

Evaluation of the Antioxidant Activities of Organic Extracts from *Ammodaucus leucotrichus* Coss & Dur Fruit Part Harvested from the Algerian Sahara

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Abstract

Aromatic and medicinal plants are a good source of natural preparations containing effective bioactive compounds which can be used for different applications. This work aims to evaluate the antioxidant activity of some organic extracts of *Ammodaucus leucotrichus* Coss & Dur fruit part. The whole plant was collected from the region of Beni Abbas (Bechar-Algeria). Five organic extracts were obtained and the evaluation of the antioxidant activity was performed by six conventional methods. Polar organic extracts exhibited more antioxidant power than non polar extracts. The level of phenolic compounds was moderate in all extracts. The investigation of the antioxidant activity of organic extracts from fruit part of *Ammodaucus leucotrichus* revealed a moderate activity tested by six conventional methods.

Keywords: *Ammodaucus leucotrichus*; Medicinal plant; Antioxidant activity; Organic extracts; Phenolic compounds

Introduction

Ammodaucus leucotrichus Coss & Dur is a medicinal plant used in folk medicine in Algeria. *Ammodaucus leucotrichus* is a small annual plant, 10-12cm high, glabrous with erect, finely striated stems. The leaves are finely dissected and slightly fleshy. The flowers are small, with 5 free petals. The fruit is a diachene, 6-10mm, 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 (Bechar, Djanet, and El Golea), its presence is also observed in the Canary Islands [1-3]. *Ammodaucus leucotrichus* is used for the treatment of many diseases (Table 1). In the continuity of our works on this species, the present study reports the determination of the phenolic and flavonoid contents and, also, the assessment of the antioxidant activity of different organic extracts of the fruit part of this plant [4].

Table 1: Different uses of *Ammodaucus leucotrichus* (Coss & Dur) in traditional folk medicine in Algeria.

Arabic Denomination		Traditional Uses
<i>Ammodaucus leucotrichus</i> Coss & Dur (Apiaceae)	Arabic: kammûn es-sofi, el massoufa	Leaves are used for chest complaints. In the Tassili (South of Algeria), leaves are used to aromatise tea, and its powder is a much appreciated spice for food Fruits are used either as a powder or in a decoction to treat gastric-intestinal pain and indigestion. It is also frequently used, as an infusion, for diverse infantile diseases of the digestive apparatus: dysentery, nausea, regurgitation, vomiting. It is used also to treat patients suffered with high blood pressure

Material and methods

Plant materials

The whole plant was collected from the province of Beni-Abes (west-southern of Algeria-region of Bechar). The fruits of *Ammodaucus leucotrichus* were dried away from direct sunlight. Dried plant material was then crushed into a mortar and stored at very low temperature until further use [5].

Sample preparation

A powder (10g) of the fruit part of *Ammodaucus leucotrichus* was extracted by 100mL of different organic solvents with increasing polarity, under reflux for 3h. The extracts were then filtered and concentrated under reduced pressure at 60 °C using a rotary evaporator. The obtained residue was recovered in methanol and stored at +4 °C.

Total phenolic contents

Total phenolic was estimated by the Folin-Ciocalteu method [6]. 0.1mL of each sample was mixed with 2mL of sodium carbonate (2%) freshly prepared, the whole was mixed. After 5min, 100 μ L of Folin-Ciocalteu reagent (1N) were added to the mixture and left for 30min. The values of absorbance were recovered at 750nm against a blank. A calibration curve was performed under the same operating conditions using gallic acid. The results were expressed as mg gallic acid equivalent per gram of dry extract (mg GAE/g).

Total flavonoid contents

The total flavonoid content was determined by the method described by Ardestani & Yazdanparast [7]. Each sample (500 μ L) was mixed with 2mL of distilled water and subsequently with 150 μ L of a NaNO₂ solution (15%). 150 μ L of aluminum chloride (AlCl₃) solution (10%) was added and allowed to stand for 6min. Then, 2mL of NaOH solution (4%) was added to the mixture. Immediately, distilled water was added to bring the final volume to 5mL and the mixture was thoroughly mixed and allowed to stand for 15min. Absorbance of the mixture was determined at 510nm. Results were expressed as catech in equivalent per gram of dry extract (mg CEQ/g).

Study of the Antioxidant Activity

Evaluation of the total antioxidant capacity (TAC)

An aliquot of 0.1mL of sample solution was combined with 1mL of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate, and 4mM ammonium molybdate). The tubes were capped and incubated at 95 °C for 90min. After the samples had cooled to room temperature, the absorbance was measured at 695nm. A typical blank solution contained 1mL of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under the same conditions as the rest of the samples. For positive control, acid ascorbic was used at the same conditions [8].

