

REGULAR ARTICLE

Evaluation of antioxidant potential of two *Daphne* species (*D. gnidioides* and *D. pontica*) from Turkey

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ABSTRACT

Daphne species have been used traditionally for treatment of several ailments including rheumatism, abscess and diarrhoea. Purpose of present study was to evaluate antioxidant activities of methanol and water extracts of *Daphne* parts (roots, stems and leaves). Different experimental models including phosphomolybdenum, DPPH, β -carotene/linoleic acid, ferric and cupric reducing power assays were performed to characterize the antioxidant potentials of the extracts. Folin-Ciocalteu and AlCl_3 methods were also used to quantify total phenolic and flavonoid content, respectively. *D. pontica* leaf extract showed high phenolic contents (123.09 mgGAEg⁻¹ in methanol and 146.94 mgGAEg⁻¹ in water extract) therefore has the strongest antioxidant potential. Present study showed that *Daphne* extracts (*D. gnidioides* and *D. pontica*) can be considered as good sources of natural antioxidants for medicinal and food applications.

Keywords: Antioxidants; *Daphne*; Phenolics; Flavonoids; DPPH

INTRODUCTION

Oxidative stress is defined as the imbalance between production of free radicals and antioxidants. The situation is responsible for causing several diseases including cancer, cardiovascular diseases and neural disorders (Tolluec et al., 2010). Antioxidants are substances present at low concentrations, which significantly inhibit or delay the oxidative process. Besides, playing an important role in physiological systems, antioxidants have been used in food processing to prolong the shelf life of foods, especially those rich in polyunsaturated fats. Therefore, several types of synthetic antioxidants (BHA, BHT, PG or TBHQ) are widely used food industry to prevent lipid oxidation. However, the use of these compounds is restricted due to potential health risks such as carcinogenic (Ito et al., 1986; Kim et al., 2010; Sherwin et al., 1990). Hence, development of novel antioxidants from natural resources continues to arouse great attention, and in recent years, medicinal plants have attracted great attention in the search of natural antioxidants.

The genus *Daphne* belongs to the family Thymelaceae and is represented by 70 species, of which seven species

grow in Turkey. These species are *D. gnidioides*, *D. mezereum*, *D. mucronata*, *D. oleoides*, *D. pontica* and *D. serica* (Tan, 1982). *Daphne* species are used as folk herbal medicine in many countries for the treatment of several ailments. For example, *D. giraldii*, *D. tangutica*, *D. retusa* and *D. koreana* are used in China for the treatment of aches and rheumatism, especially for rheumatoid arthritis (Yang, 1991). Fruits of *D. mezereum* are used for ulcers, rheumatism and skin diseases (Grieve and Leyel, 1967). In Turkey, *D. oleoides* subsp. *oleoides* is used as remedy for rheumatism, wound healing and abscess (Sezik et al., 2001). *Daphne pontica* L. is a shrub grown in the northern Anatolia. Moreover, the stem bark of *D. pontica* is used against diarrhea (Yesilada et al., 1999). Phytochemical investigations showed that the *Daphne* species contain several types of natural compounds including flavonoids, coumarins and diterpenoids (Zhang et al., 2006; Zhang et al., 2008). Also, in our previous study (Zengin et al., 2013) antioxidant properties of different extracts of *D. oleoides* subsp. *oleoides* and *D. serica* were reported. To the best of our knowledge, no antioxidant investigation has been reported on extracts of *D. gnidioides* and *D. pontica* parts yet.

The aim of the current study was to investigate the *in vitro* antioxidant effects of these species in order to determine

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the significance in terms of pharmacological and food industry. For this purpose, two extracts (methanol and water) obtained from roots, stems and leaves of two *Daphne* species were investigated with different *in vitro* antioxidant models including phosphomolybdenum, DPPH, β -carotene/linoleic acid, ferric and cupric reducing power assays.

MATERIALS AND METHODS

Plant materials

Daphne species were collected from different localities in Turkey during vegetation season of 2011. The plants were identified by botanist Dr. M. Aydin Sanda. The voucher specimens were deposited in Selcuk University Education Faculty Herbarium, Konya. The complete list of plant materials with taxonomical information, location and voucher number is given below:

1. *Daphne gnidioides* Jaub. & Spach: Antalya: Akseki, Murtiçi, Çukurköy, 673 m, 36S 395817E; 4082048N, Zengin and Sanda 1001.
2. *Daphne pontica* L.: Bursa: 409 m, 291230.3E; 400951.4N, Zengin and Sanda 1002.

