INTERNATIONAL JOURNAL OF SCIENCES AND NATURAL RESOURCES (IJSNR)
NUMBER 1 JUNE (2022)

# Antioxidant activities of *Hypochaeris laevigata* var. *hipponensis*: Endemic species from Algeria

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## Abstract

*Hypochaeris laevigata* var. *hipponensis* (Asteraceae) is an endemic plant from Algeria. In the current study, we analyzed for the first time the antioxidant activities by five methods (*β*-Carotene bleaching test, DPPH test, ABTS radical cation reduction test, CUPRAC test, Ferrous ions chelating test) of three fractions of aerial part of plant: dichloromethane (DCM), ethyl acetate (EA) and n-butanol (BuOH). We also determined the total phenolic and flavonoid contents. The fraction EA showed the highest values, followed by BuOH and DCM fractions. Furthermore, the antioxidant action was dictated by five methods and the tested plant fractions demonstrated a noteworthy antioxidant action.

**Key words:** *Hypochaeris laevigata* var. *hipponensis*, Asteraceae, phenolic compounds, antioxidants activities.

## 1. Introduction

A large number of medicinal and aromatic plants grow there spontaneously in the Edough Peninsula such as plants of family Asteraceae which are rich in phenolic compounds, volatile oils and other bioactive compounds. It is fundamental to extend the knowledge of the chemical composition of some plants of this family **(Hamel., 2013).** According to **Stebbins (1971),** *Hypochaeris* is a small genus of Asteraceae family, that contains about 50 species. On the other, the genus of *Hypochoaeris* contains 100 species, the majority of which are native to South America. The species of *Hypochaeris laevigata* var. *hipponensis* is a perennial plant with a bitter root, endemic to Algeria, very common everywhere and on the coast, usually develops on wet rocks **(Quezel and Santa, 1962).**

Until today, any phytochemical study is mentioned for the *Hypochaeris laevigata* var. *hipponensis*, except that some studies on the species *H. radicata* by **Jamuna *et al.* (2014)** on the presence of alkaloids, flavonoids, glycosides, cardiac glycosides, phenols, resins, saponins, steroids, tannins, terpenoids and triterpenoids. *Hypochaeris radicata* is medically important and has anti-inflammatory, anticancer, antioxidant **(Jamuna *et al.*, 2012),** antibacterial **(Jamuna *et al.*, 2013a),** antifungal **(Jamuna *et al.*, 2013b)** properties and antidiuretics. It is used for the treatment of jaundice, rheumatism, dyspepsia, constipation, hypoglycemia and kidney problems in the traditional medicinal practice of Tamil Nadu, India **(Pullaiah, 2006).** However, no scientific validation has been made for this species for medicinal purposes.

To the best of our knowledge, the aim of the present work was to study the chemical composition of *Hypochaeris laevigata* var. *hipponensis,* which is an endemic species of Algeria, has not been reported before and to evaluate its antioxidant activities.

**Figure.1.** *Hypochaeris laevigata* var. *hipponensis*

## 2. Materials and Methods

### 2.1. Plant material and extraction method

Samples of the plant (*Hypochaeris laevigata* var. *hipponensis*) are collected in full bloom in Sérraïdi (Annaba), in northeastern Algeria during the month of May 2015. The plant was identified by Dr. Tarek Hamel, Lecturer at the Department of Plant Biology and Environment, Badji Mokhtar University (Annaba, Algeria). A reference specimen was deposited in the herbarium of the laboratory under the reference code: ChifaDZUMCAPBC000038. The samples were dried in the shade at room temperature in a ventilated place, and cut into small pieces.

The aerial parts powder (800 g) were macerated in a mixture of methanol/water (70/30, v/v) at a ratio of 1:10 (w/v) for 24 h with a constant stirring speed of 200 rpm, at room temperature. The suspension was then filtered on whatman paper. The extraction is repeated three times till exhaustion, then the solvent was evaporated at 40 °C using Rota Vapor (Büchi R-200, Germany) to afford 29.86 g extract. The crude extract was dissolved in 90% aqueous methanol for fractionation with different solvents such as dichloromethane (DCM), ethyl acetate (EA) and n-butanol (BuOH). Briefly, first fractionation was carried out with 100 ml DCM three times (3x). DCM fraction was collected and evaporated under reduced pressure to give a semisolid paste. Then the residual aqueous phase of dichloromethane was further fractionated with EA and BuOH solvents. The resulting fractions were evaporated to dryness. The yields of DCM, EA and BuOH fractions were found to be 8.73, 6.30 and 13.04 g, respectively. Dried fractions were dissolved in methanol and kept at a temperature of 4 °C for further analysis

### 2.2. Quantification of total phenols

The total phenolic content was evaluated according to the method described by **Li *et al*. (2007).** a 1.5 ml of the Folin-Ciocalteu reagent previously diluted ten times with distilled water was added to300 µl of the extract. After 4 minutes a 1.2 ml of 7.5% sodium carbonate ($Na_2CO_3$) was poured onto the solution. The samples were placed in the dark. After 2 hours, the results were read on a spectrophotometer at 750 nm, the concentration of total phenols is deduced from a calibration curve established with gallic acid and the results were expressed in mg of gallic acid equivalent per g dried extract (mg GAE/ g extract).

