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Partial chemical composition and antimicrobial activity of *Daucus crinitus* Desf. extracts.

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RESUMEN

Composición química parcial y actividad antimicrobiana de extractos *de Daucus critinus* Desf.

La composición química de los ácidos grasos y la fracción insaponificable de raíces, hojas, y tallos de *Daucus crinitus* Desf. fueron establecidas utilizando cromatografía de gases (GC) y cromatografía de gases-espectrometría de masas (GC-MS). La fracción de ácidos grasos de los diferentes órganos (hojas, tallos y raíces) se caracterizó por el ácido láurico (17.9, 17.5 y 18.1% respectivamente) y otros ácidos grasos de cadena larga (hasta C22). Diferencias cualitativas y cuantitativas se registraron entre las fracciones insaponificable de los diferentes órganos de *D. crinitus*. De hecho, las fracciones insaponificable de la raíz, de la hoja y del tallo mostraron cantidades altas de componentes alifáticos (83.4%, 87.2% y 91.4%, respectivamente). Los componentes monoterpénicos, diterpénicos y sesquiterpénicos solo estuvieron presentes en un pequeño porcentaje. Las propiedades antimicrobianas de los extractos de *D. critinus* fueron ensayadas en cuatro microorganismos diferentes. Estos extractos fueron activos contra *Bacillus* cereus, *Staphylococcus aureus, Escherichia coli* y *Candida albicans.*

PALABRAS CLAVE: Ácidos grasos – Actividad antimicrobiana – Daucus crinitus Desf. – Fracción insaponificable – GC-MS.

SUMMARY

Partial chemical composition and antimicrobial activity of *Daucus crinitus* Desf. extracts.

The chemical composition of fatty acids and the unsaponifiable fraction of the roots, leaves and stems from *Daucus crinitus* Desf. were, determined using gas chromatography (GC) and gas chromatography-Mass Spectrometry (GC-MS). The fatty acid fractions of different organs (leaves, stems and roots) were characterized by lauric acid (17.9, 17.5 and 18.1 % respectively) and other long chain fatty acids (until C_{22}). Qualitative and quantitative differences were reported between the unsaponifiable fractions of different organs from *D. crinitus*. The unsaponifiable fractions of the leaves, roots and stem showed high amounts of aliphatic components (83.4%, 87.2% and 91.4%, respectively). The monoterpen, diterpen and sesquiterpen components were only present in small percentages. The antimicrobial properties of the *D. critinus* extracts were tested on four different microorganisms. These extracts were found to be active against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*.

KEY-WORDS: Antimicrobial activity – Daucus crinitus. Desf. – Fatty acid – GC/MS - Unsaponifiable fraction.

1. INTRODUCTION

The carrot is a plant belonging to the Apiaceae family and is grown throughout the temperate regions for its roots that are consumed raw and cooked. The carrot is reported to be endowed with medicinal properties, i.e. hypotensive, diuretic, carminative, stomachic and antilipemic (Gilani *et al*., 2000; Kumarasamy *et al*., 2005; Nicolle *et al*., 2004). In Algerian folk medicine, the leaves are used raw or in an infusion as depurative and diuretic agents.

In Algeria, the *Daucus* genus is represented by some species living in dry and uncultivated areas and, among them, *D. carota* is widespread together with its many subspecies. In this group *D. crinitus* is different from the other species. The morphological features observed in the leaves of the *Daucus* genus are alternately arranged, except in *D. crinitus*, in which they appear to be pseudoverticillate. Also, *D. crinitus* is characterized by the presence of many subspecies that colonize sands and cliffs (Quezel and Santa, 1963; Sáenz Laín, 1981).

The species studied is known by two botanical classifications that are considered synonyms: *D. crinitus* Desf. and *D. meifolius* Brot. This species grows along the Central-Western Mediterranean coasts; in Algeria it can be found along the Western coast from Tlemcen to Mascara (Quezel and Santa, 1963; Sáenz Laín, 1981). In folk medicine, it is also used to expel the placenta after childbirth or to give some stoutness. The mixture is also galactogene and depurative.

