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ORIGINAL PAPER

Correlation between chemical composition and antioxidant activity of the essential oils from leaves and berries of *Schinus molle* L. growing in two areas of Bejaia (Algeria)

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Abstract
Schinus molle L. is a medicinal and aromatic plant used in traditional medicine for his therapeutic properties. The leaves and berries of *Schinus molle* L. were collected in Iheddaden and Amriw (Bejaia, Algeria) and the essential oils were isolated by hydrodistillation with yields between 0.26 and 0.80%. The chemotype of the essential oils was sesquiterpene group, cadinene and cadinols subgroups. The cadinene subgroup was obtained from Iheddaden leaves and Amriw samples (leaves and berries) with proportions between 16.1 and 23.4%, while the cadinols subgroup was only shown with Iheddaden berries (30.5%). The antioxidant activities of the essential oils from leaves and berries of *Schinus molle* L. were low: IC_{50} DPPH were between 6.9 and 8.6 mg/mL, when IC_{50} ABTS varied between 0.7 and 5.0 mg/mL. Principal component analysis indicates that high proportions of oxygenated sesquiterpenes promote the antioxidant activity of essential oils from *Schinus molle* L. against ABTS radical, while the antioxidant activity against the DPPH radical requires the combination of the different chemical families contained in the essential oils of *Schinus molle* L.

Keywords Essential oils · Chemotypes · Sesquiterpenes · Antioxidant activity · IC_{50} · Correlation

Introduction

The false pepper tree (*Schinus molle* L.) is a tree of the Anacardiaceae family having up to 15 ms in height. Its foliage is persistent with fine and pendants branches, its flowers are small and white having a dimension between 3 and 4 mm and its fruits (berries) are pink and globular having a size from 6 to 7 mm and are available from April to August. Native of the tropical and subtropical regions of South America, it is grown in avenues on the Mediterranean coast as an ornamental plant [1].

Schinus molle L. is known and used in traditional medicine as antiseptic, anti-inflammatory, antirheumatic and antidiarrheal [2, 3]. It also has a tonic, astringent, stomachic, vasoconstrictor, diuretic, expectorant and healing effect [3, 4]. Other uses were also reported to treat fever, cough, colds, bronchitis, tuberculosis, asthma, conjunctivitis, ophthalmia, stomach pain (gastrointestinal disorders) and hemorrhoids [5, 6]. *Schinus molle* L. extracts obtained with various solvents and its essential oil possess antibacterial, antifungal, antioxidant, insecticidal, analgesic, cytotoxic and trypanocidal properties [3, 7].

The essential oils of *Schinus molle* L. were classified into two groups: the first is dominated by monoterpenes and the second is dominated by sesquiterpenes. Each group was subdivided into subgroups according to its majority constituent. There are six subgroups of monoterpenes: the first one is pinenes (α- and β-), the second is that of myrcene, the third is that of phellandrenes (α- and β-), and the fourth is that of cymene (p-), the fifth is that of limonene and the last one is that of sabinene. The group of sesquiterpenes contains five

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subgroups: the first group is dominated by germacrene-D, the second is that of bicyclogermacrene, the third is that of cadinene (δ-), the fourth is that of cubenol and the last is that of cadinol (α- and *epi*-α or τ). The essential oils of *Schinus molle* L. reported in the literature were classified by origin country and according to the groups and subgroups cited above (Table 1).

The aim of this study is to determine the major constituents of essential oils extracted from the leaves and berries of *Schinus molle* L. harvested in two areas of Bejaia (Algeria) and to classify them according to their chemotype. Thereafter, the antioxidant activity of these essential oils against the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals will be evaluated by measuring the inhibitory concentrations of 50% (IC_{50}).

Materials and methods

Plant material

The leaves and the berries of *Schinus molle* L. were gathered from two areas of Bejaia (Algeria) between May and June 2012: Iheddaden (36°44′21.93″ N, 5°02′46.27″E and an altitude of 27 ms) and Amriw (36°44′57.87″ N, 5°03′03.76″E and an altitude of 19 ms). The collected samples were identified by the Museum of the National Park Gouraya (Bejaia, Algeria) and a specimen was deposited, under KBAA 104 number, in Laboratory of Organic Materials, Faculty of Technology, University of Bejaia (Algeria). The leaves and the berries were dried in the shade at ambient temperature. Once the mass of the samples becomes constant, a fine and homogeneous powder was obtained using a laboratory grinder.

Table 1 Chemotypes of the essential oils from leaves and berries of *Schinus molle* L.

