

## **Chapter 2**

# **Objectives and Plan of Work**

## 2. Objectives and Plan of Work

### 2.1. Objectives

The focus of the present work centered on exploring the potential bioactive phytoconstituents present in the bark of *Dysoxylum malabaricum* Bedd. A profiling strategy using LC-MS-based dereplication was proposed for the rapid identification of metabolites from the bark extract. First a solvent extraction method was employed to prepare crude bark extract. Then through bioassay-guided fractionation, phytochemical investigation of the extract was planned to isolate pure phytoconstituents followed by *in-vitro* cytotoxic evaluation. Furthermore, it was proposed to investigate the reactive sites of the isolated compound for introducing functionalization *via* chemical modification to create a diverse compound library around the identified lead for biological evaluation.

The objectives of the present research work are as follows:

#### **2.1.1. Phytochemical investigation of *Dysoxylum malabaricum* Bedd. bark using LC-MS-based dereplication strategy and cytotoxic evaluation**

LC-MS-based dereplication platform was proposed for the identification and isolation of metabolites present in the bark extract using the DNP database. Bioassay-guided fractionation followed by purification, structure elucidation, and cytotoxicity screening of compounds isolated from different fractions were planned.

#### **2.1.2. Chemical modification of isolated metabolites and their cytotoxic evaluation**

This part of the work hypothesized to construct a new molecular framework by incorporating diverse functionalities at the reactive sites of isolated compounds (beddomeilactone) by synthesizing nitrogenous derivatives in series 1 and halogenated derivatives in series 2.

## 2.2. Plan of work

A brief outline of the research work carried out is summarized here.

### 2.2.1. Phytochemical investigation and cytotoxic evaluation of *Dysoxylum malabaricum* Bedd. bark

#### Extraction of plant material

- Plant material selection via chemosystematics approach
- Solvent extraction of plant bark using organic solvents
- LC-MS profiling of crude extract

#### Isolation of compounds from fractions

- LC-MS DNP-Based dereplication for targeting new compounds
- Bioassay-guided fractionation
- Purification by silica gel-based column chromatography and HPLC
- Structure elucidation by conducting 1D, 2D NMR, HRMS and ECD experiments.

#### Biological evaluation of isolated compounds

- *In-vitro* cytotoxicity assay
- Microscopic assessment of nuclear morphology *via* DAPI and acridine orange staining
- Cell cycle analysis and western blotting

### 2.2.2. Chemical modification of isolated compound (beddomeilactone)

#### Synthesis of beddomeilactone derivatives

- Series 1: Synthesis of the nitrogenous derivative of beddomeilactone.
- Series 2: Synthesis of the halogenated derivative of beddomeilactone.

#### Characterization of synthesized derivatives

- Physiochemical characterization using melting point and TLC (R<sub>f</sub> values)

- Structural characterization using  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and HRMS.

### **Biological evaluation of synthesized derivatives**

- *In-vitro* cytotoxicity by MTT assay.