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Bioactivity and chemical quality of *Ammodaucus leucotrichus ssp. leucotrichus* Coss. & Durieu essential oils from Morocco

Alaoui Moulay Sidi Brahim[1,2], Satrani Badr[1,*], Ghanmi Mohamed[1], Aafi Abderrahman[1], Amusant Nadine[3], El Antry Salwa[1], Chaouch Abdelaziz[2]

[1]Chemistry and microbiology laboratories, forest research center. Bp 763. Ma-10050, agdal, rabat, (MOROCCO)

[2]Laboratory of biotechnology, environment and quality, unit of formation and research of the geniuses of the processes, faculty of science. University ibn tofail, bp 133. Ma-14000, kenitra (MOROCCO)

[3]Cirad, department environments and societies, umr ecology forests of french guiana1,bp 732, 97310 kourou cedex, (FRENCH GUIANA)

E-mail : badrsat@yahoo.fr

ABSTRACT

As part of the valorization of medicinal and aromatic plants in South Morocco, we studied the effect of provenance on the yield, chemical composition and antibacterial and antifungal activity of essential oils of Ammodaucus leucotrichus subsp. leucotrichus. The essential oils obtained by hydro-distillation of the fruits were analyzed by GC and GC/MS. The most abundant compounds identified varied depending on the origin of the plant: perillaldehyde (69.9% to 88.7%), limonene (8.3% to 16.6%) and α-pinene (1.4% to 7.1%). The oils analyzed all displayed strong antimicrobial activity against all the microorganisms tested.

KEYWORDS

Ammodaucus leucotrichus subsp. leucotrichus;
Essential oils;
Chemical composition;
Antimicrobial activity;
Origin.

INTRODUCTION

The spontaneous flora in the Saharan regions of Morocco and the relations between humans and plant species deserve particular attention. Some species have pharmacological properties making them of major medicinal interest. Natural remedies, particularly medicinal plants, were formerly the main, and sometimes even the only remedy for pathologies, and today can provide raw material for modern medicine.

Ammodaucus leucotrichus Cosson & Durieu, also known as hairy cumin or kammun es sofi belongs to the botanical family of Apiaceae. This species thrives in the desert sands and gravels of Saharan ouadi, often at the foot of hills or dunes. It grows in arid bioclimatic conditions where annual precipitation does not exceed 100 mm and flowers from March to May[1].

It is found in Morocco, the Canary Islands and in Algeria. The species comprises two subspecies:

Ammodaucus leucotrichus ssp. nanocarpus Beltrán-Tej is very small in size and is endemic to the Atlantic coast of the Moroccan Sahara and the Canary Islands.

Ammodaucus leucotrichus ssp. leucotrichus Coss. & Durieu: This subspecies is taller than the other subspecies, and is distributed in a wider area extending from Morocco, Algeria, Tunisia to Egypt[2].

Ammodaucus leucotrichus is an annual herbaceous plant with drawn up stems and finely striated. The leaves are divided into narrow, somewhat fleshy strips; the umbels have 2-4 rays and involucral bracts that are much divided. The fruits are very hairy, with long, curly, yellow-russet hair at the base, otherwise white, 8-10 mm in length. The plant has a very strong odor of aniseed[1].

Ammodaucus leucotrichus is one of the aromatic medicinal plants most commonly used to treat many different infections. Dried, crushed and mixed with water or goat's milk, or alone, applied to the skin or taken orally it is used against snake bites, to treat infected wounds, or to prevent infection. The crushed seeds are mixed with milk and inhaled to treat sinusitis. It can also be used to perfume tea[3].

Boiled in milk, the seeds are used to treat infections of the respiratory tract and laryngitis. In an infusion with tea with milk, or added to food as a spice, the seeds are used as appetizers and or as liqueurs to treat colitis. The fruits of Ammodaucus leucotrichus are also used in decoction against indigestion, anorexia, some allergies, palpitations, diarrhea and vomiting[3].

To our knowledge only one study has been undertaken to determine the chemical composition of the essential oil of the fruits of Ammodaucus leucotrichus ssp. leucotrichus from the Dakhla area (southern Morocco)[4]. No study has been conducted of the chemical profiles of the essential oils of hairy cumin from different areas in southern Morocco or to evaluate their range of antimicrobial activity. The aim of this study was thus to characterize the chemical composition and the antibacterial and antifungal activity of essential oils extracted from the fruits of Ammodaucus leucotrichus ssp. leucotrichus sampled in the south of Morocco.

