GENERAL INTRODUCTION
AND REVIEW OF LITERATURE.......
1. General Introduction and Review of Literature

"Developing nations are not mining their green gold"

Gobh Sampath

India is one of the twelve megadiversity countries of the world with a rich diversity of biotic resources (Bapat et al. 2008). A total of 560 plant species of India have been included in the International Union for Conservation of Nature and Natural Resources (IUCN) Red List of Threatened species, out of which 247 species are in the threatened category. On a global basis, the IUCN has estimated that about 12.5% of the world's vascular plants, totaling about 34,000 species are under varying degrees of threat (Phartyal et al. 2002). Many medicinal plants are also in trouble, due to indiscriminate collection and over exploitation of natural resources for commercial purposes and to meet the requirements of the pharmaceutical industry.

In terms of the number of species individually targeted, the use of plants as medicines represents by far the biggest human use of the natural world. Plants provide the predominant ingredients of medicines in most traditional systems of medicine. There is no reliable figure for the total number of medicinal plants on Earth, and numbers and percentages for countries and regions vary greatly (Schippmann et al. 2002). Estimates for the numbers of species used medicinally include: 35,000-70,000 or 53,000 worldwide (Schippmann et al. 2002); 10,000-11,250 in China (He and Gu 1997; Xiao and Yong 1998; Pei 2002); 7500 in India (Shiva 1996); 2237 in Mexico (Toledo 1995); and 2572 traditionally by North American Indians (Moerman 1998).

The World Health Organization estimates that up to 80% of people still rely mainly on traditional remedies (Kala 2005) resulting in the increasing demand for medicinal plants. In vitro propagation of rare and threatened plants
is generally undertaken to enhance the biomass and conserve the germplasm especially when population numbers are low in the wild. Tissue culture technique has been successfully used when wild grown plants are difficult to propagate through conventional ways. *In vitro* propagation or micropropagation is a viable alternative for species which are difficult to regenerate by conventional methods; where populations have decreased due to over exploitation by destructive harvesting and can effectively be used to meet the growing demand for clonally uniform elite plants. When species have been over collected by hobbyists for medicine, food or fragrance, *in vitro* propagation can provide an alternate source of plants and alleviate pressures on wild populations (Kapai et al. 2010).

Biotechnology offers avenues for maintenance, genetic improvement and efficient use of endangered plant resources and products (Bapat et al. 2008). Tissue culture is used for conservation of biological diversity by multiplication of plant species that have extremely small populations, for species with restricted reproductive capabilities and for recovery and reintroduction (Bramwell 1990). Production of phytochemicals from cell cultures is advantageous and *in vitro* studies on secondary metabolites, biotransformation, cryopreservation of valuable cell lines, immobilization and understanding enzymatic pathways will generate new data as well as reduce the production cost of photochemicals from medicinal plants (Bapat et al. 2008).

Higher plants are able to synthesize a wide range of biologically active secondary metabolites used as pharmaceuticals (e.g. morphine, codeine, atropine, scopolamine, quinine, diosgenin and digoxin), and generally of high value (Kirsi-Marja and Raimo Hiltunen 1996). Although the total synthesis of most of these compounds is chemically possible, it is usually very complicated and thus not economically viable. Plant products are currently isolated from naturally growing or cultivated plants or specific plant organs. Many medicinal plants require a special climate for growth. Secondary compounds are
synthesized in certain types of cells during a particular development stage of
the plant and are usually stored in the vacuoles of the plant cell or in other
differentiated tissues, e.g. glandular hairs. Thus, the optimal storage of the
compounds is dependent on the age of the plant and it sometimes takes several
years before the plants can be harvested. There is also a risk of over-collecting
endangered species (e.g. *Taxus bravifolia*). Alternative methods for producing
these plant-derived drugs are therefore desirable. All Secondary metabolic
pathways originate from primary precursors. Most enzymes in a given pathway
of secondary metabolism are coordinately regulated and speculated that there
are no clear rate-limiting enzymes as is the case for primary metabolism.
Catalytic activities of individual enzymes in a pathway often vary considerably,
however which may result in the accumulation of some intermediates unless
metabolic channeling or compartmentation occurs. One major limitation to
modifying an existing biosynthetic pathway by introducing a foreign enzyme is
the substrate specificity of the introduced enzyme because it must act on an
intermediate in the certain pathway. The regulation of enzyme levels and
activity is the most important factor in the control of secondary product
biosynthesis (Kirsi-Marja and Raimo Hiltunen 1996).

