CHAPTER 3

Material and Methods

3.0 Introduction:

Thorough understanding of the technical aspects in conjunction with the socio-cultural milieu of the use of the botanicals is required for this study. A review on the include various ethno-botanical studies helped to frame a multidisciplinary method and research strategy for the present study. This approach visualized integrating disciplines like ethno-botany, taxonomy, social sciences, Ayurveda, pharmacognosy including anatomy and use of select molecular techniques for better understanding of the trade scenario (Figure 3.1)

![Diagram of research strategy]

**Figure 3.1. Research strategy to study the raw drug markets of southern India**

*Quality Assurance and Good Manufacturing Practices*
3.1. Study design:

The study design primarily focuses on the comprehensive ethno-botanical documentation of medicinal plant resources traded and the dynamism, including the application of pharmacognostic tools to authenticate the raw materials found in the market. Data was collected through interviews using open-ended questionnaires from identified Knowledge Partners (KP).

3.2. Steps involved in the study:

The main aim of the study is to facilitate the process of Quality Assurance (QA) of herbal drugs and authentication of plant materials traded and used in ISM industries. The following describes the materials and method applied to undertake the entire study.

3.2.1. Step 1: Identification of the study areas:

Trade study was limited to Tamil Nadu and Kerala states in southern India, where quantum of trade is more than 5,000 MT per annum as per the trade study conducted (Sasidharan and Muraleedharan, 2003; Harilal, 2008; Ved and Goraya, 2008). From these two states, six markets were identified based on earlier works and consultation with trade experts and latest studies (Table 3.1. and Figure 3.2).

Table 3.1: Selected markets and their specialties:

<table>
<thead>
<tr>
<th>Markets</th>
<th>Specialty</th>
<th>States</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chennai</td>
<td>Intermediate Mandis Export</td>
<td>TN</td>
<td>1,3,4</td>
</tr>
<tr>
<td>Virudhunagar</td>
<td>Intermediate Mandis</td>
<td>TN</td>
<td>1,4</td>
</tr>
<tr>
<td>Thoothukudi</td>
<td>Regional Mandis and Export</td>
<td>TN</td>
<td>1,4</td>
</tr>
<tr>
<td>Thrissur</td>
<td>Regional Mandis</td>
<td>KL</td>
<td>1,2,3,4</td>
</tr>
<tr>
<td>Madurai</td>
<td>Regional Mandis</td>
<td>TN</td>
<td>4</td>
</tr>
</tbody>
</table>

3.2.2. Step 2: Identification of Knowledge Partners (KP):

For prioritization of KPs, interactions with individual/experts/agencies working in the area of trade in, Tamil Nadu (TN) and, Kerala (KL) were consulted as a reliable source of information (Table 3.2).
### Table 3.2. Knowledge Partners represents a wide range of stake holders in the sector

<table>
<thead>
<tr>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary level collectors</strong></td>
</tr>
<tr>
<td><strong>Healers- cum-collectors community</strong></td>
</tr>
<tr>
<td><strong>Agents / Facilitators in raw material collections</strong></td>
</tr>
<tr>
<td><strong>Exporters</strong></td>
</tr>
<tr>
<td><strong>Traders:</strong></td>
</tr>
<tr>
<td><strong>Cultivators:</strong></td>
</tr>
<tr>
<td><strong>Agencies/ individuals who facilitate medicinal plants trade</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Industries categories</th>
<th>Consumption MT</th>
<th>Type of industry (numbers)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;10 T</td>
<td>Very small (1)</td>
</tr>
<tr>
<td></td>
<td>0 -50 T</td>
<td>Small(1)</td>
</tr>
<tr>
<td></td>
<td>&gt;10 -50 T</td>
<td>Medium(1)</td>
</tr>
<tr>
<td></td>
<td>&gt;10 -50 T</td>
<td>Large(1)</td>
</tr>
<tr>
<td></td>
<td>&gt;50 T</td>
<td>Very Large(1)</td>
</tr>
</tbody>
</table>
3.2.3. Step 3: Building rapport with the community and individuals respondents:

In this step, initially the key partners (KPs) and communities were contacted via non-government organization (The Covenant Centre for Development, Madurai) and Grama Mooligai Company Limited (GMCL), Madurai, a Community-Based Enterprise company in the study area. Through the link established, desired information including trade transactions were elicited through frequent contacts and visits (Chapter 4).