### 2.3. Quantification of flavonoids

The content of total flavonoids was determined according to the method described by **Djeridane *et al*., (2006).** 500 µl of the extract was mixed with 500 µl of 2% aluminum chloride. The absorbance of the mixture is measured at 430 nm, after 10 minutes of incubation. The flavonoid concentrations were expressed in mg equivalent quercetin per g dried extract (mg QE/g extract) with reference to a calibration curve.

### 2.4. Antioxidant activities

#### 2.4.1. Evaluation of Antioxidant Activity by *β*-Carotene bleaching test

The antioxidant activity of the extracts was evaluated using the *β*-carotene-linoleic acid system described by **Miller (1971)** with a slight modification. Dissolve 0.5 mg of *β*-carotene in 1 ml of chloroform. The solution obtained was introduced into a flask containing a mixture of 25 µl of linoleic acid and 200 mg of Tween 40. After evaporation of the chloroform under vacuum, 100 ml of distilled water saturated with oxygen were added by vigorous stirring. From this new solution 4 ml was transferred to different test tubes containing different concentrations of the sample in ethanol. As soon as the emulsion was added to each tube, the absorbance of the zero time was measured at 470 nm, using a spectrophotometer. The emulsion system was incubated for 2 hours at 50 °C. A negative control, free of *β*-carotene, was prepared for background subtraction. The bleaching rate (R) of *β*-carotene was calculated according to the following equation: $R = \ln_{a/b} / t$.

Where ln is the natural log, a is the absorbance at zero time, b is the absorbance at time t (120 min). Antioxidant activity (AA) was calculated in terms of percent inhibition versus control, using the following equation: ***% inhibition = [R control - R sample / R control] x 100.***

Quercetin, BHT and *α*-tocopherol have been used as antioxidant standards for the comparison.

#### 2.4.2. DPPH free radical scavenging test

The anti-radical activity against DPPH of the studied extracts was measured by the DPPH test described by **Blois (1958)** with a slight modification. Briefly a 0.1 mM solution of DPPH in methanol was prepared and 4 ml of this prepared solution were added to 1 ml of sample solutions in methanol at different concentrations. After 30 minutes of incubation in the dark at room temperature, the absorbance is measured at 517 nm. Lower absorbance of the reaction mixture indicated greater free radical scavenging activity. The antioxidant activity was expressed as a percentage of DPPH radical inhibition, and calculated from the following equation: ***% inhibition = [A control - A sample / A control] × 100.***

The $IC_{50}$ value (the inhibitory concentration of the extract necessary to decrease the initial concentration of the DPPH radical at 50%) was calculated from the percentage plot of the trapping effect of the different

concentrations of each extract (Scherer & Godoy, 2009). We deduced the anti-radical activity of the extracts by calculating the inverse of the $IC_{50}$ values found (Maisuthisakul *et al*., 2007), by the following formula: $ARA = 1/IC_{50}$. Quercetin, BHT and $\alpha$-tocopherol have been used as antioxidant standards for the comparison of activity.

### 2.4.3. ABTS radical cation reduction test

The anti-radical activity against the radical $ABTS^{\cdot+}$ of the studied extracts was determined according to the method of **Re *et al*. (1999)** with slight modification. In this test, the radical cation $ABTS^{+}$ is generated by mixing 7 mM ABTS in $H_2O$ and 2.45 mM Potassium Persulfate. The mixture is then stored in the dark at room temperature for 12 hours. The oxidation of $ABTS^{\cdot+}$ started immediately, but the absorbance was not maximal and stable until more than 6 hours had elapsed. The radical cation was stable in this form for more than 2 days with storage in the dark at room temperature. Before use, the $ABTS^{+}$ solution was diluted with ethanol to obtain an absorbance of $0.700 \pm 0.02$ at 734 nm. Then 2 ml of $ABTS^{+}$ solution was added to 1 ml of sample solution in ethanol at different concentrations (5-50 mg/ml). After 30 min, the percent inhibition at 734 nm was calculated for each concentration based on a blank absorbance (methanol). The $ABTS^{+}$ scanning capability was calculated using the following equation:

***% inhibition = [Abs control - Abs sample / Abs control] × 100***

Where the Abs controls are ABTS solution absorbance plus methanol, and the Abs sample is ABTS absorbance plus extract or standard. The $IC_{50}$ value is calculated for each sample and compared with quercetin, BHT and $\alpha$-tocopherol, which were used as antioxidant standards for activity comparison.

