Total Phenolic Contents and Antioxidant Activity of Methanol Extract from Leaves, Flowers and Stems of Warionia saharae L. from Morocco

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Abstract: The phenolic contents of metabolic extracts of Warionia saharae L. leaves, flowers and stems from Morocco were measured in this work. The antioxidant activities of these extracts were also evaluated. The leaves methanol extract afforded the highest yield (28.2 g/100 DW) while the lowest yield was obtained from the stems (5.6 g/100 DW). Total phenolic and flavonoid contents were (73.0 ± 0.52 mg Gallic acid/g extract) and (2.02 ± 0.22 mg quercetin equivalents (QE)/g extract). Moreover, flowers methanol extract has remarkable DPPH radical scavenging activity with IC₅₀ = 31.45 μg/mL. A result indicates that methanol flower extracts of W. saharae L. have marked amount of total phenols which could be responsible for the antioxidant activity and could be further investigated by detailed phytochemical study.

Key words: Warionia saharae L, methanol extract, total phenolic content, total flavonoid content, DPPH, radical scavenging activity.

Introduction

The use of traditional medicines remains widespread in developing countries and the plant species still serve as a rich source of many natural antioxidants intended to be a key bridge for the development of new drugs. For this reason, a thorough investigation of natural sources, namely effective anti-radical compounds, receives great attention in scientific research related to human health. The DPPH (2,2-diphenyl-1-picryl-hydrazyl) assay is one of the most commonly employed methods because, in general terms, it is simple, efficient and inexpensive ¹.

It was reported that Warionia saharae L. extract has a considerable antioxidant activity ²,³,⁴ which may be partially due to the existence of several antioxidants including phenols and flavonoids compounds. In the present study, the antioxidant activities of methanol extract of W. saharae L. was evaluated in terms of activities of scavenging DPPH radicals. The contents of phenolics and flavonoids compounds of this extract were also determined. The results will be helpful to understand this plant and here significant utilization for industrial development.

Experimental

Plant material

Leaves, flowers and stems of W. saharae L. were previously air-dried in the laboratory, in the shade until constant weight before use for extract preparation. 85.0 g of each organ was extracted by Soxhlet method in methanol for 06 hours. The resulting extracts were clarified by filtration. The clear filtrates were concentrated under reduced pressure.

Determinations of total phenolics contents

The total phenolic content (TPC) was determined by the Folin-Ciocalteu method ⁵. For the
preparation of calibration curve 1 mL aliquots of 25, 30, 50, 80, 150 and 200 μg/mL methanol gallic acid solutions were mixed with 5 mL of the Folin-Ciocalteau reagent (2M) (diluted ten-fold) and 4 mL of 7.5 % sodium carbonate (added 2 min after the Folin-Ciocalteau reagent). After incubation at 40°C for 30 min, the absorbance of the reaction mixtures were measured at 760 nm by using a spectrophotometer (Rayleigh UV-2601). 1 mL methanol leaf extracts (0.1 %) was mixed with the same reagents as described above, and after 1h the absorbance was measured for the determination of *W. saharae* L. leaf phenolics. All determinations were performed in triplicate. Total content of phenolic compounds in methanol extracts was expressed as mg gallic acid equivalents (GAE)/g methanol extract.

**Determinations of total flavonoid contents**

The AlCl₃ method was used for the determination of the total flavonoid content of the sample leaf extracts. The methanol leaf extracts (1.5 mL) was added to 10 mL volumetric flask filled with 5 mL H₂O and 0.3 mL 5 % NaNO₂ and mixed. A reagent blank using H₂O instead of sample was prepared. After 5 min., 2 mL of 2 % methanol AlCl₃ solution was added. 2 mL of NaOH (1M) was added 10 min later and then the volume was made up to 10 mL with H₂O. The mixture was vigorously shaken on orbital shaker for 5 min and after 10 min of incubation; the absorbance was read at 510 nm. All determinations were performed in triplicate. Flavonoid contents were calculated using a standard calibration curve, prepared from quercetin. The calibration curve range was 0.5-20 μg/mL. The flavonoid contents were expressed as mg quercetin (QE)/g methanol extracts.

**Evaluation of antioxidant activity with DPPH radical scavenging assay**

Gallic acid, The free radical scavenging activity of different parts of *W. saharae* L (leaves, flowers and stems) and that of the reference antioxidants (vitamin C and α-tocopherol), was determined using the method described by Popovici *et al*.

