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Research Article

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Antioxidant potentials of the extracts from 14 selected medicinal plants

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ABSTRACT

Most of the medicinal plants possess interesting antioxidant properties. The present study aimed to evaluate the antioxidant capacity of some medicinal plants from Turkey, such as *Anthemis tinctoria* L. (Compositae), *Inula britannica* L. (Compositae), *Malabaila secacul* Banks & Sol (Apiaceae), *Zosima absinthifolia* (Vent) Link (Apiaceae), *Thymus sipyleus* Boiss. (Lamiaceae), *Phlomis armeniaca* Willd. (Lamiaceae), *Sideritis galatica* Bornm. (Lamiaceae), *Sedum acre* L. (Crassulaceae), *Potentilla erecta* Uspenski ex Ledeb. (Rosaceae), *Digitalis lamarckii* Ivan (Scrophulariaceae), *Glaucium grandiflorum* Boiss. & Huet var. *grandiflorum* (Papaveraceae), *Fumaria asepala* Boiss. (Papaveraceae), *Centranthus longiflorus* Stev. (Valerianaceae), *Allium rotundum* L. (Amaryllidaceae). The ethyl acetate and methanol extracts of the 14 species were screened by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, superoxide anion (SO) radical scavenging, and lipid peroxidation (LPO) assays. The methanol and ethyl acetate extracts of *Potentilla erecta* have the highest DPPH scavenging activity (IC_{50}=0.014 and 0.03 mg/mL, respectively). The maximum inhibition of LPO has been exhibited by ethyl acetate extract of *Glaucium grandiflorum* var. *grandiflorum* (IC_{50}=0.34 mg/mL) followed by methanol extracts of *T. sipyleus* (IC_{50}=0.38 mg/mL). The methanol extract of *A. rotundum* demonstrated the highest SO activity (IC_{50}=0.11 mg/mL). In conclusion, these extracts have a high potential for antioxidant activity may be considered to use free radical-related diseases.

KEYWORDS: Antioxidant, DPPH, extract, lipid peroxidation, medicinal plants, superoxide

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INTRODUCTION

Free radicals, reactive oxygen (ROS), and nitrogen (RNS) species are derived from exogenous and endogenous sources and produced by normal metabolism. Moreover, free radicals are involved in various physiological and pathological conditions. When there are imbalance oxidants and antioxidant system, the reactive species accumulate, causing extensive damage to cells and tissues may lead to the development of chronic diseases in various conditions [1-5].

In recent years, there has been a worldwide trend towards the use of natural products and natural antioxidants. These natural antioxidants from plant materials are mainly polyphenols (phenolic acids, flavonoids, anthocyanins, lignans, and stilbenes), carotenoids (xanthophylls and carotenes) and vitamins (vitamin E and C) [6,7]. Antioxidant-rich diets are thought to reduce the oxidative damage of DNA [8]. Plants have been used in traditional medicines throughout the world for thousands of years [9]. The medicinal plants have always been used, and still, remain a significant source in the treatment of several diseases, including inflammatory and oxidative-stress associated chronic diseases. In addition, the medicinal plants are considered as valuable sources of potential therapeutic agents and a significant source of natural antioxidants that might serve for the development of novel drugs [10]. The antioxidant capacity of medicinal plants depends on their components which they have possessed, in particular, phenolic compounds to interrupt and migrate oxidation [11]. The high interest in natural products is not only due to toxic concern but also because of its consumption in natural food [12].

In the present study, we focused on using available and fundamental experimental techniques to identify natural antioxidants from plants. Therefore, the current research deals with a preliminary screening of some medicinal plants for their antioxidant activities. The ethyl acetate and methanol extracts obtained from the selected plants growing wild, which are almost known for their beneficial effects, have been investigated. At this moment, this study describes the antioxidant capacity of various plant species, such as *Anthemis tinctoria* L. (Compositae), *Inula britannica* L. (Compositae), *Malabaila secacul* Banks & Sol (Apiaceae), *Zosima absinthifolia* (Vent) Link (Apiaceae), *Thymus sipyleus* Boiss. (Lamiaceae), *Phlomis armeniaca* Willd. (Lamiaceae), *Sideritis galatica* Bornm. (Lamiaceae), *Sedum acre* L. (Crassulaceae), *Potentilla erecta* Uspenski *ex* Ledeb. (Rosaceae), *Digitalis lamarckii*

Ivan (Scrophulariaceae), *Glaucium grandiflorum* Boiss. & Huet var. *grandiflorum* (Papaveraceae), *Fumaria asepala* Boiss. (Papaveraceae), *Centranthus longiflorus* Stev. (Valerianaceae), *Allium rotundum* L. (Amaryllidaceae) using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, superoxide anion radical scavenging assay, and lipid peroxidation assay.

