Est. 1984

ORIENTAL JOURNAL OF CHEMISTRY
An International Open Free Access, Peer Reviewed Research Journal

www.orientjchem.org

ISSN: 0970-020 X
CODEN: OJCHEG
2011, Vol. 27, No. (4):
Pg. 1509-1516

In vitro Evaluation of Antioxidant Properties of Different Solvent Extracts of *Rumex acetosella* Leaves

HIRA BAIG*, DILDAR AHMED, SAMAN ZARA, MUHAMMAD IKRAM AUJLA and MUHAMMAD NADEEM ASGHAR

Department of Chemistry, Forman Christian College University, Ferozpur Road, Lahore (Pakistan).
*Corresponding author: E-mail: baighira@gmail.com

(Received: August 20, 2011; Accepted: October 02, 2011)

ABSTRACT

A wide range of antioxidant studies were conducted on fractions in various solvents of the leaves of *Rumex acetosella* including estimation of total phenolics and flavonoids, DPPH free radical scavenging, FRAP, ABTS, phosphomolybdate, reducing power and lipid peroxidation assays. The butanolic fraction showed highest phenolic and flavonoid contents; 203.30 µg/mL of gallic acid equivalent and 745 µg/mL of rutin equivalent respectively. In linoleic acid emulsion and reducing power assays, fractions in all solvents showed strong antioxidant potential. All the fractions efficiently scavenged the DPPH free radical and butanolic fraction had the lowest EC_{50} (74.95 µg/mL) and T_{EC50} (2 min). The phosphomolybdate antioxidant activity of the plant extracts ranged from 107.84 - 42.24 µg/mL of AAE (Ascorbic Acid Equivalent). The butanolic fraction had the highest FRAP value (104.23 µg/mL of AAE) and the highest TEAC value (1588.203 mM) in ABTS assay. The chloroform fraction showed the lowest TEAC value (405.359 mM). The polar fractions, having higher phenolics and flavonoids, showed remarkable antioxidant potential, the butanolic fraction being the most potent.

Keywords: *Rumex acetosella,* Free radical Scavenging, Antioxidant.

INTRODUCTION

Oxygen which is necessary for living can have adverse effects on the human body, due to the formation of reactive oxygen species.[1] These reactive oxygen species are unstable free radicals including hydroxyl ($OH^{\cdot}$), superoxide ($O_2^{\cdot}$), nitric oxide ($NO^{\cdot}$) and lipid peroxyl ($LOO^{\cdot}$) radicals.[2] Free radical reactions produce progressive undesirable changes that accumulate with age throughout the body and cause life threathening diseases like cancer and atherosclerosis.[3] The National Cancer Institute of the USA has stated that preliminary research in lab animals has shown that "antioxidants help prevent the free radical damage that is associated with cancer". Antioxidants in the body can slow down the process of aging, and may even increase longevity. They destroy the free radicals by chelating catalytic metals and by acting as oxygen scavengers.[4] To combat toxic free radicals, various antioxidants are used as drugs and as ingredients in various food products.[5] Vitamins A, C, E, carotenoids, polyphenolic compounds and flavonoids are common natural

antioxidants. Natural antioxidants are most desirable since they not only scavenge free radicals, also have very little or no side effects.[6]

