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Research article

Establishment of quality control protocols and antioxidant activity of *Urtica dioica* L.

Bhuwan Chandra Joshi[1,*] and Sushmita Uniyal[2]

[1]*Department of Pharmacognosy, Sardar Bhagwan Singh Post Graduate Institute of Biomedical Sciences and Research, Balawala, Dehradun-248001, Uttarakhand, India.*
[2]*Gyani Inder Singh Institute of Professional Studies, Dehradun-248003, Uttarakhand, India.*

*Corresponding author: E-mail: bhuwan.joshi000@gmail.com; Tel: +91-7060172840.

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ABSTRACT

The present work establishes the pharmacognostic characters for the correct identification and quality control of *Urtica dioica* L., which is a species found commonly in the Himalayas. It is a folklore medicine, used for the treatment of many diseases. The pharmacognostic studies that were carried out included organoleptic, microscopy, physicochemical, fluorescence analysis, phytochemical evaluations, antioxidant and TLC fingerprinting. The microscopic studies showed the general characteristic of leaf. Physicochemical investigation performed for the total ash, acid insoluble ash, and water soluble ash showed the result 7.91±0.17%, 1.40±0.11% and 3.63±0.15% w/w, respectively. The alcohol and water soluble extractives values of the plant material were 18.53±0.42% w/w and 21.32±0.61% w/w, respectively. The phytochemical investigation revealed the presence of various phytochemical groups. The ethyl acetate extract of *U. dioica* has showed most significant free radical scavenging activity with IC_{50} of 82.56±0.11 µg/mL. The pharmacognostic characters of *U. dioica* established in this study may help in the pharmacopoeial standard for the identification and standardization.

INTRODUCTION

Urtica dioica L. is a perennial plant belonging to family Urticaceae and is commonly known as stinging nettle. The vernacular names of *U. dioica* are Vrishchhiyaa-shaaka in Sanskrit, Bichu Butti in Hindi and Shisuun in Kumaon (Khare, 2007). The traditional use of the roots and leaves of this plant include its use internally as a diuretic, blood purifier, in menstrual and nasal haemorrhage, rheumatic pain, as emmenagogue, in colds and cough, liver problems, stomachache, diarrhea eczema, anemia, hematuria, nephritis, menorrhagia and jaundice (Joshi et al., 2014; Chopra et al., 1956; Kirtikar and Basu, 2008; Joshi et al., 2015).

The plant contains diverse types of therapeutic phytoconstituents such as steroids, terpenoids, phenylpropanoids, coumarins, polysaccharides lectins and flavonol glycosides. The plant has been reported to have anticarcinogenic, antioxidant, anti-inflammatory, immunostimulatory, hypoglycemic, anti-allergenic, antiandrogenic, hepatoprotective, neuroprotective and antiviral activities (Joshi et al., 2015; Akbay et al., 2003; Nahata and Dixit, 2014; Golalipour and Khori, 2007; Balzarini et al., 2005; Bisht et al., 2016). The literature survey and scientific data revealed that no systematic pharmacognostic parameters were performed on *U. dioica*. Therefore, the purpose of the present study is to assess various pharmacognostic parameters and TLC finger-printing together with antioxidant potential of different extracts of *U. dioica*.

MATERIALS AND METHODS

Chemicals and instruments

Safranine, phloroglucinol, hydrochloric acid, glycerin, potassium hydroxide and all other chemicals used in the study were of analytical grade, and purchased from Rankem Ltd, New Delhi.

Plant material

The leaves of *Urtica dioica* were collected from Ranikhet, Uttarakhand. The plant was authenticated from NISCAIR, New Delhi (Ref. NISCAIR/2008-9/1192/ 224). The plant material was shade dried (<40 °C), coarsely powdered and stored in an air-tight container.

Macroscopic and microscopic analysis

The macroscopic evaluation was performed with a simple microscope. The microscopic study of the fresh leaf was done by preparing a thin transverse section and staining it with safranine. Thereafter, the stained sections were observed under microscope followed by photomicrograph. The dried leaves were powdered and treated with 5% KOH solution followed by staining with concentrated hydrochloric acid-Phloroglucinol (1:1) for 5 min and mounted in 50% glycerin solution (Brain and Turner, 1975; Kokate, 1994; Khandelwal, 2007).

