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The effect of extraction method on antioxidant activity of *Atractylis babelii* Hochr. leaves and flowers extracts

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Abstract: In this study, leaves and flowers of *Atractylis babelii* were chosen to investigate their antioxidant activities. Thus, a comparison between the antioxidant properties of ethanolic crude extracts obtained by two extraction methods, maceration and soxhlet extraction, was performed using two different tests; DPPH and ABTS radical assays. Besides, total polyphenol, flavonoid and condensed tannin contents were determined in leaves and flowers of *Atractylis babelii* by colorimetric methods. The results showed that there was no correlation between phenolic contents of plant parts and their antioxidant activity. Whereas, leaves and flowers of *Atractylis babelii* showed that both had almost similar phenolic contents, while their antioxidant activity depended on the plant parts. Furthermore, the antioxidant activity of plant parts was also depended on extraction method. Such a result may be likely ascribed to a variety of chemical composition can be found in *Atractylis babelii* extracts which has been related to its antioxidant properties.

Keywords: *Atractylis babelii; antioxidant activity; polyphenol contents; DPPH; ABTS*

I. Introduction

Atractylis babelii Hochr. (Asteraceae family) is a kind of endemic plant of North Africa [1, 2]. In Algeria, this plant is quite rare and its geographic range covers a short territory in the south-west of the country [2].

A small number of *Atractylis* spices have been studied for their phytochemical composition. Therefore, the knowledge about phytochemical investigations of *Atractylis* plants is very scanty, amongst these plants; *A. flava*, *A. macroceohala*, *A. carduus*, and A*. gummifera* from which flavonoids, polysaccharide, triterpenes, and diterpenes were identified [3-9]. In particular, atractyloside and carboxyatractyloside are among of terpenoids found in this genus, this diterpenoid glucosides isolated from *Atractylis gummifera* are mainly responsible for its hepatotoxic properties [10, 11]. However, therapeutic properties such as antimalarial and antileishmanial activities of *Atractylis gummifera* have been also reported [12]. moreover, *Atractylis* species are used as general remedies

in folk medicine for treatment of various diseases such as cholelithiasis, ulcer, tumor and circulatory disorders, hepatitis, when *Atractylis babelii* is particularly known in traditional North African medicine for its diuretic effects [4,13,14].

Recently, the evaluation of medicinal plants for their antioxidant properties has been received more attention because of increased concerns about oxidative stress in the human body which is found throughout in various pathologies. Whereas, the main role of antioxidants is to help the body protect itself against oxidative damage and lowering the risk of degenerative diseases [15-17]. So far, we know that the antioxidant activity of plants is attributed to the presence of antioxidant metabolites namely polyphenols, vitamins, terpenes and also with the presence of certain minerals (selenium, zinc) which enhance the effectiveness of the antioxidant system [18-20].

Generally, the bioactive compounds isolated depend mainly on the extraction methods being adapted [21-22]. Additionally, depending on the plant parts, the nature of the bioactive compound present also varied [23]. Hence, this study aimed to provide a comparison between two methods, soxhlet and maceration, used for the extraction of antioxidant compounds from leaves and flowers of *Atractylis babelii* when antioxidant capacity of obtained extracts was evaluated using DPPH and ABTS tests.

II. Materials and methods

II.1. Chemicals and Apparatus

HPLC grade ethanol absolute was obtained from Sham Lab and the other solvents are from analytical-reagent grade from Sigma-Aldrich. Folin–Ciocalteu's reagent, catechin, gallic acid, ascorbic acid, DPPH and other chemicals were also obtained from Sigma-Aldrich. Sodium carbonate was obtained from Fluka and ABTS was obtained from Bio Basic. All spectrophotometric measurements were performed using UV–VIS spectrophotometer Lamda 11 Perkin Elmer.

