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Original Article

In Vitro Screening of Natural Drug Potentials for Mass Production

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Abstract

Methanolic extracts of 16 plant species, used in herbal and traditional medicinal preparations, collected from the same geographic region (Golcuk-Seben/Bolu, Turkey) were evaluated for their relative total phenolic contents, total antioxidant activities and their mineral contents in both dried plant samples and methanolic extracts. These species included *Hypericum perforatum* L., *Thymus sipyleus* Boiss., *Equisetum arvense* L., *Achillea millefolium* L., *Tanacetum parthenium* L., *Thymus leucostomus* Hausskn. & Velen., *Usnea longissima*, *Herniaria incana* Boiss., *Teucrium polium* L., *Stachys bithynica* Boiss., *Anthemis nobilis* L., *Sambucus ebulus* L., *Berberis vulgaris* L., *Malus communis* Desf., *Matricaria chamomilla* L. and *Scorzonera laciniata* L. Spectrophotometric Folin-Ciocalteu and DPPH free radical scavenging activity methods were performed to detect the total phenolic contents and total antioxidant activities of the methanolic extracts, respectively. The results show that the fruits of *Berberis vulgaris* L., have the highest antioxidant activity and aerial parts of *Tanacetum parthenium* L. have the highest total phenolics (92.62%). Mineral contents were determined by inductively coupled – mass spectrometry (ICP-MS). All dried plant samples and methanolic extracts contained high amounts of Si, Cu, Fe and Ca. The highest levels of Si, Cu, Fe and Ca were found in *Equisetum arvense* L., *Anthemis nobilis* L., and *Thymus sipyleus* Boiss. dried plant samples, whereas Se was only detected in *Usnea longissima*. The highest levels of Si, Cu, Fe and Ca were found in the methanolic extracts of *Usnea longissima*, *Anthemis nobilis* L. and *Thymus sipyleus* Boiss. respectively.

Keywords: antioxidant, medicinal aromatic plants, mineral contents, phenolics

Introduction

Medicinal plants have a growing economic value in clinical, pharmaceutical, cosmeceutical, and other wide areas of international trade, although this varies widely among countries (Schmidt, 2012).

The plant extracts and phytochemicals isolated from these medicinal plants have been shown to exert biological activity *in vitro* and *in vivo*, which justified further research on traditional medicine focused on the characterization of their mostly antimicrobial and antioxidant activities (Deliorman Orhan *et al.*, 2012). It is well accepted that, reactive oxygen compounds such as singlet oxygen, superoxide radicals, hydrogen peroxide, hydroxyl radicals and nitric oxide are unstable and extremely reactive (Ames *et al.,*1993). Oxidative stress-induced reactive oxygen species are considered to be the indicators of the development and progress of various diseases. Antioxidants prevent the negative impacts of free radicals and reactive oxygen species and protect the body (Gavamukulya *et al.*, 2014). The widely known synthetic antioxidants are butylated hydroxyltoluene (BHT), butylated hydroxylanisole (BHA), propyl gallate and tertiary butylhydroquinone. However, the reliability of these synthetic antioxidants has been debated due to their toxic and carcinogenic effects, which can result in liver injury. Therefore, discovery of new and reliable antioxidants from natural resources has become a prominent research topic (Birman, 2012).

Flora of Gölcük-Seben, 100 km distance from Istanbul and Black sea region, which has been a little studied by scientific means before. This study provides an identification of potential, bioactive species that can be used as raw materials for plant derived products in several industries. The objectives of this study were to determine the relative total phenolic contents and antioxidant activity of 16 medicinal plant species including *H. perforatum* L., *T. sipyleus* Boiss., *E. arvense* L., *A. millefolium* L., *T. parthenium* L., *T. leucostomus* Hausskn. & Velen., *U. longissima*, *H. incana* Boiss., *T. polium* L., *S. bithynica* Boiss., *A. nobilis* L., *S. ebulus* L., *B. vulgaris* L., *M. communis* Desf., *M. chamomilla* L. and *S. laciniata* L. found in the Gölcük-Seben/Bolu area. The healing properties of these plants are very well known and used by local people in treatments of various diseases. Furthermore, some minerals,

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known to be useful for human health at certain dose levels, and which have a number of physiological functions in living organisms, were quantitatively determined both in dried plants and methanolic extracts. All these data will be used to help select the plant species, to be cultured in that region, if commercialization is warranted.

