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Chemical Analysis, Antioxidant, Antimicrobial and Enzyme Inhibitory Effects of *Daucus Virgatus* (Poir.) Maire Essential oil from Algeria

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Essential oils (EO) represent an important source of bioactive molecules and are widely used for their great efficacy relating to their different therapeutic properties. The present study aims to contribute to the valorization of *Daucus virgatus* (Poir.) Maire, an aromatic and native plant spontaneously growing in the North-Eastern regions of Algeria, by analyzing the chemical composition of its essential oil and evaluating the antioxidant and antimicrobial activities, together with the assay of the enzyme inhibitory effects against α -glucosidase and cholinesterase. *Daucus virgatus* EO was extracted by hydrodistillation and analyzed by gas chromatography-mass spectrometry analysis (GC-MS). Twenty-one constituents accounting for 98% of the whole components were identified. β -Pinene (77.9%) and α -pinene (7.6%) were the most abundant components. The antimicrobial activity against the pathogen microorganisms *Listeria innocua*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli* and *Candida albicans* was investigated. The best antimicrobial effect was observed against *S. typhimurium* and *C. albicans*, which showed high sensitivity, with inhibition zones ranging between 27.3 and 25 mm; the minimum inhibitory concentrations were 15.63 and 31.25 $\mu\text{g ml}^{-1}$, respectively. In the DPPH test, the essential oil showed a moderate antioxidant effect, with an IC_{50} value of 39.61 mg ml^{-1} . *D. virgatus* EO exhibited an interesting inhibitory effect against α -glucosidase enzyme ($\text{IC}_{50} = 0.35 \text{ mg ml}^{-1}$) compared with the positive control Acarbose ($\text{IC}_{50} = 0.24 \text{ mg ml}^{-1}$), and a moderate inhibitory effect against cholinesterases enzymes, with IC_{50} values of 0.33 and 0.20 mg ml^{-1} for acetylcholinesterase and butyrylcholinesterase, respectively. *D. virgatus* EO might be used as a promising source of natural products with potential antimicrobial, antihyperglycemic and anti-Alzheimer effects.

Keywords: *Daucus virgatus*, Essential oil, Antimicrobial, Anti-cholinesterases, α -Glucosidase inhibition, Antioxidant

INTRODUCTION

There is currently a clear trend towards the use of natural products as alternative compounds to prevent and

treat human diseases. Furthermore, many volatile compounds are currently ingredients in pharmaceutical preparations as antioxidant, bactericidal, antifungal and antiviral agents. These biological proprieties are due to their chemical structures and their functional groups that play a key role in the generation of pharmacological effects [1]. It

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has been already assessed and confirmed the biological properties as well as the pharmaceutical and therapeutic capacities of numerous species belonging to many plant families that grow wildly or cultivated in different areas of the planet [2]. Indeed, essential oils are a high concentrate of aromatic constituents extracted from plants by steam distillation, hydrodiffusion, or pressure. Rich in terpenes and other non-terpene compounds, essential oils constitute an interesting source of new bioactive molecules [3]. In this regard, the plant species of the Apiaceae family are recognized for their high content of essential oils. This family, formerly known as Umbelliferae because of their umbrella-shaped inflorescence, comprises about 600 species widely distributed around the world, in particular in the Mediterranean basin. *Daucus*, the most popular and important genus of the Apiaceae family, contains more than twenty-seven species, living in dry and uncultivated areas [2], occurring particularly in North Africa. Its main species is *D. carota* L. (carrot), reported with eight subspecies in Algeria [2]. Several investigations deal with the chemical composition and biological properties of essential oils obtained from *Daucus* species and subspecies from different regions of the world endowed with potential antimicrobial and antioxidant activities [4-16]. A number of previous studies reported the chemical composition and biological activities of essential oils of some species belonging to the *Daucus* genus from Algeria, i.e. *D. reboudii* [17], *D. muricatus* L. [18], *D. crinitus* Desf [19,20], *D. gracilis* [21], rich in monoterpene hydrocarbons, mainly β -pinene, α -pinene, limonene, *p*-cymene and β -myrcene. The oxygenated monoterpenes represent the second major fraction of essential oil and exhibit interesting antibacterial and antifungal activities.

Daucus virgatus (Poir.) Maire belongs to the *Daucus* genus and it is classified as an endemic aromatic species of Algeria-Tunisia [22]. It is an annual/biennial herb naturally growing in the North-East of Algeria. It has stiff-solid stems of 30-70 cm, very thin, draw up, and decumbent. It is characterized by bipinnate leaves having sharp leaflets on each side, in the form of lance segments. The flowers are small, white or pink, and clustered in very short terminal umbels 2-3 to 10-15 cm wide [2,23]. According to the literature, only one study on the essential oil of *D. virgatus* from Tunisia was previously conducted. It reports antiviral,

antioxidant and antimicrobial effects, attributed to its content of volatile compounds, the most predominant being methyl eugenol and β -bisabolene. Other works reported the characterization of three sesquiterpenoids in the extract obtained from the aerial parts of *D. virgatus* from the North-West of Tunisia [1,24] and led to the isolation of eight new germacranolides [25,26]. To the best of our knowledge, no chemical investigation about the *D. virgatus* essential oil from Algeria has been previously reported. Additionally, no study was carried out on the pharmacological potential of *D. virgatus* essential oil for the anti-hyperglycemic and anticholinesterase effects. In this perspective, this work aims to elucidate the chemical composition of the essential oil extracted from the Eastern Algerian native plant *D. virgatus* and to study some of its biological properties, especially the antimicrobial and *in vitro* antioxidant ones. In addition to these effects classically studied for EO, we report for the first time the study of the possible anti-hyperglycemic effect through the inhibition essays of α -glucosidase, a key enzyme in the carbohydrates metabolism and strongly implicated in post-prandial hyperglycemia in diabetic patients. Also, we investigate for the first time the effect of the essential oil for the inhibition of both acetylcholinesterase and butyrylcholinesterase. These enzymes are classically involved in Alzheimer's disease, a progressive and degenerative neurologic disorder leading to impaired memory and behavior. Most treatment methods are supported by the cholinergic hypothesis that postulated that memory impairments in patients plagued by this illness result from a deficit of cholinergic operate in the brain [27]. The cholinesterase inhibitors are one of the most promising approaches for treating this disease. Amongst them, galantamine, a plant-derived compound, has increased the interest for enzyme inhibitors from natural resources [28]. The progress of knowledge and understanding of this herbaceous orphan plant species will create new possibilities for its use in other promising fields.

