Turk J Chem
34 (2010) , 911 – 919.
© TÜBİTAK
doi:10.3906/kim-0912-370

Determination of trace element contents of *Thymus* species from Turkey

F. Zehra KÜÇÜKBAY*, **Ebru KUYUMCU**
İnönü University, Faculty of Pharmacy, Department of Basic Pharmaceutical Sciences, Division of Analytical Chemistry, 44280 Malatya-TURKEY
e-mail: zkucukbay@inonu.edu.tr

Received 14.12.2009

Eleven mineral and trace elements (Mg, Ca, K, Fe, Mn, Al, Zn, Cu, Cd, Ni, and Pb) were determined in the *Thymus* species growing in Turkey. The total concentrations were measured in samples mineralized with concentrated nitric acid and hydrogen peroxide in a microwave system. The determination of mineral and trace elements in the leaves of the *Thymus* species was carried out by flame and graphite furnace atomic absorption spectrometry. The accuracy of the method was ensured by using standard reference material (NIST-SRM 1515 apple leaves). The concentration of elements in the *Thymus* species and their biological activity on humans is discussed.

Key Words: Thymus species, trace elements, minerals, atomic absorption spectrometry, microwave digestion

Introduction

Herbal medicines have been an important source of natural product drugs and the root of modern pharmacology and drug development. Therefore, the determination of the elemental composition of medicinal plants continues to be an important research area.[1] The reason for that is the need to monitor the level of elements; intakes of some are too high, resulting in toxicity, while intakes of others are too low, resulting in nutritional problems.[2] Moreover, heavy elements, such as Hg, Pb, or Cd, have potentially negative effects on human health.[3]

An important link in the transfer of trace elements from soil to humans is plants. Element content of essential elements in plants is conditional and the content is affected by the geochemical characteristics of soil and by the ability of plants to selectively accumulate some of these elements. Through the roots, plants readily

*Corresponding author

assimilate such elements, which can be dissolved in waters and occur in ionic forms.[4] Additional sources of these elements for plants are rainfall, atmospheric dusts, plant protection agents, and fertilizers, which could be adsorbed through the leaf blades.[5]

Knowledge about the total element concentrations in biological samples such as leaves provides information about the daily intake of essential elements. Element concentrations in leafy materials show enormous variations due to sampling parameters, such as sampling period, season, leafy age, weather conditions, and sampling height.[6]

Since the concentrations of mineral elements constitute a minute fraction in leafy samples, a sensitive, selective, and reliable instrumental method is necessary to collect precise and accurate data. A number of techniques such as spectrophotometry, atomic absorption spectrometry, voltammetry, and neutron activation analysis are routinely used to analyze leafy samples.[7] Flame/graphite furnace atomic absorption spectrometry is the main instrument used for the determination of trace heavy metal contents in food analysis laboratories.

Thymus aerial parts are the most frequently used herbs in different regions of Turkey. The genus *Thymus* is represented in Turkey by 38 species, and the rate of endemism is 53%.[8] Several *Thymus* species are locally known as "kekik" or "taş kekik", and their dried herbal parts are used as herbal tea and condiment in folk medicine. The essential oils of some *Thymus* spp. are characterized by the presence of high concentration of the isomeric phenolic monoterpenes thymol and/or carvacrol.[9]

The genus *Thymus* has numerous species and varieties, and their essential oil composition and antimicrobial activities have been studied earlier.[10] However, macro- and microelements of *Thymus* species have rarely been studied.

In the present work, the following macro- and microelements were determined in 10 specimens of 7 *Thymus* species' leaves: Mg, Ca, K, Fe, Mn, Al, Zn, Cu, Cd, Ni, and Pb. The concentrations of metals were determined by means of the Flame-AAS and GF-AAS methods. Samples for determination were dissolved with the use of microwave digestion. The analytical characteristics of the proposed method, and its accuracy and precision were tested and verified by a certified reference material (NIST-SRM 1515 apple leaves). The certified reference material was chosen for the use of leaves of *Thymus* species as an analyte.

Materials and methods

Sampling and sample treatment

The list of specimens examined is given in Table 1. The voucher specimens of species are deposited in the INU and the herbarium of the Balıkesir University, Balıkesir, Turkey.

The samples were washed and cut to simulate the human intake conditions better. They were dried 25 $^{\circ}$C for 2 days on a sheet of paper to eliminate excess moisture. The samples were oven-dried at 80 $^{\circ}$C for 12 h and ground in an agate mortar until they could pass a 60 mesh sieve. Then they were stored in a clean, dry, and stoppered glass container.

