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ORIGINAL ARTICLE

Identification and Comparison of Essential Oil Components in Leaf and Stem of Garden Thyme Grown Under Greenhouse Conditions

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Shahram Sharafzadeh, Morteza Khosh-Khui, Katayoon Javidnia, Omid Alizadeh, Kourosh Ordookhani: Identification and Comparison of Essential Oil Components in Leaf and Stem of Garden Thyme Grown Under Greenhouse Conditions

ABSTRACT

Garden thyme (*Thymus vulgaris* L.), member of Lamiaceae family, is one of the important medicinal plant species. The plant is used in medicine, cosmetic and food industry. In this study thyme plants were grown in greenhouse. Hydrodistillation was used to isolate the essential oils and chemical analyses were performed by GC and GC-MS with three replications. The yield of the oils of leaves and stem were 1.5% and 0.03% respectively. Twenty six components in leaf and 18 components in stem essential oils of thyme were identified. The major components of leaf essential oils were thymol (56.9%), γ-terpinene (10.5%), *p*-cymene (8.2%), carvacrol (5.5%), linalool (3.3%) and β-caryophyllene (3.1%). Similarly the major components of stem essential oils were thymol (55.5%), borneol (10.4%), *p*-cymene (6.0%), carvacrol (4.6%), linalool (3.6%), caryophyllene oxide (2.2%), γ-terpinene (2.1%) and eicosane (1.8%).

Key words: *Thymus vulgaris*, thymol, carvacrol, GC-MS

Introduction

Thyme (*Thymus vulgaris* L.) is a herbaceous perennial plant belonging to the Lamiaceae family. The plant is native to the western Mediterranean region and southern Italy [21]. There are 350 species of thyme cultivated all over the world [31]. The green part of thyme plant constitutes the most popular herbal medicine and spice, used in all developing countries. The beneficial effects of thyme are well known from ancient times and consumption of its extract is recommended all over the world [1]. It is considered as the main ingredient of many phytopreperations and commonly used as water extracts for its pharmacological activities and thus, have a very important role in phytotherapy [30]. Recently, thyme has become one of the most important medicinal plants used as a natural additive in poultry and livestock feeding studies [6,13]. Such studies have shown that thyme plant could be considered as an alternative natural growth promoter for poultry instead of antibiotics [22].

Essential oil content of thyme has been reported from 0.32% [26] to 4.9% [8].

Thymol and carvacrol, which are the principal constituents of thyme oil [3] have been reported to act as antioxidant [19], antimicrobial agent [9,29], antifungal agent [18] treatment for respiratory tract diseases [16], wound healing, a stomachic carminative, diuretic, urinary disinfectant and vermifuge [7].

The composition and quantity of essential oil from a particular species of thyme plant could be markedly affected by harvesting season [3], geographical environment and other agronomical factors [17,23].

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The published results reveal that major volatile constituents obtained from the aerial parts of the plant are geranial, linalool, carvacrol, thymol and trans-thujan-4-ol/terpinen-4-ol [24,25,27,28]. In samples of thyme were collected during the flowering period in eastern Morocco (Taforalt) in May, essential oil yield was 1.0% and camphor (38.54%), camphene (17.19%), α-pinene (9.35%), 1,8-cineole (5.44%), borneol (4.91%) and β-pinene (3.90%) were the major oil components [15]. Howewer, characteristic compounds of *T.vulgaris* essential oil are thymol (44.4 – 58.1 %), *p*-cymene (9.1-28.5%), γ-terpinene (6.9 – 18.9%) and carvacrol (2.4-4.2%) [4,5,10,11].

Various organic fertilizers were applied in North Sinai, Egypt and major components of essential oils were thymol (34.50-71.12%) and limonene (0.69-30.30%) [2]. In another experiment in Cairo, Egypt several organic fertilizers were used to grow the plant, and thymol (44.12-52.10%) and *P*-cymene (23.14-30.50%) were the main compounds of the oil [12].

This study focuses on identification of essential oil components in leaf and stem of thyme when the plants grown under greenhouse conditions.

Materials and Methods

This study was conducted in experimental glasshouse of Islamic Azad University, Firoozabad Branch (28°35' N, 52°40' E; 1327 m above sea level). Breeded seeds were sown and the seedlings were transplanted in pots containing 1/3 soil, 1/3 sand and 1/3 peat (v/v) at 4-6 leaf stage and kept at 27±3/17±3°C day/night temperatures. The soil of pots were tested before transplanting and soil texture was sandy-loam with pH=7.73, organic C=2.27%, total N=0.22%, available P=20.19 mg/kg, available K=176.7 mg/kg and EC=1.94 dS/m. After six months plants were cut to 10 cm above the pot soil surface and were dried at room temperature. Leaves were separated from the stem by hand.

Isolation of essential oils was performed using hydrodistillation of 50 g sample of leaves and stems separately by using a Clevenger-type apparatus over 4 hours. The oils were dried over sodium sulphate and the yields of the essential oils (w/w) were calculated.

