Essential Oil Composition of Thymus Vulgaris L. and their Uses

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ABSTRACT

The composition of the essential oil from the *Thymus vulgaris* L. type growing wild in northern Italy were identified by GC/MS . Identified were thirty components, the main essential oils are as follows:- thymol, γ-terpinene, ρ-cymene, linalool, myrcene, α-pinene, eugenol, carvacrol and α -thujene. Among these twelve aroma constituents of thyme were examined for their antioxidant activities using the aldehyde/carboxylic assay. Eugenol, thymol, carvacrol and 4-allylphenol showed stronger antioxidant activities than did other components tested in the assay. Twenty one essential oils were tested for insecticidal activity against spodoptera litura. Carvacrol has shown mutagenecity. ρ -cymene, linalool, terpinene -4-ol and thymol exhibited antifungal activity against Botrytis Cinerea and Rhizopus Stolonifer, two common storage pathogens of strawberries (*Fragaria ananassa*). Effects of thymol on the spontaneous contractile activity have been found in in-vitro experiments with circular smooth muscle strips from guinea pig stomach and vena portae. Thymol was found to possess an agonistic effect on α_1-α_2 and β-adrenergic receptors. Thymol has shown analgesic effect through its action on the α2 adrenergic receptors of the nerve cells.

***Key words**: Thymus vulgaris* L., essential oil, composition, smooth muscle fibres, α_1-α_2 and -adrenergic receptors, natural anti oxidants, *Fragaria ananassa*, Rosaceae, volatile components, antifungal, *Botrytis cinerea*, *Rhizopus stolonifer, Spodoptera litura*

INTRODUCTION

Thyme (*Thymus vulgaris* L.) belonging to the lamiaceae family is a pleasant smelling perennial shrub, which grows in several regions in the world (Davis, 1982). Its well known aromatic plant and its essential oil and aromatic water are used in the mountain regions of the Mediterranean parts of Turkey. Thyme was used by the Greeks as an incense in their temples and by the Romans in cooking and as a source of honey. Essential oils extracted from fresh leaves and flowers can be used as aroma additives in food, pharmaceuticals and cosmetics (Simon *et al.,* 1999 and Senatore, 1996). Traditionally basil has been used as a medicinal plant in the treatment of headaches, coughs, diarrhea, constipation, warts, worms and kidney malfunction (Simon *et al*., 1999). Thyme also possesses various beneficial effects as antiseptic, carminative, antimicrobial and antioxidative properties (Baranauskiene *et al*, 2003). The main essential oil in thyme, thymol is active against *Salmonella* and *Ataphylococcus* bacteria. The main constituents of thyme include thymol, carvacrol and flavonoids. The known primary constituents of thyme include essential oil (borneol, carvacrol, linalool, thymol), bitter principle, tannin, saponins and triterpenic acids. Its used to suppress coughing ease chest congestion and stimulate production of saliva (Baytop, 1984; Jellin *et al.*, 2000; Lueng and Foster,1996; Barnes *et al.,* 2002). Thyme also known as creeping thyme, mountain thyme and wild thyme a small shrubby plant with a strong spicy taste and order is extensively cultivated in Europe and US for culinary use. Thymol shows spontaneous contractile activity (SCA) of smooth muscle strips (SCA) from the stomach and vena portae of guinea pigs (Beer *et al.,* 2007).

MATERIAL AND METHODS

Plant material: Dried aerial parts of *Thymus vulgaris* were collected at the flowering stage. Plants were identified and authenticated by plant taxonomist.

Recovery of the essential oil: Dried over ground parts of plants (about 100g) were cut into small pieces and subjected to hydrodistillation for 3hrs using a Clevenger type apparatus; the oils obtained were dried using anhydrous sodium sulphate. Essential oil yielded from the air dried over ground parts of *Thymus vulgaris* was 1.6%.

The other methods used for extraction of essential oils were the oven drying and the wire basket dryer respectively. Here the percentage of essential oils extracted were calculated on 100g (dry wt. basis) and found to be 0.5 and 0.6%. The density of the extracted essential oil was lighter than water (0.925g/ml at 20^0c). The difference is due to the continuous heating in the oven for 2h. Dehydration date from drying of Thyme (*Thymus Vulgaris* L) herbs were shown in Table 1.

