SATURREJA L. ESSENTIAL OILS IN PREVENTION AND PHYTOTHERAPY OF SALMONELLA INFECTION

T. Mihajilov-Krstev¹, D. Radnović² and D. Kitić³
¹University of Niš, Faculty of Science and Mathematics, Department of Biology and Ecology, Niš, Serbia
²University of Novi Sad, Faculty of Science and Mathematics, Department of Biology and Ecology, Novi Sad, Serbia
³University of Niš, Faculty of Medicine, Department of Pharmacy, University of Niš, Serbia
Correspondence to: Tatjana Mihajilov-Krstev
E-mail: nis_mikrobi@yahoo.com

ABSTRACT

In wide regions of the Balkan Peninsula, plant species of the genus Satureja L. (Lamiaceae) are traditionally used as natural preservatives for meat products, and as antimicrobial agents in phytotherapy of food borne diseases. The present study describes the antimicrobial activity of eight Satureja species essential oils against Salmonella enteritidis. The aerial parts of wild growing plant material of eight Satureja species were collected from the central part of Balkan Peninsula. After drying, essential oils were produced by hydrodistillation in a Clavenger-type apparatus. The antimicrobial activities of the tested essential oils were evaluated using the following two methods: agar disc diffusion method and broth micro-well dilution method. The results of disc diffusion method showed especially high activity of S. subspicata ssp. subspicata, S. montana ssp. montana and S. hortensis essential oils. The minimum inhibitory/bactericidal concentration (MIC/MBC) of the essential oils was in the range from 0.20 – 6.25 μl/ml. S. horvatii, S. hortensis and S. montana ssp. montana exhibited the highest antimicrobial activity. Also, it has been recorded that each essential oil, except S. subspicata, in the same concentration had both inhibitory and bactericidal effect (MIC=MBC).

Keywords: antimicrobial activity, essential oils, Salmonella enteritidis, Satureja L.

Introduction

Members of the genus Salmonella are flagellated, Gram-negative bacteria. Most strains of Salmonella are Lac-, and produce acid and gas during fermentation of glucose. They also produce H₂S from sulfur-containing amino acids. Salmonella classification has undergone numerous revisions; currently, all strains are grouped in a single species, S. enterica. This species is further divided into over 2000 serotypes based on the cell wall (O), flagellar (H), and capsular (Vi, analogous to K) antigens. Lipopolysaccharide (both lipid A and O antigen) and the Vi antigen are virulence factors. All these organisms produce exotoxins. Salmonella invade epithelial cells of the small intestine. Disease may remain localized or become systemic, sometimes with disseminated foci. The organisms are facultative, intracellular parasites that survive in phagocytic cells. Salmonella infection can cause both intestinal and extraintestinal diseases (20). It has been estimated that as many as 30% of people in industriated countries suffer from food borne disease each year and in 2000 at least two million people died from diarrheal disease worldwide (22). There is therefore still need for new methods of reducing or eliminating food borne pathogens.

In Western medicine 74% of the 121 bioactive plant-derived compounds currently in worldwide use were identified via research based on leads from ethnomedicine (6). In the area of the Balkan Peninsula, different Satureja species have been used in traditional medicine to treat bronchitis, skin, respiratory, digestive and urinary inflammation (8). This has been confirmed by scientific data which pointed out high antimicrobial activity of essential oils isolated from different species of genus Satureja (1-4, 7, 9, 10, 14-16). Major active constituents of their essential oils
are phenolic compounds carvacrol and thymol but, there is some evidence that minor components have a critical part to play in antibacterial activity (synergistic effect) (23). For this reason, the present study was carried out using whole essential oils.

Considering traditional medicine experiences, existing data about high antimicrobial activity of the *Satureja* species essential oils and expressed appearance of *Salmonella* multi-resistance to the antibiotic therapy (which can lead to the germ-carriage), we searched for the alternative possibilities for the prevention and treatment of salmonellosis.

