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Pharmacognostical standardization of roots of *Imperata cylindrica* Linn (Poaceae)

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Abstract:

Imperata cylindrica Linn. Beauv (Poaceae), commonly known as Darbh in Hindi, is an important drug of "Trinpanchmool" and used extensively for the treatment of various ailments viz. urinary calculi, retention of urine, diabetes, cardiac disorder, gout, common cough and cold, inflammation, anemia, as aphrodisiac, etc. The present study was aimed to undertake pharmacognostical studies to fulfill the work required for the identification of the drug. The macroscopic studies revealed that the roots are fibrous, arising from nodes of stolons having uneven surface with fine wrinkles, light brown to dark brown in colour, fibrous fracture, taste and odour indistinct. Microscopically the epidermal cells are thin walled, semi circular with projection on outer walls. The cortex is differentiated into outer thin continuous cylinder of subepidermal sclerenchymatou cells. Inner cortex consists of several radially elongated wide air chambers and thin uniseriate partition filaments comprising of spherical cells. The stele has an outer covering of endodermal layer of tabular cells and depositional silica bodies. The entire pith tissue of the stele consists of sclerenchymatous cells. The metaxylem vessels are circular and thin walled, but protoxylem are not well developed. In between the metaxylem elements occur two small crystals of phloem. Moisture content, total ash, acid insoluble ash, alcohol soluble extractive, water soluble extractive were 7.3, 8.3, 5.77, 4.8 and 5.6% respectively. Thin layer chromatography (TLC) of alcoholic, chloroform and aqueous extracts showed 5, 3 and 2 spots respectively. All there diagnostic features may be useful for proper identification of the crude drug.

Keywords: *Imperata cylindrica*, cytomorphological studies, physicochemical parameters, poaceae.

Introduction:

Imperata cylindrica Linn. Beauv (Poaceace), is a perennial, erect, tall tufted grass distributed from Punjab to southwards. In Hindi it is known as Darbh and Thatch grass in English [1, 2]. It is an important drug of "Trinpanchmool" [3], used for treatment of various ailments viz. urinary calculi, retention of urine, cardiac disorder, gout, common cough and cold, menorrhegia, inflammation, burning sensation, fever, anemia, and as aphrodisiac, rejuvenator etc [1]. The drug contains 5 triterpenoids viz. cylindrin, arundorin, ferneon, soburneol and simiarenol [1, 4, 5]. It was observed that the drug is helpful in abolishing the symptom of UTI i.e. burning in urine frequency, pain in parineal region, fever and dribbling to a great extent. Due to its various Rasas it is not harmful to the digestive system which has been observed in clinical cases. The drug is well tolerated in patients and has no diarrhoea or burning in abdomen in any form. The potassium loss is minimal with this drug in comparison to modern diuretic drugs [6]. The literature survey showed inadequate pharmacognostical work on Darbh. Therefore it has been decided to undertake the pharmacognostical studies on Darbh roots to bring out their salient diagnostic features which will enable to identify the drug.

Materials and Methods:

Collection and authentification of plant material

The drug has been collected from Banaras Hindu University campus, Varanasi and authenticated by Dr. V.K. Joshi, Dean of Faculty of Ayurveda, Institute of Medical Science, B.H.U., Varanasi and also through National Botanical Research Institute (NBRI), Lucknow. A Voucher specimen has been preserved in the Department of Pharmacognosy, College of Pharmacy, IFTM, Moradabad for further references.

Macroscopic and microscopic studies

The macroscopical characters of the root were studied following standard methods [7, 8]. For the microscopical studies, cross sections were prepared and stained as per the procedure of Johansen [9] and the representative diagrams were taken with the help of inverted microscope for photodocumentation (Leitz, Japan).

Table 1: Fluorescence analysis of root powder of *Imperata cylindrica*

Treatment	Normal light	UV light	
		(254 nm)	(365 nm)
Powder (P)	Cream	Light brownish cream	Black
P + 5% KOH	Light yellow	Dark green	Dark brown
P + 5% NaOH	Yellowish brown	Dark green	Dark brown
P + 5% $FeCl_3$	Orange yellow	Yellowish green	Dark reddish brown
P + Iodine solution	Blood red	Dark green	Dark reddish brown
P + dil. H_2SO_4	Cream	Yellowish green	Light black
P + conc. H_2SO_4	Cream brown	Creamish green	Brownish black
P + conc. HCl	Cream brown	Light green	Brownish black
P + dil. HCl	Cream yellow	Light green	Light black
P + conc. HNO_3	Reddish yellow	Green	Dark brown
P + dil. HNO_3	Light brown	Light green	Light black
P + Ammonia solution	Creamish yellow	Green	Black

Table 2: Preliminary phytochemical investigation of different extracts of I*mperata cylindrica* root

