BIOLOGICAL ACTIVITIES OF DIFFERENT EXTRACTS OF *Ammodaucus leucotrichus* subsp. *leucotrichus* COSSON & DURIEU FROM ALGERIAN SAHARA

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Abstract. This study describes the chemical composition, the antimicrobial and antioxidant activities of the aqueous and ethanolic extracts as well as the essential oil obtained from the fruits of the Algerian endemic subspecies *Ammodaucus leucotrichus* subsp. *leucotrichus* Cosson & Durieu. The analyzed aqueous and ethanolic extracts by HPLC technique showed the presence of ferulic acid. The most important phenolic content was observed in the ethanolic extract with 146.18±5.82 mg GAE/g DW compared to the aqueous extract with 77.51±2.21 mg GAE/g DW. The same results were obtained for flavonoids where the ethanolic extract showed a significant amount of 43.55±2.46 mg QE/g DW. The results of DPPH assay showed IC50 values of 20.64±0.52 mg/mL for the essential oil, 337.6±16.73 µg/mL for the aqueous extract and 97.89±2.91 µg/mL for the ethanolic extract. The percent inhibition bleaching of β-carotene capacity of the essential oil (43.78±1.82) was found to be higher in comparison with the two tested extracts. The obtained results of the antimicrobial activity showed remarkable inhibition zones of the tested essential oil (5 µL/disc) against many tested microorganisms. The results suggest that *A. leucotrichus* subsp. *leucotrichus* fruits constitute a novel source of natural antioxidants and antimicrobials and can continue to be used as a food additive.

Keywords: *Ammodaucus leucotrichus*; ethanolic and aqueous extracts; essential oil; antioxidant activity; antimicrobial activity; HPLC.

INTRODUCTION

Medicinal and aromatic plants have been and continue to be used in many applications because of their ability to synthesize secondary metabolites of high chemical diversity and many biological properties [19]. One of the spontaneous aromatic and medicinal plants that is commonly used by the local population of Algeria is *Ammodaucus leucotrichus* subsp. *leucotrichus* Cosson & Durieu [57]. This plant is locally known as kammûnes-sofi, el massoufa, oum draiga, moudrayga, kamoune l'ibel [12, 17, 33, 40], it is also called akamman by the Targui population in Algeria [33]. *A. leucotrichus* belongs to the Apiaceae family (Umbelliferae) [55], it grows and thrives in the desert environments, besides Algerian Sahara, this plant is encountered in other North African countries such as Morocco and Tunisia, extending to Egypt and tropical Africa [12, 69]. In addition to the leaves of this plant that are used to perfume tea and its powder that is used as a spice for food [11-12, 16, 25], this plant has been extensively used by indigenous Algerian and Moroccan populations to treat many ailments. This plant is used against stomach pain and vomiting [12, 33], digestive disorders and complains [10, 17, 51], indigestion [11-12, 53], gastro-intestinal antiseptic [11], cold and fever [51], allergy [17, 33, 53], palpitations [17, 53], anorexia [33, 53], diarrhea [11, 53], chest complains [12]. Also, this plant has been used for treating cardiac diseases [38], urinary stone problems [9, 30] and for type 1 diabetes mellitus [67]. In addition, a decoction from the aerial parts of this plant is prepared by some traditional Algerian healers for the treatment of thyroid gland problems [2]. Also, *A. leucotrichus* is used as an emmenagogue, abortive and aphrodisiac by the population of Tassili N'Ajjer (Algeria) [33]. Several studies have shown that the studied plant is very rich in essential oils that show antimicrobial activities against bacteria, yeasts and filamentous fungi [2, 5, 21, 29, 44-45, 47-48, 52, 69]. Furthermore, the essential oil of *A. leucotrichus* has shown interesting anti-inflammatory activity and could be used as a natural drug against inflammatory diseases or as bioactive pharmaceutical molecules [52].

Therefore, this study aims to investigate the chemical composition of different extracts of the fruits of *A. leucotrichus* subsp. *leucotrichus*, collected from the Béchar region and to evaluate the potential of the essential oil and ethanolic and aqueous extracts as a source of phenolic and flavonoid compounds as well as natural antioxidants and antimicrobials.

