GYMNEMA SYLVESTRE: A COMPREHENSIVE REVIEW

Triveni K.B*, Lakshmi V. K, Shashidhara S, Anitha S

Department of Pharmacognosy, Government College of Pharmacy, Bangalore-560027, Karnataka India.

ABSTRACT
Diabetes mellitus is a systemic metabolic disease, which has become common. The worldwide prevalence of diabetes for all age groups was estimated to be 2.8% in 2000 and it is projected to be 5.4% in 2025. Herbal medicines have been the highly esteemed source of medicine throughout human history. Although attempts at the isolation and structure elucidation of the Gymnemic acids have continued for more than a century, this area of endeavour has caused a great deal of difficulty for phytochemical researchers, and has been somewhat controversial. So an attempt has been made to summarize the isolation procedures, including Pharmacognostical, Phytochemistry and pharmacological review.

Keywords: Gymnema sylvestre, Phytochemistry, Gymnemic acid, isolation, pharmacology.

INTRODUCTION
Diabetes mellitus is a systemic metabolic disease characterized by hyperglycemia, hyperlipidemia, hyper aminoacidemia and hypoinsulinaemia[1]. It is frequently associated with the development of micro and macro vascular diseases which include neuropathy, nephropathy, cardio vascular and cerebrovascular diseases[2]. Currently available therapies for diabetes include insulin and various oral Antidiabetic agents such as sulfonylureas, biguanides, α-glucosidase inhibitors and glinides. In developing countries as products are expensive and not easily accessible. Presently there is growing interest in herbal remedies due to the side effects associated with the oral hypoglycemic agents for the treatment of diabetes mellitus[3]. Herbal products or plant products are rich in phenolic compounds, flavonoids, terpenoids, coumarins and other constituents which show reduction in blood glucose levels[4, 5, 6]. Due to their perceived effectiveness, fewer side effects in clinical experience and relatively low costs, herbal drugs are prescribed[7].

Gymnema sylvestre (Retz) Schult R.Br is one of the most popular anti-diabetic plant consists of leaves, is commonly known as periploca of woods, madhunashini belongs to
the family Asclepiadaceae. It is locally called as gurmar, and is distributed throughout India, in dry forests up to 600 meter height in peninsular India and malwa region.

**Vernacular names**:

- Kannada: sannagerasehambu, kadhasige
- Sanskrit: meshashringi, madhunashini, vishani.
- Bengali: mera-singi
- Gujarati: dhuleti, mardashingi
- Hindi: gurmar, mera-singi
- Malayalam: cakkarakkolli, madhunashini
- Marathi: kavali, kalikardori, vakundi
- Tamil: adigam, cherukurinja
- Telugu: podapatri
- English: periploca of woods, cow plant, Australian cow plant.
- Oriya: gudmari

**SYNONYMS**:

- Periploca sylvestris Retz
- Gymnema affine Decaisne
- Gymnema formosana Warburg
- Gymnema alternifolium (Lour) Merr.

**Distribution**:

The species is widely distributed in throughout India in a dry forest up-to 600 meter height. It is found in Banda, konkan, Western Ghats, and Deccan extending to the part of the northern and western India, in Tamilnadu, Karnataka and Uttarpradesh. It is
distributed in Asia, tropical Africa, Malaysia and Sri Lanka. It is occasionally cultivated as medicinal plant\textsuperscript{[8]}.

**CULTIVATION & PROPAGATION\textsuperscript{[14]}:**

**Soil and Climate**

The plant grows in a variety of soil and agro-climatic conditions in tropical and subtropical regions up to 600 m.

**Nursery Raising and Planting**

Mature seeds are collected during October-December and sown in poly-boxes/bags or small plots in nursery. The raised seedlings are transplanted in field during February-March. The plant grows well with the onset of rainy season. The climber is given proper support for its better growth and development. It can also be planted in between trees as intercropping.

The plant can also be propagated through cuttings and planted during rainy season.

