Pharmacological Activities, Phytochemical Investigations and in vitro Studies of *Gymnema sylvestre* R.Br. – A Historical Review

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ABSTRACT

*Gymnema sylvestre* R.Br., a liane belonging to the family Asclepiadaceae, has been employed to control diabetes, obesity, asthma etc., by traditional medicinal practitioners of India for nearly two millennia. The active principle from the leaf extracts is assumed to be gymnemic acid and its derivatives which are of triterpenoids in chemical nature have the ability to renew the islet cell mass for the possible cure of diabetes. The present review deals with botanical description, phenology, geographical distribution, chemical constituents and medicinal properties, pharmacological, phytochemical and in vitro plant tissue culture studies from the past to recent developments. Other aspects such as current status, progress and future challenges are also discussed.

Key words : *Gymnema sylvestre* R.Br, antidiabetic, botanical description, geographical distribution, phenology, medicinal uses,
INTRODUCTION

Gymnema sylvestre R.Br. is a well known and highly valuable medicinal plant in folk, Ayurvedic, Siddha and Homeopathy systems of medicine for the treatment of diabetes (Dixit & Pandey, 1984; Mitra et al., 1996). More than 148 plant species of 50 families have shown hypoglycaemic activity (Handa et al., 1989). All the species were helpful only in balancing the blood glucose level, while G. sylvestre brings blood glucose homeostasis through increased serum insulin levels provided by repair or regeneration of the endocrine pancreas (Shanmugasundaram et al., 1983, 1990). This character gives a distinct advantage over other plant and synthetic drugs used for diabetes treatment. Besides antidiabetic activity, G. sylvestre has various medicinal uses discussed in the later part of this review. Several products, under brand names such as Body Slatto Tea®, Gymnema®, Gymnema Diet®, Sugar Off®, Glucoset™, Cinnidrome X™, and Plisoft™ are commercially available in markets of Japan, Germany and USA as health foods and cosmetics.

Several scientists during the period 1907 – 2009 have attempted and succeeded in the pharmacology, phytochemical studies (isolation and structure elucidation) and in vitro plant tissue culture studies (Figs. 1 & 2). The composition of triterpenoids of this plant has been thoroughly investigated. However, there are many references about the traditional uses of this plant, often they are contradictory. A systematic review related to pharmacognosy, phytochemistry, pharmacology and clinical applications of G. sylvestre was made (Shailendra Gurav et al., 2007). However, it deals only with minimum literature and not discussed about the contradictory views, structure of all the gymnemic acid derivatives, biotechnological aspects and future prospects.

Alternative in vitro (plant tissue culture) methods would be beneficial in accelerating large scale multiplication of secondary metabolites and conservation of the plant. Gymnemic acid is an important source of new drug for diabetes but to attain that numerous challenges are encountered including the procurement of plant material, the selection and implementation of appropriate high-throughout screening bioassays and the scale-up of active compounds. Hence an attempt is made to review G. sylvestre historically which helps the plant to utilize completely for the near future.
Fig 1. Number of publication in *Gymnema sylvestre* during the year 1967 – 2009

Fig 2. Benchmark studies of *Gymnema sylvestre* (1967 - 2009)
Gymnema sylvestre (syn. Asclepias geminata Roxb, Periploca sylvestris Retz) is a woody climber running over the tops of high trees. The vernacular names of G. sylvestre are in Tamil-Sirukurinjan, English-Periploca of the woods, Hindi-Gurmar and Sanskrit-Ajabolli. The young stems and branches are pubescent and often densely so, terete. Leaves 3.2-5 by 1.3-3.2 cm, elliptic to obovate, base obtuse, apex abruptly acute, penninerved; petioles 6-13 mm long, pubescent, flowers dull yellow, small, in pedunculate or nearly sessile cymes; peduncles densely pubescent, shorter than the pedioles and arising between them, sometimes producing successive umbels or whorls of flowers; pedicels 3-13 mm long, pubescent; bracts min, ovate-oblong, hairy ciliate; calyx lobes obovate, 1 mm, scabrous without, obtuse; corolla 4 mm across, pollinial bags 0.2 mm; fruits of 2 (or 1), dark green smooth follicular mericarps; seeds ovate, margined, ending in a silky coma, cotyledons elliptic, radicle cylindric (Gamble, 1956; Caius et al., 1975; Mathew & Rani, 1983). Flowering: August-March; Fruiting: from October onwards; The Gymnema species are diploid with a chromosome number of 2n = 22 (Nadkarni et al., 1967; Sredeevi & Namboodiri, 1977).

GEOGRAPHICAL DISTRIBUTION

G. sylvestre native to the tropical forests of central and southern India had wider distribution and it grows in the plains from the coast, in scrub jungles and in thickets at an altitude ranging from 300 - 700 m (Gamble, 1956; Mathew & Rani, 1983). The genus Gymnema comprises 40 species distributed from Western Africa to Australia (Caius et al., 1975). G. acuminatum (Roxb.) Wall, G. aurantiacum, G. balsamicum, G. elegans W&A, G. hirsutum W&A, G. lactiferum, G. latifolium, G. montanum Hook.f., G. sylvestre R.Br., G. tingens W&A, G. indorum, G. yunnanse and G. spartum are some of the important species of genus Gymnema (Gamble, 1956; Caius et al., 1975; Mathew & Rani, 1983). They are mainly distributed in the Deccan peninsula parts of northern, western India, Tropical Africa, Australia, Vietnam, Malaysia and Sri Lanka (Nakamura et al., 1997; Ye et al., 2000).

