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Phyto-pharmacognostic evaluation and HPLC study on *Sariva* (*Hemidsmus 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 diabetes mellitus. As per Acharya Charaka has mentioned suitable season for collection of Moola i.e. *Greeshma* and *Shishira Rutu.* Here an attempt to study to check the potency of root collected as per classical reference. The present study focused on the pharmacognostical, phytochemical investigation as well as HPLC study were performed by taking different solvent extracts of *Hemidesmus indicus* root. This study highlights the detailed HPLC 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, HPLC.*

INTRODUCTION

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.[1] *Hemidesmus indicus* belonging to family Asclepiadaceae. 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.[2-7] It is also used in combination with other drugs for snake bite.[8] The pharmacognostical parameters, phytochemical screening and HPLC study are major reliable criteria for the confirmation of the identity and determination of quality and purity of the drugs.

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MATERIALS AND METHODS

Plant Collection

- *Sariva Moola* was collected and authenticated by botanist from FRLHT (Foundation for Revitalization of local health traditions, Bangalore. Root of *Hemidesmus indicus* were collected in *Greeshma* and *Shishira Rutu*, washed thoroughly with water to remove physical impurities like mud.
- They were then dried in shade for a period of 15-20 days until they were completely dry. After drying, they were made into a coarse powder and kept preserved in air tight container for phytochemical and physicochemical analysis. 50 gm of the drug was kept apart for macroscopic and microscopic studies.Then the drug was made

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into coarse powder and kept preserved in air tight container for physic chemical analysis. The time of collection of root as per Acharya Charaka is *Greeshma* and *Shishira Rutu*.[9]

- 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. Dried powdered samples was used for the physico-chemical and phytochemical investigations according to the standard method.[10]

Table 1: Organoleptic characters of *Hemidesmus indicus* root.

Character	Observation
External Colour	Dark Brown
Internal colour	Pale yellow
Odour	Characteristic pleasant smell
Taste	Sweetish
Size of the Root	Variable in size 20-30cm in length, less than 1cm in diameter
Shape	Cylindrical
Fracture	Short at the periphery and fibrous at the Centre

Identity, Purity and Strength

Table 2: Identity, purity and strength of *Sariva*[10]

Foreign matter	Not more than 2 percent, Appendix 2.2.2
Total Ash	Not more than 4 percent, Appendix 2.2.3.
Acid-insoluble ash	Not more than 0.5 percent, Appendix 2.2.4
Alcohol- soluble extractive	Not less than 15 percent, Appendix 2.2.6
Water- soluble extractive	Not less than 13 percent, Appendix 2.2.7

Table 3: Qualitative phytochemical analysis of *Hemidesmus indicus* root extract.

SN	Test	Reagent	Observation	*Greeshma*		*Shishira*	
				Aq	Alc	Aq	Alc
1.	Alkaloids	Mayer's Test	Cream or pale yellow	+	-	+	-
2.	Flavonoids	Alkaline reagent test	yellow colour	-	-	-	-
3.	Triterpenoids	Salkowski test	Lower layer turning golden yellow	-	-	-	-
4.	Glycoside	Kellerkillani test	Reddish brown	-	+	-	+
5.	Steroids	Liebermann-Bruchard test	Brown ring	-	-	-	-
6.	Saponnins	Foam and froath test	Honeycomb like froth indicates the presence of Saponins	+	+	+	+
7.	Tannins and phenolic compounds	Ferric chloride test	Blue/black/brown precipitate	+	-	+	-
8.	Carbohydrates	Molisch's test	Purple ring	-	-	-	-
9.	Proteins	Biuret test	Blue colour	-	-	-	-

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10.	Starch	Iodine test	Blue colour	+	-	+	-
11.	Resin	Acetone-water Test	Turbidity	-	-	-	-

Organoleptic evaluation

Organoleptic evaluation refers to evaluation of the formulation by colour, odour, tase, texture etc. The organoleptic characters of the fresh root and market sample were evaluated based on the method described by Ayurvedic pharmacopoeia of India.

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.

