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Review Article

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Some Aspects of Investigation of the Indian Medicinal Plant *Hemidesmus indicus* R. Br.: Chemical Constituents and Anti-Diabetic Activity

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ABSTRACT

The medicinal plant Hemidesmus indicus R. Br. is extensively used in Ayurveda, the Indian school of traditional medicine. A large amount of work on different aspects of H. indicus has been carried out and reported over the years. The present review covers recent work on H. indicus which have not received adequate coverage in the reviews on this plant so far. The two aspects covered in this review are (i) the chemical constituents of H. indicus and (ii) anti-diabetic properties of H. indicus.

Keywords: Hemidesmus indicus; Ayurveda; Phytochemical screening; Anti-diabetic properties

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INTRODUCTION

Recently there has been a global resurgence of interest in the traditional Indian school of medicine of Ayurveda. The Chemistry Department, University of Calcutta and the Calcutta unit of the Central Council for Research in Ayurveda and Siddha, which has developed to the present Central Ayurveda Research Institute for Drug Development (CARIDD) have been carrying out investigations on different aspects of chemistry, pharmacognosy, pharmacology and biomedical uses of Indian medicinal plants for many years. Joint investigations [1-9] have been carried out on several medicinal plants including *Hemidesmus indicus*, *Murraya koenigii*, *Pongamia pinnata*, *Wrightia tinctoria*, *Ferula assafoetida and Zizyphus jujube*. Work has been resumed on some new aspects of *Hemidesmus indicus* at the CARIDD. Hence, a thorough literature search on *H. indicus* was carried out.

It was seen that much work has been published since the earlier reviews [10-18]. It was therefore felt pertinent to prepare a review covering recent work on *H. indicus*, which have not received adequate coverage in these reviews. Hence in the present communication, two aspects have been reviewed:

Section I - The chemical constituents of *H. indicus*, as no detailed review exists on these.

Section II - Anti-diabetic properties of *H. indicus*.

The next section briefly overviews the characteristics of *H. indicus* and information in the earlier reviews on *H. indicus*.

Hemidesmus indicus R. Br.: Characteristics and earlier reviews

Hemidesmus indicus R. Br. (*Anantamul*, *Sariva*, Indian *sarsaparilla*) is a well-known Indian plant [10-12]. It is a climbing plant that grows almost all over India. It is common in hedges and also grows wild. Its root and root-bark are extensively used in traditional Indian medicine. Its usage has been recorded in Indian Ayurvedic texts of centuries past. *Hemidesmus indicus* was formerly placed under the family Asclepiadaceae, but recently based on the pollinical characters it has been transferred to Periplocaceae [13]. *Periploca indica* [14] is synonymous with *Hemidesmus indicus*. The vernacular names of *Hemidesmus indicus* are *Anantamul* (literally endless root) – Bengali, Hindi, Punjabi, Marathi; *Sugandhipala* – Telugu; *Namdaberu*, *Sogadaberu* – Kannada; *Onontomulo* –

Oriya; *Nannari* – Tamil, Malayalam; *Sariva* – Sanskrit, Gujrati. *Zaiyana* and *Ushbanidi* are respectively the Arabic and Persian names testifying to its use in other countries from ages past.

Anantamul can be distinguished by its slender, tortuous, rigid, and cylindrical root; its bark is rust-colored and corky, as well as furrowed with annular cracks. Anantamul's leaves are opposite one another – smooth, shiny and firm, and vary in shape and size according to their age. The flowers are small, externally green, and internally deep purple. Its root and root bark have a pleasant mild aroma. Recently, genetic fingerprinting has been used to identify the plant [13]. There are a number of leading articles and reviews on *Hemidesmus* indicus [10-18]. Its therapeutic properties have been summed up in a Sanskrit *sloka*, quoted in the Treatise of Indian Medicinal Plants, Vol. 4 [10]. These are given in the following translation – this plant is svādu (madhura, sweet), snigdha (cool), spermatopoietic; effective in anorexia, dyspnea, dyspepsia, cough; cures vitiated 'tridosha', menorrhagia, fever and diarrhoea; diuretic, invigorating and rejuvenating; a remedy for skin diseases, rheumatoid arthritis, gout and gonorrhea and diseases caused by mercury poisoning. The uses mentioned in ancient literature in the above-mentioned source2 have been listed: root and root-bark – alterative, demulcent, diaphoretic, diuretic; prescribed in fever, dyspepsia, anorexia, leucorrhoea, chronic rheumatism, skin disease and ulcerations; for chronic cough as well as diarrhoea a hot infusion of root-bark with milk and sugar is given; for constitutional debility and kidney trouble a paste is applied to swellings and urinary calculi. There are several reports regarding the use of *Hemidesmus indicus* in various ethnomedical practices in different states [10-13]. In addition to Bengal (now divided by political borders into West Bengal in India and Bangladesh), it finds use in Odisha (formerly called Orissa), Goa, Madhya Pradesh, Maharashtra, Assam, Uttar Pradesh, Karnataka (formerly called Mysore) and Tamilnadu (formerly called Madras).

Hemidesmus indicus is reported by S.R. Iyer [15] to form an ingredient of about 46 Ayurvedic preparations either alone or in combination with other drugs [16,17]. The publications Treatise of Indian Medicinal Plants, Vol. 4 [10] and relevant volumes of Wealth of India, give a large amount of information on *Hemidesmus indicus*. Since these were published decades ago, much further information has accumulated.

A detailed review by George [13] appeared in 2008. This contained a very short section on the chemical constituents in addition to detailed surveys on botanical, therapeutical, pharmacological and other aspects. Since publication of this review a large number of publications have appeared regarding *Hemidesmus indicus*.

T. Lakshmi (Chennai) and R. Rajendran (Bengaluru) in their review entitled '*Hemidesmus indicus* commonly known as Indian sarsaparilla – an update' [18] have given an account of some aspects of recent research on its biomedical properties, *viz*. antibacterial, anticancer, antinioceptive, natriuretic and salinuretic, hepatoprotective, wound-healing, anti-arthritic, cytotoxic, anti-venom, anti-inflammatory activities.

Anantamul was introduced to European medicine in 1831 [19]. In current years, in the USA and Europe Anantamul is receiving renewed attention as an Ayurvedic medicinal and herbal product. A recent overview entitled 'Anantamul (*Hemidesmus indicus*): A Review of Biomedical Studies and US Products' has been published by Wendy Weissner [20]. This overview summarises facts given in earlier reviews up to 2008, without significant additional inputs to those mentioned earlier. This overview has missed a number of earlier core references of Indian origin, and uses the earlier classification of *Hemidesmus indicus* as belonging to the family Asclepiadaceae. Weissner has mentioned that there is a paucity of aggregated information on Anantamul that consumers, practitioners, and manufacturers in the USA can turn to for product substantiation. The intention of her review was to provide a meaningful overview of Anantamul as an herbal supplement/drug, drawing evidence from textual, classical, biomedical, anecdotal, and theoretical data. The main importance of Weissner's article is that it lists the various Anantamul-containing products available in the USA. She mentions the renewed interest in Anantamul in the USA for its potential use in novel treatments or preventatives for cardiovascular disease, cancer and diabetes, which are leading causes of mortality in the USA, according to a 2011 report by the Centers for Disease Control [21]. In these instances, the study of Anantamul is being limited to its cardioprotective, antihyperlipidemic, diuretic, antioxidant and anti-cancer activities in laboratory assays as well as animal models.

Aim of present review

Much work has been published since the reviews by George [13] and by Lakshmi and Rajendran [18]. It is therefore felt pertinent to prepare a review covering recent work on *H. indicus*, which have not received adequate coverage in recent reviews [13,18,20]. Hence in the present communication, two aspects have been reviewed:

Section I - The chemical constituents of *H. indicus*, as no detailed review exists on these.

Section II - Anti-diabetic properties of *H. indicus*.

