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GYMNEMA SYLVESTRE R. BR. EX ROEMER AND SCHULTES; A REVIEW WITH SPECIAL REFERENCE TO CONSERVATION THROUGH PROPAGATION

KKIU Arunakumara[1], BC Walpola[2], & Faiz MMT Marikar[3*]

[1]Department of Crop Science, Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya, Sri Lanka.

[2]Department of Soil Science, Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya, Sri Lanka.

[3]Staff Development Centre, General Sir John Kotelawala Defence University, Ratmalana, Sri Lanka[4]

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*Corresponding Author
Email: faiz@kdu.ac.lk

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Abstract

Gymnema sylvestre R. Br. Ex Roemer & Schultes (Asclepideceae) has been used for centuries in traditional medicine for the treatment of diabetes. Inhibition of glucose absorption, stimulation of insulin release and increased glucose tolerance are the key mechanisms by which *G. sylvestre* produce antidiabetic effects. The plant reversibly inhibits the sensation of sweet presumably by blocking sucrose receptors. Its impairment of sweet sensation is profound and dramatically alters the perception of sweetness altering the perception of the other primary tastes. The plant is also considered to be antiviral, diuretic, antiallergic, hypoglycemic, astringent, anti-arthritic, thermogenic, anti-inflammatory, anodyne, digestive, liver tonic and hypolipidemic. Despite many aspects including physiochemical characterization, biological evaluation, toxicity studies, molecular mechanism of action(s) of isolated phytoprinciples and their clinical trials have been extensively studied; little attention has so far been paid on the conservation strategies of the species. Due to exponential demand in the market, the natural habitats of the species are tremendously under pressure as wild harvest is continued to be the key source of supply. The species is now becoming endangered, due to unsustainable wild harvest and the poor natural regeneration. No alternative mode of multiplication is found under natural conditions; thus, the species is totally dependent upon the seed germination for survival. However, vegetative propagation through stem cuttings along with micro-propagation offer a viable source of planting materials needed for *ex situ* conservation by means of cultivation. As *in vitro* extraction could also be possible, propagation through tissue culture is commercially attractive too. However, an integrated approach, which include involvement of government, private sector and local public in conservation strategies could only be effective in

1. Introduction

Natural products remain a prolific source of discovery of new drugs due to their chemical diversity and ability to act on various biological targets (Bhutani & Gohil, 2010). Therefore, search for natural products to cure diseases is becoming an area of great interest, in which plants have been the most important source. The value of the medicinal plants lies in some chemical substances that produce a definite physiological action on the human body (Musthafa *et al.*, 2017). In fact, herbs have traditionally been considered to be non-toxic, thus have been used for treating various diseases and health related problems (Odula *et al.*, 2007). Herbal formulation, a rapidly growing industry (Calapai & Caputi, 2007), controls 30 % of the global drug market and draws almost 1 billion dollars in profit every year (Ritchie, 2007; (Tsuji & Tsutani, 2008). Herbal medicines are the undeniable root of modern pharmacology also (Oricha, 2009) as it has been estimated that around 75 % of all orthodox medicines are of herbal origin (Luzhetskyy, *et al* 2007). Medicinal herbs have been used to treat diabetes for many hundreds of years, in particular, in Asia and Africa, where these plants grow abundantly (Liu et al., 2009). There have been many reports of insulin tropic actions of such plant extracts in vitro demonstrating direct stimulatory effects on β-cells of human body (Saxena & Vikram, 2004). In this context, Gymnema sylvestre, a medicinal plant species widely distributed throughout India and Sri Lanka (Khanna & Kannabiran, 2009) has been studied extensively for its antidiabetic properties due to the fact that the aqueous extracts of the leaves are reported to stimulate insulin secretion and increase glucose uptake in vitro and in vivo (Liu et al., 2009). The main chemical constituents of Gymnema sylvestre are a group of triterpenoid saponins known as gymnemic acids, which are considered to be the active compounds responsible for the anti-diabetogenic effects of the extracts (Kanetkar,, *et al* 2007). The plant is rich in phytochemicals such as alkaloids, flavonoids, carbohydrates and phenols also (Khana & Kannabiran, 2007). The recent discoveries about the medicinal value of the species have led to increase the demand across the world. However, as substantial cultivation is yet to be started to meet the demand, pressure is mounting on the wild stocks. Continuous exploitation from the wild and substantial loss of their habitats has resulted in the population decline of many high value medicinal plant species over the years. Cultivation of medicinal plants is thus advocated as a measure to take the pressure off wild stocks, especially for species collected in large quantities for trade (Bodeker *et al*, 2002). Cultivation can be commercially attractive to drug producing companies also, because they then have greater control over quality and supply (Harnischfeger, 2000). However, successful cultivation could only be expected if reasonable agro-technological package has been developed for the species of concern. Such package generally includes all the techniques from propagation to harvesting and processing. Natural strands of G. sylvestre are fast disappearing and are threatened with extinction due to its indiscriminate collection and over exploitation.