Ferric reducing antioxidant power (FRAP)

Samples and ascorbic acid were used at different concentrations. 1mL of each sample was mixed with phosphate buffer (2.5mL, 0.2mol L⁻¹, pH 6.6) and potassium ferricyanide [K₃Fe(CN)₆] (2.5mL, 30mmol L⁻¹). The mixture was incubated at 50 °C for 20min. A 2.5mL of TCA (0.6 mol L⁻¹) was added to the mixture, which was then centrifuged for 10min at 3000g. The supernatant (2.5mL) was mixed with distilled water (2.5mL) and FeCl₃ (0.5mL, 6 mmol L⁻¹), and the absorbance was measured at 700nm [9].

DPPH- scavenging activity

A methanolic solution (50 μ L) of each sample at different concentrations was added to 1.95mL of DPPH- (2,2-Diphenyl-1-Picrylhydrazyl) solution (6 \times 10⁻⁵M in methanol). The studied compounds were tested with methanol as control, BHA (Beta Hydroxy Acid), ascorbic acid and quercetin as antioxidant references. The absorbance at 515nm was determined after 30min.

The absorbance (A) of the control and samples was measured, and the DPPH- scavenging activity (SA), in percentage, was determined as follow:

$$SA\% = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

IC₅₀ (inhibition concentration of 50%) was obtained graphically from nonlinear regression analysis [10].

H₂O₂ scavenging activity

A solution of H₂O₂ (20mM) was prepared in phosphate buffered saline (PBS, 0.1M, pH 7.4). 1mL of sample or positive control (BHA and α -tocopherol) in methanol was added to 2mL of H₂O₂ solution in PBS. The absorbance was measured at 230nm, after 10min [11]. The percentage of H₂O₂ scavenged was calculated:

$$\% \text{ of scavenged } H_2O_2 = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

Hydroxyl scavenging assay

OH- radicals were generated from FeSO₄ and H₂O₂, and detected by their ability to hydroxylate salicylate. The reaction mixture contained 1mL of FeSO₄ (1.5mM), 0.7mL of H₂O₂ (6mM), 0.3mL of sodium salicylate (20mM) and different concentrations of organic extracts tested. After incubation for 1h at 37 °C, the absorbance was measured at 562nm. The percentage of scavenging effect was calculated as:

$$\% \text{ of Scavenging} = [1 - (A_1 - A_2) / \times A_0] \times 100$$

Where A₀ was the absorbance of the control (without extract) and A₁ was the absorbance in the presence of the extract and A₂ was the absorbance without sodium salicylate. IC₅₀ (inhibition concentration of 50%) was obtained graphically from nonlinear regression analysis [12].

β -Carotene bleaching method

A stock solution of β -carotene was prepared with 0.5mg of β -carotene in 1mL of chloroform, 25 μ L of linoleic acid and 200mg of Tween 40. The chloroform was evaporated and 100mL of oxygenated distilled water were added to the residue. 350 μ L of each sample were added to 2.5mL of the above mixture. The test tubes were incubated at 50 °C for 2h, together with two blanks, one contained BHA as a positive control and the other contained the same volume of methanol instead of the extracts. The absorbance was measured at 470nm. Antioxidant activities (inhibition percentages, I%) of the samples were calculated using the following equation:

$$I\% = (A \beta\text{-carotene after 2h assay} / A_{\text{initial } \beta\text{-carotene}}) \times 100$$

where A β -carotene after 2h assay is the absorbance of β -carotene after 2h assay remaining in the samples and A initial β -carotene is the absorbance of β -carotene at the beginning of the experiments. IC₅₀ (inhibition concentration of 50%) was obtained graphically from nonlinear regression analysis [13]. All tests were carried out in triplicate and results were reported as means \pm SD of triplicates.

Results and Discussion

Table 2: Yield of extraction, phenolic contents and antioxidant activity (DPPH- scavenging, OH- scavenging and β -carotene bleaching methods) of the different organic extracts of fruit part of *Ammodaucus leucotrichus*.

