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Review Article

A Ready Reckoner Document Sheet on Roots of *Hemidesmus indicus* R.Br

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Abstract

Medicinal plant extracts are the main constituents of the innumerous ayurveda, siddha and homeopathy medicines that cure various ailments and also contribute to the general well being of mankind. Herbal medicines must contain standardized extracts for gaining market value and global acceptability. Existing standardization strategies involve documenting the physico-chemical, chemical and biological characteristics of authentic medicinal plants and their extracts.

A document sheet that provides all standardized data about a medicinal plant as a ready reckoner reference to herbal manufacturers will greatly contribute to preparing standardized herbal medicines with global acceptability. In view of this the present review to document the data on various standardization parameters reported for the medicinal plant *Hemidesmus indicus* (Nannari), a plant well known in Indian traditional system of medicine.

Keywords: *Hemidesmus indicus*; Fact Sheet; Herbal Formulation; Standardized Data; Ready Reckoner

Introduction

The name Hemidesmus is derived from the Latin word "Hemidesmos" which means 'half bond'. It is so named in allusion to sub connate filaments at their base-joints pods and connected stamens. The word "indicus" stands for "of India". Hemidesmus indicus belonging to the family of Apocynaceae, is the well known traditional medicinal plant distributed throughout India growing under mesophytic to semi dry conditions, plains and upto an altitude of 600m. It is quite common in open shrub jungles, hedges, and uncultivated soils. It also found in Sri Lanka, Pakistan, Iran, Bangladesh and Moluccas.

In spite of the folkloric use of the plant and its immense medicinal potential, there are only 300 reports of scientific work on Hemidesmus indicus. There is paucity of documents on standardization protocols for herbal formulations. In order to aid herbal formulation manufacturers to prepare formulations containing standardized extracts of roots of Hemidesmus indicus and to increase the global market value and acceptability of herbal medicines containing this medicinal plant, the present review document is prepared with special focus on roots of the plants since the roots find more medicinal and folkloric use.

Fact sheet

Template of documentation

To aid in ready reference, all data available in reports on scientific studies on roots of Hemidesmus indicus are presented based on the following template. All references to data and figures reported and are duly cited in this document sheet. The extractive value, spectral fingerprinting and elemental analysis of the root extracts reported in this review were done in our laboratory.

Scientific name	***Hemidesmus indicus* (L.) R.Br**
Family	*Apocyanaceae*
Vernacular names	Tamil - Nannari English - Indian sarsaparilla Hindi and Bengali - Anantamul, Kapuri Marati - Upalasari , uparasal Kannada - SogadeBeru Malayalam - Naruninti
Synonyms	Ananta, Asphota, Utpala sariva, Canadana
Distribution	The species is distributed throughout the tropical and sub- tropical especially in gangetic plains, Bengal, Madhya Pradesh and South India
Description and Habitat	It is a climber found throughout India. Leaves are 2.5" long and alternatively arranged in pairs. The woody roots are underground and are very aromatic. Commonly found in hedges and in waste places almost throughout India
Propagation	Propagated from stem and root stock cutting obtained from more than one year old plant [1] Through auxiliary bud culture [2] Through organogenesis and somatic embryogenesis [3]
Constituents reported Source - A recent review 'Some Aspects of Investigation of the Indian Medicinal plant *Hemidesmus indicus* (R.Br) Chemical Constituents and Anti-Diabetic Activity' [Banerji *et al.,*2017]	Flowers- flavonoids-hyperoside, isoquercetin, rutin [4] Leaves- hyperoside, rutin, hemidesmine, hemidesmin-1, hemidesmin-2 [4,5,6] Stem- indicine and hemidine [7] hemidescine, and hemidine [8].demicunine, heminine,mesidesmine [9,10] Roots- hemidesmol,resin, glucoside [11] Lupeol, α- amyrin, β-amyrin, β-sitosterol [12] Lupeol, α-amyrin, lupeol acetate, β-amyrin acetate, hexatriconate acid, lupeol octacosonate [13] Coumarinolignoid-hemidesminine,hemidemin-1, hemidesmine-2 [14] Root oil- hemidesmol, hemidesterol, 2-hydroxy 4-methoxy benzaldehyde [15] α-amyrin triterpenes, β-amyrin and benzaldehyde, 2-hydroxy 4-methoxy benzenoid [16] 2-hydroxy 4-methoxy benzaldehyde [17] Twigs- desinine [18]
Compounds quantified	Lupeol Octacosanate, 2-hydroxy 4-methoxy benzoic acid, 2-hydroxy 4-methoxy benzaldehyde, ferulic acid, isovanillin
Bioactive markers	2-hydroxy 4-methoxy benzoic acid- anti viper venom and anti-inflammatory [19,20] Lupeol acetate-anti-serum against albino mice [21] β-amyrin palmitate- anti-diabetic in rats [22]
Ethnobotanical reports	As treatment for scorpion sting and snake [23] As bite blood purifier [24], As cooling agent [25]
Parts mostly used in medicinal preparations	Roots
Pharmacognostical investigation	Microscopic studies on root [26,27] The highest concentration of glycosides, flavonoids, tannins and sterols in the entire plant during summer [23] Macroscopic and microscopic studies on root [28]
Dosage and safety aspects extracts	Water decoction 50-100 ml; Root paste 5g; Root powder 1-3 g
Mention in database	Indian Pharmacopoeia [29] Indian Systems of Medicine [30] British Pharmacopoeia [31] Dravya database [32]
History	Dates back to the 18thcentury when it was discovered by Linneous and Robert brown

