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Medicinal plants as sources of selenium and natural antioxidants

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Abstract. The beneficial synergism between selenium (Se) and other antioxidants on human health stimulates the search of new medicinal plants with high antioxidant activity (AOA) and Se accumulation levels. Using fluorimetric method of Se analysis and titrimetric method of total AOA determination monitoring was achieved of 53 species of herbs, 24 species of deciduous and evergreen trees and shrubs and 11 species of conifers grown on the Southern and South–Eastern coast of the Crimean peninsular. Two new Se–hyperaccumulators were indicated among deciduous trees: *Tamarix* ramosissima and *Paliurus spina–christi* with leaves Se content 6236 and 1800 μg·kg^{-1} d.w. respectively. Among herbs 5 new hyperaccumulators were revealed: *Artemisia dracunculus* cv. Izumrudny, *Anthemis tranzscheliana, Cota tinxtoria*, *Astragalus arnacantha* and *Limonium gerberi* with Se accumulation levels of 23460, 9479, 960, 4110, and 1926 μg·kg^{-1} d.w. respectively. Medians of Se levels reached 97 μg·kg^{-1} for conifers, 86.5 μg·kg^{-1}–for deciduous plants and 61 μg·kg^{-1} – for herbs with the appropriate AOA values: 67, 70. and 49.3 mg–GAE.g–1 d.w. respectively. *Tamarix* was the only plant with both Se hyperaccumulation abilities and high AOA level (212 mg–GAE·g^{-1} d.w). High AOA (188–256 mg–GA^{E}·g^{-1} d.w) and relatively high Se levels (193–236 μg·kg^{-1}) were demonstrated for *Pistacia atlantica* and *Cotinus coggygria*.
Keyword: *Selenium; antioxidant activity; deciduous evergreen trees and shrubs; conifers; herbs.*

Introduction

Antioxidants are an important group of compounds capable to protect human organism from many diseases, suitable for production of food additives and inhibiting oxidation processes. Several groups of phenolics (anthocyanins, tannins, flavonones, resveratrol, ellagic acid) are already used in industry as nutriceutics and/or functional food products [ESPIN *et al.*, 2007; LOURENÇO *et al.*, 2019]. Despite antioxidant properties, these compounds demonstrate wide spectrum of biological activity, including anti–allergic, anti–inflammation, antithrombotic, cardio–protective, anticarcinogenic and vasodilator effect [PANICKAR, 2013, AKINYEMI *et al.*, 2018]. All medicinal plants compose an important source of antioxidants for human beings, effectively blocking the oxidation processes and neutralizing free radicals [PATEL CHIRAG *et al.*, 2013]. Special attention is paid to the role of plants antioxidants in cancer prevention [GHOLAMIAN–DEHKORDI *et al.*, 2017].

Utilization in food, pharmaceutical and cosmetic industry, wide possibilities in medicine utilization form the basis of constantly growing interest in search of new natural sources of antioxidants [HUANG *et al.*, 2005; LOURENÇO *et al.*, 2019].

In addition to plants secondary metabolites important for human health, the elemental composition of plants is also of great interest, as it is known to affect plants biological activity. In this respect, special attention is paid to selenium known to demonstrate powerful antioxidant properties, immune–modulating effect, being a part of enzymes with antioxidant activity, including triiodothyronine deiodinases [SHARIFI-RAD J *et al.*, 2010; OKLO *et al.*, 2017].

Se deficiency is widespread in the world increasing risks of cardiovascular and oncological diseases, disturbances in reproductive function and brain activity [GUPTA, GUPTA, 2016]. Especially important is the fact that synergistic interactions between natural antioxidants including Se [SONAM, GULERIA, 2017; GOLUBKINA, PAPAZYAN, 2006] decrease the requirement of doses of different drugs in combination thus reducing the side effects caused by the high concentrations of a single drug.

Most of agricultural crops and herbs are not Se accumulators and the only way to get products with high Se and antioxidants content is biofortification. Se hyperaccumulators among plants are rather rare and, as a rule, are grown on soils rich with selenium [PILON–SMITS, 2019; WHITE, 2016]. Overall, there are a few data regarding the plants ability to hyperaccumulate Se and the question needs special investigations. Up to date, few studies devoted to the search of selenium hyperaccumulators with high antioxidant activity have been achieved [ZOTEK *et al.*, 2019].

The aim of the present investigation was to monitor medicinal plants in the absence of significant anthropogenic interferences in the territories of Nature Reserves of the Southern and South–Eastern Crimean coast using Se and AOA parameters.

Material and methods

Sample collection and preparation

Samples of plant leaves were gathered at the end of May– beginning of June 2017–2018 in the territory of Karadag Nature Reserve (44º56'10''с.ш.; 35º14'00''в.д.), Martyan Cape Nature Reserve and Nikitsky Botanical Garden (N 44°30.725; E 34°14.089), and Ayu–Dag protected area (N 44.55831503; E 34.33527650) (Figure 1).

