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ZESZYTY NAUKOWE UNIWERSYTETU SZCZECIŃSKIEGO

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ANTIBACTERIAL PROPERTIES OF ESSENTIAL OIL OF *SATUREJA HORTENSIS* L. (*LAMIACEAE*) FROM PELAGONIAN REGION

Abstract

The aim of this study was to investigate whether essential oil of the plant *Satureja hortensis* (savory) (*Lamiaceae*), cultivated in Pelagonija region (Bitola, Macedonia), manifested certain antibacterial activity against unsporulated pathogens *Salmonella enteritidis* and *Escherichia coli* as well as a sporulated apathogenic species *Bacillus subtilis.*

The essential oil was obtained from the aerial parts at the flowering stage by the method of hydrodistillation. Unger apparatus was used to obtain the essential oil from the plant. Antibacterial activities of the oil were evaluated by a disc diffusion method.

Essential oil from the plant *Satureja hortensis* L. manifested varied antibacterial activity against *Escherichia coli*, *Salmonella enteritidis*, and *Bacillus subtilis*, depending on the concentration of essential oil used as well as the type of bacteria.

Keywords: savory, essential oil, antibacterial activity, inhibition zones, bacteria

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Introduction

According to the investigations of Jain & Kar (1971), Inouye et al. (1983), Garg & Dengre (1986), Janssen et al. (1987a), Rios et al. (1987), Sherif et al. (1987), Deans & Svoboda (1988, 1989), Cruz et al. (1989), Recio et al. (1989), Crespo et al. (1990), Larrondo et al. (1995), Pattnaik et al. (1995), Carson et al. (1996), Nenoff et al. (1996) and Lis-Balchin & Deans (1997), the antimicrobial activities of plant essential oils and their constituents are well known. These studies clearly show that secondary metabolites of these plants have the potential for use not only in medicine, cosmetic and food industry (Ueda et al. 1982; Baratta et al. 1998; Youdim et al. 1999), but also in the pharmaceutical industry (Pelissier et al. 1994; Shapiro et al. 1994; Cai & Wu 1996).

Satureja is a genus belonging to the aromatic plants of *Lamiaceae* family. The genus Satureja L. (Lamiaceae) comprises more than 30 species of aromatic herbs and shrubs, widely distributed over the Mediterranean region. These are annual aromatic plants that grow in arid, sunny, rocky habitats (Slavkovska et al. 2001). According to Hajhashemi et al. (2000), Güllüce et al. (2003), Dorman & Dengre (2004) and Souri et al. (2004), extracts of Satureja uhortensis manifest antimicrobial, antioxidative, antispasmolitic and sedative activity.

The aim of this study is to investigate whether essential oil of the plant *Satureja hortensis* (savory) (*Lamiaceae*), cultivated in Pelagonija region (Bitola, Macedonia), manifests certain antibacterial activity against unsporulated pathogens *Salmonella enteritidis* and *Escherichia coli*, as well as, a sporulated apathogenic species *Bacillus subtilis.*

Materials and methods

Materials

We have used essential oil of *Satureja hortensis* L. (*Lamiaceae*) cultivated in Pelagonija region (Bitola, Macedonia), for the above stated purposes.

Plant material

The aerial parts of *Satureja sp*. were collected in June 2008, from Pelagonija region, at an altitude of 675 m, average temperature of 20.65 °C and average relative humidity of 75.25% (Hydro-meteorological Institute – Bitola, 2008). Botani-

cal determination of the plant material was made by Silvana Manasievska-Simić, at the Department of Botany and Microbiology at the Faculty of Agricultural Sciences and Food in Skopje. Samples were determined as *Satureja hortensis* L. (savory). A voucher specimen was deposited in the Herbarium of the same Faculty.

Plant material was dried in a dryer, under strictly controlled drying temperature conditions, at a constant temperature of 30 °C, for the period of four days.

