

MINERAL COMPOSITION, PHYTOCHEMICAL EXPLORATION AND ANTIOXIDANT ACTIVITIES OF AN ENDEMIC TAXA: *HYPOCHAERIS LAEVIGATA* VAR. *HIPPONENSIS* MAIRE

BOUASLA, I.^{1,2} – BOUASLA, A.^{1,3*} – BAROUR, C.^{3,4} – ZEKRI, S.⁵ – HAFIDI, Y.⁵ – HAMEL, T.⁶

¹Laboratory of Organic Chemistry and Interdisciplinarity, Faculty of Sciences and Technology,
Mohamed Chérif Messaâdia University, Souk Ahras 41000 Algeria

²Biochemistry Department, Faculty of Sciences, Badji Mokhtar University, BP 12 Sidi Amar,
Annaba, Algeria

³Biology Department, Faculty of Natural and Life Sciences, Mohamed Chérif Messaâdia
University, Souk Ahras 41000, Algeria

⁴Laboratory of Aquatic and Terrestrial Ecosystems, Faculty of Natural and Life Sciences,
Mohamed Chérif Messaâdia University, Souk Ahras 41000, Algeria

⁵Badji Mokhtar University, BP 12 Sidi Amar, Annaba, Algeria

⁶Laboratory of Plant Biology and Environment, Faculty of Sciences, Badji Mokhtar University,
BP 12 Sidi Amar, Annaba, Algeria

*Corresponding author

e-mail: asmabouaslauniv@yahoo.fr; a.bouasla@univ-soukahras.dz; phone: +213-557-326-141

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Abstract. The aim of the present work is the scientific evaluation of an Algerian endemic taxa (*Hypochaeris laevigata* var. *hipponensis* Maire) widely used in folk medicine. For this objective, the active compounds, mineral element composition and antioxidant effects of three different extracts prepared from leaves were determined. Essays were started by a phytochemical screening, followed by mineral elements determination and leaf extract preparation using three organic solvents. The concentration of polyphenols, flavonoids, tannins, flavanols and orthodiphenols was determined in each extract as well as the radical scavenging activities and the total antioxidant properties. Correlation between bioactive compounds and antioxidant activities was also evaluated. Ethyl acetate extract showed the highest levels of polyphenols while the lowest one was found in raw extract, which contains the most important tannins. However, the lowest rate in anthocyanin was noted in butanol extract. Our data indicated that antioxidant properties varied according to extract type and radical scavenging used assay, indeed the half-maximal inhibitory concentration (IC₅₀) value ranged between 0.71 ± 0.02 and 7.00 ± 0.62 mg/g extract. Several positive correlations between secondary metabolite contents and antioxidant activities were registered. The obtained results support the influence of solvent extracts on bioactive compounds. These phytochemical constituents as well as mineral elements provide substantial antioxidant activities and explain the effectiveness of the studied species as traditional remedy.

Keywords: *Hypochaeris laevigata* var. *hipponensis* Maire, radical scavenging, Algerian species, bioactive compounds, solvent extraction

Introduction

Several scientific papers have indicated that reactive oxygen species (ROS) could be possibly involved in the etiology of large panoply of human pathologies and/or the development of their complications. In parallel, numerous studies have proven the antioxidant effects of many botanical species. It has been reported that the protective actions

of plants against oxidative stress depend on their contents in bioactive compounds and/or mineral elements (Montagnier et al., 1998; Hajam et al., 2022; Twaij and Hasan, 2022).

Algeria has a considerable floristic richness comprising thousands of medicinal species, of which 15% are endemic. This country is considered as a hot spot that constitutes a line of promising scientific research (Myers et al., 2004; Senouci et al., 2019).

The species *Hypochaeris laevigata* var. *hipponensis* Maire is an endemic Algerian taxa described for the first time by Maire (1931) on the sandstone rocks of Edough Mount near Annaba City (ex Bône, Hippone, Hippo-Regius) (Quézel and Santa, 1963).

Literature data reported the wide traditional uses of other species belonging to the same genera (*H. radicata*, *H. radicata* subsp. *neapolitana*) to treat jaundice, rheumatism, dyspepsia, constipation, hypoglycemia and kidney problems. The anti-inflammatory, anticancer, antibacterial, antifungal and antidiuretic properties were also reported (Senguttuvan et al., 2014; Abu-Izneid et al., 2018; Shulha et al., 2019).

