

American Journal of
Drug Discovery
and Development

ISSN 2150-427X



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Evaluation of Antibacterial and Antioxidant Activities of Different Methanol Extract of *Rumex vesicarius* L.

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ABSTRACT

This study is designed to examine the antibacterial and antioxidant activity along with total phenolic content and flavonoid content of different extract i.e., WPH (whole plant hot), WPC (whole plant cold), LH (leaves hot) and LC (leaves cold) of *Rumex vesicarius* L. Antibacterial activity of all the extract was evaluated against five clinically significant bacterial cultures by agar well diffusion method. The different extract of *R.vesicarius* L. exhibited moderate to potent broad spectrum antibacterial activities targeting both gram+ve and gram-ve bacteria. The inhibition zone, relative% inhibition, MIC and MIB showed highly significant variations according to variations of extraction method, plant part and tested bacterial isolates. The WPH extract was found to be most effective against *Pseudomonas aeruginosa* (11.66 ± 0.41), *Staphylococcus aureus* (9.33 ± 0.577) and *Escherichia coli* (8.00 ± 0.00), respectively, WPC showed the moderate activity against *Pseudomonas aeruginosa* (7 ± 0.00). The LH extract exhibited significant activity against *Escherichia coli* (12.33 ± 0.417) and *Staphylococcus aureus* (11.750 ± 0.404) respectively and LC showed good antibacterial activity against *Pseudomonas aeruginosa* (10.33 ± 0.932). The highest MIC value is 250 mg mL^{-1} and the lowest MIC value is found to be 31.25 mg mL^{-1} . The MIC index calculated for each extract shows that $\text{MBC/MIC} < 4$ thus all the extract of *R.vesicarius* are bactericidal. Antioxidant activities of the samples were determined by three different test systems, DPPH, lipid peroxidation, reducing power assay. In all the systems, WPH and LH extract exhibited excellent activity potential than those of other extract. A positive correlation was observed between the pharmacological activity and phenolic, flavonoid levels of the extract.

Key words: *Rumex vesicarius* L., antimicrobial activity, DPPH, lipid peroxidation, reducing power assay

INTRODUCTION

Energy management is an intrinsic oxidation process in all living organism (Halliwell and Gutteridge, 2007). However the excessive production of free radicals which results from biochemical reactions or from external factors is involved in a range of physiological and pathological process (Collins, 1999). Excess free radicals generated in the body beyond its antioxidant capacity can even cause DNA strand breakage, carbonylation of cellular proteins and lipids peroxidation leading to chronic health problems such as cancer. Intake of antioxidants in the form of diet or drug/formulations may be an important strategy for inhibiting or slowing down the oxidation of susceptible cellular substrates and is thus relevant in disease prevention. Antioxidants interfere with the production of free radicals scavengers and inhibit and inhibit or slow down lipid

peroxidation and other free radical mediated process. Therefore they have a tendency to project the body from various disorders which are attributed to free radicals. Since last 3 decades more importance is given on antioxidant based drugs/formulations for the prevention and treatment of complex diseases. Currently the synthetic antioxidants have been reported to have toxic side effects making the way for the search of antioxidant and free radical scavengers based on natural products (Rashid *et al.*, 2013). Oxidation mediated by free radical reactions is also responsible for food poisoning, spoilage and rancidity which is also one of the growing problem that has not yet been overcome despite the use of many robust preservation techniques. The screening of plant for antioxidative activity has revealed their potential as a source of new antioxidant agent. Synthetic antioxidants have been in use in the food industry since the 1940's but now trends has shift and preferences is given to natural compounds and also FDA recognizes them in the category of GRAS Generally Regarded as safe (Leong and Shui, 2002; Rios *et al.*, 1988).

On the other hand, new sources of antimicrobial agents need to be discovered due to the existence and continuous evolution of resistant micro-organisms, the emergence of new infections diseases and the toxicity concerns of some of the currently used antimicrobial drugs. The indiscriminate use of conventional antibiotics and synthetic antimicrobial drugs has resulted in the emergence of resistance microbes such as Methicillin Resistance *Staphylococcus aureus* (MRSA). Vancomycin resistance enterococci and multidrug resistance strain of *Klebsiella pneumonia* and *Pseudomonas aeruginosa* which have become a cause of concern for scientists to find alternative way for treating such infection. In most of the Asian countries 90-95% of *S.aureus* are penicillin resistance and 75% are methicillin resistance (Bozin *et al.*, 2006; Hemaiswarya *et al.*, 2008). In addition, the high costs of the effective western medicines have also pushed people in the developing countries to turn to alternative therapy as source of drug for the management and treatment of diseases (Houghton *et al.*, 2005).

