PHENOLIC AND FLAVONOID CONTENT AND ANTIOXIDANT ACTIVITY OF *DAPHNE BLAGAYANA* GROWING IN SERBIA

Nedeljko Manojlovic[1], Miroslav Sovrlic[1], Pavle Maskovic[2], Perica Vasiljevic[3] and Marina Juskovic[3]
[1]Faculty of Medical Sciences, University of Kragujevac, 34000 Kragujevac, Serbia
[2]Faculty of Agronomy, University of Kragujevac, 32000 Cacak, Serbia
[3]Faculty of Sciences and Mathematics, University of Nis, 18000 Nis, Serbia

SADRŽAJ FENOLA I FLAVONOIDA I ANTIOKSIDATIVNA AKTIVNOST BILJKE *DAPHNE BLAGAYANA* KOJA RASTE U SRBIJI

Nedeljko Manojlović[1], Miroslav Sovrlić[1], Pavle Mašković[2], Perica Vasiljević[3] i Marina Juškoviić[3]
[1]Fakultet medicinskih nauka, Univerzitet u Kragujevcu, 34000 Kragujevac, Srbija
[2]Agronomski fakultet, Univerzitet u Kragujevcu, 32000 Čačak, Srbija
[3]Prirodno-matematički fakultet, Univerzitet u Nišu, 18000 Niš, Srbija

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ABSTRACT

The aim of this study was to examine the phenolic and flavonoid contents and antioxidant activities of methanol and chloroform extracts of leaves and twigs of Daphne blagayana. The total phenolic content in the chloroform extract of plant twigs (90.26 ± 0.69 mg GA/g) was higher than that of the other extracts (from 76.56±0.89 to 77.45±0.43 mg GA/g). In the case of flavonoids, a greater value was also obtained for the chloroform extract of twigs (35.24±0.55 mg RU/g). Several different methods were used to determine the antioxidant activity of the tested extracts, including total antioxidant capacity, metal chelating activity, hydroxyl radical scavenging activity and inhibitory activity against lipid peroxidation. Our results showed that although secondary metabolites of the plants may contribute significantly to their antioxidant activities, those antioxidant activities were not directly related to the phenolic and flavonoid amounts. The results of the present analysis demonstrated, for the first time, that Daphne blagayana leaves and twigs possess high phenolic and flavonoid contents, as well as potential antioxidant activity. This study suggests that Daphne blagayana twigs and leaves may potentially be used as an accessible source of natural antioxidants.

Key words: *Daphne blagayana, phenols, flavonoids, antioxidant activity.*

SAŽETAK

Cilj ovog rada je bio da se ispita fenolni i flavodnoidni sadržaj, kao i antioksidativna aktivnost metanolskih i hloroformskih ekstrakta lišća i grančica biljke Daphne blagayana. Ukupan sadržaj fenola u hloroformskom ekstraktu grančica (90.26±0.69 mg GA/g) bio je veći od sadržaja u ostalim ekstraktima (od 76.56±0.89 do 77.45±0.43 mg GA/g). U slučaju flavonoida, maksimalna vrednost je takođe zabeležena kod hloroformskog ekstrakta grančica (35.24±0.55 mg RU/g). Nekoliko različitih metoda su korišćeno za određivanje antioksidantne aktivnosti testiranih ekstrakata uključujući ukupan antioksidantni kapacitet, metal helacionu aktivnost, aktivnost hidroksi radikala i inhibitornu aktivnost prema lipidnoj peroksidaciji. Naši rezultati su pokazali da, iako sekundarni metabolite biljaka mogu značajno doprineti antioksidantnim aktivnostima, ove aktivnosti nisu bile uvek direktno povezane sa količinom fenola i flavonoida u ekstraktima. Rezultati ovih ispitivanja su pokazali, po prvi put, da grančice i lišče biljke Daphne blagayana poseduju visok sadržaj fenola i flavonoida i potencijalno antioksidantno delovanje. Ova studija je pokazala da se lišće i grančice ove biljke mogu potencijalno koristiti kao pristupačan izvor prirodnih antioksidanasa.

