CHAPTER 1

INTRODUCTION

1.1. General Introduction

“What exists in medicinal plants are, a design of nature developed over millenniums much like fractal geometry a system within nature and this is one reason all life is valuable for the chemistry or DNA within” - Russel Ade., Scientist.

Company of nature itself is big healer and has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. These plant based traditional medicine systems continue to play an essential role in health care, with about 80% of the world’s inhabitants relying mainly on traditional medicines for their primary health care. At least 119 chemical substances derived from 90 plant species are important drugs currently in use (Ashwani Kumar, 2009).

It is our pride that, ‘Indian medicine’ is the birthplace of all modern medicines exported to the world over time. India is sitting on a gold mine of well-recorded and well-practiced knowledge of traditional herbal medicine. It is hoped that, with the right combinations of drugs, medicinal plants and lifestyle, the quality of life and life expectancy can be increased. There has been explosion of scientific information concerning plants, crude plant extracts and various substances from plants as medical agents during last few decades. In Indian system of medicine the phytochemistry and
mechanism of plants in treating ailments remains largely unexplored. This has prompted researchers to focus their investigations to understand the holistic information specially their functional properties of such plants.

Herbs are the major source of natural products used as pharmaceuticals, agrochemicals, flavoring agents, ingredients in food additives and pesticides (Balandrin et al., 1988). Over time humanity found plants suitable for consumption as functional foods, and medicinal purposes. The fact is that the plants rich in GABA-active flavones are not common food plants, but fall within the category of medicinal plants (Anna K Jager, 2011). Many researchers have discussed the importance of medicinal plants as sources of new therapeutic agents (Cragg and Newman, 2005) and others have effectively focused on the potential of specific chemical classes (e.g., Flavonoids, Alkaloids) in drug discovery.

Nowadays chemicals decide the importance of herb and several plants and its products consumed as food has been slowly dragged into medicinal category (Fig. 1.1). For example curcumin (turmeric), resveratrol (red grapes, peanuts and berries), Genistein (soybean), diallyl sulfide (allium), S-allyl cysteine (allium), allicin (garlic), lycopene (tomato), capsaicin (red chilli), diosgenin (fenugreek), 6-gingerol (ginger), ellagic acid (pomegranate), ursolic acid (apple, pears, prunes), silibinin (milk thistle), anethol (anise, camphor, and fennel), catechins (green tea), eugenol (clove), indole-3-carbinol (cruciferous vegetables), limonene (citrus fruits), beta carotene (carrots), dietary fiber had started exploring for their medicinal value. So the current research scenario admits priority in search for new plant derived chemicals towards sustainable conservation and rational utilization of biodiversity.
Phytochemicals mostly fall in the category of alkaloids, terpenoids, phytosterols, saponins and flavonoids which are responsible for the biological potency of respective herbs. Secondary metabolites have many applications in pharma industry. Flavonoids have been discovered for their roles in plants and as medicine.

Phenolic compounds are aromatic organic compounds with at least one hydroxyl group attached directly to a benzene ring. These are hydroxylated derivatives of benzoic acid, present in the form of esters and glycosides. On the basis of C-skeleton, polyphenols are classified as flavonoid and phenolic acid (Slade et al., 2005). Polyphenols are the major secondary metabolites present in plants and have various beneficial effects in multitude of disease by their antioxidant property (William et al., 2004).

Flavonoids are secondary metabolites characterised by flavan (2-Phenylbenzopyran) nucleus (Heim et al., 2002) and C_6-C_8-C_6 carbon-skeleton. These are group of structurally related compounds with a chromane-type skeleton having phenyl substituent in C_2-C_3 position. The basic structural feature of flavonoid is 2-phenyl-benzo-γ-pyrane nucleus consisting of two benzene rings (A and B) linked through a heterocyclic pyran ring (C). Flavonoids differ in their arrangement of hydroxyl, methoxy and glycosidic side groups and in the conjunction between A and B rings. A variation in C ring provides division of subclasses. Based on molecular structure (Fig. 1.2), they are classified as follows flavone, flavonon, flavonol, isoflavone, anthocyanidine, catechin, dihydroflavonol and chalcone (Peterson J Dwyer, 1998, Tsuchiya, 2010, Rijke et al., 2006).
Flavonoids are widely distributed among the plant kingdom (Cushine et al., 2005). Flavonoids are found in vegetables, fruits, nuts, seeds, stems, flowers, tea, wine etc. These are an integral part of our daily diet (Cook et al., 1996, Sahu et al., 1996, Prey et al., 2003). They have been reported to exert wide range of biological activities. These includes: anti-inflammatory, antibacterial, antiviral, antiallergic (Cushnie et al., 2005, Murray, 1998, Cook et al., 1996) cytotoxic, antitumour, treatment of neurodegenerative diseases, vasodilatory action (William et al., 2004, Murray, 1998, Tsuchiya, 2010, Chebil et al., 2006).

In addition flavonoids are known to inhibit lipid-peroxidation, platelet aggregation, capillary permeability and fragility, cyclo-oxygenase and lipoxygenase enzyme activities. They exert these effects as antioxidants, free radical scavengers, chelators of divalent cation (Cook et al., 1996, Chebil et al., 2006, Middleton et al., 2000). These are also reported to inhibit variety of enzymes like hydrolases, hyaluronidase, alkaline phosphatase, arylsulphatase, cAMP phosphodiesterase, lipase, α-glucosidase and kinase (Narayana et al., 2001).

