Available online on 30.06.2020 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Total Phenolic Contents, DPPH Radical Scavenging and β-Carotene Bleaching Activities of Aqueous Extract from *Ammoides atlantica*

Karima Loucif[1]*, Hassiba Benabdallah[1], Fatima Benchikh[1], Soulaf Mehlous[1], Chawki Ben Souici[2], Smain Amira[1]

[1] Laboratory of Phytotherapy Applied to Chronic Diseases, Department of Animal Biology and Physiology, Faculty of Nature and Life Sciences, University Ferhat Abbas, Setif-1, 19000, Algeria

[2] Biotechnology Research Center (CRBt), UV 03 BP E73, Nouvelle Ville Ali Mendjli, Constantine, Algeria

ABSTRACT

Phytotherapy has known a great evolution all the world and some medicinal plants are important remedies of some diseases. *Ammoides atlantica* is one of the medicinal plants used in folk medicine. This study aims to estimate the total phenolics and flavonoids contents then to investigate both *in vitro* antioxidant activity models of aqueous extract (AqE) from *Ammoides atlantica.* Total polyphenol contents were determined using Folin Ciocalteu's reagent; flavonoids were quantified employing the $AlCl_3$ Method. The *in vitro* antioxidant property was assessed by DPPH-scavenging radicals and lipid peroxidation assays. The results revealed that *Ammoides atlantica* aqueous extract presented a high total phenolic and flavonoid contents with values of 85.56±4.71 µg GAE (gallic acid equivalent)/mg and 40.55±4.09 µg QE (quercitin equivalent)/mg dry extract, respectively. This extract shows a good DPPH radical scavenging and β-carotene bleaching activities with an IC_{50} of 107.48±5.9 µg/mL and 130.17±5.52 µg/mL, respectively. This study indicates that the aqueous extract from *Ammoides atlantica* has potent antioxidant effects and may prove to be of latent health benefit as well as supplementary sources for natural antioxidants drugs.

Keywords: *Ammoides atlantica*, aqueous extract, antioxidant activity, phenolic compounds.

Article Info: Received 03 April 2020; Review Completed 08 June 2020; Accepted 21 June 2020; Available online 30 June 2020

Cite this article as:

Loucif K, Benabdallah H, Benchikh F, Mehlous S, Souici CB, Amira S, Total Phenolic Contents, DPPH Radical Scavenging and β-Carotene Bleaching Activities of Aqueous Extract from *Ammoides atlantica*, Journal of Drug Delivery and Therapeutics. 2020; 10(3-s):196-198 http://dx.doi.org/10.22270/jddt.v10i3-s.4151

***Address for Correspondence:**

Karima Loucif, Laboratory of Phytotherapy Applied to Chronic Diseases, Department of Animal Biology and Physiology, Faculty of Nature and Life Sciences, University Ferhat Abbas, Setif-1, 19000, Algeria

1. INTRODUCTION

The excessive production of reactive oxygen species (ROS) overwhelming the antioxidant defense mechanisms of the cells has been shown to oxidize biological molecules and induce damage to the cell membrane, proteins, carbohydrates, and DNA. This oxidative stress is involved in several pathological situations including hypertension, heart failure and diabetes [1]. Antioxidants are compounds that can protect cells against the damage arising from unstable molecules known as ROS and free radicals [2]. The use of synthetic antioxidants in the prevention of free radical damage had many toxicological side effects including carcinogenicity. For this, there is an increasing interest in the potential benefits of natural antioxidants with the potent capacity to inhibit lipid peroxidation and scavenge ROS [3]. Recently, the exploration of natural antioxidant agents from plants is an important and essential step in the evolution of effective alternative medications [4]. Several polyphenols compounds are usually produced in plants and have attracted substantial recognition because of their antioxidant capabilities and free radical scavenging capabilities which are likely to be of concern to human health [5]. The *Ammoides atlantica* (coss. et Dur.) Wolf, of the family Apiaceae, is widespread in the Mediterranean region and it is endemic in Algeria [6]. Traditionally, this plant is known to be used for the therapy of fever and headache, besides its use as antidiarrheic [7]. Given the interest of *Ammoides atlantica* in folk medicine, this study aims to assess the polyphenolic contents of the aqueous extract from aerial parts of *Ammoides atlantica* and evaluate the *in vitro* antioxidant activity using DPPH radical scavenging and β-carotene bleaching assays.