Country	Group of monoterpenes (%)						Group of sesquiterpenes (%)				
	sg-Pi	sg-Ph	sg-My	sg-Sa	sg-Cy	sg-Li	sg-Cn	sg-Co	sg-Bi	sg-Ge	sg-Cu
Brazil [8–15]	24.8–50.7^{L} 56.6^{B}	–	–	19.7–48.6^{L} 51.7^{B}	–	24.2–41.4^{L}	–	23.7^{L} and 27.3^{L}	–	–	27.1^{L}
Argentina [16–21]	22.5^{L}	39.8–45.8^{L}	–	34.8–51.0^{L}	–	15.7^{L} 40.3^{B}	–	–	–	–	–
Uruguay [22]	–	–	–	–	–	–	–	–	29.2^{L}	–	–
Peru [23]	–	–	26.4–42.0^{B}	–	–	–	–	–	–	–	–
Mexico [24, 25]	–	32.8^{L} 45.0^{B}	– 39.7^{B}	–	–	–	–	–	–	–	–
Costa Rica [26]	53.8^{L}	–	–	–	–	–	–	–	–	–	–
USA [27]	24.6^{L}	–	20.4^{B}	–	–	–	–	–	–	–	–
Algeria [28, 29]	–	24.3^{L} and 38.9^{L} 23.3^{B}	–	–	–	–	–	–	–	–	-
Tunisia [30–33]	–	85.5^{L} 56.6 –82.8^{B}	–	–	–	–	–	–	–	–	–
Egypt [34–36]	–	27.7–51.0^{L} 23.4^{B}	–	–	–	–	–	–	–	–	–
Ethiopia [37]	–	31.4^{L}	–	–	–	–	–	–	–	–	–
Saudi Arabia [38]	–	–	–	–	69.4^{L} 32.8^{B}	–	–	–	–	–	–
Yemen [39]	–	–	–	–	–	–	–	–	–	16.7^{L}	–
India [40]	–	28.1^{L}	–	–	–	–	–	–	–	–	–
Turkey [41, 42]	–	59.3^{L} 35.2^{B} and 49.8^{B}	–	–	–	–	11.3^{L}	–	–	–	–
Italy [43, 44]	–	39.8–67.9^{L} 70.8^{B}	–	–	–	–	–	–	–	–	–
Portugal [45]	–	36.4^{L}	51.3^{B}	–	–	–	–	–	–	–	–

sg subgroup, *L* leaves, *B* berries, *Pi* pinenes, *Ph* phellandrenes, *Mi* myrcene, *Sa* sabinene, *Ci* cymene, *Li* limonene, *Cn* cadinene, *Co* cadinol, *Ge* germacrene, *Bi* bicyclogermacrene, *Cu* cubenol

Extraction of essential oils

A sample of 100 g of powder from each studied organ (leaves and berries) of *Schinus molle* L., added to 750 mL of distilled water was loaded into the Clevenger apparatus to extract the essential oil by hydrodistillation for 4 h. The essential oil was recovered by decantation and the organic phase was dried over anhydrous sodium sulfate (Cheminova Int. S.A., Madrid, Spain; 99% purity). The obtained essential oil was kept at 4 °C in an opaque and sealed bottle. Each extraction was repeated three times, and the yields with standard deviations were calculated. Extraction yield was calculated as the mass of the essential oil per mass unit of the dried plant.

Gas chromatography analysis

The gas chromatography (GC) was performed on an Agilent 6890 apparatus (Agilent Technologies Manufacturing Gmbh & Co. KG. Waghäusel, Germany) provided with a capillary column HP5-MS (5%-phenyl)-methylpolysiloxane (30 m length, 0.25 mm internal diameter and 0.25 µm film thickness). The column was programmed at 50 °C for 5 min then increased to 320 °C with a rate of 5°C/min and kept at this temperature for 5 min. The injector and flame ionization detector (FID) were programmed respectively to 280 and 300 °C. The sample was diluted in acetone (Sigma-Aldrich. St. Louis/Missouri, USA; 99.8% purity) to 1/25 (v/v) and 1 µL was injected in split mode with a fraction of 1/60. Hydrogen was used as carrier gas with a rate flow of 1 mL/min. A series of C_7–C_{30} alkanes (Sigma-Aldrich. St. Louis/Missouri, USA; 99.9% purity) was injected in the same conditions as our samples, in order to calculate the retention indices of each constituent. The proportions of the compounds were calculated by internal normalization.

Gas chromatography/mass spectrometry analysis

The gas chromatography/mass spectrometry (GC/MS) was performed on an Agilent 7890/5975 instrument (Agilent Technologies Manufacturing Gmbh & Co. KG. Waghäusel, Germany) under the same conditions as GC. The acquisition of the mass spectra were obtained for m/z values between 33 and 550 in electron impact mode with an ionization energy of 70 eV and the ion multiplier at 1800 V. The temperature of the ion source and MS quadrupole were 230 and 150 °C respectively. Identification of essential oil components was performed on the MSD ChemStation G1701EA E.02.02.1431 by comparing the retention indices and mass spectra of each element with different databases (Adams and Wiley) [46, 47].

Antioxidant activity

Evaluation of the antioxidant activity of essential oils from *Schinus molle* L. was performed in vitro using two methods: scavenging DPPH and ABTS radicals. DPPH and ABTS were provided from Sigma-Aldrich (St. Louis/Missouri, USA) with 85 and 98% purities respectively. The essentials oils were diluted in methanol (Sigma-Aldrich. St. Louis/Missouri, USA; 99.7% purity) and the various solutions were tested at 100 µg/mL. Then, according to the obtained results, a series of concentrations was selected to calculate the 50% inhibitory concentrations (IC_{50}) using a polynomial regression of order two. For all tests, the measurements were performed three times and the average with standard deviations were calculated.

Scavenging DPPH radical

An antioxidant has the ability to give hydrogen in synthetic radical DPPH (2,2-diphenyl-1-picrylhydrazyl) violet color at oxidized form to reduce it to DPPH-H (2,2-diphenyl-1-hydrazine picryl) yellow-green color. The evaluation of the anti-radical activity of essential oils against the radical DPPH was performed according to Hemalatha et al. protocol [48]. Three mL from different concentrations of each essential oil were mixed with 1 mL of the solution of DPPH at 0.1 mmol /L. Three mL of methanol and 1 mL of DPPH were used as negative control. After incubation at 37 °C in the dark for 30 min, the absorbance was determined at a wavelength of 517 nm. The percentage inhibition of DPPH radical was determined by the formula below:

$$\%\ \text{inhibition of DPPH} = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100$$

with $A_{control}$ is the absorbance of negative control; A_{sample} is the absorbance of the essential oil.

