ANTIOXIDANT PROPERTIES AND ELEMENTAL COMPOSITION OF *WITHANIA SOMNIFERA* L.

Helene Kekina[1], Oksana Shevchuk[2], Nadezhda Golubkina[3], Lidia Logvinenko[2], Ludmila Khlipenko[2], Ann Molchanova[3], Gianluca Caruso[4]

[1]Medical Academy of Postgraduate Education, Moscow 123995, Russia

[2]Nikita Botanic Gardens, National Scientific Center of the RAS, Yalta, 298648, Russia

[3]Federal scientific center of vegetable production, Moscow region 143072, Russia

[4]Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici (Naples), Italy

Abstract

Withania somnifera (L.) Dunal is popular in the traditional medicine of many areas of the world due to its high biological activity. Research was carried out in order to characterize the plants of this species in terms of antioxidant content and activity, as well as macro- and microelements concentration in leaves and roots plants grown along Crimea sea coast, which receive an intensive element transfer from the sea surface. The leaves of Withania somnifera showed higher levels of polyphenols, flavonoids and total antioxidant activity compared to roots (7.52 mg eq. GA g^{-1} d.w., 2.30 mg eq. quercetin g^{-1} d.w. and 38.2 mg eq. GA g^{-1} d.w. in leaves; 3.83 mg eq. GA g^{-1} d.w; 0.48 mg eq. quercetin g^{-1} d.w. and 10.8 mg eq. GA g^{-1} d.w. in the roots). Withanolides content in roots reached 0.62 %. The determination of 25 macro- and microelements revealed that Withania somnifera intensively accumulated Ca, K, Mg, P, B, I, Li, Mn, Mo, Si and Zn in leaves, whereas the concentration of V, Cr, Fe and Al in roots exceeded those recorded in leaves by 5.5, 5.86, 4.35 and 5.26 times respectively. The highest difference between leaves and roots concentration was detected for Li whose content in leaves was 10 times higher than in roots. Among other cultivated herbs (14 species belonging to Artemisia and Myrthus), Withania somnifera showed the highest levels of Li which makes it suppose this species is a good Li source for humans. Moreover, the leaves of W. somnifera contain high levels of iodine.

Keywords: *Indian ginseng, leaf and root properties, antioxidants, macro- and microelements*

INTRODUCTION

Medicinal plants are widely used throughout the world due to safety and high efficiency of their use, fewer side effects compared to synthetic drugs, and a wide range of biological properties. According to WHO, more than 80% of the population in developing countries use medicinal plants as the main components in the treatment of several diseases [1]. Moreover, about a quarter of all known medicinal preparations contain extracts of medicinal plants or active ingredients isolated from plant sources.

W. somnifera (L.) Dunal, also named Indian ginseng, is popular in the traditional medicine of many areas of the World such as India, China, Asia, Africa and Mediterranean Countries due to its wide biological activity [2].

Indian ginseng shows strong immunomodulating, hematopoietic, antioxidant, cardioprotective, antiinflamatory, neuroprotective, anticarcinogenic, antiaging, hepatoprotective, antimicrobial effects [2-8].

All parts of *W. somnifera* plant, i.e. leaves, roots and fruits, have medicinal properties and, in this respect, the most important biologically active compounds are alkaloids and steroidal lactones of triterpenoids, the so-called withanolides [9-11]. Different phenolic compounds were detected in Indian ginseng leaves, including quercetin, kaempferol, rutin and related glycosides [12,13]. To date, 62 secondary metabolites and 48 compounds from roots, among which 29 are characteristic of both leaves and roots, have been isolated and identified.

The investigations carried out in Nikita Botanic Gardens demonstrated that in dry subtropical climate *Withania somnifera* undergoes a full development cycle as a one-year culture and forms full-fledged seeds that have no rest period [13].

Taking into account that the biological activity of most herbs is strictly connected with the biogeochemical characteristics of their habitat, the present study was aimed to assess the antioxidant profile as well as macro- and microelements composition of *Withania somnifera* grown at the Southern coast of Crimea.

