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Evaluation of antimicrobial activity of solvent extracts from different parts of *Daucus crinitus* Desf.

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ABSTRACT

The objective of this work was to examine the phytochemicals present in different aqueous and organic extracts from different organs of *Daucus crinitus* Desf. and to evaluate their antimicrobial activity. Phytochemical screening was done to characterize the secondary metabolites in various solvent extracts, and antimicrobial activity was evaluated by agar disk diffusion and broth microdilution to determine inhibition zone diameters and MICs. A remarkable antimicrobial effect was observed in organic extracts of stems and seeds (MIC = 0.31-0.83 mg/ml on *S. aureus*, *B. cereus*, and *C. albicans*) containing many phytochemical families such as coumarins, flavonoids, reducing sugars, steroids, tannins, and terpenes. *Daucus crinitus* may be a good source of bioactive molecules endowed with antimicrobial activity.

INTRODUCTION

Seeking healing powers in plants is not a new concept. Many people across the world have since antiquity exploited native plants and taken their extracts as an infusion (Cowan, 1999). That was an act of routine for either disease prevention or treatment. It should also be noted that many plants possess poisoning effects. This has contributed to the formation of a knowledge balance allowing a distinction between what is useful and what is harmful from all of these plants and in which satisfactory condition they could be used.

Daucus crinitus Desf. is a plant species belonging to the *Apiaceae* family. This plant has other scientific names as synonyms which are *Daucus meifolius* Brot. and *Daucus verticillatus* Schousb. ex Ball. It also has several local names such as “buzaffur” and “erq Sidi Messaoud” among people in western Algeria. The plant seems to have its center of distribution in the western Mediterranean, more specifically in the southern region of the Iberian Peninsula (Portugal and Spain) and northwestern Africa (Morocco, Algeria, and Tunisia) (Sáenz Laín, 1981).

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In Algeria, this species grows commonly around Tlemcen and Mascara (Sáenz Laín, 1981) near the Moroccan border. According to the population of Tlemcen region, the plant has since long been traditionally employed for various usages. What seems to be strikingly important at first is almost the limited use of its roots. People proceed either by boiling the roots in water, and then using the resulting extract, especially by pregnant women and young mothers, or by grinding them and adding the derived powder to foods as spices, especially to pasta. Pulverized roots are sometimes added to beverages such as milk for young children or are used as colloid with honey, whereas cut in the form of circle, they are utilized as a bracelet around the arms of babies for inhalation. Used in soaked state, the traditionally known benefits are dyspepsia avoidance, vomiting assistance, bowel discharge and reconstitution of the intestinal flora, delivery facilitation, and expulsion of lochia. In crushed form, it may protect young children against diuresis and common cold. It also seems that its fragrance may help night-awakened and agitated babies to get composed. This plant is poorly investigated in a chemical and biological point of view. There are only the works on essential oils (Dib *et al.*, 2010; Lanfranchi *et al.*, 2010) and antioxidant activity of solvent extracts (Bendiabdellah *et al.*, 2012). Therefore, it is worth carrying out a quantitative as well as a qualitative study on its other phyto-chemicals from different organs to demonstrate its usefulness and

to possibly identify the compounds for which these nutritional and health beneficial effects are accounted. In this work, the objectives were to examine the phytochemical composition and antimicrobial potential of aqueous and organic extracts (chloroform and ethanol) from different organs of *Daucus crinitus* grown in western Algeria.

MATERIALS AND METHODS

Plant material

The plant material was harvested in May 2012 corresponding to the period of full maturation from the station of Bensekrane located in Tlemcen province. The five organs of the harvested plant material, namely, roots, leaves, stems, flowers, and seeds were individually separated and allowed to dry in open air and under light-free condition for the extraction by different solvents.

Phytochemical screening

Three solvents with increasing polarity (chloroform, ethanol, and water) were successively used to extract the different families of chemicals in a continuous Soxhlet extractor. Twenty to thirty grams (20-30 g) of powdered plant material were prepared in a filter paper cartridge and placed in the Soxhlet apparatus. The flask was filled with 300 ml of solvent and placed over a heating mantle for 2-3 h, until total exhaustion. Recovered solutions were evaporated using a Büchi Rotavapor® R 110-type rotary evaporator to measure the amount extracted. The products were dissolved in appropriate solvents for various phytochemical analyses. Characterization was carried out according to the methods of Harborne (1998) and Raaman (2006).

