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Schinus molle: Chemical Analysis, Phenolic Compounds and Evaluation of Its Antioxidant Activity

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ABSTRACT

Essential oils (EOs) and various extracts of Tunisian Schinus molle L. were screened for their chemical composition and antioxidant activities. EOs were obtained by hydrodistillation from leaves, stems and fruits of S. molle and characterized by GC-FID and GC-MS. Leaf EO was characterized mainly by β-eudesmol (14.82%), elemol (13.71%), α-eudesmol (12.76%), d-limonene (9.25%) and spathulenol (7.21%). Stem EO was characterized mainly by elemol (20.7%), 6-epi-shyobunol (20.36%), d-limonene (16.19%) and α-eudesmol (7.01%). Fruit EO was characterized by 6-epi-shyobunol (16.22%), d-limonene (15.35%), spathulenol (8.16%) and 4-epi cubebol (7.84%). Phenolic components of various extracts were evaluated. The antioxidant activities of EOs and various extracts were assessed by DPPH and ABTS assays. Our results showed that the fruit essential and the methanol extract expressed the highest antioxidant activities in the ABTS assay (IC_{50} of 32.6 ± 0.6 mg/L and 7.1 ± 0.2 mg/L, respectively). Suggestion on relationships between chemical composition and antioxidant activity is outlined.

Keywords: *Schinus molle*; essential oils (EOs); extracts; GC-MS; antioxidant

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INTRODUCTION

The genus *Schinus* L. (Anacardiaceae*)* is native to South America and encompasses about 30 species [1]. *Schinus molle* (pepper tree) was introduced in Tunisia, as an ornamental plant, by French colonisers in the late 1900s. It's a dioecious and female plant growing up to 7–10 m that produces large crops of small pink to reddish fruits arranged in bunches on pendulous stems.

S. molle has been used in folk medicine in several countries. It was used as antiviral, antibacterial, antioxidant and topical antiseptic [2], antitumoral [3], also as analgesic and central depressant [4] and as repellent and insecticidal against several pathogenic herbivores and insect pests [5], [6], [7]. It was also used in the treatment of toothache, rheumatism, menstrual disorders, respiratory and urinary tract infection [8], [9].

The essential oils of *Schinus molle* from different provenances have been previously undertaken and some differences in their chemical profile and biological activities have been observed [10], [11], [12], [13]. Other studies were interested in evaluating the antioxidant properties of EOs and extracts of *Schinus molle* [14], [15], [16], [17].

To our knowledge, our study is the first dealing with the chemical composition of *S. molle* stems EO. Furthermore, little is known about the phenolic profile of *S. molle* fruits exctracts. Thus, the present investigation was undertaken to characterise the chemical composition of essential oils from leaves, stems and fruits from *S. molle* collected in the northen of Tunisia. The phenolic content of various extracts (hexane, ethyl acetate, ethanol and methanol) were also

determined. Furthermore, we evaluated the antioxidant activities of the essential oils and various extracts by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonate (ABTS) assays.

EXPERIMENTAL SECTION

2.1. Plant material

Schinus molle leaves, stems and fruits were collected on March 2012 from trees growing in the Lac 2 region, located in Tunis governorship in Tunisia. The harvested materials were air-dried at room temperature (20 ± 2 °C) for 1 week. Dried materials were hydrodistilled and various extracts prepared. Samples were identified by Mr. Ridha El Mokni and voucher specimens were deposited in the herbarium of the Department of Biology of the Faculty of Sciences of Bizerte.

2.2. Extraction methods

2.2.1. Essential oils

Three lots of 50 g of each air-dried organ type (leaves, stems and fruits) were separately hydrodistilled (500 mL water) in a Clevenger type apparatus for 2 h (time fixed after a kinetic survey during 15, 30, 45, 60, 75, 90, 105, 120 and 135 min). This extraction procedure gave pale yellowish essential oils which were dried with anhydrous sodium sulfate and kept in amber vials at 4 °C for further analysis. Na_2SO_4 was removed before use of the essential oils. All experiments were done in triplicate and the extraction yields were calculated.

2.2.2. Various extracts

The extraction method was sequential extraction with solvents of increasing polarity. Solvents used were: hexane, ethyl acetate, ethanol and methanol. 5 g of harvested fruits were placed in hexane (50 mL) for 24 h under frequent agitation at ambient pressure and temperature. The mixture was filtered using Wattman paper (GF/A, 110 mm). The solvent was evaporated using a rotary evaporator under vacuum at 35 °C. Then, the firstly extracted powder was extracted with ethyl acetate under the same conditions as with hexane. The same procedure was applied for the following solvents. Extracts were kept in amber vials and stored at 4 °C for further analysis.

