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RESEARCH ARTICLE

ANTIOXIDANT ASSAY OF *Rumex vesicarius* L.

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ABSTRACT

The main characteristic of an antioxidant is its ability to trap free radicals and also reduces the risk of chronic diseases. The information on antioxidant properties of various natural sources is still rather scared. The purpose of this study was to evaluate and to compare the antioxidant activity of hexane, chloroform, ethyl acetate, ethanol and aqueous extracts of *Rumex vesicarius* L. (Polygonaceae) by DPPH free radical scavenging assay and total antioxidant capacity by Phospomolybdenum method. Preliminary phytochemical screening revealed that the extracts possess flavonoids, phenols, tannins and saponins. The extracts showed significant antioxidant activity, on compared to standard antioxidant in a dose dependent manner. The ethyl acetate extract showed maximum inhibition percent of 94.82, followed by other extracts while hexane extract showed minimum inhibition percent of 76.40 at same concentration of 1000µg/ml. Compared with positive standard Quercitin and vitamin E. The results suggest that the extracts can be a vital source of antioxidant phytochemical.

INTRODUCTION

Antioxidants prevent the damage done to the cells by free radical-molecules that are released during the normal metabolic process of oxidation. Antioxidants protect unsaturated fats in the body from oxidation by peroxides and other free radicals (Habila *et al.,* 2010). Antioxidants widely used are synthetic origin and have recently been suspected to their toxicity and causes carcinogenic effects (Rao *et al.,* 2010). Consequently antioxidants may be used as protective (or) therapeutic agents. Recently there has been an upsurge of interest in the therapeutic potential of medicinal plants, vegetables and leafy greens as antioxidants in reducing such free radical induced tissue injury (Suresh Kumar *et al.,* 2008). In the human body, Reactive Oxygen Species (ROS) are derived from normal metabolic activity (or) from external sources like exposure to radiations, smoking cigarette, air pollutants and industrial chemicals. The ROS formed may cause cellular and sub cellular damage by peroxidation of membrane lipids by denaturing cellular proteins and by breaking DNA strands disrupting cellular functions. The ROS are major cause of human cancer and other diseases. The risk of disease can be reduced by increased consumption of antioxidants which are abundant in food. Therefore attention is focused on the development of new, safe and cheap antioxidants of natural origin (Shanab, 2007). *Rumex vesicarius* L. is an annual herb belongs to family Polygonaceae, it is a wild edible plant eaten fresh (or) cooked (Mostafa *et al.,* 2011). It is distributed in several parts of world, commonly known as "Bladder dock", in tamil it is called as "Chukkan keerai" is an annual, glabrous herb of 15-30cm in height, branched from root, with long elliptic, ovate (or) oblong leaves with monoecious flowers. It is widely cultivated as green leafy vegetable in many parts of India (Panduraju *et al.,* 2009). The green leafy vegetables are in general a good source of vitamins, minerals, and fibres. According to unani system of medicine, the herb is an analgesic, astringent, antiulcer, hepatoprotective agent and is useful in scabies, leucoderma, toothache, asthma, heart troubles, tumors and scurvy. The leaves are used as asperient, diuretic and considered as antitiode for snake venom and seeds are considered as antitode for scorpion venom (Shankar Gopal Joshi, 2000). In the present study we have evaluated the antioxidants potential of hexane, chloroform, ethyl acetate, ethanol and aqueous extracts of *Rumex vesicarius* L. The evaluation of antioxidant activity was performed in *vitro* by DPPH (1, 1-diphenyl, 2-picryl hydrazyl) radical scavenging activity and total antioxidant capacity by Phospho molybdenum method.

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MATERIALS AND METHOD

Chemicals

DPPH, trichloroacetic acid, ferric chloride and quercitin were purchased from Sisco research laboratories Pvt., Ltd., India.

All other chemicals and solvents used were of analytical grade.

Plant material

Rumex vesicarius L. was collected from plains of Tiruvannamalai, Tiruvannamalai Distict, Tamilnadu, India. Taxonomic identification was authenticated by Dr. G.V.S. Murthy, scientist "F", BSI, South regional centre, Coimbatore, India. The voucher specimen was deposited at botany department, Government Arts College (autonomous), Kumbakonam, Tamilnadu, India. The plant material was shade dried, powdered coarsely and well preserved for further use. The dried powder was subjected to extraction with different solvents viz., hexane, chloroform, ethyl acetate and ethanol. Aqueous extract was prepared by maceration method (Mukerji, 2007). All the extracts were filtered through Whatmann number 1 filter paper and then concentrated in vacuum and dried. The extracts thus obtained were directly used in DPPH assay and total antioxidant activity.

Preliminary phytochemical tests

The preliminary phytochemical screening (Kokate, 1994) of all the extracts were carried out to know the different constituents present in *Rumex vesicarius* L. as per the standard procedure.

