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# Total phenolic content and in vitro antioxydant activity of extracts from the endemic medicinal plant warionia saharae

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## ABSTRACT

The AcOEt fractions prepared from 80% EtOH extract of the endemic Saharan medicinal plant *Warionia saharae* exhibited the most potent antioxidant capacity ($IC_{50}$ = 3.08 ± 0.40 µg/mL for DPPH and 8.95 ± 0.23 µg/mL for superoxide anion radical), compared to the *n*-BuOH and $CHCl_3$ fractions. Thus, this extract fraction exhibited a strong antioxidant activity and had the most potent scavenging abilities which may be caused by the presence of Phenolic compounds. 

## KEYWORDS

Antioxydant;
Total Phenolic;
Warionia saharae;
Endemic;
Sahara;
Algeria.

## INTRODUCTION

Oxidative damage is caused by free radicals and reactive oxygen species, mostly generated endogeneously, they are recognized to be involved in the pathogenesis of various diseases such as atherosclerosis, cancer, diabetes mellitus and reperfusion disorder[1]. Recently some synthetic antioxidants such as BHT (butylated hydroxyl toluens) and BHA (butylated hydroxyl anisole) have been suspected to dangerous to human health. Therefore, there is great interest in finding antioxidants from natural sources, which could be used in medicine and additive to nutraceuticals to prevent such deleterious effect and to minimize oxidative damage to cells[2-5].

A direct relationship has been found between the content of total phenolics and antioxidant capacity of plants and phenolic compounds, naturally distributed in plants, are effective to counteract deleterious action of ROS[6,7]. It is noted that, plant extracts sometimes have better antioxidant activities than those of pure molecules and there is a growing interest for the use of plant extracts as bioactive agent[8].

As a part of our investigation into medicinal plants growing in Algerian Sahara[9-14]; In this study we investigate the antioxydant activity and compare phenolic and flavonoids contents of extracts from the aerial part of the *Warionia saharae* an endemic Saharan specie.

*Warionia saharae* Benth & Coss. (local name " Efessas or Kabar Lem'aiz") a genus of the family Asteraceae, is an endemic herbaceous medicinal plant represented by only one species which is widely distributed in the south west of Algeria and south east of Morocco[15]. The aerial part of this plant was used in Sahara folk medicine for treating gastrointestinal tracts, icter and as anti-inflammatory[16-20].

## EXPERIMENTAL

### Plant material

The leaves of *Warionia saharae* were collected from Bechar (south west Algeria) in May 2008. The plant was identified by Pr A. Marouf (Department of

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Biology, University Es Senia, Oran – Algeria) and a voucher specimen is kept in the Herbarium of POSL Laboratory, Faculty of Sciences (University of Bechar, Algeria) under N° CA 02/07.

**Preparation of the extracts**

Dried and powdered leaves (500 g) of *Warionia saharae* were exhaustively extracted with 80% EtOH solution in Soxhlet apparatus for 6 h. The obtained hydro-alcoholic extract was concentrated by a rotary evaporator and diluted with water (200 mL). The resulting 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 $CHCl_3$ (2.30 g), EtOAc (3.10 g) and *n*-BuOH (5.91 g) extracts.

**Antioxidant activity**

**DPPH radical scavenging activity**

The stable radical DPPH° (1,1-diphenyl-2-picryl hydrazyl) was used for determination of free radicals cavenging activity of the extracts obtained from leaves of *Warionia saharae* based on the described methods[21-23], with minor modifications. 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 control contained all reagents without the sample was used as blank. 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:

$$\mathbf{I\ (\%) = [1 - Ab_s / Ab_c] \times 100}$$

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.

**Superoxide anion radical scavenging activity**

The superoxide anion radical scavenging activity was determined by NBT (nitro blue tetrazolium) reduction method as described early[23-25] with minor modifications. The superoxide anion radical reduces the yellow dye $NBT^{2+}$ to produce the blue formazan, whose absorbance was measured at 560 nm. The antioxidants compounds are able to inhibit the formation of purple NBT. The extract of *Warionia saharae* 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:

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

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

**Determination of total phenolic content (TPC)**

The total phenolic contents (TPC) were determined according to the early described procedures [26] with the slight modification of using a Folin and Ciocalteu's phenolic reagent. Briefly, 1 ml of extract solution 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).

