Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2011;3(3): 74-79
ISSN: 0975 4873

Research Article

Pharmacognostical and Phytochemical Studies on *Hemidesmus Indicus* Root

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ABSTRACT

Plants have been the basis of many traditional medicines throughout the world for thousands of years and continue to provide new remedies to mankind. Plants are one of the richest sources of bioactive compounds. Roots of *Hemidesmus indicus* is one of the plant drugs used as a tonic, demulcent, diaphoretic and diuretic, in the treatment of syphilis, chronic rheumatism and urinary disorders. Scientific evidences further supports traditional uses of this traditional drug. Present work aims to highlight the phytochemical and pharmacognostical standards of *Hemidesmus indicus* root. Organoleptic, macroscopic, microscopic, physicochemical and fluorescence features were studied along with their phytochemical features using methods given in Indian Ayurvedic Pharmacopoeia. Market samples were also analysed pharmacognostically and phytochemically and compared with authentic samples.

Keywords: Microscopy, physico-chemical parameters, phytochemical analysis, quantitative determination, *Hemidesmus indicus* root

INTRODUCTION

Hemidesmus indicus Linn.R.Br is a twining shrub, belonging to the family Asclepiadaceae. Roots of this taxon has been used in folk medicine as well as in ayurvedic and unani preparations. They have been prescribed against the diseases of blood, inflammation, diarrhoea, respiratory disorders, skin diseases, syphilis, fever, bronchitis, asthma, eye diseases, epileptic fits in children, kidney and urinary disorders, loss of appetite, burning sensation and rheumatism[1,2]. It has also been used in combination with other drugs for snake bite[3,4]. Recently, this plant was used to treat viper venom (haemotoxic)-induced lethality[5] and against hypercholesterolaemia in hyperlipidaemic rats[6]. However, sufficient scientific standards or parameters are not

available to ascertain the identity and to determine the quality of this crude drug. The pharmacognostical parameters are major reliable criteria for the confirmation of the identity and determination of quality and purity of the drugs[7]. The present work attempts to report various necessary phytochemical and pharmacognostical standards of *Hemidesmus indicus* root, so as to further strengthen the pharmacopeal standards prescribed for this traditional drug source.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

Table - 1Organoleptic Characters of *Hemidesmus indicus* root

S. No	Character	Observation	
		Fresh root	Market root
1	Colour	Brownish yellow	Brownish orange
2	Odour	Pleasant aromatic	Pleasant aromatic
3	Taste	Bitter taste	Bitter taste
4	Size of the root	Length3.95±0.43cm Width-0.21±0.6mm	Length4.23±0.74cm Width-1.21±0.9mm
5	Texture	Fine	Fine
6	Fracture	Nil	Rough

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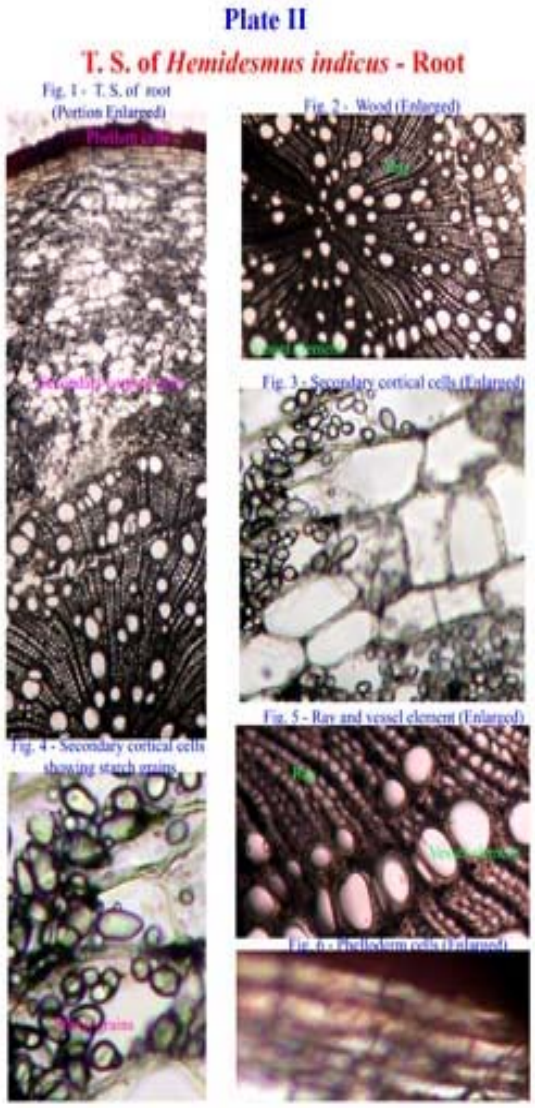
Table – 2 Physicochemical contents of *Hemidesmus indicus* root powder

