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Chemical variability of the essential oils from *Rosa canina* L. and *Rosa sempervirens* L. flowers collected at Tunisia

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The chemical variability of the essential oils of *Rosa canina* L. and *R. sempervirens* L. flowers collected at seven localities from northern Tunisia was investigated by gas chromatography (GC) and GC–mass spectrometry (GC–MS). The essential oils yields ranged between 0.7% and 1.4% (*v*/f.w.) for *R. canina* and *R. sempervirens*, respectively. Forty-one components were identified in *R. canina* and twenty-six in *R. sempervirens* oils. *Rosa canina* essential oil, from plants collected at Ain Draham, was dominated by β-caryophyllene (32%) and geraniol (21%), whereas in the oils from plants collected at Boussalem *n*-heneicosane (29%), *p*-cymene (12%) and β-caryophyllene (11%) predominated. However, the oil isolated from the samples collected at Fernana was dominated by 2,4,6-trimethyl-octane (9%), *n*-undecane (8%) and geraniol (8%). *p*-Cymene (14%), limonene (11%) and γ-terpinene (11%) were the main components of the oil isolated from Feija samples. *Rosa sempervirens* oils, from plants collected at Ain Draham and Fernana, were characterized by a high 2-phenylethyl alcohol content (29% and 93%, respectively). The major components of the oil isolated from Tabarka samples were *p*-cymene (16%) and γ-terpinene (12%). Cluster analysis of the essential oils composition from the studied populations, confirmed the major chemical variability.

Keywords: *Rosa canina*; *Rosa sempervirens*; Rosaceae; essential oils; chemical variability; cluster analysis

Introduction

The genus *Rosa* L. (Rosaceae) grows naturally throughout the temperate and subtropical regions of the northern hemisphere and is divided taxonomically into four subgenera: *Hulthemia* (Dumort) Focke, *Platyrhodon* (Hurst) Rehder, *Hesperhodos* Cockerell and *Rosa* (1). The subgenus Rosa comprises nine sections: *Cinnamomeae* (DC. Ser.) (incl. *Carolinae* Crép.), *Caninae* (DC. Ser.), *Synstylae* DC., *Pimpinellifoliae* (DC. Ser.), *Banksianae* Lindl., *Bracteatae* Thory, *Indicae* Thory, *Laevigatae* Thory, and *Rosa* (1). The sections *Cinnamomeae* and *Caninae* are the largest and comprise around ninety and sixty species, respectively (1).

Tunisian flora records eight species for this genus: *Rosa gallica* L., *R. agrestis* Savi, *R. sicula* Tratt., *R. sempervirens* L., *R. stylosa* Desv., *R. micrantha* Borrer ex Sm., *R. canina* L., and *R. moschata* Herrm. (2).

Rosa canina, belonging to the section *Caninae*, is an erect shrub of up to 3.5 m, sometimes climbing; branches often curved or arched. Petals are white to pale pink, rarely deep pink and fruit ripens late. Its main range of distribution includes Europe, North Africa, Asia Minor, Syria, Iran, west and north Asia (3).

Rosa sempervirens, belonging to the section *Synstylae*, is a shrub with long scrambling stems, 5–10 m tall with dark leathery evergreen leaves and few or no prickles. Petals are white and flowers appear from May to July. This species occurs in southern Europe in Spain and eastwards to Greece, Turkey and North Africa (3, 4).

Although *Rosa canina* fruits (rose-hips with seeds) are considered to possess prophylatic and therapeutic activities against inflammatory disorders including arthritis, rheumatism, gout and sciatica and can be used against diseases with fever, colds and influenza, in the prevention of inflammation of the gastric mucosa and gastric ulcer, against gallstones, dropsy, kidney and lower urinary tract disorders, as laxative, diuretic and as an astringent (5–7); for *R. sempervirens* no uses are reported in the literature (3).

In addition to the ornamental value and to the use of *Rosa canina* fruits in folk medicine, the floral fragrance finds also application in cosmetic, food, aromatherapy and household products (8). Although less is

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known on *R. sempervirens*, the flower volatiles may be a good alternative to explore mainly in those places where this species can be easily found, under sustainable conditions.

A recent study reported the fatty acid and phenolic constituents of leaves, flowers and fruits of Tunisian *Rosa canina* (9). Nevertheless, a survey of the existing literature showed no information on the essential oil of this species or on that of *R. sempervirens*. Given the lack of knowledge on the variability of these species essential oils, the aim of the present work was to study the chemical composition of the essential oils from *R. canina* and *R. sempervirens* flowers collected at diverse regions of Tunisia.

