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Antibacterial Activities and Composition of the Essential Oils of *Salvia sericeo-tomentosa* Varieties

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Abstract: The essential oil compositions and antimicrobial activities of two varieties of a new endemic *Salvia* species growing in Turkey were compared. The essential oils (EOs) were obtained from the aerial parts by hydrodistillation and were analysed by gas chromatography (GC), and gas chromatography-mass spectrometry (GC-MS). The major constituents of essential oils were sabinyl acetate (79.9 - 80.1 %) and α-pinene (3.2 - 3.8 %) in both varieties. Such high sabinyl acetate content of an essential oil of a *Salvia* species has not been reported hitherto. The essential oil of *Salvia sericeo-tomentosa* var. *sericeo-tomentosa* (ST) (having MIC/MBC values of 0.3/1.25 mg/mL) showed better activity than the essential oil of *Salvia sericeo-tomentosa* var. *hatayica* (SH) (having MIC/MBC values of 0.6/1.25 mg/mL) especially against *Staphylococcus aureus* and *Bacillus subtilis*. The ST and SH have exhibited significant antimicrobial activity against *Escherichia coli* and, methicillin-resistant *S. aureus* (MRSA).Whereas only modest antimicrobial activity has been observed against *Pseudomonas aeruginosa*, and any activity against *Enterococcus faecalis*, *Proteus mirabilis* and *Klebsiella pneumoniae*.

Keywords: *Salvia sericeo-tomentosa* var. *sericeo-tomentosa; Salvia sericeo-tomentosa* var. *hatayica*; essential oils; antibacterial activity.

1. Introduction

Due to the inherent toxicities of commonly used synthetic antioxidants such as butyl hydroxyanisole (BHA) and butylate hydroxytoluene (BHT) [1, 2], many natural plant extracts and essential oils with antioxidant and antimicrobial properties have been considered as alternative food additives and systematically evaluated for the use as a food and phytopharmaceutical preservative [3-6].

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Salvia, with more than 900 species worldwide, is the largest genus of Lamiaceae family. Anatolia is the gene centre for *Salvia* in Asia [7, 8]. The genus comprises 90 species with 50% endemism in Turkey [9]. Members of the genus known as "sage" have been traditionally used in folk medicine since ancient times. Dioscorides describes, the use of decoction of the leaves and branches of *Salvia* spp. is able to induce movement of the urine and the menstrual flow, is an abortifacient in his "De Materia Medica". The bioactivity studies performed on the essential oils and extracts of *Salvia* species show multiple pharmacological effects such as antimicrobial, antioxidative, anti-inflammatory, hypoglycemic, cardiovascular, anxiolytic, antitumor and sedative activities [10].

In this study was investigated the chemical composition and antimicrobial activity of essential oils of *Salvia sericeo-tomentosa* var. *hatayica* Celep & Doğan (SH) and *Salvia sericeo-tomentosa* Rech. f. var. sericeo-*tomentosa* (ST) for the first time.

2. Materials and Methods

2.1. The Plant Material

The plant materials were collected from Arsuz, Hatay in May 2015. The voucher specimens have been identified and deposited in the Herbarium of the Faculty of Pharmacy, Istanbul University, Turkey (*Salvia sericeo-tomentosa* var. *hatayica* Celep & Doğan (SH) ISTE: 107535, *Salvia sericeo-tomentosa* Rech. f. var. *sericeo-tomentosa* (ST) ISTE: 107536).

2.2. Isolation of the Essential Oil

The essential oils from air-dried plant materials were isolated by hydrodistillation for 3 h, using a Clevenger-type apparatus. The obtained oils were dried over anhydrous sodium sulphate and stored at +4°C in the dark and analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The detailed information and chromatograms regarding GC and GC-MS were reported in the supporting information.

2.3. Antimicrobial Activity Assay

The antimicrobial activity of the EOs was evaluated against Gram positive and Gram negative reference standard microorganisms; *S. aureus* ATCC 25923, Meticillin Resistant *S. aureus* (MRSA) ATCC 43300, *E. faecalis* ATCC 29212, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 4352, *B. subtilis* ATCC 6633, and *P. mirabilis* ATCC 7002 by using standard microbroth dilution method modified with rezasurin and disc diffusion method [11-13]. Experimental details of the antimicrobial activity testing were described in the supporting information.

