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Essential Oil from Aerial Parts of Wild Algerian Rosemary: Screening of Chemical Composition, Antimicrobial and Antioxidant Activities

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Abstract: Essential oils from the aerial parts of 15 samples of Algerian *Rosmarinus officinalis* L. were analysed by gas chromatography (GC) and Gas chromatography/mass spectroscopy. Thirty eight components have been characterized; among the monoterpene hydrocarbons α-pinene, camphene and limonene were individuated as the main components; camphor, 1,8-cineole and borneol were the principal oxygenated compounds, caryophyllene, α-bisabolol and partly humulene were the most represented sesquiterpenes. The scavenging activity of the oils was determined by the DPPH model system. The SC_{50} (Scavenging Concentration) values were in the range 120.4-326.1 µL/mL, representing a moderate antioxidant effectiveness. Essential oils were evaluated for their antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*; as well as against ten fungal strains belonging to *Aspergillus, Alternaria, Candida, Fusarium*, *Penicillium* and *Saccharomyces* genera. The results showed a moderate antimicrobial activity. The Algerian rosemary essential oils could be promising sources of biologically active compounds if they receive further studies on their biological properties.

Keywords: *Rosmarinus officinalis*, essential oil, antimicrobial, antioxidant, GC/MS.

Introduction

Extracts of plants, spices and herbs play an important role in promoting human health by their anticancer, antioxidant and anti-inflammatory properties, as well as in the food sector for their preservative action [1]. Most essential oils derived from plants are known to possess insecticidal, antifungal, acaricidal, antibacterial and cytotoxic activities. Therefore, they are intensively screened and applied in pharmacology, pharmaceutical botany, medical and clinical microbiology, phytopathology and food preservation [2,3].

Rosemary is a dense, aromatic, and evergreen perennial small shrub which grows up to 2 m high. It is native to the Mediterranean regions. As an aromatic species, rosemary is widely used in the Mediterranean cuisine as spice and as food flavoring. The parts used include leaves and essential oil [4]. As medicinal plant rosemary belongs to the pool of herbs, whose efficacy is largely ac-

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knowledged, being, in fact, listed in the official Pharmacopoeia of several countries. Several biological activities have been ascribed to rosemary: as a memory improving tool [5], against neurodegenerative diseases [6,7] and hypertension [8], as antidepressant [9], anticancer [10], anti-inflammatory [11,12], antimicrobial [3,13,14,15], antithrombotic[16] and antioxidant [17,18,19,20,21]. Concerning this last property, rosemary is the sole herb spice used as a source of commercial antioxidant derivatives, marketed as oil and/or water miscible formulations in Europe and United States [22]. From a chemical point of view, rosemary essential oil is normally classified in three chemotypes, namely, cineoliferum (high content in 1,8-cineole), camphoriferum (camphor > 20 %) and verbenoniferum (verbenone > 15 %) [23]. Other chemotypes have been recognized according to the relative abundance of different compounds such as α-pinene (Italy and Morocco), myrcene (Portugal, Argentine, Brazil), comparable amounts of 1,8-cineole and camphor (India) or 1,8-cineole and α-pinene (Lebanon) [18,24,25,26,27].

Rosmarinus officinalis is one of the oldest known medicinal plants in Algeria; it is used for its antispasmodic properties. The leaves are used for flavouring foods as condiment [28,29]. This study aimed to analyze and evaluate the antioxidant and antimicrobial activities of the essential oil of *R. officinalis* growing wild in Algeria.

Materials and methods

Plant material

Fifteen different populations of *R. officinalis* L. were collected from several regions of Algeria (table 1) and belonging to different bioclimatic zones: humid (R31 and R06 samples); sub-humid (R34 and R41 samples); and semi-arid (R28KA, R28O, R28HD, R28M, R28B R28B3, R17, R17City, R17HS, R26 and R04 samples). The samples (3 to 4 kg fresh weight each) were representative of the species and their geographic area of distribution. Voucher specimens were deposited in the Herbarium of the Nature and life Sciences Department, University Mohamed Boudiaf of M'Sila - Algeria.

Isolation of the essential oil

Air dried aerial part of each plant (100 g) was subjected to hydrodistillation until there was no significant increase in the volume of collected oil (3 h). The procedure was repeated at least 3

Table 1. Collection sites, collection period, GPS data and essential oil yield of the 15 rosemary samples

No.	Sample	Origin(collection place)	Collection date	Latitude/ Longitude	E.O. yield (% v/w dry wt.)
1	R04	Ain Mlila (Oum-El-Bouaghi)	March 2011	36°02'N 6°42'E	1.92±0.13
2	R06	Kherrata (Bejaia)	April 2011	36°27'N 5°19'E	1.40±0.26
3	R17	Djelfa	April 2011	34°41'N 3°11'E	1.62±0.18
4	R17HS	Had-Shary (North Djelfa)	March 2011	35°17'N 3°23'E	1.83±0.21
5	R17City	Djelfa	April 2011	34°37'N 3°18'E	1.63±0.15
6	R26	Boughar (South West Médéa)	May 2011	35°55'N 2°40'E	1.38±0.16
7	R28B	Djbel messaâd (South M'sila)	April 2011	34°58'N 4°12'E	1.93±0.19
8	R28KA	Kaf-El-Assal (NorthWest M'sila)	April 2010	35°56'N 4°25'E	1.50±0.10
9	R28O	Ouenougha (West M'sila)	March 2011	35°59'N 4°09'E	1.81±0.22
10	R28HD	Hammam Dalâa (North West M'sila)	April 2011	35°58'N 4°23'E	1.21±0.19
11	R28M	Mâadid (East M'sila)	March 2011	35°49'N 4°47'E	1.12±0.07
12	R28B3	Djbel messaâd (South M'sila)	April 2012	35°02'N 4°06'E	1.63±0.13
13	R31	Ain Turk (Oran)	March 2011	35°43'N 0°43'W	0.70±0.05
14	R34	Bibans (BorjBou Arreridj)	March 2011	36°10'N 4°23'E	1.62±0.11
15	R41	Souk Ahras	March 2011	36°19'N 8°00'E	1.82±0.08

times. The oils, whose yield is listed in Table 1, were dried over anhydrous sodium sulphate and stored under N_2 in sealed vials until further use.

GC and GC/MS analyses

Gas chromatographic (GC) analyses were run on a Hewlett-Packard gas-chromatograph model 5890, equipped with a flame ionization detector (FID) and connected with an electronic integrator. GC-FID analyses were carried out with the following analytical conditions: ZB-5 capillary column (30 m x 0.25 mm i.d. x 0.25 µm film thickness); helium as carrier gas; injection in split mode (1:50); injector and detector temperatures 250 and 280°C, respectively. The oven temperature was programmed from 40°C to 300 at 2°C/min.

Gas chromatography-mass spectrometry (GC/MS) was carried out on the same gas chromatograph connected to a Hewlett-Packard mass spectrometer model 5971A, ionization voltage 70 eV, electron multiplier 1700 V, ion source temperature 180°C, mass spectra data were acquired in the scan mode in *m/z* range 40-400. Gas chromatographic conditions were the same as above.

The identity of components was based on the comparison of their GC retention index (retention time normalised to the retention times of adjacently eluting n-alkanes on the ZB-5 column) [30], with those reported in literature. In addition, each chemical identity were confirmed by computer matching of spectral MS data with those from Wiley 275 library and the comparison of the fragmentation patterns with those reported in literature. Moreover for the validation of the whole method and the correct attribution of chemical identities for dubious cases, whenever possible, co-injections with authentic standards was done (see Table 2).

Measurement of the scavenging activity against the stable radical DPPH•

The free radical-scavenging capacity of extracts was determined by the DPPH assay [31], a method based on the reduction of the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•). The reagent mixture consisted in 1.5 mL of 100 mM DPPH• in methanol, to which 37.5 µL of solutions containing various concentrations of the oils to be tested, were added to negative control tubes. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as positive control. After 20 min of incubation at room temperature, the absorbance was recorded at 517 nm in a UV-vis spectrophotometer. Each determination was carried out in triplicate. The scavenging concentrations (SC_{50}) values were obtained through extrapolation from linear regression analysis and denoted the concentration of sample required to scavenge 50 % of DPPH radicals.

Antimicrobial activity

The antimicrobial activity of essential oils was evaluated by disc diffusion method and minimum inhibitory concentration (MIC).

Organisms and media

Test organisms used in this study were as follows: *Staphylococcus aureus* ATCC 6538P, *Escherichia coli* ATCC 10536, *Aspergillus niger* ATCC16404, *A. westerdijkiae* NRRL5175, *A. flavus* (wild type), *A. parasiticus* (wild type), *Penicillium* spp. (wild type), *P. frequentans* (wild type), *Fusarium oxysporum* (wild type), *Alternaria alternata* (wild type), *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* (wild type). The strains were maintained on Tryptone Soya Agar (bacteria) and Sabouraud Dextrose Agar (SDA) (mycetes). For the antimicrobial tests, cells were grown overnight in Muller Hinton Broth (bacteria) and Sabouraud Dextrose Broth (SDB) (yeasts) at 37 and 30°C, respectively. The moulds were cultivated on SDA for 7 days at 30°C in order to harvest the mature spores.

