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Original Article

Yield and Chemical Composition of the Essential Oils Extracted from Marjoram (*Majorana hortensis*), Peppermint (*Mentha piperita*) and Thyme (*Thymus capitatus*).

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Abstract

The objective of this research was to investigate the yield and the chemical composition of the essential oils extracted from three aromatic plants; marjoram, peppermint and thyme. The essential oils were extracted by steam distillation method and their yield was calculated. GC-MS technique was used to isolate, identify, and quantify the essential oils chemical constituents. The essential oils yield of marjoram; peppermint and thyme were 0.3, 0.6 and 1.0 % respectively. The major components in tested essential oils were terpinen-4-ol (19.27%), γ-terpinene (13.75%), cis-saboinene hydrate (13.36%) for marjoram essential oil; menthol (24.35%), *L*-menthone (18.28%), cineole (13.88%) for peppermint essential oil; 1.8 cineol (9.90%), camphor (9.85%), camphene (9.59%), thymol (8.24%), linalool (7.23%), terpinene-4-ol (7.06%), *D*-limonene (6.32%), cymene (5.66%), *P*-menthan-1-ol (5.61%), carvacrol (5.00%), α-terpinolene (3.82%) and α-pinene (3.10%) for thyme essential oil.

Key words: Chemical Composition, Essential Oils, Marjoram, Peppermint, Thyme, Yield.

Introduction

Essential oils, also called volatile, aromatic or ethereal oils, are oily liquids extracted from some parts of aromatic plants including flowers, buds, seeds, leaves, twigs, bark, herbs, woods, fruits and roots. Steam distillation is one of the most widely used methods for the commercial production of aromatic oils, in addition to other methods used such as expression, fermentation, enffleurage or extraction (Guenther, 1948 and Van de Braak and Leijten, 1999). Essential oils can be modified to meet particular needs for solubility, dispersibility, aroma, flavor, and color. They have standardized, flavor, color, and physical attributes and are microbiologically sterile (Dziezak, 1989). Aromatic oils extracted from plants vary considerably in their chemical composition as a result of a combination

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of factors including climatic, season, geographical location, geology, part of the plant and extraction method (Viuda-Martos *et al*., 2007).

Monoterpenes, monoterpenoids , phenylpropanoids, Alcohols, sesquiterpenes, sesquiterpenoids, aldehydes, and esters are the main chemical groups present in essential oils (Calín-Sánchez *et al*., 2013 and Angioni *et al*., 2004).

There are different species of marjoram herbs and the best known is *Origanum majorana* L., syn. *Majorana hortensis* Moench belonging to the family of Lamiacea. It is originated from Egypt and cultivated in several areas of Europe, North Africa, America and Asia. (Jelali *et al*., 2007). The chemical composition of essential oils extracted from marjoram were determined in many studies (EL-Ghorab *et al*., 2004; Vagi *et al*., 2005; Verma, 2010; Hussain *et al*., 2011).

Mint has been used for centuries as flavors in cooking and as traditional medicine in many societies in the world for their antimicrobial and antioxidant properties. Mentha genus belongs to the Lamiaceae family and contains about 25 species and some hybrids (Tsai *et al*., 2013). Singh *et al*. (2015) reported that Peppermint oil consists primarily of menthol (29–48%), menthone (20–31%), menthofuran (6.8%) and menthyl acetate (3–10%). Other pharmacologically active ingredients include bitter substances, caffeic acid, flavonoids (12%), polymerized polyphenols (19%), carotenes, tocopherols, betaine, choline and tannins.

The genus Thymus, member of the Lamiaceae family, divided into eight sections contains about 250 species of perennial aromatic and about 110 grow in the Mediterranean area. The main constituents of thyme include thymol, carvacrol and flavonoids (Mabberley, 1997; Zeljković and Maksimović, 2015 and Aziz *et al*., 2008).

Essential oils have been used for centuries in the treatment of infections and diseases, in different parts of the world (Mohadjerani *et al*., 2016), because essential oils and their active components possess antiviral, antimycotic, antitoxigenic, and insecticidal properties (Perricone *et al*., 2015). Nowadays, the use of essential oils is growing and there are noticeable range of applications for them (e.g. in food and beverages industry, as fragrances in perfumes and cosmetics) (Mohadjerani *et al*., 2016).

