PAPER

CHEMICAL CHARACTERIZATION AND BIOACTIVE POTENTIAL OF ESSENTIAL OIL ISOLATED FROM *RHANTERIUM SUAVEOLENS* DESF. SPECIES GROWING IN TUNISIAN ARID ZONE

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ABSTRACT

The purpose of this work is to assess the antioxidant and the antibacterial activities of essential oil from flowers of *Rhanterium suaveolens* Desf. and to investigate its chemical composition. The GC-MS analysis revealed the identification of a total of thirty-one compounds representing 98.4% of the total oil. Spathulenol (18.3%), carvacrol (12.1%), linalool (9.4%), α-terpineol (7.10%), α-terpinolene (6.3%) and pinocarvone (5.6%) were identified as major constituents. The tested oil exhibited weak activities in both DPPH and ABTS radical scavenging assays and ferric reducing power test. However, it showed a good lipid peroxidation activity using the β-carotene/linoleic acid assay with an IC_{50} value of 26.20±1.01 μg/mL. In addition, the highest antibacterial effect was recorded against *Staphylococcus aureus* ((MIC=37.5 μg/mL). These findings show that essential oil of *R. suaveolens* flowers can be used as a promising source of natural food and drug preservatives.

Keywords: antioxidant, antibacterial, essential oil, *Rhanterium suaveolens*, chemical composition

1. INTRODUCTION

Food preservatives are usually used to extend the shelf life of food products and to limit their deterioration caused by oxidation and growth of foodborne pathogens (RUSSELL and GOULD, 2003). As harmful effects caused by the extensive use of chemical preservatives and the increase of microbial resistance to a wide number of antibacterial drugs (SHAN *et al.*, 2007), the search of new bioactive substances with interesting biological activities is required. In this purpose, several studies have been carried out for the prospection of new products derived from plants and their potential use as ingredients in food and pharmaceutical industries (BEN SALAH *et al.*, 2019).

Essential oils are natural products known for their multi-propose applications (DE MARTINO *et al.*, 2015). They have shown a big interest as agents with several healthy-promoting activities such as antibacterial, antioxidant, anti-carcinogenic and antimutagenic properties (GUTIERREZ *et al*., 2009). Therefore, their investigation proves to be a relevant choice in order to limit the use of chemical or synthetic preservatives and minimize their toxic effect (CAILLET and LACROIX, 2007). The use of essential oils can improve food safety and protect our body against bacteria causing food poisoning (ULTEE *et al*., 2000). In fact, many studies have demonstrated the potential use of essential oils as natural antimicrobial agents in cheese-making industry (KHORSHIDIANA *et al*., 2018). As an alternative of specific applications, the essential oils can also be prepared in a large number of formulations, which can be used in food preservation. Recently, GIRARDI *et al.* (2018) reported that the application of microencapsulated *Peumus Boldus* essential oil was useful to prevent peanut deterioration caused by food spoilage microorganisms. This biological potential is mainly attributed to the presence of several constituents such as oxygenated derivatives and terpenoids (ABERRANE *et al.*, 2019; BIDA *et al*., 2019).

Tunisian flora is characterized by a wide variety of aromatic and medicinal species producing several bioactive substances with multiple interests (SALEM *et al.*, 2018). However, only few of these species have been investigated for their antioxidant and antibacterial potential. For example, *Rhanterium suaveolens* Desf. from the *Asteraceae* family is an endemic species from North Africa growing in Algerian Sahara (QUEZEL and SANTA, 1963) and arid zone of Tunisia (CHAIEB and BOUKHRIS, 1998). Three species of the genus *Rhanterium*; namely, *R. epapposum* Oliver, *R. adpressum* Coss. & Durieu and *R. suaveolens* Desf. have been reported in literature. *R. suaveolens* commonly known as "Arfadj" is a forage plant, grazed on by sheep and camel in the desert. It is used by the local population in the production of cheese and in folk medicine as an antidiuretic (HAMIA *et al.*, 2013).

To the best of our knowledge, only few studies have been conducted on the phytochemistry of the *R. suaveolens* essential oil (RSEO) and information on its biological activities, particularly, antioxidant potential, are still scarce in literature. Therefore, the main purpose of this study was to investigate the chemical profile of essential oil collected from the flowers of *R. suaveolens* growing in arid zone of Tunisia and to evaluate its antioxidant and antibacterial activities.

