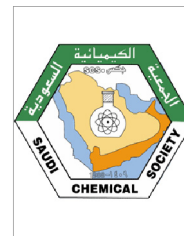




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ORIGINAL ARTICLE

Chemical constituents and antimicrobial activity of essential oils of *Ammodaucus leucotricus*

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KEYWORDS

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Abstract *Ammodaucus* belongs to the family *Apiaceae* (Umbelliferae), subfamily *Apioideae*, trib *Caucalideae*, and comprises one species in Algeria (Quezel and Santa, 1962).

The biological importance of members of this genus promoted us to reinvestigate the volatile constituents of the fruits of *Ammodaucus leucotricus* Coss. et Dur., and to carry out a biological study as far as the antibacterial activity is concerned. The present work deals with the chemical composition and antibacterial activities of the hydrodistilled oils. After extraction, the oil was analyzed by gas chromatography–mass spectrometry to afford 14 components. The major components were found to be, 1-cyclohexene-1-carboxaldehyde (56.40%), D-limonene (28.82%), and 2-pentanone-4-hydroxy-4-methyl (5.73%). Moreover, the antimicrobial activities of the volatile oils were evaluated by disk diffusion method against Gram-positive and Gram-negative bacteria. The results showed a strong antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*.

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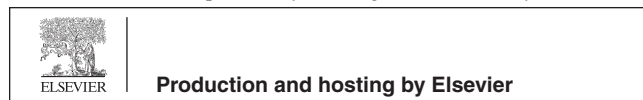
1. Introduction

Ammodaucus leucotricus Coss. et Dur. (endemic plant) belongs to the family *Apiaceae* (Umbelliferae), subfamily *Apioideae*, trib *Caucalideae*, and comprises one species in Algeria (Quezel and Santa, 1962).

Essential oils are important natural products used for their flavor and fragrances in food, pharmaceutical and perfumery industries. They are also sources of aroma chemicals, particu-

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larly of enantiomers that are useful as chiral building blocks in syntheses. Biological and pharmacological activities of essential oils and their constituents have been gathering momentum in recent years. Essential oils therefore will continue to be indispensable natural ingredients (Zoubiri et al., 2010).

Essential oils are components obtained from different plant parts. They are usually synthesized to combat infectious or parasitic agents or generate in response to stress conditions. In the last few years, there has been target interest in biologically active compounds, isolated from plant species for the elimination of pathogenic microorganisms, because of the resistance that microorganisms have built against antibiotics (Derwich et al., 2010).

2. Experimental

2.1. Plant material

The fruits of *A. leucotricus* Coss. et Dur. were collected in May 2008 in Ouargla, Algeria. The plant was identified by Dr. A.M. Chahma, faculty of Sciences, University of Ouargla Algeria, a voucher specimen was deposited at the chemistry department University of Mentouri-Constantine under the code number ZA106.

2.2. Extraction

Essential oils were obtained by hydrodistillation of 100 g of dried fruits using a Clevenger-type apparatus for 3 h. Diethyl ether (10 ml) was used as the collector solvent as reported in the literature. After evaporation of the solvent, the oil was dried over anhydrous sodium sulfate and stored in sealed vials, protected from the light at $-20\text{ }^{\circ}\text{C}$ before analyses, to give 0.7 g (07%) of crude oil. The oils were subsequently analyzed by GC-MS (Dey and Harborne, 1997).

2.3. Analytical conditions

2.3.1. Gas chromatography/mass spectrometry (GC/MS)

The oil was analyzed by GC/MS using an Agilent 5973EI mass selective detector coupled with an Agilent GC6890A gas chromatograph, equipped with a cross-linked 5% PH ME siloxane HP-5MS capillary column (25 m \times 0.20 mm, film thickness 0.33 μm). Operating conditions were as follows: carrier gas, helium with a flow rate of 1 mL/min; column temperature $50\text{ }^{\circ}\text{C}$ for 1 min, $50\text{--}150\text{ }^{\circ}\text{C}$ ($3\text{ }^{\circ}\text{C}/\text{min}$), $150\text{--}250\text{ }^{\circ}\text{C}$ ($5\text{ }^{\circ}\text{C}/\text{min}$) then isothermal for 5 min. Injector and detector temperatures, $280\text{ }^{\circ}\text{C}$; split ratio, 1:50. The MS operating parameters were as follows: ionization potential, 70 eV; ionization current, 2 A; ion source temperature, $200\text{ }^{\circ}\text{C}$; resolution, 1000.