Previous studies refer mainly to the different subspecies and varieties of *D. carota*. In this species, a considerable qualitative and quantitative variability has been observed. In the fruits of *D. carota* ssp. *sativus* (Benecke *et al*., 1987) and *D. carota* (subspecies non reported) (Ashraf *et al*., 1979; Hilal *et al*., 1977; Meshcheryuk *et al*., 1983; Nigam and Radhakrishnan, 1963; Perineau *et al*.,1991; Stahl, 1964; Talwar *et al*., 1963; Toulemonde *et al*., 1987) the main constituents were carotol, daucol, geranyl acetate and terpinyl acetate. For *D. carota ssp. gummifer* high percentages of geranyl acetate have been reported (Pinilla *et al*., 1995). In the essential oil obtained from the fruits, leaves and stems of *D. carota ssp. maximus* (Saad *et al*., 1995), trans-methylisoeugenol, methyleugenol, basarone, shyobunone and preisocalamendiol were identified among the main constituents; in disagreement with other varieties, carotol, daucol and geranyl acetate were completely absent. Sabinene, linalool, linalyl acetate, carvone and carotol were the main compounds identified in the volatile oil of the leaves of *D. carota ssp. sativa* (Khanna *et al*., 1989). Kameoka *et al*. (1989) identified cis- and trans-asarone, β-bisabolene, asarone aldehyde, eugenol, 2-hydroxy-4-methoxyacetophenone, 3-carene and methyleugenol among the volatiles of the fruits of *D. carota* (subspecies not reported).

The essential oils obtained from the leaves and fruits of *D. gingidium* ssp. *gingidium* have been studied (Guido *et al*., 2007). The main constituents of the essential oil from the leaves were sabinene (26.8%), α-pinene (10.8%), germacrene-D (6.9%) and limonene (5.7%). Sabinene (60.6%) was the main compound identified in the essential oil of the fruits, followed by α-pinene (12.2%) and 4-terpineol (5.4%).

In Algeria, many patients used medicinal plants, aside from conventional treatments, as an alternative therapy for many diseases. Recently, special attention has been paid to the bioactive elements extracted from plants in order to discover new drugs for the treatment of several pathologies (Kivçak and Mert, 2001). Therefore, the aim of the present work was: (i) to establish the chemical composition of solvent extracts (fatty acids and unsaponifiable fractions) of the stems, leaves and roots from *D.crinitus* for the first time. For this purpose, the analysis of extract composition was carried out by Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS); (ii) to test the antibacterial and antifungal activities of unsaponifiable, methanolic and aqueous fractions against four species of micro-organisms using a microdilution method. The assessment of the *D. crinitus* potential as a source of natural compounds for pharmaceutical application is expected as a possible outcome of this study.

2. MATERIAL AND METHODS

2.1. Plant material

Samples from each organ (stems, leaves and roots) of *D. crinitus* were collected in the Bensekrane forest area (at about 30 km north west of Tlemcen - Algeria) [287 m, 35°07'N 1°22'O] in September, 2009. Voucher specimens were deposited in the herbarium of the Tlemcen University Botanical Laboratory (Voucher number: UBL 05.09). A portion of each organ was stored at 4°C for further analysis .

2.2. Sample preparation

Samples were air-dried and crushed. Obtained powders were sieved in size grading 0.355 and 0.600 mm for roots, leaves and stems, respectively. 50 g of each organ were extracted with 2x200 mL of methanol and petroleum ether under reflux (Soxhlet apparatus) separetely. After evaporation of the solvent under reduced pressure, extracts were gathered and dried on $MgSO_4$. The extraction was performed in triplicate and the yields (average of three extractions) in methanol were 2.5% for stems, 6.1% for leaves and 1.8% for roots and in petroleum ether were 1.2% for stems, 5.8% for leaves and 1.5% for roots. For aqueous extracts, 5 g of each organ were extracted with 100 mL of wáter and were lyophilized.