2.3. Chemical components analysis GC-FID and GC-MS

Essential oils analysis were carried out by gas chromatography (GC) on a Hewlett–Packard 6890 gas chromatograph (Agilent Technologies, Palo Alto, California, USA) equipped with a flame ionization detector (FID) and an electronic pressure control (EPC) injector. A 5% diphenyl, 95% dimethylpolysiloxane apolar HP-5 capillary column (30 m x 0.25 mm, 0.25 μm film thickness; Hewlett-Packard, CA, USA) was used. Injector and detector temperatures were set, respectively, at 250 and 300° C. Analyses were performed using the following temperature program: oven temps isotherm at 35°C for 10 min, raised from 35 to 205°C at the rate of 3°C/min and then kept isothermally at 205°C for 10 min. For analysis, 1 μL of diluted essential oils were injected in 60:1 split mode. The flow rate of the carrier gas (N2, U) was 1.6 mL/min.

Essential oil analysis was performed on an Agilent 7890A GC system, coupled to an Agilent 5975C mass spectroscopy detector with electron impact ionization (70 eV). A HP-5 MS capillary column (30 m x 0.25 mm, coated with 5% phenyl methyl silicone, 95% dimethylpolysiloxane, 0.25 mm film thickness; Agilent Technologies, Hewlett-Packard, CA, USA) was used at a temperature programmed to rise from 60 to 260 °C with a 5 °C/min rate then rise to 340°C with a 40 °C/min rate, the carrier gas was helium N60 with a 1.2 mL/min flow rate; split ratio was 60:1. Scan time and mass range were 1s and 40–300 m/z, respectively. The identification of volatile components was assigned by comparison of their KI (retention indices) relative to (C_6- C_{22}) *n*-alkanes obtained on a non polar HP5-MS column, with those provided in the literature or with those of authentic compounds available in literature and the authors' laboratory. Further identification was made by comparison of their recorded mass spectra with those recorded in the Wiley 09 NIST 2011 mass spectral library of the GC/MS data system and other published mass spectra [18] and by-coinjection of available reference compounds (>99% purity) provided from commercial suppliers (Sigma–Aldrich, Darmstadt, Germany) and dissolved in dimethyl sulfoxide (DMSO) Sigma–Aldrich (USA). Data were expressed as relative percentage of the total peak area.

2.4. Determination of total phenolic content

The total phenolics amount of each extract was determined by the Folin–Ciocalteu method [19]. Briefly, the diluted aqueous solution of each extract (0.5 ml) was mixed with 2.5 ml of the Folin–Ciocalteu reagent (0.2 N). This mixture was allowed to stand at room temperature for 5 min and then sodium carbonate (Na_2CO_3) solution (75 g/L in water, 2 ml) was added. After 60 min of incubation, the absorbances were read at 765 nm against water blank. A standard calibration curve was plotted using gallic acid (0 to 300 mg/L). Results were expressed as mg of gallic acid equivalents (GAE) per gram of dry weight (mg of GAE/g of dw).

2.5. Total condensed tannin content
The condensed tannin content of *S. molle* extracts were determined by the vanillin method [20] with modifications: 50 µl of each extract solution was mixed in a test tube with 150 µl of vanillin (1% in 7 M H_2SO_4) in an ice bath. The mixture was kept at 25°C for 15 min. Then the absorbance was read at 500 nm versus methanol as a blank. Results were expressed as mg catechin equivalents (CE) per kilogram of dw (mg of CE/kg of dw) from a calibration curve.

2.6. Total flavonoid content
Total flavonoids were estimated according to the Dowd method [21] using a microplate reader. To 96 well plate, 100 µl of each variety extract was mixed with a solution (100 µl) of aluminium trichloride ($AlCl_3$) in methanol (2%). The absorbance was measured at 510 nm against a reagent blank sample consisting of methanol (100 µl) and extract (100 µl) without $AlCl_3$. Quercetin was used as reference compound to produce the calibration curve, and results were expressed as milligrams of quercetin equivalents (QE) per gram of dry weight (mg of QE/g of dw).

2.7 Total anthocyanin content
Total anthocyanin contents were measured using the pH differential absorbance method [22] using two buffers: hydrochloric acid-potassium chloride (pH 1.0, 0.2 M) and acetic acid-sodium acetate (pH 4.5, 1 M) using 96-well plates. 20 µl of *S. molle* extract was mixed with 180 µl of corresponding buffers and the absorbances were measured at 510 and 700 nm after 15 min of incubation. A molar extinction coefficient (ε) of 29600 (cyanidin-3-glucoside) and absorbance of: A = [(A510 – A700) pH 1.0 – (A510 – A700) pH 4.5] were used for anthocyanin calculation. The final results were expressed as milligram of cyanidin-3-glucoside equivalent (C3GE) per gram of dry weight (mg C3GE/g dw).

