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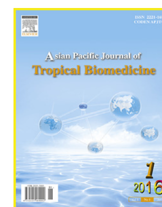

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Chemical analysis, antimicrobial and anti-oxidative properties of *Daucus gracilis* essential oil and its mechanism of action

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ABSTRACT

Objective: To evaluate the essential oils (EO) composition, antimicrobial and antioxidant power of a local plant, *Daucus gracilis* (*D. gracilis*).

Methods: The aerial parts of *D. gracilis* were subjected to hydro distillation by a Clevenger apparatus type to obtain the EO which had been analyzed by gas chromatography and gas chromatography coupled with mass spectrometry, and screened for antimicrobial activity against five bacteria and three fungi by agar diffusion method. The mechanism of action of the EO was determined on the susceptible strains by both of time kill assay and lysis experience. The minimal inhibitory concentrations were determined by agar macro-dilution and micro-dilution methods. Anti-oxidative properties of the EO were also studied by free diphenyl-2-picrylhydrazyl radical scavenging and reducing power techniques.

Results: The EO yielded 0.68 (v/w). The chemical analysis presented two dominant constituents which were the elemicin (35.3%) and the geranyl acetate (26.8%). *D. gracilis* EO inhibited the growth of *Bacillus cereus* and *Proteus mirabilis* significantly with minimal inhibitory concentrations of 17.15 μg/mL by the agar dilution method and 57.05 μg/mL and 114.1 μg/mL, respectively by liquid micro-dilution. A remarkable decrease in a survival rate as well as in the absorbance in 260 nm was recorded, which suggested that the cytoplasm membrane was one of the targets of the EO. The EO showed, also, important anti-oxidative effects with an IC_{50} of 0.002 mg/mL and a dose-dependent reducing power.

Conclusions: *D. gracilis* EO showed potent antimicrobial and anti-oxidative activities and had acted on the cytoplasm membrane. These activities could be exploited in the food industry for food preservation.

1. Introduction

Apiaceae/Umbelliferae is one of the best known families of flowering plants, which comprises 300–450 genus and 3 000–3 700 species. They are aromatic plants and have a distinctive flavor which diverse volatile compounds from the fruits and leaves. The plants of this family are occurring throughout the world, but they are most common in temperate regions [1]. Species of this family are widely distributed around the world and have a great history in the medicinal use. They are popularly used in medicine and in cooking (*Anethum graveolens*, *Angelica archangelica*, *Apium graveolens*, *Carum carvi*, *Coriandrum sativum*, *Foeniculum vulgare*), although this family also includes the most toxic species of the world causing digestive problems, neurological poisoning, even death (*Cicuta maculata*, *Conium maculatum*) [2]. The genus *Daucus* seems to have its center of dispersion in the

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Mediterranean region, particularly in the north of Africa. Outside *Daucus carota* (*D. carota*), the common core, which is grown around the world [3]. Since the plant-derived antimicrobials became a source of novel therapeutics and in order to exploit local species, we aimed to study the antimicrobial activity of the essential oil (EO) and its mechanism of action, and we tested the anti-oxidative activity of this one knowing that the two species were never studied before.

2. Materials and methods

2.1. Plant material

Aerial parts of *Daucus gracilis* (*D. gracilis*) were collected from the mountain Felfla (Skikda, Algeria) in June 2013. The plant was identified by Prof. H. Laouer (Laboratory of Natural Biological Resources, University of Sétif, Algeria) next they were freed of impurities and after that dried in the shade at room temperature.

2.2. EO extraction

Air-dried parts were cut into thin parts and were subjected to a hydro distillation for 3 h by using a Clevenger-type apparatus. The oil was stored in the refrigerator (4 °C) until use.

2.3. Analysis of the EO

Gas chromatography (GC) and GC coupled with mass spectrometry (GC–MS) analyses were carried out by using an Agilent 6890N gas chromatograph apparatus equipped with a flame ionization detector and coupled to a quadruple Agilent 5973 network mass selective detector working in electron impact mode at 70 v. The gas chromatograph was equipped with two fused silica capillary columns HP-1. Analytical parameters were the following: the carrier gas was helium at a flow rate of 1 mL/min, the oven temperature was programmed from 60 to 250 °C at 2 °C/min and held isothermal for 40 min and the injector temperature was 250 °C. The flame ionization detector temperature was set at 250 °C, and in the GC–MS analyses, temperatures of the ion source and transfer line were 170 and 280 °C, respectively. The identification of constituents was assigned on the basis of comparison of their retention indices and mass spectra with those given in the literature [4].