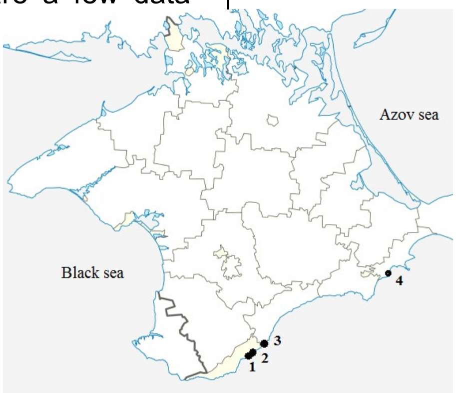

Figure 1. Plants sampling sites: 1– Nikitsky Botanical Garden, 2–Martian cape, 3–Ayu–Dag, 4–Karadag

Among deciduous and evergreen trees and shrubs 23 plant species were investigated, belonging to 12 families: Anacardiaceae, Cornaceae, Fabaceae, Elaeagnaceae, Fagaceae, Myrtaceae, Moraceae, Oleaceae, Rhamnaceae, Rosaceae, Tamaricaceae, and Vitaceae.

Among conifers 11 evergreens species were studied. And among herbaceous species we investigated 54 taxa (including 3 cultivars of the same species) belonging to 20 families: Asparagaceae, Asteraceae, Fabaceae, Asphodelaceae, Boraginaceae, Brassicaceae, Hypericaceae, Capparidaceae, Caryophyllaceae, Lamiaceae, Ephedraceae, Euphorbiaceae, Papaveraceae, Rosaceae, Paeoniacea, Plumbaginaceae, Polygonaceae, Ranunculaceae, Solanaceae, and Urticaceae.

Status and nomenclature of taxa are presented according to modern international databases. The 2013 Plant List, 2020 Catalogue of Life and IPNI.

Samples of leaves were cut with plastic knife and dried at room temperature to constant weight without direct sunlight. Dried samples were homogenized and the resulting powders were kept in hermetically closed polyethylene bags without direct sunlight.

Extraction of antioxidants was achieved using 70 % ethanol. Half gram of dry leaves powder were mixed with 15 mL of 70 % ethanol, heated at 80 ºC during one hour and cooled to room temperature. Mixtures were quantitatively transferred to volumetric flasks and the volume was adjusted to 25 mL with 70 % ethanol and samples were filtered through a pleated filter. Despite the fact that each biological object requires a special selection of optimal conditions for antioxidants extraction [XU *et al.*, 2017], 70 % ethanol is used most frequently which allows to simplify the process of monitoring.

Antioxidant activity (AOA) of medicinal plants leaves was assessed using a redox titration method [GOLUBKINA *et al.*, 2020] via titration of 0.01 N $KMnO_4$ solution with ethanolic extracts of dry samples. The reduction of $KMnO_4$ to colorless Mn^{+2} in this process reflects the quantity of antioxidants dissolvable in 70% ethanol. The values were expressed in mg gallic acid equivalents (GAE) g^{-1} d.w. The $KMnO_4$ acidic solution has been successfully used earlier for the determination of the *Ocimum basilicum* antioxidant potential [SRIVASTAVA *et al.*, 2015] and the antioxidant capacity of serum [ZHAN *et al.*, 2014]. Simplicity of the method and its cheapness makes it more and more popular in modern biochemical laboratories [KASOTE *et al.*, 2019].

Selenium was analyzed using the micro fluorimetric method previously described for tissues and biological fluids [ALFTHAN, 1984]. The method includes wet digestion of dried homogenized samples via heating with a mixture of nitric–chloral acids, subsequent reduction of selenate (Se^{+6}) to selenite (Se^{+4}) with a solution of 6 N HCl, and formation of a complex (piazoselenol) between Se^{+4} and 2,3–diaminonaphtalene. The calculation of Se concentration was achieved by recording piazoselenol fluorescence values in hexane at 519 nm λ emission and 376 nm λ excitation. Each determination was performed in triplicate. The precision of the results were verified using in each determination a reference standard–lyophilized cabbage with Se concentration of 150 μg·kg^{-1}.

Statistical analysis

Data were processed by analysis of variance and mean separations were performed through the Duncan multiple range test, with reference to 0.05 probability level, using SPSS software version 21.

Results and discussion

Literature data indicate several plant orders and families where Se hyperaccumulators are revealed [GUPTA, GUPTA, 2017] (Table 1).

Table 1.

