CHEMOTYPIC OF *ROSMARINUS OFFICINALIS* L. ESSENTIAL OILS FROM EASTERN SIDE OF IRAQ

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INTRODUCTION

*Rosmarinus officinalis* L. is an aromatic plant from the Lamiaceae family, and the primary origin is the Mediterranean area, but also it is cultivated around the world [1-3]. Rosemary is mostly used as a spice in food preparation and as a food preservative. Furthermore, rosemary extract and its essential oil have been used in herbal medicine as antioxidant, antimicrobial, and anti-inflammatory agents [1-3]. Besides, one of the major constituents of rosemary essential oil such as eucalyptol has been shown to have great potential in treating chronic inflammatory diseases [4].

Many studies have determined the chemical constituents of *R. officinalis* essential oils, leaves and flowers grown in different countries and geographical origins. Each of these studies showed variations in the constituents within the monoterpenes and oxygenated monoterpenes [5-7]. Similarly, two studies from two different locations in the Baghdad, capital of Iraq, have been performed to identify the chemical constituents of the rosemary leaves essential oil and presented significant variations in the percentage constituents, as well as in the chemical identification of monoterpenes and oxygenated monoterpenes. For instance, one study obtained a high percentage of oxygenated monoterpenes, linalool (11.58%), whereas the other second did not report any [8,9]. Therefore, it is essential to characterize the chemical constituents of rosemary essential oils in different geographical locations within the same country.

Since (a) the demand of using the essential oil of *R. officinalis* in complementary medicine as well as in the food and pharmaceutical industry and (b) the constituents varied between countries and within the same country's geographical areas, it is determined to establish the chemical constituents of rosemary essential oil from Diyala Province, an agricultural industry with 7685 km² area, in the eastern side of Iraq, northeast of Baghdad. Furthermore, the present study compares the rosemary essential oil constituents from similar geographical areas as well as different world locations.

METHODS

Plant material

Rosemary samples were harvested during October 2017 from the Diyala Province of the eastern side of Iraq, which has the following coordinates: 33°53′N 45°4′E. The samples consisted of the leaves parts, washed extensively with tap water, and kept drying for 7 days. Identification of the leaves was performed by microscopic investigations, and a voucher specimen is retained under the code AATR1 at Faculty of Pharmacy, Uruk University, Baghdad, Iraq.

Extraction of essential oils

The extraction method used was hydrodistillation using a Clevenger apparatus. Briefly, a 100 g of Rosemary leaves sample was immersed in 800 ml distilled water in a 2 L round bottom flask. The mixture was put in heated mantel to boil, and then, hydrodistillation is performed for 3 h. The samples were boiled with water and the volatile oils evaporated along with the water into the condenser connected to a flask at 100°C and atmospheric pressure. To prevent overheating, however, the heating mantel was then set to 60°C. At the end of the distillation, a less dense phase than water (essential oil) and an aqueous phase were established. The oil phase was collected, dried, and stored in sealed vials. To remove the traces of water in the essential oils, 1 g of magnesium sulfate was added. The vials were kept in the dark at 4°C until used. The extraction method was repeated twice.

Yield of essential oils

The percentage yield of rosemary essential oil from the above extraction method was calculated from the equation below:

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\text{Percentage yield of essential oils} = \frac{\text{Amount of oil extracted (g)}}{\text{Amount of dry powder (g)}} \times 100
\]

Chromatographic analyses of essential oils

Following extraction, identification of chemical constituents of the rosemary essential oils was performed by gas chromatography...
coupled with mass spectrometry (GC–MS) (Shimadzu, GCMS-QP2010 Ultra, Tokyo, Japan). The column used was a 30 m × 0.25 mm with an internal diameter and film thickness of 0.25 μm, with helium as a carrier gas. The instrument was set to the following: Column oven temperature was initially set at 70°C and then gradually increased within minutes to 240°C; injection temperature: 240°C; sampling time: 1.0 min; pressure:100.0 kPa; total flow:19.9 mL/min; column flow: 1.53 mL/min; linear velocity: 45.4 cm/s; purge flow: 3.0 mL/min; and split ratio: 10.0. As for the GC–MS program, it was set to ion source temperature: 200.00°C; interface temperature: 240.00°C; solvent cut time: 4.00 min; ionization mode: SEI; detector gain mode: Relative; detector gain: 1.20 kV +0.00 kV; start time: 4.50 min; end time: 27.00 min; event time: 0.30 s; scan speed: 2500; start m/z: 45.00; and end m/z: 700.00. The MS pattern of each constituent was compared and matched with MS patterns of known compounds and with those of other essential oils using the database library of the National Institute of Standards and Technology (NIST08).