EXPERIMENTAL

Plant Material

The aerial parts of *Daucus virgatus* (Poir.) Maire were collected on the mountain of Djebel Edough in Annaba, Eastern Algeria, during the flowering stage in July 2014.

The plant was identified by Laouer. H (Natural and Biological Resources Valorization Laboratory, University of Ferhat Abbas Setif 1, Algeria). A sample was deposited at the biological and ecological department herbarium. After removal of impurities, the plant material was dried in air and in shade until constant weight.

Essential Oil Extraction

The dried aerial parts were hydro-distilled for 3 h in Clevenger-type equipment. The obtained oil was kept at +4 °C until use.

Analysis of the Essential Oil by the GC-MS Method

Gas chromatography-mass spectrometry (GC-MS) analyses were performed using a Varian CP-3800 instrument equipped with a DB-5 capillary column (30 m × 0.25 mm; coating thickness 0.25 µm) coupled with a Varian Saturn 2000 ion trap mass detector. Injector and transfer line temperatures were set at 220 and 240 °C, respectively and the oven temperature programmed from 60 °C to 240 °C at 3 °C min⁻¹, using as a carrier gas helium at 1 ml min⁻¹; split ratio 1:30 [29]. The constituents were identified by comparison of their retention times with those of authentic samples, of their linear retention indices relative to the series of *n*-hydrocarbons, and on computer matching against commercial [30] and home made library mass spectra built up from pure substances and MS literature data [30-33].

Free Radical DPPH Scavenging Assay

In vitro antioxidant activity was determined by DPPH radical-scavenging assay to estimate the antioxidant power of the compounds through a reduction reaction that discolors the DPPH solution [34]. The effect of volatile compounds on the DPPH free radical was measured in terms of hydrogen donating or their ability to scavenge 2, 2-diphenyl-1-picrylhydrazyl (DPPH), a stable radical that absorbs at 517 nm; the decrease of the absorbance values indicates the antioxidant effect of the essential oil. The radical scavenging abilities of *D. virgatus* EO were evaluated according to the slightly modified method of Que *et al.* [35]. In brief, 600 µl of various dilutions of the samples were mixed with 600 µl of a methanol solution of DPPH 0.004%. After 30 min of incubation in the dark,

absorbances were measured at 517 nm. Methanol was used as a blank, whereas the DPPH methanol solution was used as a control. Butylatedhydroxytoluene (BHT), a synthetic antioxidant, was used as a reference control. The ability to scavenge DPPH radical was calculated as follows [36]:

$$\text{Radical scavenging (\%)} = \frac{(\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}})}{\text{Abs}_{\text{Control}}} \times 100$$

Where Abs_{Control} is the absorption of the control sample and Abs_{Sample} is the absorption of the DPPH solution containing tested Essential oil (t = 30 min).

Scavenging of DPPH free radicals was represented by IC₅₀ values expressed as the mean at least three measurements.

Antimicrobial Activity

Microbial strains. *D. virgatus* EO was assayed against six bacterial strains from the American Type Culture Collection (ATCC): *Staphylococcus aureus* ATCC 25923 (*S. aureus*), *Bacillus subtilis* ATCC 11778 (*B. subtilis*), *Listeria innocua* CLIP 74915 (*L. innocua*), *Escherichia coli* ATCC 25922 (*E. coli*), *Salmonella typhimurium* ATCC 19430 (*S. typhimurium*), *Pseudomonas aeruginosa* ATCC 27853 (*P. aeruginosa*) and one yeast: *Candida albicans* ATCC 1024 (*C. albicans*). Strains of bacteria and fungi were obtained from the Laboratory of Microbiology (Faculty of Nature and Life Sciences, University of Ferhat Abbas Setif 1, Algeria). Strains culture were routinely incubated for 24 h at 37 °C in Mueller Hinton broth (MHB) for the bacteria and 48 h at 28 °C in Sabouraud dextrose agar (SDA) for the yeast [37].

Disc Diffusion Assay

The antimicrobial activity of *D. virgatus* EO was screened using the disc agar diffusion method that was carried out according to the Bauer *et al.* method [38]. The inoculums containing 2.0 × 10⁶ CFU/ml of bacteria and 10⁷ CFU/ml yeast were spread on a Muller-Hinton agar and Sabouraud dextrose agar (SDA), respectively. Sterile absorbing paper discs (6 mm diameter) were impregnated with 10 µl of different oil dilutions (1:2, 1:5 and 1:10 v/v) in DMSO (Sigma-Aldrich), then placed on the surface of inoculated Petri dishes (90 mm) that were pre-incubated for 30 min, allowing complete dispersion of the essential oil,

afterward the incubation at 37 °C for 24 h. The antimicrobial activity was determined by measuring inhibition zone diameters (mm). The diameter of inhibition was measured after 24 h of incubation at 37 °C for bacteria and after 7 days of incubation at 28 °C for the fungi [39]. Gentamicin (10 µg ml⁻¹, Sigma Aldrich) was used as a positive control for bacterial strains, nystatine as a positive control for fungal strains. DMSO was used as a negative control. All the inhibition growth tests were realized in triplicate.

Determination of Minimal Inhibitory Concentration

The minimal inhibitory concentration (MIC) of the *D. virgatus* essential oil was determined by macrodilution broth method according to the Clinical and Laboratory Standards Institute method [39]. A series of twofold dilutions of the oil were prepared to range from 1000-15.625 µg ml⁻¹. Standardized inoculums (0.5 McFarland) were prepared. 1 ml of adjusted inoculum was added to each tube containing 1 ml of dilutions of essential oil. A positive control tube contains only inoculated broth. The results were determined after 24 h (72 h for yeast), and MIC values were determined as the lowest concentration of the oil that inhibited the visible growth of each microorganism [39].