Plants as a source of medicine have a rich tradition in all human cultures. In Pakistan, India, China they constitute an alternative solution to health problems, due to easy availability, low costs and rare side effects. Pakistan is rich in medicinal plant owing to its diversity in climatic zones but so far only a small number of plants have been studied chemically.[7] *Rumex acetosella* var. *acetosella,* commonly known as sheep's sorrel, is found in many parts of the world.[8] The genus Rumex is reported to possess many pharmacological properties including antimicrobial, larvicidal, anti-inflammatory, antianalgesic, antioxidant etc.[9,10] Many phytochemical compounds have also been isolated from *R. acetosella* which include various flavonoids, phenolic compound and terpenoids.[11,12,13] *R. acetosella* mainly grows in hilly grasslands and moist valleys. In Pakistan, *Rumex acetosella* is found in the northern hilly areas including Hazara and Swat. It can grow up to 1 m in height. Its leaves are green, fleshy and long with fringed cone at the base. They are alternate on reddish grooved stem. It has green flowers. *R. acetosella* has high chlorophyll content. In traditional healing, it is used for curing liver, digestive, and bowel functions and is used for the treatment of inflammatory diseases, tumors, cancers, and urinary/kidney diseases.[14] The literature does not report any antioxidant activity on the leaves of *R. acetosella.* The main aim of this study was to evaluate the leaves extracts in different solvents for various antioxidant parameters.

MATERIAL AND METHODS

Plant collection and extract preparation

Leaves of *Rumex acetosella* were collected from the hills near Abbottabad, Pakistan, in June 2010. A specimen of the plant is kept in the Department of Chemistry, Forman Christian College, Lahore. After drying the leaves under shade for 15 days, they were ground, and the powder (100 g) was extracted in 100% methanol at room temperature (300 mL x 15 days x 3). The three extracts were filtered and the filtrates were combined and concentrated on rotary evaporator under reduced pressure at 30 °C. This crude methanolic (8.068 g) was suspended in water (30 mL) and extracted with hexane, ethyl acetate, chloroform and 1-butanol respectively. In this way the following samples were obtained: crude methanolic, hexane, ethyl acetate, chloroform, 1-butanolic and aqueous after partition. Each fraction was dried under reduced pressure, and weighed.

Chemicals

Sodium hydroxide, sodium nitrite, rutin, Folin-Ciocalteu reagent, sodium carbonate, sulfuric acid, ammonium molybdate, ferric chloride, potassium thiosulfate, sodium chloride, iron (II) sulfate, sodium acetate trihydrate, hydrochloric acid, iron (II) chloride, Tween 20, dipotassium phosphate, potassium thiocyanate, butylated hydroxyanisole (BHA) and all solvents used were of analytical grade and were purchased from Merck (Germany). Aluminium chloride was obtained from BDH Labs., (England). Gallic acid was purchased from Scharlau, (Switzerland). 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH), ascorbic acid, 2,2'-azinobis 3-ethylbenzothiazoline-6-sulfonic acid– (ABTS) were purchased from MP Biomedicals, (France). 2,4,6-Tripyridyl-s-triazine (TPTZ), linoleic acid and Trolox were obtained from Sigma-Aldrich, (Germany). Potassium ferricyanide and trichloroacetic acid were of Unichem, (China) and glacial acetic acid was purchased from PRS Pancreac, (France).

Total Flavonoid Content

The total flavonoid content was determined using the method by Sahreen *et al.* (2010).[15] Briefly, 30 mg of each plant fraction was dissolved in 10 mL of methanol to obtain working samples. In a glass vial, 300 µL of plant extract or standard solution, 3.4 mL of 30% aqueous methanol and 150 µL of $NaNO_2$ (0.5 M) solution was added and mixed. After an interval of 5 min, 150 µL of $AlCl_3$ (0.3 M) solution, and after another 5 min, 1 mL of NaOH (1 M) solution was added. The absorbance of the mixture was then measured at 506 nm. Rutin was used as a standard and the total flavonoid content of various fractions of *R. acetosella* were expressed as micrograms per milliliter of Rutin Equivalents (µg/mL of RE)

Total Phenolic Content

The total phenolic content was determined by the method followed by Slinkard *et al.* (1977).[16]

Briefly, 30 mg of each plant fraction was dissolved in 10 mL of methanol. In a glass cuvette, 40 µL of the plant extract or standard solution, 3.16 mL of distilled water and 200 µL of Folin–Ciocalteu reagent was added and the solutions were mixed thoroughly. After an interval of 8 min, 600 µL of sodium carbonate (7%) solution was added and mixed. The glass cuvettes containing the samples were incubated at 40 °C for 30 min. Absorbance of the mixture was determined against a blank solution at 765nm. The total phenolic content of *R. acetosella* extracts were expressed as micrograms per milliliter of Gallic Acid Equivalents (µg/mL of GAE) .

