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Original Article

IN VITRO ANTIOXIDANT PROPERTIES AND TOTAL PHENOLIC AND FLAVONOID CONTENTS OF *RUMEX VESICAIUS L.*

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ABSTRACT

Objective: The aim of the study was to investigate the total phenolic and total flavonoids contents and evaluate the antioxidant potential of different solvent extracts of *Rumex vesiarius L.*

Methods: The antioxidant potential of the hexane, ethyl acetate and methanol extract by using FRAP, LPO and Peroxidase assays and free radical scavenging by using DPPH and ABTS were determined. These activities were determined by using standard protocols with some modifications. Aluminium chloride colorimetric method was used to estimate TFC (total flavonoid content) and TPC (total phenolic content) was measured by Folin-ciocalteu method.

Results: Among the test extracts methanol extract exhibited strong antioxidant activity than that of hexane and ethyl acetate. Free radicals, DPPH and ABTS were significantly inhibited by methanol extract of leaf and fruit (IC_{50} 174.91±17.96 µg/ml and 526.791±91.85 mM $min^{-1}g^{-1}$DW), whereas methanolic extract of leaves showed good antioxidant potential using FRAP (306±14.8 mM $min^{-1}g^{-1}$DW) and LPO (30.57±5.65 MDA g-1DW) methods, while Peroxidase was were strongly inhibited by ethyl acetate extract of flower (0.624±0.013 mM min-1g-1DW). The maximum total phenolic and total flavonoids content was observed in leaves (0.53±0.31 mg GAE/gm DW and 2.15±0.72 mg QE/gm DW).

Conclusion: The present investigation suggested that methanol extracts of *Rumex vesicarius* has significant antioxidant activity. These results clearly indicate that *R. vesicarius* is effective against free radical mediated diseases. These crude extracts can further purify and may be considered as a new source for antioxidant pharmaceutical compounds.

Keyword: *R. vesicarius*, Free radical scavenging activity, Peroxidase, FRAP, LPO, DPPH, ABTS, Methanol.

INTRODUCTION

In response to the increased popularity and greater demand for medicinal plants, a number of conservation groups are recommending that wild medicinal plants be brought into cultivation. Ethno pharmacological surveys conducted and revealed that a large number of indigenous plant species are being used as a source of herbal therapies [1]. Since very old times, herbal medications have been used for relief of symptoms of disease. plants still make an important contribution to health care. Much interest, in medicinal plants however, emanates from their long use in folk medicines as well as their prophylactic properties, especially in developing countries. Large number of medicinal plants has been investigated for their antioxidant properties. Natural antioxidants either in the form of raw extracts or their chemical constituents are very effective to prevent the destructive processes caused by oxidative stress [2]. Antioxidants are the substance that when present in low concentrations compared to those of an oxidisable substrate significantly delays or prevents oxidation of that substance. [3]. Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer [1].

Rumex vesicarius L. is a wild edible plant used as a sorrel and collected in spring time and eaten fresh, or cooked. *Rumex vesicarius* L. has many important medicinal uses such as treatment of tumors, hepatic diseases, bad digestion, constipation, calcules, heart troubles, pains, diseases of the spleen, hiccough, flatulence, asthma, bronchitis, dyspepsia, piles, scabies, leucoderma, toothache and nausea. The plant also used as cooling, laxative, stomachic, tonic, analgesic, appetizer, diuretic, astringent, purgative, antispasmodic and antibacterial agents [4, 5]. The medicinal importance of R. Vesicarius is reflect the presence of various bioactive substances such as flavonoids, polyphenols, anthraquinones, carotenoids, vitamins (especially vitamin C), proteins, lipids and organic acids. The intake of dietary antioxidant phytochemicals like carotenoids, phenolic compounds and flavonoids may lead to the protection against non-communicable diseases in human being [6].

Antioxidant activity of R. vesicarius studied by many researchers. Beddou *et al.*, 2014[7] evaluated antioxidant activity of hydroalcoholic extract by DPPH, TAC method. El-Bakary *et al.*, 2012[8] performed DPPH assay and HPLC analysis for quantification of quercetin and emodin, at vegetative stage of growth. *In vitro* grown seedlings can be considered rich sources of many biologically active constituents, especially flavonoids and phenolics, the formation of these substances varied with seedling ages[9]. Elfotoh *et al.*, 2013[10] demonstrated chemical profiling by using different chromatographic and spectroscopic techniques and DPPH radical scavenging assay of lipophilic extracts. Khan *et al.*, 2014[6] and Tukappa *et al.*, 2013[11] determined antioxidant ability by DPPH, LPO and hydrogen peroxide assay.

Majorly the DPPH assay was used for antioxidant activity determination. There was a need to use more method to evaluate antioxidant potential of plants. Therefore, this study was designed to access antioxidant by using five different methods like DPPH, ABTS, LPO, FRAP and peroxidase and it provided a comparative analysis of different methods.

