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Research Article

Antibacterial Activity of *Daucus crinitus* Essential Oils along the Vegetative Life of the Plant

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The essential oils from the aerial parts of *Daucus crinitus* Desf. were analyzed at three developmental stages (early vegetative, early flowering, and full flowering). Oil yield was found to vary depending on the stage of development, and the highest content of oil (0.15% w/w) was obtained at full flowering. The chemical composition of essential oils studied by GC and GC-MS showed a total of 71 compounds: 27 aliphatic compounds, 18 sesquiterpene hydrocarbons, 9 hydrocarbons monoterpene, 5 oxygenated monoterpenes, 5 phenolic compounds, 4 oxygenated sesquiterpenes, 2 oxygenated diterpenes, and 01 diterpene hydrocarbons. Whatever the analyzed stage, phenolic compounds were the most abundant group. Their level significantly increased during ripening and varied from 36.4 to 82.1%. Antimicrobial activities of oils were tested on four different microorganisms. The oils of various phenological stages showed high activity against *Candida albicans* (30 mm) and *Staphylococcus aureus* (11–28 mm) bacteria strains which are deemed very dangerous and very difficult to eliminate. Thus, they represent an inexpensive source of natural antibacterial substances that may potentially be used in pathogenic systems.

1. Introduction

In the last years, interest in medicinal plants as an alternative to synthetic drugs is more and more increasing, particularly against microbial agents because of the growth of antibiotic resistance. Essential oils of various species of edible and medicinal plants, herbs, and spices constitute a wide source of natural biologically active agents [1]. Their components have many applications in ethnomedicine, food, beverages, preservation, cosmetics as well as in the fragrance and pharmaceutical industries [2–4]. For a long time, plants from the Apiaceae family have been used as spices or drugs, particularly due to their essential oils. A dozen important herbal medicinal products from this botanic family are described in some pharmacopoeias, having antiseptic, expectorant, diuretic, carminative, vasodilator, or spasmolytic actions [5]. Variation in chemical composition of essential oils may be due to many factors, such as the genetic factors, phenological stages, the environmental conditions, and the pedoclimatic conditions. Antimicrobial activity of an essential oil is attributed mainly to its major components, although the synergistic or antagonistic effect of one minor compound of the mixture has to be considered [6]. Therefore, antimicrobial and other biological activities may vary, based on the variations in the chemical composition [7, 8]. *Daucus crinitus* Desf. is characterized by the presence of many subspecies that colonize the sands and cliffs [9]. A survey conducted by herbalists identified that, in folk medicine, a drink made from the roots of *D. crinitus* is used in decoction to expel the placenta after childbirth, as a tonic and coldness. From a chemical point of view, *D. crinitus* has been only the subject of two studies on the chemical composition of essential oil. The aerial parts oil were characterized by isochavicol isobutyrate, octyl acetate, α-pinene, and isochavicol 2-methylbutyrate [10, 11]. However, roots oil was mainly composed of aliphatic compounds [10]. In fact, during ontogenesis a number of

transformations occur, revealed by morphological changes and variability of physiological processes [12]. The aim of this research is to study the essential oil compositions of aerial parts of *D. crinitus* during three vegetative stages and its coherence with antibacterial activity in order to find new bioactive natural products.

2. Material and Methods

2.1. Plant Material. *D. crinitus* were collected in Chelaida forest area (at about 8 km northeast of Tlemcen, Algeria). Stems/leaves essential oils used for the comparative study were obtained from wild plants collected at the end of March (early vegetative), at mid-May (early flowering), and at the end of July (full flowering).

2.2. Essential Oil Extraction. *D. crinitus* essential oils were isolated by hydrodistillation (400–450 g of dried plant per sample) for 6 h using a Clevenger-type apparatus [13] according to the European Pharmacopoeia.

2.3. Gas Chromatography Analysis (GC). GC analyses were carried out using a Perkin-Elmer (Waltham, MA, USA) Autosystem XL GC apparatus equipped with a dual flame ionization detection system and fused-silica capillary columns (60 m × 0.22 mm I.D., film thickness 0.25 μm), Rtx-1 (polydimethylsiloxane). The oven temperature was programmed from 60°C to 230°C at 2°C/min and then held isothermally at 230°C for 35 min. Injector and detector temperatures were maintained at 280°C. Samples were injected in the split mode (1/50), using helium as the carrier gas (1 mL/min); the injection volume was 0.2 μL. Retention indices (RIs) of the compounds were determined relative to the retention times of the series of n-alkanes (C_5–C_{30}) with linear interpolation, using the Van den Dool and Kratz equation and software from Perkin-Elmer [14]. Relative amounts of individual components were calculated on the basis of their GC peak areas on two capillary Rtx-1 and Rtx-Wax columns, without FID response factor correction.