One of the present authors (AB) has been involved in earlier investigations of this plant. Work was done at the Department of Chemistry, University of Calcutta, in collaboration with the CCRAS unit at Calcutta (the predecessor of CARIDD), Dhaka University and BIRDEM, Dhaka. The work with the Dhaka groups involved extensive work on the anti-diabetic properties of the extract of the roots. This was the first time that the anti-

diabetic properties of *Hemidesmus indicus* extracts were investigated in details (see section on anti-diabetic properties of *Hemidesmus indicus*). The resultant publication [22], triggered a series of investigations on this aspect, and subsequently about 20 publications have resulted on the anti-diabetic properties of *H. indicus*.
Further investigations *H. indicus* roots are being carried out at present at the CARIDD. Other aspects of our recent work on *H. indicus* roots are (i) the isolation and characterisation of a new triterpenoid designated Hemidesterpene from *H. indicus*; and (ii) Phytopharmacognostic studies on the roots – this includes extraction of *Hemidesmus indicus* roots by different solvents, and preliminary analysis of these extracts – by chemical tests and HPTLC.

Section I - Chemical constituents of *Hemidesmus indicus* R. Br.

In view of its widespread occurrence in India and its pre-eminent position among Indian medicinal plants, various research groups at different locations in India have investigated different aspects of the *H. indicus* plant. Investigations on the chemical constituents have been carried out by research groups at Calcutta (Kolkata), Lucknow, Madras (Chennai) among others. Extraction methods have differed from group to group. Further no comparative studies of the constituents of the plant collected from different locations, as well as at different seasons seem to have been done. Much of the isolation and characterisation work has been done on the constituents extracted by organic solvents.
This section summarises the present status of knowledge on the chemical constituents of different parts of the plant *H. indicus*.
The following discussion is divided into several sections:

1. Preliminary screening for different classes of phytochemicals in extracts, without separation of individual constituents,
2. Volatile constituents;
3. Terpenoids;
4. Steroids;
5. Polyphenolics - Coumarino-lignoids, flavonoids.
6. Other Constituents.

Finally a consolidated list of compounds isolated from *H. indicus* is given.

The Treatise on Indian Medicinal Plants, Vol. 4 [10] mentions the following compounds isolated from different parts of *H. indicus*.
Flowers – Hyperoxide, isoquercitin, rutin – all flavonoids.
Leaves – Hyperoxide, rutin; tannin.
Roots – the coumarino-lignoids hemidesminine, hemidesmine-1 and hemidesmine-2; β-sitosterol; the terpenoids α-amyrin, β-amyrin, β-amyrin acetate, lupeol, lupeol acetate and lupeol octacosanoate; 2-hydroxy-4-methoxy-benzaldehyde; drevogenin-β-3-O-β-D-olandropyranosyl (1-4)-β-D-oleandropyranoside (desinine).

Preliminary screening for phytochemicals

A number of earlier publications reported preliminary screening of *H. indicus* for different classes of phytochemicals. These publications were mainly from research groups working in Pharmacology and Pharmacognosy; the work involved the use of standard colour reactions to determine the broad classes of constituents in crude extracts. The results of these studies as reported are summarised below.

1. Subramanian et al. [23] – *H. indicus* roots collected from Tirunelvali, Tamilnadu. Ethanolic extract by soxhletting petrol-defatted material. Classes of constituents present: phenols, alkaloids, flavonoids, glycosides, saponins, tannins, phytosterols, terpenoids. Negative tests for anthraquinones.
2. Sowmia and Divya Priya [24] – *H. indicus* roots collected from Maruthamalai Hills, Coimbatore district, Tamilnadu. Ethanolic extraction in the cold. Classes of constituents present: phenols, alkaloids, flavonoids, glycosides, saponins, tannins, steroids, terpenoids, resins, proteins. Negative tests for carbohydrates.
3. M. M. Moideen et al. [25] – *H. indicus* leaves collected from Cuddalore, Tamilnadu in April-May. Leaves extracted with ethanol by cold maceration. Classes of constituents present: phenols, alkaloids, flavonoids, glycosides, saponins, tannins, steroids, lignins. Negative tests for coumarin, proteins and free amino-acids, fixed oils gums and mucilage.
4. Vijaylakshmi et al. [26] – *H. indicus* roots collected from Thanjavur district, Tamil Nadu. Ethanolic extraction by soxhletting. Classes of constituents present: phenolic compounds, glycosides, saponins, tannins, phytosterols, volatile oils, proteins, free amino-acids. Reported absence of alkaloids, flavonoids, lignins, carbohydrates, fixed oils and fats, gums and mucilage. Their results are at some variance with other reports.

5. M. Sayyed et al. [27] – *H. indicus* whole plant obtained from drug suppliers in Hyderabad. Methanolic extraction by soxhletting. Classes of constituents present: phenolic compounds, glycosides, tannins, flavonoids, carbohydrates, lignans, proteins. Negative tests for alkaloids, terpenoids, saponins.
6. R. Saryam et al. [28] – *H. indicus* roots collected from Bhopal, Madhya Pradesh in January. Root extracts were prepared by successive soxhlet extractions with petroleum ether, ethanol and distilled water. Classes of constituents present: ethanol extract - alkaloids, glycosides, carbohydrates, polyphenols and saponin; aqueous extracts - glycosides, carbohydrates, polyphenols and saponin; petroleum ether extract - steroids and triterpenoids only. TLC of ethanol and aqueous extracts were reported without identification of any component.

Volatile constituents

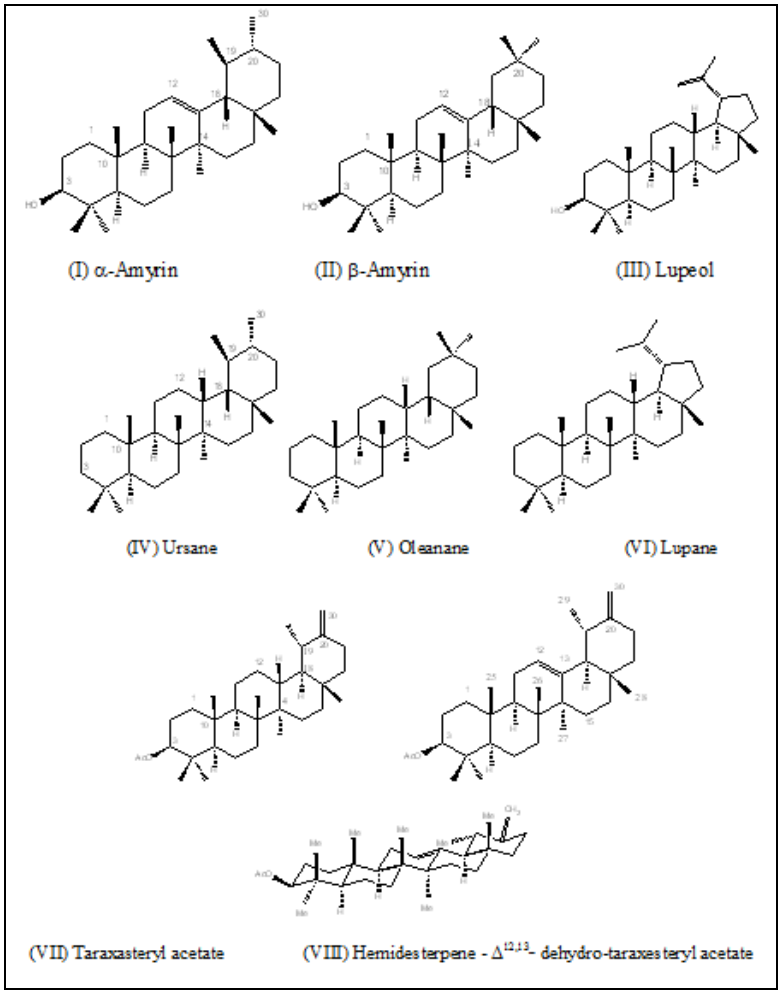
Nagarajan and Gurudutt (CFTRI, Mysore) [29] analysed the volatiles of *H. indicus* roots. The extraction procedures involved steam-distillation methods. Silica-gel column chromatography yielded 2-hydroxy-4-methoxy benzaldehyde (91% of steam-distilled mixture) and ledol (4.5%). GC-MS analysis showed the presence of at least 40 constituents, of which comparatively the most abundant were salicylaldehyde, camphor, borneol, linalyl aetate, dihydrocarvyl acetate, nerolidol, iso-caryophyllene, 1,8-cineol, α-terpinyl acetate, dodecanoic acid, hexadecanoic acid. Nagarajan and Rao [30] have determined the amounts of 2-hydroxy-4-methoxy benzaldehyde in roots of *Decalepis hamiltonii* and *H. indicus*. Sreekumar et al. [31,32] reported the production of 2-hydroxy-4-methoxy benzaldehyde using root cultures of *H. indicus*. HPTLC quantitation of 2-hydroxy-4-methoxy benzaldehyde in *H. indicus* root powder extract has been reported by Dareker et al. [33]. Sircar et al. [34] developed a validated HPLC method for simultaneous determination of 2-hydroxy-4-methoxy benzaldehyde and its corresponding oxidation product 2-hydroxy-4-methoxy benzoic acid. The latter has been found to be one of the active principles of *H. indicus* having anti-diabetic activity by K. Kannabiran and M. Gayathri (see Section on anti-diabetic activity for an account of their several publications on this aspect). Jirovitz et al. [35] reported essential oil analysis of *H. indicus* roots collected in South India GC-MS analysis revealed that 2-hydroxy-4-methoxy benzaldehyde was the major component (95.8%); other minor components (less than 1% each) were vanillin, salicylic acid derivatives and (*E,Z*)-nonadienal. Comparative study of the essential oils from *H. indicus* and *Decalepsis hamiltonii* has been subsequently reported by Sreelekaha and Jirovitz [36]. The steam-distillate of the dried and powdered roots of these plants was extracted with ether, and analysed by GC-MS; 2-hydroxy-4-methoxy benzaldehyde was the major component in both plants (~98% and ~95% respectively). Additionally seven minor components in *H. indicus* (all present to the extent of less than 1%) were detected - anisaldehyde, octanoic acid, decanoic acid, thymol, isobutyl anilide, palmitic acid, *m*-guiacol. Five of these were also present in the steam-distillate of *Decalepsis hamiltonii.*

