

ANTIOXIDANT ACTIVITY OF *RUMEX VESICARIUS* L. AT THE VEGETATIVE STAGE OF GROWTHEL-BAKRY, A.A.<sup>1</sup>, MOSTAFA, H.A.M.<sup>2</sup> AND EMAN, A. ALAM<sup>2</sup><sup>1</sup>Botany Department, Faculty of Science, Helwan University, Helwan, Egypt,<sup>2</sup> Botany Department, National Research Centre, Dokki, Giza, Egypt. Email: eg\_flower2011@yahoo.com, good\_people2022@yahoo.com, ae.bakry@yahoo.com

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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<sub>s</sub>/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 stages of growth) was found to contain the highest amount (174.793±1.148 µg/g D.W., respectively). Quantitative estimation of Quercetin (using HPLC analysis) revealed that, all plant parts contained Quercetin in high amounts. Leaves extract, at early vegetative stage of growth was found to contain the highest amount of Quercetin (66.360 ±0.575 µg/g D.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 GAE as ppm). Results of DPPH scavenging activity studies revealed that, the least IC<sub>50</sub> (the highest the effectiveness) was obtained using leaves (at early vegetative stage) extract (IC<sub>50</sub>= 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 plant is also used as antioxidant, cooling, laxative, stomachic, tonic, analgesic, appetizer, diuretic, astringent, purgative, antispasmodic, aphrodisiac and antibacterial agents. The roasted seeds were eaten for the cure of dysentery. Finally, the plant can be used also to reduce biliary disorders and control cholesterol levels. The medicinal importance of this plant is a reflection to its chemical composition since this plant contains many bioactive substances such as flavonoids (vitexin, isovitexin, orientin and isorientin), anthraquinones particularly in roots (emodin and chrysophanol), quinones, 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, Quatamia- 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 from the standard Gallic acid graph.

$$\text{Absorbance} = 0.0167 \text{ Gallic acid (ug)} + 0.017 \text{ (R}^2\text{: 0.99)}$$

## 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.. Concentration of flavonoid contents were calculated according to the following equation that was obtained from the standard Quercetin graph:

$$\text{Absorbance} = 0.0228 \text{ Quercetin (ug)} - 0.0045 \text{ (R}^2\text{: 0.9979)}$$

## 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 (0.4 µm); 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, 2:1 v/v); Flow rate: 1 ml/minute; Agilent 1100 series (Waldborn, Germany); Quaternary pump (G1311A); Degasser (G1322A); Thermostated Autosampler (G1329A); Variable wave lengths detector (G1314A); Column Zorabax 3005 B C<sub>18</sub> column"25X4.6 mm, 5 $\mu$ "(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  $\mu$ L. 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, 28M 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  $\pm$  SE for all experiments.

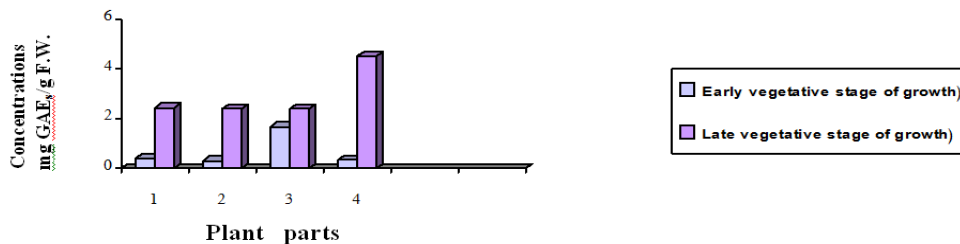
#### RESULTS

##### 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<sub>s</sub>/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 $\pm$ 0.180), followed by leaves (0.405 $\pm$ 0.045), while stems were found to contain the least amount in this regard (0.340 $\pm$ 0.036). Meanwhile in case of late vegetative stage, whole plant parts were found to be the richest organ in this regard (4.518 $\pm$ 0.018), followed by leaves (2.436 $\pm$ 0.129), while stems were found to contain the least amount in this regard (2.421 $\pm$ 0.025). There were non significant variations between all investigated plant parts in this regard.

