Identification and Classification of Libyan Rosmarinus Officinalis Essential Oil Components by GC-MS and Predication of Its Antioxidant Activity

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Abstract: The aim of this investigation is identification and classification of Rosmarinus Officinalis essential oil components by GC-MS and prediction of their antioxidant activity. GC-MS analysis of Libyan Rosemary essential oil indicated that in total, 44 compounds were detected comprising 98.3% of compounds in the oil. The results also revealed that the oil contained some constituents with great antioxidant activity such as eugenol (0.04%), diethylphalate (0.28%), myrtenol (0.46%), γ-Terpinene (2.2%), α – Terpenolene (1.96%), with total percentage of (4.94%) which was considered to be very small. However, about the half of the essential oil contained of constituents with very weak antioxidant activity. These include trans-β-caryophinen (2.2%), α- humulene (0.34%), 1.8- cineol (6.15%), 1.8 – cineol isomer (11.8%), linalool (3.29%), 4-terpinol (5.65%), 1α – terpine (7.93%), isoborneol (0.29%), perillaldehyde (0.09%), β- citronellal (0.03%), α – pinene (3.4%), comphen (3.04%) and β-pinene(4.21 %), our result shows that the essential oil of Libyan Rosemary might have a weak antioxidant activity.

Keywords: Rosmarinus Officinalis, essential oils, GC-MS analysis of essential oil, antioxidants.

INTRODUCTION

Essential oil yield and chemical composition vary, considerably due to different factors, both intrinsic and extrinsic (Stahl-Biskup, 2002). Intrinsic factor includes genetic and sexual variations, plant organ (roots, leaves, stem, etc.), age and vegetative cycle stage. On the other hand, extrinsic factors and described by ecological and environmental aspects such as attitude, soil composition, climate and light, all these factors can drastically affect the chemical composition of essential oils (Stahl-Biskup, 2002). Thus, these variations are of distinct important when studying the biological activity, such as, antioxidant capacity of essential oils as the degree of activity has to be related to its chemical composition (Panizzi et al, 1993; Lahlou and Berrada, 2003; Alvarez et al., 2019).

Essential oils are commercially important especially for the pharmaceutical, cosmetic, perfume industries and medical properties, as well as in food and beverages, as flavoring agents and preservatives (Van De Braak and Leijen, 1999; Burt, 2004; Fitsiou et al., 2016). Nowadays, essential oils and their components are gaining increasing attention, because of their relatively safe status, their wide acceptance by customers, and the possi-
bility of their exploitation for potential multi-purpose functional uses (Ormancey et al., 2001). In this context, many essential oils and constituents have been studied (Ruberto and Barata, 2000; Nieto et al., 2018). For natural antioxidants with the virtue of being non-toxic has given rise to a large number of studies on the potential of the essential oils of several aromatic plants, among them, Rosemary. (Miura et al, 2002; Wang et al, 2008; Ojeda et al., 2013; Alvaraz et al., 2019).

In Libya, the Al-Jabal Al-Akhdar mountain, which is a region located in the North-East of Libya, is a rich habitat of many aromatic plants that grow wild, among them *Rosemary Officinalis*. These aromatic plants are used fresh or dry as spicy herbs, in many Libyan dishes, and for medical purposes (Jafri and El-Gadi, 1985). So, the aim of this study is extraction of the essential oils of Libyan *Rosemary Officinalis* by hydro-distillation, identification and classification of its chemical constituents by using gas chromatography coupled to mass spectroscopy (GC-MS) and the prediction of its antioxidant activity.

**MATERIALS AND METHODS**

**Chemical and reagent:** All chemicals and reagents were highly pure which purchased from Fisher scientific (Loughborough, UK) and Sigma-Aldrich (Pool, Dorset, UK).

**Plant material:** Leaves of wiled plant *Rosmarinus Officinalis* (Rosemary) were collected during the flowering stage in April 2019) at Al-Jabal Al-Akhdar region in North-East of Libya, specimens of collected plant were confirmed and deposited at the Herbarium of the department of Biology, University of Tripoli, Libya. The fresh sample were frozen in sealed sample bags at -18°C till extraction.

**Essential oil extraction:** The classic methodology of hydro-distillation using Clevenger-type apparatus was used for the isolation of the essential oil from rosemary (*Rosmarinus Officinalis*). The fresh leaves of the aromatic plants (100 g) were blended with water (1500 ml) in a blender. The mixture was transferred into the hydro-distillation unit for 3-4 hours, until no more essential oil was obtained. The essential oil was separated from water, dried over anhydrous sodium sulphate and stored in sealed airtight amber glass flasks at 4°C until analyzed (Daferera et al, 2003).