Materials and Methods

Site characterization

Collection site is located in an area of Euro-Siberian, Mediterranean and Irano-Turanian Floristic Regions together with Sub-Euxine and Xero-Euxine zones. The coordination of research area is given in Table 2. The soil type of the research area is mainly brown forest soil, which is extremely calcified. The structure of the soil is fine-pored or granulated and the pH of the soil is alkaline and rarely neutral. The study site, dating back to upper-Eocene (35-40 mya) of the Tertiary in the Cenozoic era, consists of different types of rock, including basalt, andesite, turf and aglomera of a variety of colours from pink to black to claret red. The surface morphology, formed by erosion and tectonic activities, is elaborated by metamorphite and volcanic rocky types (Ikinci and Guneri, 2007). According to the data obtained from the Bolu Meteorology Station, the mean annual temperature of the area is expected to be between 7.1 °C and 9.6 °C. Mean annual precipitation of the research area is expected to be between 599.6 and 869.6 mm. Of this precipitation 32% is in winter, 29% is in spring, 21% is in autumn and 18% is in summer. The rainiest months of the year are December, January and February whereas the least rainy month is August.

Plant materials and extraction of the plants

A list of the studied plants, including the botanical name, voucher specimen and data related to therapeutic usage and the plant parts used are listed in Table 1. Voucher specimens were identified by Dr. Mecit Vural and they were deposited in the Herbarium of Field Crop Department, Abant İzzet Baysal University (Bolu, Turkey). Plant samples were air dried at room temperature and 20 g powdered plant materials extracted by chloroform and evaporated till dryness. Then 80% methanol (2×250 mL) was added to the remaining material after chloroform filtration and again vaporized. Chloroform and methanolic extracts were used for further analyses.

Determination of total phenolic compounds

Total phenolic contents of sixteen extracts were determined by using Folin-Ciocalteau Method (Singleton and Rossi, 1965). From each extract, 2 mg mL^{-1} sample was weighed and dissolved in 75% ethanol. Reference gallic acid dilutions and each extract samples were mixed with 100 µL Folin-Ciocalteau reagent, sodium carbonate (20%) and distilled water. Mixtures were incubated for 30 minutes at 40°C and their absorbances were measured at 765 nm. According to the calibration curve, the total phenolic concentration was calculated as gallic acid equivalents using the absorbance of the samples.

Determination of total flavonoids

To determine total flavonoids, 2 mg mL^{-1} of each extract samples and the reference quercetin solutions were mixed with 75% EtOH, 10% $AlCl_3$, 1M sodium acetate and 2,800 µL distilled water separately. Mixtures were incubated for 30 minutes at room temperature and their absorbance was measured with a spectrophotometer at 415 nm. According to calibration curves, total flavonoids were calculated as quercetin equivalents using the absorbance of the samples (Woisky and Salatino, 1998).

Determination of radical scavenging activity

The method of diphenyl picrylhydrazyl (DPPH) was utilized to determine *in vitro* radical scavenging activity (Burda and Oleszk, 2001). 1 mg mL^{-1}, 0.5 mg mL^{-1}, 0.25 mg mL^{-1}, 0.125 mg mL^{-1}, 0.0625 mg mL^{-1} of extracts and butylated hydroxyanisole reference (BHA) were prepared in methanol. 1 mL of each extract and BHA were mixed with 2 mL of DPPH (0.1 mM in 70% methanol) and incubated for 30 minutes in the dark, at room temperature and their absorbances were measured at 517 nm. Antiradical activity was calculated as follows:

Scavenging activity (%) = (1-A1/A0) × 100, where:
A0: absorbance of control;
A1: absorbance of sample.

