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Chemical Composition of Ethanolic Extracts of Thymus Syriacus Boiss of Three sites of Syrian Coast by using Gas Chromatography (GC/MS)

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Abstract *Thymus syriacus* Boiss is a wild plant naturally found in the of Syrian Coast at varying altitudes up to more than 1000 m above the sea level, and is an important medicinal plant widely used in traditional systems of medicine. In the present work, the chemical composition of ethanolic extracts of Pneumatic parts of T.S was study by using gas chromatography system equipped with mass spectrometry detector (GC-MS).

Remarkable differences were note between the composition and the constituent ratio of the different studied organs. The results of chemical analysis showed that ethanolic extract contains 30 compounds with different ratios in the three sites,Carvacrol percentage ranged between 62.61 and 73.28 %, and p-Cymene percentage between 5.60 and 7.72 % while γ-Terpinene content ranged between 9.15 and 19.45%, and β-Caryophyllene percentage content between 1.94 and 2.96%, While Thymol percentage ranged between 0.16 and 0.21%.The highest percentage of both compounds Carvacrol and p-Cymene is record at A3 site (867 Meter) above sea level. On the other hand, A1 site (250 Meter) above sea level has the highest value of compounds γ-Terpinen, β-Caryophyllene and thymol compared with the other sites.

Keywords: *Thymus Syriacus*, Ethanolic extract, Carvacrol, Thymol, GC/MS

1. Introduction

Aromatic plants are important owing to their applications in folk medicine and their commercial value in view of their potential use in spices, beverages, perfumes, cosmetics, pharmaceutics, and aromatherapy [1].

Furthermore, their essential oils can be a source of alternative natural treatment of diseases, and essential oils consist of a number of active compounds, some of them comprising more than 60 components, which are virtually a complex mixture of hydrocarbons, alcohols, esters, aldehydes, carboxylic compounds and in cases phenylpropanoids. Most hydrocarbons are monoterpene compounds, however sesquiterpenes can also be found. Antibacterial properties of essential oils, are mostly because the presence of phenolic compounds [2,3,4].

Researchers have been interested in biologically active compounds isolated from plant species for the elimination of pathogenic microorganisms [5].

Syrian flora is well known for its diversity and richness, among plants grown in Syria *Thymus Syriacus* [2,6].

Thymus Syraicus belongs to the mint family (Lamiaceae), it is from herbaceous perennials and small shrubs in the world, and grows in the wild and is cultivated for agricultural, medical, ornamental and edible purposes. Stem is square section list, coarse texture. Leaves are simple narrow gray, rigid 1-3 cm, inflorescences are Rasimah found on the terminal pregnant, with flowers are small purple or white in color. More or less shrubby 30-50 cm, The Mediterranean region can be described as the center of the genus Thymus [2,7,8].

It is generally used as fresh, bruised, dried product or as Aromatic oil; it grows at different altitudes starting from sea level to a height of 2,000 meters above sea level [9].

Thyme green leaves are rich of essential oil, which is responsible for its characteristics of flavor and a strong aromatic fragrance, reddish brown color, drawn from the flowering tops and fresh and dried leaves. The main chemical components are "Thymol, Carvacrol, α-pinene, β-pinene, Borneol, Linalool, p-cymene and Camphene". Moreover, its extracts were found to have strong biological activity, due to the presence of phenols, thymol and carvacrol as major constituents of thyme oil in the plant [10,11].

Thyme oil is very effective and should not be used during pregnancy or in cases of high blood pressure due to the phenols (Thymol and Carvacrol), which can cause mucous membranes and skin irritation [8], and this oil is classified among the top ten essential oils [12].

Extraction of essential oils from Sage cultivated and wild in southern Lebanon was done by steam distillation, oil content of Thymol and Carvacrol was determined by GC-MS. Carvacrol was the highest component in all samples (49.8%) compared to Thymol (31.5%) [13].

The importance of this research lies in the economic, environmental and medical use of wild thyme and the continuous degradation, which affects it, in addition to the current trend towards herbal remedies and the absence of a similar study on wild thyme in the Syrian Coast.

Hence, the aim of this research is to determine the chemical composition of the ethanolic extracts of Pneumatic parts of T.Sin Syrain by using gas chromatography system equipped with mass spectrometry detector (GC/MS).

Materials and Methods

Plant Material

The plant parts of *Thymus Syraicus* collected from three different locations from the Syrian coast, whene the plants were in the beginning stage of flowering during May and June 2018 depending on the height and distance from the sea.