2.3. Extraction of unsaponifiable compounds

1 g of petroleum ether extract was saponified with 50 mL of methanolic potassium hydroxide solution (2 mol/L) for 1 h under reflux; the unsaponifiable components were then extracted three times with 100 mL of ether. The pooled extracts were washed three times with 50 mL of deionized water. The solvent was subsequently removed at 35°C under reduced pressure with a rotary evaporator. The unsaponifiable fractions of the leaves, stems and roots represent 54.1%, 69.1% and 55.3% (w/w) of petroleum ether extract, respectively.

2.4. Extraction of fatty acids

The soapy aqueous solution obtained previously was acidified (HCl 1N) until precipitation of fatty acids (pH 5-6). The freed fatty acids were extracted with ether (3x 50 mL) and dried on $MgSO_4$ and then weighed. Fatty acids were transformed into their methyl ester derivatives by the addition of a methanolic solution of BF_3 10% (Metcalfe and Schmitz, 1961). Thereafter, fatty acids were extracted three times with 50 mL of hexane at room temperature. The organic layer was evaporated and dried on Na_2SO_4. The acid fractions of the leaves, stems and roots represent 21.2%, 29.4% and 11.4% (w/w) of diethyl ether extract, respectively.

2.5. 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 a fused-silica capillary column (60 m x 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 indexes (I) of the compounds were determined relative to the retention times of the series of n-alkanes (C5-C30) with linear interpolation, using the Van den Dool and Kratz equation (Van Den Dool and Kratz, 1963) and software from Perkin–Elmer. Component relative concentrations were calculated based on GC peak areas without using correction factors.

2.6. Gas chromatography-Mass spectrometry analysis (GC-MS)

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.

2.7. Component identification

As previously reported (Paolini *et al*., 2005), the methodology used for the identification of individual components was based on: (a) a comparison of calculated retention indices I_a on an apolar column with those of authentic compounds or literature data I_l (Jennings and Shibamoto, 1980; National Institute of Standards and Technology, 2005; National Institute of Standards and Technology, 2005) and (b) computer matching with commercial mass spectral libraries (König *et al*., 2001; Adams, 2001; Mc Lafferty and Stauffer,1994; Mc Lafferty and Stauffer,1988; National Institute of Standards and Technology, 1999) and a comparison of mass spectra with those of our own library of authentic compounds or literature data (Jennings and Shibamoto,1980; Joulain and König,1998).

2.8. Bacterial and yeast strains and media

The bacterial strains used in this study, i.e. *Bacillus cereus*, *Staphylococus aureus* and *Escherichia coli*, were isolated at the Medical Reanimation Department of the Hospital University Center of Tlemcen in Algeria. The yeast *Candida albicans* was isolated at the Dermatology Department of the same hospital.

Bacterial strains preserved in nutrient agar at 4°C were revived in a nutrient solution and incubated at 37±1°C for 18 to 24 h. 0.1 mL of each culture was added to 10 mL BHIB (Brain Heart Infusion Broth, Pronadisa Hispanalab). *C. albicans* preserved at 4°C in the Sabouraud agar supplemented with chloramphenicol was revived in a nutrient solution and incubated at 30±1°C for 24 to 48 h. 0.1 mL of each culture was added to 10 mL sterile physiological water.

For the 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 of 10^6 to 10^7cfu/mL.

2.9. Antimicrobial activity

Two different techniques were used to test the anti-microbial activity: the paper disc diffusion and the dilution agar method. The minimum inhibitory concentration (MIC) was determined by the later method.

Paper-disc diffusion method

Paper discs (6 mm in diameter) saturated with a 40 mg/mL solution of plant extract were applied to the surface of agar plates that were previously seeded by spreading 0.1 mL of culture overnight. The plates were incubated overnight at the appropriate temperature (see above), and the diameter of the resulting zone of inhibition was measured in millimeters. The results indicated in Table 3 and in the text represent the net zone of inhibition including the diameter (6 mm) of the paper disk. The scale of measurement was the following (disk diameter included) : > 20 mm zone of inhibition is strongly inhibitory; < 20–12 mm zone of inhibition is moderately/mildly inhibitory; and <12 mm is not inhibitory. All the data collected for each assay are the averages of three determinations.

