CHAPTER - 3

Materials & Methods
(Mucuna pruriens)

2.8. Identification of Valepotriates from roots of *Nymphoides macrospermum*

The bioactive Dichloromethane extract of roots of *Nymphoides macrospermum* was subjected to HPTLC and GC-MS analysis for the identification of valepotriates. Methods are described in section 4.4 of chapter 4 (Results and Discussion).

2.9. Determination of content of marker in methanol extract (NMR) of roots of *Nymphoides macrospermum*

Betulinic acid is one of the marker constituent identified from the roots.Therefore, to ensure identity and quality of this plant a simple, sensitive, specific and reproducible HPTLC method was developed for the quantification of betulinic acid. The method is described in detail in section 4.6 of Chapter 4(Results and Discussion).

2.10 Isolation and characterization of constituents from bioactive extract.

The bioactive dichloromethane extract of roots of *Nymphoides macrospermum* was subjected to column chromatography, method is described in section 4.7 of Chapter 4 (Results and Discussion)

Present section deals with description of methods employed for carrying out different studies, on the roots of *Mucuna pruriens.*

Pharmacognostical parameters

- Macroscopical studies
- Microscopical studies
- Determination of physicochemical constants

Determination of inorganic elements including the heavy metals

Determination of Microbial content

Phytochemical parameters

- Preliminary phytochemical studies
- HPTLC fingerprint profile of the successive extracts of the selected plant materials
- Determination of Phenolic content.
- Estimation of suitable marker content in the extract.
- Isolation and characterization of the compounds from bioactive extract/fraction.
- Development of HPTLC method for quality control of the selected plant materials using a suitable marker.

Biological parameters

- Toxicity studies of the extracts of the selected plant materials
- Evaluation of the extracts for anti-stress and immunomodulatory activitites using suitable models .

3.1 Pharmacognostical parameters

3.1.1 Collection and identification of plant material

Roots of *Mucuna pruriens* were collected from the outfield of Vadodara city, Gujarat, India and were authenticated from Botanical Survey of India, Southern Circle, Coimbatore. Voucher specimen (MP/05-06/05/KM) have been deposited in the Pharmacy Department of The M.S University of Baroda, Vadodara, India.

The roots of *Mucuna pruriens* were subjected to macroscopic, microscopic evaluation, proximate analysis, heavy metals including inorganic trace elements and microbial content determination as per the methods described in section 2.1 of Chapter 2.

3.2 Preliminary phytochemical studies

Successive solvent extraction and qualitative chemical analysis of successive extracts to identify the presence of various chemical constituents were carried out as described in section 2.2 of chapter 2.

3.2.1 Preparation of Total Methanolic extract

Coarsely powdered roots of *Mucuna pruriens* (500g) was extracted with Methanol by hot extraction process (Soxhlet). After completion of extraction, the solvent was removed by distillation and concentrated *in vacuo*.

3.3 Acute toxicity studies

Toxicity study of total methanol and successive extracts were performed as per OECD guidelines in female albino mice. Animals were dosed with single oral dose of 2000 mg/kg body weight and observed for mortality. Mice were observed for any reactions like tremors, convulsions, salivation, and diarrhoea. 1/10th of the highest tolerable dose was used as a safe dose for further in vivo studies .

3.4 Evaluation of total methanolic extract and successive extracts of roots of *Mucuna pruriens* for Adaptogenic activity.

The investigations for anti-stress and immunomodulatory activities of total methanol extract and successive extracts was done using different models as described in section 2.3 of Chapter 2.

Immunomodulatory activity was proved with Antibody titre, DTH response, Phagocytic function, E coli induced abdominal sepsis, and Cyclophosphamide induced myelosuppression models in mice and Anti-stress activity by swimming endurance test.

Total methanol extract (MPR) was screened at 100, 200 & 400mg/kg b.w, and successive petroleum ether extract (MPRPE) at 25, 50 & 100mg/kg, ethyl acetate extract (MPREAE) and methanol extract (MPRME) at 50,100 & 200 mg/kg b.w and aqueous extract (MPRWE) at 100 & 200 mg/kg dose levels.

3.5 Development of comparative HPTLC fingerprint profile of the extracts of roots and seeds of *Mucuna pruriens*

Preliminary phytochemical screening showed the presence of phytosterols, phenolic compounds and amino acids. Therefore, total methanol extract containing the above constituents and the successive extracts were used for fingerprint studies. A comparative fingerprinting study was also performed with extracts of seeds of *Mucuna pruriens*. Compounds of varying polarity in the extracts were separated using various solvent systems on TLC. The HPTLC fingerprint profile comprising of typical spectra, Rf values, UV λ_{Max} and relative percentage of the separated compounds were then recorded.

Table 3.1. Solvent systems used for recording the HPTLC finger print profiles of extracts of roots of *Mucuna pruriens*

	Solvent system
1	Toluene: Chloroform: Ethyl acetate: Acetic acid (10:2:1:0.03) v/v
2	Toluene :Ethyl acetate: Methanol: Water (10:5:2.5:1)
3	Ethyl acetate: Formic acid:Acetic acid:Water (8:1:0.4:1)
4	Toluene: Formic acid:Ethyl formate (5:1:4)
5	n-Butanol:Acetic acid:Water (4:1:1)

Solvent systems 1 & 2 were used to resolve the non polar compounds and the separated compounds (steroids & terpenoids) were detected by derivatization with anisaldehyde sulphuric acid whereas solvent systems 3 & 4 were used to resolve medium polar and polar compounds. Solvent system 5 was used to resolve amino acids and amines, which were detected by derivatization with Ninhydrin.

3.6

Determination of content of marker in methanol extract of roots of *Mucuna pruriens*

The phytochemical and co-TLC studies confirmed the presence of L-dopa and β-sitosterol in roots of *Mucuna pruriens* therefore simple, sensitive, selective and precise high-performance thin-layer chromatographic (HPTLC) methods for the analysis of β-sitosterol and L-dopa in bioactive extracts of roots of *Mucuna pruriens* were developed and validated.

The methods are described in detail in the results section (Chapter 5).