3.2.4. Step 4: Market survey:

One of the key objectives of this study is to systematically document the existing knowledge about the diversity of medicinal plants species traded in the select raw drug markets of Tamil Nadu (TN) and Kerala (KL), southern India. Regular visits to the market (shops, whole sale units, go downs, collection centres), enabled the preparation of a comprehensive resource inventory. An open-ended questionnaire was used to collect information (Annexure 1, given at the end of the thesis). To ensure the data consistency and reliability, recall techniques, personal observations, recording oral history and repeated questioning were employed (Alexiades and Sheldon, 1996; Yow, 2005). The survey was conducted for the period from Jan 2011 till Dec 2016.

In the present studies, primary collectors at village clusters were engaged in a Focused Group Discussions (FGD) to obtain ‘objective facts’ about the perceptions, attitudes and opinions of the participants in the group by employing moderating skills (Morgan and Krueger, 1993; Morgan, 2002).

In this study, a list of botanicals traded in the selected southern India markets were compiled from the field documentation. The list was further subjected to identification of high volume traded drugs based on the all India trade study conducted during 2006-08 (Ved and Goraya, 2008). An inventory of 298 botanical species and their 779 botanicals (different parts) were recorded. Authenticated specimens were deposited in the FRLH herbarium, Bangalore (Suma et al. 2017; Chapter 4).
3.2.5. Step 5: Documentation of fresh herbs sold in Tilagar tidal market (a special case):

In this study, an initiative has been made to document the unorganized system of healthcare service offered by the local shanties, especially at the Tilagar tidal market, in Madurai City of Tamil Nadu, which is popular for dispensing “Plant Drugs” and “Remedies”. During 2011-2013, recurrent visits were also made to this market, and all the five shop owners belonging to Valaiyar (Moopanar) community were interviewed to document the diverse medicinal plant species that are dispensed along with the prescriptions. Through ethno-botanical documentation, 136 species were recorded as being used for managing health care by a traditional snake catcher’s communities. This is the first time documentation of such a market in Madurai (Suma et al., 2017; Chapter 5).

3.2.6. Step 6: Preparation of herbarium and raw drug voucher specimen:

Samples of fresh plant drugs sold in the market were procured, processed into herbarium and raw drugs voucher specimen as per the international protocols (Bridson and Forman, 2010; Jain and Rao, 1977) and deposited at FRLH herbarium for authentication (Chapter 4).

3.2.7. Step 7: Authentication of the reference materials/voucher specimen:

The specimens were systematically identified by referring to regional, national and international flora (Gamble, 1987, 1993; Matthew, 1983; Henry et al., 1989 & 1987; Nair et al., 1983; Daniel, 2005; Vajravelu, 1990; Sivarajan and Balachandran, 1994; Sasidharan, 2004; Subramanian and Nallaswamy, 1987; Nayar et al., 2006; The Plantlist, 2013). Further, authenticated by the expert taxonomists at FRLH herbarium and raw drug repository, FRLHT-TDU, Bengaluru. These specimens were given accession numbers. Apart from these, the raw drug voucher specimens collected from the markets, were counter checked by comparing the features with the collections from wild and market sources in the Raw Drug Repository of FRLH-Herbarium, at FRLHT, Bengaluru. Subsequently, botanical names correlation for unidentified specimen using ‘FRLHT’s database on Indian Medicinal Plants
Nomenclature browser’ available on the website (Ved et al. 2016), based on trade name and photo-library comparison was done for the “yet to be identified” categories. Those specimens, which could not be identified, due to lack of proper plant parts and support of database were labeled as ‘yet to identified’ (Chapter 4).

