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Original Research Article

ANTIOXIDANT POTENTIAL AND TOTAL PHENOLIC CONTENT OF *URTICA DIOICA* (WHOLE PLANT)

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ABSTRACT

Whole plant of *Urtica dioica* Linn. (Urticaceae) were subjected to extraction with different solvent according to polarity and further to obtain antioxidant rich extract. Different concentrations of different solvent extracts were subjected to antioxidant assay by DPPH, Nitric oxide NO scavenging method and Total phenolic contents. The IC_{50} values for different solvent extracts (Petroleum ether, ethyl acetate, n-butanol, ethanol) of *Urtica dioica* Linn.(UD) were found to be 215.96 ± 0.066, 78.99 ± 0.171, 168.24 ± 0.346 and 302.90 ± 0.141 respectively in comparison to L-Ascorbic acid as standard with IC_{50} values of 26.24 ± 0.193 respectively in DPPH model. In nitric oxide radical scavenging activity the IC_{50} values were found to be 172.38 ± 0.635, 101.39 ± 0.306, 141.23 ± 0.809, 202.26 ± 0.67 and 55.38 ± 0.56 for different extracts and L-Ascorbic acid respectively. The highest Total phenolic content was found to be 13.06 ± 0.15 mg GAE/g in ethyl acetate extract. However, the ethyl acetate extract showed a better free radical scavenging activity as compared to other extracts.

Keywords: *Urtica dioica* Linn.(UD), DPPH assay, NO assay, Total phenolic content.

RUNNING TITLE: Antioxidant potential of *Urtica dioica* Linn. (whole plant).

INTRODUCTION

The free radicals usually act by attacking the unsaturated fatty acid in the biological membranes which extend to membrane lipid peroxidation, decrease in membrane fluidity, and reduction of antioxidant defense enzymes, receptor activity and damage to membrane protein. These destructive processes finally triggers the cell inactivation or death [1,2]. The antioxidants generally scavenge the free radicals and detoxify the physiological system. The free radicals are generated in normal metabolic function and also can be acquired from the environment. Free radicals can be oxygen radicals, such as hydroxyl radical, superoxide radical and non free radical species, such as singlet oxygen, hydrogen peroxide and are generated in various redox processes [3]. The endogenous antioxidant like superoxide dismutase, catalase and glutathione peroxidase etc. are the enzymes of antioxidant defense system which trap and destroy these free radicals [2]. The excessive production of free radicals, a decreased level of antioxidant defense enzymes and increased lipid peroxidation are responsible for producing oxidative stress and linked with various pathological conditions [4]. The antioxidant defences that shield against oxidative damage and repair enzymes to remove or repair damaged molecules. However, the natural antioxidant mechanisms can be inefficient hence, consumption of dietary antioxidant compounds is important [5,6,7]. The synthetic antioxidants like butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tertiary butyl hydroquinone (TBHQ) have been used in food processing; have shown formation of possible toxic and carcinogenic components during degradation [8]. Dietary intake of antioxidant rich foods are related in preventing a number of human diseases [9,10].

Urtica dioica Linn. (Urticaceae) is a perennial plant which is commonly known as Vrishchhiyaa-shaaka in Sanskrit, Bichu Butti in Hindi, and Shisuun in folk language. The plant is available in many South Asian countries and Indian subcontinent. It has been known in the world as a medicinal herb for a long time. The plant is used traditionally as diuretic, stomachache, emmenagogue, blood purifier, anthelminthic, rheumatic pain and for colds and cough. It is also used in nephritis, haematuria, jaundice and menorrhagia. The plant has been reported to contain lectins, linolenic acid, lutein, lutein isomers, b-carotene and b-carotene isomers, neoxanthin, violaxanthin and lycopene. In the studies plant reported to have antidiabetic, hepatoprotective, antiinflammatory, antihypertensive

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activity, diuretic and natriuretic effects [11]. The present study was designed to examine the antioxidant activities of different extract of *Urtica dioica* Linn. (UD) and to check out scientifically it's uses in folk medicine.

