

CHAPTER 3

SCOPE OF INVESTIGATION

The study entitled “**Consumption pattern of *Calamus tenuis* Roxb. shoots of the forest village natives of Dibrugarh, Assam and investigation of its cytotoxicity activity on cancer and normal cells (A549, MCF7 and L132)**” conducted under the following given heads is divided in five phases with a range of interest to establish the documentation of consumption pattern of *Calamus tenuis* Roxb. edible shoots, its traditional therapeutic practices and beliefs; and its cytotoxicity activity on human lung carcinoma cells (A549), breast carcinoma cells (MCF7) and lung normal cells (L132).

Phase-I Plant identification, sample collection and survey:

- Preparation of plant herbarium and identification.
- Collection of tender shoots of the plant and primary processing.
- Survey among selected forest village natives of Dibrugarh district of Assam regarding consumption pattern of *Calamus tenuis* Roxb. shoots and beliefs on health benefits/issues.

Phase-II Crude extraction of *Calamus tenuis* Roxb. shoots, cytotoxicity assay on human carcinoma cells, and qualitative phytochemical screening:

- Crude extraction of the sample involving successive use of different solvents (Hexane, Ethyl acetate, Methanol).
- MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay of crude extracts on human lung carcinoma cells (A549) and breast carcinoma cells (MCF7).
- Qualitative phytochemical screening of the extracts.

Phase-III Fractionation of methanolic precipitate (MPCT) extract of *Calamus tenuis* Roxb. Shoots, Cytotoxicity assay of fractions (F-2, F-3, F-8) on human lung carcinoma cells (A549) and breast carcinoma cells (MCF7) and Qualitative phytochemical screening of the fractions (F-2, F-3, F-8):

- Fractionation of the crude extract showing highest cytotoxicity potential (MPCT).
- MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay of the fractions (F-2, F-3, F-8) on human lung carcinoma cells (A549) and breast carcinoma cells (MCF7).
- Qualitative phytochemical screening of the fractions (F-2, F-3, F-8).

Phase-IV Cytotoxicity of Methanol precipitate (MPCT), Methanol supernatant (MSCT) extracts and fractions (F-2, F-3 and F-8) of *Calamus tenuis* Roxb. shoots on human lung normal cells (L132):

- MTT assay on human lung normal cells (L132) for cell viability and cytotoxicity potential evaluation of Methanol precipitate (MPCT) and Methanol supernatant (MSCT) extracts of *Calamus tenuis* Roxb. shoots.
- MTT assay on human lung normal cells (L132) for cell viability and cytotoxicity potential evaluation of Fractions (F-2, F-3 and F-8) of *Calamus tenuis* Roxb. shoots.

Phase-V Comparison of lethal concentration (LC₅₀) of methanolic precipitate (MPCT) and supernatant (MSCT) extracts; and fractions (F2, F3 and F8) of *Calamus tenuis* Roxb. shoot among human carcinoma and normal cells:

- Evaluation and comparison of Lethal Concentration (LC₅₀) of methanolic precipitate (MPCT) and supernatant (MSCT) extracts of *Calamus tenuis* Roxb. shoot against human carcinoma and normal cells.
- Evaluation and comparison of Lethal Concentration (LC₅₀) of Fractions (F2, F3 and F8) of *Calamus tenuis* Roxb. shoot extracts against human carcinoma and normal cells.