Dilution-agar method

A dilution agar method was used to determine the Minimum Inhibitory Concentrations (MIC). Stock solutions were obtained by dissolving extracts in dimethylsulfoxide (DMSO 1%). Serial dilutions were made to obtain concentrations ranging from 0 to 4000 µg/mL of unsaponifiable, methanolic and aqueous extracts. Each mixture was added to Mueller–Hinton agar for bacteria and Sabouraud dextrose agar with Chloramphenicol for yeast (cooled to 45– 50°C) (Cowan, 1999; Lennette and Balows, 1985). Two controls were included in this test. The Petri dishes contained a sterile solution of DMSO and the culture medium, respectively. The experiments were performed in triplicate. After incubation at 37°C for 24h for bacteria and at 30°C for 48h for the yeast.

3. RESULTS AND DISCUSSION

3.1. Fatty acids of the leaves, stems and roots from *D. crinitus*

Fatty acids from the leaves, stems and roots, their retention indexes and their relative abundance

are given in Table 1; the components are listed in the order of elution on the Rtx-1 column. 25 acid constituents were identified from leaf, stem and root extracts amounting to 84.1%, 85.7% and 86.6% of the total acid fraction, respectively (Table 1). The three fractions were qualitatively similar. However, propanoic acid (0.9%) was reported only in the stem extract and 15 methyl hexadecanoic acid (0.6%) in the root extract. The leaf, stem and root extracts were characterized by high contents of saturated fatty acids with lauric acid (17.9%, 17.5% and 18.1%, respectively) as the major compound followed by palmitic acid (16.8%, 14.3% and 10.7%, respectively), myristic acid (10.2%, 14.5% and 16.1%, respectively), behenic acid (7.2%, 9.1% and 6.5%, respectively), and stearic acid (3.2%, 2.7% and 1.8%, respectively). The other main compounds were unsaturated fatty acids: - linolenic acid (12.5%, 13.8% and 14.6%, respectively), 9-hexadecenoic acid (5.9%, 4.1% and 4.6%, respectively), and cis-11-eicosenoic acid (5.4%, 2.5% and 1.6%, respectively).

3.2. Unsaponifiable fraction of leaves, stems and roots from *D.crinitus*

The combined analysis (GC and GC-MS) of *D. crinitus* unsaponifiable fractions revealed the presence of 33 components: 33 in leaf fraction, 29 in stem fraction and 15 in root fraction accounting for 91.5%, 92.8% and 91.4% of the total composition, respectively (Table 2). Even if aliphatic compounds

Table 1
Fatty acids of leaf, stem and root extracts from *D. crinitus* (proportion w/w expressed according to the total fatty acids)

N[a]	Acid components	I_l [b]	I_a [c]	Leaves[d]	Stems[d]	Roots[d]
1	Propanoic (C_3 : 0)	618	611	–	0.9	–
2	Enanthic (heptanoic, C_7 : 0)	1006	1005	0.2	0.2	0.1
3	Undecanoic (C_{10} : 0)	1407	1406	0.8	0.7	1.1
4	Lauric (dodecanoic, C_{12} : 0)	1501	1508	17.9	17.5	18.1
5	Tridecyclic (tridecanoic, C_{13} : 0)	1608	1605	0.3	0.9	0.4
6	Dodecanoic 2-hydroxy (C_{12} : 0)	1627	1619	0.8	0.1	0.3
7	Myristic (tetradecanoic, C_{14} : 0)	1708	1708	10.2	14.5	16.1
8	Farnesyl(3,7,11-trimethyl-2,6,10-dodecatrienoic,C_{15} : 3	1765	1770	Tr	0.3	0.2
9	12-Methyl tetradecanoic (C_{15} : 0)	1771	1777	0.1	0.2	0.3
10	(Z) Methyl pentadec-10-enoic (C_{15} : 1)	1786	1787	0.4	TR	0.1
11	Pentadecanoic (C_{15} : 0)	1808	1806	0.4	0.8	0.6
12	Tetradecanoic 2-hydroxy (C_{14} : 0)	1835	1826	0.2	0.1	0.3
13	Palmotoleic (9-Hexadecenoic, C_{16} : 1)	1895	1889	5.9	4.1	4.6
14	Palmitic (hexadecanoic, C_{16} : 0)	1908	1910	16.8	14.3	10.7
15	15-Methyl hexadecanoic (C_{17} : 0)	1974	1970	–	–	0.3
16	Margaric (heptadecanoic, C_{17} : 0)	2008	2006	0.3	0.4	0.4
17	Linoleic (9,12-octadecadienoic, C_{18} : 2)	2083	2072	12.5	13.8	14.6
18	Linolenic (9,12,15-octadecatrienoic, C_{18} : 3)	2081	2081	0.5	0.8	7.1
19	Oleic ((Z)-octadecenoic, C_{18} : 1)	2106	2084	0.4	0.7	0.7
20	Stearic (octadecanoic, C_{18} : 0)	2109	2108	3.2	2.7	1.8
21	Nonadecanoic (C_{19} : 0)	2209	2207	0.3	0.4	0.1
22	Arachidonic (5,8,11,14-eicosatetraenoic, C_{20} : 4)	2217	2216	0.1	0.2	tr
23	Cis 11-eicosenoic (C_{20} : 1)	2248	2247	5.4	2.5	1.6
24	Arachidic (eicosanoic, C_{20} : 0)	2311	2312	0.2	0.5	0.6
25	Behenic (docosanoic, C_{22} : 0)	2511	2509	7.2	9.1	6.5
Total identified				*84.1*	*85.7*	*86.6*

