Summary

Ethno botanical bioprospection takes advantage of traditional medicinal knowledge for identifying potential phytochemical drugs (Figure 1). The commercialization of medicinal plant resources has become rapid due to the identification, purification and characterization of active bio-molecules for various therapeutic purposes which have been vital for the present scenario. Because, the synthetic drugs exhibit severe side effects along with their mode of action. Hence, “bioprospection” plays an important role in the identification of the novel bio-molecules from the natural resources, especially from plants, having less side effects and they are cost effective. Hence, an active research is required for the identification of the plants which produces active compounds with therapeutic value. The current research aimed at evaluating potential role of *Strychnos genus* in bioprospection and targeted towards identifying lead molecules for the Multiple myeloma (MM) therapy.

Genus *Strychnos* is commonly seed propagated, as vegetative propagation through conventional methods is inefficient, reduced span of seed viability and low germination rate while commercial exploitation for production led to dwindling in natural population. Consequently micropropagation techniques are the ideal for rapid production of these plants. Attempts were made to propagate the recalcitrant *S. nux-vomica and S. potatorum* by *in vitro* and *in vivo* techniques. In natural conditions, because of its hard seed coat, the percentage of germination is very poor and lengthy (70-120 days to
germinate). This problem can be solved by \textit{in vitro} culture of embryos, there by reducing the germination time and increasing the germination rate. \textit{In vitro} shoot multiplication from \textit{in vitro} raised explants was successful. Sporadic rooting was obtained in \textit{S. potatorum} where as \textit{S. nuxvomica} did not respond. Acclimatization to natural habitat was poor and further experimentation is required.

\textit{S. wallichiana} is one of the rich sources of important bioactive indole alkaloids. In the present study, strychnine and brucine were isolated from the roots and seeds of \textit{S. wallichiana}. Strychnine and brucine were separated by RP-HPLC. The purified strychnine and brucine were characterized by subjecting to the spectral analysis to reconfirm identity and elucidate the structure. The spectral data (IR, $^1$H-NMR, $^{13}$C-NMR and LC –MS) correlates with the existing literature and reconfirms the presence of strychnine and brucine in south Indian \textit{S. wallichiana’s} roots and seeds. The described HPLC procedure could be useful for the qualitative and quantitative analysis of alkaloids of the Loganiaceae family. Evaluation of the pharmacological importance of ethanolic root extracts (\textit{S. nux-vomica, S. wallichiana}) on the MM cell lines (RPMI 8226, and U226B1) showed dose and time dependent growth inhibition. Qualitative and quantitative apoptotic studies were carried out thereby, the elucidation of the underlying molecular mechanisms. Morphological assessment of the cells (Light, Phase, SEM, and TEM) revealed cells were undergoing apoptosis. Nuclear staining assays (by using fluorescent probes like DAPI and Hoechst 33258) were performed by the confocal laser microscope. The flow cytometer analysis revealed loss in DNA content indicating that cell
cycle arrest at G0/G1 phase and preventing cells from entering S or G2/M phase and finally apoptosis occurred. Mitochondrial depolarization was assessed by the flowcytometry using Rhodamine123 derivatives and found that loss in membrane potentials, eventual leakage of mitochondrial proteins, cytochrome c, into cytosol and was detected by the Western blot analysis. Syndecan-1 (CD138) expressed on the surface of MM cells, acts as a multifunctional regulator of the cell behavior in the tumor microenvironment and was used as a standard marker for identification of the tumor cells in the MM patients. It is also used as standard marker for differentiating the viable and apoptotic cells. The present study was undertaken to check the CD138 expression levels in the U226 B1 cell line treated with the plant extracts. The flowcytometer analysis of CD138 expression level using FITC labeled CD 138 antibody showed dose dependent decrease in expression levels with increase in concentration of plant extracts indicating cells under apoptosis.

In order to understand the role of active principles in *S. nux-vomica* and *S. wallichiana* root extracts, attempts were made to analyze the extracts by the various analytical Methods (TLC, HPLC, IR spectra, $^1$H-NMR, $^{13}$C-NMR and LC-MS). The two potential bio-molecules (Strychnine and brucine) were identified, which are common in these plants. Strychnine and brucine showed dose and time dependent growth inhibition on the MM cell lines (RPMI 8226, and U226B1). Qualitative and quantitative apoptotic studies were carried out similarly as said above. The above results confirmed *Strychnos* extracts and its bio-molecules have anti proliferative properties. The eventual
accomplishment of this study is to find a more effective anti-cancer drug by properly modifying the structure of these active bio-molecules. This will provide new information for the design of new cancer chemo preventive agents. Thus, the experimental model was established in this work and will surely accelerate the understanding of the structure–activity relationships in these bio-molecules and pave the way for drug discovery. The executive summary of work carried out on “Bioprospecting Indian Strychnos” was demonstrated in the figure (59).
Figure 59. Executive summary of work carried out on “Bioprospecting Indian Strychnos”