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Influence of cultivation on the chemical composition and antimicrobial activity of *Sideritis* spp.

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ABSTRACT

Hexane extracts of wild *Sideritis scardica* Griesb. and of cultivated hybrid *S. scardica* x *S. syriaca* were analyzed by GC-MS and tested for antimicrobial activity. Main components of all the extracts were diterpenes and *n*-alkanes. Cultivated and wild plant extracts demonstrated significant activity against *S. aureus*. The results demonstrate the possibility to find cultivation conditions that will lead not only to preservation but to increasing of the quantity of biologically active constituents in *S. scardica* and the biological activity of the extracts.

KEY WORDS: *Sideritis*, cultivation, diterpenes, antibacterial activity.

INTRODUCTION

Sideritis scardica Griesb. is a Balkan endemic encountered in Southwest Albania, Greece, Macedonia, and Bulgaria. In Balkan countries, it is a very popular medicinal plant, used for treatment of inflammations, coughs due to cold, gastrointestinal disorders, as stimulant and for many other purposes (1). For this reason it is a subject of intensive exploitation. The negative changes in the resource potential and reproduction capacities of the species have determined it as one of the most threatened components of the Bulgarian spontaneous flora, for this reason it is included in Red Book of Bulgaria and is protected by the National Protected Low (2). The important role of *Sideritis scardica* as traditional remedies tea and its conservation status has required its cultivation as market production. *S. scardica* is cultivated very difficult; as it grows in high mountains up 1200 m. Another closely related species, *S. syriaca*, grows in much lower locations, so interspecific hybrids have been produced in earlier studies, using wild *S. scardica* and *S. syriaca*. The interspecific selected hybrids demonstrated higher adaptogen properties, biomass production and flavonoid yield than their parental species. They have been cultivated since the last 10 years in Bulgaria from 0 to 1500 m a.s.l. (3).

In this article we report on the preliminary study of the chemical composition of hexane extracts of two wild populations of *S. scardica* and of cultivated plants from three locations. This type of study is especially important with respect to the well known variability of the essential oil composition of *Sideritis* species from different localities, caused by ecologo-geographic factors (4). As far as diterpenoid content of *Sideritis* species can be correlated with their pharmacological activites (5), we preferred to analyze hexane extracts because of the higher content of diterpenes in them, compared to essential oils. We also determined the antibacterial activity of the extracts.

MATERIALS AND METHODS

Plant Material.

Aerial parts of wild *Sideritis scardica* Griesb. were collected in the region of Trigrad, Rodopi Mountain and in Pirin Mountain. Aerial parts of cultivated hybrid plants *S. scardica x S. syriaca* were collected at three locations: Beglica and Pashovo, Rodopi Mtn., and near Sofia. The interspecific hybrids were produced by using wild *Sideritis scardica*, colected from Pirin mountain and *Sideritis syriaca*, colected from Strandja mountain

(80 m). From the F1 produced individual interspecific hybrids, 20 superior plants were selected on the basis of their yield production and quality of flavonoids and were remaind for crossing again. From the F2 hybrids, 250 trials were selected on the same basis and continue to be propagated in the culture.

All plants were collected at flowering stage in July 2005. Voucher specimens are deposited in the Herbarium of the Institute of Botany, Bulgarian Academy of Sciences (*S. scardica*, Rhodopes - Co 1125; *S. scardica*, Pirin - Co 1126; hybrid, Beglica - Co 1127; hybrid Sofia - Co 1128; hybrid Pashovo - Co 1129).

Extraction.

Fresh aerial parts (10 g) were cut into small pieces and extracted with *n*-hexane for 24 h. The extracts were filtered, evaporated to dryness under reduced pressure (the yields of dry extract are represented in Table 1), and analyzed by GC-MS.

GC-MS analysis.