2. MATERIALS AND METHODS

2.1. Plant material

2.1.1. Plant collection and identification

The aerial parts from *Ammoides atlantica* were collected from Jijel North-Eastern part of Algeria, during the flowering stage. The plant was identified and authenticated by Prof.

Laouer H., a botanist at the Department of Biology and Vegetal Ecology, University of Sétif, Algeria.

2.1.2. Extraction procedure

100g of *Ammoides atlantica* powder was mixed with 1L of boiling distilled water (100 °C) and after 20 minutes, it was removed from the heat. The mixture was filtered using Wattman filter paper n°1 and then dried at 45 °C to obtain aqueous extract which was stored at 20°C until further analysis [8].

2.2. Determination of total phenolic and flavonoid contents

2.2.1. Total phenolic content

The total phenolic content was evaluated by utilizing the reagent of Folin-Ciocalteu [9], according to a method of microplate described par Muller et al. [10]. Herein, 20 µl of a sample (1 mg extract/1ml water) were blended with100 µl of Folin-Ciocalteu reagent (1:10) and 75 µl of sodium carbonate solution (7,5%). The microplate was incubated for two hours at room temperature in darkness. Absorbance at 765 nm was measured by using the microplate reader. The total phenolic content was evaluated as micrograms of gallic acid equivalents per milligrams of extract.

2.2.2. Total flavonoids content

Total flavonoids content was determined by the method of Topçu *et al*. [11] with some modifications to adapt it to the microplate. Briefly, 130 µl of methanol were added to 50 µl of a sample (1mg extract/1ml water). Subsequently,10 µl of 1M potassium acetate (CH_3COOK) and 10 µl of 10% aluminum nitrate ($Al(NO_3)_3, 9H_2O$) were added and the microplate was incubated at room temperature for 40 minutes. Absorbance was read at 415 nm. Data were represented as micrograms of quercetin equivalents per milligrams of extract.

2.3. Antioxidant activity assays

2.3.1. DPPH free radical-scavenging assay

The free radical-scavenging capacity was determined spectrophotometrically employing the DPPH assay [12]. Briefly, 40 µl of the sample at various concentrations was added to 160 µl DPPH (0.1 mM). The reaction mixture was shaken forcefully, and the absorbance of the remaining DPPH was read at 517 nm after 30 min. BHT was used as an antioxidant standard for comparison of the activity. The scavenging capability of DPPH radical was calculated using the following equation: DPPH scavenging effect (%) = $[(A_{Control}-A_{sample})/A_{Control}] \times 100$. The results were given as IC_{50} values (µg/ml) corresponding to the concentration of 50% inhibition.

2.3.2. β-Carotene/linoleic acid assay

The β-carotene bleaching activity was evaluated using the β-carotene-linoleic acid system described by Marco [13]. Thus, a solution of β-carotene (0.5 mg) in 1 ml of chloroform is combined with 25 µl of linoleic acid and 200 µl of Tween 40. After evaporation in vacuo of the chloroform, 50 ml of hydrogen peroxide (H_2O_2) is added under vigorous agitation. The absorbance of the solution is then adjusted to 0.8-0.9 nm. Amounts of 160 µl of this solution are added to 40 µl of a sample at different concentrations. The absorbance was measured at 470 nm, using a 96-well microplate reader. The emulsion system was incubated for 2 h at 50°C. BHT was used as a reference. The bleaching rate (R) was assessed as follows: R = (ln c/d)/t. Where: c= absorbance at time zero, d= absorbance at time 120 min (t) and ln= natural log. The antioxidant activity (AA) was calculated in terms of percent of inhibition relative to the control, using the following equation: AA (%) = $[(R_{Control} - R_{Sample})/R_{Control}] \times 100$.

2.4. Statistical analysis

Results are represented as the mean ± standard deviation (SD) and all measurements were conducted in three determinations (n=3). The statistical interpretation was directed by the help of a one-way analysis of variance (ANOVA) for significance with the aid of Graph Pad Prism 7.00. differences were examined significant at $p \leq 0.05$.

3. RESULTS

3.1. Total phenolics and flavonoids contents

The results showed that the *Ammoides atlantica* aqueous extract (AqE) was rich in polyphenols and flavonoids (141.74±0.44 µg GAE/mg dry extract and 61.87±6.7µg QE/mg dry extract, respectively) as shown in Table 1.