Tocopherol, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were used as standard (positive controls) and were purchased from Sigma-Aldrich (St. Louis/Missouri, USA) with 95.5, 98.5 and 98% purities respectively. The absorbance measurements were performed on a spectrophotometer UNICO model 1200 (Dayton/New Jersey, USA).

Scavenging ABTS radical

The decrease in absorbance caused by the antioxidant reflects the capture capacity of the cationic radical $ABTS^{\bullet+}$ (green blue color) transforming the cation $ABTS\text{-}H^{+}$ (colorless) by a hydrogen donation. Capture of radical cation $ABTS^{\bullet+}$ was determined by the method of Re et al. [49]. The solution of the radical cation $ABTS^{\bullet+}$ was prepared by

mixing 2.45 mmol/L of ABTS with 7 mmol/L of potassium persulfate (Biochem Chemopharma. Cosne-Cours-sur-Loire, France; 98% purity). After 16 h of incubation the solution $ABTS^{\bullet+}$ was diluted with methanol to obtain an absorbance of 0.70 ± 0.02 to 734 nm (negative control). A volume of 10 µL of essential oil at the tested concentration is added to 1 mL of the $ABTS^{+\bullet}$ solution. Absorbance was measured at 734 nm after 6 min incubation in the dark. The percentage inhibition of the radical cation $ABTS^{+\bullet}$ was determined using the following formula:

$$\% \text{ inhibition of ABTS} = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100$$

with $A_{control}$ is the absorbance of negative control; A_{sample} is the absorbance of the essential oil.

Trolox (Sigma-Aldrich. St. Louis/Missouri, USA; 97% purity) was used as a positive control (standard). The absorbance measurements were performed on a spectrophotometer UNICO Model 1200 (Dayton/New Jersey, USA).

Statistical analysis

The statistical analysis (Anova/Manova) was performed with Statistica software (StatSoft, Inc. 1999. STATISTICA, version 5.5). The analysis of variance (Anova) was performed by applying the Post Hoc comparisons with $p < 0.05$. The hiearchic ascending classification (HAC) was obtained by calculating the distances in Euclidean aggregates (EA). The principal component analysis (PCA) was determined using the Spearman correlation coefficients (r) at $p < 0.05$. The Spearman test was also applied to different errors ratio ($p < 0.01$ and $p < 0.001$) to estimate the significance of correlations. All data represent the mean of three tests ± standard deviations (SD).

Results and discussion

Essential oils yields

Table 1 shows the yields of the essential oils from *Schinus molle* L. depending on the region and the organs used for the extraction. The yields obtained were expressed in mass of the essential oil per 100 g of dry plant material.

The statistical analysis (Anova) gave a significant difference between the yields of the essential oils from *Schinus molle* L. The lowest yield was that of the berries of Iheddaden region (0.26 ± 0.03%) followed by that of Iheddaden leaves (0.42 ± 0.06%), while the Amriw berries have given a higher yield (0.64 ± 0.04%) and finally the highest value was obtained with Amriw leaves (0.80 ± 0.06%). A significant difference between the yields of the essential oils from *Schinus molle* L. was shown between the two regions (Iheddaden and Amriw). The lowest yield was obtained at Iheddaden (0.34%) and the highest was that of Amriw (0.72%). Regarding the organ no difference was shown between the yields of leaves and berries. The lowest yield was that of berries with 0.45% and the highest was obtained with leaves (0.61%).

Compared to the literature, the yields obtained from the leaves of *Schinus molle* L. were variable ranging from 0.1 to 3.0%. The lowest value was obtained in Yemen (0.1–0.3%) [39], followed by Brazil (0.3%) [11] and Italy (0.7%) [44]. Intermediate yields of between 1.1 and 1.7% were obtained in Portugal [45], in Tunisia [30], in Costa Rica [26], in Uruguay [22], in Brazil [8, 13–15], in Italy [43] and in Turkey [41]. Values round 2% (between 2.1 and 2.3%) were obtained in Algeria [28], in Saudi Arabia [38] and in India [40]. The highest yield was obtained in Turkey with 3.0% [42].

The yields obtained from leaves of *Schinus molle* L. remain relatively low compared to the literature: 0.41% at Iheddaden and 0.80% at Amriw.

The yields of essential oils from *Schinus molle* L. berries reported in the literature were also fluctuating, ranging from 0.5 to 4.3%. The lowest value was obtained in Brazil (0.5%) [8], followed by 0.8% in Tunisia [32] and 0.9% in Portugal [45]. Another extract from Brazil was obtained with 2.3% [15] and others essential oils from Tunisia were extracted with yields between 1.2 and 4.1% [30, 31, 33]. The highest yields were obtained in Turkey [41] and Saudi Arabia [38] with the same result (4.3%).

The yields obtained with *Schinus molle* L. berries were low with an average yield of 0.45% between the two studied regions (Iheddaden and Amriw).

Chemical composition of essential oils

After integration of the chromatograms and identification of the constituents of the various essential oils from *Schinus molle* L., the constituents were classified by terpene groups. The results were summarized in Table 2.

