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Chemical composition and antimicrobial activity of *Satureja hortensis* L. essential oil

Communication

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Abstract: Essential oil of *Satureja hortensis* L. was analyzed by GC and GC/MS and tested by a broth micro-well dilution method for activity against multiresistant clinical isolates of pathogenic bacteria from 10 different genera: *Klebsiella*, *Escherichia*, *Proteus*, *Staphylococcus*, *Streptococcus*, *Pseudomonas*, *Enterococcus*, *Enterobacter*, *Citrobacter* and *Acinetobacter*. The main compounds in the oil were carvacrol (67%), γ-terpinene (15.3%) and p-cymene (6.73%). The oil showed activity against all tested strains. MIC/MBC values were in the range of 0.78-25 μl/ml, with the exception of the strain *P. aeruginosa*. Microbicidal concentration for this particular strain (50 μl/ml) was the highest tested concentration. The oil showed inhibitory and bactericidal effect at the same concentration (MIC=MBC) for all but three strains.

Keywords: *Satureja L.* • *S. hortensis* • *Essential oil* • *Antimicrobial activity* • *Wound infection*

1. Introduction

Satureja hortensis L. (Lamiaceae) is a widely distributed, annual plant, cultivated in many parts of the world. It is one of the most important of nine classified *Satureja* species on the territory of the central part of Balkan Peninsula [1]. *Satureja hortensis* L. (summer savory) is an aromatic herb, which is, beside its application as a spice and natural food preservative, used in the traditional folk medicine in the treatments of cardiovascular diseases and thrombosis [2], muscle pain, stomach and intestinal disorders [3], and as an anti-inflammation agent in the treatment of rhinosinusitis [4]. Extracts and essential oils of this plant species have antioxidant [5], antibacterial [5,6] and antifungal activities [5,7].

Using a broth micro dilution method, antibacterial activity of *S. hortensis* essential oil against clinical isolates from wounds was tested. The test showed high antimicrobial activity of *S. hortensis* essential oil. Considering that the tested bacterial strains were multiresistant and the possibility of the local application, essential oil of this species can be recognized as a potential natural antimicrobial agent. Its application could be synergistic with the standard antibiotics in wound infection treatment.

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2. Experimental Procedures

2.1 Plant material

The aerial parts of cultivated *Satureja hortensis* L. were collected at the beginning of the flowering stage. Voucher specimens No.UTM34TEN89 were confirmed and deposited at the Herbarium of the Department of Biology and Ecology (BUNS Herbarium), Faculty of Natural Sciences, University of Novi Sad.

2.2 Isolation of the essential oil

Air-drying of plant material was performed in a shady place at room temperature for 10 days. Dried aerial parts (100 g) were cut and subjected to hydro-distillation for 3 h, using a Clevenger-type apparatus. The resulting essential oil was dried over anhydrous sodium sulfate and stored at 4°C. The oil solution (1%) in ethanol was used for chromatographic analysis.

2.3 Analytical gas chromatography (GC/FID)

GC/FID analysis of the oil was carried out on a Hewlett-Packard HP-5890 Series II GC apparatus, equipped with split-splitless injector and automatic liquid sampler (ALS), attached to HP-5 column (25 m · 0.32 mm, 0.52 μm film thickness) and fitted to a flame ionization detector (FID). Carrier gas flow rate (H_2) was 1 ml/min, split ratio 1:30, injector temperature was 250°C, detector temperature 300°C, while column temperature was linearly programmed from 40-260°C (at rate of 4°/min). Solutions of essential oil samples in ethanol (~1%) were consecutively injected by ALS (1 μl, split mode). Area percent reports, obtained as a result of standard processing of chromatograms, were used as a base for the quantification purposes.

