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Research Article

Antifungal and Anti-Yeast Activity of *Thymus Tosevii* vel. Subsp. *Tosevii* var. *Degenii* (*Lamiaceae*) Essential oil From Pelister, Baba Mountain (Bitola, Macedonia)

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Abstract

The aim of this study was to investigate whether essential oil of the plant *Thymus tosevii* Vel. subsp. *tosevii* var. *degenii* (Lamiaceae), endemic plant species in Pelister, Baba Mountain (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 *Thymus tosevii* Vel. subsp. *tosevii* var. *degenii* showed different antifungal and anti-yeast activity against *Aspergillus niger* and *Candida albicans*, depending on the concentration of essential oil used, as well as, the type of microorganism.

Keywords: Thyme; Inhibition Zones; Essential Oil; Fungus; Yeast

Introduction

Among the aromatic plants belonging to the family *Lamiaceae*, the genus *Thymus* is noteworthy for the numerous species and varieties of wild-growing plants [1]. For centuries, the genus *Thymus* (*Lamiaceae* family) is known as a spice and food preservative as well as a protective and curative remedy for many ailments. It is widely used in folk medicine in the world for numerous biological activities including antimicrobial [2-4] and antifungal effects [5]. The antimicrobial properties of essential oils are utilized in the food industry to prolong shelf life of food products. The essential oils of many *Thymus* species are widely used as flavoring agents in food processing and many pharmacological preparations, and particularly thyme oil is still among the world's top 10 essential oils [6].

The aim of this study was to investigate whether essential oil of the plant *Thymus tosevii* Vel. subsp. *tosevii* var. *degenii* (*Lamiaceae*), endemic plant species in Pelister, Baba Mountain (Bitola, Macedonia) manifest certain antifungal activity against the fungus *Aspergillus niger* and anti-yeast activity against the yeast *Candida albicans*.

Materials and Methods

Materials

Essential oil of thyme - *Thymus tosevii* Vel. subsp. *tosevii* var. *degenii* (*Lamiaceae*) was used for these purposes, originating from Pelister, Baba Mountain (Bitola, Macedonia).

Plant material

The aerial parts of *Thymus* sp. were collected from Pelister, Baba Mountain (Bitola, Macedonia) at an altitude of 900 - 950 m, average temperature of 21,7°C and average relative humidity of 62,6%.

The botanical determination was made by Academic Vlado Matevski, Ph.D. at the Faculty of Biology, University "St. Cyril and Methodius"- Skopje, Macedonia. Samples were determined as *Thymus tosevii* Vel. subsp. *tosevii* var. *degenii*. 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 a constant temperature of 30 °C, with a duration of four days.

Plant samples were grinding in electric blender with granulation of 0.25 mm.

The essential oil was obtained from the aerial parts at the flowering stage by hydrodistillation method 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.

Figure 1: *Thymus tosevii* Vel. subsp. *tosevii* var. *degenii* (*Lamiaceae*) from Pelister, Baba Mountain (Bitola, Macedonia).

Microorganisms

In our examinations, the fungus *Aspergillus niger* was used to determine antifungal activity and the yeast *Candida albicans* was used to determine anti-yeast activity of *Thymus tosevii* Vel. subsp. *tosevii* var. *degenii* essential oil. For this purpose, clinical isolates from patients at the Centre for public health – Bitola were used.

Methods

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 radius of the inhibition zones was marking and measuring. 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 were 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.

Results and Discussion

The present study was designated to evaluate the antifungal and anti-yeast activities of the *Thymus tosevii* Vel. subsp. *tosevii* var. degenii essential oil, obtained by using Unger distillation apparatus.

Essential oil from the plant *Thymus tosevii* Vel. subsp. *tosevii* var. *degenii* (thyme) showed different antimicrobial activity against tested microorganisms, depending on the concentration of essential oil used, as well as the type of microorganism.

Based on data obtained from the examination of the anti-yeast activity of Thymus tosevii Vel. subsp. tosevii var. degenii essential oil, originating from Pelister, Baba Mountain (Bitola, Macedonia) against Candida albicans, we obtained following results that are shown in Table 1.

