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1, 8-Cineole, α-Pinene and Verbenone chemotype of essential oil of species *Rosmarinus officinalis* L. from Saudi Arabia

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Abstract
The chemical composition of the essential oil of the flowering branches and fresh leaves of *Rosmarinus officinalis* L. from Saudi Arabia was analysed by gas chromatography-based techniques (GC and GC/MS). 63 compounds were identified and amounted to 94.64 % of the total oils. The presence of oxygenated monoterpenes (51.70%) characterized the oil of Saudi Arabian rosemary. *R. officinalis* oil was also rich with monoterpene hydrocarbons (28.45%) and acyclic monoterpenes (8.64%). Sesquiterpenes (1.28%) were minor compounds in the oils of this plant. The dominant constituents (56.09 %) were 1,8-Cineole (23.16%), α-Pinene (19.48%) and Verbinone (13.45%).

Keywords: *Rosmarinus officinalis* L. essential oil, Saudi Arabia, monoterpenes, 1, 8-Cineole, α-Pinene, Verbinone.

1. Introduction
Rosemary (*Rosmarinus officinalis* L.), is a small evergreen plant which reaching a height of 1.5 m [1]. *R. officinalis*, a member of the Lamiaceae family is a flowering plant that grows, wildly, in Mediterranean countries, southern Europe and in the littoral region through Minor Asia areas [2]. Rosemary, a pleasant-smelling perennial shrub, is a very important medicinal and aromatic plant, which widely used in folk medicine, culinary, cosmetic virtues and for the flavoring of food products [3].

In traditional medicine, Rosemary is used to treat different diseases including: depression, insomniac and arthritic pains [4]. Essential, volatile or aromatic oils are generally obtained by the steam or hydro-distillation of plants. Different parts of plants have been used to obtain essential oils. These include flowers, leaves, seeds, roots, stems, bark, and wood though secretionary parts. Rosemary essential oil is of immense medicinal importance for its powerful antimutagenic, antiphlogistic, antioxidant, chemo-preventive and antibacterial properties, anti-inflammatory, antiseptic, antispasmodic and anti-diabetic [5-7]. The essential oil of *Rosmarinus officinalis* has been the object of several studies as antioxidant activity [8-11], antibacterial [3, 12-15], toxicity insecticidal [16-17], anti-inflammatory and Antinociceptive [18], antifungal [19-20] and recently as a pest control products.

Essential oil of rosemary has been widely studied and multiple studies, belonging to different regions in the world, have been reported: in Mediterranean region [1-2, 7, 21], in Middle East especially in Iran [22], genotypes of the species were investigated chemically. Recently chemical composition of rosemary grown in Taif region in the central west of Saudi Arabia have also been studied but yet rosemary that grow in northern part of Saudi Arabia haven't studied.

In Saudi Arabia, the species grows widely in southern region of the country, in the mountains of Asir, in the region of Taif and in Northern part in Tabuk Mountains.

In this paper we report the chemical composition of rosemary essential oils from specimens growing in Camel and Range Research Center of Ministry of Agriculture, Al-Jouf.

2. Materials and methods
2.1. Plant material
The starting material consists of flowering branches and fresh leaves collected from specimens growing in Camel and Range Research Center of Al-Jouf (Kingdom of Saudi Arabia). The centre is located in the Northern region of Saudi Arabia (latitude 29◦49′18.04 ′′ N, longitude 40◦07′ 47. 52′′ E, located at 1035 km NW from Riayadh).

The site belongs to the Mediterranean desert –continental zone with a rainfall ranging between 50 and 150 mm/year and situated at an altitude of 350 m.

2.2. Essential oils identification

The essential oils have been extracted from (100 g) air-dried flowered stems by hydrodistillation for 3 h, using a Clevenger-type apparatus. Oil yields were then estimated on the basis of the dry weight of plant material (v/w).

