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Phytochemical content, in vitro antioxidants and antimicrobial activities of *Daphne gnidium* L. leaves

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ABSTRACT

Daphne gnidium is an endemic plant of Mediterranean basin, widely used for hair care and dermatological diseases in this area. This investigation aims to evaluate the phytochemical composition of *D. gnidum* leaves extracted by ultrasounds assisted extraction using aqueous and organic solvents (methanol, ethanol, chloroform and ethyl acetate) and to determine their antioxidant and antimicrobial activities. The ethanolic extract showed a higher concentration of total phenolic content (93.24 ± 0.07 mg GAE/g of dry extract), while the chloroform extract had a higher flavonoid content (60.94 ± 0.18 mg QE/g of dry extract) and condensed tannins (91.52 ± 2.23 mg CE/g of dry extract). Antioxidant potential was assessed by three methods (DPPH, ABTS and FRAP). Aqueous extract showed stronger antioxidant potential in DPPH test (IC_{50} = 37.99 ± 1.51 µg/mL). Methanolic extract was the most potent in ABTS assay (IC_{50} = 190 ± 4.86 µg/mL), while aqueous extract performed best in FRAP method (9.1 ± 0.08 mg AAE/g). The Pearson correlation test revealed a strong positive correlation between total flavonoid content, condensed tannins and antioxidant activity (DPPH, ABTS and FRAP assay), while a negative correlation was demonstrated between the DPPH, ABTS methods and total phenolic content. The antimicrobial activity was examined against four strains (*Staphylococcus aureus, Bacillus subtilis, Proteus mirabilis* and *Candida albicans*). At a concentration of 50 mg/mL, all tested extracts (aqueous, methanolic, ethanolic, chloroform and ethyl acetate) exhibited inhibitory effect against the four strains with inhibition diameters ranging from 8.67 ± 0.47 to 19.33 ± 0.94 mm, the latter achieved by the ethanolic extract against *Proteus mirabilis*. In conclusion, *D. gnidium* contains compounds with potential antioxidants and antimicrobial activities against certain harmful bacteria.

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Introduction

The genus Daphne comprises exceeding 70 species [1], among which *D. gnidium,* a Mediterranean species, widespread in the countries of this area such as, the North Africa, Southern Europe, and the Middle East [2]. It is commonly used in the traditional medicine of these regions as well as in pharmaceutical field for treating, diabetes [3], dermatological diseases [4], and bone fracture [5]. Applications in cosmetics, such as, hair care [6–8]. Also used for fishing [5,9], and as insecticide [10].

Various studies on the *D. gnidium* plant have confirmed that it contains diverse classes of compounds, including flavonoids, coumarins, terpenoids, tocopherol and phenolic compounds which have been extracted from diverse parts, using aqueous, organic, and essential oil extracts [11–16]. These compounds appear to be accountable for several biological activities such as anti-inflammatory, antioxidant, antibacterial, anti-corrosion antifungal, antiviral, insecticide and phytotoxic [11,17–24].

In Morocco, a few pharmacological studies were conducted involving the *D. gnidium* species in comparison between different countries of the Mediterranean Sea [25]. The current research aims to characterize the phytochemical composition of *D. gnidium* leaves extracted using ultrasound-assisted extraction and to examine their in vitro antioxidant and antimicrobial activities using both aqueous and organic extracts.

Materials and methods

Plant material

D. gnidium was harvested from the National Park of Tazekka locality in the North-eastern Morocco, during the flowering season (between May and October 2023). Taxonomic identification was carried out at Plant, Animal, Production and Agro-Industry Laboratory, Department of Biology, Faculty of Sciences at Ibn Tofail University Kenitra, Morocco. The voucher specimen has been deposited in the Herbarium with the assigned code 205. The collected samples were dried in the dark and preserved for future analysis.

Preparation of extracts

Leaf powder (1:10, *w/v*) was extracted by ultrasound-assisted extraction for 60 min at 40 °C using aqueous and organic solvents: methanol, ethanol, chloroform, and ethyl acetate. The plant residues were removed by filtration and then all organic extracts were concentrated by evaporator (Buchi Rotavapor R-210). Aqueous extract was centrifuged at 6120 rpm for 30 min, filtered and lyophilized. All the samples were stored at 4 °C.

Determination of phenolic contents

Total phenolic content (TPC) of *D. gnidium* leaves extract was measured according to the Folin-Ciocalteu protocol [26]. 100 µL of extract (prepared in methanol), 500 µL of the Folin-Ciocalteu (10 %) reagent and 400 mL (7.5 %, *w/v*) of sodium carbonate (Na_2CO_3) were combined. The solution obtained was stored in the dark, at room temperature (20–25 °C) for 60 min and then, the absorbance was determined at 765 nm by a UV–visible spectrophotometer (Specuvisi UV/VIS, No RE1701008). The concentration of TPC was estimated by the calibration curve found by gallic acid with different concentration, and the results were described in mg of gallic acid equivalent per g of extract (mg GAE/g).

Determination of total flavonoids (TF)

Total flavonoids (TF) in different extract was measured using Aluminum Trichloride ($AlCl_3$) colorimetric method [27]. 1 mL of extract, 0.3 mL of sodium solution nitrite ($NaNO_2$) solution (5 %, *w/v*), and 4 mL of distilled water were introduced in the test tube. 0.3 mL of $AlCl_3$ (10 %, *w/v*) was added after 5 min, and then 2 mL of sodium hydroxide NaOH (1 M) was added after 6 min. The result solution was mixed and stored for 30 min in the dark. The absorbance was carried out at 510 nm. Quantification of flavonoid content was performed using quercetin standard curve with result given in mg quercetin equivalent per gram of extract (mg QE/g).

Determination of total tannins condensed (TTC)

The tannins condensed were evaluated using vanillin method [28]. 50 µL of each sample and 1.5 mL of vanillin prepared in methanol (4 %, *w/v*) were mixed. A volume of 750 µL of HCl (hydrochloric acid) was introduced to the solution and kept reacting 20 min at room temperature (20–25 °C). Absorbance reading was taken at 500 nm, and the result given in mg catechin equivalent per g extract.

Determination of antioxidants activity

DPPH assay

The ability of *D. gniudm* leaves extracts on the DPPH test was evaluated as described by Sharma and Bhat [29]. 3 mL of different concentration (6.25–100 µg/mL prepared in methanol) of extracts were mixed with 1 mL of the DPPH solution (0.2 mM). The sample was incubated in the dark at 30 °C for 30 min. Absorbance reading was carried out at 517 nm by a spectrophotometer (SPECUVIS1,

UV/VIS, No RE1701008) verified with controls. Ascorbic acid was employed as standard antioxidant. The percentage of inhibition was determined following this equation

$$\% \text{ Scavenging} = \left[\left(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}\right)/\text{Abs}_{\text{control}}\right] \times 100$$

ABTS assay

Radical scavenging activity against ABTS+ of *D. gnidium* leaves extracts was performed according to the protocol of Benali and colleagues [30]. A 7 mM ABTS+ solution containing potassium persulfate (2.45 mM) was prepared and stored in the dark at room temperature (20–25 °C) for 16 h. The ABTS+ solution obtained was diluted by methanol to get a range of absorbance (0.75 to 0.8, at 734 nm). Then, 75 µL of extract (prepared in methanol) at varying concentrations (15.62 to 500 µg/mL) were combined with 925 µL of ABTS solution. The solution was incubated in the dark for 10 min at room temperature (20–25 °C), and the absorbance was taken at 734 nm using a spectrophotometer (SPECUVIS1, UV/VIS, No RE1701008).

