

CHEMICAL COMPOSITION, ANTIMICROBIAL, ANTIOXIDANT AND ANTICANCER ACTIVITIES OF ESSENTIAL OIL FROM *AMMODAUCUS LEUCOTRICHUS* COSSON & DURIEU (APIACEAE) GROWING IN SOUTH ALGERIA

Benchikha Naima^{1*}, Rebiai Abdelkrim¹, Brahmia Ouarda², Neghmouche Nacer Salah¹ and Ben Amor Mohammed Larbi¹

¹University of El-Oued, Department of chemistry, P.O. Box 789, 39000, El-Oued, Algeria

²University of Constantine 1, Laboratory of Innovative Techniques for Environment Preservation, Algeria

(Received April 22, 2019; Revised May 24, 2019; Accepted May 25, 2019)

ABSTRACT. The chemical composition of the essential oil obtained by hydrodistillation from aerial parts of *A. leucotrichus* Cosson & Durieu (Apiaceae) grown in the south of Algeria (El-Oued) was determined by GC-MS analysis. The oil was found to be rich in perillaldehyde 64.66% and D-Limonene 26.99%. The biological activity of *A. leucotrichus* Cosson & Durieu essential oil has been investigated. The in vitro antimicrobial activity of the essential oil sample was tested on eight strains, one yeast and one fungi. The test showed interesting antimicrobial properties, especially on *Salmonella enterica* and *E. coli*, the antioxidant capacity of the oil was measured using the cyclic voltammetry, and the AAT value of *A. leucotrichus* essential oil was evaluated 47.84 mg α -TE/L. In addition, the antitumor activity showed that the oil of *A. leucotrichus* was very significant against the HCT116 colon cancer cell line.

KEY WORDS: *Ammodaucus leucotrichus*, Antioxidant activity, Anticancer activity, Cyclic voltammetry

INTRODUCTION

Ammodaucus leucotrichus Cosson & Durieu belonging to Apiaceae family, is one of the huge plant's diversity of the Sahara desert [1]. It is a small annual plant, 10-12 cm in height, glabrous with erect, finely striated stems [2]. The leaves are finely dissected and slightly fleshy. The flowers are grouped in umbels of 2 to 4 bran. The flowers are small, with five free petals. The fruit is a diachene, 6-10 mm, long and is covered with dense silky white hairs. It usually flowers in early spring (February to April). The plant is common in the Algerian Sahara (El-Oued) [3]. In the Algerian traditional medicine, *A. leucotrichus* is used for the treatment of common cold and fever [4], stomach diseases, vomiting pains and allergies [5]. Moreover, emmenagogue, abortive and aphrodisiac properties are attributed to this species. The flowers are used for the treatment of cardiac diseases [6]. *A. leucotrichus* seeds decoction plays an important role in traditional medicine in North African countries (Algeria, Morocco, Tunisia, Libya, Egypt, Mauritania and upper Niger valley). Its best implantation is in desert regions, often down a hill or a dune [7]. The leaves are used to aromatize tea. Powdered, it is much-appreciated as a spice food in the El-Oued area [8]. Even though, this plant is known to grow wild in Algeria and in spite of its wide uses in traditional medicine, a full investigation of the chemical composition of the essential oil extracted from the aerial parts, has never been mentioned before. This study is considered as the first original report giving full details of the composition as well as the antimicrobial, anticancer and antioxidant properties using spectrophotometrical (DPPH) and electrochemical methods (cyclic voltammetry) of the aerial parts oil obtained from *Ammodaucus leucotrichus* Coss & Dur, to effectively exploit of the usage of this plant. The essential oil was also evaluated for its anticancer activity against HePG2 (Hepatic) and HCT116 (Colon) human cancerous cell lines.