The essential oils were analyzed by Gas chromatography–mass spectrometry (GC–MS) using a HP 5975 C mass spectrometer (Agilent technologies) with electron impact ionization (70 eV). A HP-5MS capillary column (30 m×250 µm coated with 5% phenyl methyl silicone, 95% dimethylpolysiloxane, 0.25 µm film thickness) was used. Oven temperature was programmed to rise from 60 to 220 ^{0}C at a rate of 4 ^{0}C/min; transfer line temperature was 230 ^{0}C. The carrier gas was He with a flow rate of 0.8 ml/min and a split ratio of 50:1. Scan time and mass range were 1 s and 50–550 m/z, respectively.

The identification of oil components was assigned by a comparison of their retention indices (RI) relative to (C8–C22) n-alkanes with those of literature or with those of authentic compounds available in the authors' laboratory. Further identification was made by matching their recorded mass spectra with those stored in the Wiley/NBS mass spectral library of the GC/MS data system and other published mass spectra [23]. Determination of the percentage composition was based on peak area normalization without using correction factors. Analyses were performed in triplicate for the studied species.

3. Results and discussion

The average percentage of the essential oil of the aerial parts (leaves and flowered stems) of Saudi Arabian rosemary was light yellow with yields of 1.45% (v/w). [1-2, 24] obtained, respectively, a yield of 1.5-2% (w/w), 1.65% (w/w) and 1.43% (w/w).

Typical CG-MS chromatogram of the essential oil of Saudi Arabian rosemary is shown in Figure 1.

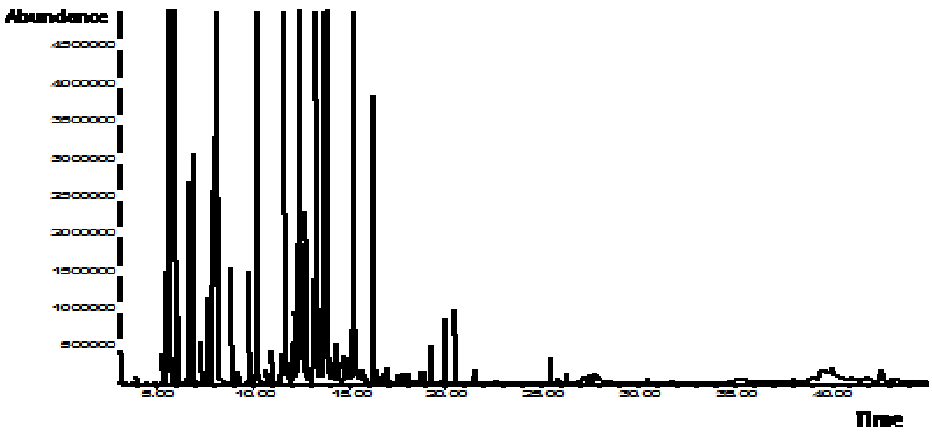


Fig 1: Typical CG-MS chromatogram of the essential oil of Saudi Arabian rosemary (*Rosmarinus officinalis* L.)

Qualitative and quantitative analyses of the essential oils volatile profiles are listed in Table 1. The table includes the retention indices and the area percentage of 63 identified components, representing 94.64% of all constituents. The components are grouped into five classes as well: Monoterpene Hydrocarbons, Oxygenated Monoterpenes, Acyclic Monoterpenes, Sesquiterpene Hydrocarbons and Other compounds.

The highest percentage of compounds (Table 1) were oxygenated monoterpenes (51.70 %), followed by monoterpenes hydrocarbons (28.45%), acyclic monoterpens (8.64%), other compounds (3.0%) and hydrocarbon sesquiterpenes (1.28%).

In oxygenated monoterpenes, the main components is 1, 8-Cineole with 23.16% of the total oil followed by verbinone (13.45%). Other constituents present in appreciable amounts are: Borneol (4.51%), Camphor (4.10%), α-Terpineol (2.43%) and Bornyl acetate (1.66%).

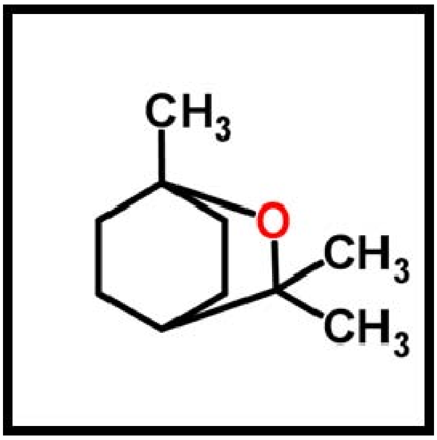
Among monoterpene hydrocarbons (28.45%), α-Pinene is the major compound and represents 19.48%. Four other constituents (Table 1) present in appreciable amount with percentage varying between 1.09% (α- Terpinolene) and3.30% (Camphene). Acyclic monoterpenes represent 8.64% of the total identified compounds.