Group of phytoconstituent	Extract			
	Petroleum ether	Chloroform	Alcoholic	Aqueous
Alkaloids	-	-	-	-
Carbohydrates	-	-	+	+
Gums and mucilage	-	-	-	-
Proteins	-	-	-	-
Fats and oils	+	-	-	-
Amino acids	-	-	-	-
Steroids	+	+	+	-
Glycosides	-	-	+	-
Cardiac glycosides	-	-	-	-
Anthraquinone glycosides	-	-	-	-
Saponin glycosides	-	-	-	-
Coumarins	-	-	-	-
Flavonoids	-	-	+	+
Tannins and phenolic compounds	-	-	-	-

+ indicates present and – indicates absent

Table 3: TLC of different extracts of roots of *Imperata cylindrica*

S.No.	Extracts	Solvent System	R_f value
1.	Alcoholic	Ethyl acetate : Propanol : Pyridine (2 : 2 : 1)	0.18, 0.35, 0.49, 0.60 & 0.98
2.	Chloroform	Ethyl acetate : Pyridine (3 : 2)	0.5, 0.75 & 0.87
3.	Aqueous	Ethy acetate : Benzene (1 : 1)	0.35 & 0.5

Determination of physicochemical parameters

Physicochemical parameters i.e. percentage of moisture content, percentage of ash values and extractive values were performed according to the official methods [10] and the WHO guidelines on the quality control methods for medicinal plant materials [11]. Fluorescence analysis was carried out following reported methods [12, 13].

Thin layer chromatography

Thin layer chromatography of alcoholic, chloroform and aqueous extracts was performed using standard method of Stahl [14] and the R_f values were determined.

Preliminary phytochemical screening

Preliminary phytochemical screening of petroleum ether, chloroform, alcoholic and aqueous extracts was carried out for different group of phytoconstituents following standard procedures described by Harborne [15] and Khandelwal [16].

Results:

Macroscopic characters *(Figure 1)*

Macroscopically the roots are fibrous, up to 2.2 mm diameter having uneven surface with fine wrinkles, light brown to dark brown in color, taste and odour indistinct, fracture fibrous.

Figure 1: Roots of *Imperata cylindrica*

Microscopic characters

Root measuring 700 µm thick was studied and the various tissue zones were:

The epidermis is a continuous layer (Figure 2A). The epidermal cells are thin walled, semicircular with projection outer walls (Figure 2B & 2C). The epidermal layer is 10µm thick.

The outer cortex consists of thin, continuous cylinder of sub epidermal sclerenchymatous cells. This zone has two or three layers of cells (Figure 2B & 2C). Inner cortex is arenchymatous and consists of several radially elongated wide air chambers and thin uniseriate partition filaments comprising of spherical cells (Figure 2A, 2B, 2C & 2D). The air chambers are 150µm radially and 50µm tangentially.

The stele is covered by an outer endodermal layer of tabular cells (Figure 2A & 2D); the inner tangential wall has U-shaped thickening and deposition of silica bodies (Figure 2C, 2E, 2F, 2G & 2H). The number of metaxylem elements varies from five (Figure 2D) to seven (Figure 2A). The entire pith tissue of the stele consists of sclerenchymatous cells (Figure 2C, 2E & 2F), the metaxylem vessels are circular and thin walled, measuring up to 30µm wide, but protoxylem are inconspicuous. In between the metaxylem elements occur two small clusters of phloem (Figure 2E & 2G).

Measurement of different cells and tissues of the root of *Imperata cylindrica* are given below in microns:

Epidermis:	11.12-16.68-19.46 x 8.34-11.12-13.9
Hypodermis:	5.56-8.34-11.12
Cortex region:	13.9-25.02-36.14
Pericycle:	13.9-16.68-19.46
Xylem vessel:	33.36-36.4-41.7
Pith cells:	5.56-8.34-11.12

Physicochemical parameters

Moisture content, total ash, acid insoluble ash, alcohol soluble extractive and water-soluble extractive were found to be 7.3, 8.3, 5.77, 4.8 and 5.6% respectively. The fluorescence behavior of the root powder was furnished in Table 1.

Preliminary phytochemical screening

Preliminary phytochemical analysis revealed the presence of carbohydrate, glycosides, fats & oil, reducing sugar,

