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GC-MS CHEMICAL COMPOSITION OF VOLATILE OIL AND MINERAL ELEMENT CONTENT OF *ALLIUM URSINUM* AND *NECTAROSCORDUM SICULUM*

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Abstract

Since ancient times, essential oils are recognized for their medicinal value and are very interesting and powerful natural plant products. The present study aimed at investigating and comparing the micro- and macro- element content and volatile oil constituents of *Allium ursinum* L. and *Nectaroscordum siculum* ssp. *Bulgaricum* (Janka) Stearn. These two plant species were traditionally used in folk medicine, and since *N. siculum* is barely investigated, it is interesting to compare to *A. ursinum*. The current research represents a first detailed investigation report on the mineral element content and chemical composition of *N. siculum*. The ramsons essential oil yield was established to be 0.37 %, while the *N. siculum* – 0.24 %. The main constituents of *A. ursinum* leaves essential oil as detected by GC-MS were disulphides (44 %), trisulphides (41 %), and tetrasulphides (5 %). In comparison the GC-MS analysis of the *N. siculum* leaves oil extract revealed mainly the presence of ethyl benzoate (1.3 %) and lipoic acid (1.1 %). Both examined plants were rich in calcium, magnesium, and potassium. Based on the results *A. ursinum* and *N. siculum* leaves could be recommended as a daily source of biologically active substances of natural origin and could serve as health contributing agents.

Key words: *Allium* spp., Chemical composition, Food ingredients, Mineral elements, Spices.

Introduction

Aromatic plants and spices have great importance for food, cosmetics and pharmaceutical industries (Satyal *et al.*, 2017). They have been in use since ancient times, and despite many of them are substituted by synthetic ones, the demand for natural products is increasing (Guillén *et al.*, 1996).

Essential oils are the odorous principles found in the various parts of plant, and when exposed to the air at ordinary temperatures they substantially evaporate, which also explains the use of terms like volatile oils or ethereal oils (Rassem *et al.*, 2016). Different parts of plants have been used to obtain essential oils. These include the leaves, roots, flowers, seeds, steams, and wood through secretionary parts and internal multicellular cavities which are of particular value. Volatile compounds obtained from plants, have known antimicrobial, antifungal and insecticidal activities (Janssen *et al.*, 1987; Oka *et al.*, 2000).

Minerals are naturally chemical elements occurring in the body which form an integral part of functionally important organic compounds such as iron (Fe) in hemoglobin and Cytochrome, or zinc (Zn) in insulin (Chaney, 2006). They are essential for the normal functioning of muscles, heart, nerves, and in the maintenance of body fluid composition among others, as well as for building strong bones. Mineral deficiencies have manifested in forms of different disease conditions as goiter, rickets, and one form of metabolic dysfunction or the other (Soetan *et al.*, 2010).

Minerals are divided into two groups: major minerals and trace minerals. The body needs larger amounts of major minerals than trace minerals, although trace minerals can be just as important for good health (Chaney, 2006).

The major minerals include calcium, chloride, phosphorus, potassium, sodium, sulphur, and magnesium, while the trace minerals include iodine, iron, zinc, selenium, fluoride, chromium, copper, molybdenum, and manganese (Chaney 2006; Crook, 2006).

Magnesium plays an important role in forming and functioning of bones, muscles and prevents high blood pressure and depression (Smith and Hammarsten, 1958); plays an important role in enzyme activity (Scelig, 1989). Potassium helps in release of chemicals which acts as nerve impulses, regulates heart rhythms, its deficiency causes nervous irritability, mental disorientation, low blood sugar, insomnia and coma (Suttle, 1999). Calcium plays an important role in building and maintaining strong bones and teeth, and is also necessary for normal functioning of cardiac muscles, blood coagulation, and regulation of cell permeability (Heaney, 1994). Calcium deficiency causes back pain, osteoporosis, irritability, premenstrual tension and cramping (Hasling *et al.*, 1991). Zinc maintains various reactions of the body which help to construct and maintain DNA, required for growth and repair of body tissues (Diaz-Gomez *et al.*, 2003). Zinc deficiency causes clinical consequences, including diarrhea, pneumonia, distributed neuropsychological performance and abnormalities of fetal development (Hambiadge, 2000). Phosphorous maintains blood sugar level, normal heart contraction (Linder, 1991), cell growth and repair, and kidney function. It also plays an important role in maintaining the body's acid-alkaline balance (Johns & Duquette, 1992).