2. MATERIALS AND METHODS

2.1. Plant material

Flowers of *R. suaveolens* were collected during the flowering period in April 2014 from a single population of this species growing in Gorthab from the Tataouine region situated in the South East of Tunisia. Taxonomic identification of the plant material was confirmed by a local botanist at the Institute of Arid Zone Research in Medenine (Tunisia). A voucher specimen (IRABS1865) was prepared and deposited in the Herbarium of the Laboratory of Pastoral Ecology. The collected plant material was cleaned and then air-dried at room temperature for eight to ten days. .The dried flowers were ground to powders and stored in air-tight glass.

2.2. Essential oil extraction

Air dried flowers (100 g) of *R. suaveolens* were subjected to hydrodistillation in a Clevenger-type apparatus for 3h. The obtained oil was dried over anhydrous sodium sulfate (Na_2SO_4) to remove water traces and stored in amber glass vials at 4°C. The oil yield (%) was expressed as volume of essential oil vs. dry weight basis (v/w).

2.3. Gas Chromatography/Mass spectrometry (GC-MS) analysis

The GC-MS analysis of the essential oil was carried out using an Agilent 6890N Network GC system combined with Agilent 5975 B Inert MSD detector (quadrupole) with electron impact ionization (70 eV). AHP-5MS (5% phenyl methyl siloxane) column (30 m×0.25 mm i.d, film thickness 0.25 mm). The analysis was performed using helium (purity > 99.99 vol.%) as a carrier gaz at a flow rate of 1.0 $mL.min^{-1}$. The column temperature was programmed to rise from 50 to 280°C at a rate of 7 °C/min. Injector and detector temperatures were maintained at 220 and 240°C, respectively. Essential oil (1 μL) was injected in a split mode ratio of 1:10. Scan time and mass range were 2.2 s and 50–550 m/z, respectively.

2.4. Identification of the essential oil constituents

Identification of the *R. suaveolens* essential oil (RSEO) components was based on their linear retention indices (RIs) and comparison of their mass spectra with those of the computer library (Wiley 275 library and NIST98 database/ChemStation data system) provided by the instrument software and MS literature data (JOULAIN *et al.*, 2001; ADAMS, 2001). RIs were calculated using n-alkane series (C_6–C_{22}) analysed under the same GC–MS conditions as for the samples.

2.3. Antioxidant assays

2.3.1 Scavenging effect on DPPH (2,2-diphenyl-l-1-picrylhydrazil) radical

The DPPH assay was estimated as described by DHAOUADI *et al*. (2014), with slight modifications. Different concentrations of the RSEO were prepared in pure methanol, then 50 μL of each of them were added to 950 μL of a 40 μmol/L (v/v) DPPH methanolic solution in methanol. After vigorous shaking, the resulting mixtures were left in the dark

at room temperature for 30 min. The absorbance of the resulting solutions was measured at 517 nm. And the radical scavenging ability of RSEO was measured as shown below:

$$DPPH\ scavenging\ effect\ (\%) = \frac{Ao - At}{Ao} \times 100$$

Where $A0$ is the absorption of the control sample after 30 min and At is the sample absorption after 30 min. The antioxidant activity was expressed as IC50 value (mg/mL).

2.3.2 Scavenging effect on ABTS (2,20 azinobis-3-ethylbenzthiazoline-6- sulphonic acid) radical cation

The ABTS+ assay was performed according to a slight modified version of the method described by TUBEROSO *et al*. (2007). The radical cation was produced by mixing the ABTS+ solution (7 mmol/L) with potassium persulfate aqueous solution (2.45 mmol/L). The ABTS+ solution was kept in the dark at room temperature for 12-16 h, then, was diluted with phosphate buffer to the absorbance of 0.7±0.02 at 734 nm. Different concentrations of RSEO were prepared in methanol. To 50 μL of each test concentration, 950 μL of diluted ABTS solution were added. The resulting mixtures were allowed to incubate in the dark for 10 min at room temperature. The absorbance of the mixtures was recorded at 734 nm. The antioxidant activity was calculated as follows:

$$Inhibition\ (\%) = \frac{1 - (\mathrm{A} - \mathrm{B})}{\mathrm{C}} \times 100$$

where, A is the absorbance of the mixture containing the sample, B is the absorbance of the blank reagent and C is the absorbance of the blank sample.The concentration providing 50% of inhibition (IC_{50}) was calculated using a calibration curve in the linear range by plotting the extract concentration.