Compared to the available data on previous cited genera, less interest has been given so far to *H. laevigata* var. *hipponensis* M. although it may constitute a natural antioxidant wealth that could bring several benefits in different fields. Therefore, this endemic Algerian taxa was chosen to be subject of our study.

At our knowledge, the mineral composition of *H. laevigata* var. *hipponensis* M has never been recorded before, neither the relation between their bioactive compounds and antioxidant activities. We were interested in this work to determine mineral contents, bioactive compounds of three extracts (water/methanol, ethyl acetate, and butanol) and their correlations with antioxidant properties.

Material and Methods

Chemicals

All reagents and chemicals used in this study were obtained from Sigma-Aldrich corporation.

Plant material

The collection of aerial parts of *H. laevigata* var. *hipponensis* M. (Fig. 1) was carried out in March to April 2019 from Edough Peninsula.

Referring to Quézel and Santa (1963), the official nomenclature was confirmed by Dr. T. Hamel (Plant taxonomist at the biology department, Badji Mokhtar University). The specimens were stored at the laboratory of plant biology and environment under a voucher number-L.P.B.E.24.

The harvested parts were dried, after a gentle clean, in the shadow at $21 \pm ^\circ\text{C}$. 20 days later, the dried samples were stored in dark glass box until use.

Qualitative assays

Phenols test

A few drops of ferric chloride (2%) were added to a macerate of 2 mg of *H. laevigata* var. *hipponensis* M. in 200 ml of methanol. Presence of phenols was detected by formation of blue/black colour (Békro et al., 2007).



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

Flavonoids test

First the mixture of 10 mg of *H. laevigata* var. *hipponensis* M. and 150 ml of hydrochloric acid were warmed for 24 hours. Then, NH_4OH was added. Positive reaction was revealed three hours later by the formation of a light yellow colour (Okmu, 2005).

Tannins test

Few drops of ferric chloride (2%) and 100 ml of ethanol were added to 10 g of *H. laevigata* var. *hipponensis* M. formation of blue /black colour indicates the presence of gallic tannins, however green colour indicates the presence of catechenic tannins (Karumi et al., 2004).

Anthocyanins test

The mixture of 0.5 mg of *H. laevigata* var. *hipponensis* M. and few drops of hydrochloric acid was heated for 15 min in a water bath. Change of purplish or red cherry colour after addition of few drops of ammonia indicates a positive reaction (Bruneton, 1998).

Saponins test

Two grams of *H. laevigata* var. *hipponensis* M. were boiled in 80 ml of distilled water. After that, they were stirred vertically. Appearance of foam indicates a positive reaction (Karumi et al., 2004).

Mucilages test

5 ml of methanol were added to 1 ml of the macerate of *H. laevigata* var. *hipponensis* M. in distilled water. Reaction is positive if flaky precipitate forms (Karumi et al., 2004).

Sterols and terpens test

Mixture of 0.5 ml of acetic acid and the same volume of chloroform were added to the heated macerate of 5 g of *H. laevigata* var. *hipponensis* M. in 20 ml of petroleum ether. Appearance of purple colour after concentrated sulfuric acid addition indicates positive reaction (Dohou et al., 2003).

Alkaloids

100 ml of 1% diluted HCl were added to 10 g of the plant sample. The appearance of a cloudy solution after adding Mayer's reagent few drops indicates the presence of alkaloids (Bouquet, 1972).

Reducing compounds

1 ml of Fehling liquor was added to 10% of *H. laevigata* var. *hipponensis* M methanol macerate. The reducing compounds presence was confirmed by the formation of red precipitate after heating for 3 min (Karumi et al., 2004).

Mineral composition

The determination of mineral rate contents (Ca, Na, K, S, P and Mg) was made by an atomic absorption spectrometry (AAS: ZEE nit 700P following Zeeman technique, Germany). In this purpose, dried samples were incinerated at 550°C until constant weight. Before the addition of hydrochloric acid to ashes, their yields were calculated.

In order to estimate the trace elements levels in our samples, an inductively coupled mass spectrometry Agilent 7700 ICP-MS was used.