DPPH Radical Scavenging

The radical scavenging activity of DPPH was determined using the method reported by Brand–Williams *et al.* (1995).[17] Briefly, 10 mg of each plant fraction was dissolved in 10 mL of methanol. The stock solution was prepared by dissolving 24 mg of DPPH in 100 mL of methanol and kept in a refrigerator until used. The working solution was obtained by diluting the DPPH stock solution with methanol to obtain an absorbance of about 0.98 (±0.02) at 517 nm. In a glass vial, 3 mL of the working solution was mixed with 100 µL of the plant extract or the standard solution and its absorbance was measured at 517 nm for a period of 30 min. The percent scavenging activity was calculated using the following formula:

$$\% \text{ DPPH}_{\text{rem}} = \frac{[\text{DPPH}]_{\text{T=t}}}{[\text{DPPH}]_{\text{T=0}}}$$

Where % $DPPH_{rem}$ is the percent of unreacted DPPH, $[DPPH]_{T=0}$ is the concentration of DPPH before reaction with antioxidant sample. $[DPPH]_{T=t}$ is the concentration of DPPH after reaction with antioxidant sample at time t. Ascorbic acid was used as a standard. EC_{50} value was also determined which is the effective concentration that has the potential to scavenge 50% of the DPPH radicals. T_{EC50} or the time taken by the sample to scavenge 50% of the DPPH radicals was also determined.

Phosphomolybdate Antioxidant Assay

The phosphomolybdate antioxidant assay was carried out according to the procedure reported by Umamaheswari and Chatterjee (2008).[18] Briefly, 25 mg of each plant fraction was dissolved in 10 mL of methanol. Phosphomolybdate reagent was prepared by mixing 0.6 M sulfuric acid (100 mL), 4 mM ammonium molybdate (100 mL) and 28 mM sodium phosphate (100 mL) solution. In a test tube, 3 mL of phosphomolybdate reagent, 300 µL of the plant extract or standard solution or methanol was taken and mixed. The test tubes were capped with silver foil and incubated in water bath at 95 °C for 90 min. After the contents of the test tubes were cooled down, the absorbance of the test tube contents were measured at 765 nm against a blank. Ascorbic acid was used as a standard. The antioxidant activity of *R. acetosella* fractions were expressed as micrograms per milliliter of Ascorbic Acid Equivalents (µg/mL of AAE).

Ferric Reducing Antioxidant Potential – FRAP

The total antioxidant activity of each plant extract was measured by ferric reducing antioxidant power assay of Benzie and Strain (1999).[19] Fresh FRAP reagent was prepared by mixing 25 mL of 300 mM of acetate buffer pH 3.6, 2.5 mL of 10 mM TPTZ solution made in 40 mM of HCl and 2.5 mL of 20 mM ferric chloride solution. The mixture was then warmed at 37 °C for 15 min before use. The FRAP reagent (2.85 mL) was mixed with 150 µL of a plant extract or standard. The mixture was incubated for 30 min in dark. The absorbance of the mixture was then noted at 593 nm. The FRAP values of samples were expressed as micrograms per milliliter of Ascorbic Acid Equivalents (µg/mL of AAE).

Reducing Power Assay

The Reducing Power Assay was carried out by the method of Oyaizu (1986).[20] A plant extract or gallic acid solution (2.5 mL) was mixed with 2.5 mL of 0.2M sodium phosphate buffer and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min and 2.5 mL of trichloroacetic acid solution (100 mg/L) was added. The mixture was centrifuged at 650 rpm for 10 min, and 5 mL of the supernatant was mixed with 5 mL of distilled water and 1 mL of 0.1% ferric chloride solution. The absorbance was measured at 700 nm.

