

ANTIFUNGAL AND ANTI-YEAST ACTIVITY OF *SATUREJA HORTENSIS* L. (LAMIACEAE) ESSENTIAL OIL FROM PELAGONIAN REGION

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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), manifest certain antifungal activity against the fungus *Aspergillus niger* and anti-yeast activity against the yeast *Candida albicans*.

The essential oil was obtained from the aerial parts at the flowering stage by method of hydro-distillation and Unger apparatus was used to obtain the essential oil from this plant.

Antifungal and anti-yeast activities of the oil were evaluated by a disc diffusion method.

Essential oil from the plant *Satureja hortensis* L. showed different antimicrobial activity against *Aspergillus niger* and *Candida albicans*, depending on the concentration of essential oil used, as well as, the type of microorganism.

Key words: Savory, antifungal activity, anti-yeast activity, inhibition zones, essential oil, fungus, yeast.

1. Introduction

According to the investigations of Jain and Kar [6], Inouye *et al.* [5], Garg and Dengre [9], Janssen *et al.* [3], Rios *et al.* [7], Sherif *et al.* [8], Deans and Svoboda [10] [11], Cruz *et al.* [13], Recio *et al.* [14], Crespo *et al.* [12], Larrondo *et al.* [17], Pattnaik *et al.* [15], Carson *et al.* [16], Nenoff *et al.* [19] and Lis-Balchin and Deans [18], 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.* [22], Baratta *et al.* [20], Youdim *et al.* [21]), but also in the pharmaceutical industry (Pelissier *et al.* [23], Shapiro *et al.* [25], Cai and Wu [24]).

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 in the Mediterranean region. These are annual aromatic plants that grow on arid, sunny, rocky habitats (Slavkovska *et al.* [27]). According Hajhashemi *et al.* [28], Güllüce *et al.* [26], Dorman *et al.* [29] and Sourı *et al.* [30]) extracts of *Satureja hortensis* manifest antimicrobial, antioxidative, antispasmodic 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), manifest certain antifungal activity against the fungus *Aspergillus niger* and anti-yeast activity against the yeast *Candida albicans*.

2. Materials and Methods

Materials

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

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). Botanical determination was made by botanist at the Department of Botany and Microbiology at the Faculty of Agricultural Sciences and Food - 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, with strongly controlled drying temperature conditions, at constant temperature of 30 °C, with duration 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 hydro- distillation and Unger apparatus was used to obtain the essential oil from this plant.

The essential oils dilution was made in a chemically defined medium, Dimethyl Sulfoxide (DMSO) in concentrations of: 10, 50, 100, 150, 200 and 600 mg/mL, accordingly.

Microorganisms

Antifungal activity was determined against the fungus *Aspergillus niger* and anti-yeast activity against the yeast *Candida albicans*. In our case, we used clinical isolates from patients at the Centre for public health – Bitola.

Methods

Microbiological examinations were performed in the Microbiology laboratory at the Faculty of Agricultural Sciences and Food - Skopje. Antifungal and anti-yeast activities of the oil were evaluated by a disc diffusion method using Sabouraud agar for *Aspergillus niger* and *Candida albicans*. 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 antifungal and anti-yeast activity of this plant was made in three repeats, for each microorganism and each concentration, accordingly. Measures of any repeat was made in a period of ten days, as well as the essential oil influence against fungus and yeast and forming and moving of the inhibition zones in a period of ten days was considered.

3. Results and Discussion

The present study was designated to evaluate the antifungal and anti-yeast activities of the *Satureja hortensis* L. essential oil, obtained by using Unger distillation apparatus. Based on data obtained from examination of the anti-yeast activity of *Satureja hortensis* L. essential oil, cultivated in Pelagonia region, against *Candida albicans*, we obtained following results, that are shown in Table 1.

Table 1. Average values for anti-yeast activity of *Satureja hortensis* L. against *Candida albicans* (radius of inhibition zones /mm)

Microorganism	Essential oil - Savory (<i>Satureja hortensis</i> L.) (mg/mL)						
	K*	10*	50*	100*	150*	200*	600*
1 day	0.00	0.16	0.33	0.33	3.83	4.83	14.00
2 day	0.00	0.00	0.16	0.16	2.16	2.66	14.00
3 day	0.00	0.00	0.16	0.16	1.83	2.33	14.00
4 day	0.00	0.00	0.16	0.16	1.50	2.00	14.00
5 day	0.00	0.00	0.16	0.16	1.50	2.00	14.00
6 day	0.00	0.00	0.16	0.16	1.50	2.00	12.66
7 day	0.00	0.00	0.16	0.16	1.50	2.00	11.66
8 day	0.00	0.00	0.16	0.16	1.50	2.00	11.66
9 day	0.00	0.00	0.16	0.16	1.50	2.00	11.66
10 day	0.00	0.00	0.16	0.16	1.50	2.00	7.83