2.4. Antibacterial assay

2.4.1. Microbial strains

Five bacterial strains from the American Type Culture Collection (ATCC) were tested: *Acinetobacter baumanii* ATCC 19606 (*A. baumanii*), *Staphylococcus aureus* ATCC 25923 (*S. aureus*), *Bacillus cereus* ATCC 10876 (*B. cereus*), *Lysteria monocytogenes* ATCC 15313 (*L. monocytogenes*), *Proteus mirabilis* ATCC 35659 (*P. mirabilis*), and three fungi: *Aspergillus niger* 2CA936 (*A. niger*), *Aspergilus flavus* NRRL 391 (*A. flavus*) and *Candida albicans* ATCC 1024 (*C. albicans*).

2.4.2. Disc diffusion assay

A preliminary antibacterial activity of the EO was determined with the agar diffusion method by using a 6-mm diameter discs. Briefly, the Petri dishes were seeded by swabbing areas and preincubated for 1/2 h at room temperature, allowing the complete diffusion of the EO and then incubated at 37 °C for 24 h [5]. The antibacterial activity was determined by measuring of inhibition zone diameters (mm). Gentamicin was used as a positive control for bacterial strains and miconazole as a positive control for fungal strains.

2.4.3. Determination of minimal inhibitory concentration (MIC) by dilution methods

2.4.3.1. Agar dilution method

This method allows the determination of the MICs from a range of concentration of EO in agar culture media. A solution of sterilized Tween 80 in distilled water (10%) was added to an amount of EO so that the ratio EO/Tween was 80/20 (v/v). The mixture was stirred for 2–3 min to disperse in the EO stock solution (S). Next, two-fold series dilutions were made to obtain the range of dilutions. In test tubes, each containing 18 mL of sterilized agar medium and kept molten at 50 °C in a water bath, 50 μL of the solution S or various dilutions were added aseptically. After solidification of the medium, containing the EO or not (negative control), seeding of bacteria was performed on the surface by a bacterial suspension (10^5 CFU/mL) [6,7].

2.4.3.2. Broth micro-dilution method

This method involves the use of small volumes of broth dispensed into sterile plastic micro-dilution trays. A two-fold dilution of the EO volumetrically in broth was made. Then, it was dispensed into the wells so that each well contained 0.1 mL. A standardized inoculum of 5×10^5 CFU/mL was inoculated in each well. The inoculated micro-dilution trays were incubated at (35 ± 2) °C for 24 h [7].

2.4.4. Time kill assay

This method allows the characterization of the antibacterial EO activity over time. It assesses the decrease of bacteria, which are subject to a given EO concentration over several hours. A standardized suspension of 10^8 CFU/mL was diluted on 1/20. A total of 1 mL of this inoculum was introduced into 9 mL of Muller-Hinton broth-Tween 80 (0.01%, v/v) in the absence (growth control) or in the presence of a concentration corresponding to the MIC of the EO in the liquid medium. The suspension obtained contained approximately 5×10^5 CFU/mL and was maintained under stirring at 37 °C. A total of 100 μL of the suspension were removed at different time (0, 2, 4, 6, 8 and 24 h) to carry out a counting on methionine hydroxy analog agar after incubation at 37 °C for 24 h. The quantification of the number of bacterial colonies was limited to the value of 10^2 CFU/mL. Results were interpreted by a bactericidal curve representing time intervals on the abscissa axis and the number of survivors on the ordinate axis [7,8].

2.4.5. Bacterial lysis

This method determines if there is a bacteriolytic action of EO by measuring the absorbance at 620 nm [9]. Indeed, nonlysed bacteria absorb in 620 nm, so if there is a bacteriolysis, absorbance at 620 nm over time will decrease. A young bacterial suspension was standardized at 3.10^{10} CFU/mL (OD620 ~ 0.3), placed in a sterile tube in the absence (negative control) or in the presence of EO at two concentrations, one corresponding to the MIC and the other two times the MIC. Suspensions obtained were subjected to agitation. On time 0 s,

30 s, 30 min, 60 min, 90 min and 120 min, they were diluted to 1/100 and absorbencies were measured at 620 nm. The results were expressed as the relative optical density (OD_{620}) in each time interval.

