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INVESTIGATION OF SELECTED MEDICINAL PLANTS FOR ANTIOBESITY PROPERTIES

SEÇİLİ BAZI TIBBİ BİTKİLERİN ANTİOBEZİTE ÖZELLİKLERİNİN İNCELENMESİ

Running Title: Antiobesity Properties of Some Medicinal Plants
Kısa Başlık: Bazı Tıbbi Bitkilerin Antiobezite Özellikleri

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ABSTRACT
Background: Obesity which is a risk factor for diabetes, hypertension, cardiovascular diseases, 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 have the potential to be used in the treatment of obesity.
Objective: The aim of the study is to research the antiobesity potentials of *Amaranthus albus* L. (Amaranthaceae), *Helichrysum compactum* Boiss. (Asteraceae), *Chenopodium album* L. (Chenopodiaceae) ve *Agrimonia eupatoria* L. (Rosaceae).
Material and methods: To detect the antiobesity potentials of the plants, *in vitro* lipase inhibitory effect studies by spectroscopic method and quantitative analysis studies of some antiobesity effective secondary metabolites by 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 analysis, *p*-coumaric acid (0.27 mg/g) for *A. albus*; benzoic acid (0.33 mg/g) for *C. album*; vanillic acid (7.32 mg/g), syringaldehyde (14.97 mg/g) and quercetin (4.66 mg/g) *p*-coumaric acid (0.71 mg/g) and benzoic acid (3.43 mg/g) for *H. compactum;* coumaric acid (0.71 mg/g) ve benzoic acid (3.43 mg/g) for *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 the treatment of obesity.

Keywords: *Agrimonia eupatoria*, *Amaranthus albus, Chenopodium album, Helichrysum compactum*, Obesity

ÖZET

Giriş: Diyabet, hipertansiyon, kardiyovasküler hastalıklar, kanser için risk faktörü olan obezite, küresel ölçekte ciddi sağlık sorunlarına ve ekonomik maliyetlere neden olmaktadır. Günümüzde, pankreatik lipaz inhibitörleri, lipit sindiriminin engellenmesine neden olur ve lipit emilimi, obezite için sınırlı tedavi yaklaşımlarından biridir. Bitkisel kaynaklar ve bitkisel kaynaklı sekonder metabolitler, obezite tedavisinde kullanılma potansiyeline sahiptir.

Amaç: Bu yaklaşımdan hareketle gerçekleştirilen ilgili araştırmada, *Amaranthus albus* L. (Amaranthaceae), *Helichrysum compactum* Boiss (Asteraceae), *Chenopodium album* L. (Chenopodiaceae) ve *Agrimonia eupatoria* L.'nin (Rosaceae) antiobezite potansiyelinin ortaya çıkarılması amaçlanmıştır.

Gereç ve Yöntem: Bitkilerin antiobezite potansiyelini tespit etmek için spektroskopik yöntemle *in vitro* lipaz inhibitör etki çalışmaları ve RP-YPSK tekniği ile bazı antiobezite etkili sekonder metabolitlerin kantitatif analizi yapılmıştır.

Bulgular: *In vitro* lipaz inhibitör çalışmaları, tüm bitki ekstrelerinin lipaz inhibitör etkiye sahip olduğunu ve en yüksek lipaz inhibitör potansiyelinin *H. compactum* (IC50 = 45.70 ± 2.3618 µg/mL)'da gözlendiğini göstermiştir. YPSK analizine göre *A. albus* için *p*-kumarik asit (0.27 mg/g); *C. album* için benzoik asit (0.33 mg/g); *H. compactum* için vanilik asit (7.32 mg/g), siringaldehit (14.97 mg/g) ve kersetin (4.66 mg/g); *p*-kumarik asit (0.71 mg/g) ve benzoik asit (3.43 mg/g); *A. eupatoria* için *p*-kumarik asit (0.71 mg/g) ve benzoik asit (3.43 mg/g) tespit edilmiştir.

Tartışma: Sonuç olarak, *H. compactum*, çalışma için en dikkat çekici bitkisel kaynak olmakla birlikte, çalışılan tüm bitkiler obezite tedavisi için umut verici bir kaynak olabilir.

