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Original Article

Investigating the Storage Conditions of the Essential Oil Compounds of Garden Thyme

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ABSTRACT

The changes in the compositions of essential oils (EOs) from the aerial parts of *Thymus vulgaris* (garden thyme) were determined at different temperatures and storage times. In this study, the effect of time and temperature on the quality of essential oils was investigated. The essential oil of air-dried samples was obtained by hydro-distillation and was analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). Changes in essential oil compositions were detected during storage for three months in a refrigerator (4 °C), a freezer (−20 °C), and at room temperature. In thyme, the amount of the important thymol compound was stable until the first two months, but when entering the third month, a significant decrease was observed in all three temperature conditions. At the same time, a significant increase was observed in the amount of *p*-cymene in each of the temperature conditions. In the end, we can introduce the best temperature maintenance conditions for thyme at room temperature that can have a lower cost from an economic point of view than other conditions.

INTRODUCTION

In recent years, the use of essential oils in medical science to treat a variety of diseases in humans, including migraine [1], stroke [2] and depression [3], has been confirmed. Essential oils are a very important part of plant fragrances that are extracted from different parts of plants, such as leaves, flowers, skin, roots and fruits, each of which has special healing effects and provides energy [4]. In general, the production of aromatic substances in plants under stress is one of the important metabolic processes of plants. That is, essential oils are part of the plant's immune system [5]. *Thymus vulgaris* (thyme) is an herbaceous plant with many branches and wood that grows in mountainous areas between boulders, especially in European countries. This plant also belongs to the mint family, which is a shrub species called Shirazi Avishan in Iran. Shirazi Avishan is a plant with bright blue flowers that grows in high mountainous areas and has a very good aroma [6]. The medicinal organs of this plant are its leaves and flowers. The main compounds in the essential oil of this plant are carvacrol, thymol, linalool and *p*-cymene, whose antimicrobial effects have already been shown [7]. The essential oil of this plant is one of the most effective plant essential oils, and it has antioxidant and antimicrobial properties due to its phenolic monoterpene compounds [8]. Thymol is one of the most important compounds of oxygen monoterpene with antibacterial, antifungal and inhibitory growth and production of mycotoxins [9]. This plant has beneficial therapeutic properties; for example, it strengthens the gastrointestinal tract and acts as an ant flatulent, sedative, laxative, respiratory and gastrointestinal antiseptic and gastrointestinal antispasmodic [10]. Therefore, the aim of the present study was to investigate the influence of storage on the chemical compositions of essential oils in *T. vulgaris*. In this regard the composition of *T. vulgaris* was measured in different time periods stored at 4 °C, −20 °C, and room temperature.

MATERIALS AND METHODS

Plant Material

Samples of thyme (*T. vulgaris*) were collected (1000 gr) from Marvdash Shiraz in Iran, and all plants were harvested at full flowering stage. The plants were shade dried for 14 days at room temperature (20–25 ◦C). The EOs of all dried samples were isolated by hydro-distillation for 3 h, using a Clevenger. The distillated oils were dried over anhydrous sodium sulfate and put in tightly closed dark vials for further investigations. Plants were identified by Ahmad Hatami. Voucher sample of thyme (No. 14828) was deposited in the Herbarium.

Volatile Oils Storage Conditions

In order to investigate the impacts of different storage conditions on the compositions of distilled oils, the oil samples were subjected to different storage temperatures such as, refrigerator (4 °C), freezer (−20 °C) and at room temperature (25 °C) for three successive months until analysis. The oils analysis of all storage treatments performed monthly. Moreover, to determine the exact effects of storage conditions on EOs compositions during the experiment period, the fresh extracted oil was analyzed immediately after extraction.

Oil Analysis Procedure

GC analysis was performed using an Agilent gas chromatograph series 7890-A with a flame ionization detector (FID). The analysis was carried out on fused silica capillary HP-5 column (30 m × 0.32 mm i.d.; film thickness 0.25 μm). The injector and detector temperatures were kept at 250 °C and 280 ◦C, respectively. Nitrogen was used as carrier gas at a flow rate of 1 ml/min; oven temperature program was 60–210 °C at the rate of 4 °C/min and then programmed to 240 °C at the rate of 20 °C/min and finally held isothermally for 8.5 min; split ratio was 1:50. GC–MS analysis was carried out by use of Agilent gas chromatograph equipped with fused silica capillary HP-5MS column (30 m × 0.25 mm i.d.; film thickness
0.25 m) coupled with 5975-C mass spectrometer. Helium was used as carrier gas with ionization voltage of 70 eV. Ion source and interface temperatures were 230 °C and 280 °C, respectively. Mass range was from 45 to 550 amu. Oven temperature program was the same given above for the GC.

