Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(9):180-184

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Chemical Composition and Antioxidant Activity of the Essential Oils Isolated from Greek and Albanian *Thymus* Species

Kontogiorgis Christos[1], Mpompou Evlampia-Maria[2], Papajani-Toska Vilam[3], Hadjipavlou-Litina Dimitra[4] and Lazari Diamanto[2*]

[1]Laboratory of Hygiene and Environmental Protection, Democritus University of Thrace, Dragana (Campus), Alexandroupolis, Greece
[2]Department of Pharmacognosy-Pharmacology, School of Pharmacy, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece
[3]Department of Pharmacy, Faculty of Medicine, University of Tirana, Albania
[4]Department of Pharmaceutical Chemistry, School of Pharmacy, Faculty of Health Sciences, Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece

---

ABSTRACT

The genus Thymus (family Lamiaceae) is one of the eight most important genera as regards number of species within the Lamiaceae family. It is a perennial herbaceous plant indigenous to central and southern Europe, and is now widely cultivated as a tea, spice, and herbal medicine. Its leaf is listed in the German and British Herbal Pharmacopoeia, and has been used as a stomachic, carminative, diuretic, urinary disinfectant, and vermifuge. The essential oil composition from Thymus sibthorpii Bentham collected from North Greece and Thymus striatus Vahl. collected from South Albania have been investigated by Gas Chromatography-Mass Spectroscopy. α-terpinyl acetate (68.9%), α-terpineol (11.0%) and thymol (7.9%), were found to be the major compounds of T. sibthorpii. Thymol (53.6%), p-cymene (18.6%) and γ-terpinene (9.4%), were found to be the major compounds of T. striatus. Furthermore, the oils and thymol, the major component of T. striatus, were evaluated for their antioxidant activity using the DPPH interaction, as well as for their anti-inflammatory activity using the soybean lipoxygenase bioassay.

Keywords: *Thymus sibthorpii* bentham; Essential oil; Terpenes; Antioxidant activitity; Lipid peroxidation

---

INTRODUCTION

Thyme (*Thymus*) is a genus of about 350 species of aromatic perennial herbaceous plants and sub-shrubs to 40 cm tall, in the family Lamiaceae and native to Europe, North Africa and Asia. A number of species have different chemotypes. The ancient Greeks used it in their baths and burnt it as incense in their temples, believing that thyme was a source of courage [1].

Thyme is widely cultivated as it was grown for its strong flavor, which is due to its content of thymol. The essential oil of common thyme (*Thymus vulgaris*) is made up of 20-55% thymol. Thymol, an antiseptic, is the main active ingredient in widely known mouthwash available to the market. Before the advent of modern antibiotics, it was used to medicate bandages. Medicinally thyme is used for respiratory infections in the form of a tincture, tisane, salve, syrup or by steam inhalation [2].

Thyme oil is the important commercial product obtained by distillation of the fresh leaves and flowering tops of *T. vulgaris*. Its chief constituents are from 20 to 25 per cent of the phenols Thymol and Carvacrol, rising in rare cases to 42 per cent. The phenols are the principal constituents of Thyme oil. Thymol is the most valuable for medicinal purposes, while Carvacrol is an isomeric phenol, preponderate in some oils. Two commercial varieties of Thyme oil are recognized, the 'red,' the crude distillate, and the 'white' or colorless, which is the 'red' rectified by re-distilling. The value of Thyme oil depends so much upon the phenols it contains, that it is important that these should be estimated, as the abstraction of Thymol is by no means uncommon. Red oil of Thyme is frequently imported and sold under the name of oil of *Origanum*: it is often adulterated with oils of turpentine, spike lavender and rosemary, and coloured with alkanet root, and is not infrequently more or less destitute of Thymol. Further properties that are well documented by experimental research are: anti-inflammatory, antioxydative, spasmolytic, secretolytic and tonifying effects [3].

Antioxidants minimize oxidation of the lipid components in foods. There is an increasing interest in the use of natural and/or synthetic antioxidants in food preservation, but it is important to evaluate such compounds fully for both

antioxidant and pro-oxidant properties. The properties of thymol, carvacrol, 6-ginerol, hydroxytyrosol and zingerone were characterized in detail. Thymol, carvacrol, 6-gingerol and hydroxytyrosol decreased peroxidation of phospholipid liposomes in the presence of iron (III) and ascorbate, but zingerone had only a weak inhibitory effect on the system. The compounds were good scavengers of peroxyl radicals (CCl_3O_2; calculated rate constants > 10^6 M^{-1} sec^{-1}) generated by pulse radiolysis [4].

MATERIALS AND METHODS

Plant material

The aerial parts of the two Thymus taxa were collected when in flower, as follows: T. sibthorpii from Chortiatis Mountain, on June 2006 and T. striatus from South Albania, on July 2006. Voucher specimens have been deposited with the Herbarium of the Laboratory of Pharmacognosy, Aristotle University of Thessaloniki.

