Quantitative ICP-OES Determination of Trace and Essential Elements in the Plant Species of Genus *Ferula*

Sermin Yelkenci^a, Huseyin Altundag^{a*}, Mehmet Sagiroglu^b, and Abdul Razaque Memon^c

^a Sakarya University, Faculty of Arts and Sciences, Department of Chemistry,
54187 Sakarya, Turkey

^b Sakarya University, Faculty of Arts and Sciences, Department of Biology,
54187 Sakarya, Turkey

^c International University of Sarajevo, Faculty of Engineering and Natural Sciences,
71000, Sarajevo, BiH

INTRODUCTION

Apiaceae is a family of aromatic plants that constitutes approximately 3,700 species spread across 434 genera. The genus *ferula* (Apiaceae) contains 180–185 species with the highest diversity in central and southwest Asia. It has about 130 species, around 100 of which are endemic (1). In Turkey, the first investigation on *ferula* was carried out by Pesmen (2) who identified 18 species, one of which was known and nine of them were endemic. Four new species have since been added to the flora of Turkey (3,4,5) and additionally one of the previously known species was redescribed by Sarioglu, and Duman, (6). Around 55% of the Turkish species (12 of 22 in total) are endemic, showing that Turkey is possibly one of the major gene centres of this genus. The Turkish *ferula* species are monocarpic or polycarpic herbaceous perennial plants growing up to a height of 20–500 cm, with hollow stems. The leaves are tri-seven pinnate, with generally stout basals heath clasping the stem. The flowers are yellow, with or without reduced sepals in large umbels. Mericarps are dorsally compressed with generally 1–3 vittae pervallecula on the dorsal surface.

Species of the genus *ferula* are usually referred to as "Caksir" in Turkish and are used today as a tonic (aphrodisiac), germicide,

ABSTRACT

Some of the species belonging to the genus *ferula* are known to be used as traditional medicine for treating various diseases in South Asia, the Middle East, and in North Africa. In this study, samples from 20 varieties of plants belonging to 20 species of the genus *ferula* L. (Apiaceae) were collected from various regions of Turkey. After air drying, the samples were solubilized by using the microwave dissolution technique. The concentration levels of the different elements (Al, B, Ba, Cr, Cu, Fe, Mn, Ni, Pb, Sb, Se, Sr, and Zn) were determined by ICP-OES. The accuracy of the proposed method was verified by analyzing certified reference materials SRM 1573a Tomato and INCT-TL-1 Tea Leaves. The results showed a significant variation of elemental content, especially for Al, B, Ba, and Fe amongst the fruit- and flower-bearing plants of the different plant species of the same genus. This clearly indicates that each plant species has a different absorption and accumulation capacity for different metals. Further work on the accumulation of these metals in the leaves and shoots of the plants is in progress.

constipation relaxant, an antitussive antidote against poisoning as well as an agent for treatment of hemorrhoids and urinary tract diseases. Moreover, they are also used as a nutrient and an animal feed in different regions of Turkey and are

widely consumed in the vicinity of Erzurum, Van (East Anatolia), Hatay, Osmaniye, Kahramanmaraş (South Anatolia), Nigde, and Malatya (Central Anatolia).

Certain mineral elements that are non-toxic and necessary for human health may exhibit toxic effects if taken in high quantities or for a long period of time (7). The heavy metal content in commercial products has particularly increased in recent years. Also, high concentrations in edible plants are a threat to human health (8) because these metals accumulate in the body through inhalation, ingestion, and skin absorption (9, 10). On the other hand, the elements of this study (Al, B, Ba, Cr, Cu, Fe, Mn, Ni, Pb, Sb, Se, Sr, and Zn), which are in the second row in the periodic table, may also lead to various diseases if their intake is insufficient. Therefore, it is necessary to classify these plant species according to their metal content in order to use appropriate material for herbal medicine.

The aim of this study was to determine the metal composition of these important plant species and to determine the levels in the different parts of the plants used for natural herbal medicine. For this study, 20 varieties of plants belonging to the genus *ferula* (Apiaceae), collected from various regions of Turkey, were first solubilized by microwave dissolution technique, then the concentrations of Al, B, Ba, Cr, Cu, Fe, Mn, Ni, Pb, Sb, Se, Sr, and Zn in these plants determined by ICP-OES. This study is important,

*Corresponding author.
E-mail: altundag@sakarya.edu.tr

because there is no reported data available regarding the trace metal content in these medicinal plants.

