

Original Research Article

Polyphenol content, antioxidant, antihemolytic and anticoagulant potentials of *Ammodaucus leucotrichus* seed extracts

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Sent for review: 28 February 2023

Revised accepted: 24 May 2023

Abstract

Purpose: To investigate the therapeutic potentials of different extracts from *Ammodaucus leucotrichus* seeds through various *in vitro* antioxidant assays.

Methods: Seeds of *Ammodaucus leucotrichus* were subjected to liquid-liquid scale extraction using solvents of varying polarities. Polyphenols and flavonoids were evaluated in these extracts using colorimetric methods. Antioxidant potential was investigated through established *in vitro* assays including free radical scavenging potency against DPPH, β -carotene bleaching assay for lipid peroxidation, chelating, and transition metal reducing assays. Antihemolytic, antithrombotic, and anticoagulant activities were also determined.

Results: Maximum amount of total phenolics and flavonoids were recorded in crude extract (CrE). Ethyl acetate extract (EAE) possessed the highest scavenging activity against DPPH radical ($p < 0.05$) while the aqueous extract showed significant ferrous ion chelation among the extracts with EC_{50} equal to 0.883 ± 0.019 mg/mL. EAE exhibited the highest antioxidant activity in β -carotene/linoleic acid (81.99 ± 2.942 %), and ferric reducing ($EC_{50} = 0.116 \pm 0.002$ mg/mL) assays. The 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) oxidative action revealed that the extracts protected the erythrocyte membrane from hemolysis. Moreover, EAE and chloroform extract (ChE) increased clotting time while only EAE and CrE caused a high percentage of clot lysis when added to blood samples.

Conclusion: *Ammodaucus leucotrichus* is a promising source of natural antioxidants, as all extracts possess significant antioxidant activities irrespective of solvent polarities.

Keywords: *Ammodaucus leucotrichus*, Phenolics, Antioxidants, Scavenging, Hemolysis, Clotting time

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Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, Web of Science, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Reactive oxygen species (ROS) is a term that encompasses a wide variety of free radicals, that

are considered to be highly reactive with cellular matrix components in their self-stabilization process and to achieve a non-radical state [1]. Oxidative stress (OS) occurs from an imbalance

between ROS production and antioxidant defenses, ultimately contributing to the pathogenesis of a wide range of degenerative diseases including brain dysfunction, vascular disorders, and diabetes [2]. Since there is a link between several diseased states and the production of ROS, antioxidant agents (polyphenols and flavonoids) were found to be effective in the prevention of several ailments by acting at various levels: scavenging or inhibiting free radicals and their precursors or binding to metal ions that catalyze ROS creation [3].

As a part of the objective to find medicinally active agents from plants, *Ammodaucus leucotrichus* Coss & Dur is one of the spontaneous aromatic and medicinal plants belonging to the genus of *Ammodaucus* in the Apiaceae family [4]. This last taxon is referred to as “Kammûnes-sofi”, or “Moudrayga” in Algeria, and it thrives in desert environments [5]. Ethnobotanical survey reveals that *A. leucotrichus* is used as a food spice and for various medicinal applications. In the southern Algerian Sahara, the fruits and leaves are often used for flavoring and seasoning during culinary preparation [4]. In addition, this species was extensively used to treat many ailments, including allergies, vomiting, preventing wound infections, digestive disorders, gastrointestinal pains, cardiac diseases, urinary stone problems, liver diseases, and especially type 1 diabetes mellitus [4,6]. The current paper aims to provide a relationship between the ethnobotanical uses along with recent studies concerning the ethnopharmacology, and therapeutic activities of *Ammodaucus leucotrichus* methanolic extract and its fractions.

EXPERIMENTAL

Chemicals and instruments

Solvents (methanol, hexane, petroleum ether, chloroform, and ethyl acetate) were of analytical grade and afforded from Sigma-Aldrich, Biochem Chemopharma, and VWR International. Folin-Ciocalteu reagent, aluminium chloride, 1,1-diphenyl-2-picryl-hydrazyl (DPPH); β -Carotene, ferrous sulfate (FeSO_4), 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-p, p'-sulfonic acid (ferrozine); potassium ferricyanide ($\text{K}_3\text{Fe}(\text{CN})_6$); 1,10 phenanthroline; 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) and butylated hydroxytoluene (BHT) were purchased from Sigma-Aldrich chemicals. All extracts were concentrated using a rotary evaporator (BÜCHI, Germany). Spectrophotometric values were recorded on DRAWELL (DV-8000) spectrophotometer.