In flavonoidal classifications Isoflavones form a group of distinct secondary metabolites produced predominantly in leguminous plants (Oliver Yu et al., 2000). Isoflavones establish the symbiotic relationship between the plant and rhizobial bacteria for the formation of nitrogen-fixing root nodules, where isoflavones act as chemo-attractants for the rhizobial bacteria and as inducers of nod gene expression (Van Rhijn and Vanderleyden, 1995, Pueppke, 1996). Another major role is during the disease resistance response, where the synthesis of isoflavones is induced to provide defence compounds. The isoflavone Daidzein is the precursor to the major phytoalexins including
medicarpin and glyceollins, which are produced in alfalfa and soybean, respectively (Blount et al., 1992, Graham, 1995). The isoflavone Genistein has antifungal activity (Rivera Vargas et al., 1993), which is also the precursor to phytoalexin kievitone made by *Phaseolus vulgaris* (Garcia Arenal et al., 1978). There are evidences for isoflavones play a role in human health as a dietary component (Davis et al., 1999, Messina, 1999).

Sustaining the nutritional requirements is one of the important tasks for any developing as well as developed countries. Nevertheless, combating several existing and newly spreading dreadful diseases is a major problem. In resolving these combined factors, potential herbs should be identified and their properties need to be evaluated. Many herbs in current use have any one of the above resolving capacity. But from the traditional and scientific data available, legumes have promising potential source in terms of nutrition, medicine and agricultural development in developing countries.

One such known legume is *Mucuna pruriens*. Its L-dopa content is scientifically proved to be a very effective in neurodegenerative disorders. It is also a best nutritional source as it contains rich nutrients especially protein and carbohydrate. *Mucuna* can be processed properly and can be utilized as best nutrient and medicine. L-dopa isolated from *Mucuna* was found to be more effective than the synthetic product (Hussain et al., 1997). Extracts of *M. pruriens* has been evaluated worldwide and concluded as a potential medicinal herb. Approximately 120 species of *Mucuna* had been reported so far (Evans, 2002) and 130 species according to the Zipcodezoo Data Base. In India, 15 species were identified and reported (Anonymous, 2005).
Fig. 1.1. Chemicals from functional food identified as medicine
Various works in taxonomical and nutritional characters on different geographical accession were reported by many scientists (Gurumoorthi et al., 2003). The herb is very much acceptable as livestock feed after removing the anti-nutritional constituents.
Scanty information is available on pharmacological efficacy other than antiparkinson and aphrodisiac activities for *Mucuna* genus. Apart from *Mucuna pruriens* almost all other species were underexplored. Keeping this in view the present investigations were carried out to explore the pharmacological potency of an unexplored species in *Mucuna* genus- *Mucuna cochinchinensis*.

1.1.1. Cancer

Cancer is an uncontrolled growth of cells and it is the major public health burden in both developed and developing countries and a serious threat to human life with complex pathogenesis. The transformation of normal to cancerous cell involves three distinct phases i.e., initiation, promotion and progression. Dietary habits are known to modify each of these phases. Plant derived agents are being used for the treatment of cancer. Several anticancer agents including taxol, vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan and etoposide derived from epipodophyllotoxin are in clinical use all over the world. A number of promising agents such as flavopiridol, roscovitine, combretastatin A-4, betulinic acid and silvestrol are in clinical or preclinical development (Mohammad Shoeb, 2006).

Many chemotherapeutic drugs have been developed to treat cancer, including DNA-alkylating agents and antimitotic agents. However, their complex synthesis, difficult formulation, lack of bioavailability in oral administration and chemoresistance by cells, makes these drugs sub-optimum for clinical treatment of cancer (Wenlong Fang et al., 2011). The factors need to be addressed by the researchers to combat the treatment complications in cancer.
The International Agency for Research on Cancer (IARC) reported that in 2012, an estimated 14.1 million new cancer cases and 8.2 million cancer related deaths occurred in 2012, compared with 12.7 million and 7.6 million respectively in 2008. Prevalence estimates for 2012 show that there were 32.6 million people (over the age of 15 years) alive, diagnosed in the previous five years.

The most commonly diagnosed cancers worldwide were those of the lung (1.8 million, 13.0% of the total), breast (1.7 million, 11.9%) and colorectum (1.4 million, 9.7%). The most common causes of cancer death were cancers of the lung (1.6 million, 19.4% of the total), liver (0.8 million, 9.1%) and stomach (0.7 million, 8.8%). Projections based on the GLOBOCAN 2012 estimates predict a substantive increase to 19.3 million new cancer cases per year by 2025, due to growth and ageing of the global population. More than half of all cancers (56.8%) and cancer deaths (64.9%) in 2012 occurred in less developed regions of the world and these proportions will increase further by 2025.

Breast cancer is a malignant tumor that starts from cells of the breast. A malignant tumor is a group of cancer cells that may grow into (invade) surrounding tissues or spread (metastasis) to distant areas of the body. The disease occurs almost entirely in women, but men can get it, too. In 2012, 1.7 million women were diagnosed with breast cancer and there were 6.3 million women alive who had been diagnosed with breast cancer in the previous five years. Since the 2008 estimates, breast cancer incidence has increased by more than 20%, while mortality has increased by 14%. Breast cancer is also the most common cause of cancer death among women (522 000 deaths in 2012) and the most
frequently diagnosed cancer among women in 140 of 184 countries worldwide. It now represents one in four of all cancers in women (WHO, 2013).

“Breast cancer is also a leading cause of cancer death in the less developed countries of the world. This is partly because a shift in lifestyles is causing an increase in incidence and partly because clinical advances to combat the disease are not reaching women living in these regions,” - Dr David Forman, Head, IARC

Generally, worldwide trends show that in developing countries going through rapid societal and economic changes, the shift towards lifestyles typical of industrialized countries leads to a rising burden of cancers associated with reproductive, dietary and hormonal risk factors.

