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RESEARCH ARTICLE

Evaluation of Antioxidant and Enzyme Inhibitory Activities of Some Daphne Species

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SUMMARY

Daphne species are distributed in many parts of the world and are traditionally used in the treatment of various diseases. This study was carried out to investigate the antioxidant, anti-obesity, and antidiabetic activities of leaf and branch extracts of Daphne gnidioides Jaub. & Spach, D. oleoides Schreb. and D. sericea Vahl. In vitro antidiabetic (α-glucosidase and α-amylase inhibitory effects) and antiobesity (pancreatic lipase inhibitory effects) activities of D. oleoides and D. sericea were reported for the first time in this study. Antioxidant effect was evaluated by DPPH and ABTS radical scavenging activity, total antioxidant capacity, and ferric reducing power tests. The total flavonoid content of the extracts ranged from 11.26 ± 0.55 to 81.97 ± 1.84 QE/g extract. D. oleoides leaf extract was found to have the highest ferric reducing power absorbance value (0.406 ± 0.02). D. oleoides branch extract (90.05 ± 0.57%) was found to have the strongest DPPH radical scavenging activity and this result was similar to the reference substance ascorbic acid at the same concentration. The analysed Daphne species showed either weak or no effect on the pancreatic lipase enzyme. Among the tested extracts, D. oleoides branch extract was found to have the strongest α-glucosidase inhibitory activity (IC50 = 28.53 ± 1.35 µg/mL). Similarly, D. oleoides branch extract inhibited α-amylase enzyme at the highest rate (52.09 ± 1.91%). In conclusion, D. oleoides branch extract was found to have high antioxidant activity and remarkable antidiabetic activity in experiments with different mechanisms.

Key Words: *Daphne, antioxidant, antidiabetic, pancreatic lipase.*

Bazı Daphne Türlerinin Antioksidan ve Enzim İnhibitör Aktivitelerinin Değerlendirilmesi

ÖZ

Daphne türleri dünyanın birçok bölgesinde dağılım göstermekte ve çeşitli hastalıkların tedavisinde geleneksel olarak kullanılmaktadır. Bu çalışma Daphne gnidioides Jaub. & Spach, D. oleoides Schreb. ve D. sericea Vahl'ın yaprak ve dal ekstrelerinin antioksidan, anti-obezite ve antidiyabetik aktivitelerinin araştırılması amacıyla gerçekleştirilmiştir. D. oleoides ve D. sericea'nın in vitro antidiyabetik (α-glukozidaz ve α-amilaz inhibitör etkileri) ve anti-obezite (pankreatik lipaz inhibitör etkileri) aktiviteleri ilk kez bu çalışmada rapor edilmiştir. Antioksidan etki DPPH ve ABTS radikal süpürücü aktivite, total antioksidan kapasite ve demir indirgeme gücü testleri ile değerlendirilmiştir. Ekstrelerin total flavonoit içeriği 11,26 ± 0,55 ile 81,97 ± 1,84 QE/g ekstre aralığında bulunmuştur. D. oleoides yaprak ekstresinin en yüksek demir indirgeme gücü absorbans değerine (0,406 ± 0,02) sahip olduğu bulunmuştur. D. oleoides dal ekstresinin (%90,05 ± 0,57) en güçlü DPPH radikal süpürücü aktiviteye sahip olduğu ve bu sonucun aynı konsantrasyonda referans madde olan askorbik asit ile benzer olduğu görülmüştür. İncelenen Daphne türleri pankreatik lipaz enzimi üzerinde ya zayıf etki göstermiş ya da hiç etki göstermemiştir. Test edilen ekstrelerinden en güçlü α-glukozidaz inhibitör aktiviteye D. oleoides dal ekstresinin sahip olduğu bulunmuştur (IC50 = 28,53 ± 1,35 µg/mL). Benzer şekilde D. oleoides dal ekstresi α-amilaz enzimini en yüksek oranda (%52,09 ± 1,91) inhibe etmiştir. Sonuç olarak D. oleoides dal ekstresinin farklı mekanizmalar ile gerçekleştirilen deneylerde yüksek antioksidan aktiviteye ve dikkate değer antidiyabetik aktiviteye sahip olduğu bulunmuştur.

