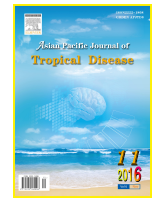




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Daucus aristidis Coss. essential oil: Volatile constituents and antimicrobial activity in pre-flowering stage

Mebarka Lamamra^{1*}, Hocine Laouer¹, Abdenour Adjaoud², Farida Sahli³, Smain Amira⁴, Salah Akkal⁵

¹Laboratory of Natural Resources Valorization, Department of Biology and Plant Ecology, Ferhat Abbas University, Setif-1, Algeria

²Departement of Biology, Abderrahmane Mira University, Bejaia, Algeria

³Laboratory of Microbiology, Abdenour Saadna Hospital, Setif, Algeria

⁴Laboratory of Phytotherapy Applied to Chronic Diseases, Department of Animal Biology and Physiology, Ferhat Abbas University, Setif-1, Algeria

⁵Laboratory of Chemistry, Chemical and Biological Analyze, University of Mentouri, Constantine, Algeria

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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

*Corresponding author: Mebarka Lamamra, Laboratory of Natural Resources Valorization, Department of Biology and Plant Ecology, Ferhat Abbas University, Setif-1, Algeria.

Tel: (00213)550352141

E-mail: lamamramebarka@yahoo.fr

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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 °C 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 °C for 10 min and programmed to 220 °C at a rate of 4 °C/min, and kept constant at 220 °C for 10 min and then programmed to 240 °C at a rate of 1 °C/min. Split ratio was adjusted at 40:1. The injector temperature was set at 250 °C. 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 °C. 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 faecalis* 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 10⁸ CFU/mL by comparison with tube No. 1 of Mac Farland scale. The young fungal inoculates were standardized to obtain a final concentration of 10⁵ 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 °C for 24 h, fungi were incubated at 30 °C for 72 h and yeast was incubated at 37 °C 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.

3. Results

The yield of essential oil of the aerial part of *D. aristidis* in pre-flowering stage was about 0.35% (w/w) and it was analyzed by gas chromatography coupled to a mass spectrometer (GC-MS) to isolate and identify 27 compounds (Table 1) representing 98.33% of the essential oil constituents. The major components were α -pinene (20.14%), the cedrol (20.12%) and E-asarone (18.54%). This oil was characterized by a significant oxygenated fraction (65.49%) represented mainly by the cedrol (20.12%) and the E-asarone (18.53%) and oxygenated monoterpenes (15.22%). The hydrocarbon fraction was 32.84%, where α -pinene represented 20.13% of this fraction.

Table 1

Chemical composition of essential oil of *D. aristidis* in pre-flowering stage.

No.	Compounds	RT	%
1	α -Pinene	5.2345	20.14
2	Camphene	5.6061	0.43
3	2,4(10)-diene-Thuja	5.7373	0.92
4	Sabinene	6.2313	0.21
5	β -Pinene	6.3318	3.81
6	Myrcene	6.6903	0.47
7	O-Cymene	7.7351	0.64
8	Limonene	7.8706	0.52
9	α -Campholenal	11.3547	1.27
10	Trans-carveol	11.8313	1.23
11	Cis-verbenol	11.9362	0.53
12	Trans-verbenol	12.0761	2.71
13	NI	12.2247	0.60
14	Pinocarvone	12.7799	0.59
15	Myrtenal	14.1482	1.43
16	Verbenone	14.6859	0.44
17	Geranyl acetate	22.0258	7.07
18	β -Cedrene	23.3809	0.86
19	NI	25.1646	0.41
20	β -Bisabolene	27.0094	4.84
21	Caryophyllene oxide	29.7810	3.51
22	Cedrol	30.4411	20.12
23	NI	30.7689	0.28
24	NI	31.5471	0.38
25	β -Eudesmol	32.2815	0.59
26	E-asarone	33.5449	18.54
27	Eudesm-7(11)-en-4-ol	33.9471	7.47

NI: Non identified compounds; RT: Retention time.

The antimicrobial activity of the essential oil of *D. aristidis* assessed by diffusion method revealed a limited activity on the growth of *E. coli* and *B. cereus* (inhibition diameter of 13 mm and dilution of 1/2), The same results are found with the other strains but with less inhibition diameters: *P. aeruginosa* (inhibition diameter of 11 mm and dilution of 1/2), *S. aureus* (inhibition diameter of 10 mm and dilution of 1/2) and *K. pneumoniae* E47 (inhibition diameter of 9 mm and dilution 1/2). All bacteria were inhibited only with the exception of *S. aureus* that was destroyed. However, *P. mirabilis* ATCC 35659, *Enterococcus faecalis* ATCC 49452 and *K. pneumoniae* ATCC 700603 K6 were shown to be resistant against all dilutions tested. This oil was shown no activity on all tested fungi in the various dilutions (Table 2). The diameters of the zones of inhibition showed that the antimicrobial capacity was inversely proportional

to the dilution, that is to say, the effect decreased with increasing dilution of the essential oil.

Table 2

The sensitivity of the microorganisms tested against *D. aristidis* essential oil.

Parameters	Inhibition Zone (mm)					
	1/2	1/5	1/10	GN	CTR	
Tested microorganisms	<i>E. coli</i>	13	7	6	24	-
	<i>S. aureus</i>	10	8	6	28	-
	<i>P. aeruginosa</i>	11	9	6	26	-
	<i>Enterococcus faecalis</i> ATCC 49452	6	6	6	25	-
	<i>K. pneumoniae</i> K6 ATCC 700603	6	6	6	18	-
	<i>B. cereus</i>	13	11	7	27	-
	<i>K. pneumoniae</i> E47	9	8	6	18	-
Homopathogenic fungi	<i>P. mirabilis</i>	6	6	6	24	-
	<i>Aspergillus niger</i> 2 CA 936	6	6	6	-	15
	<i>Aspergillus flavus</i> NRRL 391	6	6	6	-	25
Phytopathogenic fungi	<i>C. albicans</i>	6	6	6	-	45
	<i>Phytophthora cinnamomi</i>	6	6	6	-	28
	<i>Phytophthora cactorum</i>	6	6	6	-	22
	<i>Colletotrichum acutatum</i>	6	6	6	-	21
	<i>Verticillium dahliae</i>	6	6	6	-	19
	<i>Botrytis cinerea</i>	6	6	6	-	23
	<i>Botrytis fabae</i>	6	6	6	-	21

GN: Gentamycin; CTR: Clotrimazole.

4. Discussion

The yield of *D. aristidis* [0.35% (w/w)] can be seen as the lowest compared to that obtained by steam distillation from the fruits of *Daucus gingidium* L. subsp *gingidium* (*D. gingidium* L. subsp *gingidium*), which is 1.21% (w/w) and that obtained from the flowers of *Daucus carota* subsp *halophilus* which is between 0.6% to 1% (v/w)[1,11]. Similarly, a yield of 1.1% was found from the flowers of *Daucus carota* subsp *sativum*[12]. However, this yield is higher than that obtained from the leaves of *Daucus gingidium* L. subsp *gingidium* which is around 0.04%, but very close to the yield obtained from flowers of *Daucus carota* subsp *halophilus* which is 0.4% and the same value was obtained after extraction of the aerial parts of *Daucus reboudii*[1,11,13]. It should be noted that all of these essential oils were obtained by hydrodistillation in a Clevenger.

The results of chemical analysis showed that the essential oil of *D. aristidis* contains α -pinene (20.14%), cedrol (20.12%) and E-asarone (18.53%) as major constituents and the α -pinene is the main component of the most essential oil species of the genus *Daucus*. The main components of the essential oil of the aerial part of *Daucus muricatus* from western Algeria are α -pinene (9.9%–21%) and limonene (14.2%–24%) and this component is present in very small amount (0.5%) in the oil of *D. aristidis* studied[14]. Similarly, the essential oil of the aerial part of *Daucus reboudii* contains α -pinene as majority compounds which constituents 39.7% followed by sabinene (21.2%), in contrast to these results, our oil tested contains sabinene with a small percentage (0.2%)[13]. Chemical analysis of the essential oil of *Daucus carota* ssp. *carota* from Poland showed that the major proportion of this oil is represented by α -pinene with a percentage of 41%[5]. However, the essential oil analysis of leaves and fruits of *Daucus sahariensis* revealed the presence of an appreciable amount of myristicin (34.3% and 43.9%) respectively, which is completely absent in the oil of *D. aristidis*[15]. The α -pinene is also present in oil of *Daucus sahariensis* but with lesser amounts (5.4%–13.1%), respectively[15]. The essential oils of leaves and fruits of *D. gingidium* ssp. *gingidium* contain sabinene

