The Effect of Distillation Methods and Plant Growth Stages on the Essential Oil Content and Composition of *Thymus daenensis*

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Abstract

Background: ***Thymus daenensis*** **is on e of the endemic aromatic species in Iran that its essential oil is used in food and pharmaceutical industries.**

Objective: In this study, the effects of plant growth stages and distillation methods on essential oil content and thymol percentage of this species were evaluated.

Methods: The aerial parts of *Th. daenensis* were collected in three stages of plant growth from Tehran province of Iran. The plant materials were dried in shade and their essential oils were isolated by hydro-distillation in three replications. In addition, the essential oils of plant materials at full flowering stage were obtained by hydro-distillation, water&steam distillation and steam distillation. The essential oils were analyzed by capillary GC and GC/MS.

Results: The result showed that the different plant growth stages and distillation methods had significant effect on essential oil content. The highest essential oil content was obtained at full flowering stage (2.28% w/w). Among distillation methods, the highest essential oil content was obtained by hydro-distillation (2.27%) that was significant difference with other distillation method. There was no significant difference between essential oil composition at different plant growth stages. Also, thymol, p-cymene and γ-terpinene were the main components. The highest percentage of thymol was obtained by hydro-distillation.

Conclusion: Generally, it can be concluded that harvesting of *Th. daenensis* at full flowering stage and extraction of its essential oil by hydro-distillation were more suitable for obtaining the higher oil content and thymol percentage.

Keywords: ***Thymus daenensis*, Distillation methods, Essential oil, Plant growth stages, Thymol**

Introduction

The genus of *Thymus* (Lamiaceae) is mainly distributed in Mediterranean region. There are 350 species of *Thymus* L. all over the world. The genus *Thymus* L. represents 14 species in Iran [1], which four of them are endemic. One of these endemic species is *Th. daenensis*.

Thyme volatile phenolic oil has been reported to be among the top 10 essential oils, showing antibacterial, antimycotic, antioxidative, natural food preservative, and mammalian age delaying properties [2 - 4]. The essential oils of *Thymus* species are used in the food and drug industries [5 - 8]. These essential oils were previously evaluated in the many studies in respect of quality and quantity. Essential oil composition of *Th. kotschyanus* [9] (with 41.4% carvacrol and 19.6% thymol), *Th. carnosus* [10] (with 36.1% thymol and 21.3% *p*-cymene), *Th. pubescens* [11] (with 48.8% carvacrol and 13.9% thymol), *Th. persicus* [12] (with 27.1% thymol and 9.4% geraniol), *Th. serpyllum* [13] (with 22.7% γ-terpinene and 18.7% thymol), *Th. eriocalyx* [14] (with 1.8 – 60.4% linalool, trace – 50.5% geraniol and 1.6 – 58.4% thymol) and *Th. daenensis* [15] (with 49.7% thymol, 15.2% carvacrol and 6.4% p-cymene) have been reported previously.

The effect of different distillation methods on oil content and composition of aromatic plants have been also reported previously. The rose-scented geranium (*Pelargonium* sp.), was processed by various distillation methods, which revealed that water distillation of the herb gave a higher oil content (0.16-0.22%) than the water-steam distillation (0.09-0.12%) and steam distillation methods (0.06-0.18%). The distillation methods had also effect on the percentage of oil components [16].

The effect of distillation methods and stage of plant growth on the essential oil content and composition of *Thymus kotschyanus* were also studied, previously. The highest oil content was obtained by hydrodistillation method and the lowest by steam distillation. The essential oil content, related to distillation method and stage of plant growth, was 0.28-1.80% w/w (the highest for complete flowering stage by hydro-distillation method) [17]. The main components of all oils were carvacrol (46.7%-61.2%), thymol (7.5%-26.9%), γ-terpinene, p-cymene and borneol. Previous researches showed that *Th. vulgaris* produced the most oil content and thymol content at the beginning of flowering by hydro-distillation [18].

Due to the use of *Thymus* species or their essential oils in the food and drug industries [5 - 8] and because of the importance of thymol in the essential oil of *Th. daenensis*, we were interested in studying the effect of distillation methods and plant growth stages on the essential oil and thymol content. In this research, the effect of distillation methods and plant growth stages on the essential oil and thymol percentage of *Th. daenensis*, as Iranian aromatic and medicinal plant was investigated.

Materials and Methods

Plant material

The aerial parts of *Thymus daenensis* were collected at three stages of plant growth (before, at the beginning of and full flowering), from Tehran province in spring and summer of 2008. A voucher specimen has been deposited in the national herbarium of Iran (TARI).