Gas Chromatography analysis was performed on an Agilent technologist model (6890 USA) series II gas chromatograph equipped with flame ionization detector and capillary column HP-5 (30 m´ 0.25 mm, 0.25 μm film thicknesses). The chromatographic conditions were as follows: The oven temperature increased from 60 to 240ºC at a rate of 3ºC/min. The injector and detector temperatures were 240 and 250ºC, respectively. Helium used as the carrier gas was adjusted to a linear velocity of 32 cm/s. The samples were injected using split sampling technique by a ratio of 1:20. Quantitative data was obtained from electronic integration of peck areas without the use of correction factors. Essential oil was also analyzed by Hewlett- Packard GC-MS (model 6890 series II) operating at 70e V ionization energy, Equipped with a HP-5 capillary column (phenyl methyl siloxane (30 m´ 0.25 mm, 0.25 μm film thickness) with He as the carrier gas and a split ratio of 1:20. The retention indices for all the components were determined according to the Van Den Doll method using n-alkanes as standard. The compounds were identified by comparison of retention indices (RRI- AP-5) with those reported in the literature and by comparison of their mass spectra with the Wiley and mass finder 3 libraries or with the published mass spectra. The data was collected from three replications and standard deviation was calculated by excel software.

Results and Discussion

The yield of essential oils of leaves and stem were 1.5% and 0.03% respectively. Results obtained from qualitative and quantitative analysis of essential oils have been shown in Table 1. Twenty six compounds representing 99.0% of the oil of leaves and 18 components representing 93.7% of the oil of stems were identified. Thymol was the major component of both leaf (56.9%) and stem (55.5%) oils. Various reports have revealed that thymol is one of the major components in thyme oil [14,20,24,25,27,28]. Results revealed that there is 22% monoterpene hydrocarbons (α-thujene, α-pinene, camphene, myrcene, α-phellandrene, *p*-cymene, γ-terpinene, terpinolene), 7.1% monoterpene alcohols (1,8-cineole, cis-sabinene hydrate, linalool, borneol, menthol), 62.4% monoterpene phenols (thymol, carvacrol), 1.2% monoterpene phenol derivatives (thymol methyl ether, carvacrol methyl ether), 4.2% sesquiterpene hydrocarbons (β-caryophyllene, α-humulene, β-bisabolene, γ-cadinene, δ-cadinene), 0.5% oxygenated sesquiterpenes (caryophyllene oxide, 10-epi-γ-eudesmol) and 1.6% aliphatic alcohol (1-octen-3-ol) in leaf oil. Comparison of leaf and stem oil components by t-test shown significant differences in percentages of γ-terpinene, borneol and caryophyllen oxide. α-pinene was not identified in stem oil and borneol was in high level in stem oil. The percentage and composition of essential oil could be markedly affected by the geographical environment, places that plants is grown, physical and chemical characteristics of soil, seed source, plant age, parts of plant that which is used for oil isolation and oil isolation method.

Table 1: Amounts of the different chemical components in leaf and stem of thyme.

RI	Component name	% in leaf oil	% in stem oil	t-test
928	α-thujene	0.5 ± 0.1	n.d.	
936	α-pinene	0.4 ± 0.1	n.d.	
949	Camphene	0.2 ± 0.1	n.d.	
981	1-octen-3-ol	1.6 ± 0.2	n.d.	
991	Myrcene	0.8 ± 0.2	n.d.	
1003	α-phellandrene	0.1 ± 0.0	n.d.	
1015	α-terpinene	1.2 ± 0.2	n.d.	
1025	*p*-cymene	8.2 ± 1.9	6.0 ± 3.6	ns
1030	1,8-cineole	0.7 ± 0.2	0.5 ± 0.1	ns
1060	γ-terpinene	10.5 ± 2.0	2.1 ± 1.3	**
1069	Cis-sabinene hydrate	1.4 ± 0.2	1.2 ± 0.1	ns
1086	Terpinolene	0.1 ± 0.0	n.d.	
1102	Linalool	3.3 ± 0.3	3.6 ± 0.4	ns
1171	Borneol	1.2 ± 0.4	10.4 ± 3.6	**
1175	Menthol	0.5 ± 0.2	n.d.	
1233	Thymol methyl ether	0.6 ± 0.3	0.7 ± 0.2	ns
1243	Carvacrol methyl ether	0.6 ± 0.1	0.6 ± 0.2	ns
1280	Isopulegyl acetate	n.d.	0.3 ± 0.3	
1294	Thymol	56.9 ± 1.4	55.5 ± 5.2	ns
1303	Carvacrol	5.5 ± 3.8	4.6 ± 0.5	ns
1381	Isobornyl propionate	n.d.	0.3 ± 0.1	
1417	β-caryophyllene	3.1 ± 0.2	2.5 ± 1.1	ns
1450	α-humulene	0.1 ± 0.1	n.d.	
1505	β-bisabolene	0.3 ± 0.2	n.d.	
1512	γ-cadinene	0.3 ± 0.0	0.5 ± 0.2	ns
1521	δ-cadinene	0.4 ± 0.1	0.8 ± 0.2	ns
1580	Caryophyllene oxide	0.4 ± 0.1	2.2 ± 0.5	**
1621	10-epi-γ-eudesmol	0.1 ± 0.1	n.d.	
1917	Farnesyl acetone	n.d.	0.1 ± 0.0	
2000	Eicosane	n.d.	1.8 ± 1.5	
	Total (%)	99.0	93.7	

RI, retention index
Means of three replications ± SD
ns, not significant
n.d., not detected
**,significant (P<0.01)

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