Anahtar Kelimeler: *Daphne, antioksidan, antidiyabetik, pankreatik lipaz.*

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INTRODUCTION

People have been using plants against various diseases for centuries (Nwozo, Effiong, Aja, & Awuchi, 2023). Fruits, roots, flowers, seeds, and leaves of plants contain bioactive compounds. Medicinal plants and herbal products are advantageous because they are easily accessible, inexpensive and have fewer side effects/toxicity and contraindications compared to synthetic drugs (Petrovska, 2012; Sun & Shahrajabian, 2023). In recent years, the number of studies on plants and plant-derived active compounds in new drug discovery has increased (Miller, 2001; Parasuraman, 2018). Although the mechanisms of action of plants on diseases are not fully understood, many of them have antioxidant activity. Thanks to this activity, plants and plant-derived compounds are effective in diabetes, atherosclerosis, cardiovascular diseases, cancer, and memory problems (Karimi, Majlesi, & Rafieian-Kopaei, 2015).

Diabetes mellitus is a disease caused by problems in the secretion or action of insulin and characterised by hyperglycaemia. It is reported that more than 360 million people worldwide will be affected by diabetes by 2030 (Patel, Kumar, Laloo, & Hemalatha, 2012). In the treatment of diabetes, insulin, insulin analogs, oral hypoglycaemic and genetic agents are prescribed. These drugs cause stability problems, bioavailability problems, and various side effects in oral use (Zhao et al., 2020). Therefore, the number of studies on medicinal plants in the management of Type 2 diabetes has increased (Unuofin & Lebelo, 2020). In this context, reducing glucose absorption by inhibiting α-amylase and α-glucosidase is desired in the treatment of diabetes (Cardullo et al., 2020). Moreover, obesity or overweight is also effective in the progression of Type 2 diabetes. In this context, diabetic patients should not be overweight (Chandrasekaran & Weiskirchen, 2024). Pancreatic lipase has a particularly active role in lipid absorption and digestion. Therefore, improvement in obesity can be achieved by inhibiting the pancreatic lipase enzyme (Hou, Qin, Hou, Tang, & Ge, 2022).

The genus *Daphne* belongs to the Thymelaeaceae family and has about 100 species. This genus is distributed in Europe, Asia and Africa, and species of the genus are traditionally used in the treatment of various diseases (Moshiashvili, Tabatadze, & Mshvildadze, 2020). When literature data is examined, it has been reported that these species are generally used internally and externally against rheumatic diseases, stomach disorders, hypertension, skin diseases, malaria and gonorrhea (Moshiashvili et al., 2020; Suntar et al., 2012). However, when these plants are consumed internally by humans, various toxic and side effects are observed (Tongur, Erkan, & Ayranci, 2018; Tosun, 2006). Plants belonging to this genus have been reported to have antioxidant, anti-inflammatory, anti-HIV, antiproliferative, antifungal and antibacterial effects in various activity studies (Kupeli, Tosun, & Yesilada, 2007; Grubešić, Kremer, Končić, Rodríguez, & Randić, 2012; Hajji et al., 2017; Tundis, Loizzo, Bonesi, Peruzzi, & Efferth, 2019; Lutfullah, Shah, Ahmad, & Haider, 2019; Tang et al., 2021; Tan et al., 2022; Muzammil et al., 2023).

To our knowledge, there is no report on the *in vitro* antidiabetic (α-glucosidase and α-amylase inhibitory effect) and antiobesity (pancreatic lipase inhibitory effect) activities of *Daphne oleoides* Schreb., and *D. sericea* Vahl. In this study, antioxidant, anti-obesity and antidiabetic effects of three *Daphne* species (*D. oleoides*, *D. sericea* and *D. gnidioides* Jaub. & Spach) distributed in Türkiye were reported.