**Identification of essential oil by gas chromatography mass spectroscopy (GC-MS):** Essential oil of Libyan Rosemary obtained by hydro-distillation was analyzed, and their constituents identified using GC-MS. The analysis was performed using a Shimadzu GC-MS-QP 5050 A, software class 5000, with a DBI column (30m x 0.53 m i.d), 1.5 µm film thickness. The carrier gas was helium (flow rate 1ml/min). For GC-MS detection, an electron ionization mode (EI) with ionization energy 70ev was used. The temperature program as follows: 30°C (static, for 2 min) then gradually increasing (at a rate of 2°C/min) up to 250°C (static for 5 min). Injector temperature was 280°C. Qualitative identification of the essential oil constituents was achieved using a willey 299 LIB database, and by comparing mass fragmentation patterns with those from the available published data. Quantitative estimation of the volatile constituents was determined by computerized peak are measurements using an internal normalization method. This analysis was carried out on the Regional Center for Mycology and Biotechnology, Al-Azhar university, Cairo (Egypt).

**RESULTS AND DISCUSSION**

The yield of the essential oil from *R. Officinalis* growing wild in Libya was 0.47% ± 0.18%, on a dry weight basis. This yield is slightly higher than those obtained from the wild Portuguese *R. Officinalis* 0.3% (Mata et al., 2007). However, it was lower than these obtained from Egyptian cultivated *R. Officinalis* 1.2% (Viud-Martos et al., 2010). Serbian
R. Officinalis 1.18% (Bozi n et al., 2007) and also Tunisian wild R. Officinalis 1.2% (Hosni et al., 2013). It is quite often that the yield of essential oil is affected by many factors, among them the species of the plant, the part of the plant and geographical and climate conditions (Sthl-Biskup, 2002).

**GC-MS analysis of Libyan essential oils from R. Officinalis** To rationalise the antioxidant activity of Libyan endemic plant R. Officinalis, it is necessary to address composition, which to the best of our knowledge has not been studied before. Compound identification was carried out using GC-MS. GC-MS analysis of the Libyan essential oil indicated that in total, 44 compounds were detected in rosemary oil comprising more than 98.3% of total compounds in the oil. The pattern of chemical contents of R. officinalis essential oils indicated that this oil were complex mixtures of several compounds. For this reason, the components were divided into six classes, namely monoterpenes hydrocarbons, oxygenated monoterpenes, sesquiterpenes hydrocarbons, oxygenated sesquiterpenes, heterogeneous hydrocarbons and oxygenated heterogeneous hydrocarbons.

**GC-MS analysis of Libyan R. officinalis essential oil**

1. **Monoterpene hydrocarbons**
   The results showed that the monoterpene hydrocarbons group of the R. officinalis oil from Libya consisted of eight compounds (table 1) and represented 25.5% of the contents. The highest content was of the β-pinene isomer and the lowest content was of γ-terpinene. Although the monoterpene hydrocarbons group makes up about a quarter of the total contents of the oil, the compounds in this group were found in small concentration. In addition, this group of compounds contained some constituents that could be considered to be active as antioxidants, ranging from very weak to very strong (Ruberto and Baratta, 2000; Wang et al., 2008). γ-Terpinene and α-terpinolene, which are known to be strong antioxidant compounds, were found in small quantities, whereas α-pinene, camphene and β-pinene were found in measurable amounts in this group, but they are characterized by their very weak antioxidant activity.

| Retention time (min) | Molecular ion | Base Peak | Chemical compound | Molecular formula | Percentage % |
|----------------------|---------------|-----------|-------------------|-------------------|--------------|
| 13.15                | 136           | 93        | α-pinene          | C10H16            | 3.4          |
| 13.44                | 136           | 93        | α-pinene isomer   | C10H16            | 3.17         |
| 13.59                | 136           | 93        | camphene          | C10H16            | 3.04         |
| 13.82                | 136           | 93        | camphene isomer   | C10H16            | 2.87         |
| 14.28                | 136           | 93        | 1-β-pinene        | C10H16            | 4.29         |
| 14.47                | 136           | 93        | 1-β-pinene isomer | C10H16            | 4.61         |
| 15.93                | 136           | 93        | γ-terpinene       | C10H16            | 2.2          |
| 16.96                | 136           | 93        | α-terpinolene     | C10H16            | 1.96         |
| Number of identified compounds | 8 |
| Percentage of the total composition | 25.54% |