The experiments were performed with two replications and the results were expressed as average values for the both methods (micro dilution and disc diffusion).

3. Results and Discussion

3.1. Essential Oil Composition

The essential oils were obtained by hydrodistillation from the aerial parts and were analysed by gas chromatography (GC), and gas chromatography-mass spectrometry (GC-MS).

The GC analysis of the essential oils of both species led to the identifications of 28 components, representing 98.8 % for SH, 100 % for ST of total oil constituents. The major constituents of oils were sabinyl acetate (79.9 - 80.1 %) and α-pinene (3.2 - 3.8 %). The analysis results are given in Table 1.

Table 1. Chemical composition of *Salvia sericeo-tomentosa* var. *hatayica* (SH) and *Salvia sericeo-tomentosa* var. *sericeo-tomentosa* (ST) essential oils

RRI[a]	RRI[b]	Compound	SH %[c]	ST %[c]
1025[d]	1032	α-pinene	3.2	3.8
1077[e]	1076	camphene	0.2	0.2
1117[d]	1118	β-pinene	0.4	0.7
1122[f]	1132	sabinene	0.3	0.5
1122[f]	1135	thuja-2,4(10)-diene	0.3	0.2
1160[f]	1174	myrcene	0.1	0.2
1212[g]	1203	limonene	t	0.1
1213[h]	1213	1,8-cineole	0.4	1.8
1282[e]	1280	p-cymene	0.8	0.7
	1499	α-campholene aldehyde	t	t
1515[f]	1532	camphor	t	t
1579[f]	1591	bornyl acetate	0.4	t
1601[f]	1611	terpinen-4-ol	0.5	0.4
	1642	thuj-3-en10-al	0.2	t
1631[f]	1648	myrtenal	0.3	t
1651[f]	1651	sabina ketone	0.3	t
	1658	sabinyl acetate	79.9	80.1
1680[f]	1683	trans-verbenol	0.8	1.2
1717[f]	1720	trans-sabinol	2.5	1.6
1720[f]	1725	verbenone	1.2	1.2
1784[f]	1802	cumin aldehyde	1.3	1.3
1812[f]	1838	2-phenylethyl acetate	0.2	t
1836[f]	1845	trans-carveol	0.4	t
1848[f]	1864	p-cymen-8-ol	0.5	0.5
	1981	cuminyl acetate	0.5	0.3
	2073	p-mentha-1,4-dien-7-ol	t	t
	2113	cumin alcohol	2.5	3.6
2227[f]	2255	α-cadinol	1.6	1.6
		Monoterpene hydrocarbones	**5.7**	**8.2**
		Oxygenated monoterpenes	**91.3**	**90.2**
		Oxygenated sesquiterpenes	**1.6**	**1.6**
		Others	**0.2**	**-**
		Total	**98.8**	**100**

[a] RRI indices from literature d) [13], e [14], f [15], g [16], h [17],

[b] RRI: Relative retention indices calculated against *n*-alkanes for a polar column, % calculated from FID data, t Trace (< 0.1 %)

Numerous investigations about the chemical composition of *Salvia* have resulted in the isolation of various biologically active terpenoids and phenolic compounds [14-23].

In this study, the isolated major components of both *Salvia sericeo-tomentosa* varieties (SH and ST) are sabinyl acetate (respectively 78% and 80%) and α-pinene (respectively 3.2% and 3.8%). Such high sabinyl acetate content of a *Salvia* essential oil has not been reported previously. In contrast, reported amount of sabinyl acetate in the volatile oils of *S. lavandulifolia* [24], *S. yosgadensis* [25], *S. multicaulis* var. *simplicifolia* [26] and *S. pilifera* [27] were 12.8%, 10.1%, 5.3%, 0.3%, respectively. The varieties of the *S. sericeo-tomentosa* are belong to the section of *Hymenosphace* and the closely related species of them

are *S. euphratica, S. kronenburgii* [28]. In the literature there are reports regarding EO compositions of these both species but without sabinyl acetat content [29, 30].

Sabinyl acetate is a toxic substance, its fetotoxic, abortifacient and skin irritant effects are well documented in the literature [31, 32]. Due to the high sabinyl acetate content of essential oils of *Salvia sericeo-tomentosa* varieties, pregnant women should not use them.