II.2. Plant materials and preparation of extracts

Leaves and flowers of *Atractylis babelii* were collected in May 2012 from the area of Bechar in the South of Algeria (Latitude: 30.91666, Longitude: -2.03333. 30°55'0" North, 2°1'60" West). The harvested plant was identified in the Botany and Plant Ecology Laboratory of sciences faculty of Bizerte. Leaves and flowers were dried at room temperature in a dry place with good air circulation and away from direct sunlight. Then, the air dried plant materials were ground to a fine powder with a coffee grinder (Sayona) and stored in a dry place prior to analysis. The extraction of powdered plant samples was assayed with two different methods. In the first extraction method (method N°1), plant materials were macerated at room temperature with 70% (v/v) aqueous ethanol for 24 hours. The macerates were filtered and the extracts were stored at 4°C in dark until determination of polyphenol contents and antioxidant activities. While in the second extraction method (method N°2), powdered leaves and flowers were extracted with ethanol in a Soxhlet apparatus for 6 to 8 hours until the solvent became colorless and the extracts were stored at +4°C in dark until the determination of the antioxidant activities.

II.3. Determination of total phenols content

Total phenolic content (TP) of the extracts was quantified using Folin–Ciocalteu's reagent as previously described by Singleton with slight modification [24]. 0.2 mL of diluted extract and 1mL of ten-fold diluted Foline-Ciocalteu's reagent were mixed. After 3min, 0.8 mL of a 7% aqueous sodium carbonate solution (Na_2CO_3) was added to the mixture and the contents were mixed vigorously. The sample was incubated in the dark for 30 min at 40°C and then cooled at room temperature. The absorbance was measured at 760 nm using a spectrophotometer against blank. Gallic acid (20-120 mg L^{-1}, n= 6 concentrations) was used as the standard to produce the calibration curve y=9.17x-0.01, R^2=0.98. The results are expressed in mg gallic acid equivalents per g of dry weight plant material (mg GAE/g DW). All samples were analyzed in triplicate.

II.4. Determination of total flavonoids content

Total flavonoid content (TF) was determined by the aluminum complexation method described by Zhishen et al. (1999) [25]. 0.250 mL of extract solution was added to a test tube containing 1.25 mL of distilled water. At time zero, 0.075 mL of 5% sodium nitrite ($NaNO_2$) solution was added and well mixed. After 6 min of incubation, 0.150 mL of 10% aluminium chloride hexahydrate ($AlCl_3.6H_2O$) solution was added, and then the mixture was left for a further 5 min before addition of 0.5 mL of 1 M sodium hydroxide (NaOH) solution. The total volume was immediately made up to 2.5 mL with distilled water and the solution was mixed well again. The absorbance was measured at 510 nm using a spectrophotometer against blank. Catechin (30-210 $mg.L^{-1}$, n=6 concentrations) was used as the standard to produce the calibration curve y=3.51x+0.01, R^2=0.99. The results are expressed in mg catechin equivalents per g of dry weight plant material (mg CE/g DW). All samples were analyzed in triplicate.

II.5. Determination of condensed tannins content

The condensed tannins content (TTC) was determined by the vanillin essay according to the method of Broadhurst et al. (1978) [26]. A 0.5 mL of extract solution was mixed with 3 mL of 4% vanillin–methanol solution and then 1.5 mL of concentrated hydrochloric acid (HCl) was added. The mixture was left to stand for 15 min at 20°C in the dark and then the absorbance was measured at 500 nm using a spectrophotometer against blank. Catechin (30-250 mg L^{-1}, n=6 concentrations) was used as the standard to produce the calibration curve y=2.90x+0.01, R^2=0.99. The results are expressed in mg catechin equivalents per g of dry weight plant material (mg CE/g DW). All samples were analyzed in triplicate.

II.6. DPPH radical scavenging activity

The method of Brand-Williams et al. (1995) [27] was used for measuring the DPPH radical scavenging activity. 0.050 mL of various concentrations of each extracts were mixed with 1.950 mL of a 6×10^{-5}M DPPH ethanolic solution. The mixture was shaken vigorously and left to stand for 30 min in the dark at room temperature. The absorbance of the resulting solution was then measured at 515 nm with a spectrophotometer against blank.
The ability to scavenge the DPPH radical was calculated as the inhibition percentage of free radical DPPH (I %) using the following equation:

$$[\mathrm{I}\ \% = [(\mathrm{A_{DPPH}} - \mathrm{A_S})/\mathrm{A_{DPPH}}] \times 100] \quad (1)$$

Where:
A_{DPPH} is the absorbance of the DPPH solution without an antioxidant.
A_S is the absorbance of the sample at 30 min of incubation.
The radical scavenging activity of extracts was expressed as IC_{50}. The IC_{50} value is the concentration (in mg mL^{-1}) of the sample required to scavenge 50% of the DPPH free radical. Ascorbic acid was used as a standard. All samples were analyzed in triplicate.

