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Essential Oil from the Leaves of *Ammodaucus leucotrichus L* : Chemical Composition and Antimicrobial Activity

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ABSTRACT

Essential oils (EOs) are volatile, natural, fragrant liquids that can be extracted from different parts of the plants (especially leaves and flowers) presenting anti-inflammatory, antiviral, and antibacterial properties. The main aim of the study was to investigate the chemical composition, and antimicrobial activity of *Ammodaucus leucotrichus* L leaves of Bechar region in the South of Algeria. . The essential oil as analyzed by GC/GC- MS and resulted in the identification of Twenty compounds representing 88.02 % of the oil were identified. The main compounds identified were: Cineole (18.56), Linalool (10.80%) Decanal (9.50%) p-Vinylguaiacol (8.50%), Geraniol (5.04%) ,β-pinene (04.4%).

Gram positive bacteria were the most susceptible to the effects of the essential of *Ammodaucus leucotrichus* leaves.

Keywords: *Ammodaucus leucotrichus* , leaves, Essential oil, GC/SM, antimicrobial activity

I. INTRODUCTION

Bioactive compounds are an area of dieticians and food technologists' interest. They are used

For the production of enriched food with enhanced health-promoting properties, which is an important element of the human diet. The plant extracts that are the source of active compounds are attributed to antimicrobial activity and, therefore, may be potential preservatives [1].

Aromatherapy is the use of essential oils to promote the treatment of diseases and injuries. Linalool is frequently used in aromatherapy [2].Essential oils (EOs) have gained increasing attention due to their pharmacological effactiveness', and they also constitute some of the most popular natural products, These molecules are applied due to their several functional properties, including anti-proliferative, anti-inflammatory [3], as also antimicrobial [4].

II. METHODS AND MATERIAL

Plant material collection

Plant Material was collected in August 2018 from the locality of Bechar in Sahara of Algeria. The plant

material was dried at room temperature in a thin layer on filter paper for about 14 days. The voucher specimen was deposed at the Department of biology, University of Mascara Algeria.

Essential oil distillation

The leaves of *Ammodaucus leucotrichus* was hydro-distilled (with 1.2 L of water) in a Clevenger-type apparatus by recirculating the condensed water. The distillation was terminated after 250 min. The resulting EOs were, collected, and treated with anhydrous sodium sulfate to remove excess water. The EOs were stored in sealed vials at 4 ◦C until analysis. [5]

3.4.1. Analysis of the essential oils

Chemical Analysis

Chemical Analysis of the Essential Oil The chemical compositions of the essential oil of *Ammodaucus leucotrichus* leaves were analyzed by GC-MS techniques. A 6890N Network GC system (Agilent, CA, USA) equipped with a HP-5 MS capillary column (30m × 0.25mm, 0.25µm film thickness) was used. Carrier gas helium was run at a flow rate of 1.0 mL/min, and the temperature program run from 50 °C (2 min), increased 10 °C/min up to 100 °C, and 2 min at 180 °C, increased 20 °C/min up to 250 °C. Moreover The GC-FID analysis of the EOs was conducted with a 7890A gas chromatograph (Agilent Technologies Inc., Santa Clara, CA, USA) coupled to a FID and HP-5 silica fused capillary column (30 m length × 0.32 mm i.d. 0.25 µm film thickness). The oven temperature was programmed as mentioned above, whereas the detector and injector temperatures were 280 °C and 220 °C, respectively[6]. The carrier gas was helium at a flow rate of 1.0 mL/min. EOs (1.0 µL) were injected using the split mode. The percentage composition of EO samples was calculated using the peak normalization method. The compounds were identified by comparison of their retention indexes (RI) [7[retention times (RT) and mass spectra with those of authentic samples and/or the NIST/NBS, NIST02.

Microbial strains

Microbial Strains and Culture Media All microbial strains were obtained from the Microbiology Laboratory, University of mascara ALGERIA. Stock cultures of gram-positive and gram-negative *Bacillus cereus ATCC- (*49444)S. aureus (ATCC 29213), gram-negative E. coli (ATCC 25922), and were sub-cultured and maintained on nutrient agar at 37 ◦C for 24 h; subsequently, they were diluted in sterile saline solution (0.85% w/v) to reach a final concentration of 0.5 McFarland (1.5×10^7 colony-forming units [CFUs]/mL).

Determination of the Minimum Inhibitory Concentration

The disc diffusion assay IN Mueller-Hinton broth .These dilutions were added to 96-well microplates, and 20 µL of the microbial cultures were added at a concentration of 1.5×10^8 CFU/mL to reach a final volume of 200 µL/well. Gentamicin was used as the positive control as it is a broad-spectrum antibiotic, and normal saline/dimethyl sulfoxide (DMSO) was used as the negative controlFor the agar disc diffusion method, a 100 µL of 107 CFU/mL bacterial suspensions after incubation was spread on the Mueller Hinton Agar.[8] Filter paper discs (6 mm in diameter) were infused with 15 µl of the EO tested and placed on the inoculated MHA. MHA was kept at 4 °C for 2 h and then at 37 °C for 24 h. After the incubation period, the diameter of inhibition zones was measured (mm). Growth inhibition was compared with the standard drugs. Tests were performed in three separate experiments, and the means were calculated.

