextracts prepared from TOU-M. As a result of 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 data, no previous isolation studies on *T. orientalis* were found. Rosmarinic acid was determined in the roots of the plant by high performance liquid chromatography (HPLC).[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 root of *Symphytum officinale* and danshensu from the leaves of *Cordia americana*.[36,37] Danshensu, globoidnan B, and rabdosiin have been shown to be found in the Boraginaceae plants (the aerial parts and the roots of *S. officinale*; the leaves and the roots of *S. ibericum*).[38,39]

In this study, 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 TOU-A. It was observed that the activity of rosmarinic acid was close to the activity of these extracts. It is thought that the activity of the extracts is higher owing to the synergistic effect of the isolated phenolic compounds. In an *in vitro* study evaluating the pancreatic lipase inhibition of *Rosmarinus officinalis* extract and its phenolic compounds, including rosmarinic acid, the IC_{50} value of the extract was found to be 13.8 µg/mL and that of rosmarinic acid as 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] There are a few studies showing that rosmarinic acid plays a role in different obesity-related mechanisms apart from pancreatic lipase inhibition. In a study, it was 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 found to be better than the extracts. It was observed that the activity of rosmarinic acid was the best among the isolated compounds. The isolated compounds are thought to be responsible for the activity of the extracts. In a study,

authors attributed the high antioxidant activity of 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 has antioxidant activity close to TOU-E. Compounds from this subextract may be responsible for the activity. In a study, it was shown that danshensu has a higher scavenging activity of 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 to vitamin C.[44] It has been reported that root extracts of *S. officinale* rich in compounds such as rosmarinic acid, globoidnan B, rabdosiin and 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 globoidnan B and rosmarinic acid.[37] Phenolic compounds show radical scavenging, metal chelators and hydrogen donors properties.[32] *T. orientalis* shows high antioxidant and antilipase activity via its phenolic compounds, and therefore it may be a promising therapeutic agent that can contribute to the treatment of obesity.

Study Limitations

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

CONCLUSION

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

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 *T. 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*.

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 *T. 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*.