Plate 1: *Hemidesmus indicus* – Botanical Description

Root

Twining Shrub

Flower

Powder

Determination of Alkaloids

Quantitative determination of alkaloid was according to the methodology by Gravimetry. Exactly 200cm^3 of 10% acetic acid in ethnol was added to each wood powder sample (2.50g) in a 250cm^3 beaker and allowed to stand for 4 hours. The extract was concentrated on a water bath to one-quarter of the original volume followed by addition of 15 drops of concentrated ammonium hydroxide drop wise to the extract until the precipitation was complete immediately after filtration. After 3 hours of mixture sedimentation, the supernatant was discarded and precipitates were washed with 20 cm^3 of 0.1M of ammonium hydroxide and then filtered using gem filter paper (12.5cm). Using electronic weighing balance Model B-218, the residue was dried in an oven and the percentage of alkaloid is expressed mathematically as;

$$\%\ Alkaloid\ = \frac{Weight\ of\ alkaloid}{Weight\ of\ Sample} \times 100$$

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Determination Glycosides

Weigh accurately 4g sample in to 250ml conical flask. Add 100ml 50% aq.Ethnol and sonicate for 20min. Filter the solution using Whatman filter paper 1. Add 10ml of 10% lead acetate solution. Centrifuge the solution and discard the residue. Add 5ml of 10% sodium oxalate solution. Filter and wash the residue with water. Evaporate the filterate to dryness and weigh the residue.

Glycoside content = Weight of residue × 100 Weight of sample

HPLC Analysis of Tannins

The analysis was made (Waters model no. 550; Waters Corp., Milford, MA, USA) on C18 column (symmetry, 4·6mm×250mm) in isocratic mode with the mobile phase methanol and water in the ratio 1:1 with the RP-HPLC C-18 column at a flow rate of 1mL/min. The standard tannic acid with the concentration 0.4mg/mL and sample (10mg/mL) were dissolved in mobile phase and 20µL was injected and the elution was monitored at 270nm. The amount of tannins present in the sample was estimated using the formula,

$$\frac{Sample\ area}{Standard\ area} \times \frac{standard\ amount}{dilution\ of\ standard} \times \frac{dilution}{sample\ amount} \times Mean\ weight$$

HPLC Analysis of Saponins

The analysis was made (Waters model no. 550; Waters Corp., Milford, MA, USA) on C18 column (symmetry, 4·6mm×250mm) in isocratic mode with the mobile phase acetonitile and water in the ratio 4:6 with the RP-HPLC C-18 column at a flow rate of 1mL/min. The standard saponin with the concentration 0.4 mg/mL and sample (10mg/mL) were dissolved in mobile phase and 20µL was injected and the elution was monitored at 203nm. The amount of saponins present in the sample was estimated using the formula,

$$\frac{Sample\ area}{Standard\ area} \times \frac{standard\ amount}{dilution\ of\ standard} \times \frac{dilution}{sample\ amount} \times Mean\ weight$$

HPLC Analysis of Vanillin[11]

The analysis was made (Waters model no. 550; Waters Corp., Milford, MA, USA) on C18 column (symmetry, 4·6mm×250mm) in isocratic mode with the mobile phase methanol and water in the ratio 6:4 with the RP-HPLC C-18 column at a flow rate of 1mL/min. The standard vanillin with the concentration 0.1mg/mL and sample (100mg/mL) were dissolved in mobile phase and 20µL was injected and the elution was monitored at 231nm. The amount of vanillin present in the sample was estimated using the formula;

$$\frac{Sample\ area}{Standard\ area} \times \frac{standard\ amount}{dilution\ of\ standard} \times \frac{dilution}{sample\ amount} \times Mean\ weight$$

RESULT

Pharmacognostical Study

Macroscopy

Root occurs in pieces, about 30mm in diameter, cylindrical, thick, hard, somewhat tortuous, sparcely branched, provided with few thick rootlets and secondary roots, external appearance dark brown, sometimes with violet grey tinge, centre yellow, woody, surrounded by a mealy white cortical layer, dark brownish, corky, marked with transverse cracks and longitudinal fissures and easily detachable from the hard central core, odour, characteristic, taste, sweetish, slightly acrid and aromatic.

Powder Microscopy

Light brown in colour. Shows fragments of parenchyma containing starch grains and prism, vessels pitted wall, lignified xylem fibres, tracheids, radially cut medullary rays, containing starch grains and prism of calcium oxalate, fragments of latex tubes and reddish brown cork cells.

