CHAPTER- 5

SUMMARY AND CONCLUSIONS

5.1. Summary

Current research entitled “Pharmacognostical, Phytochemical and Biological Evaluation of unexplored Mucuna cochinchinensis (Lour) Cheval Seeds” was chosen and carried out with the objective of standardizing the pharmacognostical characters, extraction of phytoconstituents from seeds using methanol and ethyl acetate solvents and to understand the major phytoconstituents present in the ethyl acetate extract by modern analytical techniques. Further to quantify and to determine various biological activities of M. cochinchinensis seeds. To fulfill the above objective, a systematic approach was adopted and executed.

The whole thesis is divided into five chapters as follows

Chapter 1 dealt with general introduction on phytomedicine research, selected herb description and related literature review which supports the entire concept in this thesis.

Chapter 2 dealt with our first aim, to standardize the pharmacognosy of Mucuna cochinchinensis seed and its nutritional value.

Chapter 3 dealt with our second aim, to understand the chemical architect, to confirm the presence and to quantify the target molecules in the selected seed.
Chapter 4 dealt with our prime as well as our third aim to evaluate the potency of *Mucuna cochinchinensis* seed in various ailments like cancer, microbial infection and diabetes mellitus emphasizing the resistance factor in antibreast cancer (Bcl-2) treatment.

Chapter 5 dealt with the summary and conclusions.

*Mucuna* genus belongs to the family Fabaceae and the entire parts of the plant are used by ethnic groups in treating various ailments. In Ayurvedic system, powder of *Mucuna* seeds is used for treating Parkinson’s disease. The content, isoflavones in soyabean, proved to prevent and treat breast cancer. *Mucuna* is reported to have higher content of isoflavones. The scientific works on *Mucuna cochinchinensis* are very meager and that is why this plant was selected for the present study.

*M. cochinchinensis* seeds were collected from Mundanthurai forest of Western Ghats, Tamilnadu, India and were identified and authenticated by renowned Botanist. Pharmacognostical studies of *M. cochinchinensis* seeds have been studied for its microscopic features, nutritional potency and preliminary phytochemical as well. By these studies the selected taxa was confirmed.

Methanolic (MMC) and ethyl acetate (EMC) extractions of *M. cochinchinensis* seeds were done and evaluated. The phytochemical screening of MMC and EMC revealed the presence of alkaloids, phenols, flavonoids, amino acids, quinines, steroids and carbohydrate in both the extracts. EMC showed higher quantity of flavonoidal content (16.884 ± 0.157) and phenolic content (6.56 ± 0.051). Phenolic and flavonoidal content of MMC was found to be 4.96 ± 0.048 and 4.093 ± 0.031 respectively.
In order to realize a qualitative analysis on the compounds present in EMC, the extract was analyzed by Liquid Chromatography / Electron spray Ionization / Mass Spectroscopy (LC/ESI/MS) using Bruker UHPLC 3000 chromatograph coupled to a quadrupole-ToF mass selective detector (microToF QII, Bruker, Germany). Genistein, Daidzein, Kaempferol-3-O-rutinoside and 6-C-pentosyl-8-C-hexosyl apigenin were confirmed. This is the first study to report Kaempferol-3-O-rutinoside and 6-C-pentosyl-8-C-hexosyl apigenin in *Mucuna* genus. The concentration of Genistein, Daidzein and L-dopa were quantified using HPTLC and found to be 1.19, 1.27 and 5.18 % w/w respectively. The quantification methods were also validated using ICH guidelines and found to be simple, precise and linear.

MMC and EMC exhibit good antioxidant property under various mechanisms tested. EMC proved to exhibit potent lipid peroxidation (29.63 ± 0.57 µg/ml) even when compared to standard (232.33 ± 2.52 µg/ml).

Extract was subjected to acute, sub-acute and chronic toxicity evaluations using suitable animal models and found to be very safe. From the experimental observations, LD$_{50}$ and ED$_{50}$ were calculated as 2000mg/kg and 200 mg/kg respectively.

MMC and EMC were preliminarily screened for anticancer activity (*in-vitro*) using MTT assay, tested at various concentrations ranging from 62.5 - 1000 µg/ml against HeLa - Human cervical cancer cell line, HEP-2 - Human laryngeal epithelial carcinoma cell, MCF7- Breast cancer, NTH 3T3- Mouse embryonic fibroblasts (Standard) and IC$_{50}$ values were calculated. The results revealed that EMC showed potent
IC$_{50}$ value for all tested cell line except NIH 3T3 normal cells (IC$_{50}$ > 1000 µg/ml) whereas MMC doesn’t produce any significant effect compared to EMC.

