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Research Article

Chemical Composition and Heavy Metals in Wild Edible Scorzonera Incisa DC

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Abstract

Alapouk or *Sheng Asbi* is the native name of *Scorzonera incisa* DC, a plant with two or more leaves and is wildly grown in Fuladshar -Isfahan in the center of Iran. It grows within the period starting at about middle March and ending late in April. It is used as an edible vegetable and leaves are sometimes cooked before they are served. The natives of the area ground the nodes of the plant in a mortar and mixed water, wheat or barley flour, some salt and such things to bake bread. In the present study, nutritive value and mineral compositions of some *S. incisa* that wild grown in Isfahan, Iran were determined in the all used plant parts. As nutritional value; dry matter, total ash, % N, crude protein, crude fiber and protein contents and pH were determined. Se, Li, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, P, Pb, S and Zn were the investigated minerals in the aerial parts and root of plant samples. The samples were analysed by standardized international protocols in Nutrition and Food Sciences Research Center in Pharmaceutical Sciences Branch, Islamic Azad University. Obviously in the order of amount of some mineral element contents such as iron, zinc and manganese are high in comparison by other vegetables studied in Iran in other studies. The crude protein content of Iranian *S. incisa* examined in this study which reached 8.14 and this value is significantly higher and superior than other edible vegetables reported in other countries especially in Turkey samples. Therefore, due to the high nutritive and mineral elements and low contents of heavy metals such as Pb, Cd and Ni, it could be a new source of edible vegetable after the future toxicological studies.

Keywords: Scorzonera Incisa; Heavy Metals; Chemical Composition; Edible Vegetable

Introduction

Confirmed the influence of plants in environmental studies, identifying vegetation cover of different areas could play significantly role in planning a variety of programs notably in the preservation, restoration, management and identification of plant species, including endemic plants. Floristic studies in Iran for conservation of natural resources, biodiversity and making the management planning are concerned. Iran has one of the most attractive rich floras in the South - Western Asia, and this is because of the large area, diversity of climate and topography [1-7]. Endemic plants are not few in Iran, so their study plays a significant role in the preservation of natural resources. Iran, with 1,640,000 square kilometres area, in the south-west of Asia- of the northern hemisphere, has its specific combination of different elements of life and a special ecosystem and biodiversity due to various factors including different climatic conditions, high mountains all around and a large desert in center. Different phytogeographic regions in Iran's plateau cause massive genetic flow in this area which result in a variety of plant species and in comparison, with neighbour countries and some others has very interesting points.

Some plant species have been walled beyond the natural fences (as endemic), and some are scattered in other lands. Most part of Iran is occupied by Deserts and semi-deserts. Residents of these areas have always been strongly dependent on vegetation cover, and where the vegetation cover could create good micro-climate, people have settled there.

Due to the diversity of climate, topography and edaphic conditions, limited areas of vegetation in Iran, are very different and heterogenous. Due to social, economic and cultural reasons, indigenous and non-indigenous residents of these regions, consciously or unconsciously have exploited from these natural resources excessively [3-7].

Scorzonera species are used in different folk medicines to combat many diseases, including the illnesses connected with inflammation. *Alapouk* or *Sheng Asbi* is the native name of *Scorzonera incisa* DC, a plant with two or more leaves and is grown in Fuladshar -Isfahan in the centre of Iran. Its leaves occasionally measure a few centimetres in length. The leaves vary in colour from dark green to dark brown. The plant provides yellow flowers in continu-

ation of its life period. This plant grows at various zones of southern Khorasan, south Khorasan Razavite and Mehrdasht and Lenjan of Isfahan Province. Stem leaves at most c. 1/2 size of basal leaves. Capitula 1-2(-4) per stem, (35-)40-50 mm long. Inner phyllaries c. 25 mm [8-24].

