ANTIOXIDANT ACTIVITY OF RUMEX VESICARIUS L. AT THE VEGETATIVE STAGE OF GROWTH

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ABSTRACT

The present work has been carried out to investigate some biologically active constituents and antioxidant activity of different plant parts of Rumex vesicarius L., at the vegetative stages of growth (early and late vegetative stages). There were variations in the presence and/or amount of these active ingredients within different plant parts in these two stages of growth. Total phenolics in different plant parts, showed that whole plant parts (at late vegetative stage) were the richest organ in this regard (4.518±0.018 mg GAE/g F.W.). It was found that, all plant parts, at both early and late vegetative stages of growth were rich in anthraquinones and whole plant parts extract (at early vegetative stage) was found to contain the highest amount of anthraquinones (2054±39.600 µg/g F.W.). It was found also that, all plant parts, at early vegetative stage of growth were rich in flavonoids. Leaves contained the highest amount of flavonoids (28350.000±31.110 µg/g F.W.). Quantitative estimation of Emodin (using HPLC analysis) revealed that, all plant parts (at both early and late vegetative stages of growth) contained Emodin in high amounts, whole plant parts extract (at early vegetative stage of growth) was found to contain the highest amount of Quercetin (66.360 ±0.73 µg/g F.W.). Regarding antioxidant activity studies, using total antioxidant activity and DPPH scavenging activity methods, it was found that, root (at late vegetative stage) extract had the highest amount of total antioxidants (428606.000±4792.885 GAEs as ppm). Results of DPPH scavenging activity studies revealed that, the least IC₅₀ (the highest the effectiveness) was obtained using leaves (at early vegetative stage) extract (IC₅₀= 0.345±0.005 mg/ml).

Key words: Rumex vesicarius L., antioxidant activity, anthraquinones, flavonoids, phenolics, Emodin, Quercetin, HPLC.

INTRODUCTION

The genus Rumex, (family: Polygonaceae) comprises about 150 species widely distributed around the World. The main chemical constituents of Rumex are anthraquinones and flavonoids (1).

The genus includes many edible plant species that have medicinal importance for the treatment of some most dangerous diseases (2, 3).

Rumex vesicarius L. is a wild edible plant used as a sorrel and collected in spring time and eaten fresh, or cooked. The species has many important medicinal uses such as treatment of tumors, hepatic diseases, bad digestion, constipation, calculi, heart troubles, pains, diseases of the spleen, hiccup, flatulence, asthma, bronchitis, dyspepsia, piles, scabies, leucoderma, toothache and nausea. The use of this species as a food is spreading and becomes widely distributed around the World. The main chemical composition since this plant contains many bioactive substances such as polyphenols, flavonoids, carotenoids, vitamins (especially vitamin C), proteins, lipids, carbohydrates, reducing sugars, phenols, tannins, saponins, triterpenoids and organic acids. This plant is also a good source of minerals, such as; K, Na, Ca, Mg, Fe, Mn, Cu (4-7).

The previously mentioned bioactive phytochemicals found in Rumex vesicarius L. (such as polyphenols, flavonoids, carotenoids, tocopherols and ascorbic acid) have a role as antioxidant and detoxifying agents. The intake of dietary antioxidant phytochemicals like carotenoids, phenolic compounds and flavonoids will lead to the protection against noncommunicable diseases in human beings: cancer, cardiovascular diseases and cataract (8-9).

We aim in this study to investigate some biologically active constituents and antioxidant activity of different plant parts of Rumex vesicarius L., at the vegetative stages of growth.

MATERIALS AND METHODS

Plant materials

Rumex vesicarius L. samples were collected at early vegetative stage of growth (February), late vegetative stage (March) from 60 km away from Ain Sokhna, Quattamia- Ain Sokhna desert road, Egypt. Plant specimens were botanically identified and authenticated by comparing with herbarium specimens (10). Sample was deposited in the Herbarium of the Botany and Microbiology Department, Faculty of Science, Helwan University, Helwan, Egypt (Number : 1057). All experimental studies on the plant were carried out in Botany Department and Central Services Labs., National Research Centre, Dokki, Giza, Egypt.

