CHAPTER 7

MATERIALS & METHODS
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Collection, authentication of voucher specimens and preservation of collected specimen for proper identification.

In the present study the following methods and techniques were adopted.

In the course of research work, the first step has been taken for the collection of information from literature which were available locally. This literature pertains to any kind of information, printed or unprinted which can be found to give information on the traditional use of medicinal plants. From this literature, different plants and their medicinal uses were noted. These reports are systematically sorted out and arranged; then after scrutiny, they were once again compiled in the field note/data book which served as an aid in making it easy in the field investigation. Conducting interviews either personal or group were also carried out during the field study.

The chief informants i.e., Ojha, Baidya, Bhopa, Headman, Kabiraj, Old women, School teacher, forest dwellers and the practitioners of folk, medicine settled in different parts of Cachar district were interviewed (Plate : 20 - 23, 25 - 30.) They are known to use their knowledge of medicinal plants on their immediate families, and others. Besides, some Homeopaths, Naturopathis, veterinary doctors and faith – healers were also contacted and interviewed.

For conducting interviews every possible opportunites were taken. Some people were contacted at their homes, at times staying with them and their uses of medicinal plants were observed. Some people were interviewed at public meetings and conferences, social gatherings (Plate : 18 -19) etc.

All the informations were recorded carefully in the notebook which was used later to confirm the authenticity of the uses of medicinal plants at large.

COLLECTION AND PROCESSING OF SAMPLES:

The following equipments / materials and chemicals were used during the collection and processing of plant materials / samples:

(A) List of Equipments:

(B) Laboratory Equipments:

(i) Dissecting Box, (ii) Plastic or Enamel tray, (40 cm X 30 cm X 3 cm)  
(iii) Specimen jars, (iv) Cane forceps, (v) Quick fix / Dendrite/ Fevicol.

(C) Chemicals:

(i) Formaldehyde, (ii) Ethyl alcohol (98%), (iii) Specimen jars, (iv) Rectified spirit,  
(v) Mercuric chloride, (vi) Sodium carbonate, (vii) Methanol,  
(viii) Paraformaldehyde.

(D) Herbarium Equipments:

(i) Genus cover (48 cm X 32 cm), (ii) Species cover (42 cm X 30 cm),  
(iii) Absorbers or blotters (46 cm X 30 cm), (iv) Mounting boards (42 cm X 30 cm),  
(v) Corrugated cardboards (45 cm X 30 cm), (vi) Straps & needles,  
(vii) Labels, (viii) Naphthalene, (ix) Pigeon – hole herbarium cabinets (170 cm X 62 cm X 50 cm).

Field trip Programme:

Before starting the actual field work, a programme for a field trip was chalked out, by using the map of important places/areas were demarcated for the collection of samples to be undertaken (fig.- 2). Then the intensive and extensive field work was undertaken covering almost all the seasons of the year so as to gather detailed information on each and every species which are found useful in herbal medicine by the different communities of the Cachar district.

Actual field work:

The field collection was carried out throughout the study area. The study area was divided into different blocks i.e., Lakhipur, Dholai, Sonai, Katigora, Udarband & Silchar etc. In each block different villages i.e., H'mar - Khawlien, Sonabari, Jorkha, Srikona, Damcherra, Saron, Hebron, SaiH'mar, Chikur, Hounveng, Uttar Lalpani, Kharzawl, Doglang, Kamrangphailien, Jiriphai, New Tuolpui, Longkawa, Joypur Muolkawi, Diphucherra, Kumba, LowerThenzo, Nagadum, Boroidisa, Vapuol, Muolthawizawl, Tupidhor, Khanthuom, Jurkhal, Chekarcham, Ningpu, Arda, Bombaithol, Nagathal, Pana punji, Inbuon, Diger compound, Kapakhal, Mirpur, Bagkhal, Saisel, Lalpani Haokip, Biete, Digli, Diglangmukh, Ramthalai, Neithag punji, Ngete, Bubon, Upper Thenzo, Tuitha, Rawpuizawl, Khawthlir, Muoldam,
While taking interviews with the local specialized people, the approaches and methodologies have been followed as suggested by Jones (1941), Schultes (1960, 1962), Jain (1964a, 1967a, 1989), Ford (1978), Rao & Hajra (1987), Rao (1989), Lipp (1989) & Cotton (1996). But it is important to mention here that the techniques applied varied from one place to another depending on the social background of the people. The interviews have to be held in private as most of the medicine men do not want to disclose their knowledge in front of their own people. To overcome this situation the scholar had to stay along with them and it is found that if the local medicine men felt that he is talking to a trustworthy person, he is cooperative and does not hesitate to divulge these secrets. However, interviews with those local medicine men/herbalists went off smoothly and the information gathered were most helpful during the of field study of the present research work.

