Chapter - 1

General Introduction
1. General Introduction and Review of Literature

"The human conditions have been enhanced by those biochemists and pharmacologists who have applied the scientific method to probe ancient myths and legends."

..........Davis (1983)

TRADITIONAL medicine based on herbal remedies has always played a key role in the health system of many countries. An estimated three quarters of prescription drugs are derived from plants (Zenk, 1978; FAO report, 1993). Taking its cue from China, India has launched a scheme for placing time-honored traditional remedies on a scientific footing in collaboration with laboratories in the West.

Medicinal plants play a key role in the human health care. About 80% of world populations rely on the use of traditional medicine which is predominantly based on plant materials (WHO, 1993). Plant-based remedies have always been an integral part of traditional medicine throughout the world. There are more than 100 drugs of known structure that are extracted from higher plants and used in allopathic medicine (Fransworth, 1990; Cox, 1994).

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 brevifolia). 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. The in vitro mode of production however has yet to prove its profitability and may in future be surpassed by medicinal and industrial plants improved by plant breeding and genetic engineering. Still the demonstration of secondary
metabolites in plant cell cultures and strategies to exploit their potential is valid (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 32 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 germplasms (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. Importance of Legumes

Legumes are a fascinating group of plants valued for food, fodder, ornamentals and raw materials for industry and also for their role in biological nitrogen fixation. Apart from several conventional grain legumes, as many as 95 Leguminosae taxa are traditionally used by the locals of north-east India for food, medicine, fodder, ornamentals, industrial raw materials, dye, fish poison and biological nitrogen fixation.

Legumes, broadly defined by their unusual flower structure, podded fruit and the ability of 88% of the species examined to-date to form nodules with
Rhizobia (Faria et al., 1989), are second only to the Graminiae in their importance to humans. The 670 to 750 genera and 18,000 to 19,000 species of legumes (Polhill et al., 1981) include important grain, pasture and agroforestry species. Cohen (1977; cited by Bryan [2000]) reported domestication of lentils (Lens esculenta) at a site in Iran dating to 9,500 to 8,000 BP.

Roosevelt et al., (1996) noted the use of Hymenaea as a food source in Amazonian pre history. Bean (Phaseolus vulgaris) and soybean (Glycine max), staple crops in the Americas and Asia, respectively, were each domesticated more than 3,000 years ago (Hymowitz and Singh, 1987; Kaplan and Lynch, 1999). Use of legumes in pastures and for soil improvement dates back to the Romans, with Varro (37 BC; cited by Fred et al. [1932]) noting “Legumes should be planted in light soils, not so much for their own crops as for the good they do to subsequent crops”.

Legumes account for 27% of the world’s primary crop production, with grain legumes alone contributing 33% of the dietary protein nitrogen (N) needs of humans (Vance et al., 2000). Under subsistence conditions, the percentage of legume protein N in the diet can reach twice this figure. In rank order, bean, pea (Pisum sativum), chickpea (Cicer arietinum), broad bean (Vicia faba), pigeon pea (Cajanus cajan), cowpea (Vigna unguiculata), and lentil constitute the primary dietary legumes (National Academy of Science, 1994). Legumes (predominantly soybean and peanut [Arachis hypogaeae]) provide more than 35% of the world’s processed vegetable oil, and soybean and peanut are also rich sources of dietary protein for the chicken and pork industries.

Nutrient depletion of soil is a particular problem for small landholders in developing countries, where much grain-legume production occurs, and many farmers cannot afford to use fertilizers. Soil acidity affects more than 1.5 billion ha worldwide, with acid soil constraints to legume production likely to increase as the result of acid rain, long-term N fertilization, and natural weathering.
(Graham and Vance, 2000). Hydrogen ion concentration per se, Al and Mn toxicity and P, Mo, or Ca deficiency all contribute to the problem (Graham, 1992). Nodulation and N fixation and survival of rhizobia in soil are particularly affected under low phosphate, acid soil conditions (Coyne et al., 2003).

