Original Research Article

Radical-Scavenging Effect, Ferric Reducing Ability and Phytochemical Analysis of *Urtica urens* (L.) and *Mercurialis annua* (L.)

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Abstract: Important Moroccan medicinal plants known for their food and medicinal potential include *Urtica urens* and *Mercurialis annua*. We examined the antioxidant activity of the methanolic and aqueous extracts by several *in vitro* systems of the assay, namely the ferric reducing/antioxidant power (FRAP) assay, the DPPH radical-scavenging activity, and the ABTS free radical-scavenging capacity. In addition, all active extracts were subjected to phytochemical screening to determine the key groups of chemicals implicated in their therapeutic activity. Total phenolic and flavonoid concentrations were also measured to see how they affected the antioxidant properties of the plant extracts. The extracts of *Urtica urens* and *Mercurialis annua* were found to have different levels of antioxidant effect in the systems tested. Based on the three different antioxidant assays, the *Mercurialis annua* extracts showed the highest values of antioxidant ability, based on the three used assays.
Phytochemical testing of studied extracts revealed the presence of flavonoids, anthocyanins, and tannins. Total phenol and flavonoid contents were shown to provide the highest association with different antioxidant methods. The present study provides evidence that the extracts of *Urtica urens* and *Mercurialis annua* are a potential source of natural antioxidants, and this justifies their uses in Moroccan traditional medicine.

**Keywords:** *Urtica urens*; *Mercurialis annua*; Phytochemical screening; Phenolic and flavonoid contents; antioxidant effects.

### 1. Introduction

Free radicals produced in the body during normal physiologic functions or introduced from the entourage are highly unstable products capable of inducing several side health effects and diseases [1–5]. Medicinal herbs are known to have the ability to reduce this damage and thus prevent oxidative stress-related diseases [6–9]. *Urtica urens* and *Mercurialis annua* are the most common species worldwide, extensively used for diverse medicinal purposes.

*Urtica urens* (Urticacea), locally known as “Hurriyqa”, is a perennial plant with stinging hairs frequently used as decoctions, infusions, or powders to treat anxiety, arthritis, rheumatism, cancer, toothache, scabies, and pruritus [10–13]. Seeds soaked in milk are frequently used against cough, kidney stones, cystitis, and oliguria [10]. The aerial parts are known for diuretic, galactogenic, and aphrodisiac properties [10], and are used to treat cancer and sciatica [14], and as a styptic [15], the leaves are frequently used against burns, rheumatism, and urinary diseases [14–16]. Several pharmacological and phytochemical works have confirmed their traditional uses in folk medicine [11,17,18]. The methanolic extract of the *Urtica urens* aerial part has proved to have anxiolytic activity [11], free radical scavenging activity, and antimicrobial potential [19]. The ethanol and aqueous extracts of the leaves of *Urtica urens* have been tested on *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* and show considerable antibacterial activity [17]. Many studies have shown that the biological properties of *U. urens* are especially related to its richness on various bioactive compounds such as polyphenols [20], caffeic acid [21], and chlorogenic acid [22].

*Mercurialis annua* (Euphorbiaceae), popularly known in Moroccan folk medicine as “Hurriyqa l-melsâ”, is largely used in the treatment of a range of disorders [11,23,24]. In Morocco, the plant is extensively used as a purgative, anxiolytic, and also in treating Female Infertility [10,11,25]. In Portugal, the whole plant is known for its diuretic effect and is traditionally used to treat internal parasitosis and oral inflammation [26]. The Italians widely use leaf infusions against diuresis and constipation [24]. One cup of a leaf decoction is taken
three times/day to treat diabetes and cancer in Israeli folk medicine.\cite{27} The plant has also been used as a laxative\cite{28}, anti-warts, and antbronchial\cite{29}. The dried, powdered plant also promotes wound healing\cite{30}. Several studies have confirmed the various traditional reputations\cite{18,23,31}. \textit{Mercurialis annua} is plenty of biological effects, such as anti-cancer\cite{23}, anti-inflammatory\cite{23}, anti-microbial\cite{23}, anti-anxiety\cite{18}, and anxiolytic effects\cite{31}. Many reports have demonstrated that pharmacological activities of \textit{M. annua} are related to their rich content of phenolic compounds such as rutin, narcissin, flavonol glycosides\cite{32-34}.