Distillation and analysis of the volatile fraction

Dried aerial parts of T. sibthorpii (30g) and T. striatus (43.0 g) have been hydro-distillated for 3h, using a Clevenger apparatus according to standard procedures [5]. The essential oil, taken in 2 mL of capillary GC grade n-pentane has been dried over anhydrous sodium sulphate and stored at 2-4°C. The yield was defined as the essential oil to the dry aerial parts matter mass ratio (v/w).

Gas chromatography-Mass spectroscopy

The composition of the volatile constituents was established by GC-MS analyses. GC-MS analyses were performed on a Shimadzu GC-2010 – GCMS-QP2010 system operating in EI mode (70eV) equipped with a split/splitless injector (230^0C), a split ratio 1/30, using a fused silica HP-5 MS capillary column (30m x 0.25mm (i.d.), film thickness: 0.25μm). The temperature program was from 50 ^{0}C (5min) to 290 ^{0}C at a rate of 4 ^{0}C/min. Helium was used as a carrier gas at a flow rate of 1.0 mL/min. Injection volume of each sample was 1 μL. Arithmetic indices for all compounds were determined according to the Van der Dool approach [6], using n-alkanes as standards. The identification of the components was based on comparison of their mass spectra with those of NIST21 and NIST107 [7] and those described by Adams [8], as well as by comparison of their retention indices with literature data [9]. In many cases, the essential oils were subject to co-chromatography with authentic compounds (Fluka, Sigma).

Chemicals

1,1-Diphenyl 2-picryl hydrazyl (DPPH), Lipoxygenase (1.13.11.12) type I-B (Soybean) and linoleic acid (sodium salt), 99% purity, were purchased from Sigma (St Louis. MO, USA). Nordihydroguaiaretic acid (NDGA) was purchased from Merck. All other chemicals were of analytical grade. A Perkin Elmer Lambda 20 UV-Vis spectrophotometer has been used for the radical scavenging activity experiments.

RESULTS AND DISCUSSION

Chemical composition

The volatile fractions obtained from *T. sibthorpii* and *T. striatus* were different in yield ca. 1.33 % and 2.12 % (v/w), respectively, on dry weight basis. Table 1 gives the percentage amounts of identified compounds of both essential oils as long as the percentage amounts of some group compounds in the same samples.

Essential oil of *T. sibthorpii* was composed mainly from esters with α-Terpinyl acetate (68.9%) being the dominant compound. The fraction of oxygenated monoterpenes was 24.4%; the main components were α-Terpineol (11.0%) and Thymol (7.9%). The fractions of monoterpene hydrocarbons as well as sesquiterpene components were very low. Previous studies show that *Thymus sibthorpii* Benth. growing wild in Northern Greece seems to occur in two chemotypes: one geraniol-thymol-p-cymene type and one geraniol-linalool-citronellyl acetate type [12], while the essential oil from Turkey was found to be rich in thymol (34.8%) [13]. Comparative analyses of the winter and summer leaf essential oils in *T. sibthorpii* from North Greece showed that the winter leaves have a higher essential oil yield (1.20%, i.e. 1.20 ml /100 g leaf d.w.) compared to the summer leaves (1.11%) and the principal components of both types of oils were p-cymene, linalool, borneol, terpinen-4-ol, thymol, and β-caryophyllene, with linalool (42.4%) being the dominant compound in winter leaves and p-cymene (25.0%) being the dominant compound in summer leaves followed by linalool (19.1%) [14]. It could be suggested, that different chemotypes exist in this species and our results showed the existence of a new one in North Greece: α-Terpinyl acetate - α-Terpineol.

Essential oil of *T. striatus* was composed mainly from monoterpene oxygenate compounds with thymol (53.6%) being the dominant compound. The monoterpene hydrocarbon fraction was 31.8%; the main components were p-Cymene (18.6%) and γ-Terpinene (9.4%), while the fractions of sesquiterpenes were very low. Our results are generally in agreement with the analysis of essential oil of *T. striatus* collected from Mountain Orjen (Montenegro) [15].

Biological activity

The 1,1-diphenylpicrylhydrazyl (DPPH) assay is widely used in the evaluation of plant constituents antioxidant activity. The method is based on the spectrophotometric measurement of the DPPH concentration change resulting from the reaction with an antioxidant, where decolorization is stoichiometric with respect to the number of electrons captured.