Preparation of extract

Extracts were prepared according to Chaabi and his co-authors procedure (Chaabi et al., 2008) with minor modification. First, 25 g of dried samples were macerated in n-hexane at 24°C. After 24 hours, the mixture was filtrated. The obtained residues were macerated one more time in the same solvent. The previous procedure was repeated until obtaining a clear solution. Then, residues were macerated in methanol. Twenty-four hours later, macerate was filtered. Finally, methanol was removed by a rotary evaporator (BUCHI R-210) at 40°C.

Raw extract was obtained after addition of warmed water. Acetate and butanol extracts were obtained after four repetitive times of a liquid-liquid extraction with different solvents of increasing polarity (ethyl acetate and n-butanol respectively).

All extracts were dissolved in methanol and stored at 4°C until use.

Quantitative assays

Total phenols

1.5 ml of diluted Folin-Ciocalteu reagent (10x) was added to 200 µl of the 1 mg/ml sample solution. The mixture was incubated in the dark for 5 min at 25°C. Then 1.5 ml of Na₂CO₃ sodium carbonate (60 g/l) was added. The mixture was incubated again in the dark for 90 min at 20°C before reading the optical density at 765 nm.

Total polyphenol content was calculated from the equation of the calibration curve of gallic acid (0.2 mg/ml). This curve was carried out under the same concentration conditions. Concentrations were expressed as mg of gallic acid per 100 g of extract (mg AGE/ g extract) (Wolfe et al., 2003).

Total flavonoids

1.25 ml of H₂O and 75 µl of 5% NaNO₂ were mixed with 250 µl of each extract. Six minutes later, 150 ml of the 10% AlCl₃ solution and 0.5 ml of NaOH were added to the mixture. Optical density was read at 510 nm.

The content of total flavonoids was calculated from the equation of quercetin calibration curve previously carried out under the same conditions. Total flavonoid contents were

expressed as mg of quercetin equivalent per gram of extract (mg QE/g extract) (Zhishen et al., 1999).

Total flavanols

2 ml of AlCl_3 dissolve in methanol at 2% and 3 ml of sodium acetate solution were added to 1 ml of each extract. The mixture was incubated in dark at room temperature for 150 min. absorbance was read at 440 nm. Total flavanols contents were expressed as mg of routine equivalent (mg RE/g extract) (Yermakov et al., 1987).

Total tannins

50 μl of each extract were mixed with 1.5 ml of vanillin (dissolved in methanol at 4%) and 750 μl of HCl (12%). Solutions were incubated in the dark for 20 min. Results were expressed as mg catechin equivalent (mg CE/g extract) (Julkunen-Titto, 1985).

Total ortho-diphenols

0.5 ml of sodium molybdate (5%) was added to 2 ml of samples. The mixed solution was incubated at ambient temperature. After 15 min in the dark, absorbance was read at 370 nm. Contents in total ortho-diphenols were expressed as mg caffeic acid equivalent (mg CAE/g extract) (Ollivier et al., 2004).

Anthocyanin

400 μl of each extract were aliquoted into two tubes and 3.6 ml of potassium chloride buffer (0.03 M, pH 1) were added to the first tube, the same volume of sodium acetate buffer (0.4 M; pH4.5) was added to second one. The absorbance was measured at 520 and 700 nm, after the incubation of tubes in dark for 30 min. The anthocyanin content was calculated according to the following equation (Giusti and Wrolstad, 2001).

$$\frac{(A \times \text{MW} \times \text{DF} \times 1000)}{(\epsilon \times 1)} \quad (\text{Eq.1})$$

where: $A = (A_{520} - A_{700})$ pH 1.0 – $(A_{520} - A_{700})$ pH 4.5; MW: molecular weight for cyanidin 3-glucoside = 449.2, ϵ : is the molar absorptivity of cyanidin 3-glucoside = 26 900, and DF the dilution factor.

Evaluation of antioxidant activities

Radical scavenging assays

DPPH test

The method of Meriga et al. (2012) was followed in this assay. Four milliliters of DPPH/methanol (60 μM) solution were added to 1 ml of different dilution of each extract. Mixtures were incubated in the dark for 30 min. the absorbance was measured at 517 nm.