Organic Extracts of <i>Ammodaucus leucotrichus</i>	Yield of Extraction (%)	Phenolic Contents (mg GAE/g)	Flavonoid Contents (mg CEQ/g)	IC ₅₀ (μ g/mL)		
				DPPH• Scavenging	IC ₅₀ (μ g/mL) OH• Scavenging	IC ₅₀ (μ g/mL) β -carotene Bleaching
Methanol Extract	10.02 \pm 0.49	118.69 \pm 8.5	66.65 \pm 0.37	38.10 \pm 2.54	43.33 \pm 1.32	191.53 \pm 1.53
Ethanol Extract	9.92 \pm 0.28	160.61 \pm 6.28	87.79 \pm 0.51	29.03 \pm 1.85	89.67 \pm 3.33	nd
Acetone Extract	7.22 \pm 0.48	152.26 \pm 4.44	97.38 \pm 1.3	41.31 \pm 0.56	96.33 \pm 5.21	nd
Ethyl Acetate Extract	4.78 \pm 0.81	20.9 \pm 3.12	21.48 \pm 0.84	nd	nd	nd
Chloroform Extract	5.71 \pm 0.88	12.49 \pm 1.17	15.94 \pm 1.38	nd	nd	nd
Ascorbic Acid				2.48 \pm 0.09	61.83 \pm 1.98	nd
BHA				2.61 \pm 0.13	nd	4.26 \pm 0.98
Quercetin				2.59 \pm 0.15	nd	nd

Methanol and ethanol extracts showed yields of the order of 10.02 \pm 0.49% and 9.92 \pm 0.28%, respectively (Table 2). An extraction yield of methanol extract (maceration) of *Ammodaucus leucotrichus* fruit part of about 20.4% was reported by Sifi et al. [14]. The contents of this plant on phenolics and flavonoids in their different organic extracts were important in polar organic extracts. The most important phenolic contents were observed in ethanol extract (160.61 \pm 6.28mg GAE/g). While the most important flavonoid contents were recorded in acetone extract (97.38 \pm 1.31mg CEQ/g). Chloroform extract contained the less important contents in those compounds (Table 2). Some species in Apiaceae family contain significant levels of phenolics and flavonoids. The study of Saeed et al. [15] on *Torilis leptophylla* methanolic extract reported high contents on those compounds (121.9 \pm 3.1mg GAE/g and 60.9 \pm 2.2mg RTE/g, respectively). Another study on three species of Apiaceae: *Heracleum lasiopetalum* Boiss, *Kelussia odoratissima* Mozaff and *Echinophora platyloba* DC, reported important levels of phenolics (from 74 to 120mg TAE/g), and flavonoids (from 7.63 to 14.52mg RE/g) [16].

The evaluation of antioxidant activity of the organic extracts was made by six conventional methods. The antioxidant activity of Apiaceae was investigated by several authors in the literature. Chandran et al. [17] investigated the antioxidant activity of aqueous and methanolic extracts of leaf part of *Ardisia solanaceae* Roxb. The methanolic extract of this plant revealed more antioxidant activity compared to the aqueous extract. Interesting antioxidant activity was reported by the authors of this publication [17]. Pirbalouti et al. [16] in their study on three species of Apiaceae family, endemic in Iran, reported an interesting antioxidant power of their methanol extracts tested by: the DPPH- test, reduction of iron and the ABTS-test.

The antioxidant activity of organic extracts of the fruit part of *Ammodaucus leucotrichus* that we evaluated was moderate overall antioxidant tests used in our study (Figures 1-3). Extracts from

polar solvents showed more antioxidant activity than those from non-polar one. In TAC test, we noticed that the methanol extract has shown the most important antioxidant activity compared to the other extracts organic, but lower than that of ascorbic acid. For DPPH- scavenging test, organic extracts: ethanol, methanol and acetone, had presented a moderate antioxidant activity with values of IC₅₀ in the order of 29 \pm 1.85 μ g/mL, 38.10 \pm 2.54 μ g/mL and 41 \pm 0.56 μ g/mL, respectively. Nevertheless, these extracts remain less active compared to the used positive controls. In H₂O₂ scavenging test, methanolic and ethanolic extracts presented an important activity superior to that of the positive controls used (BHA and tocopherol). The other extracts were also active. The chemistry behind this test is based on a transfer of electrons and proton between the antioxidant and hydrogen peroxide by reducing it in a water molecule. Measurement in the UV band makes this test very sensitive and estimate with a critical eye [18].