In this paper, the results of antimicrobial activity of eight *Satureja* species essential oils against *S. enteritidis*, obtained by disc diffusion method, are presented. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of their essential oils were determined by micro-well dilution method.

### Materials and Methods

#### Plant material

The aerial parts of eight wild growing *Satureja* plant species were collected during the beginning of the flowering stage in Serbia (*S. kitaibelii* Wierzb. Ex Heuff and *S. hortensis* L.), Montenegro (*S. monatana* ssp. *montana* L., *S. subspicata* ssp. *subspicata* Vis., *S. cuneifolia* Ten., and *S. horvatti* Šilić) and F.Y.R.O.M. (*S. fuakarekkii* Šilić, *S. adamovicii* Šilić) (19). Voucher specimens were deposited at the Herbarium of the Faculty of Natural Science and Mathematics, University of Novi Sad.

#### Extraction of the essential oils

Dried aerial parts (100 g) of plants were cut and subjected to the hydro-distillation for 3 h, using Clevenger-type apparatus to produce oil. The resulting essential oils were dried over anhydrous sodium sulfate and stored at 4°C.

#### Microbial strain

The antimicrobial activity of eight *Satureja* species essential oil was evaluated using a laboratory control strain *Salmonella enteritidis* ATCC 13076. The inocula to the bacterial strain was prepared from overnight broth culture and suspension was adjusted to 0.5 McFarland standard turbidity, corresponding to 10^7-10^8 CFU/ml for bacteria, depending on genera (11).

#### Disc-diffusion assay

Antimicrobial tests were carried out by disc diffusion method using 100 μl of suspension containing 2.0 x 10^8 CFU/ml of bacteria spread on Mueller-Hinton agar (MHA, Torlak) in sterilized Petri dishes (90 mm in diameter). The discs (6 mm in diameter, HiMedia Laboratories Pvt. Limited) were impregnated with 15 μl of oil ethanol dilution (2%, 5% and 10%) and placed on the inoculated agar. Negative controls were prepared using the same solvents to dissolve the essential oil (ethanol). Streptomycin (30 μg) was used as positive reference standard to determine the sensitivity of a *Salmonella enteritidis* strain. The inoculated plates were kept at 4°C for 2 h and incubated at 37°C (24 h). Antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganism (11).

#### Micro-well dilution assay

A broth microdilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) according to the National Committee for Clinical Laboratory Standards (11). Methanol was used to dissolve the essential oils and then diluted to the highest concentration (500 μl/ml). A serial doubling dilutions of the oils were 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. Streptomycin served as a positive control. These tests were performed in duplicate. MIC was defined as the lowest concentration of essential oil at which microorganisms show no visible growth. The microbial growth was determined by absorbance at 620nm using the universal microplate reader (ThermoLabsystems, Multiskan 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 microorganism was 99.9% killed.

#### 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.

### Results and Discussion

Species of genus *Satureja* belong to the group of aromatic plants with the middle oil content. After hydrodistillation in the Clevenger-type apparatus, content of essential oils in the plant material was in the range from 0.10 v/w (%).
SS. subspicata) to 2.05 v/w (S. hortensis). Literature data about chemical composition of Satureja species essential oils, refered to the central part of the Balkan Peninsula, showed that the dominant components are monoterpenes carvacrol and thymol, their precursors γ-terpinene and p-cymene and monoterpenoidal alcohols borneol and linallole. Monoterpen-rich oil was isolated from S. hortensis (carvacrol - 67.00%) (10), S. subspicata (carvacrol – 16.76%; carvacrol – 27.90% and thymol – 28.60%) (14, 4) and S. horvatii (thymol – 63.37%) (7). Almost each previous mentioned species also contains high percent of γ-terpinene and p-cymene, which are precursors of these phenol compounds (especially S. adamovicii 40.00%) (13). Monoterpenoidal alcohol borneol is present in the oils of S. fukarekii (55.00 %) (13) and S. kitaibelii (9.80%), while S. montana ssp. montana (two localities) along with borneol (7.10 and 10.60 %) contains linallole in high percent (8.10% and 22.80%) (17). In the oil of S. cuneifolia, there is a similar percentage of the phenol compounds carvacrol (4.10%) and thymol (3.60%), as well as both monoterpenoidal alcohols borneol (3.80%) and, in smaller percent, linallole (1.60%) (1).