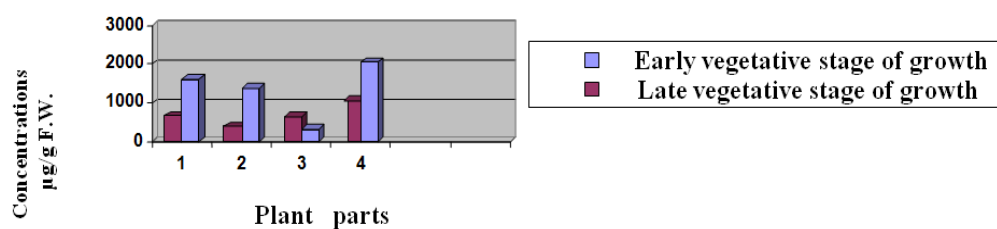
It was found that, all plant parts, at both early and late vegetative stages of growth of *Rumex vesicarius* L. (Figure: 2) were rich in anthraquinones ( $\mu$ g/g F.W.). In this regard, in case of early vegetative stage, whole plant parts were found to contain the highest amount of anthraquinones (2054 $\pm$ 39.600), followed by leaves (1620.000 $\pm$ 39.600), while roots were found to contain the least amount in this regard (342.000 $\pm$ 39.600). Meanwhile in case of late vegetative stage, whole plant parts were found to be the richest organ in this regard (1061.770  $\pm$ 15.810), followed by leaves (670.927 $\pm$ 30.985), while stems were found to contain the least amount in this regard (406.826 $\pm$ 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 ( $\mu$ g/g F.W.). In this regard, leaves were found to contain the highest amount of flavonoids (28350.000  $\pm$  30.576), followed by whole plant parts ( 1986.000 $\pm$  30.576), while roots were found to contain the least amount in this regard (458.000 $\pm$  30.576). Meanwhile in case of late vegetative stage, whole plant parts were found to be the richest organ in this regard (18.110 $\pm$ 0.186), followed by leaves (13.306 $\pm$ 0.063), while stems were found to contain the least amount in this regard (10.831 $\pm$ 0.466). There were highly significant variations between all investigated plant parts in this regard.



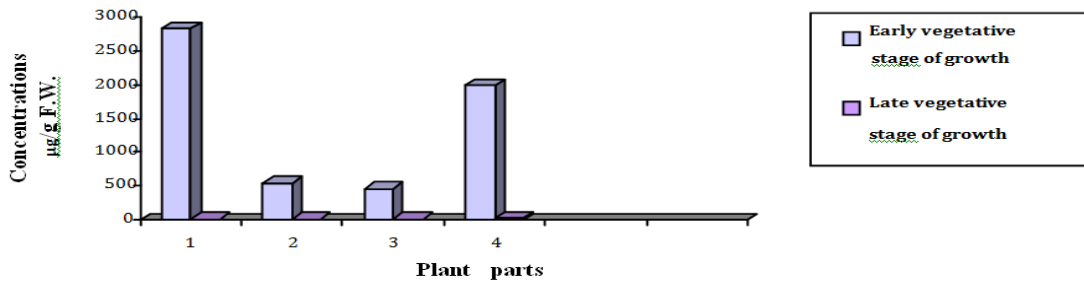
(1 = Leaves, 2= Stems, 3 = Roots, 4= Whole plant parts).

Figure 1: Total phenolics (mg GAE<sub>s</sub>/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 2: Total anthraquinones ( $\mu\text{g/g}$  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 ( $\mu\text{g/g}$  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 ( $\mu\text{g/g}$  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 sages 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 ( $174.793 \pm 1.148$  and  $121.620 \pm 1.132$ , respectively). Meanwhile results of quantitative estimation ( $\mu\text{g/g}$

D.W.) of Quercetin (using HPLC analysis) in different plant parts, at both early and late vegetative stages of growth (Table: 1 and Figures 6-7) revealed that, all plant parts contain Quercetin in high amounts, there were variations between different plant parts at these two

sages 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 ( $66.360 \pm 0.575$ ,  $17.537 \pm 0.439$  and  $0.400 \pm 0.017$ , 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.