EXPERIMENTAL

Instrumentation

In order to solubilize the plant samples, a Milestone Ethos D microwave closed system (maximum pressure 1450 psi, maximum temperature 300 °C) (Milestone, Italy) was used. A Spectro Arcos model 165 inductively coupled plasma optical emission spectrometer (ICP-OES) was used for the determination of all elements (Spectro Analytical Instruments, Kleve, Germany). The instrumental operating conditions are listed in Table I.

Standard Solutions and Reagents

A 10% HNO₃ solution was used for cleaning the glass and plastic containers used for the study. The containers were allowed to stand in 10% HNO₃ solution for 1 day before being rinsed with Milli-Q® ultra-distilled water (18.2 MΩ·cm) (Millipore Corporation, USA) and used thereafter. Suprapur® nitric acid (65%, w/w) and analytical grade hydrogen peroxide (30%, w/w) were purchased from Merck (Germany). An ICP multi-element standard solution of 1000 mg L⁻¹, as the calibration standard, was diluted to the desired concentrations using ultra-distilled water. The accuracy of the proposed method was evaluated by analyzing certified reference materials of SRM 1573a Tomato (National Institute of Standards and Technology, Gaithersburg, MD, USA) and INCT-TL-1 Tea Leaves (Institute of Nuclear Chemistry and Technology, Warsaw, Poland).

Analysis of Reference Materials

The concentrations of Al, B, Ba, Cr, Cu, Fe, Mn, Ni, Pb, Sb, Se, Sr, and Zn were determined using the certified reference materials SRM

1573a Tomato Leaves and INCT-TL-1 Tea Leaves. It can be seen from Table II that the results are compatible with the certified values, and proves the validity of the method used for the plant samples.

Sample Collection and Pre-treatment

The floral parts of *F. tingitana*, *F. Lycia*, *F. duranii* ve *meyveli* kısımlarda *F. szowitsiana*, *F. orientalis*, *F. tingitana*, *F. duranii*, *F. Lycia* plant specimens were

TABLE I
ICP-OES Instrumental Operating Conditions

Instrument	Spectro Arcos 165 ICP-OES
Viewing Height	12 mm
Wavelengths	Al: 176.641 nm, B: 182.527 nm, Ba: 455.404 nm, Co: 230.786 nm, Cr: 267.716 nm, Cu: 324.754 nm, Fe: 238.204 nm, Mn: 257.611 nm, Ni: 231.604 nm, Pb: 220.353 nm, Sr: 407.771 nm, Zn: 213.856 nm
Replicates	3
RF Power	1450 W
Spray Chamber	Cyclonic
Nebulizer	Modified Lichte Nebulizer
Nebulizer Flow Rate	0.8 L/min
Plasma Torch	Quartz, fixed, 3.0 mm Injector Tube
Replicate Read Time	50 sec per Replicate
Plasma Gas Flow	13 L/min
Auxiliary Gas Flow	0.7 L/min
Sample Aspiration Rate	2.0 mL/min
Sample Pump Rate	25 rpm

TABLE II
Comparison of Results for Certified Standard Materials and the Proposed Study (N=3)

Elements	Concentration (µg g ⁻¹)					
	TEA LEAVES (INCT-TL-1)			TOMATO LEAVES 1573a		
	Certified Value	This Study	Recovery (%)	Certified Value	This Study	Recovery (%)
Al	0.229±0.228	0.218±0.197	95	598 ± 12	580 ± 8	97
B	-	-	-	33.3 ± 0.7	31.4 ± 0.4	95
Ba	43.2±3.9	41.5±2.8	96	-	-	-
Co	0.387±0.042	0.368±0.039	95	0.57 ± 0.02	0.54 ± 0.01	92
Cr	1.91±0.22	1.98±0.25	94	1.99 ± 0.06	1.89 ± 0.05	94
Cu	20.4±1.5	19.9±1.1	98	4.70 ± 0.14	4.65 ± 0.09	95
Fe	-	-	-	368 ± 7	361 ± 6	99
Mn	0.157±0.011	0.149±0.009	95	246 ± 8	234 ± 4	98
Ni	6.12±0.52	5.94±0.48	97	1.59 ± 0.07	1.54 ± 0.04	95
Pb	1.78±0.24	1.71±0.29	96	-	-	97
Sr	20.8±1.7	19.8±1.5	95	-	-	95
Zn	34.7±2.7	34.0±2.6	98	30.9 ± 0.7	29.9 ± 0.3	97

BDL, below the detection limit.

taken from different locations (Table III). The samples from the taxa belonging to the genus *ferula* were collected from different regions in Turkey in the form of either the flower or the fruit.

