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ORIGINAL ARTICLE

Comparative evaluation of antioxidant and insecticidal properties of essential oils from five Moroccan aromatic herbs

Ayoub Kasrati · Chaima Alaoui Jamali · Khalid Bekkouche · Hans Wohlmuth · David Leach · Abdelaziz Abbad

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Abstract This study describes the antioxidant and insecticidal activities of essential oils (EOs) of *Mentha suaveolens* subsp. *timija*, *Thymus satureioides*, *Achillea ageratum*, *Cotula cinerea* and *Salvia officinalis* widely used in Morocco as flavorings, food additives and preservatives. Sixty seven components were identified accounting for more than 95.0 % of the total oils. *M. suaveolens* subsp. *timija* oil had as main components menthone and pulegone. *A. ageratum* oil was particularly rich in artemisyl acetate and yomogi alcohol. The essential oil of *T. satureioides* was characterized by high contents of carvacrol and borneol. *C. cinerea* oil contained *trans*-thujone and *cis*-verbenyl acetate as major constituents, whereas *S. officinalis* oil was characterized by *trans*-thujone and camphor. Antioxidant activities were examined by means of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), reducing power, β-carotene/linoleic acid bleaching and ABTS radical tests. In all assays, the highest antioxidant potency was observed in *T. satureioides* EO with IC_{50} values ranging from 0.15±0.36 μg mL^{-1} to 0.23±0.67 μg mL^{-1} across the four assays. The in vitro evaluation of the insecticidal activity showed that *M. suaveolens* subsp. *timija* EO present the highest insecticidal efficiency against adults of *Tribolium castaneum* with LD_{50} and LD_{90} values of 0.17 μL cm^{-2} and 0.26 μL cm^{-2}, respectively and LT_{50}, LT_{90} values ranged from 44.19 h to 2.98 h and 98.14 h to 6.02 h, respectively. Our data support the possible use of *T. satureioides* oil as potential antioxidant agent, while *M. suaveolens* subsp. *timija* oil can be developed as a new natural bio-insecticide.

Keywords *Mentha suaveolens* subsp. *timija* · *Thymus satureioides* · *Achillea ageratum* · *Cotula cinerea* · *Salvia officinalis* · Essential oils

A. Kasrati · C. Alaoui Jamali · K. Bekkouche · A. Abbad (✉)
Laboratory of Biotechnology, Protection and Valorization of Plant Resources, Faculty of Science Semlalia, Cadi Ayyad University, B.P. 2390, 40000 Marrakech, Morocco (URAC 35)
e-mail: abbad.abdelaziz@gmail.com

A. Abbad
e-mail: abbad@uca.ma

H. Wohlmuth · D. Leach
Southern Cross Plant Science, Southern Cross University, Lismore, NSW 2480, Australia

H. Wohlmuth
Integria Healthcare, Gallans Rd, Ballina, NSW 2478, Australia

Introduction

In order to protect food products against deterioration caused by oxidation and attack of insect pests during production, storage and marketing, many synthetic chemicals have widespread applications. Recent reports reveal that these synthetic chemicals may be implicated in many human health problems and may also lead to unintended side effects such as environmental pollution and pest resistance (Kumar et al. 2011; Sivasothy et al. 2011). For these reasons, the use of plant-based natural compounds instead of synthetic additives might be desirable, and the isolation and development of new natural bioactive compounds are of considerable interest. Bioactive natural compounds are considered to be attractive due to their low cost, availability in large quantity from the raw material, biodegradability and safety for human health and environment (Kong et al. 2010). Amongst plant-based natural products, essential oils (EOs) have received significant attention from the scientific community because of their wide acceptance by consumers and their exploitation for potential multi-purpose functional use (Bakkali et al. 2008). Extracted from herbs and spices through distillation, EOs, also known as volatile oils,

are considered a good source of natural bioactive compounds which, when used as food additives, increase the shelf-life of foods by minimizing rancidity and retarding the formation of toxic oxidation products (Kelen and Tepe 2008). In fact, EOs and their constituents can act as antioxidant due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, or by decomposing peroxides, quenching singlet and triplet oxygen, or by chelating the metal ions (Sarikurkcu et al. 2010). Furthermore, EOs are also widely recognized for their acute toxicity, repellency, antifeedant effect and fumigant toxicity against many stored-product insects (Tozlu et al. 2011). In fact, EOs rich in monoterpenes were reported to be effective as insecticidal agents by inhibition of respiration, reduction in growth and fecundity and by inhibiting acetylcholinesterase activity in the nervous system of insect pests (Sertkaya et al. 2010).

