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Antimicrobial and antioxidant activity of essential oil of *Ammodaucus leucotrichus* Coss. & Dur. seeds

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Abstract
This work presents the chemical composition, antibacterial and antioxidant activities of essential oils obtained from *Ammodaucus leucotrichus* Coss. & Dur. seeds. The essential oil composition was investigated by GC/MS with 34 identified essential constituents representing 96.29 % of the total amount; the main constituents were perillaldehyde (59.12 %) and limonene (23.89 %). The antioxidant activity was evaluated by β-carotene bleaching method, compared to the synthetic antioxidants. In This method oxidation of linoleic acid was inhibited by essential oil. The inhibition value was close to BHT with 88.10% inhibition. The antimicrobial potentials were determined by different techniques. In the minimum inhibitory concentration methods, the essential oil played a major role as a remarkable antimicrobial agent due to its inhibition action against most pathogenic tested with MIC values ranged from 0.37 to 0.92 mg/ml. In the disc diffusion method, the essential oil at dose 1.29 mg/disc showed significant antibacterial activity against bacteria and yeast. The antifungal results for the essential oil revealed good clear zones of growth inhibition. The activity of the essential oils could be related to the structure of the constituent components.

Keywords: *Ammodaucus leucotrichus* Coss. & Dur., antimicrobial activity, antioxidant activity, essential oil, gas chromatography/mass spectrometry (GC-MS).

Introduction
In the world, up to 500000 plant species are reported. Few of them are used in herbal medicine. The Mediterranean region is characterized by heterogeneous soil and climatic conditions that have produced more than 10000 medicinal and aromatic plant species with diverse properties worthy of further investigation [1].
Ammodaucus leucotrichus Coss. & Dur. plays an important role in traditional medicine in North African countries, especially in the southern Algerian Sahara [2]. The seeds of this plant are used to treat diseases related to the digestive apparatus and to ease stomach and liver pain. The leaves are also used for chest complaints [3]. In the Tassili region of Algeria, it is mainly used as powder or as an infusion to treat the symptoms mentioned above. It is also used in the area to recover the appetite or avoid indigestion, by crushing the seeds and mixing them with milk or millet. The leaves are used to aromatize tea. Powdered, it is much appreciated spice food in the Djanet area [4].
Ammodaucus leucotrichus Coss. & Dur. belongs to the family Umbelliferae (Apiaceae). It is a small annual plant, 10-12 cm in height, glabrous with erect, finely striates stems. The leaves are finely dissected and slightly

fleshy. The flowers are small grouped in umbels of 2 to 4 branches. The fruit is a diachene, 6-10 mm long and is covered with dense silky white hairs [5].
The medicinal properties of aromatic plants are usually attributed to their essential oils. Essential oils are heterogeneous group of complex mixtures of organic substance whose quality and quantity vary with growth stages, ecological conditions and other factors of plant from which it is extracted [6].
A number of studies on various plants reported that some plant extracts and essential oils have antioxidant activity and benefits to the human health in playing an important role in neutralizing free radicals, which can cause several disorders of immune system and gene expression [7-8]. For this reason they can be used to protect organisms and cells from damage induced by oxidative stress, the latter being considered a cause of ageing, degenerative diseases and cancer [9-10-11]. Essential oils usually show antimicrobial activity against a wide range of microorganisms including antibiotic resistant bacteria and fungi. They can affect both gram-positive and gram-negative bacteria in addition to yeasts and filamentous fungi [12-13].
This work is the first report giving the composition, antimicrobial and antioxidant properties of the seed oil obtained from *Ammodaucus leucotrichus* Coss. & Dur. in order to understand the utilization of this plant.

2. Materials and methods

2.1. Seed collection

The seeds of *Ammodaucus leucotrichus* Coss. & Dur. were collected from the southern Algerian Sahara (Béchar 31°36′59.76″N, -2°12′59.76″W, subtropical desert/low-latitude/arid hot climate) during spring season. Voucher specimens have been deposited at the Herbarium of the botanical department, high school of agronomy, Algiers.

2.2. Isolation of the essential oil

Seeds were air-dried in shade at room temperature and subsequently comminuted using an electric hammer. The oil was extracted from 100 g of plant material by hydrodistillation using a Clevenger-type apparatus until total recovery of oil (ca. 3 h). The oil was dried over anhydrous sodium sulphate and stored at 4 °C, for further analysis and tests.