The collected plant samples were dried and turned into herbarium materials, then allowed to stand at -20 °C for 2 days in order to remove any insects and insect eggs. The fruit-bearing parts of the plants were oven-dried at 105 °C for 150 minutes and then again for the fruit-bearing parts at 105 °C for 60 minutes. The dried fruit- and flower-bearing parts were homogenized in a porcelain mortar and

TABLE III
Samples from Genus *Ferula*
Collected at Different
Regions in Turkey

Genus of Flower-bearing Samples	Genus of Fruit-bearing Samples
<i>F. elaeochytris</i>	<i>F. brevipedicellata</i>
<i>F. haussknechtii</i>	<i>F. elaeochytris</i>
<i>F. tenuissima</i>	<i>F. tingitana</i>
<i>F. communis</i>	<i>F. hermonis</i>
<i>F. tingitana</i>	<i>F. haussknechtii</i>
<i>F. duranii</i>	<i>F. duranii</i>
<i>F. rigidula</i>	<i>F. halophila</i>
<i>F. durudeana</i>	<i>F. tenuissima</i>
<i>F. brevipedicellata</i>	<i>F. parva</i>
<i>F. lycia</i>	<i>F. szowitsiana</i>
<i>F. huber-morathii</i>	<i>F. orientalis</i>
<i>F. mervynii</i>	<i>F. lycia</i>
<i>F. longipedunculata</i>	<i>F. communis</i>
<i>F. tingitana</i>	<i>F. halophila</i>
<i>F. caspica</i>	<i>F. szowitsiana</i>
<i>F. lycia</i>	<i>F. orientalis</i>
<i>F. anatolica</i>	<i>F. tingitana</i>
<i>F. orientalis</i>	<i>F. duranii</i>
<i>F. hermonis</i>	<i>F. rigidula</i>
<i>F. duranii</i>	<i>F. lycia</i>
<i>F. parva</i>	<i>F. caspica</i>
<i>F. szowitsiana</i>	<i>F. huber-morathii</i>
<i>F. halophila</i>	<i>F. anatolica</i>
-	<i>F. mervynii</i>
-	<i>F. durudeana</i>
-	<i>F. longipedunculata</i>

stored in polyethylene bags at room temperature until the day of analysis. After solubilizing by microwave dissolution, the fruit- and flower-bearing parts of the plant samples were analyzed for trace and essential elements using ICP-OES.

Microwave Digestion of Samples

For microwave digestion, 0.25 g of the reference material and 1 g of the plant sample were taken and studied in duplicate. A 6-mL amount of 65% HNO₃ and 2 mL of 30% H₂O₂ was added to the samples and digested in the microwave oven.

TABLE IV
Operating Conditions
for Samples in
Microwave Digestion System

Steps	Time (min)	Power (W)	Temp. (°C)
1	2	250	100
2	2	250	100
3	5	300	120
4	5	550	170
5	6	700	200
Vent for 12 minutes			

After completion of the procedure, the digested samples were diluted to a volume of 25 mL with ultra-pure distilled water. The digestion conditions for the microwave system are given in Table IV.

RESULTS AND DISCUSSION

The trace element concentrations of the fruit- and flower-bearing plants of 21 plant species are given in Figures 1 and 2, respectively.

The quantities of trace elements in the human body have been previously reported (11). The daily amount of aluminium per kg received from food by a healthy individual weighing 70 kg on average is 0.01-1.4 mg (11). Figures 1 and 2 show that the trace elemental content of both fruit- and flower-bearing plants are much higher in some species than the daily elemental level required per person.

The daily tolerable amount of elements vs. the amount found in the flower- and fruit-bearing plants for an individual weighing 60 kg are listed in Table V.

