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AN ANTIOXIDANT POTENTIAL OF HYDRO-METHANOLIC EXTRACT OF *URTICA PARVIFLORA* ROXB.

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ABSTRACT: Antioxidant activity of hydromethanolic extract of *Urtica parviflora* Roxb. (family Urticaceae) was investigated by different *in vitro* methods, namely, nitric oxide scavenging, DPPH scavenging, and reducing power assay. In the present study, plant extract exhibited dose dependent free radical scavenging and reducing activity. The antioxidant activity of the hydromethanolic extract of *Urtica parviflora* Roxb. was compared with ascorbic acid as standard. In addition, phytochemical screening of hydromethanolic extract of the plant was undertaken to identify the phytochemicals present in the extract. Phytochemical examination revealed the presence of alkaloids, polysaccharides, saponins, flavonoids, phenolic compounds, glycosides and tannins. It was concluded that the extract contains important phytoconstituents responsible for antioxidant effect. The study indicated that *Urtica parviflora* could protect the cell injury caused by the reactive oxygen species and might be a valuable source of antioxidant both for medicine and food industry.

KEYWORDS: *Urtica parviflora*, flavonoids, alkaloids, DPPH scavenging, reducing power

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INTRODUCTION

Free radicals have aroused significant interest among scientists in the past decades because of their broad range of effects on biological systems. Reactive oxygen species (ROS) like superoxide anion radical, hydroxyl radical, hydrogen peroxide and nitric oxide are continuously formed inside the body. However, normally a balance between oxidative events and antioxidative forces maintains the status quo within living cells. When normal balance is upset, either by loss of reducing agents or protective enzymes or by both events simultaneously, the tissue is considered to be under oxidative stress. It can then cause oxidative damage of all major groups of biomolecules (DNA, proteins, lipids and small cellular molecules) leading to pathogenesis of various diseases like cancer, emphysema, cirrhosis, atherosclerosis, arthritis cardiovascular diseases, diabetes, asthma,

hepatitis, liver injury, immune deficiency diseases, neurodegenerative diseases and aging [1-4].

Due to the above-presented pathological implications of ROS, it is important to find an antioxidant, which may scavenge multiple ROS so that it can be used in multiple disease states and also to maintain a healthy states. Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are extensively used in food industries as antioxidants. However, the possible toxicity as well as general consumer rejection led to decreasing use of these synthetic antioxidants [5].

Flavonoids and other phenolic compounds of plant origin have been reported as scavengers of free radicals [6]. Nowadays search for natural antioxidant source is gaining much importance. Therefore, attempt has been made to evaluate antioxidant potential of *Urtica parviflora* Roxb. in the present study. *Urtica parviflora* Roxb. belongs to the family Urticaceae and is widely distributed throughout the India, especially in Himalaya (lower altitude) from

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Kashmir to Sikkim, in Darjeeling, West Bengal, Mishmi hills in Arunachal Pradesh and Nilgiri hills in the south [7]. It is commonly known as Himalayan stinging nettle and locally as 'Shishoon' in Kumaun and 'Kaldiya' or 'Kandali' in Garhwal. Its leaves and stems produce inflammatory rash, accompanied by a considerable burning and itching sensation attributed to the presence of histamine and 5-hydroxytryptamine [8]. The roots are employed for the treatment of fractures of bone and dislocations of joints [7]. The leaves are used in dysentery, joint pain and liver disorders [9]. The inflorescence are used as cleansing agent after parturition and in the treatment of dermatitis in alpine region of central and eastern Himalayas [7].

EXPERIMENTAL

Plant material

The plant material was collected from local surroundings of Bhimtal (Nainital), India in the month of September 2009 and was identified from Botanical Survey of India, Dehradun. A voucher specimen (No.112286) has been kept in Department of Pharmaceutical Sciences, Bhimtal Campus, Kumaun University, Nainital.