The samples were brought to the Graduate Laboratory at department of Environmental Chemistry of the Higher Institute for Environmental Research in Tishreen University, cleaned well from the of dust and the impurities attached to them, then all samples were dried in a well-ventilated, shady place (away from sunlight) With continuous stirring at room temperature (20-25 ºC) for several days, then the leaves and flowers were separated from their wooden parts then grind by using the electric mixer to get a fine powder, kept in the refrigerator in sealed and sterile glass containers until use.

Where the coordinates of the sampling locations were determined using the GPS device

A1: (250 Meter) above sea level.

A2: (420 Meter) above sea level.

A3: (867 Meter) above sea level.

Preparation of Crude Extract

50 g of dried powder was soaked in 250 ml of ethanol 95%, the flask was covered with aluminum foil and then placed on a magnetic stirrer for half hour, left for 2days into dark, stirring occasionally, then filtered with Whatman No.1., concentrated in a rotary vacuum evaporator at 40°C. The crude extract kept in the refrigerator in sealed and sterile glass containers until use.

Chemical analysis of Thymus Syriacus extracts

-Ethanol extracts from the parts of Thymewere analyzed using gas chromatography system equipped with mass spectrometry detector (GCMS-QP2010, Shimadzu, Kyoto, Japan) (Figure1).

-The device has the following specifications:

• Column: OPTIMA 5, film thickness 0.25 μm, column length 30 meters, diameter 0.25 mm.

• Gas carrier: helium with pressure of 54kPa.

• Injector: automatic, injector temperature 250 °C, injection pattern: Split less, injected sample size: 1 μl.

• Thermal program: Column temperature has been raised from 50 ^{o}C to 130 ^{o}C at a rate of 20 ^{o}C/min, then heating temperature was kept for one minute and then lifted from 130 ^{o}C to 250 ^{o}Cat a rate of 9 ^{o}C/min, then was kept on 250 ^{o}C for 8.33 minutes so that the total duration of heating program is 27.66 minutes

• Mass spectrometer: Ion source temperature: 200 °C.

-The extracts were prepared for analysis with pure ethanol to obtain 5% concentration.

- The components of the extract were identified using the Wiley and Nist electronic offices.

Figure 1: Gas Chromatography system equipped with Mass Spectrometry detect

Results and Discussion

Table (1) shows the GC-MS results of the ethanolic extracts of Thymus Syriacus plant, type of chemical compound. Figuers (2, 3, and 4) describe the Chromatogram of ethanolic extracts samples from three sites A1, A2, A3, respectively.

The results of chemical analysis showed that ethanolic extract contains 30 compounds with different ratios in the three sites, Carvacrol percentage ranged between 62.61 and 73.28 %, and p-Cymene percentage between 5.60 and 7.72 % while γ-Terpinene content ranged between 9.15 and 19.45%, and β-Caryophyllene percentage content between 1.94 and 2.96%, While Thymol percentage ranged between 0.16 and 0.21%. The highest percentage of both compounds Carvacrol and p-Cymene is record at A3 site (867 Meter) above sea level. On the other hand, A1 site (250 Meter) above sea level has the highest value of compounds γ-Terpinen, β-Caryophyllene and thymol compared with the other sites.

Most of the compounds obtained from the chemical analysis of the extracts belong to different functional groups (aromatic compounds, carboxylic acids, aldehydes, ketones, esters, hydrocarbons and alcohols) [14]. And this is consistent with the study was made from plant Thymus syriacus (leaves and flowers), where was collected during the flowering season in July at an altitude of (840 m) above sea level and extracted by water distillation using the Kelvinger method and analyzed with GC / MS technology, which revealed that the main component of oil was carvacrol (36.73%), whereas the other major components were γ-terpinene (8.97%), β-caryophyllene (6.17%), thymol (4.00%), myrcene (3.03%), and α-pinene (2.40%), and the essential oil showed anti-microbial activity [15].

And this is consistent with the study which showed presence some of that compounds in *Thymus Syriacus*extracts. Where the essential oils were obtained from the leaves of Thyme by hydrodistillation, and the oils content were analyzed by with the same previous technique. In total, 42 components representing 99.5% of the detected GCpeak areas were identified in the pre-flowering period oil, and 47 representing 98.8% of the detected components of the essential oil hydrodistilled from plant samples collected at the full flowering stage. Carvacrol (82.0-85.7%) was the main component of the essential oils in both pre-flowering and full flowering periods. It was followed by thymol (3.1-4.0%), γ-terpinene (1.0-2.3%), and β-caryophyllene (0.9-1.3%) [16].