Identification of Compounds

The constituents of the EOs were identified by calculation of their retention indices under temperature-programmed conditions for n-alkanes (C8–C25) and the oil on a HP-5 column under the same chromatographic conditions. Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those of reported in the literature [11]. For quantification purpose, relative area percentages obtained by FID were used without the use of correction factors.

Statistical analysis

Data analysis was conducted using SAS software (v.25.0). Duncan's Test was used to compare the differences between groups (*P*< 0.05) between treatments.

RESULTS AND DISCUSSION

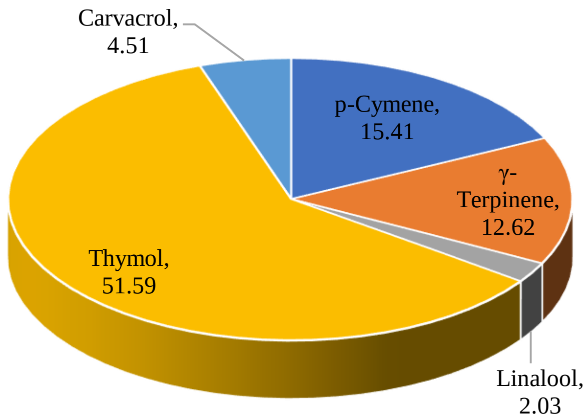

Fig. 1 Percentage of the main compounds of *T. vulgaris*

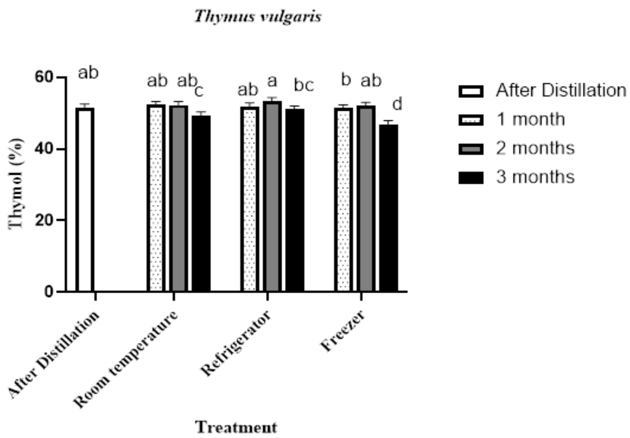

Fig. 2 The effect of different storage time and temperatures on the thymol content in *T. vulgaris* essential oil

There are little investigations about plant secondary metabolites storage especially essential oils as these metabolites are volatile and potentially could be subjected to different alterations by storage circumstances.

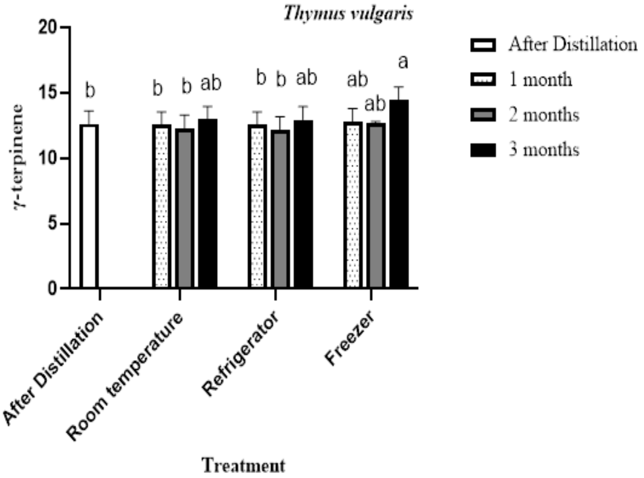

Fig. 3 The effect of different storage time and temperatures on the γ-terpinene content in *T. vulgaris* essential oil

