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Quantitative analysis of polyphenols and antioxidant activity in four *Daphne* L. species

Research Article

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Abstract: The content of biologically active phenolic compounds (total polyphenols, tannins, flavonoids, and phenolic acids) were determined using spectrophotometry in four wild Croatian species of *Daphne* L. in the family Thymelaeaceae (*Daphne alpina, D. cneorum, D. laureola*, and *D. mezereum*). The concentration of total flavonoids (TF) was highest in the leaves of these *Daphne* species (0.12–0.51% dry herb weight, DW) whereas the content of other phenolic compounds analyzed were highest in the roots, including total polyphenols (TP; 2.71–19.03% DW), tannins (T; 1.14–7.39% DW), and total phenolic acids (TPA; 0.12–0.87% DW). *D. alpina* contained the highest amount of polyphenols, with the exception of flavonoids, where maximum concentrations were found in *D. laureola*. We also examined the antioxidant activity of leaf, stem, and root extracts. All extracts analyzed demonstrated high free radical scavenging activity with the highest concentration in the leaf extracts of *D. alpina*. Leaf extracts of *D. cneorum* showed the highest antioxidant activity in a β-carotene bleaching assay.

Keywords: *Daphne • Total polyphenols • Tannins • Flavonoids • Phenolic acids • Multivariate analysis • Antioxidant activity*

1. Introduction

Plants and their preparations were the first drugs used by humans for the maintenance of health and the treatment of various diseases. Due to the increasing use of synthetic drugs, modern medicine has neglected the therapeutic values of many plants. However, given a resurging interest in the medicinal properties of natural products, the importance of phytopharmacy is increasing rapidly. The development of modern research techniques and analytical methods has enabled different procedures for isolating compounds and determining the content and the structure of active substances in plants. These new techniques allow exploring the mechanisms of action of biologically active substances and therefore discovering and introducing new phytotherapeutics. Scientists have long been interested in medicinal plant species but examining the biological basis of their medicinal properties is becoming more feasible with the advent of new technologies. The genus *Daphne* L. (family *Thymelaeaceae* Juss. 1789) includes 50 to 90 shrubs distributed in Europe, Asia, North America, Arctic, North America, Australia, and Oceania [1-3]. Seventeen *Daphne* species have been described in Europe [1], five of which occur in Croatia: *Daphne*

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alpina L., *D. blagayana* Freyer, *D. cneorum* L., *D. laureola* L., and *D. mezereum* L. [4].

The medicinal properties of *Daphne* species are mostly attributed to the bark, which, however, may also contain toxic compounds. Poisoning has occurred commonly in the past because *Daphne* species have been indiscriminantly used in folk medicine for treating aches, rheumatism, rheumatoid arthritis, gout, skin diseases and for abortion [5-8]. Several substances have been isolated from *Daphne* species, including mezerein, vesiculosin, isovesiculosin, gniditrin, gnidicin, daphnetoxin, excoecariatoxin (diterpene structure); umbelliferon, acetylumbelliferon, daphnoretin, daphneticin, isodaphneticin, daphnetin, daphnin, triumbellin, 7,8-dihydroxy-chromene-2-one, and 7-hydroxy-8-metoxycoumarin (coumarine compounds); luteolin, orientin, isoorientin, apigenin-7-O-glucoside, genkwanin, 5-O-beta-D-primeverosyl genkwanine, 2,5,7,4'-tetrahydroxyisoflavanol, and hesperidine (flavonoids), and beta-sitosterol (steroid compound) [6-10]. Many of these compounds have potential therapeutic effects: antimalarial, analgesic, anti-inflammatory [11,12], antimicrobial [10], antioxidant, and antinociceptive activities [13,14], as well as properties for treating breast and lung cancers [15,16].