It grows within the period starting at about middle March and ending late in April. It is used as an edible vegetable. Its leaves are sometimes cooked before they are served. About 70 years ago the natives of the area ground the nodes of the plant in a mortar and mixed water, wheat or barley flour, some salt and such things to bake bread. Interestingly the other plant (scorzonera paradoxa fisch and CA Mey) which is very similar to S. incise in Khorasan region is called Peyghambar bread, Galvac or Naghoudashak and native people are used to eat it as edible vegetable. It has excrescence root in various forms, which is edible as well. Based on a study carried out in scorzonera paradoxa fisch and CA Mey plant, the leaves of this plant contain higher percentages of protein, ash and crude fat compared to those in root [25]. Highest rates of energy and crude fiber are found in the root. The leaves contain considerable values of zinc, manganese, chrome, magnesium and cadmium while the root contain significant values of iron, zinc and manganese.

Prolonged uses of considerable amounts of this plant bring reduction to blood sugar; however, it may result in hepatic injury in diabetic mice. Healthy mice may develop symptoms of mild toxicity with an increase in dosage [26-28]. The aim of current study was determination of proximate composition, mineral elements (Calcium, Potassium, iron, Lithium, Zinc, Sodium, Selenium, Magnesium and Copper), and on the other hand determination of toxicity and analysing some heavy metals such as Pb, Cd, Ni of *Scorzonera incisa* DC different parts (root and aerial parts) as an edible vegetable in Isfahan, Iran.

Experimental

Sampling Plant

S. incisa plant gathered from Fuladshahr in March and April of 2017 randomly. Fuladshahr is situated 25 kilometers (16 mi) south of Isfahan. Isfahan is located on the main north-south and east-west routes crossing Iran, and was once one of the largest cities in the world [29]. Fuladshahr is under the effect of local steppe climate. No much rain in Fuladshahr all through the year. Its climate is classified as BSK within Coupon's system. Mean annual temperature is 15.2 degrees Celsius and man annual precipitation is 154 mm. the soil is clay in plains and it is a combination of sands and soil on mountainous areas. The sampling zone has the following geographical coordinates: 32°29′27″N 51°25′16″E, which is shown in Figure 1.

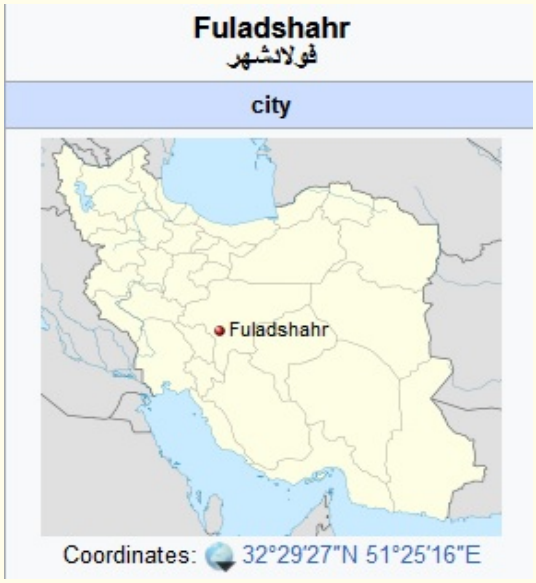

Figure 1: Location of S. Incisa Samples Collection.

pH determination and Total crude fiber determination

pH values were determined by pH-meter in the plant samples according to the AOAC method 981.12 [30]. Crude fiber analyses were accomplished by AOAC method 962.09 [31].

Total nitrogen determination and crude protein calculation

Kjeldahl apparatus and method were used to find of total nitrogen content of the samples [4]. After total nitrogen content determination, crude protein contents were calculated [4].