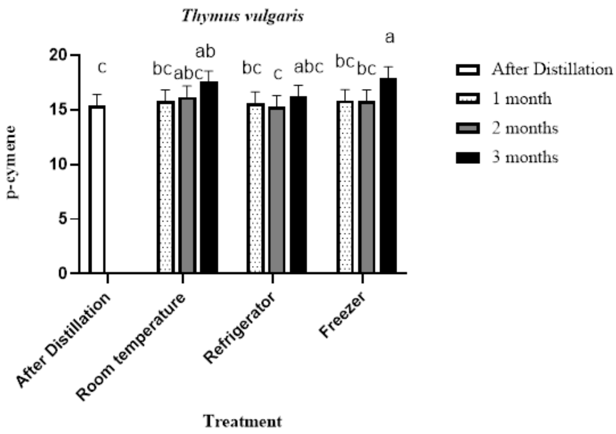

Fig. 4 The effect of different storage time and temperatures on the *p*-cymene content in *T. vulgaris* essential oil

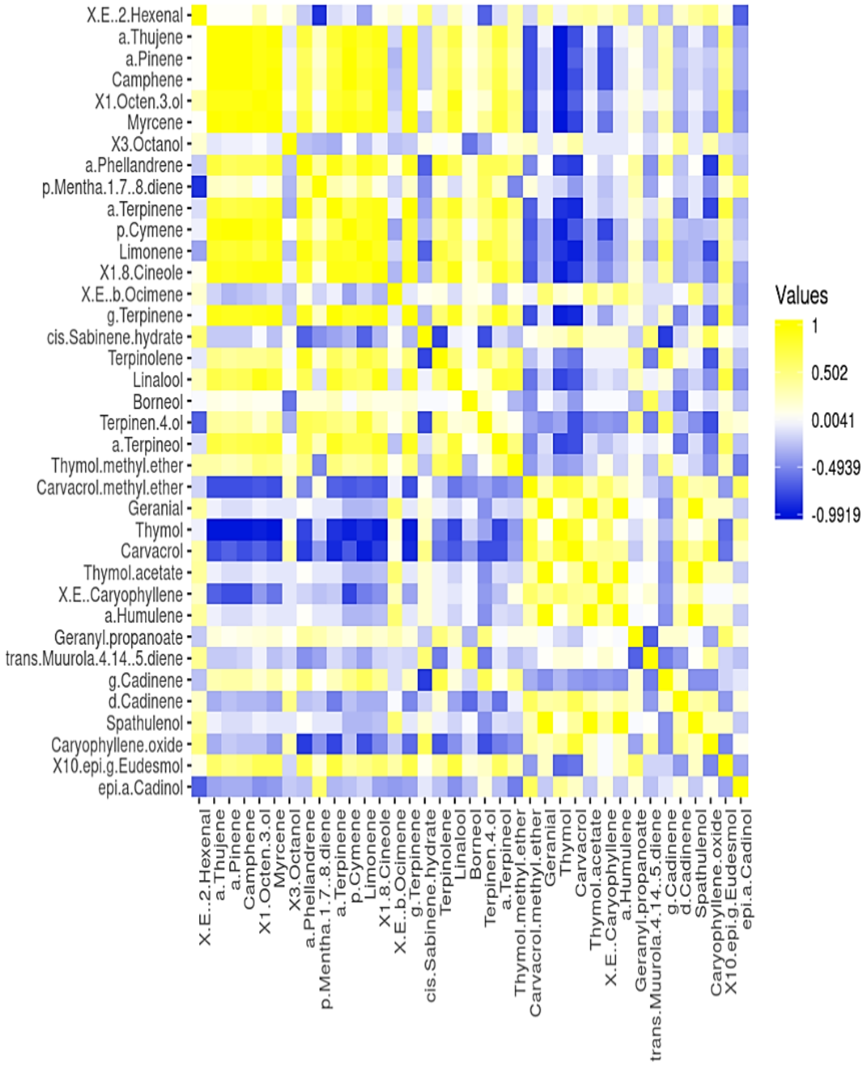

Fig. 5 The correlation heat map of essential oil compounds of *T. vulgaris*

In this study, the compositions of hydro-distilled essential oils of *T. vulgaris* were determined at different temperatures and storage times. In total, 37 constituents were identified and quantified in the *T. vulgaris* EOs samples representing more than 99.6% of the total (Fig.1 and Table 1- 3).