As far as possible sufficient information have been recorded of the plants with regard to their (i) vernacular name (almost all, except a few which people could not provide), (ii) Plant / Plant parts used, (iii) Process of preparation of medicine
either individually or in combination with other plants, (iv) mode of application and dosages for the treatment of particular disease or diseases, (v) domestication of such plants used as herbal medicine for their subsistence and livelihood & (vi) commercial exploitation of wild plants based on the market survey of selling items. At the time of collection of the plant samples and the herbarium technique principles/ guidelines followed are as suggested by Jain & Rao (1977); Womersley (1981); Mehrotra (1989); Martin (1995). The Therapeutic value of plants as suggested by tribal and non-tribal medicine-men have also been recorded. The seasonal pattern for collection of plants were as followed.

Seasonal pattern for collection of plants of therapeutic value:

<table>
<thead>
<tr>
<th>Plants parts</th>
<th>Time</th>
<th>Season</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root and tuber</td>
<td>Only at daytime but before 12 noon.</td>
<td>Winter &amp; Summer season.</td>
</tr>
<tr>
<td>Leaves &amp; branch</td>
<td>After sunrise in the morning but before 9 A.M.</td>
<td>Rainy &amp; Spring Season.</td>
</tr>
<tr>
<td>Whole plant</td>
<td>In the morning time</td>
<td>After flowering and fruitsing.</td>
</tr>
<tr>
<td>Bark, latex, resin, gum and flower</td>
<td>At day time but between 12 noon to 3 P.M.</td>
<td>Rainy and Autumn season.</td>
</tr>
<tr>
<td>Seed and fruit both tender and ripe</td>
<td>Only at day time between 12 noon to afternoon</td>
<td>As per the season &amp; when ever available.</td>
</tr>
</tbody>
</table>

After the verification of authenticity of the uses of a particular medicinal plant, they were brought to the laboratory for further processing.

Collection of ferns:

Ferns were collected with their basal portion, because the shape of rhizome, hairs and scales on rhizome are important taxonomic characters. In case of big-fronds the basal portion was also collected and the exact size was noted in the field note-book, along with other details. The habits, habitat, phenological characters etc. of the medicinal plants were also studied. Soil samples were collected from the
different selected sites of Cachar district to analyse the $P^H$, N, P, K, states of the respective site.

**PROCESSING OF PLANTS:**

Processing of Plants includes collection of plant, herbarium techniques, identification and preservation of plant specimen / samples.

Plants have been collected in its flowering and fruiting stages from its natural habitat and serially tagged with the collection numbers. Some plants were collected in their vegetative form, they have been brought under cultivation in the Home garden to study their flowering and fruiting stages.

**COLLECTION OF PLANTS HAVING MUCILAGE, GUMS AND RESINS:**

Some plants having mucilage or resins, stick to the dryers and cause difficulty while changing. Such specimens were placed in a folder or muslin or any other thin cloth and pressed. Only the dryers were changed and not the muslin cloth, till the plants were fully dried to be made into herbarium specimens. Thorough observations have been made on the spot of collection of the individual plant species and field data were recorded relating to location, natural habitat, ecological adaptations, distribution pattern, the nature of roots or tubers, rhizomes / corms and bulbs etc. The colour of the flower and fruits are also recorded during the field collection.