3.2.8. Step 8: Data digitization and curation for analysis:

The data gathered were systematically compiled and computerized using Microsoft Excel and Access version 10. The database comprised of trade names (regional or Sanskrit names), binomial nomenclature, habit, habitat, specific botanical sources, annotated notes on striking features of the raw drugs (Suma et al. 2017).

3.2.9. Step 9: Botanical correlations with vernacular and trade names:

Further, the botanical correlation with Tamil/Malayalam/trade names, were carried out using multi-dimensional databases on Indian medicinal plants species (FRLHT Database, 2017).

3.2.10. Step 10: Tagging of botanical names to medical systems:

Additionally, the usage of these plants in other medical systems viz. Ayurveda (A), Siddha (S), Unani (U), Folk (F), Tibetan (T), Modern (M) and Traditional Chinese Medicine (C) were also tagged (envis.frlht.org). The inventory of medicinal plants recorded was overlapped with the database on Indian Medicinal Plants nomenclature browser to connect to the plants recorded in various Indian Systems of Medicine (ISM).

3.2.11. Step 11: Folk Medicinal uses and classical references:

In the special case study pertaining to Tilagar tidal market, medicinal uses recorded were closely examined by the physicians of Indian Systems of Medicine (ISM), who are familiar with Local Health Traditions (LHT) adopting the Documentation and Rapid Assessment methodology (Hafeel et al. 2004; Shankar et al. 2004). The experts consulted various classical medical publications related to medicinal uses of the studied species for direct or indirect references. Besides these, physicians also
contributed their experience of using these species for treating health conditions (Chapter 5).

3.2.12. **Step 12: Identification of alternatives/substitutes in the practice:**

In order to understand the demand and supply chain, it is important to know the alternatives used in the practice to meet the need in the herbal sector. In this process, based on field level documentation as explained in step 4, personal communication with traders, ISM physicians, collectors, industries and published sources including classical literature related to concept of alternatives, especially in Ayurveda resulted in listing of alternatives and substitutes. Additionally, during 2015, a consultative workshop of multi-stake holder (included collectors, traders and manufacturer) conducted which helped in expanding the list of alternatives/ substitutes as per business-as-usual. Through this process, approximately 60 trade name complexes with their multiple species were derived (Chapter 6).

3.2.13. **Step 13: Prioritization of Balaa complex:**

From the exhaustive list of species that are traded in the market, an attempt has been made to prioritize one of the well-known Ayurvedic drug complexes Balaa, called as Kurunthoti in Malayalam. In the Ayurveda concept of Pratinidhi Dravyas (alternatives) for Balaa complex, there are more than 5 species as per scholarly works. Many of these are accepted alternatives as per concepts specified in classical texts, scholarly works and Pharmacopeia (Chapter 8, Table 8.1). The multiple species used shows taxonomic affinities; regional complexities in Kurunthothi / Balaa trade (Aiyer and Kolammal,1972; Sivarajan and Balachandran,1994). Annual consumption of Balaa as raw drug by the medicine manufacturing units in North Kerala shows 6,96,943 tons of Balaa / Kurunthoti (Sida rhombifolia L. spp. retusa (L.) Borss. (Sasidharan and Muraleedharan, 2003). At all India level, Sida rhombifolia L. (Balaa) was recorded to be in high volume consumption (>5,000-10,000 MT (Harilal, 2008). Wide range of products for nerve disorders depend largely on Balaa classical and proprietary medicines. Hence, this group was though a commonly found weed, due to heavy demand, chances of alternatives is seen. Example: in Kerala, one of the raw drug trader alone sells 100 MT of Sida retusa L. and Sida
acuta Burm. f. to one of the popular company per year. In the preliminary understanding of the market, personal communication with KPs indicated that there has been demand for Kurunthoti (Balaa) in past 30 years in the study area. They have observed changing scenario of usage from the official part (roots of Sida cordifolia L.) to entire plant being used. Price also has risen from Rs. 8/- to Rs. 80/- per kilogram; over three decades. The use of alternative species such as Sida acuta Burm. f. and Sida retusa L. by industries has been seen as a business practice. As a regional choice for Tamil Nadu industries, Sida acuta Burm. f. is the first choice, while Kerala industries prefer Sida retusa L. and Sida rhombifolia L. If there is non-availability of these species, then Tamil Nadu variety is preferred. In business recorded, only one industry was found to use the original source of drug ie. Sida cordifolia L. The godown in charge said, this happens during available season, else Sida acuta Burm. f. / Sida retusa L. (imported from Kerala) is much sought and used.

