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Original Research Article

Elemental Analysis of *Galium incanum* SM subsp *Centrale* Ehrend by X-ray Fluorescence Spectroscopy

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Abstract

Purpose: *To evaluate the content of trace elements in Galium incanum SM. subsp. centrale Ehrend.*
Methods: *The air-dried aerial parts of the plant material were used and its water extract (2 % w/v infusion) was analysed for trace elements using x-ray fluorescence (XRF) spectrometry.*
Results: *The aqueous extract depicted significant concentrations of macro- and micro-nutrients with heavy metal and metal oxide content of 4.07 - 6.02 and 3.19 - 4.01 % for powdered plant material and water extract, respectively. The contents of Ca (22840 ppm) and K (8204 ppm) were the highest among all the elements while Zn (45.9 ppm) and Fe (328 ppm) were also detected in significant amounts. Zn, Mn and Cu showed the highest content while those of Mg, Al, K, Ca, Fe and P lowest in the water extract.*
Conclusion: *The presence of significant levels of Ca, K, Na, Fe, Zn, Mg, Mn and Cu in G. incanum subsp. centrale showed that this plant has notable nutrient elements. The traditional use of Gallium species as a diuretic may be attributed to its rich content of potassium.*

Keywords: *Rubiaceae, Galium incanum, Trace elements, Macronutrients, Micronutrients, X-ray fluorescence.*

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INTRODUCTION

Rubiaceae, comprising 640 genera and over 10000 species in about 10 tribes distributed in the world, chiefly in tropical regions, form the fourth largest angiosperm family after Asteraceae, Orchidaceae and Leguminosae [1]*Galium* with 400 species distributed in both temperate and tropical regions is one of the largest genera of Rubiaceae [2]. *Galium* is represented in Turkey by 101 species [3]. These species are used to coagulate milk because of an enzyme in their chemical composition [4]. *Galium aparine* L., *G. cruciata* (L.) Scop. and *G. verum* L. are used as diuretic, chloretics, against diarrhea and in the treatment of some stomach complaints, gout and epilepsy in folk medicine [5]. Iridoids, anthraquinones, triterpenic saponins, naphtalene derivatives, flavonoids, lignans and alkaloids have been reported from *Galium* species [6-9].

It is well known that many trace elements play a vital role in human health. Most of the studies on medicinal plants pertain to their secondary metabolites viz. glycosides, alkaloids, phenolic componds, essential oils and other active components and their biological effects. Considering the importance of trace elements and for adequate safety evaluation, the present study was aimed to determine the presence and quantity of metallic elements in *G. incanum* SM. subsp. *centrale* Ehrend., an endemic species known as “yoghurt herb” in Turkey.

EXPERIMENTAL

The aerial parts of *Galium incanum* subsp. *centrale* were collected in July 2012 from Ödemiş, Bozdağ, İzmir, Turkey. Plant material was identified by Dr. Cenk Durmuşkahya, Celal Bayar University, Faculty of Education, School of Applied Science, Demirci, Manisa. A voucher specimen (no. 1432) was deposited in the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Ege University, Izmir, Turkey.

The air-dried plant materials were cut into small pieces and ground into fine powder. The water extract of plant material was prepared as a 2 %w/v infusion and then filtered using Whatman filter paper no. 1 paper. The filtrate was concentrated using vacuum evaporator and kept at -20 °C until use. The powdered plant material and water extract were analysed for content of trace elements by energy dispersive x-ray fluorescence (XRF) spectroscopy. The elemental composition was determined by using SPECTRO IQ II (Ametek, Germany) with silicon drift detector SDD with resolution of 145 eV at 10 000 pulses. The primary beam was polarized by Bragg crystal and Highly Ordered Pyrolytic Graphite - HOPG target. The samples (0.1 g) were measured during 300 s at voltage of 25 kV and 50 kV, at current of 0.5 and 1.0 mA under helium atmosphere.

RESULTS

The elemental range for XRF spectroscopy is from sodium to uranium. Generally, it is difficult to quantify elements lighter than sodium. In this study, the concentrations of trace elements from sodium to uranium in the periodic table were determined in the water extract (WE) and powdered plant material (PM) of *Galium incanum* subsp. *centrale* by using XRF spectroscopy. The results showed various concentration of macroelements and microelements in the samples. An examination of the data from Table 1 shows that PM and WE contain Na, Mg, Al, Si, S, K, Ca, Fe, Cl, Mn and Zn and their oxides in various proportions. The results indicate that heavy metal and metal oxide content was 4.07 - 6.02 and 3.19 - 4.01 % for powdered plant material and water extract, respectively.