The percentage inhibition was calculated following this equation:

$$\% \text{ Scavenging} = \left[\left(\text{ Abs}_{\text{control}} - \text{ Abs}_{\text{sample}}\right)/\text{Abs}_{\text{control}}\right] \times 100$$

The result was expressed in terms of IC_{50} which means the concentration required for 50 % radical inhibition.

FRAP assay

Reducing power was performed according to Benali and coworkers [31]. The solution was prepared by 1 mL of extract, 2.5 mL of (0.2 M, pH 6.6) phosphate buffer and 2.5 mL of potassium ferricyanide, and then incubation for 20 min at 50 °C (in water bath). 2.5 mL of (10 %) trichloroacetic acid was joined to the solution and centrifuged at 3000 rpm for 10 min after which 2.5 mL of the supernatant was combined with distilled water (2.5 mL) and 0.5 mL of 0.1 % ferric chloride. Absorbance was recorded at 700 nm. Ascorbic acid at concentrations (50 to 450 µg/mL) was used as a standard curve and the results were presented as milligram equivalence per gram of extracts (mg AAE/g of extract).

Antimicrobial activity

Microorganismes strains and growth conditions

The antibacterial activity of *D. gnidium* leaves extracts was performed against the following bacteria *Staphylococcus aureus* CECT 976, *Bacillus subtilis* DSM 6633, and *Proteus mirabilis*. Antifungal activity was performed against *Candida albicans* ATCC 10,231. Bacterial strains were cultured in Mueller-Hinton Agar or Broth (MHA or MHB) at 37 °C for 24 h, while fungi culture was maintained in YPGA (Yeast Extract-Peptone-Glucose-Agar) medium or YPG (Yeast Extract-Peptone-Glucose) broth and incubated at 30 °C for 48 h. Inoculum concentrations were standardized at 10^6 CFU/mL and 10^5 spores/mL for bacteria and fungi respectively [32].

Agar disc diffusion method

The antimicrobial activity of *D. gnidium* leaves extracts was performed by agar disk diffusion method according to Benali [32] and Rota [33]. Sterile disc (6 mm diameter) filled by 20 µL of each extract (50 mg/mL) dissolved by dimethylsulfoxide (DMSO) (10 %) were implemented to the agar medium and seeded by inoculum concentration test. Following incubation, diameter zones of inhibitory were recorded. Colistin CT (50 µg) and Amikacin AK (30 µg) were positive control, while 10 % of DMSO was negative control.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC was assessed using a sterile 96-well microplate assay [34]. In each plate, 100 µL of MHB was dispensed into each test wells, excluding the first column of microplates which received 200 µL of 50 mg/mL extract in 10 % DMSO. From the first to the ninth well, serial concentrations (0.195 to 50 mg/mL) were prepared by transferring 100 µL into subsequent wells with 10th well serving as a sterility control. From each well, 10 µL of the suspension was taken and substituted by the inoculum test concentration. The 11th well served as the positive control with MHB only, while the last well was the negative control contain (10 % DMSO, *v/v*) only. Then, all the plates were incubated at the same conditions as described above. 25 µL of MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) served as an indicator of microbial growth to each well and re-incubated for 30 min at 37 °C and 28 °C for bacterial and fungal strains respectively. The solution in the wells with absence of color change following MTT addition revealed no microorganism's growth. Minimum bactericidal/fungicidal concentration was carried out by transplanting 10 µL of broth derived from uncolored wells in MHA (bacteria) and LPGA (fungi), poured into petri dishes and incubated for 24 h at 37 °C and 72 h at 28 °C for bacteria and fungi respectively. The MBC was noted as having the lowest concentration that illustrated no bacterial growth after incubation.

Statistical analysis

All the tests in this study were done at least 3 times. Statistical analysis was carried out by IBM SPSS Statistics 23. Results are expressed as mean ± SD. Comparison between groups was carried out by one-way ANOVA test, followed by Tukey post hoc. Statistical significance was set at $p < 0.05$. The Pearson correlation test was performed to determine the relationship between phenolic, flavonoids contents, condensed tannins and antioxidant power.

Results and discussion

Phytochemical composition

Total phenolic, flavonoids contents and condensed tannins of *D. gnidium* leaves extracts were quantified, and the outcomes are summarized in Table 1. Ethanolic extract had the highest amount of phenolics compounds (93.24 ± 0.07 mg GAE/g of extract), while aqueous extract had lowest quantities (25.37 ± 0.14 mg GAE/g of extract). This result slightly exceeds those obtained by Bouyahya and teams using the ethanolic leaves extract (82.46 ± 1.12 mg GAE/g of extract) [35], and those noted by Chaves and collaborators using the methanolic leaves extract (0.014 mg GAE/mg extract) [17], and the result mentioned by Chelef and collaborators who obtained 23.02 ± 2.02 mg GAE/g extract by the methanolic extract [36].

Chloroform extract had the highest concentration of flavonoids and condensed tannins (60.94 ± 0.18 mg QE/g of extract and 91.52 ± 2.23 mg CE/g of extract) respectively. While the lowest quantities of flavonoids (7.44 ± 0.02 mg QE/g of extract) and condensed tannins (4.42 ± 0.00 mg CE/g of extract) were observed in the aqueous extract. Our results exceed other studies in literature. Chelef reported the quantities of flavonoids in order of 8.64 ± 0.87 and 5.94 ± 0.19 mg QE/g of extract on methanolic and aqueous extract respectively [36]. Similarly, Bouyahya found a concentration of 17.68 ± 1.23 mg QE/g of extract in the ethanolic leaves extract [35]. Regarding the condensed tannins, our findings are in agreement with the result of Chelef, who found the values of 6.37 ± 0.26 and 4.14 ± 0.17 mg EC/g of extract in methanolic and aqueous extracts, respectively [36]. In contrast, Chaabane and coworkers found no trace of condensed tannins in the chloroform extract of *D. gnidium* leaves [15]. This differentiation could be due to the difference in the extraction methods used in each study. The ultrasound-assisted extraction could facilitate the release of intracellular compounds, such as phenolic compounds and flavonoids, promoting their dissolution by the solvent [37,38]. Our result confirms that the ultrasound assisted extraction could be the effective method to extracting the phenolic compounds in *D. gnidium* plant.