2.3.2. Identification of components

Identification of oil components was achieved on the basis of their retention indices RI, (determined with reference to a homologous series of normal alkanes), and by comparison of their mass spectral fragmentation patterns with those reported in the literature (Adams, 2007) and stored on the MS library (NIST database). The concentration of the identified compounds was computed from the GC peak total area without any correction factor (see Table 2).

Table 1 Phytochemical survey of *Ammodaucus leucotricus*.

Chemical group	Presence
Volatile oils	+++
Carotenoids	+
Alkaloids	–
Flavone aglycone	+
Coumarins	+++
Tanins	++
Saponins	–
Flavone glycoside	+++

Table 2 Volatile oil composition of *Ammodaucus leucotricus*.

Components	Rt	%
Pyranon	3.093	5.73
α -Pinene	5.392	0.31
β -Myrcene	7.206	0.37
3-Carene	7.770	1.42
D-limonene	8.417	28.82
L-perrillaldehyde	17.671	56.40
Perillol	19.167	0.61
Acetic acid, (2-isopropenylcyclopentylidene)-, methyl ester	23.052	2.24
1-Pentadecene	24.283	0.79
Rosifolol	29.911	0.99
z-5-Nonadecene	32.156	0.60
Caotol	34.263	0.33
1-Nonadecene	39.278	0.28
		98.89

2.3.3. Antimicrobial activity

The anti-microbial activity test was carried out on essential oils of *A. leucotricus* Coss. et Dur. using the disk diffusion method against three human pathogenic bacteria, including Gram positive and Gram-negative bacteria (NCCLS, 1999). The bacterial strains were first grown on Muller Hinton medium (MHI) at $37\text{ }^{\circ}\text{C}$ for 24 h prior to seeding onto the nutrient agar. A sterile 6-mm-diameter filter disk (Whatman 3) was placed on the infusion agar seeded with bacteria, and each extract suspended in water was dropped onto each paper disk (40 μL per disk) for all prepared concentrations (8, 4, 2, 1, 0.5 and 0.25 mg/ml). The treated Petri disks were kept at $4\text{ }^{\circ}\text{C}$ for 1 h, and incubated at $37\text{ }^{\circ}\text{C}$ for 24 h. The antibacterial activity was assessed by measuring the zone of growth inhibition surrounding the disks. Each experiment was carried out in triplicate (Singh et al., 2011).

3. Results and discussion

The present work focused essentially on the phytochemical and antibacterial studies from *A. leucotricus* Coss. et Dur. The species has been screened for 7 chemical groups (Ikhir and Boureima, 1992). It is worth noting that the presence of volatile oils, flavonoids, saponins, tannins, carotenoids, and coumarins has not been previously reported in the literature (Table 1).

Table 3 Antimicrobial activity of volatile oils at different concentrations on the three bacteria strains.

Strains	Concentration					
	0.25 mg/ml	0.5 mg/ml	1 mg/ml	2 mg/ml	4 mg/ml	8 mg/ml
<i>E. coli</i> ATCC 25922	8 ± 1.0	10 ± 00	11.33 ± 0.86	12.66 ± 0.57	13.5 ± 1.80	15.5 ± 0.86
<i>Klebsella pneumonia</i>	06 ± 00	08 ± 00	08.50 ± 00	09.50 ± 1.80	11 ± 2.0	12.66 ± 1.15
<i>Staphylococcus aureus</i> ATCC	07.5 ± 0.86	07.5 ± 0.86	07.50 ± 0.86	08.00 ± 01.00	010.0 ± 01.00	14.66 ± 01.15

4. Results and discussion

The diffusion test was applied to three microorganisms including Gram-positive and Gram-negative bacteria. The results summarized in Table 3 shows that the volatile oil from *A. leucotricus* prevented the growth of all the tested microorganisms and it has been revealed that the medium diameter of inhibition zone increases proportionally with the increasing concentrations.

The obtained inhibition zone varied from 6.00 to 15.50 mm with a highest inhibition zone recorded with *Escherichia coli* ATCC 25922 at the concentration of 8 mg/L (21 and 20 mm).

In general, volatile oil exhibited a higher stronger activity against bacterial strains. The antimicrobial activity of the crude extract of *A. leucotricus* Coss. et Dur. is apparently related to its terpene type components such as oxygenated monoterpenes, since there is a relationship between the chemical structures and their antimicrobial activities.

5. Conclusion

The results of antimicrobial assays justified and supported partly the popular usage of the all organs especially fruits as traditional remedies for some infections. It was of interest to note that the strong antimicrobial activity of the volatile oil from *A. leucotricus* Coss. et Dur. against clinical and standard

microorganisms, especially *E. coli* which were established as major pathogens responsible for a wide variety of infections, suggested that this volatile oil could be new medicinal resource for antibacterial agents.

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