The present paper reviewed Gymnema sylvestre paying special attention to conserve the species through propagation.

1.1 Geographical Distribution of Gymnema sylvestre

Naturally, Gymnema sylvestre grows in secondary forest, riverine forest and dry shrub savanna, usually on sandy or loamy soils. The species prefers well distributed rainfall of 600 – 1000 mm annually (Tafokou, 2010). G. sylvestre is widely distributed in India, where the species is reported to be originated. This vulnerable species is naturally found in many Indian dry forests up to 600 m (Sharma & Bansal, 2010). However, it becomes rare in Madhya Pradesh, Maharashtra and Andhra Pradesh, mainly due to excessive collection of entire plant for making medicinal preparations and for the trade (Jain & Patole, 2001; Udayan *et al*., 2009). The species is reported to be distributed also in Vietnam and Southern China and from Japan (Ryukyu Islands) to the Philippines, Malaysia, Indonesia and Australia (Saneja *et al*., 2010). In Africa, it grows naturally throughout most of West Africa and East to Ethiopia and South to South Africa (Tafokou, 2010). G. sylvestre has long being used in Sri Lanka, where the species is now considered to be threatened due to unsustainable harvesting practices (Figure 1 A)

1.2 Vernacular Names

The plant is commonly known as Periploca of the woods (English); Gurmar, Gudmaar, Medhaa singee (Hindi); Meshashringi, madhunashini (Sanskrit); Adigam, cherukurinja, Shirukurum, Kaay, Shakkaraikkolli (Tamil); Podapatri (Telgu); Cakkarakkolli, Madhunaashini (Malayalam); Kaavalee, Medhaashingi (Marathi) (Kanetkar, Singhal, & Kamat, 2007) and Masbedda (Sinhala). The word "Gymnema" is derived from a Hindu word "Gurmar" meaning "destroyer of sugar" and it is believed that it might neutralize the excess of sugar present in the body in diabetes mellitus.

1.3 Botanical Description

Gymnema sylvestre R.Br. belongs to the family Asclepiadaceous, is a large woody, much branded, climber (The Wealth of India, 2005). It grows over the tops of trees under various ecological conditions (The Wealth of India, 2005). The cylindrical stem and branches are pubescent. The internodes, depending on the growing environment, are 0.7 - 18 cm long and 2 - 10 mm in diameter. Leaves are opposite, 3 - 5 cm long and upto 3 cm broad, ovate-elliptic, acute or shortly acuminate, rounded at base, ciliate along margin, pubescent on both sides; base rounded or heart shaped with 6 - 13 mm long pubescent petioles (The Wealth of India, 2005). The petioles of leaves are terete, pubescent, 6 - 12 mm long and 1 - 1.5 mm in diameter. Flowers are small (The Wealth of India, 2005), occur in umbellate cyme inflorescences. Calyx is pubescent, five lobed, ovate, obtuse and ciliate (Figure 1 B). Corolla is yellow, campanulate, 3.5 mm long, corolla tube 1.5 mm long, about equaling the lobes, ovate-deltoid, spreading and glabrous. Corona is corolline, of 5, fleshy processes inserted on the corolla tube, alternate with its lobes, free at the short deltoid subacute tip, which are protruding out of the mouth of corolla tube. Stamens are five. Gynostegium is 2 mm long. Style apex is thick, sub hemispherical,

much exerted beyond the anthers and follicles are paired in all plants collected from different localities (Najafi & Deokule, 2011). Mature pod is around 4-7 cm long and contains 10-12 seeds. Seeds are about 1.3 cm long, narrowly ovoid-oblong, flat, with a thin, broad, brown, and glabrous marginal wing (The Wealth of India, 2005). The root system is of tap root type.