Disc diffusion test

Overnight broth cultures adjusted to yield approximately 1.0 x 10^8 CFU/ml for bacteria and 1.0 x 10^6 CFU/mL for yeasts, were streaked with a calibrate loop on plates containing appropriate solid medium. For moulds, suspensions of mature spores were obtained by gently washing the surface of solid media with 0.85 % saline. The resulting suspension was adjusted by spectrophotometer at 530 nm to obtain inocula of 10^6 spores/ml as confirmed by colony counts in triplicate on

SDA.

Filter paper discs (6 mm diameter) were placed on the inoculated agar surfaces and impregnated with 10 µL of essential oil. Vancomycin disc (30 µg), amoxicillin disc (10 µg), amphotericin B discs (20 µg) and miconazole (10 µg) were used as positive controls. The plates were observed after 24 h at 37°C for bacteria and after 48 and 96 h at 30°C for yeasts and moulds, respectively. All tests were performed in duplicate and the antimicrobial activity was expressed as the inhibition diameters (mm) produced by the essential oils.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of the essential oils was determined by a broth dilution micromethod in 96-well round-bottomed polystyrene microtiter plates according to the Clinical Laboratory Standards Institute guidelines [32,33], with some modifications. A volume of 40 % (v/v) stock solution of each essential oil in ethanol was diluted in appropriate medium in order to obtain concentrations ranging from 2 to 0.2 % (v/v). The final inoculum was $5x10^5$ CFU/mL for bacteria and $5x10^3$ CFU or spores/mL for mycetes. Growth controls consisting of medium and medium with ethanol were included. The MIC was defined as the lowest concentration of essential oil that completely inhibited visible growth after 24 h at 37°C for bacteria and after 48 h and 3-7 days at 30°C for yeasts and moulds respectively.

Statistical analysis

Results are reported as mean±SD of three independent assays and statistically analyzed by ANOVA test, followed by Tukey's HSD, using the statistical software ezANOVA (http://www.sph.sc.edu/comd/rorden/ezanova/home.html). Differences between groups were considered significant for $p < 0.05$.

Results and discussion

Chemical composition of the essential oils

The average essential oil yield of the collected Algerian *R. officinalis* samples was 1.54 ± 0.34 (% v/w dry wt.). Table 2a (samples 1-8) and table 2b (samples 9-15) lists the EO composition and Figure 1 shows a typical GC/MS profile of an essential oil. In total, 38 components have been fully characterized and grouped in three classes: monoterpene hydrocarbons, oxygenated monoterpenes, and sesquiterpenes, to make comparison of the

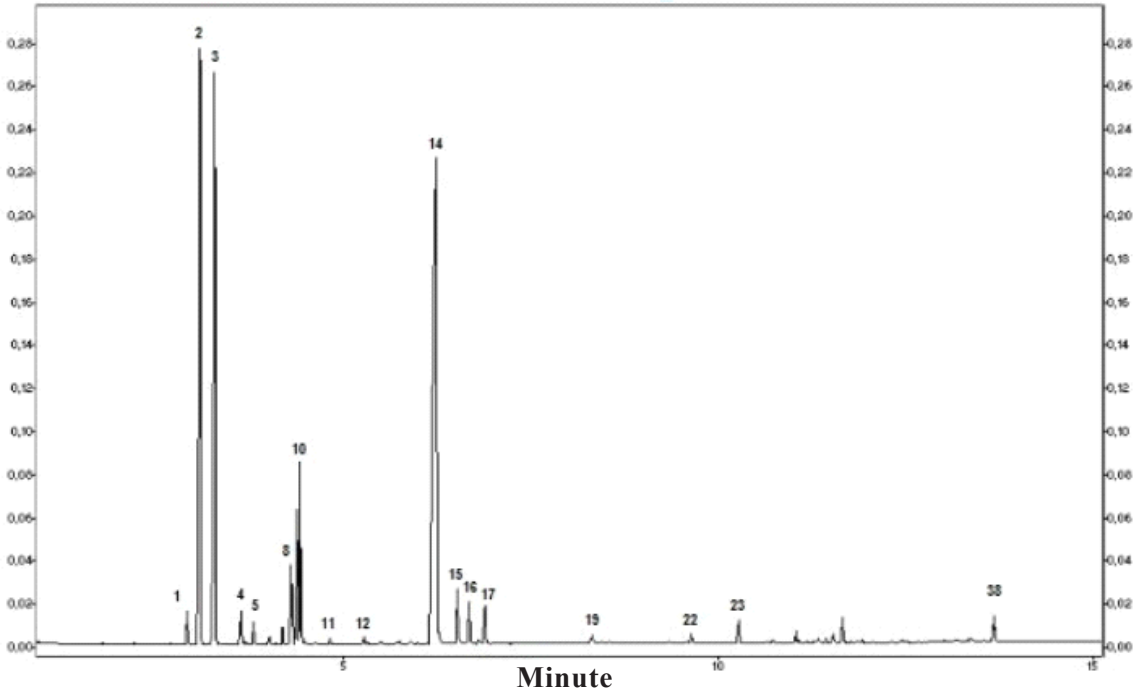

Figure 1. GC-MS profile of a representative sample (R17 city) of Algerian *Rosmarinus officinalis* essential oil.

Table 2a. Chemical composition of Algerian rosemary essential oils (samples 1-8)

