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Metal Chelating and Cupric Ion Reducing Antioxidant Capacities of *Ammoides atlantica* Aqueous Extract

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ABSTRACT

Reactive oxygen (ROS) and nitrogen species (RNS) are produced in all cells and play important roles in physiology. The loss of the redox balance, either by an increase of oxidant molecules ROS and RNS or by decreased antioxidant system activities cause a state of oxidative stress. Several studies are going on worldwide directed towards finding natural antioxidants of plant origin. Plants containing phenolic compounds have been reported to possess strong antioxidant activity. The objective of this study is to evaluate total polyphenols and flavonoids contents (TPC and TFC) as well as examine the *in vitro* antioxidative properties from aqueous extract of *Ammoides atlantica* (AqE). TPC was estimated utilizing Folin-Ciocalteu's reagent. TFC was evaluated utilizing the aluminum chloride method. The antioxidant properties were evaluated using metal chelating and cupric ion reducing antioxidant capacity (CUPRAC) assays. Indeed, results showed that the AqE is rich in polyphenols (141.74±0.44 µg gallic acid equivalents/ mg of dry weight), and flavonoids (61.87±6.7 µg quercetin equivalent/ mg dry weight). These phytochemical compounds possess significant antioxidant activities. The results showed that AqE exhibited a good Metal chelating activity with an IC_{50} of 36.57±4.73 µg/ mL. CUPRAC assay showed that AqE extract exhibited high cupric ion reducing antioxidant capacity with an $A_{0.5}$ of 8.58±0.13 µg/mL. These findings provide evidence that AqE of *Ammoides atlantica* is a potential source of antioxidant which have many benefits towards human health.

Keywords: *Ammoides atlantica*, aqueous extract, phenolic compounds, metal chelating and cupric ion reducing antioxidant capacity.

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1. INTRODUCTION

Oxidative stress is generally characterized by the excess formation of reactive molecules such as ROS (reactive oxygen species). *In vivo*, some of these ROS play a positive role such as energy production, phagocytosis, regulation of cell growth and intracellular signaling [1]. However, ROS are known to be the major cause of various chronic and degenerative diseases, including aging, coronary heart disease, inflammation, diabetes mellitus and cancer [2], and can cause cellular injuries and initiate peroxidation of fatty acids in biological membranes. ROS may damage protein [3], DNA [4], and enzymes [5]. The antioxidant compounds possess anticarcinogenic, antitumor, anti-inflammatory, antiatherosclerotic, antiviral and antibacterial activities [6]. Many plant species have been attractive to scientists as natural sources of compounds that are safer than the synthetic ones. Plant-derived antioxidants, especially, the phenolics have gained considerable importance due to their potential health benefits. Previous studies have shown that plant foods containing antioxidants are advantageous to health as it down-regulates certain degenerative processes and can significantly reduce the occurrence of cardiovascular and cancer diseases [7]. The *Ammoides atlantica* (coss. et Dur.) Wolf, of the family Apiaceae, is widespread in the Mediterranean region and it is endemic in Algeria [8]. Traditionally, this plant is known to be used for the therapy of fever and headache, besides its use as antidiarrheic [9]. This study aims to investigate the *in vitro* antioxidant Activities of

Ammoides atlantica aqueous extract using metal chelating and cupric ion reducing antioxidant capacity (CUPRAC) assays, besides to evaluate total polyphenol and flavonoids contents.

2. MATERIALS AND METHODS

2.1. Plant material

2.1.1. Plant collection and identification

Ammoides atlantica was harvested at the flowering stage from Jijel north-eastern of Algeria during spring. Aerial parts were dried in shadow at room temperature then powdered and stocked in darkness until use. The authenticity was confirmed by Pr Laouar Hocine (Department of Vegetal Biology and Ecology, University Farhat Abbas Setif 1).

2.1.2. Extraction procedure

The extraction process was done according to a method of [8]. 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 [10].

2.2. Determination of total phenolic and flavonoid contents

2.2.1. Total phenolic content (TPC)

The total phenolic content of AqE extract was determined spectrophotometrically using the Folin-Ciocalteu method [11] with some modifications. In a brief description, 100µl of 1:10 Folin-Ciocalteau reagent and 75 µl of sodium carbonate (7.5%) were added to 20 µl of aqueous extract. After 2 h of incubation in the dark at ambient temperature, the absorbance at 765 nm was measured by a microplate reader, against a control. The total polyphenol content was determined as micrograms of gallic acid equivalent per milligram of extract (µg GAE/ mg).

2.2.2. Total flavonoids content (TFC)

TFC was evaluated utilizing the aluminum colorimetric method [12] with some modifications. A volume of 130 µl of methanol was transferred into a micro-plate (96 wells) containing 50 µl of AqE and then 10 µl of potassium acetate (1 M) and 10 ml of aluminum nitrate at 10 % were added. After period incubation for 40 min at ambient temperature, the absorbance was read at 415 nm by a micro-plat reader. The standard calibration curve of quercetin at various concentrations was utilized to calculate total flavonoid concentration. The results were presented as micrograms of quercetin equivalent per milligram of extract (µg QE/ mg).