The aromatic plants (marjoram, peppermint and thyme) were chosen in this study for investigation because they are well known and much used in Libya and Egypt.

The use of essential oils as natural preservative additives in food processing needs accurate and comprehensive knowledge of their chemical composition which vary according to geographical location, environment conditions of the aromatic plant growing, plant variety, applied procedures for extraction and analysis, and chemical structure of chemical compound itself, therefore, the objectives of this study were: 1) determination of the yield of extracted essential oil from marjoram (*Majorana hortensis*), peppermint (*Mentha piperita*) and thyme (*Thymus capitatus*). and, 2) Identification and quantitative determination of the chemical components in essential oil extracted from such aromatic plants.

Materials and Methods

Materials:

Certain parts of marjoram, peppermint and thyme as shown in table 1 (from Giza Co., for seeds trade, Giza, Egypt, during 4/2012 season) were extracted and analyzed in the Food Sci. Dept., Fac. of Agric., Cairo University.

Table 1. The identification knowledge of chosen aromatic plants.

English name	Latin name	Family name	Part used
Marjoram	*Majorana hortensis*	Lamiaceae	Aerial part
Peppermint	*Mentha piperita*	Lamiaceae	Leaves
Thyme	*Thymus Capitatus*	Lamiaceae	Aerial part

Methods:

Extraction of essential oils:

The essential oils were extracted from chosen plants by steam distillation method. The parts of plant materials were cut into small pieces (ca 100 g), and placed together with distilled water (1.5 L) in a flask (2 L) with a continuous steam distillation extraction head was attached to the flask. After steam distillation (ca 3 h), the oil was separated and dried over anhydrous sodium sulfate (Farag *et al.*, 1989). The volatile compounds were fractionated, identified, and quantified using GC-MS.

GC-MS analysis of essential oils:

GC-MS system used is a Thermo scientific ISQ Single Quadrupole GC/MS (THERMO Comp., USA), coupled with a THERMO mass spectrometer detector (GC-MS ISQ) and equipped with a Trace GOLD TG-Wax MS GC Columns (30 m X 0.25 mm i.d., 0.25 μm film thicknesses). Helium was the carrier gas at a flow rate of 0.5 mL/min at a split ratio of 1:10. Temperature started at 40 º then increased at a rate of 4.0 ºC/min to 150 ºC and held for 6 min. then increased to 200 at the same rate and held for 1 min. The injector and detector temperatures were 200ºC. Extracted samples were diluted in hexane (1:10 v/v) and 0.2 μL were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 45-450. Most of the compounds were identified using analytical methods: mass spectra (authentic chemicals and Wiley spectral library collection).

Statistical analysis: Description of statistical analysis of data was carried by computing average values and standard deviations.

Results and Discussion

The average essential oil contents of the aerial part of marjoram (*Majorana hortensis*), thyme (*Thymus capitatus*) and peppermint leaves (*Mentha piperita*) were, 0.3±0.21, 0.6±0.33 and 1.0±0.71 (v/w), respectively (Table 2).

Table 2. The yield of three chosen aromatic plants essential oils.

Plants	Essential oil % (v/w)
Marjoram	0.3±0.21
Peppermint	0.6±0.33
Thyme	1.0±0.71

The results are in nearly agreement with those reported by Verma (2010), who mentioned that the essential oil yield of sweet marjoram (*Majorana hortensis*), cultivated in Kumaon region of Western Himalaya, was found to be between 0.33 % and 0.38 %, as well as with those found by Bisset (1994) who showed that peppermint contains usually 0.5 to 4.0 % essential oil and with those indicated by Benhamou *et al.* (2012) and Kazemi *et al.* (2012) who reported that the content of thyme essential oil was between 1.0 % and 1.87 %.

Composition of marjoram (*Majorana hortensis*) essential oil:

The chemical composition of marjoram essential oil was fractionated and identified by GC-MS technique. Thirty components were identified in marjoram essential oil. The identified components of marjoram essential oil represented 97% of its chemical components and were classified into eleven main groups namely; aliphatic terpenes (2.99%), monocyclic terpenes (29.82%), bicyclic terpenes (10.68%), sesquiterpenes (2.68%), aliphatic terpene alcohols (1.85%), cyclic terpene alcohols (25.30%), aromatic hydrocarbons (2.55%), oxides (0.12%), terpene esters (13.52%), phenols (5.85%) and ketones (1.64%).