Phytochemicals derived from secondary metabolism have long been
processed for pharmaceuticals, food additives, flavors and fragrances and also
for products like latex and tannins. Traditionally phytochemicals have been
obtained by extraction from plants growing in the wild or in plantations. Wild
plants however, have become the subject of environmental concerns and may
not be open to harvesting as before. Plantation crops are subject to biological
and climatic adversities, as well as economic and political instabilities.
Alternatives to collecting wild plants and growing plantations are the result of
new technology in particular the development of plant tissue and cell culture
(Constabel 1990).
One of the major problems encountered in crude plant drugs is the batch-to-batch variations in their efficacies. Such variations could arise due to natural genetic variation (Chemotypes), seasonal variation, differences in the soil and climatic conditions, nutritional status, etc., of the medicinal plants. Association of medicinal plants with other plants in their habitat can also influence the medicinal value of them in some cases. Thus, it is very often difficult to get desired plant material with uniform quality as per requirement. This problem can be solved to a large extent by biotechnological intervention such as large scale *in vitro* propagation and/or cell or tissue culture. Some of them may be cultivated under controlled ideal conditions, without loss of medicinal value.

In many plant tissue culture systems the quantitative significance of the synthesis of secondary metabolites is low. As a consequence the impact of the synthesis of these products on cell metabolism is difficult to study. However, a few types of cell suspension are able to perform such biosynthetic reactions at high rates resulting in concentrations of more than 10% on a dry weight basis (Linus et al. 1995). Given suitable culture conditions and adequate analytical methods cell culture will display compounds as known for the source plants. Non-occurrence of compounds expected may motivate variation of culture conditions or genetic manipulation of cell cultures. Greater diversity of compounds in cell cultures than in source plants has been detected on occasion (Constabel and Tyler 1994). For example, anthraquinones have been found in addition to known alkaloids in *Cinchona* spp. cultures (Mulder-Krieger et al. 1982; Koblitz 1988).

In order to increase the productivity of medicinal plants, traditional plant breeding programs have been carried out including selection, crossing and mutations. About 34 years ago it became possible to transfer foreign genes into the plant genome (Chilton et al. 1977). Since then, many attempts have been made to introduce new genes primarily into commercially important crop
species in order to improve resistance to microorganisms, pests or herbicides, to increase biomass and grain size or to improve the quality of the plants. Efficient methods of gene cloning, transformation and plant regeneration, the availability of new gene constructs, appropriate organ-specific promoters for gene expression, series of useful reporter genes, and a large number of cloned DNA fragments are the most important factors, which have made it possible to increase the number of transgenic crops during the past few years. However, much less information is available for the improvement of medicinal plants by gene transfer (Kirsi-Marja and Raimo Hiltunen 1996).

1.1. Role of Plant Biotechnology and Tissue Culture in conservation of Medicinal Plants:

Medicinal plants are of great interest to the researchers in the field of biotechnology as most of the drug industries depend in part on plants for the production of pharmaceutical compounds (Chand et al. 1997). Pharmaceutical companies largely depend upon material procured naturally occurring stands which are being depleted rapidly raising concern about possible extinction of the medicinally valuable species and providing justification for the development of in vitro propagation techniques for these crops (Rout et al. 1999).

Conventional propagation is beset with problems of poor seed viability, low germination and scanty and delayed rooting of seedlings and vegetative cuttings. There is an urgent need to apply non-conventional propagation methods for conservation and future commercial delivery of medicinally important species (Upadhyay et al. 1992). The in vitro mass propagation of the different genotypes would yield plants suited for programmes of conservation of natural genetic resources, thereby safeguarding the survival. Furthermore, if this propagation is performed with genotypes selected according to their medicinally important (pharmacologically valuable) component and its content, it will be possible to obtain a sustainable crop development. The best genotypes
already introduced *in vitro*, can also be used as basis for cell cultures that could produce the target compounds (Majada et al. 2000).

The increasing demand for herbal medicines in recent years due to their fewer side effects in comparison to synthetic drugs and antibiotics has highlighted the need for conservation and propagation of medicinal plants. Tissue culture provides efficient techniques for rapid and large scale propagation of medicinal plants and their *in vitro* conservation of germplasm (Uppeandra Dhar et al. 2002). Tissue culture techniques can play an important role in the rapid multiplication of elite clones and germplasm conservation of medicinally important plant species. Furthermore, there is a wide scope for application of biotechnology for improvement of the medicinally important plants for which standardization of an efficient direct *in vitro* multiplication protocol is a crucial prerequisite (Suchitra Banerjee et al. 1999). With an increasing world-wide demand for plant derived medicines and formulations (Parnaham 1996), there has been a concomitant increase in the demand for raw material. Hence, there is a need to develop approaches for ensuring the availability of raw material of a consistent quality from regular and viable sources (Shrivastava and Rajani 1999).

1.2 The family Asclepiadaceae

The family, *Asclepiadaceae* comprises the 80th Order of the Natural System of Plants. The name, which was bestowed upon a genus of this Order, was given in honor of ‘Aesculapius’, or ‘Asklepios’ whose priests or fabled descendants were known as the ‘Asklepiads’ or ‘priest-physicians’ and who served the god of medicine in the ancient sanctuaries at Epidauros, Sikyon, Cos, Achaia and elsewhere. This family *Asclepiadaceae* consists of some 250 genera and over 2000 species: widespread in tropical and subtropical regions, especially in Africa and southern South America, with a moderate representation in northern and southeastern Asia.
1.2.1. Habit and general anatomy