The first important compound that we considered in *T. vulgaris* is thymol. This compound showed the same trend in all three temperature conditions in the first and second months of storage, and very slight changes were observed in the third month such that the best temperature conditions for this compound were refrigerator temperature conditions, which showed a constant rate after three months. Meanwhile, in room storage conditions, the amount of this compound showed a significant decrease (Fig. 2). The third major drug compound studied was *p*-cymene. This compound showed an increase in all three storage conditions at the end of the experiment (Fig. 4). The quantity of *p*-cymene was 15.41% at the time of oil extraction and 15.76, 15.7 and 17.22 after one, two and three months of storage, respectively. The most important results of the present study were the increasing trend of *p*-cymene values during storage in all three temperature conditions.

The second important compound studied that showed a very interesting trend was γ-terpinene. After three months, the amount of γ-terpinene in all three temperature storage conditions remained steady and showed the highest durability among the important compounds. Thus, the optimal conditions with the lowest cost to maintain this compound are recommended. The quantity of this component was 12.62% at the time of oil extraction and 12.55, 12.60 and 13.31% after three months in the refrigerator, room temperature and freezer conditions, respectively. The concentration of this component was constant after three months of storage (Fig. 3).

The other two most important constituents of thyme are γ-terpinene and *p*-cymene. Thymol biosynthesis has been shown to occur through a monoterpene known as γ-terpinene and then the aromatic compound *p*-cymene as a mediator (Fig. 6.) The researchers showed that the evolution of thymol, with its precursors *p*-cymene and γ-terpinene, were completely opposite in all stages of storage [13]. The present study, in line with other studies, revealed the opposite trend of thymol with its precursors in storage conditions. Specifically, two thymol precursors showed an increasing trend with a decrease in thymol after three months. According to various studies in the field of essential oil storage in different plants, it can be concluded that the essential oil of each plant has a different reaction in storage conditions [13-16]. In lemon balm, the main constituents of essential oils, including nreal, geranial and citronellal, decreased in all three conditions after storage [15].

Table 1 Composition of *T. vulgaris* essential oils during 3 months storage at Freezer temperature.

No	Compound	RI*	RT**	After distillation (%)	After 1 month (%)	After 2 month (%)	After 3 month (%)
1	(E)-2-Hexenal	850	6.80	0.21	0.18	0.12	0.14
2	α -Thujene	926	8.63	0.78	0.78	0.77	0.93
3	α -Pinene	933	8.87	0.93	0.93	0.92	1.10
4	Camphene	949	9.35	0.41	0.41	0.41	0.48
5	1-Octen-3-ol	977	10.20	1.36	1.38	1.32	1.49
6	Myrcene	990	10.59	1.57	1.61	1.58	1.89
7	3-Octanol	995	10.75	0.10	0.10	0.09	0.10
8	α -Phellandrene	1006	11.10	0.20	0.20	0.20	0.23
9	p-Mentha-1(7),8-diene	1008	11.20	0.08	0.08	0.08	0.10
10	α -Terpinene	1018	11.55	1.82	1.86	1.85	2.14
11	p-Cymene	1024	11.80	15.41	15.86	15.80	17.93
12	Limonene	1027	11.90	0.55	0.60	0.61	0.72
13	1,8-Cineole	1032	12.10	0.53	0.55	0.54	0.61
14	(E)-β-Ocimene	1047	12.66	0.05	0.05	0.05	0.06
15	γ-terpinene	1060	13.15	12.63	12.81	12.72	14.44
16	cis-Sabinene hydrate	1071	13.55	0.47	0.47	0.41	0.42
17	Terpinolene	1090	14.28	0.17	0.17	0.17	0.18
18	Linalool	1099	14.60	2.04	2.09	2.02	2.17
19	Borneol	1169	17.50	0.79	0.85	0.84	0.84
20	Terpinen-4-ol	1178	17.85	0.31	0.68	0.68	0.68
21	α -Terpineol	1190	18.35	0.14	0.13	0.15	0.23
22	Thymol methyl ether	1235	20.22	0.57	0.57	0.57	0.58