2.8. DPPH assay
Antioxidant scavenging activity was evaluated using 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) [23] with some modifications Aliquots (1.5 mL) of various dilutions of the test material (pure antioxidant, essential oil or plant extracts) were mixed with 1.5 mL of methanolic DPPH solution (0.2 mM). The mixtures were incubated for 30 min at 25 °C, then the absorbance at 520 nm was measured. The absorbance in the presence of plant extract was recorded as $A_{(sample)}$ while the absorbance of the control reaction was recorded as $A_{(blank)}$. The free radical-scavenging activity of each solution was then calculated as inhibition percentage as follows:

$$\% \text{ inhibition} = [(A_{(blank)} - A_{(sample)})/A_{(blank)}] \times 100.$$

Antioxidant activity was expressed as IC_{50} (mg/L), defined as the concentration of the essential oil or extract required to cause a 50% decrease in initial DPPH concentration. Ascorbic acid was used as positive control. All measurements were performed in triplicate.

2.9. ABTS radical-scavenging assay
The ABTS (2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonate) radical scavenging capacity of EOs and various extracts was determined [24]. ABTS was generated by mixing a solution of ABTS (7 mM) at pH 7.4 with potassium persulfate (2.5 mM) followed by incubating the mixture in the dark at room temperature for 16 h before use. The mixture was diluted with ethanol to obtain an absorbance of 0.70 ± 0.02 at 734 nm. For each sample, diluted methanol solution of the sample (100 µL) was mixed with fresh ABTS solution (900 µL), and then the absorbance was read 6 min after initial mixing. Ascorbic acid was used as a standard. The radical scavenging activities of EOs and various extracts was expressed by IC_{50} (mg/L) values which represent the concentration of the sample required to scavenge 50% of ABTS radicals. The radical scavenging activity IC_{50} was calculated using the same equation previously used for the DPPH method. All measurements were performed in triplicate.

2.10. Statistical Analysis
All data were expressed as means ± standard deviations of triplicate measurements. The confidence limits were set at $p < 0.05$. Standard deviations (SD) did not exceed 5% for the majority of the values obtained.

RESULTS AND DISCUSSION

3.1. Chemical composition
3.1.1. Essential oils
The leaf, stem and fruit essential oils exhibited a light yellow color with pungent and pepper-like aroma. The essential oil yields in leaves, stems and fruits of *S. molle* were respectively 1.86%, 1.94% and 2.52% (w/w relative to dry material weight). They differed greatly from those reported by [25] and [13]. To our knowledge no study for essential oil composition was made on *S. molle* stems.

The components of *S. molle* essential oils have been determined by GC-FID and GC-MS analysis. The essential oil volatile compounds of different *S. molle* parts; their retention indexes and their percentages were listed in Table 1. All the constituents were arranged in order of their elution on the HP-5 column. A total of 36, 30 and 41 components representing 100% of the total content, were identified in *S. molle* leaf, stem and

Table 1. Chemical composition of leaves, stems and fruits essential oils of *Schinus molle* L

Nr	Compounds	RI	Leaf EO (%)	Stem EO (%)	Fruit EO (%)
1	α-pinene	931	0.68	0.8	1.6
2	β-myrcene	988	0.58	1.74	1.53
3	l-phellandrene	1026	2.32	4.1	5.45
4	p-cymene	1033	1.94	2.18	2.54
5	d-limonene	1036	9.25	16.19	15.35
6	trans-para-2,8-menthadien-1-ol	1122	n.d.	n.d.	0.91
7	cis-p-mentha-2,8-dien-1-ol	1128	n.d.	n.d.	0.63
8	1,8-menthadien-4-ol	1182	n.d.	n.d.	0.36
9	cryptone	1189	n.d.	n.d.	0.64
10	isopiperitenol	1196	n.d.	n.d.	3.73
11	trans-(+)-carveol	1216	0.28	n.d.	0.64
12	cis-p-mentha-1(7),8-dien-2-ol	1227	n.d.	n.d.	0.48
13	sabinyl acetate	1289	n.d.	n.d.	0.36
14	α-copaene	1376	0.68	0.39	0.73
15	β-elemene	1391	0.19	0.3	0.66
16	α-gurjunene	1410	0.5	0.52	0.74
17	α-caryophyllene	1419	1.22	0.71	4.3
18	aromadendrene	1439	n.d.	1.56	n.d.
19	α-humulene	1454	0.46	0.33	0.9
20	valencene	1477	0.17	n.d.	0.3
21	α-amorphene	1479	0.23	n.d.	0.38
22	germacrene D	1481	0.24	0.57	2.02
23	β-selinene	1485	0.36	n.d.	0.23
24	α-selinene	1493	0.68	0.43	1.13
25	α-muurolene	1499	1.36	0.36	0.75
26	germacrene A	1509	n.d.	n.d.	0.27
27	4-epi-cubebol	1512	1.42	0.49	7.84
28	ledol isomer	-	n.d.	0.34	0.27
29	β-cadinene	1524	5.03	2.93	4.27
30	elemol	1540	13.71	20.7	1.24
31	α-calacorene	1545	0.19	n.d.	n.d.
32	germacrene B	1554	n.d.	0.33	n.d.
33	palustrol	1561	1.13	0.16	0.44
34	spathulenol	1578	7.21	5.23	8.16
35	caryophyllene oxide	1580	2.85	n.d.	1.08
36	viridiflorol	1590	2.46	0.67	1.34
37	ledol	1596	1.29	0.38	0.73
38	humulene oxyde	1598	0.73	n.d.	n.d.
39	α-ylangene	1600	n.d.	n.d.	0.41
40	oplopenone	1605	0.42	n.d.	n.d.
41	γ-eudesmol	1631	5.75	2.99	0.38
42	7.alpha.-H-Eudesma-3,5-diene	-	3.97	2.35	4.25
43	β-eudesmol	1651	14.82	4.68	0.99
44	α-eudesmol	1652	12.76	7.01	-
45	6-epi-shyobunol	1680	3.03	20.36	16.22
46	7-acetyl-2-hydroxy-2-methyl-5-isopropylbicyclo[4.3.0]nonane	-	0.18	n.d.	n.d.
47	isoledene	1723	0.66	0.55	3.23
48	cis-Z-.alpha.-bisabolene epoxide	1740	1.26	0.66	1.31
	Total identified compounds		100	100	100
	Monoterpene hydrocarbons		12.83	22.83	23.93
	Aromatic monoterpenes		1.94	2.18	2.54
	Monoterpene alcohols		0.28	-	6.75
	Monoterpene ketones		-	-	0.64
	Monoterpene ethers		2.85	-	1.08
	Sesquiterpenes		81.93	75	64.72
	Others		0.18	-	0.36