**Weeding and Hoeing**

Periodical weeding and hoeing is required, particularly during and after rainy season.

**Manure and Fertilizer**

Compost or Vermi compost is preferred for application while preparing the soil for nursery and in the field plantation.

**Irrigation**

Periodic irrigation as and when required may be done weekly/fortnightly.

**Harvesting/post-harvesting**

After one-year leaves are ready for harvesting. The leaves are usually collected during October-February and are cleaned and dried in shade. The roots are collected during summer and are cleaned, washed and cut into pieces and dried.

**Flowering and fruit:** April – June\textsuperscript{[12]}. 

**Commercial varieties:** jhalawar, lotiajhir, RUBL 18050 (NKS 1343)\textsuperscript{[12]}.

**PHARMACOGNOSTIC STUDIES\textsuperscript{[10]}**
Macroscopic study:

Macroscopically the leaves are opposite, usually elliptic or ovate, cordate at base. Leaf is simple, entire, petiolate, margin is entire, apex is acute, reticulate venation, pubescent on both the surfaces, however, the dorsal one is highly pubescent; about 2 to 6 cm long and 1 to 4 cm broad, yellowish brown on axial and dark green on axial side. The texture of leaf is papery and bitter to taste. It also possesses remarkable property of paralyzing the sense of taste for few hours particularly for sweet substances\[^{10}\].

Microscopic studies

TS of petiole are horse-shoe shaped. Trichomes present profusely all over the periphery, and are uniseriate, multicellular and thick walled. The epidermis is single layered, thick walled followed by collenchymatous cortex. The vascular bundles are amphicribal and 3 in number-two lateral, and one median. Phloem well developed. Xylem consists of vessel elements, tracheids and tracheidal fibres. Rosette crystals are present more towards the centre. The starch grains are polygonal, simple or compound in two to many in groups, hilum indistinct\[^{10}\].

TS of lamina are dorsiventral, shows striated cuticle, followed by single layered epidermis. The uniseriate and multicellular trichomes are observed on the lower and upper epidermis. The upper epidermal cells are hexagonal while the lower epidermal cells are slightly wavy in surface view. Anomocytic stomata seen on lower side in surface view. The single layered closely arranged palisade cells are present just below the upper epidermis. The spongy parenchyma is 3 to 5 cells thick with large intercellular spaces. The midrib region has amphicribal vascular bundle\[^{10}\].

Powder Characteristics

Green, taste bitter with pleasant aromatic odour; thick-walled, uniseriate multicellular trichomes, anomocytic stomata, idioblast cells with calcium–oxalate rosettes, starch grains, groups of collenchymatous and parenchymatous cells, vessel elements and tracheids; when treated with 1N aqueous sodium hydroxide and 50 percent potassium hydroxide shows green fluorescence under UV at 254 nm and orange yellow colour with 50 percent nitric acid in day light\[^{10}\].
Standards\textsuperscript{[10]}

1. Foreign matter: Not more than 2.0%.
2. Total ash: Not more than 11.0%.
3. Acid insoluble ash: Not more than 2.0%.
4. Ethanol-soluble extractive: Not less than 20.0%.
5. Water soluble extractive: Not less than 29.0%.
6. Loss on drying: Not more than 7.0%.

Phyto-chemical study:
A variety of constituents have been isolated from different parts of Gymnema sylvestre. The leaves contain triterpenoid saponins belonging to oleanane and dammarane classes. Oleanane saponins are Gymnemic acids and Gymnemasaponin, while dammarane saponins are Gymnemosides. Besides this other plant constituents are nonacosane, conduritol A, gymnestrogenin, Gymnemagenin, gymnemoside a, b triterpenoid glycoside, flavones, anthraquinones, hentri-acontane, pentatriacontane, $\alpha$ and $\beta$-chlorophylls, phytin, resins, d-quercitol, tartaric acid, formic acid, butyric acid, lupeol, $\beta$-amyrin related glycosides anthraquinones and their derivatives\textsuperscript{[13]}.
TABLE 1: STRUCTURE OF GYMNEMIC ACIDS