2. **Oxygenated monoterpenes**
   The oxygenated monoterpenes group (table 2) was found to be the most representative group of the compounds present in the Libyan rosemary oil, representing 66.33% and containing 23 compounds. Camphor was at the highest level (16.07%) in rosemary oil followed by the 1, 8-cineol isomer (11.8%). On the other hand, only a very small amount of thymol was identified (0.2%) and there was a complete absence of carvacrol. Thymol and carvacrol are considered to be the most powerful members of this group responsible for the antioxidant activity of most essential oils (Youdim et al., 2002; Lee et al., 2005). Furthermore, the results showed that this group (oxygenated monoterpenes) also contained a reasonable number and amount of compounds with very weak antioxidant
activity. Such components are 1, 8-cineol, 1,8-
cineol isomer, 4-terpineol, 1-α-terpineol, and 
very small amount of isoborneol and β-
citronellall.

These results correspond well with the results 
obtained by Okoh et al. (2010) who found 
oxygenated monoterpenes and monoterpenes 
were the major constituents in R. officinalis 
oil. In plant essential oils, oxygenated 
monoterpenes and monoterpenes are mainly 
responsible for most of the antioxidant 
activities (Ruberto and Baratta, 2000).

Table:(2). Oxygenated monoterpenes identified by GC-
MS analysis of Libyan R. officinalis essential oil.

| Number | Retention time (min) | Molecular ion M+ | Base Peak | Chemical compound | Molecular formula | Percentage |
|--------|----------------------|------------------|----------|------------------|-------------------|------------|
| 1      | 15.39                | 154              | 43       | 1,8-cineole      | C10H18 O          | 6.15       |
| 2      | 16.06                | 154              | 43       | 1,8-cineole isomer | C10H18 O        | 11.8       |
| 3      | 16.81                | 154              | 93       | linalool         | C10H18 O          | 3.29       |
| 4      | 18.04                | 152              | 108      | α-pinene oxide   | C10H16 O          | 0.42       |
| 5      | 18.38                | 152              | 95       | camphor          | C10H16 O          | 16.07      |
| 6      | 19.39                | 152              | 95       | camphor isomer   | C10H16 O          | 2.01       |
| 7      | 19.55                | 150              | 81       | pinocarvone      | C10H14 O          | 4.35       |
| 8      | 19.85                | 152              | 55       | bicyclomonomote 
pene ketone        | C10H16 O        | 2.11       |
| 9      | 20.15                | 154              | 71       | 4-terpineol      | C10H18 O          | 5.65       |
| 10     | 20.46                | 154              | 59       | 1-α-terpineol    | C10H18 O          | 7.93       |
| 11     | 20.93                | 150              | 95       | monoterpane ketone | C10H14O         | 3.49       |
| 12     | 21.91                | 152              | 95       | camphor isomer   | C10H16 O          | 0.22       |
| 13     | 22.18                | 152              | 79       | myrenol          | C10H16 O          | 0.46       |
| 14     | 22.60                | 152              | 69       | aldehyde         | C10H16 O          | 0.16       |
| 15     | 22.72                | 150              | 95       | unsaturated ketone | C10H14 O        | 0.42       |
| 16     | 23.27                | 154              | 95       | isoborneol       | C10H18 O          | 0.29       |
| 17     | 23.87                | 196              | 95       | endobornyl acetate | C12H2O2        | 0.95       |
| 18     | 24.18                | 150              | 135      | thymol           | C10H14 O          | 0.2        |
| 19     | 25.07                | 154              | 41       | dihydrocarvacrol | C10H18 O          | 0.12       |
| 20     | 25.43                | 150              | 67       | prillaldehyde    | C10H14 O          | 0.09       |
| 21     | 25.77                | 150              | 121      | aldehyde         | C10H14 O          | 0.11       |
| 22     | 26.32                | 156              | 41       | β-citronellall   | C10H2O2          | 0.03       |
| 23     | 27.83                | 164              | 43       | monoterpane oxide | C10H12O2        | 0.11       |

Percentage of the total composition: 66.33%

3. Sesquiterpene hydrocarbons
The sesquiterpene hydrocarbons group (Table 
3) comprised of 3 compounds at a low 
percentage (2.62%) of the oil constituents. 
This group contained two weak antioxidant 
compounds, trans-β-caryophyllene and 
humulene.

