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RESEARCH ARTICLE

Characterization of the Essential Oil and Anticandidal Evaluation of *Thymus pallasicus* Hayek & Velen. from Turkey

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Abstract

Thymus L. of the Lamiaceae with 220 ssp. is concentrated in the Mediterranean region. *Thymus* species are well known as "Kekik" in Turkish and are used as herbal tea and spices, according to recent records, the genus is represented with 40 ssp. where 17 species are endemic to Turkey. The study material *Thymus pallasicus* is one of the endemic species for Turkey, from which the essential oil was obtained from the air dried aerial parts by hydro distillation subsequently analyzed by GC-FID and GC-MS, respectively. The main component was identified as thymol (55.5 %) along with the monoterpenes p-cymene (19.9 %), γ-terpinene (7.2%), carvacrol (5.0 %), and 1,8-cineole (1.4%). The essential oil was also evaluated for its anticandidal activity against five different standard human pathogenic strains, according to a modified in vitro CSLI micro dilution method. The minimum inhibitory activity of the essential oil was 250-500 microgram/mL against the tested *Candida* sp. suggesting rather weak inhibitory activity when compared to the standard antifungal fluconazole (0.5-16 microgram/mL). As *Thymus* species are known for their antimicrobial activities it is suggested that other clinical and pathogenic strains should be further evaluated.

Keywords: *Thymus pallasicus*, Lamiaceae, essential oil composition, anticandidal

Introduction

Lamiaceae is one of the largest families with more than 245 genera and about 7886 throughout the world (The Plant List, 2018). In Turkey, Lamiaceae has 48 genera, 603 species, 179 subspecies and varieties (782 taxa), 271 species, 75 subspecies and varieties (346 taxa) of which are endemic (ca. 44%). *Thymus* L. is centered in the Mediterranean region (Heywood et al., 2007).

According to recent records, the genus is represented by 47 taxa, 42 species and 20 species are endemic to Turkey (Jalas, 1982; Yıldız, 2012; Celep & Dirmenci, 2017). *Thymus* species are well known as "Kekik" in Turkish and are used as herbal tea and spices. It has uses for various purposes in traditional medicines for example as a stomach, sedative, antiseptic, worm lowering and blood circulation stimulating effects. Its use is particularly common as spices (Baytop, 1999; Fujita et al., 1995).

The endemic species *Thymus pallasicus* Hayek & Velen. (Syn.: *T. pectinatus* var. *pectinatus*) is a dwarf shrub, up to 20-30 cm high, endemic in Inner Anatolia, growing wild in open steppe on gypsum or calcareous fields (Jalas,1982; Yıldız, 2012). It is used in bronchitis, severe cough and upper respiratory tract colds due to bronco antispasmodic, expectorant and antibacterial effects of *T. vulgaris* and *T. zygis*. In tropical applications, it has antimicrobial usage. Due to its antimicrobial effect, *T. serpyllum* preparations are used against upper respiratory tract disorders and cold, which as found in the composition of various antitussive preparations. (WHO, 1999; Ozgen et. al, 2017).

In the present study, the anticandidal activity of the essential oil was evaluated against five different standard human pathogenic strains and the chemical composition was elucidated.

Materials and Methods

Plant material

The plant was collected from the below mentioned locality and identified by Zeki Aytaç and Murat Ekici (Gazi University, Faculty of Science, Department of Biology). A voucher specimen has been deposited in the herbarium of the Faculty of Pharmacy, Ankara University in Ankara, Turkey (AEF). Collection locality: B7 Erzincan. North west from Aşkale 3-4 km, 19.07.2013 (Voucher number: AEF 26358).

Isolation of the essential oil

Air-dried aerial parts of the plants were hydrodistilled for 3 h by using a Clevenger-type apparatus according to the method recommended in the European Pharmacopoeia (Ph.Eur., 01/2008). As small amount of essential oil was trapped in n-hexane which were dried over anhydrous sodium sulphate and stored in sealed vials at +4°C until to be analysed and tested. The analysis processes of gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) were performed with reference to Demirci et al. (2017).

GC-MS analysis

The analysis was carried out with an Agilent 5975 GC-MSD system (Agilent, USA; SEM Ltd., Istanbul, Turkey). Innowax FSC column (60 m x 0.25 mm, 0.25 μm film thickness) was used with helium as carrier gas (0.8 mL/min). GC oven temperature was kept at 60 °C for 10 min and programmed to 220 °C at a rate of 4 °C/min, and kept constant at 220 °C for 10 min and then programmed to 240 °C at a rate of 1 °C/min. Split ratio was adjusted at 40:1. The injector temperature was set at 250 °C. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 35 to 450.

GC-FID analysis

The analysis was carried out using an Agilent 6890N GC system. FID detector temperature was 300 °C. To obtain the same elution order with GC-MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts (%) of the separated compounds were calculated from FID chromatograms. The results are listed in Table 1.

Identification of components

Identification of the essential oil components were carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library MassFinder 4 Library) (McLafferty, 1989; Hochmuth, 2008) and in-house "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils.