Table 1: Total polyphenols and flavonoids content of *Ammoides atlantica* aqueous extract. AqE: aqueous extract, [(a)]: µg GAE/mg and [(b)]: µg QE/mg.

Extract	Total phenolic content[(a)]	Total flavonoid content[(b)]
AqE	141.74±0.44	61.87±6.7

3.2 Antioxidant activities

The IC_{50} values of DPPH radical scavenging and β-carotene bleaching activities from *Ammoides atlantica* aqueous extract (AqE) are presented in Table 2. The results revealed that the AqE presented a good scavenging activity against DPPH, with an IC_{50} of 204.22±12.16 µg/mL. As well, AqE displays a strong β-carotene bleaching activity with an IC_{50} of 112.45±6.66 µg/mL. This suggests a significant antioxidant activity from AqE.

Table 2 : Antioxidant activities of *Ammoides atlantica* aqueous extract (AqE). **** $p < 0.0001$ compared to correspondent standards. AqE: aqueous extract, DPPH: 2,2-diphenyl-1-picrylhydrazyl, BHA: butylated hydroxyanisole and BHT: butylated hydroxytoluene.

Extract/ standard	IC_{50} (µg/mL)	
	DPPH scavenging activity	β-carotene bleaching activity
AqE	204.22±12.16****	112.45±6.66****
BHA	5.73±0.41	0.90±0.02
BHT	22.32+1.19	1.05+0.01

4. DISCUSSION

Our results showed that the *Ammoides atlantica* aqueous extract (AqE) was a good source of polyphenol and flavonoid compounds. These results agree with the findings of [14]. Antioxidant capacity from AqE was assessed by DPPH-scavenging radicals and lipid peroxidation assays. In DPPH radical scavenging test, A freshly formulated solution of DPPH shows a dark purple color with maximum absorption at 517 nm. The purple hue usually fades/disappears in the medium contains antioxidants, Thus, antioxidant molecules can neutralize DPPH-free radicals; by provide hydrogen atoms or donate electrons and convert them to a colorless/bleached substance (2,2-diphenyl-1 hydrazine or equivalent hydrazine substituted), resulting in a decrease in absorption at the 517 nm level. The more rapidly absorbance falls, the more efficient the extract's antioxidant function in terms of atomic hydrogen-donating ability [15]. The results revealed that the AqE presented a good DPPH radical scavenging potential. This could be attributed to polyphenols and flavonoids [16]. The ability of A*mmoides atlantica* aqueous extract (AqE) to inhibit lipid peroxidation was tested by the β-carotene bleaching method. β-carotene in the absence of the antioxidant undergoes a rapid decolorization since the free linoleic acid radical attacks the β-carotene, which loses the double bonds and, consequently, its orange color [17]. The presence of a phenolic antioxidant can hinder the extent of b-carotene destruction by "neutralizing" the linoleate free radical (utilizing its redox potential) and any other free radicals formed within the system. Hence, this test can be used to examine the antioxidant potential of *Ammoides atlantica* AqE [18]. The results showed that AqE was able to inhibit lipid peroxidation. These antioxidant activities could be attributed to the richness of this extract in polyphenols and flavonoids. In fact, the literature showed that a good correlation was found between antioxidant activity and the content of polyphenols and flavonoids [19, 20]. Our results are in accordance with those of [14, 21], who demonstrated that the *Ammoides atlantica* extracts have potent antioxidant capacities.

5. CONCLUSION

The aqueous extract of *Ammoides atlantica* exhibited good antiradical activities toward 2,2′- diphenyl-1-picrylhydrazyl (DPPH) and acting as inhibiting lipid peroxidation. This may explain the medicinal use of this plant in folk medicine. These results suggest that AqE of *Ammoides atlantica* might be promising for the treatment or prevention of many diseases associated with oxidative damage. Further researches needed to identify and isolate the active principles present in this extract which could be useful for pharmaceutical purposes.

ACKNOWLEDGMENTS

This work was supported by the Algerian Ministry of Higher Education and Scientific Research (MESRS). We express our gratitude to these organizations. Authors would like also to thank Prof. Hocine LAOUER (Laboratory of Valorization of Natural Biological Resources, University of Sétif1, Algeria) for the identification of the plant material.