MATERIALS AND METHODS

Collection and Identification of the Plant Materials

The plant species were collected from Hasanoğlan village/Ankara/Turkey at an altitude of 1600-2000 m. Prof. Dr. Hayri DUMAN identified voucher specimens. The species were deposited for future reference in Herbarium of Ankara University, Faculty of Pharmacy (AEF). Therefore, the different botanical taxa studied in this work are shown in Table 1, together with some information concerning their families and herbarium numbers.

Solvents and Reagents

Solvents and chemicals used, which include ethyl acetate and methanol, were of analytical grade. Xanthine, xanthine oxidase, cytochrome c, 2,2-diphenyl-l-picrylhydrazyl (DPPH), iron (III) chloride ($FeCI_3$), thiobarbituric acid (TBA), sodium dodecyl sulfate (SDS), 1,1,3,3-tetramethoxypropane, butylated hydroxytoluene, and α-tocopherol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ascorbic acid, ethyl acetate, methanol, potassium hydrogen phosphate (K_2HPO_4), potassium phosphate monobasic (KH_2PO_4) were purchased from Merck (Germany).

Extraction of the Plants and Sample Preparation

The aerial parts of each species were separated 5 g and extracted with 150 mL ethyl acetate and 150 mL methanol, respectively. The extraction has been performed on a magnetic stirrer in an electric heater. After filtrated, each extract was evaporated under the rotavapor, and each extract was measured (Table 2) accurately.

Assessment of the Antioxidant Activity of the Plants

DPPH free radical scavenging capacity assay

DPPH (1,1-diphenyl-2-picrylhydrazine) free radical scavenging assay was conducted by using the Blois method [13] with minor modifications and α-tocopherol used as a standard. The stock solutions of the extracts were prepared at 10^{-2} M in DMSO. A series of solutions in DMSO were diluted to varying concentrations in 96-well microplates. Then, the methanolic DPPH solution (100 μm) was added to each well. The plate was shaken and placed in the dark. The optical density (OD) of the solution was measured at 517 nm, after 30 min. The methanolic solution of DPPH served as a control. The radical scavenging activities were expressed as the inhibition percentage and were calculated using the formula:

Table 1: The tested species and their herbarium numbers

No	Plant name	Family	AEF No
1	*Anthemis tinctoria* L.	Compositae	23168
2	*Inula britannica* L.	Compositae	23166
3	*Sedum acre* L.	Crassulaceae	23159
4	*Thymus sipyleus* Boiss.	Labiatae	23160
5	*Phlomis armeniaca* Willd.	Labiatae	23167
6	*Sideritis galactica* Bornm.	Labiatae	23156
7	*Potentilla erecta* L.	Rosaceae	23157
8	*Digitalis lamarckii* Ivan	Scrophulariaceae	23164
9	*Glaucium grandiflorum* Boiss. & Huet. var. *grandiflorum*	Papaveraceae	23161
10	*Fumaria asepala* Boiss.	Papaveraceae	23163
11	*Allium rotundum* L.	Amaryllidaceae	23155
12	*Centranthus longifolius* Stev.	Valerianaceae	23165
13	*Malabaila secacul* Banks & Sol.	Apiaceae	23154
14	*Zosima absinthifolia* (Vent.) Link	Apiaceae	23162

Table 2: The tested species and the amount of the extracts

Plants (Each 5 g)	EtOAc extract (mg)	MeOH extract (mg)
Anthemis tinctoria	210	1600
Inula britannica	210	1570
Sedum acre.	250	1360
Thymus sipyleus	290	950
Phlomis armeniaca	180	1820
Sideritis galactica	210	1650
Potentilla erecta	310	1540
Digitalis lamarckii	320	2850
Glaucium grandiflorum. var. *grandiflorum*	130	1250
Fumaria asepala	310	1980
Allium rotundum	190	1550
Centranthus longifolius	450	2160
Malabaila secacul	280	910
Zosima absinthifolia	140	1230

$$\% \text{ Inhibition} = (OD_{control} - OD_{sample})/OD_{control} \times 100$$

$OD_{control}$: The absorbance of the control with DMSO
OD_{sample}: The absorbance of the sample in the presence of the compounds.

A dose-response curve was plotted to determine the IC_{50} values. IC_{50} is described as the concentration sufficient to obtain 50% of a maximum scavenging capacity. All tests, and analyses were carried out in triplicate and averaged.