1.4 Photochemistry and Mechanism of Action

Leaves of G. sylvestre contain a mixture of triterpene saponins, which belongs to oleanane and dammarene classes (Saneja et al., 2010). Oleanane saponins are gymnemic acids and gymnema saponins, while dammarene saponins are gymnemasides (Spasov, Samokhina, & Bulanov, 2008). Besides this, other plant constituents are anthraquinone derivatives, pentriacontane, hentricontane, α and β chlorophyll, phytin, resins, d-quercitol, tartaric acid, formic acid, butyric acid, lupeol, β-amyrin, related glycosides and stigmasterol (Saneja et al., 2010; Kokate, 1994]. The gymnemic acids contain several acylated (tiglolyl, methylbutyroyl etc.) derivatives of deacylgymnemic acid (DAGA), which is a 3-O-β-glucouronide of gymnemagenin (Saneja et al., 2010). A flavonol glycoside namely kaempferol 3 - O - beta - D - glucopyranosyl - (1 --> 4) - alpha - Lrhamnopyranosyl - (1 --> 6) - beta - D - alactopyranoside has also been found in aerial parts of G. sylvestre (Liu et al., 2004). The antidiabetic nature of G. sylvestre leaves is a well-known character (Bhutani & Gohil, 2010). The gymnemic acids, which are known to suppress transport of glucose from the intestine into the blood stream and a small protein, gurmar, can interact with receptors on the tongue to decrease the sensation of sweetness in many foods (Osman et al., 2010). This dual action has been shown to reduce blood sugar and cholesterol levels in diabetic animals and humans and may provide some benefits in terms of regulating appetite control and food cravings (Daisy, Eliza, & Farook, 2009). The hypoglycemic activity of the species is proved to find in laboratory animals (Saneja et al., 2010). Among the possible mechanisms by which G. sylvestre exert its hypoglycemic effects, 1) enhancing the secretion of insulin, 2) promoting regeneration of islet cells, 3) increasing the utilization of glucose and 4) inhibiting the absorption of glucose from intestine have been discussed (Daisy, Eliza, & Farook, 2009). The administration of Gymnema leaf extract to a diabetic patient can stimulate the pancreas by virtue of which an increase in insulin release is resulted. G. sylvestre is also reported to exhibit the antiobesity activity, which is attributed to the ability of gymnemic acids to delay the glucose absorption in the blood (Daisy, Eliza, & Farook, 2009). The atomic arrangement of gymnemic acid molecules is pretty much similar to that of glucose, enabling them to fill the receptor locations on the taste buds preventing its activation by sugar molecules present in the food, thereby curbing the sugar craving. In addition, gymnemic acid molecules could fill the receptor locations in the absorptive external layers of the intestine preventing sugar molecules absorption by the intestine (Kanetkar, *et al*, 2007). (Rachh *et al*., 2010) have investigated the effect of G. sylvestre leaf extracts on hyperlipidaemic rats and have reported a reduction

in elevated serum triglyceride (TG), total cholesterol (TC), very low density lipoprotein (VLDL) and low density lipoprotein (LDL) in cholesterol in dose dependent manner. G. sylvestre leaf extracts have also been found to increase fecal excretion of cholesterol (Kanetkar, Laddha, & Kamat, 2004). Study further evaluated the antibacterial activity of G. sylvestre and inhibitions of Bacillus subtilis and Staphylococcus aureus were found. However, extracts had no activity against E. coli as reported by (Satdive, Abhilash, & Fulzele, 2003). Similarly, the ethanolic extract of G. sylvestre leaves showed good antimicrobial activity against Bacillus pumilis, B. subtilis, Pseudomonas aeruginosa and Staphylococcus aureus (Satdive, Abhilash, & Fulzele, 2003). According to Pasha et al (Pasha et al., 2009), the aqueous extract of G. sylvestre leaves also showed higher activity against the three pathogenic Salmonella species (Salmonella typhi, S. typhimurium and S. paratyphi). The role of G. sylvestre in countering the lipidemic-oxidative aberrations accompanying diet-induced hypercholesterolemia in rats has been investigated by Osman et al (Osman et al., 2010). G. sylvestre caused reduction in the activity of Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes in plasma of diabetic rats (Daisy *et al*., 2009). A single phytoconstituent that could be used in the treatment of both diabetes and obesity simultaneously would be Gymnemic acids (Jachak, 2002). The plant helps to promote weight loss possibly through its ability to reduce cravings for sweets and control blood sugar levels (Saneia *et al*., 2010). Rachh et al., 2009) investigated in-vitro antioxidant activity of G. sylvestre leaf extract and reported that alcoholic leaf extract possesses antioxidant activity. In addition, tannins and saponin, the chief chemical constituents present in G. sylvestre are known to possess anti-arthritic activity (Malik *et al*., 2010).