No.[a]	RI exp.[b]	RI lit.[c]	Class/compound	1 R04	2 R06	3 R17	4 R17HS % (±SD)	5 R17 city	6 R26	7 R28B	8 R28KA
Monoterpene hydrocarbons				48.1 (0.48)[a]	44.9 (0.39)[b]	42.7 (0.67)[c]	45.7 (0.37)[b]	49.8 (0.22)[xe]	45.5 (0.30)[xb]	52.6 (0.56)	42.8 (0.43)[c]
1	922	926	Tricyclene	0.8 (0.01)[a]	0.8 (0.03)[ab]	0.8 (0.04)[a]	0.9 (0.03)[d]	0.8 (0.03)[ae]	0.8 (0.03)[xa]	1.0 (0.02)	0.1 (0.01)[h]
2	936	939	α-Pinene[d]	20.3 (0.26)[a]	18.1 (0.36)[b]	18.4 (0.41)[bc]	17.3(0.36)[d]	20.5 (0.76)[ae]	18.9 (0.13)[xc]	21.0 (0.32)[e]	16.1 (0.19)
3	951	954	Camphene[d]	18.1 (0.06)[a]	18.9 (0.30)[b]	19.3 (0.49)[b]	19.5 (0.72)[bd]	19.9 (1.01)[xbe]	19.1 (0.17)[b]	22.4 (0.28)	18.7 (0.44)[ab]
4	976	979	β-Pinene[d]	0.6 (0.03)[a]	t	0.3 (0.02)	0.1 (0.02)[d]	0.8 (0.02)	0.7 (0.04)	0.5 (0.02)[g]	0.5 (0.01)[g]
5	987	990	Myrcene[d]	0.6 (0.03)[a]	0.5 (0.03) [bm]	0.5 (0.06)[acflm]	0.1 (0.02)	0.5 (0.06)[aben]	0.5 (0.02)[bf]	0.4 (0.02)[c]	0.4 (0.02)[c]
6	1001	1002	α-Phellandrene	0.4 (0.04)	0.2 (0.02) [b]	0.1 (0.01)[c]	-	0.2 (0.01)[b]	-	0.2 (0.02)[b]	0.1 (0.01)[c]
7	1015	1017	α-Terpinene[d]	0.6 (0.01)	0.3 (0.04) [b]	0.3 (0.04)[b]	0.3 (0.02)[b]	0.4 (0.02)[e]	-	0.5 (0.02)[g]	0.3 (0.01)[b]
8	1023	1024	*p*-Cymene[d]	2.1 (0.20)[eh]	2.0 (0.17) [efh]	0.5 (0.04)	2.6 (0.17)[d]	2.2 (0.20)[de]	1.8 (0.02)[f]	2.0 (0.03)[e]	2.3 (0.15)[dh]
9	1029	1029	Limonene[d]	4.1 (0.20)[abh]	3.7 (0.12) [bm]	2.3 (0.19)	4.6 (0.23)[dn]	4.2 (0.12)[ad]	3.5 (0.07)	4.3 (0.14)[ad]	3.9 (0.08)[hm]
11	1059	1060	γ-Terpinene[d]	0.2 (0.02)[hl]	0.2 (0.02)[hl]	t	-	0.1 (0.01)	t	0.2 (0.01)[hl]	0.2 (0.02)[h]
12	1088	1089	Terpinolene[d]	0.3 (0.02)[ao]	0.1 (0.02)	0.2 (0.02)[c]	0.3 (0.02)[a]	0.2 (0.02)[c]	0.2 (0.02)[c]	0.2 (0.01)[c]	0.2 (0.03)[c]
Oxygenated monoterpenes				48.0 (0.73)[a]	49.4 (0.56)[a]	48.8 (0.55)[a]	49.1 (0.40)[a]	44.5 (0.75)[e]	45.0 (0.56)[e]	41.9 (0.19)	50.6 (0.19)[h]
10	1032	1031	1,8-Cineole[d]	8.4 (0.17)[a]	8.4 (0.09)[a]	7.8 (0.20)[cf]	8.3 (0.25)[ac]	4.3 (0.21)	7.8 (0.08)[f]	2.7 (0.13)	8.2 (0.17)[ac]
13	1122	1121	exo-Fenchol	-	-	-	-	-	0.1 (0.01)[f]	-	0.1 (0.02)[fh]
14	1152	1146	Camphor[d]	35.7 (0.61)[abf]	36.7 (0.7) [abh]	36.7 (0.55)[ah]	36.3 (0.57)[af]	36.2 (0.71)[af]	35.3 (0.30)[f]	33.9 (0.09)[g]	37.6 (0.35)[h]
15	1169	1169	Borneol[d]	1.4 (0.05)	1.9 (0.04)[b]	2.0 (0.04)[b]	2.0 (0.07)[b]	1.6 (0.04)[e]	0.8 (0.02)	3.2 (0.11)[g]	2.4 (0.15)[h]
16	1180	1177	Terpinen-4-ol[d]	1.0 (0.08)[aehi]	0.9 (0.06)[agin]	0.8 (0.03)[c]	0.9 (0.04)[an]	1.1 (0.07)[eh]	0.1 (0.01)	0.8 (0.02)[cg]	1.1 (0.02)[h]
17	1192	1188	α-Terpineol[d]	1.1 (0.12)[abfi]	1.2(0.05)[b]	1.0 (0.02)[a]	1.0 (0.07)[ai]	1.0 (0.02)[a]	0.9 (0.01)[f]	0.7 (0.02)[g]	1.1 (0.08)[ai]
18	1197	1195	Myrtenol[d]	-	-	-	-	t	-	-	-
19	1290	1289	Bornyl acetate[d]	0.5 (0.04)[a]	t	0.5 (0.05)[a]	0.5 (0.03)[a]	0.3 (0.03)[e]	t	0.6 (0.02)[g]	t
20	1298	1290	Thymol[d]	t	t	t	-	t	0.1 (0.01) oq	T	t
21	1304	1299	Carvacrol[d]	0.1 (0.02)[a]	0.2 (0.03)[b]	t	-	0.1 (0.01)[ae]	0.3 (0.01)	T	0.1 (0.02)[ae]
Sesquiterpenes				2.2 (0.03)[a]	2.5 (0.11)[b]	2.3 (0.16)[ab]	1.3 (0.02)	3.1 (0.15)[e]	3.2 (0.06)[e]	3.3 (0.02)[e]	2.6 (0.09)[h]
22	1379	1376	α-Copaene[d]	0.1 (0.01)[a]	0.1 (0.00)[a]	t	0.1 (0.02)[a]	0.3 (0.01)[e]	-	0.2 (0.01)[hp]	0.2 (0.01)[h]
23	1424	1419	β-Caryophyllene[d]	0.4 (0.01)[a]	0.6 (0.04)[b]	0.7 (0.05)[c]	0.4 (0.02)[a]	0.7 (0.03)	1.0 (0.02)	0.8 (0.03)[g]	0.6 (0.03)[b]
24	1433	1434	α-*trans*-Bergamotene	-	-	-	-	-	-	-	-
25	1438	1441	Aromadendrene	-	-	-	-	-	-	-	-
26	1455	1454	α-Humulene[d]	t	0.2 (0.01)[b]	0.2 (0.02)[b]	-	-	t	-	-
27	1460	1456	β-*trans*-Farnesene	-	-	0.1 (0.01)[c]	-	0.1 (0.01)[cm]	-	0.3 (0.01)	t

table 2a. (continued).

No.[a]	RI exp.[b]	RI lit.[c]	Class/compound	1 R04	2 R06	3 R17	4 R17HS % (±SD)	5 R17 city	6 R26	7 R28B	8 R28KA
28	1482	1484	α-Amorphene	0.2 (0.01)	-	t	-	0.3 (0.02)[ef]	0.3 (0.01)[f]	0.3 (0.02)[ep]	0.3 (0.02)[ep]
29	1486	1484	Germacrene D	-	0.3 (0.02)	t	0.2 (0.02)[d]	-	0.1 (0.01)[fm]	-	-
30	1486	1495	γ-Amorphene	t	-	0.1 (0.02)[c]	0.1 (0.02)[c]	0.1 (0.02)[c]	-	0.1 (0.01)[c]	0.1 (0.01)[c]
31	1493	1496	Valencene	t	-	-	-	t	t	t	t
32	1500	1500	α-Muurolene	t	-	-	-	0.1 (0.01)[en]	0.1 (0.02)[en]	t	-
33	1505	1505	β-Bisabolene	-	-	-	-	0.1 (0.01)[e]	0.1 (0.02)[e]	-	-
34	1512	1513	γ-Cadinene	0.1 (0.01)[a]	0.1 (0.01)[a]	-	0.2 (0.03)	0.1 (0.02)[a]	0.3 (0.02)	0.1 (0.01)[ap]	0.1 (0.01)[ap]
35	1520	1523	δ-Cadinene	0.2 (0.01)[a]	0.2 (0.02)[aq]	0.2 (0.04)[aq]	0.3 (0.03)[d]	0.3 (0.02)[d]	0.3 (0.02)[d]	0.2 (0.01)[a]	0.3 (0.02)[d]
36	1538	1538	α-Cadinene	t	t	t	0.1 (0.01)[d]	t	t	t	t
37	1548	1545	α-Calacorene	-	-	-	-	t	t	-	-
38	1691	1685	α-Bisabolol	1.2 (0.02)	1.0 (0.05)[b]	1.0 (0.08)[bi]	-	1.1 (0.07)[bi]	1.1 (0.07)[bi]	1.3 (0.03)[g]	1.0(0.05)[bi]
Monoterpene hydrocarbons				48.1 (0.48)	44.9 (0.39)	42.7 (0.67)	45.7 (0.37)	49.8 (0.22)	45.5 (0.30)	52.6 (0.56)	42.8 (0.43)
Oxygenated monoterpenes				48.0 (0.43)	49.4 (0.56)	48.8 (0.55)	49.1 (0.40)	44.5 (0.75)	45.0 (0.56)	41.9 (0.19)	50.6 (0.19)
Sesquiterpenes				2.2 (0.03)	2.5 (0.11)	2.3 (0.16)	1.3 (0.02)	3.1 (0.15)	3.2 (0.06)	3.3 (0.02)	2.6 (0.09)
Total				98.3	96.8	93.8	96.1	97.4	93.7	97.8	96.0

Rosemary oils from other origins added for comparison[e]

Spanish type	α-pinene (18-26 %), camphene (8.0-12.0 %), β-pinene (2.0-6.0 %), β-myrcene (1.5-5.0 %), limonene (2.5-5.0 %), cineole (16.0-25.0 %), *p*-cymene (1.0-2.2 %), camphor (13.0-21.0 %), bornyl acetate (0.5-2.5 %), α-terpineol (1.0-3.5 %), borneol (2.0-4.5 %), verbenone (0.7-2.5 %)
Moroccan & Tunisian type	α-pinene (9.0-14.0 %), camphene (2.5-6.0 %), β-pinene (4.0-9.0 %), β-myrcene (1.0-2.0 %), limonene (1.5-4.0 %), cineole (38.0-55.0 %), *p*-cymene (0.8-2.5 %), camphor (5.0-15.0 %), bornyl acetate (0.1-1.5 %), α-terpineol (1.0-2.6 %), borneol (1.5-5.0 %), verbenone (max. 0.4 %)

[a] The numbering refers to elution order, and values (relative peak area percent) represent averages of 3 determinations (t = trace, < 0.05 %)
Values in a row followed by the same letter are not significantly different from each other ($p < 0.05$; ANOVA followed by Tukey HSD Test)
Values not followed by any letter are significaantly different from any other value in the same row
Standard Deviation (±SD) is given in parentheses
[b] Retention index (RI) relative to standard mixture of *n*-alkanes on SPB-5 column
[c] Literature Retention Index (RI) [30]; [d] Co-injection with pure component; [e] data from European Pharmacopoiea [34]

Table 2b. Chemical composition of Algerian rosemary essential oils (samples 9-15)