Our previous reports on EOs obtained from plants of the Moroccan flora have showed promising and interesting results regarding some of their biological activities (El Bouzidi et al. 2011a,b; Kasrati et al. 2013). As a continuation of the previous work of our research group, the aim of the present study was to compare some Moroccan plants EOs namely, *M. suaveolens* subsp. *timija* Briq., *T. satureioides* Coss., *A. ageratum* L., *C. cinerea* Del. and *S. officinalis* L., with respect to their antioxidant activity as well as their toxicity against *T. castaneum* Herbst, one of the most widespread and destructive stored-product pests.

Materials and methods

Plant materials

The aerial parts of *A. ageratum* L. (Asteraceae), *S. officinalis* L. (Lamiaceae), *T. satureioides* Coss. (Lamiaceae), *Mentha suaveolens* L. subsp. *timija* (Briq.) Harley (Lamiaceae) and *Cotula cinerea* Del. (Asteraceae) were harvested from different locations in south and south-west Morocco (Table 1). Selection of plants was made on the basis of ethnobotanical literature and traditional use in medicine and as food additives (Bellakhder 1997). As cited in the literature, *A. ageratum*, *T. satureioides* and *C. cinerea* were collected at full flowering phase, while *M. suaveolens* subsp. *timija* and *S. officinalis* were harvested at vegetative stage. The identification was done by one of the authors (A. Abbad) and voucher specimens were deposited at the Laboratory of Biotechnology, Protection and Valorization of Plant Resources, Faculty of Science, Semlalia, Cadi Ayyad University, Marrakech, Morocco.

Extraction of essential oils

Plant materials were dried in the shade at room temperature (≈ 25 °C) and subjected to hydro-distillation, using a Clevenger-type apparatus for 3 h until total recovery of oil. The EOs obtained were dried over anhydrous sodium sulfate and stored at 4 °C in the dark until gas chromatographic analysis and biological study.

Gas chromatography/mass spectrometry (GC/MS) analyses

The analytical GC/MS system used was an Agilent GC-MSD system (Agilent Technologies 6890/5973) with helium (high purity) as the carrier gas at a constant linear velocity of 37 cm s^{-1}. The transfer, source and quadrupole temperatures were 280 °C, 230 °C and 150 °C respectively, operating at 70 eV ionisation energy and scanning the m/z range 41–450. The column used was an Agilent DB5 ms capillary column (30.0 m x 0.25 mm ID x 0.25 μm film thickness; Model Number: 122–5532) programmed from 60 °C to 246 °C at 3 °C min^{-1}. EO samples (60 μL) were diluted with acetone (2 mL). The injection volume was 1.0 μL, the split ratio was 1:50 and the injector temperature was 260 °C. Identification of the individual components was based on; (i) comparison with the mass spectra of authentic reference compounds where possible and by reference to WILEY275, NBS75K, and Adams terpene library (Adams 2007); (ii) comparison of their retention indices (RI) on a DB5 (apolar, 5 % phenyl polysilphenylene-siloxane), calculated relative to the retention times of a series of C-9 to C-24 *n*-alkanes, with linear interpolation, with those of authentic compounds or literature data. For semi-quantitative purposes, the normalized peak area of each compound was used without any correction factors to establish abundances.