Experimental

Plant material

Rosa canina and *R. sempervirens* flowers were collected in different localities in north Tunisia, characterized by diverse geographic and climate conditions (Table 1). *Rosa canina* samples were collected at Ain Draham, Fernana, Boussalem and Feija (Figure 1) during May to mid-June 2008. *Rosa sempervirens* samples were collected at Tabarka, Ain Draham and Fernana, during May to mid-June 2008. Each collective sample was constituted of a mixture of 10–15 individual plants. The petals were stored at 4°C until extraction. The identification of plants was confirmed by Professor Dr Hasnaoui Brahim, in the Department of Ecology, Sylvo-Pastoral Tabarka, Tunisia.

Isolation of the essential oils

The essential oils were isolated from the undried petals (on average, at least 100 g from each sample) by hydrodistillation for 3 hours, using a Clevenger-type apparatus according to the European Pharmacopoeia (10). The oils were recovered with hexane. After concentration, the essential oils were stored at –20°C in the dark prior to analysis.

Analysis of the essential oils

Gas chromatographic (GC) analyses were performed using a Perkin–Elmer Autosystem XL gas chromatograph. Data was recorded from a DB-1 fused-silica column (polydimethylsiloxane, 30 m × 0.25 mm i.d., film thickness 0.25 μm; J&W Scientific Inc., Rancho Cordova, CA, USA). Oven temperature was programmed, 45–175°C, at 3°C/minute, subsequently at 15°C/minute up to 300°C, and then held isothermal for 10 minutes; injector and detector temperatures, 280°C and 300°C, respectively; carrier gas, hydrogen, adjusted to a linear velocity of 30 cm/second. The samples were injected using split sampling technique, ratio 1:50. The volume of injection was 0.1 μL of a pentane-oil solution (1:1). The percentage composition of the oils was computed by the normalization method from the GC peak areas, calculated as mean values of two injections from each oil, without using correction factors.

The GC–mass spectrometry (GC–MS) unit consisted of a Perkin–Elmer Autosystem XL gas chromatograph, equipped with DB-1 fused-silica column (30 m × 0.25 mm i.d., film thickness 0.25 μm; J&W Scientific, Inc.), and interfaced with a Perkin–Elmer Turbomass mass spectrometer (software version 4.1, Perkin–Elmer, Shelton, CT, USA). Injector and oven temperatures were as mentioned earlier; transfer line temperature, 280°C; ion source temperature, 220°C; carrier gas, helium, adjusted to a linear velocity of 30 cm/second; split ratio, 1:40; ionization energy, 70 eV; scan range, 40–300 u; scan time, 1 second. The identity of the components was assigned by comparison of their retention indices, relative to C_9–C_{21} *n*-alkane indices and GC–MS spectra from a home-made library, constructed based on the analyses of reference oils, laboratory-synthesized components and commercial available standards.

Statistical analysis

The standardized percentage composition of the isolated essential oils was used to determine the relationship between the different samples by cluster analysis using Numerical Taxonomy Multivariate Analysis System (NTSYS-pc software, version 2.2, Exeter Software, Setauket, New York) (11). For cluster analysis, correlation coefficient was selected as a measure of similarity among all accessions, and the Unweighted Pair Group Method with Arithmetical Averages (UPGMA) was

Table 1. Data on altitude, precipitation and temperature (average/year) at the collection sites of Tunisian *Rosa canina* and *Rosa sempervirens* flowers.

	Rosa canina				*Rosa sempervirens*		
Average/year	Ain Draham (AD)	Boussalem (B)	Feija (F)	Fernana (Fe)	Ain Draham (AD)	Fernana (Fe)	Tabarka (T)
Altitude (m)	610	238	1035	731	730	270	72
Precipitation (mm)	1113	853	1411	1198	1200	878	739
Temperature (°C)	16	17	13	15	15	17	18