Leaves are potential sources of minerals and vitamins and are reported as inexpensive and easy to cook (Ejoh *et al.*, 2007; Soliman *et al*., 2018). Culinary uses of the wild garlic are limited mainly to use the leaves. Its flavour is more delicate compared to agriculture-garlic. They can be used raw, pickled, salted or in brine with oil. They are added to salads, soup, potatoes, cabbage, stewed vegetables and meat dishes (Ivanova *et al*., 2009).

Wild garlic (*Allium ursinum*), also called ramsons or bear's garlic, is very often used as a traditional medicine (Janeczko and Sobolewska, 1995). Bear's garlic can be found in Northern and Central Europe and North Asia.

Nectaroscordum siculum subsp. *bulgaricum* is a glabrous plant, 50-100 (150) cm high. In the soil it has an ovoid bulb, 18-30 mm long and 15-30 mm in diameter. The leaves are 30-50 cm long and 10-20 mm wide, thin, narrow keeled leaves, making it look triangular in section. The plant is characterized by a powerful and heavy specific smell. It is found only in limited areas and thus there is limited information concerning its chemical composition.

Although there have been numerous studies on the genus *Allium*, however, *Allium ursinum* from different areas is still considered interesting to research. The aim of the present investigation was to determine and compare the mineral element content and the chemical composition of these two plants in order to enrich the limited knowledge about *N. siculum* and to contribute to the already known about *A. ursinum*.

Materials and Methods

Collection of plant material: Leaves of *Allium ursinum* and *Nectaroscordum siculum* subsp. *bulgaricum* were collected in the month of April 2014 from mountainous areas of the Sliven region, Bulgaria. The plant materials were transported in polythene bags to the Department of Biological science, Plovdiv University for their identification.

Essential oil extract preparation: Micro distillation (for 2h) was used to determine the essential oil content in apparatus 'Balinova-Diakov' (Balinova and Diakov, 1974) representing a modification of the British Pharmacopoeia.

Gas chromatography–mass spectrometry (GC-MS) analysis: The GC-MS analysis was performed as described by Petrova *et al*., (2015) on a Hewlett Packard 7890 instrument coupled with MSD 5975 equipment (Hewlett Packard, Palo Alto, CA, USA) operating in EI mode at 70 eV. An HP-5 ms column (30 m × 0.25 mm × 0.25 µm) was used. All samples were analyzed in three replicates. A split ratio of 1:20 was used for the injection of 1 µL of the solutions. Helium was used as the carrier gas at a flow rate of 1 ml/min. The analysis was performed using the following temperature program: 3 min at 40°C followed by a ramp of 5°C/min up to 300°C, which was sustained for 5 min.

The obtained mass spectra were read using 2.64 AMDIS (Automated Mass Spectral Deconvolution and Identification System, National Institute of Standardization and Technology (NIST), Gaithersburg, MD, USA). The separated polar and non -polar compounds were identified by comparison of their GC-MS spectra and Kovach retention index (RI) with referent compounds in NIST 08 database (NIST Mass Spectral Database, PC- Version 5.0, 2008). The RIs of compounds were recorded with standard n-hydrocarbon calibration mixture (C10 -C40, Fluka) using 2.64 AMDIS software.

Determination of mineral composition of plant samples: The mineral composition of the samples was determined according to the AOAC method (2000). For the purpose of the analysis a Milestone 1200 MEGA rotor 10 MRD 300 (10 positions) microwave was used. A quantity of 0.5 g of dried and finely homogenized sample was weighed and transferred into Teflon vessel. Then 8 mL HCl and HNO_3 in a ratio of 3:1 (v/v) was added. The program for mineralization includes three stages: (I) 5 min unpulsed irradiation, power 250 W; (II) 5 min pulsating irradiation, power 400 W, and (III) 5 min pulsating radiation, power 600 W. The sample was cooled by ventilating for 1 min, then transferred quantitatively to make up to 50 mL. In each series of mineralized samples, a blank one was included.