S.No	Parameters	Results	
		Fresh sample	Market sample
1	Foreign matter	<0.2%	<0.74%
2	Powder Particle size	2.07µm	1.95
4	Foaming index	250U	200U
5	Swelling index	2.3%	1.8%
6	Acid insoluble ash value	0.9%	1.15%
7	Water soluble ash value	1.85%	2.23%
8	Total ash	3.8%	4.23%
9	Ethanol extractive	13.20%	10.25%
10	Water extractive	14.40%	15.98%

The root was purchased from ayurvedic raw drug store, Srirangam, Tiruchirappalli – 620 006 and fresh plants were collected, identified by Professor Dr. John Britto, Taxonomist, Department of Botany, St. Joseph's College,



Thiruchirapalli, India. After authentication, the fresh

Table – 3 Qualitative Phytochemical analysis of *Hemidesmus indicus* root extracts of fresh and market samples
Chemical constituents were similar in nature

S. No	Test	Reagent	Observation	Aqueous Extract	Alcoholic extract
1	Alkaloids	Mayer,s reagent	Cream precipitate	Positive	Negative
2	Steroids	Con. Sulphuric acid	Reddish brown precipitate	Positive	Positive
3	Terpenoids	Chloroform + con. sulphuric acid	Reddish brown interface	Positive	Negative
4	Flavonoids	Alkaline reagent	Yellow colour to colourless	Positive	Positive
5	Saponins	Water + shake	Foam formation	Negative	Negative
6	Phenolic compounds	Alcohol + Ferric chloride	Bluish green	Positive	Positive
7	Tannins	Lead acetate	White ppt	Positive	Positive
8	Lignin	Safranine solution	Pink colour	Positive	Positive
9	Phlobatannins	1% Aqueous hydrochloric acid	Reddish colour precipitate	Negative	Negative
10	Fat and Oil	1%Copper sulphate + 10%sodium hydroxide	Clear blue solution	Negative	Negative
11	Inulin	α naphthol +Sulphuric acid	Brownish red colour	Negative	Positive
12	Cardiac glycosides	Glacial acetic acid + $Fecl_3$ +Conc. Sulphuric acid	Brown ring at the Interface	Negative	Positive
13	Proteins	Conc.nitric acid + 40% NaOH	Orange colour	Positive	Positive
14	Carbohydrates	Barfoed's reagent	Reddish brown ppt	Positive	Positive
15	Aminoacids	Ninhydrin reagent	Violet colour	Positive	Positive
16	Reducing sugars	Benedict's solution	Red precipitate	Positive	Positive

aerial parts of the young and matured roots were collected in bulk during summer. The roots were washed, shade dried and then milled into coarse powder by a mechanical grinder. Both purchased and collected samples were subjected to pharmacognostic and phytochemical analysis.

Organoleptic evaluation

Organoleptic evaluation refers to evaluation of the formulation by colour, odour, taste, texture, etc. The organoleptic characters of the fresh root and market samples were evaluated based on the method described by Ayurvedic pharmacopoeia of india[8].