Seeds of plant material

Ammodaucus leucotrichus seeds were collected from the arid region of Algerian Sahara during the summer period of 2021, authenticated with reference (220/SNV/ DA/UFAS/21) by Professor Chermat Sabah, University of Ferhat Abbas (Setif, Algeria). A voucher specimen was deposited at the Faculty of Nature and Life Sciences herbarium for reference. The plant material was air-dried at room temperature. Dried seeds were then powdered using an electric grinder, and thereafter the powdered sample was stored at ambient temperature in a low-moisture permeability pack until its use.

Preparation of extracts

Ammodaucus leucotrichus powder was subjected to methanol extraction according to the method described by Mayouf *et al* [7]. A 5 kg weight of seed powder was soaked in absolute methanol in a closed vessel and allowed to stand at room temperature for a period of 3 to 7 days with frequent agitation until the soluble matter has dissolved. The extract was filtered through Whatman no. 4.1 filter paper and then concentrated under reduced pressure on a rotary evaporator to obtain crude extract (CrE). The liquid-liquid scale CrE extraction was carried out using various solvents of increasing polarities: hexane for defatting, chloroform for aglycone flavonoids extraction, and ethyl acetate for glycoside flavonoids extraction. Each fraction was evaporated to dryness under reduced pressure to provide hexane (HE), chloroform (ChE), ethyl acetate (EAE), and the remaining aqueous (AqE) extracts. The extracts were stored at 4 °C and used to assess their antioxidant activity.

Quantitative phytochemical evaluation

Total polyphenols content

Total phenolic content (TPC) of the extracts was determined spectrophotometrically by using the Folin–Ciocalteu reagent as outlined by Trabsa *et al* [8] with slight modification. Briefly, 200 μL of diluted sample was added to 1 mL of 1:10 diluted Folin–Ciocalteu reagent. After 4 min, 800 μL of 7.5 % saturated sodium carbonate solution was added. The final mixture was vortexed and incubated at room temperature for 2 h in dark conditions. Absorbance was then measured at 765 nm. The calibration curve was established using gallic acid (0 - 200 $\mu\text{g}/\text{mL}$) and results were determined and expressed as μg of gallic acid equivalents per milligram of dry weight of each sample (μg GAE/mg DW).

Total flavonoids content

The trichloride aluminium colorimetric method was used to quantify total flavonoid content (TFC) as described by Krache *et al* [9]. Aliquots of 1 mL of each sample were mixed with an equal volume of trichloride aluminium solution (2 %). After 10 min incubation at room temperature and under dark conditions, the absorbance of the mixture was determined at 430 nm. Total flavonoid content was determined and expressed as µg of quercetin equivalents per milligram dry weight (µg GAE/mg DW) using a standard curve as reference.

Determination of antioxidant activity

DPPH radical scavenging activity

The ability of the investigated extracts to scavenge the stable free radical DPPH was carried out according to the method described by Guemaz *et al* [10]. Succinctly, 50 µL of various concentrations of the extracts dissolved in methanol were added to 1250 µL of methanolic solution containing 0.004 % DPPH radicals. The final mixture was kept in darkness at room temperature for 30 min, and the absorbance was read at 517 nm, where quercetin and BHT were used as standards. DPPH radical inhibition (H) was calculated using Eq 1.

$$H (\%) = \{(Abs_{control} - Abs_{sample}) / Abs_{control}\} 100 \dots (1)$$

where $Abs_{control}$ is the absorbance of the DPPH solution, and Abs_{sample} is the absorbance in the presence of the extract.