III. RESULTS AND DISCUSSION

Natural plant materials have been used as food preservatives against both bacteria to control organoleptic changes, off-flavors. The chemical composition of essential oil was determined by GC-MS analysis. The results from this study shown that essential oil obtained in the hydro distillation was 0.56 mL, with a yield of 1.42% (db). Regarding the chemical profile of the essential oil of density was done by double weighing d = 0.750, the Specific rotation = +0.25 by polarimetry and the refractive index n = 1.4560 by an interferometric method.

Table 1. Physicochemical composition of *Ammodaucus leucotrichus*

A. *Specification*	*Ammodaucus leucotrichus*
Density D20	0.750
Refractive index	1,456
Optical activity N20	+0.25
Solubility in ethanol 90(%)	1:2
Freezing Point (°C)	-18

The GC-MS analysis of *Ammodaucus leucotrichus* essential oil showed the presence of main peaks identified by the libraries. They revealed 28 volatile compounds in each type of EOs (Table 1) this compounds are Cineole (18.56), Linalool (10.80%) Decanal (9.50%) p-Vinylguaiacol (8.50%), Geraniol (5.04%) ,β-pinene (04.4%). A comparison with the linear retention indices (LRI) reported in literature allowed us to identify the major of these peaks. By other finding the essential oil was extracted with a yield of 2.58 ± 0.17%, being perilla aldehyde identified as the main component, accounting for 85.6% of the total composition. Concerning the compounds érillaldéhyde (17,5%) et le limonène (12,5%) thymol (19,6%), le carvacrol (17,1%) et l'e-anéthol (15,3%). [9]

Table 2. The major identified components in essential oil from *Ammodaucus leucotrichus* analyzed by GC-MS technique with retention indices on HP-5MS capillary Column.

Volatile compounds	Ri[a]	RI[b]	AREA %
. β-pinene	974	978	04.40
myrcene	988	989	02.5
sylvestrene	1050	1053	1.3
Carene	1060	1063	0.02
α-Terpinene	1015	1016	1.05
Limonene	1025	1030	12.80
Cineole	1037	1039	18.56
endo-Borneol	1184	1185	0.63
Nonadecane	1900	1900	0.56
Terpinen-4-ol	1193	1195	0.89
Linalool	1105	1185	10.80
Dodecane	1200	1203	0.77
Decanal	1206	1209	9.50
α-cubebene	1335	1336	0.63
trans-2-Decenal	1260	1262	0.58
Cyclocitral	1217	1222	1.23
Geraniol	1249	1251	05.76
p-Vinylguaiacol	1308	1309	08.6
Eugenol	1356	1351	11.56
γ-cadinene	1513	8.19	0.92
Bourbonene	1525	1526	00.5
trans-cadina-1,4-diene	1532	1533	3.23
Drimenol	1596	1597	0.69
Linolenic acid	2137	2138	1.26
Linalyl	2157	2159	3.65

anthranilate			
Tricosane	2300	2331	2.30
Manoyl oxide	2370	2378	1.2
Tetracosane	2400	2401	0.56
Total			**97.20**

T*h*e essential oil was effective against the tested microbial strains (*Bacillus cereus ATCC- (*49444)*S. aureus* (ATCC 29213), gram-negative E. coli (ATCC 25922), the most sensitive bacteria was S. aureus (ATCC 29213). In general, Gram-positive bacteria were the most sensit.ive to the effects of essential oil (EO), and this may be related to the fact that Gram-positive bacteria are more susceptible to the effects of volatile components compared to the Gram-negative ones.[10]

The global concerns on antimicrobial resistance and health-related consequences facilitate a necessity to look for natural alternatives with antimicrobial properties. Our study has demonstrated the antimicrobial effect of essential oil of *Ammodaucus leucotrichus* that could be applicable for the food industry and health care needs.

Table 3. Inhibition zone (mm) using direct contact technique in agar medium and MIC (mg/mL) for the essential oil using microdilution method in 96 multiwall microliter plates.

Microorganism	Diameter of inhibition zones (mm)	MIC (mg/mL)
Escherichia coli ATCC 25922	13.5±10.20	1.58
Staphylococcus aureus (G+) ATCC 25923	18 ±0.01	12.50
Bacillus subtilis ATCC-6633	10.5±0.8	11.00

IV. CONCLUSION

The main aim of the study was to investigate the chemical composition, and antimicrobial, activity of *Ammodaucus leucotrichus* .

Our study has demonstrated the antimicrobial effect of essential oil that could be applicable for the food industry and health care needs.

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