Grinding was done in electric blenders with granulation of 0.25 mm. The essential oil was obtained from the aerial parts at the flowering stage by method of hydrodistillation. Unger apparatus was used to obtain the essential oil from the plant in question. The essential oil dilution was prepared in a chemically defined medium, Dimethyl Sulfoxide (DMSO) in concentrations of: 10, 50, 100, 150, 200 and 600 μg/ml.

Microorganisms

The examination of antibacterial activity was determined against unsporulated pathogens: *Salmonella enteritidis* and *Escherichia coli*, as well as a sporulated apathogenic species *Bacillus subtilis.* In our case study, we used clinical isolates from patients at the Centre for public health in Bitola.

Methods

Antibacterial activities of the oil were evaluated by a disc diffusion method using Salmonella-Shigella agar for *Salmonella enteritidis*, Endo agar for *Escherichia coli* and Nutrient agar for *Bacillus subtilis.* The incubation conditions applied were 24 hours at a temperature of 37 °C for the bacteria. Filter-paper impregnated in pure DMSO was set as a control. The final step was marking and measuring the radius of the inhibition zones. The examination for antibacterial activity of the plant in question was performed in three repeats, for each microorganism and each concentration, accordingly. Measures of every repeat were conducted within the period of ten days. The essential oil influence against bacteria, as well as the formation and movement of inhibition zones, were also considered within the same period.

Results and discussion

The present study was designated to evaluate antibacterial properties of the essential oil of *Satureja hortensis* L., obtained by using Unger distillation apparatus. Our findings are based on data obtained by examining the antibacterial activity of essential oil extracted from *Satureja hortensis* L., cultivated in Pelagonia region, against *Escherichia coli*. The results that we have obtained are displayed in Table 1.

The essential oil of *Satureja hortensis* L. manifested weak to moderate antimicrobial activity against *Escherichia coli* (Table 1). During ten days of measuring and monitoring the inhibition zones formed under the influence of this oil, we determined that the examined properties were permanent and stable within the period. Thus, we concluded that the effectiveness of *Satureja hortensis* essential oil against *Escherichia coli* was not very high, but it was permanent, i.e. there were no changes to the initially formed zones of inhibition.

Table 1. Average values for antibacterial activity of *Satureja hortensis* L. against *Escherichia coli* (radius of inhibition zones/mm)

Essential oil	Savory (*Satureja hortensis* L.) (μg/ml)						
Escherichia coli	K	10	50	100	150	200	600
1 day	0.00	0.33	0.66	0.83	1.66	1.83	7.00
2 day	0.00	0.33	0.66	0.83	1.66	1.83	7.00
3 day	0.00	0.33	0.66	0.83	1.66	1.83	7.00
4 day	0.00	0.33	0.66	0.83	1.66	1.83	7.00
5 day	0.00	0.33	0.66	0.83	1.66	1.83	7.00
6 day	0.00	0.33	0.66	0.83	1.66	1.83	7.00
7 day	0.00	0.33	0.66	0.83	1.66	1.83	7.00
8 day	0.00	0.33	0.66	0.83	1.66	1.83	7.00
9 day	0.00	0.33	0.66	0.83	1.66	1.83	7.00
10 day	0.00	0.33	0.66	0.83	1.66	1.83	7.00

The results obtained from our tests, in which the oil manifested some antimicrobial activity, differed from the results obtained by Adiguzel et al. (2007), in which *Escherichia coli* showed resistance to the examined essential oil. This disparity is probably due to different climatic and geographic factors that charac-

terize the regions where the plant is cultivated, as they have a major impact on the composition and percentage of these essential oil components which are carriers of its antimicrobial properties. Our results are conformable to studies of Azaz et al. (2005) and Özkan et al. (2003) in which *Satureja hortensis* essential oil originating from Turkey manifests antimicrobial activity against the investigated bacteria. In all the above cases, the antimicrobial activity manifested by the oil is probably due to similar composition and percentage of essential oil components in plants growing in the investigated locations.

Based on data obtained from the examination of the antimicrobial activity of *Satureja hortensis* L. essential oils against *Salmonella enteritidis* cultivated in Pelagonija region, we obtained the following results, displayed in Table 2.