- : not determined; n.d. : not detected. RI : retention index relative to (C6–C22) n-alkanes on the HP5-MS column*

The leaf essential oil consists of sesquiterpenes (81.93%), monoterpene hydrocarbons (12.83%), monotepene ethers (2.85%), aromatic monoterpenes (1.94%), monoterpene alcohols (0.28%) and other compounds (0.18%). On the other hand, the stem essential oil consists sesquiterpenes (75%), monoterpene hydrocarbons (22.83%) and aromatic monoterpenes (2.18%). Otherwise, the fruit essential oil consists of sesquiterpenes (64.72%), monoterpene hydrocarbons (23.93%), monoterpene alcohols (6.75%), aromatic monoterpenes (2.54%), monoterpene ethers (1.08%), monoterpene ketones (0.64%) and other compounds (0.36%). Leaf EO was characterised mainly by β-

eudesmol (14.82%), elemol (13.71%), α-eudesmol (12.76%), d-limonene (9.25%) and spathulenol (7.21%). Stem EO was characterised mainly by elemol (20.7%), 6-epi-shyobunol (20.36%), d-limonene (16.19%) and α-eudesmol (7.01%). Fruit EO was characterised by 6-epi-shyobunol (16.22%), d-limonene (15.35%), spathulenol (8.16%) and and 4-epi cubebol (7.84%).

No data was reported in the literature regarding the chemical composition of *S. molle* stem essential oil. Our results showed that the three studied essential oils were characterized by the predominance of sequiterpenes. This is in disagreement with previous works that showed that *S. molle* essential oils were characterized by the predominance of monterpene hydrocarbons [26], [10] and [25].

By referring to the literature Table 5, the investigated *S. molle* leaf and fruit essential oils showed a marked difference in composition, by comparison to essential oils from the same organs collected at Mograne (Zaghouan), in the North-Eastern of Tunisia [25]. The major components in fruit and leaf oils were limonene and β-phellandrene (35.9–65.4%), α-phellandrene (24.3–20.1%), myrcene (12.8–7.7%) and α-pinene (5.9–1.7%). Leaf essential oil from *S. molle* grown in the Évora region, in southeast Portugal contained mainly α-phellandrene (25.9%), limonene (11.7%), β-myrcene (11.1%), β-phellandrene (10.5%) and elemol (9.0%). While Fruit EO was characterised mainly by β-myrcene (51.3%), limonene (14.1%), α-phellandrene (14.0%) and β-phellandrene (11.0%) [10]. Furthermore, the Costa Rican *S. molle* leaf essential oil was found to be rich in α-pinene and β-pinene [12]. In a recent study, [27] founded that the major compounds of *S. molle* dried leaves EO from Brazil were cubenol (27.1%), caryophyllene oxide (15.3%) and spathulenol (12.4%), while in the fruit oil the main components were β-pinene (36.3%) α-pinene (20.3%), germacrene D (12.1%) and spathulenol (11.4%).

The qualitative and quantitative analysis showed variability in the essential oils extracted from *S. molle* leaves, stems and fruits. It is well known that such variations are due to several factors such as species, geographical origin, harvesting time, climatic and soil-growth conditions, plant part used and isolation method [28].

3.1.2. Various Extracts

Extraction yields of various *S. molle* extracts are presented in Table 2. Ethanolic extract had the highest yield (6.87%), followed by methanolic extract (6.35%), hexanic extract (2.72%) and finally ethyl acetate extract (2.25%). [8] used *S. molle* aerial parts, the yield of hexanic extract was 4.6%. Moreover, the yield of our *S. molle* ethanol fruits extract (6.87%) is less important than the *S. molle* ethanol fruits extract (10.82%) obtained by [29].