Statistical analysis: All measurements were carried out in triplicates. The results were statistically analyzed using MS-Excel software.

Results and Discussion

The chemical composition of the *A. ursinum* essential oil is presented in Table 1. 14 substances were identified in *A. ursinum* sample (40 % of the total composition of the oil), 4 of which were more than 3 %. The essential oil showed a high content of disulphides, trisulphides and tetrasulphides. The yield of essential oil was 0.37 %. Sulphur compound were predominant in the oil with the prevalence of allyl polysulphides.

It was difficult to make a quantitative comparison of the elements contained in the *A. ursinum* essential oil, because their levels depend on the soil, geographical location, and the part of plant being used. This was proved by the results of the qualitative analyses of essential oils of wild garlic collected from different locations in Europe (Schmitt *et al.*, 2005). The samples from Poland were found to contain methyl-2-propenyl disulphide (16.05%) and dimethyl trisulphide (12.07%) (ecotype Roztocze); phytol (17.03 %) and n-hexane acid (16.57%) (ecotype Dukla); phytol acetate (16.40%) and (E)-B-ionone (13.33 %) (ecotype Bieszczady) (Blazewicz-Wozniak *et al.*, 2011). In comparison researchers from Serbia have identified prevailing disulphides (TD), trisulphides (TC), and tetrasulphides (TTC) in the *A. ursinum* essential oil (Godevac *et al.*, 2008) in a DS 1.2: 1; TC 0.9: 1 and TTC 1: 1 ratio to the current results. GC/MS analysis of samples of *Allium ursinum* flowers (Bulgaria) showed that the main components were sulphur compounds (Ivanova *et al.*, 2009), which were also in line with the identified components of the *A. ursinum* essential oil from this study.

The chemical composition of the *N. siculum* essential oil is presented in Table 2. Since now, there has been no detailed investigation on the plant's essential oil composition. The yield of essential oil was 0.24%, which is much less compared to the oil obtained from *A. ursinum*. 14 substances were identified in *N. siculum* oil sample (9% of the total mass of the oil), 4 of which were more than 1%. The predominant compounds were ethyl benzoate, thioctic acid and benzaldehyde (Fig. 1).

It is known that various volatiles as benzaldehyde and ethyl benzoate have been recorded as growth suppressors. Moreover, the use of thioctic acid, both fat and water soluble (Thinunavukkarasu & Anuradha, 2004), as antioxidant in food supplements to prevent or cure human diseases associated with oxidative stress has become immensely popular (Coleman *et al.*, 2001; Shay *et al.*, 2009). It anti-aging activity has been also reported (Jiang *et al.*, 2013). Thioctic acid was shown to improve memory in animals (Stoll *et al.*, 1993). In addition, its anti-toxin, anti-inflammatory, anti-proliferative effects and anti-depressant activities is of interest (Kapoor, 2013; Kwiecien *et al.,* 2013; Sokolowska *et al.,* 2013). Furthermore, blood glucose regulating activity of thioctic acid has been reported (Nebbioso *et al.,* 2013), but these reports have not yet been extensively elucidated.

Until 2017, only the isolation and identification of (SSRC)-S-n-butylcysteine sulfoxide from the bulbs of *Allium siculum* was reported by Kubec *et al.,* (2002). Arcus *et al.,* (2011) discovered carotene, saponins and steroid glycosides in *N. siculum* bulbs. Therefore it appears that there is no data in the literature regarding the chemical composition of *N. siculum* leaves, which makes any comparison difficult. Therefore, the present research could be indicated as a new contribution to the knowledge of this plant and the first detailed report regarding *N. siculum.*

Table 1. Chemical composition of *A. ursinum*.

