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ORIGINAL ARTICLE

Investigation of Selected Medicinal Plants for Their Anti-Obesity Properties

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ABSTRACT

Objectives: Obesity, which is a risk factor for diabetes, hypertension, cardiovascular diseases, and cancer, is caused serious health problems and economic costs on a global scale. Nowadays, pancreatic lipase inhibitors that cause inhibition of lipid digestion and lipid absorption are one of the limited treatment approaches for obesity. Plant-derived secondary metabolites can be used for treating obesity. The aim of this study was to research the antiobesity potential of *Amaranthus albus* L. (Amaranthaceae), *Helichrysum compactum* Boiss. (Asteraceae), *Chenopodium album* L. (Chenopodiaceae), and *Agrimonia eupatoria* L. (Rosaceae).

Materials and Methods: To detect the antiobesity potentials of the plants, *in vitro* lipase inhibitory activity studies by spectroscopic method and quantitative analysis studies of some anti-obesity effective secondary metabolites by reversed-phase high performance liquid chromatography (RP-HPLC) technique were carried out.

Results: *In vitro* lipase inhibitory studies showed that all plant extracts possess lipase inhibitory effect, and the highest lipase inhibitory potential was observed for *H. compactum* (IC_{50}: 45.70 µg/mL ± 2.3618). According to HPLC analyses, *p*-coumaric acid (0.27 mg/g) in *A. albus*; benzoic acid (0.33 mg/g) in *C. album*; vanillic acid (7.32 mg/g), syringaldehyde (14.97 mg/g), quercetin (4.66 mg/g), *p*-coumaric acid (0.71 mg/g), and benzoic acid (3.43 mg/g) in *H. compactum*; *p*-coumaric acid (0.71 mg/g) and benzoic acid (3.43 mg/g) in *A. eupatoria* were detected.

Conclusion: In conclusion, *H. compactum* is the most remarkable natural source for the study. The fact remains that all plants may be promising candidates for treating obesity.

Key words: *Agrimonia eupatoria*, *Amaranthus albus*, *Chenopodium album*, *Helichrysum compactum*, obesity

INTRODUCTION

Obesity is expressed as abnormal or excessive fat accumulation resulting from the imbalance between energy intake and consumption.[1-3] Obesity is directly related to coronary artery diseases, cerebrovascular diseases, hypertension, hyperlipidemia, diabetes, pulmonary embolism, sleep apnea, gynecological abnormalities, osteoarthritis, psychiatric diseases, and many cancers.[4-9] It also causes high economic expenses globally.[10] Some medicinal treatment approaches have been developed to treat and prevent obesity except for classical approaches like decreasing diet calories and increasing physical activity.[11,12] Inhibition of adipocyte differentiation, stimulation of energy consumption, suppression of the *FAS* gene, lipase enzyme inhibition, appetite suppression, and anti-inflammatory approach can be given as examples of these medicinal approaches.[13]

Pancreatic lipase (PL) is a 449 amino acid single chain glycoprotein. 90% of dietary fat consist of triglycerides (TG) and must be hydrolyzed in order to be absorbed. PL converts 50-70% TGs into fatty acids (FA) and monoglycerides (MG). MGs released by lipid hydrolysis and free FA are absorbed by adipose tissue to form mixed micelles with bile salts, cholesterol, and lysophosphatidic acid.[14] PL inhibition, one of the therapeutic approaches for prevention and treatment of obesity, creates an antiobesity effect through decreasing intestinal lipid absorption.[15]

Natural products have become popular for development of safe and effective antiobesity drugs.[9] Herbal sources included

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effective secondary metabolites like benzoic acid, vanillic acid, syringaldehyde, *p*-coumaric acid, sinapic acid, and quercetin, have therapeutic potential for treating obesity.[16] It has been proven that the consumption of some plant-based foods with phenolic content is associated with the prevention of obesity.