Acetylcholinesterase and Butyrylcholinesterase Inhibitory Assay

Acetylcholinesterase and butyrylcholinesterase inhibitory assays were performed using the method of Ellman *et al.* (1961) [40]. 150 µl of 100 mM sodium phosphate buffer (pH 8.0) was mixed with 10 µl of a solution of the extract of *D. virgatus* EO in ethanol at different concentrations, then 20 µl AChE or BuChE solutions were added. The mixture solution was incubated at 25 °C for 15 min. Then, 10 µl of 0.5 mM dithiobisnitrobenzoic acid (DTNB) and 10 µl of acetylthiocholine iodide (0.71 mM) or 10 µl of butyrylthiocholine chloride (0.2 mM) were added. The reaction between DTNB and thiocholines, catalyzed by enzymes, permits the formation of the yellow 5-thio-2-nitrobenzoate anion, which control the hydrolysis of acetylthiocholine iodide/butyrylthiocholine chloride. The

absorbance was measured at 412 nm, immediately and after 15 min using a microplate reader. The percentage inhibition of AChE or BuChE enzymes is determined relative to the blank (ethanol with phosphate buffer pH 8) using the following formula:

$$\%Inhibition = \frac{(\Delta A_{Control} - \Delta A_{Sample})}{\Delta A_{Control}} \times 100$$

The inhibitory results were expressed as the half-maximal inhibitory concentration (IC₅₀). Galantamine was used as a reference drug.

α-Glucosidase Inhibitory Assay

The α-glucosidase assay was realized using the chromogenic method described by Schäfer and Högger (2007) [41]. Briefly, a 3 mM solution of *p*-nitrophenyl-α-D-glucopyranoside (*p*NPG) was prepared in 0.1 M phosphate buffer and adjusted to pH 6.9 to simulate a model of intestinal fluid. Yeast α-glucosidase was dissolved in 0.1 M phosphate buffer, pH 6.7, to yield a final stock-solution of 1 IU/ml. For each assay, 0.075 IU of enzyme solution was premixed with *D. virgatus* EO at various concentrations and pre-incubated for 10 min at 37 °C. Then, a 0.95 mM solution of *p*NPG was added and the enzymatic reaction mixture was incubated for 10 min at 37 °C. The inhibition of the enzyme was determined by measuring the *p*-nitrophenol released from *p*NPG using a microplate reader (405 nm) and compared to that of the control (buffer solution in place of the extract). The α-glucosidase inhibitory activity was expressed as inhibition percent and was calculated as follows:

$$\%Inhibition = \frac{(\Delta A_{Control} - \Delta A_{Sample})}{\Delta A_{Control}} \times 100$$

Acarbose, a synthetic inhibitor of α-glucosidase was used as a positive control. The inhibitory results were expressed as the half-maximal inhibitory concentration (IC₅₀).

Statistical Analysis

All experimental tests were conducted in triplicate and the results were expressed as mean ± standard deviations (SD). The antimicrobial activity was statistically analyzed

by one-way analysis of variance (ANOVA 1-way) to test the effect of *D. virgatus* EO concentrations on inhibition zone diameter from all microbial strains studied. Then, a post hoc test was done with Tukey's multiple comparison test at $P < 0.05$ significance. Parametric conditions were verified initially with the use of Shapiro-Wilk and Levene tests for normality and homoscedasticity, respectively. The Student's *t*-test was used to evaluate the significance of differences of the inhibition zone of bacterial strain *L. innocua*. Statistical analysis was carried out using IBM SPSS 23.0 version software. The IC_{50} values were calculated using Graph Pad Prism 7 software, whose model used provides the best fit for all curves, with a value of R^2 greater than 0.95.

RESULTS AND DISCUSSION

Chemical Composition

The essential oil obtained from the aerial part of *D. virgatus* by hydrodistillation was yellow and with a strong aromatic fragrance, it was highly soluble in methanol and DMSO. The yield was 0.39% and twenty-one components were characterized, representing 98.0% of the total *D. virgatus* EO (Table 1). The gas chromatogram is shown in Fig. 1. The most abundant chemical class was represented by monoterpene hydrocarbons (92.5%), followed by oxygenated monoterpenes (4.1%) and sesquiterpene hydrocarbons (1.2%). The major components of the EO were β -pinene (77.9%), α -pinene (7.6%), myrcene (3.9%), limonene (2.3%), and myrtenol (1.1%) (Table 1). According to previous studies, the yield of the hydrodistillation of other species of the same genus was relatively higher. The fruits oil yield of *D. carota* L. was reported as 1.6% [42] while that of *D. gingidium* fruits was 1.21% (w/w) [21]. A yield of 0.16% was reported in the study of the same species *D. virgatus* that grows naturally in Tunisia [1]. In comparison, the Algerian *D. virgatus* recorded a relatively higher rate (0.39%). Furthermore, many *D. gracilis* populations that belong to the same taxonomic genus of *D. virgatus* and share some aspects with them shown similar or even lower yields (0.03% at least and 0.41% at most) [43]. Several factors can be responsible for variations in yields, such as the collection period of the plants, the geographical area, climate and the extraction

method [44]. Furthermore, essential oil yield and bioactive components of aromatic and medicinal plants change in response to the environmental stress that plants face naturally such as drought and salt stress [45,46]. The phenylpropanoidmethyl eugenol has been reported as the major constituent (33.0%) of the Tunisian *D. virgatus* EO [1], while the major components of the present Algerian EO were β -pinene (77.9%) and α -pinene (7.6%). In the case of *D. muricatus*, Bendiabellah *et al.* [18] reported the composition of the essential oil obtained from the aerial parts (leaves, stems, flowers, and umbels). The major components were monoterpene hydrocarbons (58.5%), particularly limonene (24.0%) and α -pinene (21.8%). Their relative abundance was followed by sabinene (8.1%) in stems, leaves and flowers, and *trans*-sabinyl acetate in umbels (12.1%). Conversely, the main components of the root oil were non-terpene aliphatic compounds [18]. The chemical composition of *Daucus* species reported by previous studies showed that hydrocarbon monoterpenes are the most represented derivatives, with sabinene, β -pinene, α -pinene, β -myrcene, α -terpinene, limonene, γ -terpinene, *p*-cymene, and α -terpinolene among the main ones [4,5,7,47,48]. This is in good agreement with the results obtained in the present study. Occasionally, phenylpropanoids such as apiol, myristicin, and isochavicol are reported in appreciable amounts [4]. The same is true for sesquiterpene hydrocarbons, *i.e.* β -caryophyllene, *trans*- γ -bisabolene [49]. Leaf and fruit oils presented α -pinene and sabinene as the main compounds, followed by myrcene, limonene, and germacrene D [10,11].

