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Chemo-typing of Three Egyptian Rosemary Oil for Their Chemical Composition and Anti-oxidant Activity

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MFL and HNB designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors ME and MIE managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To study the chemical composition of volatile oil samples from different place in Egypt (Mansoura, Gamsa and Assuit) and study their antioxidant activity determined by DPPH assay.
Study Design: Hydro distillation of volatile oil samples and their GC/MS analysis and determination of their antioxidant activity by DPPH assay.
Place and Duration of Study: Department of Pharmacognosy, Faculty of pharmacy, Mansoura university, Egypt, between June 2015 and November 2017.
Methodology: The essential oil was isolated by hydro-distillation for 5 h using a Clevenger-type all-glass apparatus according to the standard procedure of the European pharmacopeia and volatile oils analysis was performed by GC and GC-MS. GC analysis was carried out using Focus-DSQ-II GC/MS instrument (Thermo Scientific, MA, USA) equipped with TR-5 fused silica column (30 m × 0.25 mm, film thickness 0.25 µm).
Results: The yield of the essential oils of three rosemary plants growing in Mansoura (RM), Gamsa (RG) and Assiut (RA) were 0.20% v/w, 0.32% v/w and 0.24% v/w, respectively. Their chemical

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composition was analyzed by GC/MS, RM and RG were found to be α-pinene dominated chemotypes, 32.4% and 29.6%, respectively, meanwhile RA was camphor dominated chemotype (17.2%). Furthermore, their antioxidant activity was determined by DPPH assay. Their IC_{50} values of essential oils of RM, RG and RA were 8.66 ± 0.7, 8.18 ± 0.5 and 9.74 ± 0.2, respectively.
Conclusion: The GC/MS spectral data revealed the considerable difference between the chemical composition of essential oil constituents of RM, RG and RA which lead to different chemotypes. The present results also demonstrate that REO obtained from different areas in Egypt exhibited free radical scavenging activity determined by DPPH assay due to the synergistic effect between their constituents.

Keywords: Rosmarinus officinalis L.; essential oil; GC/MS; anti-oxidant activity.

ABBREVIATIONS

GC/MS : Gas Chromatography and Mass Spectroscopy
DPPH : 2.2 -Diphenyl-1-picryl-hydrazyl
IC : Inhibitory Concentration
RI : Retention Index
BHT : Butyl Hydroxy Toluene
REO : Rosemary Essential Oil
RM : Essential Oil Sample of Mansoura
RG : Essential Oil Sample of Gamsa
RA : Essential Oil Sample of Assuit

1. INTRODUCTION

Rosmarinus officinalis L. is a very important aromatic and medicinal plant, which belongs to the Lamiaceae family [1]. It grows primarily in the Western Mediterranean region and it is now cultivated all over the world as aromatic and ornamental plant [2].

Essential oils are a complex mixture of volatile compounds with strong odor. They are synthesized in several plant organs and possess various biological activities [3].

Rosemary essential oils (REO) are commonly used as a condiment, but it has been widely used in the treatment of gastritis, dermatitis, inflammation and bronchitis, also used as antioxidant, anticancer and antimicrobial agents [4].

Chemotype describes the species of plant have the same morphological characters, but produce different quantities of the chemical components in their essential oil [5]. REO chemical composition differed significantly but the major constituents are 1,8 cineole, α-pinene, camphor, limonene, camphene and linalool [6], associated with variable amounts of other compounds such as borneol, terpinol and verbenone [2].

REO composition is affected by various factors, such as extraction method, the distance between plants, harvest time, soil humidity and drying method. Therefore, researchers have sought to obtain information about the yield, composition, and chemical properties of REO following a variety of extraction methods and harvesting times [7].

More than 13 different rosemary oil chemotypes have been reported based on the relative percentages of α-pinene, 1,8-cineole, camphor, borneol, verbenone and bornyl acetate [8].

The aim of the study is to determine the different chemotypes of the essential oil of various rosemary plants growing in Egypt. Rosemary plants were collected from Mansoura, Gamsa and Assiut. Mansoura and Gamsa lie on the east bank of the Damietta branch of the Nile, about 56 kilometers from each other, in the delta region. They are about 120 kilometers northeast of Cairo. Meanwhile, Assiut is located in Upper Egypt and lies about 375 kilometers south of Cairo. The essential oils obtained were investigated for their yield, chemical composition and antioxidant activity.

2. MATERIALS AND METHODS

2.1 Plant Material

Rosmarinus officinalis L. plants were collected from the botanical garden of Mansoura university, Mansoura (RM), botanical garden of delta university, Gamsa (RG) and botanical garden of Assuit University, Assuit (RA) in June 2015. A voucher specimen has been deposited at the herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Mansoura University (06-15-RM-Mansoura), (06-15-RG-Mansoura) and (06-15-RA-Mansoura). Samples were kindly identified by Prof. Ibrahim Mashaly,

Department of Ecology, Faculty of Science, Mansoura University, Egypt.

