Family Asclepiadaceae: A reservoir of medicinal plants with special importance on *Gymnema sylvestre* R.Br. -An Overview.

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**Abstract**

The present study documents the traditional knowledge and micropropagation strategies of medicinal plants belonging to family Asclepiadaceae with particular emphasis on a well-studied medicinal plant *Gymnema sylvestre* R.Br. The family Asclepiadaceae comprises about 320 genera and 2700 species. Due to the elevated market demand of the medicinal plants belonging to family Asclepiadaceae, most of the species are endangered, threatened, rare or critically endangered. The species of the family are also exploited locally due to their high medicinal value and other ethnobotanical properties. There are other constrains as well which impede their abundance in nature including low seed germination and poor vegetative propagation. In this direction various in vitro micropropagation protocols have been established from time to time on a range of species of the family Asclepiadaceae. Present review thus emphasizes on medicinal importance and in vitro micropropagation studies on various plants of family Asclepiadaceae with special reference to *Gymnema sylvestre*. *Gymnema sylvestre* (Hindi: Gurmar) is a medicinal plant of immense pharmaceutical value, but it is disappearing fast from its natural habitat. The species is threatened with extinction due to its indiscriminate collection as raw material for pharmaceutical industry, where it is used for manufacturing of drugs for diabetes, asthma, eye complaints, etc. So, present study was designed to provide an overview that can serve as a data base and prospective guide on medicinal importance and micropropagation studies on family Asclepiadaceae in general and *Gymnema sylvestre* in particular.

**Keywords:** Asclepiadaceae, Medicinal plants, Conservation, Micropropagation, Herbal market, *Gymnema sylvestre*.

**Introduction**

Every civilization has a history of using medicinal plants and their products since time immemorial. In ancient times, Egyptian, Indian, Chinese, Africans and others used a variety of plant products for curing most of the ailments [1]. Moreover, modern pharmacopoeia still contains at least 25% of drugs derived directly from plants, and many others, which are synthetic analogues, built on the prototype compounds isolated from plants. The WHO estimates have reported that 80% of the world’s populations rely on plant based traditional medicine for their primary health care [2].

It is well known that plants possess an unlimited source of phytochemicals, which exhibit remarkable bioactive compounds used for medicines to cure various ailments like cancer, AIDS, hepatitis, diabetes, rheumatoid, arthritis and malaria [3]. World is endowed with a rich wealth of medicinal plants and herbs have always been the principal form of medicine in India [4].
Medicinal plants are gaining popularity throughout the world and among the developed nations, as everyone strives to stay healthy on the face of chronic stress and pollution and to treat illness with medicines that work in concert with the body’s own defense system. Nevertheless, with increasing realization of the health hazards and toxicity associated with the indiscriminate use of synthetic drugs and antibiotics, the interest in the use of plant-based drugs has been globally revived [5]. People in Europe, North America, and Australia are consulting trained herbal professionals of developing countries and using herbal medicines [6]. Folk medicines are also gaining popularity nowadays, and significantly contribute to Ayurvedic, Unani, and traditional Chinese medicines to a great extent.

India has 2.4% of the world’s area with 8% of global biodiversity. It is one of the 12 mega diversity centers having over 45,000 plant species. Its diversity is unmatched due to the presence of 16 different agro-climatic zones, 10 vegetative zones, and 15 biotic provinces [7]. The country accounts for 17,500 species of higher plants. Of these, more than 2,000 species are documented for medicinal value vis-à-vis 1,100 species are used in different systems of medicines. Out of these, 600-700 species are locally used in the country and 150 species with commercial usage [8]. Macro-analysis of the distribution of medicinal plants shows that they are distributed across diverse habitats and landscape elements. Around 70% of India’s medicinal plants are found in tropical areas mostly in various forest types spread across the western and Eastern Ghats, the Vindhyas, Chotta Nagpur plateau, Aravalis, and Himalayas and 30% of the medicinal plants, in the temperate and alpine areas and higher altitude [9].

Furthermore, from last century, there has been a rapid extension of the allopathic system of medical treatment which has led to the generation of commercial demand in national and international markets for pharmacopoeia drugs and their products of plant origin. In some developing countries, plant-based traditional medicines are main source for primary health care Fig. 1 [10].

International trade in medicinal plants and phytopharmaceutical preparations is a major force in the world economy and demand is increasing in both developing and industrialized nations. A report prepared by the Export-Import Bank of India has estimated that the international market of medicinal plant-related products is in the range of US$ 60 billion with an annual growth at the rate of 7%. The current global market is pegged at US$ 62 billion [11]. A dramatic increase in exports of medicinal plants attests to India’s interest in these products.

According to the Export-Import Bank of India, India’s share in the global herbal market in the year 2001 was of the order of US$ 1 billion. India is the second largest exporter, next to China. USA is the principal market for Indian medicinal plants, accounting for about 50% of exports. The value of traded medicinal plants in the domestic market was about Rs. 348 crore, in the year 2001. The value of domestic trade, along with the export level of Rs. 463 crore, made the commercialization of Indian medicinal plants to the tune of Rs. 847 crore in the year 2001-02 (www.vasundharaorissa.org). Thus, the export of medicinal from India appears to be growing very fast [12]. Consequently, the National Medicinal Plant Board, under the Union Government of India Ministry of Health and Welfare has decided to set up Export Promotion zone exclusively for medicinal plants [13].

