

CHEMICAL COMPOSITION OF ESSENTIAL OIL THYMUS VULGARIS L AT SYRIAN COAST

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ABSTRACT

Thyme is a wild plant naturally found in the AL-Qadmous area at varying altitudes up to more than 1000 m above the sea level. Essential oil of thyme dry leaves was extracted by water distillation method using Clevenger apparatus. Gas Chromatography – Mass Spectrometry technique (GC-MS) was used to determine essential oil content of Thymol, Carvacrol and Pinene compounds. The results of chemical analysis show that Thymol percentage ranged between 1.28 and 4.86 % and that of Carvacrol ranged between 78.08 and 83.27 %, while Pinene content ranged between 11.86 and 20.65%. Statistical analysis shows significant differences between the compounds contents studied in the three sites. The highest percentage of both Thymol and Carvacrol is recorded at A3 site (320 Meter) above sea level. On the other hand A₁ site (200 Meter) above sea level has the highest value of Pinene compounds compared with the other sites.

KEYWORDS: *Thymus vulgaris*, Essential oil, Thymol, Carvacrol, Pinene.

INTRODUCTION

Medicinal plants represent an economically important and healthy component of biodiversity components. It is necessary to do a full inventory of medicinal plants in any country flora in order to protect and ensure the continuity of use and therefore protect endangered medicinal species in the wild. The use of medicinal plants is considered to be more common in traditional medicine and pharmaceutical tool supplementary worldwide. Many communities support natural products collected from ecosystems for medicinal purposes as well as for food purposes (Al-Said *et al.*, 2004) (Youssef, 2013).

Wild thyme "*Thymus vulgaris* L." is an Herbaceous Perennial to dendritic Plant that belongs to Lamiaceae Family, 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, inflorescences are Rasimah found on the terminal pregnant, and flowers are small purple or white in color. Height ranges 10-35 cm, native to the Mediterranean region. It is generally used as fresh, bruised, dried product or as Aromatic oil (Anonymous, 1996). Economic and medical importance is increasing in North America, Europe and North Africa (Letchamo & Gosselin, 1996). It grows at different altitudes starting from sea level to a height of 2,000 meters above sea level (Morales, 1986). Thyme was used in medicine by the Greeks, Romans and ancient Egyptians, whose name is derived from the Greek word (thymos) means fragrance.

Thyme oil is a volatile oil that has a strong aromatic smell, reddish brown color, drawn from the flowering tops and fresh and dried leaves by distillation with water or steam and yield 0.7-1.0%. The main chemical components are: "thymol, Carvacrol, α -pinene, β -pinene, Borneol, Linalool, β -Simine and Camphene". 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. Therapeutic properties: antibacterial, antispasmodic, antiseptic, anti-rheumatic, repelling gas, diuretic, expectorant, hypertensor, insecticide, stimulant and tonic (Esoteric Oils, 2011).

This oil is classified among the top ten essential oils and found that the best amount of oil and the highest content of Thymol was obtained at the beginning of flowering (Badi *et al.*, 2004). As some studies suggest that the thyme plant widely used for respiratory diseases (Cough) especially its aqueous extract, which is used in some countries as a raw material to prepare many pharmaceutical plant (Al- Sheibany *et al.*, 2005), Survey of more than 60 component of thyme was done, including Thymol (72.900 - 482.600 ppm), Carvacrol (10.000- 63.800 ppm) (Nijssen *et al.*, 1999). Thymol or 2-Isopropyl-5-methylphenol (C₁₀H₁₄O) gives the thyme used as food eaten a strong distinct flavor (Fenaroli, 2005), and 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 (Holley & Palaniappan, 2010).

Carvacrol and Thymol have shown a strong antidote genetic mutations influence (Mezzouga *et al.*, 2007), In addition, there is evidence that Thymol has counter tumor properties (Andersen, 2006). Naturally, There is Carvacrol or 5-isopropyl-2-methylphenol (C₁₀H₁₄O) in the essential oil of thyme and each type contains the percentage of 5-75% of it. It is used as an additive to foods to prevent bacterial contamination (Ultee & Smid, 2001).

