



The Antioxidant Activities and Total Phenol Contents of Eleven Turkish Medicinal Plants

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Article info:

Received: 05.04.2019

Accepted: 04.11.2019

Keywords:

antioxidant activity,
total phenol content,
medicinal plant

Abstract

In this study, we assessed antioxidative activities and total phenol contents of *Achillea biebersteinii*, *A. setacea*, *Anthemis pseudocotula*, *A. tinctoria* var. *tinctoria*, *Artemisia austriaca*, *Cistus laurifolius*, *Nepeta italica*, *Paliurus spina-christi*, *Primula vulgaris*, *Rubus sanctus*, and *Salvia verticillata* used as folk medicine in Anatolia. The antioxidant activities of decoctions or infusions prepared from plants were evaluated by *in vitro* models including, ABTS radical scavenging assay, metal chelating assay, and ferric reducing power. Except for *A. tinctoria* var. *tinctoria* infusion in reducing power capacity assay and *P. spina-christi* decoction in metal chelating activity, all extracts displayed high antioxidant activity in all three antioxidant assays. In particular, *C. laurifolius* infusion showed a potent antioxidant effect as well as all references used to evaluate activity. The phenol contents of the extracts were in the range 262.70 ± 2.06 - 48.10 ± 0.68 mg gallic acid equivalent/g extract. The findings justify folkloric use of these plants used in the treatment of diseases such as colds, cough, colds, tonsillitis and bronchitis.

1. Introduction

Living organisms generate reactive oxygen species (ROS) as by products through several physiological and biochemical pathways. Over production of ROS can induce cellular damage and is associated with many pathological conditions including cancer, cardiovascular diseases, diabetes, neurological disorders, autoimmune diseases (Uttara, Singh, Zamboni, & Mahajan, 2009). Antioxidants minimize the damage caused by ROS or have protective effect against oxidative damage. On the other hand, serious side effects can be observed in long-term use of synthetic derived antioxidants. Today, medicinal plants used by traditional healers are valuable sources for the discovery of new antioxidant sources.

In this report, we evaluated the antioxidant activities of eleven medicinal plants used in the traditional Turkish folk medicine. *Anthemis* species are popularly used in pharmaceuticals, cosmetics and food industry. For example, the flowers of *A. tinctoria* have been used as natural dyes in colour industry (Hartl, & Vogl, 2003). *Salvia* species are important plants in the family Lamiaceae. They are utilized as herbal tea and for food flavouring, as well as in cosmetics, perfumery and the pharmaceutical industries (Baratta, Dorman, Deans, Figueiredo, Barroso, & Ruberto, 1998; Chalchat, Michet, & Pasquier, 1998). Also, *Cistus* and *Salvia* species have been used against high fever, rheumatic pain, peptic ulcer, stomachache, catarrh, cold, wounds in Turkish folk medicine (Üstün, Özçelik, & Baykal, 2016). On the other hand, the genus *Artemisia* has been utilized in the liqueur-making industry (Sengul, Ercisli, Yildiz, Gungor, Kavaz, & Çetin, 2011).

The decoctions or infusions of *Artemisia* and *Achillea* species are widely used in treatment of cold, cough, catarrh, tonsillitis and bronchitis in Anatolia. *Nepeta italica* and *Rubus sanctus* are used in treatment of cold as herbal tea (Yeşilada, Honda, Sezik, Tabata, Goto, & Ikeshiro, 1993; Başer, Tümen, Malyer, & Kırimer, 2006). Utilization of *Paliurus spina-christii* against sore throat, *Primula vulgaris* as expectorant in the form of an infusion in traditional Turkish folk medicine (Sezik, Zor, & Yeşilada, 1992). These findings indicate that selected plants have both medicinal properties and industrial potential.