Table 2. Extraction yields (%) of essential oils and various fruits extracts of *S. molle*

Samples	Yield (%)
Leaf EO	1.86 ± 0.01^{a}
Stem EO	1.94 ± 0.03^{b}
Fruit EO	2.52 ± 0.03^{b}
Hexane	2.72 ± 0.00^{c}
Ethyl acetate	2.25 ± 0.04^{d}
Ethanol	6.87 ± 0.24^{e}
Methanol	6.35 ± 0.12^{f}

Values within rows with different superscripts (a–f) were significantly different ($p < 0.05$); ±: Standard deviation.

The chemical composition of the various *S. molle* fruits extracts was depicted in Table 3. For phenolic compounds, ethyl acetate extract was the richest (123.7 ± 1.7 mg GAE /g dw), followed by methanol (86.2 ± 2.3 mg GAE/g dw) and ethanol extracts (82.5 ± 1.6 mg GAE/g dw).The lowest value was 9.8 ± 0.4 mg GAE/g dw in hexane extract.

The amount of total tannins showed that it was concentrated in the hexanic extract (280.6 ± 10.5 mg CE/kg dw) followed by the ethyl acetate extract (85.2 ± 3.4 mg CE/kg dw). No tannins were found in the ethanol and methanol extracts.

Flavonoids were also detected in *S. molle* extracts. The results showed a strong variation with the solvent of extraction. The ethanol extract (74.6 ± 2.54 mg QE/g dw) being the richest. Methanol and ethyl acetate extracts showed the lowest value with 4.3 ± 0.15 and 1.7 ± 0.04 mg QE/g dw, respectively. Hexane extract contained no flavonoids. Flavonoids were presented in small amounts compared to the two families mentioned above.

Anthocyanins were also found, but in small quantities compared to the other families. The hexane extract contained the highest amount of total anthocyanins (3.37 ± 0.05 mg C3GE/g dw) and the ethanol extract was found to be the poorest with 0.45 ± 0.02 mg C3GE/g dw. Anthocyanins pigments were not detected in ethyl acetate and methanol extracts.

Table 3. Chemical composition of *S. molle* Fruit extracts

Extracts	Phenolics (GAE) [a]	Tannins (CE) [b]	Flavonoids (QE) [a]	Anthocyanins (C3GE) [a]
Hexane	9.8 ± 0.4^{a}	280.6 ± 10.5^{a}	nd^{a}	3.37 ± 0.05^{a}
Ethyl acetate	123.7 ± 1.7^{b}	85.2 ± 3.4^{b}	1.7 ± 0.04^{b}	nd^{b}
Ethanol	82.5 ± 1.6^{c}	nd^{c}	74.6 ± 2.54^{c}	0.45 ± 0.02^{c}
Methanol	86.2 ± 2.3^{d}	nd^{c}	4.3 ± 0.15^{d}	nd^{b}

[a]: mg/g dw; [b]: mg/kg dw; nd: not detected; Values within columns with different superscripts (a–d) were significantly different ($p < 0.05$); ±: Standard deviation

Little is known about the phenolic profile of the *S. molle* fruits extracts. [30] founded that the amount of total phenolics extracted with aqueous solutions from *S. molle* fruits was 7.6 mg of GAE/g dw. The phenolic compounds were mainly chlorogenic acid 0.19 ± 0.01 (mg/g dw), ellagic acid 0.124 ± 0.002 (mg/g dw) and quercetin dervatives 0.42 ± 0.06 (mg/g dw). Three anthocyanins: (cyanidin-3-galactoside, cyanidin-3-rutinoside and peonidin-3-glucoside) were detected in the fruits of *Schinus molle* var. *areira* by paper chromatography using standards and UV analysis [31]. In other investigations on *S. molle*, [32] and Ono [33] had identified the biflavonoids chamaejasmin, agathisflavone and tetrahydroamentoflavone as well as the dihydroflavonol engeletin and the flavonol quercetin-3-rhamnoside. Tannins content of *S. molle* has not been reported in the literature.

Our findings showed that *S. molle* is rich in phenolic components which are responsible for several therapeutic effects in particular for the antioxidant activity. They could trap free radicals and activate other antioxidants in the body. Phenolics could activate the natural anticancer mechanism defense. Tanins (hydrolysable and condensed) not only increase the resistance against several cancers, they even have anticancer activity that reduces, and sometimes even totally eradicate tumors.

3.2. Antioxidant activity

Essential oils and different extracts were individually assessed for antioxidant activity using two tests: ABTS and DPPH free radical scavenging. Results are summarised in Table 4.