Known selenium hyperaccumulators among angiosperms

Order (Family)	Species	References
Asterales (Asteraceae)	*Haplopappus fremonti; Machaeranthera* (4 taxa); *Oonopsis* (4 taxa); *Xylorhiza* (8 taxa)	[REEVES, BAKER, 2000] [BEATH *et al.*, 1940]
Brassicales (Brassicaceae)	*Stanleya bipinnata; Stanleya pinnata*	[Beath *et al.*, 1940]
Caryophyllales (Chenopodiaceae)	*Atriplex confertifolia*	[REEVES, BAKER, 2000]
Fabales (Fabaceae)	*Astragalus* (24 taxa)	[BEATH *et al.*, 1940]
	Neptunia amplexicaulis	[REEVES, BAKER, 2000]
Gentianales (Rubiaceae)	*Coelospermum decipiens (Morinda reticulata)*	[REEVES, BAKER, 2000]
Lamiales (Orobanchaceae)	*Castilleja angustifolia* var. *dubia (Castilleja chromosa)*	[REEVES, BAKER, 2000]

Among them 6 families should be mentioned: Asteraceae, Brassicaceae, Fabaceae, Rubiaceae, Chenopodiaceae, and Orobanchaceae (Table 1). Se hyperaccumulators are considered to be plants containing more than or equal to 1000 µg Se/kg d.w. [PILON–SMITS, 2019].

In the present work monitoring of Se accumulation and levels of AOA in plants was achieved regardless of plants family, but grown in unpolluted areas of Nature Reserves (Karadag, Martyan Cape), Nikitsky Botanical Garden and specially protected area Ayu–Dag (Figure 1). Karadag Nature Reserve is situated in the territory of paleovolcano, while Ayu–Dag mountain is a laccolite.

Objects of investigation included herbs, deciduous and evergreen trees and shrubs and conifers.

Deciduous and evergreen trees and shrubs

Among 24 species of deciduous and evergreen trees and shrubs of the Southern and South–Eastern coast of the Crimean Peninsula only two demonstrated Se hyperaccumulating ability: *Tamarix ramosissima* Ledeb. and *Paliurus spina–christi* Mill. (Table 2).

Table 2.

Selenium content and antioxidant activity of deciduous and evergreen trees and shrubs

Family	Species	Se, µg·kg^{-1} d.w.	AOA, mg GAE·g^{-1} d.w	Sampling site*
Anacardiaceae	*Pistacia atlantica* Desf. (*Pistacia mutica* Fisch. et C.A. Mey)	236±76c	188±2c	1
	Cotinus coggygria Scop.	193±33c	256±19b	4
Cornaceae	*Cornus mas* L.	150±10c	125±19de	3
Elaeagnaceae	*Elaeagnus angustifolia* L.	87±7h	41±3j	4
Fabaceae	*Styphnolobium japonicum* (L.) Schott	266±118c	48±4j	1
Fagaceae	*Quercus petraea* Liebl.	21±1j	75±4f	1
	Quercus pubescens Willd.	19±2j	81±13fh	1
Moraceae	*Ficus carica* L.	108±10d	45±3j	1
	Maclura pomifera (Raf.) C.K.Schneid.	68±5i	53±4aj	1
	Morus alba L.	34±3g	48±3j	4
Myrtaceae	*Myrtus communis* L.	37±4c	110±.5	1
Oleaceae	*Fraxinus excelsior* L.	86±9h	52±9a	3
	Jasminum fruticans L.	33±2g	75±6fh	2
Rhamnaceae	*Paliurus spina–christi* Mill.	**1800±200a**	56±3a	4
Rosaceae	*Rubus caesius* L.	123±6d	45±4j	3
	Prunus spinosa L.	124±10d	55±3a	4
	Prunus dulcis (Mill.) D.A.Webb	109±6d	40±3	4
	Pyrus elaeagrifolia Pall.	75±12i	70±9f	4
	Prunus armeniaca L.	50±4ef	86±5h	4
	Crataegus monogyna Jacq.	56±2f	70±15fh	3
	Crataegus orientalis subsp. *pojarkovae* (Kossych) J.I. Byatt (*Crataegus pojarkovae* Kossych)	49±2e	70±10f	4
	Rosa canina L.	30±7g	27±2g	4
Tamaricaceae	*Tamarix ramosissima* Ledeb.	**6236±220b**	212±12b	1
Vitaceae	*Vitis vinifera* L.	119±11d	83±27c	4

Values in columns with similar indexes do not differ statistically according to Duncan test at $P<0.05$; *– numbers correspond to Fig.1 data

Both are medicinal plants though the AOA of *Tamarix* was 6 times higher than that of *Paliurus spina–christi* Mill. Antioxidant and anti–bacterial activities of *Tamarix* are considered to be connected with high content of polyphenols and tannins [SULTANOVA *et al.*, 2001; BAHRANSOLTANI *et al.*, 2020]. *Tamarix* spp. is traditionally used for treatment of gastrointestinal disorders, wounds healing, diabetes, and dental problems [KSOURI *et al.*, 2009].

Tamaricaceae family is composed of 5 genera among which *Tamarix* L. genus includes 70 species. Se accumulation ability of this tree has never been studied earlier.