This study was conducted to assess selected Turkish medicinal plants (*Achillea biebersteinii* Afan., *A. setacea* L., *Anthemis pseudocotula* Boiss., *A. tinctoria* var. *tinctoria* L., *Artemisia austriaca* L., *Cistus laurifolius* L., *Nepeta italica* L., *Paliurus spina-christii* Mill., *Primula vulgaris* Huds., *Rubus sanctus* Schreb., and *Salvia verticillata* L.) for their antioxidant activity and total phenolic content. We investigated the antioxidant potentials of the extracts using three different models viz. DPPH, ABTS radicals scavenging and metal chelating activities.

2. Materials and methods

2.1. Plant material

The collection sites and times, herbarium numbers of the plant materials were given in Table 1. All the species were identified by Professor Dr. Mecit Vural of Department of Biology, Faculty of Science and Art, Gazi University, Ankara, Turkey. Voucher specimens are stored in the Herbarium of the Department of

Table 1. Collection sites and herbarium numbers, families, parts of the plants.

Plant name	Family	Part used	Herbarium number	Collection site
<i>Achillea biebersteinii</i> Afan.	Asteraceae	Inflorescence	2930	Palandöken, Erzurum
<i>Achillea setacea</i> Waldst& Kit.	Asteraceae	Inflorescence	3034	Akşehir, Konya
<i>Anthemis pseudocotula</i> Boiss.	Asteraceae	Inflorescence	2966	Akşehir, Konya
<i>Anthemis tinctoria</i> var. <i>tinctoria</i> L.	Asteraceae	Inflorescence	2967	Akşehir, Konya
<i>Artemisia austriaca</i> L.	Asteraceae	Inflorescence	2945	Palandöken, Erzurum
<i>Cistus laurifolius</i> L.	Cistaceae	Leaves	2489	Kurtboğazi, Ankara
<i>Nepeta italica</i> L.	Lamiaceae	Aerial parts	2953	Işık Mountain, Ankara
<i>Paliurus spina-christi</i> Mill.	Rhamnaceae	Flower	2955	Gülнар Plateau, Mersin
<i>Primula vulgaris</i> Huds.	Primulaceae	Leaf	2958	Işık Mountain, Ankara
<i>Rubus sanctus</i> Schreb.	Rosaceae	Aerial parts	2960	Beypazarı, Ankara
<i>Salvia verticillata</i> L.	Lamiaceae	Leaf	2061	Işık Mountain, Ankara

Table 2. Extraction types and extraction yields, total phenolic contents of the plants.

Plant name	Extract type	Extraction yield (%)	Total phenolic content mg GAE/g extract
<i>A. biebersteini</i>	Decoction	24.83	62.10± 0.75
<i>A. setacea</i>	Decoction	14.52	194.50 ± 2.32
<i>A. pseudocotula</i>	Infusion	11.91	64.70 ± 0.83
<i>A. tinctoria</i> var. <i>tinctoria</i>	Infusion	27.73	91.50 ± 1.71
<i>A. austriaca</i>	Decoction	20.74	83.50 ± 1.35
<i>C. laurifolius</i>	Infusion	13.50	402.10 ± 3.81
<i>N. italica</i>	Infusion	32.73	48.10 ± 0.68
<i>P. spina-christii</i>	Decoction	30.65	171.90 ± 1.22
<i>P. vulgaris</i>	Infusion	30.00	75.50 ± 0.81
<i>R. sanctus</i>	Decoction	23.42	262.70 ± 2.06
<i>S. verticillata</i>	Decoction	35.39	232.10 ± 2.11

* Values are mean ± SEM of 3 replications

Pharmacognosy, Faculty of Pharmacy, Gazi University, Ankara, Turkey (GUE). The collection sites, collection times and herbarium numbers of the plant materials are given in Table 1.

2.2. Preparation of plant extracts

For decoctions, 1 g of air-dried and powdered plant material was added to 100 ml distilled water and boiled on slow heat for 30 minutes. Infusions were prepared by pouring 100 ml of boiling water onto 1 g of dried plant material. The extraction continued for 30 min while cooling. Aqueous extracts were then

filtered and concentrated using a rotary evaporator at 45°C. Extraction yields after freeze-drying were given on Table 2.

