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Chemical profiling, antimicrobial and antioxidant activity of Albanian thyme essential oil

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Abstract

The present study shows the chemical composition, antifungal, antibacterial and radical scavenging effect of the essential oil from wild *Thymus vulgaris* L. Chemical analysis of essential oil was done by gas chromatography mass spectrometry (GC-MS). Antibacterial activity was tested using disc diffusion method on three food borne diseases while antifungal activity was tested on colony growth of three postharvest pathogens in volatile phase. The free radical scavenging activity of the oil was measured *in vitro* by 2,20- diphenyl-1-picrylhydrazyl (DPPH) assay. GC-MS analysis identifies 30 components with thymol and p-cymene as main component, 35.4 and 26.93% respectively. The oil of thyme (5 μL/disc) gave higher inhibition zones on the tested bacterial strains, *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028 and *Staphylococcus aureus* ATCC 6538 compared with antibiotic cefazolin (30 μg/disc). In volatile phase, thyme oil (0.14 g/L) showed good antifungal activity on *Penicillium italicum*, *P. digitatum* and *P. expansum*, with percentages of mycelial inhibition 60, 71.4 and 73.5% respectively. The activity of the oil was fungistatic. Scavenging effect of the oil on DPPH radical varied from 36.4, 50.5 and 76.1% depending the concentrations used (1, 2 and 5 mg/mL). The *in vitro* biological activity of thyme oil can be attributed to aromatic monoterpenes thymol and p-cymene.

Keywords: *Thymus vulgaris*, thymol, p-cymene, antibacterial, antifungal, DPPH

1. Introduction

The use of synthetic antimicrobial and antioxidants in food industry is related with some problematics. Inapropriate use of these chemicals have been associated with the development of resistant spoilage microbes and food pathogens [1]. Some of these chemicals have been reported to be toxic and cancerogenic [2, 3]. In addition there is an increased consumer demand for products free of chemicals. As a result, there is a trend in alimentary industry to use natural compounds in order to replace (partially or totally) synthetic compounds. The most promising compounds are plant secondary metabolites. These plant secondary metabolites are bioactive components of essential oils and plant extracts of different plants, reported to have antimicrobial and antioxidant activity. In food industry the essential oils have been studied for their flavoring properties of foods, drinks and also for their antibacterial and antioxidant activity. *Thymus vulgaris* essential oil is a good candidate because wild and cultivated thyme apart from culinary and medicinal uses has been described to have different biological properties.

Thymus vulgaris L. is a native specie of Mediterranean Basin which belong the *Lamiaceae* family. In literature the extracts, essential oils and compounds extracted from this plant have been tested for their antimicrobial and antioxidant activities [4]. Main constituents found in thyme oil are thymol, p-cymene, γ-terpinene, carvacrol and linalool [5]. Chemical composition of wild thyme essential oil depend on factors such as chemotype, location were it

was collected (environmental condition) etc. The antimicrobial and antioxidant activity of the thyme oil have been attributed to monoterpenoid fenols such as thymol and carvacrol, and other monoterpenes (p-cymene, γ-terpinene). A few studies has been conducted on chemical and biological activity of Albanian *T. vulgaris* essential oil.

This study is aimed at assessing the essential oil composition, antimicrobial and antioxidant activities of Albanian *T. vulgaris*.

2. Material and Methods

Essential oil were provided from Albanian company "Mediterranean Spices & Imports" Tiranë. The wild thyme plant was harvested during July and the oil was steam distilled for four hours.

2.1. Chemical analysis of essential oils

The extracted thyme oil was analyzed by gas chromatography mass spectrometry (GC-MS) using an Shimadzu GC-2010 coupled to a Shimadzu GCMS-QP2010 Ultra mass detector (electron ionisation, 70eV) and equipped with a Teknokroma TRB-5(95%) Dimetil-(5%) diphenylpolisiloxane, 30m×0.25mm i.d. capillary column (0.25 µm film thickness). Working conditions were as follows: split ratio (20:1), injector temperature 300°C, temperature of the transfer line connected to the mass spectrometer 250°C, initial column temperature 70°C, then heated to 290°C at 6°C/min. Electron ionisation mass spectra and retention data were used to assess the identity of compounds by comparing them with those of standards or found in the Wiley 229 Mass Spectral Database.