[a] Order of elution are given on a polar column (Rtx-1). [b] Retention indexes of literature of corresponding esters on the apolar column (I_l) (Jennings and Shibamoto. 1980; König and al.. 2001). [c] Retention indexes of corresponding esters on the apolar Rtx-1 column (I_a). [d] Relative percentages (%) on the a polar column (Rtx-1); tr = trace (< 0.1%).

Table 2
Chemical composition of unsaponifiable fractions of leaves stems and roots from *D. crinitus*

N°[a]	Components	I_l [b]	I_a [c]	Leaves[d]	Stems[d]	Roots[d]	Identification
1	Nonane	900	900	0.2	Tr	0.9	RI, MS
2	α- pinene	936	931	0.4	–	–	RI, MS
3	Sabinene	973	966	0.6	–	–	RI, MS
4	β-pinene	978	971	0.1	–	–	RI, MS
5	Myrcene	987	981	0.3	–	–	RI, MS
6	Decane	1000	1000	tr	0.1	0.1	RI, MSº
7	p-Cymene	1015	1012	0.2	0.1	–	RI, MS
8	Limonene	1020	1021	0.9	1.1	–	RI, MS
9	(E)-β-Ocimene	1041	1037	0.5	0.9	–	RI, MS
10	γ-terpinene	1051	1049	1.6	0.8	–	RI, MS
11	α-Terpinolene	1082	1079	0.4	0.2	–	RI, MS
12	Undecane	1100	1100	5.5	8.5	4.1	RI, MS
13	Decanal	1188	1187	1.8	2.2	2.3	RI, MS
14	Tridecane	1300	1300	tr	0.1	0.8	RI, MS
15	Undecanol	1367	1368	0.3	0.2	–	RI, MS
16	Dodecanal	1389	1390	3.2	2.8	16.7	RI, MS
17	Isocaryophylene	1407	1416	0.6	0.5	–	RI, MS
18	Dodecanol	1474	1474	30.2	35.4	36.2	RI, MS
19	Germacrene-D	1479	1472	0.1	0.1	–	RI, MS
20	Pentadecane	1500	1500	4.2	3.3	4.7	RI, MS
21	Dodecyl acetate	1606	1606	2.5	1.9	3.7	RI, MS
22	α-bisabolol	1673	1667	tr	Tr	–	RI, MS
23	Heptadecane	1700	1700	3.8	2.6	0.2	RI, MS
24	Hexadeanal	1814	1826	1.1	1.8	0.3	RI, MS
25	Nonadecane	1900	1900	0.1	0.2	0.1	RI, MS
26	Heptadecanol	1968	1965	7.1	9.5	1.2	RI, MS
27	Eicosane	2000	2000	0.4	0.1	–	RI, MS
28	Phytol-(Z)	2080	2074	0.7	0.8	–	RI, MS
29	Heneicosane	2100	2100	tr	0.2	–	RI, MS
30	Phytol-(E)	2107	2105	1.1	0.7	–	RI, MS
31	Tricosane	2300	2300	9.9	7.1	10.5	RI, MS
32	Pentacosane	2500	2500	13.1	11.2	9.8	RI, MS
33	Neophytadiene	1830	1836	0.6	0.4	–	RI, MS
	Total identified			*91.5*	*92.8*	*91.4*	
	Aliphatic			83.4	87.2	91.4	
	Monoterpenes			5	3.1	–	
	Diterpenes			2.4	1.9	–	
	Sesquiterpenes			0.7	0.6	–	