Figure 1: Percent use of traditional medicine for primary health care in some developing countries.

Global trends of herbal market

Herbal drugs are not prescribed in the western countries, unless they are adequately standardized in the allopathic way. On the other hand, the scope of herbal drug marketing is better in the developing as well as underdeveloped countries, where the norms are not so strict and also the modern system is yet to completely dominate over the traditional systems. Whenever the global trend of the herbal market is discussed, although in the first place it seems to be concerned about the quantitative increase or decrease in the demand and supply status of herbal materials; the actual emphasis is on the value of trading. It is in this latter context that the role of western countries becomes important as the value of trading becomes significantly higher with respect to the marketing in these countries.
During the past decades, the western people have increased their interest in herbal medicines for a safe and natural health care. At the same time, the western scientists have intensified their research on the medicinal properties of the plants of developing and third world countries so as to develop more efficient and secure drugs for diseases of larger apprehension. In fact, more than 60% of the new anti-cancer drugs approved since 1983 were derived from plants (www.vasundharaorissa.org). Hence, the countries in Asia, Africa and Latin America see a great scope in earning valuable foreign exchange through export of their plant wealth to the western countries. The global exports of medicinal plants were US$ 759 million in the year 2001. China stood as the world’s No.1 exporter of medicinal herbs with an export value of US$200 million in the same year 2001 (www.vasundharaorissa.org).

In terms of the value of export-import, Hong Kong (17%) plus mainland China (4%) had the largest share (21%) in the import market followed by USA (14%) and Japan (10%) in 2001 (www.vasundharaorissa.org). Leading markets of herbal products in Europe are Germany followed by France, UK and Italy. Germany has the largest herbal extraction industry in Europe and USA, the major market for essential oils and herbal tea (www.vasundharaorissa.org). While 80% of the world population still uses traditional medicines, the interest in alternative medicines in the developed countries has increased by 60% since 1989. In USA, consumer use of herbal products was less than 5% in 1991 which had increased to 50% in 2004.WHO estimated the world market for herbal products to be the worth of US$ 62 billion and would hit US$ 5 trillion by 2050. The market is growing @ 7% per annum [13]. The pharmaceutical industry is growing @ 12% per annum (US$ 108 billion in 2001) while the personal care (cosmetics) industry at more than 6% (US$108 billion in 2001). The flavors’ and fragrance industry was valued at US$18 billion in 2001 as against US$7 billion during the same year 2001 (www.vasundharaorissa.org).

**The Indian scenario**

In India, traditional systems of medicine have recognized and used over 7500 species of medicinal plants out of which Ayurveda uses about 1769 species. The uses of approximately 289 medicinal plant species were documented by 1500 BC to 500AD and the number rose to approximately 650 by 1900 AD (www.vasundharaorissa.org)

Medicinal raw materials are exported from India under two categories: specified and unspecified. In 1987-88, the export was around 20 lakh kg, which declined steeply in 1988-89, but subsequently picked up to the initial level by 1989-90. Since then the export of medicinal herbs continuously gained and made a sharp rise from 1995-96 to 1998-99, which remained static till 2000-01. By 2003-04, the export sharply declined and reached below to 1987-88. Figure 2 shows the trend in the export of Ayurvedic and Unani herbs under the unspecified category.

Thus, the record indicates dwindling export of medicinal herbs from India possibly due to non-systematic collection and processing of the produce by disorganized sector, involving tribals and market regulators. In this direction authors attempt to review the status, medicinal properties, conservation approach and judicious use of some medicinal plants belonging to family Asclepiadaceae. The current endeavor can act as a prospect for sustainable use of some medicinally important plants of this family in current future. The medicinal values of some of the important members of family Asclepiadaceae is given in tabulated form in Table 1.

The conservation of medicinal plants belonging to family Asclepiadaceae is very important due to their poor regenerative capacity through vegetative propagation and stumpy seed germination. The conservation approach through in vitro regeneration and multiplication is thus only viable option. Taking this under consideration, authors here described various media with different phytoharmonal combinations used for the in vitro regeneration of family Asclepiadaceae.
### Table 1: Important medicinal plants of family Ascalpiadaceae, their medicinal properties and part used.