The results in Table 3 and Figure 1 showed that the three major components (›10%) identified in marjoram essential oil were terpinen-4-ol (19.27%), γ-terpinene (13.75%), cis- Saboinene hydrate (13.36%). Moreover, there are 14 minor (10-1%) chemical compounds namely, α-terpinene (9.84%), sabinene (8.53%), carvacrol (4.34%), α-terpineol (4.12%), *D*-limonene (3.39%), β–Phyllandrene (2.84%), O-cymene (2.48%) β–Myrcene (2.99%), Caryophyllene (2.51), Thymol (1.51%), Carvone (1.2%), Menthanol (1.04%), 1-Terpinenol (1.40%) and L-Epidozene (1.43%), besides, the presence of 13 trace compounds (‹1.0%). According to literature, there exist two main chemotypes for marjoram oil composition, one consists mostly of monoterpene alcohols and the other of phenols. In the first chemotype, terpinene-4-ol, either alone or together with other substances, such as cis- and/or trans-sabinene hydrate, was found to be the main volatile component; and second, marjoram oils rich in phenols (thymol and/ or carvacrol) (El-Ghorab *et al.*, 2004). The findings of this study showed that the tested marjoram essential oil present characteristics of the first chemotype. Many authors reported similar results, Verma (2010) found that the major components of sweet marjoram (*Majorana hortensis* Moench) oil which was cultivated in Kumaon region of western Himalaya were (Z)-sabinene hydrate, terpinen-4-ol, (E)-sabinene hydrate, sabinene, α terpinene, γ-terpinene and α-terpineol. Similarly, Hussain *et al.* (2011) found that the major constituents of *origanum majorana* essential oils growing in Pakistan were terpinene-4-ol (20.9%), linalool (15.7%), linalyl-acetate (13.9%), limonene (13.4%) and α-terpineol (8.57%). Also Zawislak (2008) reported that the Trans-sabinene hydrate, terpinen-4-ol, and sabinene dominated in marjoram essential oil.

It is worth to mention that there were variations in results of previous investigations concerning the chemical compounds profile in marjoram essential oil. This could be attributed mainly to variations in geographical location, environmental conditions of marjoram plant growth, applied extraction and analysis methods used, variety and the portion of plant used, and the chemical compound itself (Viuda-Martos *et al.*, 2007 and Zawislak, 2008).

Composition of peppermint (*Mentha piperita*) essential oil:

Twenty-nine components were identified in peppermint essential oil, Table 4. and Figure 2. These compounds represent 97.99% of the whole oil constituents. The identified chemical components were classified into eleven main chemical groups namely; aliphatic terpenes (0.53%), monocyclic terpenes (35.37%), bicyclic terpenes (2.97%), sesquiterpenes (0.63%), monocyclic terpene ketones (1.36%), aliphatic terpene alcohols (1.98%), monocyclic terpene alcohols (27.28%), oxides (14.0%), aromatic hydrocarbons (0.78%), phenols esters (5.13%) and ketones (7.96%).