MATERIALS AND METHODS

Plant material and experimental conditions

Research was carried out on *Withania somnifera* in Crimea in 2017, in order to perform the plant characterization in terms of antioxidant properties and elemental composition. The plants were grown at Nikita Botanical Gardens (southern Coast of Crimea, 44° 30.7' N, 34° 14.4' E) in agro-brown carbonate, strongly siliceous, light-clay soil with 3-5% humus, 12-17 % carbonates, pH 7.5-7.8. The lowest temperature was recorded in January (-15 °C), the highest in August (39 °C) and the total rainfall accounted for 611 mm. The mean monthly values of meteorological parameters are reported in Table 1.

Table 1. Mean monthly values of meteorological parameters in Nikita Botanic Gardens in 2017.

Month	Air temperature (˚C)	Rainfall (mm)	Air humidity (%)	Duration of sunshine (hours)
January	2.2	114.3	79	82
February	3.6	12.9	75	136
March	8.6	45.6	72	196
April	9.8	24.4	66	265
May	15.6	44.6	69	275
June	20.9	45.7	65	319
July	23.9	39.5	60	359
August	26.3	26.7	51	334
September	21.0	58.4	60	292
October	13.5	40.6	71	162
November	9.0	81.1	80	80
December	8.5	76.8	75	103

Three experimental plots, each of 20.2 m^2 (4.2 x 4.8 m), were arranged with plants spaced 60 cm along the rows which were 70 cm apart. An organic fertilization with 15 $q \cdot ha^{-1}$ manure (6 N - 5 P_2O_5 - 13 K_2O) before transplant and irrigations during the crop cycle were practiced.

The different parts of *Withania somnifera* plants were collected as follows: leaves in August, at the stage of plant blooming; roots in early October 2017 at the fruiting stage. Then, the plants were air dried and powdered.

Analytical determinations

Polyphenols

They were determined in water extract, using Folin-Ciocalteu colorimetric method [14]. One gram of dry leaves and roots powder was extracted with 20 ml of 70 % ethanol at 80 °C in 1 h. The mixture was cooled, quantitatively transferred to a volumetric flask and the volume was adjusted to 25 ml. The mixture was filtered through filter paper and 1 ml of the resulting solution was transferred to 25 ml volumetric flask, to which 2.5 ml of saturated Na_2CO_3 solution and 0.25 ml of diluted (1:1) Folin-Ciocalteu reagent were added and the volume was brought to 25 ml with distilled water. One hour later the solutions were analyzed through spectrophotometer (Unico 2804 UV, USA) and the concentration of polyphenols was calculated according to the absorption of reaction mixture at 730 nm. As an external standard, 0.02 % gallic acid was used.

Antioxidant activity (AOA)

The antioxidant activity of *W. somnifera* was assessed using the redox titration method [15], via titration of 0.01 N $KMnO_4$ solution with ethanolic extracts from *Whitania* leaves and roots. The reduction of $KMnO_4$ to colourless Mn^{+2} in this process reflects the amount of antioxidants dissolvable in 70 % ethanol. The values were expressed in mg GAE g^{-1} d.w. The acidic solution of $KMnO_4$ is successfully used for the determination of *Ocimum basilicum* antioxidant potential [16] and antioxidant capacity of serum [17].

Flavonoids

Total flavonoids content was determined by spectrophotometric method, based on flavonoid-aluminum chloride ($AlCl_3$) complexation [18]. The standard of quercetin dehydrate (98% HPLC, Sigma Co., St. Louis, MO, USA) was used. Ten mL of methanol were added to 1 g of dried and homogenized samples and the latter were left at room temperature for 2 h. The resulting mixture was filtered through pleated filter. Then, 0.2 mL of the extract were diluted with 1.8 mL of methanol, and 0.1 mL of 2 % $AlCl_3$, 0.5 mL of 1 M sodium acetate solution and 1 mL of distilled water were added. The mixture was incubated for 30 min at room temperature and absorption at 415 nm was measured. Total flavonoid content was determined by standard curve built using five different concentrations of quercetin-$AlCl_3$ complex. Quercetin was purchased from Fluka (Switzerland).