β-Carotene/linoleic acid bleaching assay

In this assay, the antioxidant capacity of extracts was determined by measuring the inhibition of the conjugated diene hydroperoxides arising from linoleic acid oxidation according to Amraoui *et al* [11]. In the first step, an emulsion was prepared by dissolving 0.5 mg β-Carotene in 1 mL of chloroform, and 200 mg of tween 40 and 25 µL of linoleic acid were added. Chloroform was evaporated completely under reduced pressure and the mixture is shaken vigorously with 100 mL distilled water saturated with oxygen to form an emulsion. A volume of 2.5 mL of this mixture was added to 350 µL of 2 mg/mL of each tested extract and BHT as a positive control. The emulsion system was incubated at room temperature in dark, and the absorbance was monitored spectrophotometrically at 490 nm at different times ranging from 1, 2, 4, 6, and 24 h. The antioxidant activity (A) was calculated as the

inhibition percentage of lipid peroxidation using Eq 2.

$$A (\%) = \{Abs_{sample} / Abs_{control} (t_0)\} 100 \dots (2)$$

Hydroxyl radical scavenging activity

The assay to determine hydroxyl radical (OH[•]) scavenging activity was conducted as outlined previously by Mayouf *et al* [7] with slight modification. The reaction volume consisted of 150 µL of samples at varying concentrations mixed with 300 µL of a stock solution containing 9 mM FeSO₄ and 0.3 % hydrogen peroxide (H₂O₂). Then hydroxyl radicals were generated by the Fenton reaction at low pH. The mixture was incubated at 32 °C for 15 min followed by the addition of 75 µL of salicylic acid (20 mM). Thereafter it was kept for another 15 min at 32 °C. The absorbance was determined spectrophotometrically at 562 nm, and BHT was used as the standard. Eq 3 was used to calculate the hydroxyl radical scavenging activity (S).

$$SI (\%) = \{1 - (Abs_{sample} - Abs_{blank}) / Abs_{control}\} 100 \dots (3)$$

where $Abs_{control}$ is the absorbance of the control without a sample. Abs_{sample} is the absorbance after adding the tested sample, and Abs_{blank} is the absorbance of the sample without salicylic acid.

Hydrogen peroxide scavenging assay

The scavenging effect of extracts on hydrogen peroxide (H₂O₂) was evaluated using the 1,10-phenanthroline reagent based on the method reported by Madoui *et al* [12]. The reaction mixture contained 250 µL of ferrous ammonium sulfate, 1.5 mL of extracts/ standard at different concentrations, and 62.5 µL of H₂O₂ at a concentration of 5 mM. After incubation at room temperature in the dark, 1.5 mL of 1,10-phenanthroline (1 mM) was added and incubated for 10 min. The absorbance of the mixture was measured at 510 nm. Hydrogen peroxide scavenging activity (P) was computed according to Eq 4.

$$P (\%) = \{Abs_{sample} / Abs_{control}\} 100 \dots (4)$$

Ferrous ion chelating assay

Metal chelating by extracts was measured as described by Aouchria *et al* [13]. Different concentrations of test compounds (250 µL) were added to a solution of 0.6 mM of ferrous chloride (50 µL) and 450 µL of methanol. After incubation for 5 min at room temperature, an aliquot of 50 µL ferrozine (5 mM) was added to the mixture

and the reaction was left standing at room temperature for 10 min. The absorbance of the Fe²⁺-Ferrozine complex was read at 562 nm, and EDTA was used as a reference chelator. The Fe²⁺ chelating activity of samples was calculated using Eq 5.

$$\text{Chelating activity (\%)} = \left\{ \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right\} 100 \dots\dots\dots (5)$$

Ferric reducing ability

Ferrous reducing power assay was conducted according to Zerargui *et al* [14] with slight modification. To 100 µL of different concentrations of the samples, an equal volume of phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide (K₃Fe(CN)₆) was added. The reaction mixtures were incubated for 20 min at 50 °C. Furthermore, 250 µL of acid trichloroacetic was added to stop the reaction, followed by centrifugation at 3000 rpm for 10 min. Next, 250 µL of the supernatant was transferred to a tube containing 250 µL distilled water and 500 µL of ferric chloride (0.1 %). The amount of ferric ferrocyanide formed was monitored by measuring the absorbance at 700 nm. The concentration of the sample giving an absorbance of 0.5 was determined from a linear regression curve.