MATERIALS AND METHODS

Plants materials

Plant material was collected from the hills of Jaipur. *Rumex vesicarius* (RUBL 21074) was authenticated by Herbarium, Department of Botany, Rajasthan University, Jaipur, Rajasthan, India.

Preparation of plant extracts

The stem, leaf and seed of *Rumex vesicarius* washed using distilled water and were dried at room temperature and ground in a mortar. Fifty grams of each plant powder was extracted in hexane, ethyl acetate and methanol by maceration (48 h), filtered through

Whatmann no. 1 filter paper and appropriately diluted with respective solvent.

Determination of total phenolic contents in the plant extracts

TPC (The total phenolic content) was determined by the Folin-Ciocalteau method [12, 13]. The reaction mixture was prepared by mixing 0.5 ml of methanolic solution (1 mg/ml) of extract, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% NaHCO3. The mixture was allowed to stand for 15 min at 45 °C and the phenols were determined by spectrophotometric method. The absorbance was determined at λmax = 765 nm. The samples were prepared in triplicate and the mean value of absorbance was obtained. Blank was concomitantly prepared, with methanol instead of extract solution. The standard curve was prepared using the standard solution of Gallic acid in methanol in the range 100-1000µg/ml. The total phenolic content was expressed in terms of gallic acid equivalent (mg of GA/g of extract), which is a common reference compound.

Determination of Total Flavonoid concentrations in the plant extracts

The concentrations of TFC (total flavonoids content) was determined using a modified Aluminum chloride spectro photometric method [14]. Plant extracts (0.5 ml) were dissolved with 1.5 ml of methanol, 0.1 ml of 10 % aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water and incubated for half an hour at room temperature. The absorbance of the reaction mixture was measured at 415 nm. All experiments were prepared in triplicate and the mean value of absorbance was obtained and values were expressed in mean±standard deviation. The standard curve was prepared using the standard solution of quercetin in methanol in the range 0.5-5.0 mg/ml. Total flavonoidal content of the extracts was expressed in milligram of quercetin equivalents/gdw.

Determination of antioxidant activity

FRAP assay (Reducing ability assay)

FRAP (Ferric Reducing ability of Plasma) assay method of Benzie and Strain, 1996 [15] is modified for determination of the total antioxidant activity in the extract of plant part. The stock solutions included 300 mM acetate buffer (0.3 M acetic acid and 0.3 M sodium acetate), pH3.6,10 mM TPTZ(2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ (ferric chloride) solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ and 2.5 mL $FeCl_3.6H_2O$. The temperature of the solution was raised to 37 °C before use. Plant extracts (100 µl each of methanolic, ethyl acetate and hexane) were allowed to react with 2900 µl of the FRAP solution for 30 min in the dark condition. Readings of the coloured product (ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 100 and 1000 µM $FeSO_4$. Results are expressed in mM Fe(II)/g dry mass.

Peroxidase assay

The method of assay measures the oxidation of pyrogallol to purpurogallin by peroxidase when catalyzed by peroxidise at 420 nm and at 20 °C. Plant extract (0.2 ml) was homogenized with 10 mL of phosphate buffer and refrigerated centrifuged at 10000 rpm for 20 min. The clear supernatant was taken as the enzyme extract. The activity was assayed after the method of Chance and Maechley, 1955 [16] with following modifications. About 2.4 mL of phosphate buffer, 0.3 mL pyrogallol (50 µM and 0.2 ml ofH_2O_2 (30%) were added. The amount of purpurogallin formed was determined by taking the absorbance at 420 nm immediately after adding 0.1 mL enzyme extract. The extinction coefficient of 2.8/mM/gm was used in calculating the enzyme activity that was expressed in terms of mill mole per minute per gram dry weight.

LPO (Lipid peroxidation assay)

The LPO activity was calculated using the protocol of Heath and Packer, 1968 [17]. About 0.5 ml of plant extract was homogenized with 10 ml of 0.1% (w/v) TCA (Trichloroacetic acid). The homogenate was centrifuged for 5 min (15000 g, 4 °C). Supernatant was collected and 1 ml of supernatant was mixed with 4 ml of 0.5% (w/v) TBA (Thiobarbituric acid) in 20% (w/v) TCA and then incubated in water bath at 95 °C for 30 min. Reaction was quickly ended by incubating on an ice bath. In case the solution is not clear, centrifuge at 10000 g 10 min and the absorbance was measured at 532 and 600 nm. The OD_{600} values were subtracted from MDA-TBA complex values at 532 nm and MDA concentration was calculated using the Lambert-Beer law with an extinction coefficient εM = 155/mM/cm. Results were presented as µM MDA/g.