Determination of the concentration of minerals

The mineral content in plant samples was determined using inductively coupled – mass spectrophotometry (Perkin Elmer Nexion 350 D / ICP-MS) according to the NMKL 186 method. Sample preparation was conducted by adding 8 mL HNO_3 (65.0%) and 2 mL hydrogen peroxide (35.0%) to a 0.25 g extract/plant sample and completing up to 50 mL by distilled pure water, in a microwave system (Berghof MWS4). The measurements were calculated as dry weight in mg/kg according to the following formula:

$W = [(a \times V \times F) / m \times 1000]$, where:

a - is the content of the element in test solution in microgram per litre;

V - is the volume of the digestion solution after being made up, in milliliter;

F - is the dilution factor of the test solution;

m - is the initial sample mass, in gram.

Results

Antioxidant activities, total phenolic and flavonoid contents

In the present study, antioxidant activities were determined by using the DPPH method and the values varied between 99.53-19.65% (Table 3). *Berberis vulgaris* fruits had the highest antioxidant activity (99.53%) and it was followed by *Achillea millefolium* (97.21%), *Equisetum arvense* (92.55%) and *Thymus leucostomus* (91.63%). Total phenolic contents varied between 92.62-1.35% the highest value in *Tanacetum parthenium* and the lowest value in *Herniaria incana* L. (Table 4). In the present study, *Tanacetum parthenium* (37.39%) had the highest flavonoid contents and it was followed by *Achillea millefolium* (31.43%), *Matricaria chamomilla* (21.03%) and *Thymus sipyleus* (20.56%), respectively (Table 4).

Mineral substance content

The mean of the level of minerals determined in 16 medicinal plant samples is summarized in table 5-6. Se has important role on thyroid metabolism, improving and sustaining of nervous system (Georgieff, 2007; Skröder *et al.*, 2015). Neurological problems have occurred due to deficiency

Table 1. Chemical constituents of the 16 extracts with their traditional usage in Turkey

Scientific name	Family	Common name	Active constituents	Therapeutic use	Parts analyzed	Reference
Achillea millefolium L.	Asteraceae	Yarrow	Phenolic acids, tannins, flavonoids, essential oil, sesquiterpene lactones	Stomachic, spasmolytic, diuretic, anti-inflammatory	Aerial parts	Trouillasa *et al*., 2003
Anthemis nobilis L.	Asteraceae	Roman, Chamomile flower	Volatile oils, flavonoids, coumarins, phenolic acids	Antispasmodic and digestive; food preservative	Leaves and Flowers	Gardiner, 1999
Berberis vulgaris L.	Berberidaceae	Barberry	Berberine, berbamine, palmitine, jatrorrhizine, isotetrandrine	Sore throat, fever, tanning skin, antiviral	Fruit	Srivastava *et al*., 2015
Equisetum arvense L.	Equisetaceae	Horsetail	Phenolic acids, flavonoids, sterols, minerals, alkaloids, terpenoids, saponins, phytosterols, aminoacids	Diuretic, antioxidant vasorelaxant, antinociceptive, antiinflammatory	Aerial parts	Gallo *et al*., 2011
Herniaria incana Boiss.	Caryophyllaceae	Rupture worts	Terpenes	Hypertension, kidney antioxidant	Aerial parts	Parmer, 2012
Hypericum perforatum L.	Hypericaceae	St John's wort	Hyperforin, adhyperforin, hypericin, flavonol glycosides	Mild and moderate, depression, epilepsy	Shoot	Briskin, 2000
Malus communis Desf.	Rosaceae	Apple	Phenols, benzoic acids, flavonoids, phenyl propanoids	Anti-microbial, antiinflammator, anti-mutagenic, anti-carcinogenic, antiallergic, anti-platelet, vasodilatory actions	Fruit	Henríquez *et al*., 2010
Matricaria chamomilla L.	Asteraceae	German chamomile	Essential oils, sesquiterpene lactones, pectic	Antiinflammatory, antispasmodic, antioxidative, antibacterial, antifungal, anti-cancer, anti-allergic, anti-pyretic	Leaves and flowers	Heidari and Sarani 2012
Sambucus ebulus L.	Caprifoliaceae	Dwarf Elder	Dimeric and mucin binding lectins, protein derivatives, flavonoids, linear Incision	High fever, rheumatic pains, snake bites and wounds treat burns, infectious wounds, eczema, urticaria, rheumatism, inflammations	Fruits	Shokrzadeh and Saeedi Saravi 2010
Scorzonera laciniata L.	Asteraceae	Cutleaf vipergrass	Hexadecanoic acid, sesquiterpenoids	Fever, carbuncle, mastitis, arteriosclerosis, kidney diseases, hypertension, diabetes mellitus rheumatism	Aerial parts	Bosgelmez *et al*., 2007
Stachys bithynica Boiss.	Lamiaceae	Lamb's ear	Tannins, phenolic acids, flavonoids, polyphenols	Anti-inflammatory genital tumor, cancerous ulcer asthma, moderate earaches pain, cramps, dizziness, fever, gout	Aerial parts	Vundac *et al*., 2007
Tanacetum parthenium L.	Asteraceae	Feverfew	Sesquiterpene lactones, canin/artecanin	Migraines, inflammation, arthritis	Aerial parts	Raal *et al*., 2014