Phenolic compounds have attracted a lot of public and scientific interest because of their health-promoting effects as antioxidants. In recent years, flavonoids have gained a lot of importance because of their potential use as prophylactic and therapeutic agents in many diseases and much work has been presented by the scientific community which focuses on their antioxidant, antimicrobial, antiviral, antiangiogenic, immunomodulatory, anti-inflammatory and antitumor benefits [17-25]. The objective of this study was to evaluate polyphenolic profiles of *Daphne* species growing in Croatia: *Daphne alpina* L., *D. cneorum* L., *D. laureola* L., and *D. mezereum* L. The content of total polyphenols, tannins, flavonoids, and phenolic acids were determined in leaves, stalks, and roots of *Daphne* species. The antioxidant activity of these compounds was also examined to determine the antioxidant potential of these species. Endogenous and exogenous antioxidants prevent reactive oxygen species (ROS) from reaching intracellular concentrations that can lead to cell damage. Among the most important exogenous antioxidants are polyphenolic phytochemicals, such as tannins, phenolic acids, and flavonoids [26,27]. Here, we examined differences in the polyphenolic content of five *Daphne* species occurring in Croatia with the objective of improving their use as medicinal plants.

2. Experimental Procedures

We extracted plant material and used spectrophotometry to determine the concentration of total polyphenols (TP), tannins (T), total flavonoids (TP), and total phenolic acids (TPA) in four *Daphne* species. The antioxidant activity of the samples was evaluated using a β-carotene bleaching assay and by estimating the radical-scavenging activity (RSA) of the extracts. The results were evaluated using univariate (ANOVA) and multivariate (PCA and UPGMA) statistics.

2.1. Apparatus

A Soxhlet apparatus was used for drug extraction. The quantitative analysis of polyphenolic substances was carried out using an Agilent 8453 UV/Vis spectrophotometer (Agilent, Germany) equipped with the PC-HP 845x UV-Visible System (Agilent, Germany) and 1 cm quartz cells. A Stat Fax 3200 (Awareness Technologies, USA) was used for absorbance measurements in antioxidant activity assays.

2.2. Chemicals

All chemicals and reagents for the poyphenolic analyses were of analytical grade and supplied by Kemika (Zagreb, Croatia), with the exception of the Folin-Ciocalteu's phenol reagent (FCR) and casein (Merck, Darmstadt, Germany), and quercetin (Roth, Karlsruhe, Germany). Butylated hydroxyanisol (BHA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), β-carotene, linoleic acid, and Tween-40 (polyoxyethylene sorbitan monopalmitate) were purchased from Sigma-Aldrich Chemical Co. (USA) and used in the antioxidant activity assays. Double-distilled water was used throughout. Sample solutions were filtered with a 0.20-μm Minisart-plus membrane filter (Sartorius AG, Germany).

2.3. Plant material

Above-ground parts of randomly selected wild growing, mature plants of four *Daphne* species were collected in Croatia in September 2010: *Daphne alpina* L., *D. laureola* L., and *D. mezereum* L. (Gornje Jelenje pass; altitude: 800 m a.s.l.) and *D. cneorum* L. (Oštrc, Samobor highlands; altitude: 700 m a.s.l.). Plant material of at least 10 plants of the same species was mixed to obtain randomly selected samples. All samples were air-dried for three weeks in a well-ventilated room at room temperature (22ºC) and 60% air humidity, single-layered and protected from direct sunlight. Air-dried samples were placed in double paper bags labeled with the sample number and stored in a dry place at room temperature protected from light for five months until analyzed. Voucher specimens of

herbal material were deposited in the Herbarium of the Department of Pharmaceutical Botany with "Fran Kušan" Pharmaceutical Botanical Garden, Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia.

2.4. Analytical procedures

2.4.1. Total polyphenol and tannin analysis (FCR procedure)

FCR procedure is based on a reaction with Folin-Ciocalteu's phenol reagent (FCR) and spectrophotometric determination of total polyphenols and tannins (indirectly, after precipitation with casein) at 720 nm [28]. The total polyphenol and tannin content were evaluated in three independent analyses and were expressed as the percentages of dry weight of herbal material (% DW). Tannin was used as a calibration standard.