The traditional use of all species for many years, their phenolic content potential, and their obesity-related effects like antioxidant and anti-inflammatory have guided the study. The purpose of this article is to investigate the antiobesity effect of *Amaranthus albus* L. (Amaranthaceae), *Helichrysum compactum* Boiss. (Asteraceae), *Chenopodium album* L. (Chenopodiaceae), and *Agrimonia eupatoria* L. (Rosaceae) through *in vitro* lipase inhibitory effect and quantitative high performance liquid chromatography (HPLC) analysis studies.

MATERIALS AND METHODS

Plant materials and preparation of extracts

Identification of *A. albus*, *H. compactum*, *C. album*, and *A. eupatoria* were carried out by Prof. Dr. Ufuk Özgen and Asst. Prof. Dr. Merve Badem. Herbarium samples of *A. albus* (1145 m, Kürekçili village, Akkuş, Ordu, AEF 26904), *H. compactum* (1190 m, Ormancık village, Akkuş, Ordu, AEF 26823), *C. album* (1145 m, Kürekçili village, Akkuş, Ordu, AEF 26902), and *A. eupatoria* (1290 m, Karaçal village, Akkuş, Ordu, AEF 26905) were deposited in the Herbarium of the Faculty of Pharmacy of Ankara University.

The aerial parts of the species *A. albus, H. compactum, C. album,* and *A. eupatoria* were powdered to prepare the methanol extracts. The powdered plant materials (~ 200 g) were extracted with ~ 500 mL methanol in a 30°C shaker incubator for 24 h and then filtered. After this process was repeated thrice, the filtrates were combined and evaporated to dryness.

Lipase enzyme inhibition

Dried methanolic extracts of the aerial parts of *A. albus, H. compactum, C. album,* and *A. eupatoria* were diluted with buffer solution (0.1 M Tris-HCl buffer, pH: 8.0) with final concentrations to be 12.5, 25, 50, 100, and 200 µg/mL in microplates. The levels of lipase inhibition were determined using a substrate *p*-nitrophenylbutyrate (*p*-NPB) (CAS: 2635-84-9). Basic principle of the method is based on determining the absorbance of *p*-nitrophenol using the spectroscopic method. *p*-Nitrophenol has a yellow color, which is formed because of the interaction of *p*-NPB with the lipase enzyme.[17] Orlistat was used as a positive control. Orlistat was diluted with buffer solution (0.1 M tris-HCl buffer, pH: 8.0) to be 6.25, 12.5, 25, 50, and 100 µg/mL final concentrations in the microplate. Absorbance measurements of the samples were carried out by a spectrophotometer (SPECTROstar Nano-BMG LABTECH).

The experimental procedure is designed by coding microplates A, B, C, and D. Microplates are designed with these ingredients: A, 90 µL enzyme solution [crudeporcine PL type II (Sigma, EC 3.1.1.3) - (200 units/mL)], 5 µL substrate solution (10 mM *p*-NPB acetonitrile solution); 5 µL buffer solution (0.1 M tris-HCl buffer, pH: 8.0); B, 90 µL enzyme solution [(crudeporcine PL type II (Sigma, EC 3.1.1.3) - (200 units/mL)], 10 µL buffer solution (0.1 M tris-HCl buffer, pH: 8.0); C, 90 µL enzyme solution [crudeporcine PL type II (Sigma, EC 3.1.1.3) - (200 units/mL)], 5 µL sample solution, 5 µL substrate solution (10 mM *p*-NPB acetonitrile solution); D, 90 µL enzyme solution [crudeporcine PL type II (Sigma, EC 3.1.1.3) - (200 units/mL)], 5 µL sample solution, 5 µL buffer solution (0.1 M tris-HCl buffer pH: 8.0). Before the substrate solutions were added to the wells containing substrate solutions, each microplate was incubated at 37°C for 15 min in the incubator (Memmert), after which the substrate solutions were added to the relevant wells, and each microplate was subjected to incubation again at 37°C for 15 min. Absorbance of samples was measured at 405 nm wavelength. Each sample was performed triple.