No.[a]	RI exp.[b]	RI lit.[c]	Class/compound	9 R28W	10 R28HD	11 R28M	12 R28B3	13 R31	14 R34	15 R41
				% (±SD)						
Monoterpene hydrocarbons				38.1 (0.07)	47.5 (0.33)[a]	43.5 (0.07)[c]	51.0 (0.41)[x]	49.3 (0.36)[e]	25.1 (0.05)[xp]	25.0 (0.13)[p]
1	922	926	Tricyclene	0.6 (0.02)	0.9 (0.01)[bd]	0.7 (0.03)	0.9 (0.02)[xbde]	0.1 (0.01)[h]	t	T
2	936	939	α-Pinene[d]	14.9 (0.06)	19.6 (0.27)[e]	17.4 (0.04)[d]	21.3 (0.15)[e]	28.5 (0.25)	13.5 (0.10)	14.1 (0.13)
3	951	954	Camphene[d]	15.1 (0.15)	19.2 (0.24)[b]	18.1 (0.11)	20.5 (0.56)[de]	7.7 (0.09)	5.3 (0.05)	4.6 (0.08)
4	976	979	β-Pinene[d]	1.8 (0.06)	0.1 (0.01)[d]	0.5 (0.04)[ag]	-	3.2 (0.02)	1.3 (0.02)	1.4(0.02)
5	987	990	Myrcene[d]	0.6 (0.02)[a]	0.5 (0.02)[elm]	0.5 (0.04)[bm]	0.4 (0.01)[cn]	0.7 (0.02)	1.2 (0.07)	1.0 (0.03)
6	1001	1002	α-Phellandrene	0.2 (0.01)[b]	0.2 (0.01)[bp]	0.3 (0.02)[m]	0.3 (0.02)[m]	0.3 (0.02)[m]	0.2 (0.01)[p]	0.2 (0.01)[bp]
7	1015	1017	α-Terpinene[d]	0.2 (0.01)	0.4 (0.02)[e]	0.5 (0.02)[g]	0.5 (0.03)[gq]	1.0 (0.02)	0.5 (0.02)[gq]	0.5 (0.01)[q]
8	1023	1024	*p*-Cymene[d]	1.3 (0.05)	2.1 (0.05)[eh]	1.5 (0.03)[m]	1.5 (0.02)[m]	3.3 (0.07)	1.6 (0.02)[m]	1.5 (0.04)[m]
9	1029	1029	Limonene[d]	2.9 (0.02)	4.2 (0.12)[ad]	3.8 (0.04)[m]	4.9 (0.02)[n]	3.2 (0.02)	0.9 (0.02)	0.8 (0.03)
11	1059	1060	γ-Terpinene[d]	0.3 (0.02)[i]	0.2 (0.01)[l]	0.3 (0.02)[i]	0.5 (0.02)	0.9 (0.09)	0.6 (0.02)[p]	0.6 (0.02)[p]
12	1088	1089	Terpinolene[d]	0.2 (0.02)[c]	0.2 (0.02)[c]	-	0.3 (0.02)[a]	0.3 (0.02)[o]	0.2 (0.02)[c]	0.3 (0.02)[a]
Oxygenated monoterpenes				51.6 (0.24)	44.6 (0.30)[e]	50.8 (0.37)[h]	40.9 (0.47)	22.6 (0.37)	68.0 (0.43)[p]	68.3 (0.19)[p]
10	1032	1031	1,8-Cineole[d]	8.8 (0.15)	5.9 (0.04)	5.0 (0.04)	1.8 (0.03)	10.9 (0.30)	47.6 (0.50)	43.2 (0.27)
13	1122	1121	exo-Fenchol	-	0.1 (0.01)[h]	-	-	-	-	-
14	1152	1146	Camphor[d]	39.1 (0.36)	32.2 (0.38)	41.2 (0.33)	33.6 (0.52)[g]	3.1 (0.10)	11.1 (0.12)	13.4 (0.17)
15	1169	1169	Borneol[d]	1.5 (0.06)[e]	4.3 (0.12)	2.5 (0.07)[h]	3.3 (0.03)[g]	3.7 (0.06)[o]	3.7 (0.09)[o]	6.5 (0.13)
16	1180	1177	Terpinen-4-ol[d]	1.0 (0.02)[ei]	0.9 (0.06)[an]	0.9 (0.02)[a]	0.9 (0.02)[n]	2.1 (0.11)	0.7 (0.04)[p]	0.7 (0.02[p]
17	1192	1188	α-Terpineol[d]	1.2 (0.10)[bi]	0.9 (0.02)[f]	1.0 (0.02)[a]	0.7 (0.02)[g]	1.5 (0.03)	3.3 (0.08)[p]	3.1 (0.17)[p]
18	1197	1195	Myrtenol[d]	-	-	-	t	1.0 (0.03)	-	t
19	1290	1289	Bornyl acetate[d]	t	0.4 (0.02)	0.3 (0.02)[e]	0.6 (0.04)[g]	t	1.6 (0.02)	1.4 (0.01)
20	1298	1290	Thymol[d]	t	-	t	t	0.1 (0.02)[o]	t	0.1 (0.01)[q]
21	1304	1299	Carvacrol[d]	t	-	0.1 (0.01)	t	0.2 (0.02)[b]	0.1 (0.01)[e]	0.1 (0.01)[e]
Sesquiterpenes				6.8 (0.04)	3.3 (0.12)[eq]	2.7 (0.04)[h]	4.6 (0.04)	15.2 (0.14)	3.6 (0.05)	3.3 (0.05)[q]
22	1379	1376	α-Copaene[d]	0.4 (0.01)	-	t	0.3 (0.02)[e]	1.0 (0.01)	0.2 (0.02)[p]	0.2 (0.01)[a]
23	1424	1419	β-Caryophyllene[d]	3.0 (0.03)	0.8 (0.01)[cg]	0.8 (0.02)[cg]	1.3 (0.02)	6.5 (0.14)	2.5 (0.05)	2.2 (0.03)
24	1433	1434	α-*trans*-Bergamotene	t	-	-	t	0.1 (0.01)	t	t
25	1438	1441	Aromadendrene	-	-	-	-	0.1 (0.01)	t	-
26	1455	1454	α-Humulene[d]	-	-	-	-	2.1 (0.01)	-	0.4 (0.01)

table 2b (continued).

No.[a]	RI exp.[b]	RI lit.[c]	Class/compound	9 R28W	10 R28HD	11 R28M	12 R28B3 % (±SD)	13 R31	14 R34	15 R41
27	1460	1456	β-*trans*-Farnesene	0.2 (0.01)	-	0.1 (0.01)[m]	0.3 (0.02)	-	0.2 (0.01)	-
28	1482	1484	α-Amorphene	0.6 (0.03)	-	t	-	1.4 (0.03)	0.3 (0.01)[p]	0.2 (0.01)
29	1486	1484	Germacrene D	0.1 (0.01)[f]	0.1 (0.02)[f]	0.1 (0.01)[m]	0.4 (0.02)	0.2 (0.03)[d]	t	-
30	1486	1495	γ-Amorphene	-	-	t	0.1 (0.02)[e]	-	-	t
31	1493	1496	Valencene	0.1 (0.01)	-	-	t	0.1 (0.01)	t	t
32	1500	1500	α-Muurolene	0.1 (0.01)[i]	-	0.1 (0.01)[e]	0.1 (0.01)[n]	0.3 (0.01)	t	0.1 (0.01)[en]
33	1505	1505	β-Bisabolene	0.2 (0.02)	-	0.1 (0.02)[e]	0.2 (0.02)	0.5 (0.02)		-
34	1512	1513	γ-Cadinene	0.4 (0.01)	-	0.1 (0.01)[apq]	0.1 (0.02)[a]	1.1 (0.11)	0.1 (0.01)[pq]	0.1 (0.01)[q]
35	1520	1523	δ-Cadinene	0.5 (0.01)	0.3 (0.02)[d]	0.2 (0.02)[aq]	0.3 (0.01)[d]	1.2 (0.04)	0.2 (0.01)[aq]	0.2 (0.01)[q]
36	1538	1538	α-Cadinene	0.1 (0.01)[d]	-	t	0.1 (0.01)[d]	0.2 (0.01)	t	t
37	1548	1545	α-Calacorene	t	-	t	0.1 (0.01)[n]	0.3 (0.02)	0.1 (0.01)[n]	0.1 (0.01)[n]
38	1691	1685	α-Bisabolol	1.1 (0.02)[i]	2.1 (0.11)	1.3 (0.02)[g]	1.5 (0.03)	0.2 (0.01)	0.1 (0.02)	-
Monoterpene hydrocarbons				38.1 (0.07)	47.5 (0.33)	43.5 (0.07)	51.0 (0.41)	49.3 (0.36)	25.1 (0.05)	25.0 (0.13)
Oxygenated monoterpenes				51.6 (0.24)	44.6 (0.30)	50.8 (0.37)	40.9 (0.47)	22.6 (0.37)	68.0 (0.43)	68.3 (0.19)
Sesquiterpenes				6.8 (0.04)	3.3 (0.12)	2.7 (0.04)	4.6 (0.04)	15.2 (0.14)	3.6 (0.05)	3.3 (0.05)
Total				96.5	95.4	97.0	96.5	87.1	96.7	96.6
Rosemary oils from other origins added for comparison[e]										
Spanish type			α-pinene (18-26 %), camphene (8.0-12.0 %), β-pinene (2.0-6.0 %), β-myrcene (1.5-5.0 %), limonene (2.5-5.0 %), cineole (16.0-25.0 %), *p*-cymene (1.0-2.2 %), camphor (13.0-21.0 %), bornyl acetate (0.5-2.5 %), α-terpineol (1.0-3.5 %), borneol (2.0-4.5 %), verbenone (0.7-2.5 %)							
Moroccan & Tunisian type			α-pinene (9.0-14.0 %), camphene (2.5-6.0 %), β-pinene (4.0-9.0 %), β-myrcene (1.0-2.0 %), limonene (1.5-4.0 %), cineole (38.0-55.0 %), *p*-cymene (0.8-2.5 %), camphor (5.0-15.0 %), bornyl acetate (0.1-1.5 %), α-terpineol (1.0-2.6 %), borneol (1.5-5.0 %), verbenone (max. 0.4 %)							