2.5. Anti-oxidative assays

2.5.1. Diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging

The DPPH radical absorbs in 517 nm and the anti-oxidative activity can be determined by recording the decrease of the absorbance of the EO. A total of 50 µL of each different EO dilution were mixed with 1 250 µL of a methanolic solution of DPPH (0.004%). Absorbencies were measured after 30 min of incubation in the dark. Synthetic antioxidant, the butylated hydroxytoluene (BHT) was used as positive control. Thus the calibration curves representing the percentage of inhibition versus concentrations were performed by using Graph-pad prism program. The ability to scavenge DPPH radical is calculated as follows:

$$I\% = [(Abs_{517}\ control - Abs_{517}\ sample)/Abs_{517}control] \times 100$$

IC_{50} values were estimated by a linear regression. Values were presented at least as the mean of triplicate measures [10].

2.5.2. Reducing power

It is a technique that measures Fe^{3+}'s reduction (ferric iron) to Fe^{2+} (ferrous iron) in the presence of the EO tested. The presence of reducers in plant extracts causes the reduction of Fe^{3+} in a complex of ferricyanide to form ferrous iron (Fe^{2+}). Therefore, Fe^{2+} can be assessed by measuring the increase of the density of the green color in the reaction medium at 700 nm [11]. In a test tube containing 1 mL of the EO, 2.5 mL of phosphate buffer was added (0.2 mol/L, pH 6.6) and 2.5 mL of potassium hexacyanoferrate [$K_3Fe\ (CN)_6$] (10 g/L). The whole was heated to 50 °C in water bath for 20 min. A volume of 2.5 mL of trichloroacetic acid (100 g/L) was then added to stop the reaction. Finally, 2.5 mL of the supernatant were mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride [$FeCl_3$] (1 g/L). A blank sample was prepared in same conditions. Absorbencies were read at 700 nm. BHT was used as positive control. An increase in absorbance corresponded to an increase of the reducing power of the EO [12–14]. Values were presented as the mean of triplicate measures.

2.6. Statistical analysis

All experiments were done in triplicate and results were reported as mean ± SD. Data were analyzed by One-way ANOVA. Statistically significant effects were further analyzed and means were compared by using Tukey test.

3. Results

3.1. EO analysis

The hydro distillation of *D. gracilis* aerial parts gave a clear limpid yellowish EO with a yield of 0.56% w/w (0.68 v/w). The analysis revealed 49 constituents representing 94.1% of the total oil. The elemicine was the major constituent (35.3%) with the geranyl acetate (26.8%) (Table 1). This oil was characterized by the dominance of phenylpropanoids and oxygenated monoterpenes (Figure 1).

3.2. Antibacterial assay

3.2.1. Disc diffusion assay

D. gracilis EO showed an antibacterial activity against three bacterial strains (Table 2). It exhibited a non-selective activity against Gram-positive bacteria; *B. cereus* (17 mm) and *S. aureus*

Table 1

Chemical composition of the EO of *D. gracilis* analyzed by GC–MS.