Alkaloids (withanolides)

The concentration of alkaloids in *W. somnifera* roots were assessed according to the method of Mishra [19]. Ten ml of chloroform and 3-4 drops of concentrated ammonia were added to 1 g of roots powder. The samples were mixed and left at room temperature for 12 hours. The mixture was filtered through a glass filter and the residue was three times washed with chloroform (3x10 ml). Chloroform was removed at 50 ^oC using rotor evaporator. The residue was dissolved in 1 ml of ethanol, 10 ml of 0.01 N solution of sulfuric acid were added and the resulting reactionary mix was titrated by a solution of 0.01 N Na0H. The alkaloid content was calculated using the difference value between volumes of alkaline used for extract titration and for titration of 10 ml 0.01 N sulfuric acid via the following formula:

Alkaloid content (%) = 0.415 x (V_{H2S04}-$V_{extract}$)

Elemental composition

Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, Hg, I, K, Li, Mg, Mn, Na, Ni, P, Pb, Se, Si, Sn, Sr, V, and Zn contents in *W. somnifera* leaves and roots samples were assessed using ICP-MS on quadruple mass-spectrometer Nexion 300D (Perkin Elmer Inc., Shelton, CT, USA), equipped with the seven-port FAST valve and ESI SC DX4 autosampler (Elemental Scientific Inc., Omaha, NE, USA) at the Biotic Medicine Center (Moscow, Russia). Rhodium 103 Rh was used as an internal standard to eliminate instability during measurements. Quantitation was performed using external standard (Merck IV, multi-element standard solution), potassium iodide for iodine calibration; Perkin-Elmer standard solutions for P, Si, and V, and all the standard curves were obtained at five different concentrations. In order to check the quality, internal controls and reference materials were tested together with the

samples on a daily basis. Microwave digestion of samples was performed with sub-boiled HNO_3 (Fluka #02650 Sigma-Aldrich, Co) in the Berghof SW-4 DAP-40 microwave system (Bergh of Products + Instruments GmbH, Eningen, Germany), diluted 1:150 with distilled deionized water. Trace levels of Hg and Sn in the samples were not taken into account and, accordingly, they were excluded from the Tables.

In order to evaluate the intensity of macro- and microelement accumulation by *W. somnifera*, other medicinal plants of Nikita Botanic Gardens collection were analyzed: *Myrthus* and 14 *Artemisia* species.

The degree of macro- and microelements transport from the sea surface to Nikita Botanic Gardens experimental fields was determined analyzing the elemental composition of *Hedera helix var. taurika* leaves collected at different heights above the sea level.

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. Data expressed as percentage were subjected to angular transformation before processing.

RESULTS AND DISCUSSION

Antioxidants

A significant part of the biological activity of *W. somnifera* is determined by the high concentration of antioxidants [2]; however, it is difficult to compare the contents of flavonoids and polyphenols in the Indian ginseng plants recorded in our investigation with those reported in literature (Table 3). These difficulties are connected: with the method of calculation, i.e. per dry weight or per amount of extracted material; with the different methods of extraction used which can affect the variability of results [20]; in some publications even no references to the extraction method are reported [21]. In this respect, the total flavonoid content in *W. somnifera* plants grown in Himalayas was 10 times lower than that recorded in our research at Nikita Botanic Gardens; however, the temperature of extraction was very low (0 ^{o}C) in Indian investigation and this may explain such great differences [22]. The comparison between 70 % ethanol and water extracts in determining flavonoids, polyphenols and AOA revealed the preference for 70 % ethanol use in the extraction (Table 2). Regardless of the close values of phenolic content in *W. somnifera* leaves detected in the present investigation to those found by Tupe et al. [23], it can be suggested that the use of both water and ethanolic extracts gives similar concentration levels of leaf polyphenols and root flavonoids. Conversely, the extraction with 70 % ethanol results in 1.3 higher content of flavonoids in leaves as well as 1.3 higher AOA both in leaves and roots, whereas root phenolics extracted with 70 % ethanol show a 1.5 times higher concentration compared to water method.

