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Article

Antioxidant, antimicrobial and phytotoxic activities of *Rhaponticum acaule* DC. essential oil

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The *in-vitro* antioxidant activity of *Rhaponticum acaule* essential oil (RaEO) was evaluated using β-carotene/linoleic acid bleaching, chelating activity, and lipid peroxidation inhibition (TBARS) assays. The antimicrobial activity of RaEO was assessed by disc diffusion and microdilution methods against 8 bacteria and 4 yeast. Finally, the allelopathic activity of RaEO on the seed germination and the shoot and root elongation of lettuce (*Lactuca sativa L.*) seedlings were investigated. According to our results, the RaEO exhibited significant antioxidant activity, similar to those of standards (BHT and ascorbic acid) with IC_{50} values of 0.042 and 0.045 mg/mL obtained by β-carotene bleaching and TBARS assays, respectively. On the other hand, despite its interesting ferrous chelating activity, RaEO possesses moderate IC_{50} value (0.35 mg/mL) as compared with that of EDTA (0.015 mg/mL). RaEO exhibited a strong antimicrobial activity against all the tested microorganisms, with IZ, MIC and MBC values being in the range of 7.67 ± 0.58 to 13.33 ± 0.58 mm, 1.25 to 5.00 and 5.00 to 10.00 mg/mL, respectively. The results reveled also that RaEO inhibited the shoot and root growth of *Lactuca sativa L.* seedlings. Our data suggested that the RaEO had pharmaceutical benefits and could be used as a potential natural herbicide resource.

Keywords: *Rhaponticum acaule* essential oil. Antioxidant activity. Antimicrobial activity. Allelopathic activity.

INTRODUCTION

Currently, although many synthetic drugs are produced, they may exhibit some side effects as well as toxic properties to human health (Zhang, Sun, Wang, 2013; Hyun, Kim, Kim, 2014). Consequently, the use of natural compounds instead of synthetic ones might be desirable, and the separation of new natural compounds is of considerable interest. In fact, natural compounds are known for being attractive due to their low cost, availability in large quantities from the raw material, biodegradability and general safety for human health and environment (Kong *et al*., 2010).

Amongst plant natural products, essential oils (EOs) have received important attention for their wide acceptance by consumers and their exploitation for multi-purpose use (Bakkali*et al*., 2008). EOs are known as complex mixtures of secondary metabolites, produced by aromatic plants and characterized by their intense odors. They contain several types of chemicals, most of which consist of low-molecular-weight mono- and sesquiterpenes. EOs are considered as good food additives. In fact, they increase the shelf-life of foods by minimizing rancidity and reducing the accumulation

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of toxic oxidation products (Kelen, Tepe 2008). Indeed, EOs can act as antioxidants 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 metal ions (Sarikurkcu *et al*., 2010).

Rhaponticum acaule DC. also known as *Leuzea acaulis L.* or *Centaurea chamaerhaponticum Ball*., is one of the most conspicuous aromatic plants of earlier spring flowering from January to March. It is a monospecific genus that belongs to the asteraceae family. It grows wild in rosette on the slopes of the hills, fields and in sandy pastures. *R. acaule* is a fragrant and perennial herb with yellow flowers and large and pinnatisect leaves. The capitula are big with fleshy and hairy receptacles. They are North African endemic species distributed in the north and central area of Tunisia (Pottier-Alapetite, 1981). In previous study, the chemical composition of R acaule aerial part essential oil was established and its inhibitory effect against xanthine oxidase, α-glucosidase, and pancreatic lipase was also evaluated to explain the mechanism of inhibition applied by the compounds of this essential oil (Mosbah *et al*., 2018). As a continuation of this previous study conducted by our research group, the aim of the present study was to investigate, for the first time, the antioxidant, antimicrobial and allelopathic activities of RaEO.

MATERIAL AND METHODS

Chemicals reagents

Thiobarbituric acid (TBA), trichloroacetic acid (TCA), iron (II) sulfate (FeSO4), β-carotene, linoleic acid, hydrochloric acid (HCl), Ferrozine and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma-Aldrich. NaCl was obtained from (CHEMI PHARMA, Tunisia). Chloroform and methanol were purchased from Merck (Darmstadt, Germany). All solvents and reagents were used in the highest purity.