Antioxidant activity

Different methods exist for antioxidant measurements, at least two methods were recommended. In our study three methods were used (DPPH, ABTS and FRAP), and acid ascorbic was used as control. The results obtained are mentioned on Table 2.

On DPPH test, aqueous extract has a high anti-free radical scavenging activity (IC_{50} = 37.99 ± 1.51 µg/mL). No difference statistically significant obtained by methanolic and ethanolic extracts with IC_{50} = 49.82 ± 0.92 and 51.55 ± 1.38 µg/mL respectively. Ethly acetate and chloroform extracts exhibited a lowest scavenging activity with IC_{50} = 68.64 ± 3.09 and 89.03 ± 0.71 µg/mL respectively. Our findings were on accord with the study of Chaabane and its teams who obtained an IC_{50} = 45 µg/mL by the methanolic extract of *D. gnidium* leaves [39], and the study by Chaves who showed an activity of 0.011 ± 0.0009 QE mg/mg dry weight performed by the methanolic extract of *D. gnidium* leaves [17]. In addition, our findings were less potent in comparison with other studies involving the *Daphne* genus. The air dried of Serbian *Daphne cneorum* showed an important antioxidant potential against the DPPH radicals (IC_{50} = 22.79 ± 0.94 µg/mL) [40], thus the chloroform and methanol extract of *Daphne alpina* leaves mentioned an IC_{50} values of 25.45 ± 0.89 and 23.15 ± 1.05 µg/mL respectively [41].

On the ABTS assay, methanolic extract showed a potent radical scavenging activity (IC_{50} = 190.77 ± 4.86 µg/mL), pursued by ethanolic extract (IC_{50} = 230.48 ± 25.93 µg/mL), ethyl acetate extract (IC_{50} = 230.32 ± 26.28 µg/mL) and aqueous extract (IC_{50} =291.31 ± 18.95 µg/mL), with no difference statistically significant. Chloroform extract shows the lowest activity with IC_{50} = 515.81 ± 54.16 µg/mL. According to Tukey test, all extracts tested are significantly less effective on both DPPH and ABTS methods than the standard antioxidant used (Ascorbic Acid). This result corroborate existing literature, Chaves reported an activity of methanolic extract of 0.003 ± 0.0006 QE mg/mg of extract [17]. Our findings were most potent in comparison with the results performed by *Daphne oleoides* from Turkey with an IC_{50} = 1.39 ± 0.21 and 0.43 ± 0.06 mg/mL obtained by methanolic extract and ethyl acetate sub-extract respectively [42], likewise, *Daphne jejudoensis* leaves shows lower anti-ABTS radical with IC_{50} = 1.21 ± 0.12 mg/mL [43].

On the FRAP test, the aqueous extract presents a capacity to reduce Fe^{3+} (ferric ions) to Fe^{2+}(ferrous ions) in order of 9.1 ± 0.08 mg AAE/g of extract, followed by ethyl acetate extract (8.4 ± 0.06 mg AEE/g), methanolic extract (7.3 ± 0.05 mg AAE/g), ethanolic extract (6.3 ± 0.03 mg AEE/g) and chloroform extract shows the lowest reducing power (5.8 ± 0.06 mg AEE/g). Also, the study of Chaves reported that the methanolic extract exhibited anti-FRAP activity with value of 0.014 ± 0.0002 FeSO4E mg/mg of extract, which seems to be a stronger result compared to our result (7.3 ± 0.05 mg AAE/g extract) [17]. The differences in findings among the methods and extracts are due to variations in their antioxidant mechanisms and bioactive compounds. Each method targets a specific

Table 1
Phenolic, flavonoids content and condensed tannins of *D. gnidium* leaves extracts.

Extract	TPC (mg GAE/g extract)	TF (mg QE/g extract)	CTC (mg CE/g extract)
AQ	25.37 ± 0.14	7.44 ± 0.02	4.42 ± 0.00
ME	67.93 ± 0.07	43.07 ± 0.41	9.02 ± 0.18
ET	93.24 ± 0.07	51.31 ± 0.04	22.71± 0.27
EA	39.54 ± 3.39	36.76 ± 0.18	47.44 ± 0.93
CH	35.79 ± 0.59	60.94 ± 0.18	91.52 ± 2.23

TPC: Total Phenolic Content; **TF:** Total Flavonoids; **CTC**: Condensed Tannins Content; **AQ**: Aqueous; **ME**: Methanol; **ET**: Ethanol; **EA**: Ethyl Acetate; **CH**: Chloroform; **QE:** Quercetin Equivalent; **CE:** Catechin Equivalent; **GAE:** Gallic Acid Equivalent.

Table 2
Antioxidant potential of *D. gnidium* leaves aqueous and organic extracts.

Extract	DPPH IC_{50} (µg/mL)	ABTS IC_{50} (µg/mL)	FRAP (mg/ AAE/g)
AQ	37.99 ± 1.51^{a}	291.31 ± 18.95^{a}	9.1 ± 0.08^{a}
ME	49.82 ± 0.92^{b}	190 ± 4.86^{b}	7.3 ± 0.05^{b}
ET	51.55 ± 1.38^{b}	230.48 ± 25.93^{a}	6.3 ± 0.03^{c}
EA	68.64 ± 3.09^{c}	230.32 ± 26.28^{a}	8.4 ± 0.06^{d}
CH	89.03 ± 0.71^{d}	515.81 ± 54.16^{c}	5.8 ± 0.06^{e}
AA	3.12 ± 0.67^{e}	33.06 ± 0.12^{d}	–

Values are expressed as means ± standard deviation (SD) from three separate measurements. Means sharing the same letter in a column do not differ significantly. **mg AAE/g**: mg of Ascorbic Acid equivalent per g. **IC_{50}**: concentration needed to decrease DPPH and ABTS radical by 50 %. **AA**: Ascorbic Acid.

type of free radical, either through electron transfer, as in the FRAP test or, through electron or hydrogen atom transfer, as in the DPPH and ABTS tests. The bioactive compounds in the extracts vary depending on the extraction technique and the polarity of the solvent used. These compounds can neutralize free radicals either individually or through interactions with other compounds. In the study by Allal and his team, carvacrol was reported as the most abundant compounds in the hydrosol extract of *D. gnidium* leaves, with a percentage of 10.9 % [14]. Various investigations have confirmed the antioxidant potential of this compound [44–46].

Correlation matrix

The relationship between phytochemical composition, Total Phenols content (TPC), Total Flavonoids (TF), and Total of Condensed Tannins (TTC) from *D. gnidium* leaves extracts and antioxidant power quantified by three tests (DPPH, ABTS and FRAP) was evaluated by Pearson correlation test. The results obtained are presented in Table 3.

No significant correlation between TPC and TF ($r^2 = 0.478$), and between TPC and TTC ($r^2 = - 0.300$). Based on this statistical analysis, the flavonoids and the condensed tannins were not the dominant compounds in total phenolics, extracted from *D. gnidium* plant. A significant positive correlation detected between TF and TTC ($r^2 = 0.66$, $p < 0.01$). This result would suggest that the flavonoids content in *D. gnidium* leaves mainly consisted of tannins.

