Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 1, 2012

Research Article

IN *VITRO* LIPID PEROXIDATION ASSAY OF *RUMEX VESICARIUS* L.

PALANI SAMY HARI PRASAD* AND N.RAMAKRISHNAN[1]

Department of Botany, Government Arts College (Autonomous), Kumbakonam, Tamilnadu, India. Email: phari.bio@gmail.com

Received: 15 Oct 2011, Revised and Accepted: 24 Nov 2011

ABSTRACT

The present investigation is aimed to carry out the invitro lipid peroxidation and total antioxidant capacity of *Rumex vesicarius* L. (Polygonaceae), *Rumex vesicarius* L. is commonly called as "Bladder dock" it is a wild green leafy vegetable cultivated in many parts of India and used in daily diet. Lipid peroxidation was monitored by the change in optical density of the prepared concentrations (1.5-1000µg/ml) and the inhibition percentage was calculated. $FeSo_4$ induced peroxidation was inhibited by standard antioxidans Quercitin. The inhibition percentage of the plant extracts (hexane, chloroform, ethylacetate, ethanol and aqueous) increased with increase in concentrations. Of all the five extracts analyzed and compared the ethanol extract of the plant showed maximum inhibition percent of 62.24 at 1000µg/ml, followed by other extracts while the ethylacetate extract showed lowest inhibition percent of 34.27. The plant extracts inhibited Lipid peroxidation, thus it could slow down aging process and can improve immune responses. The present study indicates that *Rumex vesicarius* L can be a potential source of natural antioxidant.

Keywords: *Rumex vesicarius* L., *In-vitro* Lipid peroxidation, Total antioxidant capacity.

INTRODUCTION

The immune system in living organs is subjected to free radical damage which will suppress their activity. The ability of antioxidants is to destroy free radicals and protects the structural integrity of cells and tissues. Traditional medical practition and natural food components like greens and vegetables are known to have natural antioxidants. On consuming greens and vegetables the natural antioxidants makes the body more resistant to diseases[1]. Oxygen Free Radicals (OFRs) have been implicated in the pathogenesis of an increasing number of diseases and inflammatory states. They may cause cell and tissue damage by their chemical modifications of proteins, carbohydrates, nucleotides, lipids of cell membranes and DNA. As a result they can easily initiate the peroxidation of the membrane lipids. OFRs are part of normal regulatory circuits and are neutralized by antioxidants. The ability of antioxidants is to destroy free radicals and protects the structural integrity of cells and tissues[2].

Lipid peroxidation is important in food deterioration. Oxidative stress can be assessed by measuring lipid peroxidation in the body[3]. The lipid peroxidation process is initiated by the attack of free radicals on polyunsaturated fattyacid. A lipid radical is formed that reacts with oxygen, leading to the formation of a peroxyl radical that may further react with lipids and produce a new lipid radical. There by a propagation reaction starts and is maintained, until a termination reaction occurs including for example chain breaking antioxidant [4].

Rumex vesicarius L. is an edible green used as a sorrel, eaten fresh (or) cooked. It has many important medicinal uses, used in treatment of tumors, hepatic diseases, bad digestion, constipation, calcules, heart troubles, disease of spleen, hiccough, toothache and nausea. The plant is also used as cooling, laxative, stomachic, tonic, analgesic, appetizer, diuretic, astringent, purgative, antispasmodic and antibacterial agents. It is also used to reduce biliary disorders and controls cholesterol levels[5].

The medicinal importance of the plant is a reflection to its chemical composition, since the plant contains many bioactive substances. The plant is also rich in carotenoids, vitamins, and organic acids, the plant is also good source of minerals such as K, Na, Ca, Mg, Fe, Mn and Cu [6]. The plant is rich in phytochemicals, which act as a rich antioxidant agent. The intake of dietary antioxidant phytochemicals will lead to protection against non-communicable disease in human being like cancer, cardiovascular disease and cataract [7,8].

The present study was aimed to screen the phytoconstituents of *Rumex vesicarius* L. and to evaluate the antioxidant activity by Lipid peroxide assay and total antioxidant capacity by Phosphomolybdenum method.

