CHAPTER IV

METHODOLOGY

The present chapter gives the glimpses of the methods followed in this thesis and detail methods are given separately in the concerned chapters of results and discussion. Therefore, this chapter may be taken as an outline of the methodology followed in the thesis.

Science is not limited by human imagination, but experimental research is often limited by available technology (Phillipson, 2007). Refinement and introduction of new analytical techniques greatly facilitated natural product research (Phillipson, 1995). The main heading of the present thesis is on Pharmacognosy, but then the selected botanical species are used in food purposes also. Therefore, methodology followed to fulfil the present thesis covers field visit, interviews, questionnaires, pharmacognosy, proximate, antioxidant and phytochemical methods.

To generate primary data, extensive field visit of the study area have been carried out, various methods like microscopy, spectroscopy like GC, GCMS, NMR and various reagents, solvents and apparatus like Clevenger’s apparatus, hydrodistilation plant, oven, shaker, water bath, soxlet apparatus, rotary evaporator and separating methods like TLC, CC etc. has been employed. Secondary data like related published or unpublished work and governmental documents has also been used. Thus combined documentary, observational, informal discussion, interview and laboratory generated data are used in the present study. The research concept, research outline and methods applied for the present investigation are presented in the following forms:

4.1.: Research concept

Our great ancestors were very much there in this remote area of far North East India since time immemorial, they relied totally on locally available food resources, they never used dal, wheat, milk etc. which are staple food for Indian mainland
dwellers, still they were strong and healthy. Therefore, it is hypothesised that the ethnic food or folk food must have positive contribution in the tribal folk health; the folk food must contain biologically active compounds in addition to nutritionally rich composition. The dreaded diseases like diabetic, blood pressure, eyes conjunctives, obesity were either not coon to them or there were no such health problems to them. Therefore, there is need of scientific investigation on folk food plants with medicinal uses. Let the food be your medicine and medicine be your food, once said Hippocrates some 2500 years back is kept as the centric point of research concept in the present thesis. For Indigenous people who reside far from the madding crowd, food is also a medicine. Medicinal food plants used among the indigenous people of the study area is attempted to validate scientifically in the present thesis; therefore, the selected folk medicinal food plant species was proposed to investigate under the following domains: To study proximate composition, antioxidant potential, pharmacognosy characteristics of the food plants having medicinal value and to study phytochemical contents of the few species out of the shortlisted plants as a representative species because to analysed all the selected species up to NMR level would take long time in which one would have not completed before the expiry date of the thesis submission.

Therefore, the selected species have been studied under given schematically represented concept.

Fig 4.1: Schematic representation of research concept.
4.2: Research design

FIELD VISIT → INTERVIEW → QUESTIONNAIRE → MARKET SURVEY → ENUMERATION OF FOLK MEDICINAL FOOD PLANTS → SELECTION OF SPECIES → PHARMACOGNOSY PROXIMATE ANTIOXIDANT PHYTOCHEMICAL

Fig. 4.2: Schematic research design.

4.3: Methods

The field visits and the elderly people of the village, including women folk and the local herbal practitioner, vendors is taken as the main source of information in generating folk medicinal food plants on the study areas in addition to published literatures. Accordingly, two tribes (Adi and Galo), 30 villages and 7 local markets were selected in which a total of one hundred medicinal plants were enumerated. Out of these 100 medicinal plants 36 species was listed as food plants also. Out of these listed plants following seven plants with potential medicinal food plants were selected for further study:

1. *Allium hookeri* Thw.
2. *Solanum spirale* RoXb.
5. *Clerodendrum colebrookianum* Walp.
Proximate compositions, minerals and antioxidant potential studies were carried out in all the seven selected plants. Pharmacognosy study was carried out on the leaf, stem and root of the *S. spirale*, *P. bennettiana*, *A. hookeri* and stem and leaf of *C. colebrookianum* and *Z. rhetsa*.


Mineral contents; Mn, Cu, Zn, Mg, Na, K, and Iron was studied by method described in Thimmaiah, (1999) and Iswaran (1980).

The estimation of antioxidant potential was studied by following 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and ABTS radical scavenging method by relating the Total phenolic and total flavonoid contents of the sample in which Trolox, Rutin and Gallic acids were used as standard.

To study essential oil, *Phoebe cooperiana* fruit was hydrodistilled and resulted oil was subjected to GCMS. To study phytochemistry of the selected medicinal plant food plants, sample preparation, extractions, separation including thin layer chromatography, column chromatography, Gas chromatography, GCMS and NMR has been carried out by following the following protocols and standard texts described in Harborne (1998), Plant Drug Analysis by Wagner (1996), Nuclear magnetic resonance for natural Product Researchers by Kumar *et al.*, (2007), Lampman *et al.*, (2012) and Thin layer chromatographic Atlas of Ayurvedic pharmacopoeial Drugs - Government of India, Ministry of Health and Family Welfare Publication (Anonymous, 2009).