Journal of Research in Pharmacy

Research Article

www.jrespharm.com

Antioxidant, enzyme inhibitory, and cytotoxic activity screening of *Daphne pontica* L. (Thymelaeaceae)

Eda AVCI [1,2], Gokhan ZENGIN [3], Filiz BAKAR-ATES [4], Alev ONDER[1] *

1 Department of Pharmacognosy, Faculty of Pharmacy, Ankara University, 06560 Tandogan, Ankara, Türkiye.
2 Department of Pharmacognosy, Faculty of Pharmacy, Ankara Medipol University, Ankara, Türkiye.
3 Department of Biology, Faculty of Science, Selcuk University, Konya, Türkiye.
4 Department of Biochemistry, Faculty of Pharmacy, Ankara University, 06560 Tandogan, Ankara, Türkiye.
* Corresponding Author. E-mail: pharmacogalev@gmail.com

Received: 11 November 2024 /Revised: 11 January 2025/Accepted: 17 January 2025

ABSTRACT: The genus *Daphne* L. (Thymelaeaceae), thriving in temperate and subtropical regions, is renowned for its medicinal properties. Used extensively in traditional medicine, *Daphne* species are rich in bioactive compounds that have been utilized to treat various diseases. *Daphne pontica* L., a shrub locally known as *kurtbağı*, *sırımağu*, and *sırımbağı*, is primarily distributed in Northern Anatolia. This study is the first comprehensive evaluation of the antioxidant, enzyme inhibitory, and cytotoxic activities of diethyl ether extracts obtained from different parts of this plant. MTT assays revealed selective cytotoxic effects of *D. pontica* extracts. The fruit extract demonstrated the strongest activity with IC_{50} values of 0.467 mg/ml in A-549 cells and 0.937 mg/ml in HeLa cells, while the stem extract exhibited an IC_{50} of 0.896 mg/ml in MCF-7 cells. These results suggest concentration- and extract-dependent biological potential. Additionally, enzyme inhibition assays highlighted the plant's promising antidiabetic activity. Overall, this study emphasizes the potential of *D. pontica* diethyl ether extracts as natural antioxidants and multifunctional bioactive agents. These findings provide a foundation for further research into its diverse therapeutic applications.

KEYWORDS: Thymelaeaceae; *Daphne pontica*; Antioxidant; Cytotoxicity.

1. INTRODUCTION

Plants have been a source of therapeutic medicines to improve and protect human health for centuries, with famous examples [1]. In addition, plant secondary metabolites are used for many purposes in many application areas [2]. Achievement of the new drug molecules from natural sources is of great interest on many platforms worldwide [3].

Thymelaeaceae is a cosmopolitan family of flowering plants with about 800 species from 50 genera distributed in temperate and tropical regions [4,5]. *Daphne* L. genus from the Thymelaeaceae family [6] has over 90 described species [6,7]. The *Daphne* L. grows in Anatolia and is represented by seven species in Turkish flora, including *Daphne pontica* L. [8]. *D. pontica* is described as a perennial, evergreen shrub-shaped plant up to 40-100 cm in height, with lightly fragrant yellowish flowers in pairs and black and berry-type fruits [8]. *D. pontica* (Pontic *Daphne*, twin flowered *Daphne*) [7,9], locally known as "tasma, kurtbağı, sırımağu and sırımbağı" in Anatolia [10,11], is naturally found in Southeastern Bulgaria, the Caucasus, Northern Iran, and Northern Türkiye [8].

Despite their potent toxicity, *Daphne* species have been used in the traditional medicine of many countries to treat various diseases such as cancer, rheumatism, skin diseases, and hypertension [12,13}. In Turkish folk medicine, these species are reported to be used for their antidiarrheal effects [10]. The diverse biological activities of the *Daphne* genus and its place in traditional medicine have increased interest in the phytochemical composition of these plants. To date, more than 250 compounds with medicinal significance have been isolated from *Daphne* species [14]. These compounds are primarily classified into five main groups: coumarins, terpenes, flavonoids, lignans, and other compounds. The genus *Daphne* is particularly noted for its rich diterpene content. Daphnane-type diterpenes, in particular, stand out for their anti-inflammatory, anti-HIV, neurotrophic, antihyperlipidemic, and anticancer effects [15,16]. However, studies on the phytochemistry and biological activities of these species are limited [12,17–31]. Therefore, the present

How to cite this article: Avcı E, Zengin G, Bakar-Ates F, Onder A. Antioxidant, enzyme inhibitory, and cytotoxic activity screening of Daphne pontica L. (Thymelaeaceae). J Res Pharm. 2025; 29(4): 1675-1681.