Antioxidant activity

DPPH free radical-scavenging activity

The Stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the EOs (Sahin et al. 2004). Fifty microlitres of various concentrations of the samples in methanol (EOs and control substance) were added to 2 mL of a 60 μM methanol solution of DPPH. After 20 min at room temperature, the absorbance was recorded at 517 nm. Absorption of a blank sample containing the same amount of methanol and DPPH solution acted as the negative control. Butylated hydroxytoluene (BHT) and quercetin were used as positive controls. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula:

$$\% \text{ Inhibition} = (\text{Ab}-\text{Aa}/\text{Ab}) \times 100$$

Where Ab is the absorbance of the blank sample and Aa is the absorbance of the test sample. The sample concentration

Table 1 Locality, harvesting time and essential oil yield for the five Moroccan aromatic plants studied

Species	Species code	Local name	Harvesting place	Harvesting time	Voucher specimen	Latitude/Longitude	Oil Yield (% (v/w))
M. suaveolens subsp. *timija*	MS	*Timijja*	Ourika	February 2010	MST05	31°23′N/7°42′W	0.88±0.12
S .officinalis	SO	*Salmiya*	Ourika	April 2010	SO046	31°20′N/7°45′W	2.13±0.08
A. ageratum	AA	*Terrahla*	Demnate	February 2010	ACHAG045	31°43′N/06°58′W	2.04±0.14
T. satureioides	TS	*Za-itra*	Ourika	June 2011	TS077	31°14′ N/07° 41′W	2.17±0.03
C. cinerea	CC	*al-Gertoufa*	Zagora	March 2009	COTCIN03	30°20′N/05°52′W	0.93±0.01

providing 50 % inhibition (IC_{50}) was calculated by plotting the inhibition percentages against the concentrations of the sample.

Reducing power determination

According to the method of Oyaizu (1986) the different oils and control substance were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1 %). The mixture was then incubated at 50 °C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10 %) was added to the mixture, which was then centrifuged for 10 min at 3,000 rpm. Finally, the upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1 %), and the absorbance was measured at 700 nm in a spectrophotometer. The sample concentration providing 0.5 of absorbance (IC_{50}) was calculated by plotting absorbance at 700 nm against the corresponding sample concentration.

β-Carotene/linoleic acid bleaching assay

As described by Miraliakbari and Shahidi (2008) a mixture of β-carotene and linoleic acid was prepared by adding together 0.5 mg β-carotene in 1 mL chloroform (HPLC grade), 25 μL linoleic acid and 200 mg Tween 40. The chloroform was then completely evaporated under vacuum and 100 mL of oxygenated distilled water was subsequently added to the residue and mixed to form a clear yellowish emulsion. 350 μL of various concentrations of the sample (EOs, quercetin and BHT) were added to 2.5 mL of the above emulsion in test tubes and mixed. The test tubes were incubated in a water bath at 50 °C for 2 h together with a negative control (blank) containing methanol instead of sample. The absorbance values were measured at 470 nm. Antioxidant activities (percentage inhibition, I%) of the samples were calculated using the following equation:

$$I\,\% = \left(A_{\beta\text{-carotene after 2 h assay}}/A_{\text{initial}\,\beta\text{-carotene}}\right) \times 100$$

Where $A_{\beta\text{-carotene after 2 h assay}}$ is the absorbance values of β-carotene after 2 h assay remaining in the samples and $A_{\text{initial }\beta\text{-carotene}}$ is the absorbance value of β-carotene at the beginning of the experiment. The activity was calculated as 50 % inhibition concentration (IC_{50}).

ABTS free radical scavenging assay

The ABTS method is based on the reduction of the green ABTS radical cation (7.00 mmol L^{-1}) that was obtained by its oxidation with equal volume of potassium persulphate (2.45 mmol L^{-1}), (Scalzo et al. 2005) for 12–16 h at room temperature in the dark. On the day of analysis the $ABTS^+$ solution was diluted with methanol to an absorbance of 0.7 (±0.02) at 734 nm. To determine the scavenging activity, 1 ml of diluted $ABTS^+$ solution was added to 100 μl of oil and MeOH extract (or water for the control), and the absorbance at 734 nm was measured 6 min after the initial mixing, using methanol as the blank. The percentage of inhibition was calculated by the equation:

$$\%\,\text{Inhibition} = (\text{Ab}-\text{Aa}/\text{Ab}) \times 100$$

Where Ab is the absorption of the blank sample and Aa is the absorption of the tested oils. The sample concentration providing 50 % inhibition (IC_{50}) was calculated by plotting inhibition percentages against concentrations of the sample. The test was carried out in triplicate and IC_{50} values were reported as means±SD.