N	Constituent	%	Retention index
1	α-Pinene	0.1	937
2	Neral	0.1	1 240
3	(*Z*)-isoelemicin	0.1	1 571
4	α-Phellandrene	0.2	1 006
5	γ-Terpinene	0.2	1 062
6	Geranial	0.2	1 272
7	Valencene	0.2	1 493
8	Germacrene b	0.2	1 554
9	Dill apiole	0.2	1 623
10	Selin-11-en-4-a-ol	0.2	1 655
11	*p*-Cymene	0.3	1 027
12	Terpinolene	0.3	1 090
13	α-Terpineol	0.3	1 191
14	α-Longipinene	0.3	1 352
15	α-Terpinene	0.4	1 019
16	δ-elemene	0.4	1 340
17	Myrcene	0.5	989
18	(*E*)-b-ocimene	0.5	1 052
19	(*Z*)-b-ocimene	0.6	1 041
20	Juniper camphor	0.6	1 693
21	Limonene	0.7	1 032
22	α-Humulene	0.7	1 456
23	Humulene epoxide ii	0.7	1 608
24	β-Vetivene	1.0	1 530
25	γ-Curcumene	1.1	1 480
26	β-Himachalene	1.2	1 501
27	β-Pinene	1.4	977
28	Linalool	1.5	1 101
29	Cubenol	1.6	1 645
30	Geranyl acetate	26.8	1 384
31	β-Bisabolene	3.2	1 509
32	Elemicin	35.3	1 557
33	Geraniol	4.7	1 257
34	Cedr-8(15)-en-9-a-ol	8.3	1 643
35	Heptanal	Tr	902
36	Camphene	Tr	951
37	Benzaldehyde	Tr	968
38	6-Methyl-5-hepten-2-one	Tr	987
39	1,8-Cineole	Tr	1 035
40	Phenylacetaldehyde	Tr	1 045
41	Nonanal	Tr	1 103
42	trans-Pinocarveol	Tr	1 141
43	(E)-2-nonenal	Tr	1 162
44	Pinocarvone	Tr	1 168
45	4-Terpineol	Tr	1 179
46	cis-Carveol	Tr	1 231
47	Isobornyl acetate	Tr	1 287
48	Neryl acetate	Tr	1 368
49	β-Elemene	Tr	1 392
	Total identified	94.8	
Unidentified compound		5.2	

Tr: Traces.

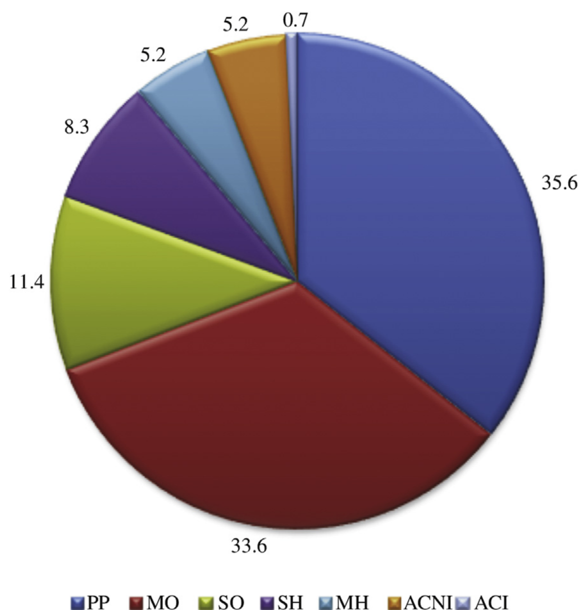

Figure 1. Percentage of the different chemical groups of components present in *D. gracilis* EO.
PP: Phenylpropanoids; MO: monoterpenes oxygen; MH: Hydrocarbon monoterpenes; SO: Oxygenated sesquiterpenes; SH: Sesquiterpenes hydrocarbon; ACI: Another identified compound; ACNI: Another unidentified compound.

(13 mm) and Gram-negative bacterium *P. mirabilis* (15 mm). However, *A. baumanii* and *L. monocytogenes* were totally resistant. *C. albicans* was the most susceptible with an important inhibition zone of 20 mm at the percentage of 50% v/v. Inhibition diameters shown by the EO were lower than those induced by gentamicin and miconazole. The recorded activity was bacteriostatic.

3.2.2. Determination of MIC

MICs values obtained by agar dilution (17.15 μg/mL) were smaller compared to those obtained by the broth micro-dilution (57.05 and 114.1 μg/mL on *B. cereus* and *P. mirabilis*, respectively).

3.2.3. Time kill assay

P. mirabilis exposed to the EO was the most affected in time showing, in first, an indifference to EO resulted by a development phase for the first 10 min. Then, there was a continuous decrease to reach the threshold of detection [(2 Log (CFU/mL)] after 24 h (Figure 2). It was found that the activity against bacteria tested is continuous over time. This activity slowly began to nearly the 5th hour, then there was a rapid decline leading to low number of bacteria, without arriving at the total absence of viable forms.

Table 2
Inhibition diameters in mm of *D. gracilis* EO.