23	Carvacrol methyl ether	1245	20.65	0.37	0.35	0.35	0.34
24	Geranial	1269	21.65	0.06	-	-	-
25	Thymol	1290	22.50	51.59	51.35	51.98	46.91
26	Carvacrol	1299	22.90	4.52	4.14	4.13	3.64
27	Thymol acetate	1356	25.20	0.02	-	-	-
28	(E)-Caryophyllene	1422	27.85	0.95	0.92	0.90	0.88
29	α -Humulene	1457	29.21	0.23	-	-	-
30	Geranyl propanoate	1475	29.90	0.08	0.15	0.07	0.10
31	trans-Muurola-4(14),5-diene	1494	30.65	0.04	-	-	-
32	γ -Cadinene	1514	31.40	0.09	0.10	0.10	0.10
33	δ -Cadinene	1524	31.76	0.16	0.15	0.15	0.14
34	Spathulenol	1579	33.80	0.06	-	-	-
35	Caryophyllene oxide	1586	34.05	0.30	0.24	0.20	0.17
36	10-epi-γ -Eudesmol-Eudesmol	1620	35.30	0.04	0.07	0.07	0.09
37	Epi-α-Cadinol	1640	36.00	0.11	0.12	0.11	0.11
-	Total	-		99.74%	99.96%	99.96%	99.95%

The specific essential oil constituent was identified through the GC-MS technique. RI*, Linear retention indices were calculated using a homologous series C8-C25 n-alkanes; RT**, Retention time. Identification***: R, Linear retention indices were calculated using a homologous series C8-C25 n-alkanes; MS, by comparison of the MS with those of the computer mass libraries NIST 05 library, Wiley and Adams.

Table 2 Composition of *T. vulgaris* essential oils during 3 months storage at refrigerator temperature.

No	Compound	RI*	RT**	After distillation (%)	After 1 month (%)	After 2 months (%)	After 3 months (%)
1	(E)-2-Hexenal	850	6.80	0.21	0.21	0.05	0.16
2	α -Thujene	926	8.63	0.78	0.76	0.73	0.79
3	α -Pinene	933	8.87	0.93	0.92	0.88	0.94
4	Camphene	949	9.35	0.41	0.40	0.39	0.42
5	1-Octen-3-ol	977	10.20	1.36	1.37	1.28	1.37
6	Myrcene	990	10.59	1.57	1.53	1.46	1.61
7	3-Octanol	995	10.75	0.10	0.10	0.10	0.11
8	α -Phellandrene	1006	11.10	0.20	0.18	0.19	0.22
9	p-Mentha-1(7),8-diene	1008	11.20	0.08	0.07	0.11	0.08
10	α -Terpinene	1018	11.55	1.82	1.81	1.77	1.91
11	p-Cymene	1024	11.80	15.41	15.64	15.29	16.22
12	Limonene	1027	11.90	0.55	0.54	0.58	0.65
13	1,8-Cineole	1032	12.10	0.53	0.54	0.52	0.57
14	(E)-β-Ocimene	1047	12.66	0.05	-	-	-
15	γ-terpinene	1060	13.15	12.63	12.54	12.18	12.94
16	cis-Sabinene hydrate	1071	13.55	0.47	0.51	0.42	0.34
17	Terpinolene	1090	14.28	0.17	0.15	0.16	0.19
18	Linalool	1099	14.60	2.04	2.05	1.98	2.07
19	Borneol	1169	17.50	0.79	1.18	0.82	0.86
20	Terpinen-4-ol	1178	17.85	0.31	0.30	0.66	0.69
21	α -Terpineol	1190	18.35	0.14	0.14	0.14	0.14
22	Thymol methyl ether	1235	20.22	0.57	0.56	0.55	0.58
23	Carvacrol methyl ether	1245	20.65	0.37	0.35	0.35	0.36
24	Geranial	1269	21.65	0.06	-	-	-
25	Thymol	1290	22.50	51.59	51.90	53.37	51.03
26	Carvacrol	1299	22.90	4.52	4.29	4.29	4.12
27	Thymol acetate	1356	25.20	0.02	-	-	-
28	(E)-Caryophyllene	1422	27.85	0.95	0.92	0.92	0.93
29	α -Humulene	1457	29.21	0.23	0.03	-	-
30	Geranyl propanoate	1475	29.90	0.08	0.31	0.10	0.09
31	trans-Muurola-4(14),5-diene	1494	30.65	0.04	0.09		-
32	γ -Cadinene	1514	31.40	0.09	0.15	0.10	0.11

33	δ -Cadinene	1524	31.76	0.16	0.03	0.15	0.16
34	Spathulenol	1579	33.80	0.06	-	-	-
35	Caryophyllene oxide	1586	34.05	0.30	0.32	0.25	0.21
36	10-epi-γ -Eudesmol	1620	35.30	0.04	-	-	-
37	Epi-α-Cadinol	1640	36.00	0.11	0.11	0.23	0.13
-	Total	-	-	99.74%	100%	100%	100%

The specific essential oil constituent was identified through the GC-MS technique. RI*, Linear retention indices were calculated using a homologous series C8-C25 n-alkanes; RT**, Retention time. Identification***: R, Linear retention indices were calculated using a homologous series C8-C25 n-alkanes; MS, by comparison of the MS with those of the computer mass libraries NIST 05 library, Wiley and Adams.