DPPH free radical scavenging activity

The In-*vitro* free radical scavenging capacity of the extracts was determined by DPPH assay (Koleva et al., 2002) about 10µL concentration (1.5-1000µg/ml) of each extract sample solution was added to 190µL DPPH (150µM) in ethanol solution. After vortexting the mixture was incubated for 20min. at 37^0C. The control blank contains solvent without extract. The decrease in absorbance of test mixture due to quenching of DPPH free radicals was measured at 517 nm and the percentage of inhibition was calculated by the below formula

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

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

The Ic_{50} values were determined as the concentration of the test mixture that gave 50% reduction in the absorbance from control blank. The experiments were repeated in triplicates. Quercitin was used as standard positive antioxidant.

Determination of total antioxidant capacity

The total antioxidant activity of *Rumex vesicarius* L. extracts was estimated by the Phosphomolybdenum method according to the procedure of Prieto *et al*. (1999). The assay is based on

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

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

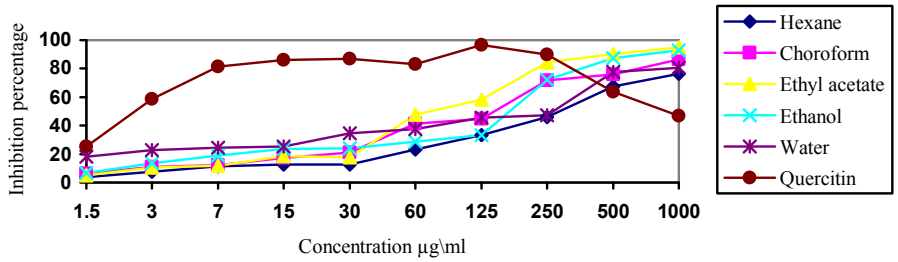

Fig. 1: DPPH radical scavenging activity of *Rumex vesicarius* L. with Quercitin

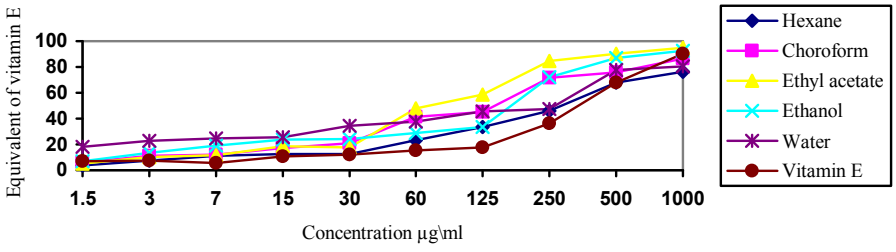

Fig. 2: Total antioxidant capacity of *Rumex vesicarius* L. with Vitamin "E"

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 3ml of 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 95^0C for 60-90min. samples were cooled to room temperature the absorbance of the aqueous solution of each 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 AND DISCUSSION

The preliminary phytochemical screening of *Rumex vesicarius* L. extracts revealed the presence of various bioactive components of phenols and flavonoids were most predominant and 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, acting as scavengers of singlet oxygen and free radicals (Moni Rani Saha *et al.,* 2008). In this present study the antioxidant activity was investigated by DPPH scavenging assay and total antioxidant capacity by Phosphomolybdenum method. The active effectiveness of the plant extracts were compared to the standard antioxidant used as reference. The DPPH (1, 1-diphenyl, 2-picryl hydrazyl) antioxidant assay is based on the ability of DPPH a stable free radical to decolorize in the presence of antioxidants. Comparison of the antioxidant activity of the extracts and standard quercitin is shown in figure 1. Of all the extracts compared the ethyl acetate extract showed maximum inhibition % of 94.82, followed by ethanol extract with 92.75%, chloroform with 86.54%, aqueous extract with 80.57% while the hexane extract showed minimum inhibition % of 76.40 all at same concentration of 1000µg/ml. The standard antioxidant Quercitin showed a maximum of 96.81% inhibition at 125 µg/ml concentration. The Ic_{50} values of the extracts was 73.5 for ethyl acetate, 175.5 for ethanol, 162.5 for chloroform, 275.5 for aqueous, hexane with 325.0, while the standard quercitin showed 13.5. The ethyl acetate extract exhibited a significant dose dependent inhibition of DPPH activity on compared to other extracts. 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 equvivalents 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 total antioxidants in the plant extracts. The assay is a quantitative one since the activity is expressed as numbers of equivalents of vitamin E. The study reveals that the antioxidant capacity of the extracts exhibits increasing trend as the concentration of the plant extracts increased the capacity of the activity also increased.

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 a treatment effect were expressed as mean $\pm$ SEM.

Conclusion

It is evident from the present study that the ethyl acetate and ethanol extracts of *Rumex vesicarius* L could be used as good source of natural antioxidants in pharmaceutical industry. However the compounds responsible for the antioxidant activities need to be isolated.

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