GC-MS analyses were performed on a GC Hewlett Packard 6890 + MS 5973 (Hewlett Packard, Palo Alto, California, USA) A HP5-MS capillary column (30 m ×0.25 mm, 0.25 µm film thickness, Agilent Technologies, Wilmington, Delaware, USA) was used. The ion source was set at 250 °C and the ionization voltage was 70 eV. The temperature was programmed from 40 °C to 280°C at a rate of 6 °C.min^{-1}. Helium was used as a carrier gas. Relative percentage amounts of the separated compounds were calculated from Total Ion Chromatograms by computerized integration. Compounds were identified using computer searches on commercial libraries. In some cases, when identical spectra had not been found, only the structural type of the corresponding component was proposed based on its mass spectral fragmentation.

Antibacterial and antifungal activity.

For the investigation of the antibacterial and antifungal activity, the agar cup method (6) was used with test strains *Staphylococcus aureus* 209 (obtained from the Bulgarian Type Culture Collection, Institute for State Control of Drugs, Sofia), *Escherichia coli* WF+ (obtained from the Collection of ZIMET, Central Institute of Microbiology and Experimental Therapy, Jena, Germany) and *Candida albicans* 562 (obtained from the Bulgarian Type Culture Collection, institute for State Control of Drugs, Sofia). An inhibitory zone with a diameter less than 10 mm corresponds to lack of activity (10 mm is the diameter of the cup). 0.1 ml of test solution containing 0.4 mg of each substance in ethanol was applied to every cup (concentration of the test solution 4 mg/ml). Control experiments with solvents showed that solvents do not have any activity. Bulgarian propolis extract (standardized) was used a positive control.

RESULTS AND DISCUSSION

The hexane extracts were analyzed by GC-MS, the results are represented in Table 2. Over 50 individual compounds were identified in the analyzed extracts. As it is obvious from the results, the main components of these extracts are diterpenes and hydrocarbons (*n*-alkanes). Mono- and sesquiterpenes are absent or are present in low concentrations. The chemical compositions of the wild populations showed significant similarity, both qualitative and quantitative, the main component being a diterpenic diol monoacetate (M=346). It has molecular mass identical with siderol, found in several *Sideritis* species (7), but its mass spectrum does not coincide with that of siderol although there are some similarities (8). This was the main diterpene also in all cultivated plants. Obviously it is an isomer of siderol. Several other diterpenes were detected but most of them were not fully identified due to the lack of matching spectra (library and literature data).

Cultivated plants did not show dramatic changes in qualitative composition. Nevertheless, the plant material cultivated at Beglica demonstrated distinct chemical features. It contained the highest amount of alkanes, fatty acids and their esters, and was the only one that contained detectable amounts of alpha-amyrine. On the other hand, diterpene concentration in the latter cultivar was the lowest one, represented with only 3 individual components.

Table 1. Yield of hexane extract of Sideritis.

Plant material (location)	Percentage of hexane extract
S. scardica (Trigrad)	0.52
S. scardica (Pirin)	0.58
Hybrid (Sofia)	0.57
Hybrid (Beglika)	0.50
Hybrid (Pashovo)	0.54

Table 2. Chemical composition of hexane extracts of S. scardica and S. scardica x S. syriaca hybrid from different locations