3.2.14. Step 14: Application of pharmacognostic tools to ease in identification of genuine Balaa materials:

Ensuring usage of right material for preparation of traditional herbal formulations is a biggest challenge and responsibility for all the stakeholders in the trade. Through employing selected pharmacognosy tools such as taxonomy, anatomy, microscopy, a practical diagnostic key for identification was developed to differentiate multiple species complex for one trade name Balaa. Along with this, molecular tools were also employed to verify whether simple or compounded formulations claiming to have Balaa (Sida cordifolia L.), really had it at all. The following paragraphs describe briefly the steps involved in the process.

a. Preparation of the sample for anatomical studies:

Collection of raw drugs from different markets of Southern India and fresh materials from the wild was undertaken. These materials were identified and authenticated by the expert taxonomists from FRLHT herbarium and raw drug repository with an accession number. Specimen was deposited in the repository as reference materials. The fresh samples were either preserved wet in Formalin Acetic Acid-Alcohol [40% Formalin (5 ml); 50% Ethanol (90 ml); Glacial
Acetic Acid (5 ml)] or dried as such in semi-shade /room temperature. It was ensured that the material is thoroughly washed before preservation.

b. Anatomical characters analysis:

Leafy and stem materials was studied under the microscope after processing as prescribed in standard methods (Krishnamurthy, 1988; Trease and Evans, 1971). They were studied under the microscope (Olympus BX 41, Tokyo) and the photographic images were captured were utilized to develop a diagnostic key using trichome characters in comparison to earlier works (Metcalf and Chalk, 1972 and 1985) was carried out (Chapter 7).

c. Exo-morphological and taxonomical studies:

Specimen collected was closely examined using tools like hand lens, microscope to observe some of the key characters like shape, texture, colour, size, appearance, odour and taste. Further, these specimens were compared with specimen deposited at FRLHT herbarium for authentication. This effort is included in the ‘Medicinal Plants Raw Drugs Catalogue’ which forms part of the ‘Market Information System (MIS) of botanicals traded in a specific market’ (Suma et al.2017; Suma and Ravikumar, 2017).

d. Molecular studies of selected single/combined drugs formulations and species:

Besides these, selected single/combined drugs formulations available in the market were chosen based on their label mentioning *Sida cordifolia* L. the accepted source of *Balaa* (API, 1999). Internal Transcribed Spacer (*ITS2*) and Maturase K (*matK*) markers based DNA barcoding technique were employed (Murray and Thomson 1980; Chen et al. 2010; Rai et al. 2012) The study suggests for completing the DNA sequence library for original source of drugs for *Balaa* (Suma et al.2018;Chapter 8).
3.2.15. Step 15: Sharing knowledge: Capacity building of the beneficiaries about the raw drug identification, sources and related aspects:

Apart from publication in journals, attempts were made to reach out to Indian Systems of Medicine community through participation in invited talks, consultative meetings, participate in the All India Trade Study, conducted by ICFRE and NMPB during 2015-16 (Annexure 2 given at the end of the thesis).

The forth-coming chapter 4, shares the understanding of trade dynamism at regional level especially from the markets of Tamil Nadu and Kerala.