Incidence has been increasing in most regions of the world, but there are huge inequalities between rich and poor countries. Incidence rates remain highest in more developed regions, but mortality is relatively much higher in less developed countries due to a lack of early detection and access to treatment facilities. For example, in Western Europe, breast cancer incidence has reached more than 90 new cases / 100,000 women annually, compared with 30 per 100,000 in eastern Africa. In contrast, breast cancer mortality rates in these two regions are almost identical, at about 15 / 100,000, which clearly points to a later diagnosis and much poorer survival in eastern Africa.

“An urgent need in cancer control today is to develop effective and affordable approaches to the early detection, diagnosis, and treatment of breast cancer among women living in less developed countries,” - Dr Christopher Wild, Director, IARC.
Breast cancer is the most frequently diagnosed cancer among adolescent and young adult (AYA) women between 15 and 39 years of age, accounting for 14% of all AYA cancer diagnoses and 7% of all breast cancer. Diagnosed Breast cancers are now recognized as heterogeneous, based on tumor expression of receptors for estrogen (ER), progesterone (PR) referred to jointly as hormone receptor (HR) and human epidermal growth factor receptor 2 (HER-2). AYA breast cancer incidence differs from that in older women, with AYAs having higher proportions of HR positive / HER-2 positive, triple-negative, and HR negative / HER-2 positive breast cancer subtypes and higher proportions of patients of non-White race / ethnicity than older women (Theresa et al., 2013).

1.1.2. Diabetes mellitus

Diabetes mellitus is chronic metabolic disorders that affect human body in terms of physical, psychological and social health. It is defined as a group of disorders characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins. It is becoming the third “killer” of the health of mankind along with cancer and cerebrovascular diseases (Chauhan et al., 2010). The prevalence of diabetes mellitus is expected to reach up to 4.4% in 2030 and the occurrence was found to be high in India, China and USA. Historical accounts reveal that as early as 200 BC, diabetes mellitus was well recognized disease in India even as distinguished in two types: a genetically based disorder and a dietary related disorder (Grover et al., 2002) Among all the cases of diabetes, type 2 diabetes was found to be more prevalent (Balaraman et al., 2010).
Many plant extracts and compounds were investigated and reported as antidiabetic. Newer molecular approaches may benefit the industry for deriving the mechanisms underlying the activity. These approaches will pave the way for economic and safety molecules to enter management in diabetes mellitus.

1.1.3. Microbial infections

Among the infinite varieties of microorganisms, relatively few cause disease in healthy individuals. Infectious disease results from the interplay between those few pathogens and the defences of the hosts they infect. The appearance and severity of disease resulting from any pathogen depends upon the ability of that pathogen to damage the host as well as the ability of the host to resist the pathogen.

According to National Institute of Allergy and Infectious diseases, microbial infections are the second most (26%) next to cardiovascular diseases (29%) common cause for overall deaths. Deaths among infections, respiratory infection stands first followed by HIV, diarrheal, tuberculosis, vaccines preventable childhood diseases, malaria, STD, meningitis, hepatitis B&C, tropical parasitic disease, dengue and other infections.

Extensive researches are being in progress to defend these infections from natural sources. However existing antimicrobial groups such as penicillin semisynthetic pencillins, quinalones, macrolides, cephalosporins, tetracyclines are effective, the need for constant and continuous research is due to the wide spectrum of chemo-resistance developed by microbes.
Antimicrobial resistance is one of our most serious health threats. Infections from resistant bacteria are now too common and some pathogens have even become resistant to multiple types or classes of antibiotics (antimicrobials used to treat bacterial infections). The loss of effective antibiotics will undermine our ability to fight infectious diseases and manage the infectious complications common in vulnerable patients undergoing chemotherapy for cancer, dialysis for renal failure and surgery, especially organ transplantation, for which the ability to treat secondary infections is crucial.

When first-line and then second-line antibiotic treatment options are limited by resistance or unavailable, healthcare providers are forced to use antibiotics that may be more toxic to the patient and frequently more expensive and less effective. To nullify these problems natural sources like plant and marine need to be bio-processed. Usually nature has defending ability to invade foreign objects and frequent changes will be there for adaptation to survive.
1.2. Literature Review

1.2.1. Review on Mucuna Genus

*Mucuna* genus belongs to the family Fabaceae. This is the second largest family of flowering plants and contains 600 genera and about 12000 species (Evans, 2002). The leaves are stipulate nearly always alternate and range from bipinnately or palmately compound to simple. The petiole base is commonly enlarged into a pulvinus that commonly functions in orientation of the leaves. The flowers are usually bisexual actinomorphic to zygomorphic, slightly to strongly perigynous and commonly in racemes, spikes or heads. The perianth commonly one or many stamens distinct of variously united sometimes. The pistil is simple often stipulate comprising a single style and stigma and a superior ovary with one locule containing two or many marginal ovules. The fruit is usually a legume sometimes a loments, follicle, indehiscent pod, achene, drupe or berry. The seeds often have a hard coat with hour glass shaped cells and sometimes bear a u-shaped line called plaerogram (Evans, 2002). *Mucuna* seeds collected from different locations show different botanical features, and environment has no interference in genetic diversities of *Mucuna* (Gurumoorthi et al., 2003).