Lipase enzyme inhibition values were calculated with the absorbance values of A, B, C, and D using the formula. The formula is given below.

% Pancreatic lipase inhibition= [[(A-B)-(C-D)]/(A-B)]*100

The chart was constituted with percentage enzyme inhibition values determined at the end of the experiment and the logarithm of the concentration (ordinate and absis). Then, the inhibitor concentration 50 (IC_{50}) values of the samples that cause 50% inhibition of the lipase enzyme were determined from the chart equation.

Statistical analysis

Three repetitions of each experiment were carried out. The results were reported as mean ± standard deviation. The Kolmogorov-Smirnov test was used to determine compatibility with the normal distribution. The analysis of Kruskal-Wallis and Mann-Whitney *U* tests were employed to compare variations between the groups. Statistical significance level was considered $p<0.05$.

HPLC analysis

HPLC (Shimadzu Corporation, LC 20AT, Kyoto, Japan) device was used in the analysis, containing a Zorbax C18 (150 x 4.6 mm, 5 µm) column.

The prepared sample solutions were dragged 3 times under the analysis conditions stated below. HPLC chromatograms prepared from sample solutions were compared with the retention times of 7 different phenolic compounds. Initially, qualitative definitions were realized with the detector signals. For the quantitative analysis of 7 different compounds in the sample solutions, during the validation studies of HPLC method, 5, 10, 25, 50, and 100 µg/mL standard mixture solutions were dragged five times under the specified HPLC analysis conditions, and then the calibration curve obtained from the peak areas and relevant concentrations were used. *p*-Hydroxybenzoic acid, vanillic acid, syringaldehyde, *p*-coumaric acid, sinapic acid, benzoic acid, and quercetin were analyzed by the HPLC method with validated analysis conditions. To separate these 7 compounds in the column, various preliminary trials were made, the appropriate gradient program was determined,

and the method was validated. It has been observed that the compounds are separated by high resolution.[6,18]

Preparation of sample

HPLC-grade methanol was added to the dry methanol extracts of the species at a final concentration of 10 mg/mL, and the solutions were transferred to HPLC vials, after being filtered through 0.45 µm membrane filters.

HPLC analysis conditions

HPLC analysis was performed using the validated method.[6,18] The flow rate is 1.5 mL/min, and the injection volume is 20 µL. For analysis, solvent systems of A solution: 100% methanol and B solution: 2% acetic acid: water (pH 2.65) were used. The gradient elution program was applied 0-3 minutes 20:80 (solution A: solution B); 4-6 minutes 30:70 (solution A: solution B); 7-9 minutes 40:60 (solution A: solution B); 10-11 minutes 45:55 (solution A: solution B); 12-13 minutes 50:50 (solution A: solution B); 14-15 minutes 60:40 (A solution: B solution) and 16 min 80:20 (A solution: B solution). Measurements were carried out at wavelengths of 200, 210, 220, 230, 240, 250, 260, 270, 280, and 320 nm.

RESULTS

Results of lipase inhibition

After *in vitro* lipase enzyme inhibition studies, IC_{50} level of orlistat was used as positive control, and dry methanolic extracts prepared from *A. albus*, *C. album*, *H. compactum*, and *A. eupatoria* species were determined on lipase. It was determined that the graph created as the logarithm of percent enzyme inhibition values of orlistat and species the concentration it belongs to was linear (Figure 1). IC_{50} value of orlistat was determined as 8.05 ± 0.8615 µg/mL.

The percentage lipase inhibition levels at different concentrations of the methanol extract prepared from *A. albus*, *C. album*, *H. compactum*, and *A. eupatoria* species are expressed in Figure 2.