Data of antioxidant activity performed by ABTS method were correlated significantly ($p < 0.01$) with DPPH and FRAP method with ($r^2 = 0.875$ and 0.789 respectively). A positive correlation was obtained between the FRAP test and DPPH ($r^2 = 0.629$) ($p < 0.05$). This strong correlation implied that the bioactive compounds are ensuring the scavenging activity against DPPH and ABTS radicals, were also contributed to the reduction of iron as measured by FRAP assay.

TPC showed a negative correlation with DPPH test ($r^2 = - 0.242$) and ABTS ($r^2 = - 0.007$) indicating that phenolics compounds of *D. gnidium* do not contribute to the DPPH and ABTS activity. These results were in line with different studies revealed that no relationship between the phenolic compounds and antioxidant power [47,48]. While a positive correlation obtained by TPC with FRAP ($r^2 = 0.366$), suggesting that TPC contribute slightly in FRAP assay. However, a significant positive correlation was shown between flavonoids content with DPPH ($r^2 = 0.714$, $p < 0.01$), ABTS ($r^2 = 0.844$, $p < 0.01$) and FRAP ($r^2 = 0.885$, $p < 0.01$). Also, the same correlation was obtained between condensed tannins and DPPH ($r^2 = 0.981$, $p < 0.01$), ABTS ($r^2 = 0.833$, $p < 0.01$) and FRAP ($r^2 = 0.647$, $p < 0.01$). This correlation indicates that flavonoid and tannins of *D. gnidium* majorly contribute to the antioxidant activity quantified by DPPH, ABTS and FRAP assays. Besides, the total flavonoids from *Daphne genkwa* demonstrated a high antioxidant activity against DPPH radicals [49].

Principal component analysis (PCA)

Principal component analysis was performed on the variables of antioxidant activities carried out by DPPH, ABTS and FRAP assays, and the total of phenolic and flavonoids content and condensed tannins of organic and aqueous extracts of *D. gnidium* leaves. Principal axis (PC1) illustrated 69.60 % of the variance, while the principal axis PC2 illustrated 25.52 % of the variance, both axes explaining

Table 3
Correlation matrix between phenolic content, flavonoids content, condensed tannins, and activity antioxidant by three tests (DPPH, ABTS and FRAP assays).

Variables	TPC	TF	TTC	DPPH	ABTS	FRAP
TPC	1					
TF	0.478	1				
TTC	- 0.300	**0.661****	1			
DPPH	−0.242	**0.714****	**0.981****	1		
ABTS	- 0.007	**0.844****	**0.833****	**0.875****	1	
FRAP	0.366	**0.885****	**0.647****	**0.629***	**0.789****	1

TPC: Total Phenolic Content; **TF**: Total Flavonoids; **TTC:** Total Condensed Tannins. **DPPH**: Diphenyl-2-picrylhydrazyl; **ABTS**: Azino bis(3-ethylbenzothiazoline-6-sulfonate); **FRAP**: Ferric ions (Fe3+) Reducing Antioxidant Power; *significant correlation with $p < 0.05$; *significant correlation with $p < 0.01$.

95.12 % of the whole variance of data, with dominate of PC1.

The factor point graph presented in Fig. 1 show that the organic and aqueous extracts of *D. gnidium* leaves (individuals) are partitioned into three groups established on the antioxidant power and the total phenolic and flavonoids content and condensed tannins.

Group1: formed by two extracts ethanolic and methanolic, FRAP assay and total phenolic and flavonoids content.

Group 2: second group contains the chloroform extract, ABTS method and condensed tannins.

Group 3: third group is constituted by two extracts (aqueous and ethyl acetate).

Antimicrobial activity

The antimicrobial activity of *D. gnidium* leaves (aqueous and organic extracts) was qualitatively and quantitatively assessed in vitro against two strains of Gram (+) bacteria (*B. subtilis* and *S. aureus*), one strain of Gram (-) bacteria (*P. mirabilis*), and one fungi *C. albicans*. The results are summarized in Table 4.

The antimicrobial activity of *D. gnidium* was classified into three categories [50]: weak inhibition activity ($\phi \leq 12$ mm), moderate activity (12 mm < $\phi \leq 20$ mm), and strong activity ($\phi \geq 20$ mm).

The qualitative assay indicates that all extracts from *D. gnidium* exhibited an antimicrobial activity against the strains tested. The AK at 30 µg showed strong antimicrobial activity against all strains tested and all extracts tested are significantly less effective than him. Ethanolic and methanolic extracts exhibited moderate activity against *P. mirabilis* at 50 mg/mL, with inhibited diameter zone equal to 19.33 ± 0.94 and 15.67 ± 0.82 mm respectively, both extracts had no difference significantly in comparison with the positive control CT (18 ± 0.81 mm at 30 µg).

Moderate activity showed by aqueous and methanolic extracts of *D. gnidum* leaves with a diameter inhibition equal to 13 ± 0.82 mm against both Gram (+) bacteria (*B. subtilis* and *S. aureus*). These findings are in line with the study conducted by Bouyahya who reported that the ethanolic extract of *D. gnidium* leaves had an inhibition zone of 15 mm against *S. aureus* CECT 976 at a dose of 25 mg/mL [35]. Also, the investigation by Allal showed similar inhibition zone of 14 ± 0.01 mm against *B. subtilis*, obtained by the hydrosol extract of *D. gnidium* leaves and stems, while the essential oil was most potent against *B. subtilis* with an inhibition diameter of 28 ± 0.00 mm [14].

The chloroform extract presents high activity against *C. albicans* in comparison between the extracts tested, with a diameter inhibition of 15.33 ± 0.47 mm, followed by ethanolic extract (14.33 ± 0.47 mm), and the lowest inhibition zone diameter was obtained by aqueous extract (8.67 ± 0.47 mm). Our results exceed those obtained by Hajji who tested the aqueous and methanolic extracts of *D. gnidium* leaves against *C. albicans* AL62 and AL73 at a dose of 64.7 mg/mL, no antifungal activity was mentioned by Hajji [51].