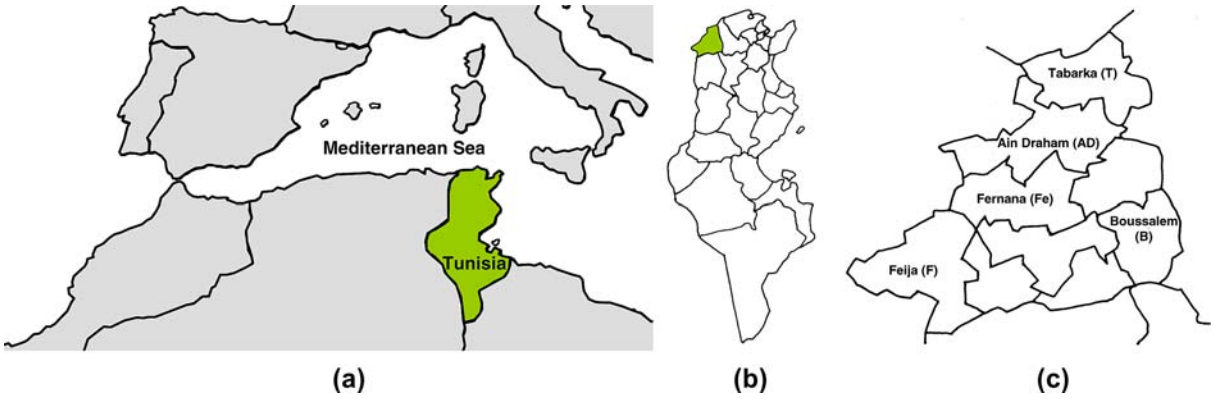

Figure 1. Tunisia geographical location (a), regions (b) and collection sites (c) of *Rosa canina* (*Rc*) and *Rosa sempervirens* (*Rs*) populations in different locations. Ain Draham (AD), Boussalem (B), Feija (F), Fernana (Fe) and Tabarka (T) (adapted from local maps).

Table 2. Composition of the essential oils isolated from the flowers of *Rosa canina* and *R. sempervirens* populations collected at different locations in Tunisia.

Components	RI[a] DB-1	RI[15] DB-1	RI[15] OV-101	RI[16] DB-1	RI[16] OV-101	RI[17] OV-101	*Rosa canina* AD	B	F	Fe	*Rosa sempervirens* AD	Fe	T
α-thujene	924		938			930	t	1.2	2.5	1.0	0.5	t	1.1
α-pinene	930	941	940[942]		942	926	t	3.4	6.1	1.9	1.1	t	4.3
Camphene	938		954		954	957	t	0.6	t	0.2	0.3	t	t
Sabinene	958	975	975[976]		976	970	t	t	t	t	0.2	t	t
β-pinene	963	978	980		981	985	t	t	t	t	0.2	t	t
β-myrcene	975	985	986	981	986	990	0.4	1.9	5.0	1.8	3.3	t	5.6
α-phellandrene	995		1002	996		1009	t	0.7	0.7	t	0.4	t	t
n-decane	1000			1000	1000	1000	t	t	t	t	t	t	t
benzyl alcohol	1000		1033	1004	1033	1117	t	t	t	t	t	t	t
α-terpinene	1002			1008		1020	t	0.7	2.8	1.0	1.0	t	2.9
p-cymene	1003	1015	1020	1014	1020	1025	1.8	11.5	14.4	4.9	10.9	t	15.8
1,8-cineole	1005	1028	1027		1027	1015	t	t	t	t	t	t	t
Limonene	1009		1030	1024	1030	1022	1.3	4.9	11.0	2.7	5.2	t	7.8
γ-terpinene	1035		1057	1048		1058	0.7	7.8	10.5	2.7	9.7	t	11.6
2,4,6-trimethyl octane[b]	1053						0.6	1.9	6.0	9.1	2.8	t	8.3
2-methyl decane	1058						0.8	0.9	2.8	4.1	0.6	t	3.8
2-phenylethyl alcohol	1064				1104	1080					28.5	93.3	t
n-nonanal	1073		1087	1082	1102	1079	t	t	t	t	t	t	t
Linalool	1074		1092	1083	1092	1085	3.0	1.9	1.1	1.7	t	t	t
n-undecane	1100			1100		1100	0.6	1.6	5.8	8.0	2.7	t	7.4
4,7-dimethyl undecane[b]	1106						0.3	6.5	3.3	4.4	0.7	t	4.1
α-terpineol	1159		1185	1170		1289	t	t	t	t	1.0	t	t
estragole (= methyl chavicol)	1163				1183	[1183]					0.6	4.7	t
Myrtenol	1168						0.2	t	t	t			
Nerol	1206			1209		1234	4.0	t	t	0.8			
Citronellol	1207		1215	1218	1215	1229	4.5	t	t	0.8			
Geraniol	1236		1243	1234	1243	1258	20.8	1.8	t	7.6			
n-tridecane	1300			1300		1300	t	t	t	0.6	6.3	t	t
geranyl acetate	1370		1364		1364	1365	0.2	t	t	t			
β-elemene	1388			1386		[1376]	t	t	t	t			
β-caryophyllene	1414		1428	1423 [1414]	1442	1438	32.0	11.0	t	4.1			
β-copaene	1426		1398	1428	1445		t	t	t	t			
α-humulene	1447		1465	1448	1465	1472	2.0	t	t	t			
γ-muurolene	1469			1469	1475	1488	0.4	0.8	t	t			
germacrene-D	1474			1473		1500	0.4	0.9	t	t			
n-pentadecane	1500				1500	1500	0.7	t	7.8	5.8	5.8	t	9.9