2.3. Gas chromatography/mass spectrometry (GC-MS) analysis

The analysis of the essential oils were performed using GC/MS system consisted of a Agilent Model 6850 gas chromatograph coupled to a Quadrupole mass spectrometer Agilent Model HP 5973. A capillary column of HP-5MS (5% phenylmethylsiloxane, length = 30 m, inner diameter = 0.25 mm and film thickness = 0.25 µm) was used with helium as the carrier gas, at a flow rate of 1.3 ml/min . The oven temperature was programmed from 60 to 246 °C at 3 °C/min and then held isothermally at 246 °C for 4 min. Injector and transfer line temperatures were, respectively, 250 °C and 280 °C. Diluted sample (1/100, v/v in hexane) of 2 µl were injected automatically. Mass spectrometry was run in the electron impact mode (EI) at 70 eV. All mass spectra were acquired in scan range m/z 35–450.
Individual components were identified by comparison of both mass spectrum and their retention indices (RI) with those of authentic compounds previously analyzed and stored in the data system. Other identifications were made by comparison of mass spectra with those in the data system library (Wiley275.L) and cited in the literature (Adams 2007). The retention indices were calculated for all volatile constituents using an *n*-alkanes homologous series (C_8-C_{42}) at the same GC conditions.

2.4. Antioxidant activity

2.4.1. β-Carotene/linoleic acid bleaching assay

In this assay, antioxidant activity was determined by measuring the inhibition of conjugated diene hydroperoxides arising from linoleic acid oxidation [10]. A stock solution of β-Carotene-linoleic acid was prepared as follows: 0.5 mg of β-carotene in 1 ml chloroform, 25 µl of linoleic acid and 200 mg Tween 40. Chloroform was completely evaporated using a rotary evaporator at 50 °C and 100 ml of oxygenated distilled water were then added to the residue. The samples (2 mg/ml) were dissolved in ethanol and 350 µl of each

sample solution were added to 2.5 ml of the above mixture in test tubes. The test tubes were incubated in a hot water bath at 50 °C for 2 h. The same procedure was repeated with a positive control BHT and ascorbic acid in the same concentration (2 mg/ml) and a blank. After an incubation period the absorbance was measured at 470 nm. Inhibition of bleaching β-carotene (I %) was calculated as follows:

Inhibition percentage (I %) = (Abs $_{initial}$ –Abs $_{sample}$ / Abs $_{initial}$) x 100

Antioxidative capacities of the essential oil were compared with those of positive control and the blank. Tests were carried out in triplicate [14].

2.5. Antimicrobial activity

2.5.1. Tested microorganisms

The essential oil was tested on different microorganisms, including Gram-positive bacteria *Bacillus subtilis*, Gram-negative bacteria *Escherichia coli* and two yeast species *Candida albicans* and *Saccharomyces cerevisiae*, in addition *Aspergillus flavus* and *Penicillium escpansum* were selected to evaluate the antimicrobial activity. All the Microorganisms were procured from the Microbial Type Culture Collection (MTCC), of *the Microbiology Laboratoy, Departement of biology*, Ecole Normale Superieure Algiers.

2.5.2. Disk-diffusion assay

The disk diffusion method was used for the determination of antibacterial activity. Bacterial species were cultured on Muller Hinton agar media, while fungi were cultured on Sabouraud Dextrose agar media. The inoculums were suspended in sterile saline and diluted according to 0.5 Mc Farland standard and then spread on solid media plates. Sterile filter paper disks (5.5 mm in diameter) were impregnated with 10 µL of tested essential oil and placed in the center of the inoculated plates then remained 2 hr at 4 ^{o}C. The cultures were incubated either at 37 °C for 24 h for bacteria or at 30 °C for 48 h for filamentous fungus. Each experiment was replicated three times. The antimicrobial activities were evaluated by measuring the inhibition zone diameters (millimeters) surrounding each disk [15].

2.5.3. MIC agar dilution assay

Minimal inhibitory concentration (MIC) was determined as the lowest concentration of various extracts able to completely inhibit visible growth of the microorganism, as detected by the naked eye [16-17].

The essential oil of *Ammodaucus leucotrichus* Coss. & Dur. was added aseptically to sterile medium, containing Tween 20 (0.5%, v/v) at the appropriate volume to produce the concentration ranging from 0.37 to 0.92 mg/ml. The resulting medium agar solutions were immediately poured into Petri plates after vortexing. The plates were spot inoculated with 1µl of inoculums (of each microorganism isolate). After the incubation period, the plates were evaluated for the presence of growth. Each test was performed in triplicate [10].

2.6. Statistical analysis

The data were presented as mean ± standard deviation of three replicates. Statistical analyses were performed using a one-way analysis of variance. Statistical significance was declared at $P < 0.05$.