The withanolides content assessed in *W. somnifera* roots in our research is close to that described by Mishra [19].

Table 2. Antioxidant activity (AOA) and compounds in leaves and roots of *Withania somnifera* (L.) Dunal.

Parameter	Leaves		Roots	
	water	70 % ethanol	water	70 % ethanol
AOA (mg eq. GA g^{-1} d.w.)	28.8±2.0 b	38.2±2.1 a	8.3±0.6 c	10.8±0.9 d
Phenolics (mg eq. GA g^{-1} d.w.)	6.88±0.4 a	7.52±0.3 a	2.60±0.1 d	3.83±0.2 c
Flavonoids (mg eq. quercetin g^{-1} d.w.)	1.75±0.1 b	2.30±0.1 a	0.50±0.03 c	0.48±0.03 c

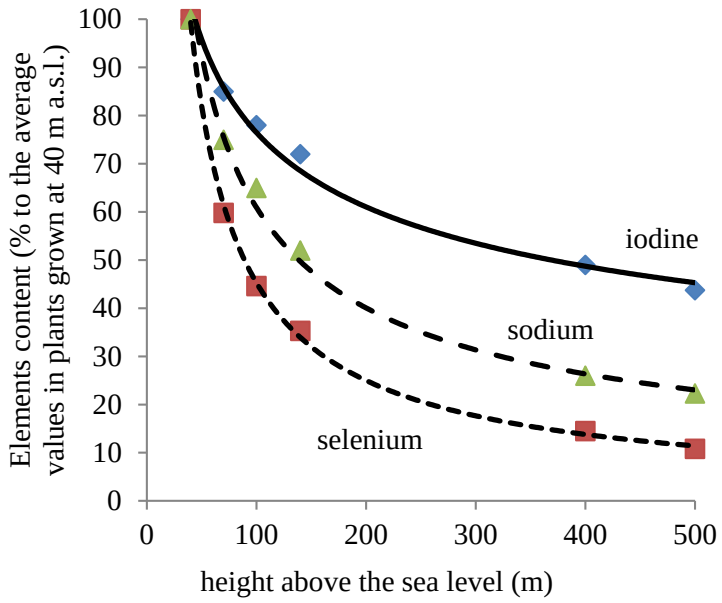
Along each line, values followed by the same letter do not differ statistically according to Duncan test at P≤0.05.

Elemental composition

The coastal zones are subject to intensive transfer of macro- and microelements, in the form of aerosols and volatile compounds (methylated forms of iodine and selenium), from the sea surface to the land [24].

The accumulation of sodium ions by *Hedera helix var. taurika* suggests relatively high levels of sodium transfer from the sea surface to the height of 150-200 m above the sea level where Nikita Botanic Gardens are located (Fig. 1). Changes in selenium and iodine concentration in *Hedera helix var. taurika* plants as a function of the a.s.l. height showed the same trends; however, the intensity of iodine accumulation in plants is the least affected by the height above the sea level.

Fig 1. Relationship between sodium, iodine and selenium content in the Crimean ivy leaves and the height above the sea level [14]



The comparison between *W. somnifera* elements concentration and both the corresponding data recorded in *Myrtus* and in *Artemisia* (average of 14 species) grown in the same unpolluted area shows slightly higher contents of I and Se in Indian ginseng, which shows additional characteristics of plants grown very closely to the sea (Table 4). Unexpectedly, *W. somnifera* leaves displayed high levels of

Li, which exceed those detected in *Myrtus* and *Artemisia* by 308 and 220 times respectively. Notably, 10 g of Indian ginseng leaves powder provides the adequate Li consumption level for humans.

