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Original Article

Extraction of Thymol Compound from *Thymus vulgaris* L. Oil

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Abstract

Essential oils consist of many chemical compounds with different structures and functional properties, which might be employed as an ingredient in many foods, drug formulations, and products. In this research, leaves and aerial part of cultivated *Thymus vulgaris* L. was isolated by hydrodistillation. The chemical compositions of the essential oil were identified by the application of GC/MS. Then thymol, as the main ingredient, was purified by hot water or microwave methods. Subsequently, the yield and degree of purity of extracted thymol were determined. The results indicated the extraction efficiency of leaves essential oil (1.16 %) was more than aerial parts (0.62 %) and thymol the main ingredient in both essential oils includes 59.47 and 53.63 %, respectively. The highest yield of thymol extracted (91.27 %) was obtained from leaves with a purity of 95.27% by microwave extraction method. Extracted thymol might be recommended for use in formulation of the food and drug supplements due to numerous functional and pharmacological activities.

Keywords: *Thymus vulgaris* L., Essential Oil, Thymol, Purification.

Introduction

Thymus vulgaris L. is belonged to Lamiaceae family with several nutritional and therapeutic properties. It is commonly used as a culinary herb for food flavoring, in cosmetics and perfumery and in the pharmaceutical industries.

Essential oils, one of the major secondary metabolites of aromatic plants, are volatile, complex compounds that contain complex mixtures of organic substances with different functional groups and mainly terpenoids [1-5]. Thymol (2-isopropyl-5-methylphenol) is the main monoterpene phenol found in thyme essential oil. This compound has revealed several biological properties, including antibacterial, antifungal, immunomodulatory, anti-carcinogenesis, anti-inflammatory, antitussive, antispasmodic, and antioxidant activities [6-8].

The antioxidant activity of thymol has been attributed to its phenolic structure ,which might play an important role in adsorbing and neutralizing free radicals or decomposing peroxides. Also it has been reported that thymol antimicrobial effects might be due to interacting with adhesiveness, a major determinant of bacterial and fungal virulence [6,7].

There are considerable researches about compositional analysis of thyme essential oil, thymol extraction, and investigation of their properties [1-3,6-8].

Bermejo *et al* extracted thymol from *Thymus vulgaris* L. and *Thymus zygis* L. by three different green solvents namely ethanol, limonene and ethyl lactate by pressurized liquid extraction and supercritical fluid extraction at different conditions and stated that the highest concentrations of thymol obtained by supercritical CO2 extraction [7].

Borgarello *et al*. obtained fractions enriched in thymol by molecular distillation of oregano essential oil which showed higher antioxidant activity than oregano essential oil [9].

Therefore, in view of the therapeutic and nutritional effects of thymol, the aim of this research was purification of thymol from different parts of *T. vulgaris*.

Experimental

Materials

Fresh aerial parts (stems and leaves) of *T. vulgaris* L. cultivated in Hamadan, the west regions of Iran were

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randomly collected in September 2018. The identity of the *Thymus* was certificated by top experts from Herbarium of the Islamic Azad University, Science and Research Branch. The plants were dried at room temperature in a shadow place for 3 days. Arial parts (stems and leaves) and leaves of *T. vulgaris* L. were ground in a mill (Triplex, France), passed through a sieve of 30 mesh separately and the powders obtained were stored in amber glass bottles at 4 °C until further analysis. Thymol standard and all the chemicals used were of analytical grade, purchased from Merck Chemical Company of Germany.

Isolation of essential oil

Air-dried aerial parts (stems and leaves) and leaves of *T. vulgaris* were hydrodistillated for 2.5h using a Clevenger-type apparatus according to the standard procedure. The essential oil volume was measured directly in the extraction burette. The obtained essential oils were dried with anhydrous sodium sulphate (Merck Co. Germany) and then stored in a sealed dark vials at 4 °C until further analysis. Yield percentage was calculated as volume (ml) of essential oil per 100 g of plant dry matter [10].

Chemical composition of essential oils

The chemical compositions of essential oils obtained from aerial parts (stems and leaves) and leaves of *T. vulgaris* by using hydrodistillation method were identified by the application of gas chromatography/mass spectrometry (GC/MS). Samples were analyzed by using an Agilent Hp-6890 gas chromatograph (Agilent Technologies, USA) with a Hp-1 Methyl silicone capillary column (60 m×0.32 mm×0.25 µm film thickness) linked to Hp-5973 mass spectrometry detector. Oven temperature was set as 60 °C for 5 min initially and increased to 220 °C, at a rate of 7 °C/min and subsequently held isothermal for 3 min. Injector temperature was set as 230 °C. Helium was used as carrier gas at a flow rate of 31.5 cm/s and 1 µL sample was injected manually in the split mode. Ionization of sample compounds was performed in the ET mode (70 ev). The identification of compounds was performed by comparison of their mass spectral pattern and their linear retention indices based on a homologous series of normal alkanes (C8–C20) with those of authentic references and the Wiley 257 mass spectra databas [10]. To determine the amount of thymol in the essential oil, different concentrations (10, 100 and 250 ppm equivalent to 0.00001, 0.0001 and 0.00025 mg thymol per 1 µL of injectable solution to GC/MS) of thymol standard were injected to GC/MS and the calibration curve plotted (Fig. 1).