Physiochemical analysis

A physiochemical parameter like ash values (total ash, acid insoluble ash, water soluble ash) and extractive values (water, alcohol soluble extractives) were determined using powdered drug (Government of India, 1996; WHO, 1998; WHO, 1992).

Fluorescence analysis

For the fluorescence analysis of leaves powder, it was treated with various chemicals and was observed exclusively at different wavelengths of ultra violet (254 nm and 365 nm) and visible light for observing characteristic colour presentation (Khandelwal, 2007; Kokoski et al., 1958; Patil et al., 2012).

Extraction

The different extracts were prepared using successive solvent extraction scheme with Pet. ether, ethyl acetate, n-butanol, ethanol and water. The coarse powder (500 g) of crushed leaves was extracted with petroleum ether (40-60 °C) using Soxhlet apparatus. The extract was filtered through Whatman filter paper and concentrated with the help of rota-evaporator. The concentrate was transferred to a pre-weighed china dish and dried in a vacuum desiccator. The marc obtained was further air dried and used for further extraction with ethyl acetate followed by n-butanol, ethanol and water. For further studies, the dried extracts were kept in desiccators and their percentage yield was calculated.

Preliminary phytochemical screening

The preliminary phytochemical screening was qualitatively tested for the presence of phytochemicals as per described standard methods (Khandelwal, 2007; Farnsworth, 1966; Harborne, 1973; Trease and Evans, 1983).

DPPH free radical scavenging activity

Different solvent extracts of *U. dioica* were assessed for their antioxidant activity by determining its ability to scavenge 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) radical. DPPH is a stable free radical which is used for the study of antioxidant activity. The 0.1 mM solution of DPPH was prepared in methanol. Further, 1 ml of this solution was added to 2 mL of test extract solution at different concentration (50–250 µg/mL). The mixture was shaken vigorously and allowed to stand for 30 min at room temperature. Then the absorbance was measured at 517 nm. Ascorbic acid was taken as a standard (Shimada et al., 1992).

The percentage of scavenging activity was determined using the following formula:

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

Where $A_{control}$ = absorbance of DPPH; A_{sample} = absorbance of DPPH with the test sample.

TLC fingerprinting of potent antioxidant extract

The potent antioxidant extract was analysed for the presence of compound by comparing with R_f value and spectral comparison with co-chromatographic standard marker i.e. ferulic acid. Chromatography was performed on precoated aluminium silica gel $60F_{254}$ (E-Merck) (4 cm ×10 cm) plates. The ferulic acid as standard marker and potent antioxidant extract of known concentrations were applied to the layers as 6 mm-wide bands positioned 15 mm from the bottom and 15 mm from side of the plate, using Camag Linomat 5 automated TLC applicator with the nitrogen flow providing a delivery speed of 90 nL/s from the application syringe. The analysis of the samples was performed by keeping the conditions constant throughout the analysis. Following sample application, layers were developed in a Camag twin through glass chamber that had been presaturated with the mobile phase of toluene: ethyl acetate: formic acid; (08:02:0.4), the developed plate was dried with a dryer and scanned at the 254, 366 nm with Camag U.V. scanner (Joshi et al., 2015).

RESULTS

Macroscopic characteristics

Among organoleptic features of the leaves on the lower surface is light green while on the upper surface they are dark green in colour. The leaves have a lightly aromatic odour with burning and unpleasant taste. The leaves are perennial, herbaceous, erect, monoecious or dioecious, pubescent, stinging hairs. Leaves are 3-15 cm long and opposite; leaf blades are ovate, lanceolate; leaf base may be sub-cordate or acuminate; leaf margin is usually crenate or serrate.

Microscopic characteristics

Leaf microscopy

Transverse section of the leaf shows a layer of upper and lower epidermis with embedded stomata. The stomata are more on the lower side, the cells of the upper epidermis covered with a striated cuticle. Simple glandular trichomes are present with unicellular stalk; meristele is centrally located and shows radially arranged rows of vessels and an arc of phloem, collenchymatous tissue are in the form of 3 to 5 rows which lie underneath. In lamina, a row of palisade cell is present under the upper epidermis, 5 to 6 rows of spongy parenchyma constitute the remaining tissue of mesophyll embedded with cluster calcium oxalate crystals as shown in Fig. 1A.