1.2.1. Biological Nitrogen Fixation

A hallmark trait of legumes is their ability to develop root nodules and to fix N\(_2\) in symbiosis with compatible rhizobia. This is often a critical factor in their suitability for the uses. Formation of symbiotically effective root nodules involves signaling between host and microsymbiont. Flavonoids and/or isoflavonoids released from the root of the legume host induce transcription of nodulation genes in compatible rhizobia, leading to the formation of lipochitooligosaccharide molecules that, in turn, signal the host plant to begin nodule formation (Long, 1996). Numerous changes occur in host and bacterial gene expression during infection, nodule development, and function (Vance, 2002), with approximately 100 host legume and rhizobial genes involved.

1.2.2. Industrial and Medicinal uses of Legumes

In addition to traditional food and forage uses, legumes can be milled into flour, used to make bread, doughnuts, tortillas, chips, spreads and extruded snacks or used in liquid form to produce milks, yogurt, and infant formula (Garcia et al., 1998). Pop beans (Popenoe et al., 1989), licorice (Glycyrrhiza glabra; Kindscher, 1992), and soybean candy (Genta et al., 2002) provide novel uses for specific legumes. Legumes have been used industrially to prepare biodegradable plastics (Paetau et al., 1994), oils, gums, dyes and inks (Morris, 1997). Galactomannan gums derived from Cyamopsis spp. and Sesbania spp. are used in sizing textiles and paper, as a thickener and in pill formulation. Many legumes have been used in folk medicine (Duke, 1992; Kindscher, 1992). Isoflavones from soybeans and other legumes have more recently been suggested both to reduce the risks of cancer and to lower serum cholesterol (Kennedy, 1995; Molteni et al., 1995). Soybean and soyfood phytoestrogens
have been suggested as possible alternatives to hormone replacement therapy for post-menopausal women.

1.2.3. Legumes with useful Phytochemicals

The USDA, ARS, PGRCU is dedicated to conserving 17 leguminous species with potentially useful phytochemicals which includes *M. pruriens*. Examples of commercially useful phytochemicals are rotenone, tephrosin and deguelin, which are used in limited quantities as pesticides (Gaskins *et al.*, 1972; Minton and Adamson, 1979; Tyler *et al.*, 1976; Beckstrom–Sternberg and Duke, 1994). The use of pesticidal plants is widespread in the developing countries (Balandrin *et al.*, 1985). The legume Tephrosia (*Tephrosia purpurea*) contains insecticidal properties and the antitumor compound, lupeol (Beckstrom-Sternberg and Duke, 1994). Rotenoid compounds derived from fish poison bean (*Tephrosia vogelii*) (Lambert *et al.*, 1993) are also used as insecticides and rotenone has been reported to have antitumor potential (Beckstrom–Sternberg and Duke, 1994).

Some legumes are potential sources of glycosides, biologics, antibiotics, and alkaloids which are used in drug manufacturing by the pharmaceutical industry (Tyler *et al.*, 1976). The glycosides include aloe-emodin, chrysophanol, emodin, and rhein. Another phytochemical with potential use as an antibiotic is prodelphinidin derived from snout bean (*Rhynchosia minima*) (Beckstrom–Sternberg and Duke, 1994). The alkaloid genistein, derived from kudzu has been found to retard cancer growth (Brink, 1995). Trigonelline, an anticancer agent is derived from jackbean (*Canavalia ensiformis*) (Beckstrom–Sternberg and Duke, 1994). Canavanine, extracted from jackbean has been found to be cytotoxic to human pancreatic cancer cells (Swaffar *et al.*, 1994; Swaffar *et al.*, 1995). Jackbean is also cultivated throughout the tropics as a cover crop, forage and green manure (Oropeza *et al.*, 1993).