Plant parts	Emodin ( $\mu\text{g/g}$ D.W.)		Quercetin ( $\mu\text{g/g}$ D.W.)	
	Early vegetative stage	Late vegetative stage	Early vegetative stage	Late vegetative stage
Leaves	$39.048 \pm 0.519$	$79.305 \pm 0.551$	$66.360 \pm 0.575$	$9.335 \pm 0.211$
Stems	$69.290 \pm 0.791$	$115.444 \pm 1.121$	$15.054 \pm 0.375$	$5.650 \pm 0.272$
Roots	$112.863 \pm 0.132$	$121.620 \pm 1.132$	$1.989 \pm 0.040$	$0.400 \pm 0.017$
Whole plant parts	$174.793 \pm 1.148$	$12.655 \pm 0.185$	$17.537 \pm 0.439$	$13.858 \pm 0.329$
L.S.D. (0.05)	2.674	2.345	1.001	0.599
L.S.D. (0.01)	3.685	3.518	1.379	0.899

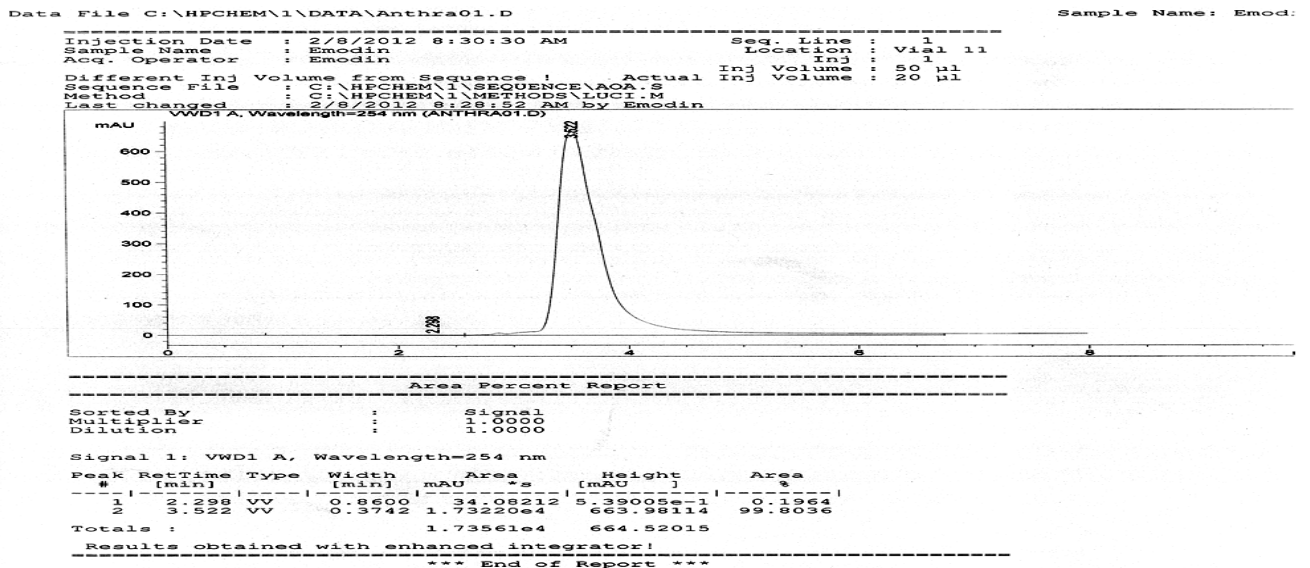


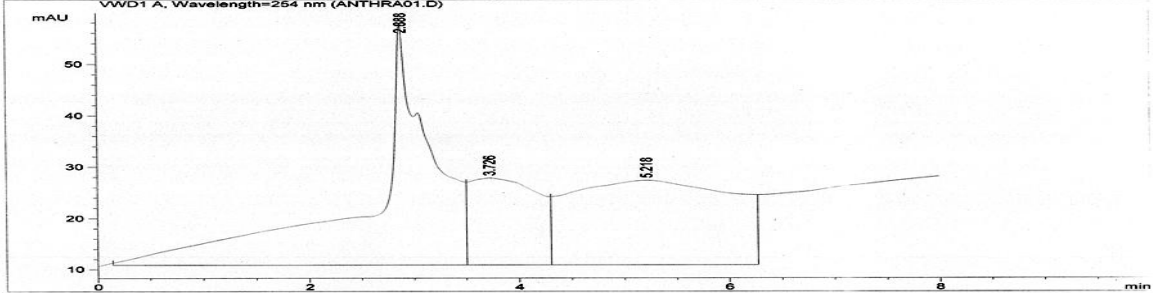
Figure 4: Chromatogram of HPLC analysis of Emodin (standard,  $R_t=3.522$ ).

Data File C:\HPCHEM\1\DATA\Anthra01.D

Sample Name: 1A

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Injection Date : 2/8/2012 8:42:04 AM      Seq. Line : 1
Sample Name   : 1A                        Location  : Vial 11
Acq. Operator : Emodin                    Inj       : 1
                                                Inj Volume: 50 µl
                                                Inj Volume: 20 µl
Different Inj Volume from Sequence !
Sequence File : C:\HPCHEM\1\SEQUENCE\AOA.S
Method        : C:\HPCHEM\1\METHODS\LUCI.M
Last changed  : 2/8/2012 8:28:52 AM by Emodin
WVD1 A, Wavelength=254 nm (ANTHRA01.D)
    
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Area Percent Report

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Sorted By      : Signal
Multiplier    : 1.0000
Dilution      : 1.0000
    
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Signal 1: WVD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	mAU	Area *s	Height [mAU]	Area %
