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Research Article

Antioxidant Activity and Polyphenols Content of Hydromethanolic Extract from *Athamanta sicula* L.

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ABSTRACT

Free radicals or highly reactive oxygen species are capable of inducing oxidative damage to the human body. Antioxidants are the compounds which terminate the attack of reactive species and reduce the risk of diseases. Plants containing phenolic compounds have potent antioxidant capacity. The objective of this study is to evaluate total polyphenols and flavonoids contents (TPC and TFC) as well as examine the *in vitro* anti-oxidative properties from hydromethanolic (Crude) extract of *Athamanta sicula* L. (ASCE) TPC and TFC were estimated by Folin-Ciocalteu and aluminium chloride methods, respectively. The antioxidant activity was evaluated using phenanthroline and ABTS assays. The results showed that the *Athamanta sicula* L. ASCE was rich in total polyphenols (149.58 ± 0.77 µg gallic acid equivalent/mg of the dry weight of plant extract) and flavonoids (40.48 ± 3.97µg quercetin equivalent/mg of the dry weight of plant extract). ASCE showed high ABTS radical scavenging activity with an IC_{50} of 31.14±1.78µg/mL. Phenanthroline assay showed that the ASC Eextract exhibited a strong effect with an $A_{0.5}$ of 167.2±20 µg/mL. These findings provide evidence that *Athamanta sicula* L. is a potential source of natural antioxidants.

INTRODUCTION

The human body produces reactive oxygen species (ROS), such as superoxide anion radical, hydroxyl radical and hydrogenperoxide by many enzymatic systems through oxygen consumption. In normal amounts, these ROS are beneficial as growth regulators and signal transducers [1]. However, during oxidative stress, large amounts of these ROS can be produced and maybe dangerous because of their ability to attack numerous molecules, including proteins and lipids. In fact, it has been reported that ROS contributes largely to cellular aging, mutagenesis, and coronary heart disease through sever always including membrane destabilization, DNA breakage and generally by oxidizing low-density lipoproteins (LDL) [2].

The cell can reduce the impact of ROS either by an endogenous system or by an exogenous system using antioxidants [3].

Antioxidants are compounds that can delay or inhibit the oxidation of lipids and other molecules and by doing so inhibit the initiation and propagation of oxidative chain reactions [4].Previous findings clearly show that the consumption of plant-derived polyphenolic compounds and natural antioxidant supplements may be used to protect the body against various diseases, including cancer, cardiovascular, and neurodegenerative diseases. Natural antioxidants help the endogenous antioxidant system to reverse oxidative damage or protect oxidative stress-induced deterioration [5]. Phenolic compounds and flavonoids have been found to have therapeutic applications against different diseases caused by oxidative stress, and several researchers demonstrated the correlation between polyphenolic compounds and the antioxidant activity of plant extracts [6]. *Athamanta sicula* L. (coss. et Dur.) Wolf,

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belonging to the Apiaceae family is endemic in Algeria [7].It is known as a diuretic, besides, to its use in dissolving kidney stones and in treating urinary tract diseases [8]. In the present study, we examined the antioxidant capacity of hydromethanolic (Crude) extract (ASCE) from this plant using phenanthroline and ABTS tests. Total polyphenol and flavonoid contents were also evaluated in the ASCE.

MATERIALS AND METHODS

Plant Material

Plant Collection and Identification

The plant *Athamanta sicula* L. (aerial parts) was collected at the Jijel region in the North-Eastern of Algeria, during the flowering period, and identified by Prof H. Laouer, Department of Ecology and Vegetal Biology, Faculty of Nature and Life Sciences, University Setif 1. A voucher number 202 AS 15/6/17 JIJ/SA/HL was deposed at the laboratory of Phytotherapy Applied to Chronic Diseases.

Extraction Procedure

The preparation of the *Athamanta sicula* L. Extract was given out according to the method of [9]. Hydromethanolic (Crude) extract (ASCE) was prepared by macerating 100g of dried plant material in 1L of methanol 80% (v/v) for three days at room temperature. Then the solution was filtered through Whatman filter paper no 1. The dried ASCE extract thus obtained was stored at 20°C for screened of its pharmacological properties.

Determination of Total Phenolic and Flavonoid Contents

Total Phenolic Content

The total phenolic content of ASCE was estimated spectrophotometrically using the Folin–Ciocalteu method [10]. A volume of 20 µl of the extract was added to 100 µl of Folin–Ciolcalteu reagent (10%). Then, a volume of 75 µl of sodium carbonate solution (7, 5%) was added. The obtained mixture was incubated for 2 h in darkness at ambient temperature. The absorbance was determined at 765 nm. Gallic acid was used as a reference to establish the calibration curve from which the concentration of polyphenols was calculated and the results were expressed in micrograms equivalent of gallic acid per milligram of extract (µg GA/mg of extract).

Total Flavonoids Content

The quantification of the total flavonoid content was performed by the trichloro-aluminum method [11] with some modifications. Briefly, 130 µl of methanol were added to 50 µl of a sample (1mg extract/1ml water). Subsequently, 10 µl of 1M potassium acetate and 10 µl of 10% aluminum nitrate were added. The mixture obtained was incubated at room temperature for 40 minutes. Absorbance was read at 415 nm. Quercetin at different concentrations was used to realize the calibration curve to estimate the concentration of flavonoids found in the aqueous extract and the results have been given in micrograms equivalent of quercetin per milligram of extract (µg EQ/mg of extract).