MATERIALS AND METHOD

Plant material

Rumex vesicarius L. was collected from plains of Tiruvannamalai, Tiruvannamalai District, Tamilnadu, India and Taxonomically identified by Dr.G.V.S.Murthy, Scientist "F", BSI, South regional centre, Coimbatore, India. The voucher specimen has been retained in Botany Department, Government Arts College (Autonomous), Kumbakonam, Tamilnadu, India. The collected material was shade dried and powered with mechanical grinder, and well preserved for further use.

The shade dried powder material was subjected to extraction with various solvents of increasing polarities such as hexane, chloroform, ethyl acetate and ethanol by soxhlet apparatus and the aqueous extract was prepared by maceration method[9]. The extracts were filtered using Whatmann number 1 filter paper and then concentrated in vacuum and dried. The extracts thus obtained were used in the analysis of antioxidants.

Chemicals

TBA, TCA, and Quercitin were purchased from Sisco research laborites Pvt. Ltd., India. All other chemicals and solvents used were of analytical grade.

Preliminary phytochemical tests

The preliminary phytochemical screening[10] of all the extracts (hexane, chloroform, ethyl acetate, ethanol and aqueous) were carried out to know the different constituents present in *Rumex vesicarius* L. as per the standard procedure.

Lipid peroxidation inhibition assay

Liver homogenate was prepared from commercial available chicken liver. The liver was quickly excised after decapitation and washed several times with ice-cold saline solution. A 10% of liver homogenate was prepared using ice-cold Kcl (0.15M) in a Teflon tissue homogenizer and the test system containing homogenate of protein content was adjusted to 500µg/ml. In the control system to 1ml of tissue homogenate, the lipid peroxidation was initiated by the addition of 0.1ml of $FeSo_4$ (25µm), 0.1ml of ascorbate (100µm) and 0.1ml of KH_2Po_4 (10mm) and the volume was made up to 3ml with

distilled water and incubated at 37⁰C for one hour. Then 1ml of 5% TCA and 1ml of TBA was added to this reaction mixture and the tubes were boiled for 30mins. in a boiling water bath. This was centrifuged at 3500rpm for 10min. In the test system homogenate was incubated with various concentrations of extracts (1.5-1000µg/ml). The extent of inhibition of lipid peroxidation was evaluated by the estimation of Thiobarbituric acid reactive substances (TBARS) level by measuring the absorbance at 532nm.[11]. the lipid peroxidation inhibition percentage was calculated by using the formula below

$$\text{Lipid peroxide Inhibition \%} = \frac{(\text{A control} - \text{A test})}{\text{A control}} \times 100$$

Where A control = Absorbance of control reaction, A test = Absorbance in the presence of the sample of extracts.

Lipid peroxidation inhibition percentage was determined by comparing the optical density (OD) of treatments with standard antioxidant. The experiments were repeated in triplicates, Quercitin was used as standard positive antioxidant.

Determination of total antioxidant activity

The total antioxidant activity of *Rumex vesicarius* L. extracts was estimated by Phosphomolybdenum method according to the procedure of Prieto[12]. The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate (or) Mo (V) complex at acidic pH. An aliquot 3ml of sample or Vitamin E (equivalent to 500µg) was combined with the reagent solution (0.6m sulfuric acid, 28mM sodium phosphate and 4mM-ammonium molybdate). In case of blank, methanol was used in place of sample. The tubes containing the reaction solutions were capped and incubated in a boiling water bath at 95⁰C for 60-90mins. Samples were cooled to room temperature the absorbance of the aqueous solution of each sample was measured at 695nm against the blank in a Perkin Elmer-UV-Visible Spectrophotometer. Vitamin E was used as standard antioxidant the total antioxidant activity is expressed as the number of equivalents of vitamin E.

RESULTS

Oxidative stress has been implicated in the pathology of many diseases and conditions including diabetes, cardiovascular disease, inflammatory conditions, cancer and ageing[13]. Antioxidants offer resistance against oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation and by many other mechanisms, antioxidants thus prevents diseases. The peroxidation of membrane lipids inhibited by oxygen radicals may lead to cell injury. Initiation of lipid peroxidation by ferrous sulphate takes place either through ferryl perferryl complex (or) through OH radicals by Fenton reaction [14] there by initiating a cascade of oxidative reactions.