Table 1. Dehydration data from the drying of thyme (*Thymus vulgaris* L.) herbs using the oven and the wire basket methods

Condition/ parameters	oven drying Method	wire basket drying Method
Initial moisture Content(% wet wt basis)	75.15	75.15
Final moisture content (% dry wt basis)	10	14
Specific drying rate (h), k	0.433	0.270
Correlation coefficient	-0.9825	-0.9855

Determined by the Dean-stark toluene method

Gc/Ms Analysis Conditions

The Gc/Ms analyses were performed in a Varian 1400 Gc coupled with a Saturn II ion trap detector. The column and column temperature programme were the same as above. The interface temperature was 280^0c, split ratio 1:100, carrier gas He, flow rate 1.0ml/min, ionization energy 7oev, mass range 40-350; volume injected was 0.4µl diluted in hexane (1:10) Identification of the individual components was based on comparison of their GC retention indices (RI) on polar columns and comparison with the mass spectra of the components by Gc/Ms.

RESULTS AND DISCUSSION

Chemical composition of the essential oil of *Thymus vulgaris* are given in Table 2; in order of the retention times and Kovats indices of the constituents. Thirty compounds were identified by hydro distillation from aerial parts of *Thymus vulgaris*; These were: thymol 46.2% γ terpinene (14.1%), P-cymene (9.9%), linalool (4.0%), myrcene (93.5%), α-Pinene (3.%) and α -thujene (2.8%).

Table 2. Composition of the essential oils if *Thymus Vulgaris* L collected at Gulnar (Mersin), Turkey

RT	RI	Components	Concentrations(%)
8.363	931	ά- thujene	2.84
8. 584	939	ά- pinene	2.97
10.159	980	βpinene	0.71
10.371	988	octan-1-en-0-o1	0.48
103.823	991	mycrene	3.45
11.237	1005	ά – phellandrene	0.42
11.716	1018	ά – terpinene	2.69
12.048	1026	p-cymene	9.91
12.177	1031	limonene	1.23
12.232	1033	1,8-cineole	1.96
13.365	1062	γ-terpinene	14.08
13.54	1068	cis-sabinen hydrate	0.19
14.296	1088	terpinolene	0.13
14.784	1096	linalool	3.99
17.290	1177	terpinen-4-ol	0.25
19.141	1235	thymol methyl ether	1.78
21.334	1290	thymol	46.21
21.334	1298	carvacrol	2.44
22.605	1352	terpinyl acetate	0.68
22.771	1356	eugenol	0.1
23.664	1386	β- bourbonene	0.09
23.830	1391	β- elemene	0.14
24.051	1401	methyl eugenol	0.21
24.641	1418	β- caryophyllene	1.64
24.880	1430	β- copaene	0.16
25.534	1454	ά humulene	0.17
26.271	1480	germacrene D	0.4
26.935	1509	β- bisabolene	0.33
27.331	1520	δ- cadinene	0.14
28.897	1581	caryophyllene oxide	0.21

Thymus specie oil was the subject of several studies conducted in the past. It was previously reported that the oil of *T. vulgaris* contained thymol (44.1-58.1%), P-cymene (9.1-18.5%), γ-terpinene (6.9-18.9%) and carvacrol (2.4- 4.2%). Zambonelli *et al.,* (2004) found thymol (22-38%), γ-terpinene and p-cymene.

Antioxidant activities of aroma chemicals

To measure antioxidant activities of the aroma chemicals in this study, the aldehyde (carboxylic acid conversion assay was used. This is a relatively simple assay that can be used to measure the antioxidant potential of a chemical in an organic, non-aqueous phase. In this assay, volatile chemicals or extracts dissolved in the organic solvent, dichloro methane can easily be evaluated for their antioxidant potential.