Table 3 Composition of *T. vulgaris* essential oils during 3 months storage at room temperature.

No	Compound	RI*	RT**	After distillation (%)	After 1 month (%)	After 2 months (%)	After 3 months (%)
1	(E)-2-Hexenal	850	6.80	0.21	0.19	0.18	0.15
2	α -Thujene	926	8.63	0.78	0.76	0.77	0.87
3	α -Pinene	933	8.87	0.93	0.92	0.94	1.07
4	Camphene	949	9.35	0.41	0.40	0.41	0.46
5	1-Octen-3-ol	977	10.20	1.36	1.37	1.36	1.40
6	Myrcene	990	10.59	1.57	1.55	1.56	1.71
7	3-Octanol	995	10.75	0.10	0.10	0.19	0.10
8	α -Phellandrene	1006	11.10	0.20	0.18	0.18	0.20
9	p-Mentha-1(7),8-diene	1008	11.20	0.08	0.08	0.07	0.09
10	α -Terpinene	1018	11.55	1.82	1.79	1.72	1.82
11	p-Cymene	1024	11.80	15.41	15.81	16.18	17.54
12	Limonene	1027	11.90	0.55	0.55	0.55	0.64
13	1,8-Cineole	1032	12.10	0.53	0.54	0.54	0.56
14	(E)-β-Ocimene	1047	12.66	0.05	-	-	-
15	γ-terpinene	1060	13.15	12.63	12.54	12.31	12.96
16	cis-Sabinene hydrate	1071	13.55	0.47	0.49	0.46	0.43
17	Terpinolene	1090	14.28	0.17	0.16	0.16	0.17
18	Linalool	1099	14.60	2.04	2.07	2.05	2.03
19	Borneol	1169	17.50	0.79	0.50	0.48	0.83
20	Terpinen-4-ol	1178	17.85	0.31	0.35	0.35	0.67
21	α -Terpineol	1190	18.35	0.14	0.15	0.15	0.13
22	Thymol methyl ether	1235	20.22	0.57	0.57	0.57	0.56
23	Carvacrol methyl ether	1245	20.65	0.37	0.37	0.37	0.35
24	Geranial	1269	21.65	0.06	-	-	-
25	Thymol	1290	22.50	51.59	52.27	52.21	49.34
26	Carvacrol	1299	22.90	4.52	4.48	4.49	4.19
27	Thymol acetate	1356	25.20	0.02	-	-	-
28	(E)-Caryophyllene	1422	27.85	0.95	0.94	0.89	0.82
29	α -Humulene	1457	29.21	0.23	-	-	-
30	Geranyl propanoate	1475	29.90	0.08	0.08	0.08	0.08
31	trans-Muurola-4(14),5-diene	1494	30.65	0.04	-	-	-
32	γ -Cadinene	1514	31.40	0.09	0.10	0.10	0.10
33	δ -Cadinene	1524	31.76	0.16	0.16	0.16	0.15
34	Spathulenol	1579	33.80	0.06	-	-	-
35	Caryophyllene oxide	1586	34.05	0.30	0.30	0.33	0.32
36	10-epi-γ -Eudesmol	1620	35.30	0.04		-	-
37	Epi-α-Cadinol	1640	36.00	0.11	0.19	0.11	0.14
-	Total	-	-	99.74%	99.94%	99.94%	99.90%

The specific essential oil constituent was identified through the GC-MS technique. RI*, Linear retention indices were calculated using a homologous series C8-C25 n-alkanes; RT**, Retention time. Identification***: R, Linear retention indices were calculated using a homologous series C8-C25 n-alkanes; MS, by comparison of the MS with those of the computer mass libraries NIST 05 library, Wiley and Adams.