### 2.4.4. Cupric reducing antioxidant capacity (CUPRAC) Test

The cupric reductive antioxidant capacity was determined according to the method of **Apak *et al*. (2004)**, with a slight modification. In each well, in a 96-well plate, 50 µl of 10 mM Cu (II) solution, 50 µl of 7.5 mM neocuprone and 60 µl of $NH_4Ac$ buffer (1 M, pH 7.0) were added. 40 µl extracts at different concentrations were added to the initial mixture to obtain the final volume of 200 µl. After 1 h, the absorbance at 450 nm was recorded against a reagent blank using a 96-well microplate reader. The results were given as $A_{0.50}$ (µg/ml) which corresponds to the concentration providing 0.500 absorbance. The concentration of the sample providing 0.50 absorbance ($A_{0.50}$) was calculated from the graph of the absorbance of cupric reductive antioxidant capacity. BHT and $\alpha$-tocopherol were used as antioxidant standards for comparison of activity.

### 2.4.5. Ferrous ions chelating test

The chelating activity of the $Fe^{2+}$ extracts was measured using Ferrin **(Decker and Welch, 1990)** with slight modifications. The extract solution (80 µl dissolved in ethanol at different concentrations) was added to 40 µl of 0.2 mM $FeCl_2$. The reaction was initiated by the addition of 80 µl of 0.5% ferene. The mixture was stirred vigorously and left at room temperature for 10 minutes. After the mixture reached equilibrium, the absorbance was measured at 593 nm. The chelating activity was calculated using the following equation: ***% of metal chelation activity = [A control - A sample / A control] × 100***

Where A control is the absorbance of the sample-free control and A sample is the absorbance of the sample in the presence of the chelator. The concentration of extract providing 50% of metal chelation activity ($IC_{50}$) was calculated from the graph of the percentage of $Fe^{2+}$ chelation effects relative to the

concentration of extract. EDTA and quercetin were used as antioxidant standards for comparison of activity.

### 2.5. Statistical analysis

All data of antioxidant activities tests were the average of three analyses. The data were recorded as mean ± standard deviation. Significant differences between means were determined by student's-t test and p values < 0.05 were considered as significant results.

## 3. Results and Discussion

### 3.1. Total phenolic and flavonoid contents

The results of the total phenolic contents (Table 2) of the three extracts of *H. laevigata* var. *hipponensis* showed that the ethyl acetate (EA) and n-butanol (BuOH) extracts have the highest value of 202.86 ± 14.64 and 200 ± 10.93 mg GAE/g extract, respectively compared to dichloromethane (DCM) extract with 184.07 ± 0.17 mg GAE/g extract. Also, the total flavonoid content (Table 2) of the BuOH extract (46.76 ± 0.36 EQ/g of extract) was greater than that of EA and DCM extracts (17.92 ± 0.12 EQ /g extract and 16.28 ± 0.16 EQ /g extract, respectively).

**Table 1.** Total phenolic and flavonoid contents of the extracts of *H. laevigata* var. *hipponensis*

| Extracts | Total Phenols[a] | Flavonoids[b] |
|---|---|---|
| **DCM** | 184.07 ± 0.17 | 16.28 ± 0.16 |
| **EA** | 202.86 ± 14.64 | 17.92 ± 0.12 |
| **BuOH** | 200 ± 10.93 | 46.76 ± 0.36 |

[a] : mg gallic acid equivalent/g extract ; [b] : mg quercitin equivalent/g extract

### 3.2. Antioxidant activities

In the present work, the antioxidant activity was determined by five methods (Table 3). For β-carotene test, a good activity was found in the three extracts ($IC_{50}$ value of 5.02 ± 0.95, 5.66 ± 2.03 and 7.60 ± 4.37 for dichloromethane, *n*-butanol and ethyl acetate, respectively), it were better than that of catechin (8.79 ± 0.89 µg/ml) and a higher of α-tocopherol, BHT and quercetin (2.10 ± 0.08, 1.34 ± 0.04 and 1.81 ± 0.11 µg/ml).