Table 3 shows the percent of hexanal remaining in each sample containing the different amounts of aroma chemicals, BHT and α-tocopherol through out a storage period of 30 days. Among the chemicals identified in the extracts from thyme and thymol carvacrol, 4-allylphenol and eugenol exhibited potent antioxidant activities among the tested materials, those aroma chemicals inhibited hexanal oxidation by 95-99% at 5μg /ml over 30 days, which is comparable to that of α- tocopherol or BHT. Both α - tocopherol and BHT inhibit hexanal oxidation by 89% and 99% at 5μg/ml over 30 days respectively. The antioxidant activity of eugenol has been reported several times, tested on various systems, thymol and carvacrol major components of aroma extracts of eucalyptus leaves have also shown strong antioxidant activities in several studies. However 4-allylphenol which was quantified in basil (0.257 mg/g) has not been investigated for antioxidant activity to date. At the lowest level of 1μg/ml, eugenol displayed the highest antioxidant activity among the chemicals tested in the assay. Eugenol inhibited hexanal oxidation by 32% at 1μg/ml over 30days. Among the other aromatic components tested for their antioxidant activity only benzyl alcohol showed slight antioxidant activity at the level of 50μg/ml in the present study. Benzyl alcohol has previously been reported to possess antioxidant activity.

An alkyl compound with a double bond, 1-Octen-3-ol, also showed slight antioxidant activity. Considering the concentration and the anti oxidative performance of each aroma chemical investigated in this study; thymol (8..55mg/g) constituted 70% of quantified total volatiles, was found to be a main contributor to the antioxidant activity of volatile extract of thyme at 10µg/ml comparable to BHT and α-tocopherol at the same concentration as found in a previous study.

Table 3. Percentage of hexanal remaining in solutions treated with different amounts of volatile components in thyme and basil, α- tocopherol and BHT through out a storage period of 30 days

Components	inhibitory effect (%)				
	0µg/ml	1µg/ml	5µg/ml	10µg/ml	50µg/ml
Eugenol	6 ± 2.7	32 ±7.6	99± 2.8	99 ± 1.3	100 ± 5.7
Thymol	5 ± 0.4	11 ± 7.1	95 ± 6.2	100 ± 0.5	100 ± 0.7
Carvacrol	6 ± 3.3	15 ± 8.2	100 ± 2.9	100 ± 0.8	100 ± 0.8
4-Allylphenol	2 ± 1.6	14 ± 10	100 ± 1.5	100 ± 0.4	99 ± 4.2
1-Octene-3-ol	2 ± 3.6	4 ± 3.2	9 ± 3.5	11 ± 2.4	19 ± 2.0
Benzyl alcohol	2 ± 1.6	8 ± 5.8	10 ± 4.5	32 ± 5.4	59 ± 10
Linalool	6 ± 1.8	7 ± 4.7	3 ± 3.4	8 ± 3.7	23 ± 4.9
Methyl salicylate	3 ± 1.6	9 ± 2.7	9 ± 0.5	9 ± 0.3	10 ± 1.4
Estragol	5 ± 0.4	8 ± 2.2	9 ± 0.8	10 ± 0.4	12 ± 2.0
1,8- Cineole	3 ± 0.5	4 ± 2.8	5 ± 4.1	7 ± 3.7	11 ± 0.9
4-Terpeneol	4 ± 0.7	2 ± 2.5	4 ± 2.6	8 ± 2.7	14 ± 0.9
Benzylaldehyde	5 ± 5.2	7 ± 5.8	5 ± 2.7	8 ± 2.5	10 ± 2.1
α Tocopherol	2 ± 0.2	17 ± 9.9	89 ± 0.6	99 ± 2.2	98 ± 3.0
BHT	5 ± 0.2	100 ± 0.8	99 ± 0.8	99 ± 0.7	100 ± 0.1

From the above discussion we conclude that the thyme exhibited varying amounts of anti-oxidative activity. In particular, eugenol, thymol, carvacrol and 4-allylphenol found in thyme exhibited potent antioxidant activity, comparable to the known antioxidants, BHT and α-tocopherol.

Use of thymus oil against insecticidal activity

The essential oils used in this study were purchased from Caesar and Loretz in Hilden, Germany. *Thymus vulgaris* (white thyme) oil was obtained from liberty natural products, Portland or USA. The studied activity was obtained from larval mortality which was recorded after 24h after topical administration of 100ug of essential oil delivered in 1ul actone. Control received acetone alone. For each oil there were three replicates each with 10 larvae. LD_{50} values were determined by probit analysis, based on a range of 4-5 doses. The results were shown in table 4.