Preparation of the extracts

All the plant materials (roots, stems and leaves) were dried at room temperature. The dried plant materials were ground to a fine powder using a laboratory mill. Fifteen grams of powdered plant parts were mixed with 250 mL methanol and extracted in a Soxhlet apparatus for 6-8 h. The extracts were concentrated under vacuum at 40°C by using a rotary evaporator. For water extraction, plant parts (15 g) were heated with 250 ml deionized water for 30 min. The aqueous extracts were filtered and freeze dried (-80°C, 48 h). Extracts were stored at + 4°C in dark until use.

Chemicals

Potassium ferricyanide, ferric chloride, Folin-Ciocalteu's phenol reagent, trichloroacetic acid, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and methanol were purchased from Merck (Darmstadt, Germany). 2,2-diphenyl-1-picrylhydrazyl (DPPH), β -carotene, linoleic acid and Tween 40 were purchased from Sigma Chemical Co. (Sigma-Aldrich GmbH, Sternheim, Germany). All other chemicals and solvents were analytical grade.

Assay for total phenolics

Total phenolic constituents of the extracts were determined by employing the methods given in the literature (Slinkard and Singleton, 1977) involving Folin-Ciocalteu reagent and gallic acid as standard. 1 mL of extract solution containing 2 mg was added to a volumetric flask. 45 mL distilled water and 1 mL Folin-Ciocalteu reagent was added and

flask was shaken vigorously. After 3 min, 3 mL of Na₂CO₃ (2%) solution was added and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 760 nm (Shimadzu-UV1800). The total phenolic content was determined as gallic acid equivalents (mg GAE g⁻¹ extract).

Assay for total flavonoids

The total flavonoid content in extracts was determined spectrophotometrically according to Arvouet-Grand et al. (1994). Briefly, 1 mL of 2% aluminium trichloride (AlCl₃) methanolic solution was mixed with the same volume of extract solution (at 2 mg mL⁻¹ concentration). The absorbance values of the reaction mixtures were determined at 415 nm after 10 min duration against a blank. Rutin was used as the standard and the total flavonoids content of the extracts was expressed as mg rutin equivalents per gram of extract (mg RE g⁻¹ extract).

Evaluation of total antioxidant capacity by phosphomolybdate assay

The total antioxidant capacities of extracts were evaluated by phosphomolybdenum method according to Prieto et al. (1999). 0.3 mL of sample extracts (2 mg mL⁻¹) were combined with 3 mL reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95 °C for 90 min and the absorbance of the solution was measured at 695 nm against a blank. The antioxidant capacity of extracts was expressed as equivalents of ascorbic acid (mg AE g⁻¹ extract).

Scavenging activity on DPPH

The hydrogen atoms or electrons donating ability of the corresponding extracts and some pure compounds were measured from the bleaching of purple colored methanol solution of DPPH. The effect of the extracts on DPPH radical was estimated according to Sarikurkcu et al. (2009). 1 mL of various concentrations of the methanolic extract was added to 4 mL of 0.004% methanol solution of DPPH. The mixture was shaken vigorously and allowed to stand for 30 min and the absorbance of the resulting solution was measured at 517 nm. Inhibition was calculated as follows:

$$I(\%) = (A_0 - A_1) / A_0 \times 100$$

Where A₀ is the absorbance of the control, A₁ is the absorbance of the extract/standard. 50% of free radical inhibition (IC₅₀) of extract was calculated. The lower the IC₅₀ value indicates high antioxidant capacity.

β -carotene-Linoleic acid bleaching assay

In this assay antioxidant capacity was determined by measuring inhibition of the volatile organic compounds and conjugated dienehydroperoxides arising from linoleic

acid oxidation (Dapkevicius et al., 1998). A stock solution of β -carotene–linoleic acid mixture was prepared as follows: 0.5 mg β -carotene was dissolved in 1 mL of chloroform (HPLC grade). 25 μ L linoleic acid and 200 mg Tween 40 were added. Chloroform was completely evaporated using a vacuum evaporator. Then 100 mL of oxygenated distilled water was added with vigorous shaking; 2.5 mL of this reaction mixture was transferred to test tubes and 0.35 mL of the extracts (2 mg mL⁻¹) were added and the emulsion system was incubated for up to 2 h at 50 °C. The same procedure was repeated with the positive control BHT, BHA and a blank. After this incubation period, absorbance of the mixtures was measured at 490 nm. Measurement of absorbance was continued until the color of β -carotene disappeared. The bleaching rate (R) of β -carotene was calculated according to Eq. (1).