2.4.2. Total flavonoid analysis (F-AlCl3 procedure)

The total flavonoids content (quercetin type) was determined following Christ and Müller [29]. This procedure includes hydrolysis of glycosides, extraction of total flavonoid aglycones with ethyl acetate and complex formation with $AlCl_3$ at 425 nm. The content of total flavonoids was evaluated in three independent analyses. The yield was expressed as quercetin and calculated toward following expression:

%=A×0.772/b;

[A = absorbance; b = mass of dry herbal material (g)]

2.4.3. Determination of total hydroxycinnamic derivates (THD procedure)

A THD procedure was performed according to the monograph of Rosmarini folium [30]. Hydroxycinnamic derivatives in the extracts were measured by spectrophotometric analysis at 505 nm (three independent analyses) using the nitrite-molybdate reagent of Arnow (mixture of sodium nitrite and sodium molybdate) in a diluted sodium hydroxide medium. Their content, expressed as a percent of rosmarinic acid, was calculated as:

A×2.5/m;

[A = absorbance; m = mass of the substance to be examined (g)], taking the specific absorbance of rosmarinic acid to be 400.

2.4.4. Validation of analytical procedures for polyphenol analysis

The quality control of the FCR, F-$AlCl_3$, and THD procedures and the evaluation of the analytical parameters were carried out using a comprehensive prevalidation strategy [31]. The efficiency of the prevalidation procedure is given by data, such as the constants of calibration and analytical evaluation function, limits of detection and quantitation, as well as precision and accuracy of the procedures.

2.4.5. Antioxidant activity assays

Extract preparation: 0.200 g of finely powdered leaf, stem or root of *Daphne* spp. was extracted with 10 mL of 30% methanol (water bath, 70ºC, 15 min). After cooling and filtration, 30% methanol was added until each extract reached a volume of 10.0 ml.

Radical-scavenging activity: Free radical scavenging activity (RSA) was evaluated by the scavenging of DPPH radicals. In its radical form, DPPH has a strong visible absorption and high molar extinction coefficient at 517 nm. Upon reaction with an antioxidant, the absorbance diminishes. The details of this procedure are given by Zovko Končić *et al*. [32]. BHA was used as the antioxidant standard. DPPH radical-scavenging activity was calculated as the concentration of the extract (dry matter) that scavenges 50% of DPPH free radical, therefore producing an RSA of 50% (EC_{50}).

β-carotene bleaching assay: The basis of β-carotene bleaching assay is degradation of β-carotene in the presence of linoleic acid. At elevated temperatures, linoleic acid forms a free radical which reacts with β-carotene and leads to its degradation and a decrease in absorbance at λ=450 nm. By reacting with linoleate radicals or any other radicals formed in the solution, compounds with antioxidant properties prevent or reduce the rate of β-carotene oxidation and degradation. The antioxidant activity of the samples was evaluated with a β-carotene-linoleic acid assay as described by Kosalec *et al*. [33].

2.4.6. Statistical analysis

The results were statistically analyzed with a multivariate approach, using a Principal Component Analysis (PCA) and an Unweighted Pair Group Method with Arithmetic Mean (UPGMA) with Euclidean distance (D_E) [34]. We used PCA to examine the variability between samples of *Daphne* species. This calculation was based on the correlation matrix between the values of the characteristics (TP, T, TF, and TPA), meaning that the contribution of each variable was independent of the range of its values [35,36]. To confirm the results of the PCA, we used an UPGMA with Euclidean distances (D_E). UPGMA generally yields results which are the most accurate for classification purposes [37,38]. Each variable was standardized prior to the cluster analysis. Statistical comparisons of phenolic content and antioxidant activity among plant species and between plant organs were conducted using a one-way ANOVA

followed by Scheffe's *post hoc* test at the $P \leq 0.05$ level. Prior to this analysis, the data were transformed using angular transformation [39]. Statistical analyses were performed using the software package Statistica 7 (StatSoft Inc., Tulsa, OK, USA).

3. Results and Discussion

Three different spectrophotometry procedures were used to quantitatively analyse the phenolic compounds in leaves, stems, and roots of four *Daphne* species: 1) total polyphenol and tannin analysis with Folin-Ciocalteu's phenol reagent (FCR procedure) [28], 2) total flavonoid determination, which includes complex formation with $AlCl_3$ ($F\text{-}AlCl_3$ procedure) [29], and 3) determination of total hydroxycinnamic derivates (THD procedure) [30].