2.3.3 Reducing power assay

The reducing power of the RSEO was assessed following the method described by SINGH *et al.* (2012). One mL of phosphate buffer (0.2 M 'w/v', pH 6.6) and 1 mL of potassium ferricyanide [$K_3Fe(CN)_6$], 1% 'w/v' was mixed with 1 mL of different concentrations of RSEO (10, 20, 30, 40 and 50 mg/mL). The obtained mixtures were incubated at 50°C for 20 min. Then 1 ml of trichloroacetic acid (TCA) (10% 'w/v') was added. The resulting mixtures were revolved at 3000 rpm for 10 min. The supernatant was recovered and mixed with 1.5 mL of distilled water and 150 μL of $FeCl_3$ (0.1% 'w/v'). The absorbance was measured at 700 nm and the butylated hydroxyanisole (BHA) was used as standard. The result was expressed as IC_{50} (mg/mL).

2.3.4 Lipid peroxidation activity

The lipid peroxidation activity of RSEO was carried out by β-carotene/linoleic method according to DAPKEVICIUS *et al.* (1998), which is based on the inhibition of the products resulting from the oxidation of linoleic acid. A stock solution of β-carotene/linoleic acid was prepared by mixing 200 mg of Tween 40, 0.5 mg of β-carotene, 25 μL of linoleic acid and 1 mL of chloroform. After chloroform evaporation, under low pressure at 40°C, 100

mL of oxygenated distilled water were added to the mixture with vigorous shaking. An aliquot of the resulting solution (2.5 mL) was dispersed to test tubes and 0.5 mL of prepared sample with different concentrations (5-40 μg/mL) in methanol and water were added. The obtained emulsion was incubated for 2 h at 50°C. Two controls were prepared, one with the standard BHA (positive control) and the other without BHA or extract (blank). The absorbance of each sample was immediately measured at 490 nm after 30 min, 60 min, 90 min and 120 min.
The bleaching rate R of β-carotene was determined according to the following equation:

$$R = \frac{\ln(\frac{A}{B})}{T}$$

Were ln=natural log, A=absorbance at time 0, B=absorbance at time T (30 min, 60 min, 90 min and 120 min). Antioxidant activity was calculated in terms of inhibition percentage using the following equation:

$$Antioxidant\ activity\ (\%) = \frac{(Rcontrol - Rsample)\times 100}{Acontrol}$$

Results were expressed as IC_{50} value (μg/mL).

2.4. *In vitro* evaluation of antibacterial activity

2.4.1 Tested bacterial strains and growth conditions

The antibacterial activity of RSEO was tested against a range of bacterial strains collected from American Type Culture Collection (ATCC, Rockville). Gram-positive: *Staphylococcus aureus* (ATCC 25923), *Listeria monocytogenes* (ATCC 19115) and *Bacillus cereus* (ATCC 14579) and Gram-negative: *Eescherichia coli* (ATCC 35218), *Salmonella Typhimurium* (NRLB 4420) and *Pseudomonas aeruginosa* (ATCC 27853). All bacterial strains were cultured at 37°C for 24h in Mueller-Hinon agar (MHA). The cultures were started by adjusting the bacterial suspension in broth to 0.5 Mc Farland turbidity. Then the bacterial suspension was diluted using 10 fold serial dilution method in order to obtain an inoculum of 10^8 colony-forming units (CFU) per plates (DHAOUADI *et al*., 2015).

2.4.2 Disk diffusion method

The *in vitro* antibacterial activity of RSEO was estimated using the disk diffusion method described by DHAOUADI *et al.* (2015) with slight modifications. 10 μL of RSEO were placed onto sterilized paper disc (6 mm ∅), and placed onto the inoculated agar surface. The petri dishes were placed at 4 °C for 1 h and then incubated at 37 °C for 24 h. After incubation, the diameters of the resulting inhibition zones were determined. Tests were performed in triplicate. Gentamicin (10 μg per disk) was used as positive control and sterile water as negative control.