Rumex vesicarius L. is a wide edible plant used as a sorrel and collected in spring time and eaten fresh (Batanouny, 1999), or cooked (Al-Qura'n, 2009). *Rumex vesicarius* L. has many important medicinal uses such as treatment of tumors, hepatic diseases, bad digestion, constipation, calculus, heart troubles and 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. The roasted seeds were eaten for cure of dysentery. Finally, the plant can be used also to reduce biliary disorders and control cholesterol levels (Elegami *et al.*, 2001; Rahman *et al.*, 2004; Abutbul *et al.*, 2005; Prasuna *et al.*, 2009). The medicinal important of this plant is due to the presence of many bioactive substances such as flavonoids and anthraquinones. The plant also contains carotenoids, vitamins, proteins, lipids and organic acids. This plant is a good source of minerals (Saleh *et al.*, 1993; Al-Rumaih *et al.*, 2002). The intake of dietary phytochemicals will lead to protection against non communicable diseases and pathogenic bacteria in human beings, thus act as a good antioxidant and antibacterial agent.

Considering the potential ethanopharmacological promises of *Rumex vesicarius* L. this study was aimed to evaluate antibacterial activity and antioxidant properties of *Rumex vesicarius* L. Additionally, the total phenolic and flavonoid content that may be responsible for these biological activities have been determined.

MATERIALS AND METHODS

Plant material collection and authentication: The leaves and whole plant of *Rumex vesicarius* L. were collected from village khusnoor in Gulbarga district Karnataka. The

specimens of plant were authenticated by Dept. of Botany, Gulbarga University, Gulbarga. A voucher specimen was submitted at Dept. of Botany Gulbarga University Gulbarga for future with the reference No. HGUG- 5012.

Processing and extraction: The thoroughly washed leaves and whole plant of *Rumex vesicarius* L. were shade dried and pulverized and stored in an air tight container for future use. Extraction of phytoconstituents was done using two techniques:

- Hot Soxhlet extraction method and
- Cold Maceration (at room temperature)

The air dried and coarsely powdered leaves and whole plant of *Rumex vesicarius* L. were successively extracted by cold maceration process and hot soxhlet extraction process using organic solvents Petroleum Ether, Chloroform and Methanol. All the extracts were filtered and evaporated to dryness and stored for future use.

Determination of total phenolic content: Total phenolic content was estimated according to the method of Alfawaz (2006), Involving Folin Ciocalteu Reagent (FCR) and Gallic acid as standard. Concentration of phenolic compound was calculated according to the following equation that was obtained from the standard Gallic acid graph and was expressed in Gallic Acid Equivalent (GAE) of each gm extract of dry weight:

$$\text{Absorbance} = 0.002x + 0.004 \quad (R^2 = 0.994)$$

Determination of flavonoids: Flavonoids content of different methanol extract of *Rumex vesicarius* L. was determined by following the method of Filho *et al.* (2008). Concentration of flavonoid content was calculated according to the following equation obtained from standard rutin graph:

$$\text{Absorbance} = 0.001x - 0.004 \quad (R^2 = 0.993)$$

Antibacterial activity

Test micro-organisms: In order to determine the antibacterial activity of the different extracts of *Rumex vesicarius* L. various Gram +ve and Gram -ve bacteria were used. *Escherichia coli* (ATTC 25922), *Klebsiella pneumonia* (ATTC 70063), *Pseudomonas aeruginosa* (ATTC 27853), *Enterococcus faecalis* (ATTC 29212), *Staphylococcus aureus* (ATTC 29122).

Preparation and standardization of inoculums: Four to five colonies from pure growth of each test organism were transferred to 5 mL of Muller Hinton Broth (MHB). The broth was incubated at 35-37°C for 4 h. The turbidity of the culture was adjusted to match the turbidity standard of 0.5 Mc farland units prepared by mixing 0.5 mL of 1.75% (w/v) barium chloride dehydrate with 99.5 mL 1% (v/v) sulphuric acid. This turbidity was equivalent to approximately 1.2×10^8 CFU mL⁻¹ (Arya *et al.*, 2010).

