Phyto-pharmacology of Hemidesmus indicus

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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 compounds. Hemidesmus indicus is one of the plants used in Ayurveda for several remedies. It is used as a tonic, alternative, demulcent, diaphoretic, and diuretic, in the treatment of syphilis, chronic rheumatism and urinary disorders. Scientific evidence suggests its versatile biological functions that support its traditional use in the orient. This review aims to highlight the ethnobotany, pharmacognostic and pharmacological uses of Hemidesmus indicus

KEY WORDS: Hemidesmus indicus, phytochemical constituents, pharmacological actions, toxicity.

INTRODUCTION
Ayurveda is a traditional system of medicine using a wide range of modalities to create health and well being. The primary aim of Ayurveda health care is to restore the physical mental and emotional balance in patients, thereby improving health, preventing disease and also treating any current illness. The number of patients seeking alternate and herbal therapy is growing exponentially. Herbal medicines are now in great demand in the developing world for primary healthcare not because they are inexpensive but also for better cultural acceptability, better compatibility with the human body and minimal side effects. Herbal medicine is still the mainstay of about 75-80% of the world population, mainly in the developing countries for primary healthcare (Kamboj 2000). However among the estimated 250,000-400,000 plant species, only 6% have been studied for biological activity, and about 15% have been investigated phytochemically (1, 2). Therefore it seems necessary to evaluate the herbs properly.

General Information
Hemidesmus indicus Linn. R. Br. (Fam. Asclepiadaceae) is a prostrate or semi-erect shrub found throughout India from upper Gangetic plains eastwards to Assam, throughout Central, Western and Southern India up to an elevation of 600m. Hemidesmus indicus Linn. belongs to a family Asclepiadaceae is commonly found throughout all parts of India. It is known as Ananta and Sariva in Sanskrit, Upalsari in Gujarati, Anantamula in Hindi and Indian sarsaparilla in English. It is widely used as tonic, demulcent, diaphoretic, diuretic and blood purifier. The plant is being used against syphilis, leucorhoea, bronchitis, chronic rheumatism, urinary diseases, leprosy, leucoderma and skin diseases, and as purgative, diaphoretic, diuretic, antipyretic and anti-diarrheal in folk medicines (3). The plant is used against diseases of blood, inflammation, diabetes, 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 etc. (4). It has also been used in combination with other drugs for snake bite (5).

Therapeutic Uses Mentioned In Ayurvedic Pharmacopoeia
The plant is used in Aruci (aversion from food), agnimandya (loss of appetite) and atisara (Diarrhoea), kasa (cough), svasa (asthma), kandu (itching), kustha (leprosy), jvara (pyrexia) and rakta-vikara (blood disorders) (6).

Therapeutic Uses As Depicted By Ethnobotanical Studies
The plant is used in traditional medicine in biliousness, blood diseases, 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 (7,8). Roots of Hemidesmus indicus R.Br. is locally named as Nannari in Kouthalai of Tamil Nadu. The powder of root along with fruit of few other plants (Calophyllum inophyllum, Diospyros ebenum, Terminalia chebula, Terminalia bellirica and Phyllanthus emblica) and honey is taken to increase the semen production. The major chemical constituents are Coumarin, hemidesmine, emidine, hemidesine and rutin etc. (9). H. indicus is used to cure 34 types of diseases therefore it is in the rising demand, the required quantity is 1.2 tonnes/annum but this species is in short supply (10). H. indicus is becoming rare and endangered species, its local name is sogada, main chemical constituents are hemidesine, emidine and mainly used as antileprotic (11).

