New Chemotype *Rosmarinus officinalis* L. (Rosemary) "*R. officinalis* ct. bornyl acetate"

Itmad Awad Elhassan[1*] and Nisreen Mohmmed Osman[2]

[1]Pharmaceutical Industries Department, Industrial Research and Consultancy Centre, P.O. Box 268, Khartoum, Sudan
[2] Faculty of Pharmacy, University of Medical Sciences and Technology, Khartoum, Sudan
***Corresponding** author (e-mail: itmad2006@yahoo.com)

Abstract

This research represents the first attempt to investigate content and chemical composition of the essential oil from leaves of *Rosmarinus officinalis* cultivated in Sudan in order to establish its chromatographic fingerprint. The essential oil (3.0%) was obtained by hydrodistillation method. GC-MS investigation of the produced oil showed detection of twenty seven compounds. The main constituents identified were bornyl acetate (20.27%), caryophllene (13.61%), eucalyptol (12.84%) camphor (6.41%), camphene (4.19%), borneol (3.61%), and α-caryophyllene (2.53%). *R. officinalis,* cultivated in Sudan revealed a new chemotype, which is *R. officinalis* ct. bornyl acetate.

Key words: *Rosmarinus. officinalis*, essential oil, chemotype, bornyl acetate

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Introduction

Rosmarinus officinalis L. (Rosemary) is a very important medicinal and aromatic plant, which belongs to the genus *Rosmarinus* of the Family Lamiaceae. Rosemary, is a perennial herb, has fragrant evergreen needle-like leaves, grows up to 2m height, and has a long flowering season (from April to August). It is native to the Mediterranean region (Socaci *et al*., 2007). Chemical composition of the essential oil from leaves of *R. officinalis* L. from different geographic locations has been investigated.

Mizrzhi *et al.* (1991) investigated the constituents of the essential oil from leaves of rosemary from Argentina and reported myrcene (17.9 %), 1, 8-cineole (14.5%), α-pinene (10.9%),camphor (9.0%), caryophyllene (8.3%) and camphene (5.1%) as the main constituents in the oil.
Essential oil composition of rosemary leaves from Italy revealed presence of α-pinene (25.16%), 1, 8-cineole (20.64%), borneol (13.7%), camphor (10.26%) and camphene (5.52%) as major constituents (Reverchon and Senatore 1992).

In a comparative study, Chalchat *et al.* (1993) investigated the comparison of Spain, Morocco and France rosemary oils. They concluded that, the main constituents of the essential oil from Spain were: α-pinene (24.7%), 1, 8-cineole (18.9%), camphor (18.9%), camphene (11.2%), myrcene (4.9%) and borneol (4.5%); that from Morocco were:1, 8-cineole (47.44%), α-pinene (12.51%), camphor (7.9 %), β-pinene (7.2%), and camphene (3.62%); whereas that from France were: α-pinene (35.80%), bornyl acetate (14.30), camphene (8.3%), 1, 8-cineole (5.3%), borneol (4.44%) and camphor (3.00 %).

The main constituents identified in the essential oil of rosemary from Cuba were camphor (34.8%), borneol (11.6%), 1, 8-cineole (11.0%), α-pinene (8.17%) and camphene (5.18%) (Pino *et al.* 1998).

Boutekedjiret *et al.* (2003) studied the constituents of rosemary essential oil from Algeria. They reported 1, 8-cineole (52.4%), camphor (12.6%), β-pinene (5.7%) and α-Pinene (5.2%) as the major constituents in the oil.

Viuda-Martos *et al.* 2007 investigated chemical composition of the essential oil of anther sample of rosemary leaves from Spain. The major constituents identified were α-pinene (36.42%), camphor (15.65%), 1,8-cineole (12.02%) and camphene (11.08%).

The major components of the *R. officinalis* essential oils from Portugal were myrcene (21.6 - 30.0 %), α-pinene (8.8-16.5 %) and 1,8-cineole (8.0-12.2 %) as investigated by Miguel *et al.* (2007).