The seeds are traditionally used as nervine tonic, emmenagogue, astringent, aphrodisiac, leucorrhrea and paralysis. The hairs of the pods are vermifuge and treated for round worm infections. *Mucuna monosperma* is used as an expectorant and sedative given in cough and asthma (Khory and Katarat, 1999). Bark powder mixed with dry ginger is used for rubbing over painful rheumatic joints (Nadkarni, 1982). The roots are bitter, thermogenic, emollient, stimulant, purgative, aphrodisiac, diuretic, emmenagogue,
anthelmintic, febrifuge, diuretic and tonic. In Ayurveda they are useful in vitiated conditions of *vata* and *pitta*, constipation, nephropathy, strangury, dysmenorrhea, amenorrhea, elephantiasis, dropsy, neuropathy, ulcers, helminthiasis, fever, delirium and for treating Parkinson’s disease. The leaves are aphrodisiac, anthelmintic and are useful in ulcers, inflammation, helminthiasis and in general debility. The seeds are astringent, laxative, anthelmintic and aphrodisiac. They are useful in gonorrhea, sterility, vitiated conditions of *vata* and general debility (Anonymous, 2002). The seeds are restorative and are sometimes consumed as a vegetable (Anonymous, 2005). Seed diet produced hypoglycemic effect in normal rats (Anonymous, 2006).

*Mucuna* form a rich source of protein, carbohydrate, lipid, fiber, minerals and amino acids. Eight different species of *Mucuna* were studied viz *M.cochinchinensis, M. jaspeada, M. veracruz, M.gigantean, M.monosperma, M. pruriens, M. solanei, M.utilis* (Ezeagu et al., 2003, Rajaram et al., 1991, Mohan et al., 1995, Siddhuraju et al., 1996, Afolabi et al., 1985, Ravindran et al., 1985) for its nutritional property. The ranges of the compositions in eight *Mucuna* accessions were as follows, crude protein (24 - 31.44 %), crude carbohydrate (42.79 - 64.88 %), crude lipid (4.1 - 14.39 %), crude fiber (5.3 - 11.5 %), ash (2.9 - 5.5 %). For minerals, 12 different species were studied (Ezeagu et al., 2003) and their constituents ranged from, 806 - 2790 mg/100g for potassium, 4 - 70 mg/100g for sodium, 104 - 900 mg/100g for calcium, 98 - 498 mg/100g for phosphorus, 85 - 477 mg/100g for magnesium, 1.3 - 15 mg/100g for iron, 0.33 - 4.34 mg/100g for copper, 1 - 15 mg/100g for zinc and 0.56 - 9.26 mg/100g for manganese. Various amino acids were reported in *M.cochinchinensis, M. pruriens* and *M. solanei* such as glutamic acid, aspartic acid, serine, threonine, proline, alanine, glycine, valine, cystine,
methionine, isoleucine, leucine, tyrosine, phenyl alanine, tryptophan, lysine, histidine and arginine supporting the genus for nutritional value (Sidduraju et al., 1996, Afolabi et al., 1985, Ravindran et al., 1985, Adebowa et al., 2005, Sridhar et al., 2007).

Extract of the whole herb is reported to have L-dopa as a major constituent and mainly in seeds (Bell et al., 1971, Dhamodharan et al., 1937). Four alkaloids in Mucuna pruriens seeds were recently reported. They are L-3-carboxy-1, 2, 3, 4-tetrahydroisoquinoline, (-)-1-methyl-3carboxy-6, 7-dihydroxy-1, 2, 3, 4-tetrahydroisoquinoline, dimethyl-3carboxy-6, 7-dihydroxy-1, 2, 3, 4-tetrahydroisoquinoline and (-)-1-3-carboxy-1, 1-dimethyl-7, 8-dihydroxy-1, 2, 3, 4-tetrahydroisoquinoline (Misra et al., 2004). Phytochemical and ethno botanical database (Dr. Dukes) describes diversified chemical constituents in Mucuna seeds like, 5-hydroxytryptamine, 5-methoxy- N, N- dimethyltryptamine- N- oxide, 5-oxyindole-3-alkylamine, 6-methoxyharman, arahidic- acid, arginine, asparticacid, behenicacid, betacarbole, betasitosterol, bufotenine, choline, cis-12, 13-poxyoctadec- trans-9-cis-acid, cis-12, 13-epoxyoctadec- trans-9-eniocacid, gallicacid, glutamicacid, glutathione, indole-3-alkylamine, linoleicacid, linoleicacid, Mucunadine, Mucunain, mucynine, myristic-acid, N, N- dimethyltryptamine, N, N- dimethyltryptamine, -N-oxide, niacin, nicotine, oleicacid, palmiticacid, prurienine, riboflavin, saponins, serotonin, stearicacid, thiamine, vernolicacid. In Mucuna leaves the data base reveals the presence of L-dopa, 6-methoxyharman, Genistein, hydroxyGenistein in minimal concentration. Recently three new lipid derivatives were also reported, triactont-5, 7, 9-triene, docos-2, 4, 6-triene-1, 8-diol and docos-5-en-1-oic acid (Misra et al., 2006).
Many *Mucuna* species have been reported processing medicinal value apart from nutritional value and as fodder crop (Caius, 1989). The main use of this herb is to treat the symptomatic effects in Parkinson’s disease. The constituents bufotenine, choline, β-carboline were reported for their antiepileptic and antineoplastic activity (Gupta *et al*., 1997). *Mucuna birdwoodiana* seeds are also used to treat joint pain and irregular menstruation (Ding *et al*., 1991). *Mucuna* pod hairs blended with honey can be used as vermifuge. *Mucuna* seed powder is used to treat leucorrhea, spermatorrhea (Nadkarni, 1982). Seeds posses anabolic, androgenic, analgesic, anti-inflammatory, antispasmodic, antivenom, aphrodisiac, febrifuge, cholesterol lowering, hypoglycemic, immunomodulator, antilithiatic, antibacterial, antiparasitic, cough suppressant, blood purifier, carminative, hypotensive and uterine stimulant properties (Sridhar *et al*., 2007).