NBT/Riboflavin test

This assay was released according to Yagi et al. (2002). Phosphate buffer (1 M, pH 7.4) were used to prepare all solutions. The absorbance was read at 560 nm after oxidation of riboflavin and reduction of nitroblue tetrazolium. Scavenging of superoxide anion activity was calculated as below:

$$\% \text{ inhibition} = 100 \times \frac{(A_{\text{blank}} - A_{\text{sample}})}{A_{\text{blank}}} \quad (\text{Eq.2})$$

where A_{blank} is the absorbance of the control reaction and A_{sample} is the absorbance in the presence of each extract or standard molecule. The half-maximal inhibitory concentration (IC_{50}) was calculated from the curve of the inhibition percentage against the extract or standard molecule concentration.

FRAP test

2.5 ml of phosphate buffer (0.2 M, pH 6.6) and the same volume of potassium ferricyanide [$\text{K}_3 \text{Fe}(\text{CN})_6$] were mixed with various dilutions of each extract. Mixture solutions were incubated for 20 min at 50°C in the dark. Then, 2.5 ml of chloroacetic acid (10%) were added. After centrifugation at 3000 rpm for 10 min, 2.5 ml of supernatant were added to 2.5 ml of distilled water and 0.5 ml of ferric chloride solution. The absorbance was measured at 700 nm (Yildirim et al., 2001).

Total antioxidant properties

β -carotene/linoleic acid bleaching test

200 μl of different dilutions of extract solutions were mixed with 5 ml of stock solution (2 ml β -carotene, 10 ml chloroform and 20 μl of tween 20) and incubated at 50°C for 120 min. the absorbance was read each 30 min for 2 h at 470 nm (Ismail and Tan, 2002).

Phosphomolybdenum test

Reagent solution was prepared by mixing ammonium molybdate (4 mM), sodium phosphate (28 mM) and sulfuric acid (600 mM). Then, extracts were diluted to obtain different concentrations. After that, 1 ml of reagent solution was added to 100 μl of each concentration. The absorbance was read at 695 nm after incubation at 90°C for 90 min (Prieto et al., 1999).

Statistical analysis

The analysis of the obtained data was performed using R (R Core Team, 2019) statistical language (Ihaka and Gentleman, 1996). Shapiro-Wilk test was used to test variables normality. The influence of the extraction solvent on the studied parameters was assessed by the non-parametric Kruskal-Wallis rank sum test; followed by the non-parametric pairwise Dunn's test (with Bonferroni adjustment) to find *post-hoc* statistical differences at $\alpha=0.05$ as significant level. Data were expressed as mean $\pm$ standard error ($m \pm se$). Correlations between secondary metabolites contents and antioxidant activities were also studied using Spearman's non-parametric correlation. The 'ggcorrplot' (Kassambara, 2019) and 'dunn.test' (Dinno, 2017), R packages were used in our statistical analysis.

Results

Qualitative assays

Our results clearly show the presence of all determined phytochemical compounds excepting alkaloids (Table 1).

Table 1. Phytochemical compounds found in *H. laevigata* var. *hipponensis* M.

Phytoconstituents	<i>H. laevigata</i> var. <i>hipponensis</i> M.
Total Phenols	++
Flavonoids	+
Tannins Catechinic	+++
Tannins Gallic	+++
Anthocyanins	+++
Saponins	++
Mucilages	++
Sterols and terpenes	+++
Alkaloids	-
Reducing compounds	+++

(+): Trace amount, (+) (+): Moderate amount, (+) (+) (+): Appreciable amount, (-): Absence

Mineral composition

From the obtained data, we could say that the most predominant minerals are sulfure (17034±7.52 mg/kg), sodium (6200±6.42 mg/kg) and potassium (3120±7.01 mg/kg). The highest concentration in trace element was registered for iron (145.5±1.61 mg/kg), copper (9.20±0.03 mg/kg) and manganese (5.00±0.03 mg/kg). Some heavy metals were also detected in trace amounts (Table 2).