Pharmacognostical Studies
The transverse section (T.S) of rhizome shows cork 4-14 layers of lignified cells containing oil globules, parenchymatous cortex containing numerous starch grains, oil globules and yellowish brown substance; single layered endodermis, pericycle parenchymatous within it 12-18 collateral vascular bundles, separated by dark medullary rays, pith large parenchymatous containing starch grains individual being 7 •
30µ, mostly 10 - 25µ in diameter. T.S of stolon shows 2-5 layers of cork, cortex upto 25 layers, parenchymatous followed by 20 collateral vascular bundles, which in young stolons separated by cellulosic parenchymatous medullary rays and in older stolons become lignified, pit wide and lacunar. T.S of root shows small, central parenchymatous pith surrounded by tetrach to polych xylem and a wide parenchymatous bark (6).

**PHYTOCHEMISTRY**

The plant is reported to contain significant amount of rutin in leaves (11), steroids in cultured tissues and mature plant (12). The plant is employed in traditional medicine for gastric ailments (8) and mainly consists of essential oils and phytosterols like hemidesmol, hemidesterol and saponins (13). A new ester identified as lupeol octacosanoate in addition to the known compounds viz., lupeol, -amyrin, lupeol acetate, -amyrin acetate and hexatriaccontane (pioneer herbs 2005, website). Coumarins, triterpenoid saponins, essential oil, starch, tannic acid, triterpenoid saponins are present (14). A stearopten smilasperic acid is also obtained by distillation with water (15). Hemindicusin was isolated from CHCl3: EtOH (3:2) fraction of *H. indicus* R.Br. by using modern spectroscopic techniques and chemical transformations. The structure of this compound was assigned as calogenin-3-o-3-o-methyl-α-L-rhamnopyranoside (16).

Leaves: 2.5% tannins is present in leaves

Stem: Two novel pregnane oligoglycosides demicunine and heminine from CHCl3: EtOH (3:2) soluble extract of dried stems of *H. indicus* (17). Desinine (18), Indicine, Hemidine (19), Indicusin (20), Hemidescine, Ermidine (21), Medidesmine, Hemisine and Demicine (22) have been reported in *Hemidesmus indicus*.

Flowers: The flavanoid glycosides in flowers are hyperoside, isoquercitin and rutin whereas in leaves only hyperoside and rutin were identified (23).

Root: Essential oil and triterpenoids are present in roots (24). The phytochemical studies on the roots of *Hemidesmus indicus* resulted in the isolation of one each of acyclic triterpenic acid; acyclic diterpenic ester and monocyclic sesterterpenic ester and their structures have been established as 2; 6, 10, 14, 18, 22-hexamethyl tetracos-1 oic acid; n-octyl-2, 6, 10, 14-tetramethyl hexadec-7-ol-10-en-13-on-1-octanoate and n-non-2′-en-1′-yl, -13 (15, 19, 19-trimethyl-cyclohex-14, 16-dienyl) -2, 6, 10-trimethyl-tetradec-6-ol-13-on-1-oate along with known β-sitosteryl glucuronate and β-sitosterol, on the basis of spectral data analyses and chemical means (25). The aqueous-ethanolic root extract is reported to contain alkaloids, tannins, phenols and saponins (26). p-methoxy salicylic acid is reported to be present in aqueous extract of roots of *H. indicus* (27). The ethanolic extract of root is reported to contain triterpenes, flavonoids, tannins, coumarins and glycosides (28). Roots are reported to contain sitosterol (29). The quantitative analysis was done on roots of *H. indicus* for saponins and tannins and showed 0.6% and 3.0% respectively and the qualitative analysis showed presence of carbohydrates, saponins, phytosterols, phenols, flavonoids, terpenoids, tannins and phlobatannins (30). Amongst the plethora of chemical entities reported, a small molecular weight aromatic compound, 2-hydroxy 4-methoxybenzoic acid (HMBA), has caught the attention as the bio-active principle of *H. indicus*. A reverse phase HPLC method was developed and validated for the simultaneous determination of 2-hydroxy-4-methoxybenzoaldehyde and 2-hydroxy-4-methoxybenzoic acid in root extracts of *H. indicus* (31).
PHARMACOLOGY