Table 4. Toxicity of essential oils to 4th instar larvae of *Spodoptera litura*

Oil	Species_family	Monograph	Mortality (%)[a]	LD_{50} (ug/larva)[b]
Oleum Saturejae hortensis	*Satureia hortensis* L. (Lamiaceae)	[1]	100.0(0	48.4 (44.2)53.1.
Oleum serpylli	*Thymus serpyllum* L. (Lamiaceae)	[2]	96.7(3.3)	~50c
Oleum Origani cretici	*Origanum creticum* L. (Lamiaceae)	[3]	93.3(3.3)	66. (58.0-75.1)0
Menthae arvensis	*Mentha ar¨ensis* L. aetheroleum var. *piperascens* Holmesex Christy (Lamiaceae)	[4]	50.0(10.0)	ND[d]
Menthae piperitae aetheroleum	*Mentha piperita* L. (Lamiaceae)	[5]	40.0(5.8)	ND
Citronellae aetheroleum	*Cymbopogon winterianus* lowitt (Poaceae)	[6]	36.7(6.7)	ND
Hyssopi aetheroleum	*Hyssopus officinalis* L. (Lamiaceae)	[7]	26.7(8.8)	ND
Oleum Absinthii	*Artemisia absinthium* L.(Asteraceae)	[8]	26.7(8.1)	ND
Salviae aetheroleum	*Sal¨ia officinalis* L (Lamiaceae)	[9]	23.3(3.3)	ND
Lavandulae aetheroleume	*La¨andula angustifolia* Miller (Lamiaceae)	[10]	20.0(5.8)	ND
Lavandulae aetherolcumf	*La¨andula angustifolia* Miller (Lamiaceae)	[10]	20.0(5.8)	ND
Matricariae aetheroleum	*Matricaria recutita* L. (Asteraceae)	[4]	20.0(5.8]	ND
Oleum Spicae	*La¨andula latifolia*	[11]	16.7(6.7)	

Villars._Lamiaceae.				
Menthae crispae aetheroleum *aquatica* Hell. var. *crispa* Benth.; *M. longifolia* Nath. var. *crispa* Benth. (Lamiaceae)	*Mentha spicata* L. var. *crispa* Benth.; *M*.	[12]	10.0(10.0)	ND
Limonis aetheroleum Burman f._Rutaceae.	*Citrus limon* (L.)	[13]	10.0(5.8)	ND
Caryophylli floris aetheroleum (Myrtaceae)	*Syzygium aromaticum*(L.) Merrill et Perry	[14]	6.7(3.3)	ND
Pini pumilionis aetheroleum	*Pinus mugo* Turra (Pinaceae.)	[15]	6.7(6.7)	ND
Cinnamoni aetheroleum (Lauraceae.)	*Cinnamomum¨erum* J.S. Presl.	[12]	3.3(3.3)	ND
Oleum Cinnamomi Cassiae	*Cinnamomum cassia* Blume (Lauraceae)	[16]	0.0(0.0)	ND
Oleum Cupressi (Cupressaceae.)	*Cupressus semper¨irens* L.	[17]	0.0(0.0)	ND

[a]Values are mean_"S.D.., *n*s3_determined at 100 mgrlarva..
[b]95% Confidence limits in parentheses.
[c] Estimated value; data from two experiments failed to adequately fit a linear dose]response model.
[d]ND, not determined.
[e]Quality 38]48% Mont Blanc.
[f]Quality 38]48% Barˆeme.

Here we got the conclusion that three of the essential oils were highly toxic to the cutworms: oils of *Satureia hortensis*, *Thymus serpyllum* and *Origanum creticum*. Oil of white Thyme (*Thymus vulgaris)* was comparable in toxicity (LD_{50}=46.7ug/$_{larva}$) to oils of *T.Serphyllum* and *S. hortensis*. Oils of *O. Creticum* were significantly less toxic. The insecticidal action of *Thymus* and *Satureia* oils are carvacrol, toxicities of which were previously established (LD_{50}=25.5 and 42.7 respectively). Evaluation of 22 essential oils for insecticidal action against the bean beetle (*Acanthoscelides obtectus)* indicated that oils of T.serpyllum and *O.marjorane* were the most toxic.