Petiole microscopy

Transverse section of the petiole shows 5 rings of vascular bundles and a peripheral ring of hypodermal collenchymatous tissue covered by epidermis having few trichomes similar to lamina as shown in Fig. 2B.

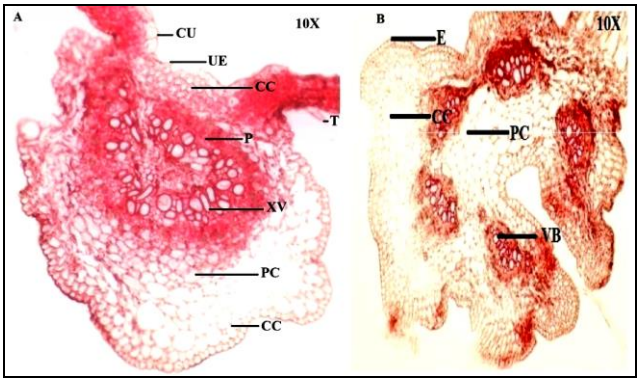

Fig.1. Transverse section of leaf (A) and petiole (B) at 10x. UE: upper epidermis; CU: cuticle; T: simple trichomes; CC: collenchyma cell; PP: parenchymatous cell; P: phloem; XV: xylem vessels; E: epidermis; PC: parenchymatous cell; VB: vascular bundle

Powder microscopy

Microscopic observation of *U. dioica* indicated the presence of parenchymatous cells, collenchyma cells, fragment of scalariform vessels, crystals of calcium oxalate, anomocytic stomata, and simple and glandular trichomes (Fig. 2).

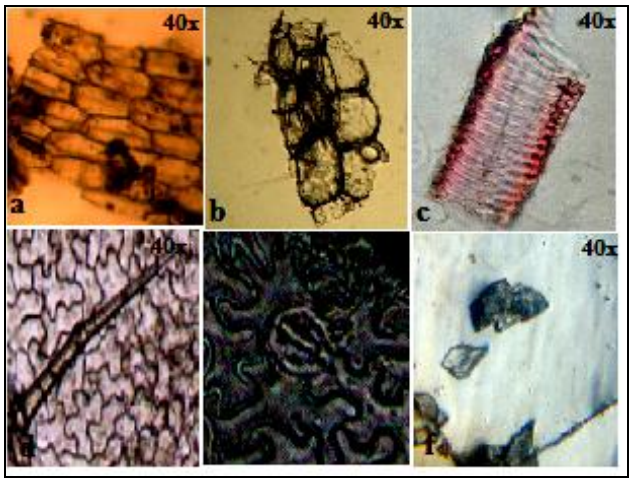

Fig. 2. Powdered characteristics of the leaf parts of *Urtica dioica*. a: parenchymatous cells; b: collenchyma cell; c: fragment of scalariform vessels; d: simple trichomes; e: anomocytic stomata; f: crystals of calcium oxalate.

Physicochemical parameters

Physicochemical parameters like total ash, acid insoluble ash and water soluble ash of *U. dioica* were found to 7.91±0.17%, 1.40±0.11% and 3.63±0.15% w/w, respectively. However, 18.53±0.42% alcohol soluble and 21.32±0.61% w/w water soluble extractives were observed (Table 1).

Table 1. Results of physicochemical studies of *Urtica dioica*

S.No.	Physicochemical parameter	Values (%)
1	Total ash	7.91±0.17
2	Acid insoluble ash	1.40±0.11
3	Water soluble ash	3.63±0.15
4	Alcohol soluble extractive	18.53±0.42
5	Water soluble extractives	21.32±0.61

Fluorescence analysis

Fluorescence analysis of stem powder was carried out after treating it with several reagents. Fluorescence was observed at 254 and 365 nm comparing its change of colour in visible light. The observations are presented in Table 2.