MATERIAL AND METHODS

Plant Materials

Whole plant of *Urtica dioica* Linn. (UD) were collected from Ranikhet hills of Almora district of Uttarakhand, India and authenticated from NISCAIR, New Delhi, India (Ref. No, NISCAIR/RHMD/Consult/-2008-09/1192/224 dated 09-04-2009). Plant drug was shade dried (<40° C), coarsely powdered and stored in air tight container.

Chemicals and Reagents

All solvents used were of analytical grade and purchased from Rankem, Jalandhar. DPPH (1, 1-Diphenyl-2-picrylhydrazyl) was procured from Sigma -Aldrich Chemical Co. L- Ascorbic acid, Tri-chloroacetic acid (TCA) and ferric chloride were procured from central drug house (CDH), New Delhi. Sulphanilic acid and napthylelene diamine dichloride from Rankem, Sodium nitroprusside and potassium ferricyanide were obtained from nice chemicals.

Extraction of plant material

Successive solvent extraction scheme was used for the preparation of different extracts. The plant was crushed to coarse powder and extracted with Petroleum ether (40-60ºC) using Soxhlet's apparatus. The extract was concentrated with rota-evaporator and transferred to a pre-weighed china dish and dried in a vacuum dessicator. The marc obtained was then air dried and used for further extraction with ethyl acetate followed by n-butanol and ethanol. The dried extracts were placed in desiccators for further studies.

Phytochemical screening

Petroleum ether, ethyl acetate, n-butanol and ethanolic extracts were subjected to phytochemical analysis for checking the presence of carbohydrates, alkaloids, glycosides, proteins, saponins, phenolic compounds, flavonoids, steroids and triterpenoids[12] etc.

In-vitro antioxidant assay

All the extracts of *Urtica dioica* Linn. (UD) were tested for their free radical scavenging property using different *in-vitro* models. All experiments were performed thrice. The Ascorbic acid was used as standard control in each experiment model.

DPPH radical scavenging activity [13-15]

The free radical scavenging activity of different extracts of *Urtica dioica* Linn.(UD) was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH•) using the method of (Shimada et al. (1992). The 0.1 mM solution of DPPH in methanol was freshly prepared. Different concentrations of standards (1-5 µg/ml) and extract (50-250 µg/ml) were added at an equal volume to methanolic solution of DPPH. After 30 min at room temperature, the absorbance was recorded at 517 nm spectrophotometer (UV-1601 Shimadzu, Japan).Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The percent DPPH scavenging effect was calculated using the following formula.

(%) DPPH· scavenging inhibition = $[(A_0 – A_t / A_0)] \times 100$

Where A_0 was the absorbance of the control reaction and A_t was the absorbance in the presence of the standard sample or extract. All the tests were performed in triplicate and graph was plotted with the mean ± SD values.

Nitric oxide radical scavenging activity [16,17]

Nitric oxide radical scavenging activity was performed according to the method of Garrat. 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract at various concentrations (50-250 µg/ml) and the mixture incubated at 25°C for 150 min from the incubated mixture 0.5 ml was taken out and added into 1.0 ml sulfanilic acid reagent and incubated at room temperature for 5 min. finally, 1.0 ml naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min before measuring the absorbance at 546 nm. The nitric oxide radicals scavenging effect was calculated using the following formula.

(%) Nitric oxide radical scavenging inhibition = $[(A_0 – A_t / A_0)] \times 100$

Where A_0 was the absorbance of the control reaction and A_t was the absorbance in the presence of the standard sample or extract. All the tests were performed in triplicate and graph was plotted with the mean ± SD values.

Determination of total phenolic content [18,19]

The total phenolic content of the all extracts of *Urtica dioica* linn. (UD) were measured by the method of Singleton et al., (1999). To 0.5 ml of test sample, 1.5 ml (1:10 v/v diluted with distilled water) Folin Ciocalteau reagent was added and allowed to stand for 5 min at 22ºC. After 5 min, 2.0 ml of 7.5% of sodium carbonate (Na_2Co_3) was added. These mixtures were incubated for 90 min in the dark with intermittent shaking. After incubation development of blue colour was observed. Finally absorbances of blue colour in different samples were measured at 765 nm using UV/VIS spectrophotometer (Schimadzu, Japan) against blank, i.e., distilled water. The phenolic content was calculated as gallic acid equivalents GAE/g on the basis of standard curve of gallic acid. The results were expressed as Gallic acid equivalents (GAE)/g of the plant material.

