REVIEW

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An overview on the advances of *Gymnema sylvestre*: chemistry, pharmacology and patents

E. PORCHEZHIAN, R. M. DOBRIYAL

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Dr. M. Dobriyal, Senior Development Scientist, Dabur Research Foundation, 22, Site IV, Sahihabad Ghaziabad – 201010 (U.P.), India
dobriyalrm@dabur.com

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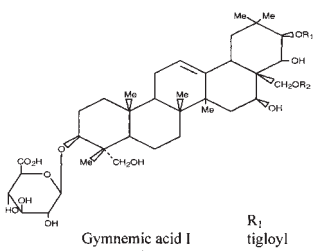
Chemistry and pharmacology of *Gymnema sylvestre* is reviewed relying on research papers and patent literature. Extracts of this plant are widely used in Australian, Japananese, Vietnamese and Indian folk medicine. *Gymnema* preparations have a profound action on the modulation of taste, particularly suppressing sweet taste sensations. It is used in the treatment of diabetes mellitus and in food additives against obesity and caries. Anti-allergic, antiviral, lipid lowering and other effects are also reported. From a technological point of view, much efforts have been made to mask the biter taste of *Gymnema* preparations.

1. Introduction

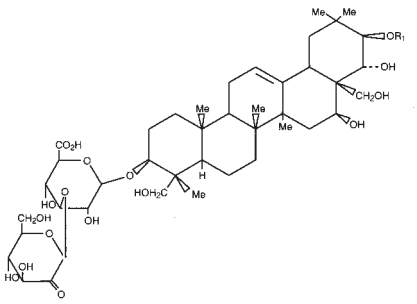
Gymnema sylvestre (Family: Asclepiadaceae) is a large, woody and branched climber, distributed in the tropical and sub-tropical regions of the world. It is extensively used in traditional systems of medicine and mentioned in the traditional literatures of Australia, Japan and Vietnam. In India, it has been used in Ayurveda, the traditional health care system, for several centuries [1–3]. *Gymnema sylvestre* is primarily used in the management of diabetes and related disorders. A sweet paralyzing property of the leaves is well recognized and used to overcome the carving for sweets. Experimental studies on the plant have started during the early 1930s with the first report on the pharmacological activity of the plant [3]. Since then much work has been carried in the field of pharmacology, chemistry and biotechnology. These days *Gymnema* based products, dietary supplements and health foods are available in the Asian, American and European markets for the management of diabetes and obesity. Many patents are also filed mainly in the fields of product formulation and analysis of constituents. This article briefs about the chemistry and pharmacology along with the patents filed on the plant.

2. Chemical constituents of *Gymnema sylvestre*

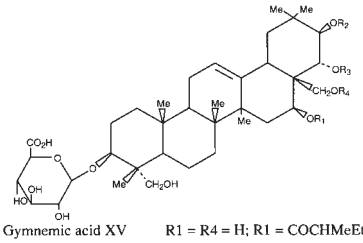
Many chemical constituents are isolated and characterized from the plant. Gymnemic acids are reported to be the main class of chemical constituents of *Gymnema sylvestre.* Gymnemic acids I–IV were the components considered to be of prime importance, first reported from the leaves of the plant followed by gymnemic acids V and VI [4–6]. All these compounds were isolated and characterized from the aqueous extract of leaves. Further investigations revealed the presence of gymnemic acids VIII and IX in addition to gymnemic acids X–XII, which were elucidated as glucosideuronic acid derivatives of gymnemagenin acylated with acetyl, tigloyl and/or 2-methylbutyroyl moieties [6–9]. The structure of gymnemagenin (Genin L), the sapogenin of the antisweet principles of *Gymnema sylvestre,* was established as 3β,16β,21β,22α,23,28-hexahydroxyolean-12-ene by means of X-ray analysis [6, 10]. Gymnemagenin, $C_{30}H_{50}O_6$, was isolated by successive extraction and chromatographic separation of acid-hydrolyzed extracts [11]. On the contrary to the observation that β-amyrin derivatives containing 16β,28-diol or 16β,22α,



	R_1	R_2
Gymnemic acid I	tigloyl	acetyl
Gymnemic acid II	2-methylbutyryl	acetyl
Gymnemic acid III	2-methylbutyryl	H
Gymnemic acid VI	tigloyl	H



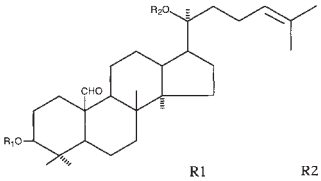
Gymnemic acid VIII: R1 = COCHMeEt; Gymnemic acid IX: R1 = COC(Me)=CHMe



Gymnemic acid XV R1 = R4 = H; R1 = COCHMeEt; R3 = COC(Me) = CHMe
Gymnemic acid XVI R1 = R3 = COC(Me) = CHMe; R2 = R4 = H
Gymnemic acid XVII R1 = R3 = R4 = H; R2 = Bz
Gymnemic acid XVIII R1 = R2 = R3 = H; R4 = Bz