Compound	*S. scardica* Trigrad	*S. scardica* Pirin	Hybrid Sofia	Hybrid Beglika	Hybrid Pashovo
MONOTERPENES	**0.1**	**0**	**0.2**	**0**	**0**
α-Pinene	0.1				
β-Pinene			0.1		
Mentha-2,4-diene			0.1		
SESQUITERPENES	**0**	**0**	**0.1**	**0**	**0**
7,11-Dimethyl-3-methylene-1,6,10-dodecatriene			0.1		
DITERPENES	**37.4**	**34.8**	**36.4**	**13.8**	**54.1**
Manoyl oxide			0.2		
7-ethenyl-1,2,3,4,4a,4b,5,6,7,9,10, 10a-dodecahydro-1,1,4a,7-tetramethyl 2-phenanthrenol	0.4		0.4		0.3
Pimara-8,15-diene-3-ol			0.4		0.4
Diterpenic alcohol, (M=288)	0.8		0.5		0.3
Diterpenic alcohol, (M=288)			0.3		
Diterpene (M=304)	4.5	1.9			
Diterpenic diol mono acetate (M=346)	28.4	30.5	30.8	11.7	48.7
Diterpene (M=286)	2.3	1.5	3.8	0.9	4.7
Diterpene (M=290)					
Diterpene (M=320)	1.0	0.9		1.2	
TRRITERPENES	**0**	**0**	**0**	**1.7**	**0**
Triterpene				0.6	
Alpha-amirin				1.1	
FATTY ACIDS AND THEIR ESTERS	**3.9**	**4.2**	**3.12**	**9.9**	**2.4**
Hexadeacnoic acid	3.5	4.2	1.9	8.9	1.5
Ethyl ester of hexadeacnoic acid			0.02		
Octadecatrienoic acid					0.1
Methyl ester of octadecatrienoic acid	0.2		0.6		
Octadecadienoic acid				1.0	
Octadecanoic acid	0.2				0.8
Methyl ester of Octacosanoic acid			0.2		
Methyl ester of hexacosanoic acid			0.4		
ALCOHOLS	**2.8**	**2.8**	**13.4**	**5.5**	**0.1**
Hexenol					
Octenol	0.6	0.5	0.1	0.7	0.1
Octadeaecatrienol		0.9	13.3	4.8	
Oleyl alcohol	1.0	1.4			
Octadecenol	1.2				
1,30-Triacontanediol	0.4				
HYDROCARBONS	**37.5**	**42**	**30.6**	**58.9**	**24.7**
Hydrocarbon				1.0	
Henicosane			0.5		0.1?
Docosane			0.8		0.2
Tricosane	0.2		0.9	0.3	0.3
Tetracosane	0.2		1.0	0.3	0.5
Pentacosane	0.5	0.7	1.1	0.7	0.5
Hexacosane				1.0	

Heptacosane	3.0	3.5	2.4	3.7	1.9
Octacosane	0.6	0.9		1.2	0.5
Nonacosane	10.5	10.7	4.8	15.2	4.6
Triacontane	0.8	1.0		1.4	0.7
Hentriacontane	15.4		10.2	24.6	10.3
Dotriacontane	1.1	0.6	0.9	1.8	0.7
Tritriacontane				6.8	4.5
OTHERS	**1.2**	**0.6**	**0.6**	**0.1**	**0.5**
Benzaldehyde				0.1	0.1
Benzene acetaldehyd	0.4	0.4	0.1		
Phenetylalcohol	0.3	0.1			
Eugenol	0.5	0.1	0.1		0.1
s-Indacene-1,77-dione,2,3,5,6-tetrahydro-3,3,5,5-tetramethyl			0.2		
4b,5,6,12-Tetrahydrochrysene			0.4		

Table 3. Antibacterial activity of hexane extracts of S. scardica and S. scardica x S. syriaca hybrid from different locations

Sample	**Antimicrobial activity** **Diameter of the inhibitory zone ± SD (mm)[a]**
S. scardica Trigrad	29±1
S. scardica Pirin	26±0
Hybrid Sofia	30±0
Hybrid Beglika	21±1
Hybrid Pashovo	25±1

[a] *Mean of three measurements*

At Pashovo locality of cultivation, the diterpene concentration was much higher, than in wild plants. This is an important fact, as far as many diterpenes are known to possess antibacterial, anti-inflammatory and anticancer activities (8, 9, 10).

The hexane extracts were tested for their antimicrobial activity. None of them was active against the gram-positive *E. coli* and the fungus *C. albicans*, but all demonstrated good activity against *S. aureus* (Table 3). It is interesting to note that cultivated and wild plants did not differ much in their antibacterial activity. The weakest sample was the one with the lowest concentration of diterpenes.

These preliminary results demonstrate the possibility to find cultivation conditions that will lead not only to preservation but to increasing of the quantity of biologically active constituents in *S. scardica* and the biological activity of the extracts. Further studies are needed to find out the influence of cultivation on other bioactive components, mainly phenolics.

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