TABLE V
Daily Tolerable Amount for Elements
(Flower-bearing Part / Fruit-bearing Part)

Element	Flower-bearing Part		Fruit-bearing Part	
	Min. Value (µg g ⁻¹)	Max. Value (µg g ⁻¹)	Min. Value (µg g ⁻¹)	Max. Value (µg g ⁻¹)
Al	9.20	717.20	7.80	498.70
B	29.60	143.00	6.10	100.90
Ba	1.00	34.30	1.00	78.80
Cr	0.54	3.10	0.10	0.94
Cu	6.90	29.30	1.20	11.20
Fe	40.30	638.20	16.90	446.30
Mn	12.50	55.00	4.50	24.20
Ni	0.40	11.40	0.10	0.89
Pb	0.50	4.40	0.07	0.89
Sb	2.60	7.80	0.01	6.20
Se	1.00	10.00	0.01	4.90
Sr	2.20	146.00	5.50	169.20
Zn	19.60	120.60	1.70	34.70

Fig. 1. Determination of trace element levels of fruit-bearing parts of plants by ICP-OES.

Fig. 2. Determination of trace element levels in flower-bearing parts of plants by ICP-OES.

Boron

For boron, the average daily amount recommended for a healthy individual weighing around 60 kg is 10–20 mg (12). It can be seen that the boron content in all fruit-bearing parts of the plants is above this value, except that the flower-bearing parts of *F. anatolica* are below this value.

Barium

The daily recommended intake of barium for an individual weighing around 60 kg through food and drink is 1.24 mg (13). The fruit-bearing parts of *F. Tingitana* (Ödemiş), *F. Duranii* (Antalya, Akseki Çukurköy), *F. parva* and *F. longipedunculata* as well as the flower-bearing parts of *F. longipedunculata*, *F. Tingitana* (Ödemiş), *F. Lycia* (Konya, Hadim) and *F. anatolica* have lower quantities of barium.

Chromium

For chromium, the daily recommended intake for an individual weighing around 60 kg is 50–200 µg (14). It was found that the results for fruit- and flower-bearing parts of the plants are above this value.

Copper

For copper, the daily recommended intake for an individual weighing around 60 kg is 3 mg (15). The fruit-bearing parts of the plants showed Cr at above this value, whereas the flower-bearing parts generally showed results close to this value.

Iron

For iron, the daily recommended intake for a healthy individual weighing around 60 kg was established as 48 mg (15). Only fruit-bearing parts of *F. Durani* (Antalya, Akseki Çukurköy) showed a lower value, while some of the flower-bearing parts were above this value and some others below this value.

Manganese

The daily tolerated intake of manganese for an individual weighing around 60 kg is in the range of 2–9 mg (16). The results showed that the amount of this element in fruit-bearing parts of the plants is above this value and is also quite high for flower-bearing parts.

Nickel

For nickel, the daily recommended amount for a healthy individual weighing around 60 kg was established as 100–300 µg (16). The results for the fruit-bearing parts of the plants are above this value, whereas some of the results for flower-bearing parts are below this range.

Lead

The daily tolerated intake of lead for an individual weighing around 60 kg on average is 3.57 mg (17). Only fruit- and flower-bearing parts of *F. tingitana* (Efes harabeleri) showed higher values.

Antimony

The daily tolerated intake of antimony for an individual weighing around 60 kg is 6 mg (18). Food usually contains small amounts of antimony. One can eat and drink about 5 micrograms (5 millionths of a gram) of antimony every day.

Selenium

The daily recommended intake of selenium established by the National Research Council (USA) is 0.5 mg. All the results for fruit-bearing parts of the plants are below this value, whereas some of the values for flower-bearing parts are above and some are below this value.

Strontium

For strontium, an individual can tolerate a daily intake of 2–4 mg from a normal diet of grain and vegetables (19). Only fruit-bearing

parts of *F. Tingitana* (Ödemiş) showed values in this range, while the flower-bearing parts of all plants show values above this range.

Zinc

The daily tolerated intake of zinc for a healthy individual weighing around 60 kg was established as 60 mg (15). Only fruit-bearing parts of *F. mervynii* have values above, while flower-bearing parts of all plants have values below this range.