Agar well diffusion method: The antibacterial bioassay was carried out following agar well diffusion method according to the standard method with slight modification (Cutler and Wilson, 2004). Briefly the bacterial inoculates were streaked on the surface of the MHA using sterile swab

stick, using different plates and different swab stick for different organisms. The seeded plates were allowed to dry at 37°C for 20 min. The wells were made on the agar plates using sterile cork borer. Fifty micro-liter of extracts at concentration 500 mg mL⁻¹ was introduced into the respective well on the agar plates. The inoculated plates were kept in the refrigerator for 1 h to allow the extracts to diffuse into the agar (Atata *et al.*, 2003). The MHA plates were incubated at 37°C for 24 h. Antimicrobial activity was determined by measuring the diameter of zones of inhibition (mm) produced after incubation. Ciprofloxacin (5 µg disc⁻¹) were used as positive control. All the experiments were done in triplicates. The relative inhibition of the test extract was calculated by using the equation (Islam *et al.*, 2011):

$$\frac{(x-y)}{(z-y)} \times 100$$

X = Total area of inhibition of test extract

Y = Total area of inhibition of solvent

Z = Total area of inhibition of standard

Total area of inhibition was calculated by using

$$\text{Area} = \pi r^2$$

r = Radius of zone of inhibition

Quantitative antibacterial evaluation: The Minimum Inhibitory Concentrations (MICs) and Minimum Bacterial Concentrations (MBCs) for the active plant extracts were determined using the standard modified method (Rios *et al.*, 1988). A Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antimicrobial that inhibits the growth of a microorganism after 18-24 h. The extracts that showed antibacterial activity were subjected to the serial broth dilution technique to determine their MIC. The minimum concentration of the extracts that showed no detectable growth was taken as the minimum inhibitory concentration. A Minimum Bactericidal Concentration (MBC) is the lowest concentration of an antibiotic required to kill a microorganism. The MBC was determined by sub-culturing 10 µL of the test dilutions from MIC tubes on to fresh Muller-Hinton agar plates. Plates were incubated for 18-24 h. The highest dilution that yielded no single bacterial colony on the plates was recorded as MBC.

MIC index: The MIC index (MBC/MIC) was calculated for each extract and standard control drug to determine whether an extract is bactericidal (MBC/MIC<4) or bacteriostatic (MBC/MIC>4) on growth of bacterial organisms (Kone *et al.*, 2004; Chattopadhyay *et al.*, 2007). Also, the range of MIC index values greater than 4 and less than 32 are considered as bacteriostatic (Cutler *et al.*, 1994).

Determination of antioxidant activity: Several methods have been developed to measure the efficiency of dietary antioxidants. These methods are based on different kinds of defense system. The antioxidants function either by scavenging various types of free radicals derived from oxidative process or by preventing free radical formation through reduction of precursors or by chelating metals. In the present study the several concentration of methanolic extract of WPH, WPC, LH and LC were tested for their antioxidant activity in different *in vitro* models.

DPPH radical-scavenging assay: 1,1 diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay for different methanol extract of *Rumex vesicarius* L. was determined as previously described by Bersuder *et al.* (1998). The scavenging of DPPH radical was calculated according to the following equation:

$$\text{DPPH radical-Scavenging activity (\%)} = [(Ac-As)/Ac] \times 100$$

Ac = Control absorbance

As = Sample Absorbance

Inhibition of lipid peroxidation: Lipid peroxidation inhibitory activity of extract and standard (ascorbic acid) were carried out according to the protocol described by Halliwell and Gutteridge, (1989).

Reducing power assay: A spectrophotometric method was used for the measurement of reducing power. The determination was carried out as per standard method described by Oktay *et al.* (2003).

Statistical analysis: Each experiment was performed in triplicates and mean values were calculated. The data recorded were Mean \pm Standard deviations. Analysis of variance for individual parameters was performed on the bases of mean value to determine the significance at $p < 0.05$.