2.2 Distillation of the Essential Oil

Two hundred and fifty grams of the fresh aerial parts of each *R. officinalis* sample was chopped into small pieces. The essential oil was isolated by hydro-distillation for 5 h using a Clevenger-type all glass apparatus according to the standard procedure of the European pharmacopoeia [9]. Each oil was transferred to a screw-capped glass vial, dried over anhydrous sodium sulfate and stored at 4° in the dark until GC/MS analysis and biological activity screening.

2.3 GC/MS Analysis of Essential Oil Content

Analysis of volatile oils by GC-MS. GC analysis was carried out using Focus-DSQ-II GC/MS instrument (Thermo Scientific, MA, USA) equipped with TR-5 fused silica column (30 m × 0.25 mm, film thickness 0.25 µm). This work has been done in Spectroscopy Lab, Faculty of Science, Mansoura university. Sample volume; 10 µl. Oven temperature was programmed from 60°C to 240°C at 5°C /min; injector temperature, 275°C; carrier gas, helium (1 ml/min); split: 1/50. The relative proportions of the essential oil constituents were expressed as percentages obtained by peak area normalization. The MS operating parameters were: interface temperature: 300°C, ion source temperature: 200°C, EI mode: 70 eV, scan range: 40 – 450 *m/z*. Compounds Identification: Mass spectra of the individual GC peaks were identified by computer search of the commercial libraries (WILEY, NIST), as well as matching with published mass spectra [10]. The identification was further confirmed by the calculation of the retention indices (RI) relative to n-alkanes [10]. The results were listed in Table 1.

2.4 DPPH (2.2 -Diphenyl-1-Picryl-Hydrazyl) Assay

The antioxidant activity was evaluated by the DPPH (1,1- diphenyl-2-picrylhydrazyl) radical method. The ability of the essential oils and main compounds to donate an electron and scavenge DPPH radical was determined by the method of Ojeda-Sana et al. [11]. Briefly, 10 µL of each sample in triplicate and 120 µL of DPPH solution in 70% methanol to start concentration of each sample of RM, RG and RA with 11.6 mg/ml, 18.3 mg/ml and 17.6 mg/ml, respectively. They were added to the well flat-bottom microtitration plate. A DPPHº solution was used as blank sample and Butyl hydroxy toluene (BHT, Sigma Aldrich, Egypt), synthetic antioxidant was used as control. The plate was covered and incubated on a shaker for 1 h at 50 rpm at room temperature. Samples were read in a plate reader (DTX 880 Multimode detector, Beckman Coulter) using a 517 nm filter. The DPPH solution employed was freshly prepared, and stored protected from light. The antioxidant activity of the tested samples, expressed as percentage inhibition of DPPH, was calculated according to the formula IC (%) = [(A0 - A)/A0] x 100, where A0 and A are the absorbance values of the blank sample and the test, respectively. Percent inhibition after 30 min was plotted against concentration, and a linear regression was applied to obtain the IC_{50} value [11]. The results were recorded in Table 2.

3. RESULTS AND DISCUSSION

3.1 Essential Oils Analysis

The yield of essential oils of RM, RG and RA obtained by steam distillation were 0.20% *v/w*, 0.32% *v/w* and 0.24% *v/w*, respectively. Their chemical composition was determined by GC/MS analysis and the results were listed in (Table 1). Eleven compounds were identified from RM, representing 91.25 %, fourteen compounds from RG, representing 96.26% and seventeen compounds from RA, representing 95.59 % of the total detected constituents. The major constituents in the essential oils were α-pinene (32.45%, 29.65%) followed by1,8-cineole (22.12%, 12.57%) and camphor (11.67%, 11.86%) in RM and RG, respectively. While in RA, camphor (17.24%) was the major constituent followed by α-pinene (14.84%) and 1,8-cineole (11.50%).

The results also showed that the major constituents in the three essential oils samples are monoterpenes while sesquiterpenes were minor, representing 6.27%, 4.01% and 0.75% in RM, RG and RA, respectively.

The dominance of 1.8-cineole over any other constituent in rosemary plant is by the data reported by several authors. For example; Jean Claude Chalchat *et al.,* reported that 1,8-cineole was the major component in the essential oils of *Rosmarinus officinalis* L. collected from Morocco and Tunisia [8]. Where in Spain, Mehmet Musa Ozcan et al., reported that α-pinene was the major component in the essential oils of

Rosmarinus officinalis [5]. Myrcene was also found as the main component in aerial parts of *Rosmarinus officinalis* L. cultivated in South America [11]. For instance, a study conducted by Robert Tisserand and Rodney Young, 2014 revealed that the prominent component of the essential oil of *Rosmarinus officinalis* L. cultivated in France was bornyl acetate [12]. For interest, verbenone was reported as the major component for *Rosmarinus officinalis* L. oils cultivated in Egypt [12]. However, this is the first report of α-pinene and camphor dominated chemotypes form Egypt.