Ključne reči: *Daphne blagayana, fenoli, flavonoidi, antioksidativna aktivnost.*

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Correspondence to: Nedeljko Manojlovic,
Faculty of Medical Sciences, University of Kragujevac, Svetozara Markovica 69, 34 000 Kragujevac, Serbia
e-mail: mtnedeljko@yahoo.com

INTRODUCTION

Medicinal plants have been widely used by both ancient and modern people of all cultures for treating different illnesses and for other purposes. Plants are a good source of biologically active natural products that are all biodegradable and, more importantly, renewable. The antioxidant properties of plants could affect a range of physiological processes in the human body, thus providing protection against free radicals (1-4). Antioxidants are micronutrients that have drawn interest in recent years due to their ability to neutralise the actions of free radicals (5). Free radicals are potentially harmful products generated during a number of natural processes in the body and are associated with ageing of cells and tissues. Failure to remove active oxygen compounds over the long term can lead to cardiovascular disease, cancer, diabetes, arthritis and various neurodegenerative disorders (6).

Phenolic compounds are ubiquitous in plants. Flavonoids and other plant phenolics, such as phenolic acids, stilbenes, tannins, lignans, and lignins, are important in the plant for normal growth development and defense against infection and injury. These compounds are commonly found in plants, and they have been reported to have multiple biological effects, including antioxidant activity (7). Various investigations have implied that the concentrations of total phenolic compounds are closely related to antioxidative activity (8), with flavonoids and tannins as the major plant compounds with antioxidant activity (9).

Components of some *Daphne* species are used in natural medicine as a laxative, diuretic, anticoagulant, and in the treatment against skin diseases, toothache and malaria. Previous studies of individual species of the genus *Daphne* indicate their potential broad applications in medicine (10-13). The aim of this study was to investigate the total phenolic and flavonoid contents as well as antioxidant activity of the methanol and chloroform extracts obtained from the twigs and leaves of *D. blagayana* which have not previously been studied.

MATERIAL AND METHODS

Chemicals used

All chemicals and reagents were of analytical grade and were purchased from Sigma Chemical Co. (St Louis, MO, USA), Aldrich Chemical Co. (Steinheim, Germany) and Alfa Aesar (Karlsruhe, Germany). 1,1-Diphenyl-2-picrylhydrazyl hydrate (DPPH), Folin–Ciocalteu, ascorbic acid, butylated hydroxytoluene (BHT) and pyrocatechol were purchased from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). Hydrochloric acid, formaldehyde, anhydrous sodium carbonate, methanol and chloroform were purchased from Centrohem (Centrohem, Stara Pazova, Serbia).

Spectrophotometric measurements

Spectrophotometric measurements were performed using an MA 9524-SPEKOL 211 (ISKRA, Slovenia).

Plant material

The plant material was collected from Mt. Kopaonik, Serbia, in July, 2007. The demonstration samples are preserved in facilities of the Department of Biology and Ecology, Faculty of Science, University of Niš, Serbia (Voucher No HMN 5517).

Preparation of the plant extracts

The air-dried leaves and twigs from the plant *Daphne blagayana* (60 g) were broken into small 2– 6 mm pieces by a cylindrical crusher, and extracted separately with chloroform and methanol (500 ml) using a Soxhlet apparatus. The mixture was filtered through filter paper (Whatman, No.1) and evaporated. The residues (7.8 g for chloroform and 8.3 g for methanol) were stored in a dark glass bottle at +4°C for further processing. The extracts were used for chemical and antioxidant analysis.

Determination of total phenolic content

Total phenols were estimated according to the Folin-Ciocalteu method (15). The extracts were diluted to a concentration of 1 mg/ml, and aliquots of 0.5 ml were mixed with 2.5 ml of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and 2 ml of $NaHCO_3$ (7.5%). After 15 min at 45˚C, the absorbance was measured against a blank sample at 765 nm. Total phenols were determined as gallic acid equivalents (mg GA/g extract), and the values are presented as means of triplicate analyses.