Plant material and essential oil extraction

The plants were collected at the flowering stage in January 2015 in the M'Saken (Sousse) area. The plant material was identified by Prof. Fethia Harzallah Skhiri (High Institute of Biotechnology of Monastir, Tunisia). A voucher specimen (N°.Ra15) has been deposited in the Herbarium of the Laboratory of Bioressources: Biologie Integrative and Valorization, High Institute of Biotechnology of Monastir, University of Monastir, Tunisia.

The inflorescences freshly collected of *R. acaule* were cut in small pieces and thereafter submitted to hydrodistillation using a Clevenger-type apparatus as previously described. The obtained total yield of the volatile fraction was 0.018% (w/w) (Mosbah *et al*., 2018).

Antioxidant activity

The RaEO was subjected to screening for their possible antioxidant activity using three colorimetric methods: β-carotene bleaching, ferrous ions chelating and TBARS assays. The obtained results were then compared to the standard products, butylated hydroxyl toluene (BHT), EDTA and ascorbic acid, respectively. The findings are expressed as EC50 values (i.e. the concentration at which the compound provided 50% inhibition).

β-carotene *bleaching assay*

The capacity of RaEO to inhibit the bleaching of β-carotene was assessed as previously described by Condelli *et al*. (2015) with minor modifications. In the first step, 5 mg of β-carotene was dissolved in 10 mL of chloroform. 750 µL of the obtained β-carotene solution, 33 µL of linoliec acid and 225 mg of Tween 40 were carefully mixed. Then, the solvent was removed using a rotary evaporator. To create an emulsion, water (75 mL) was slowly added to the mixture and vigorously agitated. Aliquots (4 mL) of the emulsion (freshly prepared before each experiment) were transferred into test tubes containing 200 µL of RaEO serially diluted in methanol to produce a concentration ranging from 0.03 mg/mL to 1 mg/mL. Thereafter, the tubes were incubated at 50 ºC in a water bath for 2 h together with a negative control (blank), which contains the same volume of methanol, instead of the sample. The absorbance was measured for all samples at 470 nm, immediately (t = 0) and at after time of 120 min, using a spectrophotometer against a blank composed by emulsion without β-carotene. The percentage inhibition was calculated using the following formula:

$$I\% = (AS_{120min} - AC_{120min} / AC_{0min} - AC_{120min}) \times 100\%$$

Where AS_{120min} is the absorbance of the sample at t = 120 min, AC_{120min} is the absorbance of the control at t = 120 min, and AC_{0min} is the absorbance of the control at t = 0 min.

Samples were read using a blank containing the emulsion of β-carotene/linoleic acid. Essential oil concentration (mg/mL), which corresponds to 50% of inhibition (EC50), was calculated from the graph plotting antioxidant activity against essential oil concentration. Butylated hydroxyl toluene (BHT) was used as a positive standard. All the tests were carried out for three sample replications and the results were averaged.

Ferrous ions chelating assay

The iron-chelating capacity of the essential oil was assessed according to the method of Zhu *et al*. (2006) with minor modifications. For each test, 100 µL of essential oil dissolved in methanol at different concentrations varying between 0.03 mg/mL and 1 mg/mL were added to 50 µL of FeSO4 (2 mM). Thereafter, the mixtures were incubated at room temperature for 5 min followed by the addition of 100 µL of ferrozine solution (5 mM) to trigger the reaction. Finally, the test tubes were then shaken and left to stand at room temperature for 10 min. Similarly, control tubes were prepared, with the substitution of the essential oil by methanol. EDTA was used as a positive control. The absorbance of solutions was measured at 562 nm, and the chelating activity (%) was calculated as follows:

Metal chelating activity **(%)** = [(ODC + ODB − ODS) / ODC] × 100,

where ODC, ODB and ODS represent the absorbances of the control, the blank and the sample reaction tubes, respectively.

Thiobarbituric acid reactive species (TBARS) assay

The inhibition power of lipid peroxidation products was carried out using thiobarbituric acid reactive substance (TBARS) quantification in homogenized sheep brain samples as described by Bellé *et al*. (2004) with minor modifications. The sheep brain was dissected, homogenized with a Polytron in ice cold Tris-HCl buffer (20 mM, pH 7.4) and was centrifuged at 3000g for10 min. An aliquot (100 µL) of the supernatant was incubated at 37 ºC for 1 h with 200 µL from each sample concentration in the presence of 100 µL FeSO4 (10 mM) and 100 µL ascorbic acid (0.1 mM). The reaction was stopped by the addition of 500 µL trichloroacetic acid (28% w/v), followed by 380 µL thiobarbituric acid (TBA, 2%, w/v), and the mixture was then heated at 80 ºC for 20 min. After centrifugation at 3000g for 10 min to remove the precipitated protein, the color intensity of the malondialdehyde (MDA)-TBA complex in the supernatant was measured at 532 nm. The inhibition ratio (%) was calculated using the following formula:

Inhibition ratio (%) = $[(Abs_C - Abs_S)/Abs_C] \times 100\%$,

where Abs_C and Abs_S were the absorbance of the control and the sample solution, respectively.