2.3. Determination of total phenolic content

Total phenolic content of extracts was determined by Folin Ciocalteu's reagent. The samples (0.25 ml) or gallic acid were put into test tubes; 2.5 ml of Folin-Ciocalteu's reagent and 2 ml of sodium carbonate were added. The tubes were vortexed and incubated at room temperature for 15 min. Afterward absorption was measured at 765 nm. The total phenol values are expressed in terms of gallic acid equivalent (GAE) (Singleton, Orthofer, & Lamuela-Raventos, 1999).

2.4. Antioxidant activity

2.4.1. ABTS^{•+} Radical Scavenging Capacity

ABTS^{•+} radical scavenging activity was determined according to Re et al. (1999). The 2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) radical cation (ABTS^{•+}) was formed by reacting ABTS aqueous solution (7 mM) with 2.45 mM of ammonium persulfate. The ABTS^{•+} radical solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. 10 µl of six different concentrations (10-3000 µg/ml) of each extract was added to 0.990 µl of diluted ABTS^{•+} radical solution. After 6 min., the absorbance reading was taken at 734 nm. by using the microplate reader (VersaMax, Molecular Devices, Sunnyvale, CA, USA). All analyses were done at least in triplicate. Trolox was used as positive control.

2.4.2. Metal chelating capacity

100 µl of six different concentrations 10, 30, 100, 300, 1000 and 3000 µg/ml of each extract, which dissolved

in methanol, was mixed with 10 µl of aqueous FeCl₂. After 5 min incubation at room temperature, the reaction was initiated by 20 µl ferrozine. After 10 min, the absorbance at 562 nm was measured against blank solution by using the microplate reader (VersaMax, Molecular Devices, Sunnyvale, CA, USA). Disodium EDTA was used as positive control (Dinish, Madeira, & Almeida, 1994).

2.4.3. Reducing power capacity

The reducing powers of the extract were estimated by described by Oyaizu (1986). Extracts (50 µl) were mixed with 0.2 M phosphate buffer (50 µl, pH 6.6) and 1% potassium ferricyanide (50 µl) in a 96 microwells plate. After 20 min. incubation at 50 °C, 10% trichloroacetic acid (50 µl) and 0.1% ferric chloride (10 µl) were added to the mixture. The reaction mixture was incubated at 50 °C for 10 min., and the absorbance was measured at 700 nm. by using the microplate reader (VersaMax, Molecular Devices, Sunnyvale, CA, USA). Ascorbic acid was used as positive control.

2.5. Statistical Analysis

All values are expressed as the mean \pm the standard error of the mean (SEM); linear regression analyses and correlation coefficients to determine the relationship between 2 variables were calculated using MS-DOS software (GraphPad InStat statistical program).

3. Results

3.1. Total phenol contents

Total phenol contents of all extracts were determined using the linear calibration curve ($y = 0.0053 \times +$

0.177, $r^2 = 0.9974$) and were expressed as GAE/g extract. Total phenol contents of the plants were presented in Table 2. The highest amount of total phenol was 262.7 ± 2.06 mg GAE/g extract in *R. sanctus* extract, while the lowest total phenol content was 62.1 ± 0.75 mg GAE/g extract in *A. biebersteinii* extract. High phenolic contents were also found in *S. verticillata* (232.1 ± 2.11) and *A. setacea* (19.45 ± 2.32) extracts. Results of the study showed that the phenolic compound of the plants extracts tested varied from 45.96 to 728.43 mg GAE/g (Table 2).

3.2. Antioxidant activity

3.2.1. Reducing power capacity

Reducing capacities of different concentrations of extracts and ascorbic acid were shown in Table 3. The reducing capacities of *S. verticillata* (2.912 ± 0.364), *N. italica* (2.472 ± 0.159), *A. austriaca* (2.452 ± 0.101), *C. laurifolius* (2.302 ± 0.078), *R. sanctus* (2.281 ± 0.167), and *P. spina-christii* (2.045 ± 0.347) extracts with increasing concentration were found to be higher or close to that of ascorbic acid (2.199 ± 0.158). Among the extracts, *A. tinctoria* var. *tinctoria* (0.029 ± 0.001) had the lowest reducing power activity.