Determination of flavonoid content

Total flavonoids were determined according to Brighente *et al.* (16). A total of 0.5 ml of 2% aluminium chloride ($AlCl_3$) in methanol was mixed with the same volume of methanol solution of plant extract. After 1 hour at room temperature, the absorbance was measured at 415 nm against the blank sample. Total flavonoids were determined as rutin equivalents (mg RU/g dry extract), and the values are presented as means of triplicate analyses.

Determination of total antioxidant activity

The total antioxidant activity of *D. blagayana* extracts was evaluated using the phosphomolybdenum method (17). This assay is based on the reduction of Mo (VI) to Mo (V) by antioxidant compounds and subsequent formation of a green phosphate/Mo (V) complex at acid pH. A total of 0.3 ml of sample extract was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solutions were incubated at 95°C for 90 min. After cooling to room temperature, the absorbance of the solution was measured at 695 nm against the

blank sample. Methanol (0.3 ml) was used as the blank in place of an extract. Ascorbic acid was used as the standard and total antioxidant capacity was expressed as milligrams of ascorbic acid per gram of dry extract.

Determination of DPPH free radical scavenging activity

The method used by Takao *at al.* (18) was adopted with suitable modifications from Kumarasamy *et al.* (19). DPPH (8 mg) was dissolved in MeOH (100 ml) to obtain a concentration of 80 µg/ml. Serial dilutions were carried out with the stock solution (1 mg/ml) of the extract. Solutions (2 ml each) were then mixed with DPPH (2 ml) and allowed to stand for 30 min to allow any reaction to occur, and the absorbance was measured at 517 nm. Ascorbic acid (AA), gallic acid and BHT were used as reference standards and were dissolved in methanol to prepare stock solutions with the same concentrations (1 mg/ml). Control samples were prepared containing the same volume without test compounds or reference antioxidants. Ninety-five percent methanol was used as a blank. The DPPH free radical scavenging activity (%) was calculated using the following equation:

$$\% \text{ inhibition} = \frac{\text{Ac-As}}{\text{Ac}} \times 100$$

The IC_{50} value, defined as the concentration of the test material that leads to 50% reduction in the free radical concentration, was calculated using a sigmoidal dose-response curve and expressed as µg/ml.

Determination of inhibitory activity against lipid peroxidation

Antioxidant activity was determined by the thiocyanate method (20). Serial dilutions were carried out with stock solutions (1 mg/ml) of the extracts, and 0.5 ml of each solution was added to a linoleic acid emulsion (2.5 ml, 40 mM, pH 7.0). The linoleic acid emulsion was prepared by mixing 0.2804 g linoleic acid, 0.2804 g Tween-20 as emulsifier in 50 ml 40 mM phosphate buffer and the mixture was then homogenised. The final volume was adjusted to 5 ml with 40 mM phosphate buffer, pH 7.0. After incubation at 37°C in the dark for 72 h, a 0.1 ml aliquot of the reaction solution was mixed with 4.7 ml of ethanol (75%), 0.1 ml $FeCl_2$ (20 mM) and 0.1 ml ammonium thiocyanate (30%). The absorbance of the mixture was measured at 500 nm and the mixture was stirred for 3 min. Ascorbic acid, gallic acid, α-tocopherol and BHT were used as reference compounds. To eliminate the solvent effect, the control sample, which contained the same amount of solvent added to the linoleic acid emulsion in the test sample and reference compound, was used. Inhibition of linoleic acid peroxidation was calculated using the following formula:

$$\% \text{ inhibition} = \frac{\text{Ac-As}}{\text{Ac}} \times 100$$

The index of lipid peroxidation was not determined as ev . limiting factor studies.