*Corresponding author. naima_chem@yahoo.fr

This work is licensed under the Creative Commons Attribution 4.0 International License

EXPERIMENTAL

Plant material

The plant material of *Ammodaucus leucotrichus* was obtained from the valley state of southeast Algeria, in February 2018. Then, we washed it thoroughly with water, and it was left to dry in the shade for 15 days. Voucher specimens (NOR-018) were deposited to the Herbarium of the chemistry laboratory, University of El-Oued.

Essential oil extraction

The aerial parts of the plant were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus. The oil obtained was collected and dried over anhydrous sodium sulfate Na₂SO₄ to remove moisture. Then, it was stored at 4 °C in sealed brown vials until analysis. The oil analysis was carried out using GC-MS.

Gas chromatography-mass spectrometry (GC-MS) analysis

Mass spectra were recorded using Shimadzu GCMS-QP2010 (Tokyo, Japan) equipped with Rtx-5MS fused bonded column (30 m x 0.25 mm i.d. x 0.25 µm film thickness) (Restek, USA) equipped with a split-splitless injector. The capillary column was directly coupled to a quadrupole mass spectrometer (SSQ 7000; Thermo-Finnigan, Bremen, Germany). The initial column temperature was kept at 45 °C for 2 min (isothermal) and programmed to 300 °C at a rate of 5 °C/min, and kept constant at 300 °C for 5 min (isothermal). The injector temperature was 250 °C. Helium carrier gas flow rate was 1.41 mL/min. All mass spectra were recorded applying the following conditions: (equipment current) filament emission current, 60 mA; ionization voltage, 70 eV; ion source, 200 °C. Diluted samples (1% v/v) were injected with split mode (split ratio 1: 15).

Compounds identification

The determination of oil components was based on comparing their spectra with those of references in the MS Library (NIST and Wiley). To confirm the identification, the corresponding retention indexes (RI) were calculated using the n-alkanes series, C₈-C₂₈, and then compared with those reported in the literature [9]. The relative amounts of individual components were calculated based on GC-FID peak area.

Cytotoxic effect on cancerous cell lines

The cytotoxic activity test (in vitro bioassay on human tumor cell lines) was conducted and determined by the Bioassay-Cell Culture Laboratory, National Research Centre, El-Tahrir St., Dokki, Cairo 12622, Egypt. In this study, we evaluated the cell viability by the mitochondrial dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to purple formazan [10]. It is to note that the following procedures were conducted in an aseptic zone using a laminar flow room biosafety class II level (Baker, SG403INT, Sanford, ME, USA). The cells were suspended in RPMI 1640 medium (for HePG2 and HCT116), 1% antibiotic-antimycotic mixture (10,000U/mL potassium penicillin, 10,000 µg/mL streptomycin sulfate and 25 µg/mL Amphotericin B) and 1% L-glutamine at 37 °C under 5% CO₂. Concentration of 10 x 10³ cells/well in complete and fresh culture medium in 96-well plastic microtiter plates at 37 °C for 24 hours under 5% CO₂, using a carbon dioxide incubator (Sheldon, TC2323), Cornelius, OR, USA. The medium was aspirated, the fresh one (no serum) was added and the cells were incubated either alone (negative control) or with different sample concentrations to give a final concentration of (-0.78-1.56-3.125-6.25-12.5-25-50-100) µg/mL. After 48 h of incubation, the medium was aspirated, 40 µL of MTT salt (2.5 µg/mL) was added

to each well and incubated for a further four hours at 37 °C under 5% CO₂. To stop the reaction and dissolve the formed crystals, 200 µL of 10% sodium dodecyl sulfate (SDS) in deionized water were added to each well and incubated overnight at 37 °C. A positive control consisting of 100 µg/mL was used as a known natural cytotoxic agent, giving 100% lethality under the same conditions [11, 12]. The absorbance was then measured using a multi-well microplate reader (Bio-Rad Laboratories Inc., Model 3350, and Hercules, California, USA) at 595 nm and a reference wavelength of 620 nm. Statistical significance was tested between the samples and the negative control (cells with vehicle) using an independent t-test by the SPSS 11 program. DMSO is the vehicle used for the dissolution of the plant extracts, and its final concentration in the cells was less than 0.2%. The percentage change in viability was calculated using the following formula (1):

$$\text{Cell viability (\%)} = (\text{Extract reading/Negative control reading}) \times 100. \quad (1)$$

A probit analysis was carried out to determine IC₅₀ and IC₉₀ using the SPSS 11 program.