Teucrium polium L.	Lamiaceae	Felty Germander	Tyrosol, caffeic acid, ferulic acid, lutein flavanoid	Anti-inflammatory, anti-nociceptive, anti-bacterial, anti-hypertensive, hypolipidemic, anti-heumatoid, hypoglycemic diuretic, diaphoretic, tonic, antipyretic, antispasmodic cholagogic properties	Leaves and flowers	Sarac and Ugur, 2007
Thymus leucostomus Hausskn. & Velen.	Lamiaceae	Thyme	Essential oil, terpinene phenol	Haemorrhoid, rheumatism, stomach kidney ailments. anti-oxidative, antibacterial antiseptic	Aerial parts	Yücel, 2011
Thymus sipyleus Boiss.	Lamiaceae	Thyme	Essential oil, terpinene phenol	Haemorrhoid, rheumatism, stomach kidney ailments. anti-oxidative, antibacterial, antiseptic, antifungal	Aerial parts	Yiğit and Kandemir, 2002
Usnea longissima	Usneaceae	Methuselah's beard	Phenolic compounds, longissiminone, Glutinol	Pain relief fever control, lower respiratory infections, anti-inflammatory, analgesic, antipyretic, anti-tumor, anti cholesterol, nematocidal properties	Whole plant	Altınterim, 2012

Table 2. Collection sources of the plant material

Sample no.	Species	Altitude (m)	Coordinates
1	*Achillea millefolium* L.	1400-1550	40.497429, 31.607366
2	*Anthemis nobilis* L	1100	40.674996, 31.634132
3	*Berberis vulgaris* L.	1170 -1200	40.659762, 31.636621
4	*Equisetum arvense* L.	900	40.669235, 31.635162
5	*Herniaria incana* Boiss.	1400	40.655432, 31.634690
6	*Hypericum perforatum* L.	1100-1200	40.674996, 31.634132
7	*Malus communis* Desf.	1010	40.453554, 31.591659
8	*Matricaria chamomilla* L.	900	40.669235, 31.635162
9	*Sambucus ebulus* L.	900	40.669235, 31.635162
10	*Scorzonera laciniata* L.	1100-1200	40.674996, 31.634132
11	*Stachys bithynica* Boiss.	1080	40.674996, 31.634132
12	*Tanacetum parthenium* L.	1080	40.674996, 31.634132
13	*Teucrium polium* L.	1350-1450	40.585104, 31.633273
14	*Thymus leucostomus* Hausskn. & Velen.	1110	40.462697, 31.595006
15	*Thymus sipyleus* Boiss.	1110	40.462697, 31.595006
16	*Usnea longissima*	1350-1450	40.585104, 31.633273

