Five Rosmarinus Essential Oils

Subjects: Agriculture, Dairy & Animal Science

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The present study reported the investigation of the chemical profile and the extraction yield of the essential oils (EOs) obtained from the dried aerial parts of four cultivars of *Salvia rosmarinus* (‘Boule’; ‘Vicomte de Noailles’; ‘Gorizia’; ‘Joyce de Baggio’) and the species *S. jordanii*, together with their antibacterial and antifungal activities. The phytochemical investigation evidenced a predominance of oxygenated monoterpenes in all the samples (57.5–77.1%), except in ‘Boule’, in which the hydrocarbon form prevailed (50.2%).

Rosmarinus officinalis | Rosmarinus eriocalyx | ‘Boule’ | ‘Vicomte de Noailles’ | ‘Gorizia’ | ‘Joyce de Baggio’ | hydrodistillation | agar diffusion test | MIC | Principal Component Analysis (PCA)

1. Introduction

The genus *Rosmarinus* L., belonging to the Lamiaceae family, was recently included in the genus *Salvia* L. [1]. *Salvia* subg. *rosmarinus* comprise three species of aromatic plants [2]: *Salvia rosmarinus* Spenn. (synonym of *Rosmarinus officinalis* L., and isonym of *Salvia rosmarinus* Schleid), *Salvia jordanii* J.B.Walker (synonym of *Rosmarinus eriocalyx* Jord. & Fourr.), and *Salvia granatensis* B.T.Drew (synonym of *Rosmarinus tomentosus* Hub.-Mor. & Maire) [3][4]; only the first two species are widely used in traditional medicine [5] and as cooking ingredients [6].

S. rosmarinus, commonly known as “rosemary”, is the most known species. It is an evergreen shrub, able to grow in every type of soil, with predilection for dry and rocky ones. It is no coincidence that it is widespread in the Mediterranean area, in particular in the coastal scrub [7]. It is widely used for its aromatic and medicinal properties [8], determined by its content in secondary metabolites. Among these, the essential oil (EO) responsible for the pleasant smell is the principal product obtained from this plant by industries [9], as it can be exploited for the treatment of skin, digestive, and renal problems, as well as for headaches [10]. On the basis of its main EO components, *S. rosmarinus* can be distinguished in different major chemotypes, including *cineoliferous* (1,8-cineole > 40%) [2], *camphoriferous* (camphor > 20%), *verbenoniferoum* (verbenone > 15%) [2][11], and *α-pinene chemotype* (α-pinene as the major component) [9]. The chemical composition of rosemary essential oil has been widely investigated in relation to different geographical locations of collection, environmental conditions, and seasonal periods. As reported by Cioni et al., soil and climatic conditions only partially modulate the biosynthesis of the main chemicals of the EO which, instead, is mainly determined by the genetic heritage of the plant [12]. Nevertheless, the differences are mainly quantitative rather than qualitative [5].

S. jordanii (ex. *R. eriocalyx* Jord. & Fourr., previously known as *R. tournefortii* De Noé), is an aromatic evergreen bush like rosemary, but it has been introduced into cultivation only in the last decades [13]. It is typical of Algeria, Spain and Morocco, preferring mountain rocky grounds and pastures. It differs from *S. rosmarinus* for some morphological characters such as the smaller leaves, the woolly calyx, and the prostrate growth [6]. As opposite to *S. rosmarinus* only few studies have been conducted on the chemical composition of *S. jordanii* essential oil, most of which also investigated its antibiotic activity [5][13][14][15].

The essential oils obtained from both *S. rosmarinus* and *S. jordanii* have been reported in numerous studies for their antibacterial and antifungal properties, as the aerial part of these plants have been widely used since ancient times as natural preservatives [9][16]. Abers et al. reported the essential oil of *R. officinalis* as a good broad-spectrum antibacterial agent [17], and Soulaimani et al. highlighted a better activity against Gram positive bacteria than against the Gram negative ones [18]. In recent times, the EOs, which are complex mixtures of secondary metabolites characterized by high volatility and strong smell [19], have received significant interest for their antibacterial and antifungal properties given the change in consumer behaviour toward a preference for natural products [16][20].

2. Phytochemical Investigation

The complete compositions and the extraction yields of the essential oils (EOs) obtained from the dried aerial part of the samples are reported in **Table 1**. The following taxa acronyms were used: Boule = *S. rosmarinus* 'Boule', Gori = *S. rosmarinus* 'Gorizia', Joyce = *S. rosmarinus* 'Joyce de Baggio', Vicom = *S. rosmarinus* 'Vicomte de Noailles', and Jord = *S. jordanii.* Overall, 65 compounds were identified, accounting for 98.6–100% of the total composition.