Determination of antihemolytic, antithrombotic, and anticoagulant activities

Free radical-induced erythrocyte hemolysis assay

The resistance of red blood cells to free radical damage was investigated by a pre-incubation of erythrocytes with plant extracts. Hemolysis induction was monitored as previously described by Djarmouni *et al* [15] with slight modification. Mice erythrocytes previously collected in a heparinized tube were centrifuged and washed 3 times with phosphate-buffered saline (PBS; 10 mM, pH 7.4). The washed blood cells were re-suspended in PBS to achieve 2 % (v/v) hematocrit. Furthermore, 300 mM of AAPH was dissolved in PBS in order to induce free radical chain oxidation. Erythrocyte suspension was assayed using a 96-well microplate coated with a free radical generator and the different extracts/Vitamin C. The AAPH-induced oxidative hemolysis was monitored at 630 nm after incubation at 37 °C, using a 96-well microplate reader (ELX 800, BioTEK instruments, Winooski, VT, USA). Erythrocytes' resistance to the free

radical attack was expressed by 50 % of total hemolysis of blood cells (half-time; HT₅₀).

Antithrombotic activity

Antithrombotic assay was carried out as previously described by Zerargui *et al* [16]. Venous blood was drawn from a healthy volunteer, transferred in different previously weighed tubes (500 µL), and incubated at 37 °C for 45 min to form the clot. Serum was completely removed without disturbing the clot and the tube was weighed again to determine clot weight. An Aliquot of 100 µL of test samples was added to each tube containing pre-weighed blood clots and citrate was used as positive thrombolytic control. Tubes were incubated for 90 min at 37 °C and observed for clot lysis. Subsequent to incubation, the fluid released was aspirated out and tubes were again weighed to observe the difference in weight after clot disruption. The differences obtained in weight taken before and after clot lysis was expressed as clot lysis (C) and calculated using Eq 6.

$$C (\%) = \left\{ \frac{W_c - W_{lc}}{W_c} \right\} 100 \dots\dots\dots (6)$$

where W_c is weight of clot and W_{lc} is the weight of lysed clot

Blood clotting time effect

Blood coagulation inhibition was evaluated as described by Zerargui *et al* [16]. Briefly, an aliquot of 74 µL of extracts was mixed immediately with fresh whole blood drawn from healthy volunteers in test tubes. The tubes were mixed and blood clotting time was recorded every 30 sec, Citrate was used as a positive control, instead distilled water was used as a negative control.

Statistical analysis

All experiments were conducted in triplicate and the results obtained are expressed as mean ± standard deviation (SD) or standard error of the mean (SEM). Data were subjected to ANOVA (one-way analysis of variance) followed by Tukey test for multiple comparisons and two-way ANOVA followed by Bonferroni post-test. *P* ≤ 0.05, *p* ≤ 0.01, and *p* ≤ 0.001 were considered indicative of statistical significance in all analyses. The IC₅₀ and EC₅₀ values were calculated from linear plotting graphs. The data were processed using Excel and GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego, CA., USA).

RESULTS

Total phenolic and flavonoids contents

In the current study, the extraction procedure resulted in various extracts with different yields; total phenolic and flavonoid contents are indicated in Table 1.

The yield calculated for methanolic extraction showed that CrE recorded a considerable quantity. The resulting yields in the percentage of fractions were in the following decreasing order: AqE > EAE > ChE. The amounts of TPC and TFC shown in Table 1 revealed that CrE contained the highest amount of both polyphenols and flavonoids. Among the extracts, AqE had the lowest polyphenolic and flavonoid content.

Scavenging and inhibitory effect of the extracts

DPPH, radical hydroxyl, and hydrogen peroxide scavenging activity

The results obtained in this study suggest that most of the extracts showed a significant radical scavenging activity increased in a dose-dependent manner. IC₅₀ of extracts are reported in Table 2. According to the results presented above, EAE was the most potent extract in all three *in vitro* systems. In the DPPH system, EAE

exhibited a similar trend compared to BHT ($p > 0.05$), followed by ChE. In addition, EAE is considered an effective OH[•] quencher with a low IC₅₀ than the synthetic standard ($p > 0.05$). Despite the high activity of ascorbic acid to stabilize 50 % of H₂O₂, EAE successfully performed a substantial quenching potency. Lower scavenging activity was registered for AqE in the three tested systems.