Superoxide anion scavenging capacity assay

The superoxide anion radical scavenging capacity of the extracts was determined by the modified method described by McCord and Fridovich [14]. The process is based on inhibition of cytochrome *c* (from horse heart, Sigma Co. St. Louis, MO) reduction spectrophotometrically. Superoxide anion was generated by the xanthine/xanthine oxidase (from milk, Sigma Co. St. Louis, MO) system. The reaction mixture has in a final volume of 1.0 mL, 0.05 M phosphate buffer pH 7.8, 0.32 Units/mL xanthine oxidase, 50 μM xanthine, 60 mM cytochrome *c* and different concentration of synthesized compounds at 100 μL solutions in DMSO/MeOH (5:95). Xanthine oxidase was finally added to this mixture to start the reaction. The absorbance was measured spectrophotometrically

at 550 nm for cytochrome *c* reduction. Each experiment was performed in triplicate. The results were expressed as % inhibition, and IC_{50} values were determined from calibration curves.

Lipid peroxidation assay

Lipid peroxidation of the extracts was determined by the modified method of Mihara et al. [15]. Lipid peroxidation was measured spectrophotometrically by the estimation of thiobarbituric acid-reactant substances (TBARS). Amounts of TBARS were expressed regarding nmol malondialdehyde (MDA)/g tissue. A typical optimized assay mixture contained 0.5 mL of liver homogenate, 0.1 mL of Tris-HCl buffer (pH 7.2), 0.05 mL of 0.1 mM ascorbic acid, 0.05 mL of 4 mM $FeCl_2$ and 0.05 mL of various concentration of crude extract, or α-tocopherol were incubated for 1 h at 37 °C. After incubation, 3.0 mL of H_3PO_4 and 1 mL of 0.6 % TBA were added and shaken vigorously. The mixture was boiled for 30 min. After cooling, *n*-butanol was added, and the mixture was shaken vigorously. Then, the *n*-butanol phase was separated by centrifugation at 3000 rpm for 10 min. The absorbance of the samples was read at 532 nm against a blank, which contained all reagents except liver homogenate.

RESULTS AND DISCUSSION

The extracts obtained from the medicinal plants were subjected to the evaluation of antioxidant activity using various *in vitro* models systems. Therefore, antioxidant potencies of methanol and ethyl acetate extracts of *Anthemis tinctoria* L. (Compositae), *Inula britannica* L. (Compositae), *Malabaila secacul* Banks & Sol (Apiaceae), *Zosima absinthifolia* (Vent) Link (Apiaceae), *Thymus sipyleus* Boiss. (Lamiaceae), *Phlomis armeniaca* Willd. (Lamiaceae), *Sideritis galatica* Bornm. (Lamiaceae), *Sedum acre* L. (Crassulaceae), *Potentilla erecta* Uspenski *ex* Ledeb. (Rosaceae), *Digitalis lamarckii* Ivan (Scrophulariaceae), *Glaucium grandiflorum* Boiss. & Huet var. *grandiflorum* (Papaveraceae), *Fumaria asepala* Boiss. (Papaveraceae), *Centranthus longiflorus* Stev. (Valerianaceae), *Allium rotundum* L. (Amaryllidaceae) was investigated in this study.

The ethyl acetate and methanol extracts of *Potentilla erecta* showed highest DPPH free radical scavenging activity (IC_{50} = 0.03 and 0.014 mg/mL, respectively). The ethyl acetate and methanol extracts of *Thymus spyleus* (IC_{50} = 0.03 and 0.019 mg/mL, respectively) and *Anthemis tinctoria* (IC_{50} = 0.06 and 0.020 mg/mL, respectively) exhibited remarkable DPPH free radical scavenging capacity (Table 3) compared to α–tocopherol (IC_{50} = 0.011 mg/mL). In addition, the ethyl acetate (IC_{50} = 0.10 mg/mL) and methanol extracts (IC_{50} = 0.042 mg/mL) of *Sedum acre* displayed DPPH free radical scavenging activity, fairly. In DPPH assay almost all tested methanolic extract of species showed radical scavenging activity.

Furthermore, the highest superoxide anion radical capacity (Table 4) was shown by the ethyl acetate extract of *Allium rotundum* (IC_{50} = 0.11 mg/mL) compared to α–tocopherol (IC_{50}=0.13 mg/mL). By the way, *Thymus sipyleus* (IC_{50} = 0.59 mg/mL), *Sedum acre* (IC_{50} = 0.63 mg/mL), *Malabaila secacul* (IC_{50}=0.88 mg/mL). *and Potentilla erecta* (IC_{50} = 0.94 mg/mL) ethyl acetate extracts also exhibited notable superoxide anion radical scavenging capacity.