For the analysis, the samples were weighed (0.50 g) in Teflon crucibles and 7 mL of HNO_3 (65%) and 1 mL of H_2O_2 (30%) were added and digested by microwave. The digestion solution was transferred to volumetric flasks of 25 mL volume and diluted with double deionized water (Milli-Q Millipore 18.2 $\mu\Omega$ cm^{-1} conductivity).

Blank digestion was carried out in the same way. Digestion conditions for the microwave system were as follows: 10 min for 500 W, 10 min for 1000 W, vent: 10 min.

Table 1. The list of examined specimens.

Taxon	Collection site	Collection date	Collector Number
Thymus eigii (Zohary & P.H.Davis) Jalas	Hatay, Serinyol	13.07.2006	Yıldız 16212
T. eigii	Hatay, Belen	15.07.2006	Yıldız 16213
T. eigii	Hatay, Yayladağ	08.07.2007	Dirmenci 3454
T. kotschyanus Boiss. & Hohen. var *kotschyanus*	Erzurum	11.08.2007	Dirmenci 3528
T. kotschyanus var *kotschyanus*	Van	26.06.2007	Dirmenci 3431
T. cilicicus Boiss. & Balansa	Mersin	07.08.2007	Dirmenci 3485
T. fallax Fisch. & Meyer	Kars	15.07.2007	Arabacı 2572
T. revolutus Celak	Antalya	07.06.2007	Akçiçek 4618
T. sipyleus Boiss. subsp. *rosulans* (Borbas) Jalas	Burdur	07.06.2007	Akçiçek 4614
T. syriacus Boiss.	Gaziantep	10.08.2007	Arabacı 2595b

Apparatus

A Perkin-Elmer AAnalyst 800 atomic absorption spectrometer (FAAS), equipped with a THGA graphite furnace and with Zeeman-Effect background corrector, was used in the experiments. For flame measurements, a 10 cm single slot-burner head, a lamp, and an air-acetylene flame were used. For graphite furnace measurements, argon was used as inert gas. The operating parameters for the working elements were set as recommended by the manufacturer. The pyrolytic-coated graphite tube (Perkin-Elmer part no. B3 000641) with a platform was used. Samples were injected into the graphite furnace using a Perkin-Elmer AS-91 auto sampler.

A Milestone Start D closed vessel microwave digestion system (maximum pressure 1450 psi, maximum temperature 300 $^\circ$C) of Teflon reaction vessels was used in all the digestion procedures. The reaction vessels were cleaned using 5 mL of concentrated nitric acid before each digestion.

Reagents

Unless stated otherwise, all chemicals used were of analytical-reagent grade. Throughout all analytical work, doubly distilled water (Milli-Q, Millipore 18.2 $\mu\Omega$ cm^{-1} resistivity) was used. In the digestion, concentrated nitric acid (65%, E. Merck, Darmstadt, Germany) and hydrogen peroxide (30%, E. Merck) were used. All the plastic and glassware were cleaned by soaking in diluted HNO_3 (1 + 9) and rinsed with distilled water prior to use. The standard elements solutions used for calibration were prepared by diluting stock solutions of 1000 mg/L of each element supplied by Inorganic Ventures/IV Labs.

Results and discussion

The basic source of essential elements for humans is the food chain and beneficial levels do not cause any disorders or harmful effects. However, there is a fine distinction between useful and toxic quantities.

In the present work, mineral and trace elements (Mg, Ca, K, Fe, Mn, Al, Zn, Cu, Cd, Ni, and Pb) were determined in the *Thymus* leaves by means of common spectroscopic techniques (FAAS/FAES and ETAAS) after the complete dissolution of their matrices with microwave assisted digestion. The reduced time required for sample preparation and reduced amounts of acids and oxidants used, minimal contamination within the laboratory, reduced loss of more volatile analytes, consequently better detection limits, and the accuracy of the method are the advantages over the numerous preparation procedures, which include classical dry or wet digestion. The reliability of the results is based on analyses of reference material (NIST-SRM 1515 apple leaves) for which the elemental contents determined were in agreement with the certified values. The certified and observed values for the SRM are given in Table 2.

Table 2. Certified and observed values of elemental concentrations in the standard reference material (NIST-SRM 1515-apple leaves).

