Chemical composition of essential oil of *Thymus vulgaris* collected from Saudi Arabian market

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**ABSTRACT**

**Objective:** To focus on the analysis of chemical constituents of the *Thymus vulgaris* L. (locally known as “Zaitra” or “Za’atar”; Family: Lamiaceae) which is available in the market of Saudi Arabia.

**Methods:** The Zaitra oil was analyzed by gas chromatography-mass spectrometry. The mass spectra were compared with the standard spectra available in National Institute Standard and Technique library.

**Results:** The results indicated that the *Thymus* oil is composed of many chemical compounds including α-pinene, thymol and caryophyllene which are biologically active and also used in various diseases.

**Conclusions:** It can be concluded that the *Thymus vulgaris* due to presence of many bioactive compounds can be used as a new potential source of medicine for the treatment of various types of illness.

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1. Introduction

In the modern era of pharmaceutical science, the extent of the degree of interest has been shifted more towards the herbal products as compared to the classical or synthetic products due to better affordability, acceptability and compatibility with the human physiology and minimal side effects [1]. The interests in herbal products for the medicines, perfumery, cosmetics and food additives are increasing among common people, patients and physicians as evident from an increased market of herbal medicine [2]. Among all these herbal drugs, the essential/volatile oils have received substantial attention due to their significant biological usefulness [3].

The composition of the aromatic and volatile oils are very much influenced by genotype (species, cultivar, clone, ecotype), ecological (geographical origin, climate condition, soil composition) and technological (cultivation, types of collection, storage of crude material and processing technique) factors [4]. For these reasons, plant of the same species, but from the different contexts can express different features and chemical compositions. In this study attempt was made to characterize the chemicals present in the locally marketed *Thymus vulgaris* L. (Lamiaceae) (*T. vulgaris*).

*T. vulgaris* is an annual plant with grassy appearance that grows in many parts of the world. It is commonly used in folk medicine for its expectorant, antitussive, antibroncholytic, antispasmodic, antihelmintic, carminative and diuretic properties. It is also used as culinary herbs and is a well-known source of flavoring agent [5].

The plant contains aromatic smell and warm pungent taste. The fragrance of this plant is due to essential oil, which gives flavoring value for culinary purposes as well as its medicinal properties [6]. The studies showed that *T. vulgaris* has carminative, antimicrobial, muscle relaxant [6] and antioxidant properties [7,8].
Keeping in view the past published work [3,4,7,9–12], on the medically important aspect of this plant, we evaluated the chemical composition of *T. vulgaris*. Additionally, the rationale behind undertaking this project was to compare what are the similarities and differences in bioactive components of the plant marketed in Saudi Arabia and rest of the world due to climate/geographical changes. Since one of the major problems associated with the herbal products and dietary supplements is its quality control [13], the data obtained by this study may be used as a tool to assess the quality of *Thymus* leaves.

It's worth noting that the *T. vulgaris* which we have studied are not necessarily grown in the Kingdom of Saudi Arabia. It may be imported from other geographical locations of the world. To the best of our knowledge, no one has characterized the chemical composition of *T. vulgaris* marketed and used by the vast majority of the Saudi population. This study is the first of its kind to demonstrate the presence of bioactive components in *T. vulgaris* that is available in Saudi Arabia.

### 2. Materials and methods

#### 2.1. Plant material

The dried leaves of *T. vulgaris* were collected from the local market in Riyadh, Saudi Arabia in December 2015. It was identified by Dr. Abuzer Ali, Department of Natural Products and Alternative Medicine, Dammam University, and the voucher specimen (voucher number P/M/014) has been deposited to Herbal Unit, Research Center, Prince Sultan Military Medical City, Riyadh, Saudi Arabia. The leaves were coarsely powdered and used for analysis.

