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GYMNEMA SYLVESTRE R. Br: A COMPREHENSIVE REVIEW ON PHYTOCHEMICAL AND ANALYTICAL STUDY

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ABSTRACT: *Gymnema sylvestre* R.Br is a valuable herb extensively used in the Ayurvedic and indigenous medicinal system, belonging to the family Asclepiadaceae. It has property of abolishing the taste of sugar and hence given name gurmar (sugar destroying). This plant has also found application in pharmaceuticals; whole plant is rich in secondary metabolites, which impart medicinal uses to the plant. The view of phytochemistry gives scope for enhancement of the quality and quantity of the bioactive secondary metabolites occurring in the plant. The present review is an attempt to highlight the various phytochemical reports on *Gymnema sylvestre*.

INTRODUCTION: During the last two decades, the changes in modern life style and unhealthy food habits have resulted in obesity and diabetes in large sector of the population. In order to cater the needs of such people, the market is flooded with many synthetic antidiabetic medicines. However, the long term use of these drugs has resulted in many toxic side effects. Natural products and plant-based drugs are gaining importance as a source of antidiabetics due to growing concern over the use of synthetic chemicals¹. A considerable amount of work has been done to study the potentials of herbal medicines. Modern science has accepted the potential of plant kingdom as a source of new biodynamic constituents. The laborious research work that has been carried out in many research institutes in different parts of the world has brought into lime light the merits and qualities of various herbal medicine.

One such medicine with a good antidiabetic activity is *Gymnema sylvestre* R.Br. It is a valuable herb from the family Asclepiadaceae. It is a woody climber has a past history of usage about 2000 years in India for the treatment of "Madhumeha"^{2, 3, 4}. The word *Gymnema* is derived from a Hindu word "Gurmar" meaning destroyer of sugar and it is believed to be chewing of the leaves neutralizes the excess of body sugar level⁵. The plant species is also known to be as Miracle fruit⁶.


Plant Profile:

Botanical Classification:¹⁰

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Gentinales
Family : Asclepiadaceae
Genus : *Gymnema*
Species : *G. sylvestre*

Synonym:^{7, 8, 9}

English : Periploca of the woods
Hindi : Gurmar
Kannada : Kadhasige

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Sanskrit	: Meshashringi
Tamil	: Shirukurum kaay
Telugu	: Gurmaar Buuti
Bengali	: Mera-singi
Guajarati	: Dhuleti
Malayalam	: Cakkarakkolli
Marathi	: Kavali
Oriya	: Gudmari

Habitat: It is found in various deciduous forest of India and common in Deccan peninsula.



FIG. 1: *GYMNEMA SYLVESTRE* R.Br

Ethno Medicinal Uses:^{11, 12, 13}

- Gynmema has played an important role in Ayurvedic medicine for centuries. Its use has been confined primarily to the management of diabetes mellitus and similar hypo / hyperglycemic conditions.
- According to the Ayurvedic Pharmacopoeia of India; both the dried leaf and root of gynmema, depending on dosage form and formulation, are also used in the treatment of svasa (bronchial asthma), kapha (cough), kustha (leprosy and other skin diseases), and vrana (wounds), among other conditions.

- Charak Samhita describes *G. sylvestre*, removes bad odor from breast milk. It is aperitive. This plant is useful as purgative and in eye troubles. The leaf extract and flower is beneficial for eyes. Bark is given in the diseases caused by vitiated kapha (phlegm). The root bark is useful in piles.
- Sushruta describes *G. sylvestre*, as a destroyer of madhumeha (glycosuria) and other urinary disease.

Phytochemical Study: Peter EM *et al.*, in 1965 isolated nonacosane, hentriacontane, and tritriacontane by vapor phase chromatography from a hydrocarbon fraction of *G. sylvestre* leaves. The cyclic alcohol, conduritol A, rather than the previously reported viburnitol, was also isolated from these leaves. The molecular weight determination of conduritol A tetraacetate by mass spectrometry has been determined¹⁴.

Joseph ES *et al.*, in 1967 examined the leaves of *G. sylvestre* for the presence of alkaloids through the establishment of quaternary and nonquaternary nitrogenous fractions. The plant bases choline, betaine and adenine were isolated and identified in a combined yield of 0.04%. The amino acids leucine, isoleucine, valine, alanine and γ -amino- η -butyric acid were also identified¹⁵.

Kurihara Y *et al.*, in 1969 carried out structural elucidation in order to elucidate the relationship between the structures of various gymnemic acids and the abilities to suppress sweetness, the anti sweet activities of different gymnemic acid components were compared in this study. A new method was devised to isolate gymnemic acid A₁ for preparative purpose. Gymnemic acid A₁ was converted into A₂ and finally into A₃ by alkaline hydrolysis. Anti sweet activity of A₁ was greatly decreased to A₂ where A₃ didn't show any activity¹⁶.

Walter stocklin *et al.*, in 1969 reported the major component, gymnemic acid A₁, which possesses the antisweet property, has been shown to be a D-glucuronide of a new hexahydroxytriterpene. On the basis of physical and chemical data the structure of the new triterpene was established¹⁷.

Joseph ES *et al.*, in 19870 isolated genins G, K, N and gymnestrogenin with the aid of selective enzyme system and shown to be in the aglycones of

gymnemic acids A-D, respectively. Genin G was found to be an acylated derivative of gymnemagenin containing formic, acetic, isovaleric and tiglic acids, while genin K differed from G by the absence of acetic acid residue. Genin N was observed to be gymnestrogenin tiglate. The sugar moieties of acids A and B are not acylated, while those of acids C and D are indicated to be esterified with ferulic acid¹⁸.

Isolation of crystalline gymnemagenin and gymnestrogenin from the leaves of *G. sylvestre*, together with preparation of various derivatives of the two aglcones, was described by Joseph ES et al., in 1971¹⁹.

Morihiko et al., in 1989 isolated two major active components of gymnemic acids in pure state. Their chemical structures were established as D-glucuronide of 3 β , 16 β , 21 β , 22 β , 23, 28-hexahydroxyolean-12-ene which is esterified with tiglic acid or 2-methylbutyric acid at 21-C hydroxyl group, respectively. The antisweet activity of these compounds was discussed in relation to their structures²⁰.

Yoshisuke T et al., in 1989 reported structure of gymnemagenin, the sapogenin of antisweet principle of *Gymnema* was firmly established as 3 β , 16 β , 21 β , 22 α , 23, 28-hexahydroxy-olean-12-ene by the x-ray analysis of the 3 β , 23; 21 β , 23 α -di-O-isopropylidene derivative. On the basis of this deacylgymnemic acid was elucidated as the 3-O- β -glucuronide by comparisons of ¹³C-NMR spectra²¹.

Kazuko Y et al., in 1991 reported the leaves of *G. sylvestre* were extracted with 50% aqueous ethanol and the extract was successively chromatographed on an amberlite XAD-2 and tyopearl HW-40 columns to give fractions I-V. Fraction I and II were further separated by ordinary phase SiO₂ and RP-HPLC on an ODS column to give five new saponins gymnema saponins I-V. Their structures were elucidated by spectroscopic studies²².

Kazuko Y et al., in 1992 isolated seven new dammarane type saponins, named gymnemasides I-VII from the leaves of *G. sylvestre*, together with the previously known dammarane-type saponins, gypenoside XXVIII, XXXVII, LV, LX11 and LXIII. Their structures were characterized by spectral data and chemical transformations²³.

Liu HM et al., in 1992 isolated five antisweet principles, gymnemic acid III, IV, V, VIII, and IX, in pure states from the hot water extract of leaves of *G. sylvestre*. The structures of gymnemic acid VIII and IX were elucidated as 3'-O- β -D-arabino-2-hexulopyranosyl gymnemic acid III and IV, respectively²⁴.

Kazuko Y et al., in 1992 isolated five oleanane-type triterpenoid saponins, gymnemic acids VIII-XII as antisweet principles from the leaves of *G. sylvestre* and characterized as glucosideuronic acid derivatives of gymnemagenin acylated with acetyl, tigloyl and 2-methylbutyryl moieties²⁵.

Kazuko Y et al., in 1993 isolated four new saponins, gymnemic acids XV-XVIII, as anti sweet substances. The structures were elucidated by spectral and chemical studies. Gymnemic acids XV - XVIII, are 21-O-2-methylbutyryl-22-O-2-methylcrotonoyl, 16-22-O-bis-2-methylcrotonoyl, 21-O-benzoyl, and 28-O-benzoyl gymnemagenin 3-O-glucuronides, respectively²⁶.

Niranjan PS et al., in 1996 isolated four new triterpenoid saponins, gymnemasins A, B, C and D, from the leaves of *G. sylvestre*, were identified as 3-O- $[\beta$ -D-glucopyranosyl(1 \rightarrow 3)- β -D-glucuronopyranosyl]-22-O-tigloyl-gymnemanol, 3-O- $[\beta$ -D-glucopyranosyl(1 \rightarrow 3)- β -D-glucuronopyranosyl]-gymnemanol, 3-O- β -D-glucuronopyranosyl-22-O-tigloyl-gymnemanol and 3-O- β -D-glucuronopyranosyl-gymnemanol, respectively. The aglycone, gymnemanol, which is a new compound, was characterized as 3 β , 16 β , 22 α , 23, 28-pentahydroxyolean-12-ene²⁷.

Wen CY et al., in 2000 isolated six oleanane-type saponins, along with two known triterpene saponins, were isolated from the leaves of *G. sylvestre*. The structures of the oleanane triterpene glycosides were characterized on the basis of hydrolysis and spectral evidence, including 1D- and 2D-NMR (TOCSY, ROESY, HMQC and HMBC) and FABMS analyses²⁸.

Wencai Y et al., in 2001 isolated three new oleanane-type triterpene glycosides (1-3), along with the sodium salt of alternoside II, from an ethanol extract of the leaves of *G. sylvestre*. The structures of these new saponins were identified and structure elucidation was accomplished by

interpretation of NMR (DQF-COSY, HMQC, and HMBC) results, FABMS, and hydrolysis²⁹.

Shu LP *et al.*, in 2005 isolated novel oleanane type triterpenic acid from the leaves of *G. sylvestre*. The structure was characterized as 3 β , 16 β , 22 β , 28-tetrahydroxy- olean-12-en-30-oic acid on the basis of spectral evidence, including 1D- and 2D-NMR (HMQC, HMBC, 1H-1H COSY and NOESY)³⁰.

Balaram M *et al.*, in 2006 reported the glycone part of the flavonoid triglycoside, kaempferol 3-O- β -D-glucopyranosyl-(1 \rightarrow 4)- α - L- rhamnopyranosyl-(1 \rightarrow 6)- β - D- galactopyranoside, has been synthesized with good yield and stereoselectivity using N-iodosuccinimide and HClO₄-silica promoted glycosylations of thioglycoside donors³¹.

The chemical constituents from the stem of *G. sylvestre* were extracted by percolation with ethanol, part of n-butanol extract was isolated and purified by macroporous adsorptive resins, silica gel column chromatography, sephadex gel column chromatography and recrystallization. Zhen HS *et al.*, in 2008 isolated eight compounds and were identified as Conduritol A, 1-Heptadecanol, Stigmasterol glucoside, 1-Quercitol, 1-Octadecanol, Potassium nitrate, Lupeol cinnamate, Stigmasterol³².

Zhu XM *et al.*, in 2008 isolated Gymnemoside-W1 and W2, together with seven known compounds from the leaves of *G. sylvestre*. By means of spectral and chemical analysis, the structures of the new compounds were elucidated as 16 beta-hydroxyl olean-12-en-3-O-[beta-D-glucopyranosyl (1 \rightarrow 6)-beta-D-glucopyranosyl]- 28- O- beta- D-glucopyranoside and 16 beta, 21 beta, 28- trihydroxyl-olean-12-ene-3-O-glucuronopyranoside³³.

Dr. Farzana *et al.*, in 2010 carried out isolation and characterization of gymnemic acid from indigenous *G. sylvestre* leaves. Four different methods of extraction were employed to obtain the maximum yield. The method where the defatted leaves were extracted under continuous hot extraction in Soxhlet apparatus with 95% ethanol gave the maximum yield of gymnemic acid (6.15% m.f.b.). The yield was minimum in aqueous extraction method (1.66% m.f.b.) Gymnemic acid was purified by preparative chromatographic method in two solvent systems. Its circular Thin Layer

Chromatography (TLC) also resulted in to a single concentric ring. Gymnemic acid was hydrolyzed by two methods to confirm its glycosidic nature. The presence of stigmasterol, β -amyrin, β -amyrin acetate and lupeol was indicated when the reference samples of these compounds were run on the same chromatoplate³⁴.

Krishna BR *et al.*, in 2012 isolated Gymnemic acid from *G. sylvestre* leaves by soxhlet using different solvents like petroleum ether, benzene, methanol and HPTLC analysis showed 30% purity with methanol³⁵.

Volatile components of flowers of *G. sylvestre* were extracted by water vapor distilling, and the 55 components were separated and 33 components were identified, accounting for 88.73% of all quantity. The principal volatile components were found to be Phytol, Pentacosane, 10-Heneicosene (c,t), 3-Eicosene, (E) -and 2-Methyl-Z-2-docosane, this study was done by Quiu Q *et al.*, in 2013 provided scientific basis for chemical component research of flowers of *G. sylvestre*³⁶.

Liu Y *et al.*, in 2014 isolated seven compounds from *G. sylvestre* and their structures were elucidated as conduritol A, stigmasterol, lupeol, stigmasterol-3-O- β -D-glucoside, the sodium salt of 22 α - hydroxy- longispinogenin- 3- O- β - D-glucopyranosyl-(1 \rightarrow 3)- β - D-glu-curono-pyranosyl-28-O- α -L-rhamnopyranoside, oleanolic acid-3-O- β -D-glucopyranosyl-(1 \rightarrow 6)- β - D- glucopyranoside, and the sodium salt of 22 α -hydroxy-longispinogenin 3- O- β -D-glucuronopyranosyl-28-O- α -L-rhamnopyranoside, on the basis of MS and spectroscopic analysis (1D and 2D NMR), as well as chemical methods³⁷.

Vishwa raj lal *et al.*, in 2015 isolated and characterized the Gymnemic acid, from five ecotypes of *G. sylvestre* leaves with different solvent systems like petroleum ether, benzene, and methanol. The defatted leaves were extracted under continuous hot extraction in Soxhlet apparatus with 90% methanol gave the maximum yield of gymnemic acid (42%) in the ecotype collected from Ranchi. Gymnemic acid was purified by preparative chromatographic methods³⁸.

Srinivasan K *et al.*, in 2016 determined the chemical composition of ethanolic extract of *G.*

sylvestre through Gas Chromatography-Mass Spectrometry technique. GC - MS analysis of leaf extracts revealed the existence of Terpenes, alcohols, fatty acids, amine and sterols. The highest % Peak area is hexadecanoic acid, α -Santoline alcohol, recorded the next highest % peak area of 9.05. Major of the compounds belongs to terpeneoid group, namely 6-Octen-1-ol, 3, 7-dimethyl, Isophytol, Squalene, Nerolidol, β -Amyrin and Cedrene-V6 which constitutes 30.7% of the peak area. The presence of α -Tocopherol- β -D-mannoside and Vitamin E also identified through this study³⁹.

Analytical Study: The molecular masses of gymnemic acid homologues were determined by Toshiaki *et al.*, in 1991 with high-performance liquid chromatography combined with atmospheric pressure ionization mass spectrometry. A mixture of gymnemic acids was chromatographed on an octadecyl silica column eluted with an aqueous methanol solution containing ammonium acetate, and directly introduced into an atmospheric pressure ionization mass spectrometer. Quasimolecular ions of gymnemic acids were detected as ammonium adduct ions. Molecular masses of thirteen different gymnemic acids and five compounds not containing glucuronic acid in their molecules were evaluated. Three pairs of geometrical isomers of gymnemic acids were found⁴⁰.

Puratchimani V *et al.*, in 2004 carried out a simple and reproducible HPTLC for the determination gymnemagenin from *G. sylvestre* was developed on silica gel 60F254 by using CHCl_3 :MeOH (9:1) and scanned using densitometric scanner in UV reflectance photomode at 293 nm. The linearity was observed in the range of 4 – 10 μg . The gymnemagenin content of 1.11% in test sample w/w was observed in test sample. The average percentage recovery value of 99.1 ± 0.27 was obtained⁴¹.

Sai Kishore S *et al.*, in 2008 carried out a comparison study for estimation of gymnemic acids by HPLC and gravimetry method for various extracts of *G. sylvestre* was an Isocratic, reversed phase HPLC procedure has been adopted by using a mixture of acetonitrile (23%) and 0.1% orthophosphoric acid as mobile phase, C18 column

as stationary phase and UV detector. HPLC method shows high resolution, accuracy and reproducibility than gravimetry estimation of Gymnemic acids in *G. sylvestre*⁴².

A reproducible and reliable HPTLC method for the indirect determination of gymnemic acids as gymnemagenin in *G. sylvestre* plant has been reported by Trivedi PD *et al.*, in 2008 Post-derivatization method was used for quantification of gymnemagenin. Linearity was observed in the range of 180 - 1440 ng/spot. Percentage recovery was found to be $98.4 \pm 1.0\%$ ⁴³.

Trivedi PD *et al.*, in 2011 developed a liquid chromatographic method for the determination of gymnemagenin in leaves of *G. sylvestre*. Gymnemagenin was obtained after acidic hydrolysis followed by basic hydrolysis of the sample and extraction into ethyl acetate. Analyte separation and quantification were achieved by isocratic reversed-phase liquid chromatography and UV detection at 220 nm. The method involves the use of an RP-18e Lichrocart reversed-phase column (5 μm , 75 \times 4 mm id) and a binary isocratic mobile-phase profile. Linearity was observed in the range of 9.18 to 720 $\mu\text{g ml}^{-1}$ with correlation coefficient of 0.998. Relative standard deviation of linearity of the method was found to be 0.015%⁴⁴.

A novel attempt has been made by Killedar SG *et al.*, in 2012 to study the various parameters for the development of *G. sylvestre* leaf extracts for injectable dosage forms. Ethanolic and water extracts of *G. sylvestre* were obtained by decoction and filtered through whatman paper-1. Stability and solubility were studied after drying the extracts in a vacuum dryer. Phytochemical screening and TLC study was carried out. Injections of different strength of dried extracts were made using normal saline and sterilized by autoclaving, where dried extracts showed 39 °C and 65% relative humidity. Liquid extracts showed presence of triterpenoids, sugars, proteins. Gymnemic acid was found to be major constituent it was confirmed by TLC by comparing with standards⁴⁵.

Dinesh KP *et al.*, in 2012 subjected methanol extracts of the leaves to TLC, FTIR, GC-MS analysis. TLC analysis with $\text{CH}_3\text{OH}:\text{CHCl}_3$ solvent revealed 6 fractions with R_f values of 0.23, 0.35,

0.45, 0.59, 0.69 and 0.85. FTIR spectroscopic investigation gave characteristic peak values with various functional compounds such as alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, carboxylic acids and aromatics. GC-MS analysis gave a spectrum of compounds with 10 major peaks, most of which are bioactive compounds which may act as good antimicrobial, antiviral, antioxidant and anti-inflammatory agents⁴⁶.

In the present study attempts were done by Manika N *et al.*, in 2012 to identify the efficient extraction procedure including solvent selection and methods, stability assessment in terms of drying as well as storage and changes occurring during annual cycle using an important analytical marker gymnemagenin. The yield of the marker was found to be highest in acid–base hydrolysis extract (1.17%). Among different drying methods adopted, gymnemagenin content was high in shade dried leaves (1.15%) while it was significantly low in sun dried material (0.54%). Estimate of yield and enriched gymnemagenin (1.01 - 1.23%) indicated that summer (April - July) is the ideal time to harvest *G. sylvestre*⁴⁷.

Tahira anjum *et al.*, in 2013 studied leaves of *G. sylvestre* is used by peoples of Vidisha district for treatment of diabetes. The active compound of this plant is a group of acids termed as gymnemic acid. Gymnemic acids have antidiabetic, antisweetner and anti inflammatory activities. The phyto-constituents of *G. sylvestre* were isolated and their chemistry and structures were studied and elucidated. The result of investigation was helpful for the correct botanical identification of plant and also different sources of medicine and pharmaceutical industry⁴⁸.

Sachin EP *et al.*, in 2014 developed a new, rapid, accurate, robust, and precise HPTLC method for concurrent quantitative determination of gymnemagenin and β -sitosterol in herbal formulation with densitometric detection. Chromatographic separation was achieved on Merck aluminum HPTLC plates precoated with silica gel 60 F₂₅₄. The optimized solvent system consisted of toluene: ethyl acetate: methanol (6.5:2.5:1.4 v/v/v) were the solvent system used. Developed plates were derivatized with 5% sulphuric acid reagent followed by heating at 110°C

for 4 min in a preheated oven and scanned at 423 nm in reflectance absorbance mode. The retention factor for gymnemagenin and β -sitosterol was found to be 0.27 ± 0.02 and 0.78 ± 0.02 , respectively. The proposed densitometric method was validated according to ICH Q2 (R1) guidelines. Results were found to be linear over a range of 100 - 1200 ng band⁻¹ and 200 - 1200 ng band⁻¹ for gymnemagenin and β -sitosterol, respectively⁴⁹.

Kusum D *et al.*, in 2014 developed and validated a simple precise and rapid HPLC method for the quantification of gymnemic acid as deacyl gymnemic acids in *G. sylvestre* extract and formulations. The analysis was performed by a reverse phase chromatography on a phenomenex C₁₈ Column with isocratic elution of acetonitrile buffer (23:77 v/v) at a flow rate of 2.0 ml/min. The linearity was found to be 50 - 800 μ g/ml with correlation coefficient of 0.9998⁵⁰.

Pawan KS *et al.*, in 2015 reported the quality control and assurance of parameters were carried out as per authenticated standard guideline to assess the quality and comparative screening of three gurmar butti leaves samples. The physicochemical data showed that the drug samples contained foreign matter (0.24%, 0.28%, 0.12%), moisture (4.24%, 4.98%, 5.20%) and ash contained (9.78%, 9.96%, 9.71%), acid in-soluble ash (1.81%, 2.04%, 1.61%), soluble extract in water (26.43%, 26.57%, 26.73%) and in alcohol (12.75%, 12.94%, 12.98%). HPTLC studies of aqueous and alcohol extracts showed various spots at 366 nm (UV region) using selected suitable solvent system of mobile phase. The quality control results revealed the absence of microbial load, Heavy metal (ppm) and Aflatoxins (ppm) contamination from the drug samples⁵¹.

A validated (HPLC-DAD) method was developed by Tushar D *et al.*, in 2015 for identification and quantification of bioactive principle gymnemagenin in a gradient elution mode using solvent mixture composing of acetonitrile (A), potassium dihydrogen orthophosphate (B) both solvent (A and B) containing orthophosphoric acid (0.05%, v/v). Results revealed significant differences for gymnemagenin content amongst the accessions evaluated. These accessions could be used in

breeding programs for development of cultivars with optimum levels of gymnemagenin, which in turn may promote the cultivation of this high value medicinal crop⁵².

Subashini MS *et al.*, in 2015 performed different phytochemical test, TLC analysis, solubility study, total phenol content, flavonoid content and antimicrobial study in the present investigation. Fingerprint analysis and quantitative analysis of quercetin were also performed through HPTLC method in the *G. sylvestre* extract. Solubility in water and alcohol, moisture content and gymnemic acid content were found to be 86.36%, 88.24%, 4.20% and 26.24% w/w. Total phenol and flavonoid content were found to be 0.80% and 1.90%. Microbiological assay was also performed⁵³.

CONCLUSION: According to literature survey the plant has extremely large medicinal, Immemorial, Ethno botanical, traditional uses and economic uses in different systems of Medicine throughout the world. 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 gives detail information of some isolation and analytical procedures to provide better scope for performing the further investigation in the plant.

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