EXPERIMENTAL

Plant materials

Fruits of Ammodaucus leucotrichus ssp leucotrichus were collected in June, 2011, in the region of Mkaimima (East Tata, South Morocco) and in the area of El Galta (East Aayoune, South Morocco).

Isolation of essential oils

Samples of plant materiel (200 g) of the two Ammodaucus leucotrichus ssp leucotrichus provenances were subjected to hydro-distillation using a Clevenger-type apparatus for two hours[5]. The method used is that described in the European Pharmacopoeia[6] and Afssaps (the French health products safety agency) 2008 recommendations. Three replicates were perfotrmed for each A. leucotrichus provenance. The yields (w/w) of essential oils were determined based on dry weight. Essential oils were dried over anhydrous sodium sulfate and stored in the refrigerator until analysis.

Analysis of essential oils

Gas chromatography (GC) analysis was performed using a Hewlett Packard Gas Chromatographer (HP 6890) with electronic pressure control, equipped with a HP-5MS capillary column (30 m x 0.25 mm, film thickness 0.25 μm), a FID detector set at 250 °C and fed with a H^2/Air mixture, and a split splitless injector set at 250 °C. The injection mode was split (1:50) and the injected volume was 1 μl. Nitrogen was used as carrier gas with a flow rate of 1.7 $ml.min^{-1}$. The column temperature was programmed from 50 to 200 °C at a heating rate of 4 $°C.min^{-1}$. The apparatus was controlled by a "Chemstation" computer system.

Gas chromatography/mass spectrometry (GC/MS) analysis was performed using a Hewlett-Packard Gas Chromatographer (HP 6890) coupled with a mass spectrometer (HP 5973). Fragmentation was performed by electron impact at 70 ev. The column used was HP-5MS (30 m x 0.25 mm, film thickness 0.25 μm). The injection mode was split (1:50). The column temperature was programmed from 50 to 200 °C at a heating rate of 4$°C.min^{-1}$. The components of the essential oils were identified based on Kováts retention indices and mass spectral database (NIST 98 library).

Microorganisms studied

The antimicrobial activity of the Ammodaucus leucotrichus oils was evaluated in 11 isolated strains. The following microorganisms were used:

- Bacteria: Escherichia coli, Bacillus subtilis, Staphylococcus aureus and Micrococcus luteus.
- Fungi: Aspergillus niger, Penicillium digitatum and Penicillium expansum.
- Wood decay fungi: Gloeophyllum trabeum, Poria placenta, Coriolus versicolor and Coniophora puteana.

The four pathogenic bacteria were chosen for their high resistance to antibiotics and for their toxicity in humans. They are encountered in many infections in Mo-

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rocco and pose a clinical and therapeutic problem. The three selected fungi are agents of decay in common food and fruits and can be toxic and pathogenic for both humans and animals. The four wood decay fungi used in this study are the most important wood-destroying fungi in buildings, in wood in contact with the soil (poles and railways) or buildings (bridges). They were chosen for the considerable damage they cause to wood and related products[7,8].

Bacteria were batches of "American Type Culture Collection" ATCC. They were subcultured on nutrient agar for 24 h at 37 °C in the dark. Mold and wood decay fungi came from the Mycothèque collection of Microbiology Laboratory in Forestry Research Centre, Rabat, Morocco. They were subcultured on PDA (Potato Dextrose Agar) nutrient medium for seven days at 25 °C in the dark.

The technique used was dispersion of EO in the 0.2% agar solution. The minimum inhibitory concentrations (MIC) of the EO of Artemisia were determined using the method reported by Remmal et al. and Satrani et al.[7,8].

Antimicrobial assays

Minimum inhibitory concentrations (MIC) of essential oils were determined according to the method reported by Remmal & al. and Satrani & al.[7, 8]. Due to the immiscibility of essential oils in water and hence in the culture medium, emulsification was obtained by using a solution of 0.2% agar to promote contact between the germ and the compound. Dilutions were prepared at 1/10th, 1/25th, 1/50th, 1/100th, 1/200th, 1/300th and 1/500th in the agar solution. Each test tube contained 9 ml of agar medium in 2% malt extract. The samples were autoclaved for 20 min at 121 °C and cooled to 45 °C. Aliquots (1 ml) of each dilution were then added to obtain final concentrations of 1/100, 1/250, 1/500, 1/1.000, 1/2.000, 1/3.000, 1/5.000 (v/v), and the tubes were stirred well before the solution was poured into Petri dishes. Negative controls containing only the culture medium and the 0.2% agar solution were also prepared.