This family consist of herbs, shrubs or rarely tree-like, with milky or, less often, clear latex. Leaves simple, opposite or occasionally whorled, very rarely alternate, usually without obvious stipules, margin nearly always entire. Inflorescences terminal, axillary, or extra-axillary, cymose, often condensed and umbel-like, occasionally a racemelike bostrychium. Flowers bisexual, 5-merous, actinomorphic. Sepals joined at base only, often with 5 or more basal glands in the sinuses. Corolla sympetalous, reflexed to urceolate or salverform; lobes valvate or overlapping in bud to right or left. Corona usually present, inserted on corolla, stamens, or both. Stamens 5, usually inserted at base of corolla tube and adhering to stigma head to form gynostegium; filaments usually connate to form a tube enclosing ovaries; anthers 4-celled (Periplocoideae and Secamonoideae) or 2-celled (Asclepiadoideae), often with a membranous apical appendage; pollen tetrads contained loosely on a spatulate translator with a basal corpusculum (Periplocoideae), or pollen united into waxy pollinia, each attached through a caudicle (stalk) to the retinaculum (gland) between adjacent anthers to form a pollinarium, pollinia 2 (Asclepiadoideae) or 4 (Secamonoideae) per pollinarium. Ovaries 2, free, superior; ovules numerous. Styles connate; stigma head fleshy. Fruit of 1 or 2 follicles. Seeds numerous, strongly compressed, with a coma (a prominent basal tuft of silky hairs). Chromosome number $x = (8–) 11$ (or 12).

1.2.2. Some of the important Medicinal plants of Asclepiadaceae:

All plant parts, especially the seeds and latex, are often poisonous. They contain various alkaloids and glycosides, many of which are used in medicine and as insecticides.

Most species of the family are endemic (e.g. *Calotropis gigantean*, *Hemidesmus indicus*, *Tylophora indica*, *Caralluma procumbens*, *Caralluma adscendens*, *Calotropis buchananii*) and wild growing (e.g. *Calotropis procera*, *S. argel*, *Leptadinea spp*) medicinal plants. Today, many medicinal
plants are facing threat of extinction and loss of genetic diversity (*Ceropegia intermedia*, *Ceropegia spiralis*, *Decalepis hamiltonii*, *Hemidesmus indicus*), due to their restricted distribution and indiscriminate exploitation for medicinal use by pharmaceutical industry. As a result, many are now listed as an endangered species by the international union for conservation of nature and natural resources. Conventional propagation of many species is hampered due to poor seed viability, low rate of germination and seasonal availability, so there is a need to conserve many members of this family.

*Bidaria khandalense* (Sant.) Jagtap and Singh is a gigantic climber reaching at the tops of tall trees in the dense forests of central and southern India. It is reported in the literature that *Bidaria khandalense* has same medicinal properties like that of *Gymnema sylvestre* in diabetes (Sumy et al. 2000).

*Calotropis gigantea* is a widely growing plant which is used traditionally as anti-inflammatory, analgesic, anti-pyretic, anti-oxidant, anti-convulsant and antidiarrhoeal agent (Himanshu Joshi et al. 2011).

*Calotropis procera* (Ait.) R.Br. wild growing tropical perennial shrub, widely distributed in Africa, South America, India and abundant in Bangladesh. It has been widely used as a traditional medicine for the treatment of many diseases like leprosy, ulcers, tumors, piles and diseases of the spleen, liver and abdomen. Leaves and roots of the plant have been used to alleviate pain under different conditions. Besides the decoction of the plant has been reported to be employed in painful muscular spasm, dysentery, fever, rheumatism, asthma and as an expectorant and purgative. Latex of the plant have been shown to confer significant anti-inflammatory activity against carageenin and formalin induced paw oedema and antipyretic effect and it is a also a rich source of several biologically active compounds including glucosides, tannins, flavonoids and many proteins (Amitav Das et al. 2011).
Caralluma Several members of the genus Caralluma have found medicinal uses in the treatment of rheumatism, diabetes, and leprosy and as antiseptics and disinfectants. The plants of the genus Caralluma have reported to possess several pregnane glycosides or their esters with antitumor activity and recently showed anti-trypanosomal activity (Abdel-Sattar et al. 2009).

Ceropegia spiralis Wight. Vulnerable climber, its tuber is used in indigestion (Pattnaik et al. 2009). The tuberous roots of many Ceropegia species are edible and many others are of medicinal value. The root tubers contain starch, sugar, gum, albuminoids, fats and crude fiber and are valuable constituents in many traditional medicinal systems in India. Active principle of tuberous roots contains an alkaloid cero-pegine which is active against diarrhoea and dysentery (Nadkarni 1976). Ceropegoin hirsute Roxb. and Diplazium esculentum (Rets). Sw., the rhizome powder of both plants are used as tonic (Rai 1987). Ceropegia candelabrum L., paste of leaves is applied for headache (Ignacimuthu et al. 2006).

Decalepis hamiltonii is a glabrous extensively climbing shrub, is an endemic and endangered medicinal plant of southern peninsula. Recently, antioxidant, antidiabetic, hepatoprotective and antiatherosclerotic properties of root extract of Decalepis has been evaluated (Naveen and Khanum 2010).

