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Investigation of the Therapeutic Value of *Verbascum pyramidatum* Bieb. for Obesity

Verbascum pyramidatum Bieb.'in Obezitedeki Tedavi Edici Değerinin Araştırılması

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ABSTRACT

Nowadays, one of the therapeutic approaches for obesity is the use pancreatic lipase inhibitors which reduce the digestion and absorption of fats. Most research indicates that natural sources which have a lipase inhibitory effect, may be utilized to treat obesity. *Verbascum pyramidatum* is one of the potential natural sources for obesity, and it has been demonstrated to have anti-inflammatory, anti-diabetic, and regulatory effects on lipid metabolism. With this study, *V. pyramidatum*'s potential lipase inhibitor effect, it is aimed to reveal its value in the treatment of obesity. In vitro spectroscopic method was used to determine the lipase inhibitory effect of *V. pyramidatum*. The quantitative investigation of *V. pyramidatum*'s phenolic metabolites with anti-obesity activity was carried out utilizing the Reverse Phase-High Performance Liquid Chromatography method. In this reported study, it was proven that extract and all fractions had an impact that inhibited lipase, with the ethyl acetate extract showing the highest inhibitory effect. Additionally, it was revealed through HPLC analysis that the species included *p*-OH benzoic acid, coumaric acid, quercetin, sinapic acid, and syringaldehyde. It has been demonstrated that *V. pyramidatum* may be a promising candidate for obesity treatment, but further investigations are required to use it as a therapeutic agent.

Key Words
Lipase, Obesity, RP-HPLC, *Verbascum pyramidatum.*

ÖZ

Obezite için günümüzde geçerliliğini koruyan tedavi yaklaşımlarından biri, lipitlerin sindirimini ve absorpsiyonunu azaltan pankreatik lipaz inhibitörlerinin kullanılmasıdır. Lipaz inhibitör etki potansiyeli bulunan doğal kaynakların obezite tedavisinde kullanılabileceği ileri sürülmektedir. Anti-enflamatuvar, antidiyabetik ve lipit metabolizması üzerinde düzenleyici etkileri kanıtlanmış *Verbascum pyramidatum*, obezite tedavisinde kullanılma potansiyeli bulunan doğal kaynaklardan biridir. Bu çalışma ile, *V. pyramidatum*'un potansiyel lipaz inhibitör etkisi üzerinden, obezite tedavisindeki değerinin ortaya çıkarılması amaçlanmıştır. *V. pyramidatum*'un lipaz inhibitör etkisi belirlemek için in vitro spektroskopik yöntemden yararlanılmıştır. Ters Faz Yüksek Basınçlı Sıvı Kromatografisi yöntemi ile V. pyramidatum içeriğindeki antiobezite aktiviteye sahip fenolik karakterde bazı metabolitlerin kantitatif analizi gerçekleştirilmiştir. In vitro lipaz inhibitör etki belirleme çalışmaları sonucunda, *V. pyramidatum*'un farklı çözücü sistemleriyle hazırlanan ekstre ve fraksiyonlarının lipaz inhibitör etkiye sahip olduğu belirlenmiştir. En yüksek lipaz inhibitör etki etil asetat fraksiyonunda gözlenmiştir. YPSK analizinde türün *p*-OH benzoik asit; kumarik asit, kersetin, sinapik asit ve syringaldehit içerdiği tespit edilmiştir. Bu çalışma ile *V. pyramidatum*'un obezite tedavisi için umut verici bir doğal kaynak olduğu ortaya çıkarılmakla birlikte terapötik bir ajan olarak kullanımı daha ileri araştırmalar gerektirmektedir.

Anahtar Kelimeler
Lipaz, Obezite, TF-YBSK, *Verbascum pyramidatum.*

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INTRODUCTION

Obesity occurs when there is an abnormal or excessive storage of fat based on the intake of energy being greater than the expenditure of energy. Globally, obesity and several chronic conditions directly related to obesity, including diabetes, hypertension, cardiovascular diseases, and cancer, result in significant health issues as well as financial losses [1,2]. Nowadays, pancreatic lipase inhibition, which prevent the breakdown and absorption of lipids, is an important therapeutic approach still available for obesity. Natural sources and their secondary metabolites with lipase inhibitory properties, own the potency to be employed for obesity treatment. To illustrate, orlistat, one of the pancreatic lipase inhibitors, is a natural compound produced by *Streptomyces toxytricini* and is marketed under the brand names Xenical® and Alli® [3-6].