The preliminary phytochemical screening of *Rumex vesicarius* L. extracts revealed the presence of various bioactive components like phenols, tannins, flavonoids etc., the results of phytochemical test has been summarized in the Table 1. The phenolic compounds and flavonoids are associated with antioxidative action in biological systems, and act as scavengers of singlet oxygen and free radicals [15].

Table 1: Preliminary Phytochemical screening of *Rumex vesicarius* L

Phytoconstituents	n-Hexane	Ethylacetate	Chloroform	Ethanol	Water
Phenols	+++	+++	+++	+++	+++
Tannins	+	+	+	+	+
Flavonoids	+	++	+	+	+
Saponins	-	-	-	+	+++
Triterpenoids	-	-	-	++	-
Anthraquinones	-	-	-	+	-
Quinones	-	-	-	+	+

The extracts of *Rumex vesicarius* L. at various concentrations decreased the amount of lipid peroxide inhibition. Figure 1 illustrates the significant decrease in lipid peroxide radical scavenging ability of plant extracts and quercitin, of all the five extracts analyzed the ethanol extract showed maximum inhibition percent of 62.24, followed by aqueous extract with 53.32% inhibition, chloroform with 39.51% inhibition, hexane with 38.11% inhibition while the ethyl acetate showed minimum inhibition percent of 34.27 respectively at same concentration of 1000µg/ml.

The positive standard antioxidant Quercitin showed 89.87% inhibition at the same concentration of 1000µg/ml.

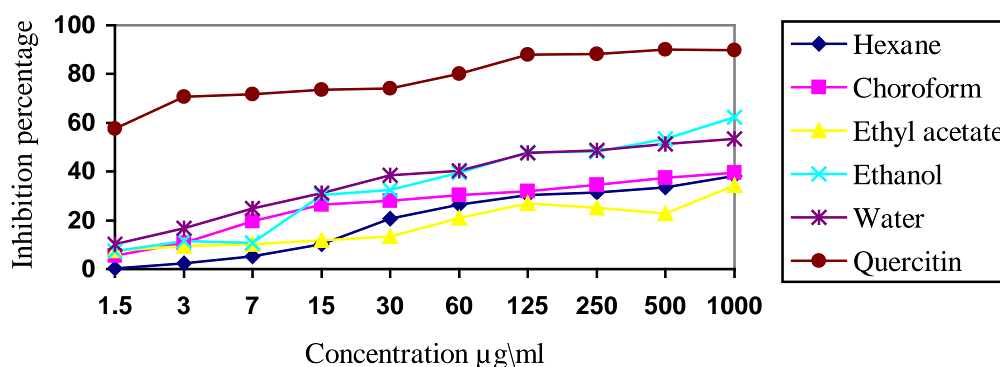

Fig. 1: Lipid peroxide free radical scavenging activity of *Rumex vesicarius* L.

The total antioxidant capacity of the extracts is given in figure 2. Total antioxidant capacity of *Rumex vesicarius* L. is expressed as the number of equivalents of vitamin E. The Phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of green phosphate or Mo (V) complex with a maximal absorption at 695nm. The assay is successfully used to quantify the total antioxidants present in the plant extracts. The assay is a quantitative one since the activity is expressed as numbers of equivalents of vitamin E. On comparing the total antioxidant capacity to standard antioxidant vitamin "E", the study reveals that the antioxidant activity of the extracts exhibited increasing trend with the increasing concentration of the plant extract. While the standard antioxidant also showed the same trend as the concentration increased the activity also increased.

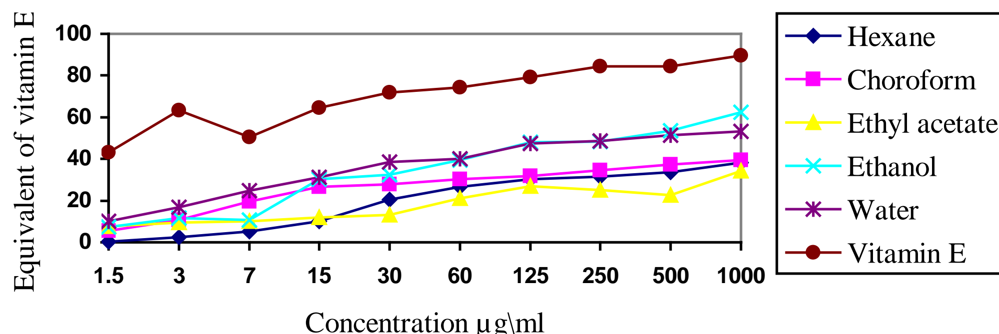

Fig. 2: Total antioxidant capacity of *Rumex vesicarius* L.