Hemidesmus indicus, commonly called Indian sarsaparilla is a climbing vine found throughout India, has long been used as a folk medicine and found to be an ingredient in ayurvedic and unani preparations which are usually prescribed against inflammation, diarrhoea, respiratory disorders, skin diseases, syphilis, fever, bronchitis, asthma, eye diseases, urinary disorders, loss of appetite, burning sensation and rheumatism and especially for epileptic fits in children (Lakshman et al. 2006).
**Heterostemma deccanense** (Talb.) Swarup & Mangaly an endangered climber, its stem bark is used in stomachache.

**Holostemma ada-kodien** K. Schum., is an important medicinal plant widely distributed in the tropical rain forests in India. The terpenoid sugars present in the root tubers of the plant are responsible for the medicinal properties. The plant is used for maintaining youthful vigour, strength and vitality, the root tubers are used as tonic, ophthalmic, alterant, stimulant, aphrodisiac, expectorant and galactagogue (Meena et al. 2011).

**Leptadaenia reticulata** (Retz.) Wt. & Arn is an endangered climber found in forests and gardens near thorny trees. It is commonly known as Jivanti, Jivanti is considered a stimulant and tonic in Ayurvedic literature. This plant is also a rich source of biologically active cardiac and pregnane glycosides which are known to possess anti tumor and anti cancer activity (Sudipta et al. 2011).

**Oxystelma esculentum** R.Br, commonly known as 'Jaldudhi', is one such plant which has not been studied sufficiently. It has many potential therapeutic uses which are of vital importance in curing the diseases of the modern world like cancer, hepatitis, kidney disorders, stress-related disorders and microbial infections. It contains two very important classes of phytoconstituents: cardenolides and pregnane glycosides, which are easily obtained from this plant and can act as precursors of many therapeutically important compounds.

**Pergularia daemia** (Forsk.) Chiov. Leaf juice is used as expectorant and to treat infantile diarrhea and asthma. Fresh leaves are boiled with water and the vapour is inhaled during headache. Root bark is mixed with cow milk and used as purgative in rheumatism.
**Sarcostemma acidum (Roxb) Voigt.** a xerophytic plant has several medicinal properties where dried stem is an emetic employed in leprosy patients, roots in snake bites and rabies (Jain and Defilipps 1991).

**Solenostemma argel,** belongs to the *Asclepiadaceae* family used for the treatment of diabetes and jaundice, some liver and kidney diseases and some allergies. It is an effective remedy for bronchitis and is used to treat neuralgia and sciatica. Also, it is used as incense in the treatment of measles and sometimes crushed and used as remedy for supporting wounds. The leaves are infused to treat gastrointestinal cramps, stomach ache, colic, cold and urinary tract infections and are effective as anti-syphilitic if used for prolonged period of 40-80 days (Tigani and Ahmed 2009).

**Tylophora indica (Burm.) Merrill** is a threatened medicinal climber shrub native to the plain and hill forests of eastern and southern India. The plant is in great demand for the production of traditional and modern medicines. Leaf extracts show anti-asthmatic properties, the major alkaloid present—tylophorine—has been reported to have immunosuppressive, anti-inflammatory and antitumor properties (Rani and Rana 2010).

**Wattakaka volubilis Linn.** is a traditional medicinal plant used to treat various diseases in Indian traditional system of medicine. Particularly, the plant material used in folk medicine for diabetes, analgesic and inflammatory activity (Arun Kumar et al. 2010). It is used for treating rheumatic pain, cough, fever and severe cold. Leaf extract has been reported to possess pharmacological activity, including anti-inflammatory activity (Arulanandam et al. 2011).
1.3 Introduction to *Gymnema sylvestre*

*Gymnema sylvestre* is a large woody climber, rooting at nodes, leaves elliptical to acuminate, base acute to acuminate, glabrous above sparsely or densely tomentose beneath. Flowers small, in axillary and lateral umbel like cymes, pedicels long; Calyx-lobes long, ovate, obtuse, pubescent. Corolla pale yellow campanulate, valvate, corona single, with 5 fleshy scales. Scales adnate to throat of corolla tube between lobes. Anther connective produced into a membranous tip, 2 erect pollinia, 2 unilocular carpels, many ovuled locules, with 1 fusiform long follicle (Wikipedia, the free encyclopedia).