RESULTS AND DISCUSSION

The hydrodistillation extraction method revealed 0.7% of essential oils from *R. officinalis* leaves. It has been shown that increasing heating time would increase the amount of essential oil extracted. For instance, heating for 20–70 min extracts 0.2–0.6%, whereas heating for 180 min acquires 1.4–4% of essential oils [10,11]. However, in the present study, the heating time was 180 min, but the amount yield was low than expected. This may be related to the rosemary plant grown in Diyala Province of the eastern side of Iraq which has also been known not to produce flowers.

The gas chromatography profile of the extracted oils showed 18 peaks in a 20 min scan time (Fig. 1).

Following MS fragmentation pattern and comparison of each to known compounds, the 18 components of the essential oils were identified and presented in Table 1.

![Gas chromatography chromatogram of essential oils of *Rosmarinus officinalis* from Diyala, Iraq](image)

**Fig. 1: Gas chromatography chromatogram of essential oils of *Rosmarinus officinalis* from Diyala, Iraq**

**Table 1: Chemical composition of essential oils of *Rosmarinus officinalis* from Diyala, Iraq**

| Peak No. | Time (%) | % composition | Compound name(s) | IUPAC |
|----------|----------|---------------|-----------------|-------|
| 1.       | 5.54     | 59.02         | Eucalyptol (1,8-Cineole) | 1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane |
| 2.       | 5.94     | 0.17          | 3-Heptanone, 5-methyl- | 5-methylheptan-3-one |
| 3.       | 6.13     | 0.39          | 3,6-Octadecadiynoic acid, methyl ester | methyl octadeca-3,6-diyneate |
| 4.       | 6.29     | 0.07          | Cosmene          | (3E,5E)-2,6-dimethyl-octa-1,3,5,7-tetraene |
| 5.       | 9.68     | 29.09         | L-Camphor       | (1S)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one |
| 6.       | 9.78     | 1.22          | Linalool        | 3,7-dimethyl-octa-1,6-dien-3-ol |
| 7.       | 9.90     | 0.08          | Cyclohexymethanol, trifluoroacetate (ester) | Cyclohexylmethyl trifluoroacetate |
| 8.       | 10.41    | 2.83          | Berylacetate    | 1,7,7-trimethyl-2-bicyclo[2.2.1]heptanylacetate |
| 9.       | 10.54    | 0.65          | β-Terpinol      | 1-methyl-4-prop-1-en-2-ykyclohexan-1-ol |
| 10.      | 10.68    | 0.07          | Preg-4-en-3-one, 17.alpha.-hydroxy-17.beta.-cyano-; | 17-hydroxy-10,13-dimethyl-3-oxo-phenanthrene-17-carbonitrile |
| 11.      | 12.01    | 3.75          | α-Terpinol      | 2-(4-methylcyclohex-3-en-1-yl)propan-2-ol |
| 12.      | 12.09    | 1.96          | Bornol          | (1S,2R,4S)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol |
| 13.      | 12.24    | 0.42          | Bicyclo[5.1.0]octane-3,8-(1-methylethylidene)- | 8-propan-2-yldenebicyclo[5.1.0]octane-3,8-(1-methylethylidene) |
| 14.      | 12.42    | 0.01          | [1,1'-Bicyclopeteny]-2-ol | 2-cyclopentylcyclopetan-1-ol |
| 15.      | 13.13    | 0.11          | Bicyclo[2.2.0]hexane-1-carboxaldehyde | Bicyclo[2.2.0]hexane-1-carbaldehyde |
| 16.      | 13.79    | 0.14          | Dihydrorange    | (3R)-3,7-dimethyl-octa-1,6-diene |
| 17.      | 15.64    | 0.02          | Verbenone       | (1R)-cis-4,6,6-Trimethylbicyclo[3.1.1]hept-3-en-2-one |
| 18.      | 19.09    | 0.02          | Longipinene epoxide | 1,3,9,9-tetramethyl-4-oxatetra cyclic[5.5.0.0.2,8.0.3.5]dodecane |
Table 2: Comparison of Rosmarinus officinalis essential oils' percentages extracted from different geographical regions