2.4. Gas Chromatography-Mass Spectrometry Analysis. Samples were analyzed with a Perkin-Elmer Turbo mass detector (quadrupole), coupled to a Perkin-Elmer Autosystem XL, equipped with the fused-silica capillary columns Rtx-1 and Rtx-Wax (ion source temperature 150°C; energy ionization 70 eV). EI mass spectra were acquired over the mass range 35–350 Da (scan time: 1 s). Other GC conditions were the same as described under GC except split 1/80.

2.5. Component Identification. Identification of the components was based on (i) the comparison of their GC retention indices (RIs) on nonpolar columns, determined relatively by the retention time of a series of n-alkanes with linear interpolation, with those of authentic compounds or literature data [15–20]; (ii) computer matching with commercial mass spectral libraries [17–22] and comparison of spectra with those of our personal library. Relative amounts of individual components were calculated on the basis of their GC peak areas on the capillary Rtx-1 columns, without FID response factor correction.

Table 1: Plant material, dates, vegetative cycle and oil yields of *D. crinitus*.

Harvest dates	Vegetative cycle	Essential oil yield (%)	Temperature (°C)
28/03/2009	Early vegetative	0.02	16
16/05/2009	Early flowering	0.05	28
28/07/2009	Full flowering	0.15	37

2.6. Antimicrobial Activity

2.6.1. Test Microorganisms. *Bacillus cereus* (ATCC 11778, gram positive), *Staphylococcus aureus* (ATCC 25925, gram positive), *Escherichia coli* (ATCC 9847, gram negative), and *Candida albicans* (IPP 444) microorganism strains were employed for determination of antimicrobial activity. Bacterial strains preserved in nutrient agar at 4°C were revivified in nutrient solution and incubated at 37 ± 1°C during 18 to 24 h. 0.1 mL of each culture was added to 10 mL OF BHIB (Brain Heart Infusion Broth, Pronadisa Hispanalab). *C. albicans* preserved at 4°C in the Sabouraud agar supplemented with chloramphenicol was revivified in nutrient solution and incubated at 30 ± 1°C during 24 to 48 h. 0.1 mL of each culture was added to 10 mL of sterile physiological water. For antimicrobial assay, bacterial strains were grown on Mueller-Hinton Agar (MHA, Pronadisa Hispanalab) while *C. albicans* was grown on Sabouraud Dextrose Agar + Chloramphenicol (SDA, Merck). Bacterial and yeast inocula reached microbial densities in the range 10^6 to 10^7 cfu/mL.

2.6.2. Evaluation of Antimicrobial Activity. The essential oil (5 μL) was applied on the paper discs (the disc diameter was 6 mm). Then disc papers were placed in the inoculated plates. After 24 h of incubation at 37°C, the diameters of growth inhibition zones were measured.

3. Results and Discussion

3.1. Chemical Characterization. The variation of the essential oil yield of aerial parts of *D. crinitus* with the three growth stages is shown in Table 1. A significant change in essential oil yield was observed during the different growth stages. During the early-vegetative stage, the essential oil yield was of 0.02%. At the early-flowering stage, the essential oil yield increased and reached 0.05%. At full-flowering stages, the essential oil yield increased significantly to reach 0.15% (Table 1).

Variation in oil yield can be attributed to many factors, such as the genetic factors, the developmental stage, the extraction method, and the pedoclimatic conditions [23, 24]. It seems that oil yield during plant growth is particularly sensitive to environmental conditions (light, nutrient availability, and day length) [25, 26].