Insecticidal activity

Insect cultures

Colonies of the red flour beetle, *T. castaneum* (Coleoptera: Tenebrionidae), were maintained in the laboratory without exposure to any insecticide. They were reared in glass containers (16 cm diameter×22 cm height) covered by a fine mesh cloth for ventilation. Each container contained a mixture of wheat flour, wheat germ, and yeast extract (13:6:1 w/w/w). The cultures were maintained in a growth chamber at 26± 1 °C, with a relative humidity (RH) of 70–85 % and 16:8 h light: dark photoperiod. Only adults were used for the test.

Contact toxicity bioassay

The insecticidal activity of plants EOs against *T. castaneum* adults was determined by the contact toxicity using filter paper discs (Whatman No. 1, 9 cm diameter). Oils were dissolved in acetone at concentrations of 0.09, 0.16, 0.24 and 0.31 μL cm^{-2} for *M. suaveolens* subsp. *timija* and *T. satureoiedes* and 0.39, 0.78, 1.17 and 1.57 μL cm^{-2} for *A. ageratum*, *S. officinalis* and *C. cinerea*. Several preliminary tests were conducted to select the doses to be used for each EO. 1 mL of each solution was dispensed on the surface of the paper that was then placed in glass petri dishes. Controls were treated with acetone. After 10 min, once the solvent had been evaporated, 10 unsexed adults (7–14 days) were deposited into each dish and stored in darkness at 26±1 °C and 70–85 % RH. Three replicates were used for each treatment, repeating each assay twice. Mortality was recorded after 2, 4, 6, 8, 24 and 48 h. Insects were considered dead when no leg or antennal movements were recorded. Bioassays were designed to assess respectively median lethal concentration (LD_{50} and LD_{90} values) (doses that kill 50 % and 90 % of the exposed insects) and median lethal time (LT_{50} and LT_{90} values) (times required to kill 50 % and 90 % of exposed insects).

Data analysis

Probit analysis (Finney 1971) was conducted to estimate lethal doses (LD_{50} and LD_{90}) and lethal times (LT_{50} and LT_{90}) with their 95 % confidence interval by SPSS 12.0 Statistical Software; LD and LT values were considered significantly different when their respective 95 % confidence interval did not overlap.

Results and discussion

Essential oils compositions

The yield of EOs, based on the dry weight of the plants, was highest (2 %) for *S. officinalis*, *T. satureioides* and *A. ageratum*. *M. suaveolens* subsp. *timija* and *C. cinerea* oil yields were significantly lower at less than 1 % (Table 1).

Between 9 and 31 constituents were identified in the EOs studied, accounting for 95.0 to 99.9 % of the total oil composition (Table 2). The EOs were shown to be characterized by a high percentage of the monoterpenoid fraction with oxygenated monoterpenes as a dominant subclass in *T. satureioides* (61.1 %), *M. suaveolens* subsp. *timija* (86.6 %), *C. cinerea* (82.3 %), *A. ageratum* (95.7 %) and *S. officinalis* (50.6 %) EOs.

A report on *M. suaveolens* subsp. *timija* EO was recently published by our research group (Kasrati et al. 2013). Menthone (39.4 %), pulegone (34.3 %) and isomenthone

Table 2 Chemical compounds of essential oils obtained from aerial parts of the species studied (%)