We controlled the quality of the spectrophotometry procedures using a prevalidation strategy [31] within the following working ranges: from 5.0 to 50.0 µg of tannin (FCR procedure), from 0.08 to 0.80 mg of quercetin ($F\text{-}AlCl_3$ procedure), and from 40 to 400 µg of rosmarinic acid (THD procedure). Thorough systematic evaluation of analytical functions over the entire analyte working range was performed using a standardized mathematical/statistical procedure [31]. Ideal linear calibration ($\hat{S}$) and analytical evaluation ($\hat{x}$) functions were found for all systems: $\hat{S} = 0.011x$ and $\hat{x} = 94.2S$ for FCR procedure, $\hat{S} = 0.85x$ and $\hat{x} = 1.17S$ for $F\text{-}AlCl_3$ procedure, and $\hat{S} = 0.004x$, and $\hat{x} = 270.3S$ for THD procedure.

Limiting values, such as the limit of detection (*LD*) and the limit of quantification (*LQ*) were estimated using analytical evaluation functions and recommended concepts of limiting values [40,41]. For all systems, the estimated limiting values were significantly lower than the amounts of tannin, quercetin, and rosmarinic acid at the lowest level of analyte. The limits of quantitation were 1.31 µg of tannin, 0.013 mg of quercetin, and 2.91 µg of rosmarinic acid for the FCR procedure, $F\text{-}AlCl_3$ procedure, and THD procedure, respectively. The systematic deviations, a measure of accuracy, ranged from -4.3% to +4.0% for the FCR procedure, from -11.5 to +1.0 for the $F\text{-}AlCl_3$ procedure, and from -10.5 to +3.2 for the THD procedure. All systems showed high precision, with the THD procedure showing the highest precision (from ±0.35% to ±3.77%) based on the prevalidation criterion of $sr < \pm 5\%$. In the other two systems, random deviations ranged from ±0.60% to ±3.42% (FCR procedure) and from ±0.81% to ±5.70% ($F\text{-}AlCl_3$ procedure). In summary, the evaluation of precision and accuracy, as well as the existence of the linear calibration and the analytical evaluation function showed a good measurement quality. Very low limiting values indicated that the procedures were sensitive and could be successfully applied to the determination of phenolic compounds in plant material.

Table 1 shows the results of the spectrophotometry analyses for the content of total polyphenols (TP), tannins (T), total flavonoids (TF), and total phenolic acids (TPA) in the four *Daphne* species investigated.

In the roots, the content of TP ranged from 2.71% (*D. laureola*) to 19.03% (*D. alpina*); T content varied from 1.14% (*D. laureola*) to 7.39% (*D. alpina*); TF ranged from 0.02% (*D. cneorum*) to 0.07% (*D. laureola*), and TPA ranged from 0.12% (*D. mezereum*) to 0.87% (*D. alpina*).

The concentrations of bioactive compounds in stems were as follows: TP from 2.47% (*D. laureola*) to 15.90% (*D. alpina*); T from 0.85% (*D. cneorum*) to 4.60% (*D. alpina*); TF from 0.03% (*D. cneorum*) to 0.20% (*D. laureola*), and TPA from 0.12% (*D. laureola*) to 0.56% (*D. alpina*).

The leaves of the four *Daphne* species contained TP in concentrations ranging from 1.42% (*D. laureola*) to 12.65% (*D. alpina*); T were measured in range from 0.36% (*D. laureola*) to 3.01% (*D. alpina*); TF were determined in concentrations of 0.12% (*D. cneorum*) to 0.51% (*D. laureola*), and TPA content were from 0.14% (*D. laureola*) to 0.84% (*D. alpina*).

Accordingly, the results of phenol determination showed that *D. alpina* samples had the highest content of TP, T, and TPA in all three of the plant organs examined (root, stem, leaf), while the highest concentrations of TF occurred in *D. laureola*. The study also showed that *D. laureola* had the lowest amounts of TP (all plant organs), T (root and leaf), and TPA (stem and leaf). All samples of *D. cneorum* had the lowest concentrations of TF, while the root specimens of *D. mezereum* contained the least amount of TPA.

The ANOVA showed statistically significant differences for TP, T, TF, and TPA content among species, as well as between plant organs (Table 1). Differences among plant organs were smallest for TPA and greatest for TF.

The Principal Component Analysis (PCA) and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) separated the *Daphne* samples as shown in Figure 1.