Despite the large flow of data of \textit{Urtica urens} and \textit{Mercurialis annua} uses, and to the authors’ knowledge, no works have so far been performed to evaluate the antioxidant effect of the Moroccan \textit{Urtica urens} and \textit{Mercurialis annua}. Accordingly, this study mainly tested the ABTS and DPPH radical-scavenging abilities, the ferric reducing power (FRAP) effect, the phenolics contents of \textit{Urtica urens} and \textit{Mercurialis annua} methanolic extracts.

\section*{2. Materials and Methods}

\subsection*{2.1. Plant Materials}

\textit{Mercurialis annua} and \textit{Urtica urens} aerial parts were manually collected from Wazzan town (Jaouna el Basra), Morocco, and were identified by Pr. M. Ibn Tatou and Pr. H. Khammar of the Scientific Institute of Rabat, Mohammed V University, Rabat, Morocco, where voucher specimens have been deposited at its herbarium (voucher number RAB78984 and RAB78983 respectively). The samples were thoroughly dried at laboratory ambient temperature, grounded into a fine powder, and kept at room temperature until further use.

\subsection*{2.2. Extracts preparation}

Methanolic extract of \textit{Mercurialis annua} (MAM) and \textit{Urtica urens} (UUM) were separately performed by cold maceration using 100 g of Dried and powdered aerial parts in 1000 mL of methanol at room temperature 24 h. Aqueous macerate of \textit{Mercurialis annua} (MAA) and \textit{Urtica urens} (UUA) were prepared by the same procedure. The macerates were filtered, and the filtrates obtained were evaporated under reduced pressure and at a temperature lower than 65 °C.

\subsection*{2.3. Phytochemical Screening}

\textit{Mercurialis annua} and \textit{Urtica urens} extracts were evaluated for the qualitative determination of major phytoconstituents, i.e., alkaloids, flavonoids, tannins, saponins, and cardiac glycosides following these methods\cite{35,36}.
2.4. Determination of Total Phenolic Content

Total phenolic content (TPC) was spectrophotometrically determined according to the Folin-Ciocalteu method [37]. A total of 20µL aliquot of the tested extract was mixed with 1.16mL of distilled water, 100µL of Folin Ciocalteu reagent, and 300µL of Na₂CO₃ solution (20%). The tubes were kept at 40°C for 30 min, and the absorbance was measured at 760nm utilizing UV–visible spectrophotometer. The total phenolic content was obtained from the extrapolation of the calibration curve, which was made by various concentrations of gallic acid solution in methanol. TPC was expressed as µg of gallic acid equivalents (µg GAE)/mg dry extract.

2.5. Determination of Total Flavonoid Content

The overall quantity of flavonoids (TFC) of Mercurialis annua and Urtica urens extracts was investigated using the method described by Ordonez et al., 2006 [37] with slight modifications. Briefly, 0.5mL of the extracts was mixed with 0.5mL of 2% AlCl₃. After 1 hour of incubation at room temperature, the absorbance was measured at 420 nm. TFC was expressed as microgram quercetin equivalent per milligram dry plant extract (µg QCE/mg dry extract).

2.6. Antioxidant Activity

The antioxidant effects of Mercurialis annua and Urtica urens extracts were tested using three complementary standard tests: DPPH free radical scavenging, ABTS scavenging, and ferric-reducing antioxidant power (FRAP) assays. All samples were analyzed in triplicate.

2.7. DPPH Free Radical-Scavenging Activity

The DPPH radical scavenging effect of Mercurialis annua and Urtica urens extracts was evaluated according to Sahin method [38]. A mixture containing 0.50 mL of various extracts concentrations and 2 mL of the DPPH methanolic solution (60µM) was incubated in the dark for 20 min. The absorbance was then measured at 517 nm and the percentage of DPPH radical scavenging performance was calculated as following Eq. (1):

\[
\text{Percentage (％) of DPPH radical scavenging ability} = \left[\frac{(A_0-A_1)}{A_0}\right] \times 100
\]

Where, \(A_0\) was the absorbance of blank, and \(A_1\) was the absorbance of the studied extract. Quercetin (QC) and Butyl hydroxytoulene (BHT) were taken as standards. The extract
concentration providing 50% of inhibition (IC$_{50}$) was calculated from the plotted inhibition curve (%) against concentrations.