DISCUSSION

XRF technique is well suited for multielemental determinations in plant samples. The samples do not need any chemical treatment and any possible contamination is therefore avoided. XRF is one of the sensitive, rapid and simple analytical technique to study the essential element content of medicinal plants [10,11].
Many trace elements play significant roles in various physiological and biochemical events. Excessive levels of these elements in medicinal plants could lead to toxicity. Food and Nutrition Board recommends calcium intake as 1000 mg/day wheras the recommended daily intake of sodium and potassium are 1500 mg/day and 2300 - 3200 mg/day, respectively [12]. Plants are important link in the transfer of trace elements from soil to humans [13]. Fruits and vegetables are safe and valuable sources of minerals [14]. In previous studies, medicinal plants are shown to posses some important elements which have both therapeutic and prophylactic properties [13-17]. In addition to expressing their nutrient contents as the uncombined elements, metals are also expressed as their oxides. Therefore, in this study, the oxide forms of elements in *G. incanum* subsp. *centrale* were also determined. The concentration of Ca was determined as 22840 and 2917 ppm for PM and WE, respectively. CaO was measured as 31950 and 4081 ppm in PM and WE, respectively. Solubility depends on the form of metal (i.e., whether the metal exists in a free or complexed form). Metal transfer from herbs to final extract is both sample-dependent and solvent-dependent. This study was also aimed to investigate the metal content of the extract prepared from *G. incanum* subsp. *centrale*. The resulting metal concentrations in WE increased or decreased when compared with PM. For example, concentrations of Zn, Mn, and Cu increased whereas that of Mg, Al, K, Ca and P decreased in WE.

The role of inorganic elements in human metabolism has long been established. Macro- and microelements influence many biochemical processes to maintain good health. Study of elements with respect to medicinal plants reveals that major and trace elements play significant roles in combatting diseases [17,18]. Some metals in health products are as a result of adulteration. Many herbs are metal accumulators and take up both essential and non-essential metals from soil and environment during the growth. Thus, knowledge of metal content is also essential to evaluating the quality of herbal products.

Table 1: Metal and metal oxide content (ppm) of water extract (WE) and powdered drug (PM) of *G.incanum* subsp. *Central*

Z	Symbol	Element	PM (ppm)	WE (ppm)	Symbol	PM (ppm)	WE (ppm)
11	Na	Sodium	788	< 810	Na_2O	1060	< 1100
12	Mg	Magnesium	1176	614	MgO	1950	1018
13	Al	Aluminum	1026	371.9	Al_2O_3	1939	703
14	Si	Silicon	2616	566.1	SiO_2	5597	1211
15	P	Phosphorus	676.7	487.8	P_2O_5	1551	1118
16	S	Sulfur	1659	1092	SO_3	4142	2726
17	Cl	Chlorine	475.5	723.2	Cl	475.5	723.2
19	K	Potassium	8204	6664	K_2O	988.3	8028
20	Ca	Calcium	22840	2917	CaO	31950	4081
22	Ti	Titanium	82.3	15.2	TiO_2	137.3	25.4
23	V	Vanadium	11.1	7.9	V_2O_5	19.8	14.1
24	Cr	Chromium	< 5.1	< 5.1	Cr_2O_3	< 7.4	< 7.4
25	Mn	Manganese	130.6	159.2	MnO	168.6	205.5
26	Fe	Iron	328	53.7	Fe_2O_3	468.9	76.8
27	Co	Cobalt	< 3	< 3	CoO	< 3.9	< 3.9
28	Ni	Nickel	< 2	< 2.4	NiO	< 2.6	< 3.1
29	Cu	Copper	22.1	1066	CuO	27.7	1330
30	Zn	Zinc	45.9	3481	ZnO	57.2	4333
31	Ga	Gallium	< 1	< 1	Ga	< 1	< 1
33	As	Arsenic	< 1	< 1	As_2O3	<1.3	< 1.3
34	Se	Selenium	< 1	< 1	Se	< 1	< 1
35	Br	Bromine	6.6	460	Br	6.6	460
37	Rb	Rubidium	6.4	206	Rb_2O	6.9	22.6
38	Sr	Strontium	23.1	707	SrO	27.3	83.6
39	Y	Yttrium	1.2	272	Y	1.2	272
40	Zr	Zirconium	< 510	< 510	ZrO_2	< 680	< 680
42	Mo	Molybdenum	12.8	1247	Mo	12.8	1247
47	Ag	Silver	32	3030	Ag	32	3030
48	Cd	Cadmium	< 5.6	< 5.1	Cd	< 5.6	< 5.1
50	Sn	Tin	< 6.1	< 6.1	SnO_2	< 7.7	< 7.7
51	Sb	Antimony	< 6.1	< 6.1	Sb_2O_5	< 8	< 8
53	I	Iodine	< 7.1	< 7.1	I	< 7.1	< 7.1
55	Cs	Cesium	< 8.1	< 8.1	Cs	< 8.1	< 8.1
56	Ba	Barium	< 68	< 4700	Ba	< 68	< 4700
57	La	Lanthanum	< 10	< 10	La	< 10	< 10
74	W	Tungsten	< 2	142.3	WO_3	< 2.5	179.4
80	Hg	Mercury	< 2	< 2	Hg	< 2	< 2
82	Pb	Lead	< 2.9	< 150	PbO	< 3.2	< 160
83	Bi	Bismuth	< 2	< 2	Bi	< 2	< 2
90	Th	Thorium	< 2	770	Th	< 2	770
92	U	Uranium	<3	487	U	<3	487

Ca deficiency produce skeletal muscle spasms and abnormality in heart beat. It is also responsible for keeping bones strong and reducing the risk of osteoporosis [19]. Based on our results, *G. incanum* subsp. *centrale* is a rich source of dietary calcium.