The most similar samples were *D. laureola* root, *D. mezereum* root and stem, and *D. cneorum* stem. The samples of *D. alpina* showed a higher degree of separation. The eigen-vector matrix with the loading of each variable in the first four principal components is presented in Table 2. The content of total polyphenols and tannins gave the highest contribution to the

Species	Total polyphenols (% DW)	Tannins (% DW)	Total flavonoids (% DW)	Total phenolic acids (% DW)
D. alpina – L	12.65±1.29Aa	3.01±1.28Aa	0.42±0.03Aa	0.84 ± 0.03Aa
D. alpina – S	15.90±1.64D	4.60±0.70D	0.11±0.01Dab	0.56 ± 0.01Dab
D. alpina – R	19.03±1.71Ga	7.39±2.30Ga	0.04±0.00Gab	0.87 ± 0.07Gb
D. cneorum – L	5.07 ± 0.03ABa	2.24±0.06Ba	0.12±0.00ABa	0.30 ± 0.03ABa
D. cneorum – S	4.09 ± 0.18Dab	0.85±0.24Dab	0.03±0.00DEab	0.42 ± 0.09DE
D. cneorum – R	5.11±0.05GHb	1.91±0.17Gb	0.02±0.00Hab	0.55 ± 0.03GHa
D. laureola – L	1.42±0.20ABCa	0.36±0.12ABa	0.51±0.01ABCa	0.14 ± 0.01ABC
D. laureola – S	2.47±0.25DEa	0.97±0.17Da	0.20±0.07DEFab	0.12 ± 0.01DEF
D. laureola – R	2.71±0.11GHIa	1.14±0.04Ga	0.07±0.03GHab	0.14 ± 0.02GH
D. mezereum – L	3.88±0.90AC	0.38±0.11ABa	0.26±0.01ABCa	0.33 ± 0.03ACa
D. mezereum – S	4.56±0.93DE	1.04±0.29Dab	0.05±0.00Fa	0.23 ± 0.01DEFab
D. mezereum – R	5.43±0.38GI	2.17±0.38Gab	0.05±0.00a	0.12 ± 0.01GHab

Table 1. Content of total polyphenols, tannins, total flavonoids, and total phenolic acids in leaves (L), stems (S), and roots (R) of *Daphne* L. species. Results are the mean and standard deviation (SD), n=3.

Note: Values marked with the same letter are statistically different according to Scheffe's post-hoc test with P<0.05;
Capital letter = difference between species for leaves (A, B, C), stems (D, E, F), and roots (G, H, I) related to specific traits (TP, T, TF, TPA).*
** Example: A represents a difference between the leaves of D. alpina and other species with regard to certain group of compounds (TP, T, TF, or TPA); D is a difference between stems of D. alpina and other species; G is a difference between compounds in the roots of D. alpina and other species, etc.*
*Lower case letter = difference within species ** for leaves, stems, and roots (a, b). ** Example: a represents a difference between leaves, stems and roots in D. alpina with regards to TF; b is a difference between stems and roots with regards to TF.*

Variable	PC 1	PC 2	PC 3	PC 4
Total polyphenols	**0.592449**	-0.084831	-0.257134	**0.758742**
Tannins	0.580079	0.076235	-0.521433	-0.621131
Total flavonoids	-0.137827	**-0.965412**	-0.209515	-0.071322
Total phenolic acids	0.541771	-0.234460	**0.786189**	-0.182809

Table 2. Eigenvectors of the principal components (PCs) obtained for the chemical traits of leaves, stems, and roots in *Daphne* species.

Note: bold values indicate the highest contribution to a PC axis.

first PC axis. Furthermore, the content of flavonoids contributed most to the second PC axis, while the maximum score for PC 3 was obtained from the phenolic acid content. The first principal component explained 68.25% of the total variance and the second one, 25.16% (Table 3).

Similar results were obtained using UPGMA (Figure 1B). All *D. alpina* samples formed a single cluster at a Euclidean distance (D_E) of 3.75 from the cluster formed by all other samples. The most similar samples were *D. laureola* root and *D. mezereum* stem, which were connected at a D_E of 0.48.