RESULTS AND DISCUSSION

Extraction process: The *Rumex vesicarius* L. components were extracted by traditional methods using solvents with different polarities (petroleum ether, chloroform and methanol). The yields of the different solvent extracts obtained are presented in Table 1 which suggest that the extraction yields with methanol are in highest amount, where as lowest yields were obtained with petroleum ether in case of hot soxhlet extraction. In case of cold extraction the lowest yields is noted in chloroform and highest is in methanol. In fact this variation is attributed to the polarities of the different compounds present in the *Rumex vesicarius* L. and the methods of the extraction used. As per our earlier findings the methanol extract consists of the good bioactive components, we selected the methanol extract of WPH, WPC, LH and LC for further studies Londonkar and Tukappa (2013).

Determination of total phenols and flavonoid: The total phenolic content was estimated by using FCR method. The total phenolic content of the different extract of *R. vesicarius* was extract dependent and expressed as mg of Gallic Acid Equivalents (GAE). Table 2 Summarizes that total phenolic content in different extract did not showed much variation and ranges from 48.66 \pm 1.247 and 52.5 \pm 1.471 mg g⁻¹ GAE. The WPH exhibited highest total phenolic content (52.5 \pm 1.471 mg g⁻¹ GAE). The flavonoid content is expressed as rutin equivalents and varied from 131.33 \pm 0.471 and 252.66 \pm 2.494 μ g rutin equivalent g⁻¹ extract (Table 2). This result is in the agreement with the previously obtained by Saeed *et al.* (2012) who reported that methanol as suitable solvent for extraction of phenolic compound from *Torillis leptophylla* L.

Antibacterial activity: Antibacterial activity of *R. vesicarius* was evaluated against a set of clinically significant bacterial strains, including gram-ve (*Pseudomonas aeruginosa*,

Table 1: Percent yield of different extract of *Rumex vesicarius* L

Solvents	WPH	WPC	LH	LC
Petroleum ether	2.35	0.65	3.40	1.0
Chloroform	1.60	1.80	3.00	3.0
Methanol	11.9	2.90	8.13	3.6

WPH: Whole plant hot, WPC: Whole plant cold, LH: Leaf hot, LC: Leaf cold

Table 2: Total phenolics (mg GAE g⁻¹), Total flavonoids (µg rutin g⁻¹)

	Total phenolic	Flavonoids
WPH	52.50±1.471	252.66±2.494
WPC	52.10±0.470	173.66±1.247
LH	51.33±1.247	188.6±2.0540
LC	48.66±1.247	131.33±0.471

WPH: Whole plant hot, WPC: Whole plant cold, LH: Leaf hot, LC: Leaf cold

Table 3: Antibacterial activity (zone of inhibition, MIC and MIB) of different extract of *Rumex vesicarius* L.

WPH			WPC			LH			LC		
Zone (mm)	MIC (mg mL ⁻¹)	MIB (mg mL ⁻¹)	Zone (mm)	MIC (mg mL ⁻¹)	MIB (mg mL ⁻¹)	Zone (mm)	MIC (mg mL ⁻¹)	MIB (mg mL ⁻¹)	Zone (mm)	MIC (mg mL ⁻¹)	MIB (mg mL ⁻¹)
11.66±0.41	125	62.5	7±0.0	125	250	6.66±0.41	31.25	31.25	10.333±0.932	125	250
8±0.00	250	500	2.33±0.471	125	250	12.33±0.471	62.5	62.5	2±0.00	250	500
5±0.816	250	250	3.66±0.471	250	500	6.66±1.247	250	250	3±0.816	250	500
6.33±0.942	125	250	6±0.00	125	125	5.66±0.471	62.5	62.5	1.33±0.471	125	250
9.33±0.577	125	62.5	5.66±0.57	125	250	11.75±0.404	250	125	4.66±0.404	250	500

¹*Pseudomonas aeruginosa* (ATTC 27853), ²*Escherichia coli* (ATTC 25922), ³*Klebsiella pneumonia* (ATTC 70063), ⁴*Enterococcus faecalis* (ATTC 29212), ⁵*Staphylococcus aureus* (ATTC 29122). WPH: Whole plant hot, WPC: Whole plant cold, LH: Leaf hot, LC: Leaf cold