Microorganism	**Essential oil - Thyme (*Thymus tosevii* Vel. subsp. *tosevii* var. *degenii*) (mg/mL)**						
Candida albicans	K*	10*	50*	100*	150*	200*	600*
1 day	0,00	0,33	1,33	2,33	4,83	7,00	13,33
2 day	0,00	0,16	0,83	1,66	3,33	4,33	7,83
3 day	0,00	0,00	0,83	1,66	3,16	3,66	6,83
4 day	0,00	0,00	0,83	1,66	3,00	3,50	6,83
5 day	0,00	0,00	0,83	1,66	3,00	3,50	6,83
6 day	0,00	0,00	0,83	1,66	3,00	3,50	6,83
7 day	0,00	0,00	0,83	1,66	3,00	3,50	6,83
8 day	0,00	0,00	0,83	1,66	3,00	3,50	6,83
9 day	0,00	0,00	0,83	1,66	3,00	3,50	6,83
10 day	0,00	0,00	0,83	1,66	3,00	3,50	6,83

Table 1: Average values for anti - yeast activity of *Thymus tosevii* Vel. subsp. *tosevii* var. *degenii* against *Candida albicans* (radius of inhibition zones/mm).

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

This yeast manifested certain sensitivity to essential oil, with inhibition zones which increased with essential oil concentration increasing. In this case came to some decrease in their radius, beginning from the first to the tenth day of the measurement, but it is not dramatically expressed. At first day, manifested anti - yeast activity of essential oil was very weak for concentrations of 10, 50 and 100 mg/ml (0,33; 1,33 and 2,33 mm), moderate for concentration of 150 mg/ml (4,83 mm) and strong for concentrations of 200 and 600 mg/ml (7,00 and 13.33 mm). The third day, complete absence of the inhibition zone was observed for lowest concentration of 10 mg/ml, which was initially very small (0,33 mm). The average values for the inhibition zones` radius at first day varied in the range of 0,33 to 13,33 mm, but after ten days, it decreased from 0,00 to 6,83 mm. It was obvious that after ten-day period, the inhibition zones were clearly expressed, except for the lowest concentration of 10 mg/ml (Figure 2 and 3).

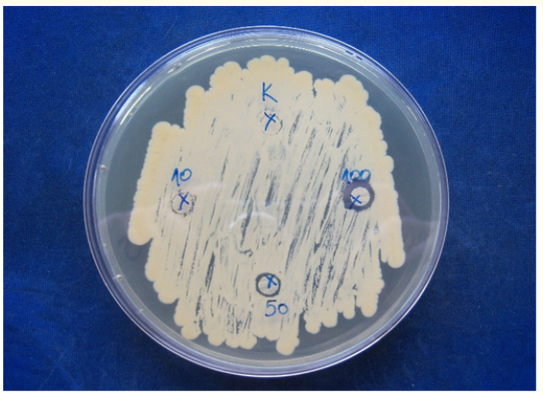

Figure 2: Inhibition zones on *Candida albicans* formed by *Thymus tosevii* Vel. subsp. *tosevii* var. *degenii* essential oil in concentrations of 10, 50 and 100 mg/ml.

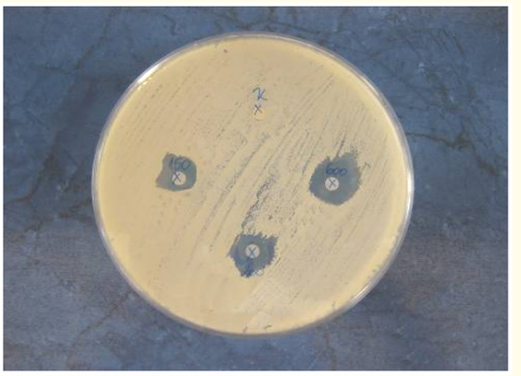

Figure 3: Inhibition zones on *Candida albicans* formed by *Thymus tosevii* Vel. subsp. *tosevii* var. *degenii* essential oil in concentrations of 150, 200 and 600 mg/ml.

Based on data obtained from the examination of the antifungal activity of *Thymus tosevii* Vel. subsp. *tosevii* var. *degenii* essential oil against the fungus *Aspergillus niger*, the following results are shown in Table 2.