Terpenoids

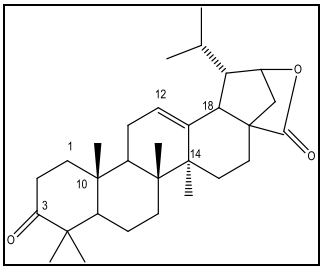
Analysis of the volatile components of *H. indicus* led to the identification of several lower tepenoids, as detailed in the previous section. The Treatise on Indian Medicinal Plants, Vol. 4 [10] and other sources cite the presence of several higher terpenoids, *viz*. α-amyrin (I) [10,37,38], β-amyrin (II) [10,37,38], β-amyrin acetate (II) [10,38-43], lupeol (III) [10,37,38], lupeol acetate (III) [10,38] and lupeol octacosanoate (III) [10,38] isolated from different parts of *H. indicus*. The investigations by several groups have led to the isolation and characterisation of a number of pentacyclic triterpenoids. The pentacyclic triterpenoids from *H. indicus* are based essentially on one of the following basic skeleta – Ursane (IV), Oleanane (V) and Lupane (VI).

Lupeol octacosanoate was isolated and characterised by Padhy, Mahato and Dutta [38]. This research group (Indian Institute of Experimental Medicine, Calcutta, renamed later as Indian Institute of Chemical Biology) was one of the first to investigate the plant *H. indicus*. Separation techniques included chromatography over the then innovative $AgNO_3$-impregnated silica gel. Petrol extract of the roots yielded lupeol octacosanoate, hexatriacontane, lupeol (III), lupeol acetate), α-amyrin (I), β-amyrin (II), β-amyrin acetate and β–Sitosterol. Lupeol octacosanoate) on saponification afforded lupeol and octacosanoic acid; the latter was methylated to its methyl ester. Subsequently, quantitation of lupeol octacosanoate in *H. indicus* root powder by HPTLC was reported by Damle and co-workers [39]. In this work, lupeol octacosanoate was used the marker compound. HPTLC was carried out on silica gel 60F-254, using isopropyl alcohol: *n*- butanol (1:1) as the developing solvent. Our own work on *H. indicus* roots has revealed the presence of α-amyrin acetate, β-amyrin acetate and taraxasteryl acetate (VII) and a new triterpene. The latter designated hemidesterpene was characterised from extensive spectroscopical studies as $\Delta^{12,13}$-dehydro-taraxesteryl acetate (VIII) [40].

Nair et al. [41] have reported that β-amyrin palmitate), obtained from *H. indicus* roots showed promising anti-diabetic activity (see Section 2 on anti-diabetic activities).



A research group from CIMAP, Lucknow investigated the hexane-soluble portion of the ethanol extract of the stems of *H. indicus* [42], and isolated a number of compounds. They characterised the new triterpene lactone 3-keto-lup-12-ene-21→28-olide (IX). Furthermore lupanone, Δ^{12}-dehydrolupanyl-3β-acetate, Δ^{12}-dehydrolupeol acetate, hexadecanoic acid, 4-hydroxy-3-methoxy-benzaldehyde and 3-hydroxy-4-methoxy-benzaldehyde were also isolated.

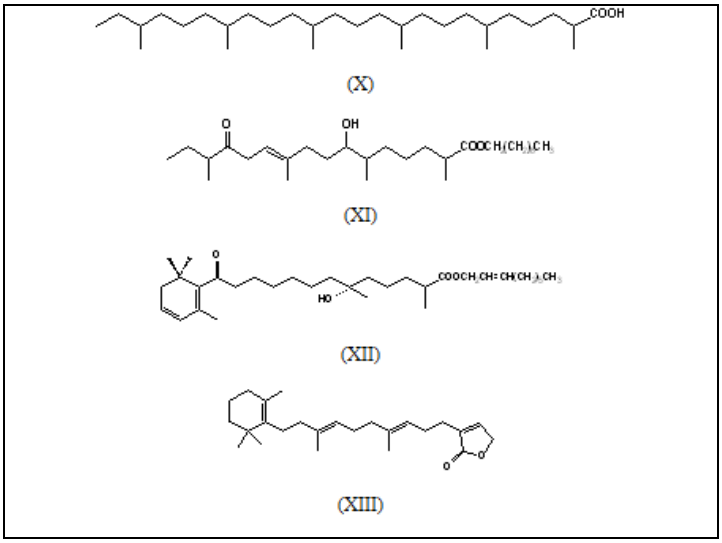


(IX)

Two publications [43,44] from Hamdard University, New Delhi, have reported the presence of a number of terpenoid constituents from the roots of *H. indicus*. They claimed the isolation and characterisation of six new pentacyclic triterpenoids [43]: two oleanenes, *viz.* olean-12-en-21β-yl acetate and olean-12-en-3α-yl acetate; three ursenes, *viz.* 16(17)-seco-urs-12,20(30)-dien-18α H-3β -yl acetate, 16(17)-seco-urs-12,20(30)-dien-18 α H-3β-ol, urs-20(30)-en-18β H-3β -yl acetate; a lupene, *viz.* lup-1,12-diene-3-on-21-ol. Their structural assignments rested on spectroscopical investigations, including one-dimensional ^{1}H- and ^{13}C-NMR studies.