Element	Certified Value	Observed values	
		Microwave %	Recovery
Cu (μg/g)	5.64	5.34 ± 0.08	95
Mn (μg/g)	54.0	49.2 ± 1.2	91
Zn (μg/g)	12.5	10.3 ± 0.4	83
Fe (μg/g)	83.0	81.0 ± 1.0	98
Pb (μg/g)	0.47	0.44 ± 0.03	94
Ni (μg/g)	0.91	0.89 ± 0.03	98
Cd (μg/g)	0.013	0.012 ± 0.001	92
Al (μg/g)	286.0	283.2 ± 1.2	99
Se (μg/g)	0.050	0.049 ± 0.002	99
K (%)	1.61	1.39 ± 0.06	86
Mg (%)	0.271	0.266 ± 0.007	98
Ca (%)	1.526	1.450 ± 0.100	95

(Xort ± SD), $N = 3$

The concentrations of 11 elements determined in each of 10 specimens of 7 *Thymus* species growing naturally in Turkey are listed in Table 3, as mean values with their absolute standard deviations based on 3 replicates. The first obvious outcome was the difference in elemental content among *Thymus* species. This variability was expected since the *Thymus* leaves selected from different parts of Turkey where the soil and climatic conditions are dissimilar. It is observed that the mineral contents of *Thymus* species are influenced by several factors, such as climatic conditions, including light and temperature, influencing the growth rates of plants and therefore the rates of mineral ion utilization. Under field conditions rain, snow, dew, mist and fog can lead to changes in minerals in plants.

Table 3. Some trace metals and minerals contents in microwave digested *Thymus* species.

Sample	Pb	Ni	Cd	Cu	Zn	Al	Mn	Fe	K	Ca	Mg
BY16212 (*Thymus eigii*)	0.70 ± 0.10	13.4 0± 1.20	0.01 ± 0.01	10.3 0± 1.60	42.00 ± 3.00	387.0 ± 48.0	42.0 ± 10.0	674.0 ± 104.0	12,853.0 ± 1031.0	8383.0 ± 905.0	5627.0 ± 2077.0
BY16213 (*Thymus eigii*)	1.20 ± 0.20	1.20 ± 0.20	0.04 ± 0.01	5.40 ± 0.20	46.00 ± 2.30	163.0 ± 23.0	22.0 ± 1.0	1728.0 ± 58.0	12,243.0 ± 2341.0	9740.0 ± 1283.0	5247.0 ± 887.0
TD3454 (*Thymus eigii*)	2.20 ± 0.20	13.80 ± 0.60	nd	6.50 ± 0.40	17.90 ± 1.20	852.0 ± 155.0	53.0 ± 2.0	1477.0 ± 232.0	10,407.0 ± 566.0	10,417.0 ± 2266.0	9453.0 ± 2822.0
TD3528 (*Thymus kotschyanus*)	0.70 ± 0.10	nd	0.08 ± 0.02	10.10 ± 1.00	36.20 ± 2.90	586.0 ± 86.0	52.0 ± 4.0	849.0 ± 122.0	8790.0 ± 1952.0	11,740.0 ± 1950.0	2690.0 ± 372.0
TD3431 (*Thymus kotschyanus*)	3.40 ± 0.20	0.10 ± 0.10	nd	14.90 ± 1.50	20.50 ± 2.40	2597.0 ± 143.0	96.0 ± 6.0	3479.0 ± 96.0	18,187.0 ± 265.0	15,740.0 ± 1959.0	4690.0 ± 255.0
TD3485 (*Thymus cilicicus*)	0.40 ± 0.02	0.20 ± 0.10	0.04 ± 0.01	10.70 ± 2.30	40.80 ± 4.70	191.0 ± 8.0	19.0 ± 2.0	189.0 ± 2.0	12,250.0 ± 672.0	14,417.0 ± 1909.0	4127.0 ± 1200.0
TA2572 (*Thymus fallax*)	1.70 ± 0.20	0.40 ± 0.30	0.20 ± 0.02	12.40 ± 1.10	45.90 ± 3.90	3547.0 ± 540.0	250.0 ± 10.0	1696.0 ± 149.0	12,957.0 ± 1559.0	13,743.0 ± 4416.0	5827.0 ± 680.0
TA2595-b (*Thymus syriacus*)	0.60 ± 0.04	0.20 ± 0.10	0.17 ± 0.02	7.50 ± 0.20	34.50 ± 1.70	1059.0 ± 48.0	40.0 ± 0.2	1340.0 ± 47.0	8470.0 ± 1225.0	23,007.0 ± 1628.0	3577.0 ± 998.0
EA4614 (*Thymus sipyleus*)	2.80 ± 0.10	0.30 ± 0.02	nd	8.10 ± 0.60	16.10 ± 1.80	2325.0 ± 424.0	98.0 ± 4.0	2515.0 ± 251.0	10,203.0 ± 508.0	25,570.0 ± 2479.0	5290.0 ± 459.0
EA4618 (*Thymus revolutus*)	2.80 ± 0.60	0.10 ± 0.02	0.25 ± 0.04	11.20 ± 1.10	32.90 ± 6.80	2553.0 ± 274.0	123.0 ± 18.0	2292.0 ± 425.3.0	11,367.0 ± 1206.0	13,630.0 ± 1753.0	3843.0 ± 190.0