Chemical composition of essential oils of rosemary from various geographic origins in Iran were determined by GC-MS. The main components detected in the oils were: α-pinene (43.9% - 46.1%), 1, 8-cineole (11.1%), camphene (8.6% - 9.6%), camphor (2.4% and 5.3%), myrcene (3.9% - 3.9%) and broneol (3.4% 6-3.4%) (Jamshidi *et al.*, 2009).

Sonia *et al.* (2010) analysed essential oils from *R. officinalis* leaves, from Romania, at different maturity stages. They found that, the main constituents of the essential oil samples are: α-pinene (60.32 – 62.18%), camphene (9.25 – 11.08%) and eucalyptol (5.83 – 7.06%), followed by 3-octanone (2.62 – 4.15%), camphor (3.10 – 3.91%), β-myrcene (2.73 – 3.13%) and β-pinene (2.10 – 2.90%) .

The major constituents of the essential oil of the leaves from Austria were 1,8-cineole (41.6%), camphor (17.0%), α-pinene (9.9%), α-terpineol (4.9%) and borneol (4.8%) as described by Tschiggerl and Bucar (2010).

Another sample from Iran was analysed by Moghtader *et al.* (2011) who identified Forty-one compounds in the essential oil, the major components of which were α-pinene (15.52%), camphor (11.66%), verbenone (11.10%) and 1, 8-cineole (10.63%).

GC and GC-MS analysis of oils from rosemary leave samples from India revealed the presence of camphor (23.1-35.8%), 1,8-cineole (21.4-31.6%) and α-pinene (6.7-15.6%) as major constituents in the oils (Ram *et al.*, 2011).

Although several studies were carried-out to investigate the chemical composition of *R. officinalis* L. essential oil, from different geographical locations, there is no study of the chemical composition of essential oil from rosemary grown in Sudan.
Therefore, the main objective of this research is to investigate the chemical composition of essential oil from leaves of rosemary grown in Sudan in order to establish its chromatographic fingerprint.

Materials and Methods

Plant material

R. officinalis leaves samples were collected from Khartoum North where it cultivated in "shambat area", Sudan, in Jan. 2012 and authenticated in Medicinal and Aromatic Plants Institute, National Research Centre, Ministry of Science and Technology, Khartoum, Sudan. The leaves were shade-dried.

Essential oil extraction

The rosemary essential oil was extracted from the sample by hydro-distillation method using Clevenger apparatus. The distillation continued until a constant volume of the essential oil was obtained (about 4 hrs). The percentage of the oil was determined (w/v) with reference to the dried sample weight.

GC-MS analysis

1 µl of the oil was diluted in 1ml hexane. 1 µl of the diluted oil was used for the analysis. GC-MS analysis was carried out on Gas Chromatography- Mass Spectrometer-Quadrupole (QP) -2010- SHIMADZU, with (30 m × 0.25 mm i.d, film thickness 0.25 µm) capillary column at flow rate 1.74ml/min . Injection temperature was 280°C and injection mode was split. The detector temperature was 280°C. Helium was used as the carrier gas with velocity 47.6 cm/sec. Oven temperature programmed at 45°C for 4min, then was raised to 240°C at rate 6°C/min, hold time 2min, then raised to 280°C at rate 39°C/min. The interface temperature was 260 °C. The quadrupole mass spectrometer scanning range was 40-350 m/z.

Identification of the oil components

Identification of the components of the oil was achieved by comparing their mass spectra with those from NIST library database (2008) and by comparison with the available references and published data.

Results and Discussion

The essential oil content of dried leaves of *R. officinalis*, which was determined by hydrodistillation method was found to be 3%. The literature showed that the oil content of the *R. officinalis* leaves varied from 1.1 to 3.2% (Boutekedjiret *et al*. 2003, Ram *et al.*2011, Moghtder *et al.*, 2011).

GC- MS investigation of *R. officinalis* essential oil revealed detection of twenty seven compounds. Their identification achieved based on the interpretation of their mass spectra. Retention time and the percentage of the separated constituents are shown in Table (1).