Table 1: Fact sheet on *Hemidesmus indicus.*

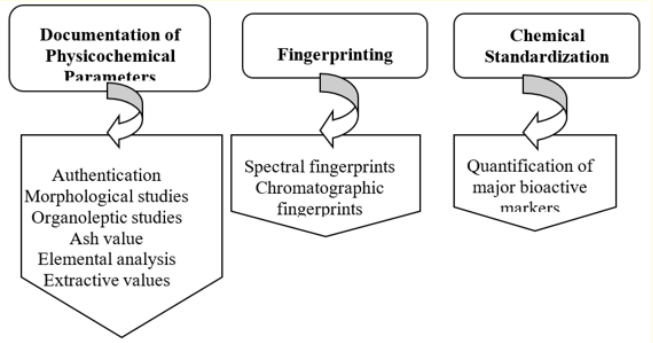

Figure a

Documentation of physicochemical parameters

Physicochemical characteristics of a plant material will aid as a reference in selecting authentic plant material for herbal formulation. Data for ready reference available in reports [33-36] on the pharmacognostic parameters of tuberous roots of Hemidesmus indicus such as morphological characteristics, organoleptic studies, ash value, elemental analysis and extractive values are reviewed and documented in this section. The bracketed numeral numbers indicate the references cited for these details.

Morphological characteristics [33-36]

Morphological characteristics of a plant provide first hand information of the authenticity of the plant. It is the first data referred to by researchers when selecting a plant for preparing herbal formulation. Macroscopic and microscopic characteristics of Hemidesmus indicus (H. indicus) root is reported with morphological characteristics as below [33].

- Length: 1 mm or more
- Width: 1-2 cms,
- Shape: Cylindrical
- Colour: Brown
- Branching: Sparsely branched
- Rootlets: Thin and wiry
- Direction of growth: Vertical
- Surface Characters: Tuberous root is dark tortuous with transversely cracked and longitudinally fissured bark, cork: thin, separates easily and peels off in flakes,
- Fracture and texture: Short, splintery
- Odour: Agreeable
- Taste: Sweetish, slightly acidic and aromatic.

Micrometrical measurement of cell/tissues of H. indicus roots [33-36]

Micrometrical measurements

Cells/Tissues	Size in Microns
Cork cells Phellogen cells	40-80 x 15-25
Parenchyma cells Xylem	40-80 x 12-201
vessels Tracheids	140-180 x 30-49
Starch grains	48-75
	28-40
	6-32

Table 2: Micrometrical Measurement of cell/tissues of *H. indicus* roots [33].

Organoleptic characteristics [35]

The color, odor and taste of a medicinal plant are organoleptic characteristics that supplement the authenticity of the plant material. Roots of H. indicus are brownish yellow in color with a pungent and pleasantly aromatic odor. It tastes bitter [35].

Behavior of root powder of H. indicus with chemical reagents

Table 3 provides the visible coloration developed when root powder is treated with chemical reagents [33]. These observations aid in selecting authentic sample of the roots of this plant.