Assay for total phenolics

Total phenolics were estimated following the method of (11) involving Folin–Ciocalteu reagent and Gallic acid as standard. 1 ml of each extract of different plant parts contains 66.7 mg F.W. Concentrations of phenolic compounds were calculated according to the following equation that was obtained form the standard Gallic acid graph.

Absorbance = 0.0167 Gallic acid (µg) + 0.0017 (R²=0.99)

Assay for total anthraquinones

Total anthraquinones were estimated using the method of (12). 1 ml of each extract of different plant parts contains 66.7 mg F.W., using Emodin as standard.

Assay for total flavonoids

Total flavonoids were determined using the method of (11). 1 ml of each extract of different plant parts contains 66.7 mg F.W., using Emodin as standard.

Quantitative estimation of Quercetin (using HPLC analysis)

Flavonoids were extracted according to (13) using HPLC- grade chemicals. HPLC analysis was carried out following the method of (14), using Quercetin (Sigma) as standard, with some modifications to fit conditions of Central Services Lab, National Research Centre, Egypt, as following: Filtration through membrane filter (0.4µm); The filtrate was subjected to separation by HPLC instruments under the following conditions (conditions in case of standard were the same with that used for all samples): Mobile phase, acetonitrile (86%): methanol
(100%), 75:25 (v/v); Buffer 14% (Pot. Dihydrogen Phosphate: Phosphoric acid, 2:1v/v); Flow rate: 1 ml/minute; Agilent 1100 series (Waldborn, Germany); Quaternary pump (G1311A); Degasser (G1322A); Thermostated autosampler (G1321A); Variable wave lengths detector (G1314A); Column Zorabax 3005 B 2.5 µm column’25x4.6 mm, Sp’ (Agilent Technologies, USA). Concentration of Quercetin in each sample = 6 mg/ml, while concentration of different plant parts in each sample = 50 mg/ml. Injection volume 20 µL. Wave length was adjusted at 370 nm for separation of different compounds.

Quantitative estimation of Emodin (using HPLC analysis)

Anthraquinones were extracted according to (15), using HPLC grade chemicals for extraction. HPLC analysis was carried out following (16), using Emodin (Aldrich) as standard, with some modifications to fit conditions of Central Services Lab, National Research Centre, Egypt, as mentioned before in case of Quercetin with little changes; Concentration of Emodin in each sample = 0.1 mg/ml, while concentration of different plant parts in each sample = 100 mg/ml. Wave length was adjusted at 440 nm, using fluorescence detector for separation of different compounds.

Antioxidant bioassay

Total antioxidant activity was performed using phosphomolybdenum reagent solution method of (17) and adopted by (18) as following: An aliquot of 0.1 ml of sample solution containing a reducing species, 1ml of ethanol extract of different plant parts (containing 66.7 mg plant material) was combined in an Eppendorf tube with 1ml of reagent solution (0.6M sulfuric acid, 28Mm sodium phosphate, and 4mM ammonium molybdate). Tubes were capped and incubated at 95°C for 90 min. After cooling the absorbance of the aqueous solution was measured at 695nm against a blank. The antioxidant capacity was expressed as Gallic Acid Equivalent (GAE) by using the standard Gallic acid graph.

DPPH (1.1 diphenyl-2 picryl hydrazyl) scavenging activity was carried out by using the method of (11).

Statistical analysis

Statistical analysis was done using Fisher analysis of variance methodology. A least significant difference test was applied at 5 and 1% probability level to determine differences among treatment means (19). The CO-STAT computerized package program was subjected to the regular statistical analysis of variance (20), using two designs -1-Anova-1 completely randomized design (CRD) -2- Factorial implemented in completely randomized design. Each reading = mean of three replicates ± SE for all experiments.