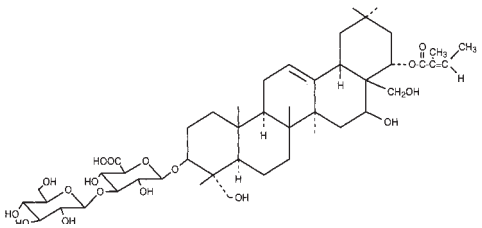
2,-triol systems do not form isopropylidene derivatives, gymnemagenin was observed to form isopropylidene derivatives involving the 16β, 28-diol structure [12–14]. Raw extract containing gymnemic acids on fermentation yielded several genins: genin J, genin K acetate, genin G acetate and genin L acetate (hexa-*O*-acetylgymnemagenin) [15]. Systemic analysis of saponin fraction of the leaves has led to the identification of gymnemic acids XV–XVIII [16].

In addition to gymnemic acids another new category of antisweet principles called gymnemasaponins have been separated and studied chemically. Five constituents (gymnemasaponins I–V) were isolated and their structures were established as novel D-glucosides of 3β, 16β, 23, 28-tetrahydroxyolean-12-ene on the basis of spectroscopic analysis [5]. The aglycon, gymnemanol of triterpenoid saponins, gymnemasins A–D that coexists with gymnemic acids was characterized as 3β,16β,22α,23,28-pentahydroxyolean-12-ene [17]. Spectral studies and chemical transformations helped in the identification of dammarane-type saponins, named gymnemasides I–VII along with gypenoside XXVIII, XXXVII, LV, LXII and LXIII of the same category [18]. Analysis of alcoholic extract of *Gymnema sylvestre* from Guangxi province of China led to the isolation of six oleanane saponins characterized as longispinogenin 3-*O*-β-D-glucuronopyranoside, 21β-benzoylsitakisogenin 3-*O*-β-D-glucuronopyranoside, 3-*O*-β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl ester, oleanolic acid 3-*O*-β-D-xylopyranosyl (1→6)-β-D-glucopyranosyl (1→6)-β-D-glucopyranoside, 3-*O*-β-D-xylopyranosyl (1→6)-β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl ester and 3-*O*-β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl oleanolic acid 28-β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl ester [19].

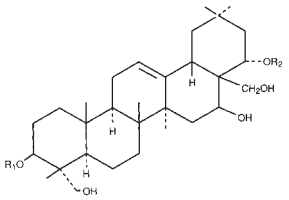


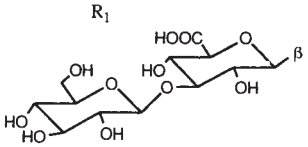
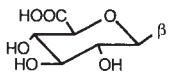
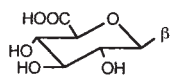
	R1	R2
Gymnemaside 1	Glc	Glc
Gymnemaside 2	Glc-Glc	Glc
Gymnemaside 3	Ara-Glc	Glc
Gymnemaside 4	Glc	Glc-Xyl
Gymnemaside 5	Glc-Glc	Glc-Xyl

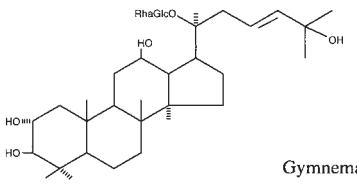
Gurmarin, a polypeptide isolated from the leaves of the *Gymnema sylvestre* suppresses the sweet taste of sucrose, glucose, glycine and saccharin [20, 21]. It is made up of 35 amino acid residues including three intramolecular disulfide bonds with an amino-terminal pyroglutamyl residue and has a molecular weight of 4,209 [20, 22]. Mass spectrometric analysis and sequencing of cystine-containing peptides obtained by thermolysin-catalyzed hydrolysis of gurmarin helped in locating the position of disulfide at Cys3–Cys18, Cys10–Cys23, and Cys17–Cys33 [23, 24]. Hydrophobic side chains of Tyr-13, Tyr-14, Trp-28 and Trp-29 in gurmarin are directed outwardly and together with the hydrophobic side chains of Leu-9, Ile-11 and Pro-12, form hydrophobic cluster. These hydrophobic amino acids are involved in the interaction with the receptor protein in suppressing the response to sweet taste [20]. Moreover nitrogenous compounds and alkaloids, gymnamine and conduritol were also reported from the leaves of the plant [25–27].



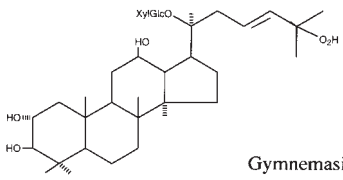
Gymnemasin A



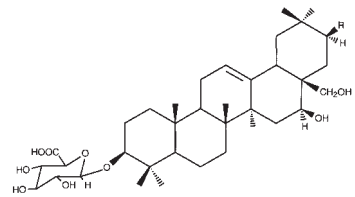
	R_1	R_2
Gymnemasin B		H
Gymnemasin C		tigloyl
Gymnemasin D		H