2.4.3 Microdilution method

The antibacterial activity of RSEO was also assessed by the determination of minimum inhibitory and bactericidal concentrations (MIC and MBC) using broth microdilution method. The minimal inhibition concentration (MIC) was determined as described by GULLUCE *et al.* (2007) with slight modifications. RSEO sample previously dissolved in 10% dimethylsulfoxide (DMSO) was first diluted to the highest concentration (3 mg/mL) to be tested. The 96 well plates were prepared by dispensing into each well 95 μL of the nutrient broth and 5 μL of the inoculum. An aliquot from the stock solution of RSEO (100 μL) was added into the first well. Then, 100 μL from the serial dilutions were transferred into eleven consecutive wells. The last well containing 195 μL of nutrient broth without RSEO and 5 μL of the inoculum on each strip were used as the negative control. After that, the plates were incubated at 37°C for 24 h. All samples were screened two times against each microorganism.The MIC is defined as the lowest concentration of the sample that did not allow any visible growth of the tested bacterial strain (BEN SALAH *et al.,* 2019).
To the determination of the MBC value an aliquot (25 μl) was spreaded onto MHA plates and then incubated for 12–16 h at 37°C. The determination of surviving bacterial strains allowed the estimation of the MBC at 99.9 % of bacterial death (FATTOUCH *et al.,* 2007).

2.5. Statistical analysis

All experiments were repeated in triplicate and the results were reported as mean values and standard deviation (mean±SD). Significance differences between the results were performed by analysis of variance (ANOVA) using Tukey's multiple comparison tests at a level of significance set at $P<0.05$. Data analysis was performed using Minitab 18 Statistical Software (Minitab Inc., U.S.A.).

3. RESULTS AND DISCUSSION

3.1. Essential oil composition

The volatile oil extracted from *R. suaveolens* flowers has yellow color with an agreeable intense smell. Its extraction yield was about 0.23%±0.02 (volume/dry weight), which was similar to that reported by BEN SALAH *et al.* (2019) (0.22%), and was slightly, higher than that obtained from the aerial parts of Algerian *R. suaveolens* (0.14%) (CHEMSA *et al.,* 2016).
As depicted in Table 1, thirty-one components have been identified in the RSEO which represent 98.4% of the total composition. This oil contains a complex mixture dominated by oxygenated monoterpenes (46%) followed by oxygenated sesquiterpenes (23.6%) and monoterpenes hydrocarbons (17.5%). The major components of the RSEO were identified as spathulenol (18.3%), carvacrol (12.1%), linalool (09.4%), α-terpineol (7.10%), α-terpinolene (6.3%) and pinocarvone (5.6%). Compared to previous studies, our findings differ from those reported by BEN SALAH *et al.* (2019), with α-pinene (25.84 %), β-pinene (17.57 %), 1-octen-3-ol (16.23 %), camphene (12.28 %), limonene (8.03 %) and β-myrcene (5.13 %) as major compounds. Also, the composition of the Algerian *R. suaveolens* essential oil showed a significant difference in the chemical composition (CHEMSA *et al.,* 2016).

Table 1. Chemical composition of the essential oil from the flowers of *R. suaveolens* analysed by GC-MS.