Zinc, Manganese, Nickel, Cadmium, Chrome, Lead, Copper and Potassium Determination

For Zinc, Manganese, Copper and Selenium concentration root and aerial parts of samples were dried in oven for 36 - 48 hours at a temperature of 80°C. The samples were then ground and sieved through 0.5 mm sieve. The powdered samples then subjected to the acid digestion using nitric acid (65% Merck, Germany), Sulfuric acid (96.5% Merck, Germany) and perchloric acid (70% Sigma-Aldrich). Two gram of air-dried of each homogeneously *S.incisa* samples accurately weighed and 20.0 mL of the digestion mixture

(3 parts by weight of concentrated nitric acid: 2 parts of concentrated Sulfuric acid and 3 parts by weight concentrated perchloric acid) and heated slowly by an oven and then rise the temperature. The remaining dry inorganic residues were dissolved in 25.0 mL of nitric acid and the solution used for the determination of mineral elements. Blanks and samples were also processed and analysed simultaneously. All the chemicals used were of analytical grade (AR). Standardized international protocols were followed for the preparation of material and analysis of heavy metals contents [32-38].

The samples were analysed by Flame Emission Spectrophotometer, using at Six standard solutions for each metal and determination of potassium content was followed by FDA Elemental analysis [38] (ORA LABORATORY MANUAL, 2013). Also, periodic testing of standard solutions was performed in order to verify of reliability of the measuring apparatus. The accuracy was checked using quality control test for fungi and their substrate samples to show the degree of agreement between the standard values and measured values; the difference was less than 5%. The samples were analysed by Flame Emission Spectrophotometer Model AA-6200 (Shimadzu, Japan) using an air-acetylene, flame temperature: 2800°C, acetylene pressure: 0.9 - 1.0 bar, air pressure: 4.5 - 5 bar, reading time: 1 - 10 sec (max 60 sec), flow time: 3 - 4 sec (max 10 sec).

Iron Determination

The aliquot was passed through the atomic absorption spectrophotometer to read the iron concentration. Standards were prepared with a standard stock of 10 mg/L using ferrous ammonium sulphate where 3 - 60 ml of iron standard solution (10 mg /L) were placed in stepwise volumes in 100 ml volumetric flasks.

2 ml of hydrochloric acid were added and then brought to the volume with distilled water. The concentration of iron in the aliquot was measured using the atomic absorption spectrophotometer in mg/L. The whole procedure was replicated three times.

Calcium, Sodium and Magnesium Determination

The contents of Ca, Mg and Na in *S.incisa* different part of plant were measured by atomic absorption spectrophotometer (AAS) (Model AA-6200 Shimadzu, Japan) according to the method of Hernandez [39].

A 5g sample was placed in a previously weighed porcelain crucible and heated. The resulting white ash was weighed, dissolved in 12 ml of concentrated nitric acid, perchloric (3:2) and diluted with nitric acid 10% in a 25-ml calibrated flask. The solution then was used to determine Ca, Na, and Mg. Standard stock solution of sodium, magnesium and calcium was prepared from AAS grade chemicals (Merck, Germany) by appropriate dilution.

Selenium Determination

Stock standard solutions for selenium were 1000 g/mL solution. All reagents and standards were of analytical grade (Merck, Germany). The palladium matrix modifier solution was prepared by the dilution (10 g/ L) Pd(NO3)2 and iridium AA standard solution, 1000 g/ mL in 20% HCl, 0.1 % V/V nitric acid prepared by dilution trace pure 65 % nitric acid and 0.1 % Triton X-100 were used. Doubly distilled water was used in all operations. The samples were analysed by Flame Emission Spectrophotometer Model AA-6200 (Shimadzu, Japan). The analyse performed according by Analytical Method ATSRD [40].

Moisture Content

All part of *S.incisa* plant samples (flowers, roots, leaves and stems) were oven dried at 70°C for 24-36 hours until a constant weight were obtained. The moisture contents were expressed as loss in weights of the wet samples [21-24].