Antioxidant capacity

Cyclic voltammetry method

The determination of antioxidant capacity was based on cyclic voltammetry (CV) experiments were performed using PGZ402 potentiostat/galvanostat (radiometer analytical SAS) controlled with the VoltaMaster4 software version 7.08 (radiometer analytical SAS). Experiments were realized in a double walled electrochemical cell of 25 mL, and conventional three electrodes system was employed. We used a glassy carbon (GC) working electrode (radiometer analytical SAS), with an area of 0.013 cm², a platinum wire counter electrode, and an Hg/Hg₂Cl₂ reference electrode (3.0 M KCl). Prior to the voltammetric experiments, the glassy carbon electrode was cleaned with cotton soaked in acetone for 2 min, and then with cotton soaked in water for several seconds. A toluene-ethanolic (50% ethanol, v/v) was used as solvent and 10 µL sulfuric acid as supporting electrolyte. All solutions were prepared using absolute ethanol. Once we cleaned the GC electrode, the required sample volume (of α-tocopherol solution or *Ammodaucus leucotrichus* oil) was added by micro-pipette into the supporting electrolyte in the electrochemical cell. The cyclic voltammograms were immediately recorded to minimize adsorption (or avoid any other electrochemically active species) onto the GC electrode surface. The cyclic voltammetric conditions used were: scans ranged from 0 to +1 V; scan rate of 100 mV.s⁻¹. All measurements were done in triplicate.

DPPH radical scavenging activity

The estimation of the remaining DPPH radical concentration in DPPH solution after reaction with essential oils was performed using slightly modified procedure [13, 14]. Pure methanol was used to adjust the zero of the spectrophotometer. Methanolic solution of the examined essential oil (1 mL) was mixed with DPPH solution (1 mL) in a 5 mL test tube. The mixture was stirred vigorously for 30 s, poured into the optical cuvettes (1 cm × 1 cm × 3.5 cm) and immediately placed in a spectrophotometer. The changes of absorbance at 515 nm were monitored in a continuous manner during 30 min. The percent of inhibition I (%) was calculated from the formula (2):

$$I(\%) = \left(\frac{A_0 - A_s}{A_0} \right) \times 100 \quad (2)$$

where A₀ is the absorbance of the control (containing all reagents except the test compound) and A_s is the absorbance of tested sample. The oil concentration providing 50% inhibition (IC₅₀) was calculated from the graph by plotting inhibition % against oil concentration. α-Tocopherol was used as reference.

Antimicrobial activity

The minimal inhibitory concentration (MIC) of essential oil values were evaluated in the Mueller Hinton Broth (MHB) by dilution method against *Bacillus subtilis* (*B.s*) (ATCC6663), *Micrococcus luteus* (*M.l*) (ATTC9314), *Listeria monocytogene* (*L.m*) (CIP82110), *Escherichia coli* (*E.c*) (CIP54.8), *Klebsiella pneumonia* (*K.p*) (CIP82.91), *Pseudomonas aeruginosa* (*P.a*) (CIPA22), *Agrobacterium tumefaciens* (*A.t*) (N°2410), *Salmonella enterica* (*S.e*) (CIP 81.3), *Mucor ramannianus* (*M.r*) (NRRL6606) and *Candida albicans* (*C.a*) (*CLM*). An aliquot (1 mL) of this suspension was transferred to a sterile tubes of MHB containing various concentrations of oils (0.1-15 μ L) [15] and the volume was adjusted to 10 mL with ethanol (5%, w/v) to obtain bacterial inoculums adjusted to a concentration of 10^6 CFU/mL, which were incubated under shaking conditions (100–120 rpm) for 24 h at 37 °C. Each test was performed in triplicate; on three separate experiments.

