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ANTIMICROBIAL EFFECT OF ESSENTIAL OILS FROM PLANTS COLLECTED FROM DIFFERENT LOCALITIES

ANTIMIKROBIÁLNY ÚČINOK SILÍC Z RASTLÍN ZBIERANÝCH Z RÔZNYCH LOKALÍT

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Antimicrobial activities of the essential oils obtained by using the hydrodistilation method from *Tanacetum vulgare* L. and *Salvia officinalis* L. were investigated using the disc agar diffusion method and by the microdilution method. The plants used in this study were collected from different localities of Slovakia. The essential oils revealed higher activity against Gram-positive bacteria, namely *Bacillus subtilis*. The essential oils with the best antibacterial properties exhibited also the highest anti-yeast effect mainly against *Candida albicans* as well as the inhibition of the growth of mycelial fungi *Aspergillus flavus* and dermatophyte *Microsporom gypseum*. Essential oils of plants belonging to the same taxa but collected from different localities showed different levels of antimicrobial activities.

Key words: essential oils, *Tanacetum vulgare, Salvia officinalis*, locality, antimicrobial effect

Essential oils (EO) and plant extracts have been used for many thousands of years, in food preservation, pharmaceuticals, alternative medicine and natural therapies (Mitscher et al., 1987). Essential oils are potential sources of novel antimicrobial compounds especially against bacterial pathogens, however they have shown to possess also antifungal, antiviral, and insecticidal and antioxidant properties (Burt, 2004; Kordali et al., 2005; Reichling et al., 2009). These properties are important for both food preservation and control of human and plant diseases of microbial origin. Some oils have been used in cancer treatment (Sylvestre et al., 2006). The quality and yield of essential oils is influenced by many factors, such as fertilizer and pH of soil (Alvarez-Castelianos and Pascual-Villalobos, 2003), chemotype or subspecies (Goren et al., 2001), choice of plant part (Keskitalo et al. 2001), harvesting season (Cornu et al., 2001), the choice and stage of drying conditions (Tateo and Riva, 1991) and extraction method (Scalia et al., 1999). The content of some components of essential oils varies according to the geographic location. Thus, 1,8-cineole was the most abundant in the oil of sage from the USA (18.0%), camphor in the oil of Romania (26.5%), caryophallene in the oil from the USA (10.0%), alpha-thujone in the oil from Italy (45.8%), beta-thujone in the oil from Romania (23.1%) (Boelens and Boelens, 1997). The composition of the essential oils from various chemotypes of *Tanacetum vulgare* was studied in detail on populations of wild growing species in Finland (Keskitalo et al., 2001), Norway (Dragland et al., 2005), Hungary, Poland, Italy, and others. In our previous work (Vaverková et al., 2006) we examined qualitative properties of the essential oil obtained from *Tanacetum vulgare* L. and we confirmed that the shares of the constituents in the essential oils differ in dependence on the location.

The aim of this work was to estimate and compare antimicrobial activity of extracts from *Tanacetum vulgare* L. and *Salvia officinalis* L. from some geographic locations in Slovakia.

Material and methods

Tanacetum vulgare L. and *Salvia officinalis* L. were used as a testing material. Air dried aerial parts (flower heads) were hydrodistiled for 3.5 hours in accordance to the European Pharmacopoea. Isolated oil was diluted in n-hexane and dried over anhydrous sodium sulphate. Oil samples were analysed using a Hewlett Packard HP 5971. A mass selective detector directly coupled to a gas chromatograph HP 5890 Series II FID was used. A capillary column DB-WAX/26m × 0.20 mm, 0.2 mm film thickness (Hewlett Packard, USA) was used.

Two strains of gram-negative bacteria *Escherichia coli* (CCM 3988), *Proteus mirabilis* (CCM 1941) and three strains of gram--positive bacteria *Bacillus subtilis* (CCM 1718), *Staphylococcus aureus* (CCM 3953) and *Micrococcus luteus* (CCM 732) as well as the yeasts *Saccharomyces cerevisiae, Candida albicans, Candida parapsilosis, Rhodotorula glutinis* and micromycetes *Aspergillus flavus* and *Microsporon gypseum* were obtained from the Czech Collection of Microorganisms, Brno, Czech Republic. The cultures were maintained in their appropriate agar slants at 4 °C throughout the study and used as stock cultures.

Disc diffusion method – nutrient agar plates were swabbed with the respective broth culture of the organisms (diluted to 0.5 McFarland standards with saline). Filter paper discs (6 mm in diameter) were impregnated with 5 L of the extract and placed on the inoculated plates. These plates, after staying at 4 °C for 2 h, were incubated at 37 °C for 24 h for bacteria and at 28 °C for 48 h for the yeasts. The diameters of the inhibition zones were measured in millimetres.