ISSN: 2630-6344

https://doi.org/10.12991/jrespharm.1734640

study investigated the extracts from various parts of *D. pontica* (stems, leaves, and fruits), using *in vitro* screening assay for the antioxidant, enzyme inhibition, and cytotoxic effects comprehensively.

2. RESULTS and DISCUSSION

The total content of phenolic substances is essential for determining the antioxidant properties of plants [32]. The diethyl ether extracts of *Daphne pontica* were examined regarding phenolic content, and the amount of phenolic substances varied between 53.47 mg GAE/g and 15.82 mg GAE/g. The stem extract had the highest amount of phenolic substance among these extracts (53.47 mg GAE/g). The lowest amount of phenolic substance was observed in the fruit extract (15.82 mg GAE/g). According to these results, *D. pontica* stem extract has higher phenolic substances than other parts (Table 1). The quantity of flavonoids in diethyl ether extracts of *D. pontica* was observed to vary between 13.99 and 0.87 mg RE/g. The highest content of flavonoids was in the stem extract (13.99 mg RE/g), similar to the amount of phenolic compounds, and the lowest one was in the fruit extract (0.84 mg RE/g) (Table 1).

Table 1. The total phenolic and flavonoid contents of the extracts from *Daphne pontica* L.

Samples	Total phenolic (mg GAE/g extract)	Total flavonoid (mg RE/g extract)
Stem	53.47 ± 1.16	13.99 ± 0.33
Fruit	15.82 ± 0.87	0.84 ± 0.11
Leaf	18.05 ± 3.08	1.58 ± 0.34

Values expressed are means ± S.D. GAE: Gallic Acid Equivalent, RE: Rutin Equivalent.

The antioxidant capacities of *D. pontica* extracts were evaluated using several methods, including DPPH, ABTS, CUPRAC, FRAP, metal chelating, and phosphomolybdenum assays (Table 2). The DPPH radical scavenging activities of *D. pontica* extracts were expressed as IC_{50} (mg/ml) values. The stem extract exhibited the best scavenging activity (1.13 mg/ml). In contrast, the fruit extract displayed the lowest activity (4.11 mg/ml), indicating that the DPPH radical scavenging capacity of the stem extract is notably greater than that of the other plant parts. Similarly, in the ABTS assay, the stem extract again showed the most potent activity (0.54 mg/ml). The fruit extract demonstrated the weakest activity (>5 mg/ml), reinforcing that the stem extract possesses superior antioxidant capacity compared to the other parts of the plant.

In the CUPRAC and FRAP assays, the stem extract of *D. pontica* further distinguished itself with remarkable EC_{50} values of 0.67 mg/ml and 0.77 mg/ml, respectively. However, in the metal chelating assay, the fruit extract exhibited enhanced activity relative to the other parts of the plant, recording an IC_{50} value of 1.31 mg/ml. In the phosphomolybdenum assay, the stem and leaf extracts yielded comparable results (EC_{50} values: 0.96 mg/ml and 0.97 mg/ml, respectively), whereas the fruit extract was markedly lower (2.43 mg/ml). These findings underscore the potent antioxidant activity of the stem extract of *D. pontica,* while revealing considerable differences in antioxidant potentials among the various plant parts.

Enzyme inhibition experiments were conducted to evaluate the inhibitory effects of *D. pontica* extracts on various enzymes, including acetylcholinesterase (AChE), butyrylcholinesterase (BChE), tyrosinase, *α*-amylase, and *α*-glucosidase (Table 3). The inhibition of enzymes *α*-amylase and *α*-glucosidase, which are responsible for elevating blood glucose levels, plays a significant role in managing type 2 diabetes [33]. The *α*-amylase inhibitory activity of the stem, leaf, and fruit extracts of *D. pontica* was assessed and found to range between 2.27 and 2.82 mg/ml. The stem extract exhibited the highest activity with an IC_{50} value of 2.27 mg/ml, while the lowest activity was observed in the fruit extract (2.82 mg/ml). In contrast, the inhibitory effects of *D. pontica* extracts on *α*-glucosidase were more potent than those observed for *α*-amylase, with values ranging from 1.09 to 1.56 mg/ml. The stem extract again demonstrated the highest activity (1.09 mg/ml). The inhibition of tyrosinase, an enzyme involved in melanin production, indicates significant potential for treating various skin disorders [34]. The best tyrosinase activity was detected in the stem extract with an IC_{50} value of 2.26 mg/ml, while no activity was detected in the leaf extract.

