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Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd

Floral research doi: 10.1016/S2222-1808(16)61151-9

Daucus aristidis Coss. essential oil: Volatile constituents and antimicrobial activity in pre-flowering stage

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ARTICLE INFO

Article history:
Received 13 Jul 2016
Received in revised form 8 Aug, 2nd revised form 29 Aug 2016
Accepted 20 Sep 2016
Available online 8 Oct 2016

Keywords:
Daucus aristidis Coss. (synonym *Ammiopsis aristidis* Batt.)
Apiaceae
Essential oil
Antimicrobial activity
α-pinene

ABSTRACT

Objective: To evaluate the essential oil composition and antimicrobial activity of an Algerian endemic plant, *Daucus aristidis* Coss. (Apiaceae) (*D. aristidis*) (synonym *Ammiopsis aristidis* Batt.) collected in pre-flowering stage in East of Algeria.

Methods: The aerial parts of *D. aristidis* Coss were collected. Essential oil (in pre-flowering stage) obtained by hydrodistillation was investigated for the first time by gas chromatograph and gas chromatograph-mass spectrometer and evaluated for their *in vitro* antimicrobial activity by the disc diffusion method at various dilutions of the oil.

Results: The main components of *D. aristidis* oil in pre-flowering stage were α-pinene (20.13%), cedrol (20.11%) and E-asarone (18.53%). *D. aristidis* oil exhibited an antibacterial activity against almost all the strains tested except for *Klebsiella pneumoniae* ATCC 700603 K6 and *Enterococcus faecalis* ATCC 49452 which exhibited a resistance against the oil with all dilutions. Also, the oil of *D. aristidis* had no activity against all fungi tested.

Conclusions: This is the first report on the volatile constituents and antimicrobial activity of *D. aristidis* in pre-flowering stage. The studied essential oil possesses moderate antibacterial activity against almost all strains tested but no antifungal activity.

1. Introduction

Since ancient times, the plants from Apiaceae family have been used as spices or crude drugs, particularly due to their essential oils. A dozen important herbal medicinal products from this botanical family are described in several pharmacopoeias, having antiseptic, expectorant, diuretic, carminative, vasodilator or spasmolytic actions[1]. *Daucus* is a genus belonging to this family, which comprises of about 300–455 genera and 3 000–3 750 species worldwide[2]. In Algeria, it is represented by 55 genera, 130 species and 27 subspecies. The species have a bipolar distribution (in temperate regions), but the majority live in the temperate Northern Hemisphere[3].

Daucus aristidis Coss. (*D. aristidis*) (synonymous: *Ammiopsis aristidis* Batt.) is an Apiaceae endemic to Algeria and has been locally known as "Noukhia", which is an annual plant with erect high and smooth stem[4]. The leaves are glabrous, pinnatisect with capillary segments. It has bracts that have many divided involucels and involucrals with white flowers and very large umbels rays that become yellow in a herbarium. The ovoid fruit is small (2–2.5 mm), grayish and finely tuberculate over their entire surface[3]. Several investigations have reported the chemical composition

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Foundation Project: Supported by the National Fund for Scientific Research, CNEPRU Projects, Ministry of Higher Education and Scientific Research, Algeria (Code N°: F01220140021).

The journal implements double-blind peer review practiced by specially invited international editorial board members.

of the essential oils from *Daucus carota* (*D. carota*) as well as its subspecies[5-8]. However, many species and subspecies of *Daucus* still remain to be examined for their essential oil components such as *D. aristidis*. In this paper, we investigated for the first time the chemical composition and antimicrobial activities of *D. aristidis* in pre-flowering stage through the study of volatile compounds extracted by hydrodistillation and by using Gas chromatography (GC) and gas chromatography–mass spectrometry (GC-MS) and the antimicrobial activity of *D. aristidis* essential oil against eight pathogen bacteria, eight fungi and one yeast using the paper disc diffusion method.

2. Materials and methods

2.1. Plant materials

The aerial parts of *D. aristidis* were collected in May 2011 and May 2012 from Ghoufi region (Algeria) at the altitude of 708 m. After taxonomic identification by Dr. Boulachaab Nacira from Department of Pharmacy, Faculty of Medicine, University Ferhat Abbas, Setif 1, a voucher specimen was deposited at the Herbarium of Department of Biology and Plant Ecology, University of Setif 1, Algeria.