No.	Compounds[a]	RI_{exp}[b]	RI_{lit}[c]	% Area	Identification methods
1	α-thujene	926	924	0.5±0.02	RI, MS
2	α-pipene	936	939	2.5±0.03	RI, MS
3	Camphene	955	956	1.8±0.01	RI, MS
4	β-pinene	981	979	1.9±0.01	RI, MS
5	β-myrcene	990	993	0.5±0.02	RI, MS
6	α-terpinene	1016	1017	2.4±0.04	RI, MS
7	Limonene	1030	1029	1.2±0.01	RI, MS
8	γ-Terpinene	1060	1059	0.4±0.01	RI, MS
9	α-terpinolene*	1088	1089	6.3±0.10[d]	RI, MS
10	Linalool*	1095	1098	9.4±0.08[c]	RI, MS
11	*Trans*-sabinol*	1140	1142	4.1±0.01[e]	RI, MS
12	p-menth-4(8)-ene	1157	1160	1.6±0.02	RI, MS
13	Pinocarvone*	1160	1164	5.6±0.21[de]	RI, MS
14	α-terpineol*	1190	1192	7.10±0.02[d]	RI, MS
15	*Trans*-carveol	1220	1217	1.5±0.01	RI, MS
16	Carvone	1242	1250	0.5±0.01	RI, MS
17	Geraniol	1255	1252	1.5±0.03	RI, MS
18	α-Thujenol	1287	1290	2.6±0.01	RI, MS
19	Carvacrol*	1298	1299	12.1±0.21[b]	RI, MS
20	*Trans*-caryophyllene	1415	1419	0.6±0.1	RI, MS
21	Aromadendrene	1437	1437	3.3±0.1	RI, MS
22	Alloaromadendrene	1463	1458	0.7±0.2	RI, MS
23	Eremophilene	1511	1512	2.4±0.1	RI, MS
24	ð-cadinene	1522	1523	0.1±0.2	RI, MS
25	α-calacorene	1541	1546	0.4±0.1	RI, MS
26	Spathulenol*	1577	1577	18.3±0.1[a]	RI, MS
27	Caryophyllene oxide*	1581	1582	4.8±0.2[de]	RI, MS
28	α-cadinol	1676	1652	0.5±0.01	RI, MS
29	Myristic acid	1762	1767	0.6±0.1	RI, MS
30	Palmitic acid methyl ester	1908	1909	0.3±0.1	RI, MS
31	Palmitic acid	1970	1968	2.9±0.02	RI, MS
	Monoterpene hydrocarbons			17.5	
	Oxygenated monoterpenes			46.0	
	Sesquiterpene hydrocarbons			7.50	
	Oxygenated sesquiterpenes			23.60	
	Others (%)			3.80	
	Total identified (%)			98.4	

[a]Compounds are listed in order of their elution from a HP-5MS column. [b]Experimental linear retention index on a HP-5MS capillary column using the homologous series of n-alkanes. [c]Linear retention index from literature. [d]Peak area of the essential oil components. [e]Compounds were identified based on their RI on HP-5MS capillary column and GC-MS data. Values are given as mean± S.D. (n=3).
*Values with different letters with in the same column indicate significant difference (p<0.05).

The presence of spathulenol, linalool and carvacrol mentioned in this work as major constituents, had never been, already, reported for the *R. suaveolens* species. The differences observed between our findings and those previsouly reported by BEN SALAH *et al.* (2019) and CHEMSA *et al.* (2016) can be attributed to the environmental, agronomic, age and geoclimatic factors (season, location, fertility regime, soil type and climate) as well as the experimental extraction conditions (BOUKHATEM *et al.*, 2014; SINGH *et al.*, 2012).

3.2. Antioxidant activity

As depicted in Figs. 1, 2 and 3, the inhibition of the DPPH and ABTS radicals, the reducing power and the inhibition of lipid peroxidation activities of the RSEO, respectively, are dose dependent.

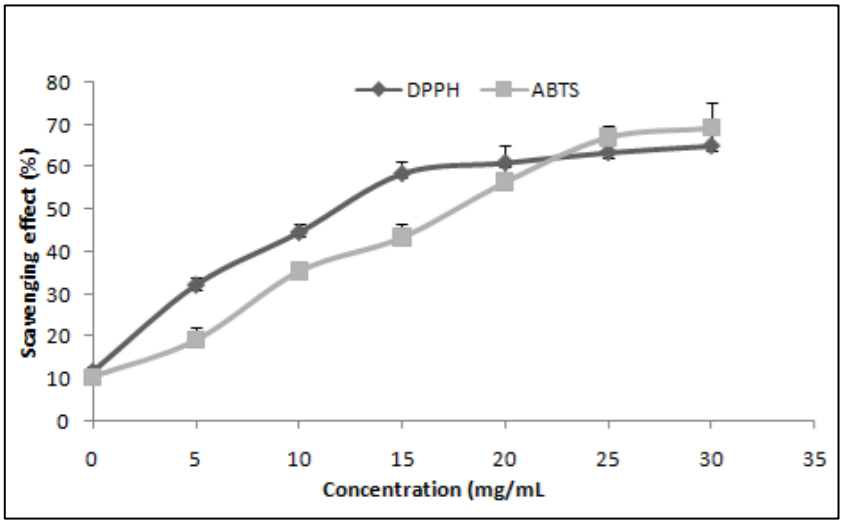

Figure 1. DPPH· and ABTS·· free radical-scavenging properties of the essential oil of the *R suaveolens* flowers. Data were presented as means±SD (n=3).