K maintains cardiac rhythm and helps in reducing hypertension. Deficiency or excess of K can affect human health [20]. Dandelion (*Taraxacum officinale* F. H. Wigg) is a significant source of potassium and other minerals [21]. Reported K levels have ranged from 23.3 to 59.9 mg/g of dried leaves [22,23]. Dandelion has been extensively employed as diuretic in traditional folk medicine and in modern phytotherapy in Europe. Additionally the Comission E approves dandelion as diuretic due to its rich content of potassium [24]. The average concentration of potassium was 8204 and 6664 ppm for PM and WE. The use of *Gallium* species as diuretics may be attributed to rich content of potassium.

The depletion of sodium is characterized by hair loss, hypotension, mood changes and muscle cramps [25]. Mineral and heavy metals in some medicinal plants collected from local market were analysed and high levels of sodium were reported for dried leaves *of Mentha spicata* L. (808 ppm), *Trigonella foenum-graceum* L. (799 ppm) and *Coriandrum sativum* L. (813 ppm) [26]. The amount of Na was found as 788 ppm in powdered aerial parts of *G. incanum* subsp. *centrale.*

Magnesium protects against diabetes and reduce blood pressure. In the body, magnesium supply is located in the bones together with calcium and phosphorous. Magnesium is also involved in insulin sensitivity and protein and nucleic acid synthesis. Phosphorous also plays important roles in every chemical reaction within the body. It forms calcium phosphate in the bones and teeth [27]. Significant concentrations of magnesium (1176 - 614 ppm) and phosphorous (676.7 - 487.8 ppm) were recorded for PM and WE.

Manganese is an essential metallic element for both plants and animals. It helps to asist the body in metabolizing protein and carbohydrates especially in treating diabetes. It plays a pivotal role in the normal growth, skeleton formation and reproductive function [28]. The average concentration in PM and WE was 130.6 and 159.2 ppm.

In a previous study, out of 24 investigated species, Na, K, Ca, Mg and Fe levels for *Taraxacum officinale* were reported as 740, 180, 106, 6.4 and 29 ppm, respectively. The blood purifying effect of *T. officinale* was attributed to the presence of high iron content [29]. *Spondias mombin* L. and *Vernonia amygdalina* Delile are reported to be good antianaemic and antidiabetic agents because of high contents of iron and chromium. The copper contents were low with 13 mg/kg in *Spondias mombin* and 11 mg/kg in *Vernonia amygdalina* [30]. The systemic decrease in Cu levels causes iron deficiency therefore it is antianaemic and essential for the formation of iron and haemoglobin [31]. Iron and copper content was determined as 328 - 22.1ppm and 53.7-1066 ppm in PM and WE respectively.

The leaves of *Mentha spicata* L. have been shown to be a rich source of zinc (49.76 mg/kg) [26]. Zinc is an essential metal for the normal functioning of various enzymes. Zn deficiency can lead to loss of appetite, growth retardation, weakness and even stagnation of sexual growth. Zinc has been reported for beneficial effects on vision, hair loss and increased milk production by pregnant women [32]. *G. incanum* subsp. *centrale* was shown to be rich source of Zn.

The presence of high element concentrations gives a new sight into its potential use to compensate for element deficiencies in both man and animals as well as medicinal plants. Researchers are trying to link the contents of the trace elements and medicinal values of the plants. From XRF analysis results, it is obvious that *G. incanum subsp. centrale* accumulates essential elements in significant concentrations.

CONCLUSION

The levels of Ca, K, Na, Fe, Zn, Mg, Mn and Cu in *G. incanum subsp. centrale* indicate that the plant is a notable source of nutrient elements with potential therapeutic benefits. Chemical constituents of *Galium* species have been published previously. To the best of our knowledge, there is no scientific report on the mineral content of this genus. The water extract prepared from the plant material accumulated various elements in higher levels than the plant material itself. It is, therefore, important for safety reasons that evaluation of both the plant material itself and its water extract are carried out. The data obtained from this study lends some support for the use of the plant in traditional medicine in Turkey. The results also suggest that the plant material may be useful in the formulation nutritional and/or therapeutic products.

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