2.4 Gas chromatography–mass spectrometry (GC/MS)

The same analytical conditions as those mentioned for GC/FID were employed for GC/MS analysis, along with column HP-5MS (30 m · 0.25 mm, 0.25 μm film thickness), using Hewlett-Packard HP G1800C Series II GCD system. Instead of hydrogen, helium was used as the carrier gas. Transfer line was heated at 260°C. Mass spectra were acquired in EI mode (70 eV), in m/z range 40-450. Sample solutions in ethanol (~1%) were injected by ALS (200 nl, split mode).

2.5 Identification of components

The components of the oil were identified by comparison of their mass spectra to those from Wiley275 and NIST/NBS libraries, using different search engines. The experimental values for retention indices (KIE) were determined by the use of calibrated Automated Mass Spectral Deconvolution and Identification System software (AMDIS ver. 2.1.), compared to those from available literature (KIL) [8], and used as an additional tool to approve MS findings.

2.6 Antimicrobial activity

2.6.1 Microbial strains

The antimicrobial activity of *S. hortensis* L. essential oil was evaluated using clinical isolated strains from wounds: *Klebsiella pneumoniae, Klebsiella oxytoca, Escherichia coli, Proteus mirabilis, Staphylococcus aureus, Staphylococcus spp., Streptococcus pyogenes, Pseudomonas aeruginosa, Enterococcus spp., Enterobacter spp., Citrobacter spp.* and *Acinetobacter spp.* (Source: Center for Microbiology, Institute for Public Health of Vojvodina, Novi Sad, Serbia).

2.6.2 Micro-well dilution assay

The broth micro-well dilution method was employed to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the *S. hortensis* essential oil against multiresistant strains isolated from wounds, according to the National Committee for Clinical Laboratory Standards [9]. The inocula of the bacterial strains were prepared from overnight broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity (corresponding to 10^7-10^8 CFU/ml for bacteria, depending on genera (consensus standard by the NCCLS M38 [ISBN 1-56238-480-8]). The investigated oil was dissolved in 1% dimethylsulphoxide (DMSO) and then diluted to the highest concentration (500 μl/ml). A serial doubling dilution of the oil was prepared in a 96/well microtiter plate over the range of 50.00-0.02 μl/ml in inoculated nutrient broth (the final concentration in each well adjusted to 2.0 x 10^6 CFU/ml). The plate was incubated for 24 h at 37°C. MIC was defined as the lowest concentration of essential oil which inhibited the visible growth of microorganisms. The microbial growth was determined by absorbance at 620 nm using the universal microplate reader (ThermoLabsystems, Multiscan EX, Software for Multiscan ver. 2.6.). To determine MBC, broth was taken from each well and inoculated in Mueller Hinton agar (MHA) for 24 h at 37°C. The MBC is defined as the lowest concentration of the essential oil at which inoculated microorganisms were 99.9% killed. Amikacin served as positive control. All determinations were performed in duplicate and two growth controls consisting of nutrient broth with 1% DMSO (v/v) were included.

2.7 Statistical analysis of data

Analysis of variance (ANOVA) was used to determine the significance (P≤0.05) of the data obtained in all experiments. All results were determined to be within the 95% confidence level for reproducibility.

3. Results and Discussion

3.1 Chemical composition of essential oil

It is well known that both environmental and genetic factors are effective in observed variations among *S. hortensis* accessions with high accuracy [10]. Because of this, yield and chemical composition of essential oil can vary among the populations of the same species from different localities.

Investigations from 20 localities in Turkey showed that wild-growing forms of *S. hortensis* had oil content in the range from 1.28-4.75%, while cultivated forms contained from 1.30-2.67% of the essential oil. Also, the material collected from the western part of the Turkey contained carvacrol as the major component of the essential oil (42-63%), whereas in the material collected from the eastern Turkey, the dominant component was thymol (29-43%) [11]. In the material collected from the different localities worldwide, carvacrol was also the major component, present in the highest percent [11]. In addition, content and the composition of the essential oil are highly dependent on the methodology of investigation. Sefidcon *et al*. [12] investigated the influence of drying methods (sun-drying, shade-drying and oven-drying at 45°C) and extraction methods (water and steam-distillation and direct steam-distillation) on yield and chemical composition of the essential oil of *S. hortensis* cultivated in Iran. It was determined that drying of aerial parts of *Satureja hortensis* in the oven at 45°C and extraction of their essential oil by hydro-distillation is most suitable and is recommended for fast drying, and high-oil yield (1.06%), as well as for a high percentage of carvacrol (48.1%).