Our study showed that limonene (7.44%) and dihydrocarveol (1.18%) were present only in RA, so it was worth to mention that the presence of limonene and dihydrocarveol constituents is good evidence for essential oils of RA chemotype. On another hand, terpinene constituent (0.61%) was characteristic for the essential oils of RG only.

α-Pinene (32.45 %) and 1.8-cineole (22.12%) were present with the highest percentage in RM over than RG and RA. While borneol (13.81 %) was the highest in RA more than RM and RG.

3.2 Antioxidant Activity

In the DPPH assay, the ability of the components of the essential oil to act as donors of hydrogen atoms or electrons for the transformation of DPPH° radical into its reduced form DPPH was investigated. The essential oils studied were able to change the stable violet DPPH° radical into yellow-coloured DPPH, reaching 50% of reduction with IC_{50} values to become 8.66 ± 0.7 µL/mL, 8.18 ± 0.5 µL/mL and 9.74 ± 0.2 µl/ml for RM, RG and RA, respectively (Table 2). Our results are in agreement with previous data reported on the antioxidant activity of essential oils of rosemary [13].

Table 1. Composition of essential oil samples from *R. officinalis* L.

No	Compound	RI	Percent composition (%) of oil		
			Mansoura (RM)	**Gamsa (RG)**	**Assiut (RA)**
1	α-Pinene	921	**32.45**	**29.65**	**14.84**
2	Camphene	935	5.04	4.80	6.49
3	α-Myrcene	968	-	-	1.20
4	3-Carene	985	1.75	2.84	1.68
5	Limonene	1010	-	-	7.44
6	Eucalyptol (1,8-cineole)	1025	**22.12**	**12.57**	**11.50**
7	Terpinene	1052	-	0.61	-
8	Terpinolene	1076	-	1.83	0.50
9	α-Linalool	1094	-	2.91	5.46
10	Camphor	1140	**11.67**	**11.86**	**17.24**
11	3-Pinanone	1152	1.30	-	0.82
12	Borneol	1164	2.41	8.19	13.81
13	Terpinol	1174	-	1.05	3.18
14	Verbenone	1201	4.89	5.74	7.93
15	Dihydrocarveol	1265	-	-	1.18
16	Isobornyl acetate	1287	3.35	10.20	1.57
17	*β*-Caryophyllene	1416	5.59	3.40	0.34
18	Caryophyllene oxide	1582	0.68	0.61	0.41
Total identified constituents			91.25%	96.26%	95.59%
-Hydrocarbon: = 44.83 % (RM), 43.13% (RG), 32.49% (RA)					
-Monoterpene = 72.88% (RM), 65.1% (RG), 65.51% (RA)					
-Sesquiterpene = 6.27% (RM), 4.01 % (RG), 0.75% (RA)					
-Total Oxygenated compounds = 46.42% (RM), 53.13% (RG), 63.1% (RA)					

Bold values are the major constituents in the essential oils, RI= Retention index, (-) absent

Table 2. Antioxidant IC_{50} values of essential oils from of *R. officinalis* L.

Sample name	IC_{50} (µg/ml)
R. officinalis of Mansoura (RM)	8.66 ± 0.7
R. officinalis of Gamsa (RG)	8 .18± 0.5
R. officinalis of Assuit (RA)	9.74 ± 0.2
BHT	1.33 ± 0.08

Antioxidants minimize oxidation of the lipid components in foods and there is an increasing interest in the use of natural antioxidants in food preservation. BHT was used as a reference since it possesses well-known antioxidant properties [13]. In mammalian cells, REO only has not proved to be mutagenic, but also it was shown to have antimutagenic properties [14]. Therefore, our results suggest that REO can be a suitable target in the search for (natural) replacements for (synthetic) antioxidant food additives.

Our study revealed that α-pinene (32.45%, and 29.65%) was the essential oil major component in RM and RG, respectively. On the other hand, Camphor (17.24%) was the major component in RA. Although the essential oils of RM, RG and RA were different chemotypes, they had comparable antioxidant activities. We suggest that the antioxidant activity may be due to a synergistic effect between their constituents.

4. CONCLUSION

In the foregoing study, the GC/MS spectral data revealed considerable difference between the chemical composition of essential oil constituents of RM, RG and RA which lead to different chemotypes. They were revealed as α-pinene and camphor dominated chemotypes, which are reported for the first time from Egypt. The present results also demonstrate that REO obtained from different areas in Egypt exhibited free radical scavenging activity determined by DPPH assay due to the synergistic effect between their constituents. So, these results showed that rosemary may allow lowering the dose of synthetic antioxidants agents in foods.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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