Compound[a]	RI[b]	MS[c]	SO	AA	TS	CC
Santolinatriene	903	–[d]	–	–	–	7.2
Tricyclene	925	–	–	–	0.3	–
α-Thujene	928	–	–	–	1.1	0.5
α-Pinene	936	0.6	3.5	–	4.3	0.8
Fenchene	948	–	–	–	–	0.1
Camphene	951	0.3	2.0	–	7.0	1.6
β-Pinene	960	0.5	1.0	–	0.9	0.5
Sabinene	989	0.4	–	–	–	1.6
Myrcene	990	0.1	1.3	–	1.0	–
Yomogi alcohol	995	–	–	12.4	–	–
α-Terpinene	1,018	–	0.2	–	1.0	0.3
p-Cymene	1,025	–	0.4	–	7.1	0.9
Limonene	1,030	0.6	0.9	–	0.8	0.3
Santolina alcohol	1,036	–	–	6.1	–	–
Z-β-Ocimene	1,042	0.4	–	–	–	–
γ-Terpinene	1,059	–	0.4	–	6.0	0.7
1,8-Cineole	1,060	0.5	10.9	–	–	8.2
Sabinene hydrate	1,067	–	–	–	0.6	–
p-Mentha-3,8-diene	1,073	–	–	–	–	0.1
Artemesia alcohol	1,081	–	–	7.1	–	–
p-Mentha-2,4(8)-diene	1,087	–	–	–	–	0.1
Terpinolene	1,088	–	0.2	–	–	–
Linalool	1,098	0.2	0.1	–	5.7	–
Isoamyl 2-methylbutyrate	1,100	–	–	–	–	0.2
cis-Thujone	1,108	–	2.0	–	–	0.3
trans-Thujone	1,121	–	20.3	–	–	41.4
Thujanol	1,133	–	0.3	–	–	–
Camphor	1,143	–	15.5	–	0.3	5.5
Artemisyl acetate	1,167	–	–	70.1	–	–
Borneol	1,169	1.4	0.5	–	17 .5	–
cis-Isopulegone	1,177	0.5	–	–	–	–
Terpinen-4-ol	1,179	–	–	–	0.9	0.1
Menthone	1,187	39.4	–	–	–	–
α-Terpineol	1,192	–	0.2	–	4.9	–
Myrtenol	1,194	–	0.1	–	–	–
Isomenthone	1,197	7.8	–	–	–	–
cis-Dihydrocarvone	1,198	–	–	–	0.2	–
Hexyl 2-methylbutyrate	1,232	–	–	–	–	0.2
Pulegone	1,242	34.3	–	–	–	–
Carvacrol methyl ether	1,244	–	–	–	2.5	–
Piperitone	1,255	0.6	–	–	–	–
cis-Verbenyl acetate	1,268	–	–	–	–	24.7
Bornyl acetate	1,288	–	0.5	–	–	1.5
Thymol	1,290	–	–	–	5.0	–
Carvacrol	1,302	–	–	–	23.7	–
Piperitenone	1,342	1.8	–[d]	–	–	–
Silphiperfol-6-ene	1,344	–	–	0.6	–	–
α-Copaene	1,381	–	–	–	0.4	–

Table 2 (continued)

Compound[a]	RI[b]	MS[c]	SO	AA	TS	CC
β-Copaene	1,390	0.3	–	–	–	–
β-Elemene	1,417	0.2	–	0.2	–	–
β-Bourbonene	1,421	0.2	–	–	–	–
(E)-Caryophyllene	1,426	3.0	11.8	–	5.6	–
cis-Jasmone	1,432	0.1	–	–	–	–
Aromadendrene	1,439	–	0.5	–	–	–
cis-β-Guaiene	1,445	0.2	–	–	–	–
trans-β-Farnesene	1,453	–	–	–	–	0.3
α-Humulene	1,460	0.3	7.6	–	0.2	–
Germacrene D	1,487	4.1	0.1	–	0.5	–
Verbenyl propionate	1,488	–	–	0.2	–	–
Viridiflorene	1,493	–	0.3	–	–	–
Alloaromadendrene	1,506	–	0.1	–	–	–
γ-Cadinene	1,519	0.1	0.1	–	0.3	–
Presilphiperfolan-8-ol	1,522	–	–	0.2	–	–
δ-Cadinene	1,524	–	0.3	–	–	–
Spathulenol	1,534	0.1	0.1	–	–	–
Bicyclogermacrene	1,541	0.7	–	–	–	–
γ-Amorphene	1,565	0.1	–	–	–	–
β-Dihydoagarofuran	1,586	0.1	–	–	–	–
Caryophyllene oxide	1,589	–	1.0	–	0.3	–
Viridiflorol	1,590	–	7.5	–	–	–
α-Cadinol	1,645	–	–	–	0.3	–
Valerianol	1,705	0.1	–	–	–	–
Manool	2,056	–	5.3	–	–	–
Monoterpene hydrocarbons		2.9	9.7	0.0	29.7	14.5
Oxygenated monoterpenes		86.6	50.6	95.7	61.1	82.3
Sesquiterpene hydrocarbons		9.2	20.8	1.0	7.0	0.3
Oxygenated sesquiterpenes		0.3	8.6	0.2	0.6	0.0
Oxygenated diterpenes		–	5.3	–	–	–
Total (%)		99.0	95.0	96.9	98.4	97.1

[a] Compounds listed in order of elution

[b] RI (retention indices) measured relative to *n*-alkanes (C-9 to C-24) on a non polar DB-5 column

tr trace (<0.1 %)

[c] Details concerning the codes of the species are given in Table 1; [d] not detected.