Cowitch (*Mucuna pruriens*) and Kudzu (*Pueraria montana* var. *lobata*) have been reported to contain multiple useful phytochemicals (Beckstrom–Sternberg...
Cell suspension cultures of Cowitch accumulated the anti-Parkinson drug, L-Dopa (Pras et al., 1993). The chemical, diadzein found in Kudzu (*Pueraria montana* var. Lobata) not only is cancer preventive, estrogenic and spasmolytic, but has also been effective in reducing alcohol consumption in hamsters (Braddock, 1995). Kudzu starch extracted from the tuberous roots in Japan is sold as a health food worldwide (NAS/NRC, 1979). Not only does winged bean (*Psophocarpus tetragonolobus*) provide useful phytochemicals such as polyunsaturated fatty acids used as an antipolyneuritic (Beckstrom-Sternberg and Duke, 1994), but it also produces edible leaves, shoots, flowers, pods and tubers as well as seeds whose composition duplicates that of soybeans. The most interesting feature of winged bean tubers is their protein content. Winged bean tubers average 20% protein as compared to 1% for cassava (*Manihot esculenta* Crantz., Euphorbiaceae) and 3–7% for potato (*Solanum tuberosum* L., Solanaceae) (NAS/NRC, 1979). To date, winged bean has not yet met expectations due in part to its intolerance of cold temperatures during the fall and winter. Lablab bean (*Lablab purpureus*) has a myriad of uses (NAS/NRC, 1979). The young pods, dried seeds, leaves and flowers can be eaten. Tyrosinase found in lablab bean has potential use for antihypertensive treatment (Beckstrom-Sternberg and Duke, 1994). Lablab bean occurs in two botanical types. The garden type is twining, late maturing and used mainly as a vegetable. The field type is erect, bushy, early maturing and used as forage, cover crop and an ornamental (NAS/NRC, 1979). Common Indigo (*Indigofera tinctoria*) has been found to contain indirubin which is useful for the treatment of chronic myelocytic leukemia (Han, 1994). Butterfly pea (*Clitoria ternatea*) contains antifungal proteins and has been shown to be homologous to plant defensins (Osborn et al., 1995). *Desmodium gangeticum* is used in Nigerian traditional medicine and has been evaluated for possible antileishmanial activity (Iwu et al., 1992). Tick clover (*Desmodium adscendens*), a medicinal herb used in Ghana has been evaluated for three active components including dehydrosoyasaponin I, soyasaponin I and soyasaponin III for potential use as antiasthma (Mcmanus et al., 1993).
1.3. Introduction to *Mucuna pruriens* L.

*Mucuna pruriens* L. is a trifoliate climbing legume, which is pseudo-racemose or umbelliform, axillary or nodose inflorescence. It bears white, lavender, or purple flowers and pods that are covered in loose orange hairs which cause a severe itch if they come in contact with skin. The beans are shiny black or brown or white (Wikipedia, the free encyclopedia). This plant has been mentioned in the treatises of ancient Indian texts such as the 'Charaka Samhiti' and the 'Susrutha Samhiti'. Its Sanskrit name is 'Atmagupta', while in Hindi it is called 'Kawach'. In southern India it is also known as 'Naikurna' (medindia.com). It is also known by a multitude of common names, including velvet bean, cowitch, cowhage, kapikachu, nescafe, sea bean, kratzbohnen, konch, yerepe (Yoruba) (Wikipedia, the free encyclopedia). The name 'nescafe' is attributed to it for its usage as a coffee substitute in South America (medindia.com).

**Taxonomy of Mucuna pruriens L.**

*Kingdom*: Plantae, Planta, Planter, Plants, Vegetal.

*Sub Kingdom*: Tracheobionta, Vascular Plants.

*Division*: Magnoliophyta. (Angiospeens)

*Class*: Magnoliopsida (Dicote, Dicotyledon)

*Sub class*: Rosidae.