The Hamdard group also reported the isolation and characterisation of another three new terpenoids from *H. indicus* [44]. The powdered roots were sohxletted with alcohol, the solvent removed under reduced pressure, the residual viscous mass taken up in methanol, and then chromatographed over silica gel. The new compounds hemidesmusoic acid (X), octyl hemidesdisterpenoate (XI) and sesterpenoid ester (XII) were isolated in addition to two known steroids. Hemidesmusoic acid, a saturated linear triterpenoid, could be methylated with

diazomethane to its mono-methyl ester. Its ^{1}H- and ^{13}C-NMR spectra showed the absence of any olefinic unsaturation, and the presence of seven methyl groups. From its molecular formula, NMR data and mass fragmentation pattern, it was assigned the structure 2, 6, 10, 14, 18, 22–hexamethyltetracosoi-1-oic acid (X). The second new compound (XI) was characterised as the acyclic diterpenoid *n*-octyl-2, 6, 10, 14-tetramethyl-hexadec-7-ol-10-en-13-on-1-octanoate from NMR investigations and chemical reactions. Its NMR data showed the presence of a trisubstituted olefinic bond, a ketonic carbonyl, an ester carbonyl and six methyl groups. It was acetylated to a mono-acetyl derivative with acetic anhydride-pyridine. Alkali hydrolysis furnished hemidesterpenoic acid where the octyl group had been hydrolysed off. The positions of the ketonic carbonyl group and olefinic bond were deduced from the mass spectral fragmentation pattern. The third new compound was a sesterterpenoid containing a cyclohexadiene moiety, a conjugated exocyclic carbonyl, a secondary hydroxyl and a 9-carbon unsaturated esterified alcohol unit. Its structure was established as *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 (XII) from detailed ^{1}H- and ^{13}C- NMR studies and its mass-spectral fragmentation pattern.



Sowmia and Divya Priya [24] reported the isolation and characterisation of a new sesterterpenoid, biogenetically derived by head-to-tail linkages of five isoprene units. Its NMR spectra showed the presence of five methyl groups, two olefinic protons and a lactonic carbonyl. From detailed ^{1}H- and ^{13}C-NMR, IR, GC-MS studies and elemental analysis the structure of this compound was proposed as (XIII).

Steroids

β–Sitosterol, an ubiquitous component of several plants, was reported in the roots by Chatterjee and Bhattacharya in 1955 [45]. This was the first report, that we could locate, on the constituents of *H. indicus.*

The known phytosteroids *β*–sitosterol and *β*–sitosterol glucuronate have been isolated from the roots of *H. indicus* [44]. Several pregnane glycosides have been obtained from *Hemidesmus indicus*. The most significant contributions in this regard have been made by Khare et al. working at Lucknow University. They have isolated and characterised eleven new pregnane glycosides from the dried stems and twigs of this plant. These are desinine, hemidescine, emidine, indicine, hemidine, medidesmine, hemisine , desmisine, indicusin, denicunine, heminine.

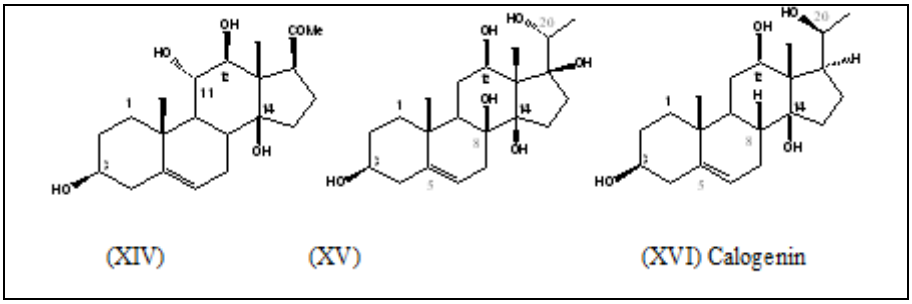
The structures of these compounds were elucidated by chemical and spectroscopical investigations (EI-MS, FABMS, ^{1}H NMR, ^{13}C NMR). The results have been summarised below. Desinine [46], a new pregnane ester diglycoside was isolated from the dried twigs of *H. indicus*, Its structure was based on 3,11,12,14-tetrahydroxypregn-5-en-20-one (XIV). Its structure was established *11-Ac*, *3-O*-[*β*-D-Oleandropyranosyl-(1→4)-*β* -D- oleandropyranoside] on 3,11,12,14-tetrahydroxypregn-5-en-20-one on the basis of chemical and spectroscopic evidence. The structure of medidesmine [47], isolated from *H. indicus*, was based on sarcostin, i.e., pregn-5-ene-3,8, 12,14, 17,20-hexol (XV).

It was characterised with the help of FAB-MS, EI-MS, ^{1}H- and ^{13}C-NMR spectroscopy, along with chemical transformations as sarcostin-3-*O*-*a*-D-glucopyranosyl (1→4)-O-*β*-D-digitoxopyranosyl (1→4)-*O*-*β*-D-oleandropyranoside; Eight of the new pregnane glycosides were derived from calogenin, i.e., pregn-5-ene-3,14, 20-triol (XVI). These are listed below –

Hemidescine [47]: 20-O-acetyl calogenin 3-*O*-*β*- D-digitoxopyranosyl(1→4)- *O*-*β*-D-oleandropyranoside.

Emidine [47]: calogenin-3-*O*-*β*- D-digitoxopyranosyl(1→4)- *O*-*β*-D-digitoxopyranosyl(1→4)- O- *β* -D-digitoxopyranoside.

Indicine [48]: calogenin-3-*O*-*β*- D-digitoxopyranoside.
Hemidine [48]: calogenin-3-*O*-*β*- D-boivinopyranoside.
Hemisine [49]: calogenin-3-*O*-*β*-D-cymaropyranosyl(1→4)-*O*-(3-*O*-methyl)-*β*-D- glucopyranosyl(1→4)- *O*-*β*-D- glucopyranosyl(1→4)-*O*-*β*-D- cymaropyranoside.
Desmisine [50]: calogenin-3-*O*-*β* -D-xylopyranosyl(1→4)- *O*-*β* -D-digitoxopyranosyl(1→4)- *O*-*β* -D-xylopyranosyl(1→4)- *O*-*β* -D-digitoxopyranoside).
Denicunine [51]: calogenin 3-*O*-3-*O*-methyl-*β*-D-fucopyranosyl-(1→4)- *O*-*β*-D-oleandropyranoside.
Heminine [51]: calogenin 3-*O*-*β*-D- digitoxopyranoside.



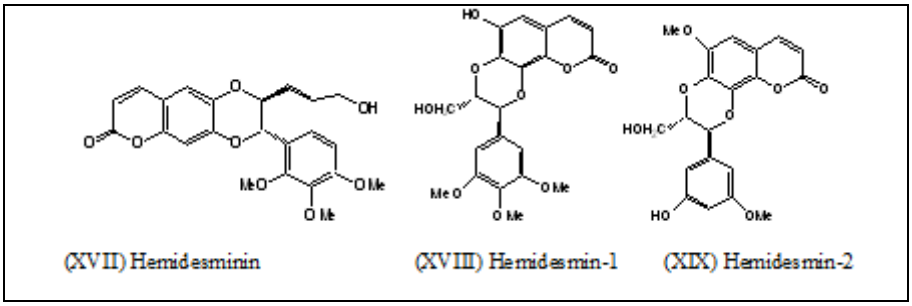
The new pregnane oligoglycoside indicusin [50] was isolated from chloroform-ethanol (3:2) fraction of *H. indicus*. With the help of FABMS, ^{1}H- and ^{13}C-NMR spectroscopy and chemical transformations, the structure of indicusin was defined as 11*α*, 12*β*-di-O-acetyl-orgogenin-3-O-*β*-D-cymaropyranosyl-(1→4)-*O*-*β*-D-cymaropyranosyl -(1→4)-*O*-*β*-D-cymaropyranoside. Heble and Chadha have carried out studies on the steroids in cultured tissues and mature plant of *H.* indicus [52]. Tissue cultures were established from shoot tip, stem and roots of the plant. Stem tissue cultures exhibited organogenetic potential whereas shoot tip and root cultures grew as unorganised callus under different hormonal stimuli. All the cultures contained phytosterols but 16-dehydropregnenolone was detected only in stem callus. Leaves, stem and roots of the plant *H. indicus* contained cholesterol, campesterol, sitosterol and 16-dehydropregnenolone. Roots contained maximum concentration of 16-dehydropregnenolone (0.04%) followed by stem (0.006%). The leaves contained trace amounts of this steroid.

A Chinese group has reported the isolation of condensed polypropanoid glucoside and pregnane saponins from the roots of *H.* indicus [53].

Polyphenolics

Coumarino-lignoids:

Three compounds of this very rare class of Natural Products were isolated and characterised from the roots of *H.* indicus [54,55]. The common structural feature of these compounds, *viz*. hemidesminine (XVII) [54], hemidesmine-1 (XVIII) [55], hemidesmine-2 (XIX) [55] is that they have a coumarin unit linked to a lignoid C6-C3 unit. Lignans contain two C6-C3 units. The combination of the lignoid C6-C3 unit is through an *ortho*-dihydroxyl bridge achieved either in a linear fashion (6,7-fusion) as in (XVII), or in a non-linear (7,8-fusion) manner (XVIII, XIX). All three compounds were isolated and characterised by research groups working at Chemistry Department, Calcutta University and the CCRAS Research Unit in Calcutta - the predecessor of the present CARIDD. Roots of *H. indicus* were purchased from the local market at Calcutta and identified by S. R. Das, RRI (Ayurvedic), Calcutta. The air-dried roots were powdered and defatted by petrol, then extracted with benzene in a Soxhlet apparatus. The concentrated benzene extract was chromatographed over silica gel; the EtOAc:MeOH (9:1) eluates furnished a mixture of three new coumarino-lignoids, which were separated by PTLC (silica gel, EtOAc:MeOH, 95:5). Structure elucidation was carried out by the extensive application of spectroscopical methods – UV, IR, ^{1}H- NMR and MS.



Structure elucidation of Hemidesminine (XVII) [54], the second linearly-fused coumarino-lignoid to be isolated, followed from detailed spectroscopic analysis (UV, IR, ^{1}H-NMR, MS). Its IR spectrum showed the presence of

conjugated carbonyl (1705 cm^{-1}) and hydroxyl (3430 cm^{-1}) groups. The ^{1}H-NMR spectrum (d_6-DMSO) showed the presence of coumarinic doublet (δ 6.30 and δ 7.90; J = 9 Hz), four aromatic protons as three singlets (1H singlets at δ 6.77, δ 6.65; 2H singlet at δ 7.31) and three methoxyls (two at δ 3.63, one at δ 3.74), two olefinic protons (δ 5.33, δ 5.51), a CH_2OH group (methylene multiplet at δ 3.60, hydroxyl at δ 5.03) and two low-field signals at δ 4.91 (doublet) and δ 4.35 (multiplet). The magnitude of the coupling constant (J = 9 Hz) between the latter two indicated *trans*-fusion.

Hemidesmin-1 (XVIII) and Hemidesmin-2 (XIX) were angularly fused coumarino-lignoids [55]. Both exhibited UV and IR data commensurate with the presence of a coumarin unit. Their structures followed from detailed analyses of their ^{1}H-NMR spectra and mass spectral fragmentation patterns.

The ^{1}H-NMR spectrum (d_6-DMSO) of Hemidesmin-1 (XVII) showed the presence of coumarinic doublet (δ 6.29 and δ 7.91; J = 9.5 Hz), three aromatic protons as two singlets (δ 6.87 for H-5; δ 6.67 for H-2',6') and three methoxyls (C-3',5' at δ 3.71, C-4' at δ 3.73), a phenolic hydroxyl (δ 8.55), a CH_2OH group (2H multiplet for methylene at δ 3.60, hydroxyl at δ 5.03) and two low-field signals at δ 4.90 (doublet) and δ 4.30 (multiplet). The latter, assignable to H-7' and H-8' respectively, exhibited a mutual coupling constant of 7.7 Hz, testifying to their *trans*-disposition. Hemidesmine-2 (XIX) was similar to Hemidesmine-1. The structural difference was that it had a 5-methoxyl group, and a 3'-hydroxyl-5'-methoxyl phenyl moiety attached to the bridging dioxane ring at C-7'. It ^{1}H-NMR (d_6-DMSO) characteristics were as follows - coumarinic doublet (δ 6.25 and δ 7.92; J=9 Hz), four aromatic protons as three singlets (δ 6.86 for H-5; δ 6.67 for H-2',6') and two methoxyls (δ 3.75), a phenolic hydroxyl (δ 8.55), a CH2OH group (2H multiplet for methylene at δ 3.95, hydroxyl at δ 5.30) and two low-field signals at δ 4.95 (H-7' doublet) and δ 4.25 (H-8' multiplet). The mutual coupling constant of 8 Hz of H-7' and H-8' indicated their *trans*- disposition.

Flavonoids:

Subramanian and Nair [56] reported the identification of the following flavones glycosides from the methanolic extract of flowers and leaves of *H. indicus*: hyperoxide, isoquercitin, rutin from the flowers; hyperoxide, rutin from the leaves. Characterisation was achieved by colour reactions, spectral properties, chemical reactions and finally chromatographic comparison with authentic samples. This was one of the earliest reports on *H. indicus*. So far as we are aware, this is the sole report so far on the constituents of the flowers of this plant.

Other compounds

Various aliphatic and aromatic compounds have been reported from *H. indicus*.

Aromatic aldehydes: 2-hydroxy-4-methoxy-benzaldehyde [29-35] (major component in steam-volatiles); salicylaldehyde [29]; 4-hydroxy-3-methoxy-benzaldehyde (vanillin) [32,32]; 3-hydroxy-4-methoxy-benzaldehyde (*iso*-vanillin) [42]; anisaldehyde [36].

Aromatic acids: 2-hydroxy-4-methoxy benzoic acid [34,35]; ferulic acid [57].

Aliphatic compounds: hexatriacontane [38]; octanoic acid [36]; decanoic acid [36]; dodecanoic acid [29]; hexadecanoic acid [29,42]; palmitic acid [36].

Also present were thymol [36]; isobutyl anilide [36]; *m*-guiacol [36]; salicylic acid derivatives [35]; (*E,Z*)-nonadienal [35]. Nagarajan and Gurudutt [29] reported the presence of at least 40 components as detected by GC-MS – some in minute quantities.

A HPTLC analysis [57] for the determination of ferulic acid in *H. indicus* roots extract was developed and validated by Chandra et al. [57]. The method employed used TLC aluminium plates pre-coated with silica gel 60 F254 as the stationary phase. The mobile phase consisted of *toluene: ethyl acetate: formic acid*; 5:5:0.2 (v/v/v). This method was found to give compact spots for ferulic acid (Rf value of 0.59 ± 0.01). Densitometric scanning of ferulic acid was carried out at λ366 nm in the absorbance/reflection mode. The method was validated for linearity, precision, limit of detection and limit of quantitation, accuracy and recovery.

Consolidated List of Reported Chemical Constituents of *Hemidesmus indicus* R. Br

Terpenoids:

α-amyrin; *α*-amyrin acetate; *β*-amyrin; *β*-amyrin acetate; *β*-amyrin palmitate; lupeol; lupeol acetate; lupeol octacosanoate; lupanone; taraxasteryl acetate; hemidesterpene; 3-keto-lup-12-ene-21→28-olide; Δ^{12}-dehydrolupanyl-3*β* -acetate; Δ^{12}-dehydrolupeol acetate; olean-12-en-21*β*-yl acetate; olean-12-en-3*α*-yl acetate; 16(17)-seco-urs-12,20(30)-dien-18*α* H-3 *β*-yl acetate; 16(17)-seco-urs-12,20(30)-dien-18 *α* H-3 beta-ol; urs-20(30)-en-18*β* H-3*β*-yl acetate; lup-1,12-diene-3-on-21-ol; hemidesmusoic acid; octyl hemidesdisterpenoate; *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; 3-{(3*E*,7*E*)-4,8-dimethyl-10-(2,6,6-trimethylcyclohex-1-enyl)deca-3,7-dienyl)furan-2(5*H*)-one; ledol; camphor; borneol; linalyl aetate; dihydrocarvyl acetate; nerolidol; iso-caryophyllene; 1,8-cineol; *α*-terpinyl acetate.

Steroids:
Desinine, Indicine, Hemidine, Indicusin, Hemidescine, Emidine, Medidesmine, Hemisine Demicine, Denicusine and Heminine, *β*–Sitosterol and *β*–Sitosterol glucuronate, cholesterol, campesterol, 16-dehydropregnenolone.

Coumarino-lignoids: hemidesminine, hemidesmine-1, hemidesmine-2.

Flavonoids: Hyperoxide; isoquercitin; rutin.

Other compounds: 2-hydroxy-4-methoxy-benzaldehyde (major component in steam-volatiles); 2-hydroxy-4-methoxy benzoic acid; ferulic acid; salicylaldehyde; 4-hydroxy-3-methoxy-benzaldehyde; 3-hydroxy-4-methoxy-benzaldehyde; anisaldehyde; octanoic acid; decanoic acid; dodecanoic acid; hexadecanoic acid; palmitic acid; thymol; isobutyl anilide; *m*-guiaacol; vanillin; salicylic acid derivatives; (*E,Z*)- nonadienal.

Section II - Anti-diabetic activity of *Hemidesmus indicus*
Introduction:
Diabetes mellitus is a major global health problem. It has been suggested by 2025 that 300 million people will have diabetes worldwide. India has more than 40 million diabetic people representing nearly 20% of total diabetes population of the whole world. Antidiabetic allopathic medicines are often overprescribed, and are found to be dangerous on long term use due to their toxicity and adverse effects on the body. World Health Organization has recommended the evaluation of traditional plant treatments for diabetes [58]. Medicinal plants are traditionally used in many countries to control diabetes mellitus [58-61]. Plant based remedies are considered to be natural, safe and remain as popular and complementary treatments for diabetes mellitus. Traditionally many plants have been used in Ayurveda, Sidha and folklore systems of medicines to treat diabetes mellitus. The hypoglycemic effect of hundreds of plant species has been reported. However, the pharmacognostic, phytochemical and pharmacological studies on many of these plants are yet to be explored.

Brief account of work done so far:
A paper was published jointly by research groups working at Calcutta University, Dhaka University and BIRDEM, Dhaka entitled 'Hypoglycemic and Hypolipidaemic effect of *Hemidesmus indicus* root on diabetic model rats' in *Diabetes Research* in 2005 [22]. The paper was authored by Sabrina Murshed, M. Mosihuzzaman, N. Nahar , B. Rokeya, A.K. Azad Khan and L. Ali from Dhaka and A. Banerji and S. Maiti from Calcutta. This was the first comprehensive report on the hypoglycemic and hypolipidemic effect of *Hemidesmus indicus* R. Br. root on diabetic model rats. Roots of *H. indicus* (Anantamul) were washed thoroughly with distilled water, dried in the shade and coarse ground. The resulting powder was extracted thoroughly (three extractions) in the cold in a percolator with 95% ethanol. From the combined ethanolic extract the solvent was removed under reduced pressure by a Buchi rotary evaporator; finally the extract was dried in a freeze-drier. The methanolic extract (HiRM) was prepared in a similar way. Male Long-Evans rats (180-220 gm) were used in the study. Ethanolic extract of *H. indicus* roots (HirE) was tested on non-diabetic and diabetic (Type I and Type II) model rats for acute hypoglcaemic effect. HirE (1.25g/kg body weight) dissolved in 10ml water was fed to nondiabetic and diabetic (Type I and Type II) model rats in fasting and postprandial (simultaneously with glucose 2.5g/kg body weight and 30 min. before glucose load) states. Methanol extract of *H. indicus* roots (HiRM) was tested for both acute and chronic effects on Type II diabetic model rats. The same dose of HiRM was used for chronic effects once a day for 28 days. In acute experiments both the extracts showed significant ($p<0.001$) hypoglycaemic effect on Type II diabetic model rats, when fed simultaneously with glucose load. After 28 days, HiRM significantly lowered fasting ($p<0.001$) and postprandial serum glucose ($p<0.001$). It also lowered triglycerides ($p<0.001$), total cholesterol ($p<0.003$) and LDL-cholesterol($p<0.01$) when compared to those in the control. HiRM also increased ($p<0.001$) the essential fatty acids (linoleic, linolenic, arachidonic and docosahexaenoic acids) and decreased significantly ($p<0.001$) the saturated fatty acids (palmitic and stearic) as compared to the control. It could be concluded [22] that both ethanol and methanol extracts of *H. indicus* roots have hypoglycaemic effects on Type II diabetic model rats. In addition the methanolic extract had a beneficial effect on dyslipidaemia. Following this report made by us, a number of groups investigated the anti-diabetic properties of *H. indicus* roots. Their work has been summarised below. Most of the subsequent reports are from the Southern states of India, others are from Northern India and Bangladesh. Krishnan Kannabiran and Mahalingam Gayathri, of Vellore, have carried out extensive work on anti-diabetic properties of *H. indicus* roots, collected in Tamilnadu (formerly Madras state) [62-64].
The hypoglycemic activity of *H. indicus* on streptozotocin-induced diabetic rats was studied by them [62]. The roots of *H. indicus* for this work were collected from the Morappur forest area, Dharmapuri district, Tamil Nadu, during April. In the experimental procedure followed the shade-dried powdered roots of *H. indicus* (100 g) were mixed with 500 ml of sterile distilled water, then a juice was obtained using a electric extractor; the

juice was filtered and the extract was concentrated *in vacuo*, then freeze-dried. Male albino rats (Wistar strain, weighing 150-200 g) were used for these experiments. The effect of aqueous extract of roots *H. indicus* roots on blood glucose was studied with fed, fasted and glucose-loaded diabetic and nondiabetic rat models. The effect of the extract on serum electrolytes, serum levels of key glucose metabolizing enzymes, hepatic microsomal protein and hepatic cytochrome P-450-dependent mono-oxygenase enzyme systems and lipid peroxidation in the liver and kidney of diabetic rats. K. Kannabiran and M. Gayathri concluded from their studies that the aqueous extract of the roots of *H. indicus* at a dosage of 500 mg/kg/day exhibited significant antidiabetic activity. It was found to restore the concentrations of serum electrolytes, glucose metabolizing enzymes, hepatic microsomal protein and hepatic cytochrome P-450-dependent mono-oxygenase enzyme systems to near normal level and also corrected the related metabolic alterations in experimentally induced diabetic rats. *H. indicus* administration also decreased liver and kidney lipid peroxidation products. They made the following comments about mode of action - they opined that prolonged administration might have stimulated the β-cells of islets of Langerhans to produce insulin. From the results they assumed that the root extract could be responsible for stimulation of insulin release and the observed restoration of metabolic activities. Further, the observed blood glucose-lowering effect of the extract in STZ-induced diabetic rats could also possibly be due to increased peripheral glucose utilization. The antihyperglycemic activity of the aqueous extract of *H. indicus* roots at the given dose was comparable with tolbutamide, a standard hypoglycemic drug. Tolbutamide has long been used to treat diabetes and is known to act by stimulating insulin secretion through action on the pancreatic β-cells. Significant reduction in lipid peroxidation can be attributed to the antioxidant activity of various phytochemicals present in the aqueous extract of the roots of *H. indicus*. Further, they felt that their results indicate the possibility that the major function of the extract may be in protecting vital tissues such as liver, kidney, pancreas, and brain, thereby reducing the complications of diabetes. These authors concluded that on the basis of their findings, *H. indicus* could be used as an antidiabetic and antioxidant agent for the prevention and treatment of diabetes mellitus.

G. Mahalingam and K. Kannabiran in another publication [63] have reported that *H. indicus* root extract ameliorates diabetes-mediated metabolic changes in rats. This study explored the anti-diabetic activity of *H. indicus* roots in streptozotocin-induced diabetic rats. They found that oral administration of aqueous extract at doses of 500 mg/kg significantly reduced the blood glucose within 5 hours. Twelve week treatment reverted the altered levels of insulin, glycosylated hemoglobin, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), g-glutamyl transferase (g—GT) and creatine kinase (CK) to near normal levels in diabetic rats. Their results suggested that *H. indicus* administration not only reduces blood glucose but also offers protection to diabetes-induced metabolic alterations in rats.

In a related study Rastogi Archit, Mahalingam Gayathri and Munusami Punnagai [64] reported *in vitro* investigation into the mechanism of anti-diabetic activity of selected medicinal plants. Their work on the anti-diabetic effect of aqueous extracts from the medicinal plants *H. indicus*, *Ficus benghalensis*, *Pterocarpus marsupium* roxb.is summarized below. The plant materials were obtained from forests in and around Chitteri Hills, Dharmapuri District, Tamil Nadu. Barks of *P. marsupium* and *F. benghalensis* and root of *H. indicus*. Aqueous extract of each of the plants were taken - 10g. of each were extracted with 250ml water at 60o C. In the actual experiments, 1mL of the extract was then placed in a bio-membrane along with a glucose solution (0.22 mM in 0.15 M NaCl); the bio-membrane was immersed in a beaker containing 40mL of 0.15 M NaCl + 10mL of distilled water. The control contained 1mL of 0.15 M NaCl containing 22 mM glucose and 1mL of distilled water. The authors made half-hourly observations of the concentration of glucose in mg/dL in the beaker. A significant hindrance to the flow of glucose across the bio-membrane was seen. *P. marsupium* and *F. benghalensis* showed a relative movement of about 16% and 78% when compared to the aqueous control after 180 minutes. *H. indicus* showed a relative movement of 84.5% after 150 minutes when compared with the aqueous control. The authors concluded that the medicinal plants were found to show potent inhibition of glucose diffusion across the membrane. These results indicate that these plants could quite possibly show hypoglycaemic activity due to this inhibitory action. However, they felt that further studies at a molecular level were essential to confirm this mechanism. C. Sowmia and R. Kokilavani [65] have reported on their investigations antidiabetic and antihypercholesterolemic effect of *H. indicus* roots in Alloxan induced diabetic rats. *H. indicus* roots were collected in Coimbatore district, Tamilnadu and extracted with ethanol. The ethanolic extracts were concentrated and then lyophilized. Male Wistar rats (7-8 weeks) were used for these experiments. Administration of HiRe (*H. indicus* root extract; 40 mg/g body weight/day) for four weeks significantly decreased the serum cholesterol, triglyceride, and free fatty acids to about the same levels as normal rats, the results were comparable with glibenclamide treatment. Four weeks treatment of diabetic rats with HiRe (40 mg/g body weight/day) showed significant hypoglycemic effect. There was significant increase in blood glucose levels in alloxan- diabetic rats. Treatment with HiRe extracts and glibenclamide separately tended to bring the parameters towards normal levels - the effects being comparable.

In a further paper, this research group [66] has reported the modulation of glycolytic and gluconeogenic enzymes by treatment with *H. indicus* ethanolic root extract in alloxan induced diabetic rats.
Further C. Sowmia et al. have assessed the glycemic potential and activity of marker enzymes of *H. indicus* ethanolic root extract in alloxan-induced diabetic rats [67].
M. Zarei et al. have also studied the effect of *H. indicus* root extract on the blood glucose level in alloxan induced diabetic rats [68]. *H. indicus* was collected from in Mysore, Karnataka. In this study, the effect of a single dose of *H. indicus* root extract (HiRe) on the blood glucose level in alloxan (150 mg/kg b.w, ip) induced diabetic rates was evaluated. Blood was collected from the tail vein in rats at zero time and after drug administration in 1st, 2nd and 4th hour and 24 hour to examine the effect. HiRe at 250 and 1000 mg/kg was used for the study. Glibenclamide (3 mg/kg, p.o) was used as the standard drug. The following results were reported –

- 2nd hr: glibenclamide- 1.82 fold decrease compared to diabetic control; HiRe 250 mg/kg treated, 1.71 fold decrease; HiRe 1000 mg/kg treated 2.69 fold decrease.
- 4th hour: glibenclamide - 1.57 fold decrease in sugar level compared to diabetic control; HiRe 250 mg/kg 1.20 fold decrease; 1000 mg/kg. 1.46 fold decrease.
- 24th hour: glibenclamide 1.17 fold decrease, HiRe 250 mg/kg 1.19 fold decrease, HiRe 1000 mg/kg it 1.24 fold decrease.

This study showed that single dose of HiRe had significantly protected the glucose level in alloxan induced diabetic rates at 1st, 2nd and 4th hour and even after 24hr, with respect to that of control animals. The authors concluded that their study had revealed that HiRe possess significant antidiabetic activity in single dose study, suggesting the potential role of this plant as antidiabetic drug. They opined that the effect was partly ascribed to the free radical scavenging activity of HiRe.
S. Subramanian et al. [69] have reported antihyperglycemic, antioxidant and antidyslipidemic properties of *H. indicus* root extract in Alloxan-induced experimental diabetes in rats. The dry powdered roots were extracted with petroleum ether, then soxhletted with ethanol. The residue from the concentrated ethanolic extract was taken in water, and the resulting aqueous solution was used for testing. The effect of oral administration of *H. indicus* root extract (400 mg/kg b.w.) on glucose tolerance, the levels of blood glucose, hemoglobin, glycosylated hemoglobin, plasma insulin, protein, lipid peroxides, enzymatic and non-enzymatic antioxidants, lipid profile, muscle glycogen content were determined in control and experimental groups of rats by these researchers. They found that altered levels of blood glucose, hemoglobin, glycosylated hemoglobin, plasma insulin, and protein in the diabetic rats were significantly reverted back to near basal values by the administration of ethanol extract of *H. indicus* root to diabetic rats for 30 days. The levels of lipid peroxides in the plasma and pancreatic tissues of diabetic rats were elevated significantly and were normalized by the administration of the extract. The activities of pancreatic enzymic antioxidants and the levels of plasma non-enzymic antioxidants markedly declined in the diabetic rats. Upon treatment with *H. indicus* root extract to diabetic rats, these decreased levels were elevated to near normal values. The reduced level of glycogen content in muscle tissues of diabetic rats was significantly improved upon treatment with *H. indicus* root extract. The altered levels of lipid profile were reverted back to near normalcy upon the extract treatment. The results of the study indicate that *H. indicus* root extract possesses antihyperglycemic, antioxidant and antidyslipidemic activity. The results were comparable with glyclazide, an oral standard hypoglycemic drug.
MA Siraj et al. of Bangladesh took a different approach. Their investigation was designed to assay the antidiabetic effect of ethanol root extract of *Hemidesmus indicus* by gut perfusion and six segment methods on Long Evans rats [70]. In the gut perfusion study the glucose absorption in control rats *vs*. rats fed with 250 mg/kg and 500 mg/kg extracts were observed at 5, 10, 15, 20, 25 and 30 minutes and the significant ($p<0.05$) change of intestinal glucose absorption was found throughout the experimental time; their results were control *vs*. dose 1 *vs*. dose 2:

- 5 minutes - 34.96 *vs*. 29 *vs*. 37.97 mmol/L,
- 10 minutes - 34.29 *vs*. 28.04 *vs*. 37.99 mmol/L,
- 15 minutes - 39.69 *vs*. 42.85 *vs*. 38.29 mmol/L,
- 20 minutes - 35.69 *vs*. 30.32 *vs*. 36.45 mmol/L,
- 25 minutes - 36.98 *vs*. 30.44 *vs*. 35.92 mmol/L,
- 30 minutes - 34.82 *vs*. 19.44 *vs*. 30.77 mmol/L.

The change of intestinal glucose absorption was found significant with 250 mg/kg than 500 mg/kg root extract of *H. indicus*. Curiously, 250 mg/kg extract depressed glucose absorption, while 500 mg/kg enhanced slightly glucose absorption compared with control rats. The authors did not offer any explanation for this. The six segment study was performed to assess the amount of glucose remaining in the six different positions of the GIT

at 30, 60, 180 and 360 minutes. The data revealed that the 500 mg/kg root extract of Hemidesmus indicus had gradually reduced the glucose absorption in GIT compared to control throughout the experimental time. These results strongly suggested that ethanol root extract of *Hemidesmus indicus* has significant dose dependent antidiabetic effect. MA Siraj et al. opined that the isolation of pure, pharmacologically active constituents from plants remains a long and tedious process. For this reason, they felt it necessary to have 'methods available which eliminate unnecessary separation procedures. Chemical screening is thus performed to allow localization and targeted isolation of new or useful constituents with potential activities. This procedure enables recognition of known metabolites in extracts or at the earliest stages of separation and is thus economically very important'. However, no such activity-oriented fractionation was attempted by these researchers. In none of the above papers, there was any attempt to isolate the components responsible for anti-diabetic property. However the research groups of Nair et al. [71], and G. Mahalingam and K. Kannabiran, have endeavored to isolate the active principles.

Nair et al. [71] (Thiruvananthapuram, Kerala), while investigating tuberous root extracts of *H. indicus*, observed glucose lowering property of the root. They proceeded to attempt the isolation of the anti-hyperglycemic principle from the root and determine its utility to develop an anti-diabetes mellitus medicine. This they endeavored to do by anti-hyperglycaemic activity guided chromatographic techniques. Glucose tolerance test in rats was used to evaluate the anti-hyperglycaemic property. Anti-diabetes mellitus property was evaluated in alloxan-induced diabetic rats as well as streptozotocin-induced (type-2 model) diabetic rats. According to these authors, the active principle which was isolated and identified by spectral data was *β*-amyrin palmitate (II). Although it was a known compound, its presence in *H. indicus* was not known previously. They observed for the first time that *β*-amyrin palmitate has remarkable anti-hyperglycemic activity in orally glucose loaded rats. Further, the authors reported that it exhibited excellent anti-diabetes mellitus activity in both alloxan-diabetic and streptozotocin-diabetic rats at a very low concentration (50 µg/kg body weight). One of the mechanisms of action of *β*-amyrin palmitate appeared to be blocking the entry of glucose from the intestine. These researchers concluded that *β*-amyrin palmitate is very promising to develop a medicine for diabetes for combination therapy and/or mono-therapy.

G. Mahalingam and K. Kannabiran have reported extensively on the effect of 2-hydroxy 4-methoxy benzoic acid isolated from the roots of *H. indicus* on streptozotocin-induced diabetic rats in a number of papers [72-76].

M. Gayathri and K. Kannabiran [72,73] found that an active principle for its antidiabetic activity from the roots of *H. indicus* was 2-hydroxy 4-methoxy benzoic acid (HMBA). This was tested on (STZ)-induced diabetic rats. HMBA was administered (500 µg/kg body weight) orally to fed, fasted and glucose-loaded diabetic and non diabetic rats. The blood glucose level was reduced significantly ($F>0.05$ and $P< 0.05$) in fed, fasted, and glucose loaded diabetic rats. The increased activities of glucose-6-phosphatase and fructose-1, 6-bisphosphatase in diabetic rats was significantly ($F>0.05$; $P<0.001$) decreased to near normal level in the liver and kidney of HMBA treated (7 weeks) diabetic rats. The decreased levels of hexokinase and phosphoglucoisomerase in the liver and kidney of diabetic rats were restored to normal level when diabetic rats were fed with HMBA. A significant reduction in glucose, glucose-6-phosphatase, and fructose-1, 6-bisphosphatase activities in diabetic rats indicate the role of HMBA in suppressing the gluconeogensis in diabetic rats. The authors felt that alternatively normalization of hexokinase and hosphoglucoisomerase activities indicates the role of HMBA in inducing glycolysis in diabetic rats. On the basis of their findings, Gayathri and Kannabiran concluded that HMBA could be used as an antidiabetic agent for prevention and management of diabetes mellitus. This research group carried out further studies to assess the mode of action of HMBA.

G. Mahalingam and K. Kannabiran reported the effect of 2-hydroxy-4-methoxy benzoic acid (HMBA) on erythrocyte membrane bound enzymes and antioxidant status in Streptozotocin-induced diabetic rats [76]. The streptozotocin-induced diabetic rats were treated with HMBA (500 µg/kg/day) for 7 weeks by oral intubation and compared with glibenclamide, a standard hypoglycemic agent (100 mg/kg). The erythrocyte membrane was isolated and the activity of Na+/K+-dependent ATPases, Ca2+-ATPases, Mg2+-ATPases were determined. These researchers also assayed superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase, vitamins C, vitamin E, plasma reduced glutathione and erythrocyte glutathione, reduced glutathione content in the tissues. Administration of HMBA to diabetic rats significantly ($F>0.05$ and $P<0.001$) elevated the activity of total ATPases, Na+/K+ ATPase, Mg2+ ATPase and Ca2+ ATPase to near normal level. The activities of catalase, superoxide dismutase and glutathione peroxidase and glutathione-S-transferase in erythrocytes were decreased significantly ($F>0.05$; $P<0.001$) in diabetic rats. Diabetic rats treated with HMBA showed a significant ($F>0.05$; <0.001) increase in the enzymic antioxidants in erythrocytes. They observed that elevated levels of vitamin E and low level of vitamin C and glutathione level in plasma and erythrocytes in diabetic rats when compared to control rats were restored significantly ($F>0.05$; $P<0.001$) after the administration of 2-hydroxy-4-methoxy benzoic acid. Their study concluded that administration of 2-hydroxy-4-methoxy benzoic acid supports the restoration of antioxidant defence, reduces the free radial production, lipid peroxidation and the glycosylation of haemoglobin in diabetic rats.

It is well-known that diabetes slows down wound-healing in affected humans and animals. In this connection of significance are the studies of M. M. Moideen et al. (Tamil Nadu) [25] on wound healing activity of ethanolic extract of *Hemidesmus indicus* (Linn) R.Br leaves on rats. This study concluded that the leaves of *Hemidesmus indicus* possess marked wound healing activity and could play a promising role in the treatment of wounds especially chronic wounds and in diabetic and cancer patients. This study was carried using four groups of six Wister strain rats each - Group 1 (positive control) was provided with nitrofurantoin ointment, group 2 (solvent control) with 70% ethanol, group 3 (test dose I) with 5% w/w *Hemidesmus indicus* ointment and group 4 (test dose II) with 10% w/w *Hemidesmus indicus* ointment. The total exposure of the study was 16 days. Excision wounds were made as described by Morton and Malone by excising the full thickness circular skin (approx. 500 mm) from the nape of the neck under ether anesthesia. Wound closure rate and epithelization time were assessed by tracing the wound on polythene sheet from wounding day, followed on 2, 4, 8, 12, 14th days and subsequently on alternate days till complete epithelization. Similarly, scars were traced on complete epithelization to assess wound contraction by noting scar size and shape. Evaluation of haematological parameters such as RBC, WBC and haemoglobin content was performed. The groups were compared for the percentage of wound healing. It was observed that the group treated with nitrofurantoin ointment showed an increase in the rate and percentage of wound contraction and period of epithelization compared to the ethanol treated group. The alcoholic extract of *Hemidesmus indicus* (5% and 10% ointment) increased rate of wound contraction and period of epithelization than solvent and even the nitrofurantoin ointment trated control groups. These researchers found that the percentage and rate of wound healing was increased at test dose I than test dose II.

CONCLUSION

A completely different approach – based on theoretical calculations – has been taken by researchers at Osmania University. Their studies are entitled '*Hemidesmus indicus* plant derived compounds as Aldose reductase inhibitors – a Molecular Docking study' [77]. They noted that the primordial role played by Aldose reductase in type 2 Diabetes is widely documented, and that the important medicinal plant *H. indicus* is known to be an of numerous uses, with special interest lying in its derived secondary metabolites to inhibit Aldose reductase activity. These researchers performed a protein-ligand interaction study of a number of components from *H. indicus* with Aldose reductase as the target protein. They described docking of these compounds from *H. indicus* into the 3D structure of 1H4G of *Homo sapiens* using FlexX of which molecular docking; top scoring compounds were vanillin, 2-hydroxy-4-methoxy benzaldehyde, hyperoside, isoquercetin, *p*-methoxysalicylic aldehyde, phenylpropanoid were identified as potential inhibitors that showed high binding affinity for Aldose reductase enzyme.

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