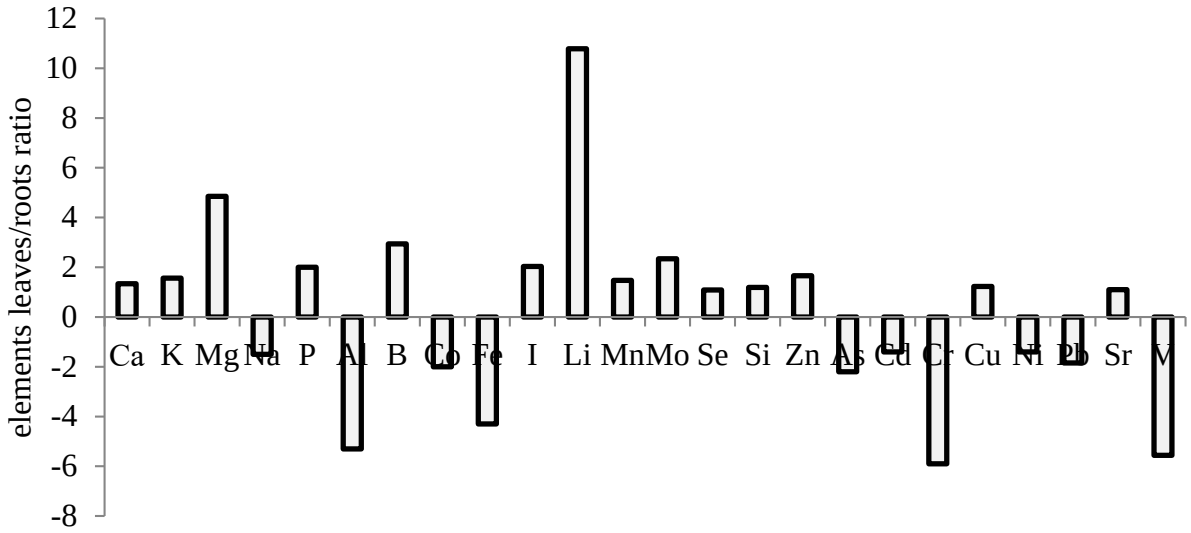
Table 3. Macro- and microelements content in *Withania somnifera*, *Myrtus* and *Artemisia*

Elements	*Withania*		*Myrtus*	*Artemisia*
	leaves	roots		(average of 14 species)
Ca	23.6±2.4 a	17.6±1.8 b	10.5±1.1	11.5±2.3
Macroelements (g kg^{-1} d.w.)				
K	35.9±3.6 a	23.0±2.3 b	8.7±0.9	30.2±7.1
Mg	5.74±0.57 a	1.18±0.12 b	1.92±0.19	2.33±0.87
Na	0.37±0.04 b	0.55±0.05 a	0.12±0.01	0.33±0.24
P	4.19±0.42 a	2.09±0.21 b	1.23±0.12	4.27±0.95
Microelements (mg kg^{-1} d.w.)				
B	34.4±3.4 a	11.7±1.2 b	43.2±4.3	47.5±11.4
Co	0.17±0.02 b	0.35±0.04 a	0.29±0.04	0.18±0.12
Cu	10.1±1.0 a	8.2±0.8 a	4.4±0.4	13.0±5.2
Fe	198±20 b	853±85 a	125±13	176±147
I	1.4±0.14 a	0.69±0.08 b	0.46±0.06	0.86±0.44
Li	92.5±9.3 a	8.6±0.9 b	0.30±0.04	0.42±0.24
Mn	41.9±4.2 a	28.6±2.9 b	33.4±3.3	75.6±26.2
Mo	0.82±0.10 a	0.35±0.04 b	0.08±0.01	0.74±0.32
Se	0.12±0.01 a	0.11±0.01 a	0.06±0.01	0.06±0.004
Si	11.3±1.1 a	9.5±1.0 a	19.1±1.9	15.4±6.7
Zn	22.8±2.3 b	37.8±3.8 a	13.9±1.4	44.9±15.3
Arsenic and heavy metals (mg kg^{-1} d.w.)				
Al	117±2 b	621±62 a	68±7	230±220
As	0.15±0.02 b	0.33±0.04 a	0.04±0.01	0.16±0.12
Cd	0.050±0.008 a	0.070±0.011 a	0.005±0.001	0.110±0.040
Cr	0.35±0.04 b	2.05±0.21 a	0.22±0.03	0.42±0.32
Ni	1.15±0.11 b	1.62±0.16 a	1.17±0.12	1.95±0.43
Pb	0.30±0.04 b	0.56±0.07 a	0.15±0.02	0.27±0.19
Sr	69.1±6.9 a	76.0±7.6 a	20.9±2.1	65.0±31.0
V	0.33±0.04 b	1.83±0.18 a	0.14±0.02	0.36±0.32