Table 2. Mineral composition of *H. laevigata* var. *hipponensis* M (m±se)

Parameters	<i>H. laevigata</i> var. <i>hipponensis</i> M.
Calcium (Ca ⁺²) mg/kg	715±4.73
Magnesium (Mg ⁺²) mg/kg	260±3.23
Phosphate (PO ₄ ⁻) mg/kg	180±1.54
Sodium (Na ⁺) mg/kg	6200±6.42
Potassium (K ⁺) mg/kg	3120±7.01
Zinc (Zn ⁺²) mg/kg	1.50±0.02
Copper (Cu) mg/kg	9.20±0.03
Manganese (Mn) mg/kg	5.00±0.03
Iron (Fe ⁺³) mg/kg	145.5±1.61
Chromium (Cr) mg/kg	0.09±0.001
Nickel (Ni) mg/kg	0.046±0.001
Cobalt (Co) mg/kg	0.015±0.002
Cadmium (Cd) mg/kg	<0.072±0.001
Sulfur (S ⁻) mg/kg	17034±7.52
Selenium (Se) mg/kg	0.0232±0.002
Arsenic (As) mg/kg	<0.05±0.003

Experiments were repeated three times (n=3)

Organoleptic characteristic and extraction yields

Table 3 summarises the different organoleptic characteristic of each extract. The highest yield was recorded for ashes (73.26 %), the lowest one is that of butanol extract (12.32%).

Table 3. Obtained yields and organoleptic characteristics of *H. laevigata* var. *hipponensis* M. various extracts and ashes

Obtained yields and organoleptic characteristics				
	Yields	Aspect	Colour	Odour
Raw Extract	23.31%	Paste	Green/Brown	Totally different from the leaves
Acetate Extract	17.08%	Opaque liquid	Dark green	Totally different from the leaves
Butanol extract	12.32%	Liquid	Green/Brown	Totally different from the leaves
Ashes	73.26%	Powder	-	-

Quantitative assays

Our data indicate notable contents of the determined secondary metabolites. Furthermore, statistical analyse of the registered data shown significant differences between extracts in total phenols, tannins and anthocyanin contents.

Indeed, the highest level of total phenols was recorded in acetate extract while the lowest one was found in raw extract, which contains the most important of tannins. However, the lowest rate in anthocyanin was noted in butanol extract. The differences in flavonoids, orthodiphenols and flavanols were statically no significant (Table 4).

Table 4. Total phenols, flavonoids, tannins, ortho-diphenols, flavanols and anthocyanins contents in *H. laevigata* var. *hipponensis* M. different extracts ($m \pm se$)

	Total phenols (mg AGE/g E)	Flavonoids (mg QE/g E)	Tannins (mg CE/g E)	Ortho- diphenols (mg CAE/g E)	Flavanols (mg RE/g E)	Anthocyanins (mg cy-3-gluE/ g E)
Raw	0.47±0.04 ^b	0.58±0.08 ^a	0.60±0.04 ^a	32.15±2.51 ^a	48.95±7.88 ^a	12.69±0.12 ^{a,b}
Acetate	3.98±0.10 ^a	1.39±0.09 ^a	0.04±0.02 ^b	67.80±2.92 ^a	77.73±4.45 ^a	17.74±0.20 ^a
Butanol	1.01±0.18 ^{a,b}	0.64±0.02 ^a	0.18±0.05 ^{a,b}	37.40±7.28 ^a	57.75±4.21 ^a	9.62±0.41 ^b

Phenols content: mg gallic acid equivalent/g extract (mg GAE/g E), Flavonoids content: mg quercetin equivalent/g extract (mg QE/g E), Flavanols content: mg rutine equivalent/g extract (mg RE/g E), Tannins content: mg catechin equivalent/g extract (mg CE/g E), Ortho-diphenols content: mg cafeique acid equivalent/g extract (mg CAE/g E), Anthocyanin content: mg cyanidin 3-glucoside equivalent/g extract (mg cy-3-gluE/g E), different lowercase letters indicate a significant difference between extracts, experiments were repeated three times ($n=3$)

Antioxidant activities

As shown in Figure 2; non-significant differences are noted between extracts in DPPH and superoxide assays. Our statistical analysis indicated that butanol extract exhibited the highest phosphomolibdenum and ferric reducing activities. In β carotene assay, acetate extract had the highest antioxidant properties.

Correlation between secondary metabolites contents and antioxidant activities

Regarding the correlation matrix (Fig. 3), we noted a positive correlation between orthodiphenols, flavonoids, flavanols contents and ferric reducing effect. The same observation was registered between phenols, tannin and DPPH, anion superoxide respectively. Furthermore, a high positive correlation was recorded between anthocyanins content and β -carotene bleaching activities.