RESULTS

Total phenolics, total anthraquinones and total flavonoids in different plant parts, at both early and late vegetative stages of growth

Concerning total phenolics (mg GAE/g F.W.) in different plant parts, at both early and late vegetative stages of growth of Rumex vesicarius L. (Figure: 1), it was found that, in case of early vegetative stage, roots were found to be the richest organ in this regard (1.659±0.180), followed by leaves (0.405±0.045), while stems were found to contain the least amount in this regard (0.340±0.036). Meanwhile in case of late vegetative stage, whole plant parts were found to be the richest organ in this regard (4.518±0.018), followed by leaves (2.436±0.129), while stems were found to contain the least amount in this regard (2.421±0.025). There were non significant variations between all investigated plant parts in this regard.

It was found that, all plant parts, at both early and late vegetative stages of growth of Rumex vesicarius L. (Figure: 2) were rich in anthraquinones (µg/g F.W.). In this regard, in case of early vegetative stage, whole plant parts were found to contain the highest amount of anthraquinones (205.4±39.600), followed by leaves (162.00±39.600), while roots were found to contain the least amount in this regard (34.200±39.600). Meanwhile in case of late vegetative stage, whole plant parts were found to be the richest organ in this regard (1061.770±15.810), followed by leaves (670.927±30.985), while stems were found to contain the least amount in this regard (406.826±35.660). There were highly significant variations between all investigated plant parts in this regard.

It was found also that, at early vegetative stage of growth (Figure: 3) all plant parts were rich in flavonoids (µg/g F.W.). In this regard, leaves were found to contain the highest amount of flavonoids (2835.00±30.576), followed by whole plant parts (1986.00±30.576), while roots were found to contain the least amount in this regard (458.00±30.576). Meanwhile in case of late vegetative stage, whole plant parts were found to be the richest organ in this regard (181.10±0.186), followed by leaves (13.30±0.063), while stems were found to contain the least amount in this regard (10.83±0.466). There were highly significant variations between all investigated plant parts in this regard.

Figure 1: Total phenolics (mg GAE/g F.W.) in different plant parts, at both early and late vegetative stages of growth.

Figure 2: Total anthraquinones (µg/g F.W.) in different plant parts, at both early and late vegetative stages of growth.

Figure 3: Total flavonoids (µg/g F.W.) in different plant parts, at both early and late vegetative stages of growth.
Figure 2: Total anthraquinones (µg/g F.W.) in different plant parts, at both early and late vegetative stages of growth.

(1 = Leaves, 2 = Stems, 3 = Roots, 4 = Whole plant parts).

Figure 3: Total flavonoids (µg/g F.W.) in different plant parts at both early and late vegetative stages of growth.

Quantitative estimation of Emodin and Quercetin (using HPLC analysis) in different plant parts:

Results of quantitative estimation (µg/g D.W.) of Emodin (using HPLC analysis) in different plant parts, at both early and late vegetative stages of growth (Table: 1 and Figures 4-5) revealed that, all plant parts contain Emodin in high amounts, there were variations between different plant parts at these two stages of growth in this regard. Whole plant parts and roots extracts, at early and late vegetative stages of growth were found to contain the highest amount of Emodin (174.79±1.148 and 121.62±1.132, respectively). Meanwhile results of quantitative estimation (µg/g D.W.) of Quercetin (using HPLC analysis) in different plant parts, at both early and late vegetative stages of growth revealed that, all plant parts contain Quercetin in high amounts, there were variations between different plant parts at these two stages of growth in this regard. Leaves extract, at early vegetative stage of growth was found to contain the highest amount of Quercetin, followed by whole plant parts extract at the same stage of growth, while roots (at the late vegetative stage of growth) extract was the least containing one in this regard (66.36±0.575, 17.53±0.439 and 0.40±0.017, respectively).

Table 1: Quantitative estimation of Emodin and Quercetin (using HPLC analysis) in different plant parts, at both early and late vegetative stages of growth.