Natural products have gained popularity to create safe and potent anti-obesity medications [6]. Natural sources that include phenolic compounds provide a variety of therapeutic and dietary advantages. It has been demonstrated that phenolic substances, such as phenolic acids, curcuminoids, flavonoids, and lignan, can prevent obesity by controlling lipid metabolism, reducing adipogenesis, increasing calorie expenditure, and inhibiting adipocyte differentiation [7,8].

Verbascum genus belongs to Scrophulariaceae has more than 2500 species. *Verbascum* genus is represented by 245 species and 194 of them are endemic in Turkey [9]. *Verbascum* species, known as "Sığır Kuyruğu" in Anatolia, is used as an expectorant, anti-inflammatory, anti-tussive, and anti-diarrheic. It is also known that the species are used in the treatment of headaches, migraines, eczema, hemorrhoids, and asthma traditionally. Previous biological activity studies on *Verbascum* species have revealed to possess wound healing, anti-inflammatory, antioxidant, anti-diabetic, antiviral, cytotoxic, antimalarial, antimicrobial, sedative, antitumor, antihepatotoxic, antioxidant, antihyperlipidemic, anthelmintic, anxiolytic, and preanesthetic effects. Additionally, the genus *Verbascum* has been proven to contain a high amount of phenolic compounds in regard to phytochemical composition [10-12]. There are preparations developed for weight control of some *Verbascum* species. The weight-loss syrup Harebell J. M. and the topical solution Harebell T. M. were created by Iran's Golden Plant Company (763/318-2006/05/12) using extracts from the species *V. songaricum* [10]. All this information has led us to the conclusion that *V. pyramidatum*, which is an endemic species to Turkey and has not been studied for its anti-obesity effect, is a researchable species for obesity.

The aim of the study is to investigation the lipase inhibitory effects of the aerial parts of *V. pyramidatum* and to perform the quantitative analysis of the phenolic compounds associated with anti-obesity effects (benzoic acid, coumaric acid, quercetin, *p*-OH benzoic acid, sinapic acid, syringaldehyde, vanilic acid) with the HPLC technique. In this study, no ethics committee approval required.

MATERIALS and METHODS

Plant material

Identification of *V. pyramidatum* Bieb. was performed by Prof. Dr. Ufuk OZGEN and Asst. Prof. Dr. Merve BADEM. *V. pyramidatum* herbarium samples were stored in the Herbarium of the Faculty of Pharmacy of Ankara University (1281 m, Yolbaşı Köyü, Akkuş, ORDU, AEF 26980).

Extract and Fractions Preparation

To prepare the methanol extract, the aerial parts of *V. pyramidatum* were first powdered up. The powdered plant material (~ 300 g) was extracted for a day at 30 °C with ~ 500 mL of methanol prior to getting filtered. The filtrates were combined after the process had been conducted out three times. Some parts of the filtrates were evaporated to dryness to obtain a dry MeOH extract. The other part of the filtrate was suspended with 300 mL of water:methanol (9:1), which was used as the solvent system. The suspended mixture was partitioned into chloroform (300 mL x 3) using a separatory funnel. The solvent of the prepared chloroform-containing phases was evaporated with an evaporator, and thus the dry chloroform fraction was acquired. The remaining aqueous fraction was partitioned with ethyl acetate (300 mL x 3) in the same way. The solvent of the ethyl acetate phases was evaporated by an evaporator, and the dry ethyl acetate fraction was obtained. The solvent of the remaining aqueous phases was evaporated and the aqueous fraction was obtained.

Lipase Enzyme Inhibition Studies

Extract and fractions of *V. pyramidatum*'s aerial parts were diluted with 0.1 M Tris-HCl buffer solution (pH =

8.0) to acquire final concentrations of 25-200 µg /mL. *p*-nitrophenylbutyrate (*p*-NPB) (CAS: 2635-84-9) was used as the substrate to evaluate the levels of lipase inhibition. The lipase inhibition method's fundamental principle is based on the spectroscopic determination of the formed *p*-nitrophenol compound's absorbance [13,14]. As a positive control, orlistat, which inhibits lipase, was utilized. Orlistat was diluted to final concentrations of 6.25, 12.5, 25, 50, and 100 g/mL with 0.1 M Tris-HCl buffer solution (pH = 8.0). Spectroscopic analysis was employed to evaluate the samples' absorbance. (SpetrostarNano-BMG LABTECH).