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<tr>
<th>Sl. No.</th>
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<tr>
<td>a)</td>
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<td>β-glc A</td>
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<td>h)</td>
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<td>i)</td>
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### TABLE 2: STRUCTURE OF OTHER CONSTITUENTS

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<tr>
<td>b)</td>
<td>Gymnestroygenin</td>
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<tr>
<td>c)</td>
<td>Gymnemasaponin III</td>
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<tr>
<td>d)</td>
<td>Gymnemasaponin IV</td>
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<tr>
<td>e)</td>
<td>Gymnemasaponin V</td>
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<tr>
<td>a)</td>
<td>Gymnemic acid XV</td>
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<tr>
<td>b)</td>
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<tr>
<td>c)</td>
<td>Gymnemic acid XVII</td>
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<tr>
<td>d)</td>
<td>Gymnemic acid XVIII</td>
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</tbody>
</table>
ISOLATION STUDIES:

1. The leaves of *Gymnema sylvestre* were investigated for new compounds by chromatographic techniques. The ethanol extract was partitioned between n-butanol and water. The butanol solution was subjected to silica gel column chromatography using chloroform: methanol as eluent and C18 column chromatography for purification. This afforded six new oleanane saponins. The compounds were subjected to spectral studies to elucidate their structure\[^{34}\].

2. The pet ether defatted leaves were continuously extracted with water or 95% ethanol. Further pH was adjusted, recrystallised three times from diethyl carbonate, purified by using adsorption chromatography on deactivated silica gel, reverse phase partition chromatography on Teflon 6 and preparative TLC. Gymnemic acids A to D were isolated and preliminary characterization of Gymnemic acid was done. The isolated compounds were characterized by spectroscopic studies and compared with literature values\[^{35}\].

3. The 50% hot ethanol extract of *Gymnema sylvestre* leaves was subjected to successive column chromatography on Amberlite XAD-2, sephadex LH-20, silica gel followed by preparative TLC and HPLC furnished four new triterpenoid saponins Gymnemasins A, B, C and D, along with known Gymnemic acids I-VI. The aglycone gymnemanol, which is a new compound, was also isolated. All the compounds isolated were characterized and identified by spectroscopic studies\[^{36}\].

4. A gymnemate salt fraction was isolated from an aqueous ethanol extract of dried leaves, by chromatography on high porosity, quarternary ammonium resin (acetate form) with 1N acetic acid in ethanol –water (7:3) furnished Gymnemic acid A\(_1\). Gymnemic acid A\(_1\) was resolved into A\(_{11}\) and A\(_{12}\) by TLC (silica gel, CHCL\(_3\): t-BuOH: OHAc: H\(_2\)O; 8:2:2:1) and were distinguishable by NMR and UV spectral studies\[^{37}\].

5. The saponins mixture obtained by Kurihara’s procedure was repeatedly chromatographed on ODS columns using different solvent systems consisting of methanol, ammonium carbonate and potassium dihydrogen phosphate buffers. Purification was done by HPLC, on ODS column. Two new Gymnemic acids,
GA-VIII and GA-IX were isolated along with known Gymnemic acids, GA III, IV and V. The structure of Gymnemagenin was established by X-ray analysis and of DAGA by C-13 NMR spectra. Compounds were characterized and structurally elucidated by spectral studies\textsuperscript{[38]}.

6. The crude saponins mixture obtained by Kurihara’s procedure was subjected for repeated chromatography on an ODS column. Final purification was done by preparative HPLC on an ODS column using MeOH-0.25% potassium dihydrogen phosphate buffer. Two new Gymnemic acids, GA VIII and GA IX were isolated. The structures were elucidated on the basis of spectroscopic and chemical means\textsuperscript{[39]}.

