Chapter 4

Plan of Work

The plan of work was chalked out in the sequence given below based on the aims and objectives presented in chapter 3.

- Collection of the selected plants authentication, pharmacognostic studies, shade drying and preparation of the extracts, phytochemical screening, chromatographic fractionation of the extracts, followed by *in vitro* antioxidant studies.
- Column chromatographic studies for the separation and quantification of the active constituents in the more active fraction.
- UV, IR, MS, NMR spectroscopic studies of the separated constituents and their interpretations.
- Rat hemidiaphragm glucose uptake studies.
- *In vivo* studies including acute toxicity studies (OECD 423) and antidiabetic evaluation using male *Wistar albino* rats.
- Docking studies using the identified constituents.
- Evaluation of mechanism of action using cell line studies (Glut-4 translocation and PPARγ upregulation studies).
- Compiling of all the results, interpreting, arriving at a conclusion and final report preparation.

It was decided to identify the plant with the help of a botanist and collect it in bulk during the period of September to December. The above time period is suggested for plant collection because of luxuriant growth after monsoon followed by good exposure to sunlight. It is thus expected to be rich in the content of secondary metabolites in all the parts of the plant including leaf and root. Shade drying is a precautionary measure followed to avoid the destruction of active principles.

Pharmacognostic studies using fresh samples were envisaged in the next step.
Fifty percent ethanol and ninety five percent ethanol extracts were separately prepared to take care of the more water soluble components also.

It was decided to perform ABTS, DPPH, nitric oxide scavenging and lipid peroxidation methods for the evaluation of antioxidant activity as the latter is nowadays accredited with much relevance in the field of antidiabetic activity. ABTS and DPPH methods work well in both lipophilic and hydrophilic media.

*In vitro* evaluation by MTT assay on L-6 cell line was also suggested to ascertain toxicity, if any, of the extract. *In vitro* antidiabetic screening by enzyme inhibition and cell line studies using L-6 cell lines were proposed in continuation to antioxidant studies. Alpha amylase inhibition, alpha glucosidase inhibition and DPP-IV inhibition studies were proposed under enzyme inhibition studies. Cell line studies proposed were glucose uptake by L-6 cell lines, Glut-4 translocation studies and PPARγ upregulation. The cell line studies were designed to locate the best active fraction in adherence to the RRR concept of CPCSEA guidelines.

Identification of the phytoconstituents present in the active fraction, their isolation and quantification, were the next stages proposed in this study. Spectral studies such as UV, shift reagent studies, IR spectral studies, NMR spectral studies and MS studies were envisaged for the confirmation of the molecules.

Docking studies of the isolated and identified molecules were the next in our plan evaluate and correlate the drug-receptor interactions.

Rat hemidiaphragm method was proposed to affirm the finding of L-6 muscle cell line results on glucose uptake. Further confirmation of activity was planned to take up using *in vivo* studies preferably using different doses in male *Wistar albino* rats (28 day study).

Mechanistic investigations on the active fraction were planned using Glut-4 translocation and PPARγ upregulation studies.

A correlation of the results of *in vitro* and *in vivo* studies, if any, was also planned.

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