Strains and suspensions

Five strains have been selected, one of them was a fungus and the rest were bacteria representing various infection sources. The fungal strain was a yeast which is *Candida albicans* ATCC 10231. Among bacterial strains, there are two Gram-positive ones which are *Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 11778, and two Gram-negative ones which are *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 70603. The purity of strains was verified by subculturing them in their selective growth media and taking distinct colonies in each time, where *C. albicans* was cultivated in Sabouraud agar (Fluka®, India) supplemented with chloramphenicol (0.5 g/l) at 37 °C for 48 h, *E. coli* and *K. pneumoniae* were cultivated in MacConkey agar (Fluka®, India) at 37 °C for 24 h, *S. aureus* in Mannitol Salt agar (Fluka®, Switzerland) at 37 °C for 24 h, and *B. cereus* in Mossel agar (Fluka®, Switzerland) at 30 °C for 24 h. To prepare microbial suspensions, previously purified colonies were taken and inoculated into test tubes containing 5 ml of Brain Heart Infusion broth (BHIB) (Conda Pronadisa™, Spain), then incubated at 37 °C for 24 h. After incubation, suspensions were shaken well using the vortex then diluted for standardizing, so that the inoculum was set to 0.5 McFarland standard turbidity, corresponding to an optical density from 0.08 to 0.1 at 625 nm wavelength. The inoculum final concentration will be approximately 10^8 cfu/ml.

Determination of growth inhibition zones

Kirby-Bauer's agar disk diffusion method was used to investigate the bioactivity of plant extracts by forming growth inhibition zones. Briefly, filter paper disks (6 mm in diameter), impregnated with 10 µl of extract, were deposited on the surface of agar medium pre-inoculated by swabbing with standardized microbial suspension. Bacterial strains were inoculated in Mueller-Hinton agar (Fluka®, India) and incubated at 37 °C for 24 h. While, fungi were inoculated in Sabouraud agar (Fluka®, India) supplemented with chloramphenicol and incubated at 37 °C for 48 h. After incubation, the results were read by measuring the diameter of inhibition zones in millimeters (mm). The experiment was performed in triplicate.

Determination of minimum inhibitory concentrations

The minimum inhibitory concentrations (MICs) were determined by broth microdilution using 96-well microtiter plates. Inocula standardized to 10^8 cfu/ml were diluted to 1/1000 by the same growth medium (BHIB) to get the concentration of 10^5 cfu/ml. A maximum of two extracts was tested in triplicate on a single microbial strain in each plate. The wells of the first vertical row were filled with 100 µl of the microbial suspension standardized to 10^5 cfu/ml as the first positive control. For chloroform extracts, the wells of the second row were filled with 80 µl of microbial suspension then 20 µl of a mixture of Tween 80 diluted to 1% in chloroform to have a final concentration of 0.2% of Tween 80 in the wells. For ethanol and water extracts, these wells were filled with 80 µl of microbial suspension, then, respectively, with 20 µl of ethanol at 96° or sterile distilled water. The wells of the second row constitute the second positive control. The remaining ten rows were filled with different concentrations of plant extracts. For chloroform extracts, a series of dilutions to 1/2 was prepared from a stock solution containing Tween 80 diluted to 1% in the extract at 50 mg/ml. Other lower concentration solutions have contained the same concentration of Tween 80 (diluted to 1%) in chloroform (in the same concentration as the stock solution), in a total volume equaling the half of the total volume of stock solution, to keep the concentration of Tween 80 constant to 1%. The ten wells in each row were filled with 80 µl of suspension then 20 µl of the corresponding concentration from the lowest to the highest concentration in a final range from 0.02 to 10 mg/ml. The final concentration of Tween 80 was 0.2%. For ethanol and water extracts, the stock solution has only contained the extract at 50 mg/ml. Other lower concentration solutions have contained the corresponding solvent, either ethanol or water. The dilution series was prepared in the same manner described previously. The wells were each filled with 80 µl of suspension then 20 µl of the corresponding concentration. The final concentration range was from 0.02 to 10 mg/ml. The last horizontal row was filled with 100 µl of BHIB in each well as a

negative control. After incubation of plates at 37 °C for 24 h, MICs were determined as the lowest concentration of extract for which no microbial growth was observed by visual inspection of the media.

RESULTS AND DISCUSSION

Yields of total solids and characterized chemical families

After extraction with different solvents in a successive manner and by increasing polarity, starting with chloroform, then ethanol, and finally, water, and after evaporation to dryness, water extract had the highest yields for all organs (5.51% for roots, 37.44% for leaves, 14.66% for stems, 17.17% for flowers, and 28.24% for seeds). Chloroform extract had the lowest yields (1.34% for roots, 3.44% for leaves, 2.26% for stems, 3.31% for flowers, and 1.18% for seeds). While ethanol has extracted 2.16% of compounds from roots, 6.85% from leaves, 2.79% from stems, 9.86% from flowers, and 3.83% from seeds. The compounds revealed in the chloroform extract were coumarins, steroids, and terpenes.