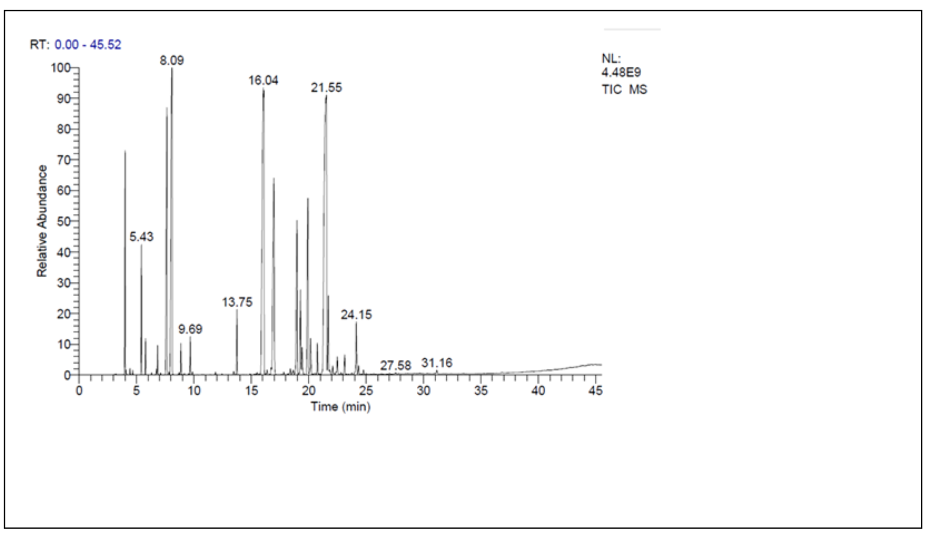

Figure 1. Chromatogram of the essential oil of *Majorana hortensis*.

Table 3. Composition of the essential oil of *Majorana hortensis*.

Chemical constituents	RT (min)	Area%	RRT
Hydrocarbons			
1-Aliphatic terpenes			
β –Myrcene	6.90	2.99	0.34
Total		2.99	
2-Monocyclic terpenes			
D-Limonene	7.65	3.39	0.37
γ –Terpinene	9.06	13.75	0.44
α –Terpinene	7.24	9.84	0.35
β –Phyllandrene	7.89	2.84	0.39
Total		29.82	
3- Bicyclic terpenes			
α –Pinene	5.44	0.62	0.27
Sabinene	5.88	8.53	0.29
L-Epidozene	23.50	1.43	1.15
1.4-dihydroxy-p-menth-2-ene	36.72	0.10	1.79
Total		10.68	
4- Sesquiterpenes			
Caryophyllene	19.06	2.51	0.93
α –Caryophllene	21.17	0.17	1.03
Total		2.68	
Total Hydrocarbons		46.17	
5- Aliphatic terpene alcohols			
α –Linalool	18.65	0.23	0.91
Citronellol	25.01	0.09	1.22
E-Pipertol	22.43	0.39	1.09
Nerol	27.37	0.1	1.34
Menthanol	21.11	1.04	1.03
Total		1.85	
6-Cyclic terpene alcohols			
1-Terpinenol	19.19	1.40	0.94
Terpinen-4-ol	20.48	19.27	1.00
α -Terpineol	23.18	4.12	1.13
P-Menth-len-3ol,trans	24.45	0.51	1.19
Total		25.30	

7-Aromatic hydrocarbons			
Ocimene	9.23	0.07	0.45
O-Cymene	9.76	2.48	0.48
Total		2.55	
8-Oxides			
Cineol	8.00	0.12	0.39
Total		0.12	
9-Terpene esters			
Geraniol acetate	24.18	0.07	1.18
Octaethylene glycol mono decyl ether	39.58	0.09	1.93
Cis-Sabinene hydrate	18.97	13.36	0.93
Total		13.52	
10- Phenols			
Thymol	16.11	1.51	0.79
Carvacrol	10.02	4.34	0.49
Total		5.85	
11- Ketones			
Carvone	19.07	1.20	0.93
Champhor	6.79	0.44	0.33
Total		1.64	
Total		97.0%	
Unknown		3.0%	

The retention time of Terpinene-4-ol (20.48) was taken as standard retention time equal one RT (min) = Retention Time , RRT=Relative Retention Time.

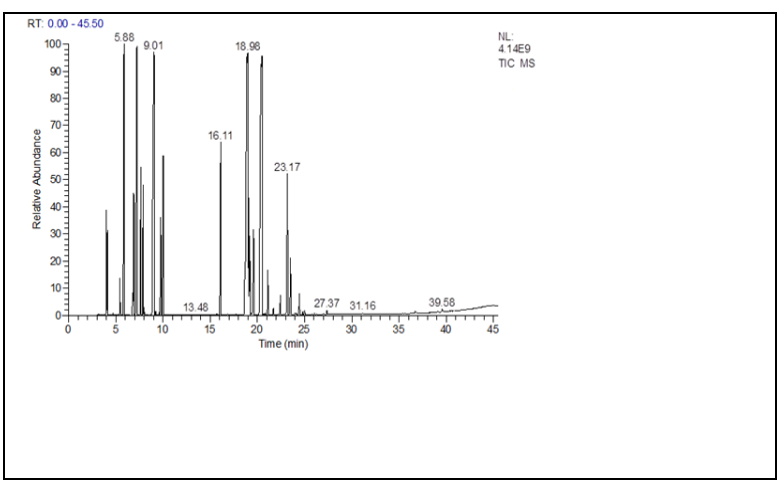

Figure 2. Chromatogram of the essential oil of *Mentha piperita*.