IC_{50} values of methanol extracts prepared from *A. albus*, *C. album*, *H. compactum*, and *A. eupatoria* species were determined as 106.02 ± 4.5125, 177.82 ± 8.6325, 45.70 ± 2.3618, and 94.18 ± 5.2569 µg/mL, respectively.

Results of HPLC analysis

The phenolic compounds and benzoic contents in the extracts of screened species were expressed as mg/g extract (Table 1). Chromatographic results of the extracts were presented in addition (Figure 3).

DISCUSSION

Obesity is expressed as abnormal or excess fat accumulation resulting from the imbalance between energy intake and consumption.[1-3] Obesity is directly related to coronary artery disease, cerebrovascular disease, hypertension,

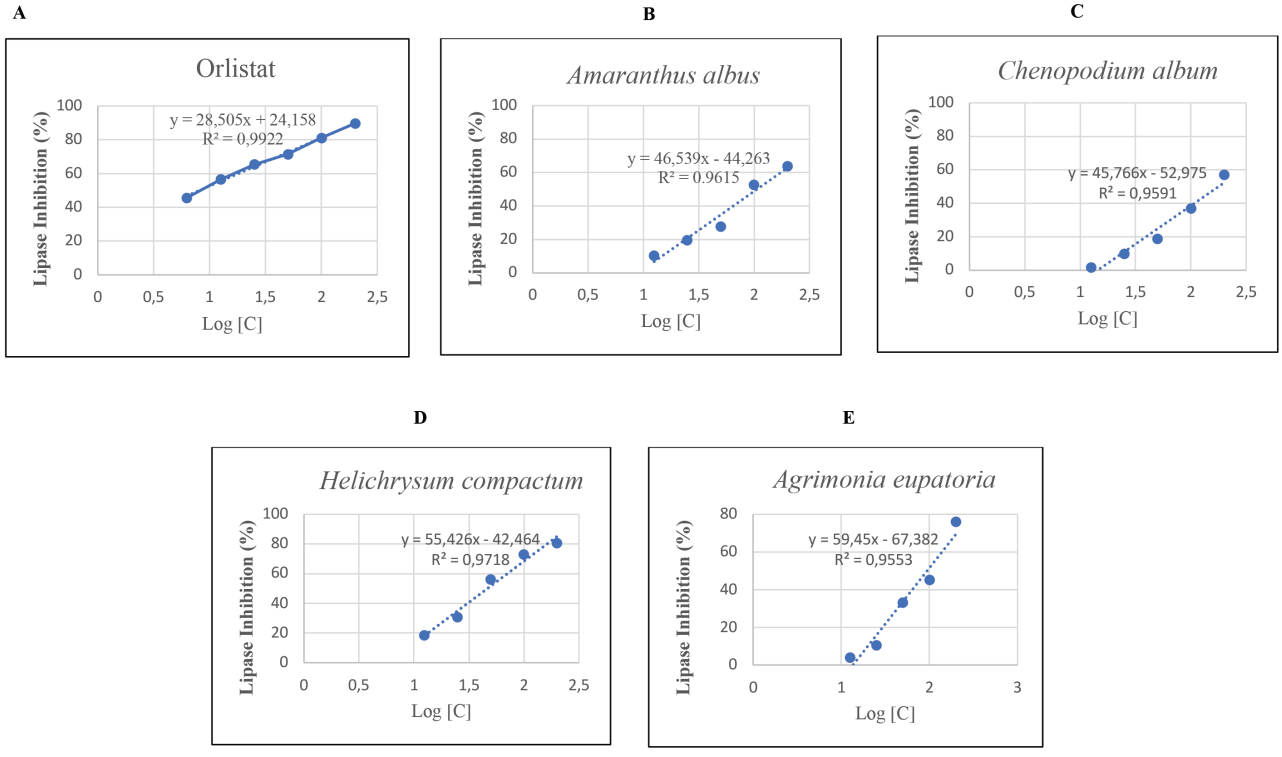

Figure 1. The graph created in the form of lipase inhibition (%) values of orlistat and the logarithm of the concentration it belongs to (A), the graph created in the form of lipase inhibition (%) values of *Amaranthus albus* and the logarithm of the concentration it belongs to (B), the graph created in the form of lipase inhibition (%) values of *Chenopodium album* and the logarithm of the concentration it belongs to (C), the graph created in the form of lipase inhibition (%) values of *Helichrysum compactum* and the logarithm of the concentration it belongs to (D), the graph created in the form of lipase inhibition (%) values of *Agrimonia eupatoria* and the logarithm of the concentration it belongs to (E)