Small herbaceous plants as a whole whether it is bulbous or rhizomatous and in case of trees, shrubs, undershrubs, woody herbs and climbers with representative twigs have been collected.

At the time of collection of certain groups of plants like succulents, aquatic plants, large bamboos and large trees etc required special methods and precautions as follows:

(i) **Collection of Succulents:**

Succulent plants like Cacti, Euphorbiaceae members and Crassulaceae, etc pose unusual difficulties in making herbarium specimens, their thick, succulent tissues take very long time to dry. They were dried by artificial heating or frequent changing of the dryers. They are also prone to rot and catch fungal infection.
Therefore, tissues were killed by dipping them in boiling water for few seconds and excess of tissues are removed by hollowing out the thick organs. The tissues were also be killed by treating them with alcohol or strong formalin, when ever required.

**(ii) Collection of very large plants:**

In this case there are very elaborate methods and instructions suggested by the specialists for these groups. The habit of the plant and the approximate size of the culm / pseudostem, leaves and inflorescences were recorded in the field note book. Either the whole plant or its main parts are photographed. Efforts were also made to collect leaves / parts of the leaves. In case of bananas, spathe with flowers were collected, at the same time photographs were also taken.

**(iii) Collection of Seeds:**

At the time of collection of plant specimen, mature seeds were also collected for experimental purposes. These were dried and mounted along with the herbarium specimens as far as possible.

**POISONING AND PRESERVATION OF SPECIMENS:**

Amongst the collected plants, the delicate ones were pressed in the field itself and the rest were brought to camp in plastic bags and were put in the press for drying. In case of rainy season specimens were pressed by spraying 10% formaldehyde on drying paper or they were sprinkled with paradichlorobenzene. Succulent plants & bulbs, rhizome and corms were boiled in water till the plants turn yellow and then they were kept under herbarium press. Necessary care has been taken for drying the specimens, then they were poisoned with the saturated solution of mercuric chloride dissolved in absolute alcohol (115 gm of HgC1₂ dissolved in 4.5 litres of Ethyl alcohol) and mounted on the herbarium sheets (42 X 28 cm) with the help of fevicol / just warmed glue. The printed label is affixed at right hand corner of the bottom of the sheet before mounting of the specimen and later field data were transferred with the help of collection number, date of collection, locality, distribution, short description as recorded in the field. The characters of dissected flowers were also recorded in the laboratory, particularly for the specimens brought under cultivation, which subsequently flowered. Their Vernacular names, scientific name and uses along with the photographic notes and collector’s name and herbarium sheets prepared were kept ready for identification.
IDENTIFICATION OF PLANTS:

Mounted herbarium specimens were used for the purpose of identification. Critical morphological studies have been made for the collected plant specimens. The specimens have been studied by making dissection of a number of flowers both live and preserved. The authentic identification of the plants were done with the help of the available floristic literature such as Flora of British India vol.1-7 (Hooker, 1872 – 1897); Bengal plants vol : I & II (Prain, 1903); Flora of British Burma (Kurz, 1877); Flora of upper Gangetic Plain, vol. I – II (Duthie, 1903 – 1922); Flora of Presidency of Madras, vol. 1 – 3 (Gamble, 1915 – 1936); Flora of India, vol. 1- 3 (Sharma etal. Edt. 1993); Flora of Assam, vol. 1-4, (Kanjilal etal, 1934 – 1940); and vol. – 5 (Bor, 1940); Flora of Lushai Hills, (Fischer, 1938); Flora of Tripura State, vol. I & II (Deb, 1981 & 1983); Flora of Nongpoh, (Joseph, 1982); Flora of Jowai vol. I & II (Balakrishnan, 1981 & 1983); Forest Flora of Meghalaya, vol. 1 & 2 (Haridasan & Rao, 1985 & 1987); Flora of Majuli (Islam, 1990); Indian Trees (Brandis, 1906); Flora of India, vol. 12 – 13 (Hajra etal., 1995); Floristic diversity of Assam (Bora etal., 2003); Assam's flora (Chowdhury,2005). Besides these floras, in order to match the specimens for further confirmation and to identify the plants up to species level, the herbarium sheets were taken to the Botanical survey of India, Eastern Circle, Shillong for consultation & confirmation of the identification.