[a] The numbering refers to elution order, and values (relative peak area percent) represent averages of 3 determinations (t = trace, < 0.05%), Values in a row followed by the same letter are not significantly different from each other ($p < 0.05$; ANOVA followed by Tukey HSD Test) . Values not followed by any letter are significaantly different from any other value in the same row; Standard Deviation (±SD) is given in parentheses

[b] Retention index (RI) relative to standard mixture of *n*-alkanes on SPB-5 column

[c] Literature Retention Index (RI) [30]; [d] Co-injection with pure component; [e] data from European Pharmacopoiea [34]

oils easier. Samples can be divided in three categories on the basis of the partition of components: 1-12 showed a content of monoterpene hydrocarbons ranging between 38.1 and 52.7 %, and a comparable amount of oxygenated monoterpenes (40.9 - 51.7 %); sample 13 was characterized by a similar value (49.4 % *ca*.) of hydrocarbons, but a lower amount of oxygenated (22.6 % *ca*.); finally, samples 14 and 15 instead showed a lower amount of hydrocarbons (average 25.1 %) and a higher content of oxygenated (68.2 %.). Sesquiterpenes ranged between 1 and 7 % in fourteen samples, with the exception of sample 13 in which these compounds reached the amount of 15.2 %. All the oils are characterized by the predominance of some components: namely α-pinene, camphene and limonene among the monoterpene hydrocarbons; camphor, 1,8-cineole and borneol among the oxygenated monoterpenes; caryophyllene, α-bisabolol and, in some cases, humulene among the sesquiterpenes. In particular, samples 1-12 showed a comparable amount of α-pinene (14.9 - 21.0 %) and camphene (15.1 - 22.4 %), and a lower amount of limonene (2.9 - 4.9 %), the oxygenated counterpart was characterized by camphor (32.2 - 41.2 %), 1,8-cineole (1.8 - 8.8 %) and borneol (1.4 - 4.3 %). Sample 13 showed 28.5 % of α-pinene, 7.7 % of camphene and 3.2, 3.3 and 3.2 % of β-pinene, *p*-cymene and limonene, respectively; among the oxygenated monoterpenes: 3.1 % of camphor, 10.9 % of 1,8-cineole and 3.7 % of borneol. This sample as previously mentioned presents a significant amount of sesquiterpenes, 15.2 %, with caryophyllene and humulene as main constituents (Tab. 2). Samples 14 and 15 showed 13.5 and 14.1 % of α-pinene and 5.3 and 4.6 % of camphene; a very high content of 1,8-cineole (47.6 and 43.2 %), 11.1 and 13.4 % of camphor and 3.7 and 6.5 % of borneol. Furthermore, in Table 2 the compositional range of the main monoterpene components of Spanish, Moroccan and Tunisian type of rosemary oils, as reported in European Pharmacopoiea [34], have been added for comparison. With respect to these oils the Algerian samples analysed in this study show a wider compositional range for the same main components, namely α-pinene, camphene, cineole and camphor, as well as for many minor compounds. A significant difference is due to the absence of verbenone in the Algerian oils, in fact, this compound is present Spanish, Moroccan and Tunisian oils although at very low extent (Table 2).

Our findings are partially in agreement with that by Meziane-Assami *et al.* [35] who studied the correlation between the chemical composition and the geographical origin of 16 Algerian rosemary oils. Three rosemary oils were obtained from plants growing in semi-arid and Saharan areas and were characterized by camphor as the main component and by remarkably high contents of camphene; however, verbenone was not detected in these oils. The other 15 rosemary oils, obtained from plants growing in the sub-humid, humid and per-humid zones had α-pinene as the principal constituent and could be considered belonging to α-pinene chemotype. In our study, all the 12 oils obtained from plants growing in semi-arid zones contained camphor as the main component. However, the 4 oils from humid or sub-humid show an heterogeneous chemical profile, since the samples R34 and R41 contain 1,8-cineole as the main component together with lower amount of α-pinene and camphor, and the sample R31 (the only one having α-pinene as main component) contains remarkably high contents of sesquiterpenes (in particular β-caryophillene), while the sample R06 shows a chemical composition similar to that of oils from semi-arid areas. Further analyses of a larger number of samples could be useful to investigate these compositional aspects, considering that an essential oil is a complex matrix influenced by genetic, environmental as well as extraction methods employed to obtain them [36].

Antimicrobial activity

The results of disc diffusion test are listed in Table 3a (i) and 3a (ii). The positive controls demonstrated the following inhibition diameters: 17 mm (vancomycin) against *S. aureus*, 19 mm (amoxicillin) against *E. coli*, 12-18 mm (amphotericin B) and 15-28 mm (miconazole) against mycetes. Generally, the essential oils showed a moderate effect on almost all tested microorganisms with inhibition diameters ranging from 7 to 15 mm. Gram positive *Staphylococcus aureus*

Table 3 a(i). Antimicrobial activity of Algerian rosemary essential oils against bacteria (*E. coli* and *S. aureus*) and yeasts (*C. albicans* and *S. cerevisiae*) as measured in the diffusion test [a]

No.	Oils Samples	*E. coli*	*S. aureus*	*C. albicans*	*S. cerevisiae*
		Inhibition zone (mm)			
1	R04	11.3±0.57 [a]	14.3±0.57 [acfil]	11.6±1.15 [a]	13.3±0.57 [a]
2	R06	12.3±0.57 [bg]	14.6±0.57 [bcdfil]	12.6±1.15	12.6±0.57 [b]
3	R17	11.6±0.57 [bc]	12.3±0.57 [c]	14.6±1.15 [acop]	11.6±1.15 [c]
4	R17HS	11.6±2.08	12.6±1.15 [d]	11.0±0.00 [cdeln]	11.3±1.52 [d]
5	R17 city	11.0±1.73	13.3±0.57 [e]	14.3±0.57 [aenop]	11.6±1.15 [e]
6	R26	10.3±0.57 [bco]	11.3±1.52 [f]	13.3±1.52	12.6±0.57 [f]
7	R28B	10.6±0.57 [g]	13.6±2.08	12.6±1.52	12.0±1.73
8	R28KA	11.3±1.15	13.6±1.15	12.6±2.08	12.3±1.52
9	R28W	10.0±1.73	11.3±1.52 [i]	ND	ND
10	R28 HD	10.3±2.08	12.3±0.57 [l]	14.0±1.00 [l]	12.0±1.73
11	R28M	11.0±1.73	13.3±1.52	14.6±0.57 [adnop]	12.6±1.15
12	R28B3	9.0±0.00 [abcgo]	11.6±0.57 [abe]	12.3±0.57 [cn]	14.3±0.57 [bcdef]
13	R31	12.0±2.00 [o]	13.6±1.15	11.6±1.15 [o]	10.6±0.57 [abf]
14	R34	11.3±1.52	13.3±0.57 [b]	11.6±0.57 [lp]	12.6±1.15
15	R41	11.6±1.52 [q]	12.3±1.52	12.3±0.57 [cde]	12.6±2.08
	Vancomycin		17.0±1.0		
	Amoxicillin	19.3±0.57			
	Amphotericin B			18.0±2.00	18.0±1.00
	Miconazole			28.0±1.00	28.6±1.15

[a] Data are reported as mean ± SD of three experiments

Values in a column followed by the same letter are significantly different from each other ($p<0.05$; ANOVA followed by Tukey HSD test)

values not followed by any letter are not significantly different from any other value in the same column

ND = Not Determined

was more susceptible respect to Gram negative *Escherichia coli*. Among mycetes, the yeasts and the *Aspergillus* species showed the highest and the weakest sensitivity respectively. *C. albicans* and *S. cerevisiae* were sensitive to all tested oils. Interestingly preliminary observations showed a significant reduction (≥ 50 %) of sporulation for *A. niger, A. parasiticus, Penicillium* spp*, P. frequentans* and *F. oxysporum,* but further confirmation is required.