nd: not detected. Elemental concentrations (□g/g), (Xort ± SD), *N = 3*

Calcium is essential for healthy bones, teeth, and blood.[11] The health of the muscles and nerves depends on calcium. It is required for the absorption of dietary vitamin B, for the synthesis of the neurotransmitter acetylcholine, and for the activation of enzymes such as the pancreatic lipase. The recommended daily allowance for Ca for children is between 500 and 800 mg and for adults is 80 mg.[7] The concentration of Ca is in the range of 8.38-25.57 mg/g. The leaves of *T. kotschyanus*, *T. syriacus*, and *T. sipyleus* are rich in Ca (15.74-25.57 mg/g) and are recommended for use in Ayurvedic medicine in calcium deficient subjects.

Among the macronutrients, the concentration of K ranges from 8.47 (*T. syriacus*) to 12.96 mg/g (*T. fallax*). Potassium has a higher concentration in the leafy materials than other nutrients and is an activator of some enzymes, in particular co-enzyme for normal growth and muscle function.[7] Plants absorb potassium in the form of K^+ ions from soil and the absorption of the K^+ usually depends on the soil type. Our data indicate that *Thymus* leaves are not deficient in potassium. The use of *Thymus* leaves might help in cases of potassium deficiency.

Magnesium is a cofactor in several enzymes and is critical for carbohydrate metabolism and is thought to play a role in glucose homeostasis, insulin action, and the development of type 2 diabetes. Experimental studies have shown that magnesium supplementation improves insulin-mediated glucose disposal and insulin secretion.[12] The Mg concentration in the *Thymus* leaves ranges from 2.69 to 9.45 mg/g. The leaves of *T. eigii* are rich in Mg (5.2-9.45 mg/g) and might reduce the incidence of type diabetes among middle-aged women, especially among those who are overweight.

Iron is an essential element for humans. It is a constituent of hemoglobin, myoglobin, and a number of enzymes, and as much as 30% of the body's iron is found in storage forms such as ferritin and hemosiderin, in the spleen, liver, and bone marrow, and a small amount is associated with the blood transport protein transferring.[2,13] Iron deficiency results in anemia. The requirement of Fe for adult is 20 mg/day and for a child is 10 mg/day.[7] The Fe content in the *Thymus* leaves is relatively high (0.67-3.48 mg/g) and might be advised to compensate for iron deficiency.

Zinc is a component of a wide variety of enzymes, including the ribonucleic polymerases, alcohol dehydrogenase, carbonic anhydrase, and alkaline phosphatase.[2] Zinc deficiency, resulting from poor diet, alcoholism, and malabsorption, causes dwarfism, hypogonadism, and dermatitis, while toxicity of zinc, due to excessive intake, may lead to electrolyte imbalance, nausea, anemia, and lethargy.[14] The Reference Daily Intake (RDI) for zinc is 15 mg/day.[15] The concentration of Zn ranges from 16.08 to 45.95 μg/g, having the highest concentration in *T. eigii* and the lowest in *T. sipyleus*.

Copper has been recognized as an essential element for many years, due to its presence in important proteins and enzymes.[14] Symptoms of copper deficiency in humans include bone demineralization, depressed growth, depigmentation, gastro-intestinal disturbances, dermatitis, and neurological disorders. In addition, toxicity due to excessive intake has been reported to cause liver cirrhosis.[16] The RDI for copper is 2.0 mg/day.[15] Human and animal studies suggest a correlation between the zinc-to-copper ratio in the diet and the incidence of cardiovascular disease.[17] The concentration of Cu ranges from 5.42 to 14.92 μg/g. The use of *Thymus* leaves can be sufficient to compensate for Cu-deficiency.