It's extremely high AOA and Se hyperaccumulation indicate great prospects for investigating other species of this family.

Paliurus spina–christi Mill. was another Se–hyperaccumulator among deciduous trees. Though its AOA and Se content were significantly lower than the corresponding values of tamarisk (4 and 3.5 times respectively), this tree also attracts significant attention due to possible participation of Se in its biological activity. *Paliurus spina–christi* leaves, fruit, florets and bark are widely used in traditional medicine for treatment of arthritis, asthma, headache, heart and muscles pains gastrointestinal disorders, wound healing, hypertension and eye inflammation [DAFNI *et al.*, 2005].

Table 3.

Selenium accumulation levels and AOA of herbs

Family	Species	Se, µg·kg^{-1} d.w.	AOA, mg GAE·g^{-1} d.w.	Sampling site
Asparagaceae	*Asparagus verticullatus* L.	68±5^{ab}	35±4^{am}	4
	Ruscus hypoglossum L.	62±3^{a}	154±13^{c}	2
	Ruscus aculeatus L.	56±5^{ad}	114±12^{d}	3
	Ornithogalum ponticum Zahar.	467±168^{e}	40±2^{alm}	2,4
Asphodelaceae	*Asphodeline taurica* (Pall.) Endl.	26±2^{f}	31±1^{b}	4
Asteraceae	*Artemisia dracunculus* L. *	**23460±232^{g}**	145±10^{c}	1
	Anthemis tranzscheliana Fed.	**9479±220^{h}**	40±2^{alm}	4
	Tragopogon dubius Scop.	**4110±2320^{j}**	41±3^{alm}	4
	Cota tinctoria (L.) J. Gay	**960±280^{i}**	56±4^{e}	4
	Artemisia dracunculus L.**	380±31^{e}	63±5^{e}	1
	Artemisia dracunculus L.***	160±20^{l}	49±3^{eg}	1
	Artemisia scoparia Waldst. & Kit.	70±7^{a}	102±10dk	1
	Artemisia annua L.	80±12b^{c}	64±5e	1
	Artemisia campestris L.	60±9^{ab}	42±3^{alm}	1
	Artemisia abrotanum L.	60±9^{ab}	210±17^{f}	1
	Seriphidium santonicum (L.) J.Soják. (*Artemisia santonica* Lam.)	60±9^{ab}	190±18^{f}	1
	Artemisia feddei H.Lév. & Vaniot	60±9^{ab}	55±5^{e}	1
	Artemisia taurica Willd.	60±9^{ab}	214±19^{f}	1
	Artemisia vulgaris L.	51±4^{a}	45±3^{gl}	1
	Artemisia argyi H.Lév. & Vaniot	50±3^{ab}	55±4^{e}	1
	Artemisia absinthium L.	42±3^{p}	42±3^{alm}	1
Boraginaceae	*Onosma cinerea* Schreb. (*Onosma taurica* Pall.)	133±30^{ln}	24±2^{h}	4
	Lithospermum officinale L.	14±1^{k}	83±6^{j}	2
Brassicaceae	*Crambe maritima* L.	282±4^{m}	42±3^{alm}	4
	Fibigia clypeata (L.) Medik.	107±5^{n}	24±2^{h}	3
Capparidaceae	*Capparis herbacea* Willd.	152±34^{l}	43±4^{al}	4
Caryophyllaceae	*Dianthus humilis* Willd. ex Ledeb.	15±11^{ko}	38±3^{am}	2
Ephedraceae	*Ephedra distachya* L.	95±6^{c}	49±3^{e}	1
Euphorbiaceae	*Euphorbia humifusa* Willd.	56±4^{ab}	27±2^{h}	4
Fabaceae	*Astragalus arnacantha* M.Bieb.	**2680±122^{j}**	44±2^{l}	4
	Bituminaria bituminosa (L.) C.H.Stirt.	159±16^{l}	58±4^{e}	4
	Vicia cracca L.	18±1^{o}	25±2^{h}	4
Hypericaceae	*Hypericum perforatum* L.	49±3^{d}	90±6^{jk}	4
Lamiaceae	*Sideritis montana* L.	276±6^{m}	31±2^{a}	2
	Prunella grandiflora (L.) Turra	62±6^{a}	35±.3^{am}	1
	Phlomis herba–venti subsp. *pungens* (Willd.) Maire ex DeFilipps	60±4^{ab}	35±.3^{am}	4
	Prunella laciniata (L.) L.	57±5^{bd}	50±3^{e}	1
	Prunella vulgaris L.	54±5^{bd}	54±3^{e}	1
	Melissa officinalis L.	51±5^{bd}	89±6^{jk}	1
	Salvia nemorosa L.	49±4^{dp}	92±13^{jk}	1
	Teucrium chamaedrys L.	29±3^{f}	21±1^{h}	1
	Thymus vulgaris L.	23±2^{f}	53±3^{e}	1
	Thymus striatus Vahl.	21±2^{f}	73±5^{i}	1
	Thýmus tauricus Klokov & Des.–Shost.	16±1^{ko}	40±3^{alm}	1
Papaveraceae	*Glaucium flavum* Crantz	246±16^{q}	60±4^{e}	4
Paeoniacea	*Paeonia daurica* Andrews	184±20^{l}	49±4^{e}	4
Plumbaginaceae	*Limonium gerberi* A.Soldano	**1926±600^{j}**	52±1^{e}	4
Polygonaceae	*Rumex confertus* Willd.	23±2f	206±12^{f}	4
Ranunculaceae	*Clematis vitalba* L.	135±13	55±15^{ei}	4
	Clematis flammula L.	67±6^{ab}	37±3^{am}	4
Rosaceae	*Agrimonia eupatoria* L.	132±15^{l}	98±7^{k}	4
Solanaceae	*Withania somnifera* L. Dunal	120±10^{n}	38±2^{m}	1
Urticaceae	*Parietaria judaica* L.	18±2^{o}	22±1^{h}	2