Table 2. Antioxidant properties of the extracts from *Daphne pontica* L. (IC_{50} or EC_{50} , mg/ml)

Samples	DPPH	ABTS	CUPRAC	FRAP	Metal chelating	Phosphomolybdenum
Stem	1.13±0.03	0.54±0.01	0.67±0.01	0.77±0.01	2.57±0.03	0.96±0.04
Fruit	4.11±0.05	>5	1.83±0.02	2.60±0.12	1.31±0.04	2.43±0.29
Leaf	3.24±0.07	1.88±0.05	1.63±0.02	1.71±0.02	1.81±0.25	0.97±0.05
Trolox	0.06±0.01	0.11±0.01	0.14±0.01	0.06±0.01	-	0.57±0.04
EDTA	-	-	-	-	0.27±0.01	-

Values expressed are means ± S.D. of three parallel measurements.

Additionally, the inhibition of AChE and BChE, considered potential treatment approaches for neurological disorders, were evaluated [35]. The highest AChE inhibition activity was determined as an IC_{50} value of 1.06 mg/ml in fruit extract, while the highest BChE inhibition activity was recorded as 1.53 mg/ml in stem extract. These findings highlight the diverse enzyme inhibition capacities of *D. pontica* extracts, suggesting their potential applications in treating various health conditions.

Table 3. Enzyme inhibitory activities of the extracts from *Daphne pontica* L. (IC_{50} , mg/ml)

Samples	AChE	BChE	Tyrosinase	Amylase	Glucosidase
Stem	1.95±0.26	1.39±0.02	2.26±0.19	2.27±0.08	1.09±0.01
Fruit	1.06±0.06	1.53±0.17	2.92±0.16	2.82±0.05	1.56±0.10
Leaf	1.41±0.11	2.24±0.31	na	2.35±0.11	1.13±0.05
Galanthamine	0.003±0.0001	0.004±0.0001	-	-	-
Kojic acid	-	-	0.07±0.01	-	-
Acarbose	-	-	-	0.62±0.02	1.53±0.03

Values expressed are means ± S.D. of three parallel measurements.
na: inactive.

Figure 1 presents the correlation matrix illustrating the relationships between the total phenolic content (TPC), total flavonoid content (TFC), antioxidant activities (measured through ABTS, DPPH, CUPRAC, FRAP, metal chelating, and phosphomolybdenum assays), and enzyme inhibitory activities (including AChE, BChE, tyrosinase, *α*-amylase, and *α*-glucosidase inhibitory activities).

Cytotoxic activity was measured by MTT assay. The MTT results, according to IC_{50} values, the highest cytotoxic activity was found in fruits (0.467 mg/ml) in the A549 cell line, in fruit (0.937 mg/ml) in the HeLa cell line, and stem extracts (0.896 mg/ml) in the MCF-7 cell line (Table 4). The severity of cytotoxic activity for the A549 cell line was observed as fruit (0.467 mg/ml) > leaf (1.329 mg/ml) > stem (2.967 mg/ml), for the HeLa cell line, fruit (0.937 mg/ml) > stem (1.424 mg/ml) > leaf (1.674 mg/ml), for the MCF-7 cell line, stem (0.896 mg/ml) > leaf (1.243 mg/ml) > fruit (3.075 mg/ml). These results indicate selective cytotoxicity, showing that extracts obtained from different parts of *D. pontica* are effective to varying degrees on various cancer cell lines. The anticancer activities of daphnane-type diterpenes have been previously determined [36]. Studies on *Daphne* species have also revealed various biological effects in different cell lines. The methanol extract of *D. gnidioides* Jaub. & Spach stems exhibited remarkable cytotoxicity only in HeLa cells (IC_{50} = 86.16 µg/ml) and did not show notable activity against MDA-MB-231 cells [17]. Similarly, the aerial parts of *D. pontica* extracts did not exhibit significant cytotoxicity on breast cancer cell lines (MDA-MB-231, MCF-7, and T47D); however, the ethyl acetate fraction showed an observable cytotoxic effect (IC_{50} = 977.46 µg/ml) [18]. The dilignan lignopontin A, isolated from the stem of *D. pontica*, was observed to induce apoptosis in Du 145 cells and both apoptosis and necrotic cell death in LNCaP cells, in addition to increasing caspase-3 activity in prostate cancer cells [19]. The methanol extract of *D. pontica* roots also demonstrated promising anticancer activity on HeLa cells, with an IC_{50} value of 203.9 µg/ml [20]. In 2024, *D. pontica* was reported to exhibit the highest chymotrypsin inhibitory activity (87.75%) and trypsin inhibitory activity (99.93%), which are essential for various physiological processes like inflammation, immune system function, and the removal of proteins surrounding cancer cells [21]. Additionally, the ethanolic extract of the flowering aerial parts of *D. sericea* significantly reduced the proliferation of MDA-MB-231 cells by 85-90%, with sinensetin identified as the most effective component [22].