Bacterial strains	EO (v/v)			Control +
	50%	20%	10%	
A. baumanii	–	–	–	20[a]
S. aureus	13.00 ± 2.08	11.00 ± 2.12	10.00 ± 1.73	10[a]
B. cereus	17.00 ± 4.04	16.00 ± 4.72	11.00 ± 1.15	19[a]
L. monocytogenes	–	–	–	19[a]
P. mirabilis	15.00 ± 0.00	13.00 ± 0.00	12.00 ± 0.00	26[a]
A. niger	–	–	–	10[b]
A. flavus	8.60 ± 1.15	–	–	30[b]
C. albicans	20.00 ± 7.50	10.00 ± 0.00	9.00 ± 1.73	26[b]

[a] : Gentamicin; [b] : Miconazole.

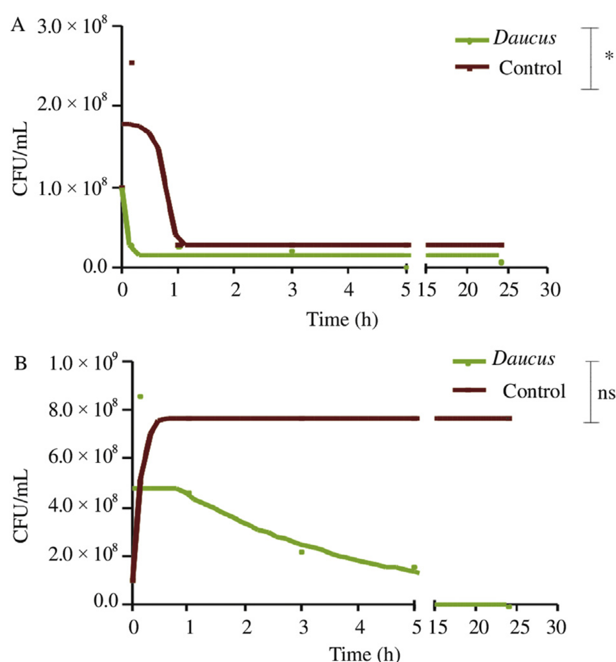

Figure 2. Time kill curves of: *B. cereus* (A) and *P. mirabilis* (B) exposed to *D. gracilis* EO.
Values were the average of three measures ±SD. Comparison was realized against the control; [*]: $P \leq 0.1$; [ns]: Not significant.

3.2.4. Bacterial lysis

Results obtained were represented by curves showing the relative percentage of the absorbance of the bacterial suspension over time (Figure 3). The decrease in absorbance was explained by cell lysis rate since only living cells absorbed at the wavelength of 620 nm, which was not the case for the lysed ones. The absorbance measurements were carried out for 2 h at different intervals. Relative absorbencies corresponding to control strains represented an increase reaching more than 400%, indicating the regular growth of bacteria in the exponential phase. Then, an absorbance drop appeared along the stationary phase. Each strain had a different absorbance after a half hour of incubation; it depended on the lifecycle of each strain. After exposure of bacteria to concentrations corresponding to the MICs, values of relative absorbencies decreased significantly ($P \leq 0.001$) from 100% to lower values; 35.3% for *B. cereus* and 40% for *P. mirabilis* (Figure 4).

3.3. Anti-oxidative assay

3.3.1. DPPH test

The EO's activity remained light until the concentration of 1.5 μg/mL where there had been a remarkable increase in inhibition concentrations (Figure 5). *D. gracilis* EO was active

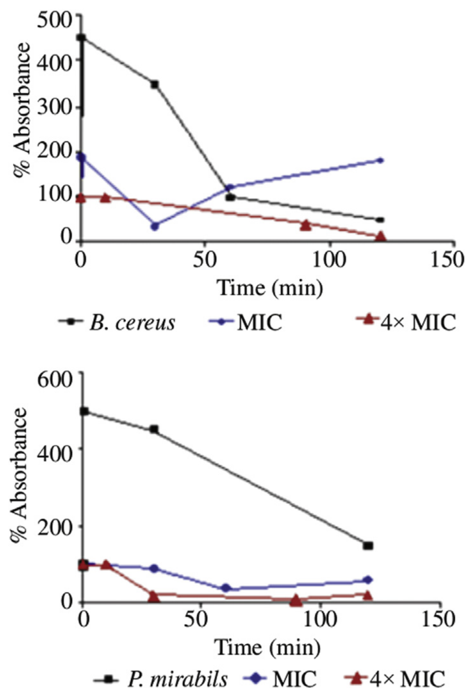

Figure 3. Curves of bacterial lysis × of *B. cereus* and *P. mirabilis* exposed to the EO of *D. gracilis*.