Anahtar Kelimeler: *Agrimonia eupatoria, Amaranthus albus, Chenopodium album, Helichrysum compactum*, Obezite

1. 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 disease, cerebrovascular disease, hypertension, hyperlipidemia, diabetes, pulmonary embolism, sleep apnea, gynecological abnormalities, osteoarthritis, psychiatric diseases, and many cancer diseases [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 increasing diet calories and 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 consists of triglycerides (TG) and must be hydrolyzed to be absorbed. PL converts 50-70% of triglycerides into fatty acids (YA) and monoglycerides (MG). MGs released by lipid hydrolysis and free fatty acids 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 effective secondary metabolites like benzoic acid, vanillic

acid, syringaldehyde, 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 the article is to investigate the antiobesity effect of *A. albus* L. (Amaranthaceae), *H. compactum* Boiss. (Asteraceae), *C. album* L. (Chenopodiaceae) and *A. eupatoria* L. (Rosaceae) through *in vitro* lipase inhibitory effect and quantitative HPLC analysis studies.

2. Material and Methods

2.1. Plant material and preparation of extract

Identification of *A. albus* (Amaranthaceae), *H. compactum* (Asteraceae), *C. album* (Chenopodiaceae), and *A. eupatoria* (Rosaceae) were carried out by XXXX and XXXX. 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 of *A. albus, H. compactum, C. album, and A. eupatoria* were first powdered to prepare the methanol extracts. The powdered plant materials (~ 200 g) were extracted with ~ 500 mL of methanol in a 30 °C shaker incubator for 24 hours and then filtered. After this process was repeated three times, the filtrates were combined and evaporated to dryness.

2.2. Lipase Enzyme Inhibition Studies

Dry 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. Lipase inhibition levels were determined using substrate of p-nitrophenylbutyrate (p-NPB) (CAS: 2635-84-9). This method's basic principle is based on determining the absorbance of the *p*-nitrophenol compound by the spectroscopic method. *p*-Nitrophenol has a yellow colour which is formed as a result of the interaction of *p*-nitrophenylbutyrate with the lipase enzyme[17]. Orlistat had lipase inhibitory effect was used as a positive control. The 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 spectrophotometer (SpetrostarNano-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 left to incubate at 37 ° C for 15 minutes 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 minutes. Absorbances of samples were 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 in below.

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

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

2.3. HPLC Analysis

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

The prepared sample solutions were dragged 3 times under the analysis conditions stated below. HPLC chromatogram prepared from sample solutions was 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 the 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 graph obtained from the peak areas and relevant concentrations were used. In Sener's doctoral thesis, *p*-hydroxybenzoic acid, vanillic acid, syringaldehyde, coumaric acid, sinapic acid, benzoic acid, and quercetin were analyzed by the HPLC method, whose analysis conditions were validated. In order 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].

2.3.1.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.

2.3.2.HPLC Analysis Conditions

HPLC analysis was performed using the validated method[6,18]. The flow rate of the HPLC method 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. minute 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 minutes 80:20 (A solution: B solution). Measurements were carried out at 200, 210, 220, 230, 240, 250, 260, 270, 280, and 320 nm with the DAD detector.

3. Results

3.1. Results of Lipase Inhibition

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

The % 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} levels 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.

3.2. Results of HPLC Analysis

The species' phenolic compounds and benzoic contents were expressed as mg/g extract (Table 1). Chromatogram results of the species were presented in addition (Figure 3-6).

4. 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, hyperlipidemia, diabetes, pulmonary embolism, sleep apnea, gynecological abnormalities, osteoarthritis, psychiatric diseases, and many cancer diseases[4-9]. Obesity also causes high economic expenses in Turkey as well as all over the world. So, reduction of obesity is important for economic burden related to obesity[10]. 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% of triglycerides to FA and MG. Inhibition of PL allows to reduce fat absorption and therefore to reduce energy intake[19].

Phenolic compounds have an important role in the treatment of obesity. It has been proven that consuming some plant-derived foodstuffs with phenolic content is associated with the prevention of obesity[20]. Vanillic acid has been proven to have an anti-inflammatory effect in rats fed a high-fat diet and provide a regulatory effect on insulin resistance-induced hyperinsulinemia, hyperglycemia, and hyperlipidemia[16]. Previous studies showed that *p*-coumaric acid and sinapic acid decreased pro-inflammatory adipokines such as TNF-α in obese mice and increased the level of anti-inflammatory cytokines such as adiponectin, thereby both phenolic compounds caused to reduce obesity and obesity-related complications[21].