Preparation of extract

The air dried leaves of *Urtica parviflora* (20 g) were extracted with 100 ml of solvent (methanol : water, 4:1). The resultant extract was concentrated under reduced pressure to yield a green residue.

Drugs and Chemicals

Potassium ferricyanide, sodium nitroprusside, ferric chloride and trichloroacetic acid. Sulfanilamide, naphthylethylene diamine hydrochloride, orthophosphoric acid and 1,1-diphenyl 2-picryl hydrazyl (DPPH) were obtained from Sigma Chemicals, USA. Ascorbic acid was procured from Ranbaxy, India. All other unlabelled chemicals and reagents were of analytical grade and were used without further purification.

Phytochemical Screening

The hydromethanolic extract was qualitatively tested for the presence of chemical constituents. Phytochemical screening was performed using following reagents and chemicals. Alkaloids with Dragendorff's reagent; phenolic compounds with FeCl_3 ; glycosides with glacial acetic acid, FeCl_3

and H_2SO_4 ; flavonoids with magnesium chip and HCl; tannins with lead acetate and 5% ferric chloride; polysaccharides with iodine test; triterpenoids with Liebermann-Burchardt's test and saponins with ability to produce suds. These were identified by characteristic color changes using standard procedures [10].

In vitro screening for antioxidant activity

DPPH radical scavenging assay

DPPH (1,1-diphenyl-2-picryl hydrazyl) scavenging activity was measured by spectrophotometric method. 2.95 ml of methanolic solution of DPPH (100 μM) was added to 0.05 ml of different concentrations (10-640 $\mu\text{g}/\text{ml}$) of hydromethanolic extract of *Urtica parviflora* dissolved in dimethylsulfoxide (DMSO). Equal amount of DMSO was added to the control. Absorbance was recorded at 517 nm after 20 min [11]. Ascorbic acid was used as a standard and all the assays were carried out in duplicate. The purple colour of DPPH changes to yellow, based on the efficacy of antioxidants. Radical scavenging activity was expressed as percent inhibition and was calculated using the following formula.

$$\text{Percentage inhibition (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100.$$

where, A_{control} is the absorbance of control reaction (containing all reagents except test compound), and A_{sample} is the absorbance of test compound. IC_{50} values (concentration of sample required to scavenge 50% of free radicals) were calculated from the regression equation, prepared from the concentration of the samples and percentage inhibition of free radical formation.

Nitric Oxide scavenging assay

Sodium nitroprusside (5 mM) in phosphate buffer saline was mixed with different concentrations of hydromethanolic extract (10-640 $\mu\text{g}/\text{ml}$) dissolved in DMSO and incubated at 25°C for 30 min. After 30 min, 1.5 ml of incubated solution was removed and diluted with 1.5 ml of Griess reagent (1% sulphanilamide, 2% orthophosphoric acid and 0.1% naphthylethylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotisation of the nitrite with sulphanilamide and subsequent coupling with naphthylethylene diamine was measured at 546 nm along with a control [12]. The percentage inhibition of nitric oxide generated was measured by

comparing the absorbance values of control and test samples using following formula.

$$\text{Percentage inhibition (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100.$$

where, A_{control} is the absorbance of the control reaction (containing all reagents except test compound), and A_{sample} is the absorbance of test compound. IC_{50} values (concentration of sample required to scavenge 50% of free radicals) were calculated from the regression equation, prepared from the concentration of the samples and percentage inhibition of free radical formation. Ascorbic acid was used as positive control and all tests were carried out in duplicate.

Reducing power

Reductive ability of the extract was measured according to the method of Oyaizu [13]. Different concentrations (10-640 $\mu\text{g/ml}$) of extract were mixed with 2.5 ml of sodium phosphate buffer (pH 6.6) and 2.5 ml of potassium ferricyanide (1%). The mixture was incubated at 50°C for 20 min. Trichloroacetic acid (2.5 ml of 10%) was added to it, the mixture was mixed and centrifuged at 650 rpm for 10 min. The upper layer (5 ml) was mixed with 5 ml of deionised water and 1 ml of ferric chloride (1%) and absorbance was measured at 700 nm. Control reaction contains all the reagents except test compound. Higher absorbance indicated higher reducing power.