1	2.888	BV	0.5322	1999.02209	46.35462	44.0420	
2	3.726	VV	0.6656	756.94214	16.98302	16.6788	
3	5.218	VV	1.4098	1782.93896	16.53027	39.2813	

Totals : 4538.90320 79.86792

Results obtained with enhanced integrator!

\*\*\* End of Report \*\*\*

Instrument 1 2/8/2012 8:50:00 AM Emodin

Page 1 of 1

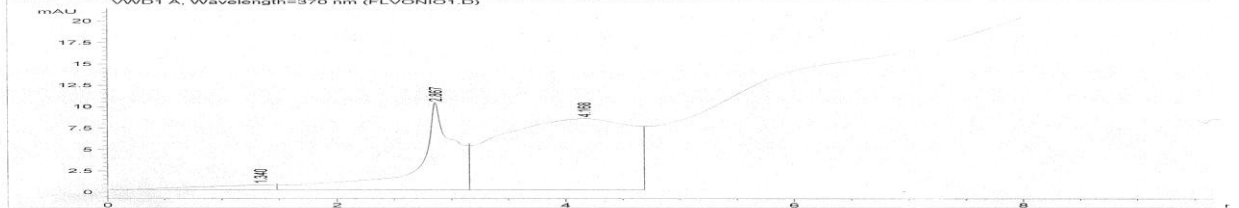
Figure 5: Chromatogram of HPLC analysis of Emodin in whole plant parts, at early vegetative stage of growth ( $R_t=3.726$ ).

Data File C:\HPCHEM\1\DATA\Flvonic1.D

Sample Name: Quercet

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Injection Date : 2/21/2012 9:13:30 AM      Seq. Line : 1
Sample Name   : Quercetin                  Location  : Vial 2
Acq. Operator : Flvoniciods               Inj       : 1
                                                Inj Volume: 50 µl
                                                Inj Volume: 20 µl
Different Inj Volume from Sequence !
Sequence File : C:\HPCHEM\1\SEQUENCE\AOA.S
Method        : C:\HPCHEM\1\METHODS\LUCI.M
Last changed  : 2/21/2012 9:11:31 AM by Flvoniciods
WVD1 A, Wavelength=370 nm (FLVONIC1.D)
    
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Area Percent Report

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Sorted By      : Signal
Multiplier    : 1.0000
Dilution      : 1.0000
    
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Signal 1: WVD1 A, Wavelength=370 nm

Peak #	RetTime [min]	Type	Width [min]	mAU	Area *s	Height [mAU]	Area %
1	1.340	BV	0.63005	29.10204	5.53263811	43.00637	
2	4.168	VV	1.1645	241.01828	10.16331	45.3735	
3	4.887	VV	1.1645	679.78064	8.35687	41.50633	

Totals : 949.90096 19.11344

Results obtained with enhanced integrator!