Antimicrobial activity

Microorganisms

The test microorganisms included the following Gram-positive bacteria: *Staphylococcus aureus* ATCC 6816, *Staphylococcus epidermidis* CECT 231, and Gram negative bacteria: *Pseudomonas aeruginosa* PAO1, *Salmonella enterica* subsp. *enterica* CECT 443, *Listeria monocytogenes* CECT 933, *Vibrio parahaemolyticus* ATCC 43996, *Vibrio parahaemolyticus* CECT 511, *Vibrio vulnificus* CECT 529. On the other hand, the antifungal effect was tested against four *Candida strains* (*C.albicans* ATCC 2091, *C. parapsilosis* ATCC 22019, *C. glabrata* ATCC90030, and *C. tropicalis* 06-085).

Disc-diffusion assay

The test of antimicrobial activity was done according to the protocol described by Vuddhakul *et al*. (2007) with same modifications. For the experiments, 10 µL of the microorganisms working stocks were enriched on a tube containing 9 mL of Mueller–Hinton (MH) broth (for bacteria) and Sabouraud (SB) Chloramphenicol broth (for Candida), then incubated at 37 ºC for 24 h. After one night, the cultures were used for the antimicrobial activity of the essential oil and the optical density was adjusted to 10^7 to 10^8 CFU/mL (0.1 at OD600 for bacteria and 0.4 at OD540 for *Candida strains*). The inoculums of the respective bacteria and fungus were streaked onto MH or SB agar plates using a sterile cotton swab as recommended by the CA-SFM EUCAST 2017. sterile filter discs (diameter 6 mm, Biolife Italy) were

impregnated with 10 µL of essential oil (20 mg/mL) placed on the appropriate agar mediums. Ampicillin (10 mg/mL; 10 µL/disc) and Amphotericin B (10 mg/mL; 10 µL/disc) were used as positive reference standards.

After 24 h of incubation at 37 ºC, the diameter (mm) of the growth inhibition zone (IZ) was measured. Each experiment was performed in triplicate and the mean diameter of the inhibition zone was recorded. The results were expressed in terms of inhibition zone (IZ) of growth around each disc in millimeters as low activity (1-6 mm), moderate activity (7-10 mm), high activity (11-15 mm) and very high activity (12-20 mm) (Parveen *et al*., 2010).

Micro-well determination of MICs and MBCs/MFCs

The minimal inhibition concentrations (MICs) and the minimal bactericidal/fungicidal concentrations (MBCs/MFCs) values were determined for all bacterial/fungal strains used in this study as described by Snoussi *et al*. (2015) with minor modifications. The bacterial/fungal inoculums were prepared from 12 h broth cultures, and suspensions were spectrophotometrically adjusted to 107 CFU/mL. The RaEO was first dissolved in 10% dimethylsulfoxide (DMSO). Then, serial twofold dilutions were made in a concentration ranging from 0.008 to 20 mg/mL in 5 mL sterile glass tubes containing nutrient broth. The plates were prepared by adding 95 µL of nutrient broth and 100 µL of stock solutions of essential oil, respectively, into each well. Finally, 5 µL from each microbial suspension were added to all wells. The first well of each plate contained 195 µL of nutrient broth (Mueller Hinton broth or Sabouraud Chloramphenicol broth) without essential oil, and 5 µL of the inoculum was used as a negative control. The final volume in each well was 200 µL. The plates were, thereafter, incubated at 37 ºC during 24 h. The MIC was defined as the lowest concentration of RaEO to inhibit the growth of the microorganisms. Consequently, no visible changes were detected in the broth medium. On the other hand, the MBC/MFC values were recorded as the lowest concentration of the essential oil, which resulted in a clear fluid with no visible growth. All tests were assessed in triplicate.