<table>
<thead>
<tr>
<th>S No.</th>
<th>Name of Medicinal Plant</th>
<th>Part Used</th>
<th>Medicinal Uses</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Asclepias curassavice</em> L.</td>
<td>Whole plant</td>
<td>Tumor, asthma, fever, osteosclerosis, inflammation, diarrhoea, cathartic, emetic, expectorant, warts, and acesodyne.</td>
<td>[14]</td>
</tr>
<tr>
<td>2</td>
<td><em>Ceropegia jainii</em>, <em>C. Bulbosa var</em></td>
<td>Leaves and tubers</td>
<td>Digestive tonic and cure urinary disorders</td>
<td>[15]</td>
</tr>
<tr>
<td>3</td>
<td><em>Ceropegia candelabrium</em> L.</td>
<td>Root tubers</td>
<td>Diarrhoea, and dysentery</td>
<td>[16]</td>
</tr>
<tr>
<td>4</td>
<td><em>Ceropegine</em> L.</td>
<td>Roots</td>
<td>Diarrhoea and dysentery</td>
<td>[17]</td>
</tr>
<tr>
<td>5</td>
<td><em>Cynanchum callialatum</em> L.</td>
<td>Areal parts</td>
<td>Wounds, headaches, infections and other skin related problems</td>
<td>[18]</td>
</tr>
<tr>
<td>6</td>
<td><em>Cryptolepis buchanani</em> Roem. &amp; Schult.</td>
<td>Leaves and roots</td>
<td>Diarrhoeal, ulcerative, anti-inflammatory, blood, anti-cough, antibacterial</td>
<td>[19]</td>
</tr>
<tr>
<td>7</td>
<td><em>Decalepis hamiltonii</em> W and A.</td>
<td>Roots</td>
<td>Stomach disorders, gastric ulcers, appetite</td>
<td>[20]</td>
</tr>
<tr>
<td>8</td>
<td><em>Decalepis arayalpathra</em> Joseph and Chandras Venter.</td>
<td>Roots</td>
<td>Peptic ulcer, cancer-like afflictions and as a rejuvenating tonic</td>
<td>[21]</td>
</tr>
<tr>
<td>9</td>
<td><em>Gymnema elegans</em> W. &amp; A.</td>
<td>Leaves</td>
<td>Eye complaints</td>
<td>[22]</td>
</tr>
<tr>
<td>10</td>
<td><em>Aristolochia indica</em> L.</td>
<td>Root bark</td>
<td>Snakebite</td>
<td>[23]</td>
</tr>
<tr>
<td>12</td>
<td><em>Ceropegia candelabrum</em> L.</td>
<td>Leaves</td>
<td>Headache</td>
<td>[25]</td>
</tr>
<tr>
<td>13</td>
<td><em>Pergularia daemia</em> (Fors.) Chiov</td>
<td>Leaves</td>
<td>Fevers, rheumatism, liver disorders, emetic and expectorant</td>
<td>[26]</td>
</tr>
<tr>
<td>14</td>
<td><em>Tylophora indica</em> (Burm. f.) Merr</td>
<td>Leaf and Root</td>
<td>Poison bites</td>
<td>[24]</td>
</tr>
<tr>
<td>15</td>
<td><em>Sarcostemma acidum</em> (Roxb.) Voigt</td>
<td>Whole plant</td>
<td>Burning micturition:</td>
<td>[24]</td>
</tr>
<tr>
<td>16</td>
<td><em>Wattakaka volubilis</em> (L.f.) Stapf</td>
<td>Leaves and roots</td>
<td>Paralysis</td>
<td>[24]</td>
</tr>
<tr>
<td>17</td>
<td><em>Oxystelma secamone</em> (L) Karst-A</td>
<td>Leaves and Root</td>
<td>ulcerations of the mouth and in sore throat, jaundice</td>
<td>[27]</td>
</tr>
<tr>
<td>18</td>
<td><em>Secamone afzelii,</em></td>
<td>Leaves</td>
<td>Antibacterial activities</td>
<td>[28]</td>
</tr>
<tr>
<td>19</td>
<td><em>Leptadenia hastata</em></td>
<td>Leaves</td>
<td>Antibacterial activities</td>
<td>[28]</td>
</tr>
<tr>
<td>20</td>
<td><em>Calotropis gigantea</em> (Linn) R.Br.</td>
<td>Latex</td>
<td>Wound Healing</td>
<td>[29]</td>
</tr>
<tr>
<td>21</td>
<td><em>Cosmostigma racemosa</em> Wight</td>
<td>Stem bark</td>
<td>Fever</td>
<td>[30]</td>
</tr>
<tr>
<td>22</td>
<td><em>Holostemma ada-kodien</em> Schultes</td>
<td>Leaf</td>
<td>Antimicrobial potency</td>
<td>[31]</td>
</tr>
<tr>
<td>23</td>
<td><em>Leptadenia reticulata</em> Wight &amp;</td>
<td>Latex</td>
<td>Skin diseases and wounds</td>
<td>[32]</td>
</tr>
</tbody>
</table>
Studies of micropropagation in Asclepiadaceae family

The family Asclepiadaceae comprises about 320 genera and 2700 species [40]. The representative genera of the Asclepiadaceae are Asclepias, Boucerosia, Calotropis, Caralluma, Ceropegia, Cosmostigma, Cryptolepis, Cryptostegia, Cynanchum, Demia, Dregea, Glossonema, Gymnema, Hemidesmus, Holostemma, Leptadenia, Marsdenia, Pentaropis, Pergularia, Periploca, Sarcostemma, Secamone, and Tylophora. The micropropagation of members of Asclepiadaceae has been reviewed below:

Asclepias curassavica

Pramanik and Datta (1986) [41] investigated clonal propagation of medicinal milkweed (Asclepiascurassavica L) by culturing excised node on Murashige and Skoog (MS) medium supplemented with different hormone combinations. Both Benzoaminopurine (BA) and kinetin (Kn) were found equally effective for shoot initiation. Indole Acetic acid (IAA) and Naphthalene acetic acid (NAA) were found suitable for root induction. Combinations of Kn and NAA resulted in the induction of both roots and shoots after 30 days of culture. Chromosomal variation was observed in the roots of in vitro regenerated plants. Regenerants with higher chromosome number (33; 2n = 22) obtained on MS medium in response to 9.2 µM Kn + 10.7 µM NAA showed vigorous and higher propagation rates than the aneuploid plants possessing less than the diploid chromosome number (2n – 2 = 20, 2n – 4 = 18). Such variations are more likely due to genetic fitness of deficiency among different aneuploids grown on particular nutrient medium.