In this paper, air-drying of plant material was performed in a shady place at room temperature for 10 days. By hydro-distillation process in the Clevenger-type apparatus, a yellow liquid of pleasant odour was isolated from the dried aerial parts of *S. hortensis*.

Constituents	KIE	KIL	RT/MS	RT/FID	% m/m	RRT	CI
α-Thujene	921.1	924	7.01	12.265	0.48	0.453	7
α-Pinene	926.6	932	7.19	12.578	0.67	0.465	10
Camphene	940.9	946	7.63	13.207	0.05	0.488	1
Sabinene	969.0	969	8.45	14.342	0.85	0.530	13
β-Pinene	974.0	974	8.52	14.763	0.84	0.545	13
β-Myrcene	986.6	988	9.03	14.917	0.18	0.551	3
α-Phellandrene	998.9	1002	9.41	15.414	0.12	0.569	2
Δ3-Carene	1004.3	1008	9.58	15.669	0.04	0.579	1
α-Terpinene	1010.8	1014	9.81	15.910	1.29	0.588	19
p-Cymene	1019.0	1020	10.10	16.232	6.73	0.600	100
β-Phellandrene	1022.5	1025	10.22	16.418	0.31	0.606	5
trans-β-Ocimene	1044.0	1044	10.96	17.100	0.05	0.632	1
γ-Terpinene	1053.4	1054	11.29	17.634	15.30	0.651	228
cis-Sabinene hydrate	1063.6	1065	11.64	18.001	0.37	0.665	6
Terpinolene	1082.8	1086	12.31	18.857	0.04	0.697	1
trans-Sabinene hydrate	1097.1	1097	12.84	19.282	0.20	0.712	3
Borneol	1161.2	1165	15.04	22.082	0.32	0.816	5
Terpinen-4-ol	1173.0	1174	15.46	22.498	0.54	0.831	8
α-Terpineol	1190.6	1186	16.06	22.993	0.15	0.849	2
Carvacrol methyl ether	1240.8	1241	17.83	24.942	0.12	0.921	2
Thymol	1291.1	1289	19.46	26.618	0.21	0.983	3
Carvacrol	1305.9	1298	19.96	27.073	67.00	1.000	1000
Carvacrol acetate	1370.0	1370	22.04	29.623	0.11	1.094	2
β-Caryophyllene	1411.6	1417	23.37	31.672	1.90	1.170	28
Aromadendrene	1431.1	1439	23.97	32.344	0.05	1.195	1
α-Humulene	1445.9	1452	24.42	32.840	0.10	1.213	1
β-Bisabolene	1501.9	1505	26.14	34.345	1.01	1.269	15
Spathulenol	1571.0	1577	28.19	36.932	0.11	1.364	2
Caryophyllene oxide	1574.5	1582	28.29	37.167	0.35	1.373	5

Table 1. Chemical Constituents of the Essential oil of *Satureja hortensis* L. (in %).

KIE: RRI experimentally determined (calibrated AMDIS)
KIE: RRI literature values (Adams, 2007)
CI: concentration indices in relation to carvacrol (which concentration was taken as $CI_{carvacrol}$=1000)

The oil yields were calculated on a dry weight basis as 2.05% (v/w). Twenty nine components (99.49%) were identified as constituents of the essential oil by combining GC and GC/MS analyses. Qualitative and quantitative data on the main constituents (and their respective RI) of herb volatiles have been summarized in Table 1. The concentrations of the components were also calculated as concentration indices in relation to carvacrol (concentration was taken as $CI_{carvacrol}$=1000). The concentration indices are shown according to the relative retention times of each component (RRT) in relation to carvacrol, retention time was taken as $RRT_{carvacrol}$=1.