Name	RI	%
Toluene as solvent	*	*
Methyl disulphide	722	0.96
3,4-Dimethyl thiophene	884	0.78
Methyl-2-propenyl disulphide	911	6.83
Methyl 1- propenyl disulphide	928	1.38
Benzaldehyde	960	1.08
Dimethyl trisulphide	972	1.66
Diallyl disulphide	1099	8.81
Diallyl tetrasulphide	1122	1.19
Di-2-propenyl trisulphide	1146	0.86
Di-1- propenyl trisulphide	1154	0.94
Methyl-2-propenyl trisulphide	1161	6.63
3-vinill-1,2-dityacyclohex-5ene	1180	2.28
Dimethyl tetrasulphide	1223	0.76
Diallyl trisulphide	1350	6.73

Mineral element content: In both plants the amounts of potassium, calcium and magnesium were significant (Table 3). As for *N. siculum* the mineral element content report is the first report. The leaves of *Allium ursinum* were richer in zinc compared to those of *N. siculum*. Wild garlic also had more calcium and potassium. These results were confirmed by the previous reports on micro- and macro- element composition of *Allium ursinum* (origin Poland) (Blazewicz-Wozniak *et al*., 2011). According to Thompson *et al*., (1997) the amount of potassium in the leaves of *Allium ursinum*, was 3.86%, and calcium - 1.23 %. The average magnesium content in the leaves of *Allium ursinum* was 0.042% dry weight. Hussain *et al*., (2011) established potassium (4010 ppm) and small quantity of selenium (0.142 ppm) in *A. sativum* along with other micro-and macro elements.

Table 2. Chemical composition of *N. siculum*.

Name	RI	%
Toluene as solvent	*	*
n-Octane	800	0.75
Butyl acetate	811	0.13
Ethyl benzoate	1173	1.29
Benzaldehyde	960	1.13
Ethyl hexanoate	995	0.12
n-Decane	1000	0.85
Methyl n-butyl disulphide	1020	0.12
Ethene,1-(ethylthio)-2-(methylthio)	1028	0.46
3,4-Diethylthiophene	1083	0.17
Dodecane	1200	0.95
Dibutyl disulphide	1318	0.16
Tetradecane	1400	0.59
3,5-Diisopropyl-1,2,4-trithiolane	1666	1.12
Thioctic acid	1697	1.14

Table 3. Mineral element content in *A. ursinum* and *N. siculum*.

Elements (mg/kg)	*A. ursinum*	*N. siculum*
Zinc	6.58	2.78
Copper	1.51	0.92
Iron	18.43	11.36
Magnesium	9775.4	9650.3
Calcium	14810.2	14800.2
Lead	< 0.01	< 0.01
Cadmium	< 0.01	< 0.01
Potassium	16250.0	13750.0
Sodium	232.0	210.4
Phosphorus	715.6	729.5
Selenium	1.905	1.900

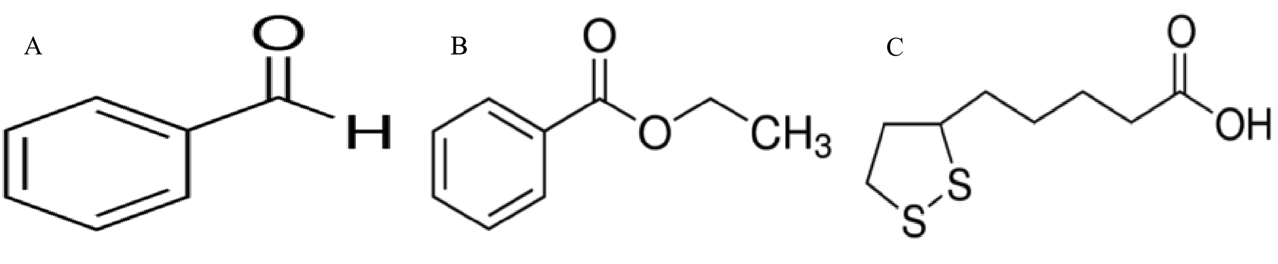

Fig. 1. Structure of the predominant compounds established in *N. siculum* essential oil: A - benzaldehyde; B - ethyl benzoate; C - thioctic acid.

Conclusions

Two Bulgarian plant species were investigated in terms of micro- and macro- element content and volatile oil constituents. *Allium ursinum* L. is a very popular plant and still the comparison to the less explored *Nectaroscordum siculum* ssp. *bulgaricum* is interesting and contributing towards complementing and extending knowledge. The present study could be regarded as the first detailed report in respect of essential oil chemical composition and mineral element content of *N. siculum.*

The mineral element content of the studied plants was relatively similar both in total and by individual components. However, the yield of essential oil was divergent and the identified components differed.

The presented results of the essential oils of the both plants could be informative regarding their possible application and benefits.

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