3.2.1. Essential oils

The results showed a promising antioxidant activity of essential oils from leaves, stems and fruits. Statistically significant differences were observed for essential oils. In the ABTS radical scavenging assay, essential oil from fruits of *S. molle* showed the best antioxidant activity with an IC_{50} of 32.6 ± 0.6 mg/Lwhich was more marked than essential oils from leaves and stems (IC_{50} of 232.8 ± 8.0 mg/Land 223.0 ± 6.0, respectively). On the other hand, the three essential oils seemed to possess low free radical scavenging activity in the DPPH assay (IC_{50} > 10 000 mg/L for fruits essential oil and IC_{50} of 3586.9 ± 119.0 and 3559.2 ± 122.0 mg/L, respectively for leaves and stems essential oils). These results were compared to Ascorbic acid used as positive control giving an ABTS and DPPH scavenging activities of 1.9 ± 0.1and 4.4 ± 0.2 mg/L, respectively.

Table 4. Antioxidant activity of *S. molle* essential oils and various extracts using ABTS and DPPH radical-scavenging assays

Samples	ABTS IC_{50} (mg/L)	DPPH IC_{50} (mg/L)
Leaves EO	232.8 ± 8.0^{a}	3586.9 ± 119.0^{a}
Stems EO	223.0 ± 6.0^{b}	3559.2 ± 122.0^{b}
Fruits EO	32.6 ± 0.6^{c}	> 10 000^{c}
Hexane	35.6 ± 0.8^{d}	539.4 ± 13.3^{d}
Ethyl acetate	19.7 ± 0.7^{e}	30.7 ± 0.9^{e}
Ethanol	8.7 ± 0.3^{f}	12.5 ± 0.4^{f}
Methanol	7.1 ± 0.2^{g}	9.2 ± 0.3^{g}
Ascorbic acid	1.9 ± 0.1^{h}	4.4 ± 0.2^{h}

Relative standard deviation < 5%.Values within columns with different superscripts (a–h) were significantly different ($P < 0.05$). ±: standard deviation

The antioxidant activity of *S. molle* essential oils was more important according to the ABTS assay, compared to the DPPH assay. This activity is significant, especially since these essential oils are composed mainly of monoterpenes and sesquiterpenes which have a moderate activity compared to phenolics and Ascorbic acid.

Our findings were in agreement with previous studies. In fact, [11] found that the fruits essential oil of *S. molle* have shown a low DPPH antioxidant activity with an IC_{50} value of 3697.6 ± 104.0 mg/L. With regard to the ABTS test, the same authors reported that IC_{50} value of the fruit essential oil from *S. molle* was 270 ± 12.0 mg/L. However, our *S. molle* fruit essential oil showed an IC_{50} of 32.6 ± 0.6 mg/L. In another study, [12] measured the antioxidant activity of the essential oil of fresh leaves of *S. molle* using DPPH assay and they obtained an IC_{50} value of 36.3 µg/mL. They have considered it as a weak free radical scavenging activity when compared to the values obtained for other plant EOs. Moreover, [10] have demonstrated that the EOs from leaves and fruits of *Schinus molle* from

Portugal (16 mg/mL) promoted a free radical scavenging effect by the DPPH assay of 4.8% and 5.5% respectively. But this was much lower than that which was recorded for ascorbic acid (14%).

The major compounds detected in our leaf EO were β-eudesmol (14.82%), elemol (13.71%), α-eudesmol (12.76%), d-limonene (9.25%) and spathulenol (7.21%). Stem EO was characterised mainly by elemol (20.7%), 6-epi-shyobunol (20.36%), d-limonene (16.19%) and α-eudesmol (7.01%). Fruit EO was characterised by 6-epi-shyobunol (16.22%), d-limonene (15.35%), spathulenol (8.16%), and α-eudesmol (7.84%). Our results are consistent with those of [34] and [35] who reported the antioxidant activity of elemol, α and β-pinene, and limonene.

Furthermore our *S. molle* EOs revealed various compounds such as α-pinene (0.68%, 0.8% and 1.6% respectively in leaf, stem and fruit EOs); l-phellandrene (2.32%, 4.1% and 2.18% respectively in leaf, stem and fruit EOs) and p-cymene (1.94%, 2.18% and 2.54% respectively in leaf, stem and fruit EOs). These findings are in accordance with those of [36] who founded α and β- pinene, p-cymene and α and β-phellandrene in *S. molle* EO that presented antioxidant activity.

3.2.2. Various extracts

For the DPPH assay (Table 4), the methanol extract possessed the most important activity (IC_{50} = 9.2 ± 0.3 mg/L), followed by the ethanol (IC_{50} = 12.5 ± 0.4 mg/L), ethyl acetate (IC_{50} = 30.7 ± 0.9 mg/L) and hexane (IC_{50} = 539.4 ± 13.3 mg/L) extracts. Ascorbic acid was used as positive control and exhibited an IC_{50} equal to 4.4 ± 0.2 mg/L.