MATERIAL AND METHODS

Plant material and extraction method

The three *Daphne* species used in this study were collected and identified by Prof. Dr. Hüseyin Fakir (Department of Forest Botany, Isparta University of Applied Sciences, Türkiye) and voucher specimens were preserved in the GUL herbarium (Isparta, Türkiye) (Table 1). After the plant samples were dried, the leaf and branch parts were separated and ground. Plant parts (leaf and branch, 10 g) were extracted using 80% ethanol (200 mL) for 18 h and filtered at the end of the time. Crude extracts were then obtained using a rotary evaporator (Heidolph) and these extracts were stored in glass vials at 2-8°C for use in activity assays.

Table 1. Herbarium numbers and collection area information of *Daphne* species

Plant name	Herbarium no	Collection sites and dates
D. gnidioides	GUL 97/1/6-1	Isparta, Eğirdir, Aşağıgökdere village, *Pinus brutia* Forest, October 2023
D. oleoides	GUL 97/1/5-1	Isparta, Sütçüler, Mount Tota, *P. nigra* Forest, October 2023
D. sericea	GUL 97/1/4-1	Isparta, Eğirdir, Aşağıgökdere village, *P. brutia* Forest, October 2023

Determination of total phenols

The total phenol content of leaf and branch extracts was determined using the Folin-Ciocalteu technique. In this experiment, Folin-Ciocalteau (10%) reagent prepared by dissolving in distilled water was added to the extracts (1mg/mL) in the microplate. After the extract-Folin mixture was kept for 5 minutes, sodium carbonate prepared by dissolving in distilled water was transferred to the mixture. At the end of half an hour, the absorbance of the mixture was measured at 735 nm. All of these procedures were also applied to the gallic acid solutions used to establish the calibration curve equation (Zongo et al., 2010).

Determination of total flavonoids

Total flavonoid content of leaf and branch extracts was investigated using the total flavonoid determination method of Kosalec et al. (Kosalec, Bakmaz, Pepeljnjak, & Vladimir-Knezevic, 2004). CH_3COONa, ethanol (95%), $AlCl_3$, and sodium acetate were transferred to the sample and the final volume of the mixture was completed to 1 mL with distilled water. After half an hour of incubation, the absorbance of leaf and branch extracts was read at 415 nm. All these procedures were also applied to quercetin solutions.

Antioxidant activity assays

Total antioxidant capacity

Firstly, the molybdate reagent was prepared in the experiment. To prepare this reagent, sulfuric acid, sodium phosphate monobasic, ammonium molybdate were dissolved in distilled water to a total volume of 10 ml and then the reagent and extract (100 µL) were mixed. The extract-reagent mixture was incubated at 90°C for 90 min. and the absorbance was measured at 695 nm. All of these procedures were also applied to the ascorbic acid solutions used to establish the calibration curve equation (Prieto, Pineda, & Aguilar, 1999).

Ferric reducing power

Branch and leaf samples were reacted with potassium ferricyanide and phosphate buffer at 37°C. The reaction was completed after 1 h and $C_2HCl_3O_2$ (10%) solution was transferred to the mixture. After measuring the absorbance of the mixture, iron (III) chloride (0.01%) was added and the absorbance was measured again at 700 nm (Orhan, Deliorman Orhan, Gokbulut, Aslan, & Ergun, 2017). The reference substance was quercetin. The extract and reference substance concentrations used in this experiment were the same as in other antioxidant activity tests.

ABTS radical scavenging activity

Firstly, the reference substance (gallic acid) and extracts were prepared at concentrations of 0.5, 1 and 2 mg/mL. In this experiment, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) and potassium peroxodisulfate solutions were prepared with distilled water at 7 mM and 2.45 mM, respectively. These two prepared reagents were mixed and the final volume was completed to 7.5 mL with distilled water. This mixture was kept in a dark cabinet at room temperature for 16 hours and then phosphate buffer (pH 7.4) was added. The final mixture was added to the branch and leaf extracts (10 µL) and absorbance values were read at 734 nm after 6 min (Orhan et al., 2017).

DPPH radical scavenging activity

In this test, the extract and reference substance (ascorbic acid) were prepared in 80% ethanol at 0.5,

1 and 2 mg/mL concentration. 1 mM 2,2-Diphenyl-1-picrylhydrazyl (DPPH) reagent was prepared and transferred to references and plant extracts (80 µL). After the mixture was kept in a dark cabinet, absorbance was measured at 520 nm (Jung et al., 2011).