CHEMICAL CONSTITUENTS AND MEDICINAL PROPERTIES

Gymnema sylvestre leaf extract (ethanol and water) contain triterpene saponins, belonging to oleane and damarane classes. Besides, flavones, anthraquinones, hentri-acontane, alpha and beta chlorophylls, phytin, resins, inositol, d-quercitol, alkaloid, tartaric acid, formic acid, butyric acid, lupeol, -amyrrins, stigmasterol were isolated and characterised (Kapoor, 1990). The dry leaves were analysed for various chemical constituents and
it consists of ash (11.45%), petroleum ether soluble residue (6.21%), ether soluble extract (1.72%), alcohol soluble residue (12.16%), albumin (0.45%), organic acids (5.50%), paraben (7.26%), calcium oxalate (7.30%), lignin (4.80%) and cellulose (22.65%). Analysis of the leaf ash consists of K$_2$O (14.73%), Na$_2$O (8.56%), CaO (20.72%), MgO (2.75%), Fe$_2$O$_3$ (5.44%), Al$_2$O$_3$ (0.92%), Mn (1.31%), CO$_2$ (11.66%), SO$_3$ (6.04%), P$_2$O$_5$ (6.73%), SiO$_2$ (insol.) (11.90%), SiO$_2$ (sol.) (5.79%) and Cl (3.35%) (Caius et al., 1975).

The various medicinal properties include antidiabetic, antisweetness, stimulant, stomachic, diuretic, laxative, acid, biliiousness, astringent, alexiteric, refrigerant, expectorant, emetic, cardiotonic and antihelmintic (Gharpurey, 1926; Sastri, 1956; Chopra, 1958; Yackzan, 1969; Liu et al., 1992).

PHARMACOLOGICAL STUDIES

Gymnema species have numerous pharmacological activities reported by several authors and many experiments were carried out in G. sylvestre. In most of the laboratory, investigations on this plant have employed on G. sylvestre leaves, which are slightly bitter and are non toxic to humans in gram quantities. The pharmacopoeia standards of the leaf of this plant have been recently published by Indian Council of Medical Research (ICMR), New Delhi, India (Anonymous, 2003).

Antisweet activity

This plant is famous for its fascinating ability to antagonize the sweet taste of sugar thus known as Gurmar in 'Hindi' (Gupta & Seth, 1962; Gupta, 1963; Gupta & Variyar, 1964; Mitra et al., 1975; Terasawa et al., 1994). It was observed that G. sylvestre crude leaf extract reduced the sweetness of eight sweeteners namely acesulfame K, aspartame, sodium cyclamate, fructose, glucose, sucrose, stevioside and xylitol to 77% in human (Frank et al., 1992). The major active compound gymnemic acid from G. sylvestre (Stocklin, 1969; Dateo & Long, 1973) binds bovine tongue and inhibits the sweet taste observed in fungiform papillae (Izutani et al., 2002), human and chimpanzee (Oakley, 1985; Hellekant et al., 1985; Hooper, 1887; Shore, 1892; Shimizu et al., 1997; Rafiullah et al., 2006). Due to anti-sweet activity of gymnemic acid, G. sylvestre appeared on the US market several years ago, and it was hyped as a “sugar blocker” (Murray et al., 1999).

Antidiabetic activity

G. sylvestre leaf extract reduces blood sugar level in diabetic rabbits (Gharpurey, 1926; Chopra, 1958; Sanjappa & Sathayanda, 1979; Kirtikar & Basu, 1975; Shanmugasundaram et al., 1983; Hirata et al., 1992; Yoshikawa et al., 1997; Agarwal et al., 2000; Rafiullah et al., 2006), rats (Srivastava et al., 1985) and human (Shanmugasundaram et al., 1990). It decreases glucose by increasing insulin releases (Shanmugasundaram et
It was found to contain an active principle which would cure the diabetes (Shanmugasundaram et al., 1990; Gupta, 1961; Jain & Sharma, 1967; Dixit & Pandey, 1984). There has been a lot of research on its involvement in carbohydrate metabolism from the viewpoint of phamacognosy, nutrition and food science. Various effects have been reported, such as suppression of glucose absorption in the small intestine of rats, reduction of plasma glucose absorption in the small intestine of rats, reduction of plasma glucose increment in the oral sucrose tolerance test, significantly lowered blood glucose and insulin values in dogs as well as suppression of insulin increase in glucose tolerance tests in men and the alleviation of diabetic symptoms in patients with non-insulin dependent diabetes mellitus (Shanmugasundaram et al., 1990).

The beneficial effect was observed in oral treatment to cure Non-Insulin Dependent Diabetes Mellitus (NIDDM) to use the 400 mg/kg of leaf extract, where there is a significant reduction of blood glucose, glycosylated haemoglobin and plasma protein and increase in serum insulin levels (Shanmugasundaram et al., 1990). Single dose oral administration of leaf powder (1.0 g/kg) to normal and Streptozotocin (STZ) induced rats led to significant reduction of blood glucose of OGTT (Oral Glucose Tolerance Test) and no significant effect in immuno reactive insulin (IRI) (Dixit & Pandey, 1984). Administration of leaf extract of G. sylvestre (120 mg/kg/day P.O) for 7 days in STZ induced rats reduced amylase activity in serum, increased b-cell function, regenerated b-cells in pancreatic islets and showed higher levels of serum C-peptide (Shanmugasundaram et al., 1990).