β-Carotene/ linoleic acid bleaching

The inhibitory effects of extracts against the coupled oxidation of linoleic acid and β-Carotene were monitored, and the results were illustrated in Figure 1.

All extracts showed a high protective ability of β - Carotene compared to the reference antioxidant BHT (98.157 ± 0.754 %). The highest antioxidant activity was observed with EAE at 81.989 ± 2.942 %, followed by CrE and ChE (78.341 ± 2.892 % and 78.236 ± 1.462 %, respectively). Even AqE exhibited a remarkable protective activity at 72.269 ± 10.831 %. Whereas, samples without antioxidant components lead to a rapid decrease in the inhibition activity.

Ferrous ion chelating activity

Ferrous ion chelating activity of extracts was evaluated and results are shown in Figure 2.

Table 1: Extraction, fractionation yields, and total phenolic and flavonoid content of *A. leucotrichus* extracts

Extract	Yield (%)	Total phenolic content (µg GAE/mg E) ¹	Total flavonoids content (µg QE/mg E) ²
CrE	8	77.143±0.010	19.593±0.084
ChE	4.143	69.833±0.163	6.062±0.039
EAE	4.624	64.571±0.045	16.92±0.105
AqE	89.371	31.738±0.010	5.265±0.009

Note: Values expressed are mean ± SD (n=3). CrE: crude extract; ChE: chloroform extract; EAE: ethyl acetate extract; AqE: aqueous extract. ¹Gallic acid equivalent/milligram of extract; ²Quercetin equivalent/milligram of extract

Table 2: Inhibitory potentials of different *A. leucotrichus* extracts compared to standard antioxidants using different scavenging techniques

Sample	IC ₅₀ values (mg/mL)		
	DPPH	OH	H ₂ O ₂
CrE	0.104±0.002***	2.387±0.007***	0.146±0.003***
ChE	0.074±0.003***	0.713±0.049 ^{ns}	0.119±0.001***
EAE	0.021 ±0.0003 ^{ns}	0.295±0.012 ^{ns}	0.042±0.001 ^{ns}
AqE	0.299±0.006***	12.877±0.245***	0.672±0.023***
BHT	0.020±0.0001	0.624±0.004	NT
Quercetin	0.003±0.0003***	NT	NT
Vitamin C	NT	NT	0.016±0.0002

Note: Results are mean of three measurements ± SD. Comparison was realized against standards (BHT and Vitamin C), ^{ns}: no significant $p > 0.05$, *** $p \leq 0.001$. CrE: crude extract; ChE: chloroform extract; EAE: ethyl acetate extract; AqE: aqueous extract. NT: not tested

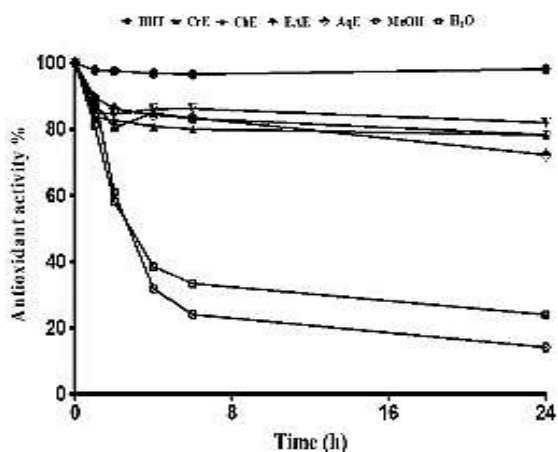


Figure 1: Bleaching kinetics of β -Carotene in the presence of *A. leucotrichus* extracts, methanol, water, and standard BHT during 24 h (values are expressed as the mean of triplicate). CrE: crude extract; ChE: chloroform extract; EAE: ethyl acetate extract; AqE: aqueous extract; MeOH: methanol