* *Izumrud cv; ** spicy aromatic form; *** Travyanysti cv;* *** sampling sites are indicated on Fig,1; Values in columns with similar indexes do not differ statistically according to Duncan test at P<0.05

Antioxidant and anti–inflammation properties of *Paliurus spina–christi* Mill. are described [SEN, 2018]. Se accumulation abilities of these plants have never been studied so far. Among deciduous and evergreen trees and shrubs, species belonging to Fabaceae and Anacardiaceae families compose a special group, capable to accumulate twice higher Se levels that the median value (86.5 µg.kg^{-1} d.w.; Table 3).

These are primarily *Pistacia atlantica* Desf. (pistachio) and *Cotinus coggygria* Scop. (smoke tree) with a recorded high AOA. *Cotinus coggygria*

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Scop is known to contain extremely high tannins content and leaves of this shrub are used as antiseptic, anti–inflammatory, anti–microbial, hepatoprotective, anti–hemorrhagic and wound healing agent. This plant demonstrates beneficial effect in treatment of periodontal disease, diarrhea and ulcer [MATIC *et al.*, 2015].

Pistachio leaves are characterized by high concentrations of polyphenols and leaves extracts and are used as tonics and appetite enhancer [TAGHIZADEH *et al.*, 2018].

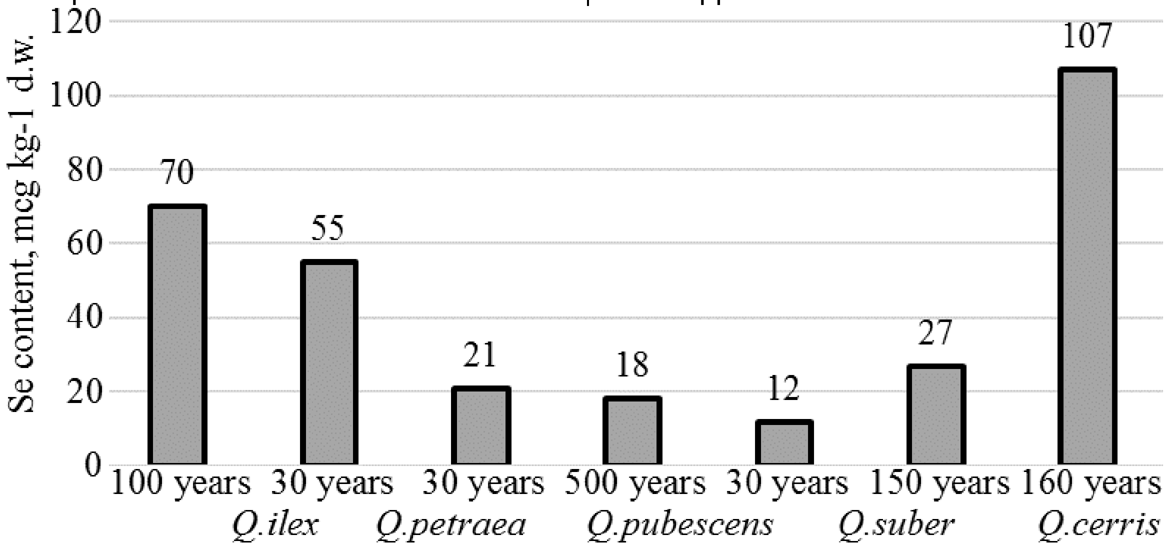

Figure 2. Interspecies differences and age effect on selenium accumulation by *Quercus* species (Nikitsky Botanical Garden)

Relatively high concentration of Se in leaves of these plants may have an additional positive effect on human health.