The hydrocarbon and oxygenated monoterpenes contained in the essential oils of *Schinus molle* L. were minorities, varying between 7.5 and 16.6%. On the other hand, sesquiterpenes constitute the largest fraction in the chemical composition of essential oils from *Schinus molle* L. (between 69.9 and 85.4%). Hydrocarbon sesquiterpenes are the major constituents of essential oils extracted from the leaves of Iheddaden, leaves and berries of Amriw with proportions between 44.7 and 51.8%. The oxygenated sesquiterpenes predominate only in the essential oil of the berries from Iheddaden with 53.8%.

The essential oils from *Schinus molle* L. of the region of Bejaia belong to the group of sesquiterpenes, subgroups of cadinene and cadinols. The cadinene subgroup was obtained

Table 2 Chemical compositions of essential oils extracted from *Schinus molle* L.

No	RI[a]	LRI[b]	Compound	Composition (%)			
				Iheddaden		Amriw	
				Leaves	Berries	Leaves	Berries
01	933	932	α-Pinene	0.4	–	1.3	0.4
02	991	988	Myrcene	1.1	2.1	0.8	1.5
03	1003	1002	α-Phellandrene	3.0	0.4	1,3	3.0
04	1024	1020	*p*-Cymene	1.6	1.9	3.6	2.8
05	1028	1024	Limonene	2.0	1.5	2.5	2.1
06	1028	1025	β-Phellandrene	1.4	0.9	1.4	1.6
07	1100	1102	Perillene	–	–	–	0.1
08	1117	1112	β-Thujone	–	–	0.5	0.5
09	1124	1123	Methyl octanoate	–	0.3	–	–
10	1146	1141	Camphor	–	–	0.3	0.3
11	1171[c]	–	*2-Isopropyl*-Furan	–	–	–	1.1
12	1179[c]	–	Octanoique acid	–	–	–	1.7
13	1187	1179	*p*-Cymen-8-ol	–	–	–	0.1
14	1189	1183	Cryptone	–	0.2	0.4	0.5
15	1205	1207	*trans*-Piperitol	–	0.2	–	–
16	1240[c]	–	α-Phellandrene epoxide	–	–	0.1	0.6
17	1249	1234	Ascaridole	0.1	0.2	–	–
18	1251	1244	Carvotanacetone	–	–	–	0.2
19	1256	1249	Geraniol	–	–	–	0.7
20	1279	1287	Phellandral	–	–	0.3	0.5
21	1302	1297	Carvacrol	0.1	0.3	0.9	2.2
22	1323	1322	Methyl geranate	–	0.7	–	–
23	1353	1345	α-Cubebene	0.2	–	0.3	0.3
24	1355	1350	Citronellyl acetate	–	0.2	–	–
25	1358	1359	Neryl acetate	–	0.4	–	–
26	1380	1374	α-Copaene	0.8	0.3	0.7	0.7
27	1385	1379	Geranyl acetate	–	0.3	–	0.1
28	1387	1387	β-Bourbonene	0.1	–	–	–
29	1395	1389	β-Elemene	0.9	0.6	1.1	0.7
30	1414	1409	α-Gurjunene	1.1	1.2	1.9	1.3
31	1425	1417	β-Caryophyllene	1.4	1.0	1.8	1.2
32	1434	1430	β-Copaene	0.2	–	–	0.1
33	1452	1448	Cadina-3,5-diene	0.2	–	0.4	0.2
34	1459	1452	α-Humulene	1.1	1.0	1.5	1.1
35	1466	1458	*allo*-Aromadandrene	0.8	0.6	0.9	0.6
36	1468	1465	*cis*-Muurola-4(14),5-diene	–	–	0.4	0.4
37	1479	1475	*trans*-Cadina-1(6),4-diene	0.4	–	0.9	0.8
38	1481	1478	γ-Muurolene	1.2	1.3	1.6	1.3
39	1485	1484	Germacrene-D	9.3	0.6	0.7	0.2
40	1491	1486	β-Selinene	–	–	1.7	0.2
41	1492	1487	Bicyclosesquiphellandrene	0.5	–	–	1.1
42	1494	1493	*trans*-Muurola-4(14),5-diene	–	0.8	–	–
43	1495	1494	γ-Bulgarene	–	–	1.6	–
44	1496	1495	γ-Amorphene	0.7	–	–	–
45	1500	1498	α-Selinene	–	0.3	1.0	0.7
46	1502	1500	Bicyclogermacrene	1.6	–	–	–
47	1505	1500	α-Muurolene	3.5	4.0	6.2	4.7
48	1511	1508	Germacrene A	0.3	–	–	–

Table 2 (continued)