Table 1. Complete composition and extraction yield (% *w/w* dry weight) of the essential oil obtained from the samples of *S. rosmarinus* and *S. jordanii*.

Peak	Compounds	l.r.i.	Class.	Relative Abundances (%) ±SD				
				Boule	Gori	Joyce	Vicom	Jord
1	tricyclene	922	mh	-	0.1 ± 0.00	-	-	0.1 ± 0.01
2	α-thujene	926	mh	-	0.2 ± 0.02	0.1 ± 0.00	0.2 ± 0.02	-
3	α-pinene	933	mh	37.3 ± 3.09	6.4 ± 0.10	25.6 ± 0.02	4.1 ± 0.04	3.1 ± 0.17
4	camphene	948	mh	2.9 ± 0.09	4.3 ± 0.04	2.0 ± 0.09	3.3 ± 0.10	3.9 ± 0.06
5	thuja-2,4(10)-diene	954	mh	0.3 ± 0.02	-	0.4 ± 0.07	-	-

Peak	Compounds	l.r.i.	Class.	Relative Abundances (%) ±SD				
				Boule	Gori	Joyce	Vicom	Jord
6	β-pinene	977	mh	0.5 ± 0.04	4.2 ± 0.40	1.8 ± 0.07	1.6 ± 0.03	0.9 ± 0.01
7	3-octanone	985	nt	-	1.0 ± 0.17	-	-	-
8	myrcene	991	mh	2.0 ± 0.16	0.6 ± 0.01	0.9 ± 0.09	0.4 ± 0.03	0.2 ± 0.02
9	α-phellandrene	1006	mh	-	1.2 ± 0.05	0.2 ± 0.00	-	-
10	δ-3-carene	1011	mh	-	0.4 ± 0.01	-	-	-
11	α-terpinene	1017	mh	0.2 ± 0.01	0.5 ± 0.02	0.5 ± 0.01	0.3 ± 0.01	0.7 ± 0.01
12	*p*-cymene	1025	mh	2.8 ± 0.22	1.0 ± 0.23	0.8 ± 0.08	1.8 ± 0.09	1.6 ± 0.06
13	limonene	1029	mh	3.3 ± 0.11	3.6 ± 0.05	2.1 ± 0.12	1.7 ± 0.14	1.7 ± 0.03
14	1,8-cineole	1031	om	11.4 ± 0.22	20.5 ± 0.73	23.9 ± 0.42	20.0 ± 0.64	11.5 ± 0.11
15	*(Z)*-β-ocimene	1036	om	-	-	-	-	1.2 ± 0.10
16	γ-terpinene	1058	mh	0.4 ± 0.03	1.0 ± 0.10	0.9 ± 0.02	0.6 ± 0.02	0.7 ± 0.02
17	*cis*-sabinene hydrate	1066	om	-	0.3 ± 0.02	-	0.1 ± 0.01	0.1 ± 0.01
18	terpinolene	1089	mh	0.5 ± 0.02	0.6 ± 0.01	0.7 ± 0.03	0.4 ± 0.00	0.2 ± 0.01
19	*trans*-sabinene hydrate	1098	om	-	0.1 ± 0.02	-	-	-
20	linalool	1101	om	1.5 ± 0.01	0.4 ± 0.04	2.1 ± 0.14	0.2 ± 0.01	-
21	filifolone	1108	om	0.2 ± 0.00	-	-	-	-