Microplates A, B, C, and D are coded to create the experimental protocol. These components are used in the creation of microplates:

Microplates are designed with appropriate amount of these ingredients: A, buffer solution (5 µL), enzyme solution (90 µL), substrate solution (5 µL); B, buffer solution (10 µL), enzyme solution (90 µL); C, enzyme solution (90 µL), sample solution (5 µL), substrate solution (5 µL); D, buffer solution (5 µL), enzyme solution (90 µL), sample solution (5 µL). Each microplate was incubated at 37 °C for 15 minutes before the substrate solutions were added. Then, the substrate solutions were added to the appropriate wells, and then microplates were again incubated at 37 ° C for 15 minutes. At a wavelength of 405 nm, samples' absorbances were measured. There were three runs on each sample.

The absorbance values designated as A, B, C, and D were used to calculate the levels of lipase enzyme inhibition. The following contains the formula.

$$\text{Lipase inhibition } \% = \frac{(A-B)-(C-D)}{(A-B)} \times 100$$

Graphs were created using the percent enzyme inhibition values that were detected at the conclusion of the experiment and the concentration logarithm. Then, using the chart equation, the values of lipase enyzme 50% inhibition concentration (IC_{50}) of the samples were calculated.

HPLC Analysis

The validated technique was used to conduct for HPLC analysis [15]. The dry extract and fractions of the species were combined with HPLC grade methanol to prepare sample solutions at a final concentration of 10 mg/mL. The injection volume for the HPLC method was 20 µL, and the flow rate was 1.5 mL/min. The solvent systems for gradient elution, 100% methanol as A solution and 2 % acetic acid (pH 2.65) as B solution were used. Measurements were made at the following wavelengths: 200, 210, 220, 230, 240, 250, 260, 270, 280, and 320 nm with the DAD detector.

Statistical Analysis

Three repetition of each experiment were carried out. The results were reported as mean ± standard deviation (SD).

RESULTS

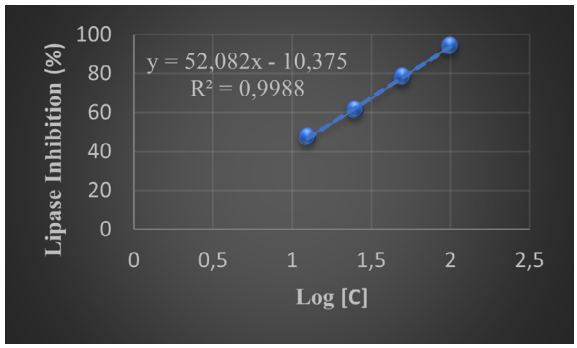
Lipase Inhibition Results

The IC_{50} levels of orlistat, dry methanolic extract, and fraction from *V. pyramidatum* were established. It was found that the graph made using the logarithm of orlistat's percent enzyme inhibition values and the species it belongs to was linear (Figure 1). Orlistat's IC_{50} level was calculated to be 14,4278 ± 0,8547 µg/mL.

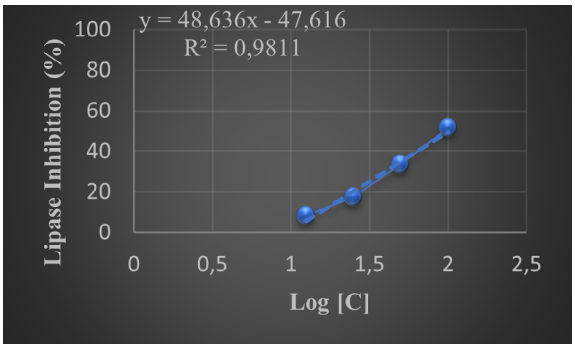
The percent lipase inhibition values of the methanol extract and all fractions obtained from *V. pyramidatum* at various concentrations were displayed in Figure 2. The IC_{50} levels for *V. pyramidatum*'s methanol extract, EtOAc fraction, $CHCl_3$ fraction, and remaining aqueous fraction were found to be 101.6419 ± 2.3745, 86.7246 ± 1.9543, 212.0416 ± 2.8118 and 94.5837 ± 2.9183 µg/mL, respectively.