*Order*: Fabales

*Family*: Leguminoseae

*Sub Family*: Fabaceae

*Genus*: Mucuna

*Species*: pruriens

**Synonyms** (The Wealth of India, 1995):

*Carpopogon pruriens*, *Dolichos pruriens*, *Mucuna aterrima*, *M. atropurpurea*, *M. cochin chinensis*, *M. cyanosperma*, *M. deeringiana*, *M. esquirolii*, *M. prurita*, *M. utilis*, *Stizolobium aterrimum*, *S. deeringianum*, *S. pruriens*, *S. pruritum*, *S. niveum*, *Negretia pruriens*. 
Common Names (The Wealth of India, 1995)
Nescafé Mucuna, Fava-coeira, Cabeca-de-frade, Cowage, Cowhage, Cow-itch, Velvet Bean, Bengalbean, Mauritius bean, Itchy bean, Krame, Picapica, Chiporro, Buffalobeans.

Vernacular names (Warrier et al., 1995)
Hindi-Kiwach, Daunch, Goncha; Bengali- Alkushi, Bichchoti; Marathi- Kavacha, Kuhili, Kanchkuri; Gujarathi- Kivanch, Kavatch; Kannada- Nasukunni; Malayalam- Naicorn; Oriya- Kaincho.

1.3.1. Distribution and Habitat:
*Mucuna pruriens* is a tropical legume, annual, climbing shrub with long vines that can reach over 15 m. It is originated in Southern China and Eastern India, but is now found extensively all over the world. In India 14 species of *Mucuna* are found in the foothills of the Himalayas, the plains of west Bengal, Madhya pradesh, Karnataka, Kerala, Andhra Pradesh, Uttar Pradesh, the Andaman and Nicobar islands (Farooqi et al., 2001).

The calyx is campanulate, four lobed and the resupinate explosive. Corollas are showy, purple, red, greenish, white or yellow. Leaflets broadly ovate, elliptic or rhomboid ovate, unequal at base (Sathyanarayanan and Arulmozhi, 2007). Its flowers are white to dark purple and hang in long clusters or pendulous racemes, grow in racemes in 2 or 3. The fruit of the plant is pod, which is thick and leathery. It is covered with reddish-orange coloured long stiff hairs that are readily dislodged and responsible for itching. Pods curved, 5-10 cm x 1.5-1.8 cm, longitudinally ribbed, turgid, densely clothed with persistent pale brown or gray, irritant bristles. The itch-producing property is attributed to the trichomes (hair) present on the pods. It has been established that this unique property is accounted by the presence of 5-hydroxy tryptamine (5-HT) in the hair (Armstrong et al., 1953). Some reports show that anti-histaminics afford protection against the itch (Broadbent, 1953).
Seeds, known as *Mucuna* beans, 4-6 in a pod, ovoid (6-12 mm long) with funicular hilum (Sastry *et al.*, 1990; Agharkar, 1991; Rastogi *et al.*, 1994). The seed is characterized by the presence of a single layered palisade with irregularly thickened cell wall and light line passing through the upper part, single layer of parallely arranged bone shaped columnar cells, presence of substantial number of irregularly shaped stone cells just below the palisade layer in the hilum region around vascular sac and abundance of large, round to oval starch grains measuring 17 to 25 µ in parenchyma cells of cotyledons (Satyavati *et al.*, 1987). Characteristic feature in root is the presence of interxylary phloem, which alternates with the fibre groups.