**Taxonomic position of Gymnema sylvestre R. Br.**

- **Domain**: Eukaryota
- **Kingdom**: Plantae
- **Subkingdom**: Viridaeplantae
- **Phylum**: Magnoliophyta
- **Subphylum**: Spermatophytina
- **Infraphylum**: Angiospermae
- **Class**: Magnoliopsida
- **Subclass**: Lamiidae
- **Superorder**: Gentiananae
- **Order**: Gentianales
- **Family**: Asclepiadaceae
- **Subfamily**: Asclepiadoideae
- **Tribe**: Marsdenieae
- **Genus**: Gymnema
- **Species**: *G. sylvestre*

**Synonyms** (The Wealth of India 1956):

**Vernacular names** *(The Wealth of India 1956)*

**Sanskrit:** Meshashringi, madhunashini; **Hindi:** Gur-mar, merasingi;

**Kannada:** Sannagerasehambu; **Telugu:** Podapatri;

**Tamil:** Adigam, cherukurinja; **Marathi:** Kavali, kalikardori, vakundi;

**Gujratli:** Dhuleti, mardashingi;

**Common names:** Gymnema, Miraele Fruit

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**Figure-1.1:** *Gymnema sylvestre* R.Br.
1.3.1. Distribution and habitat

*G. sylvester*, belong to family of *Asclepiadaceae*, is a perennial plant originated in India. *Gymnema* is a woody climbing plant that grows in the tropical forests of central and southern India. The woody *Gymnema* plant also grows in parts of Africa. *Gymnema* prefers tropical and sub-tropical type of climate, it is found growing even in dry areas also. The areas with high or medium well distributed rainfall are suitable for its growth (Wikipedia, the free encyclopedia).

Stems grows upto 8 m, sparsely lenticellate; young branchlets pubescent, glabrescent. Petiole 3-12 mm; leaf blade obovate to ovate, 3-8.5 x 1.5-5.5 cm, thick papery, adaxially pubescent to glabrous except for groove of midvein, abaxially tomentose to glabrous except for veins; lateral veins 4 or 5 pairs, convergent . Cymes much shorter than leaves, pubescent; peduncle 2-5 mm, rachis with close-spaced spiral of pedicel scars. Sepals ovate, ciliate. Corolla greenish white; lobes ovate, glabrous; appendages exserted. Stigma head exserted. Follicles mostly solitary or paired, broadly lanceolate in outline, 5-9 x ca. 2 cm, glabrous, beak acuminate. Seeds ovate, ca. 8 x 4 mm; coma silky white, ca. 3.5 cm. Fl. Apr-Nov, Fr. Sep-Dec. 2n = 22.

1.3.2. Chemical and phytochemical composition

The active principles of this plant suppress the sweet taste hence the name 'Gur-mar', meaning 'sugar destroying'. The total saponin fraction of the leaves, commonly known as Gymnemic acid belongings to a group of triterpene glycosides that constitutes the active principles of this plant (Maeda et al. 1989; Sahu et al. 1996), although GA is not one single compound but consist of several related compounds (Kiuchi et al. 1992; Kurihara et al. 1992), still we use the traditional singular form. A number of triterpene saponins along with oleanane-type saponins (gymnemic acids I-VII and gymnemasaponins I-V) (Ye et al. 2000; 2001) as well as dammarane-type saponins (gymnemasides I-VII) (Yoshikawa et al. 1992a) have been reported from this plant time-to-time
by different group of workers (Hooper 1889; Stöcklin 1967; Rao and Sinsheimer 1968; 1971; Sinsheimer et al. 1970; Sinsheimer and Rao 1970; Dateo et al. 1973; Chakravarti and Debnath 1981; Yoshikawa et al. 1989a; 1989b; 1991a; 1992a; 1992b; 1993a; Liu et al. 1992). Hooper (1887a; 1887b; 1889) was the first to isolate the active compound(s) from the leaves of *Gymnema sylvestre* reported the occurrence of gymnemic acid in the form of potassium salt and concluded that gymnemic acid is a glycoside, upon acid hydrolysis of complex mixture. Yackzan (1966) suggested that gymnemic acid could be a saponin. Stöcklin et al. (1967) reported D-glucuronide of a new hexahydroxy-Δ\(^{12}\) -oleannene triterpene named gymnemagenin(1) from gymnemic acid which was esterified with various combinations of formic, acetic, n-butyric, isovaleric and tiglic acids and proposed gymnemic acid was composed of 4 components, A\(_1\)-A\(_4\) (Kurihara 1969; Kurihara et al. 1969). Gymnemagenin structure was assigned as 3β,16β,21β,23,28-hexahydroxyolean-12-ene (Rao and Sinsheimer 1968; Stöcklin et al. 1969a). Gymnestrogenin(2) a new triterpene structure was proposed as 3β,16β,21β,23,28-pentahydroxyolean-12-ene (Stöcklin et al. 1968; Rao and Sinsheimer 1971). Sinsheimer et al. (1970) isolated gymnemic acids A-D and V, and established A and B were identical to gymnemic acids A\(_1\) and A\(_2\) while C and D were different from A\(_3\) and A\(_4\) as they contain both glucuronic acid and galactose in their structures. Gymnemic acids A-D revealed to contain genins G, K, N and gymnjestrogenin, as their aglycones. Genin G was gymnemagenin esterified with formic, acetic, isovaleric and tiglic acids, genin K was identical to G, except in the absence of acetic acid residue. Genin N was gymnjestrogenin tiglate. The glucuronic acid moiety in gymnemic acids A and D was not acylated, while those of C and D were esterified with ferulic acid.

Yoshikawa et al. (1989a) isolated gymnemic acids I (3), II (4), III (5), IV (6) all of which contains gymnemagenin and a glucuronic acid and are differentially acylated with acetyl, tigloyl or 2-methylbutyryl groups (Maeda et
al. 1989; Yoshikawa et al. 1989a) and they observed that the anti-sweet activity of these saponins decreases with decreasing number of acyl groups in their structures. They also reported gymnemic acids V (7), VI (8) and VII (Yoshikawa et al. 1989b), where VII possesses gymnestrosteogenin as its aglycone connected to β-glucuronic acid at its 3-hydroxy position and exhibits no anti-sweet activity. In next series of anti-sweet compounds, gymnemasaponins III (9), IV (10) and V (11) their structure consist of 23-hydroxylongispinogenin (12) as aglycone, glycosylated with either one or two glucose molecules at both the 23- and 28-hydroxy group and another point is none of these is acylated (Yoshikowa et al. 1991a). Liu et al. (1992) isolated gymnemic acids VIII (13) and IX (14), the structures of which were elucidated as 3'-O-β-D-arabino-2-hexulopyranosyl gymnemic acid III and IV, respectively. Other forms of gymnemic acids X (15), XI (16), XII (17), XIII (18), XIV (19), XV (20), XVI (21), XVII (22) and XVIII (23) were also isolated (Yoshikowa et al. 1992b; 1993a). Sahu et al. (1996) isolated four new triterpenoid saponins, gymnemasins A, B, C and D all of which contains a new aglycone, gymnemasol which was characterized as 3β,16β,22α,23,28-pentahydroxyolean-12-ene from Gymnema sylvestre leaves. Six new oleanane saponins were isolated from the leaves of Gymnema sylvestre (Ye et al. 2000; 2001). Besides the gymnemic acids, a new flavonol glycoside along with known four flavonoids were isolated for the first time from this genus (Liu et al. 2004). Besides this, other plant constituents are anthraquinones, hentriacontane, pentatriacontane, α and β-chlorophylls, phytin, resins, d-quercitol, tartaric acid, formic acid, butyric acid, lupeol, β-amyrin related glycosides and stigmasterol. The plant extract also tests positive for alkaloids. Leaves of this species yield acidic glycosides and anthroquinones and their derivatives (Dateo and Long 1973).
### Chapter 1: General Introduction

#### Gymnemagenin

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<tr>
<td>Gymnemic acid XII (17)</td>
<td>β-glcA-β-glc</td>
<td>tga</td>
<td>H</td>
<td>Ac</td>
<td>Yoshikawa et al. 1992b</td>
</tr>
<tr>
<td>Gymnemic acid XIII (18)</td>
<td>β-glcA</td>
<td>H</td>
<td>H</td>
<td>mba</td>
<td>Yoshikawa et al. 1992b</td>
</tr>
<tr>
<td>Gymnemic acid XIV (19)</td>
<td>β-glcA</td>
<td>H</td>
<td>H</td>
<td>tga</td>
<td>Yoshikawa et al. 1992b</td>
</tr>
</tbody>
</table>

**Figure 1.2:** Structures of anti-sweet compounds analogues (1-19) from *Gymnema sylvestre*
Gurmarin (a low molecular weight peptide) is another constituent of the leaves, and gymnemic acid (20) have been shown to block sweet taste in humans. This novel sweet suppressing 35-amino acid peptide (Imoto et al. 1991; Kamei et al. 1992) is quite intriguing, since it is very rare for a plant derived compound outside of the triterpenoid saponin group to be discovered as exhibiting a sweetness suppressing property.

1.3.3. Medicinal properties

Sushruta describes *Gymnema sylvestre*, as a destroyer of madhumeha (glycosuria) and other urinary disorders. On account of its property of abolishing the taste of sugar, it has been given the name of gur-mar meaning sugar destroying and it is believed therefore that it might neutralize the excess of sugar present in the body. The plant is also reported to be bitter, astringent, acrid, thermogenic, anti-inflammatory, anodyne, digestive, liver tonic emetic, diuretic, stomachic, stimulant, anthelmintics, laxative, cardiotonic, expectorant, antipyretic and uterine tonic. It is useful in dyspepsia, constipation, jaundice, haemorrhoids, renal and vesical calculi, cardiopathy, asthma, bronchitis, amenorrhoea, conjunctivitis and leucoderma. *Gymnema sylvestre* finds traditional use in a number of diseases and its various parts are used for various purposes.

**Plant parts used** - Root, Leaf and whole plant

**Roots** Expectorant and emetic. Used in stomach pain. Reputed as a remedy for snake bite.

**Leaves**

Extract of leaves is stimulant, diuretic, cardiovascular and hypoglycemic and has purgative action. Used in diabetes, chewed to reduce glycosuria. Used as an antidiabetic. Gymnemic acids are useful for prevention of the formation of dental plaque and caries. Leaf powder Stimulates heart and the circulatory system, increases secretion of urine and activates uterus.