2.8. ABTS Free Radical-Scavenging Assay

The ability of water and methanolic extracts of *Mercurialis annua* and *Urtica urens* to scavenge the ABTS radical was estimated using the method described by Pukalskas et al. [39]. A radical solution was produced by combining 10 mL of ABTS (7 mM), and 10 mL of potassium persulphate (70 mM) was kept in the dark at room temperature for 16 h. After the incubation period, the ABTS solution was diluted to obtain an absorbance of 0.700 ± 0.002 at 734 nm. A total of 100 µL of the diluted extract was added to 2mL of ABTS solution freshly prepared, and the absorbance was measured at 734 nm after 1 min. The percentage inhibition and the half-maximal inhibitory concentration (IC$_{50}$) of the extract were calculated with the same procedure described above.

2.9. Reducing Power Determination

The ferric-reducing ability of *Mercurialis annua* and *Urtica urens* extracts was investigated using the method of Tundis [40]. A mixture containing 0.2mL of each extract at various concentrations, 2.5 mL of potassium ferricyanide (1%), and 2.5 mL sodium phosphate buffers (0.2M, pH 6.6) was incubated at 50 °C for 20 min. After the incubation was completed, 2.5 mL of trichloroacetic acid (10%) was added, and the resulting mixture was centrifuged at 1000 r/min for 10 min; then, 2.5 ml of the supernatant was mixed with 2.5 mL of distilled water and 0.5 ml of FeCl$_3$ (0.1%). The absorbance was measured at 700 nm, and the IC$_{50}$ was calculated by plotting the absorbances versus the sample concentrations.

2.10. Statistical Analysis

All analyses were carried out in triplicate, and these values were then presented as mean values ± their standard derivations (SD). Data were analyzed using the Graph pad prism 6.0. Statistical comparisons were performed with one-way analysis of variance (one-way ANOVA), and (p < 0.05) were regarded as significant. The correlation coefficients (R) between DPPH, ABTS, FRAP, TPC, and TFC were calculated to determine their relationship.
3. Results and Discussion

3.1. Extraction Yield

The extraction yields of *Mercurialis annua* and *Urtica urens* aerial parts in methanol and water were determined and are shown in Figure 1. Depending on the species and the extraction solvent, the dry weight yield in the studied samples was remarkably affected. *Mercurialis annua* aqueous extracts showed the highest extraction yield (32.7%), followed by the methanolic extract from the same plant (21.23%). While *Urtica urens* samples extracted with water and methanol gave extract yields of 12.12% and 11.92%, respectively. The difference observed can be explained by the solvents’ polarity and the solubility of bioactive compounds \[41\].

![Figure 1. Yield (%) of *Mercurialis annua* and *Urtica urens* extracts.](image)

3.2. Phytochemical Screening

Phytochemical screening of the crude extracts of *M. annua* and *U. urens* revealed the major classes of compounds present to be polyphenols, flavonoids, and tannins (Table 1). These constituents are known to possess various pharmacological activities including antioxidant, anticancer, antifungal, antibacterial, anti-inflammatory, and antidiabetic \[42–46\]. Thus, the presence of these compounds may be responsible for the antioxidant abilities of the extracts.
Table 1. Phytochemical screening of crude extracts of *Mercurialis annua* and *Urtica urens*.