Great differences in Se content and AOA were demonstrated for Rosaceae species (30–124 μg Se.kg^{-1} d.w. and 27–86 mg GAE g^{-1} d.w. respectively). Similar phenomenon was indicated for Moraceae (34–108 μg Se.kg^{-1} d.w.) and Oleaceae families (33–86 μg Se.kg^{-1} d.w.). A good example of interspecies variations may be observed in *Quercus* species (Figure 2).

Evaluation of Se accumulation abilities of Nikitsky Botanical Garden oats collection revealed significantly higher Se levels in leaves of *Q. cerris* L. differing with *Q. pubescens* Willd. data by 6–9 times (Figure 2).

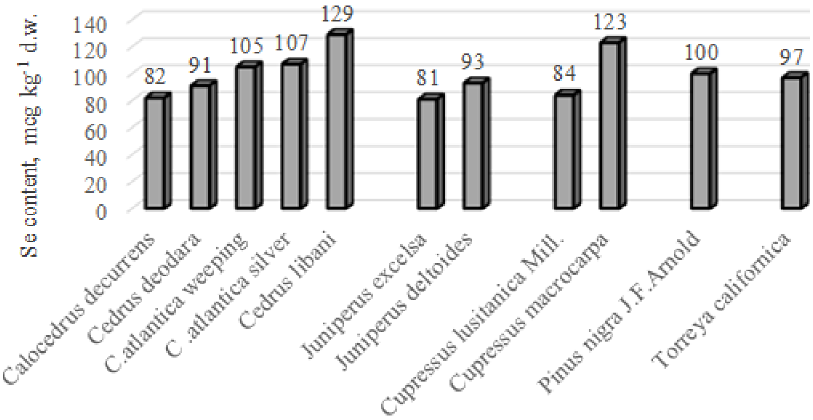

Figure 3. Se content in needles of conifers grown in the Nikitsky Botanical Garden

The results indicate that old trees accumulate Se only slightly more intensively than young trees.

Conifers

Monitoring plants for Se accumulation abilities revealed that among 11 conifer species none demonstrated Se hyperaccumulation.

At the same time it draws attention to the fact that contrary to deciduous plants Se concentrations in conifers needles are relatively constant (99 μg

Se·kg⁻¹ d.w.) and have low CV value (less than 12%) (Figure 3).

Herbaceous species

Among 20 families of this group, three contained Se hyperaccumulators. Indeed, these data are in agreement with the known Se hyperaccumulation abilities of Asteraceae and Fabaceae species [WHITE, 2016]. New Se hyperaccumulators were revealed in Asteraceae family: *Artemisia dracunculus* (tarragon) cv. Izumrudny [PLUGATAR *et al.*, 2018], *Anthemis tranzscheliana* Fed., *Astragalus arnacantha* M. Bieb. and *Cota tinctoria* (L.) J.Gay. Among 11 *Artemisia* species only *A. dracunculus* cv. Izumrudny in particular demonstrated extremely high Se accumulation values, whereas other 10 species and even two other tarragon cultivars accumulated relatively low Se levels. Table 3 data also indicate high antioxidant activity of *Artemisia abrotanum* and *Artemisia santonica*.

Tragopogon dubius Scop. Is another Se hyperaccumulator of Asteraceae family and it is widely used in traditional medicine, due to powerful antioxidant, anti–bacterial and anti–fungi properties. High levels of phenolics including rosmarinic and chlorogenic acids were identified [UYSAL *et al.*, 2019].

Cota tinctoria and *Anthemis tranzscheliana* Fed are of special attention. The first one accumulates up to 1000 μg·kg⁻¹ d.w. while the second proved to be a real Se hyperaccumulator with Se content exceeding 9000 μg·kg⁻¹ d.w. The whole plant of *Cota tinctoria* is used as antispasmodic, diaphoretic, emetic and vesicant agent. It is used internally as a tea, which can be made either from the flowers, or the whole plant. Applied externally, it is used as a poultice on piles and can also be applied to the bath water. The leaves are rubbed onto insect stings. Essential oil of *Anthemis palestina* showed anticarcinogenic effect and antibacterial activity [BARDAWEEL *et al.*, 2014]. The species belonging to *Anthemis* genus are known to possess various biological properties and have found broad use in pharmaceutics, cosmetics, and food chemistry. The flowers of *Anthemis* species are well–documented for their use as antiseptic and healing herbs, with flavonoids, and essential oils being the main active components [ORLANDO *et al.*, 2019]. Extracts, tinctures, salves, and tisanes are extensively used as antispasmodic, anti–inflammatory, antibacterial and sedative agents, in Europe [AKGÜL, SAGLIKOGLU, 2006]. Extracts are also used to clean wounds and ulcers, and as therapy for irradiated skin injuries, cystitis and dental actions [*ORLANDO et al., 2019*]. The antimicrobial activity of essential oils of several *Anthemis* species has been previously reported [BARDAWEEL *et al.*, 2014].