(7.8 %) were the most abundant individual compounds. The high content of these oxygenated monoterpenes found in the *timija* subspecies was also reported in some other mint species (Mkaddem et al. 2007; Santoro et al. 2011).

The EO compositions of *A. ageratum* and *C. cinerea* have previously been reported by our research group (El Bouzidi et al. 2011a,b). As shown in Table 2, the predominant constituents in *A. ageratum* EO were artemisyl acetate (70.1 %), yomogi alcohol (12.4 %), artemesia alcohol (7.1 %) and santolina alcohol (6.1 %). The EO isolated from the aerial part of *C. cinerea* consisted of mainly *trans*-thujone (41.4 %), *cis*-verbenyl acetate (24.7 %) and 1,8-cineole (8.2 %). This composition is qualitatively similar to that of *C. cinerea* plants of Egyptian origin, but with important quantitative differences (Fournier et al. 1989).

Turning on attention to *S. officinalis* oil, the most abundant compounds were found to be *trans*-thujone (20.3 %), camphor (15.5 %), 1,8-cineole (10.9 %) and (*E*)-caryophyllene (11.8 %). These compounds are frequently found in other *S. officinalis* EOs, but with different percentage composition depending on geographic origin, environmental factors, extraction methods, phenological stage, sampling techniques and genetic differences (Raal et al. 2007). The EO isolated from the aerial parts of *T. satureioides* was characterized by carvacrol (23.7 %), borneol (17.5 %), camphene (7.0 %), γ-terpinene (6.0 %) and *p*-cymene (7.1 %) as major components (Table 2). This result concurs with what has been previously reported by Alaoui Jamali et al. (2012) and El Bouzidi et al. (2013).

Antioxidant activity

As shown in Table 3, the best contribution in DPPH, reducing power and ABTS assays was achieved by *T. satureioides* with IC_{50} values of 0.21±1.17 mg mL^{-1}, 0.23±0.67 mg mL^{-1} and 0.15±0.36 mg mL^{-1}, respectively. The lowest activities were observed for oil extracted from *A. ageratum* with IC_{50} values of 7.49±0.41 mg mL^{-1}, 40.00±0.54 mg mL^{-1} and 1.19± 1.24 mg mL^{-1}, respectively. Similarly, *T. satureioides* oil exhibited the best performance in β-carotene/linoleic acid bleaching assay (IC_{50}=0.21±1.74 mg mL^{-1}), while *M. suaveolens* subsp. *timija* oil had the weakest potency (IC_{50}=1.40±0.05 mg mL^{-1}). For the four test systems applied, EOs from *C. cinerea* and *S. officinalis* showed in general moderate to weak activity. Although, for all assays, EO samples expressed less potency than the reference antioxidants butylated hydroxytoluene (BHT) and quercetin (IC_{50} values ranged from 2.80±0.01 μg mL^{-1} to 7.09± 0.10 μg mL^{-1} and from 0.95±0.02 μg mL^{-1} to 2.70± 0.21 μg mL^{-1}, respectively).

The data presented in this study showed that the EO samples investigated produced different antioxidant effectiveness in the test systems employed. It seems to be a general agreement that the oxygenated monoterpenes, especially two well-known phenolic compounds thymol and carvacrol, are mainly responsible for the strong antioxidant potential of plant oils that contain them (Tepe et al. 2005). From this point of view, the higher content of carvacrol may explain the superior antioxidant potency of *T. satureioides* oil observed in all test systems. The antioxidant potency observed in *T. satureioides* EO is comparable to what has been reported for other members of *Thymus* genus (Tepe et al. 2005; Alaoui Jamali et al. 2012; El Bouzidi et al. 2013) and highlights the considerable potential of the plant as an antioxidant food additive. The level of antioxidant activity determined by DPPH and reducing

