Chapter 3

Aim and Objectives

Aim of the study: The research program described here aims to screen selected plants by *in vitro* and *in vivo* methods, isolate phytoconstituents from the active plants and to ascertain their cytotoxic potential / molecular mechanism of the isolated compounds.

The main objectives are,

- To evaluate the cytotoxic, antiproliferative and DNA damaging potential of the selected South Indian medicinal plants by different *in vitro* techniques.
- To confirm the activity of the selected plant extracts by *in vivo* solid and liquid tumour models.
- To isolate the phytoconstituents present in the selected plant extract.
- To characterize the isolated compounds by spectral and chemical methods.
- To screen the chemically characterized compounds for the cytotoxic potential.
- To establish the possible molecular mechanism of action of the isolated compounds.
PLAN OF WORK

IDENTIFICATION & COLLECTION OF THE SELECTED PLANTS
- *Juvenia simplex* (D. Don)
- *Monoclepyra malabaricum* (cogn.)
- *Litsea quassiflora* (Donnet)
- *Mussaenda philippica* (Wall. Blume)

**EXTRACTION**
- Petroleum ether (60-80)
- Ethanol

- Cytotoxicity
- Antiproliferative activity
- DNA Damage
- Brine shrimp lethality assay
- Trypan Blue Dye exclusion assay (DLA & EAC)
- MITT assay (C6, HeLa, MCF7, T47D, MBAMB231)
- Microtumus assay
- Onion root assay

**In vitro cytotoxic potential of all the extracts**

**In vivo study of the selected plant extracts**
- Liquid tumor model
- Solid tumor model
- *Ex vivo*-Antioxidant enzyme
- VEGF Gene Expression

**Isolation of active constituent from the potential extract**
- Column chromatography, UV and TLC

**Characterization of the isolated compounds**
- Melting point, UV, IR, NMR, GCMS/LCMS/MS

**In vitro cytotoxic potential of the isolated compounds**
- MITT assays (HeLa & MCF 7, HaCat cell lines)

**Elucidation of Mode of Action**
- Apoptosis: Acridine orange and Ethidium bromide double staining method, FITC-Annexin V/PI using flow cytometry
- DNA content analysis and cell cycle distribution by Flow cytometry
- Gene expression by Aagarose gel electrophoresis technique, ELISA
Chapter 4

Preparation of extracts and its cytotoxic potential by *in vitro* methods

4.1 INTRODUCTION

In recent years, there has been growing interest in the alternative system of medicine, especially the therapeutic use of medicinal plants. This particular interest towards plants may be due to the limitation associated with conventional therapy that they are relatively inefficient, have side effects and impart toxicity to the normal cells (Kaur 2012). Moreover, the ecological awareness also suggests that natural products are harmless and have the immense potential as lead compounds for the development of unknown drugs, biomimetic synthesis development and discovery of new therapeutic agents (Rates 2001). Thus, it is now certain that plants are the most vital source of several compounds, which possess significant medicinal value for the treatment of cancer.

4.2 MATERIALS AND METHODS

4.2.1 Identification, collection and authentication of selected medicinal plants

The aerial part of *Justicia simplex* D. Don of family Acanthaceae, leaves of *Myxopyrum smilacifolium* (Wall) Blume. of Oleaceae, *Memecylon malabaricum* of Melastomataceae and *Litsea quinqueflora* (Dennst) of Lauraceae were collected from Kottayam (Dist.) of Kerala State during October 2010 and were authenticated by Dr. Jomy Augustine, Post Graduate and Research Department of Botany, St. Thomas College, Pala. Authenticated specimens of these plants are preserved in the Dept. of Pharmaceutical Sciences, MG University, Kottayam with voucher specimen no.1505, 1507, 1508 and 1509 respectively dated 07.01.2011.

4.2.2 Preparation of extracts

**Preparation of petroleum ether extracts:** All the plants were shade dried, milled into coarse powder (1 kg) and soaked in petroleum ether (60-80°C) for a day. The extractions were carried out in a batch of 150 g each in a 5 L soxhlet apparatus. The combined extracts were dried over sodium sulphate (anhydrous) and distilled under reduced pressure until the solvent was completely removed.