Ceropegia spp

Vishwanath (1998) [42] developed in vitro techniques for conserving wild and endemic species of Ceropegia (C. jainii, C. Bulbosa var. bulbosa and C. bulbos). Nodal explants of all species were cultured on 0.5x MS medium with 8.8 µM BA to regenerate the axillary buds. These buds produced multiple shoots when transferred to multiplication medium consisting of 0.5x MS medium with 2.2 µM BA, or micro-tubers when transferred to 0.5x MS medium with 22.2 µM BA and 23.2 µM Kn. In vitro flowering occurred in Ceropegia jainii on spermine at 0.25 µM as an additive and not in C. bulbosa. Rooting of the shoots was possible both by in vitro and ex vitro means.

Botta et al. (2003) [43] developed a rapid micropropagation system for Ceropegia bulbosa. First five nodes of 1.0 cm each, harvested from young healthy shoots from garden raised plants, were cultured on B5 medium supplemented with different concentrations of BA and Adenine sulphate (Ads) each in combination with 0.268 µM NAA. Multiple shoot formation of up to 12 shoots was observed within one week in the presence of 13.32 µM BA and 0.268 µM NAA. Shoots were multiplied by subculture on the same medium. Shoots of 3-4 cm length were rooted in the medium supplemented with 9.84 µM IBA. The rooted plantlets were hardened and successfully established in pots with 70% success rate. In vitro flowering was observed at 2.22 µM BA + 2.89 µM GA3. Shoots transferred to the medium containing 0.232 µM Kn + 9.84 µM IBA showed formation of micro-tubers in 28 days, while the shoots cultured in the presence of 2.89 µM GA3 + 2.22 µM BA showed 76% flowering leading to seed production with 65% germinability.
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Beena et al. (2003) [44] proposed protocol for in vitro propagation of Ceropogia candelabrum L. through axillary bud multiplication. MS medium with 8.87 µM BA and 2.46 µM IBA was best suited for axillary bud proliferation, inducing a mean of eight shoots per node. Excision and culture of the nodal segments from in vitro shoots on fresh medium with the same concentrations of BA and IBA facilitated 10 shoots node. Subsequent cultures enhanced the shoot proliferation rate. Shoots developed were rooted on half strength MS medium with 0.49 µM IBA. Starting from a single node explants, 250 rooted explants, i.e. plantlets were obtained within 120 days. Plantlets established in the pots exhibited 90% survival. Plantlets successfully established in the field exhibited morphological characters identical to mother plants.

Cynanchum callialatum

Ramulu et al. (2002) [45] obtained a rapid in vitro clonal propagation of Cynanchum callialatum Buch.-Ham., using shoots from aseptic seedlings. Defoliated nodal segments of 60 days old aseptic seedlings were cultured on MS medium supplemented with different concentrations of BA/Kn alone or in combination with various auxins and growth adjuvants. The highest number of shoots (3.05 ± 0.94) was obtained on the medium fortified with 4.44 µM BA + 2.32 µM Kn + 1.44µM GA₃ + 284 µM ascorbic acid + 10% coconut milk. The regenerated shoots were rooted the best on half strength MS medium containing 5.71 µM IAA+ 0.93 µM Kn. Plantlets with well-developed roots were successfully transferred to soil and 85% of the transferred plants survived.

Cryptolepis buchanani

Prasad et al. (2004) [46] standardized in vitro regeneration protocol for multiplication of Cryptolepis buchanani by using shoot tip, cotyledonary node and nodal explants derived from seedlings grown in vitro. The best response was achieved with nodal explants. Cultures were established placing the nodal explants on MS medium supplemented with various cytokinins singly or in combination with auxin and gibberellin. Of the various cytokinins used singly or in combination with auxins, BA was found to be the most effective for shoot proliferation. The maximum number of shoots (12.5-13.0 shoots per explant with shoot length of 4.5-5.0 cm) was produced on MS medium fortified with 8.88 µMBA, 0.465 µM Kn, 0.268 µM NAA and 0.144 µM GA₃ with 60% response. Individual shoots (grown on shoot proliferation medium) were rooted on MS medium supplemented with various auxins IAA, IBA, NAA singly or in combination. Of these, IBA at 4.92 µM resulted in 80% rooting and about 6.5-7.0 roots per shoot of length 4.0-4.5cm. The in vitro raised plantlets were acclimatized successfully to pots containing a mixture of autoclaved peatmoss and compost in 1:1 ratio.

Decalepis hamiltonii

Reddy et al. (2002) [47] investigated the effect of triacontanol (TRIA) on shoot multiplication and rooting of in vitro derived shoot tips of Decalepis hamiltonii. Triacontanol (TRIA) was administered at 0.004-0.0456 µM. TRIA resulted in the highest promotion of axillary shoot proliferation at 0.004 µM and maximum number of roots at 0.01 and 0.022 µM for Decalepis hamiltonii. TRIA enhanced shoot growth and chlorophyll content of leaves and also influenced root induction and supported growth of roots. The work revealed the effectiveness of TRIA for micropropagation of Decalepis hamiltonii.