hyperlipidemia, diabetes, pulmonary embolism, sleep apnea, gynecological abnormalities, osteoarthritis, psychiatric diseases, and many cancer diseases.[4-9] Obesity also causes high economic expenses in Türkiye as worldwide. So, the reduction of obesity is important for the economic burden related to obesity.[10]

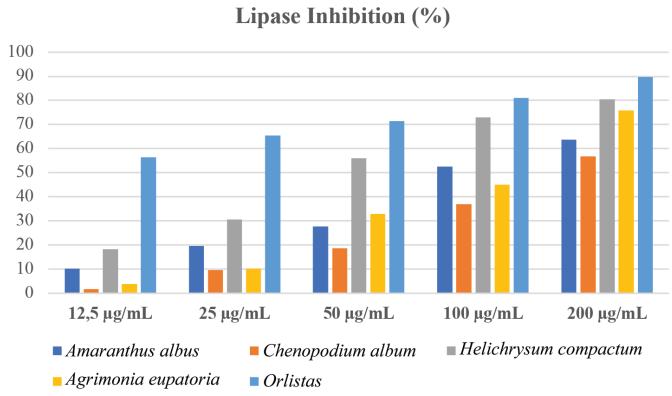

Figure 2. Lipase inhibition (%) values of the species and orlistat at different concentrations

Some treatment approaches have been developed to treat and prevent obesity except for diet or increasing physical activity.[11,12] Inhibition of adipocyte differentiation, stimulation of energy expenditure, suppression of *FAS* gene, inhibition of PL, suppression of appetite, and anti-inflammatory approach can be given as examples of these strategies.[13]

PL is responsible for the conversion of 50-70% TG to FA and MG. Inhibition of PL allows reduces fat absorption and therefore to reduces energy intake.[19]

Phenolic compounds play an important role for treating obesity. It has been proven that consuming some plant-derived food with phenolic content is associated with the prevention of obesity.[20] Vanillic acid has been proved to have an anti-inflammatory effect in rats fed a high-fat diet and provides a regulatory effect on insulin resistance-induced hyperinsulinemia, hyperglycemia, and hyperlipidemia.[16] Previous studies showed that *p*-coumaric