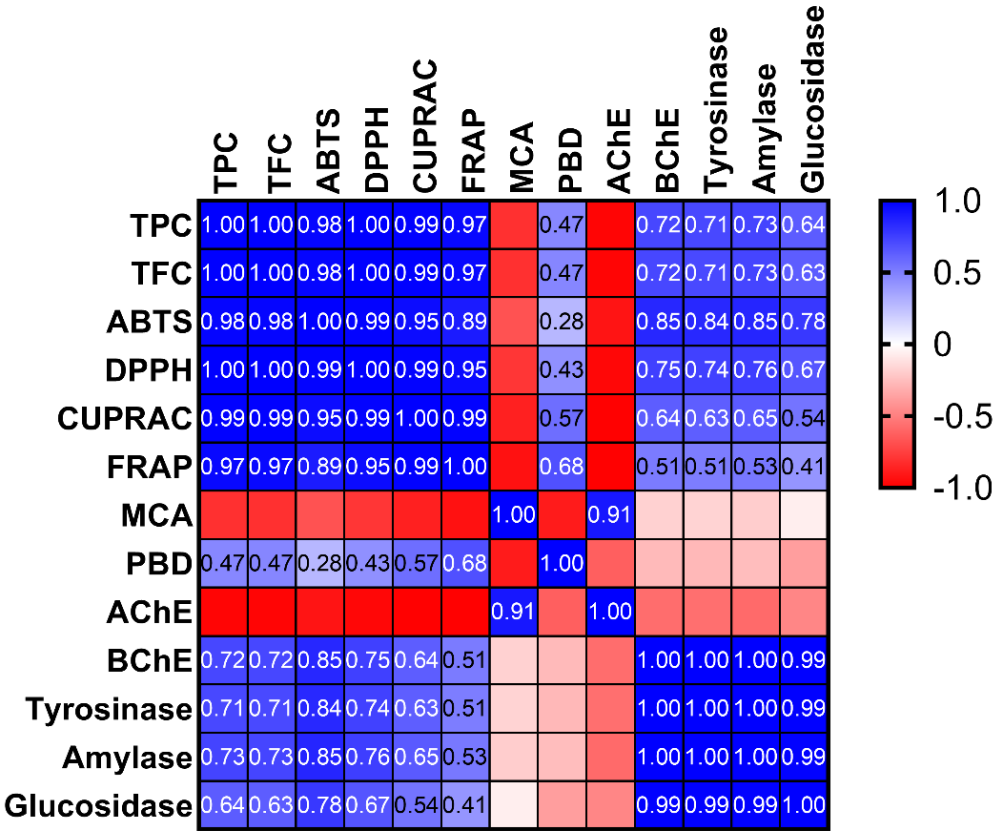

Figure 1. Correlation matrix. 0-0.20: Very weak relationship; 0.20-0.39: Weak relationship; 0.40-0.59: Medium relationship; 0.60-0.79: High relationship; 0.80-1.0: Very high relationship. TPC: Total phenolic content; TFC: Total flavonoid content; MCA: Metal chelating; PBD: Phosphomolybdenum.

Table 4. Cytotoxicity analyzes results of *Daphne pontica* L. extracts (IC_{50} , mg/ml)

Samples	A549	HeLa	MCF-7
Leaf	1.329	1.674	1.243
Stem	2.967	1.424	0.896
Fruit	0.467	0.937	3.075

In a study where the cytotoxic activities of enriched fractions obtained from $CHCl_3$ and methanol extracts of the aerial parts of *D. glomerata* Lam. and *D. pontica* species, along with the isolatedcompounds, were investigated, it was concluded that the activities of the fractions might be attributed to the minor compounds [23]. These findings suggest that *Daphne* species could be considered potential therapeutic agents in cancer research.