Inoculation of the bacteria and molds was performed by streaking with a platinum loop calibrated to collect the same volume of inoculum. The bacterial inoculum consisted of a broth cultured for 24 hours, and for the molds, of spores from a 7-day culture in the PDA suspended in physiological saline.

Inoculation of the wood-decay fungi was performed by depositing fragments (1cm in diameter) taken from the periphery of a mycelium cultured for 7 days in PDA. Samples were incubated in the dark for 24 h at 37 °C for bacteria and for seven days at 25 °C for mold and wood decay fungi. Each test was repeated three times. MIC was determined as the lowest concentration of oil able to inhibit visible growth of each microorganism on the agar plate.

RESULTS AND DISCUSSION

Yield and chemical composition

The average yield of essential oil of fruits of Ammodaucus leucotricus ssp. leucotricus is expressed in milliliters of 100 g of dry matter of the aerial part of the plant. Yields of essential oil from plants originating from El Galta were higher than yields of essential oil from plants originating from Mkaimima (1.5% compared to 1%).

Chromatographic analysis of the essential oils identified 16 compounds representing a total of 99.5% for Ammodaucus leucotricus ssp. Leucotricus from Mkaimima versus only 7 compounds (for a total of 99.9%) from El Galta (TABLE 1).

The essential oil of the fruits of Ammodaucus leucotrichus ssp. leucotrichus originating from Mkaimima was rich in perillaldehyde with 69.9%, limonene (16.6%) and less α-pinene (7.14%). The hydro-distilled essence of the fruits of A. leucotrichus originating from El Galta mainly contained perillaldehyde (88.7%) whereas limonene content did not exceed 8.3% and α-pinene content was only 1.4%.

The essential oil of the fruits of hairy cumin from Mkaimima was characterized by low concentrations of components that were completely absent in the hairy cumin originating from El Galta: α-fenchene, β-pinene, myrcene, α-terpinene, terpinolene, cis-oxide of limonene, terpin-1-ol, perillalcool and α-copaene.

A study conducted by Velasco-Negueruela et al.[4] on the fruits of Ammodaucus leucotrichus ssp. leucotrichus from the region of Dakhla (southern Morocco) showed that the essential oil mainly contained

TABLE 1: Percentage chemical composition of essential oils from fruits of *Ammodaucus leucotricus ssp. leucotricus* as a function of the location the plants were harvested.

IK	Components	Mkaimima (%)	El Galta (%)
928	α-pinene	7.14	1.44
935	Camphene	0.17	0.14
942	α-fenchene	0.48	-
967	Sabinene	0.63	0.28
971	β-pinene	0.82	-
985	Myrcene	0.23	-
1006	α-phellandrene	0.67	0.46
1019	α-terpinene	0.17	-
1023	limonene	16.62	8.26
1093	terpinolène	0.50	-
1128	Cis-oxyde limonene	0.70	-
1132	Terpin-1-ol	0.41	-
1273	Perillaldehyde	69.88	88.72
1293	perilla alcool	0.26	-
1373	α-copaene	0.39	-
1386	β-cubebene	0.50	0.68
Total		99.57	99,98

IK : Kováts index, % : percentage, - : absence.

perillaldehyde (63.6%) and limonene (26.8%). This species thus contains less perillaldehyde but more limonene than the species originating from our two regions (Mkaimima and El Galta). In addition, α-terpineol, methyl perillate, 3-hydroxyperillaldehyde and γ-decalactone were specific to the essential oil from the hairy cumin from Dakhla while the essence originating from Mkaimima was characterized by α-fenchene, cis-oxyde of limonene, terpin-1-ol, copaene α-and β-cubebene.

Essential oils hydro-distilled from two aromatic medicinal plants Dracocephalum polychaetum and Dracocephalum surmandium originating from Iran were shown to qualitatively resemble our two essences extracted from hairy cumin with chemical profiles dominated by perillaldehyde (respectively 63.4% and 54.3%) and limonene (respectively 22% and 30%)[9]. Similarly, the essence of the fruits of Laser trilobum (L.) Borkh. originating from Turkey, mainly contained perillaldehyde (62%) and limonene (26.7)[10].