**Anti inflammatory activity**
Inflammation induced by Viper venom and *Propionobacterium* acne are reported to be treated by root extract possibly by reducing reactive oxygen species and inflammatory cytokines IL8 and TNFα(32). A paste of root powder applied topically is used to treat swellings, inflammation and chronic rheumatism (29, 33, 34). The ethanolic extracts of roots also reported to show significant anti-nociceptive effects in mice and the effects were studied by inducing pain through acetic acid (writhing test), formalin (Paw licking test) and hot plate test in mice. The dose was administered orally in dose range of 25, 50 and 100mg/kg. The extract showed dose-dependent anti-nociceptive effect in all models for anti-nociception and it blocked the neurogenic and inflammatory pain (35). The methanolic extracts of roots possess potential dose-dependent anti-inflammatory and anti-pyretic activity (36). However, at molecular level, the most compelling evidence so far is its ability to inhibit the binding of transcription factor the nuclear factor kappaB (NF-κB) to DNA at low concentrations (37). Using electrophoretic mobility shift assay, the investigators elegantly prove the potential use of this extract.

**Anti - microbial activity**
Aqueous extract of roots of the plant exhibited bacteriostatic activity in mice infected with *Mycobacterium leprae*. P-methoxy salicylic aldehyde present in the extract was considered to be responsible for the activity (27). Essential oil of *H. indicus* exhibited marked antibacterial activity against both gram positive and gram negative bacteria even at concentration of 0.2%. The oil however failed to show appreciable antifungal activity against fungi tested (38). Chloroform and ethanol (95%) extracts of *H. indicus* showed antifungal activity against *A. niger* (39). The methanolic extract of root was proved to possess anti-diarrhoeal activity in in-vivo and in-vitro studies (40). The chloroform and methanol extracts of *H. indicus* root have demonstrated potent antitubercular activity, the presence of minerals in methanol extract might supplement the antidiarrhoeal activity of this plant therefore it can be used as a complementary alternative medicine for antibiotics or as a supplement to antibiotics to treat diarrhea and other food-borne diseases caused by multidrug resistant strains and seems to be an interesting and effective remedy for Salmonellosis and also for other forms of gastroenteritis (41). The glycosides of *H. indicus* roots inhibited *S. typhimurium* induced pathogenesis non-specifically, by reducing bacterial surface hydrophobicity and perhaps also by mimicking host cell receptors, thereby blocking its attachment to host cell and further pathological effects (42). The ethanolic extract of *H. indicus* stem showed broad spectrum antibacterial activity against all Anti-methicillin-resistant *Staphylococcus aureus* (MRSA) and the MSSA strains with inhibition zone size of 11-44mm. The antibacterial activity was maximum with acitone fraction. Similarly, the synergistic interaction was also evaluated with certain antibiotics like β-lactam antibiotics. *H. indicus* alongwith extracts of *A. calamus* and *P. zeylenica* showed synergism with cefuroxime (43, 44).

**Anti-ulcerogenic activity**
The aqueous ethanolic extracts of roots of *Hemidesmus indicus var. indicus* was screened for bio-chemical studies in anti-ulcer activity on animal models in winstar strain albino rats. The roots collected during flowering and vegetative periods showed anti-ulcer activities and significantly reduced the formation of gastric and duodenal lesions in rats induced by various ulcerogenic procedures and cyto-destructing agent. It has muco-protective activity by selectively increasing prostaglandin (26). Therefore it provides another alternative for ulcer treatment. It aims at enhancing the defensive factors so that the normal balance between offensive and defensive factors is achieved (45).

**Anti hyperlipidemic activity**
The cell culture extract of *H. indicus* was studied in normal and hypercholesterolemic rats for various lipid profile in serum, tissues and fecal matter. Alongwith the atherogenic diet cell culture *H. indicus* lowered the levels of serum, tissue and fecal lipid levels (46). Recent in vivo studies show that 2-hydroxy 4-methoxy benzoic acid (H MBA) is the bioactive molecule using ethanol-induced experimental model of hyperlipedemia. Oral treatment of H MBA at 200μgkg⁻¹ was
shown to significantly reduce the ethanol-induced elevated plasma and hepatic levels of total cholesterol, triglycerides, lipoproteins, phospholipids and free fatty acids in rats. This was accompanied by elevated lipoprotein lipase (47).