DPPH radical scavenging activity

The antioxidative activity of the plant extracts were determined by DPPH (2, 2-diphenyl-1-picryhydrazyl) free radical scavenging activity [18, 19, 20]. Experiments were initiated by preparing a 0.004% w/v solution of DPPH and 1 mg/ml solution of different plant parts extracts in methanol. Two ml of the methanolic solutions of DPPH was added to a sample solution (0.1 ml). An equal amount of alcohol was added to the control. The setup was left at dark in room temperature and the absorption was monitored after 30 minutes at 515 nm. Ascorbic acid was used as a control. Experiment was performed in triplicate and the percentage of radical scavenging activity was calculated from the following equation where Abs. control is the absorbance of the DPPH solution without sample and Abs. sample is the absorbance of the tested sample.

$$\% \text{ Radical scavenging} = [1 - (Abs_{\text{SAMPLE}}/Abs_{\text{CONTROL}})] \times 100$$

Linear graph of concentration Vs percentage inhibition was prepared and IC_{50} values was calculated. The antioxidant activity of each sample was expressed in terms of IC_{50} (micromolar concentration required to inhibit DPPH radical formation by 50%), calculated from the inhibition curve [19, 20].

ABTS radical scavenging assay

To determine ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging assay, the method of *Re et al.*, 1999 [21] was adopted. The stock solutions included 0.002 M ABTS solution and 0.07 M potassium persulphate solution. The working solution was then prepared by mixing the 25 ml of ABTS stock and 0.1 ml of potassium persulphate stock and allowing them to react for 12h at room temperature in the dark. The solution was then diluted by mixing ABTS solution with ethanol to obtain an absorbance of 0.706±0.001 U at 734 nm using the spectrophotometer. Fresh ABTS solution was prepared for each assay. Plant extracts (1 ml) at varying concentration were allowed to react with 3 ml of the ABTS solution and the absorbance was taken at 734 nm after 6 min using the spectrophotometer. The ABTS scavenging capacity of the extract was compared with that of BHT and percentage inhibition calculated as:

$$\text{Inhibition (\%)} = [1-(Abs_{\text{Sample}}/Abs_{\text{Control}})]X\ 100$$

Whereas Abs_{Control} is the absorbance of ABTS radical+methanol, Abs_{Sample} is the absorbance of ABTS radical+sample extract/standard.

Statistical analysis

All **e**xperimental results were carried out in triplicate and were expressed as average of three analyses±SD(Standard Deviation). The IC_{50} values were also calculated by linear regression analysis.

RESULTS AND DISCUSSION

In the present study we have investigated the antioxidant activity of *Rumex vesicarius L. Table* 1 shows the total phenolic content maximum in leaves (0.53±0.31 mg GAE/gm DW) while minimum in flower (0.18±0.65 mg GAE/gm DW) and total flavonoid content was seen maximum in leaves (2.15±0.72 mg QE/gm DW) while minimum in stem (0.96±0.38 mg QE/gm DW).

Table 2 shows FRAP, LPO and Peroxidase activity. Ethyl acetate extract of stem(244±11.2 $mMg^{-1}DW$), methanolic extract of leaf (306±14.8 $mMg^{-1}DW$) and ethyl acetate extract of flower (209±3.5 $mMg^{-1}DW$)show highest activity in FRAP assay while minimum activity of stem(71±3.6 $mMg^{-1}DW$), leaf(50±3.1 $mMg^{-1}DW$) and flower(40±2.1 $mMg^{-1}DW$) extract exhibited in hexane solvent.

Methanolic extract of stem(14.82±2.76 µM MDAg^{-1}DW), leaf (30.57±5.65 µM MDAg^{-1}DW)and flower(11.01±4.20 µM MDAg^{-1}DW) have maximum activity in LPO assay while minimum showed by hexane extract of stem(9.92±1.2 µM MDAg^{-1}DW) and flower(6.975±1.87 µM MDAg^{-1}DW), ethyl acetate (12.48±2.0 µM MDAg^{-1}DW)extract of leaf. In Peroxidase assay maximum antioxidant activity demonstrated by methanol extract of stem(0.22±0.012 mM min^{-1}g^{-1}DW) and leaf(0.39±0.007 mM min^{-1}g^{-1}DW), ethyl acetate(0.07±0.008 mM min^{-1}g^{-1}DW) extract of flower while minimum exhibited by hexane extract of stem(0.36±0.017 mM min^{-1}g^{-1}DW) and leaf (0.53±0.011 mM min^{-1}g^{-1}DW)and methanolic extract of flower(0.13±0.014 mM min^{-1}g^{-1}DW).

Table 3 shows the IC_{50} values of DPPH and ABTS assay. Flower extract (526.791±91.85 mM min^{-1}g^{-1} DW) shows highest inhibitory affect while stem (548.092±40.08 mM min^{-1}g^{-1}DW) show lowest affect in ABTS assay. For the IC_{50} value of DPPH, leaf (174.91±17.96 µg/ml) possesses highest and stem (205.26±26.09 µg/ml) shows lowest inhibitory activity.