RESULTS

Phytochemical screening

The yield of residue obtained after extraction was found to be 8.9% w/w. Phytochemical screen-

ing revealed the presence of alkaloids, polysaccharides, saponins, flavonoids, tannins, phenolic compounds and glycosides in the *Urtica* leaves (Table 1).

DPPH radical scavenging assay

Free radical scavenging potential of extract at different concentrations was tested by DPPH method and the results are depicted in Figure 1. The results showed that the hydromethanolic extract of *U. parviflora* has reduced the free radical (1,1-diphenyl-2-picrylhydrazyl) to corresponding hydrazine in a concentration dependent manner. IC_{50} values were found to be 808 $\mu\text{g/ml}$ for hydromethanolic extract and 22.43 $\mu\text{g/ml}$ for ascorbic acid. The results thus demonstrated good free radical scavenging activity of the extract.

Nitric oxide scavenging effect

The hydromethanolic extract of *U. parviflora* effectively reduced the generation of nitric oxide radicals from sodium nitroprusside solution in a concentration dependent manner (Figure 2). This showed significant nitric oxide scavenging activity of the extract ($IC_{50} = 519.15 \mu\text{g/ml}$). IC_{50} of ascorbic acid was found to be $30 \pm 1.82 \mu\text{g/ml}$.

Reducing Power

The extract exhibited concentration dependent increase in absorbance. Absorbance indicated by all the concentrations of extract was significantly higher than the absorbance of control reaction (0.031). Higher absorbance indicates high reducing power due to formation of reduced intermediate.

Table 1: Phytochemical screening of hydromethanolic extract of leaves of *U. parviflora*

Chemical Constituents	Test	
Alkaloids	Dragendroff's reagent	++
Saponins	Foam test	++
Flavonoids	Magnesium and HCl	++
Phenolic compounds	FeCl_3 test	++
Glycosides	Test with glacial acetic acid, FeCl_3 , H_2SO_4 dropwise	++
Tannins	FeCl_3 Lead acetate	++
Steroids/triterpenoids	Liebermann-Burchardt's test	-

++ = copiously present; - = absent.

Figure 1: Effect of different concentrations of hydromethanolic extract of *U. parviflora* on DPPH scavenging assay. Values are average of duplicate experiments and represented as mean \pm standard error mean

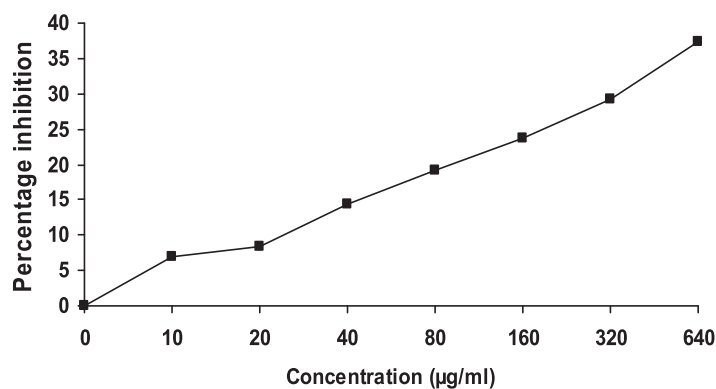


Figure 2: Effect of different concentrations of hydromethanolic extract of *U. parviflora* on nitric oxide scavenging assay. Values are average of duplicate experiments and represented as mean \pm standard error mean