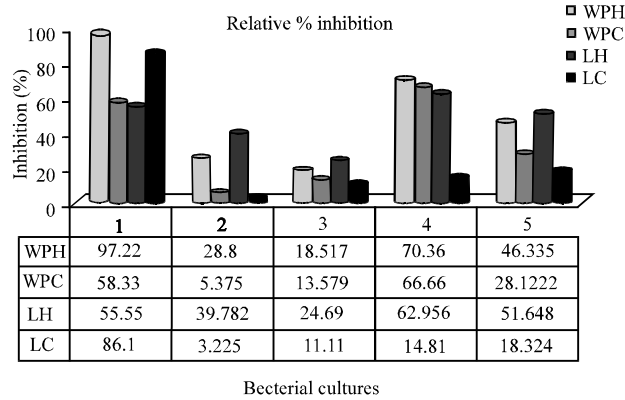


Fig. 1: Antibacterial activity (relative% inhibition) of different extract of *Rumex vesicarius* L., ¹*Pseudomonas aeruginosa* (ATTC 27853), ²*Escherichia coli* (ATTC 25922), ³*Klebsiella pneumonia* (ATTC 70063), ⁴*Enterococcus faecalis* (ATTC 29212) and ⁵*Staphylococcus aureus* (ATTC 29122). WPH: Whole plant hot, WPC: Whole plant cold, LH: Leaf hot, LC: Leaf cold

Escherichia coli and *Klebsiella pneumonia*), gram +ve (*Enterococcus faecalis*, *Staphylococcus aureus*). Potency was assessed by measuring the inhibition zones, relative percent inhibition, MIC, MIB values and the results are reported in Table 3, Fig. 1. From the data it is

evident that the different extract of *Rumex vesicarius* displayed a broad spectrum and variable degree of antibacterial activity against the tested strains. Among all the extract of *R. vesicarius*, WPH and LH extract demonstrated significant activity against *Pseudomonas aeruginosa* (11.66 ± 0.41 and 6.66 ± 0.41) and *Staphylococcus aureus* (9.33 ± 0.577 and 11.750 ± 0.404), respectively. The LH extract is also effective against *E.coli* (12.33 ± 0.471) where as the LC extract showed the inhibition zone of 10.33 ± 0.932 against *Pseudomonas aeruginosa*. However all the extracts of *Rumex vesicarius* L. (WPH, WHC, LH, LC) also showed the activity against *Klebsiella pneumonia* (5 ± 0.816 , 3.66 ± 0.471 , 6.66 ± 1.247 , 3 ± 0.816 , respectively) and *Enterococcus faecalis* (6.33 ± 0.942 , 6.00 ± 0.00 , 5.66 ± 0.47 , 1.33 ± 0.47) (Table 3). The results of antibacterial activity of methanolic extract of WPH, WPC, LH and LC of *R. vesicarius* were compared with positive control (ciprofloxacin) for evaluating their relative percentage inhibition (Fig.1). The maximum percentage inhibition was found with WPH against *P. aeruginosa* (97.22%) which is followed by *E. faecalis* (70%), *S. aureus* (46.335%), *E.coli* (25.8%) and *K. pneumonia* (18.517%) compared to other extract. However from the Table 3 it is evident that the *P. aeruginosa* is more sensitive towards the methanolic extract of *Rumex vesicarius* L. compared to other organisms and also the WPH and LH extract showed good antibacterial activity compared to WPC and LC. The results of MIC are reported in Table 3. The highest MIC value is 250 mg mL^{-1} and the lowest MIC value is found to be 31.25 mg mL^{-1} . The MIC index calculated for each extract shows that $\text{MBC/MIC} < 4$ thus all the extract of *R. vesicarius* L. is bactericidal.

These results of antibacterial activity studies were parallel to findings of Panduraju *et al.* (2009) who found that, methanol, petroleum ether, aqueous extract of *R.vesicarius* L. leaves have variable effects against both gram+ ve and gram- ve bacteria. Several previous experiments on different plant parts of different species of *Rumex* confirm that they were potent antibacterial against both gram+ ve and -ve bacteria (Nishina *et al.*, 1993; Yildirim *et al.*, 2001; AlZoreky and Nakahara, 2002; Harshaw *et al.*, 2010).