Lipid Peroxidation Assay

The lipid peroxidation values of various

extracts were determined according to the method described by Mitsuda *et al.* (1996).[21] An emulsion of linoleic acid was prepared by mixing 175 µg of Tween 20 and 155 µL of linoleic acid and the volume was made 50 mL by adding 0.05 M of potassium phosphate buffer (pH 7). In a test tube, 100 µL of a plant extract was mixed with 2.4 mL of potassium phosphate buffer and 2.5 mL of linoleic acid emulsion. The mixture was incubated at 37°C for 25 min. Then, 100 µL of this solution was regularly taken at 24 h intervals and dissolved in 3.7 mL of ethanol. It was reacted with 100 µL of 20 mM ferrous chloride solution and then 100 µL of 30% potassium thiocyanate solution was added and absorbance was measured at 500 nm. A 5 mL solution consisting of linoleic acid emulsion (2.5 mL) and potassium phosphate buffer (2.5 mL) was used as blank. BHA was used as a standard antioxidant.

$ABTS^{\bullet+}$ Antioxidant Assay

$ABTS^{\bullet+}$ (ABTS radical cation) assay protocol, reported by Re *et al.* (1999) was followed for measuring the antioxidant activity.[22] ABTS stock solution was prepared by mixing ABTS with potassium persulfate, so that the final concentration was 2.45 mM potassium persulfate and 7 mM of ABTS. The solution was allowed to stand in the dark, at room temperature, for 18 h before use. The ABTS working solution was obtained by diluting the ABTS stock solution with 0.1 M PBS buffer (pH 7.4) to an absorbance of 0.70 (±0.02) at 745 nm. Then, 10 µL of plant extract was mixed with 2.99 mL of working solution and the absorbance was measured at 734 nm, after 8 min. The percent inhibition of the sample was calculated by the following formula:

$$\text{Antioxidant Effect \%} = [(1 - (\text{Sample absorbance}/\text{control absorbance})] \times 100$$

Where the control absorbance is the absorbance of ABTS radical without the sample solution and the sample absorbance is the absorbance 8 min after the addition of antioxidant sample in the ABTS solution. The antioxidant activity of plant fractions were expressed as Trolox equivalents.

Statistical Analysis

All the experiments were carried out in triplicate and the results were expressed as mean of three readings unless mentioned otherwise. One way ANOVA was applied and the results were correlated. The EC_{50} values were calculated using the Graph Pad Prism 5 software.

RESULTS AND DISCUSSION

Percent yield of the various extracts

The crude methanolic extract of leaves of *R. acetosella* was partitioned in different solvents of varying polarity. The percent yields of the various fractions obtained are shown in Table 1. The highest yield was obtained in the butanolic fraction (45.79%) while the lowest yield was in the chloroform fraction (1.9%). The percent yield obtained showed that the extract had a greater proportion of polar compounds as most of the extract was soluble in polar solvents like 1-butanol and water.

Total Flavonoid Content

The results of total flavonoid content of various extracts were calculated by the equation obtained from the standard curve of rutin:

Concentration of Rutin Equivalent = (Absorbance - 0.005251)/ 0.000248; R^2= 0.9977

The results are shown in Table 1. All the tested fractions had high flavonoid content; the butanolic fraction had the highest (745 µg/mL of rutin equivalent, RE) and the chloroform fraction had the lowest content (281.26 µg/mL of RE). Flavonoids are known to show antioxidant activity having considerable effects on human nutrition and health. The mechanism of flavonoid action is based on scavenging or chelating process.[23]

Total Phenolic Content

The phenolic content of different fractions of *R. acetosella* was found using the equation obtained from the standard curve of gallic acid.