In DPPH test, maximum scavenging activity was found in *n*-butanol extract ($IC_{50}$ value : 8.12 ± 1.47 µg/ml) and ethyl acetate extract ($IC_{50}$ value : 8.70 ± 1.87 µg/ml) in comparaison to catechin, quercetin and α-tocopherol (4.32 ± 0.15, 2.07 ± 0.10 and 7.31 ± 0.17 µg/ml). While, dichloromethane extract (47.24 ± 0.11 µg/ml) showed a bit important activity with previous standards. Studies reported that anti-radical activity is correlated with the level of polyphenols and flavonoids in medicinal plant extracts **(Mariod *et al.*, 2009).**

For the ABTS+ method, the ethyl acetate extract (EA) exhibited the highest activity with an $IC_{50}$ value of 4.32 ± 0.09 µg/ml in comparaison to α-tocopherol and BHT (4.31 ± 0.10 and 4.10 ± 0.06 µg/ml). While, dichloromethane extract (DCM) and *n*-butanol extract (BuOH) showed a bit important activity ($IC_{50}$ value of 13.10 ± 0.97 and 15.02 ± 0.73 µg/ml, respectively). The results proved that the extracts have the ability to trap the various free radicals in the different systems, indicating that they can be useful therapeutic agents for the treatment of radical-related pathological lesions **(Wang *et al.*, 1998).**

The results of CUPRAC test, shows that the EA and BuOH extract exhibited the highest activity ($A_{0.50}$ value : 1.48 ± 0.33 and 3.00 ± 0.98 µg/ml), and it were better than the BHT and α-tocopherol (3.80 ± 0.00 and 10.20 ± 0.01 µg/ml). **Prior *et al*., (2005)** classify the CUPRAC antioxidant method as one of the electron transfer methods, and summarize the superiority of the CUPRAC method over other antioxidant tests. **Gorinstein *et al*. (2006)**, also note that the highest capacities of polyphenolic compounds are measured with CUPRAC. For the ferrous ions chelation test, all extract were not active.

**Table 3:** Antoxidant activities of the three extracts of *H. laevigata* var. *hipponensis*

| Extract | β-carotene IC50 (µg/ml) | DPPH IC50 (µg/ml) | ABTS+ IC50 (µg/ml) | CUPRAC A0.50 (µg/ml) | $Fe^{+2}$ Chelation $IC_{50}$ (µg/ml) |
|---|---|---|---|---|---|
| **Dichloromethane** | 5.02 ± 0.95 | 47.24 ± 0.11 | 13.10 ± 0.97 | 16.86 ± 3.02 | > 800 |
| **Ethyl acetate** | 7.60 ± 4.37 | 8.70 ± 1.87 | 4.32 ± 0.09 | 1.48 ± 0.33 | > 800 |
| ***n*-Butanol** | 5.66 ± 2.03 | 8.12 ± 1.47 | 15.02 ± 0.73 | 3.00 ± 0.98 | > 800 |
| **(+)-Catechin [a]** | 8.79 ± 0.89 | 4.32 ± 0.15 | 1.16 ± 0.02 | NT | NT |
| **Quercetin [a]** | 1.81 ± 0.11 | 2.07 ± 0.10 | 1.18 ± 0.03 | NT | NT |
| **α-Tocopherol [a]** | 2.10 ± 0.08 | 7.31 ± 0.17 | 4.31 ± 0.10 | 10.20 ± 0.01 | NT |
| **BHT [a]** | 1.34 ± 0.04 | 45.4 ± 0.47 | 4.10 ± 0.06 | 3.80 ± 0.00 | NT |
| **EDTA [a]** | NT | NT | NT | NT | 6.50 ± 0.07 |
| **Ascorbic acid [a]** | NT | NT | NT | NT | NT |

[a] Standard compounds, NT : Not Tested.

## 4. Conclusion

This study was performed to investigate the chemical composition of phenolic compounds in dichloromethane, ethyl acetate and *n*-butanol extracts of *H. laevigata* var. *hipponensis*. The total phenolic contents indicated that EA fraction and BuOH presented the highest value. The flavonoid contents showed that BuOH exhibited the highest value. Furthermore, the antioxidant activity was determined by five methods, such as: *β*-carotene bleaching method, DPPH radical scavenging activity, ABTS cation radical scavenging activity, metal chelating activity and cupric reducing antioxidant capacity CUPRAC. The tested extracts showed significant antioxidant activity with all assays except ferrous iron chelation assay which showed a negative result. In fact, the ethul acetate extract was more potent as antioxidant than the n-butanol and dichloromethane extracts. A continuation of this work in the future is desirable to study the components present in the extracts and to evaluate more biological activities for this promising plant.

## Conflict of Interest

The authors declare that there are no conflicts of interest.

## Acknowledgments

The authors are grateful to the both Department of Chemistry in University of Mugla (Turkey) and University of Constantine 1 (Algeria) and University of Dicle (Turkey) for providing funds to perform the present research.

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