Figure 1: Powder microscopy of *Hemidesmus indicus* (G)

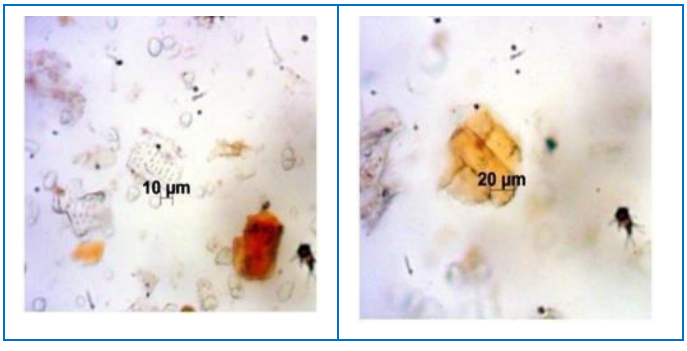

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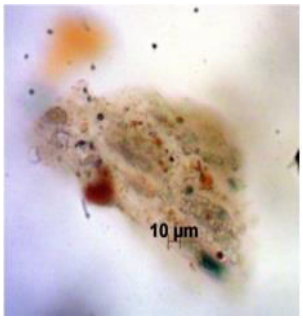
Fig 1.1: Pitted vessels	Fig 1.2: Brown content
	
Fig 1.3: Group of starch grains	**Fig 1.4: Cortical parenchyma with starch**

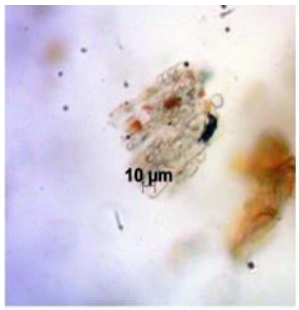
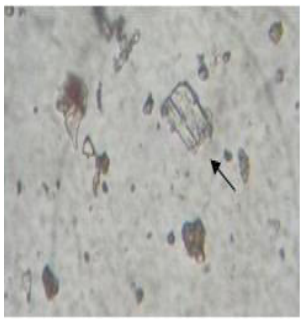



Fig 1.5: Cells containing starch	**Fig 1.6: fibres with vessels**

Shishira Rutu

	
Fig 1.7: Resinous block	**Fig 1.8: Spiral vessel**

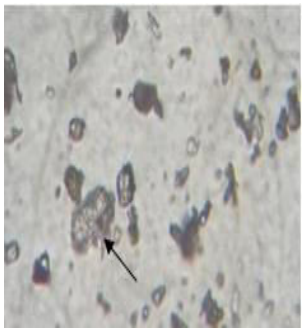
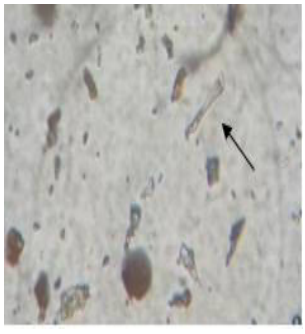

	

Fig 1.9: Pitted vessel	**Fig 1.10: Fiber**

Physicochemical parameters

Table 4: Physicochemical content of *Hemidemus indicus* root powder.

SN	Parameters	*Greeshma*	*Shishira*
1.	Loss on drying	5.78	8.51
2.	Ash value	3.37	3.49
3.	Total ash value	0.34	0.34
4.	Ethnol extractive	19.47	17.88
5.	Water extractive	18.93	17.2

Total Alkaloid Quantification

Table 5: Showing total Alkaloids

Rutu	*Greeshma*	*Shishira*
%	5.633	4.218

Total Glycoside Quantification

Table 6: Showing total Glycosides

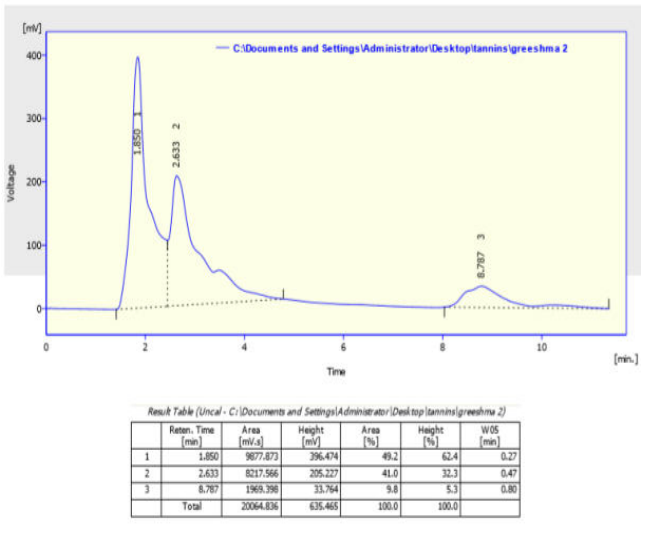
Rutu	*Greeshma*	*Shishira*
%	23.067	21.576