All three oils were analyzed by GC and GC-MS and their chemical compositions are presented in Table 2, 18 sesquiterpene hydrocarbons, 9 hydrocarbons monoterpene, 5 oxygenated monoterpenes, 5 phenolic compounds, 4 oxygenated sesquiterpenes, 2 oxygenated diterpenes, and 01 diterpene hydrocarbons. However, in the early-vegetative stage 56 components accounting for 93.8% of the total composition were identified. The most abundant chemical groups of this oil were aliphatic compounds (49.9%), phenolic compounds (36.4%), sesquiterpene hydrocarbons (4.3%), and oxygenated sesquiterpenes (2.1%). The main aliphatic compounds components were decanol (10.4%), heptadecane (9.8%), dodecanal (6.3%), pentadecane (5.4%), undecane (4.0%), followed by decanal (2.7%), hexadecane (1.8), hexadecanoic acid (1.5%), and hexadecanal (1.1%). However, isochavicol isobutyrate (26.4%) and isochavicol 2-methyl butyrate (7.9%) were the major constituent of phenolic compounds. The terpenic compounds were represented only by α-humulene (1.9%), geranyl butyrate (1.2%), and α-cedrol (1.2%). Regarding the early-flowering stage, the composition of essential oil is similar from a qualitative point of view with that observed in the early-vegetative stage, characterized by high percentage of phenolic compounds (54.0%) but with a slight difference in oil composition. We noted the presence of zizaene (2.3%), caryophyllene oxide (2.1%), bornyl acetate (2.1%), and myrcene (1.4%). On the other hand, the oil produced in full-flowering stage was characterized by isochavicol isobutyrate (76.1%), isochavicol 2-methyl butyrate (5.4%), myrcene (1.9%), zizaene (3.1%), limonene (1.5%), and dodecyl pentanoate (1.3%).

A comparison of chemical compositions of *D. crinitus* essential oils obtained from three stages of development shows significant differences. Phenolic compounds were the most abundant components identified in aerial parts of *D. crinitus*. The amount of isochavicol isobutyrate significantly increased to early-flowering and full-flowering stages (41.4% and 76.1%, resp.). Relative to early-vegetative stage, the fraction of terpenic hydrocarbons was increased, mainly due to an increase of zizaene, myrcene, and limonene percentages in the full-flowering and early-flowering stages. However, fraction of aliphatic compounds was higher at the early-vegetative stage (49.9% of total oil, resp.), while its level decreased to 19.6% at the early-flowering stage and then to 3.6% at the full-flowering stage. According to our results, it seems that chemical composition of *D. crinitus* essential oil varied significantly with the physiological stage of the plant. Along the vegetative life of the plant, *D. crinitus* produces three essential oils which differed by the percentage of their class compounds. (Figure 1).

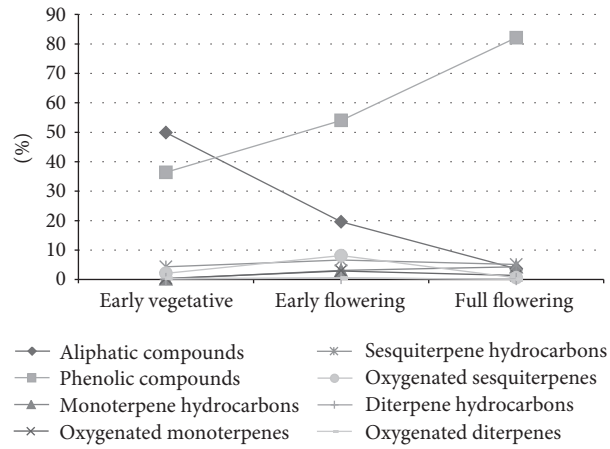

Figure 1: Percentage variation of the main *D. crinitus* classes of compounds throughout the vegetative cycle.

3.2. Antimicrobial Activity. Moreover, the antibacterial activity of essential oil of the three vegetative stages was assessed by disc diffusion assay. The results indicated in Table 3 and in the paper represent the net zone of inhibition including the diameter (6 mm) of the paper disk. Biological activity was affected to essential oil as follows: strong activity: inhibition zone >20 mm, moderate activity: inhibition zone <20–12 mm and no inhibition: zone <12 mm. As shown in Table 3, the diameters of the inhibition zones of the studied essential oils ranged from 6 to 30 mm with the highest inhibition zone values observed against the medically important pathogens *C. albicans* (30 mm) and *S. aureus* (28 mm). *C. albicans* is a microbe responsible for most clinical yeast infections, for example, in mouth infections. However, in early-flowering and full-flowering stages, the essential oils have a strong antibacterial activity against *S. aureus* with diameters of inhibition zones of 21 and 28 mm, respectively. The other bacterial strains (*E. coli* and *B. cereus*) showed no inhibition, with diameters of inhibition zones ranging from 6 to 10 mm (Table 3).