Table 3: DPPH radical scavenging activity of ethyl acetate and methanol extracts

Plant extracts	IC_{50} (mg/mL) Ethyl acetate extracts	IC_{50} (mg/mL) Methanol extracts
Glaucium grandiflorum var. *grandiflorum*	0.25	0.055
Inula britannica	0.19	0.033
Digitalis lamarckii	0.17	0.069
Anthemis tinctoria	0.06	0.020
Phlomis armeniaca	0.13	0.052
Fumaria asepala	0.24	0.064
Thymus sipyleus	0.03	0.019
Sideritis galactica	0.18	0.049
Sedum acre	0.10	0.042
Centranthus longiflorus	0.31	0.045
Malabaila secacul	0.25	0.140
Allium rotundum	0.23	0.139
Zosima absinthifolia	0.22	0.134
Potentilla erecta	0.03	0.014
α-Tocopherol	0.011	

Table 4: Superoxide anion radical scavenging capacity of ethyl acetate and methanol extracts

Plant extracts	IC_{50} (mg/mL) Ethyl acetate extracts	IC_{50} (mg/mL) Methanol extracts
Glaucium grandiflorum var. *grandiflorum*	1.30	-
Inula britannica	1.12	8.50
Digitalis lamarckii	2.66	-
Anthemis tinctoria	1.78	1.92
Phlomis armeniaca	1.25	-
Fumaria asepala	1.96	-
Thymus sipyleus	0.59	8.14
Sideritis galactica	1.36	-
Sedum acre	0.63	2.87
Centranthus longiflorus	2.32	-
Malabaila secacul	0.88	-
Allium rotundum	0.11	-
Zosima absinthifolia	1.21	-
Potentilla erecta	0.94	6.11
α-Tocopherol	0.13	

However, the ethyl acetate extract of *Glaucium grandiflorum* var. *grandiflorum* (IC_{50} = 0.34 mg/mL) following by methanol extracts of *Thymus sipyleus* (IC_{50} = 0.38 mg/mL) showed the maximum lipid peroxidation activity (Table 5) compared to α–tocopherol (IC_{50}=0.084 mg/mL). In addition to these species, the ethyl acetate and methanol extracts of *Inula britannica* (IC_{50} = 0.39 and 0.41 mg/mL), *Phlomis armeniaca* (IC_{50} = 0.40 and 0.85 mg/mL), *Potentilla erecta* (IC_{50} = 0.85 and 0.41 mg/mL) and *Digitalis lamarckii* (IC_{50} = 0.43 and 0.54 mg/mL) have also exhibited pretty LPO inhibition. Antioxidant profile of the ethyl acetate and methanol extracts of the selected plants are shown in Tables 3-5.

In this assay, almost all tested methanolic extracts of the species showed inhibition of lipid peroxidation (Table 5). The mentionable effect were observed by *Thymus sipyleus* (IC_{50}=0.38 mg/mL), *Inula britannica* (IC_{50}=0.41 mg/mL), *Allium rotundum* (IC_{50}=0.44 mg/mL) extracts.

Table 5: Lipid peroxidation inhibition effects of ethyl acetate and methanol extracts

Plant extracts	IC_{50} (mg/mL) Ethyl acetate extracts	IC_{50} (mg/mL) Methanol extracts
Glaucium grandiflorum var. grandiflorum	0.34	0.76
Inula britannica	0.39	0.41
Digitalis lamarckii	0.43	0.54
Anthemis tinctoria	0.74	0.84
Phlomis armeniaca	0.40	0.85
Fumaria asepala	0.58	0.57
Thymus sipyleus	0.43	0.38
Sideritis galactica	1.01	3.44
Sedum acre	0.66	0.57
Centranthus longiflorus	0.67	0.52
Malabaila secacul	0.99	1.12
Allium rotundum	1.98	0.44
Zosima absinthifolia	2.10	0.87
Potentilla erecta	0.85	0.41
α-Tocopherol	0.084	

CONCLUSION

The screening of antioxidant potential of collected medicinal plants has been presented in this study. Some basic tests such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, superoxide anion radical scavenging assay, and lipid peroxidation is used to evaluate the characterization and potential range of antioxidant activity of plant extracts. This study discussed medicinally significant plant species have notable antioxidant activity when compared to synthetic antioxidants. It is known that many of these species have been considered with their prominent phenolic contents. We have focused on plants belonging to several different families to understand their therapeutic value and their potential antioxidant activities. According to our results, *Thymus sipyleus*, *Potentilla erecta*, and *Inula britannica* have the significant antioxidant potential. Of course, screening with *in vitro* assays has little meaning if there is no clear evidence of the effectiveness of the extracts *in vivo*. Therefore, further *in vivo* studies of these species are required, and a systematic investigation of these antioxidant-rich species is needed before they can be used in industry and medicine.

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COMPETING INTERESTS

The authors declare no conflict of interest.

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