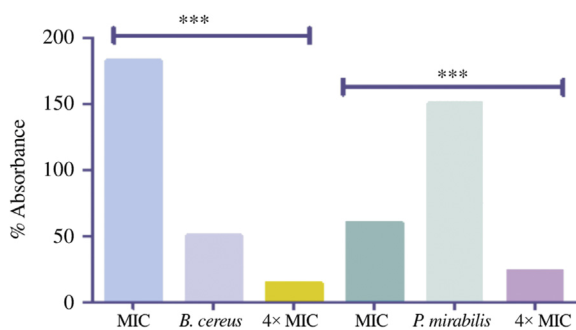

Figure 4. The percentage of bacterial lysis as a function of time at 120 min. Values were the average of three measures ±SD; ***: $P \leq 0.001$.

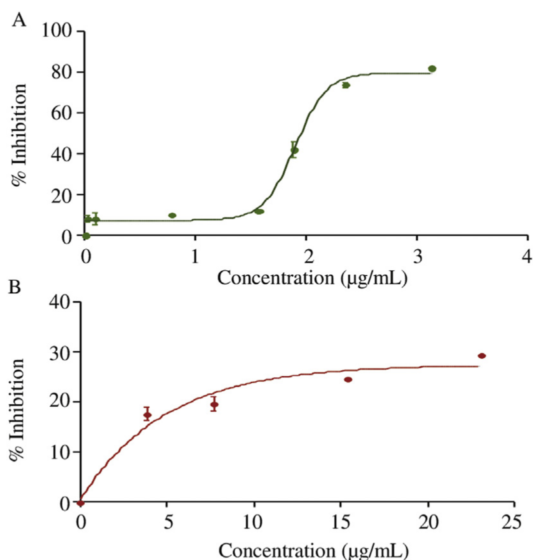

Figure 5. DPPH scavenging effect of the EO (A) of *D. gracilis* and this of BHT (B).
Values were means ± SD of three replicates.

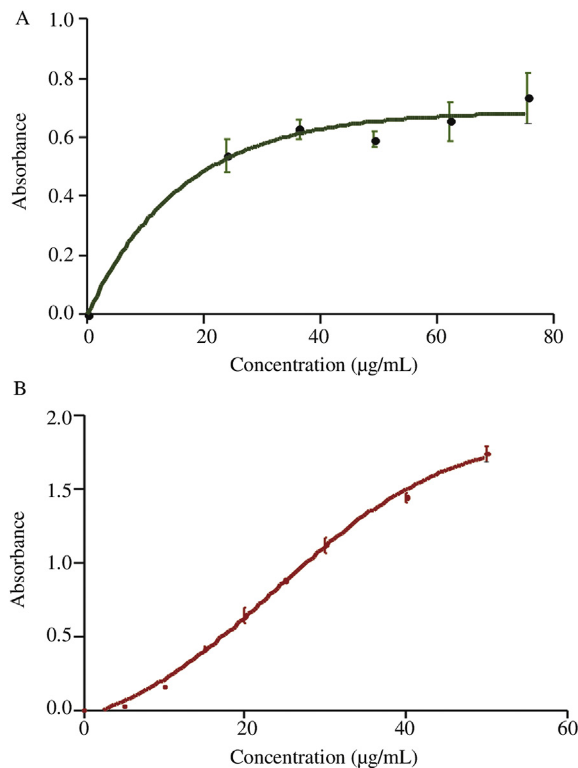

Figure 6. Reducing power of the EO of *D. gracilis* and this of BHT. Values are means ± SD of three replicates.

(Figure 5) with the lowest IC_{50} [(2.36 ± 0.2) μg/mL], this activity was concentration-dependent.

3.3.2. Reducing power assay

The difference in reducing power of the EO and the control was statistically not significantly different (Figure 6), but it exhibited a reductive activity which increased with concentrations.