Thymol Purification Methods

Hot water method. 0.5 mL of 0.1 M NaOH was added to 1 mL of essential oil in a small test tube which alkaline solution was obtained and pH reached to 13. Then 2 mL of boiling distilled water at 100 °C was added and mixed with vortex shaker for 2 minutes after that the test tube placed on a stationary surface which a two-phase mixture created. Oily phase was separated and the pH adjusted to 7 with 2 M HCl. After that, 2 mL n-Hexane was added to the solution and shaked with vortex for completely dissolving of thymol in n-Hexane. The surface phase (hexane + thymol) was isolated and its solvent evaporated that pure thymol was obtained [11,12].

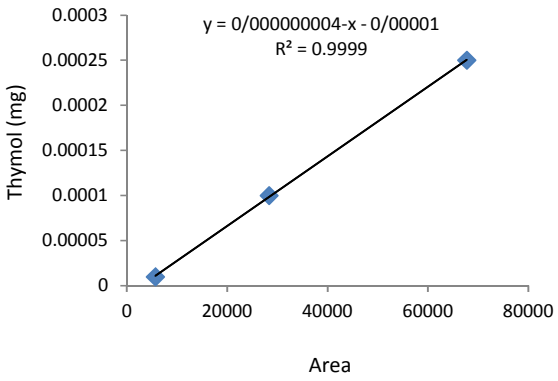

Fig. 1 Thymol standard calibration curve

Microwave Method

4 mL distilled water and 0.5 mL of 0.1 M NaOH was added to 1 mL of essential oil in a two-necked flask. The flask was placed in a microwave at 50 °C and a power of 400 w for 5 minutes. Then the solution was poured into the test tube which two-phase mixture created. Oily phase was separated and the pH adjusted to 7 with 2 M HCl. Then, 2 mL n-Hexane was added to the solution and shaked with vortex for completely dissolving of thymol in n-Hexane. The surface phase (hexane + thymol) was isolated, its solvent evaporated and yield purification calculated.

Fig. 2 The standard and purified thymol samples spotted on TLC

S: Thymol Standard, T1: Thymol extracted from Leaves, T2: Thymol extracted from aerial parts

The standard and purified thymol samples were spotted on a 20×20 cm thin layer chromatography (TLC) plate covered with 0.5 mm thickness of silica gel G type 60 (Merck). The plate was developed in Toluene: ethyl acetate (9.7:0.3), dried and observed under UV lamp. As shown in Figure 2, the Rf (Relative mobility factor) of the thymol standard and purified samples were identical. Then, to ensure the accuracy of thymol extraction, the purified samples were analyzed with GC/MS which a purity of 90.49% was obtained.

Statistical Analysis

All the experiments and measurements were carried out in triplicate. The data were statistically analyzed using SPSS 12.0 (SPSS Inc., Chicago, IL, USA). Variance analyses were performed by application of the ANOVA procedure.

Results

Table 1 Chemical composition of *Thymus vulgaris* L.

Composition	KI (Kovats Index)	Aerial parts essential oil (%)	Leaves essential oil (%)
1-Octen-3-ol	974	0.88	0.01
p-Cymene (Benzene)	1024	6.11	4.17
1,8-Cineole	1031	1.81	1.45
γ-terpinene	1059	6.86	3.64
Cis-sabinenehydrate	1070	0.85	0.44
Terpinolene	1088	5.55	6.02
Camphor	1146	1.15	0.94
Borneol	1169	4.48	4.01
Terpinen-4-ol	1177	1.88	1.64
1-Terpineol	1188	0.66	0.59
Thymol methyl ether	1235	4.29	4.85
Carvacrol methyl ether	1244	1.69	1.76
Thymol	1290	53.63	59.47
Phenol	1349	4.65	5.81
Caryophyllene -β	1424	4.33	4.06
Delta-cadinene	1523	0.71	0.67
Caryphyllene oxide	1583	0.46	0.47

The results indicated that leaves contain 55% of the total aerial parts of *T. vulgaris*. The yield of aerial parts (steam and leaves) and leaves essential oils were 0.62 and 1.16%, respectively. Chemical compositions of the essential oils are presented in Table 1.Thymol extraction efficiency in both hot water and microwave treatments have been compared in Table 2.

Based on the results, although the amount of thymol in essential oil extracted from aerial parts of *T. vulgaris* was higher than leaves essential oil, in both extraction methods the thymol extraction efficiency from leaves essential oil was more than twice of aerial parts which might be due to the presence of other components as impurity.