Antioxidants are crucial in the prevention of human diseases. Herbal compounds with antioxidants activity may function as free radical scavengers, reducing agents and quenchers of single oxygen formation or reactive oxygen species, thereby protecting the body from degenerative disease such as cancer. The reactive oxygen species are damaging byproducts generated during normal cellular metabolism or from toxic insult. They lead to a state of oxidative stress that contributes to the pathogenesis of a number of human diseases by damaging lipids, proteins and DNA [6].

The compounds such as flavonoids, which contain hydroxyls, are responsible for the radical scavenging effect in the plants [22, 23]. According to our study, the high contents of these phytochemicals in leaf extract can explain its high radical scavenging activity. The result of the present study showed that the extract of leaves, which contain highest amount of flavonoid and phenolic compounds, exhibited the greatest antioxidant activity as compared to stem and flower. The high scavenging property of leaves may be due to hydroxyl groups existing in the phenolic compounds. Our results have shown that methanol extract of *R. vesicarius* displayed strong antioxidant activity. Antioxidant activity of *Rumex* corroborated the finding of El-Bakry *et al.*, 2012 [8] and Khan *et al.*, 2014 [6].

Phenolic and flavonoidal content have shown a good correlation with antioxidant activity, this may be due to structural differences [12]. Phenolic compound, such as flavonoids, phenolics acid and tannins, possess antiinflammatory, anticarcinogenic, anti-atherosclerotic and other properties that may be related to their antioxidant activities [24]. Flavonoids and flavonols are two polyphenolic compounds that play an important role in stabilizing lipid oxidation and are associated with antioxidant activities [25].

All of the extracts in this research exhibited different extent of antioxidant activity in different assay. It is evident from the present study that the methanolic and ethyl acetate 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.

Table 1: Total phenolic and flavonoids content in different plant parts of Rumex vesicarius L

Plant part	Total phenolic content (mg GAE/gm DW)	Total Flavonoidal cotent (mgQE/gm DW)
Stem	0.25±0.15	0.96±0.38
Leaf	0.53±0.31	2.15±0.72
Flower	0.18±0.65	1.38±0.16

Each value in the table is represented as mean±SD (n=3). mg GAE/gm DW**:** milligram gallic acid equivalent per gram dry weight mgQE/gm DW: milligram quercetin equivalent per gram dry weight

Table 2: Antioxidant activity (FRAP, LPO, Peroxidase) of methanol, hexane and ethyl acetate extracts of different parts of Rumex vesicarius L.

Plant parts	Solvent	FRAP (mMg^{-1}DW)	LPO (µM MDAg^{-1}DW)	Peroxidase (mM min^{-1}g^{-1}DW)
	Hexane	71±3.6	9.92±1.2	0.36±0.017
Stem	Ethyl Acetate	244±11.2	11.00±0.7	0.28±0.008
	Methanol	161±24	14.82±2.76	0.22±0.012
	Hexane	50±3.1	13.95±3.26	0.53±0.011
Leaf	Ethyl Acetate	163±11.5	12.48±2.0	0.62±0.013
	Methanol	306±14.8	30.57±5.65	0.39±0.007
	Hexane	40±2.1	6.975±1.87	0.12±0.012
Flower	Ethyl Acetate	209±3.5	7.905±2.63	0.07±0.008
	Methanol	140±20.7	11.01±4.20	0.13±0.014

Each value is expressed as mean±S. E. (Standard Error) (n=3). FRAP values are indicated as weight (g) of $FeSO_4$ in 100g of the plant extracts. LPO values are indicated as MDA content in micro mole per miligram. Peroxidase expressed in terms of mill mole per minute per gram dry weight.

Table 3: IC_{50} values of different plant parts of Rumex vesicarius of ABTS and DPPH radical scavenging assay

Plant Part	ABTS (mM min^{-1} g^{-1} DW)	DPPH(µg/ml)
Stem	548.092±40.08	205.26±26.09
Leaf	536.232±84.62	174.91±17.96
Flower	526.791±91.85	176.19±48.23

Each value is expressed as mean±S. E.(Standard Error) (n=3)

CONCLUSION

From the results, it can be concluded that methanol extracts of *R. vesicarius* possess potent antioxidant activity and can be used as a source of natural antioxidants for medicinal uses against cancer, ageing, autoimmune diseases, diabetes and other related to free radicals, thus replacing the synthetic ones.

The methanol extracts of plant exhibited very good antioxidant activity for different assays. Further investigation of individual compounds with their *in vivo* antioxidant activities and different antioxidant mechanisms is needed.

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CONFLICT OF INTERESTS

Declared None

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