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Table 4. Composition of the essential oil of *Mentha piperita*.

Chemical Constituents	RT (min)	Area%	RRT
Hydrocarbons			
1-Aliphatic terpenes			
β-Myrcene	6.83	0.53	0.32
Total		0.53	
2-Monocyclic terpenes			
Limonene	7.64	7.96	0.36
γ-Terpinene	8.86	0.6	0.41
L-Menthone	16.09	18.28	0.75
Isomenthone	16.97	8.18	0.79
α-Phyllandrene	6.74	0.1	0.31
D-Carvone	24.34	0.25	1.13
Total		35.37	
3-Bicyclic terpenes			
α-Pinene	5.43	2.36	0.25
Sabinen	5.78	0.61	0.27
Total		2.97	
4-Sesquiterpenes			
Caryophyllene	19.93	0.63	0.93
Total		0.63	
Total Hydrocafrbons		39.50	
5- Monocyclic terpene ketones			
Piperitone	24.15	1.36	1.12
Total		1.36	
6- Aliphatic terpene alcohols			
Linalool	18.64	0.17	0.87
3-Hexen-1-ol	13.47	0.09	0.63
3-Octanol	13.75	1.45	0.64
1-Decanol	16.37	0.2 7	0.76
Total		1.98	
7- Monocyclic terpene alcohols			
α-Terpineol	23.12	0.53	1.07
γ-Terpineol,dihydro	22.09	0.23	1.03
Menthol	21.54	24.35	1.00
P-menthan-1-ol	20.75	0.78	0.96

Lavandulol	22.49	0.47	1.04
Iso Pulegol	20.16	0.92	0.94
Total		27.28	
8-Oxides			
Caryophlleneoxide	31.16	0.12	1.45
Cineole	8.09	13.88	0.38
Total		14.00	
9- Aromatic hydrocarbons			
P-Cymene	9.69	0.78	0.45
Total		0.78	
10-Phenol Esters			
Menthol acetate	18.4	0.17	0.85
Menthylacetate	18.98	4.96	0.88
Total		5.13	
11-Ketones			
Carvone	19.93	5.89	0.93
Champhor	19.42	2.07	0.90
Total		7.96	
Total		97.99%	
Unknown		2.01%	

The retention time of menthol (21.54) was taken as standard retention time equal one. RT (min) = Retention Time, RRT = Relative Retention Time.

It could be observed from the results in Table 4 that the three major chemical components (›10%) identified in the peppermint essential oil were menthol (24.35%), *L*-menthone (18.28%), cineole (13.88%). Moreover, there are 8 minor (10-1%) iso-menthone (8.18%), limonene (7.96%), carvone (5.89%), methyl acetate (4.96%), α-pinene (2.36%) camophor (2.07%), Piperitone (1.36%) and 3-Octanol (1.45%), as well as, the presence of 18 trace compounds (‹1.0%). The present findings are nearly in agreements with those obtained by Yadav *et al.* (2006); Agarwal (2008); Hussain (2009), Sokovic *et al.* (2009) and Sokovic *et al.* (2010), who reported that the major chemical compounds separated and identified in peppermint essential oil were menthol, menthone, methylacetate, sabinene hydrate, limonene, iso- menthone, α-terpinene, carvone and camophor.

There were small differences between the current results and those reported in the aforementioned investigation in content peppermint essential oil of chemical compounds and their percentages. This is attributed to the differences in the geographical location and environmental conditions of the aromatic plant growing, plant variety, the applied procedures for extraction and analysis (Aflatuni, 2005; Viuda-Martos *et al.*, 2007 and Hussain, 2009; Tsai *et al.*, 2013).