No	RI[a]	LRI[b]	Compound	Composition (%)			
				Iheddaden		Amriw	
				Leaves	Berries	Leaves	Berries
49	1520	1513	*γ*-Cadinene	2.4	3.0	4.3	4.0
50	1530	1522	*δ*-Cadinene	16.1	13.0	23.0	23.4
51	1525	1528	Zonarene	1.0	1.3	–	–
52	1538	1533	*trans*-Cadina-1,4-diene	0.3	–	0.5	0.7
53	1543	1537	*α*-Cadinene	0.7	0.7	0.9	0.8
54	1549	1544	*α*-Calacorene	0.1	0.3	0.4	0.2
55	1555	1548	Elemol	3.0	0.6	4.0	1.6
56	1563	1559	Germacrene-B	0.5	–	0.3	–
57	1564	1562	Geranyle butanoate	–	0.3	–	1.4
58	1570	1564	*β*-Calacorene	–	0.2	–	–
59	1575	1565	Ledol	–	0.8	1.0	0.9
60	1582	1574	Germacrene D-4-ol	0.7	–	–	0,9
61	1581	1577	Spathulenol	2.2	0.3	3.5	–
62	1587	1582	Caryophyllene oxide	–	0.6	–	–
63	1591	1586	Gleenol	0.4	0.5	1.0	0.7
64	1599	1592	Viridiflorol	1.3	1.8	1.5	1.5
65	1611	1606	*epi*-Globulol	0.8	1.2	0.9	0.9
66	1617	1608	Humulene-1,2-epoxyde	–	0.4	0.3	0.2
67	1622	1618	*1,10-diepi*-Cubenol	0.3	0.4	0.3	0.4
68	1628	1622	*10-epi-γ*-Eudesmol	0.2	–	–	–
69	1635	1627	*1-epi*-Cubenol	0.7	1.4	0.7	0.8
70	1639	1630	*γ*-Eudemol	2.3	1.1	2.1	1.0
71	1646	1638	*epi-α*-Cadinol (*τ*-Cadinol)	3.2	5.4	–	–
72	1649	1640	*epi-α*-Muurolol (*τ*-Muurolol)	4.9	9.8	2.5	3.0
73	1653	1644	*α*-Muurolol	1.2	2.2	3.1	–
74	1659	1649	*β*-Eudesmol	–	–	1.1	1.1
75	1663	1652	*α*-Cadinol	12.9	25.1	11.3	11.1
76	1664	1660	*cis*-Calamenen-10-ol	0.2	0.6	–	–
77	1669	1668	*trans*-Calamenen-10-ol	0.2	0.6	–	–
78	1711	1687	Eudesma-4(15),7-dien-1-β-ol	–	–	–	1.1
79	1723	1714	*(2E,6Z)*-Farnesol	–	0.4	–	–
80	1748	1739	Oplopanone	–	0.4	–	–
81	1756	1753	Geranyl hexanoate	–	–	–	0.4
Total (%)				89.6	93.7	98.8	91.7
Hydrocarbon monoterpenes (HM)				9.5	6.8	10.9	11.4
Oxygenated monoterpenes (OM)				0.2	0.7	2.1	5.2
Hydrocarbon sesquiterpenes (HS)				44.9	30.0	51.8	44.7
Oxygenated sesquiterpenes (OS)				35.0	53.8	33.6	25.2
Non terpenic compounds (NT)				0.0	2.4	0.4	5.2

[a]Calculated retention indices on HP5-MS column

[b]Literature retention indices on DB-5 column (Adams library) [46]

[c]Identification only with mass spectra (Wiley library) [47]

with Iheddaden leaves, Amriw leaves and berries with proportions between 16.1 and 23.4%. The subgroup of cadinols (*α*- and *epi-α*-) was obtained with the berries of Iheddaden with 30.5%.

The cadinene chemotype was obtained with leaves of *Schinus molle* L. in Turkey with 11.3% [42], Brazil with 23.7% [14] and 27.3% [13]. The values obtained with the leaves of Iheddaden and Amriw (16.1 and 23.0%

respectively) are greater than the value obtained in Turkey and lower than the values found in Brazil.

For essential oils extracted from the berries of *Schinus molle* L., no predominantly sesquiterpenes chemotype has been reported in the literature. Our extracts are part of the cadinene chemotype for berries of Amriw (23.4%) and cadinols for those of Iheddaden (30.5%).

Hierarchical Ascending Classification enables classifying the different chemical families of the essential oil from *Schinus molle* L. in the different analyzed samples (Fig. 1). The first group contains the two most widely presented chemical families: oxygenated sesquiterpenes (OS) and hydrocarbon sesquiterpenes (HS) at distances from non terpenic compounds (NT) of 127 AE and 145 AE respectively. The two least present families form another group between NT and the oxygenated monoterpenes (OM) with a distance of 4 AE, to which was attached the intermediate family (hydrocarbon monoterpenes HM) with a distance of 27 AE Relative to OM and a distance of 28 AE from NT. The classification of chemical families of essential oils from *Schinus molle* L. was given by the following increasing order: NT < OM < HM < OS < HS.

The Spearman significance test revealed a highly significant correlation ($p < 0.01$) between yields (YLD) of essential oils of *Schinus molle* L. and OS. Very highly significant correlations ($p < 0.001$) were obtained between YLD and HM, YLD and HS (Table 3).

The application of PCA demonstrates the relationship between yields of essential oils and the different chemical families (Fig. 2). The correlation circle shown that NT were in a minority. In addition, there is an opposition between OS and YLD, indicating that OS were in important proportions regardless of the obtained yields. This confirms the belonging of the essential oils from *Schinus molle* L. to the sesquiterpenes chemotypes.

Scavenging DPPH radical

The antioxidant activity of the samples studied was expressed in IC_{50}. It was defined as the concentration of the antioxidant necessary to reduce or inhibit 50% of the DPPH radical in solution. The best activity against the DPPH radical was obtained with the lowest IC_{50} value.

For antiradical activity tests against DPPH: tocopherol, BHA and BHT were used as positive controls (standard). The results of the antioxidant activities against the DPPH radical obtained with the essential oils of *Schinus molle* L. were summarized in Table 4.