MATERIAL AND METHODS

Plant material

Fruits of *A. leucotrichus* subsp. *leucotrichus* were collected (1 Kg) from Béchar province which is located in the South West of Algeria. This region is characterized by a desert climate with very high temperatures in summer (+45°C), intense solar radiation, a cold winter (2°C to 3°C) and low precipitations (<60 mm/year) [72]. Fruits that were

collected during the spring of 2015 were stored in a refrigerator at 4±2°C pending subsequent use.

Essential oil extraction

Fruits of *A. leucotrichus* subsp. *leucotrichus* were crushed with a mechanical grinder and 100 g of the obtained powder was subjected to 3 h hydrodistillation using a Clevenger-type apparatus. The obtained essential oil was then stored at +4°C in a sealed dark vial pending further experiments. Oil yield was calculated as follow:

$$Yield\ \% = [(B\text{-}C)/A)] \times 100$$

where Yield %: Yield percentage, A: plant material mass (g), B: bottle mass + essential oil (g), C: empty bottle mass.

Plant extracts preparation

Fruits of *A. leucotrichus* subsp. *leucotrichus* that were pulverized into a powdered form were used for the preparation of the extracts. The hydroalcoholic and the aqueous extracts of *A. leucotrichus* subsp. *leucotrichus* fruits were prepared according to the method described in [18] with slight modifications: 50 g of the crushed fruits were macerated in 500 mL of ethanol/water mixture (80/20 v/v) or 500 mL of distilled water at ambient temperature for 48 h. The obtained aqueous and hydroalcoholic macerates were subjected to double filtration on hydrophilic cotton and Whatman No. 1 filter paper. The obtained filtrates were concentrated on a rotary evaporator at a temperature of 45 and 50°C for the ethanolic extract (ETOH Ext) and aqueous extract (AQ Ext), respectively.

HPLC analysis

High-Performance Liquid Chromatography (HPLC) analysis was carried out on an Agilent 1100 Series equipped with Diode Array Detector (HPLC-DAD), manual injection, quaternary pump, thermostatted column compartment and a C18 Hypersil BDS column (250 x 4.6 mm; 5 µm). The temperature was kept stable at 30°C throughout the analysis. Using a solution (A) of ultra-purified water + acetic acid (0.2%) (v/v) and an acetonitrile solution (B) at a flow rate of 1 mL/min, HPLC analysis started with 95% of A followed by a linear gradient to 100% of B for 30 min. Chromatograms were obtained after eluting 15 µL of samples and standards injected at 300 nm because of most phenolic compounds show reasonably high absorbance at this wavelength value [60].

Determination of total phenolic content

The total phenolic content of the two tested extracts was determined spectrophotometrically using the Folin-Ciocalteu reagent which is composed of a mixture of phosphomolybdic-phosphotungstic acids as described by [63]. Total phenolic content was determined and expressed as mg of Gallic Acid Equivalents per gram of Dry Weight (mg GAE/g DW) using a standard curve as reference. Briefly, 250 µL of each extract were diluted with distilled water (3.75 mL) and 250 µL of Folin-Ciocalteu reagent. After 3 min, 750 µL of sodium carbonate (20% Na_2CO_3) was added. After shaking the mixture and 40 min of incubation time at 40°C, the absorbance was measured at λ = 760 nm using a spectrophotometer. This experiment was performed in triplicates. The concentrations of total phenolic compounds were calculated according to the following equation that was obtained from the standard gallic acid graph:

$$Absorbance = 0.1055 \times (R^2=0.9973)$$

Determination of flavonoid content

The amount of flavonoids of the two tested extracts (ETOH Ext and AQ Ext) was determined by $AlCl_3$ [56]. One milliliter of each extract at a suitable dilution was added to the same volume of $AlCl_3 \cdot 6H_20$ (2% dissolved in methanol). The mixture was vigorously shaken and incubated for 10 min at room temperature. The absorbance was measured at 440 nm. The contents were expressed as Quercetin Equivalents per Dry Weight (mg QE/g DW), it represents the average of three determinations and calculated according to the following equation that was obtained from the standard quercetin graph:

$$Absorbance = 0.1875 \times (R^2=0.9815)$$

Determination of antioxidant activity using DPPH radical scavenging method

Based on the method of [13], the free radical-scavenging activity of the essential oil and prepared extracts were tested using the stable radical 2,2'-diphenyl-1-picrylhydrazyl (DPPH). When DPPH (a stable purple color) reacts with an antioxidant compound, it is reduced to yield a light-yellow color of diphenylpicrylhydrazine and the changes of the color can be spectrophotometrically measured. In this test, 1.5 mL of various concentrations of the essential oil (100 mg/mL) and different concentrations of the prepared extracts (1 mg/mL) were added to 1.5 mL of a 0.004% methanolic solution of DPPH. The mixtures were strongly shaken and left to stand at room temperature for 30 min in the dark. The absorbance was measured at 517 nm against a blank. Butylated Hydroxy Toluene (BHT) and ascorbic acid (Vit C) were used as positive controls. The radical-scavenging activity was expressed as a percentage of inhibition (I%) according to the following formula:

$$I\ (\%) = 100 \times [(A\ control - A\ sample)/A\ control]$$

where I%: DPPH scavenging effect (%), A control is the absorbance control reaction (which contained equal volumes of DPPH solution and methanol without any test compound), and A sample is the absorbance of the tested compound with DPPH solution.

Concentration (IC50) values (the concentration in µg/mL or mg/mL required to scavenge 50% of the DPPH free radical for the extracts and the essential oil, respectively) were determined graphically by linear regression.

Determination of antioxidant activity using β-Carotene/linoleic acid bleaching method

The antioxidant activity was determined using the β-carotene bleaching method described by [61], which is based on the oxidative decomposition of β-carotene when confronted with linoleic acid. One milliliter of a solution composed of 2 mg of β-carotene that was dissolved in 10 mL of chloroform was added to 20 mg

of linoleic acid and 200 mg Tween 80. Chloroform was completely evaporated using a rotary evaporator at 50°C and 50 mL of distilled water was then added to the residue. Samples (2 mg/mL) were dissolved in methanol and 200 µL of each sample solution was added to 4.8 mL of the above mixture in test tubes. The test tubes were incubated in a hot water bath at 50°C for 120 min and the same procedure was repeated with positive controls; BHT and Vit C and the blank prepared in the same way but without β-carotene. All the tests were repeated three times. After an incubation period (0, 30, 60, 90, 120 min), the absorbance was measured at 470 nm. The percentage of antioxidant activity (AA) was calculated as follows:

The β-carotene bleaching rate (R) was calculated according to the equation:

$$R = ln(a/b)/t$$

where, ln = natural log, a = absorbance at time t (0), b = absorbance at time t, t is the time in 0, 30, 60, 90, 120 min.

The antioxidant activity (AA) was calculated as the percent inhibition relative to the control using the equation:

$$AA = [(R\ control - R\ sample)/R\ control] \times 100$$ [27]

where, R control and R sample are average bleaching rates of the negative control and the antioxidant (plant extract, Vit C or BHT), respectively.

***In vitro* antimicrobial activity**

The disc diffusion method of [8] was used to evaluate the antimicrobial activity of the essential oil and the aqueous and ethanolic extracts of *A. leucotrichus* subsp. *leucotrichus*. All tested microorganisms were obtained from the Laboratory of Microbial Systems (LBSM) of ENS Kouba. The microorganisms were cultured on Muller Hinton (MH) agar media for bacteria and Sabouraud dextrose for yeasts and fungi. The inoculums were suspended in 10 mL sterile saline water (0.9% NaCl) and diluted to 0.5 McFarland, mixed by vortex and then 100 µL was spread on the semi-solid media plates. Sterile Whatman paper disks N°3 (5.5 mm in diameter) were soaked with 5 µL of the tested essential oil and 10 µL of ETOH Ext (100 mg/mL) and AQ Ext (100 mg/mL). The culture plates were put in a refrigerator at 4°C for 1 h then they were incubated at 37°C for 24 h for bacteria and at 30°C for 48 h for yeasts and fungi. Positive controls (10 µg/disc of Nystatin and Levofloxacin), as well as negative controls that were prepared using the same solvents employed to dissolve the samples, were tested in the same conditions. The antimicrobial activities were evaluated by measuring the inhibition zone diameters (mm) surrounding each disk.