The main constituents identified were bornyl acetate (20.27%), caryophllene (13.61%), eucalyptol (12.84%), camphor (6.41%), Camphene (4.19%), Borneol (3.61%), and α-caryophyllene (2.53%).

From the Previous studies of *R. officinalis* essential oils, it could be observed that, there are mainly four *R. officinalis* chemotypes: *R. officinalis* ct. α-Pinene **I** (from Iran, Spain, France , Italy and Romania), *R. officinalis* ct.1,8-cineole **II** (from Algeria, Austria and Morocco), *R. officinalis* ct. camphor **III** (from Cuba and India), and *R. officinalis* ct. myrcene **IV** (from Argentina and Portugal).

It is surprising that, the essential oil from leaves of *R. officinalis* cultivated in Sudan is characterized by domination of bornyl acetate **V** as the major constituent. Bornyl acetate has not been found in high concentration in previously investigated *R. officinalis* essential oils (Fig. 1).

This deviation from the common chemotypes may be attributed to the effect of the factors that specifically affect the composition and yield of the essential oils, which include seasonal and maturity variation, geographical origin, genetic variation, growth stages, postharvest drying and storage (Marotti *et al.* 1994; Hussain *et al*., 2008; Verma *et al*., 2009).

From the present findings and the previous studies, a new chemotype *R. officinalis* is developed (*R. officinalis* ct.bornyl acetate) under Sudan climatic conditions.

Table 1. *R. officinalis* essential oil composition

No	R.Time	Name of constituent	Area%
1	6.162	α-pinene	7.67%
2	7.009	Camphene	4.19%
3	8.109	β--pinene	1.06%
4	9.856	D-limonene	0.41%
5	10.594	Eucalyptol	12.84%
6	15.447	Borneol	3.61%
7	15.631	Camphor	6.41%
8	18.188	Bornyl acetate	20.27%
9	18.677	Copaene	0.30%
10	20.239	Caryophyllene	13.61%
11	21.265	α-caryophyllene	2.53%
12	21.817	β--cubebene	0.78%
13	22.442	α-muurolene	0.52%
14	22.893	Germacrene D	0.82%
15	23.070	γ-cadinene	1.87%
16	23.682	Calamenene	0.27%
17	24.801	Caryophyllenyl alcohol	0.18%
18	25.416	Caryophyllene oxide	2.02%
19	26.002	Cubenol	0.28%
20	26.117	12-oxabicyclo[9-1-0]dodeca-3,7-diene	0.30%
21	26.453	Tau-cadinol	0.50%
22	26.732	Unidentified	0.57%
23	27.152	Longipinene epoxide	1.67%
24	30.127	Hexadecanoic acid,methyl ester	4.71%
25	31.331	Farnesyl acetate	0.67%
26	33.344	9-octadecenoic acid(z)-,methyl ester	7.88%
27	33.632	9,12-octadecadienoic acid (z,z)-, methyl ester	4.05%

