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Essential Oil Composition of *Thymus vulgaris* L.

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Abstract

This study was carried out at the research field of Tarbiat Modares University, Peykan Shahr, Tehran to determine the changes in the chemical compositions of essential oil of thyme *(Thymus vulgaris* L.*)* at full flowering stage. The essential oils of the aerial parts were extracted by hydrodistillation method using Clevenger apparatus, and analyzed by GC and GC-MS. The main constituents of the EO were thymol and β-Cymene.

Keywords: *Thymus vulgaris* L.; Essential Oil; Thymol; β-Cymene.

Introduction

Thymus vulgaris L.(Thyme) is a little perpetual therapeutic botanical herb belongs to lamiaceae family which is one of the biggest families and generally notable blossoming plants, with around the range of 220 genera and practically 4000 species worldwide. Additionally it is known as common thyme and it is indigenous to Mediterranean region, to the North Africa and several parts of Asia along with carefully discriminating in areas of the entire world. Typically grow as a sub-shrub from 5 to 30cm in height with fibrous ligneous root and small, greenish-grey leaves having narrow edged (Al-Rawi, 1988). It has numerous hard, branched stems (10-20cm tall) and flowers blooming from May to September having distinctive fragrance may white or purple in color. Due to its strong aroma that is because of thymol, it is grown widely as culinary herb. Thyme has long history of been used in traditional medicine for treatment of various diseases for instance to treat respiratory diseases (whooping cough, bronchitis and asthma), in the form of tea, ointment, tincture, syrup or by steam inhalation. It is also used to prevent hardening of the arteries, treatment of toothache, urinary tract infection and dyspepsia (Hashim and Gamil, 1988). It also expels fungus from stomach and intestine and it has ability to increase appetite because of its important component thymol, which has ability to kill bacteria and parasites. Different studies were carried out in last decades to reveal reported pharmacological activities of *Thymus vulgaris* L. both of plant extracts and essential oil. In mice analgesic and antipyretic properties where reported for thyme extracts (Mohsin *et al.*, 1989). Thyme has changed from a traditional herb to a serious drug rational phytotherapy. It is incredible wellspring of iron, calcium, manganese, vitamin K and likewise upgrades blood flow and pushes an invigorating impact for the entire system. This herb invigorated activity on anxious framework made it as a cure for physical and mental weakness and additionally for diminishing insomnia. The remedial potential of *Thymus vulgaris* L. is due to the presence of flavonoids, thymol, carvacrol, eugenol, phenols, luteolin and tetramethoxylated. Its controls numerous valuable effects, such as, antispasmodic, bactericides, antiseptics, antioxidants, anthelmintic properties and has late been recommended as substitute as cancer prevention agent (Monira *et al.*, 2012).

The use of thyme oil is documented for medicinal purposes since 1589. From many decades, the essential oil of *Thymus vulgaris* L. been published in pharmacopoeias and standard text books of phytotherapy (Martindale, 1972). Volatile phenolic oil of thyme has been reported amongst the top 10 essential oils (Letchamo and Gosselin, 1996).

Thyme oil contains 46 % phenols of which 44 % thymol and 3.6 % carvacrol and also important components It is confirmed by various studies confirmed that thyme oil contains polyphenolic acid (oleanic acid, rosmarinic acid, triterpene and caffeic acid). Thyme oil also contains other components such as thymol, borneol, gerniol, pinen, linalool, cineol, sabinen, myrcen limonene and cymene (Rizk, 1986; Javed *et al.*, 2013).

Materials and Methods

The experiment was carried out at the research field of Tarbiat Modares University, Peykan Shahr, Tehran. Average annual precipitation at the site is 122.2 mm, minimum air temperature is -5° C and maximum air temperature is 40.4° C. The dominant winds at the area blow from Northeast. Some chemical characteristics of the experimental soils are shown in Table 1.