Total phenolic content were expressed percentage (w/w) and calculated using following formula. Total phenolic content (% w/w) = $GAE \times V \times D \times 10^{-6} \times 100/W$

GAE-Gallic acid equivalent (µg/ml), V-Total volume of sample (ml), D-Dilution factor,

W-Sample weight (gm)

Statistical analysis

All analysis was done in triplicate and results are expressed as ± mean S.D.

RESULTS

Phytochemical screening

Phytochemical screening of different plant extracts are shown in Table 1

Table 1: Phytochemical screening of different plant extracts of *Urtica dioica* Linn.(UD)

Class of compound	EE	PEE	EAE	NBE
Carbohydrates	+	-	-	-
Glycosides	+	-	-	+
Proteins	-	-	-	-
Steroids and triterpenoids	+	+	+	+
Phenolic compounds	+	-	+	+
Flavonoids	+	-	+	-
Amino acids	+	-	-	-
Alkaloids	-	-	-	-
Saponins	+	-	-	-

(+) Present, (-) Absent (EE-ethanolic extract, PEE- petroleum ether extract, EAE- ethyl acetate extract, NBE-n-butanol extract)

Antioxidant assay

The several concentrations of different extracts of *Urtica dioica* Linn.(UD) ranging from (50-250 µg/ml) was tested for their antioxidant activity in different *in-vitro* models. It has been observed that ethyl acetate extract exerted maximum antioxidant potential and its antioxidant activity is comparable to the standards L-Ascorbic acid. The percentage inhibition of different extract in various models viz. DPPH and nitric oxide scavenging assay are shown in (figure 1, 2). The IC_{50} value of standard and different extracts in the DPPH and NO scavenging assay are shown in Table no. 2, 3.

Table 2: IC_{50} values of free radical scavenging effect by DPPH method of various *Urtica dioica* Linn. (UD) extracts and standard

S. No.	Standard / extracts	IC_{50}(µg/ml) ± S.D.
1	Ascorbic Acid	26.24 ± 0.193
2	Pet ether UD extract	215.96 ± 0.066
3	Ethyl acetate UD extract	78.99 ± 0.171
4	n-butanol UD extract	168.24 ± 0.346
5	Ethanolic UD extract	302.90 ± 0.141

(Values are mean ± S.D of 3 replicates)

Table 3: IC_{50} values of Nitric oxide scavenging activity of various *Urtica dioica* Linn. (UD) extracts and standard

S. No.	Standard / extracts	IC_{50}(µg/ml) ± S.D.
1	Ascorbic Acid	55.38± 0.560
2	Pet ether UD extract	172.38 ± 0.635
3	Ethyl acetate UD extract	101.39 ± 0.306
4	n-butanol UD extract	141.23 ± 0.809
5	Ethanolic UD extract	202.26 ± 0.670

(Values are mean ± S.D of 3 replicates)