Table:(3). Sesquiterpene hydrocarbons identified by GC-MS analysis of Libyan R. officinalis essential oil.

| Number | Retention time (min) | Molecular ion M+ | Base Peak | Chemical compound | Molecular formula | Percentage |
|--------|----------------------|------------------|----------|------------------|-------------------|------------|
| 1      | 32.1                 | 204              | 41       | trans-β-caryophyllene | C15H24        | 2.2        |
| 2      | 33.93                | 204              | 93       | α-humulene       | C15H24          | 0.34       |
| 3      | 37.37                | 204              | 161      | α-amorphene      | C15H24          | 0.08       |
|        | Number of identified compounds | 3 |
|        | Percentage of the total composition | 2.62% |

4. Oxygenated sesquiterpene and heterogeneous hydrocarbon
The oxygenated sesquiterpene and 
heterogeneous hydrocarbon groups (table 4) 
consisted of two compounds each, spathulenol 
and δ-cadinol, and nonane and 3,4-octadiene-
7-me respectively.

Table:(4). Oxygenated sesquiterpenes and 
heterogeneous hydrocarbons identified by GC-MS 
analysis of Libyan R. officinalis essential oil.

| Number | Retention time (min) | Molecular ion M+ | Base Peak | Chemical compound | Molecular formula | Percentage |
|--------|----------------------|------------------|----------|------------------|-------------------|------------|
| 1      | 41.65                | 220              | 41       | spathulenol      | C15H24 O         | trace      |
| 2      | 46.63                | 222              | 161      | δ-cadinol        | C15H26 O         | 0.21       |
| 3      | Number of identified compounds | 2 |
| 4      | Percentage of the total composition | 0.21 |

Oxygenated sesquiterpenes
41.65 220 41  spathulenol  C15H24 O  trace
46.63 222 161  δ-cadinol  C15H26 O  0.21
Number of identified compounds 2
Percentage of the total composition 0.21
Heterogeneous hydrocarbons
9.43 128 43  nonane  C9 H2O  trace
11.87 124 67  3,4-octadiene-7-me  C9 H16  0.02
Number of identified compounds 2
Percentage of the total composition 0.02 %

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5. Oxygenated heterogeneous hydrocarbons

The final group is the oxygenated heterogeneous hydrocarbons (table 5) consisting of 7 compounds representing 3.6% of the oil. This group contained very important compounds with very high antioxidant activity, namely eugenol and diethyl phthalate, present in relatively small amounts but probably affecting the overall antioxidant activity of this oil.

Table: (5). Oxygenated heterogeneous hydrocarbons identified by GC-MS analysis of Libyan R. officinalis essential oil.

| Retention time (min) | Molecular weight | Base peak | Chemical compound | Molecular formula | Percentage % |
|---------------------|------------------|-----------|-------------------|-------------------|--------------|
| 5.68 14.8 26.9 28.53 40.05 68.20 74.87 | 86 128 164 178 222 278 390 | 41 57 64 178 149 149 149 | 3-me-butanol 1-octen-3-ol me eugenolate diethyl phthalate 1,2-benzenedicarboxylic acidbutyl-2-methylpyrolyl ester 1,2-benzenedicarboxylic acid bis (2-ethylhexyl ester) | C5H10 O C8H16O C11H14O2 C12H14O2 C16H22O4 C24H38O4 | 0.02 2.13 0.04 0.53 0.28 0.54 0.03 |
| Number of identified compounds | 7 |
| Percentage of the total composition | 3.51 |

Several reports have been published (Wang et al., 2008; Viuda-Martos et al., 2010) concerning the composition of R. officinalis essential oil; these reports have emphasized the extent of marked chemical differences among essential oils extracted from the same species. These differences in the chemical composition of R. officinalis oil can be attributed to the part of the plant extracted, the season of harvesting, the geographical origin and the method of extraction (Ravid and Putrisky, 1986; Mueller-Riebau et al., 1997; Jordan et al., 2006; Bakkali et al., 2008).

The results of the GC-MS analysis in the current study were in good agreement with the results obtained by Soliman et al. (1994), who found that rosemary oil comprised 43% compounds. However, some differences were noticed with regards to the number and quantity of some compounds in rosemary oils studied from different regions, such as the cultivated Egyptian rosemary oil, when analysed 27 compounds were identified representing 90.2% of the total oil, the major constituents were 1,8-cineole (23.59%), camphor (20.70%) and α-pinene (18.21%) (Viuda-Martos et al., 2010), and the Spanish southern R. officinalis oil when investigated by Tomeia et al. (1995), they found the main components to be camphor (32.33%), 1,8-cineole (14.41%), and α-pinene (11.56%). The geographical location of where the plant grows can contribute to the content and quality of essential oils (Stahl-Biskup, 2002).

