CHAPTER II
MATERIAL AND METHODS

Most of the plant collections were made as part of a Major Research Project of the University Grants Commission, New Delhi. Members of Podostemaceae exist near the sources of rivers and in small streams which appear during the monsoon seasons. The sites of collections were often in remote forest areas and high ranges, which could be reached only by jeep. Bus and train journeys were also required to reach other sites. Often, the roads leading to the forest areas were in bad condition with uneven surface covered by coarse and loose stones which made the journey long and tedious.

Most members of this family were in bloom during December to January. The rivers, particularly near the sources started drying after January. During March to May, due to intense sunlight and hot weather, the plants became exposed and gradually they dried up. No collections could be made between June and August as the river sources were flooded during this period.

Photographs of the plants at various sites were taken during these trips. Altitude of the locality was measured using an altimeter. Entire plants as well as flowers and fruits of different stages were collected during the collection trips. The collected materials were preserved in 15% formaldehyde for morphological study. Some were fixed in FAA or FPA. Each material was given separate collection number. Field diaries showing details such as collection number, date of collection, name of the plant, family, locality, altitude, habit, habitat, important notes, details of identification and confirmation were prepared.

The numbered fresh specimens were placed in ordinary newspaper folders. These folders were then pressed in a field press. The folders required frequent changes in order to avoid blackening and decay of the plant material. The specimens were smeared with 10% aqueous solution of mercuric chloride to prevent the attack of fungi and insects. The specimens were again dried. The dried specimens were glued on herbarium sheets of standard size (34 x 27 cm). The field data were entered on the right hand side lower corner of the herbarium sheet. Museum specimens were prepared as follows :- Cotton wool was placed
on a glass plate and the specimen was placed over it. Then another glass plate of the same size was placed over it and these were tied using a twine and it was kept in 15% formalin taken in a museum jar. The jar was labelled.

The collected specimens were examined under a Carlseize binocular dissection microscope for studying morphological features. A compound microscope was also used occasionally. Drawings of the examined specimens were prepared and the measurements were taken by metric scale. These drawings were traced on gateway paper using rotring variant pens (thickness: 0.1, 0.2, 0.4, 0.6 and 1.00 mm) and black drawing ink (Rotring).

During the entire course of research, regular consultations were made with scientists of international reputation. They included Dr. Mohan Ram and Dr. Anita Sehgal of Delhi University, Delhi, Dr. Irmgard Jäger-Zürn, Germany; Dr. Rolf, Rutishauser of Switzerland, Prof. C. Thomas Philbrick of USA and Dr. Masahiro Kato of Japan. Also, some collaborative research work was done with Dr. Jäger-Zürn of Germany.

Materials of *Zeylanidium olivaceum* were sent to Dr. Rolf Rutishauser for preparation of Scanning Electron Micrographs (SEM). His comments were valuable for the research work. The illustrations and comments on *Dalzellia* spp., *Zeylanidium olivaceum*, *Podostemum subulatum* and *Podostemum munnarens* sent by Dr. Jäger - Zürn were very useful in the present study. Materials of American Podostemaceae received from Prof. C. Thomas Philbrick were compared with the Asian forms.

References for the research work were collected from Union Christian College Library, Alwaye, Central Marine Fisheries Research Institute (CMFRI) Library, Kochi, Calicut University Library, Calicut and Mahatma Gandhi University Library, Kottayam.