Isolation procedure

Dried aerial parts (60-70 g, three

replications) of *Th. daenensis,* at all harvesting times, were subjected to hydro-distillation of 3h using an all glass Clevenger-type apparatus to produce oil [19]. For investigation the effect of distillation methods on oil content and composition of *Th. daenensis*, oils were extracted by different methods including hydro-distillation, water and steam distillation and direct steam distillation, in three replications. The oils were dried over anhydrous sodium sulfate and stored in sealed vials at low temperature (4°C) until it was analyzed.

Gas chromatography

GC analyses were performed using a Shimadzu GC-9A gas chromatograph equipped with a DB-5 fused silica column (30 m x 0.25 mm i.d., film thickness 0.25 μm). Oven temperature was held at 50°C for 5 min. and then programmed to 240°C at a rate of 3°C/min. Detector (FID) temperature was 265°C and injector temperature was 250 °C. Helium was used as carrier gas with a linear velocity of 32 cm/s. The percentages of compounds were calculated by the area normalization method, without considering response factors.

Gas chromatography-mass spectroscopy

GC-MS analyses were carried out on a Varian 3400 GC-MS system equipped with a DB-5 fused silica column (30 m x 0.25 mm i.d., film thickness 0.25 μm); oven temperature was 50-240°C at a rate of 4°C/min, transfer line temperature 260 °C, carrier gas helium with a liner velocity of 31.5 cm/s, split ratio 1:60, ionization energy 70 eV, scan time 1s, mass range 40-300 amu.

Identification of components

The components of the oils were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices, either with those of authentic compounds or with data published in the literature [20, 21]. Mass spectra from the literature were also compared [20, 22]. The retention indices were calculated for all volatile constituents using a homologous series of n-alkanes.

Statistical Analysis

Analysis of variance of the results was conducted using SAS software, and means in the results were compared using the Duncan's multiple range test ($p \leq 0.01$).

Results

The results showed that different stages of plant growth had significant effect ($p \leq 0.01$) on oil content of *Th. daenensis* (Table 1). Comparing of means showed the essential oil content in full flowering stage (2.28%) was more than the other stages (Table 2).

Fourteen components were identified in the essential oil of *Th. daenensis* in all stages of plant growth and also, thymol, carvacrol, E–caryophyllene, *p*-cymene and γ-terpinene were major volatile oil constituents (Table 3).

In second experiment, analysis of variance showed that different distillation methods had significant effect ($p \leq 0.01$) on oil content of *Th. daenensis* at full flowering stage (Table 4). Comparing of means showed the highest oil content was obtained by hydro-diatillation (2.27%) that had significant difference with other distillation methods (Table 5). The lowest amount of oil content content obtained by steam distillation (1.32%).

Table 1- Analysis of variance for the effect of different plant growth stages on essential oil content of *Th. daenensis* by hydro-distillation

Source of Variation	Degree of Freedom	Means of Squers
plant growth stages	2	0.195**
Error	6	0.0037
Total	8	-

** α= 0.01

Table 2- Means of *Th. daenensis* oil content at different stages of plant growth by hydro-distillation

Plant growth stage	Essential oil content
Full flowering	2.28 a
Beginning of flowering	2.04 b
Before flowering	1.77 b

Same letters in each column means no significant difference.

Table 3- Percentage composition of the *Th. daenensis* oils obtained by Hydro-distillation in different stages of plant growth

No	Compound	RI	RT	Before Flowering (%)	Beginning of flowering (%)	Full flowering (%)
1	α- Thujene	932	942	1.1	0.8	1.0
2	α-Pinene	938	954	0.4	-	0.4
3	β-Pinene	975	969	-	-	0.15
4	Myrcene	998	984	1.7	1.6	1.4
5	α-Phellandrene	1003	1026	-	-	0.1
6	α-Terpinene	1017	1041	1.0	0.7	0.9
7	*P*-Cymene	1026	1046	3.5	3.3	3.1
8	1,8-Cineole	1030	1061	0.4	0.4	0.5
9	γ-Terpinene	1060	1080	9.0	9.02	8.1
10	Borneol	1166	1211	0.7	1.0	1.0
11	*Methyl ether* Carvacrol	1242	1262	1.1	0.9	0.9
12	Thymol	1287	1310	73.4	72.6	70.1
13	Carvacrol	1296	1321	2.2	2.3	3.1
14	E–Caryophyllene	1420	1481	3.1	2.5	4.6
	Total			**97.6**	**95.12**	**95.35**

RI = Retention indices in elution order from DB-5 column

Fourteen compounds were identified in the oil of *Th. daenensis* at full flowering stage by three distillation methods (Table 6). The main components were thymol, E-caryophyllene, *p*-cymene and γ-terpinene.