Microscopy

Specimens for microscopic observation were prepared using free – hand sections. The sections were cut with smooth strokes and transferred from the blade to a drop of water on a microscopic slide, suitably stained and observed under the light microscope. Photomicrographs were taken using Nikon compound microscope attached with digital camera. Photomicrographs of sections were taken at different magnifications depending upon the microscopic details to be observed.

Physicochemical Parameters

The determination of various physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, water soluble extractive value, alcohol soluble extractive value, swelling index, foaming index, foreign matter and microbial limit assay were determined by employing standard methods as given in Ayurvedic Pharmacopoeia of India[8, 9]. The fluorescence nature of the drug powder was determined according to the method of Kokate[10].

Preliminary Phytochemical Analysis

Quantitative phytochemical screening for the identification of various classes of active chemical constituents present in *Punica granatum* fruit rind aqueous and alcoholic extracts were studied using the standard methods. Triplicates were maintained when assessing qualitative phytochemical analysis[11,12,13].

Microbial limit assay

Dissolved 1gm of powdered plant material in 10mL of distilled water. It was serially diluted using phosphate buffer as diluent. The sample was inoculated in Nutrient agar by pour plate, Rose Bengal agar and SS agar by spread plate techniques for Bacteria, Fungi and *Salmonella* respectively. For Bacteria, the plates were incubated at 37°C for 48 hrs and for Fungi, the plates were incubated at 25°C for 96 hrs[8].

RESULTS AND DISCUSSION

Organoleptic characters

The roots used in this study occur in pieces and about 3.95 ± 0.43cm long and 0.21 ± 0.6 mm in diameter, cylindrical, thick and hard in nature. Externally brownish yellow in colour with transverse cracks and

Table – 4 Fluorescence Analysis of *Hemidesmus indicus* root extracts of fresh and market samples Similar fluorescence analysis was noted in both samples

S.No	TEST	0 HOURS		24 HOURS		48 HOURS	
		DAY LIGHT	UV LIGHT	DAY LIGHT	UV LIGHT	DAY LIGHT	UV LIGHT
1	Plant powder+ chloroform	Brown	Yellowish brown	Brown	Brownish green	Dark brown	Pale green
2	Plantpowder + Hexane	Brown	Light green	Brown	Pale green	Brown	Light green
3	Plantpowder+Benzene	Blackish brown	Brownish green	Reddish brown	Blackish green	Reddish brown	Brownish green
4	Plantpowder+Aqueous NaOH	Dark brown	Dark green	Reddish brown	Reddish green	Reddish brown	Reddish green
5	Plant powder +Alcoholic NaOH	Brown	Green	Brown	Green	Dark brown	Reddish brown
6	Plantpowder+ 1NHcl	Yellowish brown	Yellowish green	Brown	Yellowish green	Light brown	Yellowish green
7	Plantpowder+ Ethanol	Light brown	Pale green	Brown	Light green	Brown	Brownish yellow
8	Plantpowder+ Ethyl acetate	Brown	Yellowish green	Brown	Pale green	Dark brown	Yellowish green
9	Plantpowder+Acetone	Dark brown	Yellowish green	Light brown	Brownish green	Light brown	Pale green
10	Plantpowder+ 50% H_2SO_4	Brownish black	Black	Black	Blackish green	Black	Blackish green

bark was easily detachable from the hard central core. Odour was pleasant and aromatic in nature (Table 1 & Plate I). Transverse section of roots showed three major components namely outer phellem, inner cortex and wood regions. Phellem cells were brownish pink in nature, cork cells were radially flattened and rectangular in appearance filled with brownish coloured granules. Multiple layers of secondary cortical cells were very much characteristic. Secondary phloem consists of sieve elements, parenchyma, ray cells along with several lacticiferous ducts. Xylem tranversed by narrow medullary rays and central region occupied by woody tissues (Plate II).