Enzyme assays

α-Amylase enzyme inhibitory activity

This experiment was performed with minor modifications to the α-amylase inhibitory activity test method of Ali et al. (Ali, Houghton, & Soumyanath, 2006). Sodium potassium tartrate solution (5.31 M) was prepared using sodium hydroxide solution (2 M). Then, DNS solution (96 mM) was prepared by dissolving 3,5-dinitrosalicylic acid (DNS) in distilled water and mixed with the 5.31 M solution. This final mixture was called color reagent. Phosphate buffer adjusted to pH=6.9 and 4 U/mL α-amylase (Type I-A, Sigma) enzyme were added to the leaf and branch extracts and potato starch (0.5%) was added to the mixture after 5 min. Incubation was carried out for 15 min and at the color reagent the was transferred at the end of the time. Then, the mixture was kept at 80°C for 40 min and 75 µL of cold distilled water was added to the mixture at the end of the time. The change in absorbance (A) due to the mixture and maltose was measured at 540 nm. The same procedure was applied for acarbose (reference substance).

$A_{extract,\ control} = A_{Sample}-A_{Blank}$; Inhibition % = [1-(X/Y)] × 100; X: Mean maltose in acarbose and extract; Y: Mean maltose in control

α-Glucosidase enzyme inhibitory activity

α-glucosidase enzyme (*Bacillus stearothermophilus*, Type IV) purchased from Sigma was prepared in pH=6.5 phosphate buffer, and phosphate buffer and enzyme were added to the extracts transferred to the microplate. The resulting mixture was heated in an oven at 37°C for 15 min and 10 µl of 4-Nitrophenyl α-D-glucopyranoside (20 mM, substrate) was transferred to the mixture at the end of the time. Then, the microplate was kept in the oven at 37°C for 35 min and the absorbance was read at 405 nm. The same procedure was applied for acarbose (reference substance) (Lam, Chen, Kang, Chen, & Lee, 2008).

Inhibitory activity % = [1−(B−b/C−c)] × 100; B: Activity with inhibitor; b: Negative control with inhibitor; C: Activity without inhibitor; c: Negative control without inhibitor

Pancreatic lipase enzyme inhibitory activity

The method of Lee et al. (2012) was used to evaluate the enzyme inhibitory activity of the samples (Lee et al., 2012). Tris HCl (100 mM Tris-HCl, 5 mM $CaCl_2$) and pH=6.8, morpholinepropanesulfonic acid (mops) buffers were prepared to be used in this enzyme inhibition test and stored at room temperature. Lipase enzyme was transferred to the samples. Then, Tris HCl buffer (150 µL) and mops buffer were added to the mixture and the microplate was kept at 37°C for 15 min. To each well in the microplate, 10 mM substrate (*p*-nitrophenylbutyrate) prepared with acetonitrile was added and the mixture was reacted at 37°C for 30 min. At the end of the time, absorbance was measured at 405 nm and the same procedure was applied for orlistat (reference substance).

Inhibition % = [1−(B−b/C−c)] × 100; B: Activity with inhibitor; b: Negative control with inhibitor; C: Activity without inhibitor; c: Negative control without inhibitor

Statistical analysis

All experimental procedures were repeated three times. The values found in this study were shown as mean ± standard deviation (SD). GraphPad Prism and Microsoft Excel programs were used to determine activity results and IC_{50} values. The difference in the experimental results at $P<0.05$ level was considered statistically significant.

RESULTS AND DISCUSSION

Total phenolic contents of the extracts were determined according to the calibration curve equation (y = 6.8205 x - 0.1166 and coefficient of determination R^2 = 0.9924) generated from different dilutions of gallic acid. Although the investigated extracts gave similar total phenolic content results, *D. oleoides* leaf

extract had the highest phenolic content (142.01 ± 1.02 mg/gallic acid equivalent (GAE) g extract).