Phytotoxicity assay

The inhibition potential of RaEO on the seed germination as well as the shoot and root elongation of lettuce (*Lactuca sativa L*.) seedlings were investigated. Lettuce seeds were purchased from a seed shop, disinfected for 5 min with NaCl (1%), and then rinsed with dist. H2O (Mabrouk *et al*., 2013). Three replicates, each comprising 20 seeds, were prepared for the contact tests with the essential oil, using sterile Petri dishes (90 mm diameter) lined with double-sterile filter paper (Whatman No. 2). The essential oil was dispersed as an emulsion in dist. H2O using Tween 20. Four doses of the essential oil (0.01, 0.05, 0.1, and 0.25 mg/mL) were obtained by the dilution of the emulsion with deionized H2O. The dishes were then moistened with 5 mL of essential oil at different concentrations or with 5 mL of dist. H2O, used as a negative control. Thereafter, the dishes were sealed with Parafilm, to prevent the loss of moisture and to avoid contamination, and placed in a growth chamber to allow germination in the dark at an average temperature of 23 ± 2 ºC for 7 days. As a result, the seed was considered germinated when the protrusion of the radicle became evident (Mabrouk *et al*., 2013). Tests were assessed in triplicate for each treatment and control. After 7 days, the germination percentage was determined. Then, the seedlings of L. sativa were collected and the length of each shoot and root was measured to evaluate the allelopathic activity of the RaEO. The inhibitory or stimulatory effects were calculated using the following equation, with slight modifications as described by Chung, Ahn and Yun (2001):

$$\text{Inhibition } (-)/\text{stimulation } (+)\ \% = ((EOe - Ce)/Ce) \times 100$$

where EOe (essential oil effect) is the parameter measured in the presence of essential oil and Ce (control effect) is the parameter measured in the presence of dist. H2O.

Statistical analysis

Each assay was performed in triplicate. Results were expressed as the mean values ± standard deviation (n = 3). The differences were calculated using one-way analysis of variance (ANOVA) and statistically significant differences were reported at $p < 0.05$. Data analyses were carried out using the SPSS 10.0 software.

RESULTS AND DISCUSSION

Antioxidant activity

To the best of our knowledge, this is the first study on the antioxidant effectiveness of RaEO. Firstly, the antioxidant activity of RaEO was measured by the β-carotene–linoleate system and compared with that of BHT used as a positive control (Figure 1). The results indicate that RaEO prevented the bleaching of β-carotene with an effectiveness similar to that of BHT at all the tested concentrations. The antioxidant activity values for the essential oil and BHT (both at 1 mg/mL) were found to be 82.4% and 84.5%, respectively.

As a second method, the Fe^{2+} chelation was used to evaluate the ability of RaEO in metal-chelating activity. Mixing ferrozine with the Fe^{2+} ion forms complexes, that are disrupted in the presence of chelating agents, resulted, subsequently, in a decrease in color formation (Thiansilakul, Benjakul, Shahidi, 2007). Ferrous chelating activities of RaEO at different concentrations are shown in Figure2. The results indicated that this oil was able to chelate the Fe^{2+} ion with dose dependent effect. Despite its interesting ferrous chelating activity, RaEO possesses a moderate EC50 value (0.35 mg/mL) compared with that of EDTA (0.015 mg/mL) used as a positive control.

The TBARS method is considered to be one of the best approaches to evaluate the real antioxidant behavior in foods. It is well known that lipid peroxidation is caused by the generation of free radicals from several sources including organic hydroperoxides, iron-containing compounds and redox cycling compounds. The TBARS test has been used to measure the lipid peroxidation degree. TBA reacts specifically with malondialdehyde (MDA), a secondary product of lipid peroxidation to give a red chromogen, which may be subsequently measured spectrophotometrically (Coppen, 1983). The percentage of lipid peroxidation inhibition of the essential oil and the synthetic antioxidant generally used in food industry (ascorbic acid) is summarized in Figure 3. RaEO and ascorbic acid showed similar lipid antioxidant activity at all the tested concentrations, with the same EC50 value of about 0.04 mg/mL.