The major components were carvacrol (67.00%), γ-terpinene (15.30%) and p-cymene (6.73%). Except α–terpinene (1.29%), β–caryophyllene (1.90%) and β–bisabolene (1.01%), the amount of all remaining oil components was less than 1%. The monoterpene prevalence in oil (95.97%) was evident, while the most abundant were oxygenated monoterpenes (69.02%). In addition, sesquiterpenes hydrocarbons (3.06%) and oxygenated sesquiterpenes (0.46%) were isolated.

3.2 Antimicrobial activity of *S. hortensis* essential oil

The essential oil of *S. hortensis* possesses a wide antimicrobial spectrum, and it inhibits the growth of the human and phytopathogenic and food spoilage bacteria, fungi and yeasts species. *S. hortensis* essential oil was active against *E. coli* O157:H7, *S. typhimurium, S. aureus* and *L. monocytogenes* in the range from 0.013-0.003 µl/ml, as well as against *Pseudomonas putida* strain isolated from meat with a MIC of 0.05% and a MTC of 0.013% [13]. The oil showed activity against 25 (of 32 species) bacteria in the range from 15.62 – 250 µl/ml and against 3 (of 10) fungal strains in the range from 62.5 – 125 µl/ml [14]. The essential oil of summer savory (*Satureja hortensis* L.) inhibited the mycelial growth of phytopathogenic fungi *Alternaria mali* and *Botrytis cinerea*, and exhibited a fungicidal effect [7]. Also, the oil showed strong antifungal activity against *A. flavus* under *in vitro*, storage conditions [15], in liquid medium and in tomato paste [16] and inhibited the growth and aflatoxin (AFB1 and AFG1) production by *A. parasiticus* [17].

In this paper, 30 bacterial clinical isolates from 10 genera were tested by the antibiogram method, using 15 standard antibiotics. After the testing, highly multiresistant strains were selected. Antimicrobial activity of *S. hortensis* essential oil was investigated by broth micro-well dilution method against selected strains. Essential oil showed activity against all investigated clinical isolates from wounds in the range from 0.78 – 50 µl/ml (Figure 1). Oil exhibited the highest activity against *Acinetobacter spp.* (MIC=MBC=0.78 µl/ml), *K. oxytoca* (MIC=MBC=3.125 µl/ml) and *S. aureus* (MIC/MBC=3.125/12.5 µl/ml). Also, it showed some slighter activity against *E. coli* and *Staphylococcus spp.* (MIC=MBC=6.25 µl/ml). In the same concentration, the oil was active against *P. mirabilis*, *S. pyogenes, Enterobacter spp*. and *Enterococcus spp.* (MIC=MBC=12.5 µl/ml).