Anticandidial activity

The assay was performed against different pathogenic *Candida* strains. The minimum inhibitory concentrations (MIC) of *Candida* strains were determined by broth microdilution with some minor modifications (CLSI, 2002). The essential oil was used dissolved in DMSO (100%) at an initial concentration of 4000 μg/mL and fluconazole as standard control. All *Candida* strains were inoculated on Potato Dextrose Agar (PDA) prior the experiments at 37 °C. After incubation grown microorganisms were inoculated in sterile saline (0.85 %). And then standardized turbitometrically (McFarland No: 0.5) to 5 x 10^3 CFU per well in RPMI

medium under sterile conditions. Serial dilution series were prepared in 100 µL RPMI medium with an equal amount of the test samples. After serial dilution, 100 µL each microorganism suspension was pipetted into each well and incubated at 37 °C for 24 hours. For the visualization of the MICs, reassuring solution was added and incubated for 30 min at 37 °C.

Positive growth controls) were performed in wells without the antifungal standard. In addition, negative growth control (only medium) was tested. MIC (mg/mL) was defined as the lowest concentration, without visible growth of the microorganisms. All experiments were repeated in duplicate. All MIC analysis results are given in Table 2.

Results and Discussion

Essential oil from the air dried aerial part of the *Thymus pallasicus* was obtained by hydro distillation and analysed by GC-FID and GC-MS. The composition of the *Thymus pallasicus* are given in Table 1, according to their relative retention indices (RRI) and with their relative percentages.

Analytical results showed that the main components were found to be thymol (55.5 %) along with p-cymene (19.9 %), γ-terpinene (7.2%), carvacrol (5.0 %), and 1,8-cineole (1.4%).

In literature *T. pectinatus* var. *pectinatus* was analysed and show that the main components were obtained to be thymol (49.8 %) along with the monoterpenes *p*-cymene (14.8 %), γ-terpinene (16.1%) and carvacrol (3.7 %) (Vardar et.al., 2003; Başer & Kırımer, 2018).

Table 1. The Composition of the Essential Oil of *Thymus pallasicus*

RRI [a]	KI [b]	Compound	%
1032	1025	α-Pinene	0.6±0.00 [c]
1035	1012-1039	α-Thujene	0.7±0.00
1076	1043-1086	Camphene	0.4±0.00
1118	1117	β-Pinene	0.2±0.00
1174	1160	Myrcene	0.1±0.06
1176	1148-1186	α-Phellandrene	0.2±0.06
1188	1154-1195	α-Terpinene	1.2±0.00
1203	1178-1219	Limonene	0.3±0.00
1213	1186-1231	1,8-Cineole	1.4±0.00
1255	1222-1266	γ-Terpinene	7.2±0.00
1280	1246-1291	*p*-Cymene	19.9±0.06
1290	1261-1300	Terpinolene	0.1±0.00
1452	1411-1465	1-Octen-3-ol	0.3±0.00
1474	1573-1602	*trans*-Sabinene hydrate	0.7±0.00
1556	1425-1478	*cis*-Sabinene hydrate	0.3±0.00
1591	1549-1597	Bornyl acetate	0.2±0.00
1611	1564-1630	Terpinen-4-ol	0.6±0.00
1706	1659-1724	α-Terpineol	0.1±0.00
1719	1653-1728	Borneol	1.2±0.00
1741	1698-1748	β-Bisabolene	0.9±0.00
1864	1813-1865	*p*-Cymen-8-ol	0.1±0.00
1890	1868-1890	Carvacryl acetate	0.9±0.00
2181		Isothymol	0.2±0.00

2198	2100-2205	Thymol	55.5±0.11
2221		Isocarvacrol	0.3±0.00
2226	2175-2245	Methyl hexadecanoate	0.1±0.00
2239	2140-2246	Carvacrol	5.0±0.06
2431	2386-2445	Methyl octadecanoate	0.1±0.00
		Total	**99.7±0.11**

[a]RRI Relative retention indices calculated against *n*-alkanes on the HP Innowax column; [b]KI Kovats indices from literature (Babushok et al., 2011); [c]mean % calculated from Flame Ionization Detector (FID) data ± SD (n=3)

Table 2. Anticandidial activity of the Essential Oil of *Thymus pallasicus*

	C.utilis **NRRL Y-900**	***C. tropicalis*** **NRRL Y-12968**	***C. krusei*** **NRRL Y-7179**	***C. albicans*** **ATCC 90028**	***C. glabrata*** **ATCC 66032**
Thymus oil*	250	500	250	500	250
Fluconazole**	4	0.5	16	2	4

*testing range for sample: 4 µg /mL- 4000 µg /mL; ** testing range for antifungal standart: 0,125 µg /mL - 64 µg /mL

The essential oil was also evaluated for its anticandidal activity against five different standard human pathogenic strains, according to a modified *in vitro* CSLI microdilution method. The minimum inhibitory activity of the essential oil was 250-500 microgram/mL against the tested *Candida* sp. suggesting rather weak inhibitory activity when compared to the standard antifungal fluconazole as seen in Table 2.

Thymus pallasicus essential oil was tested against 5 different *Candida* pathogens. When compared to the standard antifungal agent Fluconazole (MIC 0.5-16 mg/mL) the oil was rather low in activity (MIC 250-500 mg/mL) towards the tested panel.

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