Free Radical-scavenging Assay

The antiradical activity profile shown in Fig. 2 reveals that *D. virgatus* essential oil has a dose-dependent antioxidant effect. The results obtained with the DPPH method showed moderate antioxidant activity, with an IC_{50} value of 39.61 ± 1.15 mg ml^{-1} . BHT, used as a positive control, exhibited a higher antioxidant effect, with $IC_{50} = 0.046 \pm 0.001$ mg ml^{-1} (Table 2). In general, the antioxidant activity of essential oils could be ascribed to phenols and alcohols, such as oxygenated mono- and sesquiterpenes [50,51]. *D. virgatus* EO contains 4.1% of oxygenated monoterpenes, which may explain the observed activity. On the other hand, a synergistic effect with other

Table 1. Chemical Composition of the EO of *D. virgatus* Analyzed by GC-MS

Component names ^a	L.R.I ^b	Percentage (%) ^c
α -Pinene	941	7.6
Camphene	955	0.2
β -Pinene	981	77.9
Myrcene	993	3.9
p-Cymene	1028	0.2
Limonene	1032	2.3
γ -Terpinene	1063	0.1
1-Nonen-3-ol	1082	0.1
Terpinolene	1090	0.3
<i>Trans</i> -pinocarveol	1140	0.8
Pinocarvone	1164	0.7
4-Terpineol	1178	0.2
α -Terpineol	1192	0.7
Myrtenol	1195	1.1
<i>Trans</i> -pinocarvylacetate	1299	0.6
α -Copaene	1377	0.2
β -Cubebene	1390	0.3
α -Humulene	1455	0.1
GermacreneD	1481	0.4
δ -Cadinene	1523	0.2
T-Cadinol	1641	0.1
Grouped compounds	Compounds number	Total portion (%)
Monoterpene hydrocarbons	8	92.5
Oxygenated monoterpenes	6	4.1
Sesquiterpene hydrocarbons	5	1.2
Oxygenated sesquiterpenes	1	0.1
Non-terpene derivatives	1	0.1
Total identified	21	98.0

^aEssential oil constituents classified by chemical families and identified by comparison of their retention times with those of authentic samples, of their linear retention indices relative to the series of *n*-hydrocarbons, or identified by computer matching against commercial and home-made library mass spectra.

^bL.R.I: Linear retention indices determined on a DB-5MS capillary column relative to a series of *n*-alkanes. ^cpercentage calculated by GC/ion trap mass detector.

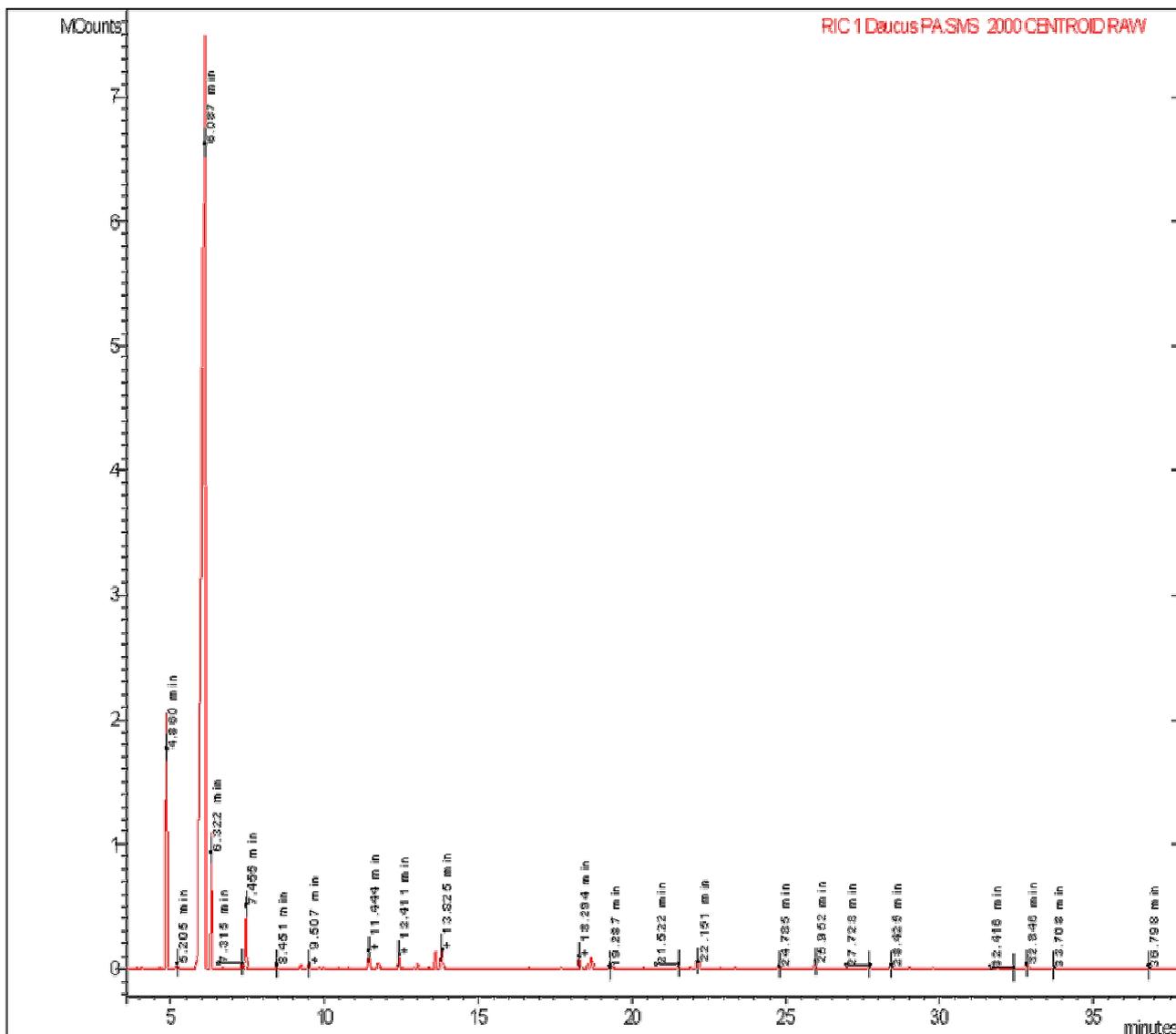


Fig. 1. Gas chromatogram of *Daucus virgatus* essential oil.