Recently rosemary oil from plants growing wild in Hammam Dalâa was reported to possess a strong clear inhibitory effect on the growth *P. digitatum* when applied as fumigant and, at a really lower extent, when applied by contact bioassay [37]; furthermore spore germination was strongly affected by the oil applied by disk diffusion method. In our study the sample R28HD from plants growing in Hammam Dalâa showed only a slight effect on growth and sporulation of some filamentous fungi, and the contrast may be explained by the different ways of exposure used. Interestingly, rosemary Algerian oils maintain a good inhibiting power against toxigenic *Aspergillus* and *Fusarium* spp. also if obtained from cultivated plants [38].

The results of MIC evaluation ranged from 0.25 % to ≥2 % with a more inhibition at the first 48-72 hours for all microorganisms (Table 3b).

Celiktas *et al.* [23] reported that *R. officinalis* presented a moderate antibacterial activity depending on location and seasonal variations and they

Table 3 a(ii). Antimicrobial activity of Algerian rosemary essential oils against moulds as measured in the diffusion test [a]

No.	Oils Samples	*A. niger*	*A. westerdijkiae*	*A. flavus*	*A. parasiticus*	*Penicillium* spp	*P. frequentans*	*F. oxysporum*	*A. alternata*
		Inhibition zone (mm)							
1	R04	LS	7.3±1.52	7.6±1.52	8.3±0.57	-	7.6±1.15^{a}	11.3±0.57^{a}	LG
2	R06	-	7.3±1.52	7.3±1.52	8.6±1.15	7.3±1.52^{bdefom}	8.0±1.73	13.6±2.08^{b}	LG
3	R17	-	8.3±0.57 LG^{c}	7.3±0.57^{c}	8.6±0.57	12.6±0.57^{bcem}	7.3±1.52^{c}	11.3±1.52^{c}	LG
4	R17HS	-	LG	7.3±1.52	8.0±1.73	12.6±1.15^{d}	7.3±0.57^{d}	11.3±0.57^{d}	LG
5	R17 city	LS	7.0±0.00^{ce}	-	8.3±1.52	10.3±0.57^{de}	-	11.6±0.57^{egm}	LG
6	R26	-	7.6±0.57	7.0±0.00 ^{f}	8.3±2.08	10.6±1.15^{f}	7.6±2.08	11.3±1.52^{f}	LG
7	R28B	7.3±1.52 LS	7.3±1.52	7.6±1.15	8.6±1.52	10.0±0.00^{bcdg}	7.0±1.00^{g}	13.3±0.57^{adglmo}	LG
8	R28KA	-	8.6±0.57 LG^{eh}	7.0±1.00	8.0±0.00 LS^{h}	10.3±0.57 LS^{bcdh}	11.0±1.00LS^{acdghp}	10.3±0.57 LS^{egmp}	LG
9	R28W	ND	ND	ND	ND	ND	ND	ND	ND
10	R28 HD	-	7.6±2.08	7.6±2.08	8.3±0.57	-	8.3±0.57^{h}	10.6±1.15^{lp}	LG
11	R28M	-	7.6±1.15 LG	7.3±1.52	9.3±0.57 LS^{h}	10.6±0.57 LS^{m}	8.6±1.15 LS	7.3±1.52 LS^{abcdflmp}	LG
12	R28B3	LS	7.0±0.00 LG^{ch}	7.0±1.00	8.0±1.73 LS	12.3±0.57 LS^{beghm}	7.0±0.00 LS^{hp}	7.6±1.15 LS^{abcdefglp}	-
13	R31	-	7.6±2.08	8.6±1.15	8.3±0.57	12.0±1.73^{o}	8.3±2.08	10.3±1.52^{o}	LG
14	R34	-	-	7.0±0.00^{p}	8.6±1.15	10.0±1.73	8.6±0.57^{dp}	13.6±1.15^{adop}	LG
15	R41	8.6±0.57 LS	-	8.6±0.57^{cfp}	8.6±0.57	8.6±0.57^{cdeghmo}	-	13.6±0.57^{adelmo}	LG
Amphotericin B		12.3±0.57	13.3±0.57	12.6±0.57	14.3±0.57	12.0±1.73	13.3±0.57	13.0±1.73	14.6±1.15
Miconazole		16.3±1.52	25.0±1.00	25.6±0.57	26.0±1.00	28.6±0.57	20.0±1.00	15.3±1.52	18.3±0.57

Data are reported as mean ±SD of three experiments

Values in a column followed by the same letter are significantly different from each other ($p<0.05$; ANOVA followed by Tukey HSD test)

values not followed by any letter are not significantly different from any other value in the same column

ND: Not Determined for insufficient material

LS: Low Sporulation

LG: Low Growth

Table 3b. Antimicrobial activity of Algerian rosemary essential oils as measured in the MIC assay and reported as modal results

No.	Oils Samples	*E. coli*	*S. aureus*	*C. albicans*	*S. cerevisiae*	*A. niger*		*A. westerdijkiae*		*A. flavus*		*A. parasiticus*		*Penicillium* spp		*P. frequentans*		*F. oxysporum*		*A. alternata*	
		MIC % (v,v) Time																			
		24h	24h	48h	48h	48-72h	7d	48-72h	7d	48-72h	7d	48-72h	7d	48-72h	7d	48-72h	7d	48-72h	7d	48-72h	7d
1	R04	2	1	2	2	2	>2	0.5	>2	LG	>2	2	>2	2	>2	LG	>2	1	2	LG	2
2	R06	2	1	1	0.25	2	>2	LG	>2	LG	>2	2	>2	0.5	>2	LG	>2	2	>2	LG	2
3	R17	2	2	1	1	1	>2	2	>2	LG	>2	1	>2	1	>2	0.5	1	2	2	2	>2
4	R17HS	2	2	1	0.5	2	>2	0.5	>2	LG	>2	1	>2	0.5	>2	LG	>2	2	2	LG	>2
5	R17 city	2	1	1	0.5	2	>2	0.25	>2	LG	>2	2	>2	2	>2	LG	>2	2	>2	LG	>2
6	R26	>2	2	1	0.25	-	>2	0.5	>2	LG	>2	2	>2	2	>2	LG	>2	>2	>2	LG	2
7	R28B	2	1	1	1	2	>2	2	>2	LG	>2	2	>2	1	>2	LG	>2	>2	>2	LG	2
8	R28KA	>2	1	1	1	1	>2	2	>2	LG	>2	1	>2	0.5	2	0.5	1	2	2	1	>2
9	R28W	>2	2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
10	R28 HD	1	1	1	1	2	>2	0.25	>2	LG	>2	2	>2	2	>2	LG	>2	2	2	LG	>2
11	R28M	>2	1	1	1	1	>2	2	>2	LG	>2	1	>2	0.5	2	0.5	1	>2	>2	1	>2
12	R28B3	2	2	1	1	1	>2	2	>2	LG	>2	1	>2	1	>2	1	1	>2	>2	2	>2
13	R31	2	1	1	1	2	>2	0.5	>2	LG	>2	1	>2	1	>2	LG	>2	1	2	LG	2
14	R34	>2	1	2	2	-	>2	0.5	>2	LG	>2	2	>2	2	>2	LG	>2	>2	>2	LG	2
15	R41	>2	2	1	1	2	>2	0.5	>2	LG	>2	2	>2	2	>2	LG	>2	2	2	LG	>2

ND: Not Determined for insufficient material
LG: Low Growth

observed high MIC and MBC values in oils with considerable contents of 1,8-cineole. Similarly, oil of rosemary growing wild in Azzaba, whose main constituent was 1,8-cineole, showed a moderate to strong antimicrobial activity against several bacterial strains including the Gram-negative *E. coli* [39].

Bactericidal effect of rosemary EO was observed on *Listeria monocytogenes* [40,41,42]. The antimicrobial activity is due to the chemical composition of the EOs with important compounds as α-pinene, bornyl acetate, camphor, 1,8-cineole [43]. But the effect of these compounds tested in pure form doesn't always show antifungal activity against fungal species [44]. The mode of action of essential oils on mycetes has not been determined and it has been shown that their antimicrobial activity is dependent on their hydrophobicity and partition in microbial membranes; the lipophilic character of their hydrocarbon skeleton and the hydrophilic character of their functional groups are of the main importance. The activity rank of essential oil components is as follows: phenols > aldehydes > ketones > alcohols > ethers > hydrocarbons [45]. In general, essential oils cause structural and functional damages by disrupting membrane permeability and osmotic balance of the microbial cells. In this context, phenolic terpenes play a primary role [46,47], but also other monoterpenes (hydrocarbons and oxygenated) can induce significant effects [48,49].

