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IN VITRO ANTIOXYDANT ACTIVITY AND TOTAL PHENOLIC CONTENT OF EXTRACTS FROM THE ENDEMIC *ARGANIA SPINOSA* (L.) SKEELS FROM ALGERIAN SAHARA

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ABSTRACT

The BuOH fractions prepared from 80% MeOH extract of the leaves from the endemic Saharan tree *Argania spinosa* (L.) Skeels exhibited the most potent antioxidant capacity (IC_{50} = 2.41 ± 0.38 µg/mL for DPPH and 7.95 ± 0.54 µg/mL for superoxide anion radical), compared to the EtOAc and $CHCl_3$ fractions. The results showed that BuOH extract fraction exhibited a strong antioxidant activity and had the most potent scavenging abilities which may be correlated to the presence of Phenolic compounds.

Keywords: *Argania spinosa*, Endemic, Sahara, Algeria, Antioxydant, Total Phenolic.

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1. INTRODUCTION

The studies on finding antioxidants from natural sources have become a subject of great interest in many areas, due to the oxidative damage caused by free radicals and reactive oxygen species. They are recognized to be involved in the pathogenesis of various diseases such as atherosclerosis, cancer, diabetes mellitus and reperfusion disorder [1-3].

It is well known that there is a direct relationship between the content of total phenolics and antioxidant capacity of plants. Thus, rich phenolic plant extracts are effective to counteract deleterious action of radical [4-6] and sometimes plant extracts have better antioxidant activities than those of pure molecules and there is a growing interest for the use of plant extracts as bioactive agent [7, 8].

Argania spinosa (L.) Skeels (Argan tree), is a woody species belonging to the Sapotaceae family and is endemic to Algeria and Morocco [9, 10]. In the southwestern Algeria, the argan tree covers a relatively area of 3 000 hectares, where it is the second most common tree after *Acacia raddiana* [11, 12].

In local traditional ethnopharmacopeae, the Argan oil has been widely used for treating gastrointestinal tracts, respiratory difficulties, rheumatism, skin diseases and for nourishing hair [13-16].

Recent studies from Moroccan team suggested that dietary argan oil could protect against cancer, atherosclerosis, improve plasma lipid profiles, paraoxonase activities and LDL peroxidation [16-19]. However, there is less study on the *Argania spinosa* leaves [20].

As a part of our investigation into medicinal plants growing in Algerian Sahara [6, 21-26]; In this study we investigate for the first time the antioxydant activity and compare phenolic and flavonoids contents of fraction from 80% MeOH extracts from the leaves of *Argania spinosa* (L.) Skeels an endemic Saharan tree.

2. RESULTS AND DISCUSSION

DPPH and superoxide anion radical have been widely used in the determination of antioxidant activity of single compounds, as well as of different plant extracts [26, 27]. The IC_{50} values (μg/mL) for radical scavenging activities tests of liquid-liquid fractionalisation of the crude

80% MeOH extract along with $CHCl_3$, EtOAc and *n*-BuOH extracts from leaves of *Argania spinosa* leaves (Sapotaceae) are summarized in Table 1.

Compared to the $CHCl_3$ and EtOAc extracts from leaves of *Argania spinosa*, the IC_{50} values of the n-BuOH extract exhibited the most potent antioxidant capacity. Thus, this extract has an important role in scavenging abilities of various radicals and IC_{50} values of antioxidant are 2.41 ± 0.38 μg/mL and 7.95 ± 0.54 μg/mL respectively for DPPH and superoxide anion radical.

Table 1. Radical scavenging activities (DPPH and superoxide) of fractions from 80% MeOH extract of *Argania spinosa leaves*

Fraction	**IC_{50} ***	
	DPPH	**Superoxide**
$CHCl_3$	27.63 ± 1.3	19.80 ± 1.6
EtOAc	5.07 ± 0.30	9.75 ± 0.21
n-BuOH	2.41 ± 0.38	7.95 ± 0.54

* IC_{50} expressed as μg/mL; Data expressed in Mean ± SD from triplicate experiments.

These results showed that the EtOAc and *n*-BuOH fractions from 80% MeOH extract of *Argania spinosa leaves* were more enriched in antiradical compounds, which suggests that phenolic derivatives present in these extracts are responsible for the scavenging activity (Table 2). According to the literature, there is a highly positive relationship between total phenols and antioxidant activity and the plant phenolic derivatives are one of the major groups of compounds having multiple biological effects and acting as antioxidants [5, 28].

The total phenolic contents of the liquid-liquid fractionalisation of the crude 80% MeOH extract from *Argania spinosa leaves*, as determined by the Folin and Ciocalteu reagent, are : 7.53 ± 0.2, 87.35 ± 1.7 and 169.21 ± 1.3 mg GAE/g extract, respectively for $CHCl_3$, EtOAc and *n*-BuOH fractions. The maximum PTC (169.21 ± 1.3 mg GAE/g extract) and TFC (14.6 ± 0.4 mg RE/g extract) in n-BuOH fraction, suggesting that this fraction extract was more enriched in phenolic derivatives that are responsible for the high scavenging activity. The present results indicate that there is a positive correlation between total antioxidant

activity and the total phenolic and flavonoids contents.

Table 2. TCP and TFC of fractions from 80% MeOH extract of *Argania spinosa leaves*

Fraction	TCP (GAE, mg/g)	TFC (RE, mg/g)
$CHCl_3$	7.53 ± 0.2	ND*
EtOAc	87.35 ± 1.7	9.4 ± 0.3
n-BuOH	169.21 ± 1.3	14.6 ± 0.4

GAE: Gallic Acid Equivalents; RE: Rutin Equivalents

* ND: Not Determined, Data from triplicate experiments.