Measurement of ferrous ion chelating ability

The ferrous ion chelating activity of the methanol extracts was measured by the decrease in absorbance at 562 nm of the iron(II)-ferrozine complex (21). One milliliter of 0.125 mM $FeSO_4$ was added to 1.0 ml of sample (with different dilutions), followed by 1.0 ml of 0.3125 mM ferrozine. The mixture was allowed to equilibrate for 10 min before measuring the absorbance. The ability of the sample to chelate ferrous ion was calculated relative to the control (consisting of iron and ferrozine only) using the formula:

$$\text{Chelating effect } (\%) = \frac{\text{Ac} - \text{As}}{\text{Ac}} \times 100$$

Determination of hydroxyl radical scavenging activity

The ability of *D. Blagayana* extracts to inhibit non site-specific hydroxyl radical-mediated peroxidation was carried out according by Hinneburg *et al.* (2006) (22). The reaction mixture contained 100 µl of extract dissolved in water, 500 µl of 5.6 mM 2-deoxy-D-ribose in KH_2PO_4–NaOH buffer (50 mM, pH 7.4), 200 µl of premixed 100 µM $FeCl_3$ and 104 mM EDTA (1:1 v/v) solution, 100 µl of 1.0 mM H_2O_2 and 100 µL of 1.0 mM aqueous ascorbic acid. Tubes were vortexed and incubated at 50°C for 30 min. Thereafter, 1 ml of 2.8% TCA and 1 ml of 1.0% TBA were added to each tube. The samples were vortexed and then heated in a water bath at 50°C for 30 min. The extent of oxidation of 2-deoxyribose was estimated from the absorbance of the solution at 532 nm. The percentage inhibition was calculated from the absorbances of the controls (Ac) and the samples (As), where the controls contained all the reaction reagents except the extract or positive control substance. The values are presented as the means of triplicate analyses.

Statistical analysis

The results are presented as mean±standard deviations of three analytical determinations. Statistical analyses were performed using Student´s t-test. IC_{50} values were calculated by nonlinear regression analysis from the sigmoidal dose-response inhibition curve. Significant differences for extracts were analysed using one way ANOVA, followed by Tukey`s HSD post hoc comparison test at $p \leq 0.05$. All computations were made using statistical software (SPSS, version 11.0).

RESULTS

The results obtained for total phenolic and flavonoid contents and total antioxidant capacity for the chloroform and methanol extracts of *D. blagayana* are presented in Table 1. The phenolic content in the chloroform extracts of twigs (90.26±0.69 mg GA/g) was higher than those in the methanol extract of leaves (77.45±0.43 mg GA/g), the chloroform extract of leaves (76.56±0.89 mg GA/g) and the methanol extract of twigs (75.88±0.54 mg GA/g). The flavonoid contents in all the tested extracts ranged from 26.79±0.34 to 35.24±0.55 mg RU/g.

Table 1. Statistical analysis of total phenols, flavonoids and total antioxidant capacity of the chloroform and methanol extracts of *D. blagayana*

Extracts of D. blagayana	Total phenolics (mg GA/g)	Flavonoids (mg RU/g)	Total antioxidant capacity (µg AA/g)
Chloroform extract of twigs	90.26±0.69	35.24±0.55	78.45±0.98
Chloroform extract of leaves	76.56±0.89	26.79±0.34	76.09±0.45
t-test	*	*	*
Methanol extract of twigs	75.88±0.54	29.95±0.39	69.50±1.00
Methanol extract of leaves	77.45±0.43	27.98±0.88	68.98±0.25
t-test	*	n.s.	n.s.
ANOVA	*	*	*

Values are the means ±SD. Data were analysed by t-test and analyses of variance (ANOVA) procedures (* $p < 0.05$; n.s. not significant)

The total antioxidant capacity was measured using the phosphomolybdenum method. The values obtained ranged from 68.98±0.25 µg ascorbic acid/g for the methanol extract of leaves to 78.45±0.98 µg ascorbic acid/g for the chloroform extract of twigs.