Statistical analysis

The data were presented as Mean±SD. Significance was tested by one-way ANOVA followed by Tukey's test. $P<0.05$ was considered to be significant.

RESULTS

Essential oil characteristics

The essential oil of *A. leucotrichus* subsp. *leucotrichus* fruits that had a blue color and a characteristic strong aromatic smell gave a percentage yield of 1.59%.

HPLC results

The identification of compounds in the aqueous and ethanolic extracts from fruits of *A. leucotrichus* subsp. *leucotrichus* was performed by HPLC using UV/visible (DAD). The obtained chromatograms for the aqueous and the ethanolic extracts are shown in Figures 1 and 2, respectively. As a result, several peaks appeared at 300 nm for the two extracts however, the only identified peak was ferulic acid.

Total phenolic and flavonoids content

The total phenolic and flavonoids content of the ethanolic and aqueous extracts of *A. leucotrichus* subsp. *leucotrichus* fruits are reported in Table 1. The most important phenolic content was observed in the ethanolic extract with 146.18±5.82 mg GAE/g DW compared to the aqueous extract with 77.51±2.21 mg GAE/g DW. The same results were obtained for flavonoids where the ethanolic extract showed a significant amount of 43.55±2.46 mg QE/g DW while we obtained a little amount for the aqueous extract 3.86±0.03 mg QE/g DW.

Antioxidant activity

The evaluation of antioxidant activities of the two prepared extracts and the essential oil were determined by two different test systems namely DPPH and β-carotene-linoleic acid. The IC50 values of the essential oil, ethanolic and aqueous extracts of *A. leucotrichus* subsp. *leucotrichus* and positive controls were determined and listed in Table 1. These results show that the ethanolic extract (97.89±2.91 µg/mL) possessed higher antioxidant activity, followed by the aqueous extract (337.6±16.73 µg/mL) and finally, the essential oil (20.64±0.52 mg/mL). After comparison with positive controls (BHT and Vit C), the essential oil and the two extracts were found to be less effective than positive controls.

We also evaluated the antioxidant activity using the β-carotene/linoleic acid bleaching assay. Based on available information, as shown in Table 1, the rate of bleaching of the β-carotene solution was measured by the difference between the initial reading in spectral absorbance at 470 nm at time 0 min and after 120 min. The antioxidant activity of the essential oil (43.78±1.82) was found to be superior to all samples however, it was less than the capacity of the positive controls BHT (95.45±2.05) and Vit C (70.18±5.65). This was followed by the ethanolic extract (40.14±2.15) while the aqueous extract showed the weakest activity potential in this test system (39.28±1.26).

***In vitro* antimicrobial activity**

The following microorganisms were used for the extensive screening for the antimicrobial activity: different *Staphylococcus aureus* strains (Sa1, Sa2, Sa5, Sa65, Sa639c and Sa4330), *Bacillus subtilis* (ATCC 30300), *Listeria monocytogenes* (ATCC 13932), *Escherichia coli* (E52), *Enterobacter cancerogenes*, *Agrobacterium tumefaciens*, *Saccharomyces cerevisiae, Candida albicans* (M2), *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus westerdijkiae*, *Aspergillus alliaceus*, *Fusarium oxysporum* f. sp. *albedinis*, *Fusarium graminearum*, *Fusarium culmorum* (Fc), *Mucor racemosus* and *Penicillium expansum*. The diffusion method was applied to test the antibacterial and antifungal activities of the aqueous and ethanolic extracts and the obtained essential oil of *A. leucotrichus* subsp. *Leucotrichus* fruits. The results are summarized in Table 2. The activity was recognized by