Giridhar et al. (2003) [48] developed a highly efficient two-stage protocol for induction of multiple shoots from in vitro shoot tip explants of Decalepis hamiltonii. It was found that phenyl acetic acid (PAA) had a synergistic effect on shoot multiplication when treated with BA. PAA was effective for multiple shoot induction from nodal explants, elongation of primary shoots, and initiation of adventitious shoot formation from primary shoot. MS medium containing 2.22-31.08 µM BA and 0.27-10.74 µM NAA or 7.34-36.71 µM PAA proved to be the best combination. The maximum number of shoots per culture was produced on a medium containing 31.08 µM BA and 14.68 µM PAA, while the longest shoot length and nodes were obtained on medium containing 22.2 µM BA and 14.68 µM PAA. Shoots sub-cultured on MS medium containing 22.2 µM BA and 14.68 µM PAA elongated along with secondary shoot formation. The shoots were rooted on MS medium containing 9.8 µM IBA. The plantlets were acclimatized in soil with an 80-90% survival rate under field.

Giridhar et al. (2003) [48] reported an efficient protocol for successful micropropagation system for Decalepis hamiltonii (‘swallow root’), through shoot multiplication. The influence of 2.5-7.5 µM 2-iP, 4.4-17.7 µM BAP, 2.3-4.7 µM Kn, 2.8-6.8 µM (Thidiazuron) TDZ and 2.3-11.4 µM zeatin alone and in combination with 0.3-0.9 µM IAA on in vitro multiple shoot production was studied by using semi-solid MS medium. The maximum number of multiple shoots (6.5±0.4) was induced from shoot tips cultured on MS medium containing 4.9µM 2-iP. But both 9.1 µM zeatin and 4.7 µM Kn in combination with 0.6 µM IAA were able to produce a maximum of 5.0 ± 0.4 and 5.1 ± 0.4 multiple shoots, respectively. Further elongation of shoots and adventitious shoot formation was obtained on the medium containing 2.5 µM GA₃. Elongated shoots were
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separated and rooted on MS medium supplemented with 9.8 \( \mu M \) IBA and various phenolic compounds within 5-6 weeks. Phloroglucinol and salicylic acid interaction with IBA stimulated in vitro rooting of shoots. Successful field transfer was achieved of plantlets.

Sudha et al. (2005) [49] reported a protocol for successful micropropagation of Decalepis arayalpathra Joseph and Chandras Venter. The nodal explants of greenhouse raised plants were more responsive than cotyledonary nodal explants of aseptic seedlings. The basal nodes (73%) of 12-16-week-old greenhouse grown plants, cultured in MS medium containing 12.96 \( \mu M \) adenine BA, 2.48 \( \mu M \) NAA, 2-iP and 2.68 \( \mu M \) NAA, formed 16-17 cm long unbranched robust solitary shoots in eight weeks. Cotyledonary nodal explants cultured in the same medium showed multiple shoot formation and axillary branching. But the shoots were thin, fragile and not suitable for mass propagation. Single nodes of a solitary shoot sub-cultured on MS medium containing 2.22 \( \mu M \) BA and 0.24 \( \mu M \) 2-iP together produced 9.8 \( \pm 0.3 \) nodes on 18.0 \( \pm 0.6 \) cm long shoots within on 5-6 weeks. The basal nodes of the shoots so formed were repeatedly subcultured to increase the stock of propagules while the 2.5-3.0cm terminal cuttings were used for rooting. The best root induction (68%) and survival (86%) was achieved on half strength MS medium with 1.07 \( \mu M \) NAA. Field established plants showed uniform growth and phenotypic similarity to parental stock.

Gymnema ssp

Komalavalli and Rao (2000) [51] reported that the nature of the explant, seedling age, medium type, plant growth regulators, complex extracts (casein hydrolysate, coconut milk, malt extract and yeast extract) and antioxidants activated charcoal, ascorbic acid, citric acid and polyvinylpyrrolidon (polyvinylpyrrolidone) markedly influenced the in vitro propagation of Gymnema sylvestre. A maximum number (57.2) of shoots were induced from axillary node explants obtained from 30-day-old seedlings on MS medium containing 4.44 \( \mu M \) BA, 2.32 \( \mu M \) Kn, 0.537 \( \mu M \) NAA, 0.01 % malt extract and 476 \( \mu M \) citric acid. High frequency of rooting (50%) was obtained in shoots, derived from axillary node explant, on half strength MS medium supplemented with 14.76 \( \mu M \) IBA. The plantlets were hardened and established successfully in the field.

Devi and Srinivasan (2008) [52] studied the need of MS salts for shoot sprouting and proliferation and demonstrated the high salt requirement for the growth of Gymnema sylvestre. Influence of plant growth regulators, IAA, BA, 2, 4-D and Kn on shoot sprouting was investigated. Synergistic effect of vitamin B2 was observed. To overcome phenolic exudation and the effects of antioxidants, the effect of activated charcoal, citric acid and ascorbic acid were investigated. Incorporation of 476 \( \mu M \) citric acid to the medium prevented phenolic exudation and increased the production of healthy normal shoots and shoot bud differentiation in Gymnema sylvestre. MS medium containing 4.44 \( \mu M \) BA + 2.85 \( \mu M \) IAA + 266 \( \mu M \) vitamin B2 + 476 \( \mu M \) citric acid was the best for the shoot proliferation and half strength MS medium with 14.76 \( \mu M \) IBA for root induction, resulted in 53% rooting.