Crude Fiber

Five grams of the ground *S.incisa* samples were digested in 75 ml of 1.25% H_2SO_4. The solutions were boiled for 45 minutes and then were filtered and washed with hot distilled water. The filtrates were digested in 100 ml of 1.25% Sodium Hydroxide solutions. These solutions were heated for 60 minutes, filtered and washed with hot deionized water and over dried. The final oven-dried residues were ignited in a furnace at 550°C. The weights of the left after ignition were measured as the fiber contents and were expressed in term of the weights of the samples before ignition.

Crude Protein

The protein nitrogen in one gram of the dried samples were converted to ammonium sulphate by digestion with concentrated H2SO4 (Merck 96.5%) and in the presence of CuSO4 and K2SO4 [41-42]. The solutions were heated and the ammonia evolved were steam distilled into Boric acid 2%. The nitrogen from ammonia were deduced from the titrations of the trapped ammonia with 0.1M HCl with Tashirus indicator (methyl red: methylene blue 2:1) until a purplish pink colour were obtained. Crude proteins were calculated by multiplying the valve of the deduced nitrogen by the factor 6.25 mg [24-26].

Ash Content

One gram of the oven-dried samples in powder from was placed in acid washed crucible by known weight. They were ignited in a muffle furnace for 3 - 4 hours at 550°C. After cooling crucibles, they were weighed and the ash contents were expressed in terms of the oven-dried weight of the sample.

Oil (Lipid) Content

The lipid contents of five grams of *S.incisa* roots by petroleum ether in a Soxhlet apparatus were extracted. The weight of the lipid obtained after evaporating off the petroleum ether from the extracts gave the weights of the crude fat in the samples [4,5].

Fatty Acid Determination

Determination of Fatty acid composition for walnut samples were done by using a modified fatty acid methyl ester method as described by Hışıl [4-5,43]. The oil was extracted three times for 2g air-dried seed sample by homogenization with petroleum ether. The oil samples (50 - 100 mg) were converted to its fatty acid methyl esters (FAME). The methyl esters of the fatty acids (1 µl) were analysed in a gas chromatography (Shimadzu GC-2011) equipped with a flame ionizing detector (FID), a fused silica capillary column (60 m x 0.25 mm i.d.; film thickness 0.20 micrometre).

Carbohydrate Content

The carbohydrate content of the samples was estimated as the differences obtained after subtracting the values of organic proteins, lipids, ashes and fibres from the total dry matter for both of root and aerial parts of samples.

Statistical Methods

All the data were analysed using the SPSS 20 statistical software for analysis of variance using ANOVA and Duncan's least significant difference (LSD at $p < 0.05$) for statistical significance. 3 duplicates with a replicate were considered in this research, and data was reported as the mean ± standard error of the mean.

Results and Discussion

The "ash content" is a measure of the total amount of minerals present within a food, whereas the "mineral content" is a measure of the amount of specific inorganic components present within a food, such as Ca, Na and K. Determination of the ash and mineral content of foods is important for a number of reasons such as nutritional labelling: The concentration and type of minerals present must often be stipulated on the label of a food and quality: The quality of many foods depends on the concentration and type of minerals they contain, including their taste, appearance, texture and stability. A proximate composition and physico-chemical characteristic of all samples has shown in Table 1.

Nutrient	Percentage (%)
Dry Matter(DM)	20.12
Crude Protein (CP)	8.14
Crude Fiber (CF)	40.22
Total Ash	14.23
N (%)	2.01
pH	6.32

Table 1: Proximate Analysis of S. Incisa Samples from Isfahan Province, Iran.