### 1.3.2. Chemical and Phytochemical composition

The plant is reported to have L-Dopa as a major constituent mainly in seeds (Damodaran and Ramaswamy, 1937; Bell and Janzen, 1971; Daxenbichler, *et al.*, 1971). Alkaloidal constituents (Mehta and Majumdar, 1994; Santra and Majumdar, 1953) viz., mucunadine, mucunine, prurienidine, prurienine (Majumdar and Zalani, 1953) are reported from seeds. Number of amino acids are reported from this plant (Pant *et al.*, 1974; Niranjan and Katuyar, 1979). Epoxy fatty acids *viz.*, *cis*-12, *cis*-13-epoxyoctadec-trans-9-cis-acid, *cis*-12, *13*-epoxyoctadectrans-9-enolic acid are reported (Hasan, *et al.*, 1980). Lecithin is reported to be present in seed (Panikkar, *et al.*, 1987). According to Dr. Duke’s phytochemical and ethnobotanical databases *Mucuna pruriens* contains many diverse phytochemicals like 1-methyl-3-carboxy-6, 7-dihydroxy-1,2,3,4-tetrahydroisoquinolone, 5-hydroxy tryptamine, 5-methoxy-n,n-dimethyltryptamine-noxide, 5-oxyindole-3-alkylamine, 6-methoxyharman, alanine, arachidic acid, arginine, aspartic acid, behenic acid, H-carboline, H-sitosterol, bufotenine, choline, cystine, leucine, linoleic acid, myristic acid, n,n-dimethyltryptamine, n,n-dimethyltryptamine-n-oxide, nicotine, oleic acid, palmitic acid, palmitoleic acid, phenylalanine, phosphorus, proline, protein, saponins, serine, stearic acid, threonine, tryptamine, tyrodine, valine and vernolic acid (Duke, 2007). Recently three new lipid derivatives were reported
from n-hexane extract of seeds of *Mucuna pruriens*, namely (Z)-Triactont-5,7,9-triene; (Z)-Docos-2,4,6-trien-1,8-diol and (Z)-Docos-5-en-1-oic acid (Misra and Wagner, 2006). This plant is a source of minerals (Rastogi and Mehrotra, 1993). Misra and Wagner (2004) reported isolation of four 1,2,3,4 tetrahydroisoquinoline alkaloids from the seed. Others such as phytoestrogens, cyanogens, cyclopropinoid fatty acids, canavanine, anti-vitamins and esters were also be found.

1.3.4. Medicinal properties

*Mucuna pruriens* finds traditional use in number of diseases and its various parts are used for various purposes.

**Plant parts used**- Root, Leaf and Seed.

1.3.4.1. Roots

Root is bitter, thermogenic, emolient, stimulant, purgative, aphrodisiac, diuretic, emmenagogue, anthelmintic, febrifuge and tonic. Roots are useful in vitiated conditions of vata and pitta in Ayurveda. The Ayurvedic usage of roots still extend for constipation, nephropathy, strangury, dysmenorrhea, amenorrhea, elephantiasis, dropsy, neuropathy, constipation, ulcers, fever and delirium.

1.3.4.2. Leaves

Aphrodisiac, anthelmintic, tonic and used in ulcers, inflammation, helminthiasis, cephalalgia and general debility.

1.3.4.3. Seeds

Seeds of *M. pruriens* have a long history of use in Indian Ayurvedic medicine, where they are used for worms, dysentery, diarrhea, snakebite, sexual debility, cough, tuberculosis, impotence, rheumatic disorders, muscular pain, gonorrhea, sterility, gout, delirium, dysmenorrhea, diabetes and cancer. In India, seed is considered as an aphrodisiac, emmenagogue, uterine stimulant, nerve tonic, diuretic, and blood purifier.
In Central America, *Mucuna* beans have been roasted and ground to make a coffee substitute for decades and is widely known as ‘nescafe’ for this reason. The bean is cooked as a vegetable. In Brazil, the seed has been used internally for Parkinson’s disease, edema, impotence, intestinal gas and worms. It is considered a diuretic, nerve tonic and aphrodisiac. Externally it is applied to ulcers. Seeds are astringent, laxative, anthelmintic, aphrodisiac, alexipharmic and tonic. They are useful in gonorrhoea, consumption, sterility, vitiated conditions of vata and general debility. The hairs and flowers are vermifuge. *Mucuna* seeds are used for treating Parkinson’s disease, because of the high concentration of L-dopa in the seeds that has been studied for its possible use in Parkinson’s disease.