Interestingly, the PCA and UPGMA did not separate samples according to plant organs (root, stem, and leaf). However, this study pointed to root samples as the valuable source of phenolic compounds in *Daphne* species (except flavonoids). However, this result is not in accordance with related studies which have shown that leaves generally contain the highest concentrations of phenolic compounds [42-44]. In order to investigate the relation between contents of polyphenols and antioxidant potential, radical-scavenging activity assay and β-carotene bleaching assay were carried out for leaf, stem, and root extracts of *Daphne* spp. (Table 4).

At high concentrations, free radicals can cause damage to cell macromolecules. The modification of nucleic acids by free radicals is particularly detrimental because the alteration of genetic material may represent the first step in mutagenesis, carcinogenesis

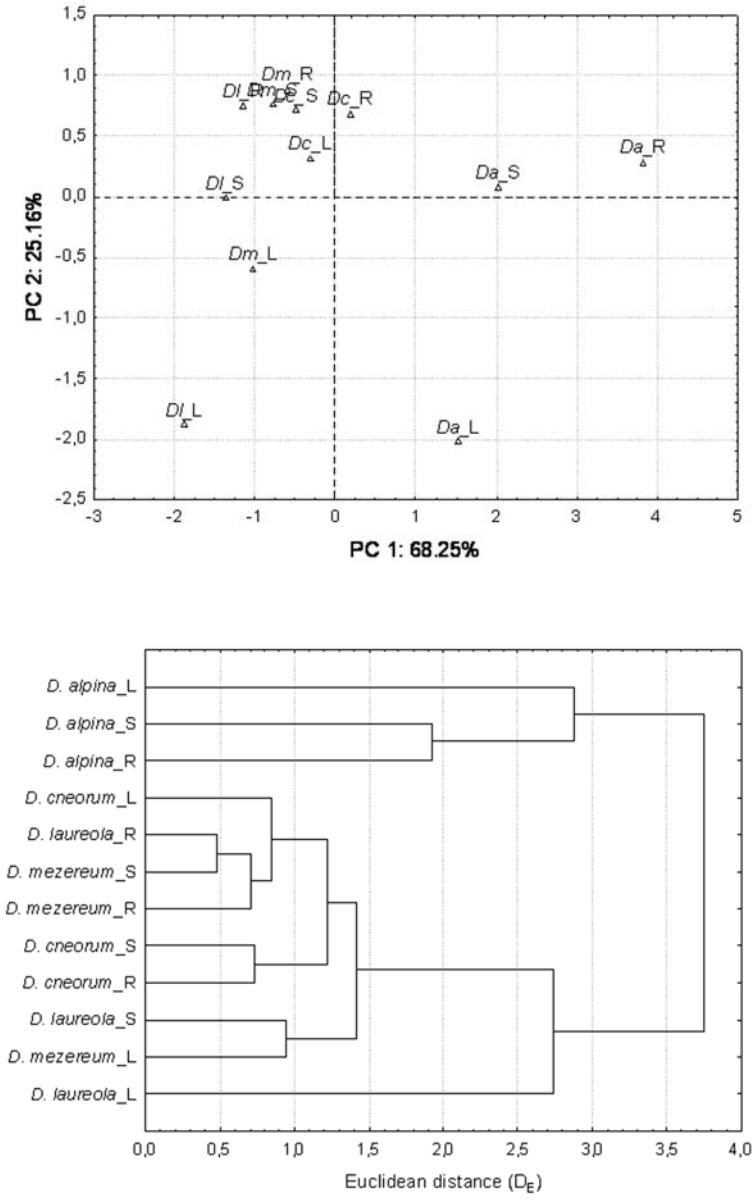

Figure 1. PCA (A) and UPGMA (B) of the content of phenolic compounds in leaves (L), stems (S), and roots (R) of *Daphne* species; *Da – Daphne alpina, Dc – D. cneorum, Dl – D. laureola, Dm – D. mezereum.*

PC	Eigenvalue	% Total variance	Cumulative eigenvalue	Cumulative % of total variance
1	2.7299	68.25	2.7299	68.25
2	1.0063	25.16	3.7362	93.41
3	0.2302	5.75	3.9664	99.16
4	0.0336	0.84	4.0000	100.00