(*Continued*)

Table 2. (*Continued*)

Components	RI[a] DB-1	RI[15] DB-1	RI[15] OV-101	RI[16] DB-1	RI[16] OV-101	RI[17] OV-101	*Rosa canina* AD	B	F	Fe	*Rosa sempervirens* AD	Fe	T
δ-cadinene	1505		1524	1519	1524	1531	2.0	t	t	t			
β-caryophyllene oxide	1561			1567			0.9	2.5	t	3.4			
n-hexadecane	1600			1600	1600	1600	t	t	3.4	3.1	1.3	t	5.3
α-cadinol	1626					1674	0.4	t	t	t			
14-hydroxy-9-*epi*-(*E*)-caryophyllene[b]	1633						8.9	t	t	1.7			
n-nonadecane	1900			1900	1900	1900	0.5	5.3	1.4	2.0			
n-heneicosane	2100			2100	2100	2100	4.7	28.9	1.6	2.9			
Percent of identification							92.1	96.7	86.2	76.3	83.1	98.0	87.9
Grouped components													
monoterpene hydrocarbons							4.2	32.7	53.0	16.2	32.8	t	49.1
oxygen-containing monoterpenes							32.7	3.7	1.1	10.9	1.0	t	t
sesquiterpene hydrocarbons							36.8	12.7	t	4.1	t	t	t
oxygen-containing sesquiterpenes							10.2	2.5	t	5.1	t	t	t
phenylpropanoids											0.6	4.7	t
Others							8.2	45.1	32.1	40.0	48.7	93.3	38.8
Oil yield (%, v/f.w.)							1.4	1.3	0.9	1.0	0.4	0.5	0.7

Notes: Ain Draham (AD), Boussalem (B), Feija (F), Fernana (Fe) and Tabarka (T). RI[a], calculated retention index relative to C_9–C_{21} *n*-alkanes on the DB-1 column; RI[15], RI[16] and RI[17], retention indices from the literature (15–17) on DB-1 or similar phase column (100% dimethylpolysiloxane); in square brackets additional retention index (RI), whenever more than one RI was listed by the same literature reference; t, trace (< 0.05%). [b]Based on mass spectra only.

used for cluster definition. The degree of correlation was evaluated according to Pestana and Gageiro (12) and classified as very high (0.9–1), high (0.7–0.89), moderate (0.4–0.69), low (0.2–0.39) and very low (< 0.2).

Results and discussion

Rosa canina and *R. sempervirens* populations studied afforded oils in a yield ranging from 0.7% (Tabarka) to 1.4% (Ain Draham) (*v*/f.w.), respectively. The identified oil components are listed in Table 2 in order of their elution on the DB-1 column. A limited number of components with relative amounts of 0.5% to 3% each and some trace components could not yet be identified; these were not included in Table 2. Together they cover a portion of 2% to 24% of the oils.

A major chemical variability was clear in all samples studied (Table 2), which was confirmed by the cluster analysis, Figure 2.

The degree of *Rosa canina* volatiles composition correlation varied between moderate (S_{corr} = 0.58), for samples collected at Fernana (Fe) and Feija (F), low (S_{corr} = 0.3), for samples from Ain Draham (AD) and Boussalem (B), to very low (S_{corr}<0.20), between these two groups of samples (Figure 2). The poor chemical correlation between these oils resulted from the fact that each oil showed different main components. *Rosa canina* essential oil, from plants collected at Ain Draham, was dominated by β-caryophyllene (32%) and geraniol (21%), whereas plants collected at Boussalem showed an *n*-heneicosane (29%), *p*-cymene (12%) and β-caryophyllene (11%) dominated oil. However, the essential oil isolated from the samples collected at Fernana was dominated by 2,4,6-trimethyl-octane (9%), *n*-undecane (8%) whereas geraniol (8%). *p*-Cymene (14%), limonene (11%) and γ-terpinene (11%) were the main components of the essential oil isolated from Feija samples.

The essential oils from two of the three *Rosa sempervirens* samples showed a high chemical correlation (S_{corr} = 0.84), but on the opposite, the oil from the sample collected at Tabarka (T) was chemically correlated to that obtained from *R. canina* collected at Feija (Figure 2, Table 2), given the high *p*-cymene (16%) and γ-terpinene (12%) of that *R. sempervirens* oil. The two correlated *R. sempervirens* oils, from plants collected at Ain Draham and Fernana, were characterized by a high 2-phenylethyl alcohol content (29% and 93%, respectively), despite being different in the remaining chemical profile.