4.2. Antimicrobial Activity

The results of MIC/MBC (mg/mL) values and disc diffusion (mm) of *Salvia sericeo-tomentosa* var. *hatayica* (SH) and *Salvia sericeo-tomentosa* var. *sericeo-tomentosa* (ST) essential oils were summarized in Table 2.

Table 2. Microdilution method MIC/MBC* (µg/mL) and disc diffusion (ZI=mm)* values results of *Salvia sericeo-tomentosa* var. *hatayica* (SH) and *Salvia sericeo-tomentosa* var. *sericeo-tomentosa* (ST) essential oils

Test strains	***S. sericeo-tomentosa* var. *sericeo-tomentosa* (ST)**		***S. sericeo-tomentosa var. hatayica* (SH)**		**DMSO**		**Ciprofloxacin**	
	ZI (mm)	**MIC/MBC (mg/mL)**	**ZI (mm)**	**MIC/MBC (mg/mL)**	**ZI (mm)**	**MIC/MBC (mg/mL)**	**ZI (5µg/disc mm)**	**MIC/MBC (µg/mL)**
E.coli ATCC 25922	9±0.71	1.25	8.5±0.35	0.625	7±0.42	6.9	31+2.12	0.25
S.aureus ATCC 25923	9±1.06	0.312/1.25	9.5±0.71	0.625/1.25	6.5±0.49	13.8	25+0.71	0.5
MRSA ATCC 43300	9±0.71	0.625/1.25	8.5±0.57	0.625	6.5±0.28	13.8	26+1.41	0.5
E.faecalis ATCC 29212	9±0.35	1.25	9±0.28	1.25	6.5±0.42	13.8	20+0.71	1
K.pneumoniae ATCC 4352	8±0.28	1.25/2.5	8±0.42	1.25/2.5	6.5±0.28	6.9	22+2.12	0.5
B.subtilis ATCC 6633	9±0.71	0.312/2.5	10±0.49	0.312/2.5	6.5±0.49	13.8	32+0.71	0.5
P.aeruginosa ATCC 27853	7.5±0.71	1.25/2.5	7±0.35	0.625/1.25	6.5±0.35	6.9	33+2.82	0.25
P.mirabilis ATCC 7002	7±0.28	1.25/2.5	7±0.42	1.25/2.5	6.5±0.28	6.9	33+2.12	0.13

** MIC = minimum inhibitory concentration, MBC = minimal bactericidal concentration and ZI=Zone of inhibition*

The essential oil of ST (having MIC/MBC values of 0.3/1.25 mg/mL) showed better activity than the essential oil of SH (having MIC/MBC values of 0.6/1.25 mg/mL) especially against *S. aureus* and both showed the same activity at 0.3 mg/mL against *B. subtilis*. Modest activities against MRSA at MIC 0.6 mg/mL were observed by both EOs. In addition, SH showed also a weak activity against Gram negative bacteria; *E. coli* and, *P. aeruginosa* at MIC 0.6 mg/mL. However, these activities were weak in comparison to the positive control Ciprofloxacin (MIC values between 0.13-1.0 µg/mL). Both, ST and SH essential oils did not show any activity against *K. pneumoniae, E. faecalis* and *P. mirabilis*.

The ST and SH indicated significant antimicrobial activity against *E. coli*, *S. aureus*, MRSA, *E. faecalis* and *B. subtilis* (between 8.5-10.0 mm inhibition zones) by disc diffusion. Between 7-8 mm inhibition zones were observed against *P. aeruginosa*, *P. mirabilis* and *K. Pneumonia* (Table 2). Similar to the MIC/MBC results, all of the above mentioned activities were modest in comparison to the positive control Ciprofloxacin (23-40 mm inhibition zones).

A good antimicrobial activity with several studies has been performed on the EOs of *S. tomentosa* against *S. aureus, B. subtilis, B. cereus, E. coli and E. aerogones* [17, 33, 34]. Our results with the antibacterial activity of EOs of SH and ST show close similarities to the both studies.

In this study was presented first time the composition and antibacterial activity of endemic varieties of *Salvia sericeo-tomentosa*. Further trials on more pathogenic microorganisms, animal tests,

pharmacological and toxicological examinations are required as a potential bactericidal agent in the treatment of infectious diseases. However, because of the high sabinyl acetate amount of the both varieties should be avoided in pregnant women and the dose depended toxicity studies are necessary.

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