There is Carvacrol (C10H14O) in the essential oil of thyme and each type contains the percentage of 5 - 75% of it [9]. It is an important mono-phenolic compound that has many medical benefits, the most important of which being anti-microbial, anti-inflammatory, anticoagulant, anti-parasitic, anti-bacterial, insecticide, analgesic, antispasmodic, hepatoprotective, and has cholesterase inhibiting properties, inhibiting the growth of several types of germs And, it is used as a food additive to prevent bacterial contamination, as the results of another study confirmed the effectiveness of the essential oil of thyme common as an insecticide to resist the rot of potatoes, due to the presence of carvacrol [15,17,18,19,20].

Thymol ($C_{10}H_{14}O$) is a prominent part of the natural compounds known as Biocides, and has the properties of an anti-bacterial when used alone or with other compounds such as Carvacrol, it also has an impact that can reduce bacterial resistance to some common drugs, penicillin [21].

β-caryophyllene is an effective compound that is anti-inflammatory [17].

The results of another study on wild thyme showed that the antimicrobial activity is associated with the presence of bioactive chemical compounds (thymol, carvacrol, γ-terpinene , p-Cymene , where p-Cymene is an effective compound and appears Its efficacy when used with phenols [18].

Another study showed that the essential oil of thyme thyme is effective as an anti-parasite due to the presence of the active compounds (carvacrol, thymol, p-Cymene, γ-terpinene), in addition to other effective compound (β-caryophyllene) [19].

Chemical Composition of Ethanolic Extracts of *Thymus Syriacus* Plant

Table 1: The GC-MS results of the ethanolic extracts of *Thymus Syriacus* plants, type of chemical compound

Peak	**Compound**	**Area 1% Ethanol T.S**	**Area 2% Ethanol T.S**	**Area 3% Ethanol T.S**
1	α-Thujene	1.35	1.14	1.03
2	α-Pinene	0.38	0.36	0.32
3	Camphene	0.04	0.04	0.04
4	β- Pinene	0.10	0.10	0.08
5	Myrcene	1.21	0.92	0.79
6	α-Phellandrene	0.23	0.12	0.13
7	δ-3-carene	0.06	-	0.05
8	α-Terpinene	1.99	0.71	0.87
9	*p*-Cymene	5.60	5.64	7.72
10	Sabinene	0.35	0.20	0.22
11	Cis-ocimene	0.07	0.03	0.01
12	γ-Terpinene	19.45	17.32	9.15
13	α-terpinolene	0.05	0.03	0.03
Monoterpene hydrocarbons		**30.88**	**9.55**	**20.44**
14	*trans*-Sabinene hydrate	0.29	0.21	0.17
15	Linalool	0.09	0.08	0.06
16	*cis*-Sabinene hydrate	-	0.09	0.06

17	Borneol	0.08	0.06	0.03
18	Terpin-4-ol	0.14	0.14	0.07
Oxygenated monoterpenes		**0.6**	**0.58**	**0.39**
19	Thymoquinone	0.18	0.22	0.07
Ketones		**0.18**	**0.22**	**0.07**
20	Thymol	0.21	0.15	0.16
21	Carvacrol	62.61	68.56	73.12
Phenolic monoterpenes		**62.82**	**68.71**	**73.28**
22	β-Caryophyllene	2.96	2.62	1.94
23	Aromadendrene	1.19	0.71	2.78
24	α-humulene	0.13	0.08	0.08
25	Allormadendrene	0.44	-	-
26	α -copaene	0.03	-	-
27	Germacrene-D	0.19	0.03	0.21
28	γ-elemene	-	0.13	-
29	δ-cadinene	0.09	0.01	0.04
Sesquiterpene hydrocarbons		**5.03**	**3.58**	**5.05**
30	Spathulenol	0.19	0.09	0.18
31	Caryophyllene oxide	0.14	0.13	0.16
Oxygenated sesquiterpenes		**0.33**	**0.22**	**0.34**

The figures (2, 3, 4) show the chromatograms of the ethanolic extracts of the studied plant parts

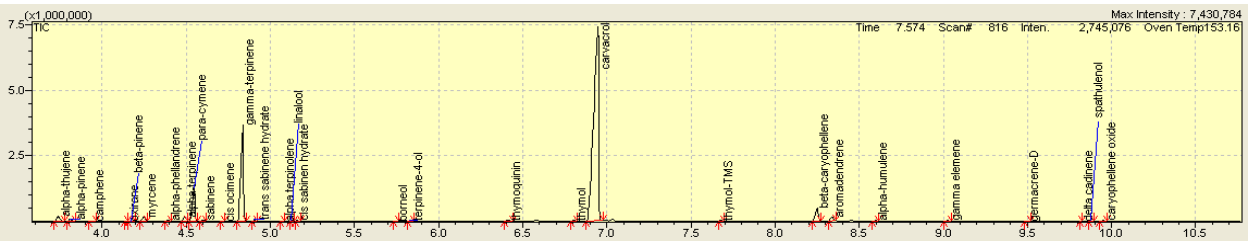


Figure 2: The chromatogram of the ethanolic extract sample from the site A1.