Table 4 Effect of temperature and time storage on Thymol, γ-Terpinene and *p*_Cymene content in the *T. vulgaris* essential oil

treatment	Thymol (%)				Mean
	After Distillation	1 month storage	2 months storage	3 months storage	
After Distillation	51.59 ab	-	-	-	51.59 A
Freezer	-	51.34 b	51.97 ab	46.90 d	50.07 B
Refrigerator	-	51.89 ab	53.37 a	51.03 bc	52.09 A
room temperature	-	52.26 ab	52.20 ab	49.34 c	51.27 AB
Mean	51.59 A	51.83 A	52.51 A	49.09 B	-
treatment	*p*_Cymene (%)				Mean
	After Distillation	1 month storage	2 months storage	3 months storage	
After Distillation	15.41 c	-	-	-	15.41 A
Freezer	-	15.86 bc	15.80 bc	17.93 a	16.53 A
Refrigerator	-	15.64 bc	15.28 c	16.22 a-c	15.71 A
room temperature	-	15.80 bc	16.17 a-c	17.53 ab	16.50 A
Mean	15.41 B	15.76 B	15.75 B	17.22 A	-
treatment	γ-Terpinene (%)				Mean
	After Distillation	1 month storage	2 months storage	3 months storage	
After Distillation	12.62 b	-	-	-	12.62 A
Freezer	-	12.80 ab	12.71 ab	14.44 a	13.31 A
Refrigerator	-	12.54 b	12.17 b	12.93 ab	12.55 A
room temperature	-	12.54 b	12.30 b	12.96 ab	12.60 A
Mean	12.62 A	12.62 A	12.39 A	13.44 A	-

Means with similar letter in % 5 level of Duncan test are not significant.

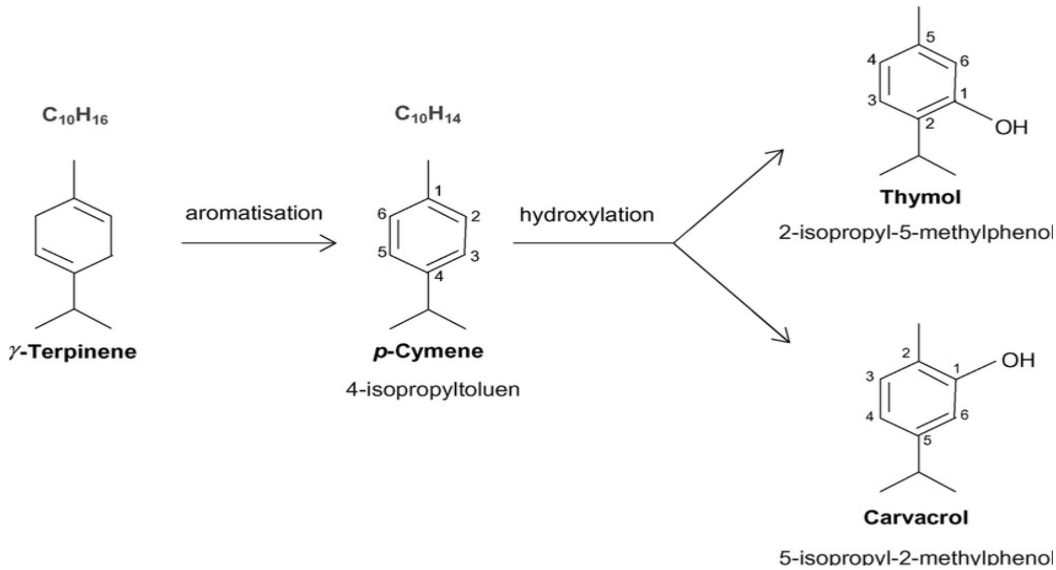

Fig. 6 Pathway for the biosynthesis of thymol and carvacrol from γ-terpinene and *p*-cymene

CONCLUSION

Interesting results regarding stability were obtained in the main compounds of garden. In most cases, the important monoterpenes of the essential oils of plants maintained their optimal quality during storage at room temperature. During this time, the experiment was maintained for three months. These findings may be extended to storage of essential oils of plants with the same chemical characteristics. Furthermore, these achievements show that essential oil producers and consumers, who utilize these compounds in pharmaceutical and cosmetic industries could benefit from this result. In conclusion, storage of secondary plant products especially essential oils, is an interesting research area which needs further studies with different aromatic plants essential oils constitute of various components.

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Disclosure Statement

No potential conflict of interest was reported by the authors.

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