#### 2.2. Essential oil extraction by steam distillation

A Clevenger apparatus was used to extract volatile oil. Thirty grams of coarsely powdered leaves were taken in a 1000 mL flask, and 750 mL of water was added. The flask was heated on a heating mantle. The volatile oil was extracted and it was evaporated along with the water vapor, which were passed through condenser and oil was accumulated. Distillation was stopped when the difference in successive readings of the oil volume remained constant. The oil was drained followed by passing over anhydrous sodium sulfate. The oil was filtered through 0.22 μm/L filter paper, and kept at 4 °C in sealed vials in dark.

#### 2.3. Instrumentation

Gas chromatography-mass spectrometry (GC–MS) analysis of the *Thymus* oil was carried out on a GC system (Agilent 7890A series, USA) as reported earlier [14]. The instrument was equipped with split/splitless injector. The auto-sampler was attached to an apolar HP-5MS (5% phenyl polydimethyl siloxane) capillary column (30 m x 0.25 mm, i.d. 0.25 μm film thickness) and fitted to Mass Detector (Agilent 5975C series, USA). Helium was used as carrier gas with flow rate of 1 mL/min. Split ratio was maintained at 1:20, injector temperature at 250 °C, detector temperature at 300 °C. The column temperature was kept at 60 °C for 2 min followed by linear programming from 60 to 250 °C (at 2 °C/min), and kept isothermal for 2 min. The transfer line was heated at 280 °C. Mass spectra were acquired in scan mode (70 eV) in range 50–550 m/z. Two microliter of 1000 μL/L oil sample diluted in hexane was injected. The components of the oil were separated and the chromatogram obtained was identified by comparing the mass spectra to those from National Institute of Standards and Technology (NIST) libraries.

#### 2.4. Identification of chemical constituents

The spectra obtained from the GC–MS were compared with the database of NIST which contains more than 62,000 standard spectra. The spectra of the compound obtained from the *Thymus* oil was compared with the spectrum of the known compounds which is stored in the NIST library.

### 3. Results

#### 3.1. GC–MS optimization

GC–MS parameters were optimized through adjusting the column, temperature program, and split ratio. The best result obtained from HP-5MS column that showed higher resolution and less analysis time. Finally, the optimized GC–MS analysis condition was developed for specific analysis of *T. vulgaris* as stated in instrumentation section.

#### 3.2. Compound identification

The GC–MS fingerprint profile of *T. vulgaris* was obtained and showed in Figure 1. By analyzing the results, hydro-distilled oil of *T. vulgaris* showed the presence of 24 chemical compounds. The compounds, its retention time, and area percentage are shown in Table 1.

![Figure 1. GC–MS chromatogram of *T. vulgaris* analyzed on GC–MS (Agilent, USA) using a capillary column (5-MS) attached with mass detector. The chromatogram showed the presence of chemical components found in the *T. vulgaris*](image-url)
A number of compounds found in essential oil of *T. vulgaris* were reported to have strong biological activities. The bioactive compounds including the molecular formula, molecular weight, chemical structure, and reported biological activity are presented in Table 2.