3.2.2. ABTS^{•+} Radical Scavenging Activity

ABTS radical scavenging activity of the extracts was compared to Trolox. Results were expressed as inhibition % of ABTS^{•+} radical formation. Except for *A. setacea*, *A. tinctoria* var. *tinctoria*, *A. austriaca*, *N. italica* and *S. verticillata* extracts, ABTS radical scavenging activity of all extracts was found to be close to that of Trolox (99.9 %). Among the extracts, *P. spina-christii* (99.9 %), *A. pseudocotula* (99.7 %),

and *C. laurifolius* (99.3 %) extracts possessed the highest radical scavenging activity (Table 4).

3.2.3. Metal chelating activity

Ferrozine forms color complexes with Fe^{2+} . In the presence of an effective chelating compound, the complex formation is disrupted and so the red color of the complexes decreases. In this work, all extracts and EDTA showed dose-dependent metal chelating activity. *C. laurifolius* extract had the highest chelating ability with 99.6 % activity when compared with EDTA which has the highest chelating ability at 3000 μ g/ml with 99.7 % (Table 5). On the other hand, *P. spina-christii* (25.6 %) extract exhibited extremely low metal chelating activity. It was observed that the metal chelating activities of the other extracts vary between 96.5 ± 0.3 and 61.9 ± 0.6 % at 3000 μ g/ml.

4. Conclusion

To our knowledge, there is no antioxidant activity study on traditionally prepared decoctions or infusions of plants used as folk remedies in this study. In general, all extracts tested showed strong and moderate effects in three antioxidant activity methods. The results showed that especially *C. laurifolius* infusion have potent antioxidant activity in all assays. As we mentioned earlier, ROS are key signalling molecules that act an important role in the progression of inflammatory diseases. In this work, in general, all extracts exhibited ABTS radical scavenging activity at different levels. These results can be considered as one of the mechanisms that explain the effects of the plants tested on the pathogenesis of diseases related to their folkloric use.

Table 3. Reducing capacities of different concentrations of extracts and ascorbic acid

Plant name	Reducing capacity (Absorbance \pm SEM)					
	3000 μ g/ml	1000 μ g/ml	300 μ g/ml	100 μ g/ml	30 μ g/ml	10 μ g/ml
<i>A. biebersteini</i>	1.169 \pm 0.138	0.938 \pm 0.045	0.363 \pm 0.026	0.133 \pm 0.003	0.047 \pm 0.001	0.021 \pm 0.002
<i>A. setacea</i>	1.540 \pm 0.045	0.824 \pm 0.026	0.320 \pm 0.005	0.152 \pm 0.033	0.048 \pm 0.015	0.016 \pm 0.001
<i>A. pseudocotula</i>	1.601 \pm 0.586	1.468 \pm 0.243	0.718 \pm 0.025	0.251 \pm 0.006	0.097 \pm 0.005	0.053 \pm 0.003
<i>A. tinctoria</i> var. <i>tinctoria</i>	0.029 \pm 0.001	0.015 \pm 0.001	-0-	-0-	-0-	-0-
<i>A. austriaca</i>	2.452 \pm 0.101	1.484 \pm 0.147	0.624 \pm 0.009	0.233 \pm 0.005	0.077 \pm 0.001	0.036 \pm 0.003
<i>C. laurifolius</i>	2.302 \pm 0.078	1.887 \pm 0.509	1.763 \pm 0.011	0.927 \pm 0.016	0.338 \pm 0.004	0.129 \pm 0.001
<i>P. spina-christii</i>	2.045 \pm 0.347	2.037 \pm 0.067	1.209 \pm 0.084	0.447 \pm 0.007	0.152 \pm 0.001	0.047 \pm 0.001
<i>N. italica</i>	2.472 \pm 0.159	1.079 \pm 0.069	0.402 \pm 0.019	0.148 \pm 0.001	0.047 \pm 0.002	0.007 \pm 0.002
<i>P. vulgaris</i>	1.868 \pm 0.186	1.119 \pm 0.007	0.482 \pm 0.006	0.168 \pm 0.002	0.055 \pm 0.003	0.018 \pm 0.001
<i>R. sanctus</i>	2.281 \pm 0.167	2.156 \pm 0.254	1.775 \pm 0.071	0.726 \pm 0.009	0.224 \pm 0.006	0.088 \pm 0.004
<i>S. verticillata</i>	2.912 \pm 0.364	1.962 \pm 0.059	1.735 \pm 0.170	0.581 \pm 0.016	0.223 \pm 0.002	0.064 \pm 0.001
Ascorbic acid	2.199 \pm 0.158	2.072 \pm 0.587	2.058 \pm 0.120	1.331 \pm 0.106	0.462 \pm 0.008	0.169 \pm 0.002