Reagent Added to Plant Powder/	Color Observed
Powder as such	Creamy brown
Picric acid (saturated solution)	Yellowish brown
Nitric acid (sp.gr.1.42)	Brownish yellow
Hydrochloric acid(sp.gr.1.16)	Dark brown
Acetic acid (glacial)	Creamy brown
Sodium hydroxide (5N) (aqueous) Ferric chloride (5% aqueous)	Pale Yellowish brown
Ferric chloride (5% aqueous)	Pale yellow
Iodine solution (aqueous)	Blackish Yellow
Antimony trichloride (5% aqueous)	Yellowish brown

Table 3: Behavior of Root Powder [33].

Ash value [33]

The proximate parameters of a plant material indicate the nature of the plant constituents. The total ash value of a plant material indicates the amount of minerals and earthy materials in the plant material. The amount of acid insoluble ash indicates the amount of silacious matter in the plant material. The alcohol soluble extractive value indicates the presence of constituents such as flavonoids, alkaloids, steroids and their glycosides; Water soluble extractive value indicates the presence of sugars, acids and inorganic components of a plant material. The proximate parameters as reported [33] for the tuberous roots of H. indicus indicate that the roots possess a higher percentage of water soluble constituents (18.6-18.8% w/w). Being a root material, the acid insoluble ash (15.5-18.8% w/w) was found to be higher.

Extractive value [33,37]

The extractive values obtained by extraction of the roots with various solvents indicate a higher extractive value with of water. The data reported are compared in table 4.

Extract	% Extractive value			
	Soxhlet Extraction			Cold Extraction
	[37]	[33]	Data reported from our lab	Data reported from our lab
Hexane	-	-	4.5	0.1
Petroleum ether	-	3.26	-	-
Ether	0.47	-	-	-
Benzene	0.39	0.32	-	-
Chloroform	0.41	0.62	-	2.4
Acetone	-	-	-	2.9
Ethyl acetate	-	-	1.4	1.6
Ethanol	12.34	-	-	6.3
Ethanol (90%)			6.2	7.2
Methanol (90%)	-	8.12	-	-
Water	9.70	4.66	-	9.3

Table 4: Percentage Extractive Value for *H. indicus* Roots.

Elemental analysis

Trace elements present in the methanol extract of roots of H. indicus was analyzed by Atomic Absorption Spectroscopy as reported [38]. The percentage of elements present in the hexane and hydro ethanol extract (90:10 ethanol -water mixture) of roots of H. indicus was analyzed by energy dispersive X-Ray analysis in our laboratory (Instrument make: MIRA3 TESCAN). The non-polar hexane extract is found to contain almost equal elemental percentage of carbon and oxygen in addition to the elements Mg, Fe, and Ca. This is indicative of the presence of oxygenated low polar molecules in the roots to a greater extent. The respective energy dispersive X-Ray Spectra depict this. Table 6 gives the weight percentage of elements present

Figure 1: Energy Dispersive X-Ray Spectra of hexane extract of roots.

Figure 2: Energy Dispersive X-Ray Spectra of hydroethanol extract of roots.

Hexane Extract		Hydro ethanol Extract	Methanol Extract [39]
Elements	**Weight (%)**	**Weight (%)**	**Weight (%)**
C	45.35	81.28	-
O	44.04	16.89	-
Mg	1.06	-	-
Si	1.59	0.35	-
Cu	2.63	-	0.82
K	2.46	0.80	-
Ca	0.47	-	-
Fe	2.40	-	10.89
Al	-	0.68	-
Zn	-	-	2.75
Mn	-	-	1.14

Table 5: Percentage of Elements Detected in Root Extracts.

Fingerprinting of Extracts

Spectral fingerprinting

Spectral fingerprints can be relied upon in assessing the authenticity of a plant. The UV, IR and NMR spectral fingerprints of hydro ethanol extract (90:10 ethanol-water mixture) of roots of H. indicus are presented below and reported from our laboratory [39].

UV-Visible spectral fingerprint

UV-Visible spectral fingerprints (Figure 3 and 4) were recorded in a double beam UV-Visible double beam spectrophotometer-Systronics (AU-2701).

Figure 3: UV- Visible fingerprint of hexane extract of roots.

Figure 4: UV- Visible fingerprint of hydro ethanol extract of roots.

