Research Article

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PHYTO-PHARMACOGNOSTICAL EVALUATION AND HPTLC STUDY ON ANANTAMUL (*HEMIDESMUS INDICUS* R.Br.) ROOT

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ABSTRACT

Plants are among the richest sources of bioactive compounds throughout the world for thousands of years and continue to provide new remedies to mankind. Roots of *Hemidesmus indicus* R. Br. is an important plant drug which is used to cure leprosy, leucoderma, itching, skin disease, asthma, bronchitis, leucorrhoea, dysentery, piles, syphilis, paralysis, urinary disorders and diabetics. The present study focused on the pharmacognostical, phytochemical investigation as well as HPTLC study on *Hemidesmus indicus* root. Organoleptic, macroscopic, microscopic, phytochemical features along with the HPTLC study were performed by taking different solvent extracts of *Hemidesmus indicus* root. This study highlights the first detailed HPTLC study on *Hemidesmus indicus* root by taking different solvent extracts with their increasing polarity which is a referential information for identification parameters and improves our confidence level of acceptability of herbal drugs.

Key words: *Hemidesmus indicus* root, pharmacognostical, phytochemical investigation, HPTLC

INTRODUCTION

We have been interested in the phytochemical and pharmacognostic studies on important Indian medicinal plants.[1,2] As a part of these investigations, we have now taken up the study of the Indian medicinal plant *Hemidesmus indicus* R. Br.

Hemidesmus indicus R.Br. (Sanskrit meaning: endless root) commonly named as 'Anantmoola or Anantamul' is slender, laticiferous and twining shrub, occurs over the greater part of India.[3] *Hemidesmus indicus* was formerly placed under Asclepiadaceae, but recently based on the pollinical characters it has been transferred to Periplocaceae.[4] It is widely recognized in folk medicine and as ingredient in a large number of Ayurvedic and Unani preparations. Roots of *Hemidesmus indicus* is used to cure leprosy, leucoderma, itching, skin disease, asthma, bronchitis, leucorrhoea, dysentery, piles, syphilis, paralysis, urinary disorders and diabetics.[4-7] It is also used in combination with other drugs for snake bite.[8] The pharmacognostical parameters, phytochemical screening and HPTLC study are major reliable criteria for the confirmation of the identity and determination of quality and purity of the drugs.

https://en.wikipedia.org/wiki/File:Hemidesmus_scandens.jpg

MATERIALS AND METHODS

Plant Collection

The root of *Hemidesmus indicus* was purchased from the local market of Kolkata and authenticated by the Pharmacognosy Department, NRIADD, Ministry of Ayush, Kolkata. The collected matured roots were washed under running tap water for 5 min followed by sterile distilled water three times. Half of the drug samples were air dried in intact condition for morphological study, the other half of these were pulverized to obtain 60 mesh size and dried in shade for 7 days and stored in airtight container to avoid any contamination due to moisture etc.

The macroscopy and organoleptic studies of the crude drug were performed in terms of its shape, size, colour, odour, taste etc. For powder microscopy, powdered samples each was treated with different solutions, stained and mounted following standard method and observed under a compound microscope at projection 10X and 40X. The Camera Lucida drawing of cellular details was done. Shade dried powdered samples was also used for the physico-chemical and phytochemical investigations according to the standard method. [9,10]

Table 1: Organoleptic characters of *Hemidesmus indicus* root

Character	Observation
Colour	Brown in colour
Odour	Pleasant aroma
Taste	Bitter
Size of the Root	Length 4.20+/- 0.68 Width-1.14+/- 0.7
Texture	Fine
Fracture	Rough

Extraction of plant material

Extractions were carried out at room temperature under normal condition. About 15 gm shade dried powder of roots of *Hemidesmus indicus* were successively extracted with petroleum ether, ethyl acetate, methanol and water.[11] The extracts obtained were filtered and concentrated by evaporating on a water bath.

Phytochemical analysis

The extracts were used for preliminary screening of phytochemicals such as alkaloids, tannins, flavonoids, proteins, saponins, carbohydrates, terpenoid, steroid. The screening was done as per the standard method.[12]

HPTLC Study

A sample and convenient HPTLC method was developed for standardization of *Hemidesmus indicus* (Root). A CAMAG HPTLC system (Switzerland) comprising CAMG Linomat 5 applicator, CAMAG TLC scanner 3, CAMAG Wincats software, version 1.44, Hamilton syringe (100µl). CAMAG Reprostar 3, CAMAG TLC plate heater, CAMAG UV Cabinet were used for the study. Silica gel $^{60}F_{254}$ Aluminium plates (Merck) was used as stationary phase.[13,14]