$$R = \ln(a/b)/t \quad (1)$$

Where, \ln =natural log, a =absorbance at time 0, b =absorbance at time t (120 min). The antioxidant activity (AA) was calculated in terms of percent inhibition relative to the control using Eq. (2).

$$AA = [(R_{Control} - R_{Sample}) / R_{Control}] \times 100 \quad (2)$$

Antioxidative activities of the extracts were compared with those of BHT and BHA at 2.0 mg/mL.

Reducing power activity (Iron (III) to iron (II) reduction)

The ferric reducing power method was applied with slight modifications of the method of Oyaizu (1986). Various concentrations of the extracts (2.5 mL) were mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. After 2.5 mL of 10% trichloroacetic acid was added. 2.5 mL of the reaction mixture was mixed with 2.5 mL distilled water and 0.5 mL of 0.1% ferric chloride. The solution absorbance was measured at 700 nm. The reducing power of samples increased with the absorbance value. The same procedure was applied with BHA and BHT.

Cupric ion reducing antioxidant capacity (CUPRAC assay)

The cupric ion reducing capacity of extracts was determined according to the method of Apak et al. (2006). 1 mL each of 10 mM CuCl₂, 7.5 mM neocuproine, and NH₄Ac buffer (1 M, pH 7.0) solutions were added into a test tube. Then, 0.5 mL different concentrations of the extracts were mixed and total volume was brought up to 4.1 mL with deionized water. The mixture absorbance was recorded against a blank at 450 nm after 30 min incubation at room temperature.

RESULTS AND DISCUSSION

Total phenolic and flavonoid contents

Leaves of *D. pontica* had the highest level of phenolics in both methanol (123.09 mgGAE/g extract) and water extracts (146.94 mgGAEg⁻¹ extract) (Table 2). The leaves contained twice the total phenolic content than leaves of *D. gnidioides* in methanol extracts. The highest total phenolic contents were observed in the water extracts, except for root extracts. Zengin et al. (2013) found high variability in the polyphenol content of different extracts from two *Daphne* taxa (*D. serica* and *D. oleioides* subsp. *oleioides*), with values ranging from 3.18 mgGAEg⁻¹ extract in *D. oleioides* subsp. *oleioides* hexan extract to 391.97 mgGAEg⁻¹ extract in *D. oleioides* subsp. *oleioides* ethanol extract. Finally, ethanol extracts from aerial parts of two *Daphne* species possessed the highest level phenolics in the extracts. Similarly, many literatures indicated that amount of total phenolic is greatly depending on the extraction solvent use (Bekir et al., 2013; Fernandez-Agullo et al., 2013).

Amounts of total flavonoids were higher in methanol extracts than in water extracts (Table 2). The values varied from 3.25 mgREg⁻¹ in water extract of leaves of *D. gnidioides* to 91.22 mgREg⁻¹ in methanol extract of leaves of *D. pontica*. Leaf extracts showed a much higher total flavonoid concentration than the rest of the parts. These values are lower than those found by Zengin et al. (2013) in ethanol

Table 1: Total phenolic, flavonoid and antioxidant capacity (by phosphomolybdenum assay) in methanolic and water extracts from different parts of two *Daphne* species

| Solvent | Assays | <i>D. gnidioides</i> | | | <i>D. pontica</i> | | |
|----------|--------|----------------------|-------------|--------------|-------------------|--------------|--------------|
| | | Roots | Stems | Leaves | Roots | Stems | Leaves |
| Methanol | TPC | 91.72±2.12* | 86.85±5.47 | 63.20±0.14 | 91.57±1.77 | 60.64±2.06 | 123.09±3.62 |
| | TFC | 49.81±0.47 | 30.72±0.12 | 91.22±12.77 | 11.11±0.90 | 42.49±0.27 | 79.40±0.20 |
| | TAC | 262.18±8.64 | 226.29±5.34 | 119.62±9.74 | 254.29±18.54 | 237.96±3.61 | 276.62±0.47 |
| Water | TPC | 89.76±0.07 | 110.99±7.53 | 92.77±1.35 | 76.15±2.84 | 76.61±4.76 | 146.94±4.97 |
| | TFC | 8.50±0.01 | 3.25±0.59 | 33.58±1.06 | 5.22±0.08 | 8.11±0.86 | 46.83±0.08 |
| | TAC | 503.84±3.46 | 512.07±0.62 | 340.18±17.13 | 624.07±17.28 | 572.84±18.70 | 682.73±19.80 |