No correlation could be drawn between the essential oils composition (Table 2, Figure 2) and the different geographic and climate conditions (Table 1).

A review of the existing literature on the essential oils, or volatile compounds of *Rosa canina* and *R. sempervirens* yielded only few results for *R. canina* and none for *R. sempervirens*.

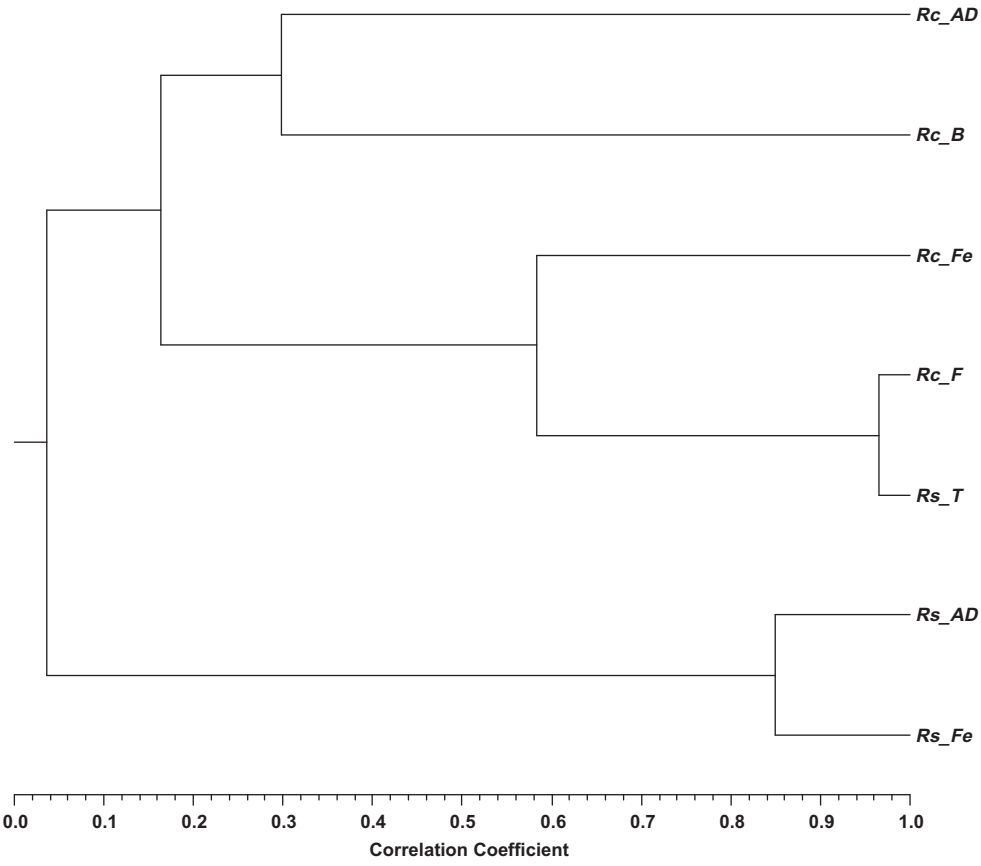

Figure 2. Dendrogram obtained by cluster analysis of the percentage composition of volatiles from *Rosa canina* (*Rc*) and *Rosa sempervirens* (*Rs*) based on correlation and using the unweighted pair-group method with arithmetic average (UPGMA). Ain Draham (AD), Boussalem (B), Feija (F), Fernana (Fe) and Tabarka (T).

Özel and Clifford (8) evaluated the floral oil of *Rosa canina* isolated using superheated water extraction (SWE) and Soxhlet extraction. At the highest SWE efficiency (100°C, 2 mL/minute flow rate and 50 bar pressure for 2 hours), benzyl alcohol (8 mg/kg sample) and benzaldehyde (7 mg/kg sample) were the main components. Octacosane (24 mg/kg sample) and benzyl alcohol (8 mg/kg sample) were the main components isolated by Soxhlet extraction. These extraction methodologies are very different from that used in the present study which may account for the qualitative and quantitative differences in the results obtained.

In the present study a marked chemical variability was detected for both *Rosa* species, even when the same extraction procedure was used. Several factors can influence the volatile composition and yield both at pre- and post-harvesting moments (13, 14). In the present study, in addition to the different harvesting locations, natural hybridization can also partly account for the observed variability. In this context, the assessment of *Rosa* species chemical polymorphism is of high importance, viewing the development of selection procedures for species that have an essential oil whose characteristics best fit the market demands.

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