The data presented in Table 3 suggest that the roots of *W. somnifera* accumulate higher levels of V, Cr, Al and Fe compared to the leaves (5.5; 5.9; 5.3 and 4.3 times respectively)

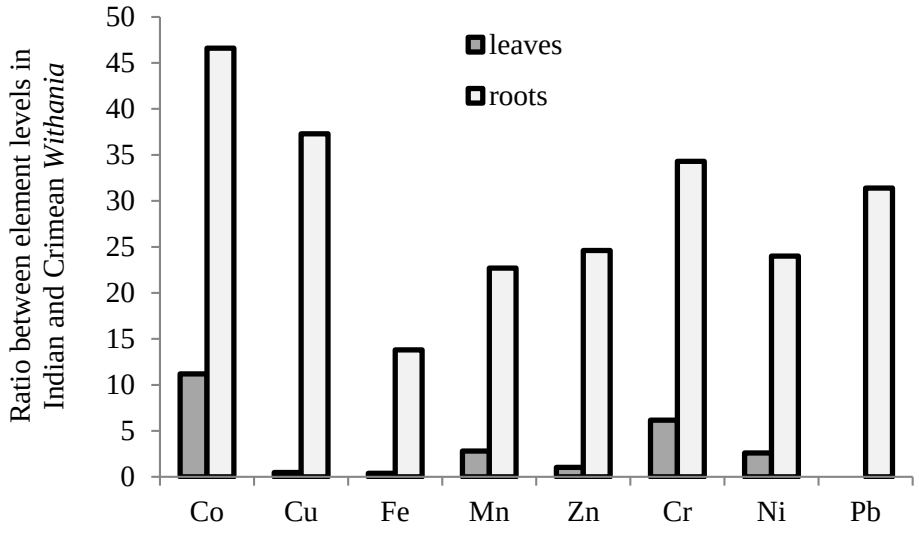
The typical profile of elements accumulation in leaves and roots of Indian ginseng is shown in Fig. 2.

Fig. 2. The typical profile of elements accumulation in *Withania somnifera* leaves and roots.



The comparison between the results of the present research and those reported in literature relevant to the elemental composition of *W. somnifera* grown in other areas of the world suggests that the leaves/roots ratios concerning the elements concentration in this species are rather stable. This is contrary to the absolute values of the elements content, whose ratios differ from 14 to 47 times in plant roots harvested in Crimea or in India [25] (Fig. 3) and up to 2500 or 2400 times for Co or Cr respectively, in plants collected in Crimea or in Pakistan [26]. In this respect, the degree of involvement of macro- and trace-elements in biological activity of *W. somnifera* is presumably highly dependent on both the biogeochemical characteristics of soil and intensity of pollution. In the unpolluted areas of the Nikita Botanic Gardens, the leaves of Indian ginseng may be regarded a significant source of Li for humans, providing neurological health. In Pakistan (Karachi) conditions, *W. somnifera* showed an outstanding ability to accumulate Fe and Cu [26], but it is characterized by high concentrations of heavy metals.

Fig. 3. Differences in elements accumulation between Indian [25] and Crimean *Withania somnifera.*



Overall, the Fig. 3 demonstrates that leaves and roots of Indian ginseng grown at Nikita Botanic Gardens are less rich in elements compared to Himalayan *W. somnifera* plants [25]. The microelement Li may represent the exception, though no literature data concerning its accumulation by *W. somnifera* is available.

CONCLUSION

The results of the present work emphasize the importance of the environmental effect on the biological activity and elemental composition of the health-beneficial *W. somnifera*, thus showing the importance of assessing the plant profiles relevant to these features.

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