Peak	Compounds	l.r.i.	Class.	Relative Abundances (%) ±SD				
				Boule	Gori	Joyce	Vicom	Jord
22	fenchol	1114	om	0.1 ± 0.01	-	-	-	-
23	*cis*-*p*-menth-2-en-1-ol	1122	om	-	-	0.2 ± 0.03	-	-
24	α-campholenal	1125	om	-	-	-	0.2 ± 0.01	-
25	chrysanthenone	1126	om	0.8 ± 0.05	0.2 ± 0.06	0.2 ± 0.01	-	-
26	*trans*-pinocarveol	1139	om	0.2 ± 0.02	0.1 ± 0.02	-	-	-
27	*cis*-verbenol	1142	om	-	0.1 ± 0.03	0.1 ± 0.03	-	-
28	camphor	1145	om	7.7 ± 0.22	16.9 ± 1.35	3.3 ± 0.50	42.2 ± 0.52	33.4 ± 0.38
29	*trans*-pinocampone	1160	om	0.3 ± 0.01	-	0.2 ± 0.01	0.2 ± 0.02	-
30	pinocarvone	1163	om	0.2 ± 0.00	0.3 ± 0.02	0.3 ± 0.03	0.3 ± 0.07	-
31	borneol	1165	om	2.5 ± 0.11	6.5 ± 0.00	3.7 ± 0.26	0.6 ± 0.13	14.6 ± 0.01
32	*iso*pinocampheol	1173	om	0.4 ± 0.04	-	-	-	-
33	*cis*-pinocamphone	1174	om	-	0.8 ± 0.02	0.8 ± 0.01	0.5 ± 0.00	-
34	4-terpineol	1177	om	1.4 ± 0.08	0.9 ± 0.08	1.1 ± 0.01	1.2 ± 0.02	2.8 ± 0.03
35	*p*-cymen-8-ol	1185	om	0.1 ± 0.01	-	-	0.2 ± 0.01	-
36	α-terpineol	1191	om	2.6 ± 0.19	2.0 ± 0.24	2.4 ± 0.09	2.8 ± 0.07	2.9 ± 0.06
37	myrtenol	1195	om	0.2 ± 0.02	0.2 ± 0.02	0.4 ± 0.14	0.2 ± 0.03	-

Peak	Compounds	l.r.i.	Class.	Relative Abundances (%) ±SD				
				Boule	Gori	Joyce	Vicom	Jord
38	verbenone	1210	om	12.8 ± 2.67	1.9 ± 0.15	14.9 ± 0.27	2.7 ± 0.03	0.6 ± 0.02
39	*trans*-carveol	1219	om	0.1 ± 0.06	-	-	0.2 ± 0.04	-
40	carvone	1244	om	-	-	-	0.1 ± 0.03	-
41	geraniol	1254	om	0.5 ± 0.08	-	3.9 ± 0.15	-	-
42	*trans*-ascaridol glycol	1268	om	-	0.4 ± 0.10	-	-	-
43	geranial	1271	om	-	-	0.3 ± 0.02	-	-
44	bornyl acetate	1286	om	3.9 ± 0.45	6.5 ± 0.50	2.6 ± 0.17	0.2 ± 0.01	10.8 ± 0.06
45	myrtenyl acetate	1326	om	-	-	0.1 ± 0.00	-	-
46	eugenol	1357	pp	-	-	-	-	0.4 ± 0.02
47	α-copaene	1376	sh	-	0.3 ± 0.05	-	-	-
48	geranyl acetate	1385	om	-	-	0.5 ± 0.03	-	-
49	*(Z)*-jasmone	1397	nt	0.4 ± 0.07	-	-	-	-
50	methyl eugenol	1407	pp	-	-	0.3 ± 0.02	-	-
51	β-caryophyllene	1419	sh	0.2 ± 0.03	6.7 ± 1.51	1.1 ± 0.15	0.6 ± 0.06	3.1 ± 0.08
52	α-humulene	1453	sh	-	1.9 ± 0.39	0.3 ± 0.04	-	3.2 ± 0.10
53	γ-muurolene	1477	sh	-	0.4 ± 0.08	-	-	-

Peak	Compounds	l.r.i.	Class.	Relative Abundances (%) ±SD				
				Boule	Gori	Joyce	Vicom	Jord
54	bicyclogermacrene	1496	sh	-	0.3 ± 0.05	-	-	-
55	*trans*-γ-cadinene	1514	sh	-	0.4 ± 0.07	-	-	-
56	δ-cadinene	1524	sh	-	0.9 ± 0.20	-	-	-
57	caryophyllene oxide	1582	os	0.4 ± 0.09	4.3 ± 0.16	0.6 ± 0.10	1.5 ± 0.24	0.8 ± 0.02
58	humulene oxide II	1608	os	0.3 ± 0.07	0.5 ± 0.08	-	-	0.6 ± 0.03
59	caryophylla-4(14),8(15)-dien-5-ol (unidentified isomer)	1633	os	-	0.2 ± 0.06	-	0.3 ± 0.04	-
60	T-cadinol	1641	os	-	0.3 ± 0.03	-	0.2 ± 0.03	-
61	α-bisabolol oxide B	1655	os	-	-	-	0.8 ± 0.06	-
62	14-hydroxy-9-*epi*-*(E)*-caryophyllene	1670	os	-	-	-	7.1 ± 1.76	0.2 ± 0.01
63	α-bisabolol	1685	os	-	-	-	-	0.4 ± 0.01
64	*trans*-ferruginol [2]	2325	od	0.2 ± 0.04	0.2 ± 0.01	-	-	-
	Total identified (%)			98.6 ± 0.06	98.7 ± 0.31	99.1 ± 0.16	96.6 ± 0.16	100 ± 0.03
				Boule	Gori	Joyce	Vicom	Jord
	Monoterpene hydrocarbons (mh)			50.2 ± 3.77 ^{A}	24.0 ± 0.54 ^{C}	36.0 ± 0.19 ^{B}	14.3 ± 0.46 ^{D}	14.2 ± 0.49 ^{D}
	Oxygenated monoterpenes (om)			46.9 ± 3.42 ^{C}	57.5 ± 2.08 ^{B}	60.9 ± 0.04 ^{B}	71.9 ± 1.55 ^{A}	77.1 ± 0.25 ^{A}
	Sesquiterpene hydrocarbons (sh)			0.2 ± 0.03 ^{C}	10.7 ± 2.35 ^{A}	1.3 ± 0.19 ^{C}	0.6 ± 0.06 ^{C}	6.3 ± 0.18 ^{B}
	Oxygenated sesquiterpenes (os)			0.7 ±	5.3 ±	0.6 ±	9.8 ±	2.0 ±