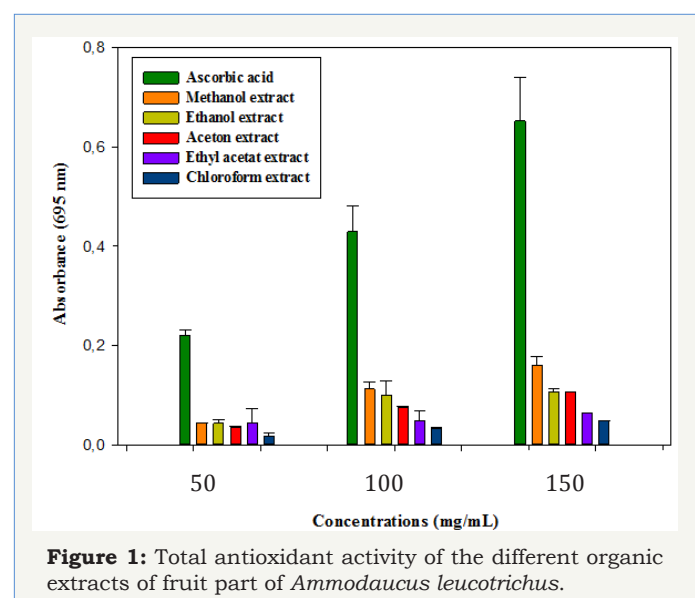


Figure 1: Total antioxidant activity of the different organic extracts of fruit part of *Ammodaucus leucotrichus*.

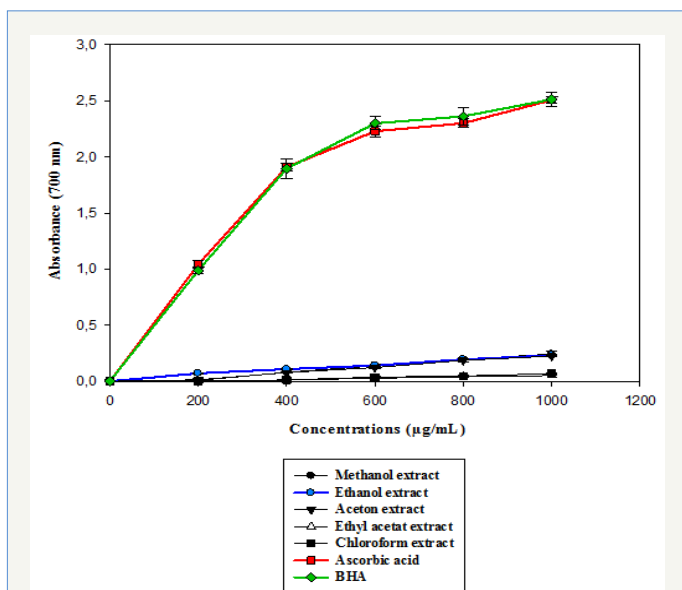


Figure 2: Reducing power activity (FRAP) of the different extract of fruit part of *Ammodaucus leucotrichus*.

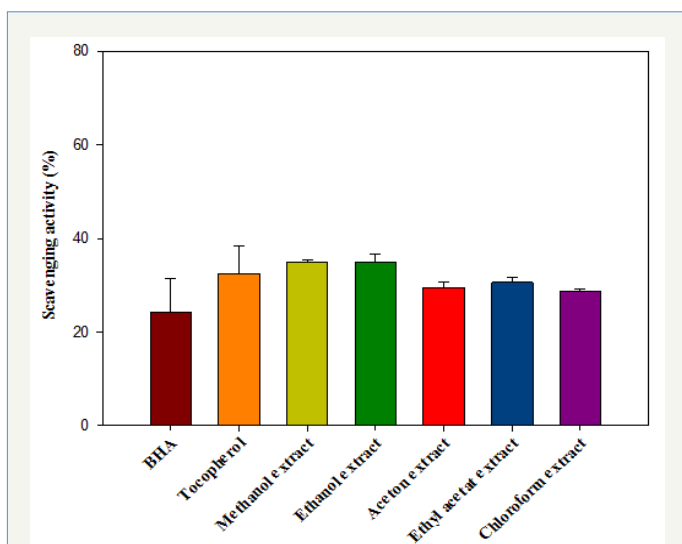


Figure 3: H₂O₂ scavenging activity of the different organic extracts of the fruit part of *Ammodaucus leucotrichus*.

Good antioxidant activity was recorded in OH⁻ radicals scavenging test for methanolic extract, more interesting than the positive control (ascorbic acid). Assessment of the activity of an extract to scavenge the OH⁻ radical is important, due to the high reactivity of this radical, which reacts with a wide range of molecules found in living cells (such as: sugars, lipids, proteins, nucleotides and so on). In the test of β-carotene bleaching, all organic extracts showed moderate activity and only the methanolic extract presented an IC₅₀ (191.53±1.53µg/mL) which is significantly lower than that of the BHA (4.26±0.98µg/mL). In the FRAP test, all organic extracts showed very modest antioxidant activity.

Conclusion

Valorization of local flora is an important priority to find new interesting compounds that can be used in different fields. One

of the endemic plants in Algeria, *Ammodaucus leucotrichus* was the subject of some scientific papers. In this study, we concluded that secondary metabolites from this plant exhibited a moderate antioxidant activity. These results help us to orientate our future studies in other directions of research.

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Conflict of Interest Statement

The authors declare that there is no conflict of interest.

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