Concentration of Gallic Acid Equivalent = (Absorbance + 0.01772)/ 0.000945; R^2= 0.9946

The results are shown in Table 1. The highest phenolic content was recorded in the butanolic fraction (203.30 µg/mL of GAE), while the

Table 1: The % yield, total flavonoid and phenolic content of different Fractions of *Rumex acetosella*

Plant Fraction	% Yield	Total Flavonoid Content (µg/mL) of RE	Total Phenolic Content (µg/mL) of GAE
Methanolic	8.0675	740.972	114.5086
Hexane	6.4	470.7931	67.94965
Chloroform	1.9	281.2647	60.01346
Ethyl Acetate	9.1	732.907	138.3172
butanolic	45.79	745.0045	203.3939
Aqueous	36.68	736.9395	156.3059

[a] Each value in the table is mentioned as mean (n=3)

[b] All the mean values are significantly different at probability level P < 0.05.

Table 2 - The antioxidant effect (EC_{50} and $T_{EC\,50}$) on DPPH radicals in different fractions of *Rumex acetosella* and Ascorbic Acid

Plant Fraction	EC_{50} (µg/mL)	T_{EC50} (min)
Methanolic	200.1442	15
Hexane	819.6968	20
Chloroform	3000	26
Ethyl Acetate	299.7524	10
Butanolic	74.9542	2
Aqueous	199.7483	15
Ascorbic Acid	99.9176	20

[a] Each value in the table is mentioned as mean (n=3)

[b] All the mean values are significantly different at probability level P < 0.05.

lowest was in the chloroform fraction (60.01 µg/mL of GAE). It is important to know the phenolic content in a sample to evaluate its antioxidant potential since phenolic compounds have great potential to scavenge free radicals as reported by Miliauskas *et al*. (2004).[24] High contents of phenolic compounds were observed in the polar extracts and it could be due to the presence of various phenolic compounds such as orcinol, gallic acid, pyragallol and others which have been reported from various species of Rumex. [25, 26]

DPPH Radical Scavenging Assay

The antioxidant potential is determined by calculating the decrease in % $DPPH_{rem}$ as a function of time is shown in Figure 1. The plot showed a sharp decrease in absorbance in the first few min

Table 3: Antioxidant Effect in different Fractions of *Rumex acetosella*

Plant Fraction	Antioxidant Effect	
	Phosphomolybdate Assay(µg/mL) of AAE	FRAP Assay(mM) of AAE
Methanolic	55.44	95.38
Hexane	42.84	30.77
Chloroform	42.24	31.15
Ethyl Acetate	72.84	58.85
Butanolic	107.84	104.23
Aqueous	85.44	62.30

[a] Each value in the table is mentioned as mean (n=3)

[b] All the mean values are significantly different at probability level P < 0.05.

after the addition of the sample and then becomes moderate for the rest of the time. This indicated that the plant sample has both slow reacting and fast reacting antioxidants. The radical scavenging activity was in the following order: butanolic > ethyl acetate > methanolic > aqueous > hexane > chloroform fraction. The EC_{50} and T_{EC50} values are shown in Table 2. The antioxidant potential of samples in general has an inverse relationship with EC_{50} which has also been reported by Kai *et al.* (2010).[27]

Phosphomolybdate Antioxidant Assay

The phosphomolybdate antioxidant assay is based on the reduction of Mo(VI) to Mo(V) by the antioxidant sample which is detected by the formation of green molybdenum(V) complex at an acidic pH. In our test, the antioxidant activity of the plant extracts ranged from 107.84 - 42.24 µg/mL of AAE. The results are shown in Table 3. The butanolic fraction showed the highest while the chloroform fraction showed the lowest antioxidant potential. The results of phenolic content in the sample were comparable to antioxidant potential in phosphomolybdate assay. This trend was also reported by Shinde *et al.* (2010).[28]

FRAP Assay

The FRAP (Ferric reducing Antioxidant Potential) assay involves the electron-transfer mechanism. The results are shown in Table 3. The highest value was found in butanolic fraction i.e. 104.23 µg/mL of AAE. The methanolic extract also showed good FRAP value (95.38 µg/mL of AAE). The fractions of nonpolar solvents had very low FRAP value.