of it (Georgieff, 2007). Only *U. longissima* dried plant sample contained Se (220.11 ± 44.02 ppm) The WHO has advised iron's grade in plants as 20 mg kg^{-1} (Shah *et al.*, 2011), which has positive effects on strengthening the immune system and cognitive performance (Domellöf *et al.*, 2001; Kon *et al.*, 2010). Low levels of iron gives rise to inflammation, pain, loss of appetite, dysphagia, exhaustion, cold limbs and impairment in nails, on the other hand, high iron intake causes lethargy, increasing blood flow of vessel and hypertension. The highest Fe content was found in *Thymus sipyleus* (3,369.20 ± 438.00 ppm) and it was respectively followed by *Thymus leucostomus* (1,246.02 ± 161.98 ppm), *S. potentilla* (912.57 ± 118.63 ppm), and *Matricaria chamomilla* (830.07 ± 107.9 ppm). Silicon has affected bone, keratinous structures, and immune system. The Si content in dried plant samples changed from 1,176.09 to 32.13 ± 4.2 ppm; with the highest value in *E. arvense* and the lowest value in *M. communis*. Calcium (Ca) is necessary for enzyme activity (Berridge, 1975), and has role on skeletal structure. Deficiency of Ca causes convulsion on muscles, and lumbago (Passwater, 1983), however, excess intake of Ca triggers gastrointestinal diseases such as dyspepsia, mouth dryness caused by hyperkalemia; weakens of physical activity (Watts, 1990). Total Ca concentrations in the dried plants ranged from 0.08 ± 0.01 to 4.74 ± 0.66, with the highest value in *E. arvense* and the lowest value in *M. communis*. Copper has an antioxidant activity (Tsuji *et al.*, 2016) and its Daily recommended intake is 10 mg kg^{-1} (Hassan *et al.,* 2012). Excessive amount of Cu uptake gives rise to pain, irritation,

Table 3. Free radical scavenging activity (DPPH)

Sample	1 mg/mL	0.5 mg/mL	0.25 mg/mL	0.125 mg/mL
BHA (control)	**98.14**	**97.67**	**96.28**	**94.88**
Achillea millefolium L.	**97.21**	88.84	88.37	87.91
Anthemis nobilis L.	68.37	46.51	45.12	43.72
Berberis vulgaris L.	**99.54**	74.88	53.023	47.44
Equisetum arvense L.	**92.56**	89.30	82.323	64.19
Herniaria incana Boiss.	67.91	63.26	56.28	38.14
Hypericum perforatum L.	77.67	88.37	94.88	95.81
Malus communis Desf.	84.65	72.09	63.72	39.07
Matricaria chamomilla L.	85.58	74.88	59.07	58.14
Sambucus ebulus L.	85.58	83.72	76.28	51.63
Scorzonera laciniata L.	73.95	69.767	66.51	57.21
Stachys bithynica Boiss.	80	74.883	61.86	60.93
Tanacetum parthenium L.	83.72	81.860	77.21	63.72
Teucrium polium L.	47.90	38.60	29.767	19.65
Thymus leucostomus Hausskn. & Velen.	**91.63**	66.51	65.581	30.23
Thymus sipyleus	13.02	12.56	-	-
Usnea longissima	51.16	44.19	42.33	26.98