Radical scavenger power

The radical scavenger power of the Algerian rosemary oils under study has been investigated using the DPPH assay. All the samples tested showed moderate antioxidant activity, being SC_{50} values in the range 120.4-326.1 µL/mL (Table 4), on the basis of the comparison with the results of similar studies on essential oils from Lamiaceae aromatic plants and tested with the same DPPH model system [37,38,39,50,51]. These finding are in agreement with many papers reported in literature which evidence the antioxidant activity of the chemicals (as chemical classes or pure compounds) contained in rosemary oils. However, no evident correlation exists between the antioxidant efficacy of these oils and their total and relative content of monoterpene hydrocarbons, oxygenated monoterpenes and sesquiterpenes. This confirms previous evidences that the biological effects of essential oils is the result of a synergism between all constituents, as the activity of the main components is modulated by other minor compounds endowed with any biological activity or also possessing antagonistic effects [52].

Conclusions

Essential oils of aromatic plants and herbs are natural products that are gaining interest as food additives and widely accepted by consumers because of their relatively low toxicity, high volatility, transient nature and biodegradability. Herein

Table 4. Antioxidant activity measured by the DPPH test of the samples of Algerian rosemary essential oils; trolox was used as positive control [a]

No.	sample	DPPH assay SC_{50} (µL/mL)	No.	Sample	DPPH assay SC_{50} (µL/mL)
1	R04	120.4±2.21 [a]	9	R28W	223.2±11.20 [bcfnl]
2	R06	200.1±3.14 [bl]	10	R28HD	207.8±0.62 [bl]
3	R17	259.0±15.95 [c]	11	R28 M	326.1±15.40 [m]
4	R17HS	195.4±2.11 [b]	12	R28B3	260.0±14.91 [chn]
5	R17city	136.5±7.30 [ae]	13	R31	143.2±8.25 [aeo]
6	R26	219.9±2.12 [cfn]	14	R34	163.2±1.89 [o]
7	R28B	176.8±15.92 [beflo]	15	R41	151.23±13.96 [aeo]
8	R28KA	313.1±11.85 [hm]		Trolox	185.21±7.23

[a] Data are expressed as mean scavenging concentrations (SC_{50}) and SD. of three independent experiments Values followed by the same letter are not significantly different from each other ($p > 0.05$)

we report the chemical characterization of 15 Algerian *R. officinalis* L. biotypes essential oils, whose composition may be related to the geographic origin. Besides antioxidant properties, our applied EO concentrations exhibit antimicrobial effect on both tested bacteria and, more interestingly, on food-contaminating fungi (*Aspergillus*, *Fusarium* and *Penicillium* spp.). Our findings could substantiate the use of these Algerian rosemary essential oils as alternatives to synthetic fungicides, in particular to improve food safety by preventing pre- and post-harvest infections and lowering the load of spores also in the storage atmosphere and on surfaces.

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Disclosure statement

No potential conflict of interest was reported by the authors.

References

1. **Erickson, M.C., and Doyle, M.P. (2017).** The challenges of eliminating or substituting antimicrobial preservatives in foods. Ann. Rev. Food Sci. Technol. 8: 371-390.
2. **Vukovic, N., Milosevic, T., Sukdolak, S. and Solujic, S. (2007).** Antimicrobial activities of essential oil and methanol extract of *Teucrium montanum*. eCAM. 4(S1): 17-20.
3. **Celiktas, O.Y., Kocabas, E.E.H., Bedir, E., Sukan, F.V., Ozek, T. and Baser, K.H.C. (2007).** Antimicrobial activities of methanol extracts and essential oils of *Rosmarinus officinalis*, depending on location and seasonal variations. Food Chem. 100: 553-559.
4. **Charles, D.J. (2013).** Antioxidant Properties of Spices, Herbs and Other Sources, 495 DOI 10.1007/978-1-4614-4310-0_48, Springer Science+Business Media, New York.
5. **Kennedy, D.O. and Scholey, A.B. (2006).** The psycopharmacology of European herbs with cognition-enhancing properties. Curr. Pharm. Des. 12: 4613-4623.
6. **Adams, M., Gmünder, F. and Hamburger, M. (2007).** Plants traditionally used in age related brain disorders - A survey of ethnobotanical literature. J. Ethnopharm. 113: 363-381.
7. **Orhan, I., Aslan, S., Kartal, M., Sener, B. and Baser, H.C.K. (2008).** Inhibitory effect of Turkish *Rosmarinus officinalis* L. on acetylcholinesterase and butylcholinesterase enzymes. Food Chem. 108: 663-668.
8. **Dias, T.R., Bernardino, R.L., Meneses, M.J., Sousa, M., Alves, M.G., Silva, B.M. and Oliveira, P.F. (2016).** Emerging potential natural products as an alternative strategy to pharmacological agents used against metabolic disorders. Curr. Drug Metab. 17(6): 582-597.
9. **Machado, D.G., Bettio, L.E.B., Cunha, M.P., Capra, J.C., Dalmarco, J.B., Pizzolati, M.G. and Rodrigues, A.L.S. (2009).** Antidepressant-like effect of the extract of *Rosmarinus officinalis* in mice: involvement of the monoaminergic system. Progr. Neuro-Psychopharm. Biol. Psychiatry. 33: 642-650.
10. **Aggarwal, B.B., Kunnumakkara, A.B., Harlkumar, K.B., Tharakan, S.T., Sung, B. and Anand, P. (2008).** Potential of spice-derived phytochemicals for cancer prevention. Planta Med. 74: 1560-1569.
11. **Altinier, G., Sosa, S., Aquino, R.P., Mencherini, T., Della Loggia, R. and Tubaro, A. (2007).** Characterization of topical anti-inflammatory compounds in *Rosmarinus officinalis* L. J. Agric. Food Chem. 55: 1718-1723.
12. **Peng, C.H., Su, J.D., Chyau, C.C., Sung, T.Y., Ho, S.S., Peng, C.C. and Peng, R.Y. (2007).** Supercritical fluid extracts of rosemary leaves exhibit potent anti-inflammatory and anti-tumor effects. Biosci. Biotech. Bioch. 71: 2223-2232.
13. **Askun, T., Tumen, G., Satil, F. and Ates, M. (2009).** *In vitro* activity of methanol extracts of plants used as spices against *Mycobacterium tuberculosis* and other bacteria. Food Chem. 116:

289-294.