The essential oil of *Satureja hortensis* L. manifested weak antimicrobial activity against *Salmonella enteritidis*, apart from the case with the lowest concentration of 10 μg/ml, where an inhibition zone was not formed, and apart from the case with the highest concentration of 600 μg/ml, where the activity of essential oil was moderate (Table 2). During ten of measuring and monitoring the inhibition zones formed under the influence of *Satureja hortensis* L. essential oil, we determined that the initially formed inhibition zones did not change in the period of ten days, thus the effect of this oil against *Salmonella enteritidis* was permanent.

Table 2. Average values for antibacterial activity of *Satureja hortensis* L. against *Salmonella enteritidis* (radius of inhibition zones/mm)

Essential oil	Savory (*Satureja hortensis* L.) (μg/ml)						
Salmonella enteritidis	K	10	50	100	150	200	600
1 day	0.00	0.00	0.33	0.50	2.50	2.83	6.50
2 day	0.00	0.00	0.33	0.50	2.50	2.83	6.50
3 day	0.00	0.00	0.33	0.50	2.50	2.83	6.50
4 day	0.00	0.00	0.33	0.50	2.50	2.83	6.50
5 day	0.00	0.00	0.33	0.50	2.50	2.83	6.50
6 day	0.00	0.00	0.33	0.50	2.50	2.83	6.50
7 day	0.00	0.00	0.33	0.50	2.50	2.83	6.50
8 day	0.00	0.00	0.33	0.50	2.50	2.83	6.50
9 day	0.00	0.00	0.33	0.50	2.50	2.83	6.50
10 day	0.00	0.00	0.33	0.50	2.50	2.83	6.50

The values obtained as a result of our study are not conformable to the values published by Adiguzel et al. (2007) in which the diameter of inhibition zones is considerably higher and equals 22 mm. These differences arise as a result of the plant's genetic predisposition and the impact of climatic factors operating in the investigated area.

Our results remain in accord with results of Güllüce et al. (2003) in which *Satureja hortensis* essential oil displays a potential antimicrobial activity against *Salmonella enteritidis*, as well as those of Özkan et al. (2003) where essential oil shows the greatest efficiency when its concentration is the highest. This is probably a result of similar chemical composition and percentage of essential oil components in plants growing in the investigated locations.

Based on data obtained from the examination of the antimicrobial activity of *Satureja hortensis* L. essential oils against *Bacillus subtilis* cultivated in the Pelagonija region, we have obtained the following results, displayed in Table 3.

Table 3. Average values for antibacterial activity of *Satureja hortensis* L. against *Bacillus subtilis* (radius of inhibition zones/mm)

Essential oil	Savory (*Satureja hortensis* L.) (μg/ml)						
Bacillus subtilis	K	10	50	100	150	200	600
1 day	0.00	0.83	1.66	2.50	2.66	3.50	8.66
2 day	0.00	0.00	0.33	0.33	2.00	2.16	7.83
3 day	0.00	0.00	0.16	0.16	1.83	1.83	5.66
4 day	0.00	0.00	0.16	0.16	1.50	1.50	5.33
5 day	0.00	0.00	0.16	0.16	1.50	1.50	5.33
6 day	0.00	0.00	0.16	0.16	0.66	1.16	4.00
7 day	0.00	0.00	0.16	0.16	0.66	1.16	4.00
8 day	0.00	0.00	0.00	0.00	0.66	1.16	3.16
9 day	0.00	0.00	0.00	0.00	0.66	1.16	3.16
10 day	0.00	0.00	0.00	0.00	0.66	1.16	3.16

Table 3 shows average values of antimicrobial activity of *Satureja hortensis* L. essential oil against *Bacillus subtilis*. The oil in question demonstrated low sensitivity against bacteria, with the exception of the cases where the concentrations were the highest. The effect of essential oil observed within the ten days period was not constant. In fact, the initially formed inhibition zones gradually

began to decrease, which meant that the sensitivity of *Bacillus subtilis* against the oil was becoming reduced. Gradually, there took place a secondary settlement of bacteria in the previously formed zones. With the lower concentrations of 10, 50 and 100 µg/ml we observed a complete absence of inhibition zones after ten days. We concluded that the antimicrobial activity of *Satureja hortensis* essential oils with regard to the bacteria in question was variable.