Table 4. Total flavonoid and phenolic contents

Sample	Total flavonoid %	Phenolic content %
Achillea millefolium L.	**22.248 ± 7.96**	18.878 ± 1.5
Anthemis nobilis L.	12.266 ± 0.22	13.366 ± 0.88
Berberis vulgaris L.	4.780 ± 0.05	14.552 ± 0.87
Equisetum arvense L.	5.363 ± 0.009	1.731 ± 0.0
Herniaria incana Boiss.	6.043 ± 0.22	1.346 ± 0.35
Hypericum perforatum L.	8.567 ± 0.16	10.641 ± 0.47
Malus communis Desf.	2.7476 ± 0.13	**21.603 ± 0.78**
Matricaria chamomilla L.	**20.711 ± 0.39**	8.782 ± 0.43
Sambucus ebulus L.	6.084 ± 0.2	10.128 ± 0.15
Scorzonera laciniata L.	6.913 ± 0.45	7.982 ± 0.19
Stachys bithynica Boiss.	8.941 ± 0.17	**39.038 ± 1.11**
Tanacetum parthenium L.	**35.986 ± 1,24**	**92.628 ± 5.44**
Teucrium polium L.	15.457 ± 0.65	21.731 ± 1.15
Thymus leucostomus Hausskn. & Velen.	13.523 ± 0.47	**31.059 ± 1.5**
Thymus sipyleus	**19.735 ± 0.72**	**45.737 ± 1.52**
Usnea longissima	2.975 ± 0.07	0.160 ± 0.05

Table 5. Mineral content of dried plant samples

Sample	Se (ppb)	Si (ppm)	Cu (ppm)	Fe (ppm)	Ca (%)
Achillea millefolium L.	ND	418.60 ± 62.79	9.43 ± 1.23	110.84 ± 14.41	1.13 ± 0.16
Anthemis nobilis L.	ND	438.67 ± 65.80	**13.18 ± 1.71**	442.52 ± 57.53	1.53 ± 0.21
Berberis vulgaris L.	ND	83.44 ± 12.52	10.55 ± 1.37	40.38 ± 5.25	0.20 ± 0.03
Equisetum arvense L.	ND	**1,176.09 ± 176.41**	3.21 ± 0.42	855.42 ± 11.20	**4.74 ± 0.66**
Herniaria incana Boiss.	ND	**1,163.27 ± 2,950.67**	6.93 ± 0.90	571.62 ± 74.31	2.10 ± 0.29
Hypericum perforatum	ND	268.58 ± 40.29	7.91 ± 1.03	169.53 ± 22.04	0.44 ± 0.06
Malus communis Desf.	ND	32.13 ± 4.82	2.24 ± 0.29	15.78 ± 2.05	0.08 ± 0.01
Matricaria chamomilla	ND	243.56 ± 36.53	10.93 ± 1.42	830.07 ± 107.91	1.77 ± 0.25
Sambucus ebulus L.	ND	106.59 ± 15.99	1.84 ± 0.24	32.53 ± 4.23	0.44 ± 0.06
Scorzonera laciniata L.	ND	382.64 ± 57.40	11.73 ± 1.53	912.57 ± 118.63	**3.41 ± 0.48**
Stachys bithynica Boiss.	ND	604.72 ± 90.71	12.19 ± 1.58	590.57 ± 76.77	2.89 ± 0.40
Tanacetum parthenium	ND	391.10 ± 58.66	13.06 ± 1.70	121.72 ± 15.82	1.24 ± 0.17
Teucrium polium L.	ND	689.35 ± 103.40	11.13 ± 1.45	680.57 ±88.7	1.33 ±0.19
Thymus leucostomus Hausskn. & Velen.	ND	751.35 ± 112.70	7.88 ± 1.02	**1,246.02 ± 161.98**	1.90 ± 0.27
Thymus sipyleus	ND	**888.9 ± 133.26**	4.75 ± 0.62	**3,369.20 ± 438.00**	1.97 ± 0.28
Usnea longissima	**220.11 ± 44.02**	**1,048.08 ± 157.21**	3.19 ± 0.41	410.74 ± 53.40	0.79 ± 0.11

ND: Non-detectable.