RESULTS AND DISCUSSION

Chemical composition

The hydrodistillation of *A. leucotrichus* areal parts gave blue oil with yield of 1.8%. A total of 24 components were identified and presented about 99.91% of the total detected constituents. The essential oil contained (Table 1) 64.8% of oxygenated monoterpenes and 34.86% of monoterpenes hydrocarbons. It could be noticed that the principal constituent was perillaldehyde (64.66%), followed by D- limonene (26.99%) and the less percentage was attributed to α -pinene (5.8%). At very low concentrations (less than 1%) of other compounds were identified such as; β -pinene 0.66%, β - ocimene 0.65% and camphene 0.4%. Methyl perillate and bornyl acetate were detected in a limited amount of 0.08%.

Our results are in agreement with those reported in the literature [14, 17], except some differences related to the quantification of the essential oil major components as well as the nature of some identified constituents. For example, according to the studies of [3, 17], they both indicated the presence of only a small amount of α -pinene, which disagree with our results [3]. In addition, indicated that Moroccan *A. leucotrichus* essential oil is constituted mainly of perillaldehyde 88.7% and limonene 8.26%, compared with our results this species contained more perillaldehyde and fewer limonene. In addition further studies reported that *A. leucotrichus* essential oil extracted from seeds collected in Algeria, contained perillaldehyde 59.12% and limonene 23.89%. Hence the oil originator from Algeria (El-Oued region) is the source of active component (perillaldehyde) used in generally in perfume, cosmetics and for its aroma [18].

Antimicrobial activity

In vitro investigation of *A. leucotrichus* essential oil was carried out to evaluate its biological efficiency against certain micro-organisms; *Bacillus subtilis*, *Micrococcus luteus* and *Lesteria monocytogene* (Gram positive bacteria), *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Agrobacterium tumefaciens* and *Salmonella enterica* (Gram negative bacteria), *Mucor ramannianus* (fungus) and *Candida albicans* (yeast). The corresponding minimum inhibitory concentrations (MICs (μ L/mL)) of various micro-organisms are shown in (Table 2). The results demonstrated that essential oil under investigation possessed antimicrobial activities against the used micro-organisms.

The different results of the antimicrobial activity of this essential oil by the MIC method are shown in (Table 2). The latter method gives more reliable results compared to the disk method but requires, however, a large oil to test [19, 20] . We can globally note the presence of an antimicrobial activity vis-à-vis the essential oil of *A. leucotrichus*.

With regard to Gram-negative bacteria; *Salmonella enterica* and *E. coli* are the most sensitive with a lowest MIC of 5 $\mu\text{L/mL}$. Of all Gram-negative bacteria, only *Pseudomonas aeruginosa* gives the highest MIC value while *Micrococcus luteus* is the most resistant gram-positive bacteria with a MIC of 15 $\mu\text{L/mL}$. The same table also shows that the yeast and the fungi manifest in the same manner, giving a MIC of 10 $\mu\text{L/mL}$. These results are in agreement with those given by [21,8] Finally, the botanical origin of the species seems to play a key role in its antimicrobial efficacy, which confirms the close relationship that exists between the chemical structures of the essential oil constituents.

Table1. Chemical composition of *Ammodaucus leucotrichus* essential oil.