components of the essential oil could not be excluded. Previous study on the antioxidant activity of Tunisian *D. virgatus* EO by DPPH assay, showed an IC_{50} of 6.23 mg ml^{-1} lower than that found in the present study. We can conclude that employing the DPPH technique, Tunisian *D. virgatus* EO exhibited a much higher antioxidant effect.

This observation could be explained by the main component of sesquiterpene hydrocarbons (25.0%) and oxygenated sesquiterpenes (20.1%) [1]. Other auxiliary

approaches based on the synthesis of nanoparticles can increase and improve the potential effect of plant extracts while ensuring the considerable stability of biological activities [52].

Antimicrobial Activity

Antimicrobial resistance in microorganisms is one of the major health problems in the world today. Aromatic and medicinal plants are useful natural resources that are

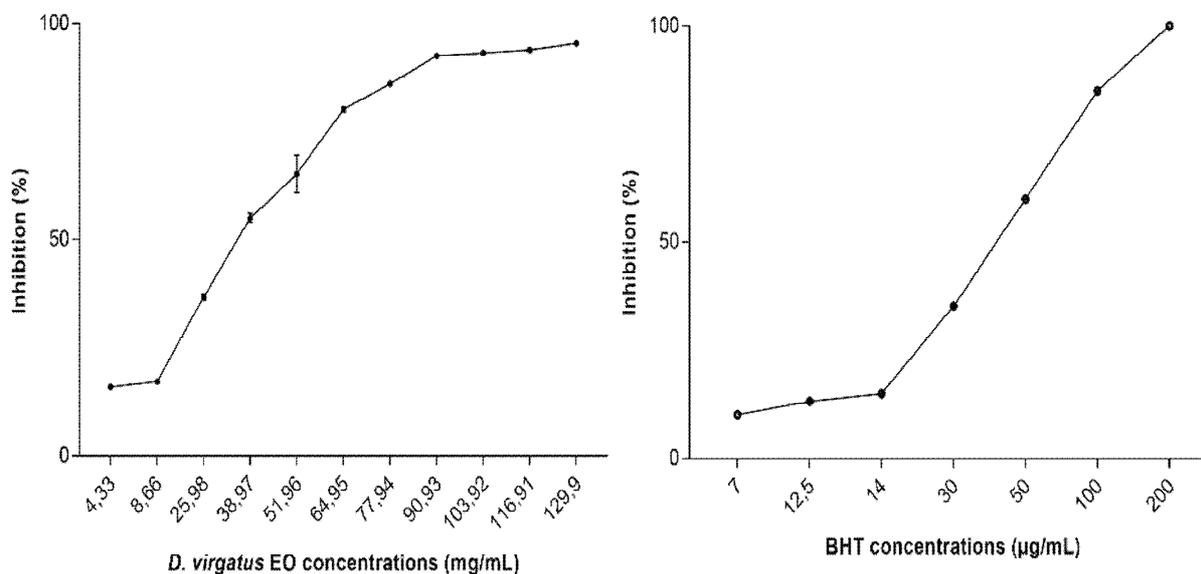


Fig. 2. DPPH scavenging effect of (A) *D. virgatus* essential oil and (B) BHT control.

Table 2. DPPH Scavenging Effect of *D. virgatus* Essential Oil

Sample	IC ₅₀
	(mg ml ⁻¹) ^a
<i>D. virgatus</i> EO	39.61 ± 1.15
BHT ^b	0.046 ± 0.001

^aConcentration of antioxidant (EO) corresponding to 50% reduction of DPPH in the reaction medium. ^bButylatedhydroxytoluene (BHT), a synthetic antioxidant used as a positive control.

efficient against infectious pathogens [53]. The disc diffusion and macrodilution broth methods allowed the qualitative and quantitative evaluation of the effects of *Daucus virgatus* EO on the different tested bacterial and fungal strains. The results that are summarized in Table 3 shows the sensitivity gradient of the strains of bacteria and fungi relative to the concentrations of essential oil. The inhibition was induced by all the EO concentrations, except for bacterium *L. innocua*, which showed an absolute resistance to 1:5 and 1:10 (v/v) dilutions. Indeed, no

antimicrobial effect can be observed beyond a concentration of 1:2 (v/v) for the EO of *Daucus virgatus* against *L. innocua*. Apart from this exception, the inhibition zone diameter of all microbial strains tested was significantly dependent on *D. virgatus* EO concentrations. It was extremely significant at the $P < 0.001$ level of probability for *B. subtilis*, *E. coli*, *P. aeruginosa* and *C. albicans*, at $P < 0.01$ for *S. typhimurium* and at $P < 0.05$ for *S. aureus*. At dilutions of 1:5 and 1:10 (v/v), a significant decrease in the diameter of the inhibition zone for all microbial strains

Table 3. Antimicrobial Activity of *D. virgatus* EO against all Tested Microbial Strains