Briefly, 3 ml methanol extracts solution of each part of *W. saharae* L., at various concentrations were respectively added to 75 μL of DPPH methanolic solution (1.3 mg/mL). The resulting mixtures were vortexed vigorously and allowed to stand in the dark for 30 min. Finally, the absorbance of these mixtures was measured by using a spectrophotometer (Rayleigh UV-2601) at 517 nm.

The antiradical activity in percentage (%) was calculated using the following formula:

\[
\text{DPPH scavenging activity} \% = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100
\]

Where \( A_{\text{blank}} \) is the absorbance of the control reaction (containing all reagents except the test compound), \( A_{\text{sample}} \) is the absorbance of the test extract.

Extract concentration providing 50 % inhibition (EC₅₀) was calculated from the graph plotted of inhibition percentage against extract concentrations. The ascorbic acid and α-tocopherol methanol solutions were used as positive control.

**Results and discussion**

**Quality control tests on UV-Vis spectrophotometers**

Control of wavelengths: The wavelength accuracy of the spectrophotometer (UV Rayleigh 2601), was evaluated using the spectrum recorded between 200 and 400 nm of potassium dichromate solution \( \text{K}_2\text{Cr}_2\text{O}_7 \), to 60.06 mg/L in 0.005M sulfuric acid. A solution was prepared to check the absorbance accuracy because wavelength standards such as holmium oxide solution couldn’t be obtained.

The ultraviolet absorbance spectrum of a 60.06 mg/L solution of potassium dichromate in 0.005 M sulfuric acid is shown in figure 1. This solution absorbs approximately between 200-400 nm and has two maxima and minima at 235, 257, 313 and 350 nm. Accepted deviation for wavelength-readings in the UV region is ±1 nm. The instrument tested, passed this test, although it cannot be assumed that the wavelengths in the visible range were also correct.

**Control of absorbance:**

The absorbance accuracy of the spectrophotom-
Table 1. Wavelength Accuracy of the spectrophotometer tested

| Wavelength required/K₂Cr₂O₇ solution | 235  | 257  | 313  | 350  |
|--------------------------------------|------|------|------|------|
| Wavelength observed by spectrophotometers* | 235.70±0.58 | 256.0±1 | 314±1 | 351.30±1.15 |

*Each value was expressed as the mean ± S.D. (n = 3)

Figure 1. A potassium dichromate solution scan

The limit stray light was detected by measuring the absorbance of potassium chloride aqueous solution to 12 g/L in a 1 cm cell between 210 nm and 200 nm, using distilled water as blank. Table 3 shows the absorbance of 1.2 % KCl solution. The recorded values were greater than 2A between 198 and 200 nm; and increase steeply between 210 nm and 200 nm.

In conclusion, and from the quality control results, it is shown clearly that the proper functioning of the spectrophotometer tested (UV Rayleigh 2601), cells and solvents used are prerequisites to ensure accurate and reliable results.

Extraction yield, total phenolic and total flavonoid content

The yield of methanol extracts from different parts of *W. saharae* L. is presented in table 4. From the table, leaves of this plant show the highest extraction yields (28.2 g/100 g of DW), flowers presented (10.4 g/100 g of DW). The Low extraction yield is observed in stem (5.6 g/100 g of DW). It is difficult to compare these results with those of the bibliography, because the yield is relative. Therefore, various factors such as experimental conditions, sample preparation methods, and physiological relevance of the assays should be considered in the evaluation of extraction 9.

The content of extractable phenolic compounds in the *W. saharae* L. leaf extracts was determined through a linear gallic acid standard curve (y = 0.005x− 0.078; r² = 0.996), and was found to be (72.99 ± 0.52) mg GAE/g methanol extracts. The significant differences were found in total phenolic compounds content of *W. saharae* L. leaf extracts. Amezouar F. and coworkers reported that total polyphenol contents found of *W. saharae* L leaf ethanol extracts was (57.85 ± 0.03) mg GAE/g DW as equivalent to 650 mg GAE/g ethanol extracts 10. This finding is also in agreement with the previous studies which reported that to-
Table 2. Absorbance accuracy of the spectrophotometer tested

| Wavelengths (nm) | Absorbance\(^a\) | Specific absorbance | Tolerance       |
|------------------|-------------------|---------------------|-----------------|
| 235              | 0.738±0.009       | 122.87              | 122.9-126.2     |
| 257              | 0.875±0.007       | 145.68              | 142.8-146.2     |
| 313              | 0.290±0.004       | 48.28               | 47.0-50.3       |
| 350              | 0.644±0.005       | 107.22              | 105.6-109.0     |