The G. sylvestre alcoholic extract also stimulates insulin secretion from rat islets of Langerhans and several pancreatic b-cells lines (Persaud et al., 1999). It was found to increase the activities of the enzyme affording the utilization of glucose by insulin dependent pathway and it controlled phosphorylase level, gluconeogenic enzymes, sorbitol dehydrogenase (Shanmugasundaram et al., 1983). It also significantly lowered the hepatoglycogen content of the glucose fed rats (Chattopadhyay, 1998; Kar et al., 1999) and it controls the blood sugar in beryllium nitrate treated rats (Prakash et al., 1986). Leaf extract of 13.4 g/kg of gymnemic acid was potentially effective in the amelioration of corticosteroid – induced diabetes mellitus (Gholap & Kar, 2003) and also potentially regulates dexamethasone induced hyperglycaemia in mice (Gholap & Kar, 2005). In Alloxan induced diabetic rabbits it decreases the activity of gluconeogenic enzymes and reversal of pathological changes in the liver initiated during the hyperglycaemic phase (Shanmugasundaram et al., 1983).

Oral administration of different concentrations of G. sylvestre leaf extract (50, 100, 200 and 400 mg/kg) with normal and STZ induced rats showed significant dose dependent hypoglycaemic activity (Prakash et al., 1986; Chattopadhyay, 1999) and it has hypoglycaemic activity in nature
(Shanmugasundaram et al., 1981; Khare et al., 1983; Shanmugasundram et al., 1990; Tripathi & Chaturvedi, 1995; Khan et al., 2005). It lowers plasma glucose and insulin levels (Kurihara, 1969; Hirata et al., 1992; Porchezhian & Dobriyal, 2003). It inhibits glyceraldehyde-3-phosphate dehydrogenase activity (Izutani et al., 2005). Gymnemic acid IV derived from G. sylvestre inhibits the glucose absorption (glycosidase inhibition), increase glucose uptake in striated muscles, lowered blood glucose and increases the insulin secretion in b-cells (Sugihara et al., 2000), and gymnemic acid IV has multidirectional antihyperglycaemic activity (Kimura, 2006).

The various herbal formulation such as Dianex (Mutalik et al., 2005), D-400 (Maji & Singh, 1995), hyponid (Babu & Prince, 2004) contain G. sylvestre extract lower blood glucose and cures diabetes mellitus. However, the absence of antidiabetic effect of G. sylvestre in non-diabetic and alloxan-diabetic rats was reported in the commercialized herbal preparations in the Brazil (Ricardo galletto et al., 2004).

**Obesity**

G. sylvestre leaf extract was found to have reduction of cholesterol and triglyceride levels in diabetic rats (Terasawa et al., 1994). The effect of G. sylvestre on body weight, glucose absorption and lipid metabolism was examined by using a breed of fatty rats with genetic obese-hyperglycaemia. Simultaneous feeding with Gymnema aqueous extract decreased the body weight in fatty and lean rats (4.2% and 6.1% respectively) compared to animals consuming only the test diet (high carbohydrate-low fat) over a 21 weeks period. Plasma glucose was lowered by 18% in the Gymnema treated animals compared to control. The plasma glucose increase following an oral glucose tolerance test was almost normalised, but not hypercholesterolemia. Two fractions of Gymnema extract (containing 160-360 mg/g of gymnemagenin) decreased body weight gain and food intake dose-dependently when given orally (0.05-1 g/kg) to rats for 22 days. Administration of Gymnema fraction (1.0 g/kg) containing 363 mg/g of gymnemagenin increased faecal excretion of cholesterol, total neutral steroids, total bile acids and cholic acid-derived bile acid (Nakamura et al., 1997). Furthermore, the lipid lowering properties (Wang et al. 1998; Shigematsu et al., 2001) hypolipidaemic and antiatherosclerotic effect in rats (Bishayee & Chatterjee, 1994) have also been reported. It is recently reported that G. sylvestre leaf contains the gymnemate promoted weight loss by its ability to reduce hyperlipidemia (Luo et al., 2006). In contradictory, dried powdered leaf prepared commercially in Brazil lacks hypolipidemic effect (Ricardo galletto et al., 2004). This may due to the adulteration present in the preparations.

In human, leaf extract (2 g thrice a day) given oral led to significant reduction of OGTT (Oral Glucose Tolerance Test) (Khare et al., 1983). In
addition, it reduces the serum lipid level to near normal levels (Shanmugasundaram et al., 1990). The mechanism behind this is that gymnemic acid inhibits the glucose uptake (Imoto et al., 1991; Shimizu et al., 1996; Shimizu et al., 1997) and maltose absorption (Imoto et al., 1991) in intestine and also inhibits glucose-stimulated gastric inhibitory peptide secretion (Shimizu et al., 1996), thereby suppressing the body weight (Agarwal et al., 2000). OB-200G polyherbal formulation extracts works against obesity of mice (Kaur & Kulkarni, 2001) and body weight managements (Preuss et al., 2004).