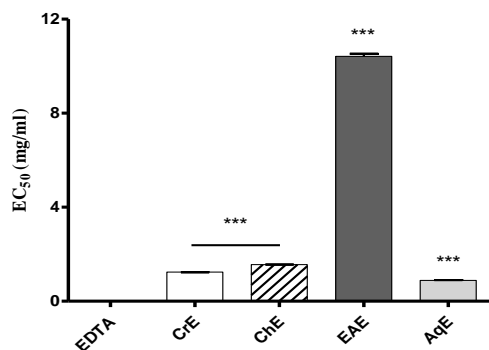


Figure 1: Graphical representation of EC₅₀ of *A. leucotrichus* extracts in ferrous ion chelating mechanism. Values are expressed as mean \pm SD (n = 3), compared to EDTA, ***p \leq 0.001. CrE: crude extract; ChE: chloroform extract; EAE: ethyl acetate extract; AqE: aqueous extract

Despite the EC₅₀ registered by EDTA (0.006 mg/mL), CrE and ChE exhibited a high capacity to chelate metal ions (1.240 \pm 0.023 mg/mL and

1.562 \pm 0.021 mg/mL, respectively). However, the aqueous extract showed more high chelating ability (0.883 \pm 0.019 mg/mL). Among the extracts, EAE was the weakest chelator, with EC₅₀ equal to 10.416 \pm 0.114 mg/mL.

Ferric reducing ability

In Figure 3, the results of the ferric reducing of *A. leucotrichus* extracts and synthetic reference are presented.

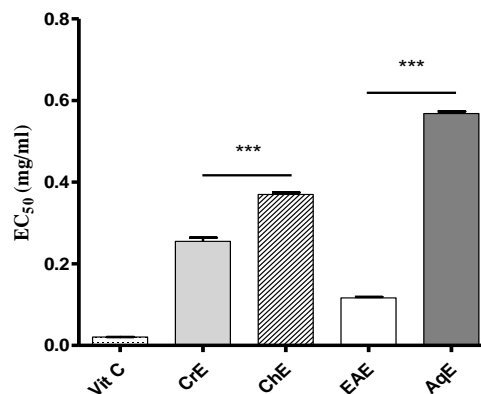


Figure 2: Total reducing power of *A. leucotrichus* extracts. Values are mean of triplicate experiment \pm SD. Comparison was realized against Vitamin C, ***p \leq 0.001. (Vit C: Vitamin C; CrE: crude extract; ChE: chloroform extract; EAE: ethyl acetate extract; AqE: aqueous extract)

The ability to reduce ferric ions was detected in all the extracts and in displayed a dose-dependent trend but weaker than Vitamin C in 0.021 \pm 0.0002 mg/mL. Significant data were shown in the decreasing order EAE > CrE > ChE > AqE. It is worth highlighting the reducing ability of EAE (0.116 \pm 0.002 mg/mL), which was significantly higher among other extracts. However, the lowest value was noticed with aqueous extract (EC₅₀ of 0.568 \pm 0.005 mg/mL).

Table 3: Pearson correlation coefficients (R) of TPC, TFC, and different *in vitro* antioxidant activities

Anti O	TPC	TFC	DPPH [•]	OH [•]	H ₂ O ₂	β -CBA	FICA	FRA
TPC	1.000							
TFC	0.643	1.000						
DPPH [•]	-0.857	-0.575	1.000					
OH [•]	-0.917	-0.539	0.986	1.000				
H ₂ O ₂	-0.918	-0.609	0.991	0.996	1.000			
β -CBA	0.782	-0.668	-0.978	-0.933	-0.954	1.000		
FICA	0.172	0.453	-0.614	-0.474	-0.516	0.745	1.000	
FRA	-0.751	-0.827	0.907	0.849	0.892	-0.967	-0.755	1.000

Anti O: antioxidant test; **TPC:** total polyphenolic content; **TFC:** total flavonoids content; **DPPH[•]/OH[•]/H₂O₂:** free-radical scavenging methods; **β -CBA:** β -Carotene bleaching assay; **FICA:** Ferric ion chelating assay; **FRA:** Ferric reducing ability

Table 4: Effect of different plant extracts, citrate, and distilled water on antithrombotic activity (AT) and Blood clotting time (BCT)