Table 4: Trolox Equivalent Antioxidant Capacity in different fractions of *Rumex acetosella*

Fraction	Trolox Equivalent Antioxidant Capacity (TEAC) (mM)
Methanolic	460.0376
Hexane	689.9858
Chloroform	405.359
Ethyl Acetate	1179.267
Butanolic	1588.203
Aqueous	1136.717

[a] Each value in the table is mentioned as mean (n=3)

[b] All the mean values are significantly different at probability level $P < 0.05$.

Reducing Power Assay

In the reducing power assay, the antioxidants present in the sample reduce the Fe^{3+} to Fe^{2+} by donating an electron and a blue coloured Iron (II) complex is formed. The antioxidant potential of the fractions of *R. acetosella* by reducing power assay was in the following order: butanolic > ethyl acetate > methanolic > hexane > aqueous > chloroform.

The increase in absorbance indicated an increase in reducing power due to the higher antioxidant potential. Most of the fractions of *R. acetosella* had a high reducing power than that of gallic acid, the standard antioxidant used. However, the aqueous and chloroform fractions had a lower value. This may be due to the high content of phenolics which are present in various species of Rumex.[25, 26] Iron (III) reduction is often used as an indicator of electron donating ability, which is an important mechanism of phenolic antioxidant action and, moreover, the reducing power of a substance appears to be related to the degree of hydroxylation and extent of conjugation in polyphenols.[29]

Lipid Peroxidation Assay

When linoleic acid undergoes peroxidation, peroxides are formed which, being quite reactive, have the potential to oxidize Fe^{2+} to Fe^{3+}. Fe^{3+} upon reaction with thiocyanate ions forms a complex which can be detected at 500 nm. The antioxidants slow down the oxidation of linoleic acid, thereby decreasing the production of peroxides which lead to a low value of lipid peroxidation indicating high antioxidant potential.

Antioxidant activity in terms of lipid peroxidation was plotted as a function of time with a standard antioxidant BHA, which is shown in Figure 2. The results showed that the *R. acetosella* had strong antioxidant activity and the samples were active even after incubation of 96 h, with a slight increase in lipid peroxidation value. This can be due to anthocyanins which are reported to be present in various species of Rumex, and can significantly inhibit peroxidation of linoleic acid and

diminish the formation of peroxides, thus implying that the anthocyanins are powerful natural antioxidants.

ABTS Antioxidant Assay

ABTS$^{\bullet+}$ (ABTS radical cation) antioxidant potentials for various fractions expressed in terms of Trolox equivalent are shown in Table 4.The TEAC (Trolox equivalent antioxidant activity) values of the sample were calculated by the equation using Trolox as standard:.

Trolox Equivalent (mM) = ((Antioxidant potential (%)) - 0.4985)/0.0459; R^2= 0.9612

The highest TEAC value (1588.203 mM) was found for the butanolic fraction while the lowest was for the chloroform fraction (405.359 mM). The polar fractions showed high TEAC values than nonpolar ones. In general, fractions with high phenolic content showed high radical scavenging and antioxidant activity, as the butanolic fraction showed highest results in both ABTS$^{\bullet+}$ decolourization assay and total phenolic content. Small and high molecular mass phenolics, including flavonoids, phenolic acids and tannins have been shown to be good quenchers of free radicals which are mostly present in polar solvents.[30]