It inhibits several types of bacteria growth, such as *Escherichia coli* (Olsen *et al.*, 2008). It also causes damage to the cell membrane of *Pseudomonas aeruginosa* bacterium (Cox & Markham, 2007). One study suggests that Carvacrol stimulates programmed death of prostate cancer cells, Carvacrol was tested with different concentrations and stages against human prostate cancer cells, Its use has led to positive inhibition effect of cancer cells, and preliminary results of this research indicates to the existence of great potential for using Carvacrol as an anti-cancer (Bavadekar, 2012).

Pinene or (1S, 5S)-6,6-dimethyl-2-methylenebicyclo [3.1.1] heptane (C₁₀H₁₆): is an organic compound of terpenes which has in nature two isomers α -pinene and β -pinene. This two figures are important components in pine resin and other conifers as well as in other plants such as Sage, thyme and others. Pinene is using in chemical industries where oxidation selectivity of Pinene with some stimuli gives many aromatic compounds, such as synthetic fragrances (Dorman and Deans, 2000).

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%) (Zein *et al.*, 2012). Thyme oil extracted from plant *Coridothymus capitatus* leaves has been analysed with the same previous technique, and essential oil mainly contained the following compounds Carvacrol (35.6%), μ - Simine (21%), Thymol (18.6%) (18.6%) (Goren *et al.*, 2003).

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. So the aim of this research is to study the variations in the chemical composition (statistically) of wild thyme plant which is widespread in the Syrian Coast in terms of aromatic oil content "Thymol, Carvacrol and Pinene" and which is evaluated by gas chromatography - mass spectrometry GC-MS method.

MATERIALS AND METHODS

1. Plant material and samples collection: Wild thyme samples were collected from three different locations at AL-Qadmous area after dividing to sectors depending on height and distance from the sea (including plains and mountain slopes and on different types of soil):

- A₁: (250 Meter) above sea level.
- A₂: (560 Meter) above sea level.
- A₃: (1000 Meter) above sea level.

Collection process was held in May and June of 2012, when plants were at the beginning of the flowering stage. All samples were dried aerielly without lightning at room temperature for two months.

2. Essential oil extraction: The amount of 200 g of dried plant from each of the studying sites was taken where essential oil extracted by distillation in accordance with the European Pharmacopia, 1996 for a period of three hours using a homemade distillation device. Oil was withdrawn and placed in a glass test sealed tubes and then tubes were wrapped blocked from light and kept at a temperature of 4C° until GC-MS analysis.

3. Analysis by Gas Chromatography-Mass Spectrometry (GC-MS): Qualitative analysis of oil samples was carried out using GC-MS QP2010 Plus device manufactured by Shimadzu Japanese company and shown in Figure (4). GC device is equipped with column of silica (30 m x 0.25 mm, and a thickness of 0.25 micrometer). MS-GC device turning conditions were: Use helium as a carrier gas, Injector temperature is 250 C°. Detector temperature is 280 C°. Column temperature has been raised from 50 C° to 130 C° at a rate of 20 C°/min, then heating temperature was kept for one minute and then lifted from 130 C° to 280 C° at a rate of 9 C°/min, then was kept on 280 C° for 8.33 minutes so that the total duration of heating program is 30 minutes. Size of Injected sample in the device is (1) microliter of oil. Chromatographic methods are used in qualitative and quantitative analysis of extracts of thyme *T. vulgaris* L. essential oil after extraction by Steam distillation (Zeković *et al.*, 2000). Identification of oil compounds depended on Retention Time (RT) and comparing these components spectrom electronically with the library included in the GC-MS system (Adams, 1995). Quantitative composition of oil components (%) was determined by calculating the areas of peaks which represented it in the Chromatogram and then the percentage of each of these values relative to the total value.

4. Statistical Analysis: Microsoft Office Excel 2007 program was used in the statistical analysis of data through a test of significant differences analysis using Anova: Single Factor and then less significant difference LSD values were calculated When significant level of 5%.