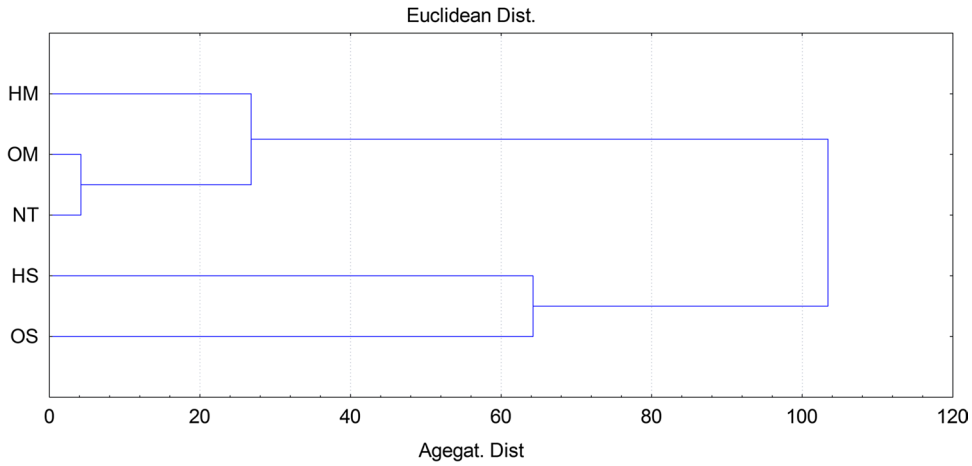

Fig. 1 HAC of chemical composition from essential oils of *Schinus molle* L.

Table 3 Correlation matrix between yields and chemical composition of *Schinus molle* L.

	YLD	HM	OM	HS	OS	NT
YLD	1.00					
HM	0.88***	1.00				
OM	0.57	0.70*	1.00			
HS	0.88***	0.89***	0.32	1.00		
OS	−0.75**	−0.97***	−0.69*	−0.82**	1.00	
NT	0.03	0.19	0.82**	−0.27	−0.25	1.00

YLD yields, *HM* hydrocarbon monoterpenes, *OM* oxyginated monoterpenes, *HS* hydrocarbon sesquiterpenes, *OS* oxyginated sesquiterpenes, *NT* non terpenic compounds

*$p < 0.05$; **$p < 0.01$ and ***$p < 0.001$

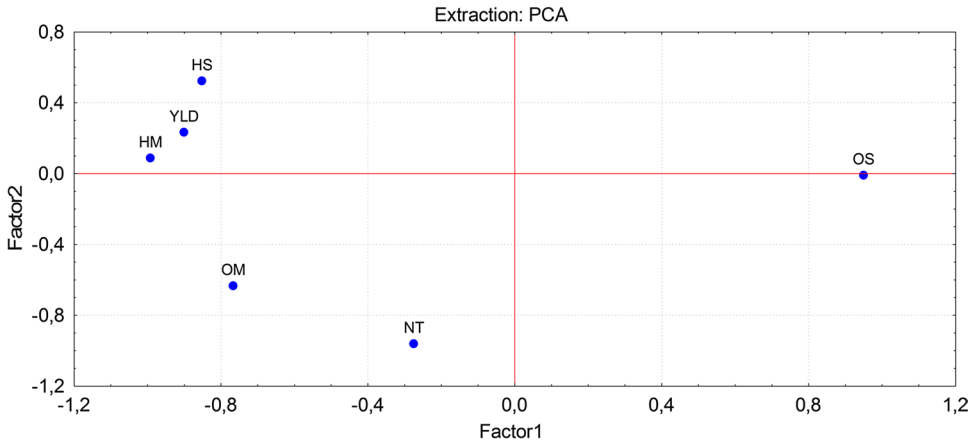

Fig. 2 PCA between yields and chemical composition of *Schinus molle* L.

Table 4 Antioxidant activity of essential oils extracted from *Schinus molle* L. against DPPH radical

	IC_{50} DPPH (µg/mL)	R^2
Iheddaden leaves EO	$7234.6 \pm 254.1^{a,b}$	0.883
Iheddaden berries EO	7513.7 ± 76.6^{b}	0.778
Amriw leaves EO	8643.4 ± 364.9^{c}	0.887
Amriw berries EO	6898.6 ± 219.1^{a}	0.911
Tocopherol	7.2 ± 0.1	0.948
BHA	2.0 ± 0.1	0.848
BHT	3.8 ± 0.4	0.887

EO essential oil

The letters a, b and c correspond to the significant difference among the IC_{50} DPPH of the essential oils

Anova analysis has shown a significant difference between the antioxidant activities of the essential oils from *Schinus molle* L. against DPPH. We note that the activities obtained with the leaves of Iheddaden and the berries of Amriw (a) do not show any significant difference, same comment with the activities of leaves and berries of Iheddaden (b). The highest antioxidant activity was obtained with Amriw berries ($IC_{50} = 6898.6$ µg/mL) and the lowest was that of the essential oil extracted from Amriw leaves ($IC_{50} = 8643.4$ µg/mL). There was no significant difference between the antioxidant activities against DPPH of the essential oils from *Schinus molle* L. of the two regions (Iheddaden and Amriw). The highest activity was found with Iheddaden extracts ($IC_{50} = 7374.1$ µg/mL) and the lowest was that of Amriw essential oil's ($IC_{50} = 7771.0$ µg/mL). For the organ, no significant difference between the activities of the leaves and fruits of the two regions. The highest was obtained with fruits ($IC_{50} = 7206.2$ µg/mL), and the lowest activity was that of leaves ($IC_{50} = 7939.0$ µg/mL).