From all the used species, *S. aureus* is one of the most common of the gram-positive bacteria that causes food poisonings. Its source is not the food itself but the humans who contaminate foods after they have been processed [27]. These observations may be attributed to the nature of biologically active components. Indeed, various chemical compounds have direct activity against many species of bacteria, such as terpenes and a variety of aliphatic hydrocarbons (alcohols, aldehydes, and ketones). The lipophilic character of their hydrocarbon skeleton and the hydrophilic character of their functional groups are of main importance in the antimicrobial action of essential oils components. Therefore, a rank of activity has been proposed as follows: phenols > aldehydes > ketones > alcohols > esters > hydrocarbons [28]. For example, some essential oils containing phenolic structures are highly active against a broad spectrum of microorganisms [28, 29]. The importance of the hydroxyl group has been confirmed [30, 31]. Aldehydes are known to possess powerful antimicrobial activity. It has been proposed that an aldehyde group conjugated to a carbon-to-carbon double bond is a highly electronegative arrangement, which may explain their activity [32], suggesting a proportional increase of the antibacterial activity with electronegativity [33, 34]. Aliphatic alcohols were reported to possess strong-to-moderate activities against several bacteria. The activity increased with the length of the carbon chain [35]. Terpenic compounds have also shown antimicrobial properties

Table 2: Chemical compositions of *D. crinitus* essential oils (%) during three developmental stages.

$N°^{a}$	Components[b]	I_l[c]	I_a[d]	I	II	III
1	Nonane	900	900	tr	—	—
2	α-pinene	936	931	—	0.6	0.5
3	Sabinene	973	966	—	0.1	tr
4	β-pinene	978	971	—	0.1	tr
5	Myrcene	987	981	—	1.4	1.9
6	Decane	1000	1000	tr	0.1	0.1
7	p-cymene	1015	1012	—	0.1	0.1
8	1,8 Cineole	1024	1020	—	0.6	0.5
9	Limonene	1025	1026	—	0.6	1.5
10	(E)-β-ocimene	1041	1037	—	0.1	0.1
11	γ-terpinene	1051	1049	tr	0.1	0.1
12	1-Octanol	1061	1056	0.1	tr	tr
13	2-Methyl-Decane	1068	1065	—	tr	0.1
14	Nonanal	1076	1074	0.2	0.1	tr
15	α-Terpinolene	1082	1079	0.2	tr	0.1
16	Linalool	1086	1086	0.2	tr	0.1
17	Undecane	1100	1100	4.0	0.1	tr
18	2-Ethyl-hexyl acetate	1144	1149	0.2	0.4	0.1
19	Terpinen-4-ol	1164	1161	0.1	0.1	0.1
20	Decanal	1188	1187	2.7	0.5	0.2
21	Dodecane	1200	1198	0.2	tr	tr
22	Decanol	1263	1259	10.4	2.1	0.7
23	Nonanoic acid	1263	1263	0.1	tr	tr
24	Bornyl acetate	1270	1269	0.1	2.1	0.7
25	Undecanal	1290	1280	0.4	0.2	tr
26	Tridecane	1300	1300	0.4	tr	tr
27	n-Octyl isobutyrate	1329	1325	tr	tr	0.2
28	Neryl acetate	1342	1336	—	0.1	tr
29	Decanoic acid	1347	1348	0.6	0.2	tr
30	Benzyl 2-methyl butyrate	1360	1352	0.6	0.2	tr
31	Undecanol	1367	1367	0.8	0.4	0.1
32	α-copaene	1379	1386	0.1	0.1	tr
33	β-Bourbonene	1386	1376	—	0.1	0.1
34	Dodecanal	1389	1390	6.3	7.3	0.2
35	Tetradecane	1400	1403	0.3	tr	tr
36	Longifolene	1411	1409	0.1	0.1	tr
37	α-Santalene	1422	1415	0.1	0.4	0.4
38	β-Caryophyllene	1420	1424	0.1	tr	tr
39	Dauca-3,8-diene	1428	1426	0.1	tr	tr
40	trans-α-Bergamotene	1432	1431	0.2	tr	tr
41	(E)-β-Farnesene	1448	1449	—	tr	tr
42	α-Humulene	1456	1451	1.9	0.5	tr
43	Zizaene	1456	1463	—	2.3	3.1
44	Dodecanol	1472	1470	2.7	0.5	0.4
45	α-Curcumene	1473	1474	0.1	0.4	0.4
46	Germacrene-D	1479	1478	0.2	tr	tr
47	β-Selinene	1486	1480	0.1	tr	tr
48	Zingiberene	1489	1483	tr	tr	0.1
49	Pentadecane	1500	1500	5.4	1.1	0.1
50	γ-Cadinene	1516	1513	0.1	0.1	tr
51	Geranyl butyrate	1534	1530	1.2	2.1	0.5

Table 2: Continued.