Total flavonoid contents of the extracts were determined according to the calibration curve equation (y = 2.1336x - 0.042 and coefficient of determination R^2 = 0.9977) generated from different dilutions of quercetin. The total flavonoid contents of the extracts ranged from 11.26 ± 0.55 to 81.97 ± 1.84 mg quercetin equivalents (QE)/g extract and the leaf extracts of all three species had higher flavonoid content than the branch extracts.

Total antioxidant capacity results of the tested samples were expressed as mg ascorbic acid equivalent (AAE)/g extract. The highest total antioxidant capacity was found in *D. oleoides* branch extract (216.47 ± 6.18 AAE/g extract) and the lowest antioxidant capacity was found in *D. gnidioides* leaf extract (65.55 ± 2.88 AAE/g extract). Total phenol, flavonoid contents, and total antioxidant capacity test results of the extracts are shown in Table 2.

Table 2. Total phenol, flavonoid content, and total antioxidant capacity assay results

Plant	Used parts	Total Phenolic Content[a] (Mean ± SD)	Total Flavonoid Content[b] (Mean ± SD)	Total Antioxidant Capacity[c] (Mean ± SD)
D. gnidioides	Branch	118.26 ± 1.45	14.13 ± 1.15	164.90 ± 9.89
	Leaf	109.02 ± 0.83	29.54 ± 2.90	65.55 ± 2.88
D. oleoides	Branch	124.66 ± 3.64	11.26 ± 0.55	216.47 ± 6.18
	Leaf	142.01 ± 1.02	62.74 ± 0.95	82.16 ± 3.30
D. sericea	Branch	108.19 ± 8.53	27.15 ± 1.49	213.46 ± 3.21
	Leaf	107.04 ± 6.95	81.97 ± 1.84	130.23 ± 7.83

[a]mg GAE/g extract, [b]mg QE/g extract, [c]mg AAE/g extract

All leaf and branch extracts analysed showed strong DPPH radical scavenging antioxidant activity with over 80% inhibition. The highest DPPH radical scavenging activity was found in *D. oleoides* branch extract (90.05 ± 0.57%) and this result was similar to ascorbic acid (91.80 ± 0.71%) at the same concentration. Both leaf and branch extracts of *D. oleoides* had the highest ABTS radical scavenging activity (78.97 ± 1.61%, 98.77 ± 1.42%, respectively). All leaf and branch extracts had weaker ABTS radical scavenging activity (<40%) at 0.5 mg/mL concentration and the weakest activity was found in *D. sericea* branch extract. The absorbance values of the extracts and quercetin were compared at three different concentrations while evaluating the results of ferric reducing power activity. At all concentrations examined (0.5, 1 and 2 mg/mL) *D. oleoides* leaf extract had the highest absorbance values (0.310 ± 0.00, 0.374 ± 0.02, and 0.406 ± 0.02, respectively). In the ferric reducing power experiment, *D. oleoides* leaf extract (0.406 ± 0.02) had a similar absorbance value to quercetin (0.570 ± 0.05). In all extracts (leaf and branch), the absorbance value of reducing power increased with increasing concentration. Antioxidant activity test results of the extracts are shown below (Table 3).