Research on various *Daphne* species has demonstrated that these plants exhibit different antioxidant properties due to their phenolic and flavonoid compound content. In a study analyzing methanol and chloroform extracts obtained from leaves and stems of *D. blagayana* Freyer, activities such as metal chelation, hydroxyl radical scavenging, and lipid peroxidation inhibition were evaluated, revealing a strong antioxidant potential in these extracts Among these extracts, the stem chloroform extract stood out with the highest phenolic (90.26 ± 0.69 mg GA/g) and flavonoid (35.24 ± 0.55 mg RU/g) content [24]. Similarly, due to their high phenolic content, *D. cneorum* L. leaf and stem methanolic extracts were identified as a natural source of antioxidants [25]. The antioxidant activities of *D. gnidioides* and *D. pontica* were assessed using phosphomolybdenum, DPPH, *β*-carotene/linoleic acid, ferric, and cupric ion reduction assays, and it was found that *D. pontica* leaves exhibited higher free radical scavenging activity with lower IC_{50} values, which was attributed to their high phenolic content [26]. Similarly, biflavonoids isolated from the roots of *D. giraldii* (dafnogirin A and B) demonstrated significant antioxidant activities in oxygen radical absorbance capacity assay (ORAC) [27]. The chloroform extract from *D. laureola* L. leaves displayed the highest DPPH

radical scavenging capacity, associated with its elevated phenolic and flavonoid content (IC_{50} 2,47 ± 0,001 mg/ml) [28]. Likewise, a strong correlation was observed between the methanolic extract of *D. mucronate* Royle leaves and its DPPH and ABTS radical scavenging capacity, aligning with its total phenolic and flavonoid content. This extract exhibited protective effects against liver and kidney toxicity induced by paracetamol, reducing elevated levels of liver enzymes (AST, ALT, ALP) and renal biomarkers (serum creatinine, uric acid, and urea) [29]. Additionally, several compounds (syringin, pinoresinol 4-O-*β*-D-glucopyranoside, daphnodorin B, lariciresinol 4,4′-bis-O-*β*-D-glucopyranoside, lariciresinol, and genkwanol A) isolated from *D. mucronata* were reported to possess potent antioxidant activities [30]. In a study investigating the *in vitro* anti-inflammatory, antioxidant, and antimicrobial activities of five different solvent fractions (*n*-hexane, dichloromethane, ethyl acetate, *n*-butanol, and water) from the methanol extract of *D. oleoides* Schreb. subsp. *oleoides* aerial parts, the dichloromethane and *n*-hexane extracts were found to inhibit NO (nitric oxide) production in LPS (Lipopolysaccharide)-stimulated Raw 264.7 cells to the greatest extent, with inhibition rates of 61.67% at 50 µg/ml and 67.91% at 100 µg/ml. The ethyl acetate fraction showed the highest antioxidant activity in both DPPH (IC_{50}: 0.35 ± 0.12 mg/ml) and FRAP (4.54 ± 0.01 mM FeSO4/mg extract) tests. Additionally, the dichloromethane fraction was notable for its high flavonoid content (0.837 ± 0.035 mg QUE/mg extract) and phenolic content (1.063 ± 0.011 mg GAE/mg extract). The study concluded that the *n*-hexane and dichloromethane fractions exhibited the most potent antimicrobial activities [31]. Mansoor et al. (2013) evaluated the antioxidant capacity of *D. retusa* Hemsl.'s methanolic extract through various tests, finding that its aqueous extract exhibited DPPH radical scavenging activity (90.89 ± 0.51 µg/ml) comparable to propyl gallate (93.01 ±0.27 µg/ml) [12]. Furthermore, the ethanolic extract from the flowering aerial parts of *D. sericea* Vahl. showed significantly higher activity (at least 40-fold) against the ABTS radical than the DPPH radical, with the antioxidant effect attributed to its polyphenolic components [22]. In addition to these effects, it has been reported that the 80% ethanol extract of *D. pontica* demonstrates more than 85% inhibitory activity against both chymotrypsin and trypsin enzymes, and HPLC analysis revealed a parallelism between the chymotrypsin and trypsin inhibition and the amount of umbelliferone [32]. These findings indicate that the richness in phenolic and flavonoid compounds contributes significantly to the *Daphne* species' antioxidant and protective potentials, suggesting that these plants could be valuable natural antioxidants. Our study corroborates previous findings and demonstrates significant results in the *α*-glucosidase and *α*-amylase inhibitory activity assays, revealing a high correlation with the plant's phenolic and flavonoid content. Moreover, a very high correlation was observed between the phenolic and flavonoid content and the antioxidant properties of the plant (Figure 1).