Quantitative analysis was presented by the MIC and MBC values as summarized in the Table 5. The outcomes indicated that the methanolic extract of *D. gnidium* leaves presented the lowest MIC value, 6.25 mg/mL against the *B. subtilis* and 12.5 mg/mL against *P. mirabilis, S. aureus* and *C. albicans.* The aqueous extract showed a MICs value of 12.5 mg/mL against *B. subtilis* and *P. mirabilis,* and 25 mg/mL against *S. aureus* and *C. albicans.* MICs values of the ethyl acetate and chloroform extracts were in order of 25 mg/mL against *B. subtilis, P. mirabilis* and *S. aureus*, and 50 mg/mL against *C. albicans.* Finally, the ethanolic extract manifested a MICs value of 12.5

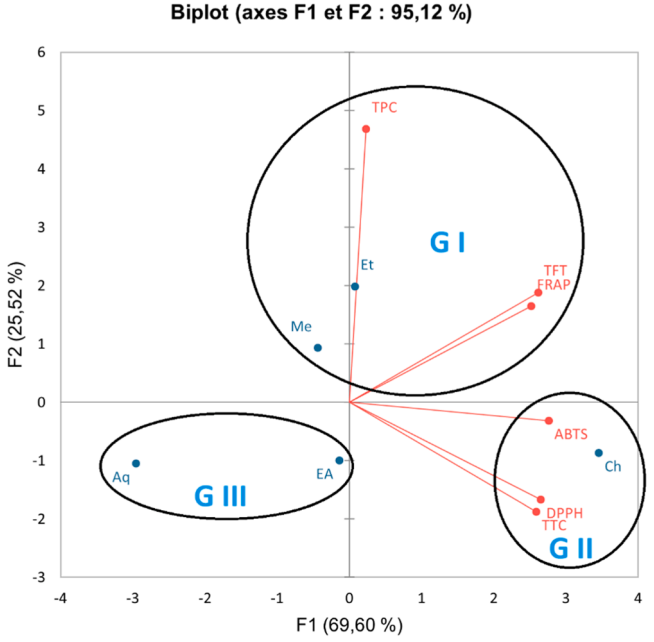

Fig. 1. Plot of variables and individuals projected on the factorial plan (F1 × F2). Aq: Aqueous; EA: Ethyl Acetate; Ch: Chloroform; Et: Ethanol; Me: Methanol; TPC: Total Phenolic Content; TF: Total Flavonoids; TTC: Total Tannins Condensed.

Table 4
Diameter of inhibition (mm) in the antimicrobial action of the extracts from *D. gnidium* leaves.

Extracts	Inhibition zone diameter (mm)*			
	B. subtilis DSM 6633	*P. mirabilis*	*S. aureus* CECT 976	*C. albicans* ATCC 10,231
Aqueous	13 ± 0.82^{a}	13 ± 0.82^{a}	11 ± 0.82^{a}	8.67 ± 0.47^{a}
Ethyl acetate	8.67 ± 0.94^{b}	10.33 ± 0.47^{b}	11 ± 0.82^{b}	10.67 ± 0.47^{b}
Methanolic	11.33 ± 0.94^{c}	15.67 ± 0.82^{c}	13 ± 0.82^{c}	11.67 ± 0.47^{c}
Chloroform	10.67 ± 0.94^{d}	10.33 ± 0.47^{d}	9.67 ± 0.47^{d}	15.33 ± 0.47^{d}
Ethanolic	12.67 ± 0.94^{e}	19.33 ± 0.94^{c}	12.33 ± 0.47^{e}	14.33 ± 0.47^{e}
CT (50 µg)	21.66 ± 1.24^{f}	18 ± 0.81^{c}	13.33 ± 1.24^{f}	16 ± 0.81^{f}
AK (30 µg)	30.33 ± 1.24 g	27.33 ± 2.05^{e}	31.66 ± 2.05 g	28.66 ± 0.94 g

*The diameter of inhibitory zones (mm), which includes the disc's diameter (6 mm), is provided as the mean ± standard deviation of triple tests. Values within a column sharing the same letter are not significantly different at $p < 0.05$.

Table 5
MIC and MBC values in (mg/mL) of aqueous and organic extract of *D. gnidium* leaves.

Extracts	Inhibition parameters	Bactria strains			
		B. Subtilis DSM 6633	*P. Mirabilis*	*S. aureus* CECT 976	*C. albicans* ATCC 1023
Aqueous	MIC	12.5	12.5	25	25
	MBC	50	50	50	50
	MBC/MIC	4	4	2	2
Ethyl acetate	MIC	25	25	25	12.5
	MBC	>50	>50	>50	>50
	MBC/MIC	–	–	–	–
Methanolic	MIC	6.25	12.5	12.5	12.5
	MBC	50	50	50	50
	MBC/MIC	8	4	4	4
Chloroform	MIC	25	25	25	12.5
	MBC	>50	>50	>50	>50
	MBC/MIC	–	–	–	–
Ethanolic	MIC	25	25	12.5	12.5
	MBC	50	50	>50	>50
	MBC/MIC	2	2	–	–

MBC: Minimum Bactericidal Concentration (mg/mL); **MIC**: Minimum Inhibitory Concentration (mg/mL).
-: when the MBC was >50 mg/mL, the ratio MBC/MIC was not calculated.

mg/mL against *S. aureus* and *C. albicans* and 25 mg/mL against *B. subtilis* and *P. mirabilis*.

Our results were in line with those obtained by the methanolic extract (MIC = 12.5 mg/mL) and chloroform extract (MIC = 50 mg/mL) of *Daphne laureola* leaves harvested from Serbia, tested against *S. aureus* ATCC 6538 [52]. On the other hand, our findings did not align with existing literature. The study by Dessi and teams tested methanolic *D. gnidium* leaf extracts from Sardinia against *C. albicans* CDC and *S. aureus* ATCC25923, finding MIC values greater than 500 µg/mL for *C. albicans* CDC and 500 µg/mL for *S. aureus* ATCC25923 [53]. Comparison between the MICs of our study with those of *Daphne alpina* and *Daphne cneorum* from Serbia showed a considerable difference. *Daphne alpina* methanolic and chloroform extract tested against *S. aureus* ATCC25923, *B. subtilis* ATCC6633, *P. mirabilis* ATCC14153, and *C. albicans* ATCC10231 revealed the MICs values of 31.25 µg/mL by both extracts against *S. aureus* ATCC25923; and 62.5 µg/mL against *P. mirabilis* ATCC14153. For *B. subtilis* ATCC6633 and *C. albicans* ATCC10231 the chloroform extract showed a MICs values of 15.62 and 31.25 µg/mL respectively, and the methanolic extract showed 125 µg/mL against both strains [41]. Similarly, *D. cneorum* methanolic leaf extract showed MIC values of 62.5 µg/mL *against S. aureus* ATCC25923 and *P. mirabilis* ATCC14153, and 31.25 µg/mL against *B. subtilis* ATCC6633 and *C. albicans* ATCC10231 [40].

The ratio of MBC/MIC can indicate the bactericidal or bacteriostatic effect of the extract (Table 5), if the MBC/MIC ratio ≤ 4 indicates that the extract has bactericidal effect. Conversely, a MBC/MIC > 4 suggests a biostatic effect [54]. Our study, the aqueous extract is bactericidal against *B. Subtilis* and *P. Mirabilis* (MBC/MIC = 4 mg/mL), *S. aureus* and *C. albicans* (MBC/MIC = 2 mg/mL). The methanolic extract is bactericidal against *P. mirabilis, S. aureus* and *C. albicans* (MBC/MIC = 4 mg/mL) and bacteriostatic against *B. subtilis* (MBC/MIC = 8 mg/mL). The ethanolic extract shows a bactericidal effect on *B. subtilis* and *P. mirabilis* (MBC/MIC = 2 mg/mL).