\*\*\* End of Report \*\*\*

Instrument 1 2/21/2012 9:21:37 AM Flvoniciods

Page 1 of 1

Figure 6: Chromatogram of HPLC analysis of Quercetin (standard,  $R_t=4.168$ ).

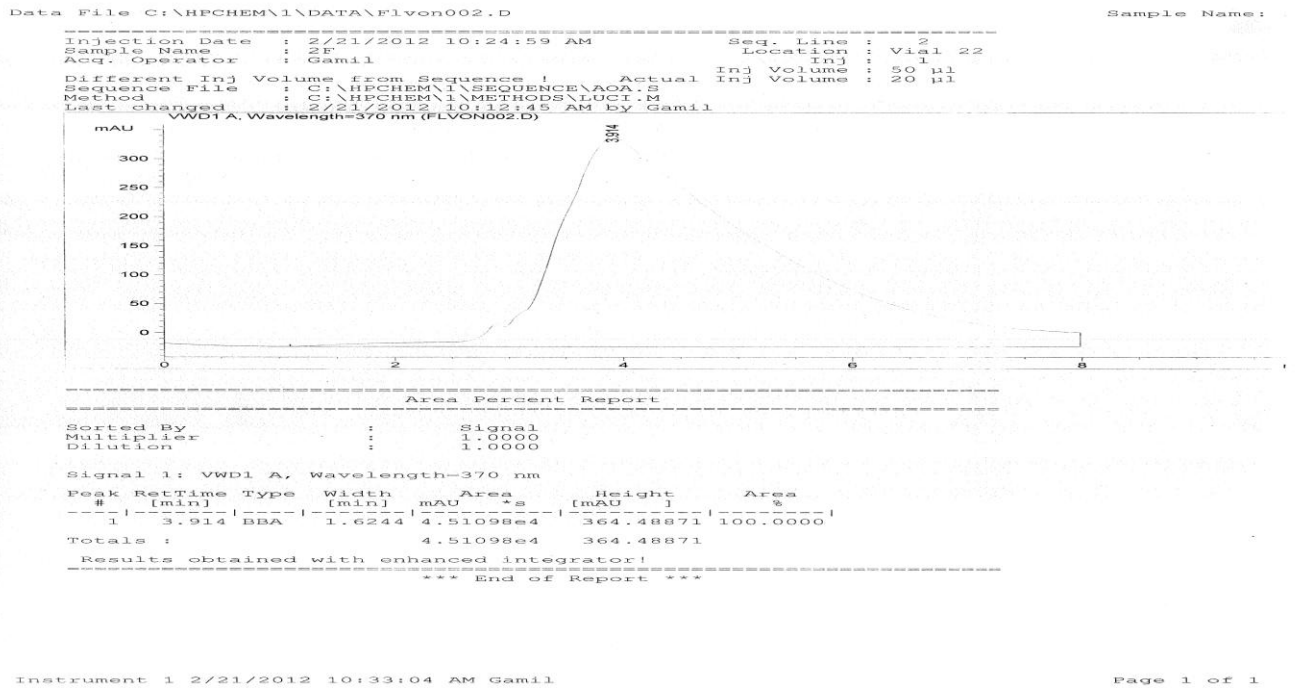


Figure 7: Chromatogram of HPLC analysis of Quercetin in leaves, at early vegetative stage of growth ( $R_t=3.914$ ).

**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 late vegetative stage of growth, roots extract was found to have the highest amount of antioxidants (428606.000±4792.885), followed by whole plant parts extract (321293.860±11585.370), while leaves extract was found to have the least amount (166645.950±1096.486).