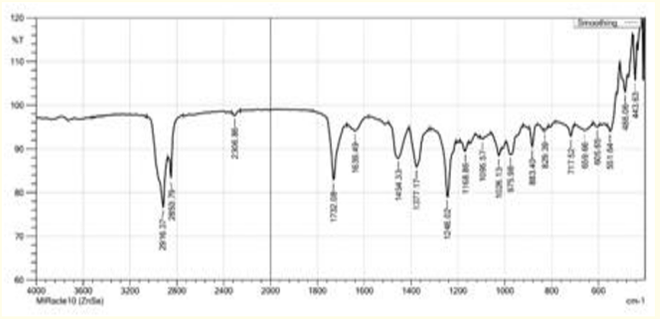

Figure 5: FT-IR Fingerprint of hexane extract of roots.

Figure 6: FT-IR Fingerprint of hydro ethanol extract of roots.

FT-IR spectral fingerprint

IR spectral fingerprints (Figure 5 and 6) were recorded in Shimadzu FT-IR spectrometer. Spectrum in the range 4000 to 750 cm^{-1}.

The IR fingerprint of the hexane extract shows prominent absorptions at 2916, 2851, 1732,1454,1377 and 1246 cm^{-1}.

The absorptions at 2916 and 2851 cm^{-1} due to saturated C-C stretch are indicative of the saturated steroidal and terpenoidal molecules in H. indicus whereas the peaks at 1732, 1454 and 1377 cm^{-1} may be representative of triglyceride esters which are constituent molecules of most plants. The hydro ethanol extract reveals prominent IR peaks at 3298 and 1033 cm^{-1} which are indicative of hydroxyl and glycosidic moieties in the metabolites.

NMR spectral fingerprint

The ^{1}H NMR spectral fingerprints (Figure 7 and 8) were recorded in Bruker 400 MHz proton nuclear magnetic resonance spectrometer at room temperature. The characteristic peak at 11.98 and 11.68 ppm may correspond to the carboxylic group and peaks at 7.6 and 6.6 ppm may correspond to the aromatic metabolites. Peaks between 0.8 - 2.0 ppm indicate aliphatic absorptions. It may be recalled that the aroma compound identified as 2-hydroxy 4-methoxy benzoic acid, the terpenoidal lupeol acetate and lupeol octacosanoate and ferulic acid have been reported as biomarkers of Hemidesmus indicus apart from flavonoids hyperoside, isoquercitin, rutin and numerous steroids and their glycosides as reported in the Treatise on Indian Medicinal Plants (Volume 4, 1996).

Figure 7: NMR fingerprint of hexane extract of roots.

Figure 8: NMR fingerprint of hydro ethanol extract of roots.

Qualitative phytochemical analysis

Qualitative phytochemical color tests carried out for the extracts revealed the presence of terpenoids, steroids and flavonoids predominantly. Of particular mention in the appearance of a bright rose red colored solution in Shinoda test (Figure 9) for the hydro ethanol extract [39].

Figure 9: Rose red colored solution in Schinoda test for the hydro ethanol extract.

Chromatographic fingerprinting

HPTLC fingerprinting

HPTLC fingerprints of various solvent extracts of roots of H. indicus developed under different solvent systems are reported by four groups of researchers [40-43]. The protocols adopted are indicated and the fingerprints reported are presented as figures 10-16. The chromatograms reported in literature are documented here. The relevant references are cited below the figures.

HPTLC protocol 1 [40,41]

System: Camag HPTLC system

Applicator: Linomat V applicator

Scanner: Camag TLC scanner 3

Software: WinCATS- 4

Adsorbent: Pre-coated silica gel sheets 60F254

Solvent system: Not mentioned.

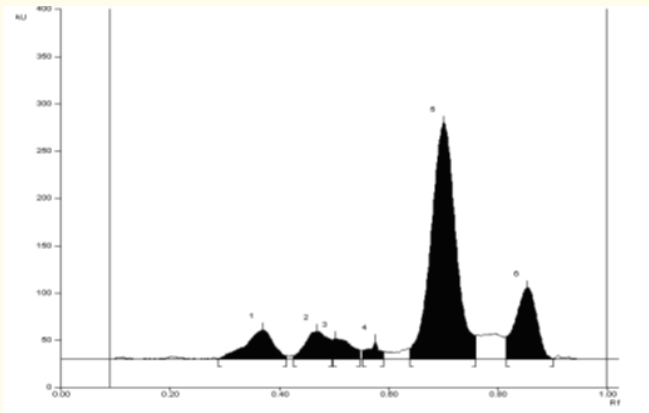

Figure 10: HPTLC fingerprint of methanol extract of *H. indicus* roots screened for steroidal saponins smilagenin and sarsapogenin at 366 nm [40].

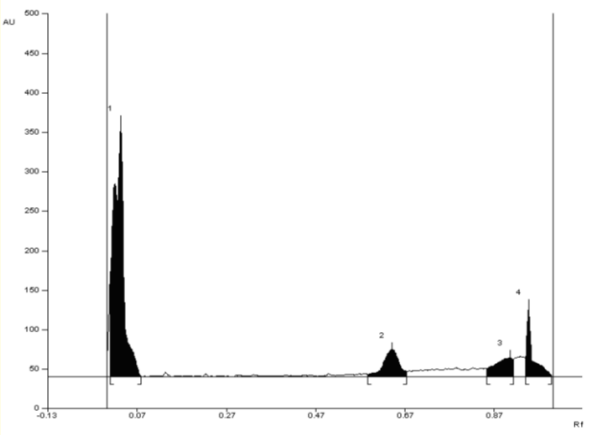

Figure 11: HPTLC fingerprint of methanol extract for *H. indicus* root screened for phenolic compounds and tannins at 366 nm [40].

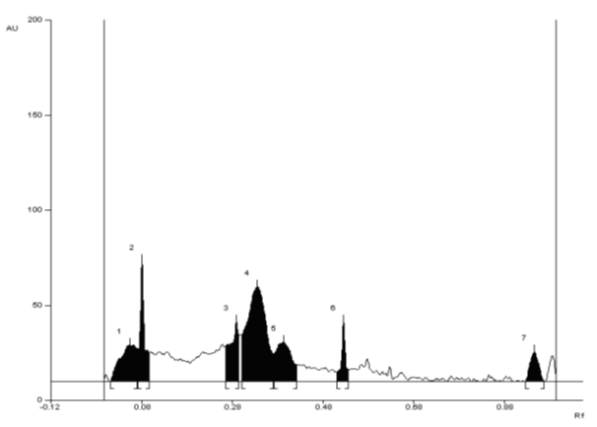

Figure 12: HPTLC fingerprint of aqueous extract of *H. indicus* roots screened for coumarins at 366 nm [40].

HPTLC protocol 2 [42]

System: Camag HPTLC system

Applicator: Linomat 5 applicator

Scanner: Camag TLC scanner

Adsorbent: Pre-coated silica gel sheets 60F254

Solvent System: chloroform: methanol: Ethyl acetate (13:1:2).

By adopting the above protocol, HPTLC fingerprinting of methanolic extract of roots of H. indicus was done. The study proposed the presence of nine peaks in the chromatogram scanned at 254nm and four peaks at 366 nm. The HPTLC fingerprints have been documented [42].

HPTLC protocol 3 [43]

System: Camag HPTLC system

Applicator: Linomat 5 applicator

Scanner: Camag TLC scanner 4

Software: WinCATS- 4

Adsorbent: Pre-coated silica gel sheets 60F254

Solvent System: Toluene: Ethyl acetate (5:1.5).

The chloroform and ethanol extract of roots of H. indicus was fingerprinted by HPTLC by protocol 3 [43]. The chloroform extract showed seven minor bands and three major bands in the HPTLC chromatogram scanned at 254nm; six bands were revealed at 366nm. The ethanol extract expressed four bands at 254 nm while at 366nm the intensity of the bands was reduced. The respective HPTLC fingerprints have been documented by the authors [43] and will serve identification and authentication of roots H. indicus.

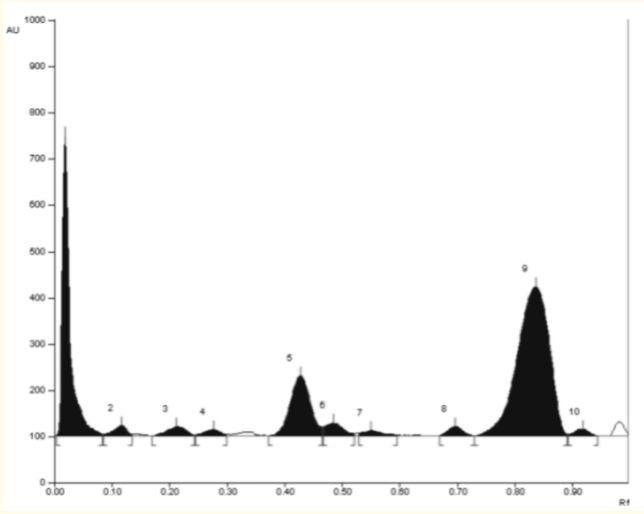

Figure 13: HPTLC fingerprint of chloroform extract of *H. indicus* roots at 254 nm [43].