Positive controls (standard) showed high antiradical activities with IC_{50} between 2.0 and 7.2 µg/mL. The antioxidant activity of the essential oils of *Schinus molle* L. against the DPPH radical is low, varies between 6.9 and 8.6×10^3 µg/mL. Compared to the literature, Diaz et al. [26] obtained a low IC_{50} against DPPH (36.3 µg/mL) with the essential oil from *Schinus molle* L., but Bendaouad et al. [33] obtained an elevated IC_{50} with a value of 3697.2 µg/mL.

The Spearman test gave a non-significant correlation between the IC_{50} DPPH and YLD. Same observation with the correlations obtained with the different chemical families of the essential oils from *Schinus molle* L. (Table 5).

The application of PCA demonstrates that OM and NT are the two chemical families of essential oils from *Schinus molle* L. that promote moderately antiradical activity against DPPH (Fig. 3).

Scavenging ABTS radical

In addition to the inhibitory concentrations of 50% of the ABTS radical cation in solution (IC_{50}), the effectiveness of the essential oils to trap this radical was evaluated in trolox equivalent antioxidant capacity (TEAC) which corresponds to the concentration of Trolox producing the same effect of reduction of ABTS per gram of essential oil. More the value of TEAC is high more the antioxidant is powerful. The results of the antioxidant activities against the ABTS radical cation obtained with the essential oils of *Schinus molle* L. were grouped in Table 6.

Analysis of the variance of the IC_{50} against the ABTS radical cation showed a significant difference between the antioxidant activities of the essential oils of *Schinus molle* L. The greatest activity was found with Iheddaden berries ($IC_{50} = 741.0$ µg/mL) and the lowest was that of Iheddaden leaves ($IC_{50} = 5048.0$ µg/mL). No significant differences between the two regions of Iheddaden and Amriw. The highest activity was obtained with Iheddaden extracts ($IC_{50} = 2894.5$ µg/mL) and the lowest was that of Amriw essential oils ($IC_{50} = 4449.7$ µg/mL). A significant difference was shown between the leaves and berries of the two regions. The highest activity was found with berries

Table 5 Matrix of correlations between IC_{50} DPPH, yields and chemical composition of *Schinus molle* L.

	YLD	HM	OM	HS	OS	NT	DPPH
YLD	1.00						
HM	0.88***	1.00					
OM	0.57	0.70*	1.00				
HS	0.88***	0.89***	0.32	1.00			
OS	−0.75**	−0.97***	−0.69*	−0.82**	1.00		
NT	0.03	0.19	0.82**	−0.27	−0.25	1.00	
DPPH	0.47	0.08	−0.26	0.38	0.14	−0.57	1.00

YLD yields, *HM* hydrocarbon monoterpenes, *OM* oxyginated monoterpenes, *HS* hydrocarbon sesquiterpenes, *OS* oxyginated sesquiterpenes, *NT* non terpenic compounds, *DPPH* IC_{50} DPPH

*p < 0.05 ; **p < 0.01 and ***p < 0.001

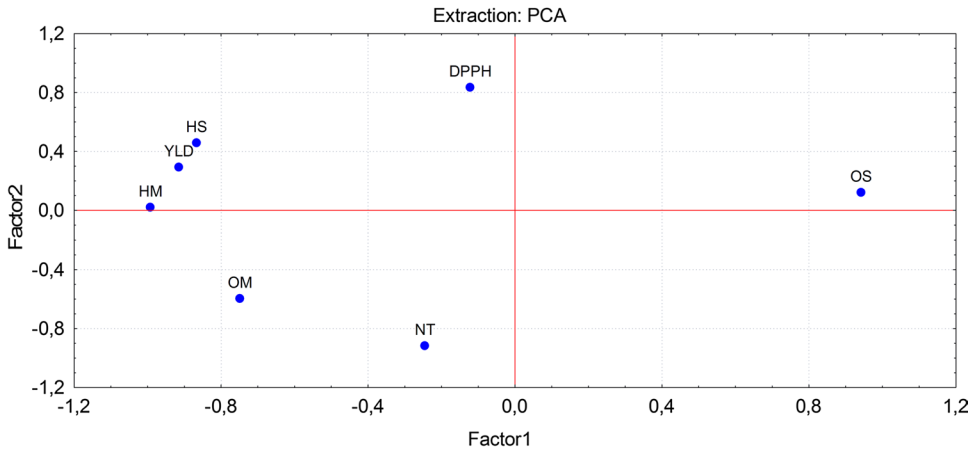

Fig. 3 PCA between IC_{50} DPPH, yields and chemical composition of *Schinus molle* L.

Table 6 Antioxidant activity of essential oils extracted from *Schinus molle* L. against the ABTS radical cation

	IC_{50} ABTS (µg/mL)	R^2	TEAC
Iheddaden leaves EO	5048.0 ± 31.5^{d}	0.994	0.01 ± 0.00
Iheddaden berries EO	741.0 ± 4.1^{a}	0.981	0.07 ± 0.00
Amriw leaves EO	4715.0 ± 91.7^{c}	0.972	0.01 ± 0.00
Amriw berries EO	4184.3 ± 38.7^{b}	0.961	0.01 ± 0.00
Trolox	50.5 ± 2.9	0.990	1.00 ± 0.06

TEAC antioxidant concentration trolox equivalent expressed in g Trolox equivalent per gram of essential oil, *EO* essential oil

The letters a, b, c and d correspond to the significant difference among the IC_{50} ABTS essential oils

(IC_{50} = 2462.7 µg/mL) and the lowest was obtained with leaves (IC_{50} = 4881.5 µg/mL).