*K-Control, 10, 50, 100, 150, 200 and 600 mg/mL

The essential oil of *Satureja hortensis* L. manifested low sensitivity against yeast *Candida albicans*, with the exception of the highest concentration of 600 mg/mL (Table 1). In this test, after ten days measurement, complete absence of the initially formed inhibition zone was observed only for the lowest concentration of 10 mg/mL, which initially wasn't so noticeable. Very small inhibition zones were formed at concentrations of 50 and 100 mg/mL. Reducing the zones of inhibition began in the second day, with the exception of the zone formed by the highest concentration of 600 mg/mL, whose radius began to reduce in the sixth day of testing. Reduction of the zones was not visible at concentrations of 10, 50 and 100 mg/mL, while at the highest concentration, the initial value of the zone which was 14.00 mm drastically reduced to 7.83 mm.

The results obtained from our tests are in correlation to the results of Azaz *et al.* [2] in which examined oil manifested certain antimicrobial activity. They are not correlated with tests of Adiguzel *et al.* [1] where *Satureja hortensis* essential oil hasn't shown effect on *Candida albicans*. Comparing our results with those of Sahin *et al.* [4] in which the hexane extract of *Satureja hortensis* manifested anti-yeast activity cannot be performed because of various chemical reagents used for essential oil dilution. The reason for such deviations should be seen in the various work techniques, as well as reagents used in microbiological tests.

Based on data obtained from examination of the antifungal activity of *Satureja hortensis* L. essential oils against *Aspergillus niger* cultivated in Pelagonija region, we obtained the following results that are shown in Table 2.

Table 2. Average values for antifungal activity of *Satureja hortensis* L. against *Aspergillus niger* (radius of inhibition zones /mm)

Microorganism	Essential oil - Savory (<i>Satureja hortensis</i> L.) (mg/mL)						
	K*	10*	50*	100*	150*	200*	600*
<i>Aspergillus niger</i>							
1 day	0.00	1.33	3.00	3.66	7.50	11.00	20.66
2 day	0.00	0.00	1.33	1.83	3.83	5.00	17.16
3 day	0.00	0.00	0.16	0.16	2.83	4.00	9.66
4 day	0.00	0.00	0.16	0.16	2.83	4.00	9.16
5 day	0.00	0.00	0.16	0.16	2.50	2.66	9.16
6 day	0.00	0.00	0.00	0.00	1.16	1.66	4.16
7 day	0.00	0.00	0.00	0.00	0.33	0.50	3.66
8 day	0.00	0.00	0.00	0.00	0.33	0.50	3.50
9 day	0.00	0.00	0.00	0.00	0.16	0.33	3.16
10 day	0.00	0.00	0.00	0.00	0.00	0.00	3.16

*K-Control, 10, 50, 100, 150, 200 and 600 mg/mL

Essential oil manifested certain effectiveness to this fungus, but only 48 hours after incubation, with the inhibition zones which increased with essential oil concentration increasing (Table 2). Manifested sensitivity of *Aspergillus niger* to this oil started to decline gradually; so after ten days, there was a complete absence of inhibition zones for all concentrations except for the highest concentration of 600 mg/mL, whose radius of 20.66 mm was reduced to 3.16 mm. Thus, we can conclude that the effect of *Satureja hortensis* essential oil is unstable, i.e. its effectiveness is reduced day by day, manifesting a reduction or complete absence of inhibition zones. The results of our tests are not in accordance with those published by Adiguzel *et al.* [1] in which the value of the diameter of inhibition zone is 18 mm. The reason for such deviations should first be seen in the different climate-geographic conditions which are characterized in both areas, as well as the genetic determination of the examined plant.

4. Conclusions

1. The average values for the radius of the inhibition zones formed by *Satureja hortensis* L. essential oil against *Candida albicans*, first day of testing, were ranging from 0.16 to 14.00 mm and after ten days, the value was reduced and was ranging from 0.00 to 7.83 mm. Initially manifested activity of *Satureja hortensis* essential oil was very weak in concentrations of 10, 50 and 100 mg/mL (0.16, 0.33 and 0.33 mm) - Figure 1, moderate in concentrations of 150 and 200 mg/mL (3.83 and 4.83 mm) and strong in concentration of 600 mg/mL (14.00 mm) - Figure 2.

