

Determination of Selenium in *Teucrium* Species by Hydride Generation Atomic Absorption Spectrometry

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Hydride generation atomic absorption spectrometry (HGAAS) was applied for determination of selenium content in dried aerial parts of wild and cultivated *Teucrium* species (*Lamiaceae*) growing in Croatia: *T. arduini* L., *T. chamaedrys* L., *T. flavum* L., *T. montanum* L., *T. polium* L., and *T. scordium* L. subsp. *scordioides* Schreb. Special attention was paid to the wet oxidation procedure for the sample dissolution. The proposed procedure involved microwave-assisted sample digestion using a mixture of HNO₃/H₂O₂. Wild specimens generally had a higher content of selenium, with concentrations of 0.030–0.095 mg/kg of the dry drug. Cultivated plants contained 0.020–0.055 mg Se/kg.

Key words: *Teucrium*, Selenium, Hydride Generation Atomic Absorption Spectrometry

Introduction

Croatian species belonging to genus *Teucrium* (*Lamiaceae*) have not been completely chemically and pharmacologically investigated so far. They have been used for centuries in folk medicine as cholagogue as well as antispasmodic, diuretic, anti-diabetic (Gharaibeh *et al.*, 1988), antiphlogistic, antirheumatic, antiseptic, anthelmintic, carminative and flavouring agents (Gharaibeh *et al.*, 1989). Previous chemical investigations of these plants revealed the presence of flavonoids (Harborne *et al.*, 1986; Kalodžera *et al.*, 1993), steroidal compounds (Ulubelen *et al.*, 1994; Kisiel *et al.*, 1995), volatile oil, tannins, bitter principles (mostly diterpenoids) (Grzybek, 1969), some microelements (Jurišić *et al.*, 2001a) and macroelements (Jurišić *et al.*, 2001b).

Although the therapeutic efficiency of plants is connected with essential oils, flavonoids, saponins

and other biologically active compounds, it is well known that the presence of trace elements could produce a synergistic effect with these constituents (Beker *et al.*, 1991; Mandić *et al.*, 1995; Lobinski *et al.*, 2000; Pavlata *et al.*, 2001). Considerable attention in that respect is paid to selenium, widely distributed trace element in the environment. Selenium has been shown to be essential for life and to be toxic at levels little above those required for health. It enters the food chain almost exclusively through plants, primarily in the form of selenates. Usually, it is unavailable in acidic soils, whereas, in alkaline soils it may accumulate to high levels in plants. (Beker *et al.*, 1991; Mandić *et al.*, 1995; Kos *et al.*, 1998; Lobinski *et al.*, 2000). HGAAS analysis of soil in Croatia showed selenium levels from 0.441–0.579 mg/kg of dry weight (Klapec, 2001).

The aim of the present study was prompted by the fact that no data on selenium in genus *Teucrium* have been provided so far. HGAAS was used to determine selenium in six *Teucrium* species growing in Croatia (*T. arduini* L., *T. chamaedrys* L., *T. flavum* L., *T. montanum* L., *T. polium* L., and *T. scordium* L. subsp. *scordioides* Schreb.) as well as to find out if there was any difference in the content of selenium between wild and cultivated forms of the same species.

Materials and Methods

Plant material

Wild plants were collected in August 2000 on three locations in Croatia: the island of Krk (*T. flavum*, *T. polium* and *T. scordium* subsp. *scordioides*), Velebit (*T. arduini*) and Gornje Jelenje (*T. chamaedrys* and *T. montanum*), while cultivated samples were obtained from the Pharmaceutical Botanical Garden “Fran Kušan”, Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia. Voucher specimens (No. 9801–9812) are deposited in the Herbarium of the Department of Pharmacognosy (Faculty of Pharmacy and Biochemistry, University of Zagreb). Samples of air-dried aerial parts of *Teucrium* species were investigated.

Standard and sample preparation

Double-deionised water was used throughout. Standard and reagents were products of Merck (Germany). Reference standard of Se (stock solution of 1000 mg/l SeO₂ in 0.5 M HNO₃) and working standard of Se (1.00 µg Se/ml) were used. 3% NaBH₄ solution in 1% NaOH was employed as a reducing agent that produced a volatile hydride of the analyte in contact with HCl.