Our study showed that the Gram-negative bacteria was more susceptible to the *D. gnidium* extract than the Gram-positive strains. It is known that the Gram-negative bacteria possess a double membrane with a hydrophilic outer layer, which suggests that the antibacterial activity may be related to hydrophobic compounds. On the other hand, the resistance of Gram-positive bacteria may be attributed to their heterogeneous cell wall structure [50]. The variation of antimicrobial activity among the different extracts could be due to differences in their phytochemical composition, such as the presence of daphnetin. This compound is recognized as an essential constituent of the *Daphne* genus [2,25]. Indeed, numerous studies have been found the daphnetin in *D. gnidium* plant [11–13], and confirm its significant antibacterial activity [11,55].

Conclusion

This study has displayed that the extract of *D. gnidium* leaves possessed antioxidant and antimicrobial activities. The phytochemicals analysis revealed that this species contains a high amount of phenol, flavonoids content and condensed tannins.

The aqueous extract exhibited the greatest potency on the DPPH and FRAP assays, while the methanolic extract has potential on the ABTS method. Pearson correlation indicates a significant and positive correlation between antioxidant activity and the flavonoids and condensed tannins. The antimicrobial activity results indicated a potential inhibitory of the aqueous extract against *B. subtilis*; the ethanolic extract against *P. mirabilis*; the methanolic extract against *S. aureus* and the chloroform extract against *C. albicans*. These findings suggest that the *D. gnidium* may be of interest in the development of natural and safe cosmetic products due to its antioxidant capacity and to its potential to inhibit certain pathogenic bacteria. Consequently, it could enhance its therapeutic applications in traditional medicine. Further studies are needed to understand the mechanisms of action, and in vivo investigations would be valuable to fully explore the medicinal properties of the *D. gnidium* species.

CRediT authorship contribution statement

El mouzazi Issam: Writing - original draft, data curation and investigation. **Mustapha Laghmari**: Data curation. **Oumayma Iraqi:** Conceptualization. **Issam Ghabbour**: Resources. **Taoufiq Benali**: Visualization. **Khalil Hammani**: Methodology and project administration. **Abdellatif Bour**: Supervision and validation. **Soad Khal-Layoun**: Supervision.

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Declaration of competing interest

I have nothing to declare.

References

[1] M. Van der Bank, M.F. Fay, M.W. Chase, Molecular phylogenetics of thymelaeaceae with particular reference to African and Australian genera, Taxon 51 (2002) 329, https://doi.org/10.2307/1554930.
[2] A. Khouchlaa, N. El Menyiy, F.E. Guaouguaou, A. El Baaboua, S. Charfi, F. Lakhdar, N. El Omari, D. Taha, M.A. Shariati, M. Rebezov, M. El-Shazly, A. Bouyahya, Ethnomedicinal use, phytochemistry, pharmacology, and toxicology of Daphne gnidium: a review, J. Ethnopharmacol. 275 (2021) 114–124, https://doi.org/10.1016/J.JEP.2021.114124.
[3] A. Ziyyat, A. Legssyer, H. Mekhfi, A. Dassouli, M. Serhrouchni, W. Benjelloun, Phytotherapy of hypertension and diabetes in oriental Morocco, J. Ethnopharmacol. 58 (1997) 45–54, https://doi.org/10.1016/S0378-8741(97)00077-9.
[4] A. Belda, B.B. Zaragozí, I.J.E. Martínez, E. Seva, Traditional knowledge of medicinal plants in the Serra de Mariola Natural Park, south-eastern Spain, Afr. J. Tradit. Complement. Altern. Med. 10 (2013) 299–309.
[5] issam El Mouzazi, K.El Oihabi, K. Faraj, S. Khal-Layoun, A. Bour, Ethnobotanical knowledge of daphne gnidium L. species in Taza (Northeastern Morocco), Ethnobot. Res. Appl. 29 (2024) 1–13. https://ethnobotanyjournal.org/index.php/era/article/view/5962.
[6] M. Eddouks, M. Ajebli, M. Hebi, Ethnopharmacological survey of medicinal plants used in Daraa-Tafilalet region (province of Errachidia), Morocco, J. Ethnopharmacol. 198 (2017) 516–530, https://doi.org/10.1016/j.jep.2016.12.017.
[7] H. Akdime, S. Boukhira, L.E.L. Mansouri, A.E.L. Hamsas Youbi, D. Bousta, Ethnobotanical study and traditional knowledge of medicinal plants in Ain Leuh region (Middle-Atlas of Morocco), Am. J. Adv. Drug Deliv. 3 (2015) 248–263. www.ojadd.com. accessed January 12, 2023.
[8] B. Benarba, L. Belabid, K. Righi, A. amine Bekkar, M. Elouissi, A. Khaldi, A. Hamimed, Ethnobotanical study of medicinal plants used by traditional healers in Mascara (North West of Algeria), J. Ethnopharmacol. 175 (2015) 626–637. https://www.sciencedirect.com/science/article/pii/S0378874115301550.
[9] C. Leto, T. Tuttolomondo, S. La Bella, M. Licata, Ethnobotanical study in the Madonie Regional Park (Central Sicily, Italy)—medicinal use of wild shrub and herbaceous plant species, J. Ethnopharmacol. 146 (2013) 90–112, https://doi.org/10.1016/J.JEP.2012.11.042.
[10] J. González, M. García-Barriuso, M. Gordaliza, F. Amich, Traditional plant-based remedies to control insect vectors of disease in the Arribes del Duero (western Spain): an ethnobotanical study, J. Ethnopharmacol. 138 (2011) 595–601. https://www.sciencedirect.com/science/article/pii/S0378874111007306. accessed June 30, 2022.
[11] F. Cottiglia, G. Loy, D. Garau, C. Floris, M. Caus, R. Pompei, L. Bonsignore, Antimicrobial evaluation of coumarins and flavonoids from the stems of Daphne gnidium L, Phytomedicine 8 (2001) 302–305, https://doi.org/10.1078/0944-7113-00036.
[12] M. Deiana, A. Rosa, V. Casu, F. Cottiglia, L. Bonsignore, M.A. Dessì, Chemical composition and antioxidant activity of extracts from Daphne gnidium L, J. Am. Oil Chem. Soc. 80 (2003) 65–70, https://doi.org/10.1007/S11746-003-0652-X.
[13] W. Nowik, HPLC-PDA characterisation of daphne gnidium L. (Thymemeacea) dyeing extracts using two different C-18 stationary phases, J. Sep. Sci. 28 (2005) 1595–1600, https://doi.org/10.1002/JSSC.200401770.
[14] A. Allal, S. Bellifa, N. Benmansour, C. Selles, M. Semaoui, H. Hassaine, A. Muselli, Essential oil and hydrosol extract chemical profile, antioxidant and antimicrobial potential of daphne gnidium L. from Algeria, J. Essent. Oil Bear. Plants. 22 (2019) 1277–1288, https://doi.org/10.1080/0972060X.2019.1673832.
[15] F. Chaabane, A. Pinon, A. Simon, K. Ghedira, L. Chekir-Ghedira, Phytochemical potential of daphne gnidium in inhibiting growth of melanoma cells and enhancing melanogenesis of B16-F0 melanoma, Cell Biochem. Funct. 31 (2012) 460–467, https://doi.org/10.1002/CBF.2919.
[16] M… Ramdani, T… Lograda, F.G. Chalard, Phytochemistry, antibacterial activity and chromosome number of two species of Daphne from Algeria, Int. J. Adv. Pharm. Biol. Chem. 4 (2015) 7–17. www.ijapbc.com. accessed October 31, 2022.
[17] N. Chaves, A. Santiago, J.C. Alías, Quantification of the antioxidant activity of plant extracts: analysis of sensitivity and hierarchization based on the method used, Antioxidants 9 (2020) 76, https://doi.org/10.3390/ANTIOX9010076.
[18] A… Horchani, F… Chaabane, M… Barboura, I… Mokdad-Bzeouich, A… Abbassi, A… Trabelsi, L. Chekir-Ghedira, Phytochemistry and biological evaluation of daphne gnidium L. butanol extract, Pharmacogn. J. 13 (2021).
[19] H. Harizi, F. Chaabane, K. Ghedira, L. Chekir-Ghedira, Inhibition of proinflammatory macrophage responses and lymphocyte proliferation in vitro by ethyl acetate leaf extract from Daphne gnidium, Cell. Immunol. 267 (2011) 94–101, https://doi.org/10.1016/J.CELLIMM.2010.12.002.