2.3. Antioxidant activity assays

2.3.1. Metal chelating activity assay

The metal chelating activity by the ferrene–Fe^{2+} complexation assay measured spectrophotometrically [13, 14] with slight modifications. 40 µl of the extract were added to 40 µl of 0.2 mM $FeCl_2$. The reaction was initiated after the addition of 80 µl of ferene solution (0.5 mM). The obtained mixture was shaken then incubated at room ambient for 10 min. The absorbance was read at 593 nm. The metal chelating potential was estimated by the utilize of the following equation. The results were given as IC_{50} value (µg/ml) (50 % inhibition):

$$\text{Metal chelating activity (\%)} = [(A_{Control}-A_{Sample})/A_{Control}]\times 100$$

2.3.2. Cupric reducing antioxidant capacity (CUPRAC) assay

The CUPRAC was determined according to the method of [15]. In each well, the reaction mixture containing 40 µl of sample and 50 µl of a copper (II) chloride solution, 50 µl of a neocuproine alcoholic solution, and 60 µl of ammonium acetate aqueous buffer at pH 7 was combined to give a final volume of 200 µl. After 30 minutes, the absorbance was measured at 450 nm. Results were recorded as absorbance ($A_{0.5}$) compared with the absorbance of BHA and BHT, which were used as antioxidant standards.

2.4. Statistical analysis

All data were the average of triplicate analyses. Data were recorded as the mean ± standard deviation. Analysis of variance was executed using Student's t-test or one-way analysis of variance (ANOVA) with the aid of Graph Pad Prism 7.00. p values < 0.05 were regarded as significant.

3. RESULTS

3.1. Total phenolics and flavonoids contents

Our results showed that the *Ammoides atlantica* aqueous extract (AqE) had high polyphenol (141.74±0.44 µg GAE/ mg dry extract) and flavonoid (61.87±6.7µg QE/ mg dry extract) contents. (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 activity

3.2.1. Metal chelating activity

The antioxidative potential was observed in *Ammoides atlantica* aqueous extract (AqE) using a metal chelating test as shown in Table 1. This assay showed that the AqE had a strong antioxidant activity with an IC_{50} of 36.57±4.73 µg/mL (Table 1).

3.2.2. Cupric reducing antioxidant capacity (CUPRAC)

CUPRAC assay showed that the *Ammoides atlantica* AqE exhibited a good effect with an $A_{0.5}$ of 8.58±0.13 µg/mL. This cupric reducing antioxidant capacity from *Ammoides atlantica* AqE was similar to that of BHT synthetic antioxidant (P>0.05, no significant difference). But this activity is relatively lower compared to the BHA ($p < 0.0001$) as standard (Table 1).

Table 2: Antioxidant activities of *Ammoides atlantica* aqueous extract (AqE). ^{ns} : no significant difference and **** $p < 0.0001$ compared to correspondent standards. AqE: aqueous extract, BHA: butylated hydroxyanisole, BHT: butylated hydroxytoluene, and EDTA: Ethylenediaminetetraacetic acid.

Extract/standard	IC_{50} (µg/mL)	$A_{0.5}$ (µg/mL)
	Metal chelating activity	Cupric reducing antioxidant capacity
AqE	36.57±4.73	8.58±0.13
BHA	/	3.64±0.19 ****
BHT	/	9.62+0.87^{ns}
EDTA	12.11+0.32****	/

4. DISCUSSION

In the current study, the antioxidant activity of *Ammoides atlantica* AqE was evaluated by using metal chelating and CUPRAC assays. Metal ion chelating activity of an antioxidant molecule prevents oxyradical generation and the consequent oxidative damage [16]. Metal ion chelating capacity plays a significant role in antioxidant mechanisms since it reduces the concentration of the catalyzing transition metal in lipid peroxidation [17]. In the presence of chelating agents, the ferrozine-Fe^2 complexes are disrupted, resulting in a decrease in the red color of the complex. *Ammoides atlantica* AqE exhibited a good metal chelating activity. This activity could be attributed to the richness of AqE in polyphenols and flavonoids. Phenolic compounds have been reported to be chelators of free metal ions [18]. CUPRAC method is based on the reaction of an electron transfer, thus the oxidant is reduced, which monitored by a color change [19]. In this assay, the *Ammoides atlantica* aqueous extract demonstrates a strong antioxidant effect. This cupric reducing antioxidant capacity could be due to phenolic and flavonoid contents in AqE. Several authors have reported that the antioxidant capacity depends on the amount of phenolic compounds of plant extracts. [20, 21]. The phenolic compounds acting as hydrogen donors, free radical acceptors, chain oxidation reaction interrupters or metal chelators [22]. This finding of the antioxidant capacity of *Ammoides atlantica* is in agreement with other studies [23, 24, 25].

5. CONCLUSION

This work revealed that aqueous extract of the aerial parts of *Ammoides atlantica* contains high levels of phenolics and flavonoids, and possesses significant antioxidant activities which may due to the presence of polyphenolic compounds. These findings provide scientific support for the traditional uses of *Ammoides atlantica*. It is also suggested that *Ammoides atlantica* be viewed as a potential source of natural antioxidants that can provide precious functional ingredients useful for the prevention of diseases related to oxidative stress.

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