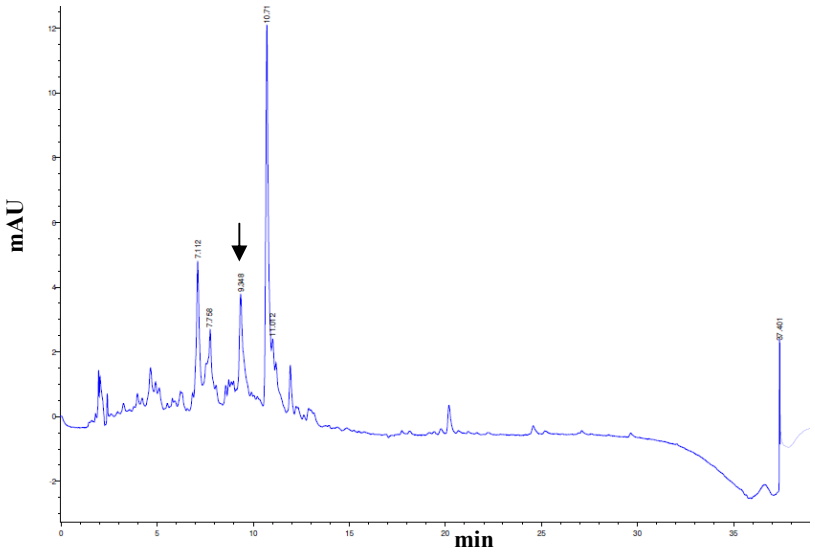

Figure 1. HPLC chromatogram of the aqueous extract of *A. leucotrichus* subsp. *leucotrichus* fruits at 300 nm

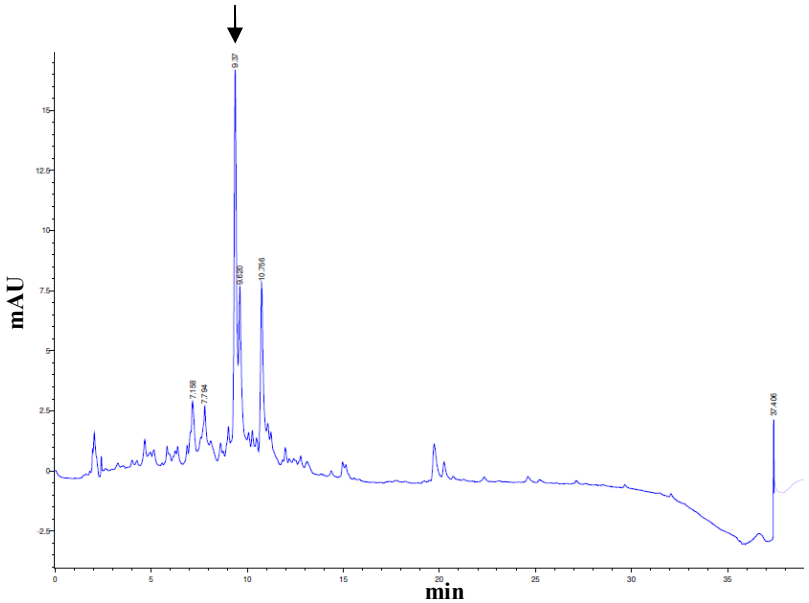

Figure 2. HPLC chromatogram of the ethanolic extract of *A. leucotrichus* subsp. *leucotrichus* fruits at 300 nm

Table 1. Total phenol, flavonoids, DPPH and β-carotene/linoleic acid bleaching assays of *A. leucotrichus* subsp. *leucotrichus*

Extracts	Total phenols content (mg GAE/g DW)	Flavonoids content (mg QE/g DW)	DPPH assay (IC50) Extract (µg/mL) Oil (mg/mL)	β-carotene/linoleic acid bleaching assay (%)
ETOH Ext	146.18^{A}±5.82	43.55^{A}±2.46	97.89^{A}±2.91	40.14^{AB}±2.15
AQ Ext	77.51^{B}±2.21	3.86^{B}±0.03	337.6^{A}±16.73	39.28^{A}±1.26
EO	ND	ND	20.64^{C}±0.52	43.78^{B}±1.82
BHT	ND	ND	72.16^{D}±0.1	95.45^{D}±2.05
VIT C	ND	ND	4.00^{B}±0.1	70.18^{C}±5.65

The values are expressed as an average±SD. The means in each column followed by a different letter are significantly different ($p < 0.05$, one way ANOVA followed by Tukey's test). ND. Non Determined.