TPC: Total phenolic content (mgGAE g⁻¹ extract), TFC: Total flavonoid content (mgRE g⁻¹ extract), TAC: Total antioxidant capacity by phosphomolybdenum assay (mgAE g⁻¹ extract). *Values expressed are means±S.D. of three parallel measurements

Table 2: Absorbances obtained from reducing power assays for methanolic and water extracts of different parts of two *Daphne* species

| Solvent | Concentration (mg mL ⁻¹) | <i>D. gnidioides</i> | | | <i>D. pontica</i> | | | Syntethic antioxidants | |
|----------|---|----------------------|-------------|-------------|-------------------|-------------|-------------|------------------------|-------------|
| | | Roots | Stems | Leaves | Roots | Stems | Leaves | BHA | BHT |
| Ferric | | | | | | | | | |
| Methanol | 0.2 | 0.327±0.01* | 0.153±0.02 | 0.283±0.04 | 0.502±0.06 | 0.291±0.013 | 0.670±0.01 | 2.225±0.092 | 1.827±0.005 |
| | 0.4 | 0.664±0.01 | 0.357±0.02 | 0.645±0.01 | 1.022±0.044 | 0.610±0.018 | 1.627±0.07 | - | - |
| | 1 | 1.480±0.07 | 0.847±0.03 | 1.402±0.10 | 2.278±0.046 | 1.362±0.189 | 3.101±0.263 | - | - |
| Water | 0.2 | 0.182±0.03 | 0.138±0.01 | 0.093±0.02 | 0.203±0.01 | 0.254±0.01 | 0.698±0.01 | - | - |
| | 0.4 | 0.457±0.052 | 0.392±0.04 | 0.311±0.01 | 0.591±0.07 | 0.693±0.05 | 1.642±0.05 | - | - |
| | 1 | 0.870±0.01 | 0.774±0.04 | 0.616±0.06 | 1.00±0.04 | 1.196±0.02 | 3.010±0.01 | - | - |
| Cupric | | | | | | | | | |
| Methanol | 0.2 | 0.483±0.081 | 0.503±0.120 | 0.497±0.113 | 0.459±0.083 | 0.269±0.034 | 0.631±0.026 | 3.508±0.192 | 3.398±0.037 |
| | 0.4 | 1.010±0.042 | 0.909±0.051 | 1.066±0.134 | 0.941±0.026 | 0.607±0.025 | 1.338±0.084 | - | - |
| | 1 | 2.103±0.250 | 1.930±0.226 | 2.388±0.088 | 2.075±0.030 | 1.422±0.071 | 3.007±0.158 | - | - |
| Water | 0.2 | 0.469±0.021 | 0.440±0.010 | 0.452±0.010 | 0.396±0.013 | 0.347±0.01 | 1.015±0.051 | - | - |
| | 0.4 | 0.781±0.010 | 0.818±0.030 | 0.857±0.021 | 0.787±0.010 | 0.697±0.035 | 2.007±0.035 | - | - |
| | 1 | 1.878±0.158 | 1.835±0.010 | 1.896±0.057 | 1.874±0.028 | 1.657±0.030 | 3.971±0.021 | - | - |

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

extracts of *D. oleoides* subsp. *oleoides* (94.17 mgREg⁻¹) and *D. serica* (180.19 mgREg⁻¹). These differences probably can be caused by several factors such as extraction procedures, climatic conditions. According to our knowledge, there is very little information on the total phenolic and flavonoid content of Thymelaeaceae species. From a study of antioxidant activity of *Thymela hirsuta* performed by Akrouit et al. (2010), total phenolic and flavonoid content was found to 463.21 mgGAeg⁻¹ and 36.80 mgREg⁻¹ for 50% ethanol extracts, respectively.