Shah et al. (2013) [53] reported the micropropagation of Gymnema sylvestre. Aseptic cultures were established on MS (Murashige and Skoog) medium supplemented 10.0\( \mu M \) BA (\( N^6 \)-Benzyladenine) and 0.5 \( \mu M \) NAA (\( \alpha \)-Naphthalene acetic acid). Five nutrient media [MS (Murashige and Skoog Medium), WPM (Woody Plant Medium), B5 (Gamborg Medium), SH (Schenk and Hildebrandt Medium) and NN (Nitsch and Nitsch Medium)], five cytokinin sources [Ads(Adenine hemisulphate), BA, Kn (Kinetin), 2-iP (\( N^6 \)-(2-isopentyl) adenine) and TDZ (Thidiazuron)], six doses and their all possible interactions in two successive experiments, NN medium and 5.0 \( \mu M \) BA significantly proved optimum for in vitro shoot multiplication and resulted in 1.82 shoot number per explant, 2.7 node number per shoot and 5.2 node number explant per one month after inoculation. The in vitro multiplied shoots were tested for in vitro root induction on different culture media (MS, WPM, B5, SH,
NN), explant types (apical bud with two nodal segments or with three nodal segments, two nodal segments, one nodal segment) and auxin (IAA, IBA, NAA)/ non-auxin (coumarin) treatment. Explants comprising apical bud with two nodal segments or three nodal segments without apical bud, inoculated on B₅ media supplemented with 5.0µM NAA, screened out to be significantly excellent for induction and growth of adventitious roots, resulting in 85% rooting and 4.27 root number per explant at 35 days after inoculation. The in vitro propagated plants exhibited excellent growth.

In our previous work a procedure for rapid in vitro shoot multiplication in Gymnema sylvestre was established. Addition of potassium nitrate and ammonium nitrate were found to be essential to induce maximum axillary bud proliferation on NN medium. Both potassium nitrate at the highest dose, i.e. 2.0x significantly enhanced shoot number proliferation on NN medium. Both potassium nitrate at the highest dose, i.e. 2.0x significantly enhanced shoot number explant¹ by 30% at 15 days and 33% at 30 days as well as node number per explant by 37% 15 days and 51% at 30 days in comparison to potassium nitrate at the lowest dose, i.e. 0.5x [54].

Authors have also reported the effect of different pH regimes (3.4, 4.0, 4.4, 5.0, 5.4, 6.0 and 6.4), NAA (0, 3.75 µM) and their all possible interactions on in vitro rooting (%) and root number per explant in Gymnema sylvestre at 21 and 28 days after inoculation. A uniform B₅ basal medium was provided. The experiment was laid out taking three replicates each of ten explants, i.e. microshoots with apical bud and two nodes. Various pH regimes and NAA at both stages of sampling and their interactions at 21 days after inoculation significantly influenced rooting (%) and root number per explant. The pH 4.4 enhanced rooting (%) by 168% and 100% at 21 days and 63% and 24% at 28 days in comparison to pH 3.4 and 4.0, respectively. Administration of 3.75µM NAA significantly promoted root number per explant both stages of sampling being 6.94 at 21 days and 5.28 at 28 days. Thus, administration of auxin at low pH facilitates induction and growth of in vitro rooting in Gymnema sylvestre [55].

*Aristolochia indica* Linn

In one of our previous studies, we have reported a protocol for successful micropropagation of *Aristolochia indica* a medicinal woody perennial climber plant of immense pharmaceutical value. Aseptic cultures were established using Murashige and Skoog (MS) with 5.0 µM BA (N⁶-Benzyladenine). Different culture media, MS, Woody Plant Medium (WPM), Gamborg Medium (B₅), Nitsch and Nitsch Medium (NN), and Schenk and Hildebrandt Medium (SH) and, three cytokinin at 10.0 µM concentration each of BA, TDZ (Thidiazuron), and Ads (Adenine hemisulphate). Ads at 10.0 µM proved optimum for in vitro shoot multiplication. The treatment resulted in 0.74 shoot number per explant at 15 days and 1.70 at 30 days in MS medium; 1.90 node number per shoot at 15 days and 3.17 at 30 days and 2.03, and 5.60 nodes per explant at 30 days in WPM medium. SH medium with 10.0 µM Ads registered the highest node number per explant (7.73) at 30 days after sampling. The in vitro multiplied shoots were used for root induction on five nutrient media (MS, WPM, B₅, NN and SH) and three auxin sources (IBA, IAA and NAA) at 10.0 µM each. SH medium along with10.0 µM NAA induced 45 % and 86 % roots and 0.44 and 1.01 roots per explant at 21 and 28 day after inoculation, respectively. The in vitro propagated hardened plants exhibited excellent growth on transfer to natural conditions [56].

**Hemidesmus indicus**

Patnaik and Debata (1996) [57] developed a procedure for rapid in vitro propagation of the aromatic medicinal plant, *Hemidesmus indicus* R.Br. from nodal explants. The highest shoot multiplication rate of 8.2 ± 0.4 shoots explant¹ with a 95% frequency was achieved in 5 week old culture on MS medium supplemented with 1.15 µM Kn and 0.054 µM NAA. Excised shoots were rooted on the same basal medium supplemented with 1.15 µM Kn and 7.35 µM IBA. The derived shoots exhibited better rooting response than those from primary cultures. After a hardening phase of two weeks, there was 70% transplantation success in the field.