Daidzein and Genistein were the main isoflavones responsible for the antioxidant activity present in soybean and *Mucuna*. The total Daidzein and Genistein in *Mucuna* was found to be higher than in soybean, while it is the opposite in Indonesian traditional food (Tempe) formulation. Factor II (6, 7, 4; trihydroxy isoflavone) and Genistein in *Mucuna* and its Tempe were higher than in soybeans. *Mucuna* and its Tempe contain higher Factor II (6, 7, 4; trihydroxy isoflavone) and lower Daidzein and glycitein than that of soybeans (Wuryani, 1994). Comparative analysis between traditional *Mucuna* seed Tempe and soybean Tempe revealed the following results; *Mucuna* Tempe had a higher dietary fibre level, but lower vitamin E content. The *Mucuna* Tempe contains 31.5% protein, 7.3% fat, 3.0% ash, 58.1% carbohydrate and 9.1% fibre. It contains 0.551 mg/L isoflavone aglucone; Daidzein is the highest, followed by Factor II (6, 7, 4 trihydroxy isoflavone) that is much higher than that of soybeans Tempe. These are much higher
isoflavone aglucone contents than found in soybeans Tempe (Sri Handjani, 2001). From these studies it is evident that *Mucuna* is a good nutritional supplement well comparable to soybean. *Mucuna* has been tested for its several pharmacological activities for the past decades. The pharmacological evidence reports that *Mucuna* is one of the major constituents in polyherbal extract formulations for treating different ailments. Few recent evidences are discussed below.

1.2.1.1. Anti-Parkinson’s effect

The extract of *Mucuna pruriens* used for Parkinson’s disease (MPE) is known to contain, among other components, 12.5% L-dihydroxyphenylalanine (L-dopa), as compared to the equivalent doses of L-dopa (Kasture *et al.*, 2009). An acute administration of MPE at a dose of 16 mg/kg (containing 2 mg/kg of L-dopa) consistently antagonized the deficit in latency of step initiation and adjusting step induced by a unilateral 6-hydroxypaminale lesion, whereas L-dopa was equally effective only at the doses of 6 mg/kg. At the same dosage, MPE significantly improved the placement of the forelimb in vibrissae-evoked forelimb placing, suggesting a significant antagonistic activity on both motor and sensory-motor deficits. The effects of MPE were moreover investigated by means of the turning behavior test and in the induction of abnormal involuntary movements (AIMs) after either acute or sub-chronic administration. MPE acutely induced a significantly higher contra lateral turning behavior than L-dopa (6 mg/kg) when administered at a dose of 48 mg/kg contains 6 mg/kg of L-dopa. On sub chronic administration, both MPE (48 mg/kg) and L-dopa (6 mg/kg) induced sensitization of contra lateral turning behavior; however, L-dopa alone induced a concomitant sensitization in AIMs suggesting that the dyskinetic potential of *M. pruriens*
is lower than that of L-dopa. *M. pruriens* (48 mg/kg) was also effective in antagonizing tremulous jaw movements induced by tacrine, a validated test reproducing Parkinsonian tremor. Furthermore, *M. pruriens* induced no compartment preference in the place preference test, indicating the lack of components characterized by rewarding effects in the extract. Finally, in a sub-chronic mice model of 1- methyl- 4- phenyl- 1, 2, 3, 6 tetrahydropyridine hydrochloride (MPTP) - induced dopamine neuron degeneration, MPE did not prove, capable of preventing either tyrosine hydroxylase decrease induced by MPTP or astrogial or microglial activation as assessed by means of glial fibrillary acidic protein (GFAP) and CD11b immunohistochemistry, supporting the absence of neuroprotective effects by *Mucuna pruriens*. Characterization of MPE strongly supports its anti-Parkinson’s activity.

Also one another study proved the neuroprotective effects of *M. pruriens* in which the neurorestorative effect of *M. pruriens* cotyledon powder on the nigrostriatal tract of 6-hydroxyl dopamine (6-OHDA) lesioned rats was evaluated (Manyam *et al.*, 2004). The results revealed that *M. pruriens* cotyledon powder significantly increased the brain mitochondrial complex-I activity but did not affect the total monoamine oxidase activity *(in-vitro)* and unlike synthetic L-dopa treatment, *M. pruriens* cotyledon powder treatment significantly restored the endogenous L-dopa, dopamine, norepinephrine and serotonin content in the substantia nigra. Nicotine adenine dinucleotide (NADH) and coenzyme Q-10 that are shown to have a therapeutic benefit in Parkinson's disease were present in the *M. pruriens* cotyledon powder. Earlier studies showed that *M. pruriens* treatment controls the symptoms of Parkinson's disease. The additional finding of a neurorestorative benefit by *M. pruriens* cotyledon powder on the degenerating dopaminergic neurons in the
substantia nigra may be due to increased complex-I activity and the presence of NADH and coenzyme Q-10.

1.2.1.2. Aphrodisiac effect

The second most potential effect proved for this Mucuna is aphrodisiac. The Mucuna pruriens, ethanolic extract administered in either sex rats significantly increased the mounting frequency, intromission frequency and ejaculation latency and decreased the mounting latency, intromission latency, post-ejaculatory interval and inter-intromission interval. The potency test significantly increased erections, quick flips, long flips and total reflex. The ethanolic extracts of M. pruriens seed produced a significant and sustained increase in the sexual activity of normal male rats at a particular dose (200mg/kg), when compared to the control (Suresh et al., 2009).