Table 1: Identified components (% v/v) in the essential oils of *Thymus sibthorpii* and *Thymus striatus*

Compound[a]	AI[b]	T. *sibthorpii*	T. *striatus*	Identification[c]
α-Thujene	925	n.d.	0.2	AI, MS
α-Pinene	931	n.d.	0.5	AI, MS, Co-GC
Camphene	945	n.d.	0.3	AI, MS
1-Octen-3-ol	978	n.d.	1.1	AI, MS
β-Myrcene	990	0.1	1.1	AI, MS, Co-GC
α-Terpinene	1014	n.d.	1.2	AI, MS
p-Cymene	1022	0.2	18.6	AI, MS, Co-GC
Limonene	1026	0.5	n.d.	AI, MS, Co-GC
β-Phellandrene	1026	n.d.	0.5	AI, MS
Eucalyptol	1028	n.d.	0.5	AI, MS, Co-GC
γ-Terpinene	1056	0.2	9.4	AI, MS, Co-GC
Terpinolene	1085	0.1	n.d.	AI, MS
Linalool	1097	1.3	3.2	AI, MS, Co-GC
1-Octen-3-ol, acetate	1112	0.4	n.d.	AI, MS
Camphor	1141	n.d.	0.5	AI, MS, Co-GC
Isoborneol	1162	n.d.	0.9	AI, MS
Terpinen-4-ol	1174	0.4	0.8	AI, MS, Co-GC
α-Terpineol	1187	11	0.2	AI, MS
Thymol, methyl ether	1232	n.d.	0.1	AI, MS
Carvacrol, methyl ether	1242	0.2	1	AI, MS
Geraniol	1252	3.6	n.d.	AI, MS, Co-GC
Isobornyl acetate	1283	0.5	n.d.	AI, MS
Thymol	1289	7.9	53.6	AI, MS, Co-GC
Carvacrol	1298	0.2	3	AI, MS
α-Terpinyl acetate	1347	68.9	n.d.	AI, MS
Geranyl acetate	1381	0.5	n.d.	AI, MS
β-Caryophyllene	1417	2.1	1.1	AI, MS, Co-GC
Geranyl propanoate	1472	n.d.	0.2	AI, MS
γ-Muurolene	1480	0.5	0.1	AI, MS
β-Bisabolene	1507	0.5	n.d.	AI, MS
γ-Cadinene	1512	n.d.	0.1	AI, MS
δ-Cadinene	1522	n.d.	0.2	AI, MS
Caryophyllene oxide	1583	0.7	0.6	AI, MS, Co-GC
epi-α-Cadinol	1640	n.d.	0.1	AI, MS
Total		99.8	99.1	
Monoterpene Hydrocarbons		1.1	31.8	
Oxygenated Monoterpenes		24.4	62.7	
Sesquiterpene Hydrocarbons		3.1	1.5	
Oxygenated Sesquiterpenes		0.7	0.7	
Esters		70.3	0.2	

[a]Compounds listed in order of elution from an HP-5 MS capillary column; [b]AI: Arithmetic indices as determined on a HP-5 MS capillary column using a homologous series of n-alkanes (C9–C25); [c]Identification method: AI arithmetic index, MS mass spectrum, Co-GC coinjection with authentic compound; n.d.: not detected

The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm. The protocol followed in the present study, includes NDGA as the reference standard and the samples were measured at twice, 20 min and 60 min after reaction was started. Two different concentrations of DPPH were also used (10 and 20 ul).
Both the essential oils as well as thymol in two different concentrations interacted with the stable free radical DPPH. In all cases the antioxidant activity of both the essentials oils and the purified thymol was very low. NDGA (nordihydroguaiaretic acid) was used as reference compound.

Table 2: Percentage (%) interaction of essential oils with DPPH and their % soybean LOX inhibitory activity

Essential Oil	% Interaction with the stable free radical of DPPH				% Inhibition of LOX	
	20 min		60 min			
	10 ul	20 ul	10 ul	20 ul	10 ul	100 ul
T. *sibthorpii*	5.3	3.5	6.2	3.8	13.3	93.8
T. *striatus*	7.3	11	9.2	11.7	0.9	10.4
Thymol	8.9	12.9	3.2	5.3	6.3	100
Thymol 0.1mM	7.3	8.2	7.4	9.1	6.1	34.9
NDGA	81	87	85	93	91	97

The samples were further evaluated for inhibition of soybean lipoxygenase (LOX). The majority of the LOX inhibitors are antioxidants or free radical scavengers and since lipoxygenation occurs via a carbon-cantered radical, the extracts were further evaluated for their possible inhibitory activity against soybean lipoxygenase (LOX). The soybean lipoxygenase assay was previously used as an indication of possible anti-inflammatory activity of these extracts (Pontiki and Hadjipavlou, 2006).
It is previously suggested that LOX inhibition is linked to the ability of the inhibitors to reduce Fe^{+3} at the active site of soybean lipoxygenase to the catalytically inactive Fe^{+2}. LOXs contain a "non-heme" iron per molecule in the enzyme active site as high-spin Fe^{+2} in the native state and the high spin Fe^{+3} in the activated state. Several LOX inhibitors, such phenolic derivatives, are excellent ligands for Fe^{+3}.
The results showed that both essential oils and thymol presented concentration dependent inhibitory activity on soybean lipoxygenase. It is quite interested that *T. sibthorpii* presented stronger inhibitory activity than *T. striatus.* This can be probably explained by the significant difference in the % quantity of the identified components (% v/v) in the essential oils In case of thymol, the lipoxygenase inhibitory activity seems not to be concentration dependent since the most concentrated sample (thymol 0.1 mM) presents less potent inhibitory activity.