Table 3 Antioxidant activity of essential oils in three different assays

	Essential oils (IC_{50} mg ml^{-1})					Standard antioxidants (IC_{50} µg mL^{-1})	
	MS[a]	SO	AA	TS	CC	Quercetin	BHT
DPPH	1.28±0.01	2.58±0.14	7.49±0.41	0.21±1.17	1.05±0.15	1.07±0.01	4.21±0.08
Reducing power	1.27±0.03	4.65±0.18	40.00±0.54	0.23±0.67	2.68±0.09	2.29±0.12	7.09±0.10
β-Carotene bleaching	1.40±0.05	0.47±0.12	0.46±0.14	0.21±1.74	0.63±0.16	0.95±0.02	4.30±0.33
ABTS	1.06±2.23	0.72±1.39	1.19±1.24	0.15±0.36	0.91±0.52	2.70±0.21	2.80±0.01

Values represent means±standard deviations for triplicate experiments

[a] Codes for the species are given in Table 1

power assays, showed that *M. suaveolens* subsp. *timija* EO has a moderate antioxidant activity. This activity may be related to the high content of menthone and pulegone, as reported in many others mint species (Mimica-Dukic et al. 2003; Teixeira et al. 2012). Concerning *S. officinalis* and *C. cinerea* EOs, the antioxidant activity observed could be explained partially by the presence of *trans*-thujone, camphor, 1,8-cineole, and α-pinene (Bozin et al. 2007; Bouaziz et al. 2009). However, the antioxidant performance of our *S. officinalis* chemotype was clearly inferior to what has been reported by Bouaziz et al. (2009) for samples obtained from Tunisian *S. officinalis* chemotype with high content of 1,8-cineole and *trans*-thujone. *A. ageratum* EO exhibited the weakest antioxidant activity, particularly in DPPH free radical scavenging and reducing power test systems, whereas it prevented the bleaching of β-carotene (IC_{50}=461.00±0.14 µg mL^{-1}). As far as our literature survey could ascertain, this is the first study to provide data regarding the antioxidant power of *C. cinerea*, *A. ageratum* and *M. suaveolens* subsp. *timija* EOs.

Insecticidal activity

The toxicity of essential oils against the important stored-product pest insect, *T. castaneum* was evaluated in the contact toxicity assay using adult insects. The lethal dose (LD_{50} and LD_{90}) and lethal time (LT_{50} and LT_{90}) values are given in Tables 4 and 5. The lower LD and LT values reflected better effectiveness of essential oils.

The results indicate that *M. suaveolens* subsp. *timija*, *T. satureioides* and *S. officinalis* EOs possess contact toxicity towards adults of *T. castaneum.* Neither *A. ageratum* nor *C. cinerea* EOs demonstrated toxicity in this assay.

As shown in Table 4, *M. suaveolens* subsp. *timija* EO had the best insecticidal activity against *T. castaneum* with LD_{50} and LD_{90} values of 0.17 µL cm^{-2} and 0.26 µL cm^{-2}, respectively. The least efficiency was observed for *S. officinalis* oil with LD_{50} and LD_{90} equal to 1.50 and 2.80 µL cm^{-2}, respectively. *T. satureoides* oil expressed intermediate toxicity with LD_{50} value of 0.31 µL cm^{-2} and LD_{90} of 0.77 µL cm^{-2}. The LT_{50} and LT_{90} mortality decreased with increasing concentrations of the oils (Table 5). Interestingly, at the lowest tested concentrations of 0.16 µL cm^{-2}, *T. castaneum* was more susceptible to *M. suaveolens* subsp. *timija* EO with LT_{50} and LT_{90} values of 44.19 and 98.14 h, respectively. The highest median lethal time values were observed when the insect was treated with *S. officinalis* EO with LT_{50} value of 56.32 h and LT_{90} value equal to 106.01 h at the lowest tested concentration of 0.78 µL cm^{-2}.

The above results show that the EOs tested possessed variable toxicity against *T. castaneum*. This can be attributed

Table 4 LD_{50} and LD_{90} values of essential oils from species studied against *T. castaneum*

Essential oils	LD_{50}[b] (95 % CL)[c]	LD_{90} (95 % CL)	Slope±SE	Chi square (χ^2)	df
MS[a]	0.17 (0.13–0.21)	0.26 (0.21–0.41)	3.30±0.34	0.63	2
SO	1.50 (1.17–4.80)	2.80 (1.83–8.99)	4.71±1.60	0.05	2
AA	–[d]	–	–	–	–
TS	0.31 (0.23–1.17)	0.77 (0.42–2.57)	2.34±0.40	0.65	2
CC	–	–	–	–	–

LD lethal dose.