Table 3. Antioxidant activity test results

			Antioxidant activity tests		
Plant	Used parts	Concentration (mg/mL)	DPPH radical scavenging activity inhibition % ± SD	ABTS radical scavenging activity inhibition % ± SD	Ferric reducing power absorbance ± SD
D. gnidioides	Branch	0.5	85.12 ± 0.33***	36.15 ± 0.43***	0.223 ± 0.02***
		1	87.26 ± 0.20***	47.56 ± 2.16***	0.260 ± 0.01***
		2	88.05 ± 0.20***	79.84 ± 1.24***	0.302 ± 0.02***
	Leaf	0.5	83.77 ± 1.93***	34.32 ± 0.43***	0.231 ± 0.00***
		1	86.78 ± 1.12***	38.09 ± 3.17***	0.293 ± 0.01***
		2	87.22 ± 0.08***	43.69 ± 3.89***	0.304 ± 0.01***
D. oleoides	Branch	0.5	82.90 ± 0.20***	37.21 ± 1.82***	0.224 ± 0.01***
		1	87.30 ± 0.45***	58.17 ± 0.89***	0.290 ± 0.01***
		2	90.05 ± 0.57***	78.97 ± 1.61***	0.297 ± 0.02***
	Leaf	0.5	85.21 ± 0.35***	32.08 ± 1.58***	0.310 ± 0.00***
		1	87.13 ± 0.15***	60.96 ± 2.04***	0.374 ± 0.02***
		2	87.61 ± 0.84***	98.77 ± 1.42***	0.406 ± 0.02***
D. sericea	Branch	0.5	86.08 ± 0.20***	18.90 ± 1.11**	0.233 ± 0.01***
		1	87.17 ± 0.23***	23.34 ± 1.52***	0.270 ± 0.01***
		2	88.09 ± 0.23***	77.33 ± 1.35***	0.307 ± 0.02***
	Leaf	0.5	86.04 ± 0.40***	31.10 ± 0.63***	0.245 ± 0.01***
		1	86.61 ± 0.87***	48.92 ± 0.85***	0.301 ± 0.03***
		2	87.65 ± 2.46***	68.75 ± 2.39***	0.332 ± 0.02***
Reference	AA GA QU	0.5	91.54 ± 0.41***	98.88 ± 0.22***	0.416 ± 0.01***
		1	89.95 ± 1.05***	99.25 ± 0.13***	0.437 ± 0.05***
		2	91.80 ± 0.71***	99.93 ± 0.26***	0.570 ± 0.05***

DPPH test reference: Ascorbic acid (AA); ABTS test reference: Gallic acid (GA); Ferric reducing power test reference: Quercetin (QU); **p<0.01; ***p<0.001

In this study, the effects of three *Daphne* species on obesity and diabetes were investigated by *in vitro* enzyme assays. The antidiabetic effect was tested using α-glucosidase and α-amylase enzymes, while the anti-obesity effect was tested using the pancreatic lipase enzyme.

Among the leaf and branch extracts tested, *D. oleoides* branch extract showed the strongest α-glucosidase inhibitory activity and inhibited the α-glucosidase enzyme with an IC_{50} value of 28.53 ± 1.35 μg/mL. After this extract, *D. sericea* leaf and branch extracts highly inhibited α-glucosidase enzyme (IC_{50} values: 48.46 ± 1.28 and 49.74 ± 0.95 μg/mL, respectively). None of the extracts inhibited the α-glucosidase enzyme as potently as acarbose (IC_{50}= 0.58 ± 0.02 μg/mL). Among the extracts analysed for α-amylase inhibitory effect, only *D. oleoides* branch extract inhibited the enzyme at IC_{50} values below 200 μg/mL. The inhibitory effect of the investigated extracts on α-amylase was extremely low compared to acarbose. In the pancreatic lipase inhibitory activity test in which the anti-obesity effect of the extracts was evaluated, none of the extracts had an IC_{50} value below 200 μg/mL. Additionally, *D. sericea* branch extract did not show inhibitory effect on the pancreatic lipase enzyme. The activity of the extracts acting on the pancreatic lipase enzyme was found to be extremely weak compared to orlistat. The α-glucosidase, α-amylase, and pancreatic lipase enzyme inhibitory activity test results of the extracts are shown in Table 4.

Table 4. Enzyme inhibitory activity test results of extracts

Plant	Used parts	200 µg/mL[a] inhibition % ± S.D. (IC_{50}: µg/mL ± S.D)		
		α-amylase	α-glucosidase	Pancreatic lipase
D. gnidioides	Branch	4.51 ± 1.91[ns]	89.74 ± 1.13*** (IC_{50}= 96.86 ± 0.22)	12.86 ± 3.27*
	Leaf	16.20 ± 3.82*	61.96 ± 1.60** (IC_{50}= 138.67 ± 3.23)	22.62 ± 1.80***
D. oleoides	Branch	52.09 ± 1.91*** (IC_{50}= 186.36 ± 4.30)	96.99 ± 0.82*** (IC_{50}= 28.53 ± 1.35)	25.95 ± 0.82***
	Leaf	18.29 ± 2.61**	86.54 ± 0.45*** (IC_{50}= 83.75 ± 1.04)	12.86 ± 1.43*
D. sericea	Branch	26.37 ± 3.24**	88.94 ± 1.37*** (IC_{50}= 49.74 ± 0.95)	-
	Leaf	27.05 ± 2.61***	87.32 ± 2.47*** (IC_{50}= 48.46 ± 1.28)	40.71 ± 3.03***
Reference	Acarbose Orlistat	94.48 ± 2.80***[b] (IC_{50}= 4.41 ± 0.02)	99.55 ± 0.01***[b] (IC_{50}= 0.58 ± 0.02)	76.09 ± 3.28***[c] (IC_{50}= 11.49 ± 0.09)