The activity of *Satureja hortensis* essential oil against *Bacillus subtilis* is unstable, i.e. its effectiveness decreases day by day, manifesting a reduction of the inhibition zone radius or even a complete absence of inhibition zones when the essential oil concentrations are lower. Terminal values obtained from our tests do not conform to the values published by Adiguzel et al. (2007) in which the diameter of inhibition zone is larger and equals 10 mm. The reason for this should be seen in the genetic predisposition of the plant which affects the manifestation of its antimicrobial properties. Our results remain partly in accord with the results published by Özkan et al. (2003), whose examinations of antimicrobial activity of the oil in question were carried out with several dilutions. The authors noted that the most effective antimicrobial activity was shown with the highest dilutions, the same as in the case studied by us. Positive results for antimicrobial activity of *Satureja hortensis* essential oil against *Bacillus subtillis* were obtained by Sahin et al. (2003), but compared to our investigations, there is a difference in the types of solvents used for the essential oil dilution.

Conclusions

1. The radius of the inhibition zones formed by *Satureja hortensis* L. essential oil against *Escherichia coli* was ranging from approximately 0.33 to 7.00 mm depending on the essential oil concentration used. This means that the zone of inhibition for the lowest concentration of 10 µg/ml was the smallest (0.33 mm), while for the highest concentration of 600 µg/ml, it equalled 7.00 mm.
2. The radius of the inhibition zones formed by *Satureja hortensis* L. essential oil against *Salmonella enteritidis* was ranging from approximately 0.00 to 6.50 mm depending on the essential oil concentration used. This means that the radius of the zone for the lowest concentration of 10 µg/ml was 0,00 mm, while the largest zone of 6.50 mm radius was obtained under the influence of the highest concentration of 600 µg/ml. A manifested antimicrobial activity against *Salmonella enteritidis* was low for the concentrations of 50,

100, 150 and 200 μg/ml (0.33, 0.50, 2.50 and 2.83 mm) and moderate for the concentration of 600 μg/ml (6.50 mm). The 10 μg/ml concentration of the essential oil did not manifest antimicrobial activity against the examined bacteria.

3. Initial average values for the radius of the inhibition zones formed by *Satureja hortensis* L. essential oil against *Bacillus subtilis* were ranging from 0.83 to 8.66 mm. The radius of the zone formed at the lowest concentration of 10 μg/ml was 0.83 mm, while the largest, 8.66 mm, zone of inhibition was formed at the highest concentration of 600 μg/ml. Zones of inhibition began to decrease on the second day, so after a period of ten days in the case of lower concentrations of 10, 50 and 100 μg/ml there was observed a complete absence of zones. For the concentration of 150 μg/ml, the zone became quite insignificant (0.66 mm). The final radius value of the zones formed with concentrations of 200 and 600 μg/ml equalled 1.16 mm and 3.16 mm, respectively.
4. Comparing the results of antibacterial activity of *Satureja hortensis* L. essential oil against *Escherichia coli*, *Salmonella enteritidis* and *Bacillus subtilis*, we can observe varying degrees of this oil's effectiveness towards these bacteria. Thus, we have concluded that the most sensitive bacteria to this oil's influence are *Salmonella enteritidis* and *Escherichia coli*, while *Bacillus subtilis* shows a moderate sensitivity to the oil's activity.
5. Due to the essential oil's antimicrobial activity, it has wide applications acting as a natural antimicrobial agent utilized in pharmacology, pharmaceutical botany, fitopathology, medical and clinical microbiology, food industry, cosmetic industry, and in many other fields.