Sreekumar et al. (2000) [58] observed caulogenic responses of various explant types from 12-month-old plants of *Hemidesmus indicus*. Second and third visible nodes (0.5 cm) from the apex on MS medium supplemented with 2.22 µM BA and 1.07 µM NAA and root segments (0.5 cm) on 4.44 µM BA and 2.69 µM NAA on half strength MS medium produced 9.37 and 2.6 shoots in 4 weeks, respectively. Caulogenic ability of the nodes decreased with increasing maturity. Shoot buds initiated upon the young nodes on day 10 developed into 7.2 cm long shoots within 4 weeks, thereby making a shoot elongation phase unnecessary. Nodal explants of the in vitro raised shoots sub-cultured in the same medium produced 9.32 shoots of 7.1 cm length in 3-4 weeks, similar to those of the mature plant-derived nodes. Sustained shoot multiplication through subculture of the nodes up to 25 passages of four weeks each was achieved without decline. Shoot cultures were rooted in quarter salt strength, i.e. 0.25x of MS medium containing 9.8 µM IBA. The rooted plants were hardened for establishment in pots with 96% success. Four months after establishment, the
micropropagated plants were stable and showed uniform morphological growth characteristics. After 12 months of cultivation in the field, micropropagated plants produced 4-5 shoots, 5-8 branches per shoot with increased root biomass (12.5g) compared to the poor growth (single shoot and 2-3 branches) and root biomass (4.6g) obtained with plants raised from conventional rooted stem cuttings. Both tissue culture and cutting raised plants exhibited the same concentration (0.12%) of the compound on dry weight basis. However, the total yield of the root specific compound, 2-hydroxy 4-methoxy benzaldehyde per plant was 2-3 folds higher in micropropagated plants.

**Holostemma ssp**

Sudha *et al.* (1998) [59] established a rapid micropropagation system for establishment of *Holostemma annulare* Roxb. Shoot tips (0.5-0.8 cm), terminal and basal nodes (1.0-1.5 cm) harvested from actively growing shoots of conventionally raised plants, were cultured on MS medium supplemented with various concentrations of BA and NAA. Multiple shoot formation (3.8) was observed in 68% of basal nodes cultured on the medium with optimum doses of 4.43 µM BA and 0.54 µM NAA after 8 weeks. Terminal nodes were not suitable for inducing multiple shoots. Irrespective of the orientation (vertical/horizontal), all shoot tip explants responded with a single shoot to all combinations of plant growth regulators tried. Effects of other cytokinins (Kn and 2-iP) and auxins (IAA and IBA) on regeneration potential of basal nodes were also analysed. Shoots were multiplied by sub-culture of basal node stumps (the original explant tissue free of shoots, but with remnant axillary meristem and two or three protruding buds) in reduced concentration of 2.21 µM BA and 0.27 µM NAA. Liquid medium was found to be ineffective for multiplication due to a high degree of hyperhydrocity. To make multiplication process cost effective, culture bottles with polypropylene caps were used for multiplication. The best root induction (75%) and survival (80%) was achieved on half strength MS medium supplemented with 1.48 µM IBA. Field-established plants had uniform growth traits in terms of height of plants and number, length, and weight of the tuberous roots.

Martin (2002) [60] developed an efficient protocol for axillary bud multiplication for *Holostemma ada-kodlen* Schult. MS medium supplemented with 8.88 µM BA and 2.46 µM IBA induced an average of 8 shoots per node and was the best for axillary bud proliferation. Subsequent cultures enhanced the number of shoots. The abscission of leaves and shoot tips of the developed shoots was prevented by the addition of AgNO₃ or CoCl₂, but with a concomitant significant reduction in number of shoots. Half-strength solid or liquid MS medium with 0.246 µM IBA exhibited the best *in vitro* rooting. The developed plantlets showed 90% survival in the field.

**Leptadenia reticulata**

Parabiaet *et al.* (2007) [61] reported multiple shoot production from axillary buds of *Leptadenia reticulata* using nodes as explants. The MS basal medium supplemented with combinations of 4.92 µM IBA and 46.5 µM Kn from the axillary bud proliferation resulted in six shoots per node. Microshoots for rooting were transferred to IBA containing medium, in which maximum (13) roots were produced in 4.92 µM IBA. Through sequential hardening process, well rooted plantlets were established in the field.

**Tylophora ssp**

Sharma and Chandel (1992) [62] reported a procedure for rapid *in vitro* multiplication of *Tylophora indica* (Burm.f.) Merrill. Addition of ascorbic acid was essential to induce sprouting of axillary buds. Optimum multiplication was observed on MS medium containing 22.2 µM BA, 2.68 µM NAA and 586 µM ascorbic acid. Rooting of *in vitro* produced shoots was readily achieved on MS medium with 5.71 µM IAA. The plantlets, thus obtained, were successfully transferred to pots in large numbers, which grew normally.

Mukundan *et al.* (2002) [63] reported micropropagation of *Tylophora asthmatica*. Nodal segments were cultured on MS medium supplemented with BA for shoot bud induction. The maximum number of multiple shoot formation was obtained on MS medium containing 6.66 µM BA. The *in vitro* grown shoots were rooted in half strength MS medium supplemented with 2.46 µM IBA. Regenerated plants were transferred to soil with survival rate of 96%.