1.2.1.3. Effect on Fertility

Mucuna pruriens improves male fertility by its action on the hypothalamus-pituitary-gonadal axis. A study on treatment with M. pruriens significantly improved serum testosterone, luteinizing hormone, dopamine, adrenaline and noradrenaline levels in infertile men and reduce the levels of follicle stimulating hormone (FSH) and prolactine hormone (PRL). Sperm count and motility were significantly recovered in infertile men (Shukla et al., 2009). The quality of seminal changes due to psychological stress was assessed after treating the case with M. pruriens seed powder at 5 g/ day orally. For carrying out morphological and biochemical analysis, semen samples were collected twice, first before starting the treatment and second after 3 months of treatment. The results demonstrated the decreased sperm count and motility in subjects who were under psychological stress. Moreover, serum cortisol and seminal plasma lipid peroxide
levels were also found elevated along with decreased seminal plasma glutathione (GSH) and ascorbic acid contents, reduced superoxide dismutase (SOD) and catalase activity. Treatment with *M. pruriens* significantly ameliorated psychological stress and seminal plasma lipid peroxide levels along with improved sperm count and motility. Treatment also restored the levels of SOD, catalase, GSH and ascorbic acid in seminal plasma of infertile men. *M. pruriens* not only reactivates the antioxidant defence system of infertile men but also helps in the management of stress and improves semen quality (Shukla *et al.*, 2007). The effects of *Mucuna urens* on the gonads of male Guinea pigs were investigated and found to be the potential male antifertility agent even at a lower dosage of 70mg/kg (Paul Udoh *et al.*, 2001).

**1.2.1.4. Antioxidant effect**

The antioxidant activity on *in vivo* models of lipid peroxidation concluded that the seed ethanolic extract of *Mucuna pruriens* has an antilipid peroxidation property which is mediated through the removal of superoxides and hydroxyl radicals (Tripathi, 2001). Experiment on *in vitro* lipid peroxidation of *M. pruriens* seeds revealed the inhibition of ascorbate/FeSO$_4$ induced peroxidation by methanolic extract of *M. pruriens* which was monitored by the changes in optical density of the prepared concentrations (10-320 μg/ml). The inhibition increased with increase in concentration of the extract (Rajeshwar *et al.*, 2005a).

**1.2.1.5. Antitumor effect**

The antitumor effect and antioxidant role of methanolic extract of *Mucuna pruriens* seed against Erlich Acites Carcinoma (EAC) bearing Swiss albino mice were studied. The effect of methanolic extract of *M. pruriens* on tumor growth and host’s
survival time was studied by the following parameters; tumor volume, packed cell volume viable and non viable cell count and life span of the host. Extract was administered at 125 and 250 mg/kg body weight once daily for 14 days, starting after 24 h of tumor inoculation. Decrease in tumor volume, packed cell volume and viable cell count were observed in extract treated animals when compared to EAC treated animals. Treatment with extract at a dose of 125 and 250 mg/kg increased the mean survival time to 29.5± 0.55 and 34± 0.2 days respectively. The extract also decreased the body weight of the EAC tumor bearing mice. There was a significant decrease in WBC count and increase in RBC counts in extract treated animals when compared to EAC treated animals. The study was also extended to estimate the liver biochemical parameters such as LPO, GSH and antioxidant enzymes like SOD, catalase etc. Treatment with extract decreased the levels of lipid peroxidation and increased the levels of glutathione, superoxide dismutase and catalase. The results suggest that the methanolic extract of *M. pruriens* seeds exhibits significant antitumor and antioxidant effects in EAC bearing mice (Rajeshwar *et al.*, 2005b).

### 1.2.1.6. Antidiabetic effect

Many reports have been established relating to antidiabetic property of *Mucuna*. The hypoglycemic activity of *M. pruriens* ethanolic extract in alloxan induced rats and streptozotocin induced mice produced the maximum activity at 6th week in 200mg/kg/day dose (Rathi *et al.*, 2002). A comparative study of the hypoglycemic effect of aqueous extract of the seeds of *M. pruriens* between normal, glucose load conditions and streptozotocin induced diabetic rats were analyzed. The results showed that in normal rats, the aqueous extract of the seeds of *M. pruriens* (100 and 200 mg/kg body weight)
significantly (p<0.001) reduced the blood glucose levels after an oral glucose load from 127.5 ± 3.2 to 75.6 ± 4.8 mg % 2 h after oral administration of MPE seed extract. It also significantly lowered the blood glucose in streptozotocin induced diabetic rats from 240.5 ± 7.2 to 90.6 ± 5.6 mg % after 21 days of treatment (p<0.001). Thus, the study concludes that *M. pruriens* has an antihyperglycemic action and it could be a source of hypoglycemic compounds (Anusha Bhaskar *et al.*, 2008). Comparative evaluation of hypoglycemic activity of some ethanolic extracts of Indian medicinal plants in alloxan induced diabetes condition was done and the plants are positioned according to the significant blood glucose lowering activities in decreasing order in the following 24 samples: *Coccinia indica, Tragia involucrata, Gymema sylvestre, Pterocarpus marsupium, Trigonella foenum-graecum, Moringa oleifera, Eugenia jambolana, Tinospora cordifolia, Swertia chirayita, Momordica charantia, Ficus glomerata, Ficus benghalensis, Vinca rosea, Premna integrifolia, Mucuna prurita, Terminalia bellirica, Sesbenia aegyptiaca, Azadirachta indica, Dendrocalamus hamiltonii, Zingiber officinale, Aegle marmelos, Cinnamomum tamala, Trichosanthes cucumerina and Ocimum sanctum* (Kar *et al.*, 2003).