2.2. Antibacterial bioassay

Antibacterial activity of the oil was investigated by disc diffusion method as already described by Sfeir et al. [6]. The bacterial suspension of *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028 and *Staphylococcus aureus* ATCC 6538 was adjusted to a density of bacterial cells of 1.0×10^8CFU/mL. A sterile swab immersed in this bacterial suspension was used to inoculate the entire surface of a nutrient agar. Five µL of thyme oil was applied on a sterile paper disc (8 mm) aseptically placed on the inoculated plates. Then, plates were incubated for 15 minutes at room temperature. Only one disc was tested per plate. After 24 h of incubation at 37°C in an incubator, the inhibition zones were measured in millimeters. Cefazolin (30 µg/disc) was used as a positive control for bacterial inhibition. All experiments were done in triplicate.

2.3. Antifungal bioassay

The antifungal activity of the oil was tested on colony growth using the methods given by Shao et al. [7] and Soylu et al. [8] with some modifications. Sterile plastic Petri dishes (90x15mm) with PDA medium were inoculated in the center with conidial suspension 10^5/mL prepared from 5-6 days cultures of *P. expansum P. digitatum* and *P. italicum*. Sterile filter paper discs (14 mm diameter) were attached to the inner surface of each Petri dish lid. The amount of 10 µL (0.14 g/L) of oil was added onto the filter paper, and the dishes were quickly covered. Petri dishes were wrapped with parafilm to inhibit the release of volatile components. The oil was allowed to volatilize inside the Petri dishes spontaneously at 24°C for three hours before the parafilm was removed. Controls were prepared similarly with the exception of the volatile treatment. Treatment was carried out with three replications. The efficacy of the oil was evaluated by measuring diameters of each colony after 7 days of incubation at 24°C.

Percentage mycelial inhibition (PMI) was calculated as follow:

PMI = $[(dc - dt)/dc] \times 100$, where *dc* is the mean colony diameter for the control sets and *dt* is the mean colony diameter for the treatment sets. All tests were repeated two times.

2.4. DPPH radical scavenging activity assay

The free radical scavenging activity of the oil was measured *in vitro* by 2,20- diphenyl-1-picrylhydrazyl (DPPH) assay according to Saeed et al. [9]. Concentrated solution was prepared by dissolving 24

mg DPPH in 100 mL methanol. The working solution was achieved by diluting DPPH stock solution with methanol to attain an absorbance of about 0.98±0.02 at 517nm using the spectrophotometer (UV-1202 SHIMADZU). Three mL aliquot of this solution was mixed with 100 µL of the sample at 1, 2 and 5 mg/mL concentration. The reaction mixture was shaken and incubated in the dark for 30 min at room temperature. Then the absorbance was measured at 517 nm. Control was prepared as above without essential oil. The scavenging activity was estimated based on the percentage of DPPH radical scavenged as follow:

Scavenging effect % = {(control absorbance - sample absorbance) / (control absorbance)} x 100.

3. Results and Discussion

3.1. Chemical analysis of essential oils

GC-MS analysis identifies 30 components with thymol and *p*-cymene as main components, 35.4 and 26.93% respectively with lesser quantities of γ-terpinene (5.16%), β-caryophyllene (3.92%), linalool L (3.68%) and carvacrol (2.71%). In table 1 are presented the other components of thyme essential oil.

Table 1. Identified compounds of thyme oil

Peak	Base m/z	R.Time	Area %	Compounds
1	93.10	4.958	0.13	β-phellandrene
2	93.10	5.104	1.36	α-pinene
3	93.10	5.426	1.05	Camphene
4	93.10	6.058	0.13	β-pinene
5	93.10	6.353	0.93	β-myrcene
6	93.10	6.753	0.13	α-phellandrene
7	93.10	7.042	1.21	α-terpinene
8	119.15	7.042	26.93	*p*-cymene
9	68.05	7.377	0.83	Limonene
10	43.00	7.43	2.32	1,8-cineole
11	93.10	8.209	5.16	γ-terpinene
12	93.10	9.13	0.15	Terpinolene
13	71.05	9.433	3.68	Linalool L
14	95.10	10.867	1.10	Camphor
15	95.10	11.57	2.31	Borneol L
16	71.05	11.977	1.67	4-terpineol
17	59.05	12.451	0.38	α-terpineol
18	149.15	13.88	1.48	Carvacrol methyl ether
19	149.15	14.2	1.41	Thymyl methyl eter
20	93.10	15.685	0.61	Isobornyl acetate
21	135.15	15.865	35.14	Thymol
22	135.15	16.177	2.71	Carvacrol
23	105.10	18.696	0.40	α-ylangene
24	81.10	19.005	0.15	β-bourbonene
25	93.10	20.131	3.92	β-caryophyllene
26	161.15	21.968	0.48	α-amorphene
27	105.10	22.731	0.13	α-murolene
28	161.20	23.16	0.72	Germacrene D
29	159.15	23.438	1.25	δ-cadinene
30	79.05	25.283	2.11	Caryophyllene oxide