Table 2. Fluorescence analysis of *Urtica dioica*

Treatment	Visible light	Under UV light	
		Short wavelength (254 nm)	Long wavelength (365 nm)
Powder	Brown black	Brown	Dark green
Powder + Methanol	Green	Pale green	Green
Powder + 70% ethanol	Green	Green	Bottle green
Powder + Pet. ether	Pale green	Pale green	Olive green
Powder + 50% H_2SO_4	Black	Black	Brownish black
Powder + 50% HCl	Pale green	Pale green	Greenish black

Powder +1N NaOH(aq.)	Light green	Green	Pale green
Powder +1N NaOH(alc.)	Light green	Green	Bottle green
Powder + 50% HNO_3	Green	Light brown	Light green
Powder + 5% KOH	Brown	Purplish green	Dark purplish green
Powder + Ammonia	Brown	Green	Black
Powder + Acetic acid	Green	Green	Brownish red
Powder + Formic acid	Dark green	Green	Green
Powder + Nitric acid	Brick red	Black	Brown

Plant extraction

The physical properties and percentage yield of different extracts i.e., petroleum ether, ethyl acetate, n-butanol, ethanol and aqueous extracts are mentioned in Table 3. The ethyl acetate extract showed highest yield of 4.50% whereas ethanol extract was found to 3.25%.

Table 3. Physical properties of different solvent extracts of *Urtica dioica*

Extract	Colour	Consistency	% Yield (w/w)
PEE	Greenish yellow	Solid mass	1.30
EAE	Dark green	Semi-solid	4.50
NBE	Dark brown	Semi-solid	2.90
EE	Light brown	Semi-solid	3.25
AE	Greenish brown	Semi-solid	1.95

Abbreviations: PEE = Pet. ether extract; EAE = Ethyl acetate extract; NBE = n-Butanol extract; EE = Ethanol extract; AE = Aqueous extract.

Preliminary phytochemical screening

Preliminary phytochemical screening of *U. dioica* leaves revealed that steroids, saponins, triterpenoids and phenolics are the important constituents (Table 4).

DPPH radical scavenging activity

The antioxidant activity of different extracts of *U. dioica* was determined by its capacity to scavenge DPPH radicals. The petroleum ether, ethyl acetate, n-butanol, ethanol and aqueous extracts showed free radical scavenging activity with IC_{50} of 212.55±0.56, 82.56±0.11, 171.22±0.53, 315.01±0.73 and 423.17±0.23 µg/mL, respectively whereas standard ascorbic acid showed activity with an IC_{50} value of 26.24±0.19 µg/mL. The activity of ethyl acetate extract has most significant free radical quenching capacity when compared to petroleum ether, n-butanol, ethanol and aqueous extracts shown in Fig. 3 and Table 5.

Table 4. Phytochemical screening of various extracts of *Urtica dioica*

S.No.	Constituents	PEE	EAE	NBE	EE	AE
1	Amino acids	-	-	-	+	+
2	Proteins	-	-	-	+	+
3	Carbohydrates	-	-	-	+	+
4	Steroids	+	+	+	-	-
5	Triterpenoids	+	+	+	-	-
6	Alkaloids	-	-	-	-	-
7	Glycosides	-	-	+	+	+
8	Saponins	-	-	+	+	+
9	Flavonoids	-	+	-	+	+
10	Tannins	-	+	-	+	+
11	Phenolics	-	+	+	+	+

Abbreviations: PEE = Pet. ether extract; EAE = Ethyl acetate extract; NBE = n butanol extract; EE = Ethanol extract; AE = Aqueous extract; (-) = Absent; (+) = Present.

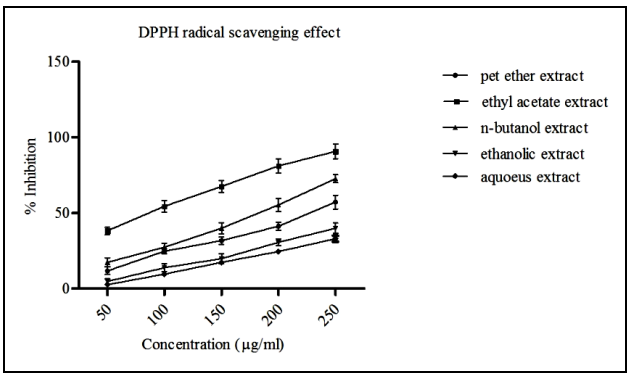

Fig. 3. DPPH radical scavenging activity of various extracts of *Urtica dioica.*

Table 5. Antioxidant activity of *Urtica dioica*.