To correlate the obtained results of antioxidant activity with the chemical composition of the RaEO, it is interesting to refer to the paper of Ruberto and Baratta (2000). The latter studied the antioxidant activity of 98 pure essential oil components, which represent the main class of typical compounds contained in essential oils. They reported that sesquiterpene hydrocarbons displayed a low scavenging activity, whereas monoterpene hydrocarbons exhibited a significant protective effect, with several variants due to their different functional groups. Indeed, according to the literature, the scavenging mechanism of sesquiterpenes may be due to the abstraction of a hydrogen atom from a methylene group (-CH2-) on the sesquiterpenes to form an allyl radical (Priyadarsini *et al*., 2003). Moreover, the allyl radical of the carbon radical tends to be stabilized by a molecular rearrangement or by the abstraction of a hydrogen atom from another methylene group to form a conjugated diene or other stable compounds (Espin, Soler-Rivas, Wichers, 2000). However, antioxidant activities were also reported for various essential oils isolated from other plants that belong to the asteraceae family (Laciar *et al*., 2009; Sokmen *et al*., 2004; Yayli *et al*., 2005; Matasyoh *et al*., 2007; Silvério *et al*., 2013). Probably, similar components detected in our essential oil could be responsible for these properties, such as germacrene D and β-caryophyllene (Matasyoh *et al*., 2007). The antioxidant activity observed for RaEO could be attributed to the presence of high percentages of germacrene D (49.2%), methyl eugenol (8.3%) and β-caryophyllene (5.7%) (Mosbah *et al*., 2018).

Essentially, it can be noted that the essential oils are quite complex mixtures, constituted by several tens of components. This complexity makes it often difficult to explain the activity pattern. For this reason, in many reports, the antioxidant potentials of essential oils are explained by specific concepts such as synergism, antagonism and additivity.

According to the literature, the asteraceae species are well documented as natural antioxidants (Maisuthisakul, Suttajit, Pongsawatmanit, 2007) but to the best of our knowledge, this is the first work performed on the essential oil of *R. acaule.*

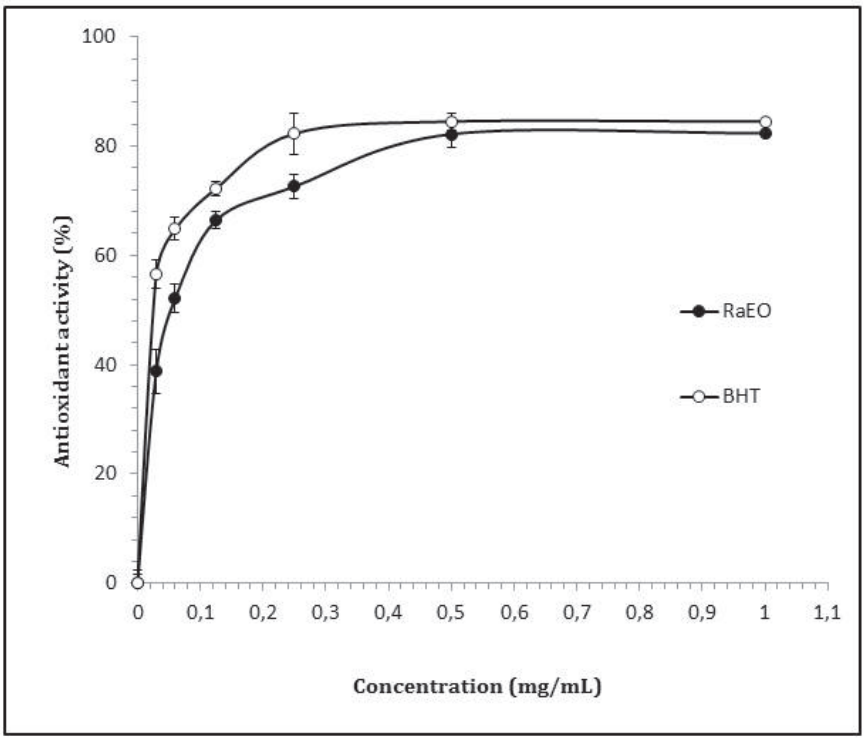

FIGURE 1 - Antioxidant activity of RaEO using β-carotene bleaching method. BHT was used as positive control. Data represent the mean (±SD) of three independent experiments.