<table>
<thead>
<tr>
<th>Plant parts</th>
<th>Emodin (µg/g D.W.)</th>
<th>Early vegetative stage</th>
<th>Late vegetative stage</th>
<th>Quercetin (µg/g D.W.)</th>
<th>Early vegetative stage</th>
<th>Late vegetative stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>39.04±0.519</td>
<td>79.30±0.551</td>
<td>11.80±0.575</td>
<td>66.36±0.575</td>
<td>9.33±0.211</td>
<td></td>
</tr>
<tr>
<td>Stems</td>
<td>69.29±0.791</td>
<td>115.44±1.121</td>
<td>15.05±0.375</td>
<td>5.65±0.272</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roots</td>
<td>112.86±0.132</td>
<td>121.62±0.132</td>
<td>1.98±0.040</td>
<td>0.40±0.017</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole plant parts</td>
<td>174.79±1.148</td>
<td>126.55±0.185</td>
<td>17.53±0.439</td>
<td>13.85±0.329</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L.S.D. (0.05)</td>
<td>2.674</td>
<td>2.345</td>
<td>1.001</td>
<td>1.379</td>
<td>0.599</td>
<td></td>
</tr>
<tr>
<td>L.S.D. (0.01)</td>
<td>3.685</td>
<td>3.518</td>
<td>1.001</td>
<td>1.379</td>
<td>0.899</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4: Chromatogram of HPLC analysis of Emodin (standard, Rt=3.522).
Figure 5: Chromatogram of HPLC analysis of Emodin in whole plant parts, at early vegetative stage of growth (R<sub>t</sub>=3.726).

Figure 6: Chromatogram of HPLC analysis of Quercetin (standard, R<sub>t</sub>=4.168).
Antioxidant activity of different plant parts, at both early and late vegetative stages of growth

Regarding antioxidant activity studies (Table 2), there were non significant variations within different plant parts, at both early and late vegetative stages of growth using total antioxidant activity and DPPH scavenging activity methods.

Total antioxidant activity (Table 2) was estimated (GAEs in ppm), it was found that, in case of early vegetative stage of growth, roots extract was found to have the highest amount of antioxidants (62461.000±20.800), followed by whole plant parts extract (20289.000±50.600), while stems extract was found to have the least amount (401.500±23.600). Meanwhile in case of the least effective plant part used (IC<sub>50</sub>= 1.83±0.156), while stems extract was found to have the least amount (16645.950±1096.486).

DPPH scavenging activity of different plant parts (Table 2) revealed that, in case of early vegetative stage of growth, the least IC<sub>50</sub> (in mg/ml, the highest the effectiveness) was obtained using leaves (IC<sub>50</sub>= 0.34±0.005), followed by whole plant parts extract (IC<sub>50</sub>= 0.36±0.011), while roots extract was the least effective plant part used (IC<sub>50</sub>= 0.47±0.000). Meanwhile, in case of late vegetative stage of growth, the least IC<sub>50</sub> was obtained using whole plant parts extract (IC<sub>50</sub>= 8.34±0.156), followed by roots extract (IC<sub>50</sub>= 8.96±0.156), while stems extract was the least effective plant part used (IC<sub>50</sub>= 14.36±0.358).

Positive controls in these experiments were Quercetin and Emodin, it was found that Quercetin is a potent antioxidant agent (IC<sub>50</sub>= 0.80±0.26), while Emodin has no effect at the used concentrations, it was found also that all plant parts under investigation were potent antioxidant agents when compared with Quercetin.

**DISCUSSION**

Phenolics and flavonoids were important biologically active constituents, since they considered to be anticancer, antioxidant and antimicrobial agents etc, (Alberto et al., 2006; Abd Ghafar et al., 2010 and Imran et al., 2011).

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**Table 2**: Antioxidant activity of different plant parts, at early and late vegetative stages of growth; Total antioxidant activity (GAEs in ppm) and DPPH scavenging activity methods (IC<sub>50</sub> mg/ml).