\(^a\): Each value was expressed as the mean ± SD. (n = 3)

Table 3. Stray light testing according to Ph. Eur. using 12 g/L KCl aqueous solutions

| Mesure no. | \(\lambda\) (nm) | Absorbance\(^a\) | Tolerance           |
|------------|------------------|-------------------|---------------------|
| 1          | 198              | 3.4010±0.0008     | > 2                 |
| 2          | 199              | 3.2500±0.0003     |                     |
| 3          | 200              | 2.7520±0.0007     |                     |
| 4          | 210              | 0.2500±0.0007     | Increase steeply between 210 nm and 200 nm |

\(^a\): Each value was expressed as the mean ± SD. (n = 3)

Table 4. The yield of methanol extracts from different parts of \(W.\) saharae L

| Part of \(W.\) Saharae | Yield extraction \((g/100\ g\ of\ DW)\) |
|-------------------------|------------------------------------------|
| Leaves                  | 28.2                                     |
| Flowers                 | 10.4                                     |
| Stem                    | 5.6                                      |

tal phenolic content of leaf aqueous extract was \((56.34 ± 3.13)\ mg\ GAE/g\ aqueous\ extract\)\(^{11}\).

Flavonoids are the most common and widely distributed group of plant phenolic compounds, which usually are very effective antioxidants\(^{12}\). In this work, the total flavonoid content of methanol extract from leaves of \(W.\) saharae L, was measured according to aluminum colorimetric method. Quercetin was used as a standard \((y = 0.07x + 0.023; r^2 = 0.997)\) and the total flavonoid content was found to be \((2.02 ± 0.22)\ mg\ QE/g\ methanolic\ extract\). The previous study showed that \(W.\) saharae L. leaves had a flavonoid content of \((19.31 ± 0.19)\ mg\ GAE/g\ aqueous\ extract\)\(^{10}\). On the other hand, Amezouar F.\(^{10}\) found that the extraction of total flavonoid with ethanol from \(W.\) saharae L. leaves contained \((15.95 ± 0.01)\ mg\ QE/g\ DW\).

The difference in the extraction yield could be the result of using different extraction solvents in other works and methanol in ours. The importance of the solvent type used in the extraction has already been mentioned\(^{13,14}\). These authors showed significant variability on phenolic contents in the same extract when using solvents with different polarities.

Kinetic study of DPPH scavenging

The evolution of reaction kinetics with DPPH depends on the nature of the antioxidant being tested. Kinetic behavior of ascorbic acid and \(\alpha\)-tocopherol is shown respectively in figure 2 and figure 3. As indicated in fig. 2, the DPPH\(^*\) ascorbic acid reaction was carried out in two steps with a first addition of ascorbic acid solution. The initiate step is characterized by a fast decay in absorbance in the first seconds, followed by a second step in which the steady state was reached.
Two zones are distinguished: high DPPH• scavenging zone, during which the absorbance of the mixture decreases very rapidly and tumbles down after five minutes from 0.792 to a very low value between 0.024 and 0.031. In this case, ascorbic acid at a concentration of 10 μg/mL, reacted with more than 97% of the initial DPPH•. The second zone describes the equilibrium where absorbance was stable after 10 minutes for all the ascorbic acid concentrations.

The kinetic profiles of α-tocopherol solutions (fig. 3), introduced in the DPPH solution, reveal an anti-radical activity largely dependent on the concentrations of this antioxidant compound. As shown in (fig. 3), more α-tocopherol solution is concentrated, more the absorbance decrease is important. At concentrations of 60 μg/mL, the available DPPH free radicals were almost completely consumed in a rapid and instantaneous reaction, resulting in a color change from deep violet to light yellow of reaction mixture. This would allow for the transformation of DPPH - free radical into its reduced form DPPH-H. The absorbance decreases from maximum value 0.8 to 0.12, which indicates that 85% of initial DPPH’s - free radical concentration is reduced. This step is immediately followed by a steady state and signals the end-point of the reduction.
reaction.

The scavenging activity of the reference antioxidants (ascorbic acid or α-tocopherol) against DPPH•, is dependent on the mobility of the hydrogen atoms at their hydroxyl groups. In methanol (polar solvents), hydrogen atom was transferred via electron transfer process as showed in earlier study 15.