Total Tannin Quantification

Table 7: Showing total Tannin

Rutu	*Greeshma*	*Shishira*
mg/grm of Extract	71.915	157.668

Graph 1: Quantification of Tannins



Result Table (Uncal - C:\Documents and Settings\Administrator\Desktop\tannins\greeshma 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.850	9877.873	396.474	49.2	62.4	0.27
2	2.633	8217.566	205.227	41.0	32.3	0.47
3	8.787	1969.398	33.764	9.8	5.3	0.80
	Total	20064.836	635.465	100.0	100.0	

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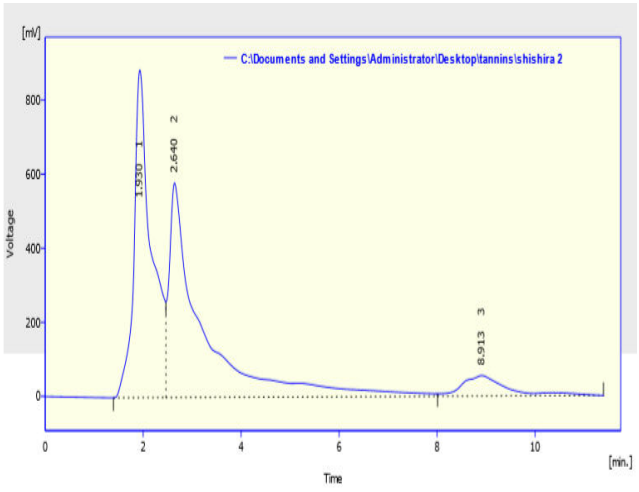


Result Table (Uncal - C:\Documents and Settings\Administrator\Desktop\tannins\shishira 2)

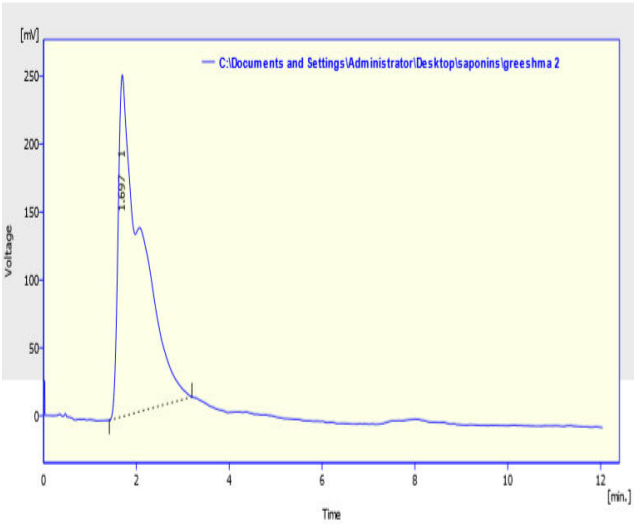
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.930	21656.574	884.651	41.4	58.2	0.28
2	2.640	27067.383	579.060	51.8	38.1	0.39
3	8.913	3533.692	55.249	6.8	3.6	0.86
	Total	52257.649	1518.960	100.0	100.0	

Total Saponin Quantification

Table 8: Showing total Saponins

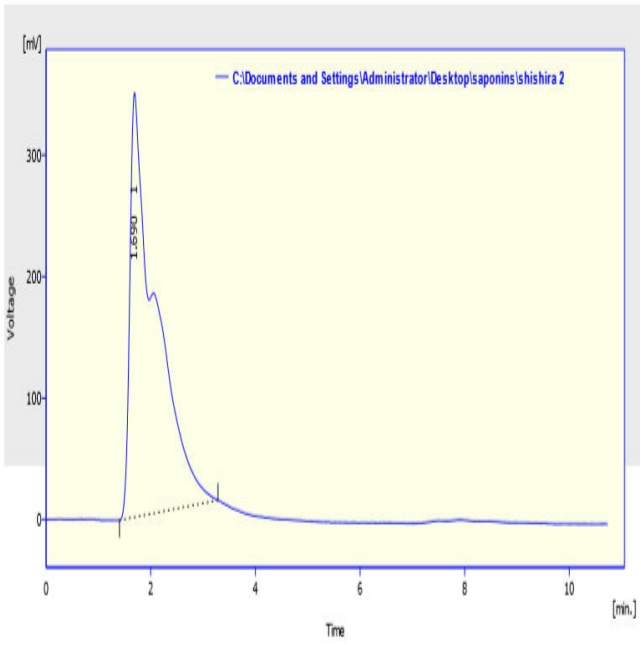
Rutu	*Greeshma*	*Shishira*
mg/grm of Extract	62.886	85.291