Coumarins and steroids were also present in the ethanol extract in addition to flavonoids, reducing sugars, and tannins. While the water extract has contained mucilages, reducing sugars, saponins, and tannins. A very important number of compounds was absent, and others were characterized in some organs but were absent in others. Table 1 reports the details of their presence and absence. In the genus *Daucus*, the species *Daucus carota* L. was the most phytochemically studied plant with its different subspecies. Flowers of the common wild subspecies (subsp. *carota*) were a source of various compounds such as sugar alcohols including iditol, flavonoids such as astragalin, and phenylpropanoids such as laserine which were extracted by a mixture of dichloromethane-methanol (Akgul *et al.,* 2009).

Polyacetylenes and starch were present in roots of other wild subspecies (subsp. *gummifer* and subsp. *maximus*) and measured *in situ*, without any preliminary sample preparation (Roman *et al.,* 2011). Root ethanol extract of cultivated subspecies (subsp. *sativus*) has contained reducing sugars (carbohydrates) and tannins (Patil *et al.,* 2012). Polyacetylenes were also extracted from seeds by trifluoroacetic acid or methanol (Metzger *et al.,* 2008), anthocyanins by hydrochloric acid-ethanol (Singh *et al.,* 2012), and steroids, flavonoids, and sesquiterpenes such as daucuside and daucusol by ethanol-water (Fu *et al.,* 2010ab).

Some chemical families characterized in different subspecies of *Daucus carota* were absent in *Daucus crinitus*. There is a certain variability in phytochemical composition between the two species. What was well noticed was the absence of starch and tannins from roots and anthocyanins from seeds.

Antimicrobial effect of plant extracts

Chloroform and ethanol extracts had an inhibitory effect especially on growth of Gram-positive bacteria and fungi, with a moderate effect on *E. coli* (Table 2). The most remarkable MICs (Table 3) were those of chloroform extracts of stems (0.63 mg/ml on *C. albicans* and 0.83 mg/ml on *B. cereus*) and seeds (0.31 mg/ml on *S. aureus* and 0.63 mg/ml on *C. albicans*), in addition to those of ethanol extracts of stems (0.83 mg/ml on *C. albicans*), and flowers and seeds (0.83 mg/ml and 0.63 mg/ml respectively on *B. cereus*).

Water extracts had no remarkable effect on all studied microbial strains. Antimicrobial activity of solvent extracts from many species of the family *Apiaceae* has been evaluated. Ethyl acetate extract of *Daucus carota* subsp. *maritimus* seeds was effective on West Nile Virus (WNV) with an IC_{50} of 0.008 mg/ml (Miladi *et al.,* 2012). Hexane extract of *Foeniculum vulgare*, rich in bergaptol, was active against *Mycobacterium tuberculosis* (MIC = 12.5 µg/ml) (Esquivel-Ferriño *et al.,* 2012). Against Gram-positive bacteria and fungi, similar results were observed. Hexane and ethyl acetate fractions of ethanol-water extract of *Ferula vesceritensis* fruits, and ethanol extract of parsley (*Petroselinum crispum*) and dill (*Anethum graveolens*), showed an activity against *Staphylococcus aureus* (Bouchouka *et al.,* 2012; Wahba *et al.,* 2010).

An MIC value of 100 µg/ml by 8-geranyloxy psoralen-rich hexane extract of *Prangos uloptera* roots was able to inhibit growth of *Staphylococcus epidermidis* and *Candida kefyr* (Razavi *et al.,* 2009). Against Gram-negative bacteria, a purified component from ethanol extract of celery (*Apium graveolens*) seeds had an activity against *Helicobacter pylori* at 3.15 µg/ml MIC and 6.25-12.5 µg/ml MBC (Zhou *et al.,* 2009). Antiplasmodial activity against *Plasmodium falciparum* was assessed by hexane extract of *Ferula pseudalliacea* roots, rich in sanandajin, with an IC_{50} value of 2.6 µM (Dastan *et al.,* 2012). These results confirm the effectiveness of organic extracts from *Daucus crinitus*.

CONCLUSION

The effects observed for the different extracts show, somehow, the ability of this species (*D. crinitus*) to be a good source of bioactive molecules. Anthocyanins and starch present in different subspecies of *Daucus carota* were absent in all extracts of all organs of *Daucus crinitus*. Other characterized families require chromatographic analysis to individually select the compounds in order to discover, perhaps, new molecules of natural origin. While these results are quite interesting and encouraging for carrying out further refined studies to possibly establish an activity-chemical structure relationship.

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Table. 1: Characterization results.