Syringaldehyde has been revealed to positively affect hyperglycemia by increasing GLUT-4 transporter and mRNA levels in muscles in diabetic rats[22]. Benzoic acid-derived compounds improved lipid metabolism and diabetes by regulating plasma insulin, glucose, LDL, and triglyceride in streptozotocin-induced diabetic rats[23]. A study about 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 inhibitor 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, gallic, quercetin, *p*-coumaric, rutin, sinapic, syringic acid, vanillic acid, and quercetin have been detected on the Amaranthus genus previously[27]. Previous biological activity studies revealed analgesic effect, anti-inflammatory effect, antifungal effect, antioxidant effect, enzyme inhibition, and hepatoprotective effects of *Amaranthus* genus[28-31]. As a result of *in vitro* lipase inhibitor activity tests, the 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 antiobesity effect of *A. albus* may be due to the *p*-coumaric acid. Also, HPLC signals except for coumaric acid may be related to phenolic content determined by previous studies about *Amaranthus* genus. Furthermore, this state may contribute to the antiobesity effect. At the same time, 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 antiparasitic, diuretic, hepatoprotective, laxative, and sedative effects[33]. Terpenoids, flavonoids, tannins, alkaloids, sterols, cardiac glycosides, and saponin were found for the content of *C. album*[32]. Lots of phenolic components like 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 with previous studies[32]. Biological activity studies revealed anthelmintic, antidiarrheal, anticancer, antimicrobial, antinociceptive, antioxidant, antipruritic, hepatoprotective, and sperm immobilization effects of *C. album*[32-40]. The IC50 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 be inhibited 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 literatures.

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 saponin[42]. It was determined that phenolic compounds of 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 inhibitor activity analysis on *H. compactum*, the IC50 value was evaluated as 45.70 ± 2.3618 µg/mL. Vanillic acid, syringaldehyde, sinapic acid, and quercetin were determined as phenolic compounds via HPLC analysis. Other HPLC signals can be related to the potential phenolic content mentioned in previous studies. Therefore, the lipase inhibition effect may be due to its potential phenolic content and phenolic compounds detected with 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 saponin 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 with 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 of lipase inhibition was stated as 94.18±5.2569 µg/mL, and coumaric acid and benzoic acid were identified via HPLC analysis. The antiobesity activity of *A. eupatoria* may be based on phenolic compounds of coumaric acid and benzoic acid. Also, other phenolic components observed on HPLC analysis can be related to phenolic content aforementioned in previous studies. At the same time, antidiabetic, anti-inflammatory, antioxidant, and hepatoprotective effects determined by literature studies may contribute to the potential therapeutic effect of the species in the treatment of obesity.

According to HPLC Analysis, the highest phenolic content was found for *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, C. album*. In this study, the relationship between phenolic compounds and lipase inhibitory effects has been proven again, and the therapeutical potential of these species has been demonstrated for obesity.

5. 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.

6. 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 in the treatment of obesity.

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Tables

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

Species	*p*-hydroxybenzoic acid	Vanilic acid	Syringaldehyde	Coumaric acid	Sinapic acid	Benzoic acid	Quercetin
mg/g extract							
A. albus	-	-	-	0.27	-	-	-
C. album	-	-	-	-	-	0.33	-
H. compactum	-	7.32	14.97	-	0.30	-	4.66
A. eupatoria	-	-	-	0.71	-	3.43	-