14. **Shan, B., Cai, Y.Z., Brooks, J.D. and Corke, H. (2007).** The *in vitro* antibacterial activity of dietary spice and medicinal herb extracts. Int. J. Food Microbiol. 117: 112-119.
15. **Weckessera, S., Engela, K., Simon-Haarhausa, B., Wittmerb, A., Pelzb, K. and Schempp, C.M. (2007).** Screening of plant extracts for antimicrobial activity against bacteria and yeasts with dermatological relevance. Phytomedicine. 14: 508-516.
16. **Naemura, A., Ura, M., Yamshita, T., Arai, R. and Yamamoto, J. (2008).** Long-term intake of rosemary and common thyme herbs inhibits experimental thrombosis without prolongation of bleeding time. Thrombosis Res. 122: 517-522.
17. **Botsoglou, N.A., Taitzoglou, I.A., Botsoglou, E., Zervos, I., Kokoli, A., Christakia, E. and Nikolaidisc, E. (2009).** Effect of long-term dietary administration of oregano and rosemary on the antioxidant status of rat serum, liver, kidney and heart after carbon tetrachloride-induced oxidative stress. J. Sci. Food Agric. 89: 1397-1406.
18. **Napoli, E., Siracusa, L., Saija, A., Speciale, A., Trombetta, D., Tuttolomondo, T., La Bella, S., Licata, M., Virga, G., Leone, R., Leto, C., Rubino, L. and Ruberto, G. (2015).** Wild Sicilian Rosemary: Phytochemical and morphological screening - antioxidant activity evaluation of extracts and essential oils. Chem. Biodiv. 12(7): 1075-1094.
19. **Posadas, S.J., Caz, V., Largo, C., De la Gándara, B., Matallanas, B., Reglero, G. and De Miguel, E. (2009).** Protective effect of supercritical rosemary extract, *Rosmarinus officinalis*, on antioxidants of major organs of aged rats. Exp. Geront. 44: 383-389.
20. **Suhai, M. (2006).** Spice antioxidants isolation and their antiradical activity: a review. J. Food Comp. Anal. 19: 531-537.
21. **Yanishlieva, N.V., Marinova, E. and Pokorny, J. (2006).** Natural antioxidants from herbs and spices. Eur. J. Lipid Sci. Tech. 108: 776-793.
22. **Bozin, B., Mimica-Dukic, N., Samojlik, I. and Jovin, E. (2007).** Antimicrobial and antioxidant properties of rosemary and sage (*Rosmarinus officinalis* L. and *Salvia officinalis* L.; Lamiaceae) essential oils. J. Agric. Food Chem. 55: 7879-7885.
23. **Carrubba, A., La Torre, R., Piccaglia, R. and Grandi, S. (2006).** Chemical and botanical characterization of a *Rosmarinus officinalis* biotype from Sicily. Acta Hort. 723: 197-202.
24. **Angioni, A., Barra, A., Ceretti, E., Barile, D., Coïsson, J.D., Arlorio, M., Dessi, S., Coroneo, V. and Cabras, P. (2004).** Chemical composition, plant genetic differences, antimicrobial and antifungal activity investigation of the essential oil of *Rosmarinus officinalis* L. J. Agric. Food Chem. 52: 3530-3535.
25. **Diab, Y., Auezova, L., Chebib, H., Chalchat, J.C. and Figueredo, G. (2002).** Chemical composition of Lebanese rosemary (*Rosmarinus officinalis L.*) essential oil as a function of the geographical region and the harvest time. J. Essent. Oil Res. 14: 449-452.
26. **Porte, A., De, O., Godoy, R.L., Lopes, D., Koketsu, M., Goncalves, S.L. and Torquillo, H.S. (2000).** Essential oil of *Rosmarinus officinalis* L. (rosemary) from Rio de Janeiro, Brazil. J. Essent. Oil Res. 12: 577-580.
27. **Serrano, E., Palma, J., Tinocco, T., Venancio, F. and Martins, A. (2002).** Evaluation of the essential oils of rosemary (*Rosmarinus officinalis* L.) from different zones of "Alentejo" (Portugal). J. Essent. Oil Res. 14: 87-92.
28. **Boutekedjiret, C., Bentahar, F., Belabbes, R. and Bessiere, J.M. (2003).** Extraction of rosemary essential oil by steam distillation and hydrodistillation. Flavour Fragr. J. 18: 481-484.
29. **Hendel, N., Larous, L., Sari, M., Boudjelal, A. and Sarri, D.J. (2012).** Place of Labiates in folk medicine of the area of M'sila (Algeria). Glob. J. Res. Med. Plants & Indig. Med. 1: 315-322.
30. **Adams, R.P. (2007).** Identification of Essential Oil Components by Gas Chromatography/Mass

Spectrometry. Allured Publ. Corp.: Carol Stream, IL, USA.
31. **Boussahel, S., Speciale, A., Dahamna, S., Amar, Y., Bonaccorsi, I., Cacciola, F., Cimino, F., Donato, P., Ferlazzo, G., Harzallah, D. and Cristani, M. (2015).** Flavonoid profile, antioxidant and cytotoxic activity of different extracts from Algerian *Rhamnus alaternus* L. bark. Pharmacogn. Mag. 11(Suppl 1): S102-S109.
32. **CLSI - Clinical Laboratory Standards Institute, (2009).** Quality Manual, Document M07-A6, Wayne, PA, USA.
33. **CLSI - Clinical Laboratory Standards Institute, (2009).** Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi. Approved standard. NCCLS Document M38-A, Wayne, PA, USA.
34. **European Pharmacopoeia (2008).** Rosemary oil: Essential oil of *Rosmarinus officinalis* L. Spanish type, Moroccan and Tunisian type. EP 1846: 1228-1229.
35. **Meziane-Assami, D., Tomao, V., Ruiz, K., Meklati, B.Y. and Chemat, F. (2013).** Geographical differentiation of rosemary based on GC/MS and Fast HPLC analyses. Food Anal. Meth. 6: 282-288.
36. **Zermane, A., Larkeche, O., Meniai, A.H., Crampon, C. and Badens, E. (2016).** Optimization of Algerian rosemary essential oil extraction yield by supercritical CO_2 using response surface methodology. C.R. Chim 19: 538-543.
37. **Hendel, N., Larous, L. and Belbey, L. (2016).** Antioxidant activity of rosemary (*Rosmarinus officinalis* L.) and its *in vitro* inhibitory effect on *Penicillium digitatum*. Int. Food. Res. J. 23: 1725-1732.
38. **Outaleb, T., Hazzit, M., Ferhat, Z., Baaliouamer, A., Yekkour, A., Zitouni, A. and Sabaou, N. (2015).** Composition, antioxidant and antimicrobial activities of Algerian *Rosmarinus officinalis* L. extracts. J. Essent. Oil Bearing Plants 18: 654-665.
39. **Boughendjioua, H. and Boughendjioua, Z. (2017).** Investigation of antibacterial and antioxidant activities of rosemary essential oil from Algeria. Res. J. Pharm. Biol. Chem. Sci. 8: 1425-1434.
40. **Aureli, A., Costantini, A. and Zolea, S. (1992).** Antimicrobial activity of some plant essential oils against *Listeria monocytogenes*. J. Food Prot. 55: 344-348.
41. **Dimitrijevic, S.I., Mihajlovski, K.R., Antonovic, D.G., Milanovic-Stevanovic, M. and Mijin, D.Z. (2007).** A study of the synergistic antilisterial effects of a sub-lethal dose of lactic acid and essential oils from *Thymus vulgaris* L., *Rosmarinus officinalis* L. and *Origanum ulgare* L. Food Chem. 104: 774-782.
42. **Giarratana, F., Muscolino, D., Ragonese, C., Beninati, C., Sciarrone, D., Ziino, G., Mondello, L., Giuffrida, A. and Panebianco, A. (2016).** Antimicrobial activity of combined thyme and rosemary essential oils against *Listeria monocytogens* in Italian mortadella packaged in modified atmosphere. J. Essent. Oil Res. 28: 467-474.
43. **Kocie-Tamackov, S.D. and Dimie, G.R. (2013).** Antifungal activity of essential oils in the control of foodborne fungi growth and mycotoxin biosynthesis in food. In A. Méndez-Vilas, ed. Microbial pathogens and strategies for combating them: science, technology and education, Formatex Research Center, Spain, 2: 838-849.
44. **Kordali, S., Cakir, A., Mavi, A., Kilic, H. and Yildirim, A. (2005).** Screening of chemical composition and antifungal and antioxidant activities of the essential oils from three Turkish *Artemisia* species. J. Agric. Food chem. 53(5): 1408-1416.
45. **Kalemba, D.A.A.K. and Kunicka, A. (2003).** Antibacterial and antifungal properties of essential oils. Curr. Med. Chem. 10(10): 813-829.
46. **Palou, L., Smilanick, J.L. and Droby, S. (2008).** Alternatives to conventional fungicides for the control of citrus postharvest green and blue moulds. Stewart Posthar. Rev. 4(2): 1-16.
47. **Prakash, B., Kedia, A., Mishra, P.K. and Dubey, N.K. (2015).** Plant essential oils as food

preservatives to control moulds, mycotoxin contamination and oxidative deterioration of agrifood commodities - Potentials and challenges. Food Control. 47: 381-391.

48. **Cristani, M., D'Arrigo, M., Mandalari, G., Castelli, F., Sarpietro, M.G., Micieli, D., Venuti, V., Bisignano, G., Saija, A. and Trombetta, D. (2007).** Interaction of four monoterpenes contained in essential oils with model membranes: implications for their antibacterial activity. J. Agric. Food Chem. 55: 6300-6308.
49. **Trombetta, D., Castelli, F., Sarpietro, M.G., Venuti, V., Cristani, M., Daniele, C., Saija, A., Mazzanti, G. and Bisignano, G. (2005).** Mechanisms of antibacterial action of three monoterpenes. Antimicrob. Agents Chemother. 49: 2474-2478.
50. **Sari, M., Biondi, D.M., Kaâbeche, M., Mandalari, G., D'Arrigo, M., Bisignano, G., Saija, A., Daquino, C. and Ruberto, G. (2006).** Chemical composition, antimicrobial and antioxidant activities of the essential oil of several populations of Algerian *Origanum glandulosum* Desf. Flavour Fragr. J. 21: 890-898.
51. **Tomaino, A., Cimino, F., Zimbalatti, V., Venuti, V., Sulfaro, V., De Pasquale, A. and Saija, A. (2005).** Influence of heating on antioxidant activity and the chemical composition of some spice essential oils. Food Chem. 89: 549-554.
52. **Horvathova, E., Navarova, J., Galova, E., Sevcovicova, A., Chodakova, L., Snahnicanova, Z., Melusova, M., Kozics, Z. and Slamenova, D. (2014).** Assessment of antioxidative, chelating, and DNA337 protective effects of selected essential oil components (eugenol, carvacrol, thymol, borneol, eucalyptol) of plants and intact *Rosmarinus officinalis* oil. J. Agric. Food Chem. 62: 6632-6639.