Samples were prepared by digesting 0.5 g of powdered and homogenized drug of each sample in a microwave furnace (CEM, MDS-2000, Matthews, North Carolina, USA) with 5 ml 65% HNO₃ (Suprapur), 1 ml 30% H₂O₂ and 2 ml water. Microwave furnace conditions are described in Table I. After cooling and filtration, each solution was made up to 100 ml with water (basic sample solution). 10 ml of each basic solution was heated for 30 min in a water bath (60 °C) with 2 ml 30% HCl (Suprapur) and put into the test tube after rinsing it with 10 ml 1.5% HCl.

Se analysis and data treatment

Se content was measured by using MHS-10 system (Mercury/Hydride Type 10) which was connected to the atomic absorption spectrophotometer Perkin-Elmer (PE) model 2380, Perkin-Elmer, Germany (Mandić *et al.*, 1995; Kos *et al.*, 1998). The MHS-10 is a manually operated accessory for the high sensitivity determination of mercury and hydride-forming elements such as As, Se, Sb, Te, Bi, Sn, etc. Sodium borohydride (NaBH₄) solution is used exclusively as the reducing agent, which liberates hydrogen on contact with acids. For determining metallic elements that form volatile hydrides, the sample solution is first treated so that the metal under study is present in ionic form in acid solution. Reductant is dispensed into the sam-

ple solution where it reacts to liberate hydrogen; this in turn reduces the metal ions to volatile hydride. The hydrogen stream flushes the hydride into the heated quartz cell, where it is decomposed and the absorption of the metal measured (Perkin-Elmer, 1978).

The detection limit of Se was 0.02 µg/l and the sensitivity of method was 2.2 ng Se per 1% absorption.

Determination requirements were: temperature 900 °C; flame: air (pressure 344.73 kPa; flow 8 l/min)/acetylene (pressure 100.00 kPa; flow 2.5 l/min); wavelength: 196.4 nm; slit: alt 2.0; lamp: electrodeless discharge lamp (EDL); power: 6 W.

Statistical analysis was carried out using Student's *t*-test. The results are given as a mean ± standard deviation.

Results and Discussion

Referring to the Table II, results obtained by described analytical method showed significant variation of selenium content among the examined species. Concentrations ranged from 0.030 mg/kg (*T. chamaedrys*, *T. flavum*) to 0.095 mg/kg (*T. montanum*) in wild *Teucrium* specimens and from 0.020 mg/kg (*T. flavum*) to 0.055 mg/kg (*T. montanum*) in cultivated forms. Selenium content in wild samples of the investigated *Teucrium* species was generally higher than those found in cultivated specimens. The sample of wild *T. montanum* had the highest amount of this trace element.

Since no hybridization under cultivating conditions was possible, we may presume that the plant ability of absorbing selenium had not changed. Accordingly, the established variations between different *Teucrium* species as well as between wild and cultivated forms of the same plant were probably caused by physiological variability as well as by different ecological, climatic and pedological factors.

Phase	Microwave furnace conditions					
	1	2	3	4	5	6
Power (%)	80	80	80	80	80	80
Pressure [kPa]	137.89	275.79	586.05	896.30	1034.20	551.57
Phase time [min]	10.00	10.00	10.00	10.00	10.00	10.00
Time at pressure [min]	5.00	5.00	5.00	5.00	5.00	5.00
Temperature [°C]	116	121	140	135	133	125

Table I. Sample burning procedure.

Sample name	Se concentration (mg/kg of dry weight) ^a	
	Wild plants	Cultivated plants
<i>T. arduini</i>	0.031 ± 0.0010 ^b	0.021 ± 0.0010 ^b
<i>T. chamaedrys</i>	0.030 ± 0.0005 ^b	0.023 ± 0.0008 ^b
<i>T. flavum</i>	0.030 ± 0.0004 ^b	0.020 ± 0.0006 ^b
<i>T. montanum</i>	0.095 ± 0.0010 ^b	0.055 ± 0.0010 ^b
<i>T. polium</i>	0.038 ± 0.0020 ^c	0.034 ± 0.0009 ^c
<i>T. scordium</i> subsp. <i>scordioides</i>	0.041 ± 0.0020 ^b	0.021 ± 0.0009 ^b

Table II. Content of selenium in wild and cultivated *Teucrium* species.

^a Mean ± SD (number of independent analyses, $n = 3$).

Significant difference between wild and cultivated forms of the same species:

^b $p < 0.01$; ^c $p < 0.05$.

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