The established reports on their (the collected plants) utilization in medicine have been compiled from a number of accounts published by Kirtikar & Basu (1933); Nadkarni (1954); Chopra (1958); Chopra etal. (1956, 1969); Anonymous (1943 – 1972); Asolkar etal. (1992) & Sinha (1996); Ambasta (1986); Basu (1975); Sharma etal. (2003); Sharma (2003).

One set each of the identified herbarium sheets have been deposited in the herbarium maintained in Assam University, Silchar.

SOIL ANALYSIS:

Collection of Soil:

Soil samples were collected from the selected different parts of Cachar district at the time of field work. The soil samples were dug with the help of a spade upto a depth of at least 10 cms. and about 1 – 1.5 kg samples were collected and packed in the sterilized polythene packets / bags.

As soon as possible the samples were brought to the soil analysis laboratory of the Agriculture Department, Silchar and these were serially registered and recorded. Informations on them were also recorded in the data sheet.
**Processing:**

The collected soil samples were air dried. Proper care was taken so that there should be no contamination. The air dried soil sample were passed through 2 mm. sieve for analysis. But before sieving, the soil clods were crushed in wooden pestle and mortar to pass it through the sieves of finer mesh size (0.2 – 0.5 mm).

**Soil pH:**

10 gm of soil samples were taken in 50 ml beaker. Wooden rack having 11 such beakers and each containing 10 gm soil sample, were kept under the multiple dispenser. 20 ml. of distilled water were added and stirred gently with glass rod for \( \frac{1}{2} \) an hour. \( P^H \) of the soil sample was then measured by the \( P^H \) meter. Utmost care was taken before the electrodes were immersed and the soil suspension was stirred well. Electrodes were washed with distilled water after each determination / observation.

**Estimation of available Potassium:**

Determination of available Potassium was done by Flame photometer method as suggested by Toth and Prince (1949) and Stanford and English, (1949).

**Estimation of available phosphorus:**

Two methods were used for the determination of available phosphorus in soil sample, viz. Bray's method No. 1 for acid soils and Olsen's (1954) method for alkaline or neutral soil samples.

**Estimation of Nitrogen:**

Organic matter content of the soil samples was calculated as par the formula given below:

- Organic matter content = Organic carbon \( \times 1.724\% \)
- Organic matter = N% \( \times 20 \)
- P.C. (%) of Nitrogen = \( \frac{\text{Organic Carbon} \times 1.724}{20} \)
- Nitrogen content in ppm = Nitrogen content in PC \( \times 10,000 \)
- Available Nitrogen in Kg / ha = Nitrogen content ppm \( \times 2.24 \)
Plate- 18 : Scholar attending the H'mar Women's gathering in front of the Church at H'markolin.

Plate – 19 : Scholar attended the Jaintia gathering for the collection of information.

Plate – 20 : H'mar women collecting their traditional medicinal plants.
Plate- 21 : H'mar Kabiraj showing the medicinal plants – *Clerodendrum viscosum* vent.

Plate- 22 : H'mar woman kabiraj showing the processing of medicine used for their day to day life.

Plate - 23 : Barman Kabiraj showing the roots of medicinal plant and a patient waiting behind him.
Plate - 24: *Ocimum sanctum* L. – a plant maintained in the House complex of Barman family for Puja and Medicinal use.

Plate - 25: Rupendra Barman – (77) – a Kabiraj & a village patient is waiting for taking medicine.

Plate - 26: A Reangmei medicine man showing a medicinal plant – i.e. *Clerodendrum colebrookianum* Walp.
Plate - 27: Investigator collecting information from an old Riang woman.

Plate - 28: Photograph showing a Jaintia Kabiraj.

Plate - 29: A Jaintia medicine man showing the medicinal plants.
Plate - 30 : A tea garden Kabiraj searching for medicinal plant.

Plate - 31 : Ananas comosus (L.) Merr.