In the earlier studies, essential oils of the other plant species with high content of the active components carvacrol and thymol, showed antimicrobial activity against Salmonella spp. (2, 12). Oregano, rosemary and garlic essential oils, which are carvacrol-rich, exhibited larger inhibitory zones on S. enteritidis (18). Precursors of thymol and carvacrol, p-cymene and gama-terpinene mainly had no antibacterial effects, but it is well known that together with thymol and carvacrol, they exhibit synergistic effect. P-cymen can incorporate into the lipid bilayer and very likely facilitates transport of carvacrol across the cytoplasmic membrane (21). Linallole and borneol are monoterpenoidal alcohols which have also exhibited some antimicrobial activity (5).

On the basis of our results, the disc diffusion method showed that essential oils of all eight Satureja species have high antimicrobial activity against S. enteritidis (Fig. 1). Slight activity of the solvent ethanol was investigated and presented on the graph. According to the size of the inhibition zones (for the oil concentrations of 2%, 5% and 10%), outstanding taxa were: S. subspicata ssp. subspicata (19-20-20 mm, respectively), S. montana ssp. montana (20-21-18 mm) and S. hortensis (19-19-18 mm). The sizes of their inhibition zones were in the level of the referent antibiotic Streptomycin effect (30 mg of the active substance), whose average inhibition zone is 20 mm. Also, the oil concentration of 10% had very similar activity as the oil concentration of 2%, which points out the fact that this method can be used only for the preliminary screening. Because of that, during the further investigation, we used more precise, broth micro-well dilution method.

The results of broth micro-well dilution method confirmed high essential oil activity of all eight Satureja species (Fig. 2). Their MIC/MBC values were in the range from 0.20 – 6.25 μl/ml, that is very good considering the values of the referent antibiotic, Streptomycin (MIC=MBC=4.0 μl/ml). S. horvatii showed the highest activity (MIC=MBC=0.2 μl/ml), while the lowest, but related to the antibiotic also high activity, showed S. cuneifolia (MIC=MBC=6.25 μl/ml). The results of the disc diffusion method refered to the high activity of S. hortensis (MIC=MBC=0.39 μl/ml) and S. montana ssp. montana (MIC=MBC=0.7 μl/ml) were also confirmed. It can be noticed that the all essential oils had inhibitory and microbicidal effect in the same concentration (MIC=MBC), with the exception of S. subspicata ssp. subspicata. This taxon showed microbiostatic and microbicidal effect on the two different concentrations (MIC=MBC=1.36/6.25 μl/ml).
Fig. 2. Minimal inhibitory (MIC) and minimal bactericidal concentrations (MBC) of *Satureja* essential oils and Streptomycin for *S. enteritidis* (in μl/ml)

Analysis of our results about antimicrobial activity of essential oils, isolated from eight *Satureja* species, against *Salmonella enteritidis* confirmed literature data referred to high antimicrobial activity of *Satureja* species against foodborn pathogens. Their phenol components carvacrol and thymol are responsible for the antimicrobial effect (23).

**Conclusions**

According to the previously described data, it can be concluded that essential oils of eight species from the genus *Satureja* from the territory of the Balkan peninsula showed high antibacterial activity against *S. enteritidis*. Especially active oils were *S. horvatii*, *S. hortensis*, *S. montana* and *S. subspicata*, which can be explained by high content of monoterpenes carvacrol and thymol in their structure. Therefore, their essential oils or particular antimicrobial components can be used for the prevention and treatment of salmonelosis. Further investigations *in vitro* on multiresistant clinical isolates, testing of sinergistic effect with adequate antibiotics together with additional investigations *in vivo* are necessary before any applying of this oils in medicine.

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