[a]: Final concentration; [b]: Acarbose; [c]: Orlistat; [ns]: Not statistically significant; -: No activity; *p<0.05, **p<0.01; ***p<0.001

During extraction, 80% ethanol with medium polarity is generally preferred to reveal active phytochemicals (Al Jaafreh, 2024). Extraction with hydro-alcoholic solvents dissolves many phytochemicals and solvents of this polarity have the highest extraction yield and total phytochemical (phenol and flavonoid) content (Kabubii, Mbaria, Mathiu, Wanjohi, & Nyaboga, 2023). Based on this information, we preferred 80% ethanol for the extraction of plant parts.

The total phenol and flavonoid contents of the ethanol extract obtained from aerial parts of *D. sericea* (collected from Italy) were 34.1 ± 2.8 tannic acid equivalents µg/mg extract and 8.4 ± 0.9 QE µg/mg extract, respectively. It has been reported that the extract has antioxidant effects with its DPPH, ABTS radical scavenging activity, and ferric ion chelating properties (Frezza et al., 2021). In a different study, Tongur et al. investigated the antioxidant activity of *D. gnidioides* and *D. sericea* leaf methanol extracts and the results showed that *D. gnidioides* methanol extract had the highest ABTS radical scavenging activity (IC_{50}= 147.2 ± 0.4) while *D. sericea* methanol extract had the highest DPPH radical scavenging power (IC_{50}= 61.6 ± 0.1). The total flavonoid content of *D. gnidioides* methanol extract was 244.5 ± 19.5 mg rutin/g extract, while the flavonoid content of *D. sericea* methanol extract was 121.3 ± 19.7 mg rutin/g extract (Tongur et al., 2018). In contrast to Tongur et al., in our study, *D. sericea* leaf extract had stronger ABTS radical scavenging activity than *D. gnidioides* leaf extract and both leaf extracts had similar DPPH radical scavenging activity. This may be related to the varying phytochemical contents of plants.

In a different study, the antioxidant activity and chemical content of hexane, ethyl acetate, methanol and ethanol extracts prepared from the aerial parts of *D. oleoides* subsp. *oleoides* (collected from Konya, Türkiye) were evaluated. The highest phenolic content (391.970 ± 1.286 mg GAE/g extract) was found in the ethanol extract, while the lowest content (3.182 ± 1.814 mg GAE/g extract) was found in the hexane extract. Ethyl acetate extract had the strongest DPPH radical scavenging activity and ferric reducing power (IC_{50}= 65.481 ± 0.613 µg/mL, EC_{50}= 160.676 ± 6.013 µg/mL, respectively) (Zengin, Arkan, Aktumsek, Guler, & Cakmak, 2013). The antioxidant activity of the extract (methanol) and its fractions (hexane, dichloromethane, ethyl acetate, *n*-butanol, water) prepared from the aerial parts of *D. oleoides* subsp. *oleoides* (collected from Karaman, Türkiye) was evaluated. The authors found the highest DPPH radical scavenging activity and reducing power in the ethyl acetate extract and attributed this to the high phenolic substance content of the extract (Balkan et al., 2017). Similar to the study

by Balkan et al., we found that *D. oleoides* leaf extract had the strongest reducing power and ABTS radical scavenging activity. At the same time, we revealed that this extract had the highest phenolic content.