| Chemical compounds | DMS latitude and longitude from where rosemary extracts were obtained |
|-------------------|------------------------------------------------------------------------|
|                   | Iraq (Diyala) | Morocco (Fez) | Alabama (Huntsville) USA | Western Cape, (Riebeeck Kasteel) South Africa | Kenya (Thika) | Victoria, (Lancefield) Australia | Nepal (Jumla) | Yemen (Dhamar) | Egypt (Northern)* | Spain (Southern) | Tunisia (Sidi Bouzid) | Lebanon (Sehaileh) | Turkey (Kahramanmaraş) | Hungary (Bordj Bou Arreridj) | Algeria (Baghdad) |
| Borneol           | 1.96 | 1.47 | 4.00 | 7.00 | 2.80 | 2.10 | 2.50 | 5.50 | 8.17 | 5.45 | 5.58 | 2.40 | 14.52 | - | 3.4 | 3.78 |
| Bornyl acetate    | 2.83 | 0.74 | 1.70 | 2.00 | 1.00 | 0.90 | 1.00 | 1.80 | -   | 1.25 | 0.71 | 1.25 | 2.90 | 2.02 | - | 1.1 | 6.96 |
| Camphene          | -    | -    | 2.50 | 7.60 | 2.60 | 4.60 | 4.60 | 1.50 | -   | 6.90 | 4.28 | 4.30 | 4.30 | 3.33 | - | 3.0 | 0.36 |
| Camphor           | 29.90| 16.54| 2.40 | 0.70 | 2.10 | 1.70 | 1.70 | 7.00 | 17.01| 25.90| 15.75| 3.30 | -   | 22.00| 12.6 | 23.04|
| p-Cymene          | -    | 4.79 | 0.90 | 1.60 | 0.40 | 1.60 | 1.50 | 1.10 | -   | 1.80 | 2.73 | -    | 4.26 | -   | 2.2 |
| Dihydromyrcene    | 0.14 | -    | -    | -    | -    | -    | -    | -    | -   | -    | -    | -    | -   | -   | - |
| Eucalyptol        | 59.02| 31.20| 18.80| 16.30| 20.90| 29.40| 23.00| 20.60| 19.60| 13.25| 35.86| 22.10| 44.52| 33.85| 52.4 | 14.01|
| Geraniol          | -    | -    | 4.80 | -    | 4.60 | 0.70 | 1.60 | 3.80 | -   | -    | 5.60 | -    | -   | -   | - |
| Limonene          | -    | 0.81 | 2.70 | 4.40 | 2.30 | 3.50 | 3.50 | 1.60 | -   | 2.90 | 3.07 | 3.20 | 3.17 | -   | - | 0.42 |
| Linolool          | 1.22 | 1.49 | 2.70 | 3.10 | 2.80 | 2.30 | 2.20 | 3.80 | 5.32| 0.65 | 1.05 | 3.15 | 1.3  | -   | - | 1.1  |
| Myrcene           | -    | 3.75 | 1.30 | 1.60 | 1.20 | 1.50 | 1.40 | 0.70 | -   | 2.70 | 1.10 | 1.50 | 1.18 | -   | - | 1.7  |
| α-Pinene          | -    | 15.82| 25.40| 33.60| 31.70| 37.90| 38.10| 13.50| 15.12| 15.90| 9.04 | 28.65| 9.55 | 11.30| 5.2 |
| β-Pinene          | -    | 3.56 | 1.40 | 2.30 | 2.10 | 3.00 | 2.80 | 1.00 | -   | 4.90 | 8.15 | 2.05 | 9.50 | 5.7 |
| α-Terpineol       | 3.75 | 7.16 | 2.90 | 1.00 | 2.60 | 0.90 | 1.50 | 3.20 | -   | 1.85 | 6.42 | 7.05 | 8.41 | -   | 2.1 |
| β-Terpineol       | 0.65 | -    | -    | -    | -    | -    | -    | -    | -   | -    | -    | -    | -    | 10.8| -   | -   |
| Terpinen-4-ol     | -    | 1.1  | 0.7  | 1.0  | 0.5  | -    | 2.2  | 1.0  | -   | 0.65 | -    | -    | -    | 13.8| -   | -   |
| α-Terpinene       | -    | 2.44 | 0.30 | 0.30 | 0.40 | 0.50 | 0.60 | Trace| -   | 0.71 | -    | -    | -    | -   | -   | -   |
| γ-Terpinene       | -    | 1.00 | 0.40 | 1.00 | 0.80 | 1.20 | 0.10 | -    | 0.62 | -    | -    | -    | -   | -   | 0.5 |
| Verbenone          | 0.02 | 0.15 | 17.10| 0.00 | 11.90| 2.50 | 2.70 | 18.60| 9.55 | 4.00 | -    | 0.11 | 6.80 | -   | 11.47|
| Total (%)         | 99.49| 89.92| 91.0 | 83.4 | 91.4 | 94.4 | 92.1 | 84.48| 74.77| 88.1 | 86.92| 92.3 | 94.42| 83.45| 91.0| 84.64|