Thyme oil was the subject of numerous studies conducted earlier. Previous works reported that the oil of *T. vulgaris* contains as main constituens thymol, p-cymene, γ-terpinene and carvacrol [10, 11, 12]. Our results are in line with those repots. Thyme oil based on chemical composition can be clasified in different chemotypes. Thyme oil in this study belongs to thymol chemotype in agreement with those reported erlier for Albanian thyme oil [13].

3.2. Antimicrobial activity

Antimicrobial activity of thyme oil is shown in table 2. The antibacterial activity is expressed as inhibition zone diameter in mm.

The oil of thyme (5 μL/disc) gave higher inhibition zones on the tested bacterial strains, *E. coli* ATCC 25922, *S. typhimurium* ATCC 14028 and *S. aureus* ATCC 6538 compared with antibiotic cefazolin (30 μg/disc).

In volatile phase, thyme oil (0.14 g/L) showed good antifungal activity on *Penicillium italicum*, *P. digitatum* and *P. expansum*, with percentages of mycelial inhibition 60, 71.4 and 73.5% respectively. The activity of the oil was fungistatic.

Table 2. Inhibition zone diameters (mm) on three bacterial species and percentages of fungal mycelial inhibition of thyme essential oil.

Pathogens	*T. vulgaris*	Control	Cefalozin
Bacterial species			
E. coli	45.7±1.5	0.0	19.3±0.6
S. typhimurium	31.7±5.5	0.0	18.3±0.6
S. aureus	48.0±2.0	0.0	33.3±0.6
Fungal species			
P. digitatum	71.4±5.1		
P. expansum	73.5±1.3		
P. italicum	60±7.9		

Values in the table represent the mean value of three replicates (± standard deviation).

T. vulgaris essential oil, thymol chemotype, have shown a broad scale of antibacterial activity against food borne pathogens. In a study conducted by [14] thyme oil (64% thymol) inhibited the growth of different bacterial species including E. coli and S. typhimurium. In an earlier study the thymol chemotype (49% thymol and 19% p-cymene) of thyme oil showed good antibacterial activity against S. aureus and S. typhimurum [15]. Our results are in agreement with those reported in literature [14, 15].

Essential oil extracted from different *Thymus* species have been studied for their antifungal activity and in many studies the oils have shown to be fungicidal or fungistatic. The antifungal activity of *T. vulgaris* essential oil and other oils are with high interes in agricultural sector in particular for the control of postharvest pathogens of fruits and vegetables. Due to the volatilization of oil it can be useful as fumigant for the control of postharvest pathogens especially in some fruits such as strawberries that are difficult to be sanitized with aqueous solution.

In a study conducted by Klarić *et al.* [16] thymol and *T. vulgaris* essential oil with main costituents *p*-cymene (36.5%) and thymol (33.0%) showed strong fungistatic or fungicidal against diferent fungal species such as *Aspergillus*, *Penicillium*, *Cladosporium*, *Trichoderma*, *Mucor* and *Rhizopus*. In these experiments thymol reduced the fungal growth three time higher than the oil. In another study with *P. digitatum* wich cause green mould in citrus fruits the thyme oil showed good antimicrobial activity in contact and volatile phase [17]. Propably the

fungistatic activity in three *Penicillium* species tested is due to phenolic costituent thymol, these confirmed also by other studies [11, 16].

3.3. DPPH radical scavenging activity

Scavenging effect of thyme oil on DPPH radical varied from 36.4, 50.5 and 76.1% depending the concentrations used (1, 2 and 5 mg/mL). Previous studies have demonstrated that the antioxidants activity of thyme oil is related to phenolic compound thymol and the monoterpene p-cymene [18, 19]. Our data is in agreement with these reports given that the oil used in this study has as main compounds thymol (35.4 %) and p-cymene (26.93%).

4. Conclusions

Chemical analysis of the essential oil of *T. vulgaris* identified different compounds with thymol and p-cymene as main compounds. *In vitro* experiments the oil showed good antibacterial activity on the three food borne pathogens and inhibited the colony growth of the three *Penicillium* species. The oil also presents good scavenging activity on DPPH radical. The biological activities of the oil maybe attributed to major compounds (thymol and *p*-cymene).

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