Microbial strains	Essential oil concentrations				Control±
	(v/v)				
	1:1	1:2	1:5	1:10	
<i>S. aureus</i>	14.33 ± 1.15 ^a	13.67 ± 1.15 ^{a,b}	12.33 ± 0.58 ^{a,b}	11.33 ± 0.58 ^b	30 ± 0.0
<i>B. subtilis</i>	15.67 ± 1.15 ^a	15.33 ± 0.58 ^a	10.67 ± 0.58 ^b	8.33 ± 0.58 ^c	35 ± 0.0
<i>L. innocua</i>	10.67 ± 0.58 ^a	9 ± 1.0 ^a	-	-	30 ± 0.0
<i>E. coli</i> ATCC	15.33 ± 0.58 ^a	14.67 ± 0.58 ^a	11.33 ± 0.58 ^b	11 ± 1.0 ^b	28 ± 0.0
<i>P. aeruginosa</i>	15.33 ± 0.58 ^a	14.33 ± 0.58 ^a	11 ± 1.73 ^b	9 ± 1.0 ^b	27 ± 0.0
<i>S. typhimurium</i>	27.33 ± 2.52 ^a	17.33 ± 4.93 ^b	13 ± 1.73 ^b	11.33 ± 0.58 ^b	38 ± 0.0
<i>C. albicans</i>	25 ± 1.0 ^a	23 ± 1.0 ^a	16.33 ± 0.58 ^b	15 ± 1.0 ^b	30 ± 0.0

The mean ± standard error. For each microbial strain test, means of inhibition zone diameters (mm) within a line between concentrations not having a common superscript letter are different at $P < 0.05$ (Tukey's multiple range tests).

resulted; however, the inhibitory effect of *D. virgatus* EO at a concentration of 1:2 (v/v) was statistically similar compared to 1:1 against all the microbial strains, except *S. typhimurium*. The best antimicrobial effect was observed against *S. typhimurium* at an essential oil concentration of 100% (27.33 ± 2.52 mm), while the 1:2 (v/v) concentration has a significantly different effect on their inhibition zone diameter compared to the 1:1 one. This half dilution resulted in a decrease of approximately 57.7% in the diameter of the inhibition zone of this bacterial strain. This implies a strong relationship between the concentration of the EO, its effectiveness and inhibitory activity against microbial strains. In comparison, *C. albicans* was most sensitive at all EO concentrations, with an inhibition zone ranging from 25 ± 1 mm to 15 ± 1 mm. Besides, moderate activity was observed at 1:2 and 1:1 (v/v) dilutions of the essential oil against *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa* (ranging from 13.66 to 15.66 mm). Using the macrodilution method, the lowest values of MIC varying between 15.63, 25.0 and 31.25 µg ml⁻¹ were observed against Gram-negative bacteria *S. typhimurium*, *P. aeruginosa* and the fungal strain *C. albicans*, respectively (Fig. 3). Thus, *D. virgatus* essential oil had a moderate MIC

against *E. coli* (62.5 µg ml⁻¹). However, the Gram-positive bacteria *S. aureus* and *B. subtilis* showed the highest MIC values (500 and 125 µg ml⁻¹). On the contrary, no *L. innocua* MIC was obtained. Previous studies have shown that the essential oils of some *Apiaceae* species, such as those of the *Daucus* genus, have good antibacterial activity. Staniszewska *et al.* [14] found that the essential oil of *D. carota* subsp. *sativus* exhibits a strong activity on gram-positive bacteria, such as *B. subtilis* and *S. aureus*. Glisic *et al.* [54] also demonstrated the activity of *D. carota* L. EO on Gram-negative strains, such as *E. coli* and *S. typhimurium*. El Kolli *et al.* [21] reported an inhibition zone similar to our results for the Gram-positive bacteria *S. aureus* and also for the fungal strain *C. albicans* with a slight difference. Thus, relative to other Umbelliferae species abundant in α-pinene (43.9%) and β-pinene (16.0%), Boucekrit *et al.* [55] observed a greater inhibitory effect of *Elaeoselinumasclepium* EO on *C. albicans* ATCC 1024 (14.5 mm) among the 12 microbial strains examined, but still lower than our value for the Algerian *D. virgatus* EO that reveals a high antifungal effect against *C. albicans* (25 mm), probably related to its different composition, in particular to the high percentages of β- and α-pinene (77.9

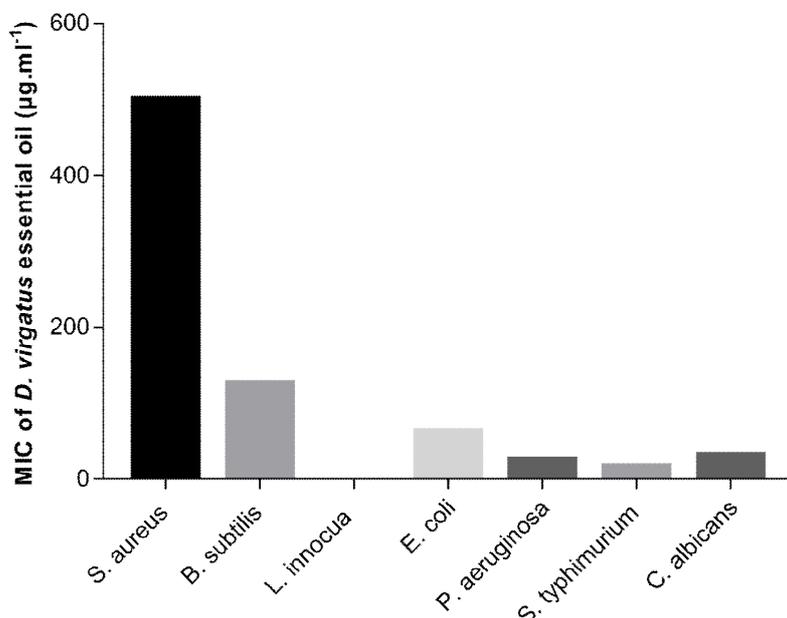


Fig. 3. The MIC of *D. virgatus* EO obtained against all microbial strains.