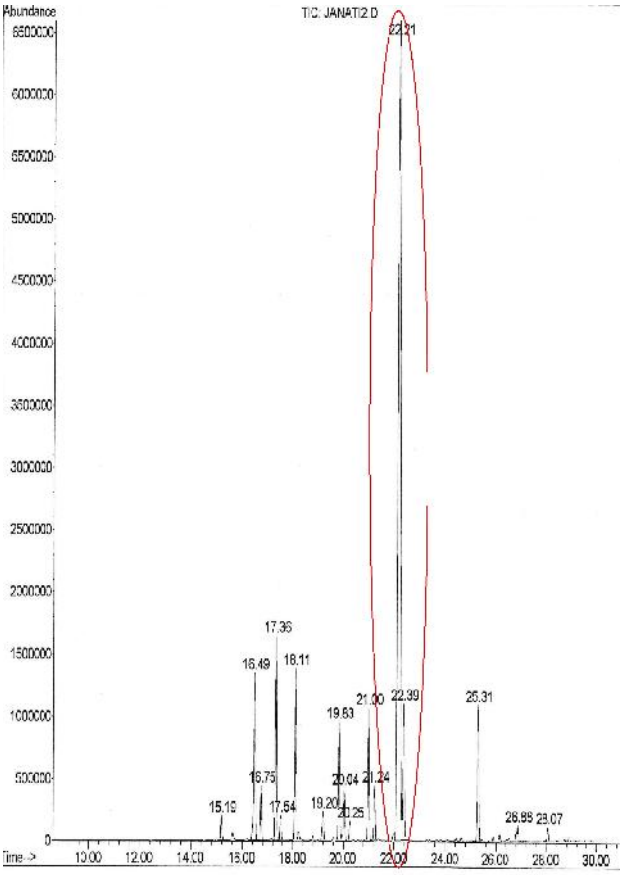

Fig. 3 GC/MS Chromatogram of *Thymus vulgaris* L. aerial parts essential oil

Table 2 Thymol extraction efficiency by hot water and microwave methods

Sample	Thymol (mg/µL essential oil)	Hot water method		Microwave method	
		Extraction yield (%)	Thymol purity (%)	Extrction yield (%)	Thymol purity (%)
Aerial parts	0.25 ± 0.06 a	30.70 ± 0.5 [b]	90.59 ± 1.1 b	34.16 ± 0.9 b	90.36 ± 2.1 b
Leaves	0.13 ± 0.02 b	71.20 ± 1.2 [a]	91.36 ± 0.7 a	91.27 ± 1.3 a	95.27 ± 1.8 a

[*] The values are expressed as means ± standard deviation, n = 3.

Different letters in each column indicate significant differences (P< 0.05).

Discussion

As shown in the Figure 3, seventeen constituents were identified by GC/MS of *Thymus vulgaris* L. aerial parts and leaves essential oils that the predominant compounds were thymol, γ- terpinene, *p*-cymene, α-terpinolene, phenol, endo bornel, β-caryophyllene and methyl thymyl ether, accounted for 89.90 and 91.84 % of the essential oils, respectively. Although the main compounds of essential oils extracted from both aerial parts and leaves are similar, the amounts of compounds are somewhat different so that thymol the main ingredient in both thyme aerial parts and leaves essential oils includes 53.63 and 59.47 % of these essential oils, respectively.

Porte and Godoy stated that thyme essential oil might be in thymol chemotype, carvacrol chemotype or other chemotypes, therefore it seems that both essential oils investigated in current study belong to thymol chemotype [13].

Lemrhari *et al.* analysed the chemical composition of the essential oils of different varieties of thyme and reported that chemical profiles of *Thymus munbyanus* Boiss. & Reut., *Thymus riatarum* Humbert & Maire and *Thymus saturejoides* Coss. are quite similar in terms of contents of Borneol and camphene. *Thymus broussonetii* Boiss. presents a profile rich and unique in carvone compared to other species. Also *Thymus leptobotrys* Murb., *Thymus zygis* L. and

Thymus vulgaris L. have a very similar profile richer in thymol and carvacrol than other species [14]. Also Satyal *et al*. stated that not only the particular chemotype of *T. vulgaris* is an important consideration, but the enantiomeric distribution may also have a profound influence on its bioactivity, flavor and aroma profile [15]. Therefore it should be noted that the composition of thyme essential oil especially the amounts of thymol and carvacrol, an important factor for determining its nutritional or pharmaceutical applications, varies considerably depending on thyme variety as well as geographical regions.

Thymol extraction yield from both essential oils was higher by microwave method as compared to the hot water method that might be due to the more efficient heat flow involved with microwaves. Also in all treatments the degree of purity of extracted thymol was more than 90% with a significant difference ($p<0.05$). The highest degree of purity was obtained in leaves samples, 95.27 and 91.36 % by microwave and hot water methods, respectively.

Conclusion

It might be concluded that leaves are the best parts of *T. vulgaris* for thymol extraction and the microwave method will present higher yields.

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