**Plant as a whole**

Used in the medicines which cures cough, dyspepsia ulcers, jaundice etc.
1.4 Review of Literature

Leaves of *Gymnema sylvestre* have been used in India for the treatment of diabetes for over 2000 years (Nadkarni 1992). Several investigators reported antidiabetic properties of *Gymnema sylvestre* R. Br., leaf extract, not only in rats (Choudhury 1988; Shanmugasundaram et al. 1990a; Okabayashi et al. 1990; Chattopadhyaya 1999) but also in humans (Shanmugasundaram et al. 1990b; Hirata et al. 1992). Many studies have shown that oral administration of *Gymnema* extract reduces serum glucose level and improves glucose tolerance in mildly diabetic rats (Shanmugasundaram et al. 1981). Shanmugasundaram et al. (1983) reported that *G. sylvestre* restored glycogen and enzymes in diabetic rabbit liver after 24 weeks of treatment. Srivastava et al. (1986) reported that pancreas tissue was completely destroyed in alloxan treatment, after *G. sylvestre* leaf extract regenerated the prolonged survival and adaptogenic activity. Shanmugasundaram et al. (1990) reported aqueous extract of *G. sylvestre* leaf (200 mg/kg body wt.) normalized blood sugar level in STZ-induced diabetic rats. Restoration of hepatic glycogen by *G. sylvestre* leaf and callus could improve the insulin secretion or inhibition of glucose-6-phosphatase in liver, and has prevented the conversion of glucose 6-phosphate to glucose (Shanmugasundaram et al. 1990). Administration of water extract of *G. sylvestre* leaves was found to increase serum insulin level, suggesting its insulin releasing effect (Baskaran et al. 1990). The water soluble part of an alcoholic extract of *Gymnema* leaves was found to have hypoglycemic and antihyperglycemic effect in normal, glucose-fed hyperglycemic and streptozotocin-induced diabetic rats (Chattopadhyay et al. 1993). Chattopadhyay (1998) reported that *G. sylvestre* leaf extract acts by potentiation of extra insulin secretion there by acting as a antihyperglycemic. However, gymnemic acid from *G. sylvestre* leaf regulated hyperglycemia (Gholap and Kar 2005) and an aqueous extract maintained the blood glucose level in normal Wistar rats (Rafiullah et al. 2006). Leaf and callus extracts of *G. sylvestre* reduced blood sugar and lipid profiles such as cholesterol, triglyceride. HDL, LDL, VLDL in alloxan-induced diabetic Wistar rats.
(Ahmed et al. 2008). Renu et al. (2009) observed no adverse effect on the health status of the subjects and it can thus be concluded that gur-mar powder is effective in lowering the fasting as well as post-prandial blood glucose levels. Srividya et al. (2010) evaluated the in vitro and in vivo hepatoprotective activity of *G. sylvestre* and reported that the cells treated with the hydro-alcoholic extract of *G. sylvestre* showed a significant restoration of the altered biochemical parameters towards the normal.

The possible mechanisms by which *Gymnema sylvestre* exerts its antidiabetic effects includes: recovery of β-cells (Baskaran et al. 1990; Shanmugasundaram et al. 1990a,b), inhibition of glucose absorption (Hirata et al. 1992; Shimizu et al. 1997), stimulation of insulin release (Persaud et. al. 1999; Sugihara et al. 2000) and increased glucose tolerance (Kar et al. 1999). Furthermore, the lipid lowering properties (Ahmed et al. 2008; Rachh et al. 2010; Shigematsu et al. 2001a; Wang et al. 1998) glucose homeostasis (Rafiullah et al. 2006), antihyperglycemic effect (Gholap and Kar 2005) have been described. It is also used in the treatment of asthma, cough, eye complaints, inflammations, family planning and snakebite (Selvanayagam et al. 1995; Uniyal 1993). In addition, it possesses antimicrobial, diuretic, stomachic, antihypercholesteremic, hepatoprotective and anti-saccharine activities (Kurihara 1969; Liu et al. 1992; Masayuki et al. 1997; Nadkarni and Nadkarni, 1976; Rana and Avadhoot 1992).

*G. sylvestre* leaf contains more than 20 saponin glycosides. The major saponin fraction comprises of gymnemic acid (the anti-sweet principle) which is a complex mixture of at least nine closely related acidic glycosides (Yoshikawa et al. 1991a). Thus it is difficult to separate gymnemic acid in pure form. Rao and Sinsheimer (1971) reported a process for isolation of gymnemagenin involving initial acid hydrolysis followed by alkaline hydrolysis. A quantitative analysis of gymnemic acids by HPLC analysis of gymnemagenin, the common aglycone obtained on hydrolysis has been
reported by Toshihiro et al. (1994). Puratchimani and Jha (2004) reported a HPTLC method for analysis of gymnemagenin. Valivarthi et al. (2006) reported an improved comparative method for HPTLC analysis of Gymnemagenin at UV range of electromagnetic radiation. These authors adopted a basic hydrolysis (1 h) followed by acid hydrolysis (1 h) and passed the hydrolysate through polyamide C-200. A reproducible and reliable HPTLC method for the indirect determination of gymnemic acids as gymnemagenin in Gymnema sylvestre plant has been reported by Priti and Pundarikakshshudu (2008).