Manganese is an essential metal of low toxicity and trace amounts occur in biological materials. Any excess is, however, toxic to plants and animals and might be hazardous.[2] Estimated safe and adequate daily dietary intake levels for manganese are 1.0-2.0 mg/day for children 4-10 years old and 2.0-5.0 mg/day for people over 10 years old.[18] In our work the lowest concentration of Mn was in the leaf of *T. eigii* (21.48 μg/g) and the highest level in the leaf of *T. fallax* (250.00 μg/g).

Aluminum is a ubiquitous element, comprising approximately 8% of the earth's crust. It is commonly inhaled as well as ingested. Recently it has attracted considerable attention due to its possible connection with several disorders, of which Alzheimer's disease is the most notable.[19-21] Previous studies[22] indicated that most investigated foodstuffs (vegetables, meat, and dairy products) contained < 5 μg/g of Al (fresh wt.); the highest concentrations were determined in cocoa/cocoa products (33 μg/g), spices (145 μg/g), and black tea leaves (899 μg/g). The daily dietary intake for Al is 2000-45,000 μg/day.[23] Al concentration of *Thymus* species is in the range of 163.28 to 3547.2 μg/g, with the highest value in *T. fallax*.

Nickel plays some roles in body functions including enzyme functions. It is present in the pancreas and hence plays an important role in the production of insulin. Its deficiency results in liver disorder.[24,25] However, Ni is a widely used heavy metal and exerts a potent toxic effect on peripheral tissues as well as the reproductive system. The maximum permissible level (MPL) of nickel 100 μg/day.[26] The nickel content in the samples was in the range of 0.009-13.81 μg/g. Even at the highest level, the level of nickel is lower than the maximum permissible level.

Lead, a ubiquitous and versatile metal, has been used since prehistoric times. It has become widely distributed and mobilized in the environment and human exposure to and uptake of this non-essential element has consequently increased. At high levels of human exposure there is damage to almost all organs and organ systems, most importantly the central nervous system, kidneys, and blood, culminating in death at excessive levels. At low levels, heme synthesis and other biochemical processes are affected, psychological and neurobehavioral functions are impaired, and there is a range of other effects.[27,28]

The maximum recommended limit of Pb is 10 μg/g.[29] The lowest and highest contents of lead were found as 0.56 μg/g, for *T. syriacus*, and 3.41 μg/g, for *T. kotschyanus*, respectively. Our values for Pb for all the samples were lower than the WHO's value.

Cadmium is a nonessential element in foods and natural waters, and it accumulates principally in the kidney and liver. Cadmium in foods is mostly derived from various sources of environmental contamination.[30-32] Cd causes lung cancers, pulmonary edema, pneumonia, respiratory distress, liver and kidney damage, and skeletal deformities commonly known as "Itai-itai" disease.[33] The concentration of cadmium was generally low in nearly all analyzed samples, ranging from 0.70 to 200 μg/kg. The acceptable daily intake (ADI) of Cd in foods is 0.21 mg/day.[34]

Conclusions

The total concentrations of macro- and microelements were measured in 10 specimens of 7 *Thymus* species growing naturally in Turkey. Nutritive values of the examined *Thymus* leaves were evaluated. The results presented here clearly show that the examined *Thymus* leaves play an important role in human nutrition as a source of micronutrients.

Acknowledgments

The authors are grateful to the Unit of the Scientific Research Projects of Inönü University for its financial support (Grant no: 2008/34). The authors are also thankful to Prof. Dr. Bayram Yıldız, Dr. Ekrem Akçiçek, Dr. Tuncay Dirmenci, and Dr. Turan Arabacı for collecting and identifying the *Thymus* species.