<table>
<thead>
<tr>
<th>Plant parts</th>
<th>Early vegetative stage</th>
<th>Late vegetative stage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total antioxidant activity (Gallic acid equivalent)</td>
<td>DPPH scavenging activity (IC&lt;sub&gt;50&lt;/sub&gt; mg/ml)</td>
</tr>
<tr>
<td></td>
<td>ppm)</td>
<td>ppm)</td>
</tr>
<tr>
<td>Leaves</td>
<td>8396.00±464.00</td>
<td>0.34±0.005</td>
</tr>
<tr>
<td>Stems</td>
<td>401.00±23.60</td>
<td>0.46±0.000</td>
</tr>
<tr>
<td>Roots</td>
<td>62461.00±20.800</td>
<td>0.47±0.000</td>
</tr>
<tr>
<td>Whole plant</td>
<td>20289.00±50.600</td>
<td>0.36±0.011</td>
</tr>
<tr>
<td>parts</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Quercetin (positive control)= 0.80±0.260</td>
<td>0.80±0.260</td>
</tr>
<tr>
<td>L.S.D. (0.05)</td>
<td>159.300</td>
<td>2.279</td>
</tr>
<tr>
<td>L.S.D. (0.01)</td>
<td>229.400</td>
<td>3.372</td>
</tr>
</tbody>
</table>

There were no significant variations within different plant parts, at both early and late vegetative stages of growth using total antioxidant activity and DPPH scavenging activity methods. Total antioxidant activity was estimated (GAEs in ppm), it was found that, in case of early vegetative stage of growth, roots extract was found to have the highest amount of antioxidants (62461.000±20.800), followed by whole plant parts extract (20289.000±50.600), while stems extract was found to have the least amount (401.500±23.600). Meanwhile in
case of late vegetative stage of growth, roots extract was found to have the highest amount of antioxidants (4286.06 ± 0.004 vs 479.2885), followed by whole plant parts extract (32129.3862 ± 11585.370), while leaves extract was found to have the least amount (16645.950 ± 106.486).

DPPH scavenging activity (mg/ml) of different plant parts revealed that, in case of early vegetative stage of growth, the least IC50 (the highest the effectiveness) was obtained using leaves [IC50 = 0.345 ± 0.005], followed by whole plant parts extract [IC50 = 0.366 ± 0.011], while roots extract was the least effective plant part used [IC50 = 0.473 ± 0.000]. Meanwhile, in case of late vegetative stage of growth, the least IC50, the highest the effectiveness was obtained using whole plant parts extract [IC50 = 8.344 ± 0.156], followed by roots extract [IC50 = 8.964 ± 0.156], while stems extract was the least effective plant part used [IC50 = 14.364 ± 0.358]. Positive controls in these experiments were Quercetin and Emodin, it was found that Quercetin is a potent antioxidant agent [IC50 = 0.801 ± 0.260], while Emodin has no effect at the used concentrations, it was found also that all plant parts under investigation were potent antioxidant agents when compared with Quercetin.

The present antioxidant activity results of R. vesicarius L. were in agreement with Nishina et al., 1991; Demirezer et al., 2001; Al-Ismail et al., 2006; Özen, 2010 and Li and Liu, 2009, since they investigated different extracts of roots of R. japonicus and R. patientia, leaves of R. pulcher and R. acetoceilla and whole plant parts of R. dentatus, respectively. Their results revealed that, these species were considered to be antioxidant agents. Chemical compositions of some of these extracts were studied, with special reference to flavonoids and anthraquinones in case of roots of R. patientia carried out by Demirezer et al., 2001, where there was a similarity between their results and the obtained results in this study. Antioxidant activity, total phenolics and flavonoids results agreed with El-Demirezer et al., 2006; Özen, 2010 and Li and Liu, 2009, since they investigated these extracts were studied, with special reference to flavonoids and anthraquinones were in agreement with Nishina et al., 1991; Demirezer et al., 2001; Al-Ismail et al., 2006; Özen, 2010 and Li and Liu, 2009, since they investigated these species were considered to be antioxidant agents. Chemical compositions of some of these extracts were studied, with special reference to flavonoids and anthraquinones in case of roots of R. patientia carried out by Demirezer et al., 2001, where there was a similarity between their results and the obtained results in this study. Antioxidant activity, total phenolics and flavonoids results agreed with El-Demirezer et al., 2006; Özen, 2010 and Li and Liu, 2009, since they investigated.

**REFERENCES**