Compound	ARI ^a	RI ^b	Area%
α -pinene	932	925	5.8
α -Fenchene	945	934	0.02
Camphene	946	941	0.4
Thuja-2,4(10)-dien	953	948	0.04
Sabinene	969	969	0.03
β -Pinene	974	972	0.66
Myrcene	988	989	0.15
α -Phellandrene	1002	1003	0.04
β -Ocimene	1009	1022	0.65
<i>p</i> -Cymene	1020	1024	0.06
D-Limonene	1024	1029	26.99
1,3,8- <i>p</i> -Menthatriene	1108	1122	0.02
α -Campholenal	1122	1127	0.02
Trans-Pinocarveolc	1135	1141	0.05
Pentyl Benzene	1152	1147	0.03
Myrtenal	1195	1198	0.01
Verbenone	1204	1212	0.02
Cumin aldehyde	1238	1244	0.02
Carvone	1239	1249	0.02
Linalool acetate	1257	1257	0.03
Perillaldehyde	1269	1285	64.66
Bornyl acetate	1287	1291	0.08
Benzyl isobutanoate	1297	1305	0.03
Methyl perillate	1392	1399	0.08
Total identified			99.91
Yield of essential oil	1.8		
Monoterpene hydrocarbons			34.86
Oxygenated monoterpenes			64.8
Other Compounds			0.25

RI^b: Retention indices, ARI^a: Adams retention indices.

Cytotoxic activity test

The cytotoxic activity of *A. leucotrichus* essential oil obtained by hydro-distillation was tested against the two-cell line as it happens HCT116 (colon cell line) and HePG2 (Human hepatocellular carcinoma cell line) [21]. The evaluation of cytotoxicity was performed for essential oil samples concentration that ranged between 0.78 to 100 $\mu\text{g/mL}$ using MTT assays. The results are shown in (table 3). The essential oil possesses cytotoxic activity on HCT116 [21]. Hence the LC₅₀ (lethal concentration of essential oil which causes the death of 50% of cells in 48 h) and LC₉₀ (lethal concentration of essential oil which causes the death of 90% of cells in

48 h) was found to be 72.6 $\mu\text{g/mL}$ and 110.6 $\mu\text{g/mL}$, respectively. The inhibition percentage of this essential oil against HCT116 colon cancer cell line is 78.2% for 100 $\mu\text{g/mL}$. In fact, the results are very promising compared with positive control Adrinamycin (Doxorubicin) 37.6 $\mu\text{g/mL}$, while the tested oil is almost ineffective against the HePG2 cell line, for 100 $\mu\text{g/mL}$ the inhibition percentage is 11.5%. Finally, the active compound in oil of *A. leucotrichus* for its significant cytotoxic activity especially for colon cancer is most likely the perillaldehyde and limonene, acting alone or synergetically with one or more components.

Table 2. MICs of essential oil from *Ammodaucus leucotrichus*.

Concentration ($\mu\text{L/mL}$)	0.1	0.2	0.3	0.4	0.5	1	2	3	4	5	10	15
Gram + Bacteria												
<i>Bacillus subtilis</i> (ATCC6663)	+	+	+	+	+	+	+	+	+	+	-	-
<i>Micrococcus luteus</i> (ATTC9314)	+	+	+	+	+	+	+	+	+	+	+	-
<i>Listeria monocytogene</i> (CIP82110)	+	+	+	+	+	+	+	+	+	+	-	-
Gram - Bacteria												
<i>Escherichia Coli</i> (CIP54.8)	+	+	+	+	+	+	+	+	+	-	-	-
<i>Klebsiella pneumoniae</i> (CIP82.91)	+	+	+	+	+	+	+	+	+	+	-	-
<i>Pseudomonas aeruginosa</i> (CIPA22)	+	+	+	+	+	+	+	+	+	+	+	-
<i>Agrobacterium tumefaciens</i> (N°2410)	+	+	+	+	+	+	+	+	+	+	-	-
<i>Salmonella enterica</i> (CIP 81.3)	+	+	+	+	+	+	+	+	+	-	-	-
Fungi												
<i>Mucor ramannianus</i> NRRL 6606	+	+	+	+	+	+	+	+	+	+	-	-
Yeast												
<i>Candida albicans</i> (CLM)	+	+	+	+	+	+	+	+	+	+	-	-

*Note: (-), total inhibition; (+), growing.