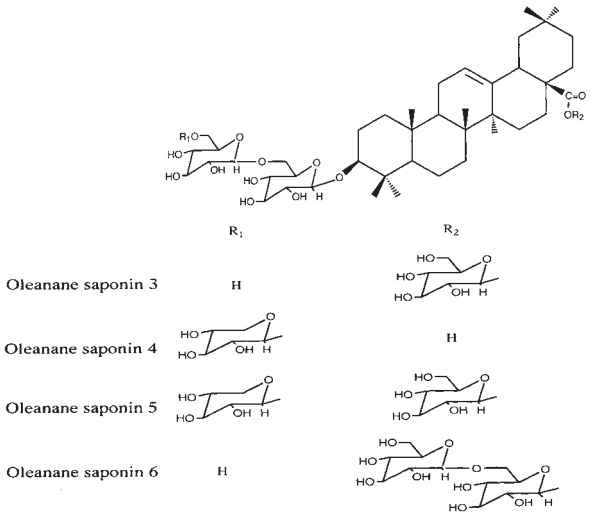
Gymnemaside 6



Gymnemaside 7



Oleanane saponin 1 R = H
Oleanane saponin 2 R =



3. Analytical assessment and processing of gymnemic acids

Various analytical methods are employed to estimate the amount of gymnemic acid in dried leaves and also in the marketed preparations. Estimation of gymnemic acid present in the leaves states that the amount varies depending on the time of collection. Reports of gymnemic acid in the leaves collected from Hepu, Guangxi of China was 0.67% and 0.97% during July and October, respectively. Samples from the province of Qinzhou showed a content of 1.06% of gymnemic acid [28]. HPLC analysis of gymnemagenin, the main sapogenin of gymnemic acids mixture from the four specimen samples of India varied between 3.9–4.6%. This result indicates the necessity of standardization procedure to ensure the presence of standard amount of gymnemic acid [29].

Report on the amount of gymnemic acids in leaves or extract used in the 21 health foods noticed a wide range of less than 10.5 μg/g to 20.5 mg/g for 11 kinds of tablets, 8.46 mg/g for a kind of powdered leaf preparation, 2.12–6.89 mg/g for 5 kinds of *Gymnema* tea, less than 0.207 μg/ml to 4.41 μg/ml for 2 kinds of soft drinks and 0.029–0.055 mg/g for 2 kinds of chewing gum [30].

Moreover, analysis of different complementary health supplements of containing gymnemic acids differed from 38 to 251 mg daily dosage. HPLC estimation of gymnemic acid in alkaline hydrolyzate as deacylgymnemic acid can be used to determine quantitatively the amount of gymnemic acid. Analysis using HPLC showed the presence of twice the amount of gymnemic acid in ethanolic extract than the aqueous extract [31]. HPLC combined with atmospheric pressure-ionization mass spectrometry helped in direct introduction of eluted fraction in to mass spectrometry and ions of gymnemic acids are detected as ammonium adduct ions and/or proton adduct ions. Application of this method helped to identity three pairs of geometrical isomers of gymnemic acid [32].

Many attempts are being made to suppress the antisweet property of *Gymnema.* Gurmarin the polypeptide with 35 amino acid residues is mainly responsible for the inhibition of sweet taste. Replacement of native disulfide bonds with alanine led to the suppression of sweetness inhibition activity by interfering in the interaction of gurmarin with the receptor protein [33]. Treatment of the mixture of gymnemic acid and starch with cyclomaltodextrin glucanotransferase resulted in the 15-fold weakening of antisweet activity. And also the addition of γ-cyclodextrin to a gymnemic acid sample was found to be effective in removing the bitterness and antisweet activity similar to glycosyl-steviate [34].

Chemical processing is also carried out to overcome the sweet masking property of gymnemic acids. Gymnemic acids from the aqueous extracts of *Gymenma* are subjected to chemical processing and eluted from a DEAE-Sephadex column to obtain gymnemic acid A3. In this process gymnemic acid A1 is converted to gymnemic acid A2 and finally to gymnemic acid A3 that lacks antisweet property [35]. In alternative bitter fraction of aqueous extract can be separated and processed to suppress the sweet inhibiting property by acidification with hydrochloric acid or by using ion-exchange resin followed by treatment with alkali at initial pH 11.0 in boiling water for 30 min, and then mixed with non-bitter fractions obtained above. This helps to remove the bitter taste without altering the activity [36].

4. Pharmacological activities of *Gymnema sylvestre*

4.1. Action on sweet receptors

Gymnema sylvestre is having a profound action on the modulation of taste. Extracts of the herb strongly suppressed the neural responses to a mixture of monosodium glutamate and disodium inosine monophosphate, and sucrose. But the action against individual solutions of monosodium glutamate and disodium inosine monophosphate was negligible which may be due to different transduction mechanisms [37]. *Gymnema* extract selectively suppressed the response to sweeteners like sucrose, fructose, saccharin, and cyclamate. Fifty percent of this action is extended against the sweet taste caused by water-after-citric acid [38]. Sweet taste of the glycoside, methyl α-D-mannopyranoside is suppressed without any alteration in the response to its bitterness [39].

Partially purified extract of the plant altered the neural response of single taste hairs of the house fly *Musca domestica.* Inhibition of just-suprathreshold sucrose and glucose stimuli resulted with the modification of neuronal firing pattern that ranged from a waxing and waning of impulse frequency to an abolition of impulses. Above changes remained positive for sodium chloride stimulus with faster restoration of firing pattern near normalcy. A similar response is witnessed in other flies like *Lucilia caesar* and *Blaesoxipha cessator* [40].

Glycoprotein in the extract of *Synsepalum dulcificum-miraculin* enhanced the response of chorda tympani to acids that is counteracted by the application of gymnemic acid [41, 42]. This suggests that miracle fruit adds sweetness to acids without directly blocking sour receptor sites. Gymnemic acid had no significant effect on the response to any of the stimuli if miraculin was not applied beforehand. The ester group of gymnemic acid may play important role to exhibit the antisweet activity [41, 43]. Gymnemic acid A1 eluted with ethanol suppressed the sweet taste of sucrose, sodium saccharin, cyclamate, D-amino acids, $BeCl_2$, and $Pb(OAc)_2$ with the change of sodium glutamate to a taste similar to that of sodium chloride [43, 44]. Apart from the acidic and glycosidic principle some more mixtures isolated also possess the antisweet property [44].

Aqueous extracts of *Gymnema* depressed the sweetness of amino acids, glycine and DL-alanine in a way very similar to that of sucrose [45]. Temporary suppressing of sweet taste is found to be more prominent although it influences the bitter taste modalities [46]. Gurmarin is the taste modifying protein from *Gymnema sylvestre.* It is a residual

protein with 35 residual polypeptides that acts on the greater superficial petrosal nerve innervating palatal taste buds to depress the phasic taste response to sugars and saccharin sodium. Its action is extended to D-amino acids that taste sweet to humans (His, Asn, Phe, Gln) without having significant action on L-amino acids series [47]. Despite striking structural homologue with δ-atracotoxin, a spider neurotoxin known to slow the inactivation of voltage-gated Na^+ channels, gurmarin had no effect on a variety of voltage-sensitive channels [48].

Electrophoresis and affinity chromatography studies of submandibular saliva of *Gymnema* fed rats showed the presence of gurmarin-binding proteins. Gurmarin-induced decreased preference to sucrose was restored by the suppression of gurmarin by salivary gurmarin-binding proteins induced by the gymnema diet. The same preference curve is reproduced for monosodium glutamate. The feeding of *G. sylvestre* did not change preferences for sodium chloride and quinine hydrochloride [49, 50]. The action of gurmarin was highly specific to sweet taste and exhibited maximum suppression at a pH of 4.5, which corresponds to the isoelectric point of the peptides.

Intravenous administration of gurmarin is found to be ineffective because of the confinement of action to the apical side of the taste cells possibly by binding to the sweet taste receptor protein [51]. The hydrophobic groups of gurmarin may act as the site for interaction with the receptor protein to suppress the responses to sucrose, glucose, fructose, or glycine [52]. Suppression of sweet response may last for several hours but anti-gurmarin serum will shorten the recovery time considerably [51]. 21β-*O*-benzoylsitakisogenin 3-*O*-β-D-glucopyranosyl (1→3)-β-D-glucuronopyranoside and the sodium salt of alternoside II, isolated from the ethanol extract of *Gymnema sylvestre* possess the same antisweet activity as gurmarin [53].

4.2. Antidiabetic activity

The water-soluble fraction of an alcoholic extract of *Gymnema* lowered the glycogen content of isolated rat hemidiaphragm in glucose fed hyperglycemic rats but failed to produce the action in normal rats. Lowering of glycogen content is accelerated by the exogenous insulin administration along with the leaf extract [54]. Intraduodenal infusion of glucose increased the portal immunoreactive gastric inhibitory peptide concentration in a dose-dependent manner. Concomitant administration of *G. sylvestre* leaf extract or purified gymnemic acid along with glucose resulted in the suppression of raise in the portal immunoreactive gastric inhibitory peptide concentrations [55]. Though *Gymnema* is having profound influence on blood sugar level and even helps in the reduction of cholesterol, it did not show any noticeable effects on the sugar-induced blood pressure elevation [56].

Gymnemic acids are the class of active constituents isolated form *Gymnema sylvestre* which are having prominent influence on blood sugar level. Given orally even at small concentrations (0.2 g/kg), they produced a reduction in the elevated levels of blood sugar induced by sucrose [57]. Overnight fasted experimental animals treated with gymnemic acid and sucrose showed a reduction of fasting blood glucose level to 53 and 68% at 15 and 30 min intervals when compared to that of control group. Gymnemic acid given 2 and 1 h before sucrose treatment and with sucrose simultaneously resulted in 58 and 60% reduction after 15 and 30 min in concurrent to single gymenmic administration [58].

Gymnemic acid mixture inhibits the glucose absorption from the small intestine and suppresses the increase in plasma glucose level. In oral glucose tolerance test crude gymnemic acid and fractions isolated from the crude gymnemic acid by using affinity chromatography showed a varying degree of response in lowering the blood sugar level. Significant activity was shown by the crude gymnemic acid and isolated fraction phase I, but the other isolated fractions (10% E, 20% E, and 50% E) failed to produce significant action. However, intraperitoneal administration of crude gymnemic acid or fractions 10% E, 20% E, and 50% E (25 mg/kg body wt.) without glucose caused a significant increase in the plasma glucose concentration, which is not observed with the administration of fraction I [59].

Gymnemic acids interfered with the absorption of glucose from the intestine in association with the Na-dependent transport system. It also inhibits the intestinal absorption of oleic acid and histidine [60]. In isolated intestinal preparation addition of gymnemic acid increased the transmural p.d. whereas in the presence of glucose, the glucose evoked transmural p.d. was decreased. In oral sucrose tolerance test, gymnemic acid also decreased the raise in blood glucose concentration after the oral administration of 2 g/kg of sucrose and resembled the activity of *Z. jujuba* leaf extract [61]. Gymnemic acid analogs were also analyzed for their influence on the intestinal absorption of sugars [54].

Some fractions isolated from the gymnemic acid inhibited the glucose uptake from intestine by interfering with glucose transport in experimental animal models [60, 62]. Ethanolic subfractions isolated from the crude gymnemic acid by affinity chromatography having γ-cyclodextrin as ligands exerted a marked inhibition of glucose absorption from the small intestine in addition to reduction of plasma glucose level. Subfraction phase I may play an active role in inhibiting the glucose absorption from the small intestine [63]. Concurrent administration of gymnemic acid along with sucrose to streptozotocin-induced diabetic animals exerted a dose dependent hypoglycemic effect [64].

Oral glucose tolerance test done in humans with the administration of 75 g glucose containing gymnemic acid (50 mg) or aqueous extract of *G. sylvestre* (2 g) reduced the absorption of glucose from intestine. Plasma glucose values observed did not show any remarkable change but serum insulin secretion was suppressed. In addition to the interference to glucose absorption from the intestine, gymnemic acid and *Gymnema* leaves probably diminish the glucose inhibitory polypeptide [65].

Gymnemic acid 1 and 2 characterized as (3β,4α,16β,21β,22α)-21-tigloxy-16,22,23,28-tetrahydroxyolean-12-en-3-yl-β-D-glucopyranosiduronic acid and (3β,4α,16β,21β,22α)-21-(2-methylbutyroxy)-16,22,23,28-tetrahydroxyolean-12-en-3-yl-β-D-glucopyranosiduronic acid, isolated form the leaves of *Gymnema sylvestre* counteracted the contraction induced by hyperosmotically added 65.4 mM KCl (H-$65K^+$) to isolated guinea pig ileal longitudinal muscle. Levels of PNred fluorescence (reduced pyridine nucleotide) and Fpox (oxidized flavin protein) were also decreased by gymnemic acid treatment, but pyruvate treatment restored the muscle contraction without any change in intracellular Ca^{2+} [Ca^{2+}] 1 level thoughout the entire experiment. Gymnemic acids performed this action through the inhibition of glucose uptake, which is an energy source of the muscle and not probably mediated by Ca^{2+} [66]. Other gymnemic acid categories like II, III, IV, V and VII are also found to have inhibitory effects on the

absorption of glucose from rat small intestinal fragments and this effect is observed to be absent with gymnemic acid I [67, 68].

GS II consumed orally found to be useful in restoring the altered levels of total protein-bound polysaccharide components and glycosaminoglycans in serum and tissues (brain, kidney, heart and liver) of experimental animals during short-term alloxan-induced hyperglycemia. GS II lowered increased levels of some protein-bound polysaccharides, hexuronic acid, hexoses, hexosamines, nonaminopolysaccharides and sialic acid and some glycosaminoglycans, hyaluronic acid, and heparan sulfate to normalcy. Decrease in 3 chondroitin sulfates and sulfated to non-sulfate glycosaminoglycans ratios were increased by oral consumption of GS II [69].

Gymnemic acid IV at the dose of 13.4 mg/kg produced a maximum of 60.0% reduction in blood sugar level 6 hours after administration and the effect was comparable to glibenclamide. Gymnemic acid IV exerted hypoglycemic acid by promoting the release of insulin. In normoglycemic mice no change in blood glucose levels were observed. Association of α-glycosidase activity in lowering the blood glucose level is found to be absent [70]. GS IV from *Gymnema* stimulated the release of insulin from HIT-T15, MIN6 and RINm5F β-cells and from islets, and not affected by blockade of voltage-operated Ca^{2+} channels with 10 μM isradipine. But the presence of 1 mM EDTA inhibited the insulin stimulating capacity of gymnemic acid. Results indicate the stimulatory action of insulin release by GS IV is mainly due to increased cell permeability, rather than by stimulating exocytosis by regulated pathways [71]. GS V is also expected to have an active role similar to GS IV [72].

Conduritol A, a polyol from the dried leaves of *Gymnema sylvestre* stopped the intestinal absorption of glucose at a dose of 0.2 mg/ml and also depressed the blood sugar level at 10 mg/kg body weight [73]. Moreover, administration of conduritol A at a dose of 10 mg/kg in a streptozotocin-diabetic experimental model prevented the development of cataract. This is because of its ability to inhibit aldose reductase, an enzyme catalyze the conversion of aldoses to sugar alcohols. *In vitro* studies confirmed this activity and have clearly shown its inability to act on other enzymes, which may be responsible for its cataract-suppressing effect [74]. Gymnemosides-a (21-*O*-tigloyl-22-*O*-acetylgymnemagenin 3-*O*-β-D-glucopyranosiduronic acid) and gymnemosides-b (16-*O*-acetyl-21-*O*-tigloylgymnemagenin 3-*O*-β-D-glucopyranosiduronic acid) are having only marginal hypoglycemic activity in oral glucose-loaded rats [68, 72]. Gymnemoside-c (21-*O*-benzoyl-28-*O*-acetylgymnemagenin 3-*O*-β-D-glucopyranosiduronic acid), gymnemoside-d (23-*O*-[β-D-xylopyrano-syl (1→6)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] gymnestrogenin), gymnemoside-e (23-*O*-[β-D-xylopyranosyl (1→6)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-28-*O*-[β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl] 23-hydroxylongispinogenin), gymnemoside-f (23-*O*-[β-D-xylopyranosyl (1→6)-β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl]-28-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] 3β,16β,23,28-tetrahydroxyolean-18-ene) are having the capability to exhibit inhibitory action on glucose uptake from intestine [67]. Triterpenoid saponins, gymnemasins A, B, C and D isolated from the leaves of *Gymnema sylvestre* are characterized as 3–*O*-[β-D-Glucopyranosyl (1→3)-β-D-glucuronopyranosyl]-22-α-tigloyl-gymnemanol, 3-*O*-[β-D-glycopyranosyl (1→3)-β-D-glucuronopyranosyl-gynmemanol, 3-*O*-β-D-glucuronopyranosyl-22-*O*-tigloyl-gymnemanol and 3-*O*-β-D-glucuronopyranosyl-gymnemanol, found in the total saponin fraction exhibit hypoglycemic and antihyperglycemic effects in rats [75].

4.3. Lipid lowering effect

Gymnemagenin containing leaf extract, acid precipitated extract and column fractionate were given to rats to find out the role of gymnemic acid in fecal steroidal excretion. All the extracts significantly increased the fecal excretion of neutral steroids and bile acids in a dose dependent manner. Fecal steroid excretion of cholesterol, total neutral steroids, total bile acids and CA-related bile acids were acute and significantly correlated with fecal gymnemagenin levels [76]. Moreover, studies using the intestinal perfusion method revealed the potent inhibition of oleic acid absorption by gymnemic acid. Intestinal inhibition of oleic acid is similar to that of glucose absorption and facilitates its application in diabetes mellitus and obesity [77].

4.4. Action on gastro-intestinal tract

Gymnema sylvestre or purified crystalline components containing the potassium salts of gymnemate suppressed the rate of acid secretion to approximately 50% [78]. In K^+-induced muscle contraction of the rat intestinal circular muscles, water soluble extract of *Gymnema sylvestre* counteracted the contraction induced by KCl and also suppressed spontaneous contraction, explaining its ability to relax the intestinal muscle owing to nitric oxide and endothelium-derived hyperpolarizing factor participation [79].

4.5. Antiallergic and antiviral properties

In vitro studies using pectic substances from *Gymnema sylvestre* inhibited the histamine release from mast cells by antigen. Gel filtration technique followed by anion exchange chromatography resulted in the separation of two active pectic substances containing 73% D-galacturonic acid. This suggests the possibility of D-galacturonic acid of *Gymnema* as an hyaluronidase inhibitor. These results suggest that pectic substances of *Gymnema sylvestre* may have antiallergic activities [80, 81].

Gymnemic acids A and B isolated form the plant exhibited antiviral activity. Gymnemic acid A at the concentration of 75 mg/kg body weight showed the maximum activity against influenza virus followed by gymnemic acid B. But other fractions from the leaves lack this activity [82].

4.6. Antidote against snake venom

K gymnemate isolated from the plant was evaluated for its activity against the ATPases isolated form venom of *V. russelli* and *N. naja.* ATPases were fractionated from venom in a single-step chromatography and characterized. Gymnemate inhibited these fractionated ATPases, toxic components of the venom by binding to the same site(s) studied by a spectrofluorimetric method [83, 84].

5. Patents on *Gymnema sylvestre*

Many patents are filed on *Gymnema sylvestre* in the areas of food supplements, processing, medicinal applications and isolation of active constituents. Out of the patents filed those related to the food supplement category mainly on antiobesity, diabetes and anti-caries are important.

5.1. Anti-obesity category

Health food containing *Gymnema sylvestre* in combination with *Commiphora mukul, Guar gum,* and *Allium sativum* is reported to be useful to overcome obesity [85]. Food containing high amounts of oils and fats is prepared with gymnemic acids from *Gymnema.* Inclusion of gymnemic acids helps in the inhibition of fat and oil hydrolyzates from the digestive tract thereby preventing obesity [86]. A water-oil-water emulsion with gymnemic acid as an antiobesity agent in the inner aqueous phase is incorporated to ice cream to exhibit action against obesity [87]. Consuming diet-containing extracts of *Garcinia cambogia* and *Gymnema sylvestre* in addition to chromium picolinate, vanadium compound, L-carnitine and conjugated linoleic acid can also control body weight. A effective dose can be taken in conjunction with daily meals or restricted-calorie diet [88]. Gymnemic acid taken as an auxiliary food reduces the sensitivity of tongue and helps in reducing the food intake. Externally it suppresses the sensibility to sweetness and internally the desire for eating. Chewing gum containing spray dried gymnemic acids 0.3–13 mg was prepared, which on chewing inhibits the sweet taste of foods like doughnut, milk chocolate, and shortcake [89].

5.2. Anti-diabetic category

A new nutritional supplement is developed for the treatment of diabetes that has to be administered on phases. The first phase consists of sources of vandate and chromium, and *Gymnema sylvestre* along with lipoic acid in the second phase. This nutritional supplement can be taken with change in phase to avoid the accumulation and desensitization, common with the continuous use over a prolonged period [90]. Compound preparation comprising of *Pterocarpus marsupium* [heartwood containing (−)-epicatechin] 30.0, *G. sylvestre* (leaves containing gymnemic acid) 27.0, *Cinnamomum tamala* (leaves) 3.0, *Aegle marvelos* (leaves) 6.0, *Momordica charantia* (seeds) 6.0, *Azadirachta indica* (leaves) 3.0, *Tinospora cordifolia* (stem) 5.0, *Trigonella foenum-graceum* (seeds) 10.0, *Ficus racemosa* (leaves) 2.0, and *Syzygium cumini* (fruit) 8.0% induces significant reduction in serum glucose levels of humans due to the regeneration of pancreatic islet cells [91].

A compound preparation containing bilberry extract, Ca (Krebs), chondroitin sulfate, chromium picolinate, Co Q10, fenugreek seed powder, Flax seed powder, folic acid, linoleic acid, *Ginkgo biloba, Gymnema sylvestre,* taurine (or homotaurine), grape seed extract, acetyl L-carnitine, lutein, Mg (Krebs), *N*-acetyl-L-cysteine, pine bark extract, phytosterol complex, K citrate, protamine sulfate, shark cartilage, soy isolate, green tea polyphenols, vitamin A, vitamin B2, vitamin B6, vitamin B12, vitamin C, vitamin E and Zn (Krebs) is found to be useful in the treatment of diabetic retinopathy and nephropathy. It can be used in retinal disorders including macular degeneration and cataracts, prevention of neuropathy, cardiovascular disease and periodontal disorders [92]. A nutrient supplement containing *Gymnema* along with antioxidant components promoting collagen synthesis and regulating blood lipids, glucose and/or insulin and lower homocysteine levels is recommended in cancer as nutrient and therapeutic supplementation [93].

Isolated compounds from the leaves on oral and parentral administration were found to be useful in the treatment of diabetes, impaired glucose tolerance, and various conditions associated with diabetes. Moreover, these compounds reduce polydipsia, polyuria and polyphagia, regenerate the pancreatic cells, increase endogenous insulin, lipase and amylase levels, increase production of proinsulin and c-peptide, and lower blood lipids and triglycerides and free fatty acids. A hydroalcoholic extract without water-soluble fraction significantly reduced fasting blood glucose levels, increased pancreatic amylase and lipase levels and number of pancreatic islets and beta cells [94].

Isolated compounds in the form of pharmaceutically acceptable salts can also be employed in the prevention or treatment of disorders related to high blood sugar, high blood lipids, or blood clotting [95]. On administration to fasting experimental animals along with glucose conduritol A extracted from the leaves stopped the rise in blood pressure [96].

Unpleasant bitter and astringent taste makes it difficult to administer *Gymnema* as an oral anti-diabetic agent. To overcome the bitter taste, dried leaf powder is mixed with powdered chitosan and packed in water-permeable bags that can be consumed as tea [97]. Mixing with chitosan can be employed to food and pharmaceutical preparations containing gymnemic acid [98]. Another way of masking bitter taste is mixing *Gymnema* with cyclodextrin. This technique can also be employed to food or food additives but addition of cyclodextrin to *Gymnema* further enhances the blood sugar-lowering effect of the extract [99]. *Gymnema* can be mixed with natural water-soluble polymers, natural oil and lecithins to prepare an encapsulated food to overcome the off-taste that can be used as antidiabetic and anticarcinogenic [100].

5.3. Dental application

Gymnemic acid I characterized from the plant is capable of preventing decomposition of sugar and production of glucan by *Streptococcus mutans,* which are responsible for the cause of dental caries. *In vitro* experiments showed *Gymnema* extract to be active in stopping plaque formation by *S. mutans* in the presence of sucrose [101]. Homologous series of gymnemic acid from the dried beans of *Gymnema sylvestre* were isolated in the salt form by HPLC [102]. The cariostatic activity of HPLC isolated fraction and gymnemic acid did not differ significantly in inhibiting the activity of glucosyltransferase from the bacterial coat of *S. mutans* indicating its possible application in the prevention of dental plaque formation and dental caries. Gymnemic acid is also added to the oral formulations containing glycyrrhizin to decrease the sweet taste of glycyrrhizin [103].