7. Dried leaves of Gymnema sylvestre were extracted with 60% ethanol, which was passed through an Amberlite XAD-2 and Toyopearl HW-20 column using methanol as eluent. The crude saponins obtained were chromatographed on Servachrome XAD-2 using 40-70% methanol as eluent. Further repeated chromatography on silica gel with CHCL\textsubscript{3}: MEOH: H\textsubscript{2}O (65:35:10) and HPLC, afforded four new saponins, GA-XV to GA-XVIII. The structures were elucidated by spectral and chemical studies\textsuperscript{[40]}.

8. The ethanol extract was passed through an Amberlite XAD-2 and Toyopearl HW-40 columns to give crude saponins, which was further chromatographed on Servachrome XAD-2 using 40-70% methanol as eluent to give four fractions. By repeated chromatography on silica gel with EA: MeOH: H\textsubscript{2}O and CHCL\textsubscript{3}: MeOH: H\textsubscript{2}O as eluent and purification by HPLC gave five new Gymnemic acids, GA-VIII to GA-XII. Their structures were elucidated on the basis of spectral and chemical evidences\textsuperscript{[41]}.

9. The methanol extract of leaves of Gymnema sylvestre was repeatedly separated by reverse-phase and normal phase sio2 column to give crude saponins. Purified by RP-SiO\textsubscript{2} column and HPLC to give two new saponins, Gymnemosides A, B along with GA I, II, III, IV, V, VII and Gymnema saponins II, IV and V. Their structures were elucidated by spectral studies\textsuperscript{[42]}.
10. The leaves were extracted with 50% aqueous ethanol and the extract was successively chromatographed on an Amberlite XAD-2 and Toyopearl HW-40 columns to give fractions I-V. Fraction I and II were further separated by ordinary phase SiO\textsubscript{2} and RP-HPLC on an ODS column to give five new saponins Gymnema saponins I-V. Their structures were elucidated by spectroscopic studies\textsuperscript{[43]}.

11. The leaves were homogenized in 30% ethanol and the residual aqueous solution was acidified to get precipitate of Gymnemic acid. The supernatant was neutralized, then deionised, passed through an active carbon column, eluted with water and further purified to yield Conduritol A. The compound was identified by physico-chemical characteristics, chemical and spectroscopic methods. The effect of Conduritol A was checked by using everted sacs prepared from rat small intestine. Glucose absorption was decreased linearly with increase in concentration of Conduritol A, which proved its absorption inhibition capacity\textsuperscript{[44]}.

12. The 50% ethanol extract of Gymnema sylvestre was passed through Amberlite XAD-2 column using methanol and then through Servachrome XAD-2 using 30-70% methanol and successively Toyopearl HW 20 and sephadex LH 20 using methanol to separate each group. The dammarane fractions were subjected to repeated silica gel and HPLC. This finally provided with seven new glycosides, Gymnemosides I-VII. Their structures were characterized by spectral data and chemical transformations\textsuperscript{[45]}.

13. The leaves were macerated and then continuously hot extracted with pet ether. The solid obtained was chromatographed over florisil, eluted with pet ether. The white semisolid obtained was recrystallised from acetone and subjected to GC analysis, which revealed the presence of nonacosane, hentriacontane and tritriacontane. Their identification was done by physical, chemical properties and mass spectroscopic analysis.
PHARMACOLOGICAL STUDY:
Normoglycemic and Hypolipidemic activity

Gymnema sylvestre leaves were subjected for isolation and identification of a putative anti-diabetic compound based on bioassay guided fractionation. A novel dihydroxy Gymnemic triacetate was isolated from acetone extract and its optimum dose was determined to be 20mg/kg body weight and was administered orally for 45 days to streptozotocin induced diabetic rats. Biochemical parameters were assessed. The compound increased plasma insulin, muscle and liver glycogen content. It decreased blood glucose and glycated hemoglobin. The serum lipids level and activities of hepatic enzymes were maintained normal. These results indicated the Normoglycemic and Hypolipidemic activities of the isolated compound\(^{[30]}\).

The role of Gymnema sylvestre leaf and invitro callus extracts in promoting β-cell regeneration and anti-diabetic effects has been characterized. Their methanol extracts were administered to alloxan induced diabetic rats and the weights of their whole body, liver, pancreas and liver glycogen content was measured. A histological examination of pancreas was done. There was increase in the weight of whole body, liver, pancreas and liver glycogen content. Degenerative changes in pancreatic β-cells were minimized and normal morphology was maintained in diabetic rats administered with Gymnema sylvestre extracts. The content of Gymnemic acid in leaf and callus extracts was analyzed by using TLC, HPTLC and HPLC methods. These results proved the anti-diabetic and β-cell regeneration activities of Gymnema sylvestre extracts\(^{[29]}\).

Anti-obesity activity

The anti-obesity effects of Gymnema sylvestre leaves were investigated in wistar rats. The saponins rich aqueous extract was administered once a day in the dose of 100mg/kg body weight to a high fat diet induced wistar rats for a period of 8 weeks. Orlistat was used as standard anti-obese agent. Changes in body weight, organ weights and other related plasma biochemical profile was monitored. There was suppression in gain of body weight, organs weight and other plasma lipids level. This showed the anti-obesity effect of saponins rich aqueous extract\(^{[18]}\).
The effects of some medicinal plants that are claimed to be useful in the treatment of obesity are reviewed. *Gymnema sylvestre* had indication in obesity, lipid and glucose metabolism alterations, due to presence of Gymnemic acid and daily dosage was mentioned as 15mg\[^{[19]}\].

**Anti-microbial activity**

*Gymnema sylvestre* leaves were checked for anti-microbial activity. Different concentrations (10-200mg/ml) of ethanolic extract of leaves were tested by agar-well diffusion method. Remarkable anti-microbial activity was seen with increasing concentration of extract against *Bacillus pumilis*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*\[^{[31]}\].

The antibacterial properties of *Gymnema sylvestre* leaves were tested against three gram positive and five gram negative bacteria. The pet ether, chloroform and ethanol extracts were tested using agar well diffusion method. All the extracts inhibited the growth of all the eight bacterial species tested, in a dose dependent manner. No antibacterial activity was noted at 10 and 20mg/ml\[^{[28]}\].

**Radio protective activity**

The radio protective effect of Gymnemic acid was evaluated on Swiss albino mice induced by radiation. Hepatic biochemical alterations were monitored. Gymnemic acid lowered lipid peroxidation, protected the endogenous GSH depletion, increased protein concentration and showed a significant hepato–protective effect against irradiation as compared to normal and control groups\[^{[32]}\].

**Anti-viral Activity**

Gymnemic acids were investigated for their anti-viral activity. Crude Gymnemic acid mixture was obtained by mineral acid precipitation of aqueous extract of the leaves. It was plated on silica gel and extracted with chloroform and ethyl acetate in a Soxhlet extractor. The EA extractive was then chromatographed over silica gel using increased proportions of acetone in EA. Gymnemic acid A to D were isolated and tested invitro against influenza virus. Tube cultures of primary chick kidney cells were treated with influenza virus. Viral growth cycle was studied in control and treated cultures. The yield
of viral hemagglutinin and infectivity was measured. GA-A and B showed demonstrable inhibition of growth of viral infected cells while GA-C and D was none\[33\].

**Anti-oxidant activity**

Preliminary phytochemical screening and invitro anti-oxidant of Gymnema sylvestre R.Br. leaf extract were done. The 55% alcohol extract was subjected to invitro anti-oxidant models like DPPH radical scavenging activity, superoxide radical scavenging activity, ferric reducing power, and hydrogen peroxide scavenging activity. Total anti-oxidant capacity was found to be 17.54mg/g expressed as ascorbic acid. The plant showed a significant anti-oxidant activity which might be due to presence of acidic compounds, flavonoids, phenols, saponins, tannins and triterpenoids found in the preliminary phytochemical screening\[22\].

**Immuno modulatory activity**

The plant was investigated for immunomodulatory activity by assessing neutrophil locomotion, chemotaxis test, phagocytosis of killed Candida albicans and nitroblue tetrazolium tests. The aqueous extract of leaves significantly increased the phagocytic function and chemotactic movement at 25µg/ml. Also intracellular reduction of nitroblue tetrazolium dye to formazan was increased indicating killing property of neutrophils at 50µg/ml concentration. All these results showed immunomodulatory activity of Gymnema sylvestre\[23\].

**Anti-inflammatory activity**

The aqueous extract of Gymnema sylvestre leaves was investigated of anti-inflammatory activity in rats at a dose of 200, 300 and 500mg/kg in carrageenan induced paw edema and cotton pellet method. At dose 300mg/kg, there was a significant decrease in paw edema volume by 48.5% while that of standard drug was 57.6%. Doses of 200 and 300 mg/kg produced significant reduction in granuloma weight, when compared to control group. Hence the aqueous extract of Gymnema sylvestre showed predominantly significant anti-inflammatory activity when compared to the standard drug phenylbutazone\[24\].
Anticancer activity

The plant was investigated for anticancer activity on MCF 7 (epithelial cells of human breast cancer) and A 549 (epithelial cells of human lung cancer) under invitro conditions by MTT assay method and also to establish the active constituents responsible. The plant was successively extracted with chloroform, EA and 95% alcohol. All three extracts have shown concentration dependent anti-cancer activity and the IC \textsubscript{50} values are almost similar, at concentrations of 50 and 100µg/ml comparable to the standard drug etoposide\textsuperscript{[20]}.

Snake venom neutralizing effect:
The substances that are identified in plants reputed to have neutralized effects of snake venom was surveyed and confirmed by biological assays. Albino mice were used as experimental animals. Extracts were administered by gastric intubation, at the dose of 100mg/kg body weight. Gymnema sylvestre was also tested and proved to have antidote property against snake venom and was tabulated under the list. Its activity was thought to be due to presence of gymnemgenin\textsuperscript{[25]}.

Wound healing activity:
The wound healing activity of carbopol gels prepared from hydro alcoholic extracts of Gymnema sylvestre and Tagetes erecta Linn were checked by excision wound model and burn wound models in albino mice. Significant increase in percentage wound contraction was observed in groups treated with both extracts, when compared with standard metrogyl. Thus wound contraction, qualitative tests and TLC support the synergistic effects of both drugs. Their stability study also paves a way for its use in topical treatment and management of wounds\textsuperscript{[27]}.

DISCUSSION

Gymnema sylvestre is one of the important medicinal plants widely used for diabetes mellitus. Study of its Pharmacognostical, Phytochemistry, isolation procedure and pharmacological studies is been summarized. Study of its isolation procedures revealed that repeated reverse phase chromatographic isolations help in crude and individual separation of compounds. In normal phase chromatographic techniques, solvent system chloroform: methanol: water ratios in increasing order of their polarity
help very much in better separation of Gymnemic acids. Preparative HPLC and other new improved techniques of separation also should be used to separate chemical constituents. Also its therapeutic considerations makes it very important drug having multiple curative actions in leading healthy life.

CONCLUSION

Gymnema sylvestre is wonderful medicinal plant used since time immemorial and researched for its chemical constituents. The active principles of the plant are present in very complex mixture which created limitation in their isolation. There has been a rapid development in the isolation and characterization techniques. But a view into past methods is worthful in understanding the properties of compounds present and makes future works easy, less time consuming and use of modern improved techniques. The wide varieties of compounds isolated from this plant need to research in depth to establish their profile. This paper aims to detail some isolation procedures to provide better scope for performing the isolation investigation in the plant. The different pharmacological actions makes it wonderful drug of choice for healthy life.

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For Correspondence:
Triveni K.B
Email: trivenigcp@gmail.com