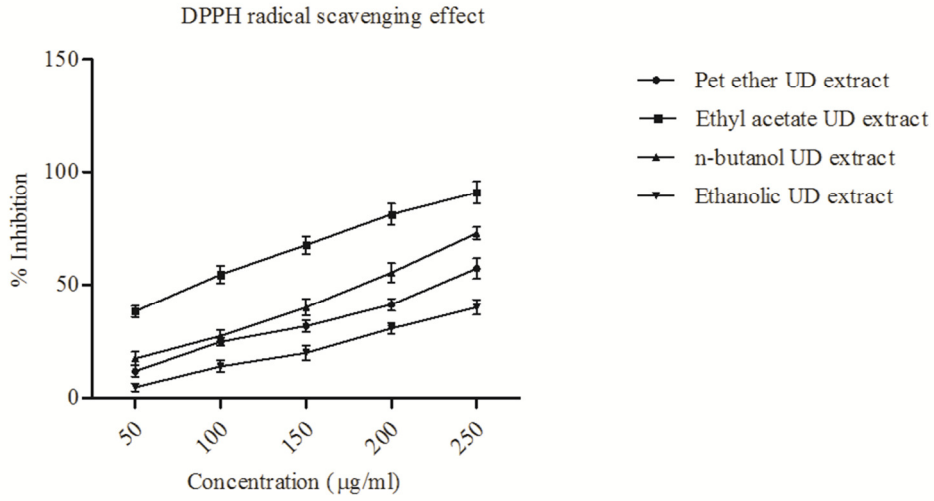
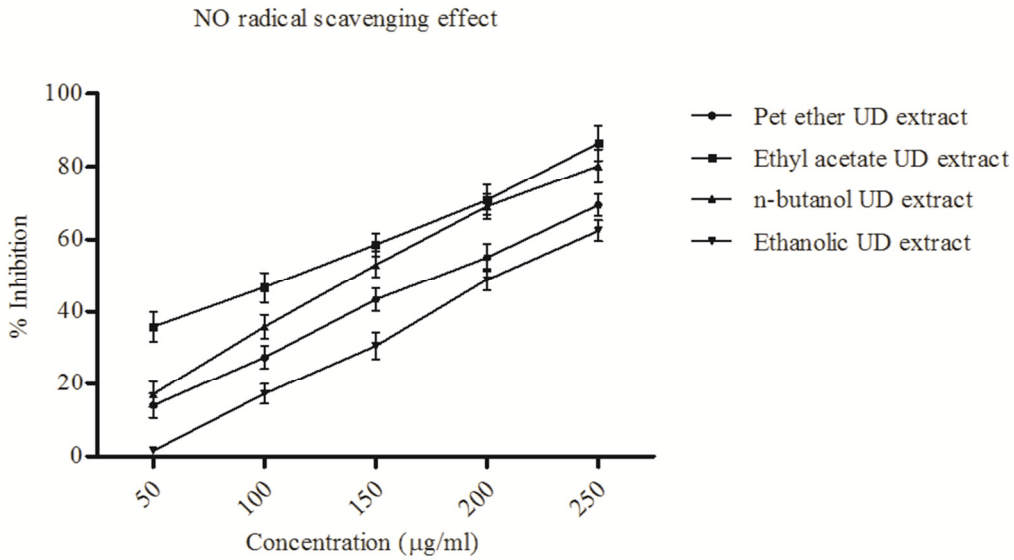


Figure 1: DPPH radical scavenging effect of various extracts of *Urtica dioica* Linn. (UD)

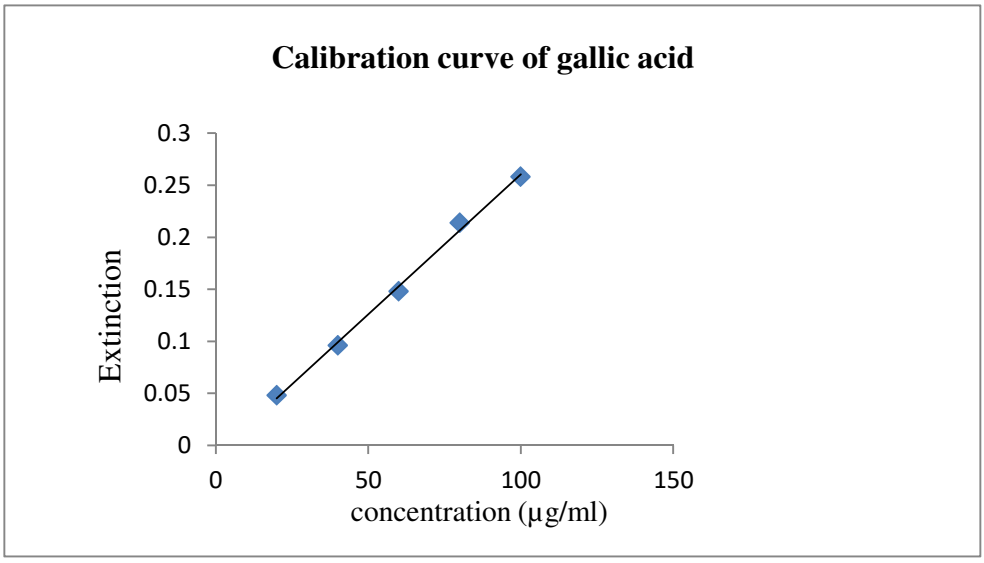
Figure 2: NO radical scavenging effect of various extracts of *Urtica dioica* Linn. (UD)