4. Discussion

4.1. Chemical analysis

The hydro distillation's yield was relatively lower compared to other species yields in the same genus: *D. carota* 0.6%, *Daucus crinitus (D. crinitus)* 0.3% (w/w), *Daucus gingidium* fruits 1.21% (w/w), *D. carota* L. var. *sativa* flowers 0.27% (v/w) and *D. carota* L. cultivar fruits 0.69% [15–19]. However, some extractions gave lower rates: *Daucus gingidium* leaves 0.04% (w/w) [17], *D. carota* L. var. *sativa* stems/leaves and roots: 0.07% and 0.01% (v/w), respectively [18]. According to Fellah *et al*. [19], variations in yields could be attributed to several factors such as the extraction technique and the collection period of the plant material. In the study of Staniszewska *et al*. [20], the highest EO yield of the wild carrot (*D. carota* L. ssp. *carota*) was observed in mature umbels (1.06% v/w). Flowering umbels contained 0.65% (v/w) and grass contained only 0.09% (v/w). The extraction technique played also an important role, in the case of *D. carota* L. cultivar fruits, and the highest yield (1.17%) was obtained by supercritical carbon, that obtained by steam distillation was 0.69% [21]. Zheljazkov *et al*. added the effect of the extraction time [22].

The main component of the EO (geranyl acetate) was also the major constituent in the EO of *D. carota* subsp. *gummifer* (37%) from Portugal, and (51.7%–76.9%) the same species from Spain [23]. Maxia *et al*. [24], analyzed the EOs of two species of *D. carota* L. subsp. *carota*, one from Portugal and the other from Italy, they found the geranyl acetate at 15% while in Italy's species, it was not listed at all. However, they found very low levels of phenylpropanoids: 0.3% and 9.7%, respectively.

Phenylpropanoids dominate at a high rate (35.6%), which is not the general case in EOs [25]. *Daucus*'s EO was largely represented by phenylpropanoids (35.6%) like the EO of *D. carota* ssp. *maximus* fruit from Egypt (56.84%) [26]. The presence of phenylpropanoids in EOs in such appreciable amounts is of a great significance in insect-plant interactions as they are known as oviposition stimulants in carrot leaves for the carrot rust fly and they are abundant in Apiaceous species [26,27]. The content and the composition in EOs in species are dependent on habitat, soil, climate (seasonal variations), vegetation period, and sunlight. Drying and improper storage can reduce the amount of EOs in plants [28,29]. Many researchers have determined that the maximum oil content is obtained when all the flowers have reached full maturity, because the cups contain the largest number of secretory glands per unit area [30]. According to Bakkali *et al*. [30], to obtain an EO with a constant composition, it must be extracted under the same conditions from the same organ of the plant which was growing on the same soil under the same climate and was harvested in the same season.

4.2. Microbial test

The non-selectivity of the EO against bacterial strains was accorded to the immense variety of the composition of EO, which did not define a particular spectrum for each oil [31]. The results obtained by the EO of *D. gracilis* were in agreement with several studies in the same genus which was widely exploited for different species. The antimicrobial activity of the EO of *D. carota* subsp. *gummifer* was due to the presence of the geranyl acetate [24]. *D. crinitus* EO acted on *E. coli* and *S. aureus* but *K. pneumoniae* remained completely resistant [17,32]. The EO of *Daucus syrticus* was also active on *B. cereus* [33]. Phenylpropanoids, widely represented in EO have been isolated from species known to exert a protective action against phytopathogens (bacteria and fungi) [34]. *A. niger* and *C. albicans* are also inhibited by the EO of *D. crinitus* [17,32]. The EO of *D. carota* L. subsp. *carota* has shown a remarkable activity on a range of fungal strains, *A. niger*, *A. flavus* and *C. albicans* [25]. The susceptibility of *C. albicans* was probably due to the high content of the elemicin (35.3%) and the geranyl-acetate (26.8%) in the EO, which was previously reported [35,36]. Villa and Veiga-Crespo have also reported that the presence of phenylpropanoids correlated with strong antifungal effects against fungi cause skin infections [37]. Moreover, thymol, carvacrol and geraniol were shown to inhibit the development of *Candida* biofilms. EOs act in different ways. Microscopic studies have shown that there may be distortion hyphae with the frequent occurrence of fragmentations and disorganization of reproductive organs, the separation of the cell membrane of the cell wall and the destruction of cellular organelles [38]. Nevertheless, it is clear that the inhibition zones do not reflect a direct measure of the antimicrobial activity because the different components do not carry all the same way in the agar medium [39].