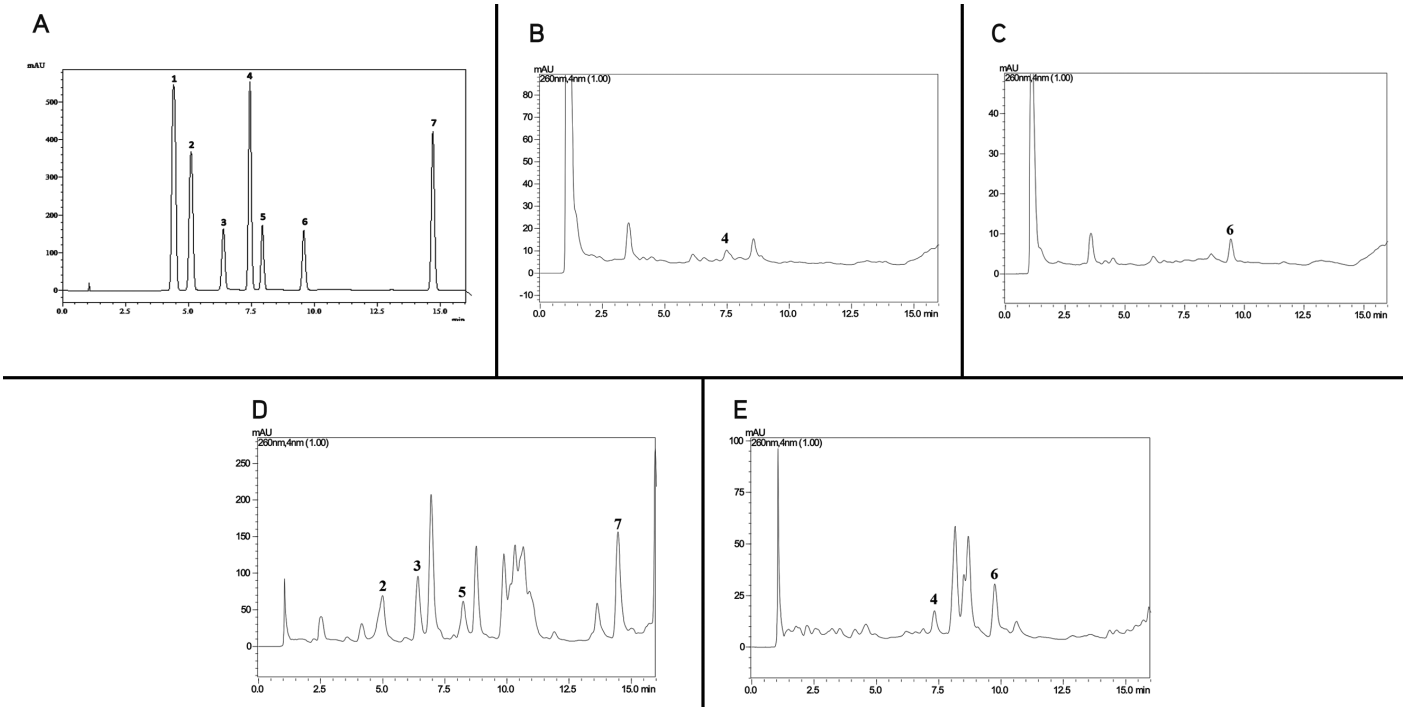

Figure 3. HPLC chromatogram of phenolic standards, Peak identification:[18] 1: *p*-hydroxy benzoic acid, 2: vanillic acid, 3: syring aldehyde, 4: *p*-coumaric acid, 5: sinapic acid, 6: benzoic acid, 7: quercetin (A), HPLC chromatogram of the methanol extract of *Amaranthus albus* (B), HPLC chromatogram of the methanol extract of *Chenopodium album* (C), HPLC Chromatogram of the methanol extract of Helichrysum compactum (D), HPLC Chromatogram of the methanol extract of *Agrimonia eupatoria* (E)

Table 1. Phenolic compounds and benzoic acid contents of their species

Species	*p*-Hydroxybenzoic acid	Vanilic acid	Syringaldehyde	*p*-Coumaric acid	Sinapic acid	Benzoic acid	Quercetin
	mg/g extract						
Amaranthus albus	-	-	-	0.27	-	-	-
Chenopodium album	-	-	-	-	-	0.33	-
Helichrysum compactum	-	7.32	14.97	-	0.30	-	4.66
Agrimonia eupatoria	-	-	-	0.71	-	3.43	-

acid and sinapic acid decrease pro-inflammatory adipokines such as tumor necrosis factor-α in obese mice and increase the level of anti-inflammatory cytokines such as adiponectin, thereby both phenolic compounds causing reduced obesity and obesity-related complications.[21]

Syringaldehyde has been presented to positively affect hyperglycemia by increasing the GLUT-4 transporter and mRNA levels in muscles of diabetic rats.[22] Benzoic acid-derived compounds improved lipid metabolism and diabetes by regulating plasma insulin, glucose, low-density lipoprotein, and TG in streptozotocin-induced diabetic rats.[23] A study on the antiobesity effect of quercetin showed that quercetin reduced body weight, liver fat accumulation, blood glucose, plasma, and liver triacylglycerol levels in a high-fat diet-fed mice.[24]

HPLC analysis was performed and lipase inhibitory effects were evaluated to reveal the potential of *A. albus*, *C. album*, *H. compactum,* and *A. eupatoria* in treating obesity with this study.