Composition of thyme (*Thymus capitatus*) essential oil:

Results stated in Table (5) and Figure (3) showed that twenty-nine chemical compounds were identified by GC-MS technique, in thyme essential oil which represent 98.69% of the chemical composition of thyme essential oil. The identified chemical compound could be classified into 10 chemical categories namely; aliphatic terpenes (0.36%), monocyclic terpenes (21.83%), bicyclic terpenes (7.98%), cyclic terpene ketones (9.85%), aliphatic terpene alcohols (7.23%), monocyclic terpene alcohols (21.29%), oxides (10.80%), aromatic hydrocarbons (5.66%), phenol compounds (13.24%) and aldehydes (0.45%).

From the results in table (5), it could be observed that 19 minor chemical compounds (10-1%) were identified in thyme essential oil namely, 1.8 cineol (9.90%), camphor (9.85%), camphene (9.59%), thymol (8.24%), linalool (7.23%), terpinene-4-ol (7.06%), D-limonene (6.32%), cymene (5.66%), P-menthan-1-ol (5.61%), carvacrol (5.00%), α-terpinolene (3.82%), α-pinene (3.10%), β -pinene (2.64%), P-Cymene-8-ol (2.03%), borneol (1.85%), α-longipinene (1.73%), terpinenol (1.73%) isoborneol (1.63%) and epoxyterpinolene (1.12%). Besides, the presence of 10 trace compounds (‹1.0%) was observed. The present results are nearly in agreement with those reported by Imelouone *et al.* (2009) who found that the major thyme essential oil components from eastern Morocco were camphor, camphene, α -pinene, 1, 8-cineole, borneol and β - pinene and Kazemi *et al.* (2012) who showed that the α -pinene, camphene, thymol, 1,8-cineole, β-pinene, and camphor were the major compounds in the thyme essential oil with some differences in the concentrations of the identified compounds.

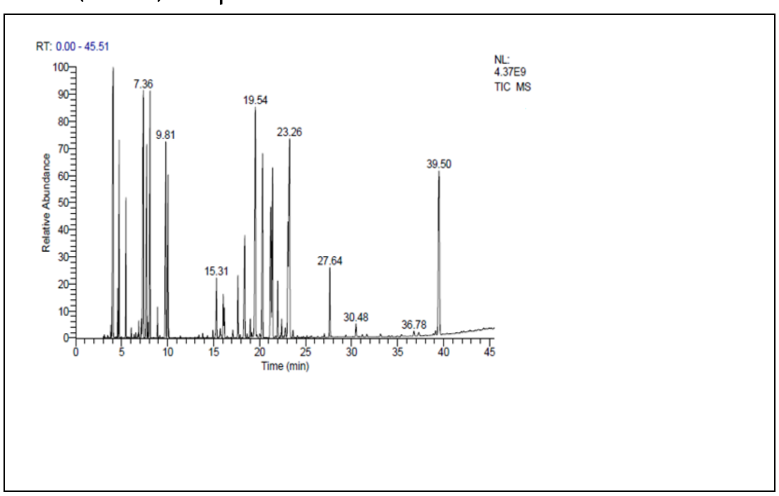

Figure 3. Chromatogram of the essential oil of *Thymus capitatus* .

Table 5. Composition of the essential oil of *Thymus Capitatus*.

Chemical Constituents	RT (min)	Area %	RRT
Hydrocarbons			
1-Aliphatic terpenes			
β -Myrcene	6.87	0.36	0.85
Total		0.36	
2-Monocyclic terpenes			
Camphene	7.36	9.59	0.91
α -Terpinene	7.15	0.33	0.88
D-limonene	7.72	6.32	0.95
γ -Terpinene	8.90	0.65	1.10
α -Terpinolen	10.04	3.82	1.24
Epoxyterpinolene	16.06	1.12	1.99
Total		21.83	
3- Bicyclic terpenes			
α -Pinene	5.47	3.10	0.68
Sabinene	7.88	0.26	0.97
α -Longipinene	15.31	1.73	1.89
Sativene	17.09	0.25	2.11
β -Pinene	18.39	2.64	2.27
Total		7.98	
Total Hydrocarbons		30.17	
4- Cyclic terpene ketones			
Camphor	19.54	9.85	2.42
Total		9.85	
5- Aliphatic terpene alcohols			
Linalool	23.26	7.23	2.88
Total		7.23	
6- Monocyclic terpene alcohols			
Terpinenol	17.65	1.73	2.18