Antifungal activity of thymol

Effect on radial growth:- In corporation of different concentrations two types of thyme oil into potato dextrose agar (PDA) showed significant reduction (P< 0.05) on the growth of two common pathogens of strawberries *B.cinerea* and *R.stolonifer* (Table 5 and 6). Complete inhibition, however was not achieved at the concentration tested in this study; indicating that the essential oils are fungistatic rather than fungicidal at these concentrations. In general, the rate of inhibition was less in *R. stolonifer* exposed to different concentrations of thyme oil. Also the growth of R. stolonifer was faster than that of *B-cinerea* and the mycelium reached the edge of the untreated PDA plates within 4 days of inocculation compared to 7 days of *B.cinerea*. There was a significant difference (P<0.05) between the oils from laval – 1 and laval – 2 in their inhibitory action, with oil from laval – 2 exhibiting a comparatively higher antifungal activity which can be related to its high thymol, carvacrol and Linalool contents. Thymol has been shown to inhibit the growth and toxin production by mycotoxigenic molds and earlier investigation showed that carvacrol completely inhibited mycelial growth of all *Rhizopus* spp. with a minimum of 5ul.

Table 5. Effect of thyme oil on the radial growth of Botrytis cinera and Rhizopus stolonifer on potato-dextrose agar

	Inhibition (%)*			
	Laval – 1		**Laval – 2**	
Concentration (ppm)	***B. cinerea***	***R. stolonifer***	***B. cinerea***	***R. stolonifer***
0	0.0	0.0	0.0	0.0
50	26.5(±0.7)	5.5(±0.9)	36.9(±0.6)	11.5(±0.5)
100	32.3(±0.6)	9.5(±0.3)	49.0(±0.9)	20.1(±0.8)
200	63.5(±0.9)	50.5(±0.6)	90.5(±1.5)	65.8(±1.3)

Table 6. Effect of thyme oil volatile exposure on the incidence of gray mold and soft rot in strawberry fruits

		Decay (%)			
		Laval – 1		**Laval – 2**	
Concentration (ppm)	**Storage (days**	***B. cinerea***	***R. stolonifer***	***B. cinerea***	***R. stolonifer***
0	7	43.5(±1.5)	55.3(±2.1)	43.5(±1.3)	55.3(±2.1)
	14	85.3(±0.9)	90.5(±2.5)	85.3(±3.1)	90.5(±3.3)
50	7	15.5(±1.3)	25.3(±1.9)	12.5(±1.1)	20.5(±1.3)
	14	18.3(±0.9)	33.7(±2.1)	17.5(±1.5)	31.7(±1.7)
100	7	12.3(±1.1)	19.7(±0.9)	10.5(±0.7)	18.7(±0.9)
	14	16.5(±1.5)	25.5(±1.3)	14.7(±1.1)	23.7(±1.1)
200	7	6.1(±0.9)	10.5(±0.7)	5.1(±0.3)	7.9(±0.6)
	14	11.7(±1.2)	17.5(±0.9)	9.5(±0.6)	15.7(±1.1)

Percentage of infected strawberries was based on five replicates of 10 fruits each values in the parentheses are standard errors of the mean

Effect on inoculated strawberries

The effect of thyme oil volatiles on the control of fungal growth in inoculated strawberries was evaluated. Thyme oil volatiles were highly effective in reducing gray mold and soft rot incidence in strawberry fruits caused by *B.cineria* and *R.stolinifer*, respectively (Table 6). Fungal infection was noticed after 6 days with thyme treatments, while in the control, the infection were evident after 2 days of storage, at 13^0. In addition significant increases in decay were noticed from day 7 to day 14 in the control experiments. Exposure to volatile oil at 200ppm from laval – 1 reduced gray mold and soft rot incidences by more than 70% after 14 days of storage. The control of decay in fruits subjected to volatiles from laval – 2 was significantly (P< 0.05) higher compared, to the oil from laval – 1 which was again related to its higher composition of antimicrobial compounds. The gray mold and soft rot incidences were reduced by 75.8 and 74.8% respectively at 14 days of storage by exposure to maximum concentration of volatiles from laval – 2. No visible phytotoxic symptoms were noticed on treated strawberry fruits. As observed in the inhibition test on PDA, the effect of thyme oil on the control of gray mold and soft rot was dependent on the oil concentration. Significant decreases in the decay with increase in the oil concentration was observed.

In conclusion, this study demonstrates the potential of thyme oil volatiles as an antifungal preservative for strawberry fruits that are quite susceptible to decay caused by *B. cinerea* and *R.stolonifer*. However further studies are required to determine the optimal concentrations of thyme oil and exposure time for decay control and the sensory quality of the treated fruits.

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