1.4.1. \textit{In vitro} propagation in \textit{Gymnema Sylvestre} 

\textit{Gymnema sylvestre}, in spite of its wide distribution the plant is considered as an endangered plant due to its overexploitation for its active principles (Choudhury 1988). Conventional propagation is hampered due to its poor seed viability and poor rooting ability of vegetative cuttings. Alternative strategies like tissue culture techniques have been undertaken by various workers. With regard to \textit{in vitro} propagation studies in \textit{Gymnema sylvestre}, Reddy et al. (1998) published first report on \textit{in vitro} propagation of \textit{Gymnema sylvestre} from mature nodal explants, using MS media containing BAP (5 mg l^{-1}) and NAA (0.2 mg l^{-1}) for shoot regeneration and half-strength MS basal medium for root induction. Komalavalli and Rao (2000) used seedling explants for \textit{in vitro} micropropagation of \textit{Gymnema sylvestre}, they obtained multiple shoots from 30 days old seedling axillary node explants on MS medium containing BAP(1 mg l^{-1}), Kinetin (0.5 mg l^{-1}), NAA (0.1 mg l^{-1}), malt extract(100 mg l^{-1}) and citric acid(100 mg l^{-1}) and high frequency of rooting on half strength MS medium supplemented with IBA (3 mg l^{-1}). The plantlets developed were hardened and successfully established on natural soil. Later in 2002, Ashok Kumar et al. reported the regeneration of \textit{G. sylvestre} through somatic embryogenesis from seedling explants, embryogenic callus was induced on MS medium with 2.0 \mu M 2, 4-D and 1.0 \mu M BAP and subsequently they were matured on medium with MS salts and B5 vitamins containing 0.5 \mu M BAP.
and 2% sucrose and regenerated plantlets were subsequently acclimatized in greenhouse condition. Subathra Devi and Srinivasan (2008) reported the need of MS salts for shoot sprouting and proliferation, they obtained best results using 1 mg l\(^{-1}\) BA+0.5 mg l\(^{-1}\) IAA + 100 mg l\(^{-1}\) Vitamin B2+100 mg l\(^{-1}\) citric acid for shoot proliferation and 1/2 strength MS medium with IBA 3 mg l\(^{-1}\) root induction.

Roy et al. (2008) studied the effect of different plant hormones on callus induction in *Gymnema sylvestre* and highest efficiency of callus formation was observed in the medium containing different concentration of 2,4-D and kinetin. Ali et al. (2009) reported somatic embryogenesis form *ex vitro* leaf explants via embryogenic suspension cultures. Callus was induced on MS medium supplemented with growth regulators (2,4 -D 0.5 mgl\(^{-1}\) (or) NAA 1.0 mgl\(^{-1}\)) and 10% coconut water. Somatic embryogenesis was induced on MS liquid medium containing NAA 1.0 mgl\(^{-1}\), BA 1.0 mgl\(^{-1}\), 3.0% sucrose (w/v), 10% coconut water, citric acid 1 mgl\(^{-1}\) and glutamine 10 mgl\(^{-1}\). Embryo maturation occurred on semi-solid medium containing MS basal medium with B vitamins, 3.0% sucrose and 0.8% agar (w/v). Sharma and Bansal (2010) reported *in vitro* propagation through apical bud culture. Highest shoot frequency was obtained in MS medium fortified with BAP (4.44 µM) and KN (4.64 µM) with 3% sucrose. Shoots obtained were rooted using half-strength MS medium with IAA, 85% of rooted shoots were successfully acclimatized in the field.

Till-date very few attempts were reported pertaining to the production of gymnemic acids in callus or cell suspension cultures. Gopi and Vatsala (2006) reported the production of gymnemic acid in callus and suspension culture. Subhatra Devi et al. (2006) attempted the gymnemic acid production using cell suspension culture by altering the combination of factors like external phytohormones, shaking speeds and medium pH. Ali et al. (2009) induced gymnemic acid production under abiotic stress through callus culture. They
obtained 4.4-fold increase in gymnemic acid content under blue light in callus culture. Kumar et al. (2010) studied in vitro salt stress induced production of gymnemic acid in callus cultures. Subathra Devi and Srinivasan (2011) reported the establishment of mass production of gymnemic acids through the shake flask and bioreactor culture with proper combinations of IAA and 6-BAP and fungal elicitor. Praveen et al. (2011) increased the biomass and gymnemic acid production by altering macro elements concentration and nitrogen source supply in cell suspension cultures of Gymnema sylvestre R. Br.

1.5 Lacuna or Gaps in the literature

Although there are attempts reported in the perusal literature about in vitro plant regeneration, somatic embryogenesis, gymnemic acid production in callus/suspension cultures and influence of certain factors of medium and blue light, till-date no published reports are available on elicitor enhanced production of secondary metabolites and genetic transformation of Gymnema sylvestre R.Br. In spite of its immense medicinal value and pharmaceutical importance of Gymnema sylvestre R. Br.