The three major predominated compounds of this population of compounds are trans-Geraniol (4.27%), β-Linalool (2.48%) and β-myrcene (1.20%).

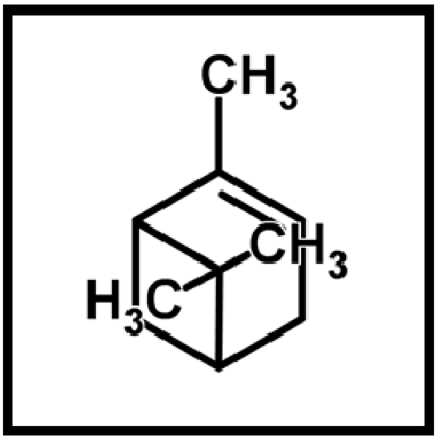
In the case of other compounds (3.0%), 16 constituents were identified with low percentages, in general less than 1%. However, p-Menth-1-en-4-ol is the only compounds with amount of 1.24%.

By analyzing the chemical composition of Saudi Arabian rosemary, the species showed a chemotype dominated by monoterpenes (oxygenated and hydrocarbons). The main components (56.09%) were1,8-Cineole, α-Pinene, Verbenone. Figure 2 depicted the molecular structure of the major constituent. These results showed that there are many qualitative similarities with previous studies focusing on the oil of rosemary from different origins, although the amounts of some corresponding compounds are different. The present study showed that the essential oil of the species in Saudi Arabia showed 63 components. Previous studies described lower number of constituents in Morocco [2], in Iran [22] and in Tunisia [1]. In the previous work dealing with chemical composition of rosemary essential oil, α-pinene is reported as the major component, followed by 1,8-cineole, camphene, ß-myrcene, camphor and borneole [15, 22, 25]. The results of GC-MS analysis of rosemary volatile oil showed that the main components of volatile oil were α-pinine, camphine, 1,8-cineol, verbinone and borneol [26].

1, 8-Cineole (23.16%)



α- Pinene (19.48 %)



Verbinone (13.45 %)

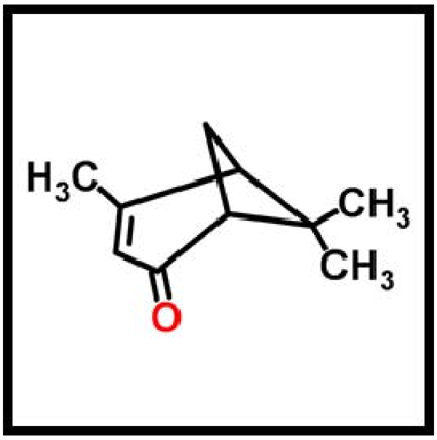


Fig 2: Major components (>56 %) of (*Rosmarinus officinalis* L.). in Saudi Arabia.

The present study revealed that rosemary from Saudi Arabia includes 1, 8-cineole, α-Pinene and verbenone chemotypes. A survey of the literature reveals that there are mainly three chemotypes: a 1, 8-cineole chemotype from France, Greece, Italy and Tunisia; a camphor borneol chemotype from Spain; and a α-pinene and verbenone chemotype from Carsica and Algeria [3, 27]. The Indian rosemary oil is characterised by relative high amounts of 1, 8- cineole, camphor and α- pinene [24]. The chemical composition of a plant essential oil generally depends on a number of factors such as heredity, part and age of plant, isolation method, environmental condition, collecting season, dehydration procedure and storage condition under which the collected plant is kept until the essential oil is extracted [28-30].

Table 1: Percentage composition of essential oil of Saudi Arabian rosemary (*Rosmarinus officinalis* L.) and their retention indices.