[20] W. Chaouki, D.Y. Leger, B. Liagre, Y. Cherrah, J.L. Beneytout, M. Hmamouchi, Roots of Daphne gnidium L. inhibit cell proliferation and induce apoptosis in the human breast cancer cell line MCF-7, Die Pharm, Int. J. Pharm. Sci. 64 (2009) 542–546, https://doi.org/10.1691/PH.2009.9502.

[21] V. Vidal, O. Potterat, S. Louvel, F. Hamy, M. Mojarrab, J.J. Sanglier, T. Klimkait, M. Hamburger, Library-based discovery and characterization of daphnane diterpenes as potent and selective hiv inhibitors in daphne gnidium, J. Nat. Prod. 75 (2012) 414–419, https://doi.org/10.1021/NP200855D/SUPPL_FILE/NP200855D_SI_001.PDF.

[22] L. Maistrello, M.A. López, F.J. Soria, R. Ocete, Growth inhibitory activity of Daphne gnidium L. (Thymelaeaceae) extracts on the elm leaf beetle (Col., Chrysomelidae), J. Appl. Entomol. 129 (2005) 418–424, https://doi.org/10.1111/j.1439-0418.2005.00993.x.

[23] Y. Zarki, M. Elmourabit, N.B. Seddik, S. Akachar, M. Achache, H.O. Idriss, F. Chaouket, K. Draoui, A. AitAghzzaf, Grafted sepiolite as smart nanocontainer for Daphne Gnidium plant extract: pH-dependent controlled release and effective green anti-corrosion coating, Surf. Interfaces 51 (2024), https://doi.org/10.1016/j.surfin.2024.104754.

[24] S… Vitalini, M… Iriti, F… Orlando, S. Garzoli, Chemical volatile composition and phytotoxic potential of daphne gnidium L. leaves, Sustain. Chem. Pharm. 25 (2022).

[25] I. El Mouzazi, A. Bour, S. Khal-Layoun, Phytochemical and biological activities of daphne gnidium L. species: a systematic review, Acta Ecol. Sin. (2023), https://doi.org/10.1016/j.chnaes.2023.06.006.

[26] F. El Mansouri, J.C.G.E. Silva, F. Cacciola, F. Asraoui, H. Tayeq, Y.M. Ben Amar, M.P. Lovillo, N. Chouaibi, J. Brigui, Evaluation of different extraction methods on the phenolic profile and the antioxidant potential of ceratonia siliqua L. Pods extracts, Mol. 2022, Vol. 27, Page 6163. 27 (2022) 6163. https://doi.org/10.3390/MOLECULES27196163.

[27] V. Dewanto, W. Xianzhong, K.K. Adom, R.H. Liu, Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity, J. Agric. Food Chem. 50 (2002) 3010–3014, https://doi.org/10.1021/JF0115589.

[28] R. Julkunen-Tiitto, Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics, J. Agric. Food Chem. 33 (1985) 213–217.

[29] O.P. Sharma, T.K. Bhat, DPPH antioxidant assay revisited, Food Chem. 113 (2009) 1202–1205, https://doi.org/10.1016/J.FOODCHEM.2008.08.008.

[30] T. Benali, A. Lemhadri, K. Harboul, H. Chtibi, A. Khabbach, S.M. Jadouali, L. Quesada-Romero, S. Louahlia, K. Hammani, A. Ghaleb, L.-H. Lee, A. Bouyahya, M. E. Rusu, M. Akhazzane, Chemical profiling and biological properties of essential oils of Lavandula stoechas L. Collected from three Moroccan sites, in: Vitro and In Silico Investigations, 12, Plants, Basel, Switzerland, 2023, https://doi.org/10.3390/plants12061413.

[31] T. Benali, K. Habbadi, A. Bouyahya, A. Khabbach, I. Marmouzi, T. Aanniz, H. Chtibi, H.N. Mrabti, E.H. Achbani, K. Hammani, Phytochemical analysis and study of antioxidant, anticandidal, and antibacterial activities of Teucrium polium subsp. Polium and micromeria graeca (Lamiaceae) essential oils from Northern Morocco, Evid. Based Complement. Altern. Med. 2021 (2021) 6641720, https://doi.org/10.1155/2021/6641720.

[32] T. Benali, H. Chtibi, A. Bouyahya, A. Khabbach, K. Hammani, Detection of antioxidant and antimicrobial activities in phenol components and essential oils of cistus ladaniferus and mentha suaveolens extracts, Biomed. Pharmacol. J. 13 (2020) 603–612.

[33] C. Rota, J.J. Carramiñana, J. Burillo, A. Herrera, In vitro antimicrobial activity of essential oils from aromatic plants against selected foodborne pathogens, J. Food Prot. 67 (2004) 1252–1256, https://doi.org/10.4315/0362-028X-67.6.1252.

[34] M. Gulluce, F. Sahin, M. Sokmen, H. Ozer, D. Daferera, A. Sokmen, M. Polissiou, A. Adiguzel, H. Ozkan, Antimicrobial and antioxidant properties of the essential oils and methanol extract from Mentha longifolia L. ssp. Longifolia, Food Chem. 103 (2007) 1449–1456.