2.2. Isolation procedure of the essential oil

A dried sample of the aerial parts (100 g) was subjected to hydrodistillation in a Clevenger-type apparatus for 3 h. The obtained essential oils were dried over anhydrous sodium sulfate and stored at 4 ℃ until tested and analyzed.

2.3. GC and GC-MS analysis

The GC-MS analysis was carried out using Agilent 5975 GC-MSD System. Innowax FSC column (60 m × 0.25 mm, 0.25 μm film thickness) was used with He as a carrier gas (0.8 mL/min). The oven temperature of GC was kept at 60 ℃ for 10 min and programmed to 220 ℃ at a rate of 4 ℃/min, and kept constant at 220 ℃ for 10 min and then programmed to 240 ℃ at a rate of 1 ℃/min. Split ratio was adjusted at 40:1. The injector temperature was set at 250 ℃. Mass spectra were recorded at 70 eV. Mass range was from 35 to 450 *m/z*.

The GC analysis was carried out using Agilent 6890N GC System. The temperature of flame ionization detector (FID) was 300 ℃. To obtain the same elution order with GC-MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

Compounds were identified by comparison of their mass spectra with those of NIST02 library data of the GC-MS system and Adams libraries spectra[9,10]. The constituents of the essential oil were identified by comparison with the elution order of compounds with their retention indices on semi-polar phases reported in the literature[10]. Retention indices of the components were determined relative to the retention times of a series of n-alkanes with linear interpolation. Percentage of individual components was calculated based on GC peak areas without FID response factor correction.

2.4. Antimicrobial activity

2.4.1. Microbial strains

A total of 17 microorganisms were used for antimicrobial activity studies including eight strains of bacteria, obtained from the Pasteur Institute (Algeria) [*Escherichia coli* ATCC 25922 (*E. coli*), *Pseudomonas aeruginosa* ATCC 27853 (*P. aeruginosa*), *Staphylococcus aureus* ATCC 25923 (*S. aureus*), *Klebsiella pneumoniae* (*K. pneumoniae*) E47, *K. pneumoniae* K6 ATCC 700603, *Enterococcus feacalis* ATCC 49452, *Bacillus cereus* ATCC 10876 (*B. cereus*) and *Proteus mirabilis* ATCC 35659 (*P. mirabilis*)], eight fungi and one yeast [*Aspegillus niger* 2 CA 936, *Aspergillus flavus* NRRL 391, *Candida albicans* ATCC 1024 (*C. albicans*), *Phytophthora cinnamomi*, *Phytophthora cactorum*, *Colletotrichum acutatum*, *Verticillium dahliae*, *Botrytis cinerea*, and *Botrytis fabae*]. The bacteria were grown on Mueller-Hinton agar (Bio-Rad, France). The fungi and yeast were grown on Sabouraud agar (Biomark, India). A volume (20 mL) of each medium was poured into 90 mm diameter Petri dishes. The bacteria used in the tests were obtained from 24 h cultures and suspended in sterile solution to obtain a concentration of approximately 10^8 CFU/mL by comparison with tube No. 1 of Mac Farland scale. The young fungal inoculates were standardized to obtain a final concentration of 10^5 CFU or spores/mL.

2.4.2. Determination of the antimicrobial activity

The disc diffusion method was used to evaluate the zone of microbial growth inhibition at various dilutions of the oil. Different essential oil dilutions (10 μL) in dimethylsulfoxide (1/2, 1/5 and 1/10 v/v) were injected into sterilized Wathman discs which had a diameter of 6 mm and pure dimethylsulfoxide (10 μL) was injected as negative control. In addition, disks with 50 μg of clotrimazole and 10 μg of gentamycin (Bio-Rad, France) were used as positive controls for antifungal and antibacterial activity, respectively. Medium surfaces were spread using a sterile swab containing the microbial suspension, dried and left for 30 min at room temperature to allow the diffusion of oil and then the bacteria were incubated at 37 ℃ for 24 h, fungi were incubated at 30 ℃ for 72 h and yeast was incubated at 37 ℃ for 48 h in order to observe the formation of clear zone around the disc. The diameter of clear zone around the disc was measured and expressed in mm as its antimicrobial activity. Three discs per plate were used and each test was run in triplicate.