DPPH free radical scavenging activity

The concentration of sample required to scavenge 50% of DPPH were determined (IC₅₀). A lower IC₅₀ value corresponds to a higher antioxidant activity of plant extract. Results are represented in Fig. 1. Water extract of *D. pontica* with IC₅₀ value of 0.08 mg mL⁻¹, showed particularly high free radical scavenging activity. The activities of the six methanol extracts used were in the order: *D. pontica* leaves>*D. pontica* roots>*D. gnidioides* roots> *D. pontica* stems>*D. gnidioides* stems >*D. gnidioides* leaves. Apparently, methanolic extract of leaves of *D. gnidioides* showed four times lower activity than *D. pontica* which possessed higher concentration of phenolics. In this direction, many studies showed positive strong correlation between free radical scavenging activity and total phenolic content. Among the studied extracts, water extract of *D. gnidioides* have very weak activity with IC₅₀ value of 0.587 mg mL⁻¹. Interestingly, water extracts of root and stems of *D. gnidioides* revealed same free radical scavenging activity. When compared to BHA (IC₅₀: 0.01 mg mL⁻¹) and BHT (IC₅₀: 0.02 mg mL⁻¹), all tested extracts was found to be less effective than synthetic antioxidants (data is not shown in Figure 1). Free radical scavenging activity of *D. oleoides* subsp. *oleoides* and *D. serica* differed significantly depending on solvent used and the highest activity was reported in ethyl acetate extract of *D.*

oleoides subsp. *oleoides* by Zengin et al. (2013). From these results, the ethyl acetate extract (IC₅₀: 0.065 mg mL⁻¹) demonstrated more potent free radical scavenging activity than the water extract of *D. pontica* leaves.

Determination total antioxidant capacity by phosphomolybdenum and β-carotene/linoleic acid bleaching assays

Phosphomolybdenum assay is an alternative to the evaluation of the total antioxidant capacity of plant extracts or compounds. Table 1 showed that both methanol and water extracts of leaves of *D. pontica* has the higher antioxidant than the other extracts (276.62 mgAEg⁻¹ for methanol extract, 682.73 mgAEg⁻¹ for water extract). The antioxidant activity of water extract of *D. pontica* leaves was 2-fold higher than that on *D. gnidioides* leaves. From the results obtained phosphomolybdenum assay it can be concluded that methanol extract has the poorest activity.

β-carotene/linoleic acid bleaching method measured the ability of an antioxidant to inhibit lipid peroxidation (Burda and Oleszek, 2011). The results obtained in this assay are an important issue in food processing and preservation. Fig. 2 shows the antioxidant activity of *Daphne* extracts as measured by the bleaching of β-carotene. In this assay, the antioxidant capacities in methanolic extracts were higher than water extracts. Methanolic extract of leaves of *D. pontica* was the most effective, with an inhibition value of 89.96% which close to the activity of the standard commercial antioxidants, BHA (93.37%) and BHT (94.32%). A relationship between the DPPH scavenging ability and β-carotene bleaching extent was found. In both assays, the polar methanolic extracts (except for *D. pontica* leaves) showed better antioxidative capacity than water extracts. Accordance with these results, the highest inhibition capacities were obtained from alcoholic extracts