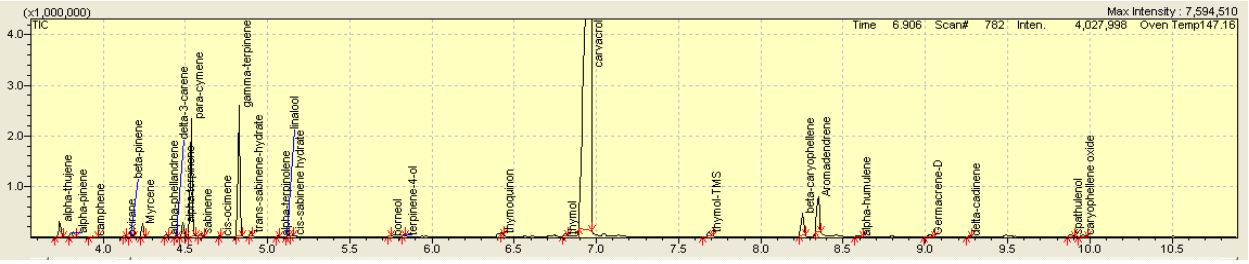


Figure 3: The chromatogram of the ethanolic extract sample from the site A2.

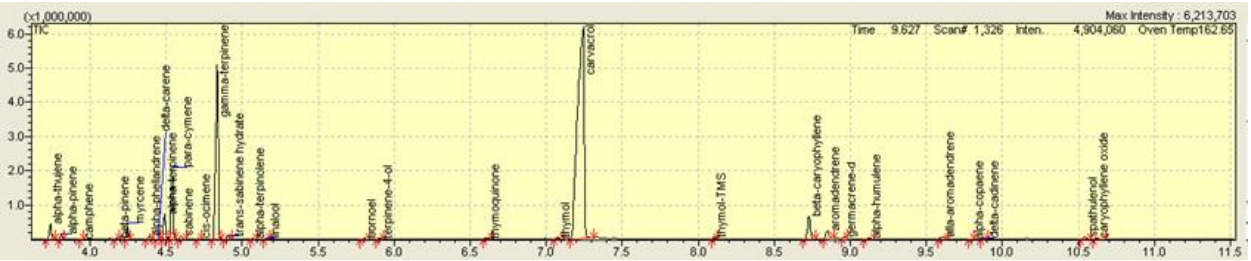


Figure 4: The chromatogram of the ethanolic extract sample from the site A3.

The following figures (5, 6, 7, 8) show the mass spectra of some chemical compounds present in the ethanolic extract of the *Thymus Syriacus* plant.