Microorganism	Essential oil - Thyme (*Thymus tosevii* Vel. subsp. *tosevii* var. *degenii*) (mg/mL)						
Aspergillus niger	K*	10*	50*	100*	150*	200*	600*
1 day	0,00	0,50	4,50	5,66	8,66	14,00	20,33
2 day	0,00	0,16	1,16	2,00	4,83	6,83	16,6
3 day	0,00	0,00	0,50	0,66	2,33	3,00	8,16
4 day	0,00	0,00	0,50	0,66	2,33	2,66	7,33
5 day	0,00	0,00	0,50	0,50	2,00	2,50	7,00
6 day	0,00	0,00	0,50	0,50	1,16	1,66	5,66
7 day	0,00	0,00	0,50	0,50	0,83	1,16	5,33
8 day	0,00	0,00	0,50	0,50	0,83	1,16	4,16
9 day	0,00	0,00	0,16	0,16	0,33	0,66	2,66
10 day	0,00	0,00	0,16	0,16	0,33	0,50	1,33

Table 2: Average values for antifungal activity of *Thymus tosevii* Vel. subsp. *tosevii* var. *degenii* against *Aspergillus niger* (radius of inhibition zones/mm).

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

This essential oil manifested strong effectiveness to *Aspergillus niger*, but only after the first 48 hours of incubation, with inhibition zones that increase proportionally with the increase of its concentration. Manifested antifungal activity immediately after incubation (1 day) was very weak for lowest concentration of 10 mg/ml (0,50 mm), moderate for concentrations of 50 and 100 mg/ml (4,50 and 5,66 mm) and strong for concentrations of 150, 200 and 600 mg/ml (8,66; 14,00 and 20,33 mm). Manifested sensitivity began to decline after the first 48 hours, so after ten days the inhibition zones were drastically reduced. In this test, after ten days of measurement, initially formed inhibition zones were visibly reduced.

Conclusion

1. The average values for the radius of the inhibition zones formed by *Thymus tosevii* Vel. subsp. *tosevii* var. *degenii*

essential oil against *Aspergillus niger* were ranging from 0,50 mm (for 10 mg/ml concentration) to 20,33 mm (600 mg/ml), 48 hours after incubation. The highest value was reduced and reached 1,33 mm (600 mg/ml) after ten days.

2. The average values for the radius of the inhibition zones formed by *Thymus tosevii* Vel. subsp. *tosevii* var. *degenii* essential oil against *Candida albicans*, first day of measurement were ranging from 0,33 mm (for 10 mg/ml concentration) to 13,33 mm (600 mg/ml). After ten days, it decreased from 0,00 mm (for 10 mg/ml concentration) to 6,83 mm (600 mg/ml).
3. Comparing the results of antimicrobial activity of *Thymus tosevii* Vel. subsp. *tosevii* var. *degenii* essential oil against *Candida albicans* and *Aspergillus niger*, we can determine the different effects of this oil on the above microorganisms. Thus, we have concluded that *Candida albicans* showed moderate sensitivity on oil's activity and *Aspergillus niger* manifested low 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.

Bibliography

1. Consentino S., *et al*. "In vitro antimicrobial activity and chemical composition of Sardinian thymus essential oils". *Letters in Applied Microbiology* 29 (1999): 130-135.
2. Dob T., *et al*. "Studies on the essential oil composition and antimicrobial activity of *Thymus algeriensis* Boiss. et Reut". *The International Journal of Aromatherapy* 16 (2006): 95-100.
3. Dorman HJD and Deans SG. "Chemical composition, antimicrobial and *in vitro* antioxidant properties of *Monaranda citriodora* var. *citriodora, Myristica fragrans*, *Origanum vulgare* ssp. *hirtum*, *Pelargonium* sp. and *Thymus zygis* oils". *Journal of Essential Oil Research* 16 (2004): 145-150.
4. Zambonelli A., *et al*. "Chemical composition and fungicidal activity of commercial essential oils of *Thymus vulgaris* L". *Journal of Essential Oil Research* 16 (2004): 69-74.
5. Soliman KM and Badea RI. "Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi". *Food Chemistry and Toxicology* 40 (2002): 1669-1675.
6. Stahl-Biskup E. "The chemical composition of *Thymus* oils. A review of the literature 1960-89". *Journal of Essential Oil Research* 3 (1991): 61-82.

Volume 2 Issue 5 May 2019