DPPH scavenging activity of different plant parts (Table: 2) revealed that, in case of early vegetative stage of growth, the least  $IC_{50}$  (in mg/ml, the highest the effectiveness) was obtained using leaves ( $IC_{50} = 0.345±0.005$ ), followed by whole plant parts extract ( $IC_{50}= 0.365±0.011$ ), while roots extract was the least effective plant part used ( $IC_{50}= 0.473±0.000$ ). Meanwhile, in case of late vegetative stage of growth, the least  $IC_{50}$ , the highest the effectiveness was obtained using whole plant parts extract ( $IC_{50}= 8.344±0.156$ ), followed by roots extract ( $IC_{50}= 8.964±0.156$ ), while stems extract was the least effective plant part used ( $IC_{50}= 14.364±0.358$ ).

Positive controls in these experiments were Quercetin and Emodin, it was found that Quercetin is a potent antioxidant agent ( $IC_{50} = 0.801±0.260$ ), 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.

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 ( $IC_{50}$  mg/ml).

Plant parts	Early vegetative stage		Late vegetative stage	
	Total antioxidant activity (Gallic acid equivalent "ppm")	DPPH scavenging activity ( $IC_{50}$ mg/ml)	Total antioxidant activity (Gallic acid equivalent in ppm)	DPPH scavenging activity ( $IC_{50}$ mg/ml)
Leaves	8396.000±464.000	0.345±0.005	166645.950±1096.486	13.483±0.405
Stems	401.500±23.600	0.469±0.000	167875.800±2662.723	14.364±0.358
Roots	62461.000±20.800	0.473±0.000	428606.000±4792.885	8.964±0.156
Whole plant parts	20289.000±50.600	0.365±0.011	321293.860±11585.370	8.344±0.156
		Quercetin (positive control)= 0.801±0.260		Quercetin (positive control)= 0.801±0.260
L.S.D. (0.05)	1589.300	2.279	35830.000	1.883
L.S.D. (0.01)	2294.000	3.372	53761.000	2.740

**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).

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 ( $428606.000 \pm 4792.885$ ), followed by whole plant parts extract ( $321293.860 \pm 11585.370$ ), while leaves extract was found to have the least amount ( $166645.950 \pm 1096.486$ ).

DPPH scavenging activity (mg/ml) of different plant parts revealed that, in case of early vegetative stage of growth, the least  $IC_{50}$  (the highest the effectiveness) was obtained using leaves ( $IC_{50} = 0.345 \pm 0.005$ ), followed by whole plant parts extract ( $IC_{50} = 0.365 \pm 0.011$ ), while roots extract was the least effective plant part used ( $IC_{50} = 0.473 \pm 0.000$ ). Meanwhile, in case of late vegetative stage of growth, the least  $IC_{50}$ , the highest the effectiveness was obtained using whole plant parts extract ( $IC_{50} = 8.344 \pm 0.156$ ), followed by roots extract ( $IC_{50} = 8.964 \pm 0.156$ ), while stems extract was the least

effective plant part used ( $IC_{50} = 14.364 \pm 0.358$ ). Positive controls in these experiments were Quercetin and Emodin, it was found that Quercetin is a potent antioxidant agent ( $IC_{50} = 0.801 \pm 0.260$ ), 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. acetosella* 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 were a similarity between their results and the obtained results in this study. Antioxidant activity, total phenolics and flavonoids results agreed with El-Hawary *et al.*, 2011, where *R. vesicarius* had antioxidant and hepatoprotective effects due to the presence of phenolics and flavonoids in this plant. Results of antioxidant activity, total phenolics and flavonoids of *R. vesicarius* L. agreed also with results of Tavares *et al.*, 2010, since they found that, flavonoids and polyphenolics in *R. maderensis* were strongly associated with antioxidant capacity, total flavonoids and phenolics content reflect the antioxidant capacity of the plant, flavonoids also have been described as antioxidant molecules that scavenge free radicals with their concomitant oxidation.

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