Figures

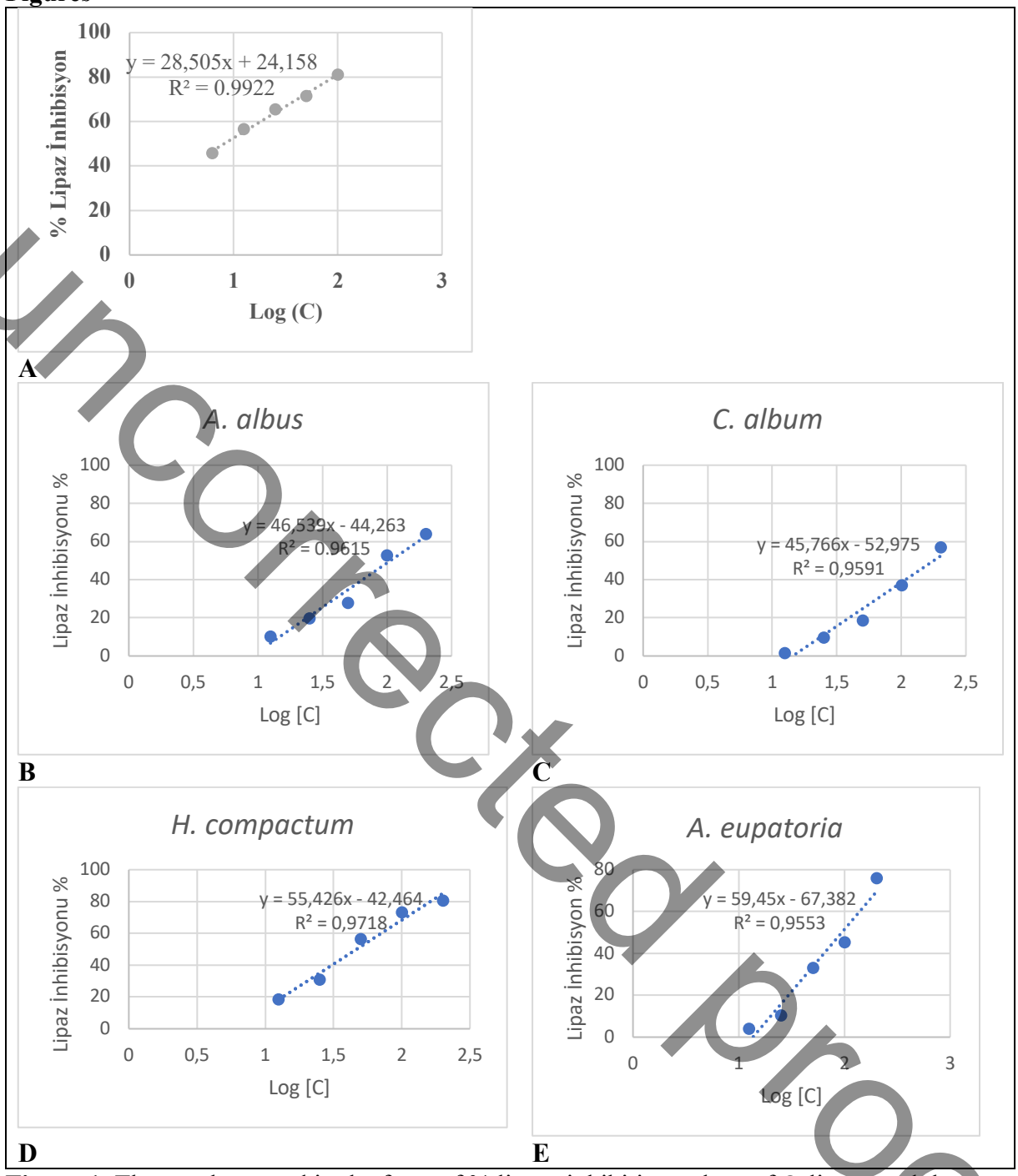

Figure 1. The graph created in the form of % lipase inhibition values of Orlistata and the logarithm of the concentration it belongs to(A), The graph created in the form of % lipase inhibition values of *A. albus* and the logarithm of the concentration it belongs to (B), The graph created in the form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