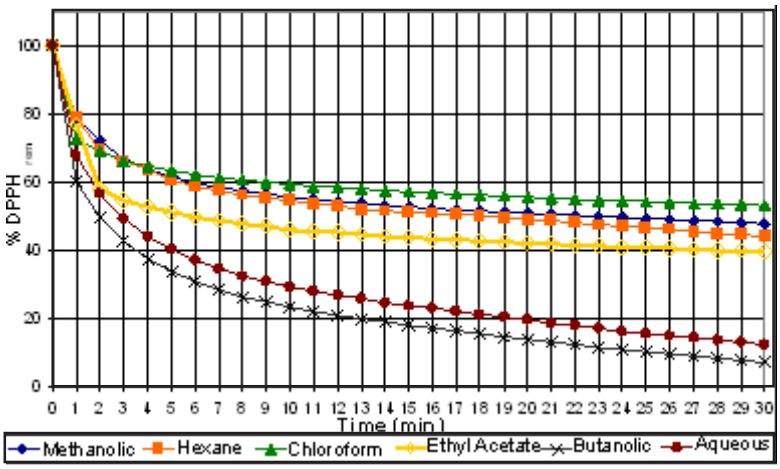

Fig. 1: The radical scavenging activity %DPPH$_{rem}$ by different fractions of leaves of *Rumex acetosella*

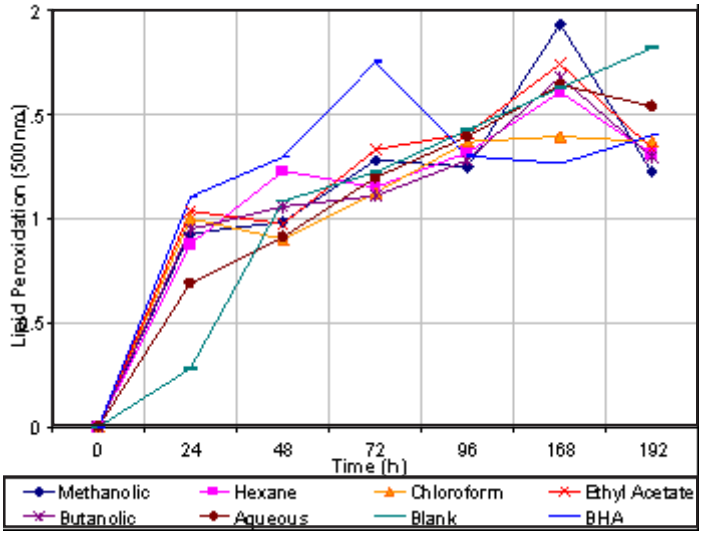

Fig. 2: Lipid Peroxidation in different fractions of leaves of *Rumex acetosella*

CONCLUSION

The wide range of assays demonstrated that the leaves of *Rumex acetosella* var. *acetosella* have remarkable *in vitro* antioxidant properties. The general trend was butanolic > aqueous > ethyl acetate > methanolic > hexane > chloroform. This can be attributed to the high phenolic and flavonoid contents found in the plant. Consequently, the results propose that leaves of the plant are significant source of antioxidant. The butanolic fraction proved to be a potent antioxidant and free radical scavenger and may lead to the discovery of a new antioxidant substance that can be used commercially.