Figure 1: Gymnema sylvestre A -; B - flower bud stage; C - mature pods

In the Ayurvedic system of medicine, both the dried leaf (mesasrngi leaf) and dried root (mesasrngi root) are used therapeutically (Malik et al., 2010). The leaves of the plant are used as antiviral, diuretic, antiallergic, hypoglycemic, hypolipidemic, for the treatment of obesity and dental caries (Malik et al., 2010). It is also used as antibiotic, in stomach pains, as a blood purifier and in rheumatism (Evans, 2002). The plant is also considered as astringent, bitter, acrid, thermogenic, anti-inflammatory, anodyne, digestive and liver tonic (Malik et al., 2008). In market, G. sylvestre is available in the form of crude plant, powder, extract paste and solid in standardized form. The plant material is also available in the form of capsule or tablets in combination with other herbal plants (American Herbal Products Association, 2001). For adults, 25 to 75 ml of the liquid form (extract) per week is recommended. However, best results of this medicine will come after 6 to 12 months of continuous use. It is also prescribed in tablet form of 8 to 12 g per day of leaf equivalent is recommended. In the case of pediatric dose, as there is insufficient evidence on the usage for pediatric population, no recommendation can still be made (Bone, 2002).

1.5. Natural Regeneration and Germination of Seeds

G. sylvestre produces flowers once a year. Under Sri Lankan conditions, flowering starts early November and lasts for two months. Pods take 2-3 months to mature (Figure 1 C). A feather like structure attached to the tiny seed takes seeds away from mother plants by means of wind. However, as this structure is easily detachable, widespread distribution is hard to seen. The weight of the seeds varies from 5115-5750 mg/1000 seeds. At the time of detaching from the mother plant, seeds contain little moisture (4-9%), which in fact hampers the germination. Therefore, number of emerging seedlings is limited in natural habitats, despite the fact that thousands of seeds fall under mother plants. No alternative mode of propagation is naturally found, thus seed propagation is recognized as the sole mechanism of natural regeneration (Arunakumara, & Subasinghe, 2004). According to Indian sources, seed germination of this species is reported to be poor and the maximum germination percentage of 50-55 % could be obtained when sown in soil mixed with vermicomposting. Commercial exploitation for production and conventional propagation is hampered due to its poor seed viability and low rate of germination (Komalavalli, & Rao, 2000). However, under Sri Lankan conditions, over 90 % seeds can be germinated when sown in coir dust media. According to Ved *et al* (Ved, Oommen, & Singh, 2002), seeds are collected from freshly harvested fruits and soaked overnight in water before been sown in the seed pans containing soil mixed with sand. The pans are watered daily. Seeds germinate in about 15 days' time and 40-50 days old seedlings are transplanted to polythene bags containing a mixture of soil, sand and farm yard manure mixed in equal proportion. About 2-3 kg seeds are required to raise the crop in one-hectare area (Ved, Oommen, & Singh, 2002). Soaking seeds in 0.2 % KNO3 for 6 hours also increases the germination (Arunakumara, & Subasinghe, 2004), which is in agreement with Harakumar, (Harakumar,

1997) who reported that leaching the seeds in tap water for 12 h followed by soaking in 0.2 % KNO3 solution enhanced the germination up to 75 per cent. The seeds do not loose the viability rapidly, thus can be stored under normal conditions (room temperature of about 250C) for two months without losing germination.

1.6. Vegetative Propagation as a Tool for Conservation

Ex situ conservation ensures long-term maintenance of the endangered species, which could be used as initial material sources for cultivation (Heywood, & Dulloo, 2005; Panayotova *et al*., 2008). However, cultivation can be practiced only for the species which can easily be propagated. Thus availability of viable propagation techniques is the key for successful ex situ conservation the species of concern. Despite the advances in tissue culture, for many conservation, domestication and breeding programs, lower cost macro-propagation methods continue to be the most convenient approaches even when human and financial resources are not scarce (Tchoundjeu et al., 2004; Atangana *et al.,* 2006). Aparicio *et al* (Aparicio *et al*., 2009) also reported that vegetative propagation ability of the species of concern and the development of practical methodologies to obtain genetic copies are essential tools for conservation and breeding programs. In this context, propagation through stem cuttings has received wide recognition though the success may vary depending on the species. Terminal and axillary cuttings with three to four nodes from one-year-old plants are the best planting material for G. sylvestre, which is in agreement with our findings (Arunakumara et al., 2006), where we reported that semi hard wood cuttings provide better results. In fact, we studied the effects of rooting hormone, indole-3-butric acid (IBA) on rooting of semi hard wood, double-nodal leafy stem cuttings. According to the findings, rooting percentage of IBA treated cuttings was around 80 % thus G. sylvestre could easily be propagated by means of cutting at an affordable cost. Our findings are in agreement with Karthic and Seshadri (Karthic & Seshadri, 2009) who also reported that both size of the planting material and IBA concentration play a major role in the induction of roots in G. sylvestre. According to (Ved et al., 2002), the species can be successfully grown using matured stem cuttings of 15 cm length, treated with 500 ppm IBA for about 18 hours. Polybags filled with soil, sand, and FYM (farmyard manure) in 1:2:1 ratio can be used in planting cuttings, where vermicompost may also be used in place of FYM. February to March is the best season for planting the cuttings in nursery, especially in North Indian conditions. The cuttings should be placed under humid conditions in shade houses or mist chambers, where rooting is initiated within a month of planting. Jiofack Tafokou, (Tafokou, 2010) studied the effects of potting media on the performance of cuttings in a pot experiment, and reported that 4 months after planting, the potting mixture supplemented with vermicompost gave significant better overall results over the other potting mixture such as coir pith, press mud or farmyard manure. Gymnema cuttings generally develop roots after 3-4 months of planting and four months later, they are ready for field planting (Ved

et al., 2002). However, (Komalavalli & Rao, 2000) reported that due to poor rooting ability of vegetative cuttings, commercial exploitation for production and conventional propagation is hampered. In fact, majority of the plants are not amenable to vegetative propagation through cutting and grafting, thus limits multiplication of desired cultivars. Moreover, many plants propagated by vegetative means contain systemic bacteria, fungi and viruses which may affect the quality and appearance of selected items (Sharma *et al*., 2010). In recent years, tissue culture has emerged as a promising technique to obtain genetically pure elite populations under in vitro conditions rather than have indifferent populations. Tissue culture has become a well-established technique for culturing and studying the physiological behavior of isolated plant organs, tissues, cells, protoplasts and even cell organelles under precisely controlled physical and chemical conditions (Sharma *et al*., 2010).

1.7. In vitro Propagation of Gymnema sylvestre

Substantial numbers of micro propagation studies have been conducted on the species. According to Reddy et al (Reddy, Gopal, & Sita, 1998), MS medium supplemented with BA (5.0 mg/L) and NAA (0.2 mg/L) could induce 7 shoots/explant. They further reported that the best root induction results in ½MS without growth regulators. According to Komalavalli and Rao (Komalavalli & Rao, 2000), MS medium containing 6-benzyladenine (1 mg/L), kinetin (0.5 mg/L), 1-napthalene acetic acid (0.1 mg/L), malt extract (100 mg/L) and citric acid (100 mg/L) is the best for shoot proliferation of G. sylvestre. They have also noted that use of axillary nodes for micro propagation is beneficial than other explant types. MS basal medium supplemented with 3 mg/L IBA is the best for root induction (Komalavalli & Rao, 2000), which is in agreement with (Devi & Srinivasan, 2008) who reported the same. However, they claimed MS medium containing 1 mg L-1 BA+0.5 mg L-1 IAA+100 mg L-1 Riboflavin (Vitamin B2)+100 mg L-1 citric acid as the best for shoot proliferation. (Devi & Srinivasan, 2008) further stressed that in vitro propagation of G. sylvestre is not very different from that of G. elegans and may be applicable for other economically important woody climbers as well. Ali Ahmed *et* (2009) have optimized a system for the somatic embryogenesis via embryogenic suspension cultures of G. sylvestre. They induced callus cultures on MS medium with growth regulators (2, 4 -D 0.5 mg/L (or) NAA 1.0 mg/L) and 10 % coconut water. The callus cultures were then transferred into MS liquid medium containing NAA 1.0 mg/L, BA 1.0 mg/L, 3.0 % sucrose (w/v), 10 % coconut water, citric acid 1 mg/L and glutamine 10 mg/L for somatic embryogenesis from callus and observed globular, heart, torpedo and cotyledonary stage of embryos in suspension cultures after 8 weeks. According to their records, the maturation embryos were significantly affected by growth regulators and photoperiod. Five to seven percent of embryos formed plantlets on semisolid medium containing basal MS medium with B5 vitamin, 3.0 % sucrose and 0.8 % agar (w/v). All plantlets established in the field exhibited morphological characters similar to those of the mother plants. Successful in vitro regeneration procedures for G.

sylvestre have been developed using stem and nodal segments as well as basal, middle and terminal cuttings on MS medium supplemented with different concentrations of various growth regulators (Tafokou, 2010). They tested the effects of chemical and physical environments on cell culture of G. sylvestre and reported that the strength of medium salt did not significantly affect cell growth, while sucrose concentration and naphthalene acetic acid increased cell growth. However, previous reports suggested 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 polyvinylpyrrolidone) could markedly influence in vitro propagation of G. sylvestre. Gopi *et al* 2009) initiated the callus cultures from nodal segments and leaf explants of G. sylvestre on MS medium containing basic salts and 30 g/L sucrose supplemented with different concentrations (0.10, 0.25, 0.5, 1.0, 2.0 and 5.0 mg/L) of 2, 4-dichlorophenoxy acetic acid (2, 4-D), naphthalene acetic acid (NAA), indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), kinetin (KN) and 6-benzyladenine (BA) and found that main components of the active principles namely gymnemic acids and gymnemagenin were present in sufficiently large amounts in the cultured undifferentiated cells. Kanetkar, *et al* (Kanetkar *et al*., 2006) also reported in vitro callus with the active compounds, gymnemic acid and gymnemagenin presenting in sufficiently large amount in the cultured undifferentiated cells. External phytohormone, shaking speeds, pH of the medium played important roles in growth and gymnemic acid production in suspension culture (Devi et al 2006). Karthic *et al*, 2009), in their experiment, young stem cuttings with an actively growing side branch were used as explants in a hydroponic system, where MS medium containing 0.5 mg/L of IBA resulted in the highest rooting (66 %) with 96 % survival. Based on the findings, they claim that their protocol can serve as an alternative to the existing in vitro and clonal multiplication protocols for G. sylvestre.

2. Conclusion

Gymnema sylvestre has contributed a great deal to the academic curiosity as it is apparent from number of publications related to physiochemical characterization, biological evaluation, toxicity studies, investigation of molecular mechanism of action(s) of isolated phytoprinciples and their clinical trials. These are necessary classical approaches in search of new drugs for management of various diseases. However, innovative studies are still needed in order to explore the other therapeutic uses. Sustainable utilization of natural habitats and development of Agro-technologies for cultivation are also needed to conserve the genetical base of the species. Though cultivation can substitute the collection of wild stock or supplements it, as there is no guaranty for such a substitution, one should not be too optimistic about the scope of cultivation as a practical strategy for ex situ conservation of the species. This does not mean, however, that cultivation efforts should be disregarded as a conservation strategy. However, development of sound propagation techniques resulting increased

availability of planting material at an affordable cost would have positive impacts on the in situ conservation of the species. In this context, as described above, Gymnema sylvestre is proved to have several means of propagation, thus practical implementation of conservation strategies is achievable.

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