* Values are mean \pm SEM of 3 replications**Table 4.** ABTS⁺ radical scavenging activities of different concentrations of extracts and Trolox

Plant name	% inhibition of ABTS ⁺ radical formation \pm SEM					
	3000 μ g/ml	1000 μ g/ml	300 μ g/ml	100 μ g/ml	30 μ g/ml	10 μ g/ml
<i>A. biebersteini</i>	97.5 \pm 0.9	70.4 \pm 0.8	27.0 \pm 2.9	16.8 \pm 0.8	0.7 \pm 0.3	-0-
<i>A. setacea</i>	62.1 \pm 0.4	26.5 \pm 1.2	16.6 \pm 1.0	9.3 \pm 1.2	23.3 \pm 0.7	12.3 \pm 1.5
<i>A. pseudocotula</i>	99.7 \pm 0.1	39.7 \pm 0.7	14.1 \pm 0.4	2.3 \pm 0.1	-0-	-0-
<i>A. tinctoria</i> var. <i>tinctoria</i>	54.8 \pm 0.3	18.7 \pm 0.4	-0-	-0-	-0-	-0-
<i>A. austriaca</i>	79.1 \pm 0.2	28.7 \pm 0.1	24.4 \pm 1.4	10.2 \pm 0.9	-0-	6.7 \pm 0.3
<i>C. laurifolius</i>	99.3 \pm 0.0	99.8 \pm 0.1	42.3 \pm 2.6	14.4 \pm 0.7	4.9 \pm 0.1	-0-
<i>P. spina-christii</i>	99.9 \pm 0.1	93.4 \pm 0.8	34.0 \pm 0.7	14.8 \pm 0.7	4.3 \pm 0.5	-0-
<i>N. italica</i>	58.3 \pm 0.2	21.6 \pm 1.1	3.1 \pm 0.2	10.4 \pm 2.3	5.7 \pm 1.1	5.0 \pm 1.3
<i>P. vulgaris</i>	97.5 \pm 0.4	42.1 \pm 0.4	9.3 \pm 0.4	-0-	-0-	-0-
<i>R. sanctus</i>	98.9 \pm 0.1	86.3 \pm 1.1	41.7 \pm 1.2	18.4 \pm 2.4	4.2 \pm 0.5	1.5 \pm 0.3
<i>S. verticillata</i>	71.5 \pm 0.5	21.8 \pm 0.2	20.8 \pm 0.7	6.7 \pm 0.2	3.8 \pm 0.6	-0-
Trolox	99.9 \pm 0.1	99.9 \pm 0.1	54.7 \pm 0.6	16.4 \pm 1.3	9.6 \pm 0.7	10.1 \pm 0.3

