CHAPTER 4: MATERIALS AND METHODS

4.1 Ethnobotanical survey of medicinal plants in Bellary district

4.1.1 Data Collection Methods

Ethnomedicinal survey of traditional medicinal plants was carried out in Bellary district from May 2008 to November 2012 using ethnobotanical and Participatory Rural Appraisal (PRA) methods. Seventy six traditional healers (68 males and 8 females) were selected from different localities of the study area based on the recommendation from elders and local people of the district (Appendix 1). The ages of the healers were between 40 years and 85 years. A brief group discussion was made with the informants at each village prior to ethnobotanical data collection to get their consent and to explain them that their cooperation is a valuable contribution to the documentation of the traditional medicinal plants of the Bellary district.

The information was recorded by means of interviews with the informants in a standard questionnaire prescribed by the Ethnobotanical Society of India (Appendix 2). Group discussions and field observation were also employed to collect data on knowledge and management of medicinal plants. The group discussions were conducted to elaborate the methods of preparation, administration and conservation of medicinal plants. Interviews were conducted in local ‘Kannada’ language. During the study period, each informant was visited two to three times in order to confirm the reliability of the ethnobotanical information.

The reported medicinal plant species were photographed and collected from natural vegetation, cultivated fields and gardens during the field walks. The collected species were authenticated with the help of floras such as, Flora of Gulbarga district (Seetharam et al., 2000), Flora of Davangere district (Manjunath et al., 2004), Flora of Karnataka (Saldanha, 1984 & 1996), Flora of The Presidency of Madras (Gamble,
1915-1934) and Flora of The Presidency of Bombay (Theodore Cooke, 1903). The Voucher specimens were pressed and deposited at the Herbarium centre, department of Botany, Gulbarga University, Gulbarga.

4.1.2 Data analysis

i. Use categories

The medicinal plant uses were classified into ailment categories following the standard developed by Cook (1995). Each time a plant was mentioned as ‘used’ was considered as one use-report. If one informