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Pharmacognostic validation of root of Hemidesmus indicus (Linn.) R.Br.

Shanthi. A*¹, R. Radha¹, N. Jayashree¹, R.Selvaraj²

¹Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai, Tamilnadu, India ²Annai Aravind Herbals, Chennai, TamilNadu, India

ABSTRACT

Hemidesmus indicus (Linn.) R.Br. Family: Asclepiadaceae, is commonly known as 'Indian sarasaparilla'. A perennial climber and growing widely in upper Gangetic plains and eastwards of Bengal and from central to southIndia. It is traditionally used for curing various ailments like stomach pain, fever, veneral disease, rheumatism and also act as a blood purifier. This traditionally useful part was standardized based on the macroscopic, microscopic, physico-chemical and chromatographic. And also estimated for its microbial limits, pesticides, Aflatoxin and heavy metals. Preliminary phytochemical screening confirms the presence of sapnonins, tannins, resins, essential oils, sterols and glycosides determination of total saponin content.

Keywords: Hemidesmus indicus, Asclepiadaceae, phytochemical screening, chromatographic identification, total saponin content estimation.

INTRODUCTION

In India, plants have been traditionally used for human and veterinary health care and medicinal plants and it also play a great role in food supplements for health care as well as in personal care of the mankind. Throughout the world, about 35,000-70,000 species of plant have been used at one time or another for medicinal, neutraceuticals and cosmetic purpose[1]. The drugs of plant origin especially of herbaceous nature are used as whole plant and are identified with their origin, common name, scientific nomenclature, family, geographical source, cultivation, collection, preservation, storage, macroscopy, microscopy, chemical composition, identity, purity, strength and assay, substitute and

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adulterants etc., The microscopic examination of root which includes Transverse section and Longitudinal section are made for identification [2]. Hemidesmus indicus is commonly known as 'Indian sarasaparilla' a perennial climber and growing widely in upper Gangetic plains and eastwards of Bengal and from central to south India[3]. The roots and woody portion has been used traditionally for curing various ailments like stomach pains, fever, veneral disease, rheumatism and also act as a blood purifier[4]. And it also posses antioxidant[5], antileprotic[6] effects. The present work is based on the standardization of Hemidesmus indicus root by pharmacognostically as per WHO guidelines. Although other species of this variety has been reported for its standards[7] this has been the complete pharmacognostic validation of this variety which may be used for formulation development in future

EXPERIMENTAL SECTION

1) Chemicals and reference drugs:[8]

All the chemicals used in this present work were analytical grade. The chromatographic estimation were done in the Asthagiri Herbal Research Foundation (Chennai).

2) **Collection of plant materials**:

The roots of Hemidesmus indicus were collected as a gift sample from the Annai Aravind Herbals, Chennai.

The sample was authenticated by Plant Anatomy Research Centre (PARC), Tambaram.

3) **Determination of foreign matter**:[9]

Weigh 100-500 g of the drug sample to be examined or the maximum quantity prescribed in the monograph, and spread it out in a thin layer. The foreign matter should be detected by inspection with the unaided eye or by the use of a lens (6x). Separate and weigh it and calculate the percentage present.

4) **Macroscopic evaluation**:[10]

For its macroscopy drugs can be identified entirely or its fragments for its sensory or organoleptic characters like colour, odour, taste, size, shape and other characters.

5) **Microscopic evaluation**:[11]

It deals with the microscopic appearance of the drug in section view and in powder form.

6) **Determination of total ash**:[12]

Incinerate about 2-3 g accurately weighed, of the ground drug in a tared platinum or silica dish at a temperature not exceeding 450° c until free form carbon, cool and weigh. If a carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ashless filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dryness, and ignite at a temperature not exceeding 450° . Calculate the percentage of ash with reference to the air-dried drug.

7) **Determination of** acid-insoluble ash:[13]

Boil the total ash obtained for 5 minute with 25ml of dilute hydrochloric acid; collect the insoluble matter in a Gooch crucible, or an ashless filter paper, wash with hot water and ignite to constant weight. Calculate the percentage of acid-insoluble ash with reference to the air dried drug.

8) Determination of water soluble ash:[14]

Boil the ash for 5 minutes with 25ml of water; collect insoluble matter in a Gooch crucible, or on an ashless filter paper, wash with hot water, and ignite for 15minutes at a temperature not exceeding 450° c. Substract the weight of the insoluble matter from the weight of the ash; the different in the weight represents the water-soluble ash. Calculate the percentage of water-soluble ash with reference to the air-dried drug.

9) Determination of alcohol soluble extractive :[15]

Macerate 5g of the air-dried drug, coarsely powdered, with 100ml of Alcohol of the specified strength in a closed flask for 24hrs, shaking frequently during 6hrs and allowing to stand for 18hrs. Filter rapidly, taking precautions against loss of solvent, evaporate 25ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105° to constant weight and weigh. Calculate the percentage of alcohol soluble extractive with reference to the air-dried drug.

10) Determination of water soluble extractive:[16]

Proceed as directed above for ethanol soluble extractive, using chloroform water instead of ethanol.

11) Determination of moisture content (loss on drying)[17]

Place about 10 g of the drug after accurately weighing it in a tared evaporating dish and dry at 105° c for 5 hrs, and weigh. Continue the drying and weighing at 1hr interval until difference between two successive weighing corresponds to not more than 0.25 percentage.

12) Quality parameters:

Limit test for heavy metals:[18]

The test for heavy metals is designed to determine the content of metallic impurities that are coloured by sulphide ion, under specified conditions. The limit for heavy metals is indicated in the individual monograph in terms of parts of lead per million of substance (by weight). It includes the limit test for Arsenic, Lead, Mercury, Cadmium etc.,

13) Pesticide residue:[19]

A pesticide is any substance or mixture of substance intended for preventing, destroying or controlling any pest. Chromatographic estimation can be used for detecting and quantifying the pesticide limits in the crude drug were detected.

14) Determination of microbial limits:[20]

Total microbial limits were detected for Total aerobic microbial count, Total bacterial count, Test for E.Coli, Salmonella species, P. aerugenosa, s. auruas and Aflatoxins.

15) Preliminary phytochemical screening:[21]

Preliminary phytochemical screening for alkaloids, glycosides, tannins, terpenoids, phenols, sterols, proteins, aminoacids, volatile oils, flavonoids, saponins, fixed oils, essential oils, coumarins, sugars and others.

16) Chemical identity:[22]

Chemical identification were done with the aid of chromatographic estimation with TLC and HPTLC. Solvent system used in this system were water :acetonitrile: methanol: ethylacetate: hexane (1.5:5.0:0.5:1.5:1.5) using CAMAG Linomat IV applicator using scanner II at the wave length of 245nm.

17) Quantitative estimation:[23]

Quantitative estimation of the active constituent were done with the determination for total saponin content in Hemidesmus indicus root.

By TLC method

To 0.5 g of the powdered drug (180) add 5 ml of a mixture of equal volumes of alcohol R and water R and heat to boiling. Centrifuge and use the supernatant liquid. Apply to the plate as bands 20 mm by 2 mm, $10 \,\mu$ l of each solution. Develop over a path of 10 cm using a mixture of 1 volume of glacial acetic acid R, 30 volumes of water R, 40 volumes of ethyl acetate R and 40 volumes of propanol R. Allow the plate to dry in air, spray with a 20 per cent V/V solution of nitric acid R and heat at 120 °C for 10 min. Allow to cool and spray with a 50 g/l solution of potassium hydroxide R in alcohol (50 per cent V/V) R until the zones appear spray with anisaldehyde solution the chromatogram

RESULTS

1. Determination of foreign matter:

Foreign matter were present in zero percentage (Limit NMT 2%)

2. Macroscopic evaluation:



Fig 1: Hemidesmus indicus root Colour - externally dark and internally yellowish brown. **Odour** - Vanillin like. Taste – Acrid Size – 0.5 to 2.0 cm in diameter. Shape – Cylindrical Extra features – Fracture outer: Short Inner: Fibrous.

3. Microscopic evaluation:

a. Transverse section:

It shows 3-15 layered cork (thick walled reddish brown), 2-3 rows of colourless phellogen, 1-2 rows of narrow thin-walled cells phelloderm, 2-3 layered thickwalled polygonal parenchymatous cells with starchgrains, prisms of calcium oxalate crystals.