s: indeed, erivatives 50.2% in α-Pinene, nsiderable pectively), class, 1,8- .0–2.9%), they were detected in all the samples, even though with a high variability in their relative abundances.

Peak	Compounds	l.r.i.	Class.	Relative Abundances (%) ±SD				
				Boule	Gori	Joyce	Vicom	Jord
				0.16 C	0.33 B	0.10 C	2.12 A	0.07 C
	Oxygenates diterpenes (od)			0.2 ± 0.04 A	0.2 ± 0.01 A	- B	- B	- B
	Phenylpropanoids (pp) [9] [5][14]			-	-	0.3 ± 0.02	-	0.4 ± 0.02
	Other non-terpene derivates (nt)			0.4 ± 0.07 B	1.0 ± 0.17 A	- C	- C	- C
	EO Extraction yield (%*w/w*)			0.57 ± 0.02 C	1.17 ± 0.16 B	0.76 ± 0.04 C	2.25 ± 0.15 A	0.71 ± 0.04 C

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This is the first time that the essential oil composition of the four cultivars of *S. rosmarinus* (‘Boule’; ‘Vicomte de Noailles’; ‘Gorizia’; ‘Joyce de Baggio’) was reported to better employ this plant material not only as ornamental display items but also as derivative products for industrial use.

Statistical Analysis

The first axis of PCA explained 62.4% of variance, the second axis (PCA2) a further 25.1% (**Figure 1**). Vicom segregated alone, while the other taxa were distributed into two groups, one formed by Gori and Jord, and the other one made up by Boule and Joyce. Nine chemical compounds showed a significant discriminative function between the taxa. Vicom was characterized by high amounts of camphor (28) and 14-hydroxy-*9-epi-(E)*-caryophyllene (62). Gori and Jord differed in their predominance of camphene (4), borneol (31), bornyl acetate (44), and α-humulene (52). Lastly, Boule and Joyce were characterized by high amounts of α-pinene (3), myrcene (8), and verbenone (38).