Antioxidant capacity

Cyclic voltammetry method

The CV method is actually based on the correlation between the area under the anodic curve of the voltammogram and the electroactivity of the compound under investigation [22]. To calculate the antioxidant capacity, we plotted the calibration curve (Figure 1) using the voltammogram area versus the concentrations of the α -tocopherol (ranged between 0-450 mg/L) used as standard. From the (Figure 1), we deduced the antioxidant capacity of *Ammodaucus leucotrichus* oil.

They were next applied to express the total antioxidant capacity of *Ammodaucus leucotrichus* oil sample as α -tocopherol equivalent. The area under peak current of the background signal (solvent and supporting electrolyte) was subtracted from the area under peak current obtained for sample and α -tocopherol which were recorded within the range of 0 to +1 V. The cyclic voltammogram of *Ammodaucus leucotrichus* oil sample clearly shows the antioxidant capacity estimated at a value of 47.84 mg α -TE/L. The obtained results are significant, but less important than the antioxidant capacity showed by *Origanum majorana* and *O. vulgare* plant oils in previous studies [13].

Table 3. Cytotoxic activity of *Ammodaucus leucotrichus* essential oil against human cell lines.

Sample code	100 $\mu\text{g/mL}$	50 $\mu\text{g/mL}$	25 $\mu\text{g/mL}$	12.5 $\mu\text{g/mL}$	IC ₅₀ ($\mu\text{g/mL}$)	IC ₉₀ ($\mu\text{g/mL}$)	Doxorubicin IC ₅₀ ($\mu\text{g/mL}$)	DMSO at 100 ppm
HCT116	78.2 \pm 2.65	29.17 \pm 1.41	9.03 \pm 1.62	0	72.6	110.4	37.6	1%
HePG2	11,5	-----	-----	-----	-----	-----	21.6	1%

*Each value represents the mean percentage of inhibition cells of three replicates \pm SEM (standard error mean).

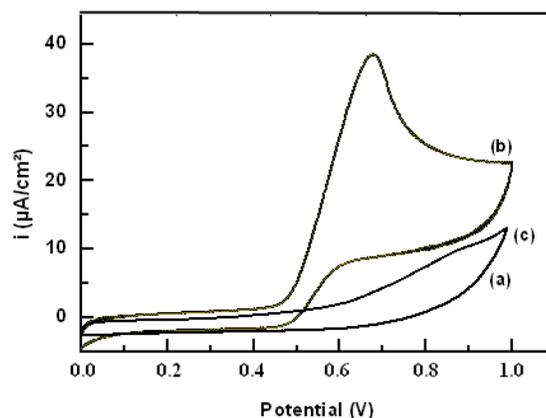


Figure 1. Cyclic voltammograms of (a) supporting electrolyte, (b) α -tocopherol, (c) *Ammodaucus leucotrichus* oil.

DPPH radical scavenging activity

The essential oil of *A. leucotrichus* was explored for antioxidant activity using DPPH free radical scavenging power characterized by IC₅₀ value. Table 4 show the percentage of DPPH inhibition of studied essential oil and α -tocopherol as positive control measured in DPPH assay at different concentrations respectively. The essential oil exhibited a less significant scavenging effect IC₅₀ = 74.53 mg/mL compared to α -tocopherol (26.426 $\mu\text{g/mL}$) used as positive control. The tested essential oil (Table 1) is markedly rich in oxygenated monoterpenes (64.8%) which may act as radical scavenging agents [23].

Table 4. DPPH radical scavenging Activity of standard solution (vitamin E).

