3. THEORETICAL ANALYSIS

Folk, tribal or indigenous medicine refers to non-codified traditional medicinal system, passed verbally from generation to generation without any written document, but can be an important and foremost source of new drug discovery. Gathering the information on indigenous medicinal plants and research on folk medicinal plants have drawn the considerable attention in the view of its great therapeutic and economic importance. Traditional system of medicines (Indian, Egyptian, Chinese, Greeks and Roman) is the evidence of use of herbs and mineral products in healthcare system. Different Texas like “Atharvaveda” from India (written in about 1200 BC), the Petrie from Kahun in Egypt (from about 1880 BC), and the “Avesta” from Persia (compiled in about the 6th century AD) are some classical traditional medical texts in world.

Herbal medicines have recognized worldwide for their safety, efficacy, cultural acceptability and lesser side effects. Natural plant based products can be promising candidates (as a lead) for drug discovery. Growing need of alternative medicines in recent years is rising and researchers mainly concentrating in traditional/folk and alternative systems of medicine. Herbal medicine is making dramatic comeback and scientists are turning their interest to natural products to find the answer of ailments like diabetes, cancer, hepatitis, renal disorders, cardiovascular diseases etc. Hence experimental evaluation of plants used by the tribal or indigenous people is the primary intention of this research attempt. The phytochemicals present in those medicinal plants and impact of those phytochemicals on their medicinal activities has to be evaluated to find better and safe treatment of those diseases and
bring some of those phytochemicals in main stream of therapy is our primary target of the study. Our study is just a contribution to achieve the same target and aimed to combine traditional knowledge and modern science to find better treatment and better lifestyle.

**3.1. SCOPE AND OBJECTIVE OF THE STUDY**

Literature was extensively searched to find the folk medicinal plants of North-East India. A large number of different ethnic groups are inhabitant in this area and they are using a number of medicinal plants in their daily life, we have found a number of plants are left over for the scientific evaluation of traditional medicinal knowledge. Several scientific investigations have been executed by taking care of those basic traditional knowledge of those plants and observed that the scientific investigation results matches with the folk medicinal uses. Some of those plants have been incorporated in the organized systems of medicines; much larger section of indigenous medicinal plants has remained endemic and not investigated thoroughly.

Primarily we have selected few plants which are common and easily available throughout North-Eastern states and used by the different group of people. After a bulk literature survey and preliminary analysis on those plants we have selected two most commonly used, traditional plant i.e. *Marsilea minuta* and *Phyllanthus acidus*. Survey showed that there are so many important folk medicinal uses of those plants are still unfolded and need to be investigating scientifically. Our aim is to prove those traditional uses and to search if any undiscovered bioactive phytochemicals present in those plants. To start up our study in this direction we have surveyed the published
reports and tried to correlate them with traditional importance, and so to bring out the result to outline the systematic study. The scheme of the study was reflected as below,

Survey of literature on traditionally important folk medicinal plant of North-East India

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Preliminary experimental investigation and selection of plants

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Survey on *Phyllanthus acidus* and *Marsilea minuta*

↓

Extraction of plant parts

(Methanol, Ethyl acetate, Petroleum ether extract)

↓

Physicochemical, and Preliminary phytochemical screening

↓

*In vitro* and *ex vivo* antioxidant activity of the extracts

Analgesic and antiinflammatory activity of *P. acidus* extracts

Antitussive and expectorant activity of *M. minuta* extracts

↓

Identify more effective extract by above studies

↓

Chromatographic fractionation of more effective extract

(Methanol, ethyl acetate and petroleum ether fraction)

↓

Pharmacological investigation of fractions

↓

*P. acidus*

In vitro antioxidant

Analgesic & antiinflammatory

Hepatoprotective & in vivo antioxidant activity

Nephroprotective activity

Anti-TB activity

↓

Chromatographic fractionation of more effective fraction

Identify most effective fraction by antioxidant study

TLC of more effective extract and its fractions

Selection of fractions for structural elucidation

Spectral analysis of selected sub fractions

↓

*M. minuta*

In vitro antioxidant

Antitussive & expectorant

Hepatoprotective & in vivo antioxidant activity

Nephroprotective activity

Anti-TB activity

↓

Figure 3.1.: Scheme of the work
3.2. PLAN OF THE WORK

- Literature review and preliminary study to select few folk medicinal plants of North-East India for scientific investigation. And selection of two plants i.e. *P. acidus* and *M. minuta* for further investigation.

- Extraction of plant parts using methanol, ethyl acetate and petroleum ether.

- Preliminary phytochemical screening and physicochemical (yield, colour, pH, density, specific gravity) investigation of extracts.

- Determination of total phenolic content and total flavonoid content in the extracts.

- Investigation of *in vitro* and *ex vivo* antioxidant activities of extracts by,
  - DPPH radical scavenging assay
  - $O_2^{•−}$ scavenging activity
  - ·OH scavenging activity
  - NO radical scavenging activity
  - $H_2O_2$ scavenging activity
  - Evaluation of reducing power ability
  - Fe$^{+2}$ ion chelating ability
  - Total antioxidant activity by ferric thiocyanate method
  - Lipid peroxidation inhibition assay using rat liver homogenate
  - Oxidative haemolysis inhibition assay using rat blood

- Chromatographic fractionation of more effective extract using petroleum ether, ethyl acetate, methanol.

- Acute toxicity study and selection of dose for extracts/fractions.

- Analgesic and anti-inflammatory activities of all extracts of *P. acidus* and fractions of more effective extract by following methods,
  - Writhing reflex induced by acetic acid in mice
  - Tail immersion test
- Formalin-induced licking response in mice
- Carrageenan-induced paw oedema in rats
- Granuloma formation induced by cotton pellet in rats
- Membrane stabilizing activity

- Expectorant and antitussive effect of all extracts of *M. minuta* and fractions of more effective extract by following methods,
  - Ammonium liquor induced cough
  - SO₂ induced cough
  - Expectorant activity using phenolsulfonphthalein

- *In vitro* and *ex vivo* antioxidant activity of fractions using
  - DPPH radical scavenging assay
  - NO radical scavenging activity
  - Total antioxidant activity by ferric thiocyanate method
  - Lipid peroxidation inhibition assay

- Thin layer chromatography of effective extract and its fractions.

- Hepatoprotective and *in vivo* antioxidant activity of fractions of more effective extract (*P. acidus* and *M. minuta*) against paracetamol induced hepatotoxicity in rats.

  Evaluation of hepatoprotective activity by estimation of following biochemical parameters,
  - SGOT
  - SGPT
  - Serum ALP
  - Total bilirubin
  - Direct bilirubin
  - Serum cholesterol
  - Serum triglycerides (TG)
Investigation of *in vivo* antioxidant activity by determining of the following parameters,

- Determination GSH in liver tissue
- Determination GSH in serum
- SOD in liver tissue
- CAT in liver tissue
- GPx in liver tissue

➤ Nephroprotective and *in vivo* antioxidant activity of fractions of more effective extract (*P. acidus* and *M. minuta*) against cisplatin induced nephrotoxicity in mice.

Nephroprotective effect was by investigation of the following parameters,

- BUN
- Creatinine
- Uric acid
- Total protein
- Albumin
- MDA

➤ Identify the more effective fractions from each plant.

➤ Chromatographic sub-fractionation of more effective fraction.

➤ Phytochemical investigation and selective antioxidant study of each subfraction.

➤ Spectral study and identification of the chemical constituent of selected pure subfraction.