3 Plant material and road map to work

3.1 Study area

Andhra Pradesh is rich in traditional knowledge districts namely Adilabad, Anantapur, Chittor, Cuddapah, Nellore, Prakasam, Kurnool, East Godavari, Karimnagar, Khammam, Mahaboobnagar, Srikakulam, Visakhapatnam, Vizayanagaram, Warangal and West Godavari, were chosen for field study (Figure 8). Plant genetic resources are rich in these areas together with folklore and traditional knowledge. Pullaiah and Chennaiah (1998) recorded 2531 species in the State of Andhra Pradesh, of which about 400 are endemic. Globalization and market economies are partly responsible to the loss of plant genetic resources (Tables 3 and 4). The following areas (district in bold face followed by locality) have been surveyed and *Strychnos nux-vomica*, *S. potatorum* and *S. wallichiana* (Figures 9-11) germplasm was collected: Srikakulam (Mahendragiri hills), Khammam (Taliperu, Kothur), Warangal (Pakala, Tandwai, Mallur, Atmukur), Medak (Narasapur), Ranga Reddy (Gachibowli), Nalgonda (Mannanur, Vatavaripally), Kurnool (Srisailam, Dornal), Kadapa (Balpally Siddhavotam, Sanipaya, Gadela, Rayachoti, Rajampet, Kodur), Chittoor (Talakona, Tirupathi, Thirumala, (Sri Venkataeswara University Campus, Bakrapeta), Nellore (Rapur, Penchalakona, Tegacherla, Somasila, Kutlamarri, Mallemadugu, Veligonda Hills), Prakasam (Chinnaruntla). Outlines of work carried out were shown in figure 12.
1. Srikakulam Mahendragiri hills
2. Khammam Taliperu, Kothur
3. Warangal Pakala, Tandwai, Mallur, Atmukur
4. Medak Narasapur
5. Ranga Reddy Gachibowli
6. Nalgonda Mannanur, Vatavaripally
7. Kurnool Srisailam, Dornal
8. Kadapa; Balpally Siddhavotam, Sanipaya, Gadela, Rayachoti, Rajampet, Kodur, Rajampet
9. Chittoor Talakona, Tirupathi, Thirumala, S.V.U. Campus, Bakrapeta
10. Nellore: Rapur, Penchalakona, Tegacherla, somasila, kutlamarri, Mallemadugu, Veligonda Hills
11. Prakasam Chinnaruntla

Figure 8: Collection of Strychnos nux-vomica, S. potatorum and S. wallichiana germplasm from different localities of Andhra Pradesh
Figure 9: Natural habitat, floral and morphological characters of *Strychnos potatorum*

*a.* Naturally grown *S. potatorum* in University of Hyderabad campus; Ranga Reddy Dist.

*b.* Fruit setting in the month of November

*c.* Flowering in the month of September

*d.* Sun dried seeds

*e.* Mature fruits
Figure 10: Natural habitat, floral and morphological characters of *Strychnos nux-vomica*

*a.* Naturally grown *S. nux-vomica* invetavarupally Kurnool Dist.

*b.* Fruit setting in the month of March.

*c.* Flowering in the month of January

*d.* Sun dried seeds.

*e.* Mature fruits.
Figure 11: Natural habitat, floral and morphological characters of *Strychnos wallichiana*

*a.* & *b.* Habit of *S. wallichiana* in Kutlamarr valley, Rapur veligonda range, Nellore Dist.

c. Fruit setting in the month of April
**In vitro differentiation for multiplication**

1. Field survey throughout Andhra Pradesh
2. In vivo germination
3. Seed coat texture study by scanning, light and fluorescent microscope
4. In vitro germination from embryos
5. In vitro shoot multiplication from in vitro raised explants
6. In vitro rooting was partially successful
7. Acclimatization was poor

- Preparation of alkaloid extracts: Roots, seeds were shade dried, and homogenized into powder - Soxlet extraction using organic solvents - Concentration of extracts by flash Rota evaporator - TLC, HPLC, separation of strychnine and brucine
- Characterization based on IR, \(^1\)H NMR, \(^{13}\)C NMR & LC –MS
- Anti-proliferative and cytotoxic properties of plant extracts and its compounds on MM cell lines
- Screening of plant extracts and compounds on multiple myeloma cell lines 8226, U226.
- Cytotoxicity assessment by MTT assays by plant extracts and compounds.
- Determination of IC\(_{50}\) values
- Morphological assessment of cells by Microscopy (Light, phase contrast, SEM & TEM)
- Nuclear fragmentation in cells were detected by DAPI & Hoechst 33258 stain using confocal microscope.
- Cell cycle analysis by FACS in RPMI 8226 cell lines treated with S.N extract and compounds.
- Mitochondria membrane damage estimation by Rhodamin 123 derivative using flow cytometer.
- Cytochrome-c leakage by western blot.
- CD 138 expression analysis in U226 in response to treatment with extracts and compounds.

**Figure 12:** Outlines of work carried out