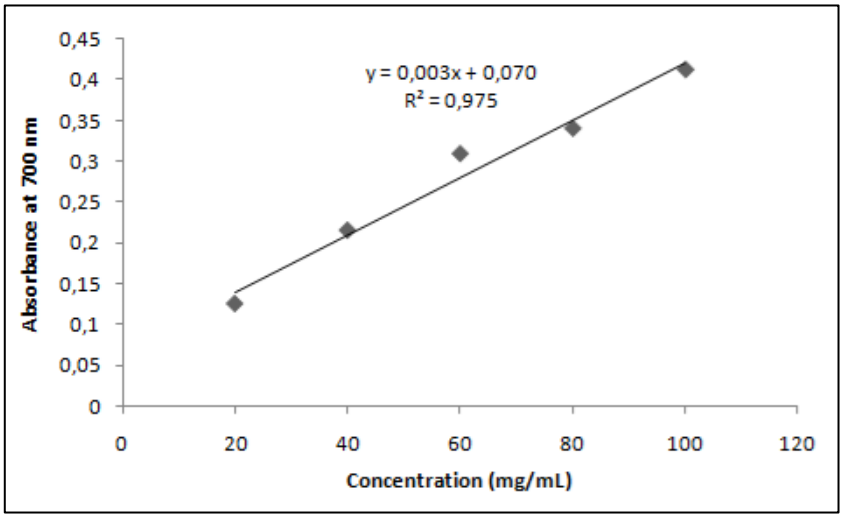

Figure 2. Reducing power of the essential oil of the *R. suaveolens* flowers.

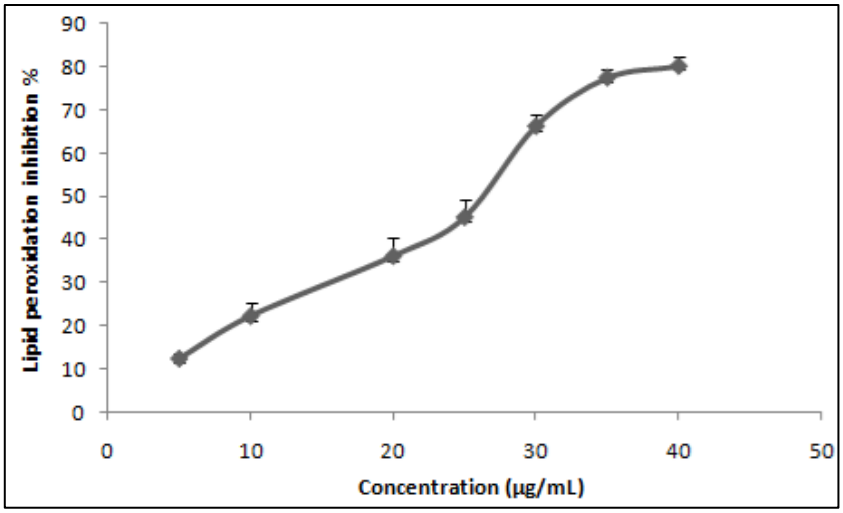

Figure 3. Antioxidant activity (%) of essential oil of the *R. suaveolens* flowers measured by β-carotene–linoleic acid method. Values expressed are means±S.D. (n=3)

As can be seen in Table 2 the IC_{50} values obtained for both DPPH and ABTS assays (11.48±0.11 mg/mL, 18.58±0.39 mg/mL, respectively) are significantly ($p<0.05$) higher than those observed for the tested standard BHA (0.041±0.002 mg/mL, 0.032±0.004 mg/mL, respectively). In other words, the activity of the selected essential oil is lower than that of the BHA standard. Our results are in agreement with the findings of the RSEO isolated from Algeria, which exhibited a weak DPPH scavenging activity (CHEMSA *et al.*, 2016).
As depicted in Table 2, the ferric reducing power of the RSEO (IC_{50}=58.95±1.21 mg/mL) was significantly ($p<0.05$) lower than that of the BHA standard (IC_{50}=0.052±0.001 mg/mL).