Table 4- Analysis of variance for the effect of different distillation methods on oil content of *Th. daenensis* at full flowering stage

Source of Variation	Degree of Freedom	Means of Squers
Distillation method	2	0.6338**
Error	6	0.0089
Total	8	-

** α= 0.01

Table 5- Means of *Th. daenensis* oil content by different distillation methods at full flowering stage

Distillation method	Oil content
Hydro-distillation	2.27 a
Water and steam distillation	1.75 b
Steam distillation	1.32 c

Different letters in each column means significant difference

Table 6- Percentage composition of the *Th. daenensis* oils obtained by different distillation methods at full flowering stage

No	Compound	RI	RT	Hydro-distillation (%)	Water &steam distillation (%)	Steam distillation (%)
1	α - Thujene	932	942	1.0	1.4	1.1
2	α - Pinene	938	954	0.4	0.5	0.4
3	β - Pinene	975	969	0.2	-	-
4	Myrcene	998	984	1.4	2.3	1.8
5	α - Phellandrene	1003	1026	0.1	-	0.2
6	α - Terpinene	1017	1041	0.9	1.0	1.1
7	*P* - Cymene	1026	1046	3.1	3.9	3.5
8	1,8 - Cineole	1030	1061	0.5	0.6	0.6
9	γ - Terpinene	1060	1080	8.1	10.6	9.5
10	Borneol	1166	1211	1.0	0.7	0.7
11	Methyl ether carvacrol	1242	1262	0.9	1.0	1.0
12	Thymol	1287	1310	70.1	67.2	68.8
13	Carvacrol	1296	1321	3.1	2.2	2.9
14	E -Caryophyllene	1420	1481	4.6	5.8	4.9
	Total			**95.4**	**97.2**	**96.5**

RI = Retention indices in elution order from DB-5 column

Twelve components were identified in the essential oil of *Th. daenensis* before flowering, representing 97.6% of the oil. The main components were thymol (73.4%), γ-Terpinene (9.0%) and p-cymene (3.5%). Chemical composition of the oil can be seen in Table 5. Eleven compounds were characterized in the oil of *Th. daenensis* at the beginning of flowering, representing 95.1% of the oil. The major constituents were thymol (72.6%), γ-terpinene (9.0%) and p-cymene (3.3%). At full flowering stage

At full flowering stage, fourteen compounds were identified in *Th. daenensis* oil, representing 95.4% of the oil. The main components were thymol (70.1%), γ-terpinene (8.1%) and p-cymen (3.1%).

Twelve components were identified in the oil obtained by water and steam distillation representing 97.2% of the oil. The major constituents were thymol (67.2%), *p*-cymen (10.6%) and E-caryophyllene (5.8%). Thirteen compounds were characterized in the oil obtained by direct steam distillation representing 96.5% of the oil. The main components were thymol (68.8%), γ-Terpinene (9.5%) and E-caryophyllene (4.9%).

So, the results showed the highest percentage of thymol, was obtained by hydro-distillation.

Discussion

This study indicated that the essential oil content at different plant growth stages was significantly different and the highest oil content was obtained in full flowering stage. Of course, there were no significant differences between oil composition and thymol percentage in different plant growth stages. So it can be concluded that harvesting of aerial parts of *Th. daenensis* in full flowering stage was better than other stages of plant growth in respect of essential oil content. The result of this research is similar to the result of Morteza-Semnani et al (2006) on *Th. pubescens*, which produced the highest oil content at full flowering stage [23].

In addition, different distillation methods had significant effect on oil content of *Th. daenensis*. The highest oil content was obtained by hydrodistillation that had significant difference with other methods. The lowest oil content was obtained by steam distillation method. This may be due to this fact that in the steam distillation method, the situation of plant material, like type of plant material, mode of comminuting, mode of charging and grade of insulation are much more important than the other distillation method. These results are in agreement with the previous studies about the effect of distillation methods on oil content and composition of other essential oil-bearing plants [17, 23].

The results showed the highest percentage of thymol, was obtained by hydro-distillation. Previous studies showed that hydro-distillation of *Th. kotschyanus* aerial parts [17] and *Eucalyptus dealbata* leaves [24] produced higher oil contents in comparison with other distillation methods.

Conclusion

Although, there are some minor differences in the relative amounts of essential oil components in different satges of plant growth and distillation methods, but totally it can be concluded that for obtaining the highest oil content and thymol percentage from aerial parts of *Th. daenensis*, full flowering stage and hydro-distillation are recommendable.

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