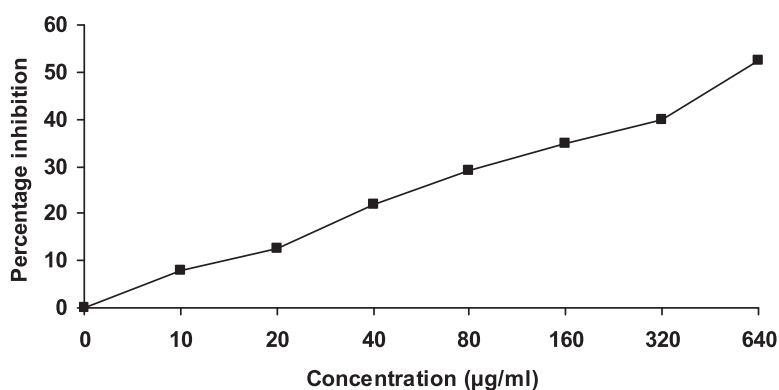


Table 2 shows the reductive capability of the plant extract compared to ascorbic acid. Ascorbic acid has much higher reducing ability than the hydromethanolic extract.

DISCUSSION

In the present study we investigated the antioxidant activity of the hydromethanolic extract of *Urtica parviflora* in some *in vitro* antioxidant models. In all the models, extract showed its ability to scavenge the free radicals in a concentration dependent manner.

The free radical scavenging activity was studied by its ability to reduce the stable radical DPPH. Antioxidants react with DPPH, a nitrogen-centered radical due to their hydrogen donating ability and convert it to 1,1-diphenyl-2-picryl hydrazine [14].

The degree of discoloration indicates the scavenging potential of the antioxidant. From the DPPH assay it may be postulated that the extract reduces the radical to the corresponding hydrazine and this scavenging ability of the extract may be attributed to its hydrogen donating ability.

Nitric oxide is a potent pleiotropic mediator of physiological processes as well as it is involved in pathogenesis of pain and inflammation. Our study demonstrated a potent nitric oxide scavenging activity of *U. parviflora* extract and offers a scientific evidence that the plant can be used in inflammatory conditions.

The reducing ability of a compound generally depends on the presence of reductants [15], which exhibit antioxidative potential by breaking the free radical chain, donating a hydrogen atom [16]. The

Table 2: Reducing ability of different concentrations of hydromethanolic extract of *U. parviflora*. Values are average of duplicate experiments. Ascorbic acid was taken as standard.

Concentration (µg/ml)	Absorbance at 700 nm	
	Ascorbic acid	<i>U. parviflora</i> extract
10	0.059	0.042
20	0.099	0.052
40	0.252	0.059
80	0.457	0.062
160	0.875	0.078
320	1.668	0.118
640	2.648	0.215

presence of reductants (i.e. antioxidants) in *U. parviflora* extract might have caused the reduction of Fe^{3+} /ferricyanide complex to the ferrous form (Fe^{2+}) which was monitored by measuring the formation of Perl's Prussian blue at 700 nm. However the reducing power of extract is much less compared to ascorbic acid.

Results of phytochemical screening revealed the presence of chemical constituents like alkaloids, polysaccharides, saponins, flavonoids, glycosides and tannins in large amount in hydromethanolic extract. Antioxidant activity of *U. parviflora* thus may be contributed to the presence of flavonoids, phenolic compounds, alkaloids and glycosides as they possess significant antioxidant activity [17-20]. This *in vitro* antioxidant activity of the extract is further supported by other workers who reported that the extract of *U. parviflora* significantly protected the experimental animal against CCl_4 -induced hepatotoxicity [21].

CONCLUSION

Overall, it could be concluded that *U. parviflora* leaves bear a potent antioxidant activity as their constituents scavenge free radicals and have reducing activities. The phenolic compounds, flavonoids and alkaloids present in the extract may be responsible for antioxidant activity. Thus it can be inferred that *U. parviflora* extract, owing to its free radical scavenging ability can be used as a source of natural antioxidants with potential application to reduce oxidative stress with health benefits. Further investigations are necessary for encompassing *in-vivo* antioxidant activity of the studied plant.

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