Antioxidant activity: Figure 2 shows the DPPH radical scavenging activity of WPH, WPC, LH, LC of *Rumex vesicarius* L. at different concentration. It can be deduced from this figure, that the scavenging activity of all the extract was concentration dependent. The results indicated clearly that the WPH and LH which contains the highest amount of total phenolics showed high antioxidant activity 75.623 and 75.123% at a final concentration of $100 \mu\text{g mL}^{-1}$. At the same concentration 81.84% of radical activity was observed with the positive control. The WPC and LC extract showed the activity of 47.761 and 58.45%, respectively. The DPPH scavenging activity of different extract of *Rumex vesicarius* L. revealed that the least IC₅₀ “the highest the effectiveness” was obtained using LH ($43.37 \mu\text{g mL}^{-1}$) followed by WPH ($45.59 \mu\text{g mL}^{-1}$) and LC ($55.98 \mu\text{g mL}^{-1}$).

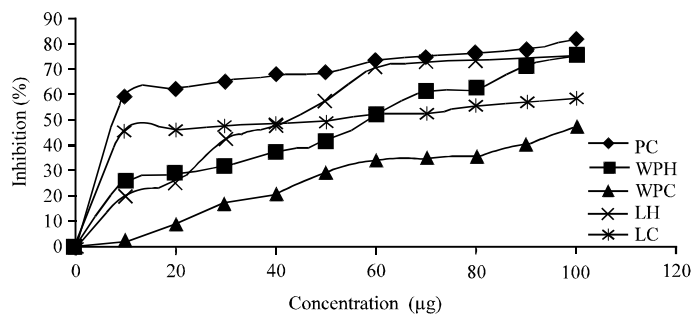


Fig. 2: DPPH scavenging activity of different extract of *Rumex vesicarius* L. PC: Positive control, WPH: Whole plant hot, WPC: Whole plant cold, LH: Leaf hot, LC: Leaf cold

While the least effective extract used was WPC (Fig. 3.). The +ve control in these experiment were ascorbic acid. It was found that ascorbic acid ($21.57 \mu\text{g mL}^{-1}$) is a potent antioxidant agent.

Lipid peroxidation inhibition assay of the methanolic extracts of WPH, WPC, LH and LC of *Rumex vesicarius* L. was compared with standard (Fig. 4). The methanolic extract of WPH and LH had higher activity than that of the other extracts. At a concentration of $100 \mu\text{g mL}^{-1}$ the scavenging activity of the WPH, WPC, LH and LC reached 72.15, 71.022, 72.15 and 26.704% while at the same concentration, that of the standard was 69.31%. The IC₅₀ of methanolic extracts of WPH, WPC, LH and LC were 54.07, 67.73 and 60.273 and $165.394 \mu\text{g mL}^{-1}$, respectively. The IC₅₀ of standard was $36.477 \mu\text{g mL}^{-1}$ (Fig. 5).

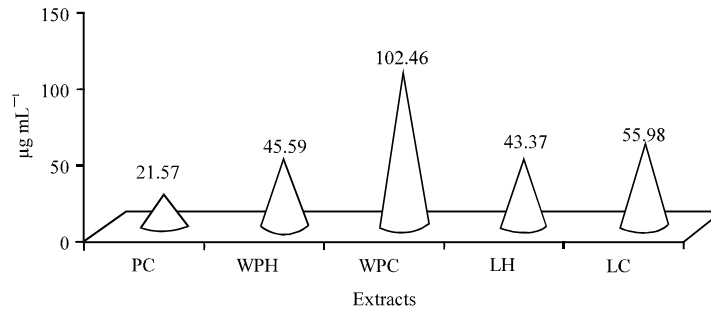


Fig. 3: DPPH scavenging activity (IC₅₀) of different extract of *Rumex vesicarius* L. PC: Positive control, WPH: Whole plant hot, WPC: Whole plant cold, LH: Leaf hot, LC: Leaf cold

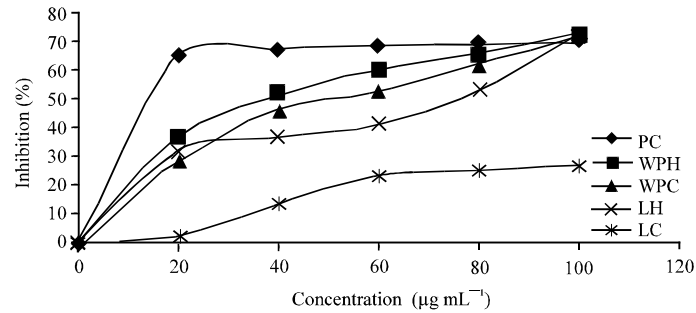


Fig. 4: Inhibition of lipid peroxidation of different extract of *Rumex vesicarius* L. PC: Positive control, WPH: Whole plant hot, WPC: Whole plant cold, LH: Leaf hot, LC: Leaf cold.