[a] Order of elution are given on a polar column (Rtx-1). [b] Retention indexes of literature on the apolar column (I_l) (Jennings and Shibamoto. 1980; König and al.. 2001). [c] Retention indexes on the apolar Rtx-1 column (I_a). [d] Relative percentages (%) on the apolar column (Rtx-1); tr = trace (<0.1%).

were in high abundance in the three fractions, these were qualitatively and quantitatively different. Indeed, the leaf and stem fractions were strongly dominated by aliphatic compounds (83.4% and 87.2% respectively), monoterpene (5% and 3.1%), diterpenes (2.4% and 1.9%) and sesquiterpenes (0.7% and 06%) respectively. Moreover, the monoterpene hydrocarbon family exhibited qualitative and quantitative differences in their composition. The γ-terpinene was identified as the major components of

the leaf fraction (1.6%) were associated with significant amounts of limonene (0.9%), sabinene (0.6%), (E)-β-ocimene (0.5%), α-pinene (0.4%), α-terpinolene (0.4%), myrcene (0.3%) and *p*-cymene (0.2%); whereas the major components of stem fraction were limonene (1.1%), (E)-β-ocimene (0.9%), γ-terpinene (0.8%) followed by α-terpinolene (0.2%) and *p*-cymene (0.1%). Moreover, the aliphatic conponent of the leaf and stem was strongly dominated by dodecanol (30.2% and 35.4% respectively) associated with significant amonts of heptadecanol (7.1% and 9.5% respectively), undecane (5.5% and 8.5% respectively), pentadecane (4.2% and 3.3% respectively), heptadecane (3.8% and 2.6% respectively), dodecanal (3.2% and 2.8% respectively) and dodecyl acetate (2.5% and 1.9% respectively). Conversely, the root fraction was characterized by a high amount of aliphatic compounds (91.4%) and no terpenic compounds have been reported. The major components of this extract were dodecanol (36.2%), tricosane (10.5%) and pentacosane (9.6%). Finally, this fraction was also characterized by aldehyde compounds (19.3%) and dodecyl acetate (3.7%).

3.3. Antibacterial activity

The in vitro antibacterial activities of root, leaf and stem unsaponifiable, methanolic and aqueous extracts of *D.crinitus* against the employed bacteria were qualitatively and quantitatively assessed by the presence or absence of inhibition zones. As shown in Table 3, the root, leaf and stem unsaponifiable extracts exhibited inhibitory effects against the tested bacterial pathogens. *C. albicans*, *S. aureus* and *B. cereus* were found to be the most inhibited pathogens by the unsaponifiable extracts with their respective diameter zones of inhibition of 30 (roots, leaves and stems), 18 (leaves) and 14 (roots) mm. The rest of the bacterial strain (*E. coli*) showed no

Table 3
Antimicrobial activity of unsaponifiable, methanol and water extracts from *D. crinitus.*

Microorganisms	Inhibition zone (mm)								
	Unsaponifiable Extracts			Methanolic Extracts			Aqueous Extracts		
Gram-positive bacterium	Root	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem
Bacillus cereus	14	8	11	14	15	10	–	–	–
Staphylococcus aureus	11	18	9	13	11	11	–	–	–
Gram-negative bacterium									
Escherichia coli	8	8	9	14	13	15	–	–	–
Yeast									
Candida albicans	30	30	30	13	16	15	–	–	–

Table 4
Minimum Inhibitory Concentrations MIC (μg/mL) of unsaponifiable, methanol and water extracts from *D. crinitus*.