Table 2. Antioxidative capacities of the essential oil of the *Rhanterium suaveolens* flowers.

	IC_{50}			
	DPPH (mg/mL)	**ABTS (mg/mL)**	**Reducing power (mg/mL)**	***B*-carotene/linoleic acid (µg/mL)**
Essential oil	11.48±0.11[b]	18.58±0.39[b]	58.95±1.21[b]	26.15±1.01[b]
BHA	0.041±0.002[a]	0.032±0.004[a]	0.052±0.001[a]	5.95±0.82[a]

BHA standard was used as a reference. All the values are means±SD (Standard Deviation) of three parallel measurements. Different letters in the same column indicate a significant difference ($p<0.05$).

The inhibition of the lipid peroxidation activity of the tested essential oil was carried out using the β-carotene bleaching test. As shown in Table 2, the activity of the RSEO was lower (IC_{50}=26.15±1.01 µg/mL) than that of the synthetic standard BHA (IC_{50}=5.95±0.82 µg/mL). Also, this activity is less important than that previously reported for the essential oil of the Algerian *R. suaveolens* aerial parts with an IC_{50} of 17.97±5.40 µg/mL (CHEMSA *et al.*, 2016). Compared to the radical scavenging effects and the reducing power, the RSEO is more active on the inhibition of the lipid peroxidation. This can be, probably, due to the

high specificity of the test to lypophilic molecules (HARKAT-MADOURI *et al.*, 2015). It has been reported that the lipid peroxidation activity may be due to the richness of the tested oil on conjugated sesquiterpenoids. Indeed, these compounds can scavenge the singlet oxygen and consequently, protect the β-carotene color against bleaching, indirectly (CHEMSA *et al.*, 2016). The weak antioxidant activity observed for the RSEO can be related to its chemical composition as well as the abundance of ineffective compounds such as the monohydroxylated compounds which are unable to chelate ferrous ions (HARKAT-MADOURI *et al.*, 2015; AIDI WANNES *et al.*, 2010; DZAMI *et al.*, 2013).
The low antioxidant potential of the tested oil can, also, be attributed to the degradation of bioactive compounds during their extraction. Indeed, during hydrodistillation process, plant material is usually extracted in boiling water for a long period which could cause thermal decomposition of the thermolabile target molecules inducing, therefore, a decrease in the antioxidant capacity of the extract (BAGHERI *et al.*, 2014).

3.3. Antibacterial activity

The antibacterial activity was evaluated against six foodborne pathogens (3 Gram-positive and 3 Gram-negative), using the dilution and disk diffusion methods. As shown in Table 3, the RSEO was sensitive to all tested bacteria and exhibited a variable antibacterial activity dependent on the tested strains. *S. aureus* was the most susceptible bacteria with the largest inhibition zone (IZ=18.25±0.35 mm) followed by *L. monocytogene* (IZ =17.37±0.53 mm) and *B. cereus* (16.0±0.0 mm). However, the highest resistance to the RSEO was observed for the *S. typhimurium* with the lowest ($p<0.05$) inhibition zone (IZ=12.25±0.35 mm). Results showed that the tested essential oil was slightly more active against Gram-positive than Gram-negative bacteria. This can be explained by the complexity of their double membrane containing cell envelope, which can limit the diffusion of hydrophobic compounds through its lipopolysaccharide covering. Generally, the bacteriostatic and/or bactericide action of the plant extracts is attributed to their ability to disrupt cell membrane structures, disturb their permeability barrier and, consequently, to cause the chemiosmotic control loss (BAGAMBOULA *et al.*, 2004). As depicted in Table 3, the MIC and MBC values of the RSEO ranged from 75 to 300 μg/mL for the tested bacterial strains. The highest antibacterial activity was observed against *S. aureus* with the lowest ($p<0.05$) MIC and MBC values (37.5 μg/mL and 75 μg/mL, respectively). There are a few reports on the antibacterial activity of the *R. suaveolens* essential oil for comparison. Recently, BEN SALAH *et al.* (2019), developed the antibacterial activity of Tunisian RSEO against a broad spectrum of bacterial strains. Our findings showed discrepancies between their published data. Larger inhibition zones values were reported against *E. coli* and *P. aeruginosa* (19 mm, 23 mm, respectively). The corresponding MICs were found 230μg/mL for *E.coli* and 46 μg/mL for *P. aeruginosa* (BEN SALAH *et al.*, 2019). In addition, a moderate antibiofilm potential has been reported against six Gram positive bacteria for the essential oil collected from the aerial parts of Algerian *R. suaveolens* (CHEMSA *et al.*, 2016). The differences between our findings and those prevesiouly reported by other authors, may result from different chemical compositions and percentage content of active constituents in the tested essential oils. Factors such as the choice of bacterial strains and their sensitivity, the experimental conditions and the choice of methods used for in vitro antibacterial activity could also be related to the variation in the experimental results (SIDDIQUE *et al.*, 2017).