Biological experiments
Collection of essential oils: All Essential oils are diluted in absolute ethanol (3mg/ml). Two solutions of thymol were used: one was 3mg/ml (final concentration 0.2 mM) and the other was 0.1 m*M.*

Interaction of the tested compounds with 1,1-diphenyl-2-picryl-hydrazyl (DPPH) stable free radical: The bioassay was performed, as previously described [10]. 10 ul or 20 ul from the stock solution of the sample diluted in absolute ethanol was added and filled with ethanol to a final volume of 1 mL and then added to 1 ml DPPH (0.1 mM, in absolute ethanol). The reaction mixture was allowed at room temperature for 20 and 60 min. The optical density (OD) of the solution was measured and the percent reduction was estimated with the following equation at 517 nm. The optical densities of the samples without the presence of DPPH were recorded and subtracted from the corresponding OD with DPPH.

Soybean lipoxygenase inhibition: The bioassay was performed according to a previously described procedure [11]. The incubation mixture (final volume 1 ml) consisted of 10 ul or 100ul of the test sample, 100 μL of sodium linoleate (0.1 mM) and 0.2 μl of the enzyme solution (1/9x10-4, w/v in saline) in buffer tris pH 9. After incubation at room temperature for 3 min the conversion of the sodium linoleate to 13-hydroperoxylinoleic acid was recorded at 234nm and compared with an appropriate standard inhibitor NDGA (nordihydroguaeretic acid 0.1 mM).

ACKNOWLEDGMENTS

The authors are grateful to Dr. N. Krigas (Laboratory of Systematic Botany & Phytogeography, School of Biology, Faculty of Sciences, Aristotle University of Thessaloniki) for the identification of the plant material. This study was financially supported by the GSRT (015-ε 5838. 14-03-06).

REFERENCES

[1] M Pećanac; Z Janjić; A Komarcević; M Pajić; D Dobanovack; SS Misković. *Medicinski pregled*, **2013,** 66, 263-267.
[2] EMA/HMPC, Assessment report on *Thymus vulgaris* L., *Thymus zygis* Loefl. ex. L., aetheroleum (131903/2009). European Medicines Agency/Committee on Herbal Medicinal Products, London **2010**. (http://www.ema.europa.eu/ocs/en_GB/document_library/Herbal_HMPC_assessment_report,2010/12/WC500100054.pdf).
[3] M Nikolić; J Glamočlija; ICFR Ferreira; RC Calhelha; A Fernandes; T Marković; D Marković; A Giweli A; Sokovic M., *Ind. Crops Prod.*, **2014,** 52, 183– 190.
[4] R Aeschbach; J Löliger; BC Scott; A Murcia; J Butler; B Halliwell; OI Aruoma. *Food & Chem. Toxicol.*, **1994,** 32**,** 31-6.
[5] Hellenic Pharmacopoeia, Ed. IV. Athens, *(I)*, **1989**191-193.
[6] H Van den Dool; PD Kratz. *J. Chromat.*, **1963,** 11, 463-471.
[7] Y Massada. *Analysis of essential oil by gas chromatography and spectrometry*. J. Wiley & Sons, New York, **1976**
[8] RP Adams, *Identification of Essential Oil Compounds by GC Quadrupole Mass-Spectometry*. 4th ed., Allured Publishing Corporation, Carol Stream, IL, USA, **2007.**
[9] NN Davies. *J. Chromat.*, **1990, 503**, 1-24.
[10] C Kontogiorgis; D Hadjipavlou-Litina. *J. Med. Chem.*, **2005,** 48, 6400-8.
[11] C Kontogiorgis; D Hadjipavlou-Litina. *J. Enz. Inhib. Med. Chem.*, **2003,** 18, 63-69.
[12] St Katsiotis; P Chatzopoulou; A Baeheim Svendsen. *Sci. Pharmaceutica*, **1990,** 58, 303-306.
[13] KHC Baser; T Özek; M Kürkçüoglu; G Tümen. *J. Ess. Oil Res.*, **1992,** 4, 303-304.
[14] AM.Bosabalidis. *J. Plant Devel*. **2013,** 20, 3 – 11.
[15] M Couladis; O Tzakou;, S Kujundzic; M Sokovic; N Mimica-Dukic. *Phytother. Res.*, **2004,** 18, 40–42.