In this study, the antioxidant activity of the chloroform and methanol extracts were evaluated using the DPPH and hydroxy radical scavenging, lipid peroxidation and metal chelating assays. The results of the antioxidant activities were compared with control antioxidants, gallic acid, ascorbic acid, BHT and α-tocopherol.

DPPH scavenging

The DPPH assay has been widely used to determine the free radical scavenging activity of various plant extracts. The IC_{50} for DPPH scavenging activity in various extract of *D. blagayana* leaves and twigs are shown in Table 2. It can be seen that IC_{50} values of all the tested extracts of *D. blagayana* were higher than 20 µg/ml and ranged from 20.25±1.55 to 25.24±0.15 µg/ml. The chloroform extract of twigs had the highest activity (IC_{50} = 20.25±1.55 µg/ml), followed in order by the methanol extract of leaves (IC_{50} = 20.95±0.99 µg/ml), the methanol extract of twigs (IC_{50} = 21.09±0.85 µg/ml) and the chloroform extracts of leaves (IC_{50} = 25.24±0.15 µg/ml).

Inhibitory activity against lipid peroxidation

Free radical scavenging is one of the known mechanisms by which antioxidant compounds inhibit lipid oxidation. Inhibitory activity against lipid peroxidation was measured using the thiocyanate method. The results of inhibitory activity against lipid peroxidation for the tested extracts of *D.blagayana* are shown in Table 3. The chloroform extract of leaves had the highest activity (IC_{50} = 33.23±0.99 µg/ml), followed in order by the methanol extract of leaves (IC_{50} = 34.65±0.89 µg/ml), the methanol extract of twigs (IC_{50} = 35.45±0.95 µg/ml) and the chloroform extract of twigs (IC_{50} = 36.46±1.68 µg/ml).

Table 2. DPPH free radical scavenging activity of the chloroform and methanol extracts of *D. blagayana*

Extracts of *D. blagayana*	IC_{50} (µg/ml)	Tukey`s HSD test
CHL extract of twigs	20.25±1.55	CHL extract of twigs/ CHL extract of leaves*
CHL extract of leaves	25.24±0.15	CHL extract of twigs/ MET extract of twigs$^{n.s.}$
MET extract of twigs	21.09±0.85	CHL extract of twigs/ MET extract of leaves$^{n.s.}$
MET extract of leaves	20.95±0.99	CHL extract of leaves/ MET extract of twigs*
Gallic acid	3.79±0.69	CHL extract of leaves/ MET extract of leaves*
Ascorbic acid	6.05±0.34	MET extract of twigs/ MET extract of leaves$^{n.s.}$
BHT	15.61±1.26	
ANOVA	*	

IC_{50} values (means ±SD) for chloroform and methanol extracts of *D. blagayana* compared with gallic acid, ascorbic acid and BHT. Data were zanalysed by analysis of variance (ANOVA) followed by Tukey`s HSD *post hoc* comparison test. (* $p < 0.05$; n.s. not significant)

Metal chelating ability

Results of metal chelating activity of the chloroform and methanol extracts are shown in Table 4. Based on the results obtained, it can be concluded that the constituents of *D. blagayana* extracts have the ability to form complexes

Table 3. Inhibitory activity against lipid peroxidation by chloroform and methanol extracts of *D. blagayana*

Extracts of *D. blagayana*	IC_{50} (µg/ml)	Tukey`s HSD test
CHL extract of twigs	36.46±1.68	CHL extract of twigs/ CHL extract of leaves $^{n.s.}$
CHL extract of leaves	33.23±0.99	CHL extract of twigs/ MET extract of twigs$^{n.s.}$
MET extract of twigs	35.45±0.95	CHL extract of twigs/ MET extract of leaves*
MET extract of leaves	34.65±0.89	CHL extract of leaves/ MET extract of twigs*
Gallic acid	255.43±11.68	CHL extract of leaves/ MET extract of leaves*
Ascorbic acid	> 1000	MET extract of twigs/ MET extract of leaves$^{n.s}$
BHT	0.23	
α-Tocopherol	0.48±0.05	
ANOVA	*	