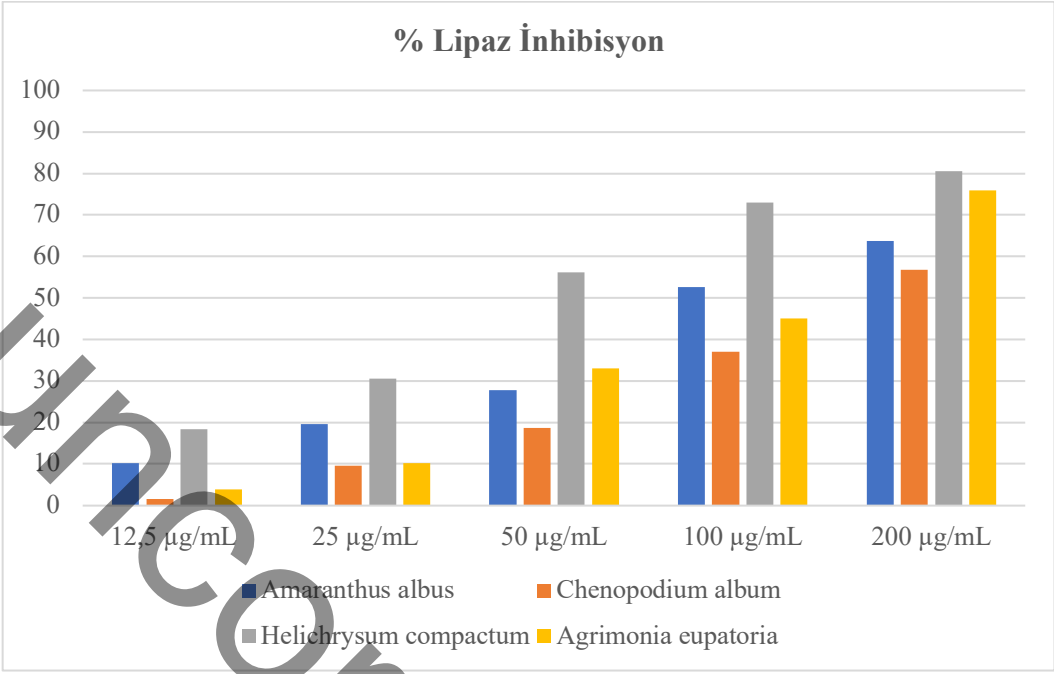

Figure 2. % Lipase inhibition values of the species at different concentrations

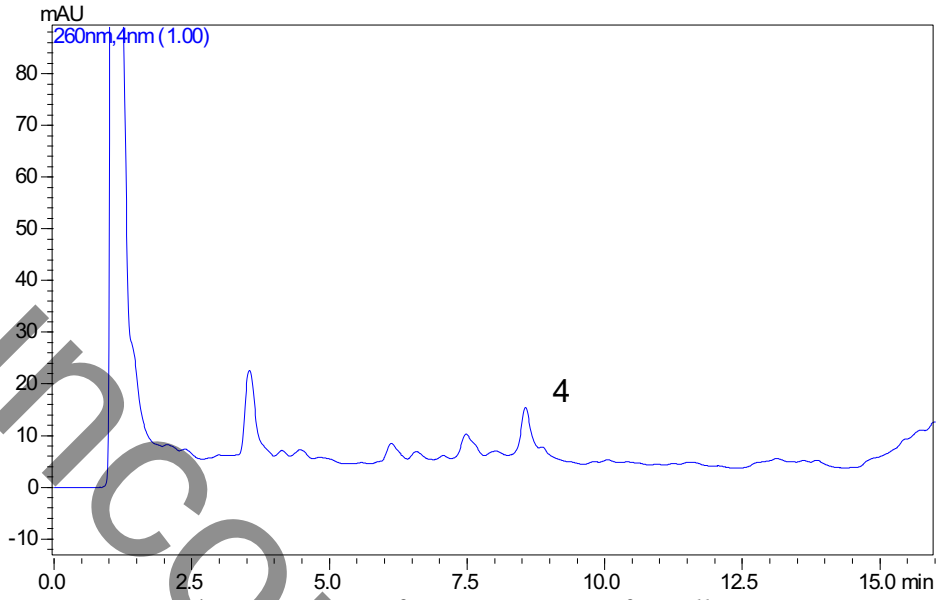

Figure 3. HPLC Chromatogram of MeOH extract of *A. albus*

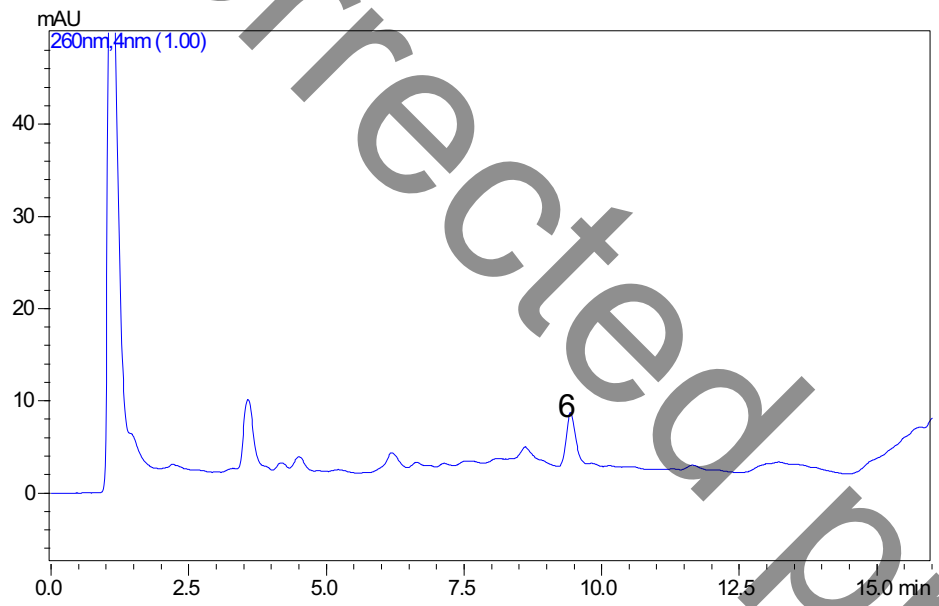