[35] A… Bouyahya, J… Abrini, A… El-Baabou, Y… Bakri, N. Dakka, Determination of phenol content and antibacterial activity of five medicinal plants ethanolic extracts from North-West of Morocco, J. Plant Pathol. Microbiol. 7 (2016).

[36] M. Chelef, H. Hemida, K. Mazrou, A. Hassani, Phytochemical analysis and evaluation of rodenticidal powder from daphne gnidium under laboratory and field conditions, Int. J. Environ. Stud. 80 (2022) 591–611, https://doi.org/10.1080/00207233.2022.2085450.

[37] L. Shen, S. Pang, M. Zhong, Y. Sun, A. Qayum, Y. Liu, A. Rashid, B. Xu, Q. Liang, H. Ma, X. Ren, A comprehensive review of ultrasonic assisted extraction (UAE) for bioactive components: principles, advantages, equipment, and combined technologies, Ultrason. Sonochem. 101 (2023) 106646, https://doi.org/10.1016/J.ULTSONCH.2023.106646.

[38] F. Chemat, N. Rombaut, A.G. Sicaire, A. Meullemiestre, A.S. Fabiano-Tixier, M. Abert-Vian, Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review, Ultrason. Sonochem. 34 (2017) 540–560, https://doi.org/10.1016/J.ULTSONCH.2016.06.035.

[39] F. Chaabane, I. Mokdad-Bzeouich, A. Sassi, N. Mustapha, R. Majouli, K. Ghedira, L. Chekir-Ghedira, Genoprotective and neuroprotective effects of Daphne gnidium leaf methanol extract, tested on male mice, Drug Chem. Toxicol. 39 (2015) 297–302, https://doi.org/10.3109/01480545.2015.1107730.

[40] N. Manojlovic, P. Mašković, P. Vasiljević, R. Jelic, M. Juskovic, M. Sovrlic, L. Mandić, M. Radojković, HPLC analysis, antimicrobial and antioxidant activities of daphne cneorum L, (2012).

[41] M. Sovrlić, P. Vasiljević, M. Jušković, P. Mašković, N. Manojlović, Phytochemical, antioxidant and antimicrobial profiles of extracts of daphne alpina (Thymelaeaceae) L leaf and twig from Mt Kopaonik (Serbia), Trop. J. Pharm. Res. 14 (2015) 1239–1248, https://doi.org/10.4314/TJPR.V14I7.17.

[42] İ.A. Balkan, T. Taşkın, H.T. Doğan, İ. Deniz, G. Akaydın, E. Yesilada, 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. 95 (2017) 695–703, https://doi.org/10.1016/J.INDCROP.2016.11.038.

[43] C.D. Lee, H. Yu, N.P. Uy, H. Yu, C.H. Lee, H. Kim, S. Lee, Phytochemical characterization and bioactive potential of daphne jejudoensis, Chem. Pap. (2025) 1–11, https://doi.org/10.1007/S11696-025-03898-9/METRICS.

[44] F.Zare Mehrjerdi, S. Niknazar, M. Yadegari, F.A. Akbari, Z. Pirmoradi, M. Khaksari, Carvacrol reduces hippocampal cell death and improves learning and memory deficits following lead-induced neurotoxicity via antioxidant activity, Naunyn. Schmiedebergs. Arch. Pharmacol. 393 (2020) 1229–1237, https://doi.org/10.1007/S00210-020-01866-6/METRICS.

[45] L.M. Fonseca, C.E. dos S. Cruxen, G.P. Bruni, Á.M. Fiorentini, E. da R. Zavareze, L.T. Lim, A.R.G. Dias, Development of antimicrobial and antioxidant electrospun soluble potato starch nanofibers loaded with carvacrol, Int. J. Biol. Macromol. 139 (2019) 1182–1190, https://doi.org/10.1016/J.IJBIOMAC.2019.08.096.

[46] J. Rúa, P. Del Valle, D. De Arriaga, L. Fernández-Álvarez, M.R. García-Armesto, Combination of carvacrol and thymol: antimicrobial activity against staphylococcus aureus and antioxidant activity, Foodborne Pathog. Dis. 16 (2019) 622–629, https://doi.org/10.1089/FPD.2018.2594;WGROUP:STRING:PUBLICATION.

[47] M.P. Kähkönen, A.I. Hopia, H.J. Vuorela, J.P. Rauha, K. Pihlaja, T.S. Kujala, M. Heinonen, Antioxidant activity of plant extracts containing phenolic compounds, J. Agric. Food Chem. 47 (1999) 3954–3962, https://doi.org/10.1021/JF990146L.

[48] A. Ismail, Z.M. Marjan, C.W. Foong, Total antioxidant activity and phenolic content in selected vegetables, Food Chem. 87 (2004) 581–586, https://doi.org/10.1016/J.FOODCHEM.2004.01.010.

[49] Y. Xiang, Z. Liu, Y. Liu, B. Dong, C. Yang, H. Li, Ultrasound-assisted extraction, optimization, and purification of total flavonoids from Daphnegenkwa and analysis of their antioxidant, anti-inflammatory, and analgesic activities, Ultrason. Sonochem. 111 (2024) 107079, https://doi.org/10.1016/J.ULTSONCH.2024.107079.

[50] F. Lv, H. Liang, Q. Yuan, C. Li, In vitro antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food-related microorganisms, Food Res. Int. 44 (2011) 3057–3064, https://doi.org/10.1016/J.FOODRES.2011.07.030.

[51] H. Hajji, F. El Makhfi, E. Abdennebi, E.A.F. Fatima, O. Laila, E.A. Aicha, In vitro evaluation of antifungal activity of Daphne gnidium extracts against six human pathogenic fungi, J. Chem. Pharm. Sci. 10 (2017).

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

[53] M. Assunta Dessí, M. Deiana, A. Rosa, M. Piredda, F. Cottiglia, L. Bonsignore, D. Deidda, R. Pompei, F.P. Corongiu, Antioxidant activity of extracts from plants growing in Sardinia, Phyther. Res. 15 (2001) 511–518, https://doi.org/10.1002/PTR.799.
[54] S.H. Al-Mijalli, N. El Hachlafi, E.M. Abdallah, M. Jeddi, H. Assaggaf, A. Qasem, S.M. Alnasser, A. Attar, M.A. Naem, L.H. Lee, A. Bouyahya, H.N. Mrabti, Exploring the antibacterial mechanisms of chemically characterized essential oils from leaves and buds of Syzygium aromaticum (L.) Merr. Et perry against Staphylococcus aureus and Pseudomonas aeruginosa, Ind. Crops Prod. 205 (2023) 117561, https://doi.org/10.1016/J.INDCROP.2023.117561.
[55] G. Wang, M. Wang, J. Pang, Q. Sun, T. Fan, Z. Li, X. You, Effect of daphnetin, the coumarin derivative isolated from Daphne genus, on Helicobacter pylori adhesion to gastric epithelial cells, Phytomedicine 137 (2025) 156357, https://doi.org/10.1016/J.PHYMED.2024.156357.