Table 6. Mineral content of the methanolic extracts

Sample	Se (ppb)	Si (ppm)	Cu (ppm)	Fe (ppm)	Ca (%)
Achillea millefolium L.	ND	**227.02 ± 34.05**	16.27 ±2.12	33.05 ± 4.30	0.10 ± 0.01
Anthemis nobilis L.	ND	279.78 ± 41.97	**51.57 ± 6.70**	15.91 ± 2.07	0.07 ± 0.01
Berberis vulgaris L.	ND	27.92 ± 4.19	5.60 ± 0.73	8.41 ± 1.09	0.02 ± 0.03
Equisetum arvense L.	ND	**334.08 ± 50.11**	10.15 ± 1.32	**135.23 ± 17.58**	0.54 ± 0.08
Herniaria incana Boiss.	ND	270.02 ± 40.50	10.72 ± 1.39	79.99 ± 10.40	0.19 ± 0.03
Hypericum perforatum	ND	155.84 ± 23.38	19.44 ± 2.53	34.85 ± 4.53	0.13 ± 0.02
Malus communis Desf.	ND	121.38 ± 18.21	1.78 ± 0.23	6.45 ± 0.84	0.06 ± 0.01
Matricaria chamomilla.	ND	152.94 ± 22.94	10.96 ± 1.42	25.29 ± 3.29	0.05 ± 0.01
Sambucus ebulus L.	ND	67.61 ± 10.14	2.66 ± 0.35	6.97 ± 0.91	0.03 ± 0.04
Scorzonera laciniata L.	ND	154.08 ± 23.11	9.88 ± 1.28	41.43 ± 5.39	0.26 ± 0.04
Stachys bithynica Boiss.	ND	**385.51 ± 57.83**	16.17 ± 2.10	121.14 ± 15.75	0.35 ± 0.05
Tanacetum parthenium	ND	126.75 ± 19.01	20.82 ± 2.71	15.77 ± 2.05	0.10 ± 0.01
Teucrium polium L.	ND	**335.48 ± 50.32**	**21.33 ± 2.77**	60.66 ± 7.89	0.24 ± 0.03
Thymus leucostomus Hausskn. & Velen.	**386.78 ± 77.36**	358.13 ± 53.72	10.43 ± 1.36	70.91 ± 9.22	0.19 ± 0.03
Thymus sipyleus	ND	ND	12.79 ± 1.66	**596.35 ± 77.53**	0.93 ± 0.13
Usnea longissima	**746.02 ± 149.20**	**568.54 ± 85.28**	2.37 ± 0.31	18.71 ± 24.33	0.04 ± 0.06

ND: Non-detectable.

anemia, diarrhea and organ damage (brain, kidney, liver etc.), (Water Treatment Solutions (Cu), 1998; Adnan Iqbal *et al*., 2011; Mebrahtu and Zerabruk, 2011; Aenab *et al*., 2013) however, deficiency of copper causes arthrosis and cardiovasculer problems and, susceptibility to infection (Passwater, 1983; Klevay, 1975; Passwater, 1983; Campbell, 2001). *A. nobilis*, *T. parthenium*, *S. bithynica* and *S. potentilla* also contained significant amounts of Cu (1.84 ± 0.24 – 13.06 ± 1.70). In addition, *A. nobilis* L. methanolic extract included more copper than dried plant sample.

In metanolic plant extract, *T. leucostomus* and *U. longissima* contained selenium (386.78 ± 77.36 - 746.02 ± 149.20 ppm) respectively. Although, dried plant samples demonstrated lower levels of Se, methanolic extracts had higher levels of Se that might due to the water solubility of the Se mineral. The highest Fe content had *T. sipyleus* (596.35 ± 77.53 ppm) and it was respectively followed by *E. arvense* (135.23 ± 17.58 ppm), *S. bithynica* (121.14 ± 15.75 ppm), *H. incana* (79.99 ± 10.40 ppm). The amount of Si content in exctract plant samples changed from 568.54 ± 85.28 to 27.92 ± 4.19 ppm; with the highest value in *U. longissima* and the lowest value in *B. vulgaris*. Total Ca concentrations in the extract of plants ranged from 0.023 ± 0.003 to 0.93 ± 0.13, with the highest value in *T. sipyleus* and the lowest value in *B. vulgaris*. In addition, *A. nobilis*, *T. polium* and *T. parthenium* also contained significant amounts of Cu (51.57 ± 6.70 - 21.33 ± 2.77 - 20.82 ± 2.71). Furthermore, dry plant samples contained higher mineral elements than methanolic plant extracts.