Terpinene-4-ol	20.33	7.06	2.51
α-Terpineol	22.40	0.51	2.77
β-Terpnol	22.79	0.35	2.82
P-Cymene-8-ol	27.64	2.03	3.42
P-Menthan-1-ol	21.42	5.61	2.65
Dihydro-α-terpinol	18.99	0.52	2.35
Borneol	23.08	1.85	2.85
Isoborneol	21.79	1.63	2.69
Total		21.29	
7-Oxides			
1.4 Cineol	16.17	0.90	1.99
1.8 Cineol	8.09	9.90	1.00
Total		10.80	
8- Aromatic hydrocarbons			
Cymene	9.88	5.66	1.22
Total		5.66	
9- Phenols			
Carvacrol	21.12	5.00	2.61
Thymol	39.50	8.24	4.88
Total		13.24	
10- Aldehydes			
2 Methl-oct-enedial	30.48	0.45	3.77
Total		0.45	
Total		98.69%	
Unknown		1.31%	

The retention time of 1.8 cineol (8.09) was taken as standard retention time equal one.

RT (min) = Retention Time, RRT = Relative Retention Time

The variation in component concentrations could possibly be related to variation in the geographical location, environment conditions of the aromatic plant growing, plant variety, applied procedures for extraction and analysis, and the chemical structure of the chemical compound itself (Van de Braak & Leijten, 1999 and Viuda-Martos *et al.*, 2007).

In general, the effect of the essential oils for preserving food depends on their chemical composition especially the active components as found by Badee *et al*, 2013. Before applying the essential oils as natural preservative materials for keeping the food, It is necessary testing their chemical composition to get the best results in terms of keeping quality and extended shell life.

Conclusion

The results showed that The essential oils yield of marjoram, peppermint and thyme were 0.3, 0.6 and 1.0 % respectively and the main components were terpinen-4-ol, γ-terpinene, cis-saboinene hydrate; menthol, L-menthone, cineole; 1.8 cineol, camphor, camphene, thymol, linalool, terpinene-4-ol, D-limonene, cymene, P-menthan-1-ol, carvacrol, α-terpinolene and α-pinene, respectively.

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الانتاجية والتركيب الكيميائي للزيوت العطرية المستخلصة من البردقوش (ماجورانا هورتينسيس) والنعناع الفلفلي (مينثا بيبريتا) والزعتر (ثايمس كابيتاتس).

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المستخلص

أجريت هذه الدراسة للتعرف على الإنتاجية وكذلك التركيب الكيميائي للزيوت العطرية المستخلصة من النباتات العطرية: البردقوش، النعناع الفلفلي، والزعتر. تم استخلاص الزيوت العطرية من النباتات المختارة باستخدام طريقة التقطير بالبخار، وحددت نوعية وكمية المكونات الكيميائية للزيوت العطرية للنباتات المختارة باستخدام التحليل الكروماتوجرافي GC-MS.بلغت الانتاجية للزيوت المستخلصة من البردقوش والنعناع الفلفلي والزعتر 0.3 و 0.6 و 1.0%. على التوالي من وزن الجزء المستخدم من النبات العطري في استخلاص الزيت. أوضحت نتائج التحليل الكروماتوجرافي إن المركبات الكيميائية الفعالة الموجودة طبيعيا في الزيوت العطرية المختبرة كانت : تربينين -4- أول (19.27%)، جاما تربينين (13.75%)، سيس سبينين هيدريت (13.36%) لزيت البردقوش العطري، منثول (24.35%)، ل- مينثون (18.28%)، سينيول (13.88%) لزيت النعناع الفلفلي العطري، 1.8 سينيول (9.90%)، كامفور (9.85%)، كامفين (9.59%)، ثيمول (8.24%)، لينالول (7.23%)، تيربينين-4- أول (7.06%)، د-ليمونين (6.32%)، سيمين (5.66%)، بارا - مينتان-1- أول (5.61%)، كارفاكرول (5.0%)، ألفا تيربينيلين (3.82%)، وألفابينين (3.10%) لزيت الزعتر العطري.

كلمات دالة : الزيوت العطرية، البردقوش، النعناع الفلفلي، الزعتر، التركيب الكيميائي، الانتاجية.

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