1.3.5. Review of Literature

Traditionally, in India, the seeds of *Mucuna pruriens* are used as a tonic and aphrodisiac for male virility, increases testosterone. The pods are anathematic seeds are anti-inflammatory (Anonymous, 1962). The plant extracts are reported to possess antidiabetic function (Grover et al., 2002), anti-snake venom properties (Roberto et al., 2008). It is also known as a muscle builder and anabolic or androgenic, stimulates growth hormone and as a weight loss aid. *Mucuna pruriens* possesses hypoglycemic and hypocholesterolemic effects in the normal rats (Pant et al., 1968). Antineoplastic activity of *M. pruriens* was evaluated by several studies. Gupta et al., (1997) reported the antineoplastic efficacy in their search for anticancer plants (Gupta et al., 1997). Tripathi and Upadhyay (2001) established the antioxidant activity on *in vivo* models of lipid peroxidation. The antidiabetic efficacy of *Mucuna pruriens* L. has been established by many researchers (Akhtar et al., 1990; Grover et al., 2002). Grover et al., (2003) reported this property in their search for antidiabetic plants in alloxan induced diabetic rats. Rathi et al., (2002) evaluated the alcoholic extract of *M. pruriens* (100, 200 and 400 mg kg\(^{-1}\) day) in alloxan induced rats and streptozotocin (STZ) mice.
*M. pruriens* cotyledon powder showed significant increase in the brain mitochondrial complex I activity (*in vitro*) on the nigrostriated tract of 6-OHDA lesioned rats. The endogenous levodopa, dopamine, norepinephrine and serotonin content in the *substantia nigra* were restored significantly. The possible mechanism, as reported for the neurorestorative activity was shown due to increased complex I activity and also the presence of NADH and Co-enzyme Q in the *M. pruriens* cotyledon powder (Manyam et al., 2004). In the study by Poornachandra *et al.*, (2005) *M. pruriens* showed significant activity on learning skills and memory enhancement in Wistar male rats.

The traditional aphrodisiac property of this plant is scientifically evaluated and proved by several experiments using seeds of *M. pruriens*. According to the studies of Amin *et al.*, (1996), *M. pruriens* stimulates sexual function in normal male rats which was observed by increase in mounting frequency, intromission frequency and ejaculation latency whereas, Rajendran *et al.*, (1997) proved the decrease in sexual function in female rats. The anti-venom activity is studied extensively and established by Guerranti *et al.*, (2002). The antivenom property of water extract of seeds was assessed *in vivo* in mice and tested for its immunological properties. Two proteins of *Echis carinatus* venom with apparent molecular masses of 25 and 16 kDa were detected. The results demonstrated that the observed anti-venom activity has an immune mechanism. Antibodies of mice treated with non-lethal doses of venom reacted against some proteins of *M. pruriens* extract (Guerranti *et al.*, 2002). Yerra Rajeshwar *et al.*, (2005) reported the antimicrobial activity of the methanolic extract of *M. pruriens* by disc diffusion method against gram-positive and gram-negative bacteria. Extract showed a broad spectrum of activity against all bacterial strains tested. Ekanem *et al.*, (2004) proved that the crude methanolic extract of leaves of *Mucuna pruriens* has potential for effective control of *I. multifiliis* infection in Goldfish. Hishikar *et al.*, (1981), has reported the anti-inflammatory activity of seeds of *M. pruriens*, while analgesic and antipyretic effects of *M. pruriens* was reported by Lauk *et al.*, (1993). Prurieninine, an alkaloidal from *M. pruriens* is reported to
slow down the heart, dilate the blood vessels, decrease blood pressure and increase the peristaltic action of intestine of frogs (The Wealth of India, 1985). The acetone extract of the root was toxic to the insects, being 1.93 times as toxic as the extract of *Jatropha curcas* Linn. seeds. The hairs on fresh/dry pods can cause intense itching on contact. It may also cause blister/dermatitis due to mucanin. The use of unprocessed, raw seed in diets for both humans and chickens is often accompanied by toxic symptoms. In human, symptoms of neurotoxicity and behavioral changes as well as severe vomiting have been reported by Miller *et al.*, (1925) and Infante *et al.*, (1990).