Amaranthus genus has been used for osteoarthritis, rheumatoid arthritis, gonorrhea, inflammation, hemorrhoids, and antibacterial effect, traditionally.[25,26] Terpenoids, flavonoids, tannins, alkaloids, sterols, cardiac glycosides, and saponins were determined for *Amaranthus* genus in previous phytochemical studies. Phenolic compounds of ellagic, ferulic, and gallic acids, quercetin, rutin, *p*-coumaric, sinapic, syringic acid, and vanillic acid, have been detected on the *Amaranthus* genus previously.[27] Previous biological activity studies revealed analgesic, anti-inflammatory, antifungal, antioxidant, enzyme inhibition, and hepatoprotective effects of *Amaranthus* genus.[28-31] Because of *in vitro* lipase inhibitor activity tests, IC_{50} value of *A. albus* was determined as 106.02 ± 4.5125 µg/mL. *p*-Coumaric acid among analyzed phenolic contents was detected *via* HPLC for the species. The anti-obesity effect of *A. albus* may be due to *p*-coumaric acid. Also, HPLC signals except for *p*-coumaric acid may be related to phenolic content determined by previous studies on *Amaranthus* genus. Furthermore, this state may contribute to the obesity effect. Simultaneously, the anti-inflammatory, antioxidant, and hepatoprotective effects of the species revealed by previous studies may contribute to the potential therapeutic effect of the plant for obesity.

C. album is traditionally used to treat peptic ulcers, dyspepsia, swelling, pharyngoplasty, splenopathy, ophthalmopathy, fatigue, liver disorders, spleen enlargement, intestinal ulcers, and burns.[32,33] Apart from these uses, it has also been observed to have antiparasitic, diuretic, hepatoprotective, laxative, and sedative effects.[33] Terpenoids, flavonoids, tannins, alkaloids, sterols, cardiac glycosides, and saponins were found in the content of *C. album*.[32] Lots of phenolic components such as quercetin, quercetin 3-*O*-glycosides, quercetin 3-*O*-xylosylglucoside, quercetin-3-*O*-rhamnoglucoside, isorhamnetin, kaempferol, kaempferol 3-*O*-glycosides, kaempferol 3-*O*-β-diglucoside, kaempferol-3-*O*-arabinoglucoside *etc*., have been defined in previous studies.[32] Biological activity studies revealed anthelmintic, antidiarrheal, anticancer, antimicrobial, antinociceptive, antioxidant, antipruritic, hepatoprotective, and sperm immobilization effects of *C. album*.[32-40] IC_{50} value of lipase inhibition was evaluated as 177.82 ± 8.6325 µg/mL for *C. album*. According to HPLC analysis, benzoic acid was determined. *C. album* may inhibit lipase inhibition potential because of benzoic acid content. Also, other HPLC signals, except for benzoic acid, may be based on phenolic components mentioned in previous literature.