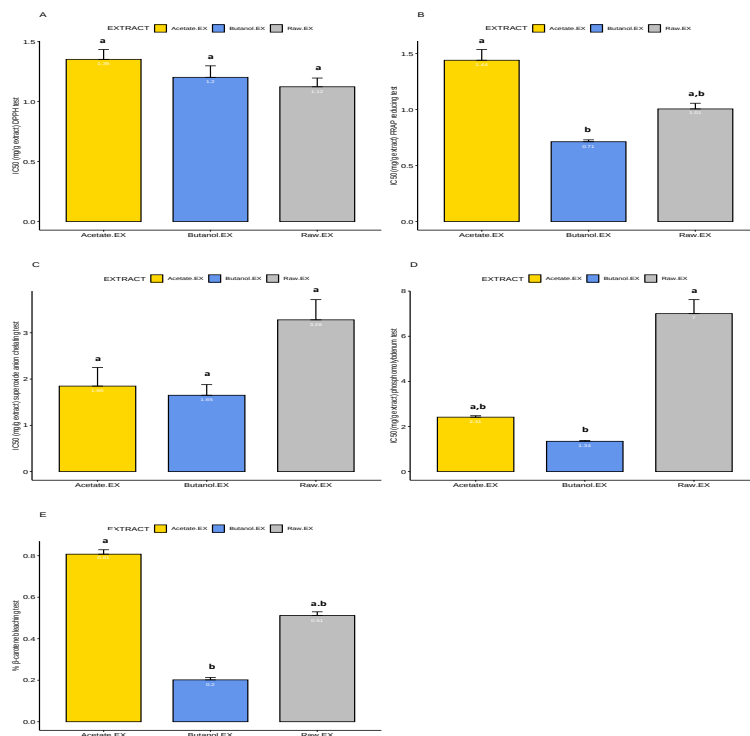


Figure 2. Antioxidant activities of *H. laevigata* var. *hipponensis* M. different extracts ($m \pm se$, $n=3$). A: DPPH: IC_{50} (mg/g extract) DPPH test. B: FRAP: IC_{50} (mg/g extract) FRAP reducing test. C: NBT/Riboflavin: IC_{50} (mg/g extract) superoxide anion chelating test. D: Phosphomolybdenum: IC_{50} (mg/g extract) phosphomolybdenum test. E: β -carotene: % β -carotene bleaching test. (Different lowercase letters indicate a significant difference between extracts, error bars correspond to the standard error of the mean (se))

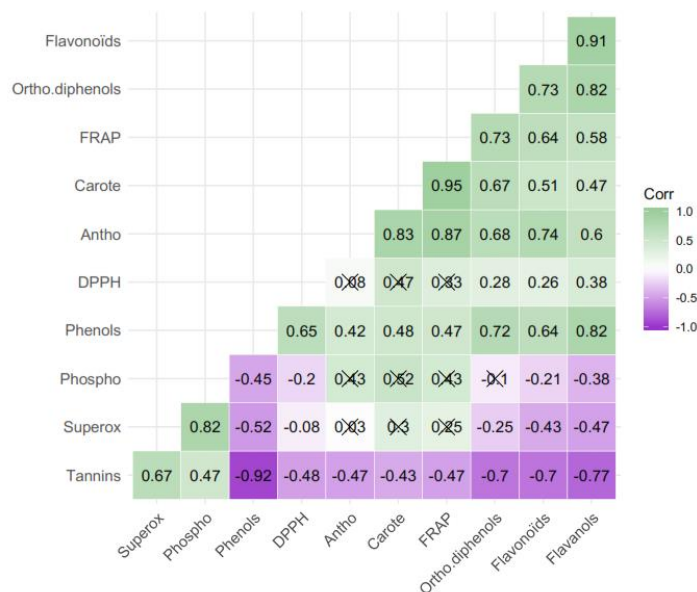


Figure 3. The Spearman's correlations (corrplot) between secondary metabolites contents and antioxidant activities (The non-crossing correlations are statistically significant, the crossing means non-significant correlation). Carote: β -carotene bleaching, Antho: Anthocyanins, Phospho: Phosphomolybdenum, Superox: Superoxide anion

Discussion

It is well known that bioactive components give plants various properties, that is why they are attracting more and more scientists interest as a potential source of biologically active substances that are found from roots to fruits. These secondary metabolites present in low doses in plants do not exercise direct effects on fundamental activities such as growth and reproduction, but provide them appreciable healing properties that no synthetic chemistry can offer us (Nyiredy, 2004; Teodoro, 2019).