**Otoprotective activity**
The 80% ethanolic extract of *H.indicus* roots was studied for its otoprotective effects in ex-vivo rat organotypic model of gentamicin toxicity. In organ of Corti organotypic cultures (OC), gentamicin can induce a fast dose-dependent apoptosis of hair cells (HC), both external and internal. The results showed that after the coadministration of gentamicin and *H.indicus* to organotypic cultures, the extract was able to significantly counteract this toxic effect on hair cells at the concentration of 25 and 50µg/ml. The studies suggest that the otoprotective activity was not merely due to non-specific inhibition of gentamicin entry but from specific inhibition of the gentamycin induced apoptosis (48).

**Antioxidant and Antithrombotic activity**
The screening of antioxidant activity of *H.indicus* has revealed its capacity to scavenge the superoxide and hydroxyl radicals at low concentrations. The methanolic extract of *H.indicus* roots was found to inhibit lipid peroxidation with IC<sub>50</sub> of 217.9µg/ml and inhibit superoxide radical and hydroxyl radicals with IC<sub>50</sub> of 73.5 and 6.3µg/ml respectively. Furthermore, the root extract was found to scavenge the superoxide generated by photoreduction of riboflavin. Although, the study does not identify specific bioactive, the activity seems to stem from fraction containing flavanoids, terpenoids, polyphenols and coumarins. This plant also showed that after the coadministration of gentamicin and *H.indicus* roots was found to inhibit lipid peroxidation with IC<sub>50</sub> of 217.9µg/ml and inhibit superoxide radical and hydroxyl radicals with IC<sub>50</sub> of 73.5 and 6.3µg/ml respectively. Furthermore, the root extract was found to scavenge the superoxide generated by photoreduction of riboflavin. Although, the study does not identify specific bioactive, the activity seems to stem from fraction containing flavanoids, terpenoids, polyphenols and coumarins. This plant also inhibited platelet aggregation. The root extract of this plant incubated with viper venom antagonized coagulant and haemorrhagic activity; the plasma recalcification time was also delayed significantly by the intravenous administration of the root extract (49). The plant also showed potent neutralizing effect against the venom of *Crotalus adamentaeus* and produced significant protection against venom induced changes in serum SOD (superoxide dismutase) and LPx (Lipid Peroxide) levels (50). The pure compound lupeol acetate from the methanolic root extract of *H.indicus* could neutralize venom induced action of *Dabola russellii* and *Naja kaouthia* on experimental animals (51). The ethanolic extract of plant has shown antioxidant effect in rats with ethanol induced nephrotoxicity. Administration of the dose of 500mg /kg body weight per day for last 30 days of the experiment to rats with ethanol induced kidney injury, significantly decreased the levels of serum urea, uric acid and creatinine as well as kidney thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides (LOOH), and conjugated dienes (CD) and significantly elevated the activities of SOD, CAT, GPx, GSH, Vitamin C and Vitamin E in kidney as compared to untreated ethanol-administered rats (52). In vivo studies confirm beneficial anti-oxidant status in response to *H.indicus* treatment. Root extract in ethanol challenged rats was found to increase the enzymic and non-enzymic anti-oxidant status like superoxide dismutase, glutathione peroxidase, catalase, ascorbic acid and vitamin E levels in serum and hepatic tissue. It was found that this plant extract offers protection against free radical-mediated oxidative stress in plasma, erythrocytes and liver of animals (53).

**Hepatoprotective activity**
Ethanol extract of *H.indicus* has been reported to alleviate ethanol-induced liver toxicity in rats. 500mg/kg dose of the extract treated for 30 days was found to decrease the elevated serum levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and lactate dehydrogenase activity. Further, the treatment was found to modulate the levels of globulin, albumin, ceruloplasmin level in addition to the liver glycogen content. This protective activity was correlated by histochemical changes seen in the liver. The “reduction in the reduction potential” due to ethanol induced hepatic injury was further found to be ameliorated by the extract led by elevated glutathione levels, decrease in oxidised glutathione and glutathione S-transferase (54). Alternately, the results also suggest the possibility of “host red-ox sparing effects” due to the ingested reductones in the form of flavonoids and other polyphenolic entities. Similarly, extract from this plant was shown to protective against rifampicin induced liver toxicity (55, 56).

The decoction prepared from *Nigella sativa* seeds, *Hemidesmus indicus* roots and *Smilax glabra* rhizome showed to protect the liver of rats against DEN-mediated expression of the P-isoform of Glutathione S-transferase (GST-P) (57,69,70). The ethanolic root extract of *H.indicus* and its active principle 2-hydroxy-4-methoxy benzoic acid (HMBA) showed inhibitory activity on liver fibrotic markers and characteristics such as collagen content, matrix metalloproteinases (MMPs) 2 and 9 in ethanol-fed rats (58).

**Anti-carcinogenic activity**
A decoction comprised of *Nigella sativa* seeds, *Hemidesmus indicus* root bark and *Smilax glabra* rhizome is being recommended for cancer patients by a family of traditional medicinal practitioners of Sri Lanka. The decoction has potential to protect against chemically induced hepatocarcinogenesis. The long term treatment (for upto 16 months) of rats with the decoction has been demonstrated to inhibit not only DEN-induced GST-P expression, but also the carcinogen mediated development of over tumours (OT) and histopathological changes leading to tumour development (HT) as assessed both by visual observations and by microscopic examinations of liver sections stained with H & E, Sweet’s silver stain and the PAS stain for glycogen. In rats treated with DEN and decoction, a marked reduction of angiogenesis was observed. The anti-angiogenesis effects of these natural products have been shown to be related to their abilities to reduce inflammation and / or vascular permeability, or production of detrimental eicosanoids and other angiogenic factors (61). The plant has been reported to possess strong anti-inflammatory, anti-oxidant and immunomodulatory properties (28, 34, 60). Recent in-vivo studies have shown that active principles isolated from *N. sativa* seeds and *H. indicus* root extract can inhibit tumour development in mouse skin (61). The roots of *H.indicus* were extracted with 80% ethanol:water in cold extraction process and tested for cytotoxicity using brine shrimp lethality assay,
sea urchin eggs assay, hemolysis assay and MTT assay using the tumor cell lines. Eleven plant extracts were studies and found that no cytotoxic activity was observed in *H.indicus* extract (62). The immunomodulatory activity of *H. indicus* extract was investigated on activities of human peripheral blood lymphocytes in vitro and it was revealed that the activity is related to immunoglobulin (IgG) secretion and Adenosine Deaminase (ADA) activity (63). The chloroform fraction of *H. indicus* root extract containing sterols and fatty acids evaluated against Salmonella typhimurium- induced cytoxicity in Int 407 cell line and it was capable of taming S. typhimurium by suppressing its cytotoxicity activity in an intestinal epithelial cell line (64).

**Miscellaneous**

The roots of *H. indicus* along with the roots of *Tinospora cordifolia*, *Gmelina asitica* and *Celastrus paniculatus* are crushed with 10gms of asafetida and black cumin. The paste is then slightly warmed in an earthen pot and made into small pills that resemble jowar seeds. Four to five pills are recommended once daily for fifteen days in the treatment of whooping cough (65). Shoot cultures and root cultures from roots and leaves of *Hemidesmus indicus* R. Br. were established on Murashige and Skoog medium with various hormonal combinations. The production of anti-oxidants (lupeol, vanillin and rutin) in shoot cultures, callus cultures derived from leaf cells and root cells was compared with root and aerial portions of the parent plant. Shoot cultures and leaf callus cultures produce more antioxidants than root callus cultures (66). The aqueous extract of *Hemidesmus indicus* roots, *Gynnema sylvestre* and *Eclipta prostrata* showed larvical effect against Culex quinquificatus larvae. Larval mortality was 100% with the use of 5% concentration of root extract of *H.indicus* (30). The suspension of *H. indicus* root in water (10mg/ml) containing 15.5mM NaCl, 3mM KCl and 12mM glucose when injected into the ligated jejunal sac (1ml/sac) of rat, increased the absorption of water, Na and K (but not glucose) from the sac. Whereas ethanol extract decreased the same. This observation could be utilized in the management of water and electrolytes loss during diarrhea (67). A rapid in vitro propagation of *H. indicus* from nodal explants is achieved with highest shoot multiplication rate of 8.2 ± 0.4 shoots/explant with 95% frequency was achieved in 5 weeks culture period on Murashige and Skoog medium. A procedure for rapid in vitro propagation of the aromatic and medicinal plant *H.indicus* R.Br. from nodal explants was developed with highest shoot multiplication rate of 8.2 ± 0.4 shoots/explant with 95% frequency in 5 weeks culture period on Murashige and Skoog medium supplemented with 1.15 µM Kinetin and 0.054 µM α-naphthaleneacetic acid. Excised shoots were rooted on the same basal medium supplemented with 1.15 µM kinetin and 7.35 µM indole-3-butyrlic acid. Shoots derived from subcultures exhibited better rooting response than those from primary cultures. After a hardening phase of two weeks there was a 70% transplantation success in the field (68). The plant is known to possess antioxidant, anticoagulant, hypolipidaemic, hepatoprotective, antiplatelet aggregation, anti-haemorrhagic and lipoprotein lipase releasing properties so considered as an effective antiatherogenic agent preventing coronary artery disease (49, 69, 70).

**PATENTS ON H. INDICUS**

The database of intellectual property office of Singapore (www.surfip.gov.sg) which comprehensively covers patent databases of US (USPTO), PCT (WIPO), European (EPO), China (SIPO), Japan (JPO), Canada (CIP0), Korea (KIP0), Taiwan (TIPO), Singapore(IPOS), UK (UK-IPO), Thailand (TIPIC) and Patentmaps (PM) was searched. Only those patents that indicated plant extract in claims were included for the review. A list of patent number and title containing *H. indicus* as part of the claim section of the patent are provided in the Table No 1.

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<td>1995</td>
<td>Cosmetic</td>
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<td>US 5,693,327</td>
<td>1997</td>
<td>Herbal compositions</td>
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<td>US2002160065A1</td>
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<td>Herbal compositions and treatment methods</td>
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<td>Extracts from plant and non-plant biomass and uses thereof</td>
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<td>US20060137702A1</td>
<td>2006</td>
<td>Anti-cigarette herbal formulation as an antidote to tobacco</td>
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applications like anti-dote to tobacco, and needs to be pursued in quest of science behind them. Further, they also offer excellent opportunities to identify bioactive(s) as lead molecules and pursue combinatorial synthesis through the semi-synthetic route for optimization or combinatorial biosynthesis through strategic engineering of gene clusters encoding secondary metabolites.

CONCLUSION

H. indicus is commonly found throughout India. Studies have revealed its use in anti-inflammatory, antimicrobial, anti-ulcerogenic, otoprotective, anti-oxidant, anti-atherogenic and anti-carcinogenic. However not much information is there to prove this plant for anti-diabetic, anti-fertility, anti-leprotic etc. therefore further studies may be carried out to prove the potential of this plant. The plant is becoming the endangered species now so more work can be done on agricultural and climatic conditions to grow this plant. The translational potential and clues to possible novel bioactivities and novel targets yet to be discovered with this amazing plant species can be gauged from the plethora of patents being awarded.

REFERENCES