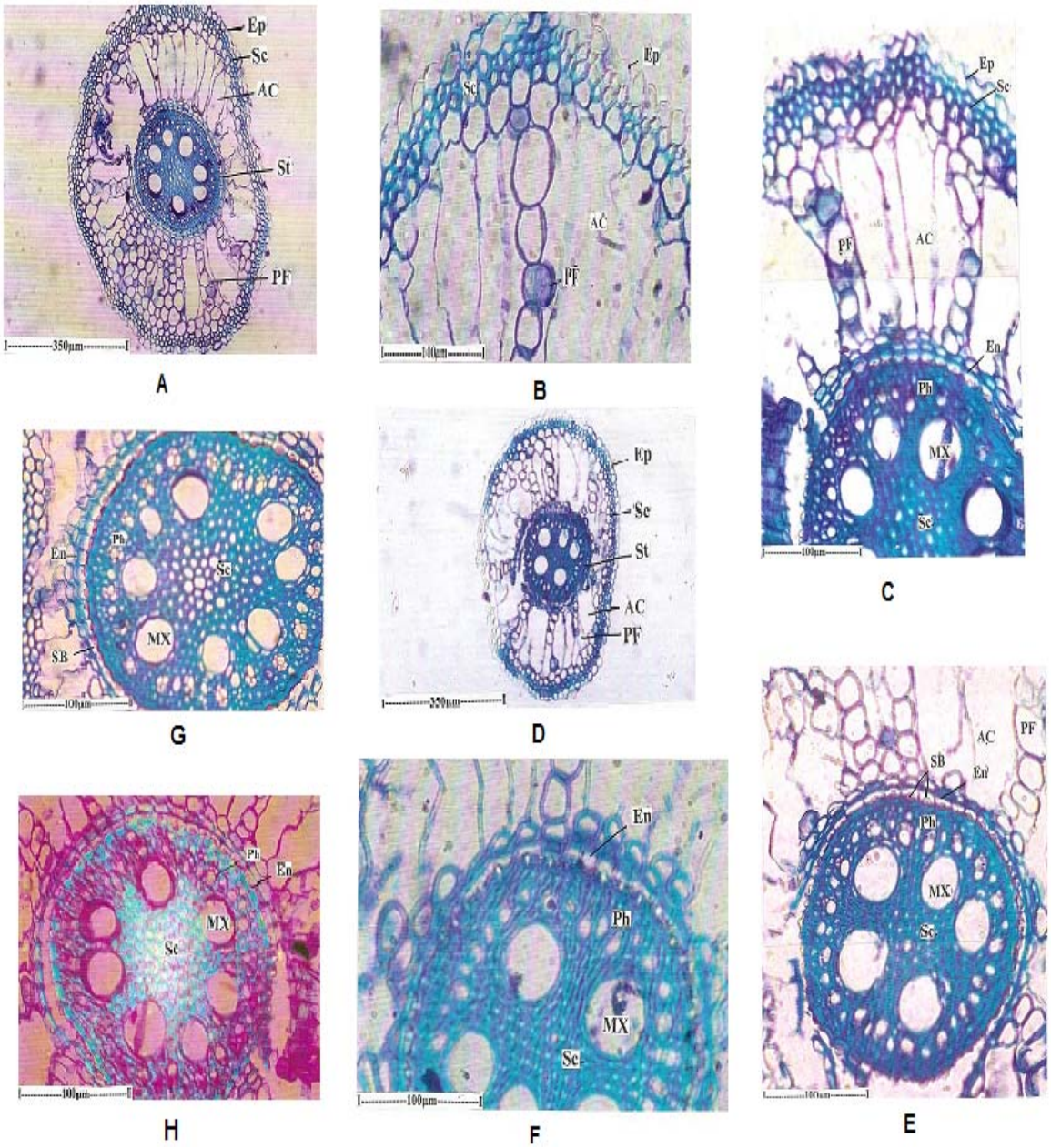

Figure 2: Microscopical characters of roots of *Imperata cylindrica*

A & D, TS of root of *Imperata cylindrica* (10x10); **B,** TS of *Imperata cylindrica* root showing cortex region (10x40); **C,** TS of root of *Imperata cylindrica* (10x40); **E,** TS of *Imperata cylindrica* root showing inner cortex and stelar region (10x40); **F,** TS of *Imperata cylindrica* root showing endodermis and stelar region (10x40); **G & H,** TS of *Imperata cylindrica* root showing stelar region (10x40).

Abbreviations: Ep, Epidermis; SC, Sclerenchyma, AC, Arenchyma; St, Stele; PF, Pericyclic Fibre; En, Endodermis; Ph, Phloem; MX, Metaxylem; SB, Silica Bodies.

flavonoids and steroids in different extracts of the roots of the plant (Table 2).

Thin layer chromatography

TLC profile of alcoholic, chloroform and aqueous extracts showed 5, 3 and 2 spots respectively (Table 3).

Discussion:

To check the potential of a crude drug with reference to its phytochemistry and pharmacology, the proper botanical identification is of paramount important [17, 18]. Hence, in the present study the detailed pharmacognostical characters of the root of *Imperata cylindrica* have been

evaluated. Fibrous root is the character in most herbs of Graminae family [19] and the roots arise from the nodes of stolon [1]. Microscopically the epidermis has projections on the outer wall. Stele is surrounded by endodermis and the inner tangential wall has casparian thickening, 5-8 metaxylem and undeveloped protoxylem, and two small clusters of phloem are present inbetween metaxylem elements which exhibits the monocotyledon structuer. The macroscopic and microscopic characters, TLC profile, preliminary phytochemical screening and physicochemical parameters developed in this study would help for botanical identification of the crude drug.

Conclusion:

The salient diagnostic features developed in the present study could be used for botanical identification of the drug in the crude form and preparation of a monograph of the plant. Further, the genuine drug may be studied for its phytochemical and pharmacological potential.

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