* Values are mean \pm SEM of 3 replications

Table 5. Metal chelating effect of different concentrations of extracts and EDTA

Plant name	Metal Chelating activity %±SEM					
	3000 µg/ml	1000 µg/ml	300 µg/ml	100 µg/ml	30 µg/ml	10 µg/ml
<i>A. biebersteini</i>	78.9±0.4	50.9±1.5	26.4±0.5	14.8±1.1	5.0±1.3	-0-
<i>A. setacea</i>	96.5±0.3	84.3±1.4	28.9±2.3	16.8±0.9	15.9±0.2	10.9±0.9
<i>A. pseudocotula</i>	83.4±0.3	49.9±0.7	19.0±0.5	9.8±0.8	5.5±1.0	3.3±0.4
<i>A. tinctoria</i> var. <i>tinctoria</i>	61.9±0.6	42.0±0.6	30.3±1.0	19.7±0.8	14.0±1.4	2.2±0.5
<i>A. austriaca</i>	72.0±0.7	37.5±0.6	15.3±0.4	4.5±0.6	-0-	-0-
<i>C. laurifolius</i>	99.6±0.1	80.4±1.2	30.1±0.9	11.4±0.5	1.2±0.8	-0-
<i>P. spina-christii</i>	25.6±1.1	16.1±0.9	3.5±0.8	-0-	-0-	-0-
<i>N. italica</i>	79.4±0.8	53.4±0.2	19.7±0.8	12.2±0.1	7.0±1.2	3.6±1.0
<i>P. vulgaris</i>	92.5±0.3	71.0±0.2	38.3±0.8	14.5±1.0	6.7±1.3	2.1±0.9
<i>R. sanctus</i>	90.1±0.5	41.6±1.4	15.8±2.0	5.7±0.9	-0-	-0-
<i>S. verticillata</i>	76.9±0.1	68.3±0.9	25.0±0.9	2.2±0.1	-0-	-0-
EDTA	99.7±0.1	99.8±0.1	99.9±0.1	55.0±2.9	-0-	-0-

* Values are mean ± SEM of 3 replications

Except for *P. spina-christi* decoction, metal chelating activity of all extracts was found to be extremely high. Oxidative damage also depends on the availability of free metal ions. An increased level of ferrous ions might result from release of these ions from body under inflammatory conditions. Antioxidant plant extracts capable of chelating Fe²⁺ will reduce the ion's concentration and inhibit its capacity to catalyze free radical formation, resulting in protection against oxidative damage and related diseases (Wu et al., 2006). Compounds or plant extracts with reducing capacity are electron donors and can reduce the oxidized intermediates of lipid peroxidation, so that they can play role as primary and secondary antioxidants. Reducing capacity of all the extracts except *A. tinctoria* var. *tinctoria* infusion was found to be strong when compared with ascorbic acid (Sharma & Joshi, 2011).

The results of the chemical study previously performed on these plants are as follows: flavonoids, terpenoids, and volatile oil for *A. tinctoria* var. *tinctoria*, *A. biebersteinii*, *A. austriaca*, *A. pseudocotula*, and *Nepeta* spp. (Öksüz, Gümüş, & Alpınar, 1991; Akgul & Saglikoglu, 2005; Kurtulmus et al., 2009; Formisano, Rigano, & Senatore, 2011); flavonoids, terpenoids, tannins and polyphenols for *C. laurifolius*; flavonoids and phenolic acid derivatives for *R. sanctus* (Süntar, Koca, Keleş, & Küpeli Akkol, 2011); flavonoids, triterpenoids, and alkaloids for *P. spina-christi* (Güner, 2005); saponin, volatile oil, flavone glycoside, and sugar for *P. vulgaris* (Ünal, Yentür, Cevahir, Sarsağ, & Kösesakal, 2003); flavonoids, catechins, diterpenoids, triterpenoids, and volatile oil for *S. verticillata* (Askun, Tumen, Satil, & Ateş, 2009).

We found no significant correlation between total phenolic content and antioxidant activity of the extracts. Non-phenolic compounds in studied plant samples may play a role in their antioxidant activities (Stępień, Aebisher, & Bartusik-Aebisher, 2018).

As a result, we can say that the antioxidant activity results of the plants tested support their folkloric uses in diseases like tonsillitis, bronchitis and cold. Additionally, this study proved that these plants can be used as a natural source of antioxidants. Further studies should be conducted on isolation of compounds responsible for activity from these plants with strong antioxidant activity.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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