RESULT

Pharmacognostical Study

Macroscopic

Roots are always fragmented, woody, slender, brown, sparsely branched, thick and hard, rigid, elongated, cylindrical with rough and wavy outer surface, externally dark tortuous with transversely cracked and longitudinally fissured bark portion and internally yellowish brown, rootlets thin and wiry; cork is thin, separates easily and peels off in flakes; root fragment is 1.0cm to 2.0cm in diameter; central core solid, 0.4cm to 0.9cm in diameter and outer surrounding cylindrical portion is 0.4 to 0.6 in breadth; length of fragment of root varies from 1.5 to 3.2 cm; fracture short, splintery; taste is sweetish, astringent or acrid; odour agreeable, very aromatic, slightly acidic, emitting a sweet scent reminiscent of a combination of vanilla, cinnamon and almonds.

Figure 1: Morphology of pieces of root of *Hemidesmus indicus*

Powder analysis

The fine powder of root yellowish brown in colour, showed the following characteristic features. Presence of profused, thick lignified walled stone cells are with prominent pit canals in various shapes and sizes (oval, semi-circular, rectangular, triangular, long tracheid like), single and in compound form (sometimes 2 semi-circular stone cells are attached together); flattened, angular to semi rectangular, lignified walled cork cells; laticiferous ducts are unique. Fibre unicellular, aseptate, long, wavy fibres with undulated inner wall; numerous prismatic crystals of Ca-oxalate having different shapes (rectangular, triangular, irregular); abundant starch grains, simple, compound by 3 to 4 components with central hilum; groups of pitted and spiral xylem vessels, sometimes attached with fibre; two types of parenchymatous cells *viz*. large squarish to rectangular to polygonal reddish brown cells in group with striated thick wall and cell contents inside and round to oval cells with cell contents inside; groups of light brownish polygonal, angular opaque cells; very few long, lignifed tracheid fibres present with present serreted inner wall; few groups of xylem parenchyma with cell contents.