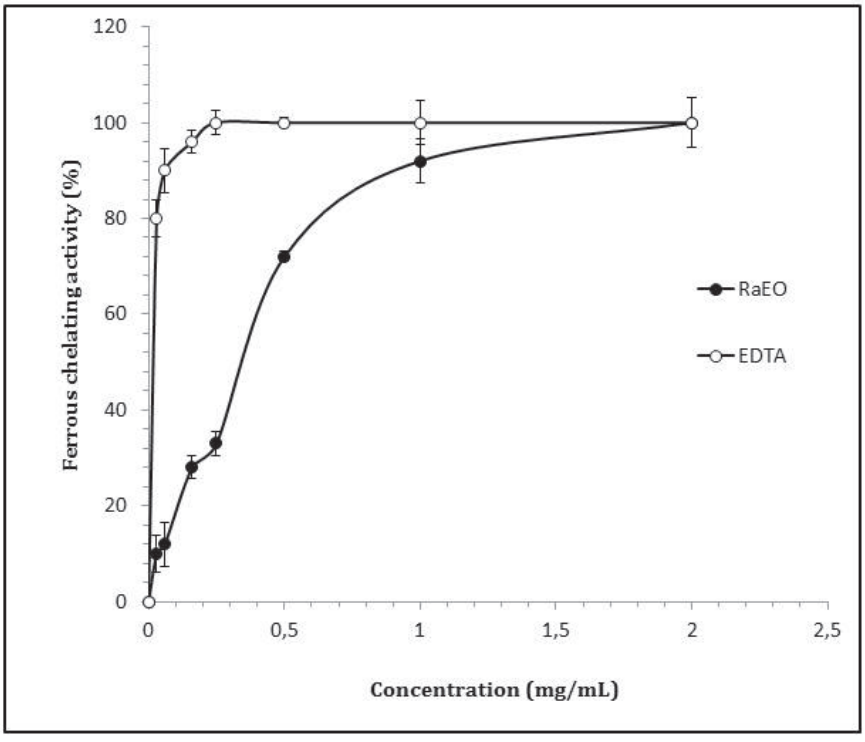

FIGURE 2 - Ferrous ions (Fe^{2+}) chelating activity of RaEO and standard antioxidant compound (EDTA). Data represent the mean (±SD) of three independent experiments.

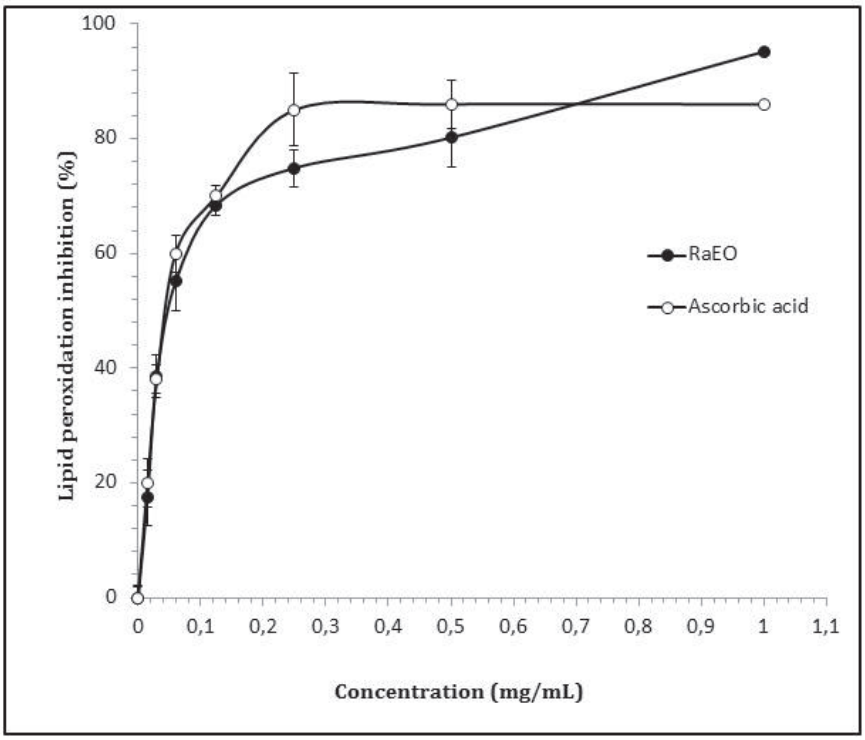

FIGURE 3 - Lipid peroxidation inhibition by RaEO using the TBARS method. Ascorbic acid was used as positive control. Data represent the mean (±SD) of three independent experiments.