Moreover, *Anthemis* species are widely used to treat intestinal disorders, kidney stones, and hemorrhoids in traditional medicine. The plant is also used as antispasmodic medications and to stimulate menstrual flow. It is documented that the seed oil has been used in the treatment of earaches and deafness. The main biologically active compounds of *Anthemis* are sesquiterpene lactones, flavonoids and essential oils [ORLANDO *et al.*, 2019]. *Cota tinctoria* is used in traditional medicine as a diaphoretic, choleretic, anti–febrile and hemostatic agent. It demonstrates insecticidal properties. The main biologically active compounds are flavonoids, glycosides and essential oil.

As far as *Anthemis tranzscheliana* Fed is concerned this plant is endemic to Karadag Nature Reserve and the plant is listed in the Red Book. Unusually high Se accumulation ability of this plant indicates the necessity of its introduction into crop culture with appropriate identification of specific biological activity.

As far as *Astragalus* is concerned Se hyperaccumulation abilities of Fabaceae family species are well documented [WHITE, 2016; CABANNES *et al.*, 2011]. Nevertheless, Se hyperaccumulation properties of *Astragalus arnacantha* M. Bieb has never been indicated earlier.

Up to date Plumbaginaceae family was not considered to contain Se hyperaccumulators. The present results indicate for the first time at least one exception, that is *Limonium gerberi* A. Soldano (*Limonium platyphyllum* Lincz.) with Se content close to 2000 μg.kg⁻¹ d.w.

Despite relatively high AOA of *Limonium gerberi* leaves due to high concentrations of tannins [TAYZUMOV *et al.*, 2019], this plant is not used in traditional medicine at present. Se accumulation ability of one more species *Ornithogalum ponticum* Zahar attracts attention. This plant belongs to Asparagaceae family and may accumulate Se in a broad concentration range (214–720 μg·kg^{-1} d.w.) depending on the distance from the seashore. According to the previous investigation [GOLUBKINA *et al.*, 2017, ROTARU *et al.*, 2010] Se accumulation levels in leaves of the Crimean ivy depends on the height above the sea level and changes according to an equation:

$$Y=2347.2X^{-0.857},$$

where X–altitude, Y–Se content, % to the value found for 20 m altitude

High responsiveness of *Ornithogalum ponticum* to Se supply suggests that this plant belongs to Se–indicators group tolerant to Se supply that may be used both in ecological monitoring and in production of Se–containing supplements. At present *Ornithogalum ponticum* is used as a cardio–stimulator being an analog of digitoxin [MULLHOLLAND *et al.*, 2013, SALEHI *et al.*, 2020]. Among other studied species *Ruscus hypoglossum* L. and *Ruscus aculeatus* also attract special attention. Young shoots and fruit of these evergreen plants also belonging to Asparagaceae family are edible and possess high antioxidant and anti–microbial activity [HADŽIFEIZOVIĆ et al, 2013]. Both species demonstrate relatively close antioxidant activity and Se accumulation levels. Similar levels of Se and AOA are also typical in *Ephedra distachya* L. from Ephedraceae family.

Table 3 data indicate a special group of herbs capable to accumulate thrice higher levels of Se than calculated median value (61 μg·kg^{-1} d.w.). The species of this group are *Glaucium flavum* Crantz (Papaveraceae), *Sideritis montana* L. (Lamiaceae). *Crambe maritima* L. (Brassicaceae), *Artemisia dracunculus* cv. Travny (Asteraceae). Mean Se concentration in leaves of these plants exceeds 200 μg·kg^{-1} d.w. while AOA values are in the range of 31–62 mg GAE·g–^{1} d.w.

Sideritis montana L. contains tannins, flavonoids, pectin and essential oil and is used as tonic tea. *Crambe maritima* L. is rich in vitamin C, mineral salts, sulphur and iodine. It contains sulphur heteroside, recognized as having anti-cancer properties. Because of its high vitamin C content, it is used to prevent scurvy as well as viral infections. It has also been used as a purifier, diuretic, antiseptic and antifungal agent. The leaves have been used for healing wounds, fruit–for removing worms and the raw juice of seeds to fight gastritis and gastric ulcers [SANYAL, DECOCQ, 2015, BUTNARIU and BUTU, 2019].

Table 4.

Mean values of AOA and selenium accumulation levels in deciduous and evergreen trees and shrubs, conifers and herbs

	Parameter	Herbs	Deciduous	Conifers
	Number of species	54	26	11
Se content μg·kg^{-1} d.w.	M±SD	877±1383	396±557	99±12
	Concentration range	14–23460	12–6236	81–129
	CV, %	157.7	140.7	12.4
	Median	61.0	86.5	97.0
	Number of hyperaccumulators	6	2	0
AOA mg GAE·g^{-1} d.w.	M±SD	64.8±32.5	85.3±41.4	69.1±12.0
	AOA range	21–210	27–256	56–80
	CV, %	50.2	48.5	17.4
	Median	49.3	70	67.0
	Number of species with AOA ≥ 200 mg–GAE g^{-1} d.w.	4	1	0

The comparison between Se concentrations median data of medicinal herbs, deciduous and evergreen trees and shrubs and conifers gathered on the Southern and South–Eastern coast of the Crimean Peninsula revealed that Se accumulation the level was the highest for

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conifers and the lowest for the herbs (Table 3).