5.4. Isolation of constituents

A new method was developed for the isolation of gymnemic acids from *Gymnema sylvestre* without undergoing hydrolysis. The step includes the initial sterilization of leaves with N_2 gas at 120 °C followed by extraction of leaves in the presence of phosphate buffer of pH 7 at 60 °C. Fat soluble ingredients are removed with an organic solvent lighter than water, such as hexane, heptane, and petroleum ether and removal of chlorophyll was achieved with an organic solvent heavier than water, such as chloroform, ethylene dichloride, and Carbon tetrachloride [104]. Solutes content in the extract can be increased from 10% to 15% by modifying the extraction process. Dried leaves were treated with ethanol and water at 30 °C for 16 h and

filtered. The residue was again extracted and extracts were combined and concentrated under reduced pressure at 40–60 °C to obtain a preparation containing 15% solutes [105]. A chromatographic method is also found to be effective in the isolation of gymnemic acids. Elution of an aqueous extract of *Gymnema* with ethanol in column chromatography containing synthetic resin as adsorber, resulted in the separation of gymnemic acids [106].
Research also helped to identify cell adhesion and metastasis inhibitors from the plant [107].

5.5. Processing of Gymnema sylvestre

More patents were filed in the field pertaining to the processing of *Gymnema.* Leaves of *Gymnema* were incubated with enzymes that degrade the cell membrane and purified by anion-exchanging cellulose chromatography and/or HPLC in the presence of γ-cyclodextrin to remove contaminants that cause side effects. Resultant gymnemic acid is good and low in cost without the use of di-Et carbonate [108]. Alternatively, extracts of gymnemic acids and starch are dissolved in acetate buffer (pH 6.0) and heated for 120 units at 54 °C for 48 h. The extracts showed significant reduction in bitter taste [109]. Hydrolysis under alkaline anaerobic conditions helps in the masking of bitter and astringent taste without any adverse effect on blood sugar-lowering activity in experimental animals [110]. After hydrolysis with hydrochloric and/or sulphuric acid, gymnemic acid is treated with porous starch and the surface of the starch particles is coated with zein. This helps in the reduction of bitterness of the product [111]. Gymnemic acid is also employed to modify the taste of beverages containing sweeteners with high sweetness. Addition to beverages containing sweeteners like aspartame, *Stevia* extract, saccharin, and acesulfame K improved the taste [112].
Recent studies focused on the stabilization and development of microgranules. Active substances are stabilized as powders with an average particle size of ≤ 5 μm. The powder was prepared from gum arabic, starch hydrolyzate and a soybean oil compound containing *Gymnema sylvestre* extract and decaglycerin decaoleate. This process helps to mask the taste of the extract wich was released slowly in the water phase [113]. Microgranules were prepared based on the dietary supplement. In this method gymnemic acid, *Garcinia* extract and chitin in an alcoholic solvent are deposited on the interior and exterior walls of insoluble microporous carrier and dried to obtain impregnated microporous granules [114].
Apart from the patents filed in the field of medicinal uses and processing, papers are filed for the application of *Gymnema* against smoking and as veterinary food. Nicotine in tobacco smoking is removed from side-stream smoke or second-hand smoke with a compound containing *G. sylvestre*, food additive vitamin, edible fat and fatty acid [115]. Even the extract of the plant is employed in veterinary purpose. Food contains oleic acid, silicic acid and extracts of plants including *Gymnema sylvestre* is recommended for the effective control of dental diseases in pets like the dog and cat [116].

6. Concluding remarks

Gymnema sylvestre, a plant of Indian origin is found throughout the tropical and sub-tropical regions of the world. Traditional systems of medicine like Ayurveda employ *Gymnema* in the management of diabetes and associated conditions. In recent years, the plant is commercially cultivated in large scale in Asia and Africa to meet the market needs of Europe, America and Southeast Asia. Most of the commercial source is used in the manufacturing of health care products and food and dietary supplements of antidiabetic and antiobesity categories. These products are available in many dosage forms like tablets, capsules, teas, chewing gums etc., mostly in combination with other ingredients and the gymnemic acids content of the daily-recommended dose differs from 38 to 251 mg [31]. Though *Gymnema sylvestre* has been used for several centuries and considered to be safe, there is a paucity of data on interaction between *Gymnema* and pharmaceuticals or minerals. Even US FDA also states some of the illness and injuries associated with the use of dietary supplements [117]. So it is imperative to carry out more research to regulate the dosage forms and recommended daily doses along with the study of possible interaction with other ingredients in multi component formulations especially those containing ingredients of non-herbal origin [88–92]. Moreover, many patents are filed in the areas of dental application of *Gymnema sylvestre* [101, 103], but systematic studies are needed to explore its possible potential oral use.

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