<table>
<thead>
<tr>
<th></th>
<th>MAA</th>
<th>MAM</th>
<th>UUA</th>
<th>UUM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total polyphenols</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthocyanins</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Terpenes</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Quinones</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Total Phenolic Content**

*Mercurialis annua* and *Urtica urens* extracts were characterized by a considerable amount of phenolic content (Figure 2). The highest TPC was found in MAM with (223.7 ± 1.59) µg GAE/mg edw, followed by MAA (168.274 ± 2.06) µg GAE/mg dry plant extract (edw), UUM (130.816 ± 2.82) µg GAE/mg edw, and UUA (109.768 ± 3.60) µg GAE/mg edw. The richness of *Mercurialis annua* and *Urtica urens* on phenolic compounds agrees with several reports [33,47]. However, the variation in total phenolic content of the different studied extracts can be explained by various parameters and conditions such as genetic factors and type of extract.

![Figure 2](image_url). Total Phenolic Content expressed as gallic acid equivalents (µgGAE)/mg plant extracts of *Mercurialis annua* and *Urtica urens*. Data are expressed as mean ± SD (n=3).
3.3. Total Flavonoid Content

Flavonoid content in investigated extracts ranged from 10.98 to 80.71 μg (QE)/mg edw (Figure 3). The high flavonoids concentration was determined in MAM extract (80.71 ± 2.27 μg (QE)/mg edw) followed by UUM (43.79 ± 2.10 μg (QE)/mg dry plant extract (edw)), MAA (29.51 ± 0.51 μg (QE)/mg edw). The lowest total flavonoid content was determined for UUA extract (10.98 ± 0.39 μg (QE)/mg edw). The richness of *Mercurialis annua* and *Urtica urens* extracts on flavonoid compounds is in agreement with several studies \cite{33,47}. However, the variation in total flavonoid Content between the studied extracts can be explained by various parameters and conditions such as genetic factors and type of extract.

![Figure 3](image_url). Total flavonoid content expressed as quercetin equivalents (μgQE)/mg plant extracts of *Mercurialis annua* and *Urtica urens*. Data are expressed as mean ± SD (n=3).

3.4. Antioxidant Activity

The antioxidant activity of the *Mercurialis annua* and *Urtica urens*, evaluated by the three assays varied significantly among the extracts (Table 2). The MAM and MAA extracts presented the most substantial reducer effect in the FRAP assay (IC$_{50}$ = 79.15 ± 0.14 and 91.52 ± 1.30 μg/mL, respectively) and the better antioxidant capacity in the DPPH (IC$_{50}$ = 51.16 ± 0.09 and 61.32 ± 0.65 μg/mL respectively) as well as in the ABTS assays (IC$_{50}$ = 59.00 ± 1.48 and 73.61 ± 0.88 μg/mL respectively). The lowest capacities were recorded for the *Urtica urens* extracts with an IC$_{50}$ ≥ 227.11 μg/mL. However, all the tested extracts exhibited low antioxidant capacities compared to Quercetin, Trolox, and BHT with IC$_{50}$ from 1.29 to 7.02 μg/mL. The antioxidant potential of *Mercurialis annua* and *Urtica urens* might
be explained partly by their richness in polyphenols such as flavonoids previously revealed in our study (Table 1, Figure 1, Figure 2). The antioxidant activities of these compounds have mainly been reported to have strong antioxidant power. Various reports have been on the antioxidant effects of several *Mercurialis* and *Urtica* species. *Urtica urens* aerial parts extract exhibited great antioxidant activity which has been strongly associated with its richness in phenolic compound. Aerial parts of *Urtica membranacea* also showed a potent antioxidant effect. The whole plant of *Urtica dioica* showing a strong antioxidant activity in the cupric reducing antioxidant capacity (CUPRAC) and the ferric reducing/antioxidant power (FRAP) assays which mainly due to its richness on phenolic compounds such as ursolic acid and quercetin. Stinging nettles provide high antioxidant activity and richness in phenolic compounds. The antioxidant ability of the nettle (*Urtica dioica*) has mainly been attributed to phenolic compounds such as rutin, quercetin 3-O-glucoside, chlorogenic acid, 2-O-caffeoylmalic acid, isorhamnetin 3-O-rutinoside, kaempferol 3-O-rutinoside, caffeic acid derivatives. In the same sense, several studies have shown the richness of *Mercurialis* spp. on phenolic compounds widely known for their potent antioxidant power, such as kaempferol, squalene, and cycloartenol.