3. Results and Discussion

3.1. Analysis of essential oil

GC-MS analysis of essential oil from the *Ammodaucus leucotrichus* Coss. & Dur. seeds powder samples were carried out. Percent compositions of major constituents identified in the oil are given in Table 1. Odour profile showed that the oil contains more than 34 constituents which represented 96.29 % of its total composition. The constituents were identified by comparing the retention time and mass spectra of authentic samples with standard library NIST and Adams 2007.

The major components of the oil from the seeds of *Ammodaucus leucotrichus* Coss. & Dur. were perillaldehyde (59.12 %) and limonene (23.89 %). Other characteristic components of the oil were δ -3-Carene (4.21 %), β-pinene (3.36 %), α-pinene (1.03 %), perilla alcohol (0.75 %) and myrcene (0.41 %).

Table 1: Chemical composition of oil from the seeds of *Ammodaucus leucotrichus* Coss. & Dur.

No	Compound	RI	ARI	Area %
1	*α*-thujene	923	924	0.01
2	*α*-pinene	931	932	1.19
3	camphene	948	946	0.11
4	benzaldehyde	959	952	0.02
5	sabinene	970	969	0.08
6	*β*-pinene	977	974	3.63
7	myrcene	987	988	0.45
8	*δ*-3-carene	1009	1008	4.41
9	*o*-cymene	1024	1022	0.14
10	limonene	1032	1024	23.89
11	*γ*-terpinene	1055	1054	0.02
12	terpinolene	1084	1086	0.02
13	*α*-pinene oxide	1099	1099	0.06
14	*p*-mentha-*trans*-2,8-dien-1-ol	1120	1119	0.06
15	*cis*-limonene oxide	1131	1132	0.67
16	cuminal	1242	1138	0.45
17	terpinen-4-ol	1179	1174	0.01
18	*trans*-p-mentha-1(7),8-dien-2-ol	1187	1187	0.06
19	perilla alcohol	1299	1294	0.78
20	perillaldehyde	1285	1296	59.13
21	*α*-copaene	1372	1374	0.09
22	*β*-cubebene	1384	1387	0.11
23	*β*-elemene	1386	1389	0.06
24	methyl eugenol	1398	1403	0.05
25	caryophyllene	1415	1417	0.03
26	*α*-caryophyllene	1450	1452	0.03
27	*γ*-decalactone	1462	1465	0.22
28	*α*-selinene	1491	1498	0.02
29	*α*-muurolene	1494	1500	0.01
30	*δ*-cadinene	1513	1522	0.10
31	spathulenol	1571	1577	0.18
32	caryophyllene oxide	1575	1582	0.04
33	*T*-muurolol	1639	1644	0.03
34	*α*-cadinol	1650	1652	0.14
Total				**96,29**

RI = Retention indices, ARI = Adams retention indices.

From the above results, it is coherent to think that *Ammodaucus leucotrichus* Coss. & Dur. drives the biosynthesis of p-memthanic constituents through the biosynthetic pathway geranyl pyrophosphate (GPP)-linalyl yrophopsphate (LPP)-*α*-terpinyl cation-limonene-perillaldehyde.

The results of our study were in accordance with those reported by Velasco-Negueruela et al, [18]. But the difference is in the proportions. The variations found in the chemical composition of the essential oils of the seeds can be due to certain ecological factors, the age of the plant, the period of the vegetative cycle and to the origin of the plant [19].

3.1. Antioxidant activity

3.1.1. β-carotene bleaching (BCB) method

The BCB method is usually used to evaluate the antioxidant activity of compounds in emulsions, accompanied with the coupled oxidation of β-carotene and linoleic acid [20]. In Table 2 the antioxidant potential of essential oil was compared with those of selected standard antioxidants.

Table 2: Antioxidative capacities of the essential oil of *Ammodaucus leucotrichus* Coss. & Dur. measured in β-carotene/linoleic acid assay

Extract and controls	β-Carotene / linoleic acid [a]
Essential oil	68.66±0.2
BHT	88.1±1.0
Ascorbic acid	11±143

[a] % inhibition rate of linoleic acid oxidation.

The antioxidant activity expressed as inhibition of bleaching β-carotene (I %) values decreased in the order BHT > essential oil > ascorbic acid. High rate percentage is an indication of a high antioxidant activity.