Table 2. Diameter of inhibition zone (mm) measured for the antimicrobial activity of *A. leucotrichus* subsp. *leucotrichus* fruits

Group of microorganism	Pathogenic strains	EO (5 µL/disc)	ETOH Ext (10 µLg/disc)	AQ Ext (10 µL/disc)	Levofloxacin (10 µg/disc)	Nystatin (10 µg/disc)
Gram-positif bacteria	*Staphylococcus aureus* (Sa1)	14	12.5	-	>60	-
	Staphylococcus aureus (Sa2)	40	-	-	36	-
	Staphylococcus aureus (Sa5)	28	-	-	28	-
	Staphylococcus aureus (Sa65)	15	13	16.5	46	-
	Staphylococcus aureus (Sa639c)	13	15	-	26	-
	Staphylococcus aureus (Sa4330)	26	-	-	30	-
	Bacillus subtilis (ATCC30300)	37	-	-	31	-
	Listeria monocytogenes (ATCC13932)	38	-	-	34	-
Gram-negatif bacteria	*Escherichia coli* (E52)	20	-	-	44	-
	Enterobacter cancerogenes	>90	-	-	46	-
	Agrobacterium tumefaciens	34	-	-	38	-
Yeast	*Saccharomyces cerevisiae*	15	-	-	22	-
	Candida albicans (M2)	>90	-	-	-	-
Fungi	*Aspergillus flavus*	48	8	9	-	25
	Aspergillus parasiticus	65	-	8	-	15
	Aspergillus westerdijkiae	38	14	11	-	20
	Aspergillus alliaceus	45	-	-	-	8
	Fusarium oxysporum f. sp. *albedinis*	35	-	-	-	-
	Fusarium graminearum	>90	-	-	-	30
	Fusarium culmorum (Fc)	>90	-	17	-	22
	Mucor racemosus	>90	-	19	-	20
	Penicillium expansum	57	-	11.5	-	36

the presence of an inhibition zone around discs and measuring its diameter. The inhibition zones were compared with two standards Levofloxacin for antibacterial activity and Nystatin for antifungal activity. The essential oil showed a broad spectrum of antimicrobial activities for the most tested microorganisms with inhibition zones ranging from 13 to >90. In contrast, the antimicrobial activity of the two tested extracts was significantly lower than that of the tested essential oil showing no inhibition or inhibition zones of 8 to 19 mm.

DISCUSSION

Yield of essential oil

After extracting the oil from the fruit powder of our studied plant, a deep blue essential oil was obtained, with an estimated yield of 1.59%. The study of [25] has shown similar results for *A. leucotrichus* fruit essential oil (1.5%). The yield of essential oil extracted from the same plant originating from El Galta area (Maroc) was similar to our result but higher than the yield of essential oil obtained from Mkaimima area (Maroc) (1.5% compared to 1%) [5]. Furthermore, the study of [52] showed yields that were ranging from 2.82% to 3.87% (w/w). These yields were expected as other members of the Apiaceae family gave resembling results, for example, the oil yield of *Pimpinella anisum* fruits was 1.91% [54].

HPLC results

The HPLC results revealed the presence of a phenolic compound that was identified in many plants of the Apiaceae family and other families which is ferulic acid. This compound exhibits many positive effects including anticarcinogenic, cardioprotective, anti-oxidative, neuroprotective, hepatoprotective and have anti-inflammatory activities [37].

Total phenolic and flavonoids contents

The results of the total phenolic compounds are in agreement with the report of [32] who showed that the ethanol extract of Turkish *Pimpinella anisum* L. fruits had the highest amount of total phenolic compounds (77.5 mg GAE/g DW) compared to the water extract (30 mg GAE/g DW). Coriander fruits included 17.04 mg of GAE/g DW of polyphenol and 11.1 mg QE/g DW of flavonoids [64]. In comparison with another study on *A. leucotrichus* of a sample taken from Ghardaïa region, the total phenolic concentration of the methanolic extract was found to be 59.24 mg GAE/g DW, which was close to the results obtained by [62]. Furthermore, [26] estimated the amount of phenols for the plant sample taken from Béni Abbas region, from the different ethanolic, acetone and methanolic extracts by 160.61, 152.26 and 118.69 mg GAE/g DW, respectively, and the amount of flavonoids were 87.76, 97.38 and 66.65 mg QE/g DW, respectively. In this case, it was observed that the amount of obtained phenols and flavonoids was very high and different according to the polarity of the solvent. The total phenolic content of the ethanolic extract was two times higher than the aqueous extract.