**1.2.1.7. Antibacterial effect**

Antibacterial activity of methanolic extract of *Mucuna pruriens* was evaluated and well documented the broad spectrum activity against all strains used (Rajeswar *et al.*, 2005a). The methanolic extract of *Mucuna monosperma* seeds were evaluated for its antibacterial activity. The result was broad spectrum as it showed activity against Gram positive *Bacillus cereus, Staphylococcus* and Gram negative *Proteus vulgaris* (Manjunatha *et al.*, 2006).
1.2.1.8. Antiprotozoal effect

Methanolic extract of leaves of *Mucuna pruriens* has the potency to eradicate *Lichthyophtirius multifilis* infection (90%) in gold fish after treatment in baths of plant extracts at 200 mg/l and parasite induced fish mortality was reduced significantly. The *in vitro* studies shows 100% mortality of parasite tested in 150 mg/l of *M. pruriens* extract (Ekanem *et al.*, 2004).

1.2.1.9. Study of analgesic and anti-inflammatory activity

*Mucuna pruriens* was evaluated for its anti-inflammatory, analgesic and antipyretic activity and found to produce significant effects (Hishikar *et al.*, 1981, Lauk *et al.*, 1993).

1.2.1.10. Anti Snake venom effect

The protective effects of *Mucuna pruriens* seed extract (MPE) against histopathological changes induced by intravenous injection of *Naja sputatrix* (Malayan cobra) venom in rats pretreated with the MPE seed extract was examined. Examination by light microscope revealed that the venom induced histopathological changes in heart and blood vessels in liver, but no effect on brain, lung, kidney and spleen. The induced changes were prevented by pretreatment of the rats with MPE. Finally it is suggested that MPE pretreatment protects rat heart and liver blood vessels against cobra venom induced damages (Fung *et al.*, 2009).

MPE pretreatment was given to rats and the animals were challenged with various snake venoms (Tan *et al.*, 2009). The effectiveness of MPE to neutralize the lethalities of snake venoms was investigated by *in vitro* neutralization and concluded as MPE
effectively protect the animal models against lethality of *Naja sputatrix* venom and moderate protection against *Calloselasma rhodostoma* venom. Indirect ELISA and immunoblotting studies showed that there were extensive cross-reactions between anti-MPE IgG and venoms from many different genera of poisonous snakes, suggesting the involvement of immunological neutralization in the protective effect of MPE pre-treatment against snake venom poisoning. *In-vitro* neutralization experiments also showed that the anti-MPE antibodies effectively neutralized the lethalities of *Asiatic cobra* (Naja) venoms, but were not very effective against other venoms tested.

**1.2.2. Review on flavonoids against cancer, diabetes and microbial infections**

Flavonoids are potent bioactive molecules that possess anticarcinogenic effects since they can interfere with the initiation, development and progression of cancer by the modulation of cellular proliferation, differentiation, apoptosis, angiogenesis and metastasis. The flavonols quercetin, kaempferol and galangin and the flavones apigenin have been reported to inhibit cytochrome P450 enzymes of the CYP1A family. Quercetin and naringin have also been shown to inhibit CYP3A4, which is the most abundant P450 enzyme in the liver and beneficial in metabolizing a significant number of carcinogens and medications (Havsteen, 2002). Quercetin is abundantly found in human diet and it gets extensively metabolized during absorption in the small intestine and in the liver, and thus exerts a dose dependent inhibitory effect on cell proliferation (Ramos, 2007). In addition, animal and *in-vitro* studies have shown that tea catechins increase the activity of several detoxifying and antioxidant enzymes, such as glutathione reductase, glutathione peroxidase, glutathione S-reductase, catalase, and quinone reductase (Havsteen, 2002, Ramos, 2007). In estrogen dependent tumor cells or animal models, this anti-proliferative
effect has been related to the antiestrogenic properties of certain flavonoids. In other in-vitro models, flavonoids have also been to affect cell signaling and cell cycle progression. For example, tea flavonoids inhibit signal transduction pathways mediated by epidermal growth factor and platelet derived growth factor, favorably affecting downstream events such as angiogenesis. Genistein and quercetin inhibit protein tyrosine kinase which is also involved in cell proliferation (Havsteen, 2002, Ramos, 2007, Lamson et al., 2000). Finally, apigenin, luteolin and quercetin have been shown to cause cell cycle arrest and apoptosis by a p53 dependent mechanism (Marchand, 2002). In a nutshell, multiple mechanisms have been identified for the anti-neoplastic effects of flavonoids, including antioxidant, anti-inflammatory and anti-proliferative activities, inhibition of bioactivating enzymes, and induction of detoxifying enzymes.

Studies suggest that flavonoids, in particular isoflavones such as Genistein might be detrimental to memory processes in the brain due to their ability to act as tyrosine kinase inhibitors. Researches revealed that isoflavones results in positive effects on neuro-cognitive functions which is apparent in post-menopausal women. Activation of both synaptic plasticity and new neural growth may act together to enhance memory and cognition. It has been found that flavonoids subclasses flavonols, flavanones, flavones and anthocyanins do not exert estrogen like effects (Spencer et al., 2009).