3. CONCLUSION

The genus *Daphne* L. from the Thymelaeaceae family has long been recognized in traditional medicine for its therapeutic properties and medicinal uses. Numerous studies have confirmed that the bioactive compounds are responsible for the biological and pharmacological effects of *Daphne* species. However, studies on *D. pontica* remain limited, and the biological potential of this species requires more comprehensive and further principle investigation. Our study provides significant insights into the remarkable pharmacological effects of this species. In our study, antioxidant and enzyme inhibition assays yielded high results, which were strongly correlated with the plant's phenolic and flavonoid content. Furthermore, the *α*-glucosidase and *α*-amylase inhibitory activity assays showed a strong correlation with the phenolic and flavonoid content of the plant, indicating a noteworthy antidiabetic potential. These findings suggest that *D. pontica* could be a promising herbal resource for managing metabolic disorders such as diabetes. Cytotoxic effects were evaluated using the MTT assay, and it was determined that different parts of the plant exhibited selective cytotoxicity against various cancer cell lines. This highlights the potential of *D. pontica* as a candidate for further investigation in anticancer research. The specific diterpenes, which are thought to play a significant role in the biological activities of this species, have been known recently. These special daphnane type diterpenes play a major role in this effect. Further phytochemical studies are essential to elucidate the chemical structures of these diterpenes and their mechanisms of action. Additionally, future research should focus on identifying which subgroups of phenolic and flavonoid compounds are more influential in *α*-glucosidase and *α*-amylase inhibitory activities as well as cytotoxic effects. The molecular elucidation of the mechanisms underlying the bioactivity of isolated compounds could provide valuable insights and contribute significantly to the development of pharmaceutical agents derived from this plant.

4. MATERIALS AND METHODS

The materials and methods section is available in the supplementary material.

Acknowledgements:

Author contributions: Concept – A.O.; Design – A.O.; Supervision – A.O.; Resource – A.O., E.A.; Materials – E.A.; Data Collection &/or Processing – A.O., E.A., G.Z., F.B.A.; Analysis &/or Interpretation - A.O., G.Z., F.B.A.; Literature Search – A.O., E.A., G.Z., F.B.A.; Writing – A.O., E.A., G.Z., F.B.A.; Critical Reviews – A.O., G.Z., F.B.A.

Conflict of interest statement: The authors declared no conflict of interest.

REFERENCES

[1] Sen T, Samanta SK. Medicinal plants, human health and biodiversity: a broad review. In: Mukherjee, J. (eds) Biotechnological Applications of Biodiversity (Vol.147). Springer, Berlin, Heidelberg. 2014, pp. 59-110. https://doi.org/10.1007/10_2014_273.

[2] Takshak S, Agrawal SB. Defense potential of secondary metabolites in medicinal plants under UV-B stress. J. Photochem Photobiol B: Biol. 2019; 193: 51-88. https://doi.org/10.1016/j.jphotobiol.2019.02.002.

[3] Hosen SMZ, Junaid M, Alam MS, Rubayed M, Dash R, Akter R, Sharmin T, Mouri NJ, Moni MA, Khatun M, Mostafa M. GreenMolBD: Nature derived bioactive molecules' database. Med Chem. 2022; 18(6): 724-733. https://doi.org/10.2174/1573406418666211129103458.

[4] Tosun A. Chemical constituents and biological activities of *Daphne* L. species. J Fac Pharm Ankara. 2006; 35(1): 43-68. https://doi.org/10.1501/Eczfak_0000000045.

[5] Marmouzi I, Bouchmaa N, Kharbach M, Ezzat SM, Merghany RM, Berkiks I, El Jemli M. *Thymelaea* genus: Ethnopharmacology, chemodiversity, and bioactivities. S Afr J Bot. 2021; 142: 175-192. http://dx.doi.org/10.1016/j.sajb.2021.06.014.

[6] Nie YW, Li Y, Luo L, Zhang CY, Fan W, Gu WY, Shi KR, Zhai XX, Zhu JY. Phytochemistry and pharmacological activities of the diterpenoids from the genus *Daphne*. Molecules. 2021; 26(21): 6598. https://doi.org/10.3390/molecules26216598.

[7] Moshiashvili G, Tabatadze N, Mshvildadze V. The genus *Daphne*: A review of its traditional uses, phytochemistry and pharmacology. Fitoterapia. 2020; 143: 104540. https://doi.org/10.1016/j.fitote.2020.104540.

[8] Tan K. *Daphne* L. In: Davis PH (Eds). Flora of Turkey and the East Aegean Islands (Vol. 7). Edinburgh University Press, Edinburgh, 1982, pp. 521-526.

[9] Sovrlić MM, Manojlović NT. Plants from the genus *Daphne*: A review of its traditional uses, phytochemistry, biological and pharmacological activity. Serb J Exp Clin Res. 2017; 18(1): 69- 80. http://dx.doi.org/10.1515/sjecr-2016-0024.

