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Research Article

Chemical and Biological Study of Essential Oils of Two Populations of Algerian *Daucus setifolius* Desf.

H. Laouer*[a], A. Bouheda[a], S. Haroutounian[b], E. Evergetis[b], M. Bouchekrit[a], F. Sahli[a], N. Boulaacheb[a], S. Akkal[c]

[a]*Laboratory of natural resources valorization, University of Setif, Algeria e-mail: hocine_laouer@yahoo.fr*
[b]*Chemistry Laboratory, Agricultural University of Athens, Athens 11855, GREECE*
[c]*Laboratory of chemistry, chemical and Biological Analyze, Department of chemistry, University of Mentouri, Constantine, Algeria*

ABSTRACT: **Introduction:** *Daucus setifolius* (Apiaceae) is a species found in east of Algeria and not known in the folk medicine. In the present study, the chemical composition, antioxidant and antimicrobial activities of *D. setifolius* essential oil obtained by hydrodistillation, collected from two sites of Algeria (Bejaia and Skikda), were investigated. **Methods:** The chemical composition of the oil was determined by GC and GC/MS. Their *in vitro* antimicrobial activities were evaluated by the disc diffusion method against a panel of eight bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633, *Klebsiella pneumonia* ATCC 700603, *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 15313, *Enterococcus faecalis* ATCC 49452 and *Acinetobacter baumannii* ATCC19606) and three fungi (*Aspergillus flavus, Aspergillus niger* and *Candida albicans*). The antioxidant activity was assessed using the DPPH test. **Results:** The essential oils of the Skikda and Bejaia populations were found to contain thirty and ten constituents respectively, which represented the 97.1% and 97.0% of their phytochemicals. The essential oils-yields varied from 0.3% (Skikda) to 0.5% (Bejaia), both containing the compounds sabinene and β-pinene as the major compounds. The IC_{50} values of DPPH assays were IC_{50} = 0.041895 µg/ml and IC_{50} = 0.00575 µg/ml for Bejaia and Skikda populations essentials oils, respectively; the Bejaia value is superior when compared with the respective value of BHT (IC_{50} = 0.0238225 µg/ml 1). **Conclusions:** The results herein indicate that the essential oils of the aerial parts of both *D. setifolius* populations investigated contain β-pinene and sabinene as major components, while the molecule of estragole (9.61%) was assessed present only in the Skikda oil. In respect of their bioactivities, the *D. setifolius* Bejaia population oil was more potent as compared to the known antioxidant compound butylated hydroxytoluene (BHT). Both oils were inactive against the test panel microorganisms except for *C. albicans*.

KEYWORDS: *Daucus setifolius*, volatile oils, antimicrobial activity, antioxidant activity

INTRODUCTION

The medicinal and nutritional properties of the Apiaceae family plants are well established for longtime and many of them are being used as spices and herbal medical preparations. Thus, they account as well-known source of essential oils and important herbal products. They are included in various pharmacopoeias as antiseptic, expectorant, diuretic, carminative, vasodilator, or spasmolytic agents.[1]

Nowadays, the consumers preference to use natural products has resulted in the exploitation of many plants, essential oils and/or extracts as potent ingredients used by the pharmaceutical, cosmetic and food industries.[2] The essential oils comprise secondary metabolites extracted from different parts of aromatic plants with potent antimicrobial[3–5] and antioxidant properties that are being used as natural remedy in phytotherapy.

The genus Daucus (Apiaceae) includes eleven species and eight subspecies in Algeria.[6] *D. setifolius* Desf.

*Correspondence
H. Laouer
Laboratory of natural resources valorization,
University of Setif, Algeria
E- mail: hocine_laouer@yahoo.fr
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(Syn. *D. setulosus* Ball. Non Guss.; *D. brachylobus* Boiss.) is a perennial aromatic species, with a stem up to 1 m erect. It is characterized by glabrous branches and subverticillata, linear and pubescent leaves. Umbels terminal and axilar, pedunculata. Its flowers are white, with very small petals, styles long, pedicels tomentose. Many plant of the genus Daucus of Algeria have become the subject of several investigations: *D. reboudii Coss*;[7] *D. sahariensis*;[8] *D. crinitus* Desf.[9–13]

Because of the side effects of some antimicrobial drugs and the resistance that pathogenic microorganisms build against the antibiotics, much recent attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine.[15] Numerous researchers have shown interest in the biologically active components isolated from plants and for their influence on the elimination of pathogenic microorganisms.[16]

Herein we present the assessment of the chemical composition, the antioxidant activity and the antimicrobial activity of the volatile oil of *D. setifolius* growing wild in Algeria, against a wide range of pathogenic microorganisms.

MATERIALS AND METHODS

Plant material

The aerial parts of *D. setifolius* Desf. were collected in August 2010 from Flifila mountain (Skikda) and from Bordj Mira site (Bejaia). The plant material was collected during its flowering stage. The air-drying of the plants was performed in the shade at room temperature. The identity of the plant was determined in our laboratory and confirmed at the Royal Botanic Garden, Kew, London. Voucher specimens of the plant material are deposited in the Herbarium at the department of biology and ecology vegetal, University of Setif (UFAS).

Microorganisms

The microorganisms used in this study were kindly provided by the laboratory of microbiology, Faculty of natural sciences and were: *Citrobacter freundii* ATCC 8090, *Bacillus cereus* ATCC 10876, *Enterococcus faecalis* ATCC 49452, *Salmonella typhimurium* ATCC 13311, *Klebsiella pneumoniae* ATCC 70060, *Acinetobacter baumannii, Proteus mirabilis* ATCC 35659, *Listeria monocytogenes* ATCC 15313, *Escherichia coli, Staphylococcus aureus, Aspergillus niger, Aspergillus flavus, Candida albicans.*

Chemicals

Dimethyl sulfoxide (DMSO), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and BHT (butylated hydroxytoluen), were purchase from Sigma-aldrich (St Louis, MO, USA). Ethanol from Fluka chemicals (Buchs, Switzerland). Mueller Hinton agar from Oxoid Lab Ltd (Hamphire, England) and Sabouraud dextrose agar from Bacto-Difco Lab Co., Ltd (Detroit, MI, USA). All other chemicals were of the finest grade available.

Determination of the antimicrobial activity

The antibacterial activity of the essential oil was evaluated using a modified agar diffusion method[17] as describe by Laouer et al.[18] A dilution of 100 µL of suspension of the tested microorganisms in 10 mL nutrient broth, containing 2.0×10^6 colony forming units (CFU/mL) for bacteria, 10^7CFU/mL for yeast, and 2.0×10^5 spore/mL for fungi, spread on Mueller-Hinton agar (LB) and Sabouraud dextrose agar (SDA) sterilized in a flask and cooled to 45–50°C were placed in 9 cm Petri dishes (20 mL). Sterile 6 mm diameter filter paper discs were impregnated with 10 µL of dilute essential oil solution (10 µL of 1/2, 1/5 and 1/10 v/v) in Dimethyl sulfoxide (DMSO) (Sigma-Aldrich), and were deposited at equal distances on the surface of the inoculated agar (LB for antibacterial essay and SDA for antifungal assay). The microbial suspension (10 mL) was spread on the solid media plates. The Petri dishes were left in at 4°C for 30 min before incubation to ensure a good diffusion of the oil in the agar. The diameter of inhibition was measured after 24 h at 37°C for bacteria, after 7 days of incubation at 27°C for the fungi and after 48 h of incubation at 37°C for the yeast. The scale of measurement[11] was the following (disk diameter included): > 20 mm zone of inhibition is strongly inhibitory; < 20–12 mm zone of inhibition is moderately/midly inhibitory; < 12 mm is not inhibitory. Gentamicin [3 µg/mL(Sigma-Aldrich)], 5-Fluorocytosine, Ketoconazole [5 mg/mL (Sigma-Aldrich)] and DMSO was used as positive and negative controls, respectively. The diameters of the inhibition zones were measured in mm. Controls were set up with equivalent quantities of ethanol. Antimicrobial activity was evaluated by measuring the zone of inhibition against each test organism. Each assay was performed in triplicate and the results were expressed as average values.

Gas chromatography

All GC analyses were carried out on a Perkin-Elmer, Clarus 500 gas chromatograph, fitted with a HP 5MS 30 m × 0.25 mm × 0.25 µm film thickness capillary column and FID detector. The column temperature was programmed from 60°C to 280°C at a initial rate of 3°C/min. The injector and detector temperatures were programmed at 230°C and 300°C respectively. Helium was used as the carrier gas at a flow rate 1 mL/min.

Gas chromatography/Mass Spectrometry

The GC-MS analyses were performed using a Hewlett Packard 5973–6890 GC-MS system operating on EI mode (equipped with a HP 5MS 30m × 0.25mm × 0.25µm film thickness capillary column), using He (1 mL/min) as the carrier gas. The initial temperature of the column was 60°C. Then was heated gradually to 280°C with a 3°C/min rate. The identification of the compounds was based on comparison of their retention indices (RI)[19] obtained, using various n-alkanes (C_9–C_{24}). Also their EI-mass spectra was compared with the NIST/NBS, Wiley libraries spectra and literature.[20,21] Additionally, the identity of the indicated phytochemicals was confirmed by comparison with available authentic samples.

Antioxidant activity

DPPH scavenging assay

The antioxidant activity of *D. setifolius* essential oil was assessed by measuring their ability to scavenging 2,2'-diphenyl-2-picrylhydrazyl stable radicals (DPPH). DPPH is a purple-colored stable free radical; it becomes reduced to the yellow-colored, diphenyl picryl-hydrazine. The DPPH assay was performed as described previously[22] with slight modification: 600 µL of various dilutions of oil or standards (BHT or methanol) were mixed with 600 µL of a 0.004% methanol solution of DPPH. The mixture was shaken vigorously and incubated in the dark. The absorbance of the samples was read at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical-scavenging activity. All measurements were performed in triplicate. The radical scavenging activity was calculated as % inhibition from the following equation:

$$Scavenging\ effect\ \% = \frac{A_c - A_e}{A_c} \times 100$$

Where: **Ac**: control absorbance, **Ae**: absorbance in the presence of oil.

RESULTS AND DISCUSSION

Table 1 summarizes the results of the chemical analyses of the essential oils of *D. setitolius*. The essential oil's chemical composition assessments and the identification of the main components were performed by GC and GC-MS analyses. Compounds were identified by comparing their retention indices with those of standard compounds. The oils tested were found to contain the monoterpenes sabinene and pinene as major components. More specifically, the yield of the essential oil obtained from *D. setifolius* Bejaia was 0.5% which was comprised of β-pinene 41.1%, sabinene 38.36% and α-pinene 4.65%. Accordingly, the yield of the essential oil isolated from the *D. setifolius* Skikda population was 0.3%, with major components the molecules of sabinene (37.7%), β-pinene (28.6%) and estragole (9.6%). It must also noted that both oils also contain in lower amounts various sesquiterpenes and are characterized by aromatic odor and a yellow-pale color, with the Bejaia oil being the darker.

The results of the antimicrobial activity of the essential oil are resented in Table 2. Oil from the Bejaia population *D. setifolius* showed much larger inhibition zones than the Skikda population oil. The dilute oils of Skikda population tested in the disc diffusion method did not affect any microorganisms tested except *C. albicans* which showed a moderate antifungal sensibility (13 mm) against the ½ (v/v) dilute oil.

The two fold dilution of Bejaia population *D. setifolius* essential oil showed distinct antimicrobial activity with a wide spectrum and wide inhibition zones when compared with several antibiotics. The essential oil exhibited antimicrobial activity of various degrees against the tested strains (Table 2). It is evident from the table that the antimicrobial activities greatly increase with increase of the oil concentrations from 1/10 (v/v) to ½ (v/v). Strong inhibition zones were obtained for essential oil against *C. albicans,* with the strongest inhibition zone (24 mm), followed by *Acinetobacter baumannii* ATCC 19606 with inhibition zone (21 mm); the other microorganisms were less sensitive to the two fold oil dilution. *Bacillus subtilis* ATCC 6633, *Klebsiella pneumonia* ATCC 700603 and, *Aspergillus niger* have a moderately zone of inhibition,

Table 1: Chemical composition of *Daucus setifolius* Desf. essential oils

Compound	X1	X2	KI[c]	Identification method
α-thujene	2.48	2.09	930	a, b
α-pinene	5.45	4.65	939	a, b
Camphene	0.58		954	a, b
Sabinene	37.65	38.36	975	a, b
β-pinene	28.62	41.1	979	a, b
Myrcene	2.27	2.85	991	a, b
para-cymene	1.6	0.83	1025	a, b
Limonene	2.73	1.52	1029	a, b
γ-terpinene	0.89	1.55	1060	a, b
α-terpineol	3.54	3.64	1189	a, b
Estragole	9.61	–	1197	a, b
E-anethole	0.55		1291	a, b
germacrene D	1.12	0.43	1480	a, b
Total	**97.09**	**97.02**		

X1= Skikda population, X2= Bejaia population, [a]Comparison of mass spectra with MS libraries and retention times [b]Comparison with authentic compounds, [c]KI, Kovats indices calculated against C_8 to C_{24} *n*-alkanes on the HP 5MS column.

Table 2: Antimicrobial activity of two populations of *Daucus setifolius* essential oils

Microorganism	X2			X1			Gent./5-FC or Ket.	DMSO
	½	1/5	1/10	1/2	1/5	1/10		
Escherichia coli ATCC 25922	11 (+)	7 (+)	–	–	–	–	22	–
Pseudomonas aeruginosa ATCC 27853	10 (+)	–	–	–	–	–	26	–
Bacillus subtilis ATCC 6633	13 (−)	8 (−)	–	–	–	–	18	–
Klebsiella pneumonia ATCC 700603	12 (+)	11 (+)	–	–	–	–	9	–
Staphylococcus aureus ATCC 25923	10 (+)	8 (+)	7 (+)	–	–	–	25	–
Listeria monocytogenes ATCC 15313	–	–	–	–	–	–	11	–
Enterococcus faecalis ATCC 49452	–	–	–	–	–	–	30	–
Acinetobacter baumannii ATCC19606	21 (−)	–	–	–	–	–	11	–
Candida albicans	24 (−)	17 (−)	14 (−)	13 (+)	–	–	26	–
Aspergillus niger	13	12	10	–	–	–	64	–
Aspergillus flavus	11	8	8	10	–	–	26	–

X1= Skikda essential oil population, X2= Bejaia essential oil population. Numbers indicate the mean diameters (mm) of inhibition of at least triplicate experiments. – indicates no growth inhibition. (–) biocide. (+) Biostatic. Gent. (Gentamicin) for all bacteria; 5-FC (5-Fluorocytosine) for *Candida albicans* and *Aspergillus niger*; Ket. (Ketoconazole) for *Aspergillus flavus* were used as the positive controls. DMSO (Dimethyl sulfoxide) was included as a negative control.

varying from 11 mm to 13 mm, meanwhile *Escherichia coli* ATCC 25922 (11 mm), *Pseudomonas aeruginosa* ATCC 27853 (10 mm), *Staphylococcus aureus* ATCC 25923 (10 mm) *Listeria monocytogenes* ATCC 15313 (0 mm) and *Enterococcus faecalis* ATCC 49452 (0 mm) are not inhibited.

The two fold dilution of *D. setifolius* essential oil showed low antimicrobial activity with respect to almost all investigated strains, while *C. albicans* showed distinct antimicrobial sensitivity to the Bejaia *D. setifolius* oil with a wide inhibition zone of 24, 17 and 14 mm, respectively. Glisic et al. (2007) have established that the fractions containing the high concentration of α-pinene and sabinene effectively inhibited the growth of microorganisms, especially against fungi. In addition, the antimicrobial activity also was dependent on β-pinene content (41.1%). However, Skikda population oil exhibited no effect of antibacterial activity against of the bacterial pathogens tested. It is possible that the estragole has decreased the antimicrobial activity of others constituents in the oil. Most of the antimicrobial activity in essential oils is found in the oxygenated terpenoids (e.g. alcohols and phenolic terpenes), while some hydrocarbons also exhibit antimicrobial effects.[23,24] Interactions between these components may lead to antagonistic, additive or synergistic effects.

The Bejaia population *D. setifolius* essential oil also showed distinct antioxidant activity $IC_{50} = 0.041895$ µg/mL superior than BHT $IC_{50} = 0.0238225$ µg/mL. Meanwhile, the antioxidant activity of Skikda essential oil is less active than of Bejaia population essential oil $IC_{50} = 0.00575$ µg/mL. This study affirms the *in vitro* antioxidant potential of *D. setifolius* essential oil Bejaia population, with results superior to those of the standard compound (BHT). However, the components responsible for the antioxidant activities of the oil were not identified and further work should be conducted to isolate these bioactive compounds. Jukié and Milos[25] have demonstrated that the thym phenolic chemotype possesses stronger antioxidant properties than the non phenolic one. On the basis of the fact, the Bejaia oil is richer in these secondary metabolites.

CONCLUSIONS

In conclusion, the results of this study demonstrate the antimicrobial potential of *D. setifolius* Bejaia population and indicate that *D. setifolius* essential oil are worthy of further study. Further evaluation of antimicrobial properties of the oil against a more extensive panel of microbial agents is warranted. Whilst the Bejaia oil examined in this report are promising as antioxidant and antibacterial agents, caution is needed before these compounds can be applied to medicinal purposes. In particular, further toxicity studies using human cell lines are needed to verify the suitability of these extracts for these purposes.

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