Discussion

In previous literatures, total flavonoid, phenolic content and antioxidant activity of *H. incana* and *T. parthenium* reported are in agreement with the result of the present study (Rabiei *et al*., 2014; Wu *et al*., 2006). Studies on *A. millefolium* demonstrated the presence of flavonoids such as apigenin, luteolin and rutin, which show powerful antioxidant properties, in this plant (Tuberoso *et al*., 2009; Kocevar *et al.,* 2012). Hanachi and Sh (2009) reported that the antioxidant activity of the ethanolic extract of *B. vulgaris* fruit has the highest (27.26 ± 1.07%). Our data indicate that *T. polium* and *U. longissima* have moderate levels of antioxidant activity in agreement with the fact that polyphenols and chemical composition are affected by different factors, such as genotype, environmental conditions and extraction procedures (Halici *et al*., 2005; Sharififar *et al*., 2009; Stankovic *et al*., 2012; Andrè *et al*., 2012). Ahmed *et al*. (2016) reported that the species such as *A. millefolium* L., *Equisetum arvense* L., *H. perforatum* L., *S. aromaticum* L., *M. chamomilla* L. exhibited high antioxidant properties. Kukric *et al*. (2013) evaluated the antioxidative effect of dried ethanolic extract of *E. arvense*, which had an IC_{50} and AAI (antioxidant activity index) of 13.5 and 3.9, respectively. Although present findings are somehow similar to results of those earlier studies, differences in extraction and activity methods, climate, soil, environmental factors, diseases, and pesticide treatments, harvest time, drying and storage methods and plant parts used in analyses may significantly affect the antioxidant activity of plants (Bergonzi *et al.,* 2001; Wang and Zheng, 2001). In previous scientific studies, all over the world on nutritive composition of wild plants high quantities of minerals, especially K, Na, Ca, P, Mg (Guil Guerrero *et al*., 1998; Agrahar-Murugkar and Subbulakshmi, 2005) are indicated. Iron values were reported between 10 and 981 mg kg^{-1} with highest value determined in sage (Zengin *et al*., 2008). Se values were reported between 0.0015 and 0.4 mg kg^{-1} with the highest value determined in *Solenostemma arghel* (Sheded, 2006). Average values for copper content of some edible plants varied from 0.05-18.4 mg kg^{-1} (Yildirim *et al*., 2001; Turan *et al*., 2003). *Equisetum arvense* had minerals content such as silica, calcium, magnesium, selenium, iron, potassium, zinc (Veit *et al*., 1995; Beckert *et al*., 1997). Ozcan (2004) indicated that the highest levels of Ca, Fe, K, Mg and S were found in *T. vulgaris*, *L. officinalis* L., *A. graveolens* L., *O. basilicum* L. and *S. alba* L., respectively while Bi, Cd, Li, Pb and Se contents of condiments were found to be very low. Our studies on mineral content of the plants were also in accordance with the previous findings.

Conclusions

In the present study, it was determined that some plants in Bolu/Turkey contained abundant phenolic compounds and mineral constituents. Antioxidant activities were also evaluated from methanolic extracts of these plants. From the experimental results, *T. sipyleus* and *Stachys bithynica* Boiss with *A. nobilis* L., *E. arvense* L., *T. parthenium* L, *T. polium* L., *T. leucostomus* Hausskn. & Velen were of particular interest. If these plants are protected by culturing, then, mass production of natural drug potential can be carried out easily and efficiently.

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