References

1. Goldman, P. *Ann. Int. Med.* **2001**, *135*, 594-600.
2. Goldhaber, S. B. *Regul. Toxicol. Pharm.* **2003**, *38*, 232-242.
3. Mamani, M. C. V.; Aleixo, L. M.; Abreu, M. F.; Rath, S. *J. Pharm. Biomed. Anal.* **2005**, *37*, 709-713.
4. Miroslowki, J.; Wiechula, D.; Kwapuliski, J.; Rochel, R.; Loska, K.; Ciba, J. *Bromat. Chem. Toksykol.* **1995**, *28*, 363-372.
5. Lozak, A.; Soltyk, K.; Ostapczuk, P.; Fijalek, Z. *Sci. Total Environ.* **2002**, *289*, 33-40.
6. Reddy, P. R. K.; Reddy, S. J. *Chemosphere*, **1997**, *34*, 22193-2212.
7. Naidu, G. R. K., Denschlag, H. O.; Mauerhofer, E.; Porte, N.; Balaji, T. *Appl. Radiat. Isotopes.* **1999**, *50*, 947-953.
8. Tümen, G.; Baser, K. H. C.; Demirci, B., Ermin, N. *Flavour Frag. J.* **1998**, *13*, 65-67.
9. Baser, K. H. C.; Demirci, B., Kirimer, N.; Satil, F.; Tümen, G. *Flavour and Frag. J.* **2002**, *17*, 41-45.
10. Baydar, H.; Sağdiç, O.; Özkan, G.; Karadoğan, T. *Food Control*, **2004**, *15*, 169-172.
11. Charles, P. *J. Int. Med.* **1992**, *231*, 161-168.
12. Song, Y.; Manson, J. E.; Buring, J. E., Liu, S. *Diabetes Care*, **2004**, *27*, 59-65.
13. El-Rjoob, A. W. O.; Massadeh, A. M.; Omari, M. N. *Environ Monit Assess.* **2008**, *140*, 61-68.
14. Onionwa, P. C., Adeyemo, A. O.; Idowu, O. E., Ogabiela, E. E. *Food Chem.* **2001**, *72*, 89-95.
15. Food and Drug Administration, 1995. *Food labeling: Reference Daily Intakes. Federal Register.* 60(249), 67163-67175.
16. Reynold, C. V., Alarcon, M. N., Serana, H. L. G., Martinez, M. C. L. *Food Addit. Contam.* **2008**, *25*, 937-945.
17. Obi, E., Akunyili, D. N.; Ekpo, B.; Orisakwe, O. E. *Sci. Total Environ.* **2006**, *369*, 35-41.
18. Amin, M. N.; Kaneco, S., Suzuki, T.; Taniguchi, Y.; Ohta, K. *Anal. Bioanal. Chem.* **2002**, *373*, 205-208.
19. Nabrzyski, M., Gajewska, R. *Nahrung.* **1998**, *42*, 109-111.
20. López, F. F.; Cabrera, C.; Lorenzo, M. L.; López, M. C. *Sci. Total Environ.* **2000**, *257*, 191-197.
21. Street, R.; Drábek, O.; Száková, J.; Mládková, L. *Food Chem.* **2007**, *104*, 1662-1669.
22. Muller, M.; Anke, M.; Illigguntherr, H. *Food Chem.* **1998**, *61*, 419-428.
23. Leniewicz, A.; Jaworska, K.; yrnicki, W. *Food Chem.* **2006**, *99*, 670-679.
24. Wróbel, K.; Wróbel, K.; Urbina, E. M. C. *Biol. Trace Elem. Res.* **2000**, *78*, 271-280.
25. Rajurkor, N. S.; Pardeshi, B. M. *Appl. Radiat Isotopes.* **1997**, *48*, 1059-1062.
26. Das, K. K.; Dasqupta, S. *Environ. Health Persp.* **2002**, *110*, 923-926.

27. Tong, S.; Schirnding, Y. E.; Prapamontol, T. *B. World Health Organ.* **2000**, *78*, 1068-1077.

28. Raži, S.; Onjia, A.; Potkonjak, B. *J. Pharm. Biomed. Anal.*, **2003**, *33*, 845-850.

29. WHO, *Monographs on selected medicinal plants.* Vol. 1, Geneva. 1999.

30. Divrikli, O.; Horzum, N.; Soylak, M.; Elçi, L. *Int. J. Food Sci. Tech.* **2006**, *41*, 712-716.

31. Das, P.; Samantaray, S.; Rout, G. R. *Environ. Pollut.* **1997**, *98*, 29-36.

32. Naithani, V.; Kakar, P. *B. Environ. Contam. Tox.* **2005**, *75*, 197-203.

33. Ansari, R.; Kazi, T. G.; Jamali, M. K.; Arain, B. M., Wagan, M. D.; Jalbani, N.; Afridi, H. I.; Shah, A. Q. *Food Chem.* **2009**, *115*, 318-323.

34. WHO, *Quality control methods for medicinal plant materials*, Geneva, Switzerland. 1998.