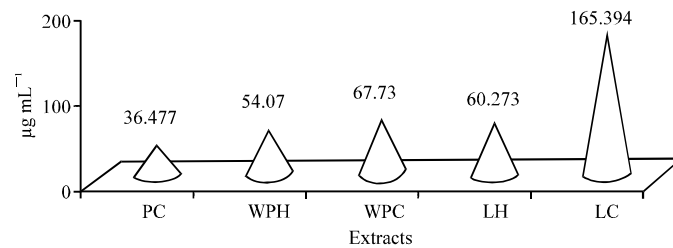


Fig. 5: IC₅₀ value of inhibition of lipid peroxidation of different extract of *Rumex vesicarius* L. PC: Positive control(Ascorbic acid), WPH: Whole plant hot, WPC: Whole plant cold, LH: Leaf hot, LC: leaf cold

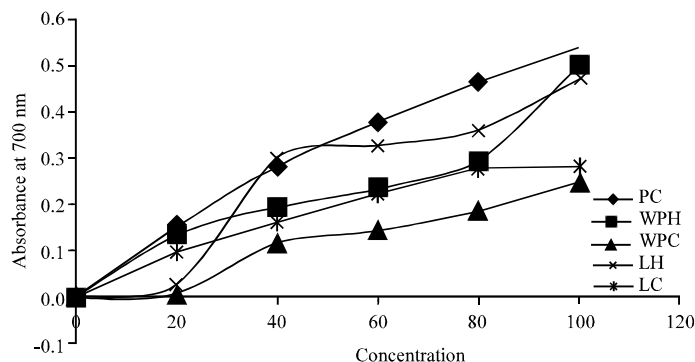


Fig. 6: Reducing power activity of different extract *Rumex vesicarius* L. PC: Positive control, WPH: Whole plant hot, WPC: Whole plant cold, LH: Leaf hot, LC: Leaf cold

The reducing power assay of various extracts of *R. vesicarius* may serve as a significant index of their potential antioxidant activity, Fig. 6 revealed that the reducing power activity of four extracts at various concentration. It can be observed that their reducing power increased with the increasing concentration of each extract. Among all the extract the strongest activity was exhibited by WPH extract with the absorbance value of 0.499 ± 0.003 . The LH, WPC and LC showed the activity with absorbance value of 0.464 ± 0.007 , 0.244 ± 0.002 and 0.281 ± 0.003 , respectively.

The present antioxidant activity results of *Rumex vesicarius* L. were in the agreement with Nishina *et al.* (1991), Demirezer *et al.* (2001), Al-Ismael *et al.* (2006), Ozen (2010) and Li and Liu (2009), since they investigated different extracts of leaves of *R. pulcher* and *R. acetosella* and whole plant parts of *R. dentatus*, respectively. Their results revealed that these species were considered as antioxidant agents. 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. The results of antioxidant activity, total phenolics and flavonoid of *R. vesicarius* agreed also with results of Tavares *et al.* (2010), since they found that flavonoids and polyphenolics in *R. moderensis* were strongly associated with antioxidant capacity.

CONCLUSION

The results reported in present studies provide an insight that the process of extraction has a significant effect on the pharmacological activity. The obtained results suggest that hot soxhlet extraction found to be more effective. The results also indicate that *Rumex vesicarius* L. have significant antimicrobial and antioxidant activity. These potential pharmacological activities of the extracts studies here could be attributed to phenols and flavonoids. As emphasized in several reports, the key role of the phenolic compounds is as a potent bioactive compound, we progress on to isolate the active bio-component responsible for the pharmacological activity.

ACKNOWLEDGMENTS

Author is grateful to UGC-RGNF, New Delhi, for the Junior research Fellowship. We also thank Dept. of botany, Gulbarga University, Gulbarga for identification and authentication of the plant.

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