Table 3. Antibacterial activity of essential oil of the *R. suaveolens* flowers using disc diffusion method and determination of MIC and MBC values.

Bacterial strains	IZ (mm±SD)	Gentamicine	MIC (μg/mL)	MBC (μg/mL)
Gram positive Bacteria				
S. aureus (ATCC 25923)	18.25±0.35^{a}	34.50±0.71^{a}	37.5	75
L. monocytogenes (ATCC19115)	17.37±0.53^{a}	31.00±0.00^{b}	75	150
B. cereus (ATCC14579)	16.00±0.00^{ab}	24.00±0.00^{d}	150	300
Gram negative Bacteria				
E. coli (ATCC35218)	15.62±0.17^{b}	26.00±0.00^{c}	75	150
S. typhimurium (NRLB4420)	12.25±0.35^{d}	21.50±0.71^{e}	150	300
P. aeruginosa (ATCC27853)	14.00±0.00^{c}	32.50±0.71^{b}	150	150

IZ: The diameter of the inhibition zones (mm), including the well diameter (6 mm), are given as mean±SD (n=3). Gentamicine: is used as positive control for bacteria. Different letters in the same column indicate a significant difference (p<0.05).

The appreciable antibacterial potential of the RSEO against some bacterial strains could be related to the presence of a high amount of phytochemicals such as monoterpenes and oxygenated monoterpenes (AGGARWAL *et al.*, 2002). Effectively, many studies have proved the presence of a relationship between the chemical composition of the major components of the essential oils and the antibacterial activity (BEL-HADJ *et al.*, 2017). The major compounds identified in the RSEO such as spathulenol, carvacrol, linalool, α-terpineol, α-terpinonene and pinocarvone have not been tested for their antibacterial activity in the present study. However, some reports have approved their antibacterial properties. Indeed, a number of researchers have shown that carvacrol and linalool are well-known substances with pronounced antimicrobial activity against several pathogenic bacteria (BOZIN *et al.*, 2006). Likewise, it was found that spathulenol and linalool exhibited moderate to strong activities against several microorganisms (MAGIATIS *et al.*, 2002). In addition, it has been revealed that interactions between the constituents of some essential oils may contribute to different effects such as additive, synergistic, or antagonistic (DELAQUIS *et al.*, 2002). A study conducted on the release of the cellular materials test, showed that α-terpineol/linalool combination treatments have shown a strong effect on the release of cell constituents both from Gram-negative and Gram-positive bacteria (ZENGIN and BAYSAL. 2014).

4. CONCLUSIONS

This paper reports the chemical composition and the *in vitro* antioxidant and antibacterial properties of the essential oil collected from the *R. suaveolens* flowers. The GC–MS analysis revealed the identification of 31 constituents. Spathulenol, linalool and carvacrol identified as major compounds, were reported for the first time in the essential oil of this species. Results obtained from the β-carotene/linoleic acid bleaching assay were found to be stronger than those obtained from DPPH, ABTS and CUPRAC systems. Apart from its weak antioxidant activity, the tested essential oil has shown an interesting antibacterial activity against foodborne pathogens, especially, *Staphylococcus aureus* and *Listeria*

monocytogenes. These findings suggest the possible use of RSEO in the food industry as a potential new source of natural additives for functional and nutraceutical food applications.

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