Concentration ($\mu\text{g/mL}$)	5	10	25	50	100	200	IC ₅₀ ($\mu\text{g/mL}$)
I (%)	33.03 \pm 0.016	36.62 \pm 0.002	47.18 \pm 0.006	92.62 \pm 0.0014	100	100	26.43

CONCLUSION

The present work pointed out the investigation of the chemical composition of the essential oil of *A. leucotrichus* growing in South Algeria on the basis of GC-MS analyzes. The results showed particularly that this oil is rich in perillaldehyde and D-limonene, added to this; many other compounds were identified and characterized. The in vitro antimicrobial activity of the essential oil was tested on eight strains, one yeast and one fungus. The test showed interesting antimicrobial properties, especially on

Salmonella enterica and *E. coli*. Moreover, *A. leucotrichus* essential oil exhibited significant antioxidant effects confirmed by two different methods as well as a notable anti-cancer activity with respect to the HCT116 colon cancer cell line.

ACKNOWLEDGEMENT

This work was supported by the ministry of higher education and scientific research of Algeria (project number B00L01UN390120180001).

REFERENCES

1. Znini, M.; Cristofari, G.; Majidi, L.; Paolini, J.; Desjobert, J, M; Costa, J. Essential oil composition and antifungal activity of *Pulicaria Mauritanica* Coss., against postharvest phytopathogenic fungi in apples. *LWT - Food Science and Technology* **2013**, 54, 564-569.
2. Hammiche, V.; Maiza, K. Traditional medicine in Central Sahara: Pharmacopoeia of Tassili N'ajjer. *J. Ethnopharmacol.* **2006**, 105, 358-367.
3. Alaoui, M.S.; Satrani, B.; Ghanmi, M.; Aafi, A.; Amusant, N.; El Antry, S.; Chaouch, A. Bioactivity and chemical quality of *Ammodaucus leucotrichus* ssp. *Leucotrichus* Coss. & Durieu essential oils from Morocco. *Nat. Prod. Indian J.* **2014**, 10, 208-214.
4. Velasco-Negueruela, A.; Perez-Alonso, M, J.; Perez de Paz, P, L.; Pala-Paula, J.; Sanz, J. Analysis by gas chromatography-mass spectrometry of the volatiles from the fruits of *Ammodaucus leucotrichus* subsp. *leucotrichus* and subsp. *nanocarpus* grown in North Africa and The Canary Islands, respectively. *J. Chromatogr. A* **2006**, 1108, 273-275.
5. Sadaoui, N.; Bec, N.; Barragan-Montero, V.; Kadri, N.; Cuisinier, F.; Larroque, C.; Arab, K.; Khettal, B. The essential oil of Algerian *Ammodaucus leucotrichus* Coss. & Dur. and its effect on the cholinesterase and monoamine oxidase activities. *Fitoterapia* **2018**, 130, 1-5.
6. Noureddine, H.; Heleno, S.A.; Costa, P.; Fernandes, I.P.; Calhelha, R.C.; Boucherit, K.; Rodrigues, A.E.; Ferreira, I.; Barreiro, M.F. Chemical profile and bioactive properties of the essential oil isolated from *Ammodaucus leucotrichus* fruits growing in Sahara and its evaluation as a cosmeceutical ingredient. *Ind. Crops Prod.* **2018**, 119, 249-254.
7. Manssouri, M.; Znini, M.; El Harrak, A.; Majidi, L. Antifungal activity of essential oil from the fruits of *Ammodaucus leucotrichus* Coss. & Dur., in liquid and vapour phase against postharvest phytopathogenic fungi in apples. *J. App. Pharm. Sci.* **2016**, 6, 131-136.
8. El-Haci, A.; Bekhechi, C.; Atik-Bekkara, F.; Mazari, W.; Gherib, M.; Bighelli, A.; Casanova, J.; Tomi, F. Antimicrobial activity of *Ammodaucus leucotrichus* fruit oil from Algerian Sahara. *Nat. Prod. Commun.* **2014**, 9, 711-712.
9. Adams, R.P. *Identification of Essential Components by Gas Chromatography/Mass Spectroscopy*, Allured Corp.: Carol Stream, IL, USA.; **2007**.
10. Abu Zarga, M.; Ihlal Al-Jaber, H.; Baba Amer, Z.; Sakhrif, L.; Al-Qudah, M.; Al-humaidi, J.; Abaza, I.; Afifi, F. Chemical composition, antimicrobial and antitumor activities of essential oil of *Ammodaucus leucotrichus* growing in Algeria. *J. Biol. A Prod. Nature* **2013**, 3, 224-231.
11. Thabrew, M.I.; Robin, D.; Mcfarlane, I.G. Screening of hepatoprotective plant components using a HepG2 cell cytotoxicity assay. *J. Pharm. Pharmacol.* **1997**, 49, 1132-1135.
12. Moustafa, S.M.; Menshawi, B.M.; Wassel, G.M.; Mahmoud, K.; Mounier, M.M. Screening of some plants in Egypt for their cytotoxicity against four human cancer cell lines. *Int. J. Pharm. Tech. Res.* **2014**, 6, 1074-1084.
13. Benchikha, N.; Menaceur, M.; Barhi, Z. In vitro evaluation of antioxidant capacity of *Algerian origanum* plant by spectrophotometrical and electrochemical assays. *Int. J. Pharm. Sci.* **2014**, 6, 577-580.