IC_{50} values (means ±SD) for chloroform and methanol extracts of *D. blagayana* compared with gallic acid, ascorbic acid, BHT and α-Tocopherol. Data were zanalysed by analyses of variance (ANOVA) followed by Tukey`s HSD *post hoc* comparison test. (*$p < 0.05$; n.s. not significant)

Table 4. Metal chelating activity of chloroform and methanol extracts of *D. blagayana*

Extracts of *D. blagayana*	IC_{50} (µg/ml)	Tukey`s HSD test
CHL extract of twigs	45.91±0.88	CHL extract of twigs/ CHL extract of leaves $^{n.s.}$
CHL extract of leaves	45.24±0.95	CHL extract of twigs/ MET extract of twigs*
MET extract of twigs	41.09±1.15	CHL extract of twigs/ MET extract of leaves*
MET extract of leaves	40.95±1.09	CHL extract of leaves/ MET extract of twigs*
Gallic acid	-	CHL extract of leaves/ MET extract of leaves*
Ascorbic acid	-	MET extract of twigs/ MET extract of leaves $^{n.s}$
BHT	-	
α-Tocopherol	-	
ANOVA	*	

IC_{50} values (means ±SD) for chloroform and methanol extracts of *D. blagayana* compared with gallic acid, ascorbic acid, BHT and α-Tocopherol. Data were zanalysed by analyses of variance (ANOVA) followed by Tukey`s HSD *post hoc* comparison test. (*p < 0.05; n.s. not significant)

Table 5. Hydroxyl radical scavenging activity of chloroform and methanol extracts of *D. blagayana*

Extracts of *D. blagayana*	IC_{50} (µg/ml)	Tukey`s HSD test
CHL extract of twigs	99.11±0.23	CHL extract of twigs/ CHL extract of leaves *
CHL extract of leaves	90.26±0.69	CHL extract of twigs/ MET extract of twigs$^{n.s.}$
MET extract of twigs	98.98±0.97	CHL extract of twigs/ MET extract of leaves*
MET extract of leaves	85.88±0.94	CHL extract of leaves/ MET extract of twigs*
Gallic acid	59.14±1.10	CHL extract of leaves/ MET extract of leaves*
Ascorbic acid	160.55±2.31	MET extract of twigs/ MET extract of leaves *
BHT	33.92±0.79	
ANOVA	*	

IC_{50} values (means ±SD) for chloroform and methanol extracts of *D. blagayana* compared with gallic acid, ascorbic acid and BHT. Data were zanalysed by analyses of variance (ANOVA) followed by Tukey`s HSD *post hoc* comparison test. (*p < 0.05; n.s. not significant)

with ferrous ions. As shown in Table 4, the chloroform extract of twigs had the highest value among the extracts examined (45.91±0.88 µg/ml), followed in order by the chloroform extract of leaves (45.24±0.95 µg/ml), the methanol extract of twigs (41.09±1.15 µg/ml) and the methanol extract of leaves (40.95±1.09 µg/ml).

Hydroxyl radical scavenging activity

The results of hydroxyl radical scavenging activity are shown in Table 5. For the tested extracts, the chloroform extract of twigs had the highest IC_{50} value (99.11±0.23 µg/ml), followed in order by the methanol extract of twigs (98.98±0.97 µg/ml), the chloroform extract of leaves (90.26±0.69 µg/ml) and the methanol extract of leaves (85.88±0.94 µg/ml).