REFERENCES

[1] Gönenç A, Hacışevki A, Tavil Y, Çengel A, Torun M, Oxidative stress in patients with essential hypertension: a comparison of dippers and non-dippers, Eur J Intern Med, 2013; 24:139-144.

[2] Saxena M, Saxena J, Pradhan A, Flavonoids and phenolic acids as antioxidants in plants and human health, Int J Pharm Sci Rev Res, 2012; 16(2):130-4.

[3] Gonçalves S, Gomes D, Costa P, Romano A, The phenolic content and antioxidant activity of infusions from Mediterranean medicinal plants, Ind Crops Prod, 2013; 43:465-471.

[4] Singh M, Pandey N, Agnihotri V, Singh KK, Pandey A, Antioxidant, antimicrobial activity and bioactive compounds of *Bergenia ciliata* Sternb.: A valuable medicinal herb of Sikkim Himalaya, J Tradit Complement Med, 2017; 7:152-157.

[5] Pereira DM, Patrícia V, Pereira JA, Andrade PB, Phenolics: From Chemistry to Biology, Molecules, 2009; 14:2202-2211.

[6] Quezel P, Santa S. Nouvelle flore de l'Algérie et des régions désertiques méridionales. Centre National de la Recherche Scientifique, Paris; 1963.

[7] Bellakhdar J. La pharmacopée marocaine traditionnelle. Médecine arabe ancienne et savoirs populaires. Ibiss Press, Paris ; 1997.

[8] Ferreira A, Proença C, Serralheiro ML, and Araújo ME, The *in vitro* screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from Portugal, J.Ethnopharmacol, 2006; 108:31-37.

[9] Singleton VL, Rossi JA, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagent, Am J Enol Vitic, 1965; 16:144-58.

[10] Muller L, Gnoyke S, Popken AM, Bohm V, Antioxidant capacity and related parameters of different fruit formulations, LWT-Food Sci Technol, 2010; 43(6): 992-9.

[11] Topçu G, Ay A, Bilici A, Sarıkürkcü C, Öztürk M, Ulubelen A, A new flavone from antioxidant extracts of *Pistacia terebinthus*, Food Chem, 2007; 103(3):816-22.

[12] Blois MS, Antioxidants determination by the use of a stable free radical, Nature, 1958; 26:1199-200.

[13] Marco GJ, A rapid method for evaluation of antioxidants, J Am Oil Chem Soc, 1968; 45:594-8.

[14] Benteldioune M, Boudiar T, Bakhouche A, Contreras MDM, Lozano-Sanchez JL, Bensouici C, Kabouche Z, Segura-CarreteroA, Antioxidant activity and characterization of flavonoids and phenolic acids of *Ammoides atlantica* by RP–UHPLC–ESI–QTOF–MSn, Natural Product Research, 2019; 1–5.

[15] Yamaguchi T, Takamura H, Matoba T, Terao J, HPLC method for evaluation of the free radical-scavenging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl, Bioscience, Biotechnology, and Biochemistry, 1998; 62:1201–1204.

[16] Miliauskas G, Venskutonis PR, Van-Beck TA, Screening of radical scavenging of some medicinal and aromatic plant extracts, Food Chem, 2004 ; 85:231–237.

[17] Benchikh F, Benabdallah H, Amira S, The Evaluation of Antioxidant Capacity of Different Fractions of *Myrtus communis* L. Leave, Annual Research & Review in Biology, 2018; 22(5):1-14.

[18] Amarowicza R, Peggb RB, Rahimi-Moghaddamc P, Barld B, Weilc JA, Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies, Food Chemistry, 2004; 84:551–562.

[19] Conforti F, Sosa S, Marrelli M, Menichin F, Statti GA, Uzunov D, TubaroA, *In vivo* anti-inflammatory and *in vitro* antioxidant activities of Mediterranean dietary plants, J. Ethnopharmacol, 2008; 116:144–151.

[20] Li HB, Wong CC, Cheng KW, Chen F, Antioxidant properties *in vitro* and total phenolic contents in methanol extracts from medicinal plants, LWT - Food Sci. Technol (Leben smittel-Wissenschaft -Technol.), 2008; 41(3):385–390.

[21] Ababsa ZEA, Benkiki N, Derouiche MT, Louaar S, Medjroubi K, Akka lS, *In vivo* anti-inflammatory activity of the species: *Ammoides atlantica* of *Apiaceae*family, Der Pharmacia Lettre, 2011; 3(6):46–48.