**Determination of total flavonoids content (TFC)**

The total flavonoid contents (TFC) in the extracts were estimated spectrophoto-metrically according to the literature[27], with minor modifications. 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)

**TABLE 1 : Radical scavenging activities (DPPH and superoxide) of fractions from EtOH extract of *Warionia saharae***

| Fraction (From 80% EtOH extract) | $IC_{50}$* | |
|---|---|---|
| | DPPH | Superoxide |
| $CHCl_3$ | 53.50 ± 9.05 | 25.2 ± 1.1 |
| EtOAc | 3.08 ± 0.40 | 8.95 ± 0.23 |
| n-BuOH | 4.40 ± 0.43 | 10.25 ± 0.63 |

*** $IC_{50}$ expressed as μg/mL**

## RESULTS AND DISCUSSION

In this work, we investigated for the first time the antioxidant activity of organic extracts from *Warionia saharae* Benth & Coss. (Asteraceae), a Saharan endemic medicinal specie. The relatively stable organic radical, 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[28,29]. The $IC_{50}$ values (μg/mL) for radical scavenging activities tests of liquid-liquid fractionalisation of the crude 80% EtOH extract along with $CHCl_3$, EtOAc and *n*-BuOH extracts from leaves of *W. saharae* are summarized in TABLE 1.

According to the $IC_{50}$ values presented in TABLE 1, the AcOEt extract from leaves of *W. saharae* exhibited the most potent antioxidant capacity, compared to the $CHCl_3$ and *n*-BuOH extracts. Thus, this extract has an important role in scavenging abilities of various radicals and $IC_{50}$ values of antioxidant activities followed 3.08 ± 0.40 μg/mL for DPPH and 8.95 ± 0.23 μg/mL for superoxide anion radical.

Data expressed in Mean ± SD (standard deviation) from triplicate experiments.

These results using the DPPH and superoxide anion radicals, suggesting that the EtOAc and *n*-BuOH fractions were more enriched in antiradical compounds, which suggests that phenolic derivatives present in these extracts are responsible for the scavenging activity of *W. saharae* (TABLE 2). Literature surveys indicated that plant phenolic derivatives are one of the major groups of compounds having multiple biological effects and acting as antioxidants[30,31]. Thus, a highly positive relationship between total phenols and antioxidant activity was found in many plant species[32].

**TABLE 2 : TCP and TFC of fractions from EtOH extract of Warionia saharae**

| Fraction (From 80% EtOH extract) | TCP (GAE, mg/g) | TFC (RE, mg/g) |
|---|---|---|
| $CHCl_3$ | 6.2 ± 0.5 | ND* |
| EtOAc | 143 ± 1.2 | 11.6 ± 0.5 |
| n-BuOH | 63.25 ± 1.1 | 5.7 ± 0.14 |

**GAE: Gallic Acid Equivalents; RE: Rutin Equivalents**

*** ND: Not Determined, Data from triplicate experiments.**

The total phenolic contents of the liquid-liquid fractionalisation of the crude 80% EtOH extract from leaves of *W. saharae*, as determined by the Folin and Ciocalteu method, are : 6.2 ± 0.5, 143 ± 1.2 and 63.25 ± 1.1 mg GAE/g extract, respectively for $CHCl_3$, EtOAc and *n*-BuOH fractions. The maximum PTC (143 ± 1.2 mg GAE/g extract) and FTC (11.6 ± 0.5 mg RE/g extract) in EtOAc fraction suggesting that this fraction extract was more enriched in phenolic derivatives that are responsible for the high scavenging activity. As can be seen in TABLE 1 and 2, the current results indicate that there is a positive correlation between total antioxidant activity and the total phenolic and flavonoids contents.

It was reported that the antioxydant activity could be due to the action of a free hydroxyl group of phenolic compounds. Specially, flavonoids with polyhydroxylated substitution on ring A or B and a free 3-hydroxyl substitution and the lower strength of the O–H bond present in these compounds corresponds to a higher scavenging activity[33,34]. However, these compounds are known to act as antioxidants not only because of their ability to donate hydrogen or electrons but also because they are stable radical intermediates[35]. These results show that in general, the rich-flavonoids plants could be a good source of antioxidants that would help to increase the antioxidant capacity of an organism, protect it against lipid peroxidation and could have a direct action on different diseases in relation with ROS[36].

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## CONCLUSION

The results obtained in this study clearly showed that both EtOAc and *n*-BuOH extracts fractions from the 80% EtOH extract of leaves of the Saharan endemic medicinal specie *Warionia saharae*, possess antioxidant activity. The EtOAc extract exhibited a strong antioxidant activity and had the most potent scavenging abilities of various radicals which may be caused by the presence phenolic compounds. The antioxidant activity of the *W. saharae* suggests that the extracts obtained by polar solvents from the leaves could be used as an effective natural source of antioxidant. Further studies should be carried out for the isolation and identification of phenolic derivatives, and antioxidant studies are also needed for an understanding of their mechanisms of action.

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