**Miscellaneous uses**

*G. sylvestre* has strong reducing effect on urinary disorder (Sanjappa & Sathayanda, 1979; Kirtikar & Basu, 1975). The *G. sylvestre* cures the major diseases such as diuretic and cough (Kirtikar & Basu, 1935) and mycosis of the toes (Reddy et al., 1989). The active constituent gymnemic acid is also useful for the prevention of the formation of dental plaque and caries (Kirtikar & Basu, 1935) and also possesses antiviral effect (Sinsheimer et al., 1968). The roots of *G. sylvestre* are macerated and drunk as a remedy for snake bite (Shanmugasundaram et al., 1983; Nagaraju & Rao, 1990) and the root extract is also found to have antioxidant activity against free radicals and LDL levels (Ohmori et al., 2005; Aldona Dembinska-Kiec et al., 2008), antimicrobial activity (Satdive et al., 2003), anti-inflammatory activity (Diwan et al., 1995) and larvicidal effect against *Culex qinquifaciatu*s mosquito larvae (Gopiesh Khanna & Kannabiran, 2007). Gastric sensation was observed in humans fed with *G. sylvestre* leaf tea extract (Schroeder and Flannery-Schroeder, 2005). Diabecon herbal formulation protects the lens against sugar induced cataract (Moghaddam et al., 2005). The pharmacology studies of *G. sylvestre* are presented in Table 1.

**Table 1. Pharmacological studies in Gymnema sylvestre**

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Pharmacological activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>Anti-sweet tested in human</td>
<td>Shore, 1892</td>
</tr>
<tr>
<td>Leaf</td>
<td>Diabetes mellitus, stomachic and diurea</td>
<td>Sastri, 1956</td>
</tr>
<tr>
<td>Root</td>
<td>Anti-sweet activity</td>
<td>Stocklin et al., 1967</td>
</tr>
<tr>
<td>Leaf</td>
<td>Decreases gluconeogenic enzyme and pathological changes</td>
<td>Shanmugasundaram et al., 1983</td>
</tr>
<tr>
<td>Whole plant</td>
<td>Reduced glucose fasting and OGTT in human</td>
<td>Khare et al., 1983</td>
</tr>
<tr>
<td>Leaf</td>
<td>Snake bite</td>
<td>Nagaraju &amp; Rao, 1990</td>
</tr>
<tr>
<td>Leaf</td>
<td>Lowered the insulin requirement in IDDM; stimulating insulin production from pancreas and Pancreatic islets production from pancreas</td>
<td>Shanmugasundaram et al., 1990</td>
</tr>
<tr>
<td>Leaf</td>
<td>Inhibitions glucose absorption in rat intestineconduritol A gymnemic acid</td>
<td>Hirata et al., 1992</td>
</tr>
<tr>
<td>Leaf extract</td>
<td>Inhibits sweetness of eight sweeteners</td>
<td>Frank et al., 1992</td>
</tr>
</tbody>
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Table 1. (Contd.)

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Pharmacological activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf extract</td>
<td>Rats – body weight, plasma glucose, serum glucose, serum triglyceride, total cholesterol and insulin.</td>
<td>Terasawa et al., 1994</td>
</tr>
<tr>
<td>Leaf extract</td>
<td>Hypolipidaemic and antiatherosclerotic effects</td>
<td>Bishayee &amp; Chatterjee, 1994</td>
</tr>
<tr>
<td>Leaf extract</td>
<td>Anti-inflammatory</td>
<td>Diwan et al., 1995</td>
</tr>
<tr>
<td>Leaf extract</td>
<td>Stimulate insulin production in rat pancreatic islet cells and the insulin secreting hamster β-cell line (HIT cells <em>in vitro</em>)</td>
<td>Diwan et al., 1995</td>
</tr>
<tr>
<td>Whole plant</td>
<td>Inflammation studies</td>
<td>Persaud et al., 1996</td>
</tr>
<tr>
<td>Whole plant</td>
<td>Triterpenoid glycosides- Inhibits glucose utilization in muscles</td>
<td>Shimizu et al., 1996</td>
</tr>
<tr>
<td>Leaf extract</td>
<td>Alcohol extract – lower hepatic</td>
<td>Nakamura et al., 1997</td>
</tr>
<tr>
<td>Leaf extract</td>
<td>Gymnemic acid – health food</td>
<td>Shimizu et al., 1997</td>
</tr>
<tr>
<td>Leaf</td>
<td>Inhibit glucose absorption in rats</td>
<td>Chattopadhyay, 1998</td>
</tr>
<tr>
<td>Leaf extract</td>
<td>Anti-microbial activity</td>
<td>Satdive et al., 2003</td>
</tr>
<tr>
<td>Leaf extract</td>
<td>Diabetes and obesity – clinical practice</td>
<td>Porchezian &amp; Dobriyal, 2003</td>
</tr>
<tr>
<td>Leaf extract</td>
<td>Diabetes and in food additives against obesity</td>
<td>Press et al., 2004</td>
</tr>
<tr>
<td>Leaf extract</td>
<td>Anti hyperglycaemic and anti oxidant studies</td>
<td>Babu &amp; Prince, 2004</td>
</tr>
<tr>
<td>Leaf extract</td>
<td>Diabecon – contract studies</td>
<td>Moghaddam et al., 2005</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>Antioxidant activity – LDL and free radicals</td>
<td>Ohmori et al., 2005</td>
</tr>
<tr>
<td>Leaf extract</td>
<td>Dianex herbal formulation – use diabetes</td>
<td>Mutalik et al., 2005</td>
</tr>
<tr>
<td>Leaf extract</td>
<td>Gustatory sensation studies</td>
<td>Schroeder &amp; Flannery-Schroeder, 2005</td>
</tr>
<tr>
<td>Leaf extract</td>
<td>Regulated – induced hyperglycemia</td>
<td>Gholap &amp; Kar, 2005</td>
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PHYTOCHEMICAL STUDIES

Phytochemical progress has been aided enormously by the development of rapid and accurate methods of screening plants for particular chemicals and the emphasis in this area is inevitably on chromatographic technique. The amount and type of compounds separated into the different fractions will, of course, vary from plant to plant. Also, such a procedure may have to be modified when labile substances are under investigation. Isolation and characterization of pharmacologically active compounds from medicinal plants continue today. More recently, drug discovery techniques have been applied to the standardization of herbal medicines, to elucidate analytical marker compounds (Balunas & Kinghorn, 2005). Since drug discovery from medicinal plants is time-consuming, faster and better methodologies for plant collection, bioassay screening, compound isolation and compound must be employed (Do & Bernard, 2004; Koehn & Carter, 2005).
In Gymnema species a number of phytochemical constituents have been reported by several authors. While considerable practical difficulty was experienced initially in purifying these labile compounds, about 20 distinct oleanane-type glycosides have now been individually characterized as sweetness inhibitory constituents. G. sylvestre cures many diseases and its constituents include two resins, gymnemic acids, saponins, stigmasterol, quercitol and the amino acid derivative of betaine, choline and trimethylamine, but its main active compound is gymnemic acid, saponins and oleanane type of triterpenoid (Kapoor, 1990). Although attempts regarding isolation and structure elucidation of the gymnemic acids have continued for many years, this area of endeavour caused a great deal of difficulty for phytochemical researchers, and somewhat controversial. The first attempt to isolate the active compound(s) from the leaves of G. sylvestre concluded that gymnemic acid is a glycoside (Hooper, 1887, 1889). Subsequently several efforts to purify gymnemic acid were unsuccessful and it able to obtain a relatively pure sample of gymnemic acid (Warren & Pfaffmann, 1969).

G. sylvestre contains oleanane type triterpene (Gymnemagenin) and gymnemic acid which itself is not pure entity, but composed of 4 components, A1 - A4, with gymnemic acid A1 as the predominant one (Stocklin et al., 1967). Gymnemagenin and gymnastogenin were isolated and crystallized (Stocklin, 1968; Kurihara, 1969). Earlier, reported the isolation of a new compound gymnemic acid A1, which could be converted to gymnemic acid A2 and it has anti-sweet activity against sucrose (Kurihara, 1969), but gymnemic acid A-D reported different forms (Sinsheimer & Rao, 1970). Others studied the gymnemic acid production (Liu et al., 1992; Stocklin et al., 1967).

Yoshikawa and co-workers (1989a, b) isolated gymnemic acids from the hot water extract of G. sylvestre dry leaves, which they named gymnemic acids I, II, III, IV, V, VI and VII respectively and the anti-sweet activity was evaluated (Sugihara et al., 2000; Stocklin, 1968; Sinsheimer et al., 1970). In contrast, the next series of anti-sweet compounds, gymnemasaponins I, II, III, IV, and V were isolated from G. sylvestre (Yoshikawa et al., 1991). Further work carried out in G. sylvestre led to isolate and characterization of other gymnemic acids VIII, IX, X, XI, XII, XIII, XIV, XV, XVI, XVII and XVIII (Liu et al., 1992; Yoshikawa et al., 1992; Yoshikawa et al., 1993; Yoshikawa et al., 1997).

The confirmed structure of gymnemagenin by X-ray crystallographic method and identified its antisweet activity (Liu et al., 1992). Gymnemic acid and deacylgymnemic acid were isolated and identified by the use of High Performance Liquid Chromatography (HPLC) and atmospheric pressure ionization mass spectrometry (Imoto et al., 1991; Suzuki et al., 1993).
In *G. sylvestre* some of the antisweet saponin compounds were reported (Yoshikawa *et al*., 1992; Ye *et al*., 2001). Gymnemasisde I, II, III, IV, V, VI and VII (Yoshikawa *et al*., 1992) gymnemoseide a, b, c, d and e (Yoshikawa *et al*., 1997), gymnesins A, B, C and D (Sahu *et al*., 1996) and some of triterpenes (Ye *et al*., 2001) were also isolated from the leaves of *G. sylvestre*. Recently, two new flavonol glycosides were isolated from the leaves of *G. sylvestre* (Liu *et al*., 2004) and 2-(trimethylsilyl) ethyl glycoside and flavonoid triglycoside from *G. sylvestre* (Mukhopadhyay & Field, 2006). High performance thin layer chromatography (HPTLC) method led to the determination of gymnestrogehin and its reference sample from *G. sylvestre* (Puratchimani & Jha, 2004). Gymnemic acid and gymnemogenin are isolated from *G. sylvestre* through HPTLC (Raju *et al*., 2006). The various aspects of phytochemical investigations and structural details of isolated phytochemical compounds from *G. sylvestre* were presented in Table 2 and Figs 3 - 6.

**Table 2.** Secondary metabolites isolated from *Gymnema sylvestre*

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Compounds</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Leaf</td>
<td>Gymnemic acid A1</td>
<td>Hooper, 1887; Yackzan, 1966; Stocklin <em>et al</em>., 1967; Sinsheimer <em>et al</em>., 1968; Warren &amp; Pfaffmann, 1969; Dateo &amp; Long, 1973</td>
</tr>
<tr>
<td>Leaf</td>
<td>Gymnemic acid A2, A3, A4 and A4</td>
<td>Kurihara, 1969; Stocklin, 1969; Rao &amp; Insheimer, 1971</td>
</tr>
<tr>
<td>Leaf</td>
<td>Gymnemogagenin and Gymnestrogenin</td>
<td>Stocklin, 1969; Rao &amp; Insheimer, 1971</td>
</tr>
<tr>
<td>Leaf</td>
<td>Gymnemasisponin A, B, C and V</td>
<td>Yoshikawa <em>et al</em>., 1991</td>
</tr>
<tr>
<td>Leaf</td>
<td>Gymnemic acid I, II</td>
<td>Imoto <em>et al</em>., 1991</td>
</tr>
<tr>
<td>Leaf</td>
<td>Gymnemic acid VII and VIII</td>
<td>Liu <em>et al</em>., 1992</td>
</tr>
<tr>
<td>Leaf</td>
<td>Gymnemic acid I - IX, X, XI, XII, XIII and XIV</td>
<td>Yoshikawa <em>et al</em>., 1992</td>
</tr>
<tr>
<td>Leaf</td>
<td>Gymnemic acid XV, XVI, XVII and XVIII</td>
<td>Yoshikawa <em>et al</em>., 1993</td>
</tr>
<tr>
<td>Leaf</td>
<td>Gymnemoseide A and B</td>
<td>Murakami, 1996</td>
</tr>
<tr>
<td>Leaf</td>
<td>Triterpenoids - saponins</td>
<td>Sahu <em>et al</em>., 1996</td>
</tr>
<tr>
<td>Leaf</td>
<td>Gymnemoseide A, B, C, D, E and F</td>
<td>Yoshikawa <em>et al</em>., 1997</td>
</tr>
<tr>
<td>Leaf</td>
<td>Six Oleanane - Saponins and Two triterpene saponins</td>
<td>Ye <em>et al</em>., 2001</td>
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<tr>
<td>Leaf</td>
<td>Triterpene glycosides (Oleanolic acid, Longispinogenin, Chichipegenin, Sitakisogenin, 30-hydroxylupeol)</td>
<td>Ye <em>et al</em>., 2001</td>
</tr>
<tr>
<td>Leaf</td>
<td>Two new flavonol glycosides</td>
<td>Liu <em>et al</em>., 2004</td>
</tr>
<tr>
<td>Leaf</td>
<td>Gymnemogagenin</td>
<td>Puratchimani &amp; Jha, 2004</td>
</tr>
<tr>
<td>Compounds</td>
<td>R1</td>
<td>R2</td>
</tr>
<tr>
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</tr>
<tr>
<td>Gymnemic Acid I</td>
<td>-gluA</td>
<td>tga</td>
</tr>
<tr>
<td>Gymnemic Acid II</td>
<td>-gluA</td>
<td>mba</td>
</tr>
<tr>
<td>Gymnemic Acid III</td>
<td>-gluA</td>
<td>mba</td>
</tr>
<tr>
<td>Gymnemic Acid IV</td>
<td>-gluA</td>
<td>tga</td>
</tr>
<tr>
<td>Gymnemic Acid V</td>
<td>-gluA</td>
<td>tga</td>
</tr>
<tr>
<td>Gymnemic Acid VI</td>
<td>-gluA(^3) -gle</td>
<td>tga</td>
</tr>
<tr>
<td>Gymnemic Acid VII</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>Gymnemic Acid VIII</td>
<td>-gluA(^3) -OG</td>
<td>mba</td>
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<td>tga</td>
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<td>Gymnemic Acid X</td>
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<tr>
<td>Gymnemic Acid XII</td>
<td>-gluA(^3) -gle</td>
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<tr>
<td>Gymnemic Acid XIII</td>
<td>-gluA</td>
<td>H</td>
</tr>
<tr>
<td>Gymnemic Acid XIV</td>
<td>-gluA</td>
<td>H</td>
</tr>
<tr>
<td>Gymnemic Acid XV</td>
<td>H</td>
<td>mba</td>
</tr>
<tr>
<td>Gymnemic Acid XVI</td>
<td>tga</td>
<td>H</td>
</tr>
<tr>
<td>Gymnemic Acid XVII</td>
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<td>Bz</td>
</tr>
<tr>
<td>Gymnemic Acid XVIII</td>
<td>H</td>
<td>H</td>
</tr>
</tbody>
</table>

* AP – Antisweet Potency x Gymnemic acid I;   ND – Not detected

**Fig 3.** Structures of Gymnemic Acid I – XVIII

\[ \text{Gymnemagenin I, II, IV, V} \quad \text{Gymnemagenin II, IV} \]

\[ \begin{align*}
    \text{I: } & R = R_1 = H; \quad \text{III: } R = R_1 = \text{Ac}; \\
    \text{IV: } & R = R_1 = \text{Bz}; \quad \text{V: } R = H; \quad R_1 = \text{C(Ph)}_3
\end{align*} \]

\[ \begin{align*}
    \text{I: } & R = H; \quad \text{VI: } R = \text{Ac}
\end{align*} \]
Fig 4. Structures of Gymnemagenin I, III – V, Gymnesticrogenin II, IV, Gymnemosides a-f
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Fig 5. Structures of Gymnemasides I – VII and Gymnemasaponins I – V
1. \( R_1 = R_3 = R_4 = R_5 = R_6 = H; R_2 = \text{O-CO-} \)

2. \( R_1 = R_2 = R_3 = R_4 = R_5 = H; R_6 = K^+ \)

3. \( R_1 = \text{OH}; R_2 = R_3 = R_4 = R_5 = H; R_6 = K^+ \)
4. R1 = R2 = H; R3 = OH; R4 = Ac, R5 = rhamnopyranosyl, R6 = Na+

1. 21-β-O-benzylsitakogenin 5-O-β-D-glucopyranosyl (1-3)-β-D-glucuronopyranoside
2. Potassium salt of 29-hydroxylongispinogenin-3-O-β-D-glycopyranosyl (1-3)-β-D-glycuronopyranoside
3. 3, 16, 28, 29-tetrahydroxyolean-12-ene
4. Sodium salt of alternoside II.

**PLANT TISSUE CULTURE**

Plant biotechnology offers an opportunity to exploit the cell, tissue, organ or entire plant by growing them *in vitro* and to genetically manipulate them to get desired compounds. Many facts of biotechnological approaches can be envisaged like plant callus culture, cell culture, shoot culture, root culture and scale up of cultures. Since the world population is increasing rapidly, there is extreme pressure on the available cultivable land to produce chemicals from plants and the available land should be used effectively. Hence, it is appropriate to develop modern technologies leading to plant improvement for better utilization of the land to meet the requirement. The search for new plant derived chemicals should thus be a priority in current and future efforts towards sustainable conservation and rational utilization of biodiversity (Phillipson, 1990). In the search for alternatives to the production of desirable medicinal compounds from plants, biotechnological approaches, specifically, plant tissue culture, is found to have potential as a supplement to traditional agriculture in the industrial production of bioactive plant metabolites (Rao & Ravishankar, 2002).

*Gymnema* species natural stands are fast disappearing and are threatened with extinction due to its indiscriminate collection, over exploitation and natural resources for commercial purposes and to meet the requirements of pharmaceutical industry (Choudhury, 1998). Commercial exploitation for production and conventional propagation are hampered due to its poor seed viability, low rate of germination and poor rooting ability of vegetative cuttings. Alternative *in vitro* propagation methods would be beneficial in accelerating large scale multiplication and conservation of the plant.

There are only a few reports available on *in vitro* propagation of *Gymnema* species using different explants (Anu *et al*., 1994; Reddy *et al*., 1998; Komalavalli & Rao, 1997; Komalavalli & Rao, 2000; Kumar *et al*., 2002). Micropropagation was observed in Murashige and Skoog (MS) medium supplemented with 6-benzyl aminopurine (BA 5.0 mg/l) combination of 1-naphthaleneacetic acid (NAA 0.2 mg/l) strength induced 7 shoots/explant and best root induction in ½ MS without growth regulators.
However, the highest number of multiple shoots (57.2 shoots/explant) observed in MS medium supplemented with 6-benzylaminopurine (BA 1.0 mg/l), 6-furfurylaminopurine (KN 0.5 mg/l), 1-naphthaleneacetic acid (NAA 0.1 mg/l), malt extract (100 mg/l) and citric acid (100 mg/l), and the best root induction was in MS medium supplemented with indole -3-butyric acid (IBA 3.0 mg/l) (Komalavalli & Rao, 2000). Micropropagation studies carried out in Gymnema species, indicated that MS medium supplemented with 6-furfurylaminopurine (KN 0.4 ppm) and indole -3-acetic acid (IAA 4.0 ppm) induced 33.3 shoots/explant (Kumar et al., 2002). It was reported that KN induced the highest shoot number in Gymnema sylvestre (Anu et al., 1994). In G. elegans the highest number of shoots were induced from seedlings and mature auxiliary node explants on MS medium supplemented with BA (13.0 mm), KN (2.0 mm), NAA (3.0 mm), ascorbic acid (100 mg/l), coconut milk (10%), GA$_3$ (1.0 mm) and rooting in MS medium with IBA (5.0, 15.0 mm) (Komalavalli & Rao, 1997). Somatic embryogenesis and plant regeneration were achieved from hypocotyl, cotyledon and leaf explants excised from seedling of G. sylvestre in MS medium supplemented with 2,4-dichlorophenoxycetic acid (2,4-D 0.5-5.0 mm), BA (0.5-2.0 mm) and 2% sucrose. The embryo germination and plantlet formation were achieved in fresh EM 8 medium (Kumar et al., 2002). In vitro organogenesis of G. sylvestre from matured decoated explants (Komalavalli et al., 2007). In vitro callus showed that the active compounds, gymnemic acid and gymnemagenin, were present in sufficiently large amount in the cultured undifferentiated cells (Gopi & Vatsala, 2006; Kanetkar et al., 2006); external phytohormone, shaking speeds, pH of the medium, played important roles in growth and gymnemic acid production in suspension culture (Devi et al., 2006); sucrose, inoculum density, auxins and aeration played important roles in cell growth in bioreactor cultures (Lee et al., 2006). In vitro production of gymnemic acid through callus culture under abiotic stress conditions was also reported (Abdul Bakrudeen Ali Ahmed et al., 2009). The various aspects of plant tissue culture of G. sylvestre is presented in Table 3.

Table 3. In vitro studies on Gymnema sylvestre

<table>
<thead>
<tr>
<th>Explants</th>
<th>Culture medium</th>
<th>Growth response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mature nodal</td>
<td>MS + BA (5.0 mg/l) + NAA (0.2 mg/l)</td>
<td>Micropropagation</td>
<td>Reddy et al., 1998</td>
</tr>
<tr>
<td>Axillary node</td>
<td>MS + BA (1.0 mg/l) + KN (0.5 mg/l) + NAA (0.1 mg/l)</td>
<td>Micropropagation</td>
<td>Komalavalli &amp; Rao, 2000</td>
</tr>
<tr>
<td>Hypocotyl, cotyledon</td>
<td>EM 8 Medium + 2,4-D (2.0 µm) + BA (1.0 µm)</td>
<td>Somatic embryogenesis</td>
<td>Kumar et al., 2002</td>
</tr>
<tr>
<td>Leaf and Nodal</td>
<td>MS + 2,4-D (0.5 mg/l) + NAA (2.5 mg/l) + BA (0.5 mg/l)</td>
<td>Callus and cell</td>
<td>Gopi et al., 2006</td>
</tr>
<tr>
<td>Leaf</td>
<td>MS + BA (1.0 mg/l) + IAA (1.0 µm)</td>
<td>Suspension culture</td>
<td>Devi et al., 2006</td>
</tr>
</tbody>
</table>
Table 3. Continue

<table>
<thead>
<tr>
<th>Explants</th>
<th>Culture medium</th>
<th>Growth response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>(0.5 mg/l) MS + BA (2.22 mg/l) + 2,4-D (0.44 mg/l)</td>
<td>Callus culture</td>
<td>Kanetkar et al., 2006</td>
</tr>
<tr>
<td>Node</td>
<td>MS + BA (1.0 mg/l) + 2,4-D (1.0 mg/l)</td>
<td>Callus culture</td>
<td>Lee et al., 2006</td>
</tr>
<tr>
<td>Seed</td>
<td>MS + BA (0.22 mg/l) + 2,4-D (0.44 mg/l)</td>
<td>Callus culture</td>
<td></td>
</tr>
<tr>
<td>Leaf</td>
<td>MS + 2,4-D (1.0 mg/l) + KN (0.1 mg/l) + Sucrose, Inoculum density, auxins</td>
<td>Batch culture</td>
<td></td>
</tr>
<tr>
<td>Mature</td>
<td>MS + NAA (8 µm) + citric acid (10 mg/l) + Casein hydrolysate (100 mg/l) + BA (22.2 µm) + Ad (35.7 µm) + GA₃ (29 µm)</td>
<td>Organogenesis</td>
<td>Komalavalli et al., 2007</td>
</tr>
<tr>
<td>decoated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>seeds</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf</td>
<td>MS + 2,4-D (1.5 mg/l) + KN (0.5 mg/l) + 3 mMol NH₄NO₃</td>
<td>Gymnemic acid production under abiotic stress</td>
<td>Abdul Bakrudeen Ali Ahmed et al., 2009</td>
</tr>
</tbody>
</table>

CONCLUSIONS

*G. sylvestre* is a multipurpose potential medicinal plant having high market potential all over the world. Hence it is utmost important to monitor the progress of pharmacological, phytochemical and plant tissue culture literature to assess the efficacy before being recommended for various therapies, commercial propagation and *in vitro* production of gymnemic acid. Thus this paper gives an overview of *G. sylvestre* from antiquity to till date. It has addressed recent advances in key areas of *G. sylvestre*, namely plant tissue culture techniques and plant secondary metabolites production. Even with all the challenges facing *G. sylvestre* from medicinal plants, large scale of new secondary metabolites isolated from *G. sylvestre* can be predicted to remain an essential component in the search for new secondary metabolites and its pharmacological activities.

There is sufficient evidence of pharmacological and phytochemical studies to draw a definite conclusion about the efficacy of the gymnemic acid for the treatment of diabetes and obesity but, there is still inadequate literature related to plant tissue culture and other activities. Moreover, refinements in protocols and field performance data are necessary to get good quality regenerants and for large scale propagation. The reported studies on the *in vitro* production of gymnemic acid are not satisfactory to obtain sufficient quantities of gymnemic acid in high yields. Therefore further *in vitro* studies such as role of bioelicitors, precursors and biotransformation are needed to enhance the gymnemic acid production at laboratory conditions and also for commercial production at large scale. With the interest that has been generated both the general public and multinationals across the globe into *Gymnema sylvestre*, there is now more
than ever a golden opportunity to continue making worthwhile contribution to healthcare.

REFERENCES


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