Parameter	Citrate	H ₂ O	CrE	ChE	EAE	AqE
AT (%)	25.36	NT	19.92	17.76	19.93	10.66
BCT (min)	NC	7±1	27±10	PC	PC	10±2

Note: Values are mean of triplicates ± SD; CrE: crude extract; ChE: chloroform extract; EAE: ethyl acetate extract; AqE: aqueous extract. NC: no observed clotting formation; PC: Partial clotting

Antihemolytic, antithrombotic, and anticoagulant activities

Antithrombotic activity and blood clotting time effect

The examined effect on platelet aggregation and clot lysis of plant extracts at a concentration of 50 mg/mL is shown in Table 4.

From the current findings, significant inhibition of coagulation was obtained with EAE and ChE compared to the Citrate, in which the two extracts showed partial clotting of the blood sample, even after 3 h observation, total blood clotting was not achieved. Regarding the percentage of clot lysis showed by the Citrate, we noted a high percentage of clot lysis with EAE and CrE. The aqueous extract recorded a low antithrombotic effect and a decrease in clotting time when added to blood samples.

Free radical-induced erythrocyte hemolysis activity

The HT₅₀ values obtained are shown in Figure 4. Results showed that when erythrocytes were treated with AAPH, a significant decrease in hemolysis time was observed compared to cells treated with *A. leucotrichus* extracts in which cells had the capacity to withstand the free radical-induced hemolysis. The extracts significantly protected the erythrocyte membrane from hemolysis in a concentration-dependent manner. At low concentrations (0.05 and 0.025 mg/mL), all studied extracts registered nearly a similar reduction in hemolysis and comparable to Vitamin C as standard ($p > 0.05$). Whereas going to high concentration (0.1 mg/mL), both ChE and EAE significantly showed high activity (HT₅₀ = 172.071 ± 12.771 and 161.865 ± 20.265 min, respectively) as more than two folds Vitamin C (HT₅₀ = 71.953 ± 12.857 min) ($p \leq 0.01$). While at 1 mg/mL, EAE had the best activity as an anti-hemolytic with HT₅₀ equal to 201.7 ± 8.3 min. Both CrE and AqE at 0.1 mg/mL gave a comparable activity (HT₅₀ = 94.7 ± 22.1 min and 78.04 ± 4.48 min, respectively) to that of Vitamin C ($p > 0.05$). However, at 1 mg/mL, AqE was the less active with HT₅₀ equal to 122.59 ± 12.25 min.

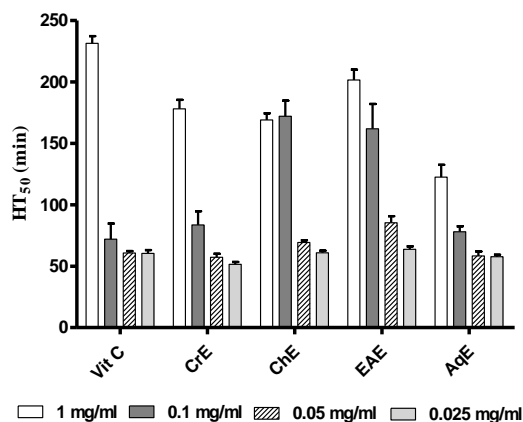


Figure 4: Effect of the studied extracts on AAPH-induced hemolysis in mice erythrocytes at different concentrations (1, 0.1, 0.05, 0.025 mg/mL). Values are expressed as mean ± SEM (n=4). (Vit C: Vitamin C; CrE: crude extract; ChE: chloroform extract; EAE: ethyl acetate extract; AqE: aqueous extract)

DISCUSSION

There are many parameters, repeated maceration, plant species, organ used in the isolation, and secondary metabolites' composition in which the variation in yield depends [17,18]. Solvents with different polarities have the ability to solubilize and recover different compounds, and the ratio of the solvent used has an impact on the quantity of extracted phenolic compounds [17,19], this could explain the difference in the amounts and the type of bioactive compounds present in our extracts.