Antioxidant activity

Antioxidant efficacy is estimated by measuring the capacity of the essential oil or extract to inhibit free radicals or stop the oxidation process. In our study, effectiveness was estimated by DPPH and β-Carotene assays. Both methods depend on coloring and discoloration at a certain wavelength [31].

In recent years, there have been many studies on natural antioxidants, especially after increasing research on the negative effects of industrial antioxidants on human health [43, 31]. These studies included many plant extracts, whether organic extracts or essential oils, in order to obtain new natural sources as antioxidants [22, 46, 71].

Our results show that the differences in the chemical composition of the extracts have a significant effect on the inhibitory ability of free radicals. Several studies indicated that the antioxidative activity of plants with medicinal properties is due to the presence of naturally occurring compounds such as phenolic compounds [48, 70]. In addition, antioxidant activities of essential oils from medicinal plants are mainly attributed to the active compounds present in them. This activity can be explained by the high percentages of the main constituents, in addition to the presence of other constituents in small quantities or to the synergy between them [31, 66]. Many studies on *A. leucotrichus* indicated the effectiveness of organic extracts. The aqueous extract of the fruits of this plant from Ghardaïa showed a high activity where the value of IC50 was estimated at 232 µg/mL. In another study of [26], the ethanolic, methanolic and acetone extracts of the fruits of this plant were very active with an IC50 that reached 29.03 µg/mL, 38.10 µg/mL and 41.31 µg/mL, respectively. The study of [58] confirmed that phenolic compounds are responsible for the antioxidant activity of many essential oils while the activity is weak for monoterpene and sesquiterpene compounds. The results reported for IC50 of essential oil of *A. leuchotrichus* was lower than the used standards, which is in agreement with our study where our essential oil did not show significant antioxidant activity [21, 25, 49]. As for the composition of the essential oil obtained from the fruits of *Ammodaucus leucotrichus*, it has been identified in several previous works of [2, 5, 21, 25, 43] by GC-MS analysis reporting perillaldehyde as the main compound.

Thus, the low antioxidant activity can be explained by its composition as it is rich in monoterpene and the presence of low amounts of molecules known for their antioxidant activities such as limonene [24].

In the β-carotene bleaching assay, the oxidation of linoleic acid produces free radicals due to the removal of a hydrogen atom from the diallylic methylene groups of linoleic acid. The highly unsaturated β-carotene then will be oxidized by the generated free radical. However, the presence of antioxidant constituents could prevent the bleaching of β-carotene because of their ability to neutralize free radicals [23].

The antioxidant properties of the different extracts of *A. leucotrichus* subsp. *leucotrichus* have been previously reported. According to [26], the organic extract showed moderate activity and significantly lower than that of the BHA. In addition, the essential oils of *A. leucotrichus* from two regions (Messaad and Debdeb) exhibited significantly lower inhibition against linoleic acid oxidation (33.10% and 19.45%, respectively) [21]. Several researchers reported that the antioxidative properties in natural sources can be mostly due to their ingredients like phenolics, flavonoids and terpenoids [6]. The study of [65] reported that flavonoids are able to scavenge hydroxyl radicals, superoxide anions and lipid peroxyl radical while [39] reported the potent antioxidant activity for terpenoids. In this regard, [6] discussed that the presence and synergism of different antioxidants in an extract will determine the antioxidative properties of a specific extract or essential oil. The DPPH results revealed that the essential oil had a low antioxidant potential when compared to the result of the β-carotene bleaching test that revealed a remarkable antioxidant power. This can be explained by the lipophilic affinity of the essential oil with the reaction mixture [41].