HPLC Analysis Results

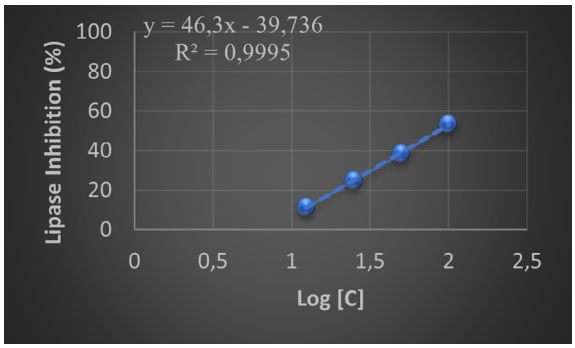
V. pyramidatum's phenolic content was expressed as mg/g extract. It was determined that the species contained *p*-OH benzoic acid (4.881 mg/g), coumaric acid (1.789 mg/g), quercetin (1.973 mg/g), sinapic acid (5.693 mg/g), and syringaldehyde (4.403 mg/g) in the HPLC analysis. Additionally, chromatogram data for the species was givenin Figure 3.



A



B



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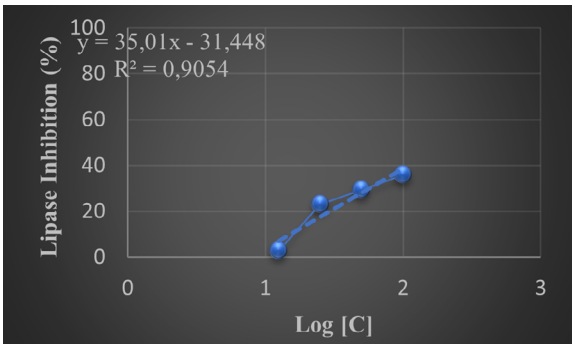
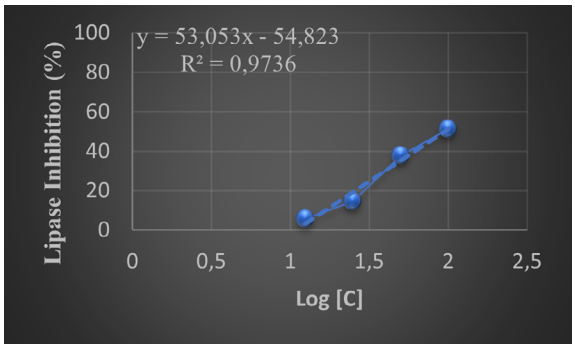
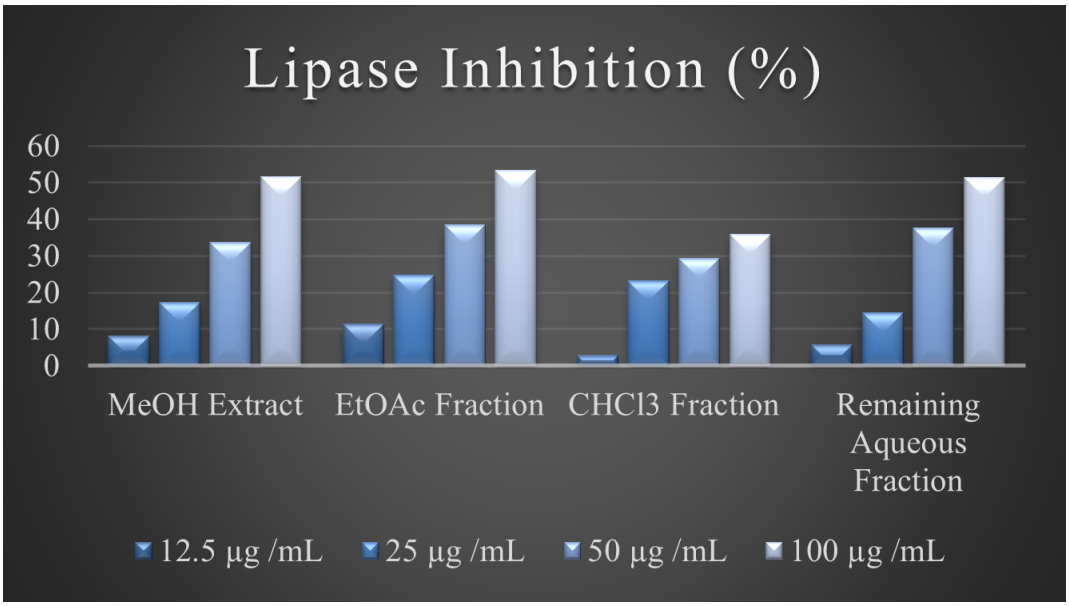


D



E

Figure 1. The graph created in the form of % lipase inhibition values of Orlistat and the logarithm of the concentrations it belongs to (A), The graph created in the form of % lipase inhibition values of MeOH extract of V. pyramidatum and the logarithm of the concentrations it belongs to (B), The graph created in the form of % lipase inhibition values of EtOAc fraction of V. pyramidatum and the logarithm of the concentrations it belongs to (C), The graph created in the form of % lipase inhibition values of CHCl3 fraction of V. pyramidatum and the logarithm of the concentrations it belongs to (D), The graph created in the form of% lipase inhibition values of remaining aqueous fraction of V. pyramidatum and the logarithm of the concentrations it belongs to (E).