Earlier data pertaining to the rosemary essential oils has pointed out the difference between the Morocco/Tunisian chemotype (containing 38-55% of 1, 8-cineol) and the Spanish chemotype characterized by high amount of the monoterpenic hydrocarbons, α-pinene (18-26%), camphene (8-12%) and 1,8-cineol (16-25%) (Bozin et al., 2007). However, the essential oil obtained from rosemary growing wild in Libya investigated here, has a specific chemical composition, 1, 8-cineol (6.15%), α-pinene (3.4%) and camphene (3.04%) that could not be categorized in one of the two previously described chemotypes.

Finally, the differences in the number and type of the compounds in the essential oils of R. officinalis could lead to differences in their antioxidant activities and should be taken into consideration when studying the antioxidant potential of the essential oil. In addition, the results of the essential oil composition give us, to some extent, a prediction about whether an
oil has a strong or weak antioxidant activity. The oil under investigation contained some constituents with great antioxidant activity such as eugenol, (0.04%), diethyl phthalate (0.28%), myrteol (0.46%), γ-terpinene (2.2%), α-terpenolene (1.96%), with a total percentage of (4.94%) which was considered to be very small. However, about half of the essential oil contained constituents with a predicted very weak antioxidant activity. These included trans-β-caryophlene (2.2%), α-humulene (0.34%), 1,8-cineol (6.15%), 1,8-cineol isomer (11.8%), linalool (3.29%), 4-terpineol (5.65%), 1-α-terpine (7.93%), isoborneol (0.29%), prillaaldehyde (0.09%), β-citronellal (0.03%), α-pinene (3.4%), camphene (3.04%) and β-pinene (4.29%). Based on the data published by Ruberto and Baratta (2000), Youdim et al., (2002) and Wang et al. (2008) the essential oil of Libyan rosemary oil might have a weak antioxidant activity.

CONCLUSION

Rosmarinus Officinalis essential oil grow wild in Libya, is a mixture of 44 compounds, including a group of six classes namely, Monoterpenes Hydrocarbons, Oxygenated Monoterpenes, Sesquiterpenes Hydrocarbons, Oxygenated Sesquiterpenes, Heterogeneous Hydrocarbons. This oil might have a weak antioxidant activity according to its chemical constituents, where the content of the phenolic compounds which are considered being strong antioxidants too low, (Thymol, 0.2% and the absent of Carvacrol, 0.0%). However, attention should be paid to study its extracts of Rosmarinus Officinalis plant using different solvents for extraction.

ETHICS

The authors declare no conflict of interest.

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تعريف وتصنيف المركبات الكيميائية لزيت نبات الإكليل النامي طبيعياً من منطقة الجبل الأخضر والتكهن بالتأثير المضاد للاكسدة لموكنته

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المستخلص: يهدف هذا البحث إلى التعرف وتصنيف المكونات الكيميائية لزيت الطيار لنبات الإكليل (Rosmarinus Officinalis)، النامي طبيعيًّا من منطقة الجبل الأخضر - ليبيا. باستخدام كروماتوجرافيا الغاز المدمج بتطبيق الكتلة (GC-MS)، وكذلك التكهن بالنشاط المضاد للاكسدة لهذه المكونات. أوضحت النتائج أن الزيت الطيار لنبات الإكليل يحتوي على 44 مركب كيميائي، شكلت حوالي 98.3% من إجمالي المركبات الموجودة في الزيت. كما بينت النتائج وجود بعض المكونات ذات النشاط العلجي كمضادات للاكسدة. شملت كل من مركب myrtenol (0.28%)، diethylphalate (0.04%)، eugenol (1.96%)، α – Terpenolene (2.2%)، γ-Terpinene (0.46%)، Mكوتات الزيت المعترف عليها. وهي تُعتبر نسبة ضئيلة جداً، ومن الناحية الأخرى، شكلت المركبات ذات النشاط الضعيف trans-β-caryophinen كمضادات للاكسدة حوالي 50% من إجمالي المركبات المعترف عليها. وهذه شملت مركبات كل من: linalool (11.8%)، 1.8 – cineol isomer (6.15%)، 1.8- cineol (0.34%)، α- humulene (2.2%)، β- citronellal (0.09%)، perillaldehyde (0.29%)، isoborneol (7.93%)، α – terpine (5.65%)، 4-terpinol (4.21 %)، β- pinene (3.04%)، α – pinene (3.4%)، و 4-terpinol (0.03%).

النتائج أن الزيت الطيار لنبات الإكليل النامي طبيعيًّا من منطقة الجبل الأخضر ربما يكون له تأثير ضعيف كمضاد للاكسدة.

الكلمات المفتاحية: نبات الإكليل، الزيت الطيار، تحليل GC-MS، مضادات الأكسدة.