RESULTS AND DISCUSSION:

The main objective of this study is to compare the essential oils extracted from wild thyme plants widespread naturally in three locations at AL-Qadmous area in terms of their containment of compounds "Thymol, Carvacrol and Pinene" as analyzed by GC-MS which has been described. Figuers (1, 2, and 3) describe the Chromatogram (ICS) of oil samples from three sites A₁, A₂, A₃, respectively.

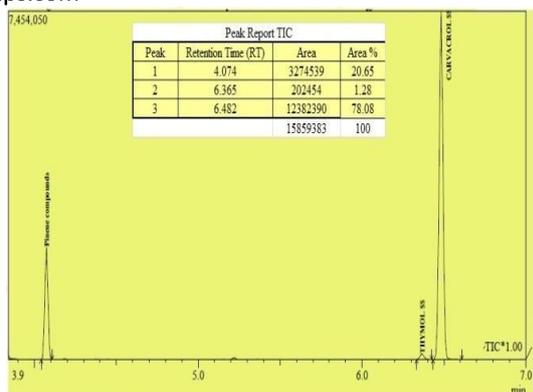


Figure.1. Chromatogram of oil sample from the site A₁.

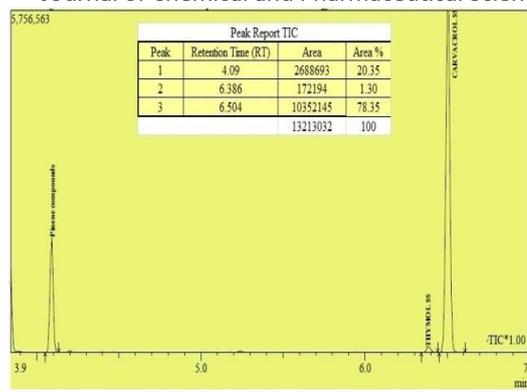


Figure.2. Chromatogram of oil sample from the site A₂.

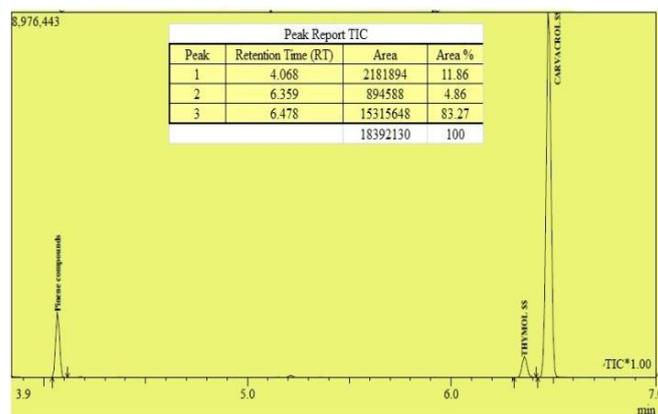


Figure.3. Chromatogram of oil sample from the site A₃.

The results in Table (1) showed that there are differences between plants chemical compounds content in these locations. Statistical analysis has improved the significant differences and the results were:

Table.1. Percentage (%) of each of Pinene, thymol and Carvacrol in the wild thyme essential oil samples in the study locations and the value of LSD (5%) of each of them.

| Compound | Site | | | LSD (5%) |
|-----------|-------|-------|-------|----------|
| | A1 | A2 | A3 | |
| Pinene | 20.65 | 20.35 | 11.86 | 0.51086 |
| Thymol | 1.28 | 1.30 | 4.86 | 0.26938 |
| Carvacrol | 78.08 | 78.35 | 83.27 | 0.48621 |

CONCLUSIONS

- 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 both Thymol and Carvacrol, whereas the site A₁ was distinguished by its plants which contained the highest percentage of pinene. Significant differences were apparent between the studied samples according to their locations.
- Thyme essential oil in the study sites within the Syrian Coast contains mainly Carvacrol compared with the other two components (Thymol and pinene compounds).

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