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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%), 1-α – 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

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wide acceptance by customers, and the possibility 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 constitutes 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 -18C^o 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 air-tight amber glass flasks at 4C^o 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: 30C^o (static, for 2 min) then gradually increasing (at a rate of 2C^o/min) up to 250C^o (static for 5 min). Injector temperature was 280C^o. 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% (Bozin et al ., 2007) and

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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-terpene. 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.

Table:(1). Monoterpene hydrocarbons identified by GC-MS analysis of Libyan R. officinalis essential oil.

Retention time (min)	Molecular ion M+	Base Peak	Chemical compound	Molecular formula	Percentage %
13.15	136	93	α-pinene	$C_{10}H_{16}$	3.4
13.44	136	93	α-pinene isomer	$C_{10}H_{16}$	3.17
13.59	136	93	camphene	$C_{10}H_{16}$	3.04
13.82	136	93	camphene isomer	$C_{10}H_{16}$	2.87
14.28	136	93	1-β-pinene	$C_{10}H_{16}$	4.29
14.47	136	93	1-β-pinene isomer	$C_{10}H_{16}$	4.61
15.93	136	93	γ-terpinene	$C_{10}H_{16}$	2.2
16.96	136	93	α-terpinolene	$C_{10}H_{16}$	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 a 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-

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cineol isomer, 4-terpineol, 1-α-terpineol, and very small amount of isoborneol and β-citronellal.

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.

Retention time (min)	Molecular ion M+	Base Peak	Chemical compound	Molecular formula	Percentage %
15.39	154	43	1,8-cineole	C10H18 O	6.15
16.06	154	43	1,8-cineole isomer	C10H18 O	11.8
16.81	154	93	linalool	C10H18 O	3.29
18.04	152	108	α-pinene oxide	C10H16 O	0.42
18.38	152	95	camphor	C10H16 O	16.07
19.39	152	95	comphor isomer	C10H16 O	2.01
19.55	150	81	pinocarvone	C10H14 O	4.35
19.85	152	55	bicyelomonoter pene ketone	C10H16 O	2.11
20.15	154	71	4-terpineol	C10H18 O	5.65
20.46	154	59	1-α-terpineol	C10H18 O	7.93
20.93	150	95	monoterpene ketone	C10H14O	3.49
21.91	152	95	camphor isomer	C10H16 O	0.22
22.18	152	79	myrtenol	C10H16 O	0.46
22.60	152	69	aldehyde	C10H16 O	0.16
22.72	150	95	unsaturated ketone	C10H14 O	0.42
23.27	154	95	isoborneol	C10H18 O	0.29
23.87	196	95	endobornyl acetate	C12H2O2	0.95
24.18	150	135	thymol	C10H14 O	0.2
25.07	154	41	dihydrocarvacrol	C10H18 O	0.12
25.43	150	67	prillaldehyde	C10H14 O	0.09
25.77	150	121	aldehyde	C10H14 O	0.11
26.32	156	41	β-citronellal	C10H20 O	0.03
27.83	164	43	monoterpene oxide	C10H12O2	0.01
Number of identified compounds					23
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.

Retention time (min)	Molecular ion M+	Base Peak	Chemical compound	Molecular formula	Percentage %
32.1	204	41	trans-β-caryophyllene	C15H24	2.2
33.93	204	93	α-humulene	C15H24	0.34
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.

Retention time (min)	Molecular ion M+	Base Peak	Chemical compound	Molecular formula	Percentage %
Oxygenated sesquiterpenes					
41.65	220	41	spathulenol	C15H24 O	trace
46.63	222	161	δ-candinol	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 ion M+	Base Peak	Chemical compound	Molecular formula	Percentage %
5.68	86	41	3-me-butanol	C5H10 O	0.02
14.8	128	57	1-octen-3-ol	C8H16O	2.13
26.92	164	164	eugenol	C10H12 O	0.04
28.53	178	178	me eugenolate	C11H14 O2	0.53
40.05	222	149	diethyl phthalate	C12H14O2	0.28
68.20	278	149	1,2-benzenedicrboxylic acidbutyl-2-meththyprolyl ester	C16H22O4	0.54
74.87	390	149	1,2-benzenedicrboxylic acid bis (2-ethylhexyl ester)	C24H38O4	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 monoterpene 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.

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The oil under investigation contained some constituents with great antioxidant activity such as eugenol, (0.04%), diethyl phtalate (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 بمنطقة الجبل الأخضر والتكهن بالتأثير المضاد للاكسدة لمكوناته

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المستخلص : يهدف هذا البحث إلى التعرف وتصنيف المكونات الكيميائية للزيت الطيار لنبات الإكليل (*Rosmarinus Officinalis*) النامي طبيعياً بمنطقة الجبل الأخضر – ليبيا. باستخدام كروموتوجرافيا الغاز المدمج بطيف الكتلة (GC-MS)، وكذلك التكهن بالنشاط المضاد للأكسدة 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 العالي كمضادات للأكسدة. شملت كل من مركب eugenol ‏(0.04%)، diethylphalate ‏(0.28%)، myrtenol ‏(0.46%)، γ-Terpinene ‏(2.2%)، α – Terpenolene ‏(1.96%)، ومجموعها شكل حوالي (4.94%) من إجمالي مكونات الزيت المتعرف عليها. وهي تعتبر نسبة ضئيلة جداً. ومن الناحية الأخرى، شكلت المركبات ذات النشاط الضعيف كمضادات للأكسدة حوالي 50% من إجمالي المركبات المتعرف عليها وهذه شملت مركبات كل من،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%)، α – terpine ‏(7.93%)، isoborneol ‏(0.29%)، perillaldehyde ‏(0.09%)، β- citronellal ‏(0.03%)، α – pinene ‏(3.4%)، comphen ‏(3.04%) ، و β- pinene ‏(4.21 %). أظهرت النتائج أن الزيت الطيار لنبات الإكليل النامي طبيعياً بمنطقة الجبل الأخضر ربما يكون له تأثير ضعيف كمضاد للأكسدة.

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

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