*Only the major constituents were reported
The two major components in rosemary extract from Diyala Province of the eastern side of Iraq were eucalyptol (1,8-cineole) and L-camphor, and they represent 59% and 29%, respectively (Table 1). In addition, α-terpineol, bornyl acetate, borneol, linalool, and β-terpineol were detected in 3.75, 2.83, 1.96, 1.22, and 0.65%, respectively (Table 1). Similarly, in 8 out of 15 studies presented in Table 2 showed that eucalyptol (1,8-cineole) (20–52%) is the major constituent. *R. officinalis* essential oils that were harvested from Morocco [10], Algeria [12], Hungary [13], Yemen [6], Egypt [14], Turkey [3], and Tunisia [15]. Besides, in the other 6 out of 15 studies presented, eucalyptol (1,8-cineole) (16–29%) is the second major constituent of rosemary from Lebanon, Alabama (USA), Western Cape (South Africa), Nepal, Victoria (Australia), and Kenya [6,16]. In addition, other studies have shown comparable results by identifying 1,8-cineole to be the major essential oil from rosemary harvested from different areas in Morocco and Algeria [17,18]. Camphor, on the other hand, is the second major essential oil constituent identified in an Iraqi, Diyala Province (29.9%), and the first in Baghdad (23.04%) (Table 2). Besides, camphor has also been shown to be a significant constituent of rosemary essential oil (16–26%) in Hungary [13], Southern Spain [1,19], Morocco [10,20], Algeria [12], Tunisia [15], and Egypt [14].

One of the surprising results in the present study is that one of the major hydrocarbon monoterpenes, α-pinene, was not detected. In some geographical locations, such as Alabama (USA), Western Cape (South Africa), Nepal, Victoria (Australia), Kenya, and Lebanon, α-pinene has been found to be a significant constituent of rosemary essential oils ranging from 25% to 38% [6]. Furthermore, α-pinene has been detected, but in a much lesser percentage (3–16%) in other countries as well (Table 2). In the present study, on the other hand, hydrocarbon monoterpenes represent <1%, whereas more than 96% of the essential constituents are oxygenated monoterpenes such as eucalyptol, camphor, α-pinene, borneol, linalool, β-pinene, and verbena (Tables 1 and 2). It has been established that monoterpenes undergo an atmospheric transformation which is likely considered the main leading geographic cause of a fundamental change within the same species. However, a good number of documents have shown that bacteria play an essential role in monoterpenes transformation as well [21]. Bacteria use monoterpenes as a carbon and energy source, and for instance, *Serratia marcescens* utilizes pinene and transforms it into α-pinene [21,22]. This opens a new insight into how bacteria play an essential role in monoterpenes transformation as well. This opens a new insight into how bacteria play an essential role in monoterpenes transformation as well.

**CONCLUSION**

The present study showed that rosemary essential oil from Diyala Province of Iraq consists mostly of oxygenated monoterpenes (>96%). The oxygenated monoterpenes are mainly eucalyptol (1,8-cineole) and L-camphor, in which they represent 88% of the essential oil extracted.

**CONFLICTS OF INTEREST**

The author has no conflicts of interest to share.

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