A

Figure 2. Lipase inhibition values (%) of the samples for different concentrations.

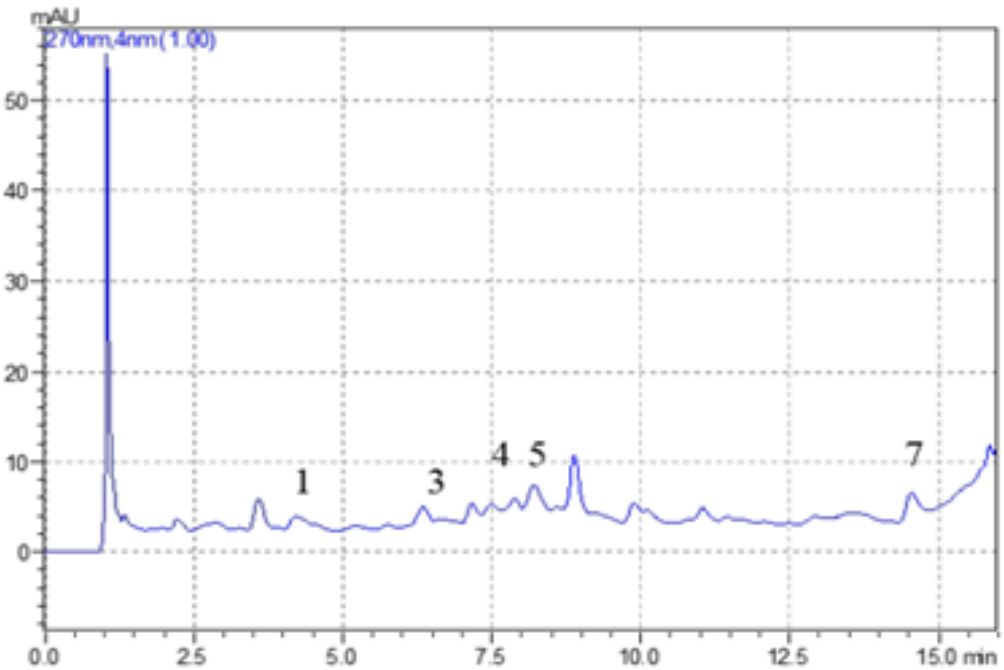


Figure 3. The HPLC Chromatogram of V. pyramidatum. 1. p-OH benzoic acid, 2. vanillic acid, 3. syringaldehyde, 4. coumaric acid, 5. sinapic acid, 6.

DISCUSSION

Obesity is regarded as an abnormal or excessive deposition of fat caused by an unbalance among both calorie intake and consumption [16,17]. Coronary artery disease, cerebrovascular disease, diabetes, hyperlipidemia, hypertension, gynecological abnormalities, osteoarthritis, psychiatric illnesses, pulmonary embolism, sleep apnea, and many cancer diseases are all closely correlated with obesity [18,20]. Also, obesity is a major contributor to high costs globally and in Turkey. Therefore, reducing obesity is crucial for reducing the financial burden associated with obesity [21].

To treat and prevent obesity, a variety of therapeutical techniques have been developed in addition to diet and increasing physical activity [19,22]. Examples of these approaches include preventing the development of adipocytes, promoting energy expenditure, suppressing the fas gene, inhibiting pancreatic lipase, reducing appetite, and using an anti-inflammatory agents [23].

Pancreatic lipase is charge of converting between 50 and 70 percent of triglycerides into fatty acids and monoacylglycerol. Inhibition pancreatic lipase activity enables lower calorie intake by reducing dietary fat absorption [24].