Microorganisms	MIC (μg/mL)								
	Unsaponifiable Extracts			Methanolic Extracts			Aqueous Extracts		
Gram-positive bacterium	Root	Leaf	Stem	Root	Leaf	Stem	Root	leaf	Stem
Bacillus cereus	250	255	255	500	500	500	>3000	>3000	>3000
Staphylococcus aureus	136	122.1	122.1	800	800	800	>3000	>3000	>3000
Gram-negative bacterium									
Escherichia coli	126	111.5	111.5	1600	1800	1750	>3000	>3000	>3000
Yeast									
Candida albicans	76.2	46.5	46.2	400	400	400	>2000	>2000	>2000

inhibition, with diameter of zones of inhibition ranging from 8 to 9 mm (Table 3).

Also the methanolic extracts exerted potential effects of antibacterial activity against the tested bacteria. However, the roots, leaves and stems exhibited inhibitory effects against *C.albicans* with diameters of zones of inhibition from 13, 16 and 15 mm respectively, *E. coli* (14, 13 and 15 mm respectively), *B. cereus* (14, 15 and 10 mm respectively) and *S. aureus* (13, 11 and 11 mm respectively). However, aqueous extracts exhibited no effect of antibacterial activity against of the bacterial pathogens tested.

3.4. Minimum inhibitory concentrations (MIC)

As shown in Table 4, the most promising results were obtained from the unsaponifiable extract which not only had the lowest MIC value (46.5, 56.2and 76.2 µg/mL for leaf, stem and root extracts respectively) against *C.albicans*, but also inhibited the growth of other micro-organisms. The unsaponifiable extract of leaves, stems and roots also shows a strong antimicrobial effect against *E. coli* with an MIC of 111.5, 111.1 and 126 µg/mL respectively and *S. aureus* with a MIC of 122.1, 122.1 and 136 µg/mL respectively and average activity on the *B. cereus* with a MIC of 255, 255 and 250 µg/mL respectively (Table 4). Using the dilution agar method technique, root, leaf and stem methanolic extracts of *D. crinitus* showed antimicrobial properties with MIC values ranging from 500 to 1800 µg/mL for bacteria and 400 µg/mL for *C. albicans*. The root, leaf and stem aqueous extracts showed no inhibition towards any of the microorganisms assayed up to the value of 3000 µg/mL for bacteria and 2000 µg/mL for *C. albicans*.

The unsaponifiable extracts of roots, leaves and stems evaluated in this work have different varieties of phytochemicals that could be considered responsible for their antimicrobial activity. Although they usually occur as complex mixtures, their activity can generally be accounted for in terms of their major components. The antimicrobial activity of the unsaponifiable extracts of roots, leaves and stems could be due to the investigated strain sensitivity to α-pinene, sabinene, limonene and the other lighter components present in unsaponifiable extracts (Magiatis *et al*., 1999; Oussou *et al*., 2008; Oussou *et al*., 2004).

4. CONCLUSIONS

The leaves, stems and roots of *D. crinitus* were characterized by the presence of fatty acids. It appears that all parts of the plant were rich in saturated fatty acids with lauric acid (17.9% for leaves, 17.5% for stems and 18% for roots) as the major compound. In all extracts, lauric acid is accompanied by fatty acids with long chain (from C_3 to C_{22}). The unsaponifiable fraction of leaves and stems were characterized by aliphatic constituent products and monoterpene hydrocarbons. Sesquiterpenic compounds were also present but in small amounts.

The antimicrobial properties of the unsaponifiable, methanolic and aqueous extracts (leaves, stems and roots) were tested on four microorganism species. The root, leaf and stem unsaponifiable and methanol extracts were active against *B. cereus*, *S. aureus, E. coli* and *C. albicans* bacteria strain which are deemed very dangerous and very difficult to eliminate.

To the best of our knowledge, the composition of fatty acid and unsaponifiable extracts and antimicrobial activity of *D. crinitus* have not been reported before and therefore our results can be evaluated as the first report about the antimicrobial properties and the chemical compositions of fatty acids and unsaponifiable factions.

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