Mineral Elements Composition

The mean content of mineral elements: Sodium, Selenium, Calcium, Iron, Magnesium, Copper and Zinc in *S. incisa* samples collected from Fuladshahr-Isfahan, Iran are shown in Table 2. The samples were analyzed by wet digestion method and standardized international protocols were followed for the preparation of material and analysis of heavy metals contents and analysed by Atomic Absorption Spectrophotometer in Research Laboratory in Pharmaceutical Sciences Branch, Islamic Azad University

Minerals	*Scorzonera incisa* DCRoots	*Scorzonera incisa* DC Flowers	*Scorzonera incisa* DC Leaves
Mg (g/kg)	8.43 ± 0.09	6.32 ± 0.16	6.76 ± 0.13
Na (g/kg)	0.71 ± 0.021	0.66 ± 0.011	0.70 ± 0.055
Se (g/kg)	0.011 ± 0.004	0.009 ± 0.003	0.023 ± 0.005
P (g/kg)	4.92 ± 0.020	3.86 ± 0.011	4.25 ± 0.061
S (g/kg)	4.55 ± 0.013	3.76 ± 0.018	4.29 ± 0.015
Zn (mg/kg)	87.22 ± 2.01	67.90 ± 1.78	82.36 ± 1.98
Cu (mg/kg)	34.78 ± 1.04	28.76 ± 1.03	34.10 ± 1.06
Cr (mg/kg)	1.77 ± 0.18	0.67 ± 0.21	0.62 ± 0.26
Fe (mg/kg)	561.09 ± 12.16	432.11 ± 14.02	398.77 ± 18.01
Mn (mg/kg)	156.40 ± 10.65	120.16 ± 11.67	176.54 ± 13.92
Co (mg/kg)	0.69 ± 0.043	0.56 ± 0.056	0.71 ± 0.023
Pb (mg/kg)	0.25 ± 0.017	0.11 ± 0.016	0.15 ± 0.011
Cd (mg/kg)	0.05 ± 0.027	ND*	0.02 ± 0.001
Ni (mg/kg)	10.48 ± 0.029	6.22 ± 0.018	7.42 ± 0.015
Li (mg/kg)	0.016 ± 0.005	0.013 ± 0.006	0.15± 0.006

Table 2: Mean Values (± SD) of Mineral Compositions of Wild Edible S.Incisa Samples.
ND* = Not Detected.

The data obtained from chemical analyses, mean values were calculated and are given in the Table 2, with their standard deviations.

The accuracy of the methods was validated by comparing the actual amounts of Cd, Cr or Pb with those of calculated concentrations. The intra-day precision and accuracy of the method were determined under the optimal working conditions by triplicate measurements of known Cd, Cr or Pb concentrations. For the determination of inter-day precision and accuracy, the same procedure was repeated over a 3-day period.

The concentrations (mg/kg) of the heavy metals in samples ranged from 0.00 to 0.05 ± 0.027for Cd in root of plant, 0.62 ± 0.26 in leaves to 1.77 ± 0.18for Cr in the roots and 0.11 ± 0.016 in flowers to 0.25 ± 0.017 for Pb in the root samples. Our findings showed that the highest concentration of heavy metals was for Pb. In accordance with the standard guideline of FAO/WHO, it was found that Cd, Cr and Pb concentrations of samples not exceeded the recommended levels. All mineral contents in roots of *S.incisa* samples were higher than leaves and flowers of the plant.

Isfahan is the third populated city in Iran with dry climate. There are several industries located at the periphery of the city which makes the possibility of contamination with heavy metals higher than that of most cities of Iran. Zayandeh-Rood River crosses Isfahan from west to east. The main source of water for the farms in this region is Zayandeh-Rood River. The study site was Fuladshahr just near Isfahan.

Conclusion

It can be concluded, from the findings of current study, that the amounts of Cd, Cr, and Pb were not higher than the acceptable levels recommended by WHO/FAO. Also, higher amount of Fe and Zn in leaves and root wildly grown samples from Fuladshahr compared to those of edible vegetables shown that this plant can be considered as a main good source of mineral elements. Due to the importance of this issue and importance of wild edible vegetables sources of nutritive value, it is recommended to conduct studies with this nature periodically and other probable toxicity such as Hg, As or some organic compounds should be followed.

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Conflict of Interest

None of the authors have any conflicts of interest associated with this study.

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