3. THEORETICAL ANALYSIS

India with its kaleidoscopic variety has rich cultural, traditional heritage and affluent biodiversity. Traditional medicinal forms of India that look at healthcare, illness and causes of disease in completely diverse ways have rich history. Indian System of Medicine or Alternative Medicine, its primary focus is on holistic health and relieves humanity of all categories of misery - physical, mental, intellect. Classical alternative medicines have been effectively used to cure or prevent many diseases for centuries. Ayurveda is thought to have initiated around 5,000 years ago in Vedic times and recognized as the most ancient medical system of India with a remarkable record of efficacy, safety and cultural acceptability.

The history of plants or plants product and healing medications goes back to the very early stages of medicine itself. Indian, Egyptian, Chinese, Greeks and Roman medicinal system are the evidence of use of herbs and mineral products in healthcare system. The Atharvaveda from India (1200 BC), the Petrie from Kahun in Egypt (1880 BC), and the Avesta from Persia (6th century AD) are some classical traditional medical texts in world.

Sophisticated knowledge of the folk medicine particularly plant based knowledge is still not confined to science fully. Human society’s mainly ethnic communities and indigenous people all across the globe have developed rich sets of knowledge skill and explanations relating to the environments they live in. These ‘other knowledge systems’ are today frequently referred to as traditional ecological or indigenous or local knowledge. They encompass the sophisticated and complicated arrays of information, understandings and interpretations that direct modern human societies and researchers around the world in their innumerable interactions with the
natural milieu. Indigenous knowledge also known as ‘local knowledge’, ‘folk knowledge’, ‘people’s knowledge’, ‘traditional wisdom’ or ‘traditional science’, and unique to a culture or society. Folk or indigenous medicine is major and key source of Ayurvedic, Unani, and other traditional medicinal system. Folk medicine incorporates crude medicinal herbs, juice, decoctions and infusions. Folk medicine is still practiced by ethnic peoples, hakims, vaids, and traditional medicinal people worldwide mainly in remote areas, many of these folk medicine/preparations are of high curative value.

Herbal medicines have stood the test of time for their safety, efficacy, lesser side effects, cultural and traditional acceptability. Natural plant based products are promising candidates (as a lead) for drug discovery and they still continue to play a significant role in future drug development programs. WHO also recognised the traditional health systems (including herbal medicine) as ‘holistic’ – ‘that of viewing human in their totality within a broad ecological spectrum, and accentuates the view that ill health or disease is brought about by an inequity or disequilibrium of human in their total ecological system and not only by the causative agent and pathogenic evolution’, probably involving that the indigenous drugs can restore the inequity or disequilibrium leading to the cure of ill health or disease. In last few decades, there has also been a growing attention in traditional/folk and alternative systems of medicine globally. In recent years herbal medicine is making spectacular comeback and scientists/researchers are turning to natural products for answer to ailments like diabetes, cancer, hepatitis, renal disorders, cardiovascular diseases etc.

Indigenous knowledge, based on our long and rich tradition, would be further extended and harnessed for the purpose of healthcare, wealth and economic development. Tripura is a small North-Eastern state but full of natural resources. It is considered as gold mine of medicinal plants. Ethnic communities of Tripura are rich
in traditional knowledge. They are using a lot of plant and folk remedies which are not confined to literature and still unknown to outer world. Investigation and development of drug or remedy from these traditional medicinal plant that add value to India's indigenous resources, and which may provide holistic and optimal solutions for different acute and chronic diseases that are suited to Indian social-cultural-economic ethos. Biological evaluation of ethno-medicinal plants is necessary for cost effective and better therapeutic treatment. In India about half of the populations are still under the poverty line, therefore cost effective herbal treatment will helpful to rural and poor people in health care system. A intensify and purposeful research on traditional/folk systems of medicine will increase the Indian share of the global herbal product market and will also help to replace existing drug with plant derived drug with less side effect and better potency. Diabetes, toxicity of liver, renal failure, parasite infections are major health problems of India. Now a day’s research on traditional and tribal medicinal plant proved as better source of medicine in this aspect. This is the need of time for India to investigate folk medicinal plants, new phytochemical or herbal formulation.

3.1. SCOPE AND OBJECTIVE OF THE STUDY

A large section of the indigenous groups, tribal communities and others, still retain a great deal of the pristine elements of their age-old lifestyles. Every tribal groups of Tripura can be considered as a repository of a distinctive body of information, knowledge, cultural traditions, community institutions and technologies, which have evolved over a long period of time. Wisdom and traditional knowledge embedded in the socio-cultural life of the tribal people of Tripura often astounding.
But the erosion of traditional knowledge and bio-diversity is observed in last year’s due to globalization, industrialization, socio-economic changes and development pressures, shrinking forest and degradation of related resources, and commodification. Ethnobotanical survey and literature review on medicinal plants of Tripura clearly shown that indigenous peoples of Tripura have vast knowledge and uses more than 200 medicinal plants in their daily life. Though some of the medicinal plants have been investigated for their biological effect and few of them have been incorporated in the organized systems of medicine, much larger section of indigenous medicinal plants has remained endemic and not investigated thoroughly.