II.7. ABTS radical scavenging activity

ABTS radical scavenging activity of extracts was determined according to Re et al. (1999) method [28]. The $ABTS^{+\bullet}$ radical was prepared by reaction of 7 $mmol.L^{-1}$ of aqueous ABTS solution and 2.45 mmol L^{-1} potassium persulfate solution in the dark at room temperature for an overnight. Before use, the $ABTS^{+\bullet}$ solution was diluted with ethanol to get an absorbance of 0.700 ± 0.020 at 734 nm. Then, 1.950 mL $ABTS^{+\bullet}$ solution were added to 0.050 mL of different concentrations of extracts. After 6 min of incubation in the dark at room temperature, the absorbance of the resulting solution was then measured at 734 nm with a spectrophotometer against blank. The radical scavenging activity was calculated using the formula (1). Ascorbic acid was used as a standard. All samples were analyzed in triplicate.

II.8. Statistical analysis

All the experiments were carried in triplicates and the experimental results represent treatment groups expressed as means ± SD.

III. Results and Discussion

III.1. Total phenolic, flavonoid and condensed tannin contents

The amounts of total polyphenols, flavonoids and condensed tannins obtained from flower and leaf extracts of *Atractylis babelii* are presented in Table 1.

Table 1. *Total polyphenol (TP), total flavonoid (TF) and total condensed tannin (TTC) contents of leaf and flower extracts of Atractylis babelii.*

	TP (mg GAE/g DW ± SD)	**TF** (mg CE/g DW ± SD)	**TTC** (mg CE/g DW ± SD)
Leaves	8.36 ± 0.06	2.29 ± 0.01	0.58 ± 0.02
Flowers	8.89 ± 0.05	2.10 ± 0.01	0.41 ± 0.01

As shown in Table 1, only small differences in total polyphenol, flavonoid and condensed tannin amounts among leaf and flower extracts of *Atractylis babelii* were observed. Polyphenol contents determined in leaves and flowers ranged from 8.36 to 8.89 mg GAE/g DW, while flavonoid contents ranged from 2.29 to 2.10 mg CE/g DW and in the case of condensed tannin contents the amounts among leaf and flower extracts ranged from 0.58 to 0.41 mg CE/g DW.

In contrast to previous studies indicating that secondary metabolites distribution may fluctuate between different plant organs and their amounts are organ dependent [29-32], the comparison between leaves and flowers of *Atractylis babelii* showed that both had almost similar contents of polyphenol, flavonoid and condensed tannin. However, the polyphenol content was slightly higher in flowers extract (8.89 mg GAE/g DW) than in leaves extract (8.36 mg GAE/g DW), similar results were obtained in flavonoid and condensed tannin contents which flowers extract showed a slightly lower amounts (2.10 mg CE/g DW and 0.41 mg CE/g DW) in comparison with the other obtained from leaves extract (2.29 mg CE/g DW and 0.58 mg CE/g DW).

Although this plant has a short flowering stage and we know that secondary metabolites have variable rates during different seasons and physiological stages [33-35], we could deemed that the ethanolic extracts of *Atractylis babelii* gave a moderate amounts of phenolic compounds when compared to others Saharan plants from Asteraceae wich have been collected during the same month in their flowering stage such as *Artemisia arboresens* (3.42 mg GAE/g DW), *Artemisia herba halba* (13.06 mg GAE/g DW), *Artemisia campestris* (20.38 mg GAE/g DW) and *Anthemis arvensis* (32.32 mg GAE/g DW) in the study of Djeridane and al. [36]. Besides, the hard environmental conditions such as hot temperatures, dryness, short growing season may be lead to varied accumulation of secondary metabolites in Saharan plants that help them cope with such conditions [1],[37].

III.2. Radical scavenging activity

The antioxidant capacity of leaves and flowers of *Atractylis babelii* was evaluated in ethanolic, macerated (method N°1) and soxhlet (method N°2), extracts using DPPH and ABTS methods and the results are expressed as IC_{50} values which are given in Table 2.