S.No.	Standard/ Sample	IC_{50} (µg/mL)
1	Ascorbic acid	26.24±0.19
2	Pet. ether extract	212.55±0.56
3	Ethyl acetate extract	82.56±0.11
4	n-Butanol extract	171.22±0.53
5	Ethanol extract	315.01±0.73
6	Aqueous extract	423.17±0.23

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

TLC fingerprinting of potent antioxidant extract

The TLC fingerprinting profile was achieved in the mobile phase of toluene: ethyl acetate: formic

acid (08:02:0.4) at wavelength of 254 and 366 nm. The TLC analysis of ethyl acetate extract of *U. dioica* confirmed the presence of ferulic acid (R_f 0.39) as shown in Fig. 4.

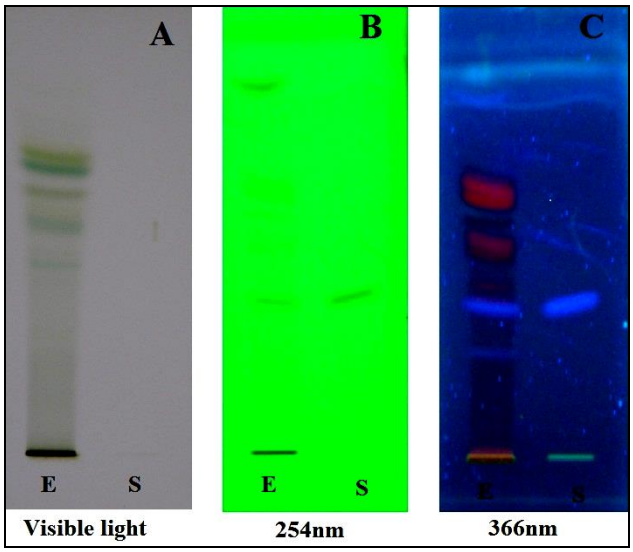

Fig. 4. TLC fingerprinting profile of ethyl acetate extract of *Urtica dioica* (E) and ferulic acid (S); A = visible light; B = 254 nm; C = 366 nm.

DISCUSSION

Quality control is an essential parameter for measurements of purity, quality and authenticity of crude drugs. *U. dioica* has extensively used in folklore medicine to cure various disorders. The present study was conducted to assess the pharmacognostic, phytochemical, antioxidant studies and TLC fingerprinting profile of *U. dioica*. The pharmacognostic and physicochemical studies, being reliable and inexpensive, play an important role in quality control issues of the crude drug. Physicochemical studies are a reliable tool for detecting adulteration (Trease and Evans, 1983). The ash values determine the physiological and non-physiological impurities present along with the drug. The extractive values give an idea about the phytoconstitution of the drug (Kumar et al., 2012). In the current study, the extractive value of alcohol was highest followed by water.

Fluorescence analysis is an alternative rapid method for a declaration of the suspicious sample. When physical and chemical methods for spotting and differentiating plant materials from their adulterants are inadequate then it can be done by analysing fluorescence characteristics and this analysis may be useful in the detection of adulterants (Kumar et al., 2012). Chemo-profile evaluation is valuable for the quality measurement of plants. Phytochemical constituents of the plant are known to have various therapeutic benefits.

DPPH is very convenient for the screening of antioxidant potential of plant materials. The measurement of the scavenging of DPPH radical allows determining the intrinsic ability of a substance to donate hydrogen atom or electrons to this reactive species in a homogeneous system. Methanolic DPPH solution gets reduced because of the presence of antioxidant substances having hydrogen-donating groups such as phenols and flavonoid compounds due to the formation of non-radical DPPH-H form. Antioxidant capacity may be associated with high phenol content as reported that most of the antioxidant activity of plants is derived from phenols (Mensour et al., 2011; Mansouri et al., 2005).

TLC fingerprint is suitable for rapid and simple authentication technique. We developed chromatographic fingerprinting of potent antioxidant extract (ethyl acetate extract) of *U. dioica* and compare with standard marker ferulic acid. Developed fingerprints are useful in confirming the identity and purity and authenticity of the medicinal plant raw material.

CONCLUSION

The present study was focused on establishing pharmacognostic standards for the identification and authentication of the *U. dioica*. Thus, the above finding will serve in the development of pharmacopoeial standards for the future studies.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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