Bendaoud et al. [33] obtained an IC_{50} of essential oil of *Schinus molle* L. against ABTS of 270.0 µg/mL. The results obtained show that the essential oils of *Schinus molle* L. have very low antioxidant activity against the ABTS cation compared with the used positive control (Trolox) that has an IC_{50} of 50.5 µg/mL and the value obtained by Bendaoud et al. [33] (270.0 µg/mL). This observation was confirmed by the TEAC obtained, which vary between 0.01 and 0.07 µg of Trolox/g of essential oil.

The Spearman test showed a significant correlation between the IC_{50} ABTS and YLD of essential oils from *Schinus molle* L. Very highly significant correlations were obtained with HM, HS and OS (Table 7).

Principal component analysis confirms that OS was the only chemical family that promotes antioxidant activity against the ABTS radical (Fig. 4).

Conclusion

The essential oils of *Schinus molle* L. present a chemical variety depending on the region and the organ. Two chemotypes were determined: sesquiterpene group, cadinene and cadinols subgroups. The cadinene subgroup was observed with Iheddaden leaves, Amriw leaves and berries with proportions between 16.1 and 23.4%. The subgroup of cadinols (*α*- and *epi-α*-) was found with Iheddaden berries (30.5%). The cadinene chemotype was already reported with the leaves of *Schinus molle* L. in Turkey and Brazil. For the essential oils extracted from the

Table 7 Matrix of correlations between IC_{50} ABTS, yields and chemical composition of *Schinus molle* L.

	YLD	HM	OM	HS	OS	NT	ABTS
YLD	1.00						
HM	0.88***	1.00					
OM	0.57	0.70*	1.00				
HS	0.88***	0.89***	0.32	1.00			
OS	−0.75**	−0.97***	−0.69*	−0.82**	1.00		
NT	0.03	0.19	0.82**	−0.27	−0.25	1.00	
ABTS	0.67*	0.84***	0.23	0.93***	−0.86***	−0.28	1.00

YLD yields, *HM* hydrocarbon monoterpenes, *OM* oxyginated monoterpenes, *HS* hydrocarbon sesquiterpenes, *OS* oxyginated sesquiterpenes, *NT* non terpenic compounds, *ABTS* IC_{50} ABTS

*p < 0.05 ; **p < 0.01 and ***p < 0.001

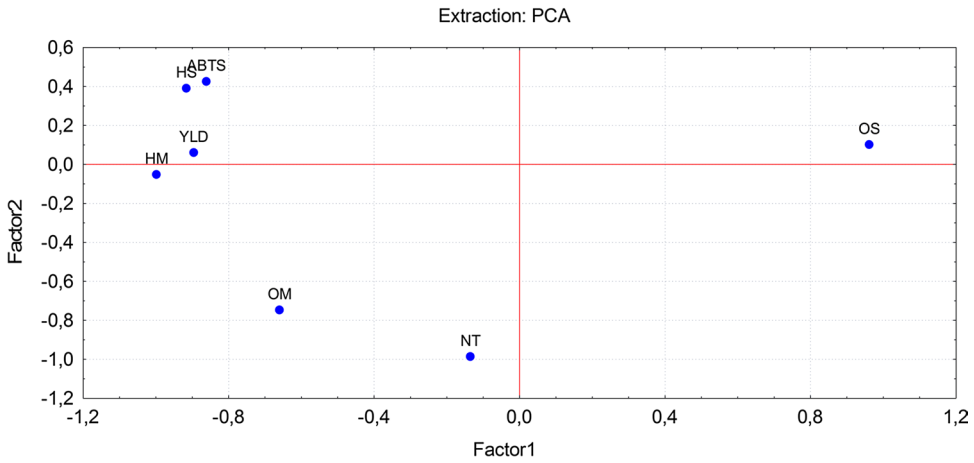

Fig. 4 PCA between IC_{50} ABTS, yields and chemical composition of *Schinus molle* L.

berries of *Schinus molle* L., the sesquiterpene chemotype was reported for the first time.

The antioxidant activities of the essential oils from *Schinus molle* L. against the DPPH radical were low between 6.9 and 8.6×10^3 µg/mL. In addition, the results obtained show that the antioxidant activities of the essential oils from *Schinus molle* L. against ABTS radical cation were very low compared with Trolox ($IC_{50} = 50.5$ µg/mL) and the value obtained by Bendaoud et al. (270.0 µg/mL). Principal component analysis (PCA) indicates that high proportions of OS (oxygenated sesquiterpenes) promote the antioxidant activity of essential oils from *Schinus molle* L. against ABTS radical, while the antioxidant activity against the DPPH radical requires the combination of the different chemical families contained in the essential oils of *Schinus molle* L.

As prospects, we wish to extend this study to other locations in Bejaia region to complete the classification of chemotypes of the essential oils extracted from *Schinus molle* L. In addition, other complementary methods: in vitro [ferric reducing antioxidant power (FRAP), total antioxidant capacity (TAC), etc.] and in vivo would be needed to explain the molecular and cellular mechanisms involved in the antioxidant activity.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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