Antimicrobial activity

Plants are important sources of potentially useful antimicrobial agents [20]. The obtained results of the *in vitro* antimicrobial assay indicated that the tested essential oil exhibited activity against all the tested microorganisms. Many of the aromatic plants have an antimicrobial activity that can be due to the presence of essential oils with the nature, composition and functional groups present in the essential oils play important roles in determining the antimicrobial activity [4]. A variety of volatile molecules that were reported as present in essential oils such as aliphatic compounds, terpenes, phenol-derived aromatic and terpenoids might have bactericidal and fungicidal activities [3]. The major components present in essential oils can penetrate the membrane of the microorganisms and react with the membrane enzymes, phospholipid bilayer and proteins which can cause an impairment of microbial enzymatic system and/or disturbance of genetic material functionality [1, 28]. Several authors agreed that good antimicrobial activities of the essential oil of *Ammodaucus leucotrichus* may be due to high percentages of monoterpenes especially the major constituent perillaldehyde [29, 59]. Results obtained by [5] revealed that the essential oils contain high perillaldehyde amounts that are very effective against bacteria and fungi thanks to their high diffusivity in the culture medium, which enables rapid direct contact with microorganisms. The perillaldehyde component is a monoterpenoid abundant in the herb perilla such as *Perilla frutescens* and frequently used in Asian traditional medicine and for flavoring purposes in many dishes [50]. It is one of the major compounds found in plants that have been reported to possess antioxidant, antidepressant, antibacterial and antifungal, hypolipidemic, anti-inflammatory, neuro-protective effects, the capability to induce apoptosis in cancer cells and other biological activities [24, 34-36, 42, 68]. Also, limonene that was previously reported in the essential oil of our plant in the study of [44], has been shown to have antimicrobial, antioxidant, anticarcinogenic, anti-inflammatory, insecticidal and insect repellent properties [15, 24].

The results of the disc diffusion test indicated that the strongest inhibitory activities of the essential oil were found against *Enterobacter cancerogenes*, *Candida albicans* (M2), *F. graminearum*, *F. culmorum* (Fc) and *Mucor racemosus* that did not grow in the presence of only 5 µL of the essential oil. In addition, good inhibitory activities were observed against the Gram-positive bacteria *S. aureus* (Sa2), *S. aureus*

(Sa5), *S. aureus* (Sa4330), *Bacillus subtilis* (ATCC 30300), *Listeria monocytogenes*, the Gram-negative bacteria *Escherichia coli* (E52) and *Agrobacterium tumefaciens* and the majority of fungi. In contrast, the lowest activity was observed against the multiresistant *S. aureus* (Sa639c).

In another hand, the antimicrobial activity of the two tested extracts were significantly lower that the tested essential oil. The ethanolic extract of *A. leucotrichus* subsp. *leucotrichus* showed an antimicrobial activity against essentially the three tested strains of *S. aureus* (Sa1, Sa65 and Sa639c) with inhibition zone diameters between 12.5 and 15 mm and fungi like *A. flavus* (an inhibition zone of 8 mm) and *A. westerdijkiae* (inhibition zone of 14 mm) however, the same extract did not show any activity against the three tested Gram-negative bacteria (*Escherichia coli* E52, *Enterobacter cancerogenes* and *Agrobacterium tumefaciens*). The aqueous extract did not inhibit the growth of all the tested bacteria with the exception of *S. aureus* (Sa65) with an inhibition zone diameter of 16.5 mm and inhibited many of the tested fungi (except three). These results are in line with what was reported by [47] who showed that ethanol is the better solvent than water for the extraction of the active ingredients of this plant. This antimicrobial activity could be related to the composition in bioactive components and their functional groups with possible synergistic interactions between them [23]. Some reports showed that the Gram-negative bacteria are less sensitive than Gram-positive bacteria due to their outer membrane barriers; the Gram-positive bacteria have only an outer peptidoglycan layer while Gram-negative bacteria have an outer phospholipids membrane [7, 14].

Some plant extracts and essential oils are an interesting source of many compounds that can have many biological activities. Our results revealed a broad spectrum activity of the obtained essential oil against many microorganisms. Chromatographic analysis showed the presence of ferulic acid in the aqueous and ethanolic extracts of the studied fruits. The ethanolic extract of *A. leucotrichus* subsp. *leucotrichus* fruits showed good antioxidant activity in DPPH and β-Carotene/linoleic acid bleaching assays. Thus, our results pointed out that *A. leucotrichus* exhibited an appreciable antibacterial and antioxidant activities. It can be concluded that the studied plant have potent antioxidant activity contributing to the use for health benefits in addition to their possible use as a food supplement.

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