and 7.6%, respectively). On the other hand, Snene *et al.* [1] found a low antimicrobial activity for *D. virgatus* EO from Tunisia. The essential oil did not affect *S. aureus* and *E. coli*, but the best antimicrobial effect was again observed against *C. albicans*. Known for their antifungal effect against yeast [56,57], volatile compounds act by inhibiting the proton pump as well as K⁺ transport, mitochondrial respiration and enhance membrane fluidity [51]. Thus, they disrupt membrane structure and inhibit ergosterol biosynthesis in *Candida* species [58]. In general, phytochemicals compounds exert a considerable antibacterial effect on Gram-positive and Gram-negative bacteria. The administration of pure β -pinene showed moderate antimicrobial activity, and it has been observed that it is ineffective on *Pseudomonas* spp. [59]. Its mixture with α -pinene enhances the antifungal properties [60]. A study shows that high doses of β -pinene can cause mitochondrial dysfunction in yeasts, and it may damage the plasma membrane's functionality [61]. Moreover, α - and β -pinenes are effective against yeasts, especially *C. albicans* [62], probably because of a synergy between the two compounds [63]. A study conducted on the enantiomers of the two isomers of pinene reveals that only the positive

enantiomers (+)- β -pinene and (+)- α -pinene exerted an inhibitory effect against *C. albicans*, but no antifungal properties with negative enantiomers were observed [64]. The enantiomer (+)- β -pinene has also been proven to effectively reduce adhesion to *Candida* spp. biofilm [65]. Likewise, β -pinene and α -pinene have considerably inhibited the growth and viability of infectious endocarditis caused by bacterial strains of *S. aureus*. [66]. High percentages of pinenes seem to make an essential oil effective against pathogenic bacteria and fungi, showing bactericidal or bacteriostatic effects [51]. It should be noted that the effect of the Algerian *D. virgatus* EO against microbial strains is mostly bacteriostatic. The research carried out by Bakkali *et al.* [67] on the mechanisms of action on bacteria, advocated that EOs generally cause static rather than toxic effects on bacteria, probably slowing down their metabolic processes [67]. Unlike most antibiotics, essential oils can access the periplasm of the bacteria through the protein porin of the outer membrane, thanks to the hydrophobicity of these substances [68]. Carson *et al.* [69] reported that membrane disruption and rupture of the ionic strength are some of the mechanisms involved in the antimicrobial properties of essential oils [69].

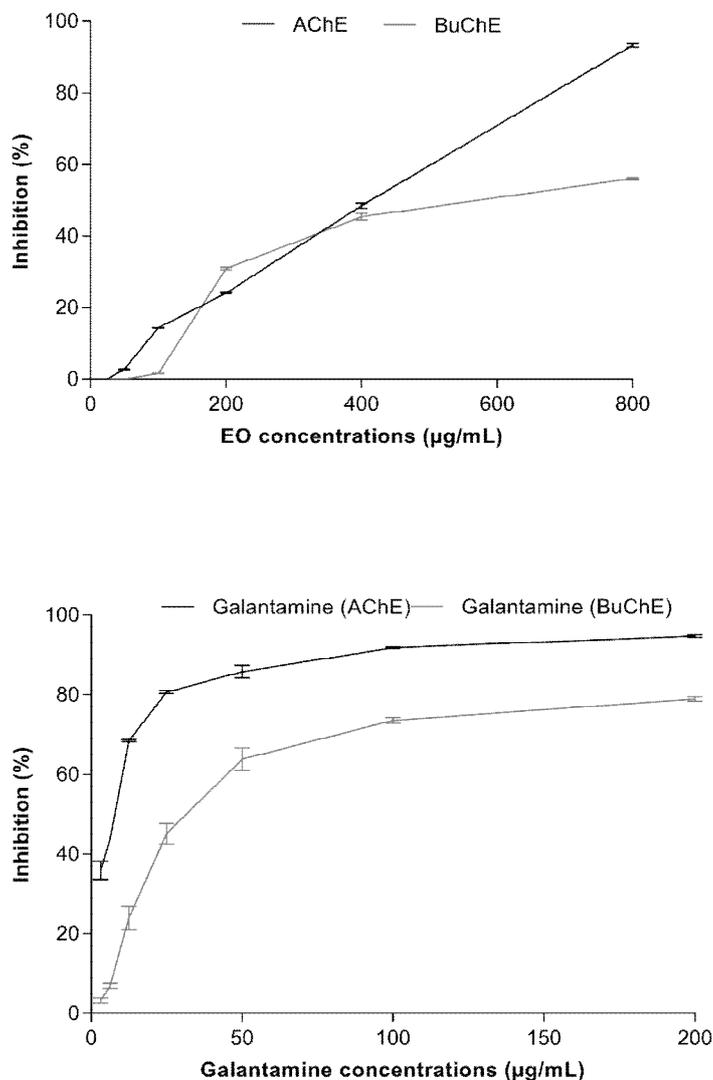


Fig. 4. *D. virgatus* EO inhibitory effect as compared to control compound galantamine against AChE and BuChE enzymes. For each concentration, inhibition (%) expressed as mean \pm SD, n = 3.

Acetylcholinesterase and Butyrylcholinesterase Inhibitory Assays

The psychological disorders and loss of memory like Alzheimer's disease have been often treated with aromatic and medicinal plants [70]. The potential of *D. virgatus* EO to inhibit cholinesterase enzymes (AChE and BuChE) was assessed for the first time in the present study. This EO exhibited an interesting inhibitory effect both on AChE and BuChE (Fig. 4), with IC_{50} of 0.33 ± 0.005 and

0.20 ± 0.003 mg ml⁻¹, respectively. Unlike Galantamine drug, which was a strong inhibitor of AChE over BuChE (0.015 ± 0.001 and 0.025 ± 0.002 mg ml⁻¹, respectively), the *D. virgatus* EO appears to have a greater inhibitory effect on BuChE than AChE (Table 4, Fig. 4). Galantamine is a natural alkaloid, which strongly inhibits acetylcholinesterase and butyrylcholinesterase, both implicated in Alzheimer's disease [70]. Some terpenes showed inhibitory properties on these enzymes. Among

Table 4. Pharmacologic Properties of *D. virgatus* Essential Oil as an Inhibitor of Acetylcholinesterase (AChE) and Butyrylcholinesterase (BuChE) Enzymes

Sample	IC ₅₀ (mg ml ⁻¹)	
	AChE inhibition ^a	BuChE inhibition ^b
<i>D. virgatus</i> EO	0.329 ± 0.005	0.202 ± 0.003
Galanthamine ^c	0.015 ± 0.001	0.025 ± 0.002

^aInhibitory concentration of 50% acetylcholinesterase enzyme. ^bInhibitory concentration of 50% butyrylcholinesterase enzyme. ^cStandard drug used as a positive control.