Figure 14: HPTLC fingerprint of ethanol extract of *H. indicus* roots at 254 nm [43].

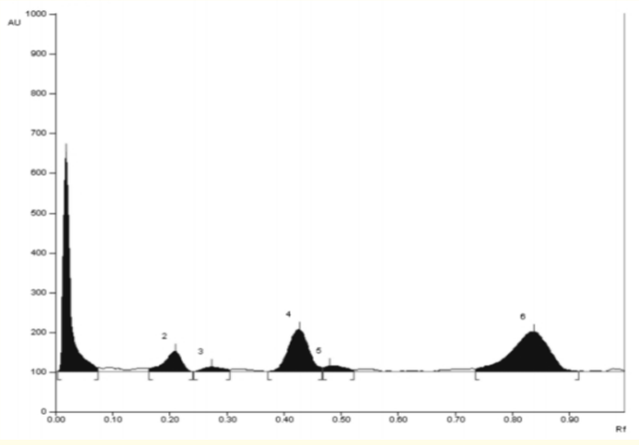

Figure 15: HPTLC fingerprint of chloroform extract of *H. indicus* roots at 366 nm [43].

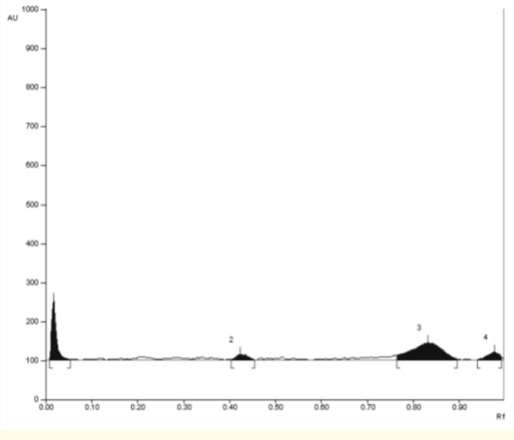

Figure 16: HPTLC fingerprint of ethanol extract of *H. indicus* roots at 366 nm [43].

Quantification of marker compounds

A total of ten review papers are available on this plant. Two of these reviews [44,45] report the isolation of compounds. The HPLC and HPTLC quantification protocols reported for the isolated compounds - 2-hydroxy-4-methoxybenzaldehyde, 2-hydroxy-4-methoxy benzoic acid, 3-hydroxy-4-methoxybenzaldehyde (isovanillin), ferulic acid and lupeol octacosante and the percentage weights of the compounds are documented in this section. The relevant references are cited below the figures. These data will be of much relevance to herbal formulation manufacturers in preparing standardized formulations of roots of Hemidesmus indicus containing a known quantity of the marker or biomarker compounds and hence reviewed in this document.

Quantification of 2-hydroxy-4-methoxybenzaldehyde and 2-hydroxy-4-methoxy benzoic acid by HPLC

HPLC protocol 2-hydroxy-4-methoxybenzaldehyde and 2-hydroxy-4-methxy benzoic acid [46]

System: JASCO HPLC system

Applicator: PU-2080 plus pump and a PU-2075 plus UV–Vis detector

Separation: Waters Symmetry C18 reversed phase column

Software: Data Apex Clarity

Adsorbent: C18 guard column

Solvent System: 1 mM aqueous TFA: methanol (70:30).

Quantification of the marker molecules 2-hydroxy-4-methoxybenzaldehyde and 2- hydroxy-4-methoxybenzoic acid in the young and mature roots of H. indicus according the HPLC protocol developed [46], indicated higher quantity of 2-hydroxy-4-methoxybenzaldehyde as tabulated in table 6 below.

Sample	2-hydroxy-4-methoxy benzaldehyde (mg g-1 dry weight)	2-hydroxy-4-methoxy benzoic acid (mg g-1 dry weight)
Young roots	2.6 ± 0.3	0.25 ± 0.09
Mature roots	3.2 ± 0.2	0.80 ± 0.1

Table 6: Quantity of 2-hydroxy-4-methoxybenzaldehyde and 2-hydroxy-4-methoxy benzoic acid in *H. indicus* roots [46].