Both deciduous plants and herbs were characterized by extremely high coefficients of variation indicating the existence of Se hyperaccumulators. Contrary, conifers group lack Se–hyperaccumulators, which explains the low CV value.

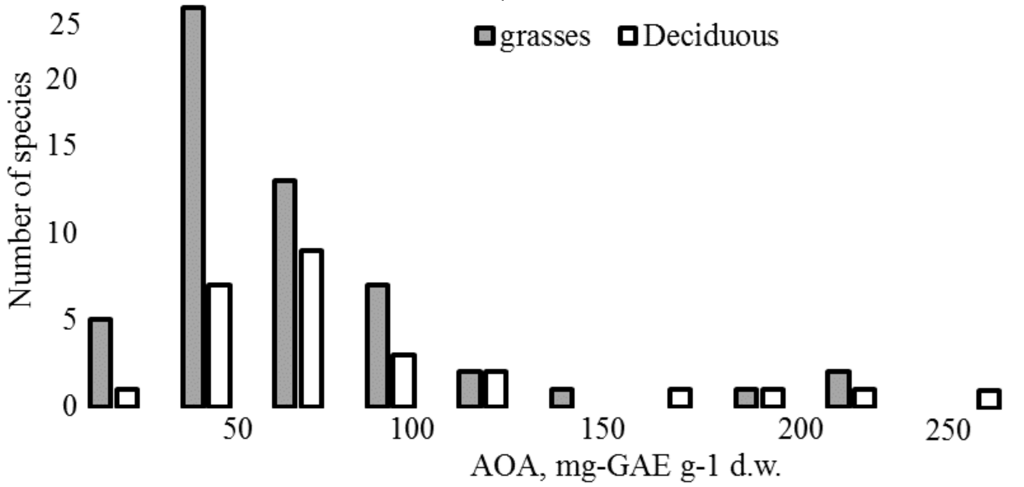

Figure 4. AOA of herbs and deciduous and evergreen trees and shrubs

According to the present data, there is no relationship between leaves Se concentrations and AOA of leaves ethanolic extracts.

Wider AOA range for herbs and deciduous plants explains relatively the high coefficients of the variations reaching 50 % (Table 4).

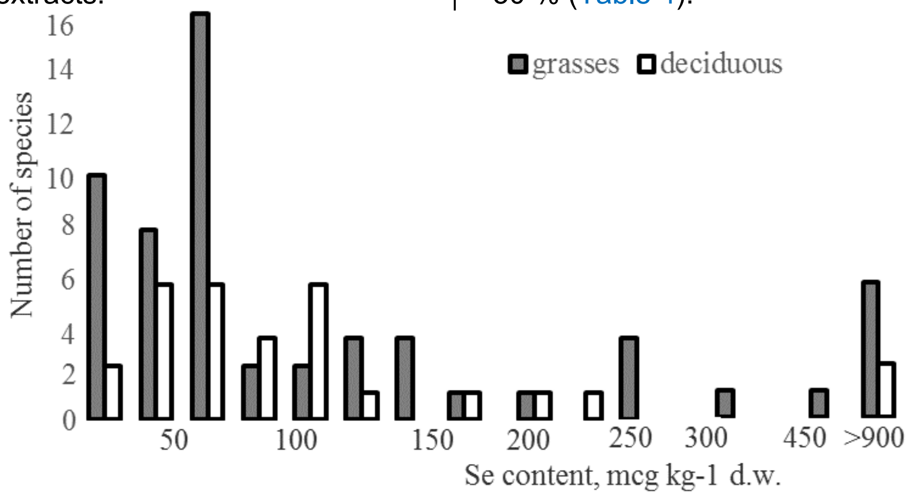

Figure 5. Selenium content in herbs, deciduous and evergreen trees and shrubs and conifers

Histogram of detected AOA values revealed distinct differences in mean AOA values for deciduous plans and herbs (Figure 4). Differences in mean Se accumulation levels are less pronounced (Figure 5).

Conclusions

The present investigation indicates high herbs and deciduous and evergreen trees and shrubs potential as significant sources of Se and antioxidants. Targeted utilization of natural Se hyperaccumulators with high antioxidant activity may become an outstanding approach for improving human health.

Further monitoring of medicinal herbs, deciduous trees, shrubs and conifers are necessary to expand our understanding of Se hyperaccumulation phenomenon and its role in the environment.

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