14. Thakkar, K.N.; Prasad, A.K.; Nayak, J.; Satish, V.; Iyer, S.K. In vitro cytotoxic activity of menispermaceae plants against HeLa cell line. *J. Phytopharmacol.* **2014**, *3*, 395-399.
15. Benchikha, N.; Djazi, F. L'activité biologique de six huiles essentielles obtenues par hydrodistillation et de leurs composés majoritaires. *Rivista Italiana EPPOS* **2007**, *44*, 39-45.
16. Louail, Z.; Kameli, A.; Benabdelkader, T.; Bouti, K.; Hamza, K.; Krimat, S. Antimicrobial and antioxidant activity of essential oil of *Ammodaucus*. *J. Mater. Environ. Sci.* **2016**, *7*, 2328-2334.
17. Sonboli, A.; Gholipour, A.; Yousefzadi, M. Antibacterial activity of the essential oil and main components of two dracocephalum species from Iran. *Nat. Prod. Res.* **2012**, *26*, 2121-2125.
18. Shioh, Y.W.; Chi-Tsun, C. Effect of allyl isothiocyanate on antioxidant enzyme activities, flavonoids and post-harvest fruit quality of blueberries (*Vaccinium corymbosum* L., Cv. Duke). *Food Chem.* **2010**, *122*, 1153-1158.
19. Ben Amor, M.L.; Benchikha, N.; Elsharkawy, E.R.; Neghmouche nacer, S. Phytochemical characterization, in-vitro cytotoxic and antibacterial activity of *Cotula cinerea* (Delile) Vis essential oil. *J. Nat. Remed.* **2018**, *18*, 107-112.
20. Josphat, C.M.; Joyce J.K.; Nicholas, M.K.; Tiffany, P.H. Chemical composition and antimicrobial activity of the essential oil of *Satureja biflora* (Lamiaceae). *Bull. Chem. Soc. Ethiop.* **2007**, *21*, 249-254.
21. Mosmann, T. Rapid Colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Nat. Remed.* **1983**, *65*, 55-63.
22. Rebiai, A.; Lanez, T. In vitro evaluation of antioxidant capacity of Algerian propolis by spectrophotometrical and electrochemical assays. *Int. J. Pharmacol.* **2011**, *7*, 113-118.
23. Zouari, N.; Fakhfakh, N.; Zouari, S.; Bougatef, A.; Karray, A.; Neffati, M.; Ayadi, M.A. Food and bioproducts processing chemical composition, angiotensin I-converting enzyme inhibitory, antioxidant and antimicrobial activities of essential oil of Tunisian *Thymus algeriensis* Boiss. et Reut. (Lamiaceae). *Food Bioprod. Process.* **2011**, *89*, 257-265.