REFERENCES

1. Kumar, S. *Adv. Appl. Sci. Res.,* **2**(1): 129-135 (2011).
2. Keaney, J.F.; Jr, F.B. "Antioxidant protection of low-density lipoprotein and its role in the prevention of atherosclerotic vascular disease.", Ed., B. Frei, Academic Press, 1994, San Diego, California,.
3. Harman, D. Mol. *Cell. Biochem.*, **84**(2): 155-61 (1988).
4. Oktay, M.; Gulcin, I.; Kufrevioglu, O.I. Lebensm. *Wiss. Technol.,* **36**: 263-271 (2003).
5. Diplock, A.T. *Free Radical Res.*, **27**: 511-532 (1997).
6. Halliwell, B.; Gutteridge, J.M.C. "Free radicals in biology and medicine" (1999), United Kingdom: Oxford University Press.
7. Ali, S.I.; Qaiser, M. Proc. Royal. Soc. Edinburg, **89**B: 89-101 (1986).
8. Hatfield, G. "Encyclopedia of folk medicine old world and new world traditions" (1st Ed.) 2004, ABC – CLIO publishers.
9. Yadav; Kumar, S.; Jain, S.; Pundir, P.; Jadon, R.K.; Sharma, S.; Khetwal, A.; Gupta, K. S.; Molan, A.L.; Faraj, A.M. Folia Parasitol (Praha), **57**(1): 62-68 (2010).
10. Tavares, L.; Carrilho, D.; Tyagi, M.; Barata, D.; Serra, A.T.; Duarte, C.M.; Duarte, R.O.; Feliciano, R.P.; Bronze, M.R.; Chicau, P.; Espírito-Santo, M.D.; Ferreira, R.B.; dos Santos, C.N. Molecules. **15**(4): 2576-2592, (2010).
11. Yuan, Y.; Chen, W.S.; Zheng, S.Q.; Yang, G.J.; Zhang, W.D.; Zhang, H.M.. Zhongguo Zhong Yao Za Zhi., **26**(4): 256-258 (2001).
12. Shuying, G.; Bo, F.; Ruonan, Z.; Jiankang, M.; Wei, W. Molecules, **16**(2): 1201-1210 (2011).
13. Abd el-Fattah, H.; Gohar, A.; el-Dahmy, S.; Hubaishi, A. *Acta Pharm Hung.* **64**(3): 83-5 (1994).
14. Kemper, K. J., Sorrel, Longwood Herbal Task Force: http://www.mcp.edu/herbal/default.htm (April 2, 2011) (1999).
15. Sahreen, S.; Khan, M.R.; Khan, R.A. *Food Chem.,* **122**(4): 1205-1211, (2010.
16. Slinkard, K.; Singleton, V.L. Am. *J. Enol. Vitic.,* **28**: 49-55 (1977).
17. Brand-Williams,W.; Cuvelier, M.E.; Berset,C. Lebensm. *Wiss. Technol.,* **28**: 25-30 (1995).
18. Umamaheswari, M.; Chatterjee, T.K. *Afr. J. Trad. CAM*, **5**(1): 61-73 (2008).
19. Benzie, F.F.; Strain, *J.J. Methods Enzymol.,* **299**: 15-23, (1999).
20. Oyaizu, M. *Japanese Journal of Nutrition.* **44**: 144-158, (1986).
21. Mitsuda, H.; Yasumoto, K.; Iwami, K. *Euyo to Shokuryo,* **19**: 210-214, (1996).
22. Re, R.; Pellegrini, N.; Proteggente,A.; Pannala,A. M.; Yang, M.; Rice-Evans, C. *Free Radical Biol. Med.,* **26**: 1231-1237 (1999).
23. Kessler, M.; Ubeaud, G.; Jung, L. *J. Pharm. Pharmacol.,* **55**: 131-142 (2003).
24. Miliauskas, G.; Venskutonis, P.R.; Van-Beck, T.A. *Food Chem.,* **85**: 231-237, (2004).
25. Mei, R.; Liang, H.; Wang, J.; Zeng, L.; Lu, Q.; Cheng, *Y. Planta Med.,* **75**(10): 1162-1164 (2009).
26. Fan, J.P.; Zhang, Z.L. *Zhong Yao Cai.* **32**(12): 1836-40 (2009).
27. Kai, W., *et al. Med. Chem. Res.*, **19**(2): 166-176 (2010).
28. Shinde, A.N.; Malpathak, N.; Fulzele, D.P. *Food Chem.*, **118**: 128–132, (2010).
29. Nabavi, S.M.; Ebrahimzadeh, M.A.; Nabavi, S.F.; Fazelian, M.; Eslami, B. *Phcog Mag,* **4**(18): 123-127 (2009).
30. Heim, K.E.; Tagliaferro, A.R.; Bobilya, D.J. *J Nutr Biochem,* **13**: 572-584 (2002).