Figure 4. HPLC Chromatogram of MeOH extract of *C. album*

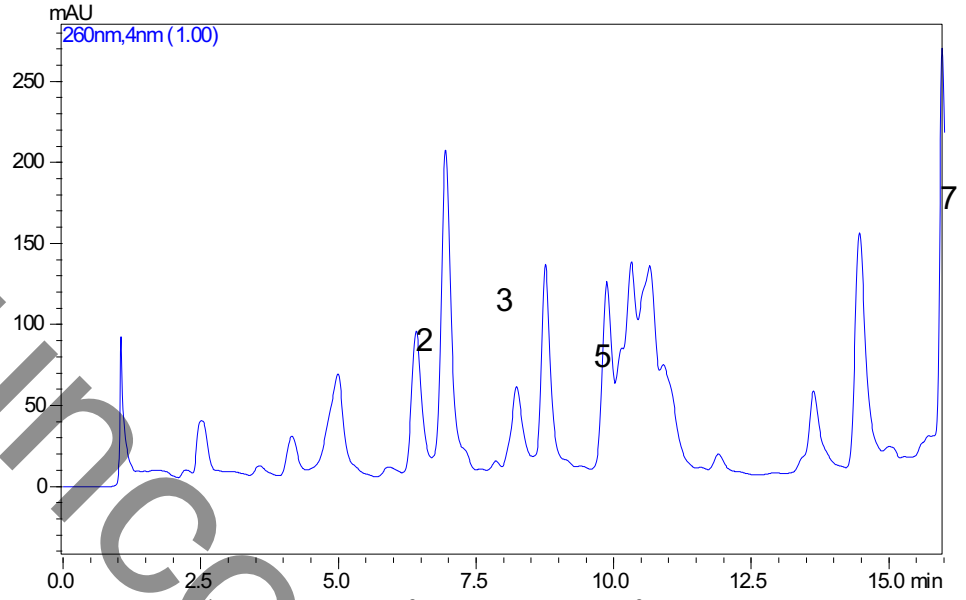

Figure 5. HPLC Chromatogram of MeOH extract of *H. compactum*

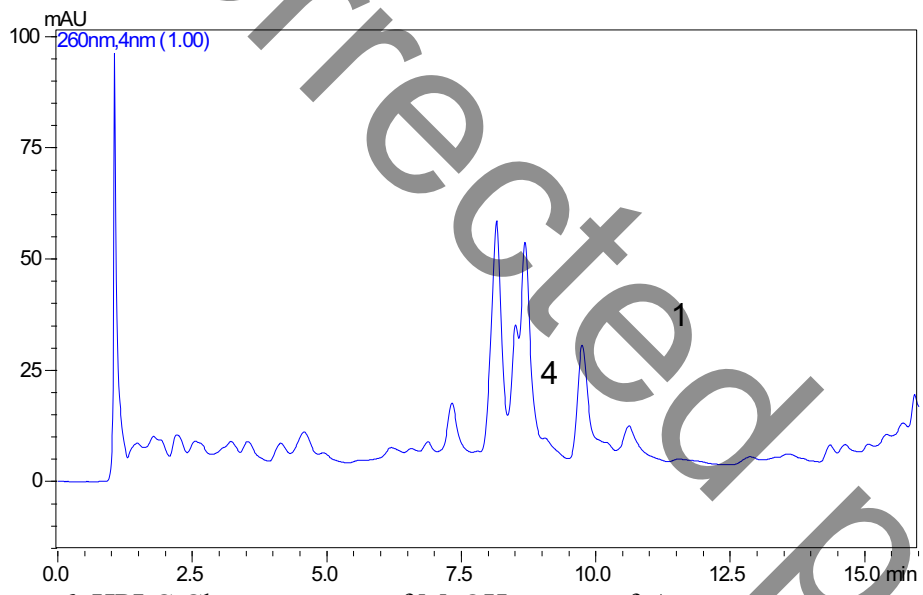

Figure 6. HPLC Chromatogram of MeOH extract of *A. eupatoria*