	Compounds	RI	Percentage of the Compounds (%)		Compounds	RI	Percentage of the Compounds (%)
1	trans-2-Hexenal	857	tr	33	Myrtenol	1203	0.65 (±0.14)
2	α- thujene	928	tr	34	Verbenone	1204	13.45 (±1.27)
3	Tricyclene	930	0.07 (±0.08)	35	p-Mentha-1,8-dien-3-one	1208	0.05 (±0.04)
4	α-Pinene	939	19.48 (±1.88)	36	Citronellol	1228	0.35 (±0.09)
5	Verbenene	951	0.51 (±0.05)	37	cis-Geraniol	1229	0.11 (±0.17)
6	Camphene	953	3.30 (±0.12)	38	car-3-en-2-one	1244	tr
7	Sabinene	977	tr	39	Eucarvone	1245	tr
8	β-pinene	980	1.20 (±0.10)	40	trans-Geraniol	1260	4.27 (±0.28)
9	β-myrcene	991	1.20 (±0.15)	41	Citral	1274	0.13 (±0.03)
10	α –phellandrene	1001	0.19 (±0.09)	42	p-Menth-2-ene-1, 8-diol	1288	0.08 (±0.12)
11	Sabinene hydrate	1103	0.11 (±0.02)	43	Bornyl acetate	1295	1.66 (±1.31)
12	1,8-Cineole	1013	23.16 (±0.85)	44	Thymol	1308	0.10 (±0.08)
13	α- terpinene	1018	0.63 (±0.04)	45	Carvacrol	1314	0.12 (±0.03)
14	*p*-Cymene	1026	1.28 (±0.87)	46	p-Cymen-3-ol	1324	tr
15	β-cis-ocimene	1031	tr	47	geranyl acetate	1352	0.19 (±0.29)
16	γ-terpinene	1053	0.84 (±0.08)	48	Eugenol	1359	0.13 (±0.05)
17	α- Terpinolene	1092	1.09 (±0.33)	49	(Z)-jasmone	1369	tr
18	β-Linalool	1098	2.48 (±0.33)	50	Geraniol acetate	1386	0.10 (±0.15)
19	trans-sabinene hydrate	1103	0.11 (±0.02)	51	Eugenyl methyl ether	1412	0.54 (±0.03)
20	α –thujone	1111	tr	52	β- caryophyllene	1419	0.71 (±0.14)
21	Fenchol	1112	0.14 (±0.05)	53	Geranyl acetone	1453	tr
22	Filifolone	1113	tr	54	α- humulene	1454	0.11 (±0.01)
23	p-Menth-1-en-4-ol	1126	1.24 (±0.16)	55	Citronellyl propionate	1478	tr
24	Exo-methyl-camphenilol	1138	0.08 (±0.07)	56	trans -Crysanthenyl acetate	1504	0.15 (±0.24)
25	Verbenol	1147	0.06 (±0.09)	57	cis- α –bisabolene	1509	tr
26	Camphor	1150	4.10 (±0.33)	58	Caryophyllene oxide	1576	0.11 (±0.17)
27	Pinocamphone	1166	0.79 (±0.24)	59	Isoaromadendrene epoxide	1594	0.10 (±0.15)
28	Borneol	1172	4.51 (±1.80)	60	α –Eudesmol	1659	0.07 (±0.10)
29	*trans*-Pinocarvone	1176	0.51 (±0.38)	61	β-Caryophyllene epoxide	1660	0.25 (±0.38)
30	Carvone	1182	tr	62	Myrtenyl acetate	1678	0.08 (±0.02)
31	p-Cymen-8-ol	1185	0.05 (±0.07)	63	Farnesyl acetone	1848	tr
32	α-Terpineol	1189	2.43 (±1.93)				
	All compounds						**94.64 % (±2.25 %)**
	Chemical classes						
	Monoterpene Hydrocarbons						**28.45 %**
	Oxygenated Monoterpene						**51.70 %**
	Acyclic Monoterpenes						**8.64 %**
	Sesquiterpene Hydrocarbon						**1.28 %**
	Other compounds						**3.0 %**

RI: Retention index on HP-INNOWAX capillary column. tr: Traces (compounds % < 0.05%).

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