H. compactum is used for kidney diseases and kidney stones, stomach pain, heart rhythm regulation, liver disease, burn and wound treatment, hand and foot cracks, diarrhea, and asthma treatment, traditionally.[41] *H. compactum* has been revealed to have terpenoids, flavonoids, tannins, alkaloids, sterols, cardiac glycosides, and saponins.[42] It was determined that phenolic compounds, *e.g.* apigenin, apigenin-7-*O*-glucoside, kaempferol, kaempferol-3-*O*-glucoside, 3,5-dihydroxy-6,7,8- kaempferol-3-*O*-glucoside, luteolin, luteolin-7-*O*-glucoside, luteolin-4′,7-di-*O*-glucoside, and naringenin were isolated from *H. compactum.*[42] Other signals observed on the HPLC chromatogram may be related to these phenolic compounds, and therefore lipase activity. Biological activity studies showed that it had antioxidant and antibacterial effects.[42-44] *In vitro* lipase inhibitory activity analysis on *H. compactum*, the IC_{50} value was evaluated as 45.70 ± 2.3618 µg/mL. Vanillic acid, syringaldehyde, sinapic acid, and quercetin were determined as phenolic compounds by HPLC analysis. Other HPLC signals can be related to the potential phenolic content mentioned in previous studies. Therefore, the lipase inhibitory effect may be due to its potential phenolic content and compounds detected in this study.

A. eupatoria is traditionally used to treat cold, bleeding, tuberculosis, skin diseases, urinary disorders, intestinal infections, ulcers, and antidiabetic, anti-inflammatory, cholagogue, hemostatic, antibacterial, fungicidal, aggregate, and diuretic effects.[45,46] In phytochemical analysis studies, terpenoids, flavonoids, tannins, alkaloids, sterols, cardiac glycosides, and saponins were found in *A. eupatoria*. Phenolic components of astragalin, apigenin 7-*O*-D-glucuronide, apigenin-7-*O*-glucopyranoside, catechin, ellagic acid 4-*O*-glucopyranoside, quercetin, kaempferol-3-*O*-rhamnoside, kaempferol-3-*O*-glucopyranoside, tiliroside, and rutin isolated from *A. eupatoria* previously may be related to other HPLC signals.[45] Biological activity studies revealed analgesic, antibacterial, antidiabetic, anti-inflammatory, anticoagulant, antinociceptive, antioxidant, antitumor, antiviral, and hepatoprotective effects of *A. eupatoria*.[46-55] In the study, the IC_{50} value for lipase inhibition was stated as 94.18 ± 5.2569 µg/mL, and *p*-coumaric and benzoic acids were identified *via* HPLC analysis. Anti-obesity activity of *A. eupatoria* may be based on phenolic compounds of *p*-coumaric acid and benzoic acid. Also, other phenolic components observed in HPLC analysis can be related to phenolic content aforementioned in previous studies. Simultaneously, antidiabetic, anti-inflammatory, antioxidant, and hepatoprotective effects reported in literature studies may contribute to the potential therapeutic effect of the species for treating obesity.

According to HPLC analysis, the highest phenolic content was found in *H. compactum,* followed by *A. eupatoria, C. album, A. albus.* When the lipase inhibitory effects of four species were evaluated, it was observed that the order of activity, respectively,

was *H. compactum, A. eupatoria, A. albus,* and *C. album*. In this study, the relationship between phenolic compounds and lipase inhibitory effects has been proven again, and the therapeutic potential of these species has been demonstrated for obesity.

Study limitations

The value of these species in treating obesity, which is a global health problem, can be demonstrated more comprehensively with toxicological analysis studies, formulation studies, standardization studies, and clinical studies on the antiobesity effect.

CONCLUSION

Because of this study, the relationship between phenolic compounds and lipase inhibitory effects has been proven and concluded that *A. albus, H. compactum, C. album, A. eupatoria* species could be used as potential therapeutic agents for treating obesity.

Ethics

Ethics Committee Approval: Ethical approval does not require for the study.

Informed Consent: Not necessary.

Authorship Contributions

Concept: S.Ö.Ş., Design: S.Ö.Ş., E.C., U.Ö., Data Collection or Processing: S.Ö.Ş., E.C., B.N.Ö., M.B., Analysis or Interpretation: S.Ö.Ş., E.C., B.N.Ö., M.B., Literature Search: S.Ö.Ş., E.C., B.N.Ö., Writing: S.Ö.Ş., E.C., U.Ö.

Conflict of Interest: No conflict of interest was declared by the authors.

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