In addition, the perillaldehyde content (83%) of the essential oil of the bark of Hemandia voyroni from Cameroon[11] resembles that in the essential oil of Ammodaucus leucotrichus fruits oil originating from El Galta (88.7%).

Thanks to its high perillaldehyde content, the essential oil from A. leucotrochus fruits originating from El Galta could be a source of this active ingredient. Perillaldehyde is used as a component of perfume, cosmetics and for its aroma[12]. Perillaldehyde can be converted into perilla alcohol, which is also used in the perfume industry.

Perillaldehyde is also sweet, 12 times sweeter than sucrose. Perillartine, an oxime with 2000 times more intense sweetness than sucrose is synthesized from perillaldehyde[13,14].

Antimicrobial activity of essential oils

The antibacterial and antifungal activities of the essential oils from the fruits of Ammodaucus leucotrichus ssp. leucotrichus from the two provenances are listed in TABLE 2. Essential oils from hairy cumin from both provenances displayed a significant inhibitory effect against all the microorganisms tested. The essential oil extracted from A. leucotrichus from El Guelta inhibited Escherichia coli, Bacillus subtilus and Micrococcus luteus at a low concentration (of the order of 1/1000 v/v) and only Staphylococcus aureus resisted up to 1/500 v/v. The high resistance of the last germ to multiple antibiotics has already been reported by several authors[15-17]. All the bacterial strains tested in this study were inhibited by essential oil from A. leucotrichus harvested in Mkaimima, starting at a concentration of 1/500 v/v.

The essential oils from the two provenances of A. leucotrichus also displayed great inhibitory activity against mold and wood rot fungi. The growth of all fungi tested was stopped at a concentration of only 1/1000 v / v. A concentration of only 1/2000 v / v essential oils of hairy cumin from the two provenances was also sufficient to inhibit the growth of C. puteana, P. placenta and G. trabeum, but not C. versicolor, which resisted up to 1/1000 v / v. The lower sensitivity of C. versicolor to both essential oils tested compared to the other fungal species could be explained by the ability of this fungus to produce laccase and other extracellular enzymes that catalyze the oxidation of phenolic compounds and causes their inactivation[18,19].

TABLE 2: Antibacterial and antifungal activity of essential oils of the fruits of Ammodaucus leucotricus ssp. leucotricus from the region of Mkaimima (M) and from the region of El Galta (G)

	1/100 v/v		1/250 v/v		1/500 v/v		1/1000 v/v		1/2000 v/v		1/3000 v/v		1/5000 v/v		Témoin	
	M	G	M	G	M	G	M	G	M	G	M	G	M	G	M	G
Bactéries																
E. coli	-	-	-	-	-	-	+	-	+	+	+	+	+	+	+	+
B. subtilus	-	-	-	-	-	-	+	-	+	+	+	+	+	+	+	+
M. luteus	-	-	-	-	-	-	+	-	+	+	+	+	+	+	+	+
S. aureus	-	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+
Moisissures																
A. niger	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
P. expansum	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
P. digitatum	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
Champignons																
C. puteana	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+
C. versicolor	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
P. placenta	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+
G. trabeum	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+

with :

E. coli : Escherichia coli, B. subtilis : Bacillus subtilis, M. luteus : Micrococcus luteus, S. aureus : Staphylococcus aureus, A. niger : Aspergillus niger, P. digitatum : Penicillium digitatum, P. expansum : Penicillium expansum, G. trabeum : Gloeophyllum trabeum, P. placenta : Poria placenta, C. puteana : Coniophora puteana, C. versicolor : Coriolus versicolor,

+ : Growth

- : Inhibition

The high perillaldehyde content of the essential oils from the two provenances of A. leucotrichus is responsible for its strong bactericidal and fungicidal activity. Indeed, the essential oils of both Dracocephalum polychaetum and Dracocephalum surmandium, with respectively 63.4% and 54.3% perillaldehyde, strongly inhibited all Gram-positive and Gram-negative bacteria tested[9]. The most sensitive microorganisms was Staphylococcus epidermidis, whose growth was inhibited at a concentration of only 1/3000 V/V of the essential oils of both plants. The Pseudomonas aerugenosa germ, known for its resistance to antibiotics, was strongly inhibited by the essence of Dracocephalum polychaetum at a concentration of 1/500 V/V[9].