Determination of minimum inhibitory concentration – was performed using broth microdilution method. The overnight growing culture of bacteria was filtered and 2% suspension of bacteria was prepared. 180 μl of this suspension and 20 μl of double diluted solutions of EO were placed into the wells of

96 well microtitre plates and cultivated for 24 h on reciprocal shaker at 37 °C. The time course of absorbance (A_{630}) was determined at 120 min intervals in triplicates.

Antifungal activity – extract was added to molten SIA medium at given concentration. The centre of each plate was inoculated with one fungal disc (5 mm diameter) of the respective fungus. The plates were incubated at 28 °C for 7 days. The antifungal activity of extracts was evaluated every 2 days by measuring the diameter of test and control colonies in millimeters. Percentage of inhibition was calculated using the formula IZc-IZt/IZt × 100 where IZc and IZt are the average diameters of mycelia colonies in control and treated fungi set.

Results and discussion

Water distiled essentials oils from flower heads of *Tanacetum vulgare* L. and *Salvia officinalis* L. collected from five (*Tanacetum*) or three (*Salvia*) localities in Slovakia have been analysed by GC/MS. The analyses showed that the main components of the oils of *Tanacetum*, which constituted 82.1% of total oils are: -pinene, camphene, sabinene, β-pinene, myrcene, 1.8-cineole, artemisia ketone, β-tujone, camphor, borneol, umbellulone, D-carvone, chrysantenyl-acetate, bornyl-acetate, thymol, germacrene and carvacrol. The shares of the constituents in the essential oils differed in dependence on the locality.

In general, activity of essential oils against the Gram-positive bacteria (*Staphylococcus aureus, Micrococcus luteus* and *Bacillus subtilis*) was higher than against Gram-negative bacteria. The strongest activity among the Gram-positive bacteria was for *B. subtilis* where inhibition zones reached up to 22 mm. From Gram-negative bacteria only *Escherichia coli* was more susceptible to essential oils from *Salvia officinalis* L. Oil from one chemotype of *Tanacetum vulgare* L. (T3) had very strong effect on both Gram-negative bacteria but oils from another chemotypes had only very slight effect. Studies of antimicrobial effects of essential oils from *Tanacetum, Salvia* and many other plants described higher activities of essential oils on Gram-negative bacteria (Kalodera et al., 1997, Nostro et al., 2000, Ouattara et al., 2000). Our results are consistent with that of El-Shazly et al. 2000, Bagci et al., 2008, Randrianarivelo et al., 2009 and others, which confirmed high activity of essential oils on Bacillus subtilis, Escherichia coli and Candida albicans.

The comparative evaluation of essential oils from two distinct species of plants (*Tanacetum vulgare* L. and *Salvia officinalis* L.) showed variations in the level of activity against bacteria. These variations were evident from zones of inhibition (Tab. 1) as well from MIC values (Tab. 2). MIC of essential oils from Tanacetum ranged in all bacteria from <0.6% to >2.5%. Three essential oils from *Salvia* had strong effect on *Staphylococcus aureus* and *Micrococcus luteus* (MIC <0.6%). In third G+ bacteria *Bacillus subtilis*, the MIC of *Salvia* oils were 1.25% and 2.5% resp. and in G- bacteria *Escherichia coli* and *Proteus mirabilis* MICs of *Salvia* essential oils ranged from <0.6% to 2.5%. G- bacteria *E. coli* and *Proteus mirabilis* MICs of *Salvia* extracts ranged from <0.6% to 2.5%.

Extracts T1-T5 originated from the same plant species, were isolated using the same procedures and differed only by the geographic location. As is evident from the comparison of inhibition zones (Tab. 1) and MIC values (Tab. 2) their

Table 1 Antimicrobial activity of essential oils from *Tanacetum vulgare* L. (T1-T5) and *Salvia officinalis* L. (S1-S3) by using the disc diffusion method. Values represent the inhibition zone in mm

	T1	T2	T3	T4	T5	S1	S2	S3
E. coli	9	9	19	7	9	11	11	13
P. mirabilis	11	7	15	9	9	7	9	7
S. aureus	9	11	13	9	11	9	7	7
M. luteus	19	9	9	9	9	13	13	9
B. subtilis	13	19	22	11	11	19	21	21
S. cerevisiae	13	9	9	11	13	7	9	7
C. albicans	11	13	15	11	11	13	15	13
C. parapsilosis	11	9	9	11	9	11	11	13
R. glutinis	11	7	11	13	13	11	11	13