Graph 2: Quantification of Saponnins



Result Table (Uncal - C:\Documents and Settings\Administrator\Desktop\saponins\greeshma 2)

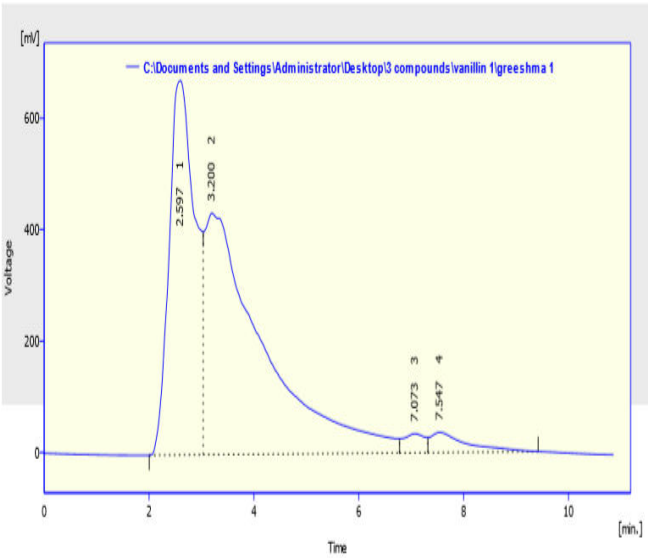
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.697	8802.047	251.635	100.0	100.0	0.59
	Total	8802.047	251.635	100.0	100.0	



Result Table (Uncal - C:\Documents and Settings\Administrator\Desktop\saponins\shishira 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.690	11938.012	349.213	100.0	100.0	0.54
	Total	11938.012	349.213	100.0	100.0	

Graph 3: Quantification of Vanillin



Result Table (Uncal - C:\Documents and Settings\Administrator\Desktop\3 compounds\vanillin 1\greeshma 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.597	23048.717	672.080	38.5	57.1	0.66
2	3.200	34027.207	433.493	56.9	36.9	1.04
3	7.073	969.156	34.183	1.6	2.9	0.54
4	7.547	1765.510	36.330	3.0	3.1	0.66
	Total	59810.590	1176.086	100.0	100.0	

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DISCUSSION

Powder Macroscopy

All the sample were similar in macroscopic and organoleptic characters. As there is no much variation, it is of not much significance.

Powder Microscopy

The powder microscopy of the samples showed the presence of Starch grains, Reddish brown Content cells, Pitted vessel, Cortical parenchyma and Pigment cells.

All the character in the powder microscopy are indicating the identity of the drug.

Discussion on pH

Shishira and *Greeshma*, sample Showed Alkaline pH (7.15,7.29,). This variation in pH may be due to inter conversion of active principle in different season.

Loss on Drying

Loss on drying was highest in *Greeshma Rutu*.

Ash Value

All the sample showed ash within the normal limit (i.e. NLT 4%). As there is no much variation, it is of not much significance.

Phytochemical Studies

The phytochemical evaluation showed the presence of following constituents, Alkaloids, Tannins, Saponins, Starch gave positive result only in Aqueous extract and Glycosoid gave positive result only in Alcoholic extract.

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, tannins and phenolic compound 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. HPLC is a valuable assessment tool for the identification of chemical constituents present in plant drugs.

CONCLUSION

Chromatographic technique HPLC was conducted, confirmed the presence of Standard compound Vanillin in *Hemidesmus indicus* (L.) Schult. in methnolic extract qualitatively and quantitatively, thus confirmed the genuinity. As water soluble extractive values showed higher yield, the drug can be used in classical forms like *Kashaya, Hima, Phanta* etc. Predominance of all the parameters were observed in *Shishira Rutu.* Thus this evidence based analytical research has reconfirmed the opinion of Acharyas Charaka to collect the root in *Shishira Rutus.*

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