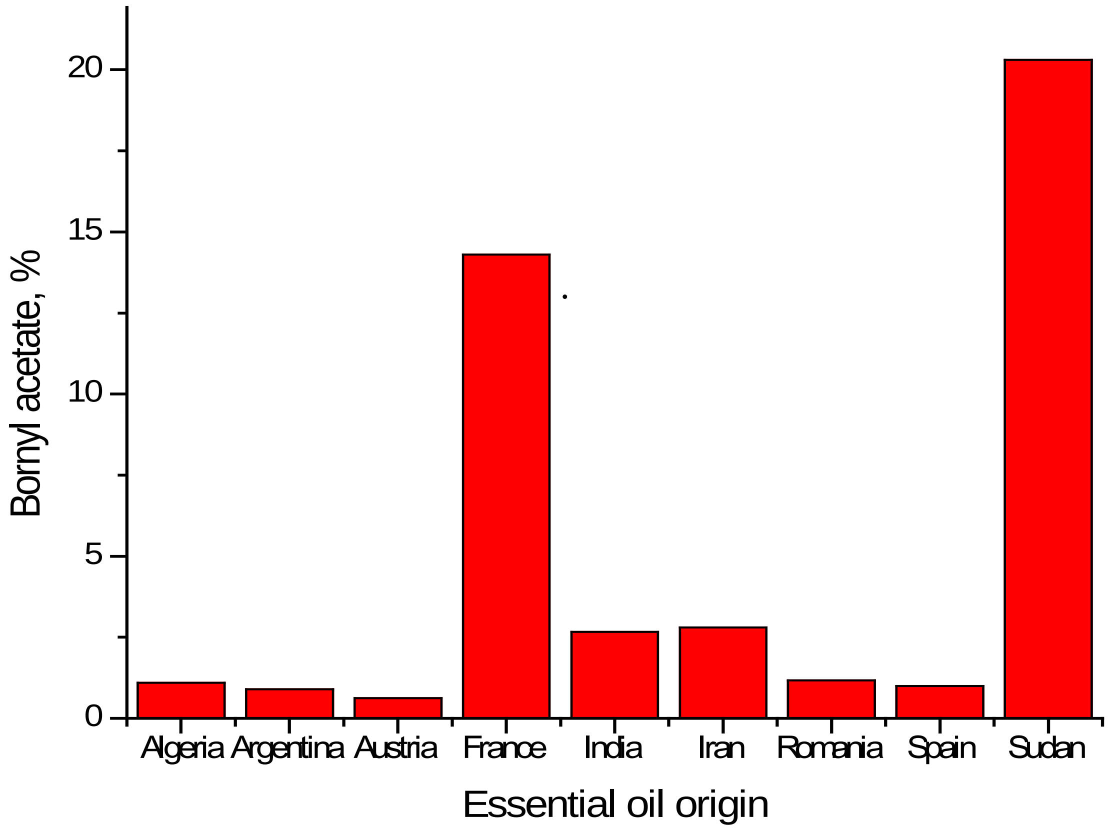

Figure 1. Bornyl acetate in *R. officinalis* essential oil worldwise.

The structures of the dominated compounds in the different *R. officinalis* chemotypes are illustrated in Fig. (2).

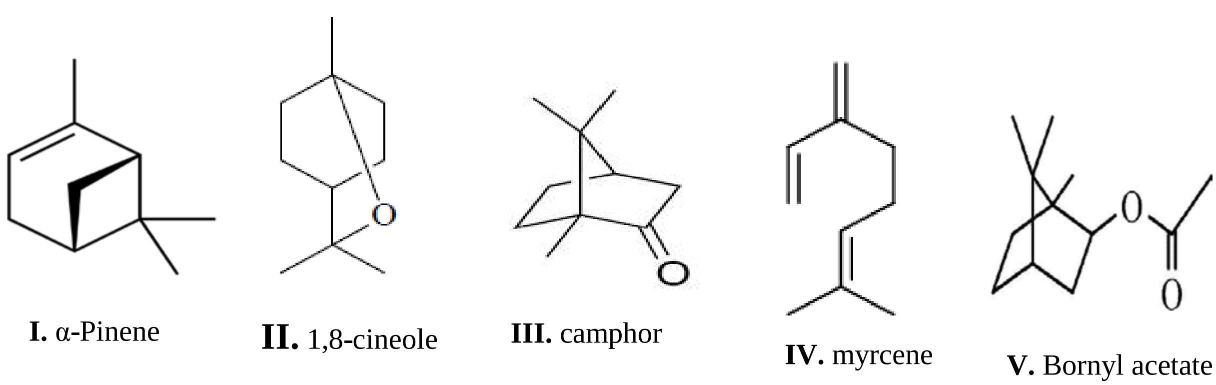

Figure 2. Dominated compounds in the different *R. officinalis* chemotypes.

Conclusion

It could be concluded that *R. officinalis* displays a wide variation in essential oil chemical composition in correlation with the climatic conditions under which it is grown, as well as the genetic variation; thus generating different chemotypes.

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