Table 3. Eigen-values of the correlation matrix obtained for the chemical traits of leaves, stems, and roots in *Daphne* species.

and ageing. Thus, it has been hypothesized that free radical scavengers could prevent or limit damage provoked by free radicals [45,46]. The ability of extracts from *Daphne* species to scavenge free radicals was assessed in reaction with DPPH, a relatively stable free radical. All the extracts examined in this study demonstrated notable antiradical activity (Table 4), albeit somewhat lower than BHA, a widely used food antioxidant. The most active extract in this assay was *D. alpina* – L, which is in accordance with the results obtained in other quantitative analyses of polyphenols. The oxidation of aqueous emulsions of β-carotene and linoleic acid is frequently employed as a test for measuring total antioxidant activity in plant extracts [47,48]. In this assay the capacity of antioxidants to inhibit the formation of conjugated diene hydroperoxide arising from linoleic acid oxidation is measured. Thus, the assay provides information on the inhibitory effect of the compound tested on lipid peroxidation [49]. Here, antioxidant activity was measured as a percentage of the total inhibition of lipid peroxidation (ANT) (Table 4). The extracts investigated in this study were able to significantly reduce the rate of degradation of β-carotene in comparison with the water control. The most active extract in this assay was *D. cneorum* – L. Yet, even though the extracts clearly showed notable activity in this assay, it was somewhat lower than the activity of BHA.

4. Conclusions

In conclusion, our spectrophotometric procedures are characterized with good prevalidation characteristics, such as high precision, accuracy, and sensitivity. We successfully applied these methods in identifying phenolic compounds in plant material. *D. alpina* samples generally had the highest polyphenol content and these plants may therefore be a valuable source of these biologically active compounds. Several *in vitro* assays were applied to evaluate the antioxidant potential leaf, root, and stem extracts from *Daphne* species. Our results suggest that *Daphne* species could be a source of polyphenolic compounds as well as other antioxidants with radical-scavenging properties.

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Species	RSA EC_{50} (mg/mL)	ANT EC_{50} (mg/mL)
D. alpina – L	318.93 ± 25.42Aa	211.10 ± 2.63Aa
D. alpina – S	961.28 ± 23.83Da	263.24 ± 3.81Dab
D. alpina – R	883.70 ± 17.21Ga	179.37 ± 1.44Gab
D. cneorum – L	460.65 ± 19.86Ba	69.57 ± 1.50ABa
D. cneorum – S	448.11 ± 16.48DEb	140.15 ± 0.93DEab
D. cneorum – R	419.75 ± 15.96GHab	128.38 ± 1.95GHab
D. laureola – L	4363.37 ± 159.35ABCa	308.56 ± 8.23ABCa
D. laureola – S	1799.06 ± 126.42DEFa	404.61 ± 15.14DEFab
D. laureola – R	1285.36 ± 30.04GHa	328.74 ± 2.59GHIb
D. mezereum – L	482.81 ± 21.51Ca	187.49 ± 3.14ABCa
D. mezereum – S	484.17 ± 24.95DFb	219.89 ± 2.52DEFab
D. mezereum – R	1302.54 ± 40.20GHab	363.78 ± 11.28GHIab
BHA	2.75 ± 0.19	3.03 ± 0.02

Table 4. Radical scavenging (RSA) and antioxidant activities (ANT) of *Daphne* species leaf (L), stem (S), and root (R) extracts. Results are the mean and standard deviation (SD), n=3.

Note: values marked with the same letter are statistically different according to Scheffe's post-hoc test with P<0.05;
Capital letter = difference between species for leaves (A, B, C), stems (D, E, F), and roots (G, H, I) with regards to RSA and ANT. * * Example: A represents a difference between leaves of D. alpina and other species with regard to certain activity (RSA or ANT); D is a difference between stems of D. alpina and other species; G is a difference between RSA or ANT in roots of D. alpina and other species etc.*
*Lower case letter = difference within species** for leaves, stems, and roots (a, b). ** Example: a represents a difference between leaves, stems and roots in D. alpina with regards to ANT; b is a difference between stems and roots with regards to ANT.*
BHA – Butylated hydroxyanisol; EC_{50} – concentration that shows 50% activity.

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