So further anti-breast cancer activity of EMC was performed in 7, 12-Dimethyl Benzanthracene (DMBA) induced rat model. 90 days study revealed the reduction in tumor size, reversion back of body weight, enzyme and non-enzyme antioxidants in serum and breast homogenate tissue parameters to near normal level significantly different from breast cancer control (p<0.01). Bcl-2 is a survival protein very well expressed in breast cancer which confers resistance to apoptosis and thereby reducing the effectiveness of chemotherapy. Inhibition of Bcl-2 leads to the apoptosis of cancer cells by inhibiting the auto-repair through mutation. Binding ability of Genistein and Daidzein with Bcl-2 was evaluated in this study by in-silico technique which indicates that these compounds occupy positions in binding pocket with low energy of 5.33 and 5.66 respectively. Expressions of Bcl-2 in experimental breast tissues were further studied by extracting total proteins from breast tissues and resolved in 12% SDS-Page and blotted (Western blot) against anti-Bcl-2 serum. The results revealed that the intensity of bands (Bcl-2) produced in blot for treatment with EMC group shows significant reduction compared to tumor control. Thereby it is confirmed the presence of apoptotic enhancing property in EMC. Estrogenic potentiality of the extract was also evaluated using alkaline phosphatase induction assay and the extract showed a clear dose-dependent estrogenic effect.

MMC and EMC shows antibacterial activity against *S. aureus, B. subtilis, B. clausii, K. pneumonia, P. vulgaris* and *E. coli* at 100 µg concentration itself and increased activity with increasing concentrations. Both the extracts do not inhibit *A. niger* and *C.*
albicans. Further MIC and MBC for both extracts determined by tube dilution and solid agar method, revealed that MIC ranges from 62.5-125µg/ml for all the tested bacterial strains and MBC ranges from 125-250µg/ml. Resistant fungal strains were subjected to efflux ability studies. Maximum effluxing ability was determined from the experimental data and it was found to be 105 min and 75 min by C. albicans for MMC and EMC respectively and 45 min and 105 min by Aspergillus niger for MMC and EMC respectively. Profound synergistic effect of inhibition was observed with combined MMC and standard Clotrimoxazole when compared to the standard alone; against C. albicans indicates the modulating efficacy of MMC.

Effects of EMC on alloxan induced diabetic rats were studied. Parameters such as blood glucose (1, 7 and 14th day) and plasma insulin levels were determined and found to revert normal by EMC significantly (p<0.01). Thereby it is proved that EMC have good antidiabetic activity.

5.2. Conclusions

The existence in development of chemo-resistance due to the disruption in multiple signaling pathways leads to the increasing attention in identifying newer target molecules from natural sources. Combined effects of target molecules with standard treatment may benefit to combat ailments. This is the current novel approach and many clinical trials are under progress. Conferring resistance to chemical substances may influenced by many factors one such is B Cell Lymphoma proteins. In this study an attempt was made to silence the Bcl-2 expression in breast cancer experimental rat models by ethyl acetate extract of M. cochinchinensis seed (EMC). The striking results
were observed with low intensity of Bcl-2 expressions in (EMC + Standard) Group IV compared to tumor control and even with standard tamoxifene treated. This substantiates the usage of extract in chemo-resistance breast tumor.

*Mucuna* is one of the major constituents in more than 200 indigenous preparations. Unique morphological and microscopical characters observed and reported in *M. cochinchinensis* facilitate the identification, cultivation and to avoid the adulteration. Spectral studies confirmed the presence of isoflavones in EMC and this is the first study to report Kaempferol -O- rutinoside and 6-C- pentosyl- 8-C apigenin, later a proven antidiabetic compound and the antidiabetic property reported in this study may be attributed to its presence. Lipid peroxidation result revealed the selected species as potent antioxidant. MMC and EMC are potent antibacterial however MMC has efflux modulating capacity in *Candida albicans*.

Upon effective processing of *Mucuna cochinchinensis*, it is possible to develop the extracts with acceptable ratio of nutritional and the bioactive constituents for the use in indigenous drug formulations. Exploration and exploitation of diversity without affecting the biodiversity and the fragile ecosystem will improve this pharma potential herb to the optimum heights.