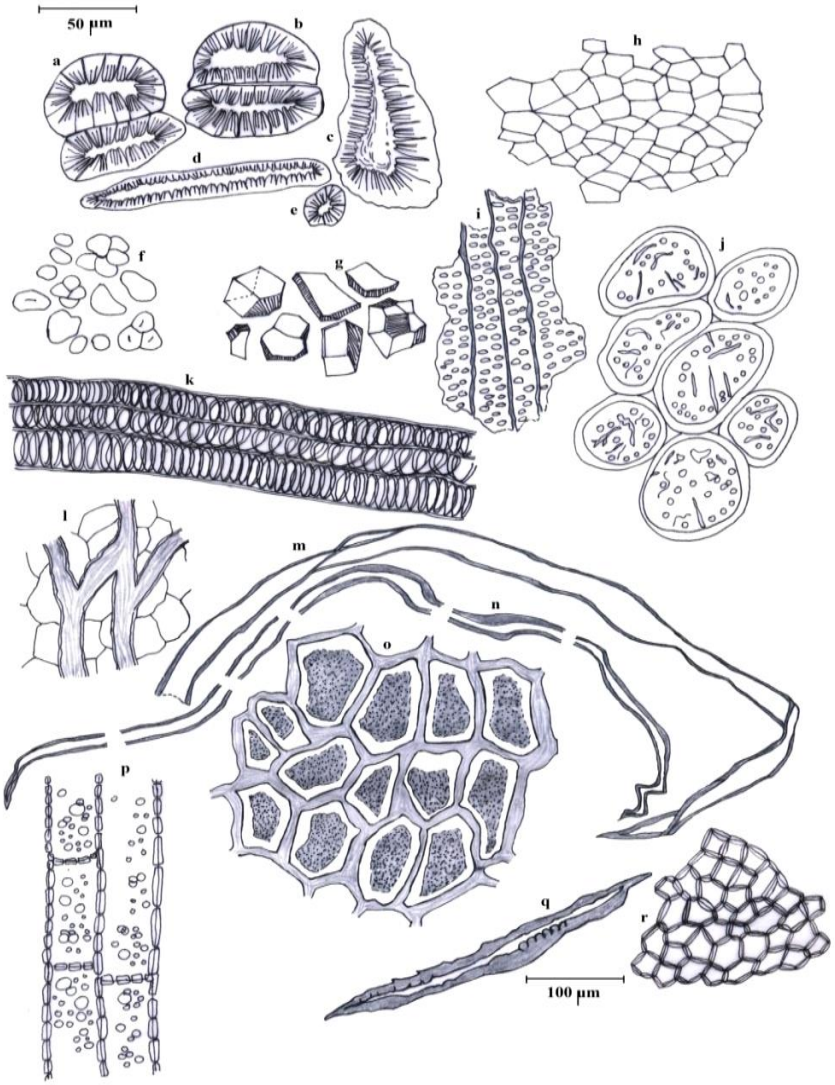

Figure 2: a,b,c,d,e: stone cells of different shape and sizes ; **f:** starch grains; **g:** prismatic crystals of Ca- oxalate; **h:** polygonal opaque cells; **i:** pitted xylem vessels; **j:** oval parenchymatous cells ; **k:** spiral xylem vessels; **l:** laticiferous ducts with ground tissue; **m,n:** aseptate fibres; **o:** rectangular to polygonal reddish brown parenchyma; **p:** xylem parenchyma; **q:** fibre tracheid; **r:** lignified cork cells in group.

Table 2: Phytochemical screening of *Hemidesmus indicus* R.Br. (Root)

S.N	Phytochemicals	Pet ether Extract	Chloroform Extract	Ethanol Extract	Aqueous Extract
1	Alkaloids (Dragendorff's Test)	Positive	Positive	Positive	Positive
2	Flavonoids (Lead acetate test)	Negative	Positive	Positive	Positive
3	Tannin	Positive	Positive	Positive	Positive
4	Terpenoids (L.B. test)	Positive	Positive	Positive	Negative
5	Steroids (L.B. test)	Positive	Positive	Negative	Negative
6	Carbohydrates (Molisch's test)	Positive	Positive	Positive	Negative
7	Poly phenols	Negative	Positive	Positive	Positive
8	Reducing Sugar (Benedict's test)	Negative	Negative	Negative	Positive
9	Glycosides	Negative	Negative	Positive	Positive
10	Saponins (Foam test)	Positive	Positive	Positive	Positive

HPTLC Profile

Chromatography experiments Sample preparation

The dried plant (Roots) were subjected to soxhlet extraction by using different solvents with gradually increasing the polarity of the solvents starting from pet ether, ethyl acetate, methanol, water for 6 hours and extracts were filtered and concentrated and taken for following HPTLC profiles.

Stationary Phase

Precoated (support on Aluminum Sheets) Silica Gel Plate. Specification: TLC Silica Gel $60F_{254}$, Mfg. by Merck, Batch No. 1.05554.0007.

Mobile Phase

Hexane: Ethyl acetate (7:3) for pet ether extract; Hexane: Ethyl acetate: Chloroform: Formic Acid (7:2:1:0.5) for Ethyl acetate extract; Chloroform: Ethyl acetate: Methanol: Formic acid (1.5:7:1:0.5) for methanol extract, Chloroform: Ethyl acetate: Methanol: Formic acid (1.5:7:1:0.5) for water extract [G R grade solvent used, manufactured by MERCK, India].

Sample application

Applied volume 5 µL as 8 mm band and applied at 10 mm from the base of the plate. Plate size was 5X10 cm.

Development

Developed up to 80 mm in CAMAG Twin trough chamber, Plate preconditioning (temp 25°C and relative average humidity was 54%)

Photography

Plate 1 (Observed at 254 nm) and Plate 2 (Observed at 366 nm)