As signaled by Rouibi *et al*. [40], a strain was called susceptible when the inhibition zone exceeded 15 mm, so the susceptible strains; *B. cereus* and *P. mirabilis* were tested to determine their MICs.

MICs obtained were very good compared to those obtained from the species *D. crinitus* on *S. aureus* (2.5×10^3 μg/mL) [17]. The differences observed in the MICs values were due to the variation usually observed in these techniques, even using standardized methods [7,41]. However, Luber *et al*. concluded that the broth micro-dilution method appeared to be a simple and reliable method for determining MICs of antibiotics for *Campylobacter* and may offer an interesting alternative to MIC determination by the agar dilution technique [42]. The choice of the method to be applied depends on the advantages and inconvenients of each one. The micro-dilution method is economic in equipment as well in extracts but delicate. However, the agar dilution is fast and economic and can be used to test several strains at once and contamination can be directly recognized but requires large amounts of EOs.

The activity of the EO against bacteria tested was continuous over time without arriving at the absence of viable forms that was probably due to the bacteriostatic effect of the EO, as it had been previously reported. The non-miscibility of the EOs keeps the bacterial growth away from EOs micelles. *B. cereus* showed an exception by restarting its growth after its dramatically decreased. This strain has proven a less susceptibility to the EO. This regrowth observed may be due to the labile nature of the EO's components [43]. The differences recorded on the two strains were significant at 99.99%. These suggest that the EO acts either on a separate target cell, either on the same target but the active molecules do not have the same efficiency due to their different compositions.

The incubation of the bacterial strains with a concentration corresponding to four times MICs did not give a significant decrease in the absorbance. This can be explained by the fact that the oil acts on the bacterial membrane. Monoterpenes or sesquiterpene hydrocarbons and their oxygenated derivatives exhibit a potential antimicrobial activity [44]. Interactions with the hydrophobic structures of the bacteria have a key role in the antimicrobial effect of hydrocarbons [10]. Bacteria are less sensitive in the stationary phase than in exponential phase. Because antimicrobial agents which act on the synthesis process often have small effects in the stationary phase, these results suggest that the main target of the EO is not the synthesis of macromolecules [10]. The penetration of active compounds of plants in the cytoplasmic membrane can have a profound effect on the physical property of the phospholipid bilayer. This change could interfere with trans-membrane transport processes leading to changes in the secretion of proteins associated with bacterial virulence in the surrounding environment [45]. Some antimicrobial agents cause large alterations in the plasma membrane causing complete lysis of the cell. Although self lytic enzyme activation may be responsible for this effect, lysis may also be due to the weakening of the cell wall and the subsequent disruption of

the cell membrane due to the osmotic pressure (rather than a specific action on the membrane) [10].

4.3. Anti-oxidative assay

D. gracilis EO was more active, by DPPH assay than BHT (87.26 ± 0.001 μg/mL) and *D. crinitus* EO (>10^3 μg/mL) [17]. Many studies on anti-oxidative activities of a wide variety of EOs showed that these properties were related to their chemical composition. However, this activity is not attributed to a single compound since a synergistic effect between the different compounds can occur [46]. Some volatiles also have the potential to preserve food. A number of studies on anti-oxidative activities of various EOs said that Oregano's EO, rich in thymol and carvacrol, had a considerable antioxidant effect on the oxidation process [47].

The reducing power is associated with the anti-oxidative activity and may serve as a significant reflection of this one [14]. The reducing power of EO was not significantly different from that of the control. Anyway, the γ-terpinene, the myrcene and the terpinolene may be responsible for this activity. The free radicals produced during inflammation, could induce gene mutations and posttranslational modifications of various proteins. If not, remove may turn injurious radicals to the whole system [48], this EO can bypass such effects.

The results of this study can be considered the first information on antimicrobial and antioxidant properties of the EO of *D. gracilis*. This EO has a moderate antimicrobial activity and it is able to disrupt membrane functions of both Gram positive and Gram negative bacteria. We conclude that this effect reduces the number of viable bacteria. Moreover, *D. gracilis* EO possesses anti-oxidative capacities which can be exploited in the food preservation after clinical confirmation and pharmacological standardization. Likewise, further studies are being made to test this oil *in vivo* and to evaluate its cytotoxicity.

Conflict of interest statement

We declare that we have no conflict of interest.

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