**Table 2.** IC50 values (μg/mL) of *Mercurialis annua* and *Urtica urens* extracts and of quercetin, BHT, and Trolox.

<table>
<thead>
<tr>
<th>Assays</th>
<th>Plant extracts</th>
<th>Positive controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAA</td>
<td>MAM</td>
</tr>
<tr>
<td>DPPH</td>
<td>61.32 ± 0.65</td>
<td>51.16 ± 0.09</td>
</tr>
<tr>
<td>ABTS</td>
<td>73.61 ± 0.88</td>
<td>59.00 ± 1.48</td>
</tr>
<tr>
<td>FRAP</td>
<td>91.52 ± 1.30</td>
<td>79.15 ± 0.14</td>
</tr>
</tbody>
</table>

3.5. Relationship between Antioxidant Assays

To evaluate the reliability and suitability of the three antioxidant assays used to determine the antioxidant abilities of *Mercurialis annua* and *Urtica urens* extracts, we performed a correlation analysis of the three used tests. As shown in Table 3, the values of DPPH were highly correlated with ABTS (R=0.997; p>0.05) and FRAP (R=0.955; p>0.05). The relation between ABTS and FRAP also showed a high and insignificant correlation.
(R=0.976; p >0.05). This suggests that DPPH, ABTS, and FRAP can be predictive assays for each other. The relationship between the three used assays has been largely reported [58,59].

**Table 3.** The correlation coefficient among antioxidant tests and total phenolic and flavonoid contents.

<table>
<thead>
<tr>
<th></th>
<th>DPPH</th>
<th>ABTS</th>
<th>FRAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABTS</td>
<td>0.997</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FRAP</td>
<td>0.955</td>
<td>0.976</td>
<td>-</td>
</tr>
<tr>
<td>TPC</td>
<td>-0.903</td>
<td>-0.908</td>
<td>-0.879</td>
</tr>
<tr>
<td>TFC</td>
<td>-0.622</td>
<td>-0.656</td>
<td>-0.710</td>
</tr>
</tbody>
</table>

### 3.6. Relationship between Antioxidant Effect and Phenolic Content

The relationship between the antioxidant effect and phenolic content of *Mercurialis annua* and *Urtica urens* extracts is described in Table 3. The TPC was negatively but highly correlated with DPPH (R=-0.903; p<0.05), ABTS (R=-0.908; p<0.05), and FRAP (R=-0.879; p<0.05). The negative relationship is due to the antioxidant activity in terms of IC50 decreases, the TPC increases. The results show that high TPC indicates a better antioxidant effect in terms of DPPH, ABTS, and FRAP assays of *Mercurialis annua* and *Urtica urens* extracts. The TFC was correlated with DPPH (R=-0.622; p<0.05), ABTS (R=-0.656; p<0.05), and FRAP (R=-0.710; p<0.05). The results of flavonoid content were quite similar to those of phenolic content; thus, both of them can be a measure to assess the antioxidant effects of *Mercurialis annua* and *Urtica urens* extracts, but still, the role of other phenolic compounds and metabolites present in the plants. Generally, individual flavonoid compounds cannot be as effective as in combination with other phenolic compounds. The high correlations between the phenolic content (TPC and TFC) and antioxidant potential have been reported for other medicinal plants such as *Hedychium spicatum*, *Azadirachta indica*, *Juniperus thurifera*, *J. oxycedrus*, *J. phoenicea* and *Tetraclinis articulata* [58,60,61]. Therefore, total phenolic and flavonoid content can be used to assess medicinal plants' antioxidant ability.

### 4. Conclusion

The results obtained in this work support the therapeutic uses of *Mercurialis annua* and *Urtica urens*. Their phenolic content contributed an increased antioxidant activity of those Moroccan species. In addition, the study suggests that the water and methanolic extracts
from the *Mercurialis annua* and *Urtica urens* constitute a valuable source of antioxidant metabolites. Further investigations are required to identify the bioactive metabolites. The study also confirms that phenolic content can predict antioxidant assays like ABTS, DPPH, and FRAP.


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**Competing interests:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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