Photography of HPTLC Plates at different wave length

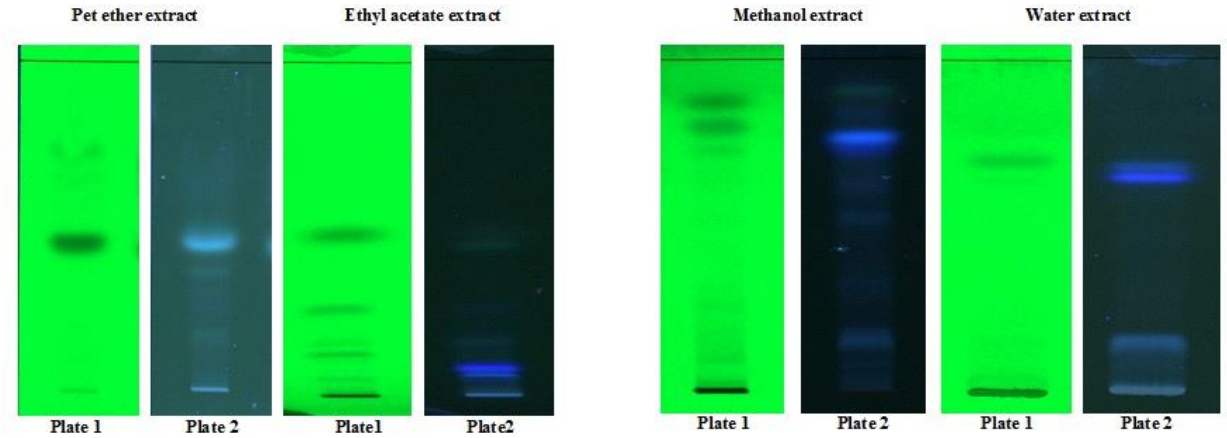

Table 3: R_F values of Pet ether extract

Observed at 254 nm		Observed at 366 nm	
R_f	Colour	R_f	Colour
0.05	Light black	0.05	Faint sky
0.09	Light black	0.09	Faint sky
0.20	Light black	0.20	Faint sky
0.45	Deep black	0.29	Faint sky
0.65	Light black	0.32	Faint sky
0.71	Light black	0.38	Faint sky
		0.45	Deep sky
		0.50	Deep sky
		0.65	Faint sky
		0.71	Faint sky

Table 4: R_F values of Ethyl acetate extract

Observed at 254 nm		Observed at 366 nm	
R_f	Colour	R_f	Colour
0.03	Light black	0.05	Deep Blue
0.05	Light black	0.08	Deep Blue
0.13	Black	0.13	Light Blue
0.16	Light black	0.16	Light Blue
0.26	Black	0.26	Light Blue
0.48	Deep black	0.44	Light Blue
		0.48	Light Blue

Table 5: R_F values of Methanol extract

Observed at 254 nm		Observed at 366 nm	
R_f	Colour	R_f	Colour
0.03	Light black	0.03	Light Blue
0.09	Light black	0.09	Light Blue
0.15	Light black	0.15	Light Blue
0.18	Light black	0.18	Light Blue
0.27	Light black	0.33	Light Blue
0.33	Light black	0.38	Light Blue
0.64	Light black	0.52	Light Blue
0.73	Light black	0.54	Light Blue
0.81	Deep black	0.61	Light Blue
0.86	Deep black	0.64	Light Blue
		0.73	Deep Blue
		0.77	Deep Blue
		0.81	Deep Blue
		0.86	Deep Blue
		0.90	Deep Blue

Table 6: R_F values of Water extract

Observed at 254 nm		Observed at 366 nm	
R_f	Colour	R_f	Colour
0.05	Light black	0.03	Light Blue
0.12	Light black	0.09	Light Blue
0.16	Light black	0.12	Light Blue
0.22	Light black	0.16	Deep Blue
0.58	Light black	0.25	Light Blue
0.64	Light black	0.31	Light Blue
0.70	Deep black	0.47	Light Blue
		0.58	Light Blue
		0.65	Deep Blue
		0.68	Deep Blue

DISCUSSION

The present study will serve as a ready reference for authentication of *Hemidesmus indicus* R.Br.(roots) through macroscopic, microscopic study as well as gives indication about the presence of secondary metabolites such as alkaloids, flavonoids, tannins, carbohydrates, terpenoids, steroids, poly phenols and saponins in different solvent extracts. Thus, the presence of large number of secondary metabolites, the roots of *Hemidesmus indicus* is so effective against several diseases. HPTLC is a valuable assessment tool for the identification of chemical constituents present in plant drugs. The retention factor (R_f) values obtained from different extracts can be used to identify each compound due to their uniqueness. In the present study, we used four different solvent extracts starting from less polar to more polar i.e. polarity of Pet ether extract < Ethyl acetate extract<Methanol extract < Aqueous extract.

Through phytochemical screening and HPTLC study it is revealed that major number of secondary metabolites are present in methanol extract rather than other three extracts. In HPTLC study it is being found that number of R_f values are maximum in methanol extract, i.e. methanol extract contains high concentrations of secondary metabolities and moderate concentrations in aqueous extract. Moreover, one more additional information we get from HPTLC study is that due to the presence of large number of secondary metabolites in methanol extract, its biological potential is more than other extracts.

CONCLUSION

The results obtained from phyto-pharmacognostical evalution and HPTLC profiling will be helpful for identification, phytochemical screening and quality control of the raw drug and ensure therapeutic efficacy. The HPTLC profile helps to differentiate the authentic root from the market drug. HPTLC profiling of methanolic extract of *Hemidesmus indicus* R.Br.(roots) shows maximum no. of R_f values i,e maximum no. of phytoconstituents are present in this extract. So, in near future by taking methanol extract researcher can isolate a large no. of biologically active compounds through column chromatography.

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