Cortex – Wide, contains thinwalled, large tangentially elongated cells contains simple and compound starchgrains, prisms of calcium oxalate.

Phloem – Narrow, with sieve tubes, phloem parenchyma, companion cells and uniseriate medullary rays.

Cambium - Narrow, distinct.

Xylem Vessels - Scattered with pitted walls, tracheids, thickwalled fibres with uniseriate medullary rays.—



Fig 2: Transverse section of Hemidesmus indicus root.

b. Powder:

Colour – Light brown

Parenchyma – Fragments with starch grains and prisms.

Vessels – Pitted wall, lignified xylem fibres, tracheids, radially cut medullary rays contains starch grains and calcium oxalate prism, fragment of latex tubes and reddish brown cork cells.



Fig 3: Microscopy of Hemidesmus indicus root a. cork cell in transverse cut; b. cork cells in surface view c. cortical cells filled with starch grains; d. starch grains; e. tracheids; f. crystals; g. latex cells; h. reddish brown content cells; I. radially; j. xylem vessel; k. vessel; l.latex canel

4. Physical constants

Table No: 1

S. No	parameter	average	Limit
1	Loss on drying	2.1%	-
2	Total ash		NMT 4.3%
3	Acid insoluble ash	0.478%	NMT 0.5%
4	Alcohol soluble extractive	31.76%	NLT 15%
5	Water soluble extractive	44.93%	NLT 13%

5. Quality parameters:

Table No: 2

S.No	Metallic element	Report (PPM)	Standard limit (PPM)
1	Arsenic	NIL	NMT 20 PPM
2	Cadmium	NIL	NMT 20 PPM
3	Lead	LT 5PPM	NMT 20 PPM
4	Mercury	NIL	NMT 20 PPM

6.

Table No: 3

S.NO	PESTICIDE	REPORT
	RESIDUE	(EXTRACT)
1	DDT	ND
2	Benzene	ND
	hexachloride	
3	Aldrin	ND
4	Dieldrin	ND
5	Lindane	ND
6	Chloropyrophos	ND
7	Enosulphan	ND

ND – not detected (concentration less than the minimum detection limit even in ng/l units). From the results it can be concluded that the plant material is totally safe and there is no traceable limit of pesticide in them

7. Microbial determination:

Table No:4

S.No	Microbial strain	Microbial count	WHO Limit
1	Total bacterial count	10cfu/g	NMT 1000 cfu/g
2	Total plate count	5 cfu/g	NMT 100cfu/g
3	Yeast and moulds	Nil	Absent
4	E. Coli	Negative	Absent
5	Salmonella species	Negative	Absent
6	S. aureus	Negative	Absent
7	Aflatoxins	Nil	Nil

As per the WHO standards, the plant material is free from microbial load and safe for further use in formulation.

8. Preliminary phytochemical screening:

Table No: 5

S. No	Chemical constituents	Extract
1	Alkaloids	-
2	Anthroquinones	-
3	Bitters	-
4	Carbohydrates	-
5	Essential oils	+
6	Flavones	-
7	Gums	-
8	Glycosides	+
9	Lignans	-
10	Phenols	-
11	Proteins	-
12	Resins	+
13	Saponins	+
14	Steroids	+
15	Tannins	+
16	Terpenoids	-

+ = present, - = absent

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Fig 4: TLC finger print of Hemidesmus indicus root

9. Chemical identity:

The result obtained from the TLC studies showed the presence of the active constituent hemidesmin in the extract has similar rf values (nm). Spots obtained in fig which

shows its presence in extract. The rf values obtained were 0.04, 0.14, 0.16, 0.26, 0.35, 0.52, 0.7, 0.78, 0.88 and the rf values states that the root part contains nine active constituents.

10.Quantification:

The total saponin content of the hemidesmus indicus root was found to be about 0.835 mg/100 gm of the root powder by TLC method of quantification.

DISCUSSION

The macroscopic and the microscopic evaluation of any plant drug are considered to be the primary steps for establishing its quality control profile and according to WHO, botanical standards should be proposed as a protocol for the diagnosis of the herbal drug. The histochemical studies give a preliminary idea about the type of compounds and their accumulation in the plant tissues. Thus, helps us in selecting the particular part or tissue of that plant where the compounds of interest are located.

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