Plants selected for the study were extensively used by the people of Tripura traditionally for health care. Traditional medicinal peoples (Ochai or Kaviraj) also recommended these plants in treatment of different diseases. These plants are also available in different part of India and well acknowledged for their medicinal uses by different local communities. After ethnobotanical survey, extensive literature survey, preliminary analysis, finally two medicinal plants viz. Meyna spinosa and Leea asiatica were selected for detail studies. There is no scientific evidence to support the folk medicinal uses of Leea asiatica and few medical literatures available on Meyna spinosa, which became the main criteria to investigate these plants in my research work.

The aim of research is to find several unexplored folk medicinal plants used by the tribes of Tripura and to investigate the therapeutic effectiveness of Meyna spinosa and Leea asiatica based on their folk uses. In addition, the active phytocostituents will be isolated through column chromatography and structure elucidation will be carried out using spectral analysis. The scheme of the (Figure 3.1) may be depicted as,
Ethnobotanical survey and review of literature regarding folk medicinal plants by tribes of Tripura, India

Selection of plants (Leea asiatica and Meyna spinosa)

Extraction of plant leaves using Methanol, Ethyl acetate, Petroleum ether

Physiochemical evaluation of extracts (yield, colour, pH, specific gravity)

Preliminary phytochemical evaluation of extracts

In vitro and ex vivo antioxidant activity of extracts

Identification of the more potent extract

Chromatographic fractionation of bio-active more potent extract using petroleum ether, ethyl acetate, methanol

In vitro and ex vivo antioxidant activities of fractions & Biological activities of potent extract and its fractions

<table>
<thead>
<tr>
<th>Leea asiatica</th>
<th>Meyna spinosa</th>
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<tbody>
<tr>
<td>Hepatoprotective &amp;a vivo antioxidant activity</td>
<td>Hepatoprotective &amp;a vivo antioxidant activity</td>
</tr>
<tr>
<td>Nephroprotective activity</td>
<td>Nephroprotective activity</td>
</tr>
<tr>
<td>Anthelmintic activity</td>
<td>Antidiabetic activity</td>
</tr>
</tbody>
</table>

Identification of the more bioactive potent fraction

Thin Layer Chromatography of potent extract and its potent fraction

Sub fractionation of bioactive potent fraction

Antioxidant activity, chemical test, melting point determination of subfractions

Selection of subfraction and structure elucidation of selected subfractions

REPORT

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

- Ethnobotanical survey on folk medicinal plants of Tripura.
- Selection of folk medicinal plants of Tripura for scientific investigation.
- Extraction of plant parts using methanol, ethyl acetate and petroleum ether.
- Preliminary phytochemical screening and physicochemical (yield, colour, pH, 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
  - Superoxide radical scavenging activity
  - Hydroxyl radical scavenging activity
  - Nitric oxide radical scavenging activity
  - Hydrogen peroxide scavenging activity
  - Evaluation of reducing power ability
  - Ferrous 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.
  - In vitro and ex vivo antioxidant activity of fractions using
    - DPPH radical scavenging assay
    - Nitric oxide radical scavenging activity
    - Total antioxidant activity by ferric thiocyanate method
    - Lipid peroxidation inhibition assay
Hepatoprotective and *in vivo* antioxidant activity of more effective extract and its fractions of *Meyna spinosa* and *Leea asiatica* leaves against paracetamol induced hepatotoxicity in rats.

Evaluation of hepatoprotective activity by estimation of following biochemical parameters,

- Serum glutamic oxaloacetate transaminase (SGOT)
- Serum glutamate pyruvate transaminase (SGPT)
- Serum alkaline phosphatase (ALP)
- Total bilirubin and Direct bilirubin
- Serum total cholesterol (TC) and total triglycerides (TG)

Investigation of *in vivo* antioxidant activity by determining of the following parameters,

- Determination glutathione (GSH) in liver tissue
- Determination glutathione (GSH) in serum
- Superoxide dismutase (SOD) in liver tissue
- Catalase (CAT) in liver tissue
- Glutathione peroxidase (GPx) in liver tissue

Nephroprotective and *in vivo* antioxidant activity of more effective extract and its fractions of both plants against cisplatin induced nephrotoxicity in mice.

Nephroprotective effect was by investigation of the following parameters,

- Blood urea nitrogen (BUN)
- Creatinine
- Uric acid
- Total protein
- Albumin
- Malondialdehyde (MDA)
Antidiabetic activity of more effective extract and its fraction of *Meyna spinosa* leave against high fat diet and alloxan induced type-2 diabetes in rats.

Antidiabetic evaluation was carried out by using following methods,

- Determination of body weight
- Determination of serum glucose level.
- Determination of serum cholesterol
- Determination of serum triglyceride
- Determination of low density lipoprotein (LDL)
- Determination of serum very low density lipoprotein (VLDL)
- Determination of serum high density lipoprotein (HDL) level
- Determination of $\alpha$-amylase activity.

Investigate the hypoglycemic effect of fractions of methanol extract of *Meyna spinosa* also carried out in NG-OGTT rat.

*In vitro* anthelmintic effect of more effective extract and its fraction of *Leea asiatica* leaves against *Pheritima posthuma*.

Identify the more effective fractions from each plant.

Thin layer chromatography of most effective extract and most effective fraction.

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.