Table 1

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>Retention time (min)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Furan, tetra hydro-3-methyl</td>
<td>2.317</td>
<td>12.19</td>
</tr>
<tr>
<td>2</td>
<td>Cyclohexane</td>
<td>2.546</td>
<td>0.13</td>
</tr>
<tr>
<td>3</td>
<td>α-Pinene</td>
<td>6.546</td>
<td>0.18</td>
</tr>
<tr>
<td>4</td>
<td>Camphene</td>
<td>6.998</td>
<td>0.13</td>
</tr>
<tr>
<td>5</td>
<td>β-Mycene</td>
<td>8.491</td>
<td>0.15</td>
</tr>
<tr>
<td>6</td>
<td>3-Octanol</td>
<td>8.663</td>
<td>0.22</td>
</tr>
<tr>
<td>7</td>
<td>Carene</td>
<td>9.389</td>
<td>0.27</td>
</tr>
<tr>
<td>8</td>
<td>p-Cymene</td>
<td>9.704</td>
<td>2.77</td>
</tr>
<tr>
<td>9</td>
<td>o-Cymene</td>
<td>9.841</td>
<td>0.39</td>
</tr>
<tr>
<td>10</td>
<td>γ-Terpine</td>
<td>11.060</td>
<td>1.18</td>
</tr>
<tr>
<td>11</td>
<td>1,6-Octadien-3-ol, 3,7-dimethyl-</td>
<td>12.828</td>
<td>0.38</td>
</tr>
<tr>
<td>12</td>
<td>Endo-Borneol</td>
<td>15.563</td>
<td>0.90</td>
</tr>
<tr>
<td>13</td>
<td>Terpinen-4-ol</td>
<td>16.090</td>
<td>0.31</td>
</tr>
<tr>
<td>14</td>
<td>α-Terpene</td>
<td>16.811</td>
<td>0.28</td>
</tr>
<tr>
<td>15</td>
<td>Thymol</td>
<td>21.354</td>
<td>3.82</td>
</tr>
<tr>
<td>16</td>
<td>o-Thymol</td>
<td>22.018</td>
<td>38.71</td>
</tr>
<tr>
<td>17</td>
<td>Phenol, 2-methyl-5-(1-methylethyl) acetate</td>
<td>24.610</td>
<td>0.34</td>
</tr>
<tr>
<td>18</td>
<td>Caryophyllene</td>
<td>26.349</td>
<td>0.91</td>
</tr>
<tr>
<td>19</td>
<td>Humulene</td>
<td>31.385</td>
<td>0.22</td>
</tr>
<tr>
<td>20</td>
<td>2-Aminopyrimidine-1-oxide</td>
<td>32.277</td>
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<tr>
<td>21</td>
<td>(−)-Spathulenol</td>
<td>32.609</td>
<td>0.13</td>
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<tr>
<td>22</td>
<td>Caryophyllene oxide</td>
<td>32.781</td>
<td>0.51</td>
</tr>
<tr>
<td>23</td>
<td>Isoaromadendrene epoxide</td>
<td>35.613</td>
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</tr>
<tr>
<td>24</td>
<td>Cyclo propane carboxamide</td>
<td>40.013</td>
<td>0.11</td>
</tr>
</tbody>
</table>

4. Discussion

The quality control of the herbal drug is a big challenge in all around the world. World Health Organization has issued guidelines to validate the herbs that are used for medicinal and therapeutics purposes. Saudi Arabia is exporting a number of herbs which are used as food products as well as for the therapeudic purposes. There is a need to develop the system through which the quality of the herbs can be evaluated in terms of presence or absence of chemical constituent.

GC is an ideal technique for the separation and identification of the herbs containing the volatile and semi volatile constitutents. MS attached with the GC has an added advantage through which each separated constituent can be fragmented and the pattern of fragmentation can be compared with the spectra available in the database. This gives the information of the available constituent present in the herbs without using the chemical/biological marker of the plants. Finally, all the available constituents can be separated, fragmented and characterized simultaneously.

The GC–MS of the *Thymus* oil resulted in the identification of 24 compounds. Chemical compounds found in *T. vulgaris* were terpenes and aromatic compounds. The major compounds were thymol, camphene, caryophyllene, humulene, α-terpeniol and para-cymene. Volatile compounds investigated in this study were found to be consistent with those of previously published studies in which volatile compounds were identified by different methods [3,7,9].

The high percentage of the thymol found in the Saudi market leaves can be seen as the desirable concentration of the phenol that could have value in the traditional market.

The analyses of this work reveal that the hydro-distilled oil of *T. vulgaris* has a number of components. These bioactive components, present in Zaitra act as a drug for various diseases. The essential oil of *T. vulgaris* reveals the presence of 24 bioactive components from GC–MS technique, and each component has specific functions and acts as a drug for various diseases. Thus from this study it can be concluded that the *T. vulgaris* may serve as a new potential source of medicines due to the presence of these phytochemicals and bioactive compounds. Further, the functional studies of these bioactive compounds will further be examined using *in vivo* models.

Conflict of interest statement

We declare that we have no conflict of interest.

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Gas chromatography
Thymus vulgaris L.
Vulgaris
Thymus hyemalis
Ocimum basilicum
Rosmarinus of