[10] Baytop T, Therapy with Plants in Turkey (Past and Present), second ed., Nobel Medical Book House, Istanbul, 1999.

[11] Yeşilada E, Sezik E, Honda G, Takaishi Y, Takeda Y, Tanaka T. Traditional medicine in Turkey IX. folk medicine in North-West Anatolia. J Ethnopharmacol. 1999; 64: 195-210. https://doi.org/10.1016/s0378-8741(98)00133-0.

[12] Mansoor F, Anis I, Ali S, Choudhary MI, Shah MR. New dimeric and trimeric coumarin glucosides from *Daphne retusa* Hemsl. Fitoterapia. 2013; 88: 19-24. https://doi.org/10.1016/j.fitote.2013.03.029.

[13] Süntar I, Akkol EK, Keles H, Yesilada E, Sarker SD, Arroo R, Baykal T. Efficacy of *Daphne oleoides* subsp. *kurdica* used for wound healing: identification of active compounds through bioassay-guided isolation technique. J Ethnopharmacol. 2012; 141(3): 1058-1070. https://doi.org/10.1016/j.jep.2012.04.001.

[14] Xu WC, Shen JG, Jiang JQ. Phytochemical and biological studies of the plants from the genus *Daphne*. Chem Biodivers. 2011; 8(7): 1215-1233. https://doi.org/10.1002/cbdv.201000117.

[15] Li F, Sun Q, Hong L, Li L, Wu Y, Xia M, Ikejima T, Peng Y, Song S. Daphnane-type diterpenes with inhibitory activities against human cancer cell lines from *Daphne genkwa*. Bioorg Med Chem Lett. 2013; 23(9): 2500 2504. https://doi.org/10.1016/j.bmcl.2013.03.025.

[16] Zhan ZJ, Fan CQ, Ding J, Yue JM. Novel diterpenoids with potent inhibitory activity against endothelium cell HMEC and cytotoxic activities from a well-known TCM plant *Daphne genkwa*. Bioorg Med Chem. 2005; 13(3): 645–655. https://doi.org/10.1016/j.bmc.2004.10.054.

[17] Can TH, Tüfekci EF, Çelik-Altunoğlu Y, Baloğlu MC, Llorent-Martínez EJ, Stefanucci A, Mollica A, Cichelli A, Zengin G. Chemical characterization, computational analysis and biological views on *Daphne gnidioides* Jaub. &

Spach extracts: Can a new raw material be provided for biopharmaceutical applications? Comput Biol Chem. 2020; 87: 107273. https://doi.org/10.1016/j.compbiolchem.2020.107273.

[18] Eskandari B, Safavi M, Sadati Lamardi SN, Vazirian M. Cytotoxic evaluation of *Daphne pontica* L. aerial part extracts on three cancerous cell lines by MTT assay. Trad Integr Med. 2019; 4(2): 58-63. https://doi.org/10.18502/tim.v4i2.1671.

[19] Nikahd M, Aghaei M, Ali Z, Sajjadi SE, Khan IA, Ghanadian M. Phytochemical analysis *of Daphne pontica* L. stems with their pro-apoptotic properties against DU-145 and LNCaP prostate cancer cells. DARU J Pharm Sci. 2022; 30(1): 85-101. https://doi.org/10.1007/s40199-022-00434-y.

[20] Çelik-Altunoğlu Y, Can TH, Tüfekçi EF, Altunoğlu BD, Baloğlu MC, Llorent-Martinez EJ, Zengin G. Comprehensive approaches on chemical composition and biological properties of *Daphne pontica* L. extracts. Plant Biosyst. 2022; 156(1): 116–129. https://doi.org/10.1080/11263504.2020.1837282.

[21] Günbatan T, Sucu M, Gökbulut A, Dilmaç E, Gürbüz I. Chymotrypsin and trypsin inhibitory activity of some medicinal plants collected from Rize (Türkiye). Chem Biodivers. 2024; 21(3): e202301879. https://doi.org/10.1002/cbdv.202301879.

[22] Frezza C, Venditti A, De Vita D, Sciubba F, Tomai P, Franceschin M, Di Cecco M, Ciaschetti G, Di Sotto A, Stringaro A, Colone M, Genitlli A, Serafini M, Bianco A. Phytochemical analysis and biological activities of the ethanolic extract of *Daphne sericea* Vahl flowering aerial parts collected in central Italy. Biomolecules. 2021; 11(3): 379. https://doi.org/10.3390/biom11030379.