Physicochemical characters

Physicochemical standards were generally used for deciding the identity, purity and strength of the drug source. These characters were also used to detect the adulterants if any. Total ash value of *Hemidesmus indicus* root was found to be 3.8%, acid insoluble ash value 0.9%, water soluble ash value 1.85% (Table 2). The extractive values of the plant material were 14.4% for water, 13.3% for ethanol (Table 2). The standards determined in the present work were in agreement with the prescribed standards in pharmacopoeia[8]. Successive extractive values determined using soxhlet extractor and cold extraction methods. This revealed that the methanol extractive values was more (12.16% and 18.7%) where as hexane extractive value was 6%. These extractive values revealed that the plant material have high molecular weight components. Higher water extractive values further indicated the presence of highly polar chemical constituents in the plant drug (Table 2).

Phytochemical Analysis

Preliminary phytochemical screening of aqueous extract answered positively for steroids, terpenoids, flavonoids, phenolic compounds, tannins, lignin, carbohydrates and proteins and negatively for alkaloids, saponins and cardiac glycosides. Gopiesh and Khannabiran[13] also reported similar results. Inulin and cardiac glycosides were also present in alcoholic and aqueous extracts along with other components. Review on *Hemidesmus indicus* root extracts also confirmed the presence of these chemical constituents[12,14,15,16] (Table 3).

Fluorescence Analysis

Fluorescence is the phenomenon exhibited by various drugs and will reveal the various chromophores of chemical constituents present in the plant material. Some constituents showed fluorescence in the visible range daylight. UV light produces fluorescence indicative of many natural products (Eg: Alkaloids like Berberine), which do not visibly fluoresce in daylight. If the substance themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence some crude drugs are often assessed qualitatively by this method and also it is an important parameter of Pharmacognostical evaluation. The data of the fluorescence features revealed various shades of brown and yellow which were presented in table 4.

Microbial limit assay

Microbial limit assay was essential to check the microbial load and to detect the pathogenic contaminations in the herbal drugs. Such contaminations

were found to be absent in the present plant drug (Table 5).

Table – 5 Microbial limit assay of *Hemidesmus indicus* root powder

S. No	Test organism	Microbial counts (CFU/g)	
		Fresh root	Market Sample
1	Total aerobic Bacteria	5×10^2	9×10^2
2	Total Fungal count	2×10^1	22×10^1
3	Total Enteric Bacteria	Nil	Nil
4	Total *E.coli*	Nil	Nil
5	*Salmonella*	Nil	Nil
6	*Shigella*	Nil	Nil

CONCLUSION

This study is in line with the quality parameters prescribed in Ayurvedic Pharmacopeia of India and also standards set by other international agencies. This work provides qualitative and quantitative standards for the identification of *Hemidesmus indicus* roots and from this study it is concluded that Pharmacognostical and phytochemical studies on *Hemidesmus indicus* roots will be highly useful in determining qualitative and quantitative standards which can ascertain the identity, quality and purity of this plant drug that inturn will result in standard herbal preparations for the betterment of human society.

Following Standards were determined in the present work

Organoleptic characters

Colour of the root : Brownish yellow
Odour : Pungent , pleasantly aromatic.
Taste : Bitter
Foreign matter : < 0.2%

Macroscopic

Roots occur as pieces about 3.95±0.43cm long and 0.21±0.6mm width, cylindrical, thick and hard.

Microscopy

Phellem cells were characteristically dark pinkish colour, presence of radially flattened cork cells with brownish granules. Laticiferous ducts were unique. Medullary ray were mostly uniseriate

Test for identity purity and strength

Total ash : not more than 3.8%
Acid insoluble ash : not more than 0.9%
Water soluble ash : not more than 2.85%
Water extractive value : not more than 14.40%
Ethanol soluble extractive value: not more than 13.20%

Phytochemical constituents

Flavonoids, Phenolic compounds and Tannins were present

Total microbial load

Total aerobic heterotropic Bacteria: $5x10^2$CFU/g
Total Fungal count : 3 CFU/g
Total enteric Bacteria : Nil
E. coli : Nil
Salmonella : Nil
Shigella : Nil

ACKNOWLEDGEMENT:

The authors thanks to the management of Srimad Andavan Arts and Science College for providing all kinds of facilities.

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