*Mucuna pruriens* significantly inhibited the oxidation of lipids and deoxyribose sugar Muralikrishnan *et al.*, (2008). *Mucuna pruriens* exhibited divalent iron chelating activity and did not show any genotoxic/mutagenic effect on the plasmid DNA. These results suggest that the neuroprotective and neurorestorative effect of *Mucuna pruriens* may be related to its antioxidant activity independent of the symptomatic effect. In addition, the drug appears to be therapeutically safe in the treatment of patients with Parkinson’s disease.

Ketan *et al.*, (2008) have shown a selective, precise and accurate high-performance thin-layer chromatographic (HPTLC) method for the analysis of L-dopa in *Mucuna pruriens* seed extract and its formulations. The method involves densitometric evaluation of L-dopa after resolving it by HPTLC on silica gel plates with n-butanol–acetic acid–water as the mobile phase. They proposed TLC method was found to be precise, specific and accurate, and can be used for identification and quantitative determination of L-dopa in herbal extract and its formulations. Okorie, (2008) studied on assessment of the haematological, serum indices and chemical constituents of eggs from laying hens fed *M. pruriens* leaf meal diets, and suggested that, the inclusion of *M. pruriens* leaf meal in diets of laying hens will enhance all the blood indices at up to 7.5% level of inclusion without any deleterious effects.
1.3.6. Micropropagation of *M. pruriens*

An extensive survey on micropropagation of *M. pruriens* revealed that very few data are available in this field. A rapid micropropagation system was developed for *Mucuna pruriens* f. pruriens using explants from 1-week old aseptically grown seedlings. Multiple shoot regeneration occurred following an initial callus growth on Revised Tobacco (RT) medium (Kaul and Staba, 1968) supplemented with 2.7 μM NAA and 9.8 μM 2-iP. Maximum number of shoot regeneration was achieved only from 6 to 7 days old seedling explant. More than 90% of the regenerated shoots could be rooted on half-strength liquid RT medium supplemented with 2.7 μM NAA. Plantlets readily adapted to greenhouse conditions. This system provides a new tool for micropropagation of *Mucuna pruriens* f. pruriens, an important medicinal plant (Sharmila *et al.*, 1995). Apart from these Sathyanarayana *et al.*, 2008 have reported a rapid and reliable method for high fidelity micropropagation on *Mucuna pruriens* var. utilis. Auxiliary bud explants from 14-day old seedlings were cultured on Murashige and Skoog's (MS) (1962) medium supplemented with different concentrations of cytokinins.


*Mucuna prurita* Hook. is synonymous member of *M. pruriens* L., but produces seeds with white seed coat.

1.4.1. Distribution and Habitat

*M. prurita* grows all over India, cosmopolitan in the tropics, in the Andaman and Nicobar islands and in other parts of the world like Srilanka, Bangladesh, Pakistan, Africa and America. It is an annual, twining herb with long cylindrical branches. Leaves alternate, 3-foliate; leaflets ovate or rhomboid, membranous and glabrescent above. Flowers purplish, solitary or 2-3 together on axillary racemes. Pods 4-6 seeded, turgid, falcately curved on both ends, densely clothed with persistent pale brown or steel grey, irritant bristles caused by histamine liberating alkaloids and a proteolytic enzyme (Du Puy *et al.*, 2002), mucunin in
the bristles. Seeds ovoid, compressed, smooth, brownish with large, oblong hilum (India). Pedicels shorter than the calyx, calyx cleft to the middle, white with adpressed hairs, segments broad-lanceolate, corolla papilionaceous, vexillum cordate, incumbent on the alae, alae oblong-linear, sometimes slightly cohering, keel straight below, slightly falcate in the upper part, terminated by an acute beak. Grow as climbing shrub on trees and shrubs along roadsides and form edges (Myanmar Medicinal Plant Database).