$N°^a$	Components[b]	I_l[c]	I_a[d]	I	II	III
52	Isochavicol isobutyrate	1546	1541	26.4	41.4	76.1
53	(Z)-3-Hexenyl benzoate	1545	1557	0.8	0.3	tr
54	Dodecanoic acid	1554	1560	0.6	0.4	tr
55	Caryophyllene oxide	1576	1572	0.6	2.1	0.1
56	Dodecyl acetate	1585	1580	—	0.4	0.1
57	Hexadecane	1600	1599	1.8	1.9	0.2
58	α-Cedrol	1603	1596	1.2	5.0	0.1
59	Isochavicol 2-methyl butyrate	1651	1648	7.9	11.3	5.4
60	α-Bisabolol	1672	1667	0.1	0.9	0.2
61	Heptadecane	1700	1700	9.8	2.9	0.1
62	Benzyl benzoate	1730	1723	0.7	0.8	0.6
63	Tetradecanoic acid	1761	1756	0.1	0.3	0.1
64	Hexadecanal	1782	1787	1.1	0.5	0.1
65	Lactarazulene	1796	1792	tr	0,5	0.1
66	Neophytadiene	1807	1807	0.1	0.5	0.1
67	Phytone	1833	1835	0.2	0.1	0.2
68	Dodecyl pentanoate	1843	1840	0.2	0.1	1.3
69	Isophytol	1946	1944	—	tr	0.1
70	Hexadecanoic acid	1951	1949	1.5	0.1	tr
71	(E) Phytol	2114	2102	0.4	0.5	tr
	Total			93.8	95.3	97.4
	Aliphatic compounds			49.9	19.6	3.7
	Phenolic compounds			36.4	54	82.1
	Monoterpene hydrocarbons			0.2	3.1	4.3
	Oxygenated monoterpenes			0.4	2.9	1.4
	Sesquiterpene hydrocarbons			4.3	6.6	5.1
	Oxygenated sesquiterpenes			2.1	8.1	0.6
	Diterpene hydrocarbons			0.1	0.5	0.1
	Oxygenated diterpenes			0.4	0.5	0.1

[a]Order of elution is given on apolar column (Rtx-1). [b]Retention indices of literature on the apolar column (lRI$_a$). [c]Retention indices on the apolar Rtx-1 column (RI$_a$). tr: trace, (<0.05%). Vegetative cycle—I: early vegetative; II: early flowering; III: full flowering.

Table 3: Antimicrobial activity of the essential oils of aerial parts of *D. crinitus*.

Microorganisms	Diameters of inhibition (mm)		
	Early vegetative	Early flowering	Full flowering
Gram-positive bacterium			
Bacillus cereus	8	11	6
Staphylococcus aureus	9	21	28
Gram-negative bacterium			
Escherichia coli	10	8	6
Yeast			
Candida albicans	30	30	30

that appear to have strong-to-moderate antibacterial activity against Gram-positive bacteria and against pathogenic fungi, but in general, weaker activity was observed against Gram-negative bacteria [36, 37]. Secondly there is some evidence that minor components have a critical part to play in antibacterial activity, possibly by producing a synergistic effect between other components. This has been found to be the case for sage [38], some species of Thymus [39] and oregano [40].

4. Conclusions

In conclusion, CG and CG-MS analysis showed that oils of *D. crinitus* are rich in phenolic compounds, whereas terpenic compounds showed marked variation with plant growth stage and the maximum amounts were detected during full-flowering stage. However, phenolic and aliphatic compounds were the main components during the early-vegetative stage. Bioassay screening of oils showed strong activity against

C. albicans and *S. aureus*. The results of the current study have shown that *D. crinitus* essential oil is potentially a good source of antimicrobial compounds and support the traditional medicinal application of this plant.

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