2. The average values of the radius of the initially formed inhibition zones by *Satureja hortensis* L.

essential oil against *Aspergillus niger* was ranging from 1.33 to 20.66 mm. For the lowest concentration of 10 mg/mL - Figure 3, it was 1.33 mm and for the highest concentration of 600 mg/mL, it was 20.66 mm - Figure 4.

3. Comparing the results of antimicrobial activity of *Satureja hortensis* L. essential oil against *Candida albicans* and *Aspergillus niger*, we can determine the different effectiveness of this oil on the above microorganisms. Thus, we have concluded that *Candida albicans* shown moderate sensitivity on oil's activity and *Aspergillus niger* manifested a strong resistance to this oil.

4. Due to its antimicrobial activity, essential oil preparations have wide applications as natural antimicrobial agents in the field of: pharmacology, pharmaceutical botany, phytopathology, medical and clinical microbiology, food industry, cosmetic industry, etc.

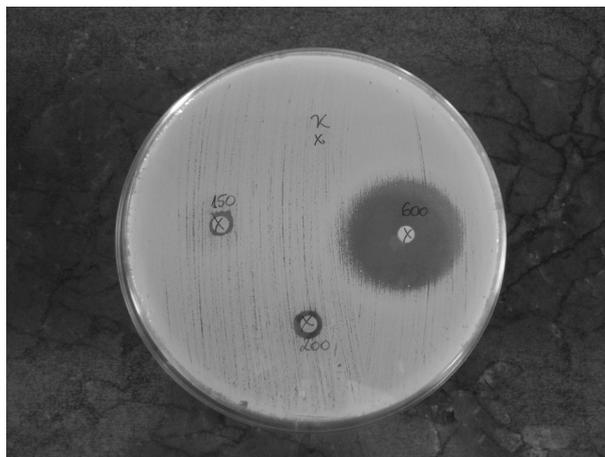


Figure 1. Inhibition zones on *Candida albicans* formed by *Satureja hortensis* L. essential oil in concentrations of 150, 200 and 600 mg/mL

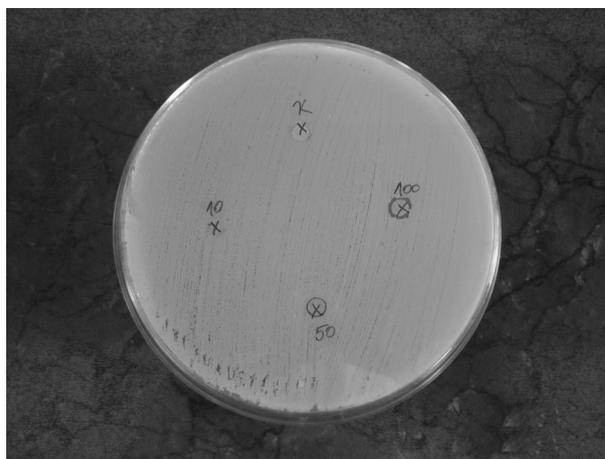


Figure 2. Inhibition zones on *Candida albicans* formed by *Satureja hortensis* L. essential oil in concentrations of 10, 50 and 100 mg/mL

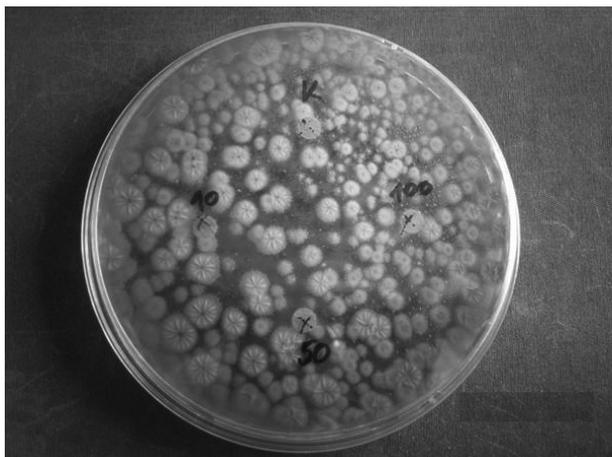


Figure 3. Inhibition zones on *Aspergillus niger* formed by *Satureja hortensis* L. essential oil in concentrations of 10, 50 and 100 mg/mL



Figure 4. Inhibition zones on *Aspergillus niger* formed by *Satureja hortensis* L. essential oil in concentrations of 150, 200 and 600 mg/mL

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