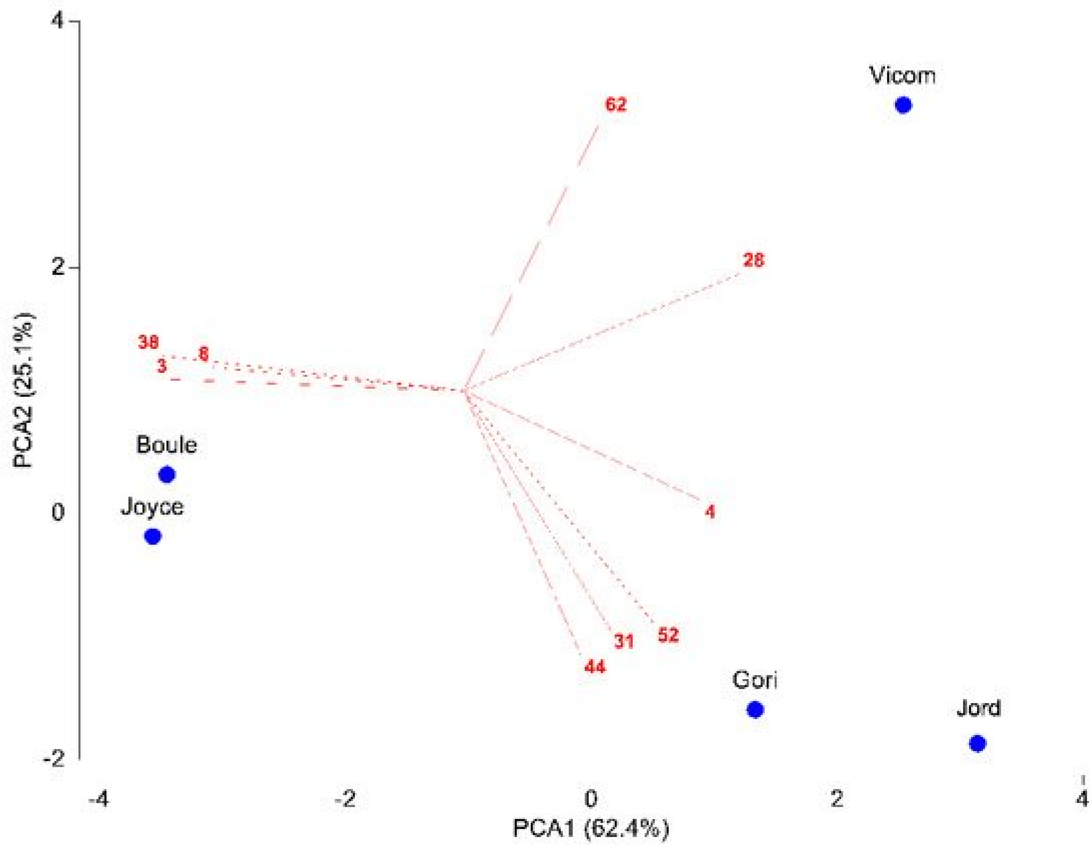

Figure 1. PCA of the matrix 5 taxa × 18 compounds. Compounds with a Pearson correlation coefficient > 0.8 with the first two PCA axes are shown. Abbreviations of chemical compounds: 3 = α-pinene, 4 = camphene, 8 = myrcene, 28 = camphor, 31 = borneol, 38 = verbenone, 44 = bornyl acetate, 52 = α-humulene, 62 = 14-hydroxy-9-*epi-(E)*-caryophyllene.

3. Antimicrobial Investigation

In vitro antibiotic sensitivity tests detected multi-resistance of the assayed bacterial isolates (**Table 2**).

Table 2. The inhibition zones expressed in millimeters resulted from the application of different antibiotics against the selected bacterial strains (S: susceptible; R: resistant; I: intermediate).

	Antibiotics					
STRAINS	**Tetracycline (30 µg/disc)**	**Ceftazidime (30 µg/disc)**	**Rifampicin (30 µg/disc)**	**Cephalexin (30 µg/disc)**	**Cefotaxime (30 µg/disc)**	**Chloramphenicol (30 µg/disc)**
S. ser. Typhimurium (S176)	18 (S)	19 (S)	15 (R)	21 (S)	25 (S)	21(S)
Y. enterocolitica (YU3)	22 (S)	27 (S)	17 (I)	0 (R)	32 (S)	22 (S)

	Antibiotics					
STRAINS	**Tetracycline (30 µg/disc)**	**Ceftazidime (30 µg/disc)**	**Rifampicin (30 µg/disc)**	**Cephalexin (30 µg/disc)**	**Cefotaxime (30 µg/disc)**	**Chloramphenicol (30 µg/disc)**
L. monocytogenes (L1)	26 (S)	0 (R)	28 (S)	21 (S)	10 (R)	22 (S)
E. durans (EU157)	24 (S)	0 (R)	33 (S)	14 (R)	0 (R)	19 (S)
E. faecium (EU107)	7 (R)	0 (R)	30 (S)	0 (R)	0 (R)	18 (S)
E. faecalis (EU37)	10 (R)	0 (R)	15 (R)	13 (R)	18 (I)	19 (S)

All the analysed EOs were characterized by a predominance of oxygenated monoterpenes (57.5–77.1%), except that of ‘Boule’, in which the hydrocarbon form prevailed (50.2%). Considering the whole chemical composition, a total of nine compounds showed a significant discriminative function between the samples. ‘Vicomte de Noailles’ was characterized by high amounts of camphor and 14-hydroxy-9-*epi-(E)*-caryophyllene; ‘Gorizia’ and Jord differed for their predominance of camphene, borneol, bornyl acetate, and α-humulene and, lastly, ‘Boule’ and ‘Joyce de Baggio’ were characterized by high amounts of α-pinene, myrcene, and verbenone.

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