It has been shown in phytochemical analysis of extracts from Argan leaves, that tannins constitute 14% of the dry leaves and the hyperoside and myricitrin were the major flavonoid components [11, 20]. The results presented in this primary study need to be conducted as bioguided results for the isolation and identification of phenolic derivatives present in *Argania spinosa* leaves.

3. EXPERIMENTAL

3.1. Plant material

The leaves of *Argania spinosa* were collected from Tindouf (south west Algeria) in March 2016. The plant was identified by Pr A. Marouf (Department of Biology, University Center Naama – Algeria) and a voucher specimen is kept in the Herbarium of POSL Laboratory, (UTMB, Algeria) under N° CA 16/01.

3.2. Preparation of the extracts

Dried and powdered leaves (300 g) of *Argania spinosa* were exhaustively extracted with 80% MeOH solution in Soxhlet apparatus for 6 h. The obtained hydro-alcoholic extract was concentrated by a rotary evaporator and diluted with water (150 mL). The obtained solution was extracted successively by liquid/liquid partition with solvents of increasing polarity: $CHCl_3$, EtOAc and *n*-BuOH. The organic layers were dried with Na_2SO_4, giving after removal of solvents under reduced pressure $CHCl_3$ (1.97 g), EtOAc (4.12 g) and *n*-BuOH (6.71 g) extracts.

3.3. Antioxidant activity

3.3.1. *DPPH radical scavenging activity*

Free radicals scavenging activity of the extracts obtained from leaves of *Argania spinosa* is determined by use of DPPH (1,1-diphenyl-2-picryl hydrazyl) based on our pervious works [8]. A solution of 0.2 mM DPPH in methanol was prepared and 1 ml of this solution was mixed with 1 ml of extract in methanol (with different concentrations 5 to 200 µg/ml). The reaction mixture was shaken vigorously and allowed to stand in the dark for 30 min at room temperature. The DPPH radical scavenging activity was determined by measuring spectrophotometrically the absorbance at 517 nm with a Unicam UV 300 spectrophotometer, using a 10 mm quartz cuvette. All measurements were made in triplicate and ascorbic acid and quercetin were used as references for comparison. The DPPH radical scavenging activity I (%) of the sample was calculated using the following equation:

$$I\ (\%) = [1 - Ab_s / Ab_c]\ x100$$

Where Ab_s is the absorbance of the plant extract containing DPPH, Ab_c is the absorbance of blank solution of DPPH without the sample.

The IC_{50} value which was defined as the concentration (in µg/mL) of the extract necessary to decrease the absorbance of DPPH by 50% was calculated from the data obtained by sigmoid non-linear regression model using plots.

3.3.2. *Superoxide anion radical scavenging activity*

The superoxide anion radical scavenging activity was determined by NBT (nitro blue tetrazolium) reduction method as described early [8, 29]. The leaves extract of *Argania spinosa* at 50, 100 or 150 µg/mL was mixed with 5 mL of 0.05 M of sodium carbonate buffer solution (pH 10.2) containing 1.3 µM riboflavin, 0.02 M methionine and 5.6 µM NBT. After 30 min at light the absorbance was then measured at 560 nm. The superoxide anion radical scavenging activity (%) was calculated according to the equation:

$$\%\ \text{Inhibition} = [1 - Ab_s / Ab_c] \times 100$$

Where Ab_s and Ab_c are the absorbance of sample and blank control (mixture without any sample) respectively.

3.4. Determination of Total Phenolic Content (TPC)

The total phenolic contents (TPC) were determined according to the early described procedures [8, 30] by using a Folin and Ciocalteu's phenolic reagent. The extract solution (1 ml) was mixed with 2 ml of Folin and Ciocalteu's reagent and allowed to react for 3 min. Then, 2 ml of saturated sodium carbonate solution was added to the mixture and it was adjusted to 10 ml with distilled water. The reaction mixture was stand for 1 h before the absorbance was read at 760 nm (spectrophotometer UV-Unicam 300). Gallic acid was used as a standard phenolic compound and the results were expressed as mg of gallic acid equivalents/g of extract (mg GAE/g extract).

3.5. Determination of Total Flavonoids Content (TFC)

The total flavonoid contents (TFC) in the extracts were estimated spectrophoto-metrically [31], briefly, 1 ml of the extract solution added to a test tube which contained 4 ml of distilled water, and then added 0.4 ml of 5% sodium nitrite solution and allowed to stand. After 5 min, 0.8 ml of 10% aluminium chloride was added and allowed to react for 5 min, then 2 ml of sodium hydroxide solution (15%) was added and the mixture was diluted with another 2 ml of distilled water. The absorbance of the mixture at 510 nm was measured immediately. Rutin was used for constructing the standard curve and flavonoids content was expressed as mg of rutin equivalents/g of extract (mg RE/g extract)

4. CONCLUSION

From the foregoing results it can be concluded that both *n*-BuOH and EtOAc extracts fractions from the 80% MeOH extract of leaves of the Saharan endemic tree *Argania spinosa*, possess antioxidant activity. The n-BuOH extract exhibited a strong antioxidant activity with IC_{50} values 2.41 ± 0.38 μg/mL and 7.95 ± 0.54 μg/mL respectively for DPPH and superoxide anion radical.

The antioxidant activity of the *Argania spinosa* leaves extracts may be caused by the presence phenolic and suggests that the extracts obtained by polar solvents from the leaves could be used as an effective natural source of antioxidant.

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