Antioxidant Activity Assays

Phenanthroline Assay

This test was carried out according to the method described by Szydłowska-Czerniak *et al*. [12]. A volume of 10 µl of a sample at different concentrations was placed into a 96 well round-bottomed plate. Then, 50 µl of $FeCl_3$ (0.2%), 30 µl of phenanthroline (0.5%), and finally, 110 µl of methanol were added. The microplate was incubated 20 minutes at 30°C in a dark. The absorbance of the solution was measured at 510 nm. BHT and BHA were used as antioxidant standards.

ABTS Radical Cation Decolorization Assay

The spectrophotometric analysis of $ABTS^{\bullet+}$ scavenging activity was determined according to the method of Re *et al*. [13]. After the preparation of the oxidation solution of ABTS, the $ABTS^{\bullet+}$ solution was diluted to get an absorbance of0.700±0.020 at 734 nm with water. Then, 160µl of ABTS solution was added to 40 µl of a sample at different concentrations. After 10 min, the absorbance was measured at 734 nm. Water was used as a control. BHA and BHT were utilized as antioxidant references for comparison of the activity. The results were given as the IC_{50} (µg/ml), which was calculated utilizing the following equation:

$ABTS^{\bullet+}$ Scavenging effect (%) = $[(A_{Control}-A_{Sample})/A_{Control}] \times 100$.

Statistical Analysis

All tests were carried out in triplicate and the results were calculated by the mean±SD. The statistical interpretation was performed by the help of Student's t-test or by one-way analysis of

variance (ANOVA) using GraphPad Prism7.00. P values < 0.05 were regarded as significant.

RESULTS

Total Phenolics and Flavonoids Contents

The results presented in Table 1, showed that the ASCE contained high total polyphenols and flavonoids (149.58 ± 0.77 µg gallic acid equivalents/mg of dry weight) and (40.48 ± 3.97 µg quercetin equivalent/mg dry weight), respectively.

Table 1: Total polyphenols and flavonoids content of ASCE extract.

Extract	Total phenolic content [(a)]	Total flavonoid content [(b)]
ASCE	149.58 ± 0.77	40.48 ± 3.97

ASCE: *Athamanta sicula* L. hydromethanolic (Crude) extract, [(a)]: µg GAE/mg and [(b)]: µg QE/mg

Antioxidant Activity

Phenanthroline Activity

The antioxidative activity was observed in *ASCE* using phenanthroline test as shown in Table 2. This assay showed that the ASCE had a strong antioxidant activity with an $A_{0.5}$ of 167.2±20µg/mL.

ABTS Radical Cation Decolorization Activity

ABTS Scavenging assay showed that the ASCE exhibited a good effect with an IC_{50} of 31.14 ± 1.78 µg/mL (Table 2). This suggests a significant antioxidant activity of ASCE.

Table 2: Antioxidant activities of ASCE.

Extract/ standard	ABTS scavenging activity	Antioxidant activity by phenanthroline assay
	IC_{50} (µg/mL)	$A_{0.5}$ (µg/mL)
ASCE	31.14 ± 1.78 ****	167.2±20****
BHA	1.81±0.10	7.13±1.01
BHT	1.29+0.30	12.14+0.79

**** p <0.0001 compared to corresponding standards. ASCE: Cude (Hydromethanolic) extract, BHA: butylatedhydroxyanisole, BHT: butylatedhydroxytoluene and ABTS: 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid).

DISCUSSION

Free radicals are thought to contribute to several disorders in the body. Hydroxyl radical (OH•) is extremely reactive, more toxic than other radical species and can attack biologic molecules such as DNA, proteins and lipids. Thus, the scavenging ability of hydroxyl radicals is widely accepted as a way to evaluate the potential of antioxidants [14]. Antioxidants are a group of compounds that inhibit oxidation and reduce free radicals directly or indirectly. Oxidative stress may be alleviated *in vivo* by exogenous administration of antioxidants. Some synthetic antioxidants showed potential adverse effects on the body. Thus, research attention is turning to find safe, effective, and natural antioxidants to resist oxidative stress [15]. There is a growing interest in natural antioxidants such as polyphenols, present in medicinal and dietary plants that could help prevent oxidative damage. The ASCE was assessed for its possible antioxidative activities by employing two complementary tests, phenanthroline (Phen) and ABTS methods. The Phen assay is based on the capacity of antioxidants (reductants) to reduce Fe^{3+} to Fe^{2+} [16]. ASCE showed high metal chelating capacity using the Phen test. Phenolic compounds have been reported to be chelators of free metal ions [17].To assay the ABTS radical scavenging of ASCE, cationic ABTS radical decolorization was carried out. The ABTS radical is relatively stable but readily reduced by antioxidants. The scavenging activity against cationic ABTS radical indicates the ability of the extract to act as electron donors or hydrogen donors in free radical reactions [18]. ASCE exhibited a strong scavenging capacity on ABTS. These antioxidant effects of ASCE are very probably attributed to its high phenolic compounds and flavonoids [19]. In fact, antioxidant activities are partially linked to the presence of phenolic compounds [20]. Also, the literature showed that a good correlation was found between antioxidant activity and the content of polyphenols and flavonoids [21].

CONCLUSION

This study demonstrated that *Athamanta sicula* L. aerial part contains high levels of phenolic compounds and was capable of directly quenching free radicals to terminate the radical chain reaction and acts as reducing agents. Phenolic compounds found in this plant are probably responsible for its antioxidant potential. These results explain the pharmacological properties of *Athamanta sicula* L. and gave a scientific base of the use of this plant in the Algerian traditional medicine. Further researches needed to identify and isolate the active principles present in this extract which could be useful for pharmaceutical purposes.

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