Tabuľka 1 Antimikrobiálna aktivita silíc z *Tanacetum vulgare* L. (T1-T5) a *Salvia officinalis* L. (S1-S3) použitím diskovej difúznej metódy. Hodnoty predstavujú inhibičnú zónu v mm

Table 2 Minimal inhibition concentration (MIC) of the essential oils from *Tanacetum vulgare* L. (T1-T5) and *Salvia officinalis* L. (S1-S3) by using the microdilution method

	T1	T2	T3	T4	T5	S1	S2	S3
E. coli	2.5%	2.5%	<0.6%	>2.5%	>2.5%	0.6%	2.5%	>2.5%
P. mirabilis	>2.5%	2.5%	<0.6%	>2.5%	>2.5%	<0.6%	2.,5%	>2.5%
S. aureus	2.5%	2.5%	2.5%	>2.5%	>2.5%	0.6%	<0.6%	<0.6%
M. luteus	2.5%	1.2%	1.2%	>2.5%	>2.5%	<0.6%	<0.6%	<0.6%
B. subtilis	1.2%	0.6%	<0.6%	2.5%	>2.5%	1.,2%	2.5%	2.5%

Tabuľka 2 Minimálna inhibičná koncentrácia (MIC) silíc z *Tanacetum vulgare* L. (T1-T5) a *Salvia officinalis* L. (S1-S3) použitím mikrodilučnej metódy

Table 3 Antifungal activity of essential oils from *Tanacetum vulgare* L.

Conc. (1)	*Aspergillus flavus*					*Microsporon gypseum*				
	0		0.5%		Inh. % (2)	0		0.5%		Inh. % (2)
T1	32 mm	100%	35 mm	109%	0%	14 mm	100%	0 mm	0 %	100%
T3	52 mm	100%	24 mm	46%	54%	22 mm	100%	0 mm	0 %	100%
T4	32 mm	100%	17 mm	53%	47%	14 mm	100%	0 mm	0 %	100%

Tabuľka 3 Fungicídna účinnosť silíc z *Tanacetum vulgare* L.
(1) koncentrácia, (2) inhibícia v %

antibacterial properties are different. Extracts T4 and T5 possess the lowest activity on bacteria and the best effect possesses extract T3 MIC of which at *E. coli, Proteus* and *Bacillus* is <0.6%, only in *Staphylococcus* and *Micrococcus* is 2.5% and 1.25% what is still lower value in the comparison with the rest extracts from *Tanacetum*.

Also, the anti-yeasts activities were different. The higher inhibition zone of essential oil was 15 mm on *C. albicans*. The maximum activity on *C. albicans* was shown (similarly like on bacteria) by the essential oils T3 from *Tanacetum* and S2 from *Salvia.*

The extracts from *Tanacetum vulgare*, which showed good antibacterial and anti-yeast activity, were further tested for their activity to mold *Aspergillus flavus* and dermatophyte *Microsporon gypseum*. The antifunga effect was monitored by measuring the diameter of fungal colony, and extracts T1, T3 and T4 inhibited the growth of *Aspergillus flavus* after 7 days of cultivation approximately 50% and totally inhibited the growth of *Microsporon gypseum* (Tab. 3).

Conclusions

The results obtained in this work confirmed, that essential oils obtained by water steam distillation from *Tanacetum vulgare* L. and *Salvia officinalis* L. possess the ability to inhibit the growth of selected bacteria, yeasts and mycelial fungi. Essential oils of plants belonging to the same taxa but collected from different localities showed different levels of antimicrobial activities.

Súhrn

Cieľom práce bolo stanoviť schopnosť silíc získaných z *Tanacetum vulgare* L. a *Salvia officinalis* L., inhibovať rast vybraných G+ a G- baktérií, kvasiniek a vláknitých húb a porovnať potenciálny antimikrobiálny účinok silíc získaných z rastlín z rozličných lokalít Slovenska. Pomocou diskovej difúznej metódy a mikrodilučnej metódy sme zistili, že silice sú v rozličnom stupni účinné voči všetkým použitým mikroorganizmom. Minimálne inhibičné koncentrácie sa pohybujú v rozpätí od 0,6% do 2,5%. Z porovnania výsledkov vyplýva, že aj keď boli silice získané z rastlín patriacich do toho istého taxónu, ale zbierané z rozličných lokalít, majú rozdielny stupeň antimikrobiálneho účinku.

Kľúčové slová: silice, *Tanacetum vulgare, Salvia officinalis*, lokalita, antimikrobiálny účinok

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