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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 3000–3700 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 pre-incubated 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, non-lysed 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,