Statistical Analysis

All the treatments were performed in triplicates and each data point in the results is the mean of three replicates. All experiments were repeated at least once. The statistical significance of the treatment effect were expressed as mean + SEM.

DISCUSSION

Unsaturated lipids in liver tissue are very susceptible to peroxidation when they are exposed to reactive oxygen species (ROS). In the present investigation the liver tissue is incubated in presence of ROS generating system $FeSo_4$ and examined the effect on the tissue homogenates by measuring the optical density (OD) at 532nm. The results of the investigations revealed that *Rumex vesicarius* L. had potent lipid peroxidation inhibition activity.

On the basis of the results obtained in the present study, we conclude that the ethanol extract of *Rumex vesicarius* L. exhibited significant in *vitro* lipid peroxide inhibition activity on compared with standard antioxidant Quercitin. The activity may be related to the presence of phenols and flavonoids in the plant extract. The ethanol extract showed highest scavenging inhibition activity on compared to other four extracts. It is concluded that the extracts of *Rumex vesicarius* L. might be useful for the development of newer and more potent natural antioxidant.

REFERENCES

1. Odukoya OA, Inya-Agha SI, Iloni OO. Immune Boosting Herbs: Lipid peroxidation in liver homogenate as index of activity. *Journal of Pharmacology and Toxicology.* 2007; 2(2): 190-195.
2. Bendich A. Physiological role of antioxidants in the immune system. *J. Dairy Sci.* 1993; 76: 2789-2794.
3. Halliwell B, Chirico S. Lipid peroxidation: Its mechanism, measurement and significance. *Am. J. Clin. Nutr.* 1993; 57:7 15S-724S.
4. Basu S. Carbon tetra chloride- induces lipid peroxidation: Eicosanoid formation and their regulation by antioxidant nutrients.*Toxicology.* 2003;189: 113-127.
5. Mostafa HAM, El-Bakry AA, Eman A Alam. Evaluation of antibacterial and antioxidant activities of different plant parts of *Rumex vesicarius* L. (Polygonacaea). International Journal of Pharmacy and Pharmaceutical sciences. 2011**;** 3(2**)**: 109-118.
6. Al-Rumaih, May M, Al Saad FA, Warsy AS. Seasonal variation in mineral content of different organs during development of *Rumex vesicarius* L. Saudi. *J. Biol. Sci.*, 2002; 9(1**)**: 69-78.
7. Rao BN. Bioactive phytochemicals in Indian foods and their potential in health promotion and disease prevention*. Asia Pacific Journal Cli. Nutr.* 2003;12(1): 9-22.
8. Matkowski A. Plant in *vitro* culture for the production of antioxidants- A review. *Biotechnology Advances.* 2008;26:548-560.
9. Mukerji PK. Quality control of Herbal drugs. Business Horizone, NewDelhi. 2007. p132, 186-195.
10. Kokate CK. Practical Pharmacognosy (IV Ed.). Vallabh Prakashan, New Delhi, 1994. p115-117.
11. Ohkawa H, Ohishi N, Yagi K. Assay of lipid peroxides in animal tissues by thio babituric acid reaction. *AnalBio-Chem.* 1979; 95**:**351-358.
12. Prieto P, Pineda M, Agulilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Anal Biochem.* 1999; 269: pp 337-341.
13. Schlesier K, Harmat M, Bohm V, Bitsh R. *Free radicals. Res.* 2000;30**:** 177.
14. Halliwell B. Superoxide-dependent formation of hydroxyl free radicals in the presence of iron and copper chelates. FEBS Lett. 1978; 92:321.
15. Moni Rani Saha, Md. Ashraful Alam, Raushanara Akter, Rumana Jahangir. In-*vitro* free radical scavenging activity of *Ixora coccinea* L. *A Journal of the Bangladesh Pharmacological Society*. 2008; 3: 90-96.