Table 5. Origin, Organ type and major compounds of some EOs of *S. molle* previously reported in the literature

Origine	Organ Type	Major compounds	References
Brazil	Leaves and fruits	**Leaves** cubenol (27.1%) caryophyllene oxyde (15.3%) spathulenol (12.4%) **Fruits** β-pinene (36.3%) α-pinene (20.3%) germacrene D (12.1%) spathulenol (11.4%)	[27]
Portugal	Leaves and fruits	**Leaves** α-phellandrene (25.9%) limonene (11.7%) β-myrcene (11.1%) β-phellandrene (10.5%) elemol (9%) **Fruits** β-myrcene (51.3%) limonene (14.1%) α-phellandrene (14%) β-phellandrene (11%)	[10]
Argentina	Leaves	elemol (12.7%) α-pinene (11.7%) β-pinene (9.3%) limonene (8.3%) α-phellandrene (8.2%)	[36]
Brazil	Leaves	α-pinene (35.28%) limonene (32.21%) β-pinene (15.42%)	[38]
Tunisia	Leaves	α-phellandrene (22.16%) α-phellandrene (6.49%) α-pinene (5.20%)	[39]
	Leaves and fruits	**Leaves** limonene and β-phellandrene (65.4%) α-phellandrene (20.1%) myrcene (7.7%) α-pinene (1.7%) **Fruits** limonene and β-phellandrene (35.9%) α-phellandrene (24.3%) myrcene (12.8%) α-pinene (5.9%)	[25]

Concerning the ABTS assay, the methanolic extract presented the most important antioxidant activity (IC_{50} = 7.1 ± 0.2 mg/L), followed by the ethanol (IC_{50} = 8.7 ± 0.3 mg/L), ethyl acetate (IC_{50} = 19.7 ± 0.7 mg/L) and hexane extracts (IC_{50} = 35.6 ± 0.8 mg/L). Ascorbic acid was used as positive control and exhibited an IC_{50} equal to 1.9 ± 0.1 mg/L. We can deduce also that ABTS assay presents more activity when we compare the results of ABTS assay to those of the DPPH one.

We noted that when polarity increases, the antioxidant activity of *S. molle* fruits extracts increased. Thus, the best antioxidant activities correspond to the polar fractions (methanol and ethanol). The IC50 values of our extracts are encouraging enough to prompt us to try to identify the molecules responsible for this activity. According to the literature, [30] found that the antioxidant activity of the aqueous fruit extract of *S. molle* using the DPPH assay was about 45%. This result may be due to the content of total phenolics which was about 7.6 mg of GAE/g. Furthermore, the leaf extract of *S. molle* prepared with 30 mL of 0.1% HCl in a solvent mixture (MeOH/water, 80:20, v/v) showed an antioxydant activity (µmol TE/g DW) of 298.2 ± 14.2, 26.9 ± 5.9 and 620.9 ± 7.5 respectively in DPPH, ABTS and ORAC assays (Chirinos et al. 2013).

CONCLUSION

Essential oils from leaves, stems and fruits of *S. molle* and various extracts were identified and investigated for their antioxidant activity. Total phenols, flavonoids, tannins and anthocyanins contents of various extracts were determined. Our data revealed that *S. molle* is rich in phenolic compounds and that the essential oils and various extracts induced promising *in vitro* antioxidant activity. These research findings suggest that *Schinus molle* essential oils and extracts could be considered as a potential alternative of natural antioxidants or flavouring additives which could be used in the food industry along with their potential applications in the pharmaceutical industry for the prevention and/or the treatment strategies of some diseases caused by free radicals. Further researches on this underutilized specie are required to target specifically the most interesting molecules responsible of the biological properties.