them, the most active is α -pinene, with an IC₅₀ of 0.4 mM [71]. It is interesting to note that *D. virgatus* EO contains pinenes among its main constituents. Other studies about the *in vitro* inhibition of essential oils on AChE, has been performed on *Salvia lavandulifolia*. It resulted that the main terpenoids of this oil were responsible for its inhibitory activity, *i.e.* 1,8-cineole, α -pinene, and camphor [72]. In the case of *Artemisia annua* oil, limonene, 1,8-cineole, borneol, α -terpineol, camphor, α -pinene, β -pinene, and α -caryophyllene were responsible for this activity [27]. In a structure-activity correlation study conducted on bicyclic monoterpenoids, those containing an allyl methyl group exerted a strong inhibition of AChE. Further confirmation about the good inhibitory activity of α -pinene was evidenced by Miyazawa *et al.* [73]. α -Pinenes are active against AChE in a non-competitive and reversible manner and they could be beneficial in the treatment of cognitive impairments related to Alzheimer's disease [51]. Essential oils are very complex mixtures of components, which act in synergy and may be responsible for a higher activity than a single compound [74].

α -Glucosidase Inhibitory Assay

The α -glucosidase inhibitory activity profiles, shown in Fig. 5, reveal that *D. virgatus* essential oil has a dose-dependent effect. The enzyme was strongly inhibited by the EO with an IC₅₀ value of 0.35 ± 0.01 mg ml⁻¹, a value comparable to that of acarbose (0.24 ± 0.001 mg ml⁻¹), as shown in Table 5. Acarbose competitively inhibits the

hydrolysis of carbohydrates to absorbable monosaccharides. This drug delays the absorption of carbohydrates and results in a reduction of postprandial blood glucose peak [75]. Other previous studies confirm the beneficial use of antihyperglycaemic drugs that may represent an important source of new bioactive molecules. Among inhibitors of α -glucosidase, the study of Benalla *et al.* [76] reports crude extracts and active natural components isolated from several medicinal plants. Several classes of natural products have shown a strong α -glucosidase inhibitory activity, such as alkaloids, myoinositol, polyphenols, triterpenes, organic acids, phytosterols, and flavonoids [76]. However, no previous study has evaluated the effect of the essential oil of *D. virgatus* on the inhibition of α -glucosidase. The observed inhibitory effect of this essential oil can be attributed to its high content in monoterpene derivatives, such as α - and β -pinene. Also, the possible synergism of the minor components should be considered.

CONCLUSIONS

D. virgatus essential oil seems to be a promising source of natural products with potential therapeutic activities. This is the first time that the essential oil of Algerian *D. virgatus* species has been studied. In addition to the anti-microbial and antioxidant effects, we have for the first time undertaken to study the inhibitory effects of essential oil on α -glucosidase and cholinesterase enzymes. Our study shows that β -pinene (77.9%) and α -pinene (7.6%) are the most

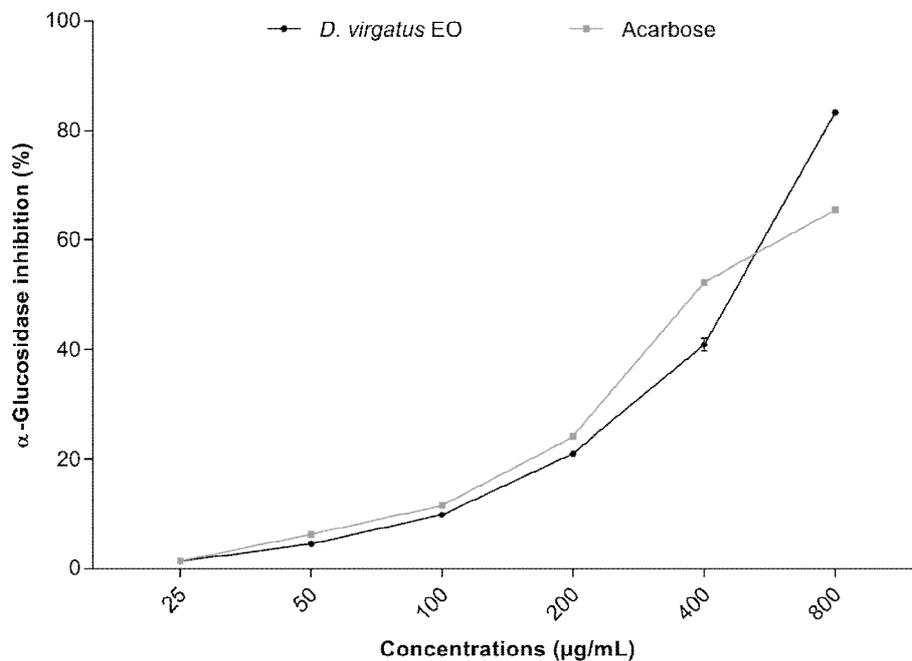


Fig. 5. *D. virgatus* EO inhibitory activity as compared to control compound acarbose against the α -glucosidase enzyme. For each concentration, α -glucosidase inhibition (%) expressed as mean \pm SD, n = 3.

Table 5. Half-maximal Inhibitory Concentration (IC_{50}) of *D. virgatus* Essential Oil Compared to Acarbose Control for Inhibition of α -Glucosidase Enzyme

Sample	IC_{50}
	(mg ml^{-1})
<i>D. virgatus</i> EO	0.354 ± 0.005
Acarbose ^b	0.237 ± 0.001

^aInhibitory concentration of 50% α -glucosidase enzyme. ^bStandard drugs used as a positive control.

abundant components of the essential oil extracted from native Algerian *D. virgatus*. The results demonstrate a significant antimicrobial activity and suggest the possible use of *D. virgatus* EO as a promising antimicrobial agent. The interesting inhibitory effect of *D. virgatus* EO on

α -glucosidase and cholinesterases reveals its potential application as an antihyperglycemic and anti-cholinesterase agent. Besides, in-depth studies are underway on the role of the constituents of *D. virgatus* EO, in particular β -pinene, in the aforementioned diseases.

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CONFLICT OF INTEREST

The authors certify that they have no competing interests are at stake and there is no conflict of interest with other people or organizations that could inappropriately influence or bias the content of the paper.

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