All flavonoids cannot cure diabetes mellitus because most types of this disease are basically genetic and no single drug can correct an inborn error. However, flavonoids can ameliorate some of the consequences of diabetes mellitus (Havsteen, 2002). Flavonoids have been identified to be good inhibitors of aldose reductase (Patra et al., 2010). It has been reported by several researchers that quercetin possess antidiabetic activity and it has
been found that it brings about regeneration of pancreatic islets and increases insulin release in streptozotocin-induced diabetes. Also, it has been reported to stimulate Ca\(^{2+}\) uptake from isolated islet cells thus suggesting it to be effective even in non-insulin dependent diabetes (Tapas et al., 2008). Flavonoids in Ipomoea batalas leaf possesses antidiabetic activity against alloxan-induced diabetes at a dose of 100 mg/kg (Li et al., 2009). Fisetin is reported to be a therapeutic agent for treatment of diabetes mellitus at a dose of 10 mg/kg (Sriram et al., 2011).

Flavonoids have been used extensively since centuries for the treatment of various diseases. Propolis has been referred even in Old Testament for its healing properties. The antimicrobial activity of propolis has been attributed to its high flavonoids content. Galangin is a flavonol commonly found in propolis. It has been reported to possess inhibitory actions against Aspergillus tamarri, Aspergillus flavus, Cladosporium sphaerospermum, Pencillium digitatum, Penicillium italicum (Cushnie, 2005). Quercetin, naringenin are reported to be inhibitors of Bacillus subtilis, Candida albicans, Escherichia coli, Staphylococcus nervous, Staphylococcus epidermis, Saccharomyces cerevisiae (Taleb Contini et al., 2003).

Morin-3-O-lyxoside, morin-3-O-arabinoside, quercetin, quercetin-3-O-arabinoside were isolated from Psidium guajava leaves and reported that these four possess bacteriostatic action against all food borne pathogenic bacteria including Bacillus stearothermophilus, Brochothrix thermosphacta, Escherichia coli, Listeria monocytogenes, Pseudomonas fluorescens, Salmonella enteric, Staphylococcus aureus, Vibrio cholera (Rattanachaikunsopon et al., 2010). Flavonones having sugar moiety showed antimicrobial activity while none of the flavonols and flavonolignans showed
inhibitory activity on microorganisms. Quercetin has been reported to completely inhibit growth of *Staphylococcus aureus* (Tapas *et al.*, 2008). Thus the minor group in flavonoid classification isoflavones are being considered by scientific community for their potential in pharmaceutical industry.
1.3. Scope and aim of current study

Anti cancer, antimicrobial and antidiabetic treatments pose risk in development of chemical resistance due to various factors as well as serious side effects. It becomes mandatory to address the resistance factors also. Multiple signaling pathways are often disrupted in ailments particularly with cancer cells. It is hoped that targeting multiple signaling pathways may reduce the development of chemical resistance and also the adverse reactions. Most targeted therapies are being developed and in clinical trials for testing combined treatment pattern to combat cancer. Mucuna seeds are well documented as analgesic, anti-inflammatory, antispasmodic, aphrodisiac, febrifuge, anti-cholesterol, hypoglycemic, immunomodulator, antilithiatic, antibacterial, blood purifier, carminative, hypotensive and uterine stimulant. It is also reported to have antiepileptic and antineoplastic property. Unique morphological characters of Mucuna cochinchinensis drew the attention, as it lacks in a specialized property of stings on the pods. So the current study focused on biological evaluation of unexplored species M. cochinchinensis.

Nutritional value of M. cochinchinensis is as good as soybeans and even better than any other legumes. Many of the legumes possess multiple uses such as food, fodder and pharmaceuticals. Some legume seeds are known for anti-cancerous compounds that retard or arrest the cancer growth. For instance, an isoflavone ‘Genistein’ derived from Pueraria lobata (kudzu beans) has the unique property to retard cancer growth.

The diversities in genus Mucuna needs further intensive attention and exploration by the researchers for their potency in pharmaceutical and nutraceutical industry. Many of the pharmacological activities, responsible bioactive constituents and their mechanisms
in *M. cochinchinensis* need to be derived. Hence this study was decided to put the plant to undergo a systematic evaluation on *Mucuna* to explore its pharmacological potency and the responsible bioactive principle.

This study aimed at standardizing the pharmacognostical characters, analyzing the phytoconstituents and to evaluate the anticancer, antidiabetic and antimicrobial potency of *M. cochinchinensis* seeds. Further the study aimed to understand the effects of *M. cochinchinensis* seed extract on silencing the chemo-resistance factor (Antiapoptotic protein) Bcl-2 in tumor tissues and to study the effluxing ability of microbes to resist the drug.
1.4. Plant Profile

Fig. 1.3. *Mucuna cochinchinensis* whole plant

Fig. 1.4. *Mucuna cochinchinensis* pods and flowers
Table 1.1 Taxonomy of *Mucuna cochinensis*. L. Cheval.

<table>
<thead>
<tr>
<th>Taxonomy</th>
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**Habitat** : Cultivated in Western Ghats of Kerala and Tamilnadu  

**Synonum** : *Mucuna nivea, Stizolobium niveum Kuntze*  

**Common Name** : Lyon Bean  

**Tamil** : Poonapidukkan  

**Bengal Name** : Khamach  

**Mundari** : Kursi  

**Use** : Nutritional and medicinal  

**Activity reported** : Antiparkinson’s and Hemagglutin  

**Chemical constituent** : L-dopa
1.5. Plan of work

**Mucuna cochinchinensis**

- **Extract**
  - Pharmacognostical characterisation
  - Phytochemical analysis
  - Biological activities

<table>
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