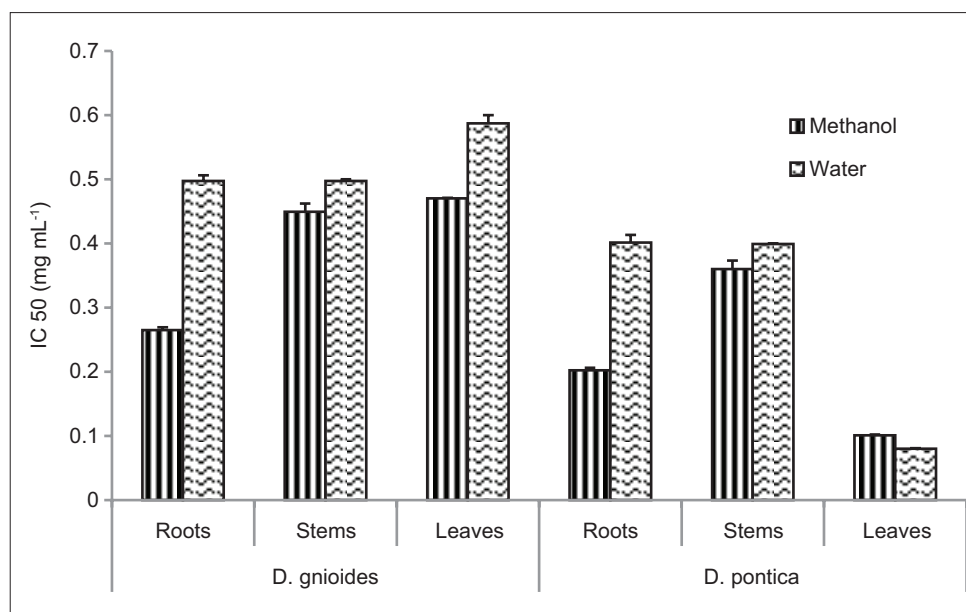


Fig 1. Free radical scavenging activities of methanolic and water extracts of different parts of two *Daphne* species by DPPH assay. (Values expressed are means \pm S.D. of three parallel measurements).

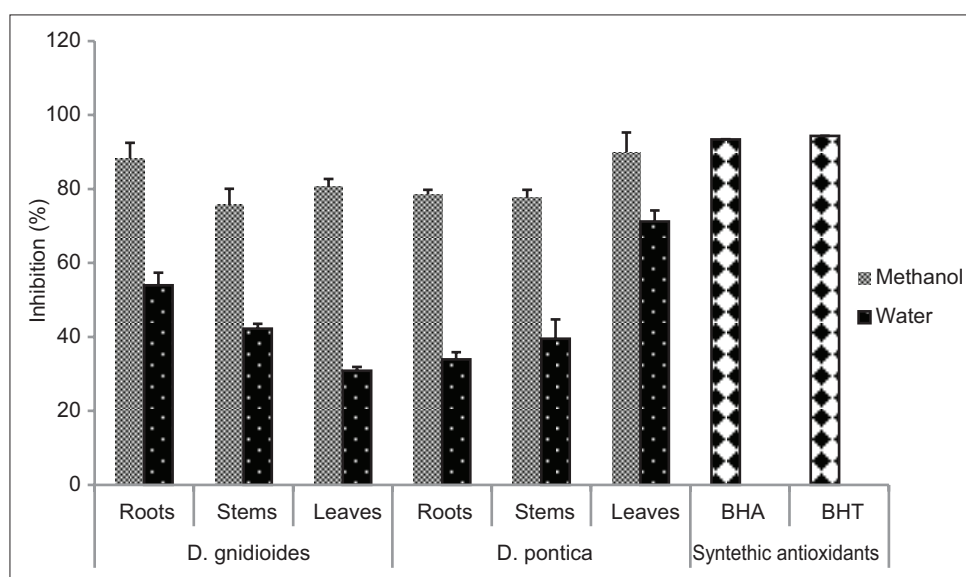


Fig 2. Antioxidant activity (%) of *Daphne* extracts by and synthetic antioxidants by β -carotene bleaching method (Values expressed are means \pm S.D. of three parallel measurements).

(ethanol and methanol) of *D. oleoides* subsp. *oleoides* and *D. serica* (Zengin et al., 2013).

Reducing power assays

Reducing power assays are often used as an indicator of electron-donating activity, which are an important mechanism of antioxidant compounds, especially phenolics. Therefore, potassium ferricyanide and CUPRAC methods were applied to evaluate reducing power potentials of *Daphne* extracts. Table 2 shows the reducing power of *Daphne* extracts using the potassium ferricyanide reducing method. The results showed that all extracts have a ferric

reducing power in a dose-dependent manner. The highest activity was noted for leaves of *D. pontica* extracts which had ferric reducing power approximately 5 times greater than that water extract of leaves of *D. gnidioides*. Consistent with this, Zengin et al. (2013) reported that methanol extract of *D. serica* had the highest ferric reducing power activity in other extracts (hexan, ethylacetate and ethanol). However, the reducing activities of all extracts were lower than BHA and BHT. The absorbance of BHA and BHT were 2.225 and 1.827 at concentration of 0.2 mg mL⁻¹, respectively. As shown in Table 2, cupric reducing power activity of the extracts was dependent on the extract concentration.