Table 1: Some physical and chemical characteristics of the experimental soil

EC ds m^{-1}	pH	OC[a] (%)	TN[b] (%)	P (mg kg^{-1})	K(mg kg^{-1})	Silt (%)	Sand (%)	Clay (%)
1.04	7.7	1.73	0.06	14	275	12	78	10

[a] Organic matter (OC), [b]Total Nitrogen (TN)

Aerial parts of flowering *Thymus vulgaris* L. grown in the open air were collected from May to July 2014. Collected plant materials were dried in the shade; the leaves and flowers of the plants were separated from the stem and ground in a grinder with a 2 mm in diameter mesh.

Essential Oils Preparation

All samples were shade-dried (during 15 days). EO was extracted by subjecting flowers and leaves together (40 g) to hydrodistillation for 2 h using an all glass Clevenger-type apparatus, according to the method outlined by the European pharmacopoeia (Anonymous, 1996). EO yield was expressed as percentage w/w on dry matter basis. The oils were dried over anhydrous Na_2SO_4 and stored in sealed vials at low temperature (4°C) before gas chromatography (GC) and gas chromatography/mass spectrometry (GC-MS) analysis.

Essential Oils Analysis by Gas Chromatography/Mass Spectrometry (GC/MS)

The EOs were analyzed by GC-MS (Agilent, USA). The analysis was carried out on a Thermoquest-Finnigan Trace GC/MS instrument equipped with a DB-5 fused silica column (60 m × 0.25 mm i.d., film thickness 0.25 mm). The oven temperature was programmed to increase from 60 to 250°C at a rate of 4°C/minute and finally held for 10 minutes; transfer line temperature was 250°C. Helium was used as the carrier gas at a flow rate of 1.1 mL/minute, with a split ratio equal to 1:50. The quadrupole massspectrometer was scanned over the 35-465 amu with an ionizing voltage of 70 eV and an ionization current of 150 mA.

GC-FID analysis of the oil was conducted using a Thermoquest-Finnigan instrument equipped with a DB-5 fused silica column (60 m x 0.25 mm i.d., film thickness 0.25 mm). Nitrogen was used as the carrier gas at the constant flow of 1.1 mL/minute; the split ratio was the same as that of GC/MS. The oven temperature was raised from 60 to 250°C at a rate of 4°C/minute and held for 10 minutes. The injector and detector (FID) temperatures were kept at 250 and 280°C, respectively. Semi-quantitative data were obtained from FID area percentages without the use of correction factors.

Identification of EO Components

Retention indices (RI) were calculated by using retention times of n-alkanes (C6-C24) that were injected after the oil at the same temperature and conditions. The compounds were identified by comparison of their RI with those reported in the literature and their mass spectrum was compared with the Wiley Library.

Results and Discussion

The compositions of EOs might be affected by the developmental stage of the plant (Saharkhiz *et al.*, 2011, Saharkhiz *et al.*, 2009). GC-MS was employed to identify the effective compounds (Table.2). In the present study, *Thymus vulgaris* L. plant contained 49 % thymol, 19.99 % β-Cymene, 7.63 % carvacrol and 6.79 % trans-caryophyllene. The composition of the essential oil of herbs and spices can vary greatly depending upon the geographical region, the variety, the age of the plant, the method of drying and the method of extraction of the oil (Jerkovic *et al.*, 2001). It is recommended to use a synergistic combination of essential oils and their compounds, e.g. carvacrol in combination with p-cymene (Ultee *et al.*, 2000), thus enabling to decrease their concentrations and minimize adverse sensorial effects.

Table 2: The major compounds in *Thymus vulgaris* L. at full flowering stage

Compound	**Peak area % in *thymus vulgaris* L.**	**RI**
α-Pinene	**3.10**	**939**
β-Pinene	**-**	**980**
α-Myrcene	**0.81**	**997**
β-Cymene	**19.99**	**1024**
1,8-Cineol	**1.22**	**1031**
γ-Terpinene	**0.87**	**1068**
Linalool	**2.31**	**1098**
Borneol	**0.01**	**1165**
4-Terpineol	**0.07**	**1189**
Anisole	**0.89**	**1197**
Carvacrol methyl ether	**4.44**	**1244**
Thymol	**49**	**1290**
Carvacrol	**7.63**	**1298**
Trans-caryophyllene	**6.79**	**1349**
Caryophyllene oxide	**1.7**	**1581**
Bisabolene	**-**	**1597**

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