The antibacterial power of the essential oil of fruits of the hairy cumin from El Galta, which had the highest perillaldehyde content (89%), was greater than that of the essence of Mkaimima A. leucotrichus (70% of perillaldehyde) (TABLE 2).

Friedman and al.[20] also tested the bactericidal activity of 96 essential oils and 23 of their pure compounds against Campylobacter jejuni, Escherichia coli, Listeria monocytogenes and Salmonella enterica. These authors reported that perillaldehyde was among the most effective substances against these pathogenic bacterial strains. Weak concentrations of perillaldehyde were also shown to be effective against bacteria that contaminate the foodstuffs[21].

In addition, among 22 monoterpenoids tested for their fungicidal efficacy, perillaldehyde displayed good inhibitory activity against the germination of the spores and the growth of the mycelium of two fungal pathogens of fruits Botrytis cineria and Monilinia fructicola[22]. McGeady et al.[23], showed that very weak concentrations of perillaldehyde and carvone can inhibit the transformation of Candida albicans into the filamentous form known for its pathogenicity. In horticulture, Smid et al.[24] investigated the effect of treating tulip bulbs with 15 terpene compounds of essential oils against rot caused by the fungus Penicillium hirsutum. These authors concluded that perillaldehyde was one of the four most effective compounds against these pathogenic fungi and did not affect the growth or flowering capacity of the tulip bulbs.

Kurita and Koike[25] showed that the synergistic effect obtained by combining very small quantities of ethanol, sodium chloride, acetic acid and perillaldehyde produced high antibacterial and antifungal activity. The authors strongly recommended applying this mixture for the preservation of food as a substitute for the synthetic chemicals that are currently used and are harmful to human health. Similarly, the oil essential from the bark of Hemandia voyroni from Cameroon, which contains 82.8% perillaldehyde, displayed remarkable antibacterial and antifungal activity against six microbial strains[11].

Inouye and al.[26] studied the correlation between the antifungal activity and the power of diffusion of the constituents of essential oils. They concluded that terpene aldehydes like perillaldehyde, citral and cinnamaldehyde, are very effective against bacteria and fungi thanks to their high diffusivity in the culture medium, which enables rapid direct contact with microorganisms.

As reported in several studies, perillaldehyde also has insecticidal properties. Omolo et al.[27] showed that perillaldehyde and perillyl alcohol, two components of the essential oil of Conyza newii (originating from Kenya), displayed high insecticidal activity against Anopheles gambiae, the main vector of malaria. In vitro, perillaldehyde extracted from the leaves of Perilla frutescens caused the total destruction of Anisakis larvae after 24 h[28]. Hierro and al.[29] tested the larvicide activity of several pure monoterpene derivatives of aromatic and medicinal plants on the same parasite and concluded that perillaldehyde is very effective against the parasitic disease caused by the L3 larva of the nematode Anisakis simplex. The authors explained the high lethal effect of perillaldehyde against the larvae of this nematode by the damage it causes to the nervous system of the parasite.

Perillaldehyde has also been shown to have a higher antioxidant activity than some terpene alcohol and phenols such as carvacrol, anethol and linalool[30].

CONCLUSION

Our qualitative and quantitative analysis of essential oils extracted from the fruits of Ammodaucus leucotrichus ssp. leucotrichus growing in the regions of Mkaimima and El Galta in southern Morocco identified 16 and 7 components respectively. The essential oil from the hairy cumin originating from Mkaimima was shown to be rich in perillaldehyde (approximately 70%) and limonene (16.60%), while the essence of A. leucotrichus from El Galta is dominated by only one compound, perillaldehyde at 89%.

In vitro, the essential oils from the two origins displayed strong antibacterial and antifungal activity against all the bacteria, molds, and timber rot fungi tested. The bioactive power of the essential oils extracted from hairy cumin from the two provenances was mainly attributed to their high percentage of perillaldehyde. Based on these results, the essential oils of the fruits of Ammodaucus leucotrichus ssp. leucotrichus could be used as a source of this natural monoterpene, paving the way for its use in the development of effective therapeutic agents in the medical field or as a preservative in the food industry.

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Full Paper

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