It can be seen that the aspects of graphs shown above, points out that all samples reveal similar trends with a different degree of absorbance decreases at 517 nm, 5 min after addition of each extract. Thus, corresponds to the reduction of a DPPH• fraction initially present, probably due to the hydrogen donating ability derived from existing compounds such as flavonoids phenols or other antioxidants 16.

At 60 μg/mL of methanol extracts of *W. saharae* L. leaves (fig. 4), flowers (fig. 5) and stems (fig. 6), the initial amount of DPPH• decreased by 33 %, 58 % and 31 % for 30 min, respectively. These results have been confirmed by a decrease in absorbance of methanol extracts from *W. saharae* L. leaves from 0.792 to 0.536, followed by flowers from 0.792 to 0.339 and stems from 0.792 to 0.549. This suggests that the different extracts might contain primary antioxidant compounds, which are able to react aggressively with DPPH• free-radicals.

**Percentage of inhibition of DPPH activity**

The percentage inhibition of DPPH• free radical scavenging activity of antioxidant standards (ascorbic acid and α-tocopherol) and methanol extracts from tree parts of *W. saharae* L. was measured and can be seen in figures 7 and 8.

![Figure 4. Kinetic profiles of DPPH• reduction by addition of methanol flowers extracts at different concentrations](image)

![Figure 5. Kinetic profiles of DPPH• reduction by addition of methanol leaf extracts at different concentrations](image)
Figure 6. Kinetic profiles of DPPH• reduction by addition of methanol stems extracts at different concentrations

The results indicate that the percentage inhibition increased if the concentration of reactants is raised. As shown in fig. 7 ascorbic acid has potent percentage inhibition greater than that of α-tocopherol, Ascorbic acid at 10 μg/mL, showed it equivalent to 96.25 ± 0.35 %, 3 times higher than that of α-tocopherol (26.35 ± 0.49 %).

From the analysis of fig. 8, we see that the scavenging effects of flowers extracts on DPPH radicals was important compared to the leaves and stems extract at all concentrations. The methanol extracts of *W. saharae* L. flowers, at 60 μg/mL possessed up to 82 % of DPPH radical percentage inhibition. The leaves and stem parts had less percentage inhibition equivalent to 44.78 ± 0.1 % and 38.51 ± 0.35 % respectively.

**IC<sub>50</sub> determination**

The IC<sub>50</sub> value, (effective concentration of sample required to scavenge DPPH radical by 50 %), was calculated out by linear regression analysis of curve plotting between % inhibition and concentrations. A lower IC<sub>50</sub> value corresponds with a higher antioxidant power 17.

In the present investigation various extracts and standard antioxidants tested, showed a wide variation in IC<sub>50</sub> values ranging from 1.28 μg/mL to 88.8 μg/mL. The figure 9 indicates different IC<sub>50</sub> values obtained. The DPPH scavenging activity is arranged in the following descending order: ascorbic acid (IC<sub>50</sub> = 1.28 μg/mL) > α-tocopherol (IC<sub>50</sub> = 11.29 μg/mL) > flowers (IC<sub>50</sub> = 31.45 μg/mL) > leaves (IC<sub>50</sub> = 63.71 μg/mL) > stem (IC<sub>50</sub> =

Figure 7. DPPH free radical scavenging activity of standard ascorbic acid α-tocopherol of α-tocopherol (26.35 ± 0.49 %)
It appears from these results that the methanol extracts of the *W. saharae* L. flowers, is the most active (IC$_{50}$ = 31.45 µg/mL) of the tested *W. saharae* L. extracts. Methanol leaf extracts (IC$_{50}$ = 63.71 µg/mL) appeared to be three times more active than ethanol and ethylacetate extracts of the same organ as demonstrated in previous study $^{10}$.

This could be explained by the presence of total polyphenols and flavonoids, in large quantities in the methanol extract, or by a synergy of these compounds with other, such as saponins, which may be involved in the antioxidant power.

**Conclusion**

High antioxidant activity is observed in the flower methanol extracts of *W. saharae* L. as compared to other tested extracts. Thus, these extracts can be regarded as promising candidates for a plant-derived antioxidant compound.

This study reveals that Moroccans species offer an interesting source of new antioxidative plant extracts, such as those of *W. saharae* L., there being a potential for their use in different fields (foods, cosmetics, pharmaceuticals). Flavonoids and phenolic acids may be the compounds responsible for the antioxidant activity in these plants.

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