**Taxonomy of Mucuna prurita Hook.**

- **Kingdom**: Plantae, Planta, Planter, Plants, Vegetal.
- **Sub Kingdom**: Tracheobionta, Vascular Plants.
- **Division**: Magnoliophyta. (Angiospeens)
- **Class**: Magnoliopsida (Dicote, Dicotyledon)
- **Sub class**: Rosidae.
- **Order**: Fabales
- **Family**: Leguminoseae
- **Sub Family**: Fabaceae
- **Genus**: Mucuna
- **Species**: prurita

**Latin Name**


**Common name**

Cowhage, Cow-Itch, Alkushi, Bichchuti, Bilaiachra, Bandarola. (Bangladesh), Kaunch (India), Wanduru me, Naula, Meegahak, Pinna (Sri Lanka), Naicorma(English), Poonaykalie(Mallyam), Peeliadagoo kaila(Tamil), KIWach (Telugu), Kanchkoorie, Kaunch, Kevanch (Hindi), Alkushee, Beng(Duk.), Kapikacchu, Markachi, Kandura(Sanskrit)
1.4.2. Chemical and phytochemical composition

*M. prurita* has been found to contain L-DOPA. The plant/seeds contain the bioactive alkaloids mucunine, mucunadine, mucuadinine, pruriendine and nicotine, besides -sitosterol, glutathione, lecithin, oils, venolic and gallic acids. The seeds with seed coat showed the presence of a number of bioactive substances including tryptamine, alkylamines, steroids, flavonoids, coumarins, cardenolides, etc., (Myanmar Medicinal Plant Database).

1.4.3. Medicinal properties

**Parts used:** Seed, Root and leaves

The root is anthelmintic, aphrodisiac, astringent, diuretic and nervine tonic, useful in cholera, dropsy, general debility, leucorrhoea, seminal weakness and as a tonic. The leaves are aphrodisiac, anthelmintic and tonic, and are useful in ulcers, inflammation, cephalalgia and general debility. The seeds are astringent, laxative, alexipharmic and tonic. They are useful in constipation, gonorrhoea, sterility and general debility (Asia Pacific Medicinal Plant Database).

The total alkaloids from seeds of *M. prurita* comprising 5 alkaloidal bases were found to bring about a note-worthy increase in the population of spermatozoa and in the weights of body testes, seminal vesicles and prostrate of the treated rats. The exhibited activity was found to stimulate testosterone-enanthate induced androgenic activity observed in another set off treated individuals. Lower dose corresponding to the clinical dose significantly decreased the sleeping time, increased the motor activity and gave equivocal results in rotarod test in experimental animals. The high dose (3 times the clinical dose) significantly increased the sleeping time, decreased the motor activity and reduced the time for falling from the rod. Thus the drug possesses CNS stimulant effect at low doses and CNS depressant effect at high doses. (Myanmar medicinal plants database).
1.4.4. Review of Literature

Much research work has not been reported on the plant *M. prurita*. A very few investigations that were carried out on this particular plant species listed hereunder. Roy *et al.*, (1988) has published paper in American society of Microbiology on aflatoxin contamination of some common drug plants, where they have used the seeds of *M. prurita* to check the aflatoxin content. Laximinarain Mishra and Hildebert Wagner (2007) have worked on and have developed suitable methods for the extraction of L-DOPA and other active components from seeds using different solvents. They showed that L-DOPA could be obtained in good yield on extraction with ethanol water (1:1) using ascorbic acid as protector. Till-date no published reports are available pertaining to micropropagation or *in vitro* propagation of this plant species.

1.5. Lacuna or Gaps in the literature

Inspite of the immense medicinal value and pharmaceutical importance of these two *Mucuna* species, till-date no published reports are available on genetic transformation of these two legumes (*Mucuna* species) and also elicitor enhanced or precursor mediated production of their secondary metabolites including L-dopa. Similarly no published reports are available even pertaining to micropropagation or *in vitro* plant regeneration of *M. prurita*. 