Antimicrobial activity

Data on the antimicrobial activity of RaEO, reported in this work for the first time, are given as inhibition zone (IZ) diameters, MICs, MBCs and MFCs (Table I). The antimicrobial activities of RaEO were evaluated using the agar diffusion and the microdilution methods against 12 test microorganisms, including 8 bacteria (two Gram-positive and six Gram-negative) and 4 yeasts. The RaEO was active against all the microbial strains, but in different degrees (Table I). The IZ varied from 11.67 ± 0.58 to 13.33 ± 0.58 mm, with MIC values ranging from 1.25 to 5.00 mg/mL for bacterial strains, and from 7.67 ± 0.58 mm to 8.67 ± 0.58 mm with MIC from 1.25 mg/mL to 5 mg/mL for yeast strains. As depicted in Table I, P. aeruginosa PAO1 was the most sensitive bacteria with IZ value of 13.33 ± 0.58 mm, which is two-time larger than that obtained with ampicillin used as a positive control. According to the literature, the IZ measured in our study are much more important than those obtained by Boussaada *et al*. (2008) with the essential oil in the same species, but collected in different areas in Tunisia.

MBC or MFC are specified as the lowest concentration of essential oil or antibiotic at which inoculated bacteria or fungi are completely killed. As shown in Table I, these values varied from 5 to 10 mg/mL for bacteria or yeasts. Again, the values of these two parameters (MBC or MFC) are different from those reported by Boussaada *et al*. (2008); this behavior can be explained by the differences in the chemical compositions of the two essential oils.

Being the most abundant component of the RaEO, germacrene D and β-caryophyllene, are known for their antimicrobial properties (Andrews, Parks, Spence, 1980; Cimanga *et al*., 2002; Burt, 2004; Bezic, Skocibusic, Dunkic, 2005). These compounds can increase the permeability of fungal cell and membrane fluidity and it can also prevent the acidification of medium. Furthermore, terpenes can induce change in cell permeability by entering between the fatty acyl chains of the membrane lipid bilayers (Sikkema, De Bont, Poolman, 1995; Christine, Brian, Thomas, 2002). However, since essential oil consists of various major and minor constituents, the synergistic effects of some compounds should be also taken into consideration.

TABLE I - Antibacterial and antifungal activities (MIC, MBC and MFC in mg/mL) of RaEO

Strains	Essential oil			Drug		
	IZ ± DS	MIC	MBC	IZ ± DS	MIC	MBC
Bacteria				Ampicillin		
Staphylococcus epidermidis CECT 231	12.67 ± 0.58	2.50	10.00	12.33 ± 0.58	5.00	10.00
Staphylococcus aureus ATCC 6816	13.00 ± 01	5.00	10.00	35.00 ± 1	0.30	5.00
Pseudomonas aeruginosa PAO1	13.33 ± 0.58	1.25	5.00	6.67 ± 0.58	1.25	5.00
Vibrio parahaemolyticus ATCC 43996	12.00 ± 0.10	1.25	5.00	11.67 ± 0.58	5.00	10.00
Salmonella enterica subsp. *enterica* CECT 443	11.67 ± 0.58	2.50	10.00	16.00 ± 1	2.50	10.00
Listeria monocytogenes CECT 933	11.67 ± 0.58	2.50	10.00	20.00 ± 1	1.25	5.00
Vibrio parahaemolyticus CECT 511	11.67 ± 0.58	5.00	10.00	17.00 ± 1.73	2.50	10.00
Vibrio vulnificus CECT 529	13.00 ± 01	2.50	10.00	23.33 ± 0.58	1.25	5.00
				IZ ± DS	MIC	MFC
Yeasts				Amphotericin B		
Candida glabrata ATCC 90030	8.67 ± 0.58	1.25	5.00	10.33 ± 1.15	0.125	0.312
Candida albicans ATCC 2091	7.67 ± 0.58	5.00	10.00	11.33 ± 0.57	0.625	0.625
Candida parapsilosis ATCC 22019	7.67 ± 0.58	2.50	10.00	11.00 ± 1	0.625	1.25
Candida tropicalis 06-085	7.67 ± 0.58	2.50	10.00	10.33 ± 1.15	0.625	0.625

IZ: Inhibition zone around the discs impregnated with RaEO (10 mg/mL), Ampicillin (10 mg/mL) or Amphotericin B (10 mg/mL) expressed as mean of three replicates (mm ± SD).
SD: Standard deviation.
MIC: Minimal Inhibitory Concentration (mg/mL).
MBC: Minimal Bactericidal Concentration (mg/mL).
MFC: Minimal Fungicidal Concentration (mg/mL).