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REFERENCES

[1] FA Barkley. A study of *Schinus* L. Lilloa Revista de Botanica, Tomo 28, Univ. Nac del Tucuman, Argentina **1957**.
[2] JA Duke. Handbook of medicinal herbs, Boca Raton, Fla.: CRC Press **1985**.
[3] MJ Ruffa; G Ferraro; ML Wagner; ML Calcagno; RH Campos; L Cavallaro, *J. Ethnopharmacol.,* **2002**, 79, 335-339.
[4] MD Barrachina; R Bello; MA Martinezcuesta; E Primoyufera; J Espulgues, *Phytother. Res*., **1997**, 11, 317-9.
[5] V Benzi; N Stefanazzi; A Ferrero, *Chilean J. of Agricultural Research*., **2009**, 69(2), 154-159.
[6] AA Ferrero; JO Werdin Gonzalez; C Sanchez Chopa, *Fitoterapia*, **2006**, 77, 381-83.
[7] AA Ferrero; Sanchez Chopa; JO Werdin Gonzalez; RA Alzogaray, *Fitoterapia*, **2007b**, 78, 311-314.
[8] DG Machado; MP Kaster; RW Binfaré; M Dias; ARS Santos; MG Pizzolatti; IMC Brighente; ALS Rodrigues, *Prog. Neuro-Psych. Biol. Psychiat*., **2007**, 31, 421-428.
[9] C Perez; C Anesini, *Fitoterapia*, **1994**, 65, 169-172.
[10] MDR Martins; S Arantes; F Candeias; MT Tinoco; J Cruz-Morais, *J. of Ethnopharmacology*, **2014**, 151, 485-492.
[11] H Bendaoud; M Romdhane; JP Souchard; S Cazaux; J Bouajila, *J. of Food Science*, **2010**, 75(6).
[12] C Diaz; S Quesada; O Brenes; G Aguila; JF Ciccio, *Nat. Prod. Res*., **2008**, 22, 1521-1534.
[13] EA Hayouni; I Chraief; M Abedrabba; M Bouix; JY Leveau; M Hammami; M Hamdi, *Int. J. Food Microbiol*., **2008**, 125, 242-251.
[14] K Hosni; M Jemlia; S Dziri; Y M'rabet; A Ennigrou; A Sghaier; H Casabianca; E Vulliet; N Ben Brahim; H Sebei, *Industrial Crops and Products*, **2011**, 34, 1622-1628.
[15] R Salazar-Aranda; LA Pérez-López; J López-Arroyo; BA Alanís-Garza; NW Torres, *Alternat. Med*., **2011**, 6.
[16] A Murray; M Gurovic; S Rodriguez; M Murray; A Ferrero, *Speg. Nat. Prod. Commun*., **2009**, 4, 873-876.
[17] A Dikshit; A Naqvi; A Husain, *Applied and environmental microbiology*, **1986**, 1085-1088.
[18] RP Adams, Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy, Allured Publishing Corporation, Carol Stream, III, USA, **2007**.
[19] VL Singleton; R Orthofer; RM Lamuela-Raventos, *Methods in Enzymology*, **1999**, 299, 152-178.
[20] M Naczk; R Amarowicz; D Pink; F Shahidi, *J. Agric. Food Chem*., **2000**, 48, 1758-1762.

[21] A Arvouet-Grand; B Vennat; P Pourrat A Legret, *J. Pharm. Belg*., **1994**, 49, 462-468.
[22] GW Cheng; PJ Breen, *J. Am. Soc. Hortic. Sci*., **1991**, 117, 946-950.
[23] MS Blois, *Nature*, **1958**, 181, 1199-200.
[24] R Re; N Pellegrini; A Proteggente; A Pannala; M Yang ; C Rice-Evans, *Free Radical Biol. Med*., **1999**, 26, 1231-7.
[25] N Zahed; K Hosni; NB Brahim; M Kallel; H Sebei, *Acta Physiol. Plant.* **2010**, 32, 1221-1227.
[26] E Abdel-Sattar; AA Zaitoun; MA Farag; SH El Gayed; Fathalla MH Harraz, *Natural Product Research,* **2010**, 24, No. 3, 15, 226-23.
[27] ADS Cavalcanti; MS Alves; LC Paulo da Silva; DS Patrocínio; MN Sanches; DS de Almeida Chaves; MA Alves de Souza, *Revista Brasileira de Farmacognosia*, **2015**, 25, 356-362.
[28] MH Alma; S Nitz; H Kollmannsberger; M Digrak; F Tuncay Efe; N Yilmaz, *J. Agric. Food Chem*., **2004**, 52, 3911-3914.
[29] C Bras; S Domínguez; S Codón; A Minetti ; A Ferrero, *J. Ethnopharmacol*., **2010**, 132, 321-327.
[30] LG Ranilla; YI Kwon; E Apostolidis; K Shetty, *Bioresource Technology*, **2010**, 101 4676-4689.
[31] AU Rahman; MA Tomas; MA Frontera, *An. Asoc. Quim. Argent*., **1974**, 62, 169.
[32] Z Yueqin; MC Recio; S Manez; RM Giner; M Cerda Nicolas; JL Rios. *Planta Med*., **2003**, 69, 893.
[33] M Ono; M Yamashita; K Mori; C Masuoka; M Eto; J Kinjo; T Ikeda; H Yoshimitsu; T Nohara, *Food Sci. Technol. Res*., **2008***,* 14, 499.
[34] SA Selim, *Grasas y* Aceites, **2011**, 62(1), 55-61.
[35] S Malhotra; S Suri; R Tuli, *Planta Med*., **2009**, 75(1), 62-64.
[36] RH Olmedo; V Nepote; NR Grosso, *J. Am. Oil Chem. Soc*., **2012**, 89, 2195-2205.
[37] R Chirinos; R Pedreschib; H Rogezc; Y Larondelled; D Camposa, *Industrial Crops and Products*, **2013**, 47, 145-152.
[38] A Pawlowski; E Kaltchuk-Santos; CA Zini; EB Caramao; GLG Soares, *South African J. of Botany,* **2012**, 80, 96-103.
[38] A Ennigrou; K Hosni; H Casabianca; E Vulliet; S Smiti, *Foodbalt*, **2011**, 90-92.