The DPPH method has been used extensively for screening the antioxidant activity of plants because of its accuracy and the relatively short time required for analysis. Aouchria *et al* [13] reported that the phenolic content of plants is responsible for their scavenging potency. Indeed, polyphenols have suitable structural for free radical-scavenging activities. Flavonoids are considered excellent chain-breaking antioxidants and it was reported that the free radical scavenging potential closely depends on their skeleton structural arrangements, mainly the configuration and the pattern of hydroxyl groups [17]. The difference in the scavenging potential could be due to the difference in the

stoichiometry of reactions between the antioxidant compounds in the extracts and the various free radicals, also the diversity in radical scavenging mechanisms shown in these assays [20]. A strong negative correlation was recorded between TPC {R = (-0.918; -0.857)} and TFC {R = (-0.609; -0.539)} with radical scavenging assays (DPPH, OH[•], and H₂O₂).

The high and comparable inhibitory effect obtained with EAE, CrE, and ChE ($p > 0.05$) showed that the phenolic compounds or flavonoids from the plant in this study strongly inhibited the oxidation of β -Carotene and this is in concordance with Pandey *et al* [21]. In addition to their suitable structure, and lipophilicity which gives them the ability to incorporate deeply into the structured lipid bilayer, contribute to the strong enhancement of the activity [10]. Correlation analysis between this assay and TPC (R = 0.782) and TFC (R = 0.668) showed a strong positive relationship. The results obtained from AqE suggest that the antioxidant activity of an extract cannot be predicted on the basis of its total phenolic content only. Perhaps other bioactive compounds or a synergism of polyphenolic compounds with other components present in an extract may contribute to the overall observed antioxidant activity [22].

The study conducted by Mayouf *et al* [7] showed that there are other molecules more effective in chelating transition metals than polyphenols. Guemaz *et al* [10] reported that in aqueous system, extracts chelating effects are more effective to inhibit the formation of the Fe²⁺-ferrozine complex; thereby the chelating capacity is directly proportional to the polarity of their solvents. In this assay, a weak positive correlation was observed between TPC (R = 0.172) and TFC (R = 0.453). The iron-reducing capacity of the studied samples is an indicator of electron-donating ability [14]. The significant differences between samples may be attributed to the increased number of bioactive agents; it is probable that the antioxidant activity increased proportionally with glycoside flavonoids. Flavonoids chelate Fe⁺² more than ferric ions. So, its reduction is a very intrinsic activity and it depends on the structure of polyphenols [23]. A strong negative correlation was exhibited in this assay with TPC (R = -0.751) and TFC (R = -0.827).

In addition to the implication of some phenolic compounds in decreasing coagulation extent [24], they have antithrombotic activity [25]. Zerargui *et al* [16] reported that flavonoids exhibit anticoagulant potential and therefore the effect of *A. eucotrichus* extracts is affected by the

presence of a considerable amount of polyphenols and flavonoids. It has been reported that flavonoids are selectively bound to the membrane of platelet thrombi due to their high lipophilicity, which leads to the biosynthesis of different factors as a result of releasing thrombolytic agents [26,27].

The antihemolytic and antioxidative properties of this plant's seed extract may be due to the presence and synergistic role of flavonoids and polyphenols. The high antihemolytic activity observed in EaE and ChE at different concentrations could be attributed to their high lipophilicity, which, leads to a decrease in fluidity of the membrane, hindering the diffusion of free radicals [28]. The observed sedimentation of erythrocytes in microplates may suggest that some phenolic compounds bind to erythrocyte membranes and exert their antioxidative properties [28].

CONCLUSION

Ammodaucus leucotrichus has antioxidant properties. Moreover, polyphenols and flavonoids are present in all extracts and they exhibit significant thrombolytic and anticoagulant activities. The findings of this study provide scientific support for the traditional use of *Ammodaucus leucotrichus* seeds in the treatment of different physiological disorders, as well as for the seed extract's potential therapeutic application where scavenging of free radicals or protection of cell damage are warranted. However, further studies should be carried out on plant fractions and purified bioactive compounds to evaluate the activity of the most abundant polyphenols.

DECLARATIONS

Acknowledgements

The authors acknowledge the Algerian Ministry of Higher Education and Scientific Research (MESRS) and the General Directorate of Scientific Research and Technological Development (DGRSDT) for their financial support during the period of the research.

Funding

None provided.

Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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