Phytotoxic potential of the essential oil

The phytotoxic effects of the tested essential oil are summarized in Table II. The allelopathic influence on *Lactuca sativa L.* seeds germination and seedlings growth varied according to the essential oil concentration. A significant inhibitory effect on the germination of L. sativa seeds was found (Table II). The germination percentage varied between 75 and 0% (values not reported in Table II) at the seventh day of germination. The RaEO showed very high phytotoxic effects against L. sativa, with an inhibition of seed germination of 100% at 0.25 mg/mL. Generally, essential oil exhibited germination and seedling growth inhibition that was concentration-dependent. Allelopathic effect was also obtained at the lowest concentration tested, 0.01 mg/mL (25%). The inhibition of the radical growth varied from 7.31 (at 0.01 mg/mL) to 100% (at 0.25 mg/mL) and that of the hypocotyl from 7.16 (at 0.01 mg/mL) to 100%

TABLE II - Allelopathic effects of RaEO on *Lactuca sativa L.* seedlings

Concentration (mg/mL)	Inhibitory effect compared to control (%)			
	Seed germination (% of control)	Root lenght (% of control)	Hypocotyl lenght (% of control)	Seedling dry weight (% of control)
0.25	-100 ± 0.0	-100.0 ± 0.0	-100.0 ± 0.0	-100.0 ± 0.0
0.1	-65 ± 1.5	-76.15 ± 1.0	-62.04 ± 1.8	-60.73 ± 0.4
0.05	-35 ± 1.3	-26.63 ± 1.2	-18.01 ± 1.03	-46.55 ± 0.3
0.01	-25 ± 1.2	-7.31 ± 1.25	-7.16 ± 1.14	-38.11 ± 0.25

(at 0.25 mg/mL). The highest activities were detected at 0.25 mg/mL concentration for the essential oil. The biomass production was slightly inhibited in the presence of the essential oil at 0.01 mg/mL, and the dry weight of the seedlings treated with 0.25 mg/mL from sample was highly reduced (100%).

The allelopathic effect is strongly related to the composition of essential oil and to the target species. The RaEO was efficient in inhibiting the seed germination and the seedling growth of L. sativa. The phytotoxic effect can be mainly due to toxic compounds identified in the essential oil (Boussaada *et al*., 2008). In line with our findings, few studies reported that some essential oils containing toxic compounds (e.g., limonene, caryophyllene oxide, spatulenol, etc.) also have interesting phytotoxic potentials (Chung, Ahn, Yun, 2001; Mabrouk *et al*., 2013). Furthermore, several researchers have shown that monoterpene compounds have strong inhibitory effects on seed germination of many crops and weeds. (Lopez, Bonzani, Zygadlo, 2009; Li *et al*., 2011). The bioassay results, reported here, demonstrated that the volatile oil of *R. acaule* possesses strong phytotoxic potential and could cause substantial germination reduction and seedling growth inhibition of L. sativa. Therefore, the decrease of the seed germination and the shoot length may be explained by the reduction of cell division rate and cell elongation due to the presence of the allelochemicals (Javaid, Anjum, 2006). Our findings indicate also that the inhibitory compounds present in Tunisian RaEO could be used as a potential natural herbicide resource.

CONCLUSION

The increasing interest of alternative and complementary medicine in the last decade shade light on essential oils, which represent a big class of plant secondary metabolites that possess diverse biological activity. For the *R. acaule* species, only a few studies were reported about its biological potentials. So, to the best of our knowledge, this is the first study to provide data on the antimicrobial and *in vitro* antioxidant activities of the essential oil obtained from aerial part of Tunisian *R. acaule*. From our results, the significant observed antioxidant and antibacterial effects of the RaEO may be due to the higher content of sesquiterpene hydrocarbons (74.2%) without neglecting also minor compounds which may make a significant contribution to the oil activity. Finally, we have investigated, for the first time, the allelopathic activities of *R. acaule* essential oil on *Lactuca sativa L.* seeds germination and seedlings growth. The obtained allelopathic effect may be strongly related to some toxic compounds in this essential oil (e.g., limonene, caryophyllene, etc.) which have interesting phytotoxic potentials (Chung, Ahn, Yun, 2001; Mabrouk *et al*., 2013).

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DISCLOSURE STATEMENT

The authors declare that there are no conflicts of interest.

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