(26.8%–60.6%) and α -pinene (10.8%–12.2%), respectively and E-asarone is also present but as minor component (0.2%) in the essential oil of the leaves[11]. It was found the presence of these abundant components in the essential oil of *Daucus carota* subsp *sativus*: carotol (10.2%–58.5%), α -pinene (21.2%–41.2%), limonene (4.4%–12.7%), sabinene (0.2%–5.3 %) and myrcene (6.4%–14.1%) and the latter component is present in trace amounts in the tested oil (0.4%)[12]. Similarly, the carotol and sabinene are major constituents of the fruit oil of *D. carota* from Northern Serbia with proportions of 20.3% and 18.7% respectively, while the α -pinene represents only 7.95%[16]. The comparative study of essential oils of *Daucus guttatus* ssp. *zahariadii* and wild *D. carota* in Balkan showed that the first oil was presented as major constituents [α -pinene (43.3%) and the β -bisabolene (34.2 %)] which presents only 4.84% of the oil of *D. aristidis* and α -pinene in a minor amount (0.3%), but the oil of the aerial parts of wild *D. carota* contains 29.3% of α -pinene which is its major component[17]. On the other hand, the chemical analysis of the essential oil of seeds of wild *D. carota* has proved the presence of the principal component geranyl acetate (53.2%) which represents 7.07% of the oil tested and a minor component of α -pinene (3.3%)[17]. In almost all of the literature on the chemical composition of the essential oil of the genus *Daucus*, no oil contains cedrol which is one of the majority compounds of the oil of *D. aristidis* components.

Variations in yield and chemical composition of the essential oil might be due to several factors: environmental factors (temperature, humidity, soil texture, altitude *etc.*), geographical origin, plant organ, stage of growth, the time of picking, storage of plant material, individual genetic variability and extraction method[18-23].

The biological activity of essential oils has a relationship with the functional groups of the components, their proportions and the interaction between them[24-26]. Indeed, the activity of the essential oil of *D. aristidis* could be attributed to the presence of α -pinene (20.14%) as major component known for its significant antimicrobial activity. The inhibitory effect of the oil of *Tetraclinis articulata* (Vahl.) of Morocco is due to the presence of α -pinene (23.54%)[27]. Indeed, the essential oil of the leaves of *Juniperus oxycedrus* showed that it has a good inhibitory activity against *C. albicans* and *S. aureus*. This essential oil is characterized by the abundance of α -pinene (about 86%)[28]. Similarly, during the work on the essential oil of *Croton stellulifer* and it was reported that the observed activity against bacteria and fungi studied is attributed particularly to the presence of α -pinene among the majority compounds of this species[29]. A study on the essential oil of *Sideritis sipylea* [containing as a main component 35.20% of α -pinene] showed that it has a high activity against the tested microorganisms[30]. The antimicrobial activity of the oil of *Mutellina purpurea* Thell. against *Staphylococcus epidermidis* was attributed to the presence of α -pinene[31]. However, the low activity of the oil of *Thymus algeriensis* was due to the presence of α -pinene (20.5%), β -pinene (8.02%) and limonene (4.85%)[32]. The α -pinene was also found inactive against *S. aureus*, *E. coli*, *P. aeruginosa* and *C. albicans*[28]. Similarly, the antimicrobial activity of the enantiomers of α -pinene and β -pinene in bacterial and fungal strains was tested and the results showed that the positive enantiomers

exhibited good activity against the tested fungi and bacteria with minimal inhibitory concentrations ranging from 117 to 4150 μ g/mL. However, no antimicrobial activity was detected with the negative enantiomers up to 20 μ g/mL[33]. Among the studied strains, *K. pneumoniae* and *Enterococcus faecalis* are shown a resistant against the oil of *D. aristidis* and in fact, these two bacteria are known in the literature by their pathogenicity and their multidrug resistance to several antibiotics[34-37]. In addition, *K. pneumoniae* has an intrinsic resistance to biocides which is related to the nature of its lipopolysaccharidic outer membrane and capsule polysaccharides which gives it its pathogenicity and forms an impermeable barrier to hydrophobic compounds[38,39]. Similarly, it was reported that a germ whose pathogenicity is high, offers almost constant resistance. When the germ is more virulent, it has more chances of large resistance species[40]. On the other hand, essential oil of *D. aristidis* showed no activity against all fungi tested. Indeed, the microbiological activity depends on the chemical composition of the oil and the strain studied. Therefore, the antimicrobial activity of the essential oil of the carrot fruit is probably due to the high sensitivity of microbial strains to carotol, which is the main component[16]. However, carotol is totally absent in the oil of *D. aristidis* oil. The absence of the antifungal activity of pine oil had also been correlated with its high content of α -pinene and β -pinene and these compounds present (23.93%) of the composition of *D. aristidis* oil[41].

In conclusion, the present study provides, for the first time, important data about the chemical composition of *D. aristidis* in pre-flowering stage grown in Algeria. The studied essential oils possess moderate antibacterial activity against almost all the strains tested but had no antifungal activity. Therefore, further investigations should be carried out on other biological activities, including antioxidant properties and insecticidal activity. Furthermore, antagonistic and synergistic effects of known and/or unknown compounds present in the volatile mixture should also be verified.

Conflict of interest statement

We declare that we have no conflict of interest.

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