It is worth to mention that the value of the essential oil of seeds (68.66%) showed a better antioxidant activity than that shown by Ascorbic acid. Therefore, essential oil exhibited antioxidant activity, by inhibiting the β-carotene bleaching due to retarding/inhibiting of linoleic acid hyderoperoxide-derived, which attack the chromophore-β-carotene. This revealed that the hyderoperoxide produced from linoleic acid autooxidation as free radicals will be neutralized by essential oil.

In addition several studies have been conducted to clarify the possible substances involved in antioxidant properties of the essential oil. Among the identified compounds in the essential oil from *Ammodaucus leucotrichus* Coss. & Dur. monoterpene hydrocarbons and oxygenated monoterpenes may be considered the main contributors to the antioxidant activity [21-22].

3.2 Antimicrobial activity

In the current study, the antimicrobial activities was determined by disc diffusion and minimum inhibitory concentration methods.

Table 3: Antimicrobial activities of seed oil using a disc diffusion method [a].

Microorganisms	Zone of inhibition /mm	Scale of inhibition[a]
Gram Positive Bacteria *Bacillus subtilis*	 14± 0.7	 ++
Gram Negative Bacteria *Esherichia col*	 9±0.2	 +
Yeast *Candida albicans* *Saccharomyces cerevisiae*	 7±0.2 18±0.5	 + +++
Fungi *Penicillium escpansum* *Aspergillus flavus*	 8±0.3 10±0.1	 + + +

a) + mild inhibitory (5-10 mm), ++ moderate inhibitory (10-15 mm), +++ strong inhibitory (>15 mm).

Table 4: Minimum inhibitory concentration of seed oil against test microorganisms.

Microorganisms	essential oil MIC mg mL-1
Gram Positive Bacteria	
Bacillus subtilis	0.37
Gram Negative Bacteria	3.68
Esherichia col	
Yeast	
Candida albicans	0.55
Saccharomyces cerevisiae	0.37
Fungi	
Penicillium escpansum	0.55
Aspergillus flavus	0.92

The essential oil showed significant antibacterial activity against Gram negative and Gram positive bacteria at doses of 1.29 mg/disc. The antibacterial activity of essential oils also tested for yeast such as *Candida albicans* and *Saccharomyces cerevisiae* showed moderate to good activity.

The antifungal results revealed good, clear zones of growth inhibition against *Aspergillus flavus* and *Penicillium escpansum*. Furthermore, the antibacterial activity was quantitatively assessed by the determination of minimum inhibitory concentration. *Ammodaucus leucotrichus* Coss. & Dur. oil exhibited strong inhibitory action against most tested organisms with MIC values ranged from 0.37 to 0.92 mg/ml.

The results of MIC indicated that the strongest inhibitory activity of oil was against *B. subtilis* and no effect activity was observed against *E. coli*. The activity of the essential oils would be expected to relate to the structural configuration of the constituent components and their functional groups and possible synergistic interactions between components [23]. The phenolic components of essential oils (monoterpene hydrocarbons and oxygenated components) showed the strongest antimicrobial activity, followed by aldehydes, ketones and alcohols [24-25]. Rehman and Pernice et al indicated that terpenoid phenolic and non-phenolic alcohols are the most bioactive compounds against fungi [26-27]. Furthermore Cowan showed that pinene-type monoterpenes could be responsible for the total activity spectrum [28]. At present, the mechanism of terpenes action is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds [29]. However, the lower efficacy of the present extract against some microorganisms in this study might have been due to the low activity of their main constituents against particular fungi or bacteria [30]. Farag and Daw suggested that this chemical structure of essential oils could play an important role for the antimicrobial activity which enable their partition between lipids of the bacterial or fungal cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable, which will lead to cell death [31-32]. Some reports showed that the Gram-positive bacteria are more sensitive to essential oil or extract than Gram-negative bacteria due to their outer membrane barriers [33]. Gram-positive bacteria have only an outer peptidoglycan layer which is not an effective permeability barrier while Gram-negative bacteria have outer phospholipids membranes [34].

Some of the major components present in essential oils can penetrate the membrane of the microorganisms and react with the membrane enzymes and proteins as well as phospholipid bilayer, which cause an impairment of microbial enzyme system and/or disturb genetic material functionality [35-36].

Conclusion

The results of this work are the first report giving the composition, antimicrobial and antioxidant properties of the seed oil obtained from *Ammodaucus leucotrichus* Coss. & Dur.

The essential oil seemed to have effective antioxidant properties and could be a better natural antioxidant. Furthermore the essential oil possessed effective antimicrobial activity against most tested microorganisms.

On the basis of the results of this work, *Ammodaucus leucotrichus* Coss. & Dur. seeds can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical applications.

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