Quantification of 2-hydroxy 4-methoxy benzaldehyde by HPTLC method

HPTLC protocol for 2-hydroxy 4-methoxy benzaldehyde [47]

System: Camag HPTLC system

Applicator: Linomat 5 applicator

Scanner: Camag TLC scanner 3

Software: WinCATS

Adsorbent: Pre-coated silica gel sheets 60F254

Solvent System: Toluene: ethyl acetate: glacial acetic acid (7:2:1).

The amount of 2-hydroxy 4-methoxy benzaldehyde present in the roots of H. indicus was estimated by the validated HPTLC method. Content of 2-hydroxy 4-methoxy benzaldehyde found in dry powder and hexane extract of root powder was 2.993 and 7.578 mg/g respectively [47].

Quantification of Isovanillin (3-hydroxy 4-methoxy benzaldehyde) by HPTLC method

HPTLC protocol for isovanillin [48]

System: Camag HPTLC system

Applicator: Linomat 5 applicator

Scanner: Camag TLC scanner 3

Software: WinCATS

Adsorbent: Pre-coated silica gel sheets 60F254

Solvent System: Toluene: ethyl acetate: acetic acid: methanol (7.5:1.5:0.5:0.5).

Isovanillin content in H. indicus was estimated by a validated HPTLC method in the root extract and a marketed formulation as 0.40%, and 0.14% respectively [48].

Quantification of ferulic acid by HPTLC method

HPTLC protocol for ferulic acid [49]

System: CAMAG (Switzerland)

Applicator: Linomat 5 applicator

Scanner: Camag TLC scanner 3

Software: WinCATs Software

Adsorbent: Pre-coated silica gel sheets 60 F254

Solvent System: Toluene: Ethyl acetate: Formic acid (5:5:0.2).

The amount of the ferulic acid in the roots of H. indicus was estimated by a validated HPTLC method. Content of ferulic acid in ethyl acetate extract of root powder was 1.694 % w/w [49].

Quantification of Lupeol octacosanote by HPTLC

HPTLC protocol for lupeol octacosanote [47]

System: Camag HPTLC system

Applicator: Linomat 5 applicator

Scanner: Camag TLC scanner 3

Software: WinCATS

Adsorbent: Pre-coated silica gel sheets 60F254

Solvent System: Isopropyl alcohol: n-butanol (1:1).

The amount of Lupeol octacosanate present in the roots of H. indicus was estimated by a validated HPTLC method. Content of lupeol octacosanate reported in petroleum ether extract of root powder is 36.5 mg/gm [47].

The above chromatographic protocols may be referred to for estimating the content of these marker compounds in a given quantity of the chosen plant extract. This will be of valuable information in labeling herbal formulations containing roots of Hemidesmus indicus.

Conclusion

This communication is a ready reckoner documentation sheet prepared through review of scientific data available in literature on roots of the medicinal plant Hemidesmus indicus (since the year 1952) and intended to aid herbal medicine formulators. Standardization of herbal formulations containing extracts of medicinal plants is of utmost importance especially in quantifying the chemical constituents in the extracts. Authentic and quantified extracts of medicinal plants will increase their market value and acceptance in the global market. This data sheet on Hemidesmus indicus will be an easy reference for this purpose. Though numerous small molecules and secondary metabolites have been isolated and characterized from this plant, the markers validated and quantified are the aroma molecules vanillin and isovanillin, ferulic acid and the anti snake venom marker 2-hydroxy-4- methoxy benzoic acid. Lupeol octacosanate is also quantified. An in silico approach carried out with a number of compounds of this plant with aldose reductase as the target protein, indicated top scoring compounds as vanillin, 2-hydroxy-4-methoxy benzaldehyde, hyperoside, isoquercetin, p-methoxysalicylic acid and the wet lab activity studies validate this especially the contribution of 2-hydroxy-4-methoxybenzoic

acid and isovanillin. Hence these molecules may be relied upon as biomarkers in extracts of roots of Hemidesmus indicus. All data cited in this review documentation are duly cited. Credit goes to the contributor authors for providing data on the valuable medicinal plant Hemidesmus indicus for preparing standardized extracts.

Conflict of Interest

None.

Acknowledgments

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None.

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