There are very few reports on the effect of *Daphne* species on diabetes. The α-amylase activity of stem, root, and leaf extracts prepared from *D. gnidioides* was investigated. As a result of the study, it was seen that all three extracts had close inhibitory activity. When α-glucosidase inhibitory activity results were analysed, it was found that root extracts had no effect on the enzyme. Stem and leaf extracts showed 3.87 ± 0.06 and 2.56 ± 0.01 mmol acarbose equivalent/g inhibition on α-glucosidase enzyme, respectively (Can et al., 2020). In another study, the *in vivo* hypoglycemic effects of methanol extract prepared from *D. oleoides* roots were evaluated and it was found that the antidiabetic effect of *D. oleoides* extract (191.50 ± 0.48 mg/dL) was less than that of metformin (111.58 ± 1.30 mg/dL) used as standard substance (Muddassir, Batool, Miana, & Zafar, 2022). Similar to the literature results, we found that the *in vitro* antidiabetic enzyme inhibitory activity of *Daphne* species was lower than that of the standard substance (acarbose). *D. oleoides* branch extract showed stronger inhibitory activity in terms of both α-glucosidase and α-amylase inhibitory activity compared to other extracts. In this study, the α-glucosidase inhibitory activity of *D. oleoides* branch extract was remarkable.

Four compounds (luteolin-7,3',4'-trimethyl ether, β-sitosterol, 5-hydroxy-7,4'-dimethoxyflavone, 5,3'-dihydroxy-7,4'-dimethoxyflavone) isolated from the barks of *Aquilaria subintegra* (Thymelaeaceae) showed pancreatic lipase inhibitory activity in the range of 6% to 53% (Ibrahim, Abdul Azziz, Wong, Bakri, & Abdullah, 2020). In another study, dichloromethane extract of *A. malaccensis* barks had significant inhibition (0.065 ± 0.147 U/mL) on pancreatic lipase (Ibrahim et al., 2018). Chloroform extract of *Thymelaea hirsute* (Thymelaeaceae) roots was reported to have 61% inhibition on pancreatic lipase at a concentration of 1 mg/mL (Abdallah, Hammoda, El-Gazzar, Ibrahim, & Sallam, 2023). To our knowledge, the anti-obesity activity of *Daphne* species has not been previously investigated. In this study, no extract had an IC_{50} value below 200 µg/mL. Therefore, the analysed *Daphne* species did not have a significant pancreatic lipase inhibitory effect.

The chemical composition of *Daphne* species may vary depending on the species or plant parts. This situation has also been reported for the species we investigated. Balkan et al. reported that the *n*-hexane extract prepared from the aerial parts of *D. oleoides* subsp. *oleoides* was rich in hexadecanoic acid and 9,15- octodecadienoic acid (Balkan et al., 2017). Oxygenated sesquiterpenes (43.0%) and nootkatone (18.5%) were found to be dominant in *D. oleoides* subsp. *oleoides* essential oil (Uysal et al., 2017). *D. gnidioides*, was reported to have high amounts of apigenin-6,8-diglucoside and syringic acid in leaf extracts (Tongur et al., 2018; Can et al., 2020). 3-*O*-*p*-coumaroyl-5-*O*-caffeoylquinic acid was detected in the root and stem extract of this plant (Can et al., 2020). The activities shown by *Daphne* species may be related to their rich chemical contents.

CONCLUSION

In this study, antioxidant, antidiabetic and anti-obesity effects of six extracts of three *Daphne* species were investigated. Although extracts of *Daphne* species had strong antioxidant activity, they showed either weak or no effect on pancreatic lipase enzyme. Our results show that these plants are important sources of antioxidants. Our findings revealed that *Daphne* species have significant α-glucosidase inhibitory activity. In this context, the branch extract of *D. oleoides* showed stronger antidiabetic effect than other extracts. As a result, *Daphne* species can be important resources for the pharmaceutical industry. Therefore, further studies should be conducted on these species.

AUTHOR CONTRIBUTION STATEMENT

Concept: SB, HF; Design: SB; Control: SB; Sources: SB, HF; Materials: SB, HF; Data Collection and/or processing: SB, HF; Analysis and/or interpretation:

SB, HF; Literature review: SB; Manuscript writing: SB, HF; Critical review: SB, HF; Other: SB

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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