Results of the preliminary qualitative analysis revealed the presence of different phytoconstituents: total phenols, flavonoids, tannins, anthocyanins, saponins, mucilage, reducing compounds, sterols and terpenes. Similar results were recorded by Senguttuvan et al. (2014), where preliminary phytochemical analysis of another botanical species from the same genera (*H. radicata* L.) exhibited the presence of total phenols, flavonoids, tannins, saponins as well as alkaloids and ascorbic acid.

It was already reported in many previous works that these secondary metabolites have several biological effects. Indeed, the antioxidant, anti-inflammatory, antimicrobial and anti-carcinogenic properties of phenolic compounds is very well documented, when saponins were reported as analgesic molecules (Mariod et al., 2009).

In addition to their role in activation of antioxidant enzymes, mineral elements constituents are strongly related to bioactive compounds levels by improving the activity of enzymes involved in their biosynthesis. According to the works of Shi et al. (2018), Fe deficiency increased both of anthocyanins and total phenols levels. Other reports confirmed that there is a correlation between several phenols and Cu contents.

During the extraction, solubility of active compounds depends on their chemical nature on one hand, in the other hand, on the used solvent polarity level. In the present study, raw extract had the highest yield. Quantitative analysis of extracts showed significant differences in the determined secondary metabolites. The most important amount of total phenols was registered in acetate extract; however, the highest level of tannins was recorded in raw extract. These results could be explained by the polarity of bioactive compounds that allows them a better solubility in the most polar solvent. Studies conducted by Senguttuvan et al. (2015) and Shulha et al. (2019) on *H. radicata* roots and leaf extracts revealed the presence of lignans, sesquiterpene, confertine and scopoletin that are prominent constituents as anti-inflammatory and antioxidant compounds.

Oxidative stress is commonly defined as a profound imbalance between the production of ROS and antioxidant defense systems in favor of first ones. This situation can result from an endogenous dysfunction or have exogenous origin (Bonnefont Rousselot et al., 2002; Pizzino et al., 2017).

The antioxidant properties of several plants have been demonstrated. In our study, both of free radical scavenging activities and total antioxidant properties were evaluated. The recorded data indicated significant differences between extracts. Indeed, butanol extract had the highest phosphomolibdenum and ferric reducing effects. However, in β carotene assays, acetate extract exhibited the highest activities. These differences could be due on one hand to the solvent polarity, on the other hand, to bioactive secondary metabolites level present in the extract, their chemical structure and/or nature (Senguttuvan et al., 2014; Bouasla et al., 2021).

Studies achieved on other *Hypochaeris* species also indicated important scavenging activities of different extracts. However, the IC₅₀ recorded in DPPH test were higher than obtained in our work. Previously, it was indicated that phytochemical composition as well as antioxidant properties are directly influenced by environmental conditions, harvest

period, storage conditions and duration, which make comparison between our results and data reported in literature very difficult.

The correlation between secondary metabolites contents and antioxidant activities has been confirmed by the correlation matrix that clearly highlighted a positive correlation between orthodiphenols, flavonoids, flavanols contents and ferric reducing effect. The same observation was registered between phenols, tannin and DPPH, anion superoxide respectively. Furthermore, a highly positive correlation was recorded between anthocyanins content and β -carotene bleaching activities.

Several other scientific papers were in agreement with our finding. In fact, Akkol et al. (2008), Shalaby and Shanab (2013) and Aryal et al. (2019) reported in their works a strong correlation between phenolic compounds and antioxidant activities. It was previously reported that these compounds could act as reductone, increase reduce glutathione levels and/or enhance antioxidant enzyme activities. This effect could depend on the number and/or the position of hydroxyl groups, which might break oxidative chain reaction after accepting an electron and forming a stable phenol radical. Many other non-phenolic compounds such as mineral elements and vitamins could be involved alone or in synergy (Shalaby and Shanab, 2013; Sarikurkcu et al., 2015).

Conclusion

The results of our work allowed us to demonstrate the influence of extraction solvent on secondary metabolite contents and antioxidant activities. They also highlighted a significant correlation between these two factors. Therefore, it will be very interesting to isolate and purify the *H. laevigata* var. *hipponensis* M. bioactive compounds and explain their mechanism of action as natural antioxidants in order to promote them in pharmaceutical, food or biotechnological industries.

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