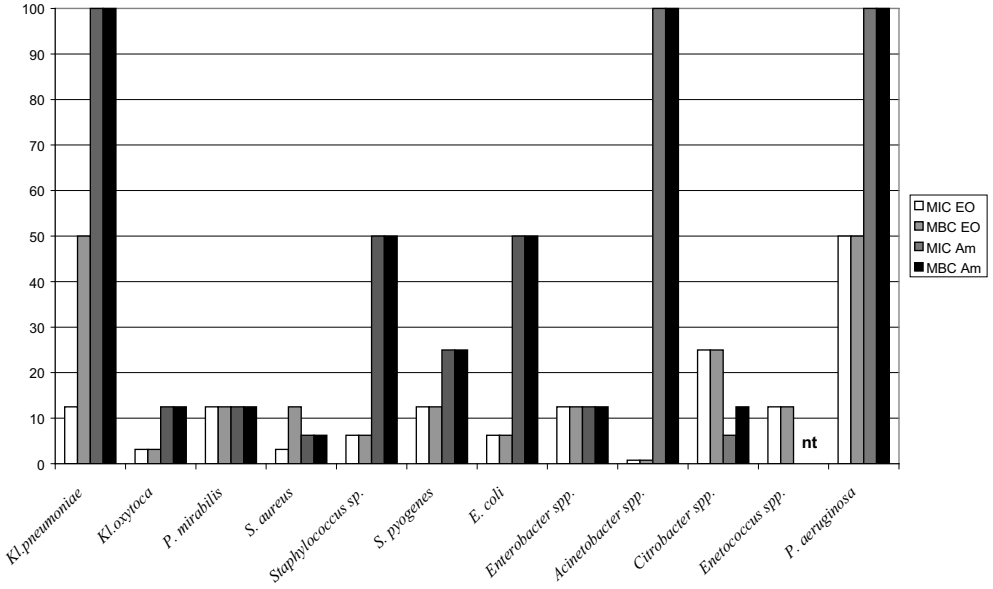

Figure 1. The MIC/MBC values (µl/ml) of the *Satureja hortensis* L. essential oil against twelve multiresistent clinical isolates from wounds: *K. pneumoniae, K. oxytoca, P. mirabilis, S. aureus, Staphylococcus spp., S. pyogenes, E. coli, Enterobacter spp., Acinetobacter spp., Citrobacter spp., Enterococcus spp.* and *P. aeruginosa*., by the broth microdilution method. (EO – essential oil; Am –amikacin µg/ml; nt – not tested).

Against the strain *K. pneumoniae*, the oil had inhibitory effect in lower concentration (MIC=12.5 µl/ml) in relation to the bactericidal concentration (MBC=50 µl/ml).

The strain with the lowest susceptibility was *P. aeruginosa* (MIC=MBC=50 µl/ml). This strain is very resistant to most of the essential oils, owing to the hydrophilicity of its cell membrane surface [18-21]. Except for the strains *K. pneumoniae* and *S. aureus*, the oil had both inhibitory and bactericidal effect at the same concentration (MIC=MBC).

High antimicrobial activity of the oil is based on the high amount of the phenol compound carvacrol (67.00%), whose antimicrobial activity is already known and confirmed. It is determined that carvacrol has much higher antimicrobial potential in relation to its chemically related compounds: eugenol (MIC=0.5-1 g/l), menthol (MIC=0.5-2 g/l), carvacrol acetate and carvacrol methyl ether (MIC≥3 g/l) [22].

Considering the activity of oregano oil (in the concentration from 0.06-0.125% v/v), carvacrol and thymol showed higher activity against tested strains *S. aureus* and *S. epidermidis* (in the concentration from 0.015-0.03% v/v and from 0.03-0.06% v/v, respectively) [23]. Their activities against *S. sonei* and *S. flexneri* strains were in the same level of thyme and oregano essential oil effect [24].

The testing of essential oil and pure oil compounds (carvacrol, thymol and p-cymen) antimicrobial activity against 5 strains of pathogenic bacteria, has determined that the activity of carvacrol was either in the same level as the entire essential oil effect (*B. cereus* and *E. coli*), or carvacrol had effect in the higher concentration than the entire oil (*P. aeruginosa*, *S. aureus* and *S. lutea*). In the last case, carvacrol had greater activity than thymol and cymene. Mixing carvacrol and thymol at proper amounts may exert the total inhibition that is evident by oregano essential oil. Such inhibition is due to damage in membrane integrity, which further affects pH homeostasis and equilibrium of inorganic ions [25]. Beside this, some very important data is that carvacrol does not have any recorded genotoxical effect at concentrations which express antimicrobial activity [26]. p-cymene barely shows any antimicrobial activity, but it has synergistic effect together with carvacrol.

Considering the fact that the referent antibiotics expressed antimicrobial effect at the concentration of 30 µl/ml, it can be concluded that essential oil of *S. hortensis* showed very high antimicrobial activity. This activity can be explained by the high amount of carvacrol in the essential oil composition. For this reason, application of this essential oil in the treatment of wound infections should be considered, together with its sinergistic effect with the standard antibiotics.

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