The above evaluation of the various elements and their recommended vs. tolerable values give an indication that before using any of the plant parts for herbal medicinal use, the elemental content should be studied before formulating a dosage for human consumption.

CONCLUSION

In the following plants used as medicinal herbs and belonging to the *ferula* gene, the concentrations of Al, B, Ba, Cr, Cu, Fe, Mn, Ni, Pb, Sb, Se, Sr, and Zn in their flower- and fruit-bearing parts were studied: *F. anatolica*, *F. arevipedunculata*, *F. caspica*, *F. communis*, *F. durani*, *F. drudeana*, *F. elaeochytris*, *F. halophila*, *F. haussknechtii*, *F. hermonis*, *F. huber-morathii*, *F. longipedunculata*, *F. lycia*, *F. mervynii*, *F. orientalis*, *F. parva*, *F. rigidula*, *F. szowitsiana*, *F. tenuissima*, *F. tingitana*. Furthermore, fruit-bearing parts of *F. durani*, *F. communis*, *F. lycia*, *F. orientalis*, *F. szowitsiana*, *F. tingitana* species and flower-bearing parts of *F. durani*, *F. lycia*, and *F. tingitana*.

The results of this study indicate that the elemental range in the plant parts were normal and that these plant parts can be safely used for medicinal purposes. People are exposed to heavy metals in a variety of ways, including by respiration, skin absorption, and nutrition. The metals in these plants do not

show toxic effects below certain threshold quantities; however, they can become harmful for the human metabolism when taken in excess. Therefore, today, it is important to have knowledge about the metal concentrations in the *ferula* genus taxa utilized as a tonic (aphrodisiac), germicide, constipation relaxant, an antidote against poisoning, antitussive and animal feed, as well as an agent for treatment of hemorrhoids and urinary tract disorders.

ACKNOWLEDGMENT

This work was supported by the Sakarya University Research Fund under Project Number 2012-50-02-021. This work was performed partly at the International University of Sarajevo, Faculty of Engineering and Natural Sciences, Sarajevo, BiH. The authors also thank Prof. Dr. Fuat Gurcan (Faculty Dean, International University of Sarajevo, Faculty of Engineering and Natural Sciences, Sarajevo, BiH) for his valuable advice during this study.

Received March 3, 2015.

REFERENCES

1. M.G. Pimenov and M.V. Leonov, Turkish J. of Botany 28, 139 (2004).
2. H. Pesmen, Flora of Turkey and the East Aegean Islands, Vol. 4. Edinburgh, U.K.: Edinburgh University Press, 440-453 (1972).
3. H. Duman and M. Sagioglu, Botanical J. of the Linnean Society 147, 57 (2005).
4. M. Sagioglu and H. Duman, Turkish J. of Botany 30, 399 (2007).
5. M. Sagioglu and H. Duman, Annales Botanici Fennici 47, 293 (2010).
6. M. Sagioglu and H. Duman, Botanical J. of the Linnean Society 153, 357 (2007b).
7. H. Altundag and M. Tuzen, Food and Chem. Toxicol. 49(11), 2800 (2011).
8. H. Altundag and M.S. Dundar, Fresenius Environm. Bull. 18(1), 98 (2009).
9. M.S. Dundar and H. Altundag, Trace Elements and Electrolytes 19(2), 55 (2002).
10. H. Altundag, M.S. Dundar, S. Doganci, M. Celik, and M. Tuzen, J. of AOAC International 96(1), 166 (2013).
11. M. Tayfur, Ünlüoglu, and Ö. Bener, Food Magazine 27(4), 305 (2002).
12. FAO. Irrigation and Drainage, Rome, Italy, 81, 29 pp. (1985).
13. IPCS Barium. World Health Organization, Geneva, Switzerland, Environmental Health Criteria (107) (1990).
14. RDA, Recommended dietary allowance (10th ed.). Washington, DC: National Academic Press, USA (1989).
15. Joint FAO/WHO, In 53rd Meeting, Rome, Italy, (June 1-10, 1999).
16. World Health Organization, WHO, 197 (1994).
17. World Health Organization, Forty-first report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Technical Report Series, 837 (1993).
18. World Health Organization, Geneva, Switzerland (2008).
19. P.S. Nielsen, Bone 35(3), 583 (2004).