Extract with	Chemical Family	Roots	Leaves	Stems	Flowers	Seeds
Chloroform	Alkaloids	−	−	−	−	−
	Anthraquinones	−	−	−	−	−
	Coumarins	+	+	+	+	+
	Emodins	−	−	−	−	−
	Steroids	+	+	+	+	+
	Terpenes	+	+	+	+	+
Ethanol	Alkaloids	−	−	−	−	−
	Anthocyanins	−	−	−	−	−
	Anthraquinones	−	−	−	−	−
	Coumarins	+	+	−	−	+
	Emodins	−	−	−	−	−
	Flavonoids	−	+	−	+	+
	Iridoids	−	−	−	−	−
	Reducing sugars	+	+	+	+	+
	Steroids	−	+	−	+	−
	Tannins	−	+	−	+	+
	Terpenes	−	−	−	−	−
	Xanthones	−	−	−	−	−
Water	Alkaloids	−	−	−	−	−
	Anthocyanins	−	−	−	−	−
	Anthraquinones	−	−	−	−	−
	Flavonoids	−	−	−	−	−
	Iridoids	−	−	−	−	−
	Mucilages	−	+	−	−	+
	Reducing sugars	+	+	+	+	+
	Saponins	−	+	−	−	+
	Starch	−	−	−	−	−
	Tannins	−	+	+	+	+
	Xanthones	−	−	−	−	−

All repeated tests have revealed the same result. −: absence, +: presence.

Table. 2: Diameters of growth inhibition zones.

Extract with	Organ	*S. aureus*	*B. cereus*	*C. albicans*	*E. coli*	*K. pneumoniae*
Chloroform (50 mg/ml) (10 µl)	Roots	7 ± 1	10 ± 2	13 ± 2	11 ± 0	7 ± 1
	Leaves	7 ± 1	8 ± 2	11 ± 0	11 ± 1	−
	Stems	10 ± 2	15 ± 2	17 ± 1	12 ± 0	−
	Flowers	9 ± 1	16 ± 1	21 ± 2	12 ± 1	9 ± 1
	Seeds	24 ± 3	10 ± 1	16 ± 1	13 ± 1	7 ± 1
Ethanol (50 mg/ml) (10 µl)	Roots	11 ± 2	14 ± 1	13 ± 0	13 ± 1	8 ± 1
	Leaves	10 ± 1	13 ± 2	14 ± 1	12 ± 1	8 ± 1
	Stems	11 ± 1	10 ± 1	15 ± 1	14 ± 2	9 ± 1
	Flowers	11 ± 2	14 ± 2	15 ± 1	12 ± 1	7 ± 1
	Seeds	12 ± 1	15 ± 1	15 ± 1	15 ± 1	8 ± 0
Water (50 mg/ml) (10 µl)	Roots	7 ± 1	−	−	−	−
	Leaves	10 ± 2	−	−	−	−
	Stems	−	−	−	−	−
	Flowers	7 ± 1	−	−	−	−
	Seeds	7 ± 1	−	−	−	−

Results are mean ± standard deviation in millimeters (mm) of three replications. −: no inhibition zone.

Table. 3: Minimum inhibitory concentrations.

Extract with	Organ	*S. aureus*	*B. cereus*	*C. albicans*	*E. coli*	*K. pneumoniae*
Chloroform	Roots	−	6.67 ± 2.89	2.50 ± 0.00	2.50 ± 0.00	−
	Leaves	−	−	10.00 ± 0.00	2.08 ± 0.72	−
	Stems	10.00 ± 0.00	0.83 ± 0.36	0.63 ± 0.00	2.50 ± 0.00	−
	Flowers	8.33 ± 2.89	0.63 ± 0.00	0.42 ± 0.18	1.25 ± 0.00	−
	Seeds	0.31 ± 0.00	−	0.63 ± 0.00	1.67 ± 0.72	−
Ethanol	Roots	11.33 ± 1.53	1.67 ± 0.72	1.25 ± 0.00	2.50 ± 0.00	−
	Leaves	8.33 ± 2.89	1.25 ± 0.00	1.04 ± 0.36	2.08 ± 0.72	−
	Stems	6.67 ± 2.89	8.33 ± 2.89	0.83 ± 0.36	4.17 ± 1.44	−
	Flowers	6.67 ± 2.89	0.83 ± 0.36	1.04 ± 0.36	3.33 ± 1.44	−
	Seeds	2.08 ± 0.72	0.63 ± 0.00	1.25 ± 0.00	1.25 ± 0.00	−
Water	Roots	−	−	−	−	−
	Leaves	10.00 ± 0.00	−	−	−	−
	Stems	−	−	−	−	−
	Flowers	−	−	−	−	−
	Seeds	−	−	−	−	−

Results are mean ± standard deviation in milligrams per milliliter (mg/ml) of three replications. −: not determined.

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