Nema *et al.* (2007) [64] established *in vitro* raised shoot culture of *Tylophora indica* on MS medium with 11.1 µM BA and 0.751 µM IAA. An excellent rate of shoot multiplication was achieved. The tissue culture-generated shoots were rooted on the medium containing IAA. A highly regenerative system was developed by using leaf explants from mature plant. This system can be effectively used to multiply the plant material.
**Gymnema sylvestre R.Br. (2N=22)**

*Gymnema sylvestre* is a vulnerable woody and highly branched climber vine run over the top of tall trees. It is a potent anti-diabetic plant used in folk, Ayurvedic, siddha and homeopathic systems of medicine. The active ingredient is gymnemic acid. Various closely related components of gymnemic acid are used for diabetes in pharmaceutical industry all over the globe. Famous pharmaceutical products known in Indian market are gymnema herbal tea, dia-botica capsules. The entire plant is used as a medicine. Following are some of the medicinal uses of *Gymnema sylvestre*.

1. Roots are used for remedies of snakebite [65].
2. Whole plant is used for the treatment of diabetes [66].
3. The aqueous extract of leaves having anti-inflammatory activity ‘Odoma’ [67].
4. The ethanolic extract of leaves demonstrated antimicrobial activity against *Bacillus pumilis, B.subtilis, Pseudomonas aeruginosa* and *Taphylococcus aureus* and ineffectiveness towards *Proteus vulgaris* and *Escherichia coli* [68].
5. The crude extract exhibits hepatoprotective activity [69].
6. It cures dental caries caused by *Streptococcus mutans* [70].
7. It makes an excellent skin cosmetic [71].
8. It reduces obesity [72].
9. It treats sore eyes, opacities of lens and cornea as well as acts as stomach stimulant and laxative, curing burning sensation in throat and stomach and family planning [51].
10. This plant is also used for controlling obesity in the form of Gymnema tea. [73].

Due to its immense pharmaceutical properties, it has gained tremendous popularity and market demand. The market cost of Gymnema concentrated extracts of one kg obtained from 25 kg or 100 kg of crude drug is Rs. 225.00 or Rs. 480.00 [74]. The leaf powder has been exported to USA, Europe, Japan and Middle-east countries. Most of the Ayurvedic as well as allopathic pharmaceutical companies also require sufficient quantity of Gymnema powder for preparation of medicine. Thus, the demand of Indian Gymnema is increasing in the international market. As revealed by (Fig. 5), the export was 70,000 kg during 1996-1997. The decreasing trend of export was observed from 1998-2002 due to short supply of *Gymnema sylvestre* powder.

**Photochemistry of Gymnema sylvestre**

The above discussed pharmacological properties of *G. sylvestre* are due to the presence of active ingredients commonly known as secondary metabolites. The leaves of *Gymnema sylvestre* contain gymnemic acid as a major bioactive component [75], which is a mixture of tri-terpene saponins of closely related structure e.g., Triterpene saponins, Oleanane saponins, Dammarene saponins, Gurmarin, Triterpenoid saponins, Gymnemasins A-D, Gymnemanol (aglycone), Gymnemestrogenin, Flavonol glycoside, Sterols, Quercitol, Lupeol, Parabin, Conduritol A and Quercitol. Other than gymnemic acid the prominent metabolite in the species, various other chemical compounds isolated and characterized in *G. sylvestre* are given below.

The low seed germination, vegetative propagation and indiscriminate use of *G sylvestre* has led it to the category of threatened species. Thus the only alternative method for conservation and large scale propagation of the species is *in vitro* micropropagation.
Family Asclepiadaceae: Reservoir of medicinal plants

Zhen et al. 2008

Gymnemasisaponins V

Triterpene saponins

Gymnemosides a, b, c, d, e, and f

Gymnemasisaponins V

Gymnemosides A, B, C, and D

[76]

[77]
**Figure 4:** Some of the important biochemical ingredients acting as potential secondary metabolites in *Gymnema sylvestre*.

**Figure 5:** Efficient *in vitro* regeneration in *G. sylvestre* and its potential medicinal properties.
Conclusion

The extensive literature survey exposed Asclepiadaceae as an important medicinal plant family with diverse ethnomedicinal and pharmacological properties. Various biological studies have been dedicated to this family, but a small number of them are useful in evaluating its traditional uses. The species of this family illustrates the occurrence of countless natural ingredients which are responsible for wide-range of pharmacological and medicinal properties. In future research work, the evaluation needs to be carried out on Asclepiadaceae family in general and *G. sylvestre* in particular. In addition the medicinal plants of the family should be characterized chemically in order to get medicinal preparations and in order to use these preparations for their practical clinical applications. The potential plants of the family like *G. sylvestre* should be selected and characterized using chemical and molecular approaches. Moreover the efficient *in vitro* multiplication of the important threatened plants of the family should be established. The better cultivars of the plants should be developed using sophisticated molecular approaches. Genetic engineering using recombinant DNA technology can serve as a useful tool for the elucidation and up regulation of the important genes associated with a particular biosynthetic pathway of the compound of interest. In nutshell, there is wide scope in pharmacological investigation of family Asclepiadaceae. The need of the hour is taking positive measures for the exploitation, conservation and sustainable use of family Asclepiadaceae.

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Conflict of interest

Authors declare that there is no conflict of interest to reveal.

References


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