Table 2. *radical scavenging activity of macerated and soxhlet extracts of Atractylis babelii leaves and flowers.*

	Leaves		Flowers		Ascorbic acid
	Method N°1	**Method N°2**	**Method N°1**	**Method N°2**	
DPPH IC_{50} ($mg.mL^{-1}$)	9.94 ± 0.03	5.13 ± 0.02	4.43 ± 0.01	7.26 ± 0.03	0.08 ± 0.01
ABTS IC_{50} ($mg.mL^{-1}$)	3.21 ± 0.02	2.21 ± 0.01	2.05 ± 0.01	3.47 ± 0.02	0.05 ± 0.01

In this study, the four extracts of *Atractylis babelii* were able to decolorize DPPH and the IC_{50} were ranged from 4.43 to 9.94 $mg.mL^{-1}$. Considering extracts obtained by method N°1, flowers extract with IC 50 value 4.43 $mg.mL^{-1}$ was two-fold more active than leaves extract which had IC 50 value equal to 9.94 $mg.ml^{-1}$. Whereas, as regards extracts obtained by method N°2, the extracts with the higher radical scavenging capacity were leaves extract with IC_{50} value 5.13 $mg.mL^{-1}$ which was 1.5-fold more active than flowers extract with IC 50 value 7.26 $mg.mL^{-1}$. Further, the free radical scavenging potentials of the extracts were found to be in the order of flowers extract (method N°1) > leaves extract (method N°2) > flowers extract (method N°2) > leaves extract (method N°1). In ABTS assay, the order of trend was somewhat similar to that found in DPPH assay. Whereas, flowers extract also showed the highest radical scavenging capacity (2.05 $mg.mL^{-1}$) of the extracts obtained by method N°1 contrary in method N°2 where leaves extract (2.21 $mg.mL^{-1}$) was more active than flowers extract 3.47 $mg.mL^{-1}$). However, the order of hierarchy of radical scavenging capacity was found to be flowers extract (method N°1) > leaves extract (method N°2) > leaves extract (method N°1) > flowers extract (method N°2), maintaining a slightly different ranking from that of DPPH assay.

The antioxidant capacity by ABTS assay was consistently higher than antioxidant capacity by DPPH assay in the total sample, which may be due to the fact that the reaction mechanisms of DPPH or ABTS are influenced by the chemical structure and conformation of the antioxidants [38].

It has been reported in many studies that antioxidant activities in many plants primarily attributed to their phenolic compounds, indicating a significant positive correlation between antioxidant activity and total phenolic content [39]. In our study, the determination of total polyphenols, total flavonoids and condensed tannins contents showed almost similar amounts in leaf and flower extracts whereas the results of antioxidant activity of the same extracts showed a difference about double puissance of radical scavenging between both plant parts. Thus, it is clear that the difference of antioxidant capacity between these plant parts and again between the results obtained with two extraction methods could not be explained only on the basis of their phenolic contents and may be due to interference of non-phenolic compounds. So far, we know that chemical constituents inside an extract are important factors governing its efficacy and the antioxidant capacity is also due to other metabolites such as ascorbic acid, terpenoids, tocoperol, carotenoids and others [18]. Hence, leaves and flowers of *Atractylis babelii* could be a source of antioxidants which also needs their characterization.

IV. Conclusions

In this study, antioxidant activity varied greatly among the different plant parts and extraction process used, but it wasn't correlated with the content of phenolic compounds. Our results suggested that higher antioxidant capacity of flowers compared to that of leaves in macerated extracts might be due to interfering non-phenolic compounds. In contrast to macerated extracts, antioxidant capacity of soxhlet extracts was higher in leaves than in flowers; such a result may be likely ascribed to the difference in chemical composition between extracts obtained by the two methods used. Therefore, we take an interest in leaves and flowers of *Atractylis babelii*, since they present a source of different natural antioxidants. After this comparative study, it will be so important to undertake research for

identification and determination of the amount of individual polyphenols and other metabolites responsible for the majority of antioxidant activity in leaves and flowers of *Atractylis babelii*.

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