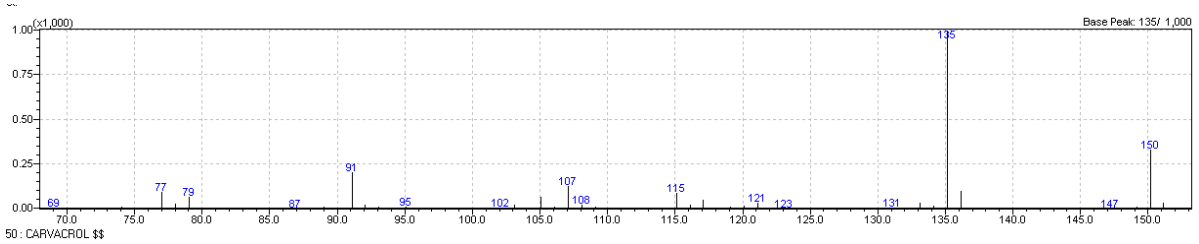


Figure 5: Mass spectrum of the compound Carvacrol

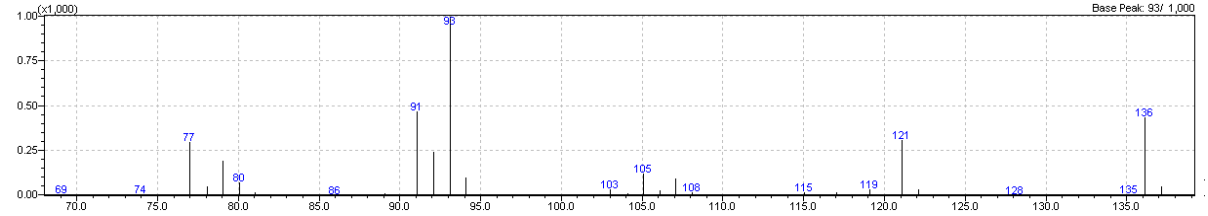


Figure 6: Mass spectrum of the compound γ-Terpinene

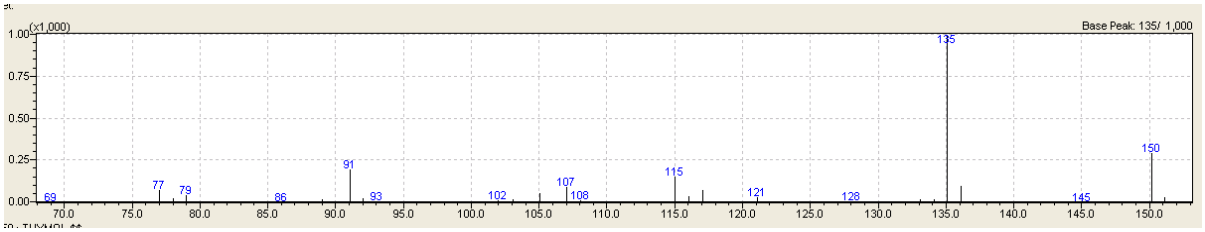


Figure 7: Mass spectrum of the compound Thymol

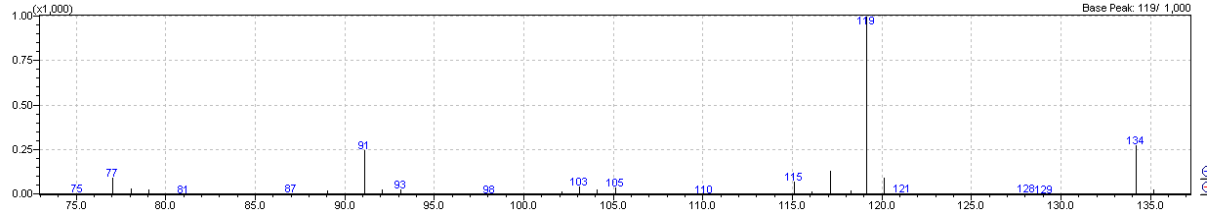
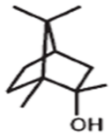


Figure 8: Mass spectrum of the compound p-cymene

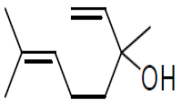
The table (2) shows the chemical formulas for some hydrocarbon and oxygen compounds for *Thymus Syriacus*

Table 2: Chemical formulas for some hydrocarbons and oxygenated compounds

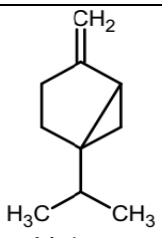
Thymoquinone	β-caryophellene	*p*–Cymene	γ–Terpinene	Thymol	Carvacrol



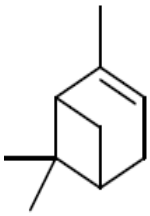
Borneol

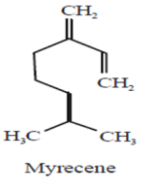


Linalool

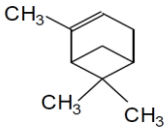


Sabinene


β- Pinene



Myrecene



Pineneα-

Conclusion

1. Chemical composition of thyme essential oil has been changed by geographic location (altitude, soil and other ...) where the Site A3 was distinguished by its plants which contained the highest percentage of bothCarvacrol and p-Cymene, whereas the site A1 was distinguished by its plants which contained the highest percentage of both γ-Terpinene and β-Caryophyllene.
2. Thyme ethanolic extracts in the study sites within the Syrian Coast contains mainly Carvacrol.
3. *Thymus Syriacus* plant extracts are contained various phytochemicals with biological activity can be of valuable therapeutic key. The presence of such a variety of phytochemicals may be attributed to the medicinal characteristics of this plant. The presence of phyto-components reveals the importance of the plant as medicinally used. Further investigations are planned to conduct the pharmacological studies to know the potency of these extracts. So, it is recommended as a plant of phyto - pharmaceutical importance.

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