[23] Moshiashvili G, Pichette A, Legault J, Mshvildadze V. Chemical composition and cytotoxic activity of *Daphne glomerate* and *D. pontica*. Chem Nat Compd. 2022; 58(2): 329-331. https://doi.org/10.1007/s10600-022-03670-5.

[24] Manojlovic N, Sovrlic M, Maskovic P, Vasiljevic P, Juskovic M. Phenolic and flavonoid content and antioxidant activity of *Daphne blagayana* growing in Serbia. Exp Appl Biomed Res. 2014; 15(1): 21-27. https://doi.org/10.2478/sjecr-2014-0003.

[25] Manojlovic N, Mašković P, Vasiljević P, Jelic R, Juskovic M, Sovrlić M, Mandić L, Radojković, M. HPLC Analysis, antimicrobial and antioxidant activities of *Daphne cneorum* L. Hem Ind. 2012; 66(5): 709-716. http://dx.doi.org/10.2298/HEMIND120114029M.

[26] Sanda MA, Zengin G, Aktümsek A, Çakmak YS. Evaluation of antioxidant potential of two *Daphne* species (*D. gnidioides* and *D. pontica*) from Turkey. Emirates J Food Agric. 2015; 27(6): 488-494. http://dx.doi.org/10.9755/ejfa.2015.04.030.

[27] Zhou GX, Jiang RW, Cheng Y, Ye WC, Shi JG, Gong NB, Lu Y. Daphnogirins A and B, two biflavones from *Daphne giraldii*. Chem Pharm Bull. 2007; 55(9): 1287-1290. https://doi.org/10.1248/cpb.55.1287.

[28] Juskovic M, Zabar-Popovic A, Matejic J, Mihajilov-Krstev T, Manojlovic N, Vasiljevic P. Phytochemical screening, antioxidants and antimicrobial potential of leaves of *Daphne laureola* L. Oxid Commun. 2017; 40(3): 1058-1069.

[29] Nazir N, Muhammad J, Ghaffar R, Nisar M, Zahoor M, Uddin F, Ullah R, Alotaibi A. Phytochemical profiling and antioxidant potential of *Daphne mucronata* Royle and action against paracetamol-induced hepatotoxicity and nephrotoxicity in rabbits. Saudi J Biol Sci. 2021; 28(9): 5290-5301. https://doi.org/10.1016/j.sjbs.2021.05.051.

[30] Bader A, Santoro V, Parisi V, Malafronte N, Al-Sheikh I, Cacciola A, Germano MP, D'Angelo, V. The anti-angiogenic effect of polyphenols from the roots of *Daphne mucronata* Royle subsp. *linearifolia* (Hart) Halda (Thymelaeaceae). Eur J Integr Med. 2022; 531: 02151. https://doi.org/10.1016/j.eujim.2022.102151.

[31] Balkan İA, Taşkın T, Doğan HT, Deniz İ, Akaydın G, Yesilada E. A comparative investigation on the *in vitro* anti-inflammatory, antioxidant and antimicrobial potentials of subextracts from the aerial parts of *Daphne oleoides* Schreb. subsp. *oleoides*. Ind Crops Prod. 2017; 95: 695-703. https://doi.org/10.1016/j.indcrop.2016.11.038.

[32] Ayaz-Seyhan S. Re-evaluating of the DPPH antioxidant assay. Batman University Journal of Life Sciences. 2019; 9(2): 125-135.

[33] Saeedi M, Hadjiakhondi A, Nabavi SM, Manayi A. Heterocyclic compounds: Effective *a*-amylase and *a*-glucosidase inhibitors. Curr Top Med Chem. 2017; 17(4): 428-440. https://doi.org/10.2174/1568026616666160824104655.

[34] Ullah S, Son S, Yun HY, Kim DH, Chun P, Moon HR. Tyrosinase inhibitors: a patent review (2011-2015). Expert Opin Ther Pat. 2016; 26(3): 347-62. https://doi.org/10.1517/13543776.2016.1146253.

[35] Das UN. Acetylcholinesterase and butyrylcholinesterase as possible markers of low-grade systemic inflammation. Med Sci Monit. 2007; 13(12): 214-221. http://dx.doi.org/10.1016/S1665-2681(19)30940-8.

[36] Hou ZL, Yao GD, Song SJ. Daphnane-type diterpenes from genus *Daphne* and their anti-tumor activity. Chin Herb Med. 2021; 13(2): 145-156. https://doi.org/10.1016/j.chmed.2020.09.006.