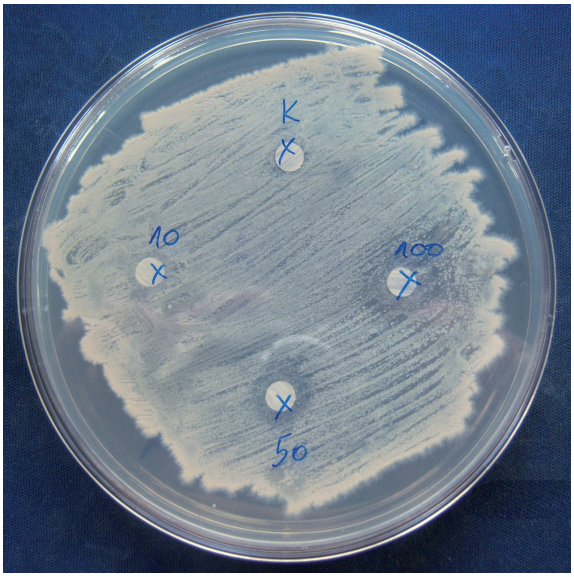

Fig. 1. Inhibition zones (and their secondary settlement) on *Bacillus subtilis* formed by *Satureja hortensis* L. essential oil in concentrations of 10, 50 and 100 µg/ml

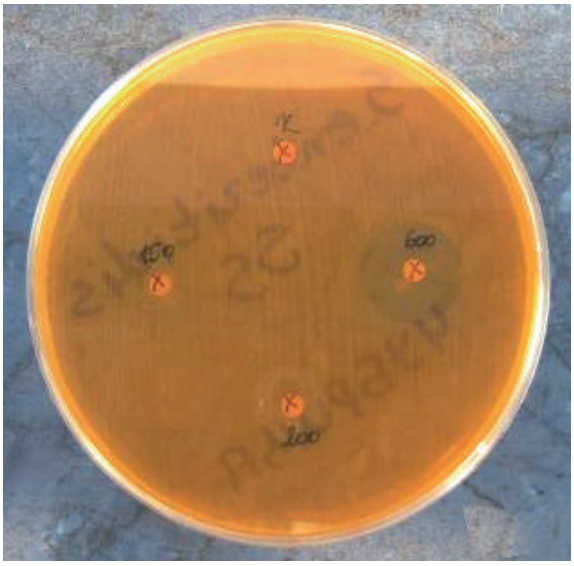

Fig. 2. Inhibition zones on *Salmonella enteritidis* formed by *Satureja hortensis* L. essential oil in concentrations of 150, 200 and 600 µg/ml

Fig. 3. Inhibition zones on *Escherichia coli* formed by *Satureja hortensis* L. essential oil in concentrations of 10, 50 and 100 μg/ml

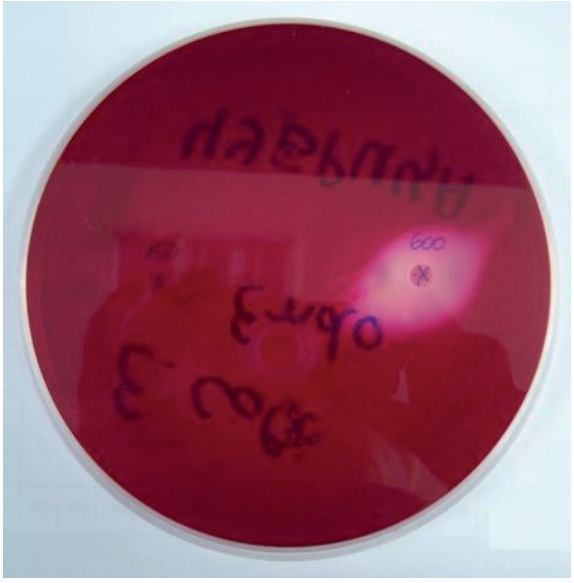

Fig. 4. Inhibition zones on *Escherichia coli* formed by *Satureja hortensis* L. essential oil in concentrations of 150, 200 and 600 μg/ml

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