Determination of Total Phenolic Content

Quantitative determination of total phenols was done on the basis of a standard curve of gallic acid and linearity of the calibration curve was achieved (figure 3). The different extracts of *Urtica dioica* Linn.(UD) was also subjected to assay for total phenolic content. The maximum phenolic content was found in ethyl acetate extract (13.06±0.15 mg/g GAE).The amount of phenolics content in different extract are shown in Table no.4

Figure: Calibration curve of Standard gallic acid (y = 0.002x - 0.008 R² = 0.996)



S. No.	Extracts	Phenolic content mg/g gallic acid equivalents (GAE)
1	Pet ether UD extract	0.87±0.042
2	Ethyl acetate UD extract	13.06±0.15
3	n-butanol UD extract	3.19±0.028
4	Ethanolic UD extract	1.06±0.031

DISCUSSION

The antioxidant activity of natural antioxidants have been attributed to different mechanisms, among which are prevention of chain initiation, decomposition of peroxides, prevention of continued hydrogen abstraction, binding of transition metal ion catalysts, reductive capacity and radical scavenging[20].There is number of antioxidant methods have been proposed to evaluate antioxidant activity. The DPPH assay, NO assay, Reducing power assay, metal chelating, active oxygen species such as H_2O_2, $O_2^{\bullet -}$ and OH• quenching assays are mainly used for the evaluation of antioxidant activities of plant extracts [21,22].In the present paper, we have evaluated the antioxidant activity of different extracts of *Urtica dioica* Linn. (UD) whole plant.

DPPH (1, 1-Diphenyl-2-picrylhydrazyl) is stable nitrogen centred free radical which can be scavenged by antioxidants and shows absorbance at 517 nm. DPPH radical accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The change in absorbance of DPPH radical caused by antioxidants is due to the reaction between the antioxidant molecules and the radical, which results in the scavenging of the radical by hydrogen donation [23]. The ethyl acetate extract showed significant DPPH scavenging activity Table 2. (IC_{50} 78.99 ± 0.171 µg/ml) and compared with the standards Ascorbic acid (IC_{50} 26.24 ± 0.193 µg/ml).

Nitric oxide (NO) is a diffusible radical and cause pathological changes in the cellular structure and functional behavior of cellular components. It is a diffusible free radical that plays various roles as an effectors molecule in diverse biological systems, even though nitric oxide is involved in defence, over production of free radical contributes to the pathogenesis of various inflammatory diseases [23, 24]. Ethyl acetate extract of *Urtica dioica* Linn. (UD) significantly inhibited NO in a dose dependent manner Table 3 with the IC_{50} 101.39 ± 0.306µg/ml and it's compared with the standards Ascorbic acid having the IC_{50} values of 55.38 ± 0.560µg/ml.

Phenols are important phytoconstituents because of their free scavenging ability due to their hydroxyl groups. In various studies it has been reported that, a highly positive relationship between total phenols and antioxidant activity was present in several plant species [3, 20].The polyphenolic compounds were reported to be associated with antioxidant activity and play an important role in stabilizing lipid peroxidation and also have inhibitory effects on mutagenesis and carcinogenesis in humans [25].

The total phenolic contents of the *Urtica dioica* linn. (UD) different extracts as gallic acid equivalents were found to be highest in ethyl acetate extract (13.06±0.15 mg/g). The findings shows that the extent of antioxidant activity of the all extracts are in accordance with the amount of phenolics present in that extract. The plant *Urtica dioica* Linn. (UD) being rich in phenolics may provide a good source of antioxidant [24].

CONCLUSION

Amongst all the extracts, ethyl acetate extracts of *Urtica dioica* Linn. (UD) showed potent antioxidant potential as determined by various procedures. The presence of phenolic compounds and flavonoids in the extracts confirmed their utility as potent antioxidant agent.

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