Table 3: Correlation analysis results of all *Daphne* extracts obtained for different antioxidant assays

| | TPC | TFC | TAC | DPPH | Ferric reducing power | Cupric reducing power | Inhibition (%) |
|-----------------------|-----|-------|--------|-------|-----------------------|-----------------------|----------------|
| TPC | 1 | 0.071 | 0.430 | 0.811 | 0.707 | 0.805 | 0.542 |
| TFC | | 1 | -0.583 | 0.384 | 0.476 | 0.345 | -0.369 |
| TAC | | | 1 | 0.243 | 0.135 | 0.346 | 0.841 |
| DPPH | | | | 1 | 0.978 | 0.890 | 0.253 |
| Ferric reducing power | | | | | 1 | 0.819 | 0.141 |
| Cupric reducing power | | | | | | 1 | 0.312 |

The highest cupric reducing power was obtained for water extract of leaves of *D. pontica* and the lowest for methanol extract of stems of *D. pontica*.

Principal Component Analysis (PCA) and correlation analysis

It would be interesting to examine the correlations between antioxidant activities assays applied in the present work. For this reason, correlation analysis was applied to the data obtained by antioxidant assays and the results are presented in Table 3. High correlations were obtained between DPPH and ferric reducing power as well as with cupric reducing power ($p < 0.05$). TPC is significantly correlated with DPPH radical scavenging activity, ferric and cupric reducing power. Therefore, phenolic compounds are the major contributors of antioxidant potential. In accordance with our results, many authors observed the strongest correlation between the total phenolic content and antioxidant capacity methods (Egea et al., 2010; Erkan et al., 2011; Sahreen et al., 2010). Ferric reducing power activities were more associated with cupric reducing power activities than inhibition activity of linoleic acid oxidation. Good correlation was observed among total antioxidant capacity assays, namely phosphomolybdenum and β -carotene/linoleic acid assays ($p < 0.05$). Conversely, a weak correlation was found among TFC and antioxidant capacity assays (DPPH, ferric and cupric reducing power). Similarly, some authors found weak correlation between antioxidant assays and TFC of several plant extracts (Muanda et al., 2011; Saeed et al., 2012). Principal component analysis (PCA) was also used to evaluate the correlation among antioxidant capacity assays (Fig. 3). The loading of first and second principle components (PC1 and PC2) accounted for 55.83% and 32.03% of the variance, respectively. PC1 was heavily loaded on TPC, DPPH, cupric and ferric reducing power assays, whereas PC2 was loaded on TFC and total antioxidant activity (TAC and inhibition activity). Similarly, Keles et al. (2011) showed that DPPH, phenolic and reducing power were heavily loaded on Factor 1 in their PCA, when methanolic extracts of wild edible mushrooms were analyzed.

CONCLUSION

The extracts of two *Daphne* species exhibited good but different levels of antioxidant activity in all *in vitro*

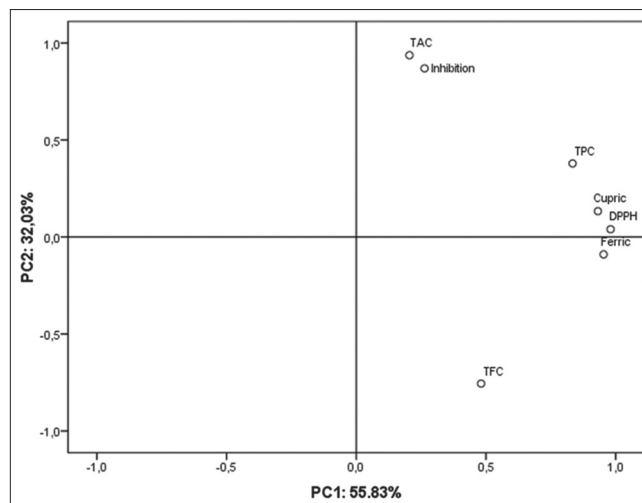


Fig 3. Principal component analysis results of TPC, TFC, TAC, DPPH, Inhibition (β -carotene/linoleic acid assay), Cupric and Ferric Reducing Powers.

models. Both methanol and water extracts of *D. pontica* leaves showed lower IC_{50} values and had higher level of total phenolic content in comparison with other *Daphne* extracts. Our study is the first report on the *in vitro* antioxidant properties of the methanol and water extracts prepared from different parts (root, stem and leaves) of *Daphne* species. From these results, *Daphne* species studied can be used as accessible source of natural antioxidants and are useful as potential food supplements. In the future efforts will be made to identify the main components responsible for the antioxidant effect in *Daphne* extracts.

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Author contributions

M. A. S. and G. Z. made a major contribution to the paper. A. A. and Y. S. C. was involved in overall planning and supervision.

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