[a] Details concerning the codes of the species are given in Table 1

[b] Concentration: µl cm^{-2}

[c] Confidence interval

[d] Not active

Table 5 LT_{50} and LT_{90} values of essential oils from aerial part of *M. suaveolens* subsp. *timija*, *T. satureioides* and *S. officinalis* applied against *T. castaneum*

Oil concentration (μl cm^{-2})	LT_{50} (h) (95 % CL)[b]	LT_{90} (95 % CL)	Slope±SE	Chi square (χ^2)	df
MS[a]					
0.09	–	–	–	–	–
0.16	44.19 (26.76–63.86)	98.14 (59.17–114.53)	2.18±0.10	3.64	3
0.24	5.08 (2.52–8.74)	25.50 (13.30–46.91)	3.70±0.49	1.79	3
0.31	2.98 (1.82–4.03)	6.02 (4.36–18.46)	3.04±1.38	0.10	3
TS					
0.09	–	–	–	–	–
0.16	71.05 (57.00–88.69)	283 (186.5–329.08)	2.06±1.03	0.73	3
0.24	45.92 (27.47–80.27)	157 (98.13–190,68)	2.59±0.92	1.26	3
0.31	30.50 (18.65–55.17)	79.38 (57.12–101.11)	2.97±1.03	0.74	3
SO					
0.39	–	–	–	–	–
0.78	56.32 (48.15–84.15)	106.01 (91.19–146.94)	2.76±0.43	0.68	3
1.17	10.07 (7.31–20.77)	34.45 (20.25–41.31)	2.64±0.36	0.79	3
1.57	8.43 (6.72–14.85)	14.71 (10.30–32.83)	2.92±0.80	3.44	3

LT lethal time

[a] Details concerning the codes of the species are given in Table 1

[b] Confidence interval

to differences in oil composition.. The potent toxic effect of *M. suaveolens* subsp. *timija* oil may be due to the high content of menthone and pulegone, two oxygenated monoterpenes well known for their strong insecticidal activity (Pavlidou et al., 2004; Liu et al. 2011). This result is in agreement with previous reports on some *Mentha* species EOs against several storage pest insects (Lamiri et al. 2001; Mohamed and Abdelgaleil 2008). The toxicity observed in *T. satureoides* EO might be explained by the presence of carvacrol, *p*-cymene and borneol. In fact, it has been previously shown that EOs rich in carvacrol and *p*-cymene possess acute toxic effects against various storage insect pests (Kordali et al. 2008; Tozlu et al. 2011). Although numerous reports exist on insecticidal activity of *S. officinalis* EO (Ateyyat et al. 2012; Tayoub et al. 2012), no data have yet been published regarding its activity against *T. castaneum*. The insecticidal property of *S. officinalis* EO has been related to the high content of camphor as was reported in many other camphor rich species such as *Salvia hydrangea* and *Achillea gypsicola* (Kotan et al. 2008; Tozlu et al. 2011). There seems to be general consensus that the insecticidal activity of EOs cannot be related to only major compounds. In fact, as EOs are very complex mixtures, synergistic and antagonistic effects of both major and minor constituents may be important determinants of the activity of the oil (Kordali et al. 2008). Concerning *A. ageratum* and *C. cinerea* EOs, to our knowledge, there has been no previous report evaluating the insecticidal properties of these species.

Conclusions

The antioxidant and insecticidal activities of five plants EOs were evaluated and compared in this work. *T. satureioides* EO exhibited strong antioxidant effects, while *M. suaveolens* subsp. *timija*, *T. satureioides* and *S. officinalis* were found to possess a significant toxicity against adults of *T. castaneum*. These findings provide additional information on the biological properties and consequently the economic values of EOs of Moroccan aromatic plants. It also provides a platform for the development of new eco-friendly and safer botanical materials, which may be used as effective alternative antioxidant and/or insecticidal natural agents for the preservation of processed food products.

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