Phenolic compounds are critical for obesity. It has been established that natural sources, including phenolic compounds, helps prevent obesity [25]. Vanillic acid has been shown to regulate insulin resistance-induced hyperinsulinemia, hyperglycemia, and hyperlipidemia as well as to have an anti-inflammatory impact in rats fed a high-fat diet [26]. Previous research has revealed that *p*-coumaric acid and sinapic acid increased the level of anti-inflammatory cytokines like adiponectin and decreased the level of pro-inflammatory adipokines like TNF-α in obese mice. Both phenolic compounds have been proven to have therapeutic potential for the reduction of obesity-related health problems [27]. Syringaldehyde has been shown to have a beneficial impact on hyperglycemia by raising the levels of the GLUT-4 transporter and mRNA in the muscles of diabetic rats [28]. In streptozotocin-induced diabetic rats, compounds derived from benzoic acid has been improved lipid metabolism and diabetes by controlling plasma levels of insulin, glucose, LDL, and triglycerides [29]. Quercetin has been displayed to have important role for obesity. It has shown in many studies, quercetin has decreased body weight and blood sugar, as well as liver fat accumulation and triacylglycerol levels [30].

In Anatolia, a species of *Verbascum* called as "Sığır Kuyruğu" are used as an expectorant, anti-inflammatory, anti-tussive, and anti-diarrheic. Additionally, it is well known that the species has been used traditionally to treat asthma, eczema, hemorrhoids, headaches, and migraines. Anxiolytic, anti-inflammatory, antiviral, cytotoxic, antimalarial, antimicrobial, sedative, antitumor, antihepatotoxic, antioxidant, antihyperlipidemic, anthelmintic, and preanesthetic activities have been discovered in previous biological activity research on *Verbascum* species. Furthermore, it has been demonstrated that the phytochemical composition of the genus *Verbascum* contains a high content of phenolic compounds [10-12]. Phenolic components of luteolin-3'-*O*-β-glucopyranoside, apigenin-7-*O*-β-glucopyranoside, chrysoeriol-7-*O*-β-glucopyranosid, luteolin-7-*O*-β-glucopyranoside were isolated from aerial parts of *V. salviifolium* [12]. Phenolic constituents of 4-hydroxybenzoic acid, 2,3-dihydoxybenzoic acid, salicylic acid, chlorogenic acid, vanillic acid, rosmarinic acid, caffeic acid, *p*-coumaric acid, ferulic acid, hesperetin, rutin, quercitrin, morin and apigenin were detected for *Verbascum glabratum* [31]. The leaf of *V. cheiranthifolium* var. *cheiranthifolium* has been demonstrated to include phenolic compounds like apigenin, apigenin glucoside, chlorogenic acid, rosmarinic acid, luteolin hexoside, and quercetin glycoside [32]. Some *Verbascum* species have preparations used for weight control. Iran's Golden Plant Company (763/318-2006/05/12) developed the topical remedy Harebell T. M. and the weight-loss syrup Harebell J. M. from extracts of the species *Verbascum songaricum* [10].

In the presented study on *V. pyramidatum*, methanol extract and all fractions have been shown to have lipase inhibitory effect. The highest IC_{50} value of lipase inhibition was observed for EtOAc fraction (86.7246 ± 1.9543 µg/ mL). Phenolic constituents of *p*-OH benzoic acid, coumaric acid, quercetin, sinapic acid and syringaldehyde were identified via HPLC analysis for *V. pyramidatum*. The phenolic compounds found in *V. pyramidatum* by HPLC analysis may be the basis for the its anti-obesity benefits. Additionally, other signals obtained by HPLC analysis can be associated with the phenolic content reported in previous studies. At the same time, hepatoprotective, anti-inflammatory, antioxidant, and anti-diabetic activities of other *Verbascum species*

discovered through previous literature studies may support the species› potential therapeutic benefit in the management of obesity. Consequently, the project›s HPLC analysis and evaluation of the lipase inhibitor›s effects revealed the potential of V. pyramidatum in the treatment of obesity.

CONCLUSION

It has been demonstrated and determined that *V. pyramidatum* may be regarded as a possible therapeutic agent in the treatment of obesity due to the relationship between phenolic compounds and lipase inhibitory effects with the presented study. However, further formulation, toxicological, and clinical research are necessary before it may be used as a medicinal agent.

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Author Contributions
Sila Ozlem SENER devised the manuscript, performed HPLC analysis and lipase inhibition studies, collected plant materials, organized data, performed statistical analysis, and conducted literature search. Merve BADEM collected plant materials, identified plant materials, generated herbarium record, revised the statistical analysis, and revised the manuscript. Mehmet CATALBAS performed extraction procedures and lipase inhibition studies and revised the manuscript. Seyda KANBOLAT performed lipase inhibition studies, edited the statistical analysis and revised the manuscript. Ufuk OZGEN identified plant materials and revised the manuscript. Nevin ULAS COLAK performed the HPLC analysis.

Conflict of Interest
All authors declare that they have no conflicts of interest.

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