DISCUSSION

In conclusion, this is the first study focused on the determination of total phenolic and flavonoid content and antioxidant activity of *D. blagayana* leaves and twigs. Our recent study investigated other similar species of the genus *Daphne* which found that methanol extracts of leaves and twigs of the plant *Daphne cneorum* have good antimicrobial and antioxidant activities (26). There are similarities between these two plants, as the methanol extract of *D. cneorum* showed a similar antioxidant activity to the methanol extract of *D. blagayana*. IC_{50} values for *D. blagayana* DPPH scavenging activity, inhibitory activity against lipid peroxidation, metal chelating activity and hydroxyl radical scavenging activity are highly comparable with the corresponding values for *D. cneorum*. Small differences between these plants (mostly less than 10%) in the examined parameters are more likely attributable to random measurement variations through repeated experiments than to genuine features of the two species. In accordance with present knowledge, it seems that phenolic and flavonoid contents play an important role in the biological activity of individual species within the *Daphne* genus. Natural phenols and flavonoids have been reported to be associated with antioxidant activity in biological systems, mainly due to their redox properties, which can play an important role in absorbing and neutralising free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (23). One of the more prominent properties of flavonoids is their excellent radical scavenging ability, which makes them valuable for therapeutic and prophylactic applications, e.g., after infection, inflammation, burns, or radiation injury (24). The activity of crude methanol extracts is due to the presence of flavonoid monomers and polymers (condensed tannins), hydrolysable tannins, and phenolics. Recently, polyphenolic compounds from plants, such as condensed and hydrolysable tannins, have been shown to be powerful antioxidants (25). The amount of total phenols varied widely in extracts and ranged from 75.88±0.54 to 90.26±0.69 mg GA/g. Among the samples tested, the chloroform extract of twigs had the highest phenolic (90.26±0.69 mg GA/g) and flavonoid (35.24±0.55 mg GA/g) contents. Analysis of variance (ANOVA) showed significant differences in the presence of total phenolics and flavonoids among the tested extracts. In order to determine statistical significance in the amount of total

phenols and flavonoids from the plant parts, Student's t-test was used. Based on the results obtained, it can be seen that there is a statistically significant difference between the total phenolic and flavonoid content of twigs and leaves, although the difference is not significant for the methanol extracts (Table 1). Variations in the total antioxidant capacity between chloroform and methanol extracts were statistically significant. Unlike chloroform extracts, methanol extracts of the twigs and leaves did not show a significant difference in the total antioxidant capacity (Table 1). In the DPPH assay, the chloroform extract of twigs showed the lowest IC_{50} value (20.25 ±1.55 μg/ml) among the samples tested, while the chloroform extract of leaves had the highest IC_{50} values. Analysis of variance of the results of DPPH activity confirmed the existence of significant differences among four tested extracts and the standards that were used. Tukey *post hoc* test for analysis of variance indicated that there are no statistically significant differences in DPPH activities between the chloroform extracts of twigs and methanol extract of twigs and leaves. For the reducing capacity of the extracts, IC_{50} values for ferrous ion chelating ability were approximately 45 μg/ml for the chloroform extracts and approximately 41 μg/ml for the methanol extracts. The metal chelating activities differ significantly, depending on the solvent. For the inhibitory activity against lipid peroxidation, IC_{50} values ranged from 33.23 ±0.99 μg/ml for the chloroform extract to 36.46 ±1.68 μg/ml for the chloroform extracts of twigs. The tested extracts showed significantly better inhibitory activity then ascorbic and gallic acids, and a lower activity then vitamin E and BHT. Tukey`s HSD *post hoc* test of the extracts showed no statistically significant difference in inhibitory activity for the parts of the plant if the same solvent is used to obtain the extract. There is a statistical significance between the chloroform and methanol extracts (Table 3). The results of hydroxyl radical scavenging activity showed higher values for the tested extracts than ascorbic acid, but lower activity than gallic acid and BHT. IC_{50} values ranged from 85.88 ±0.94 μg/ml for the methanol extract of leaves to 99.11 ±0.23 μg/ml for the chloroform extract of twigs. Statistical analysis showed that there is no statistically significant difference in activity between the chloroform and methanol extracts of twigs. The *Daphne* genus includes approximately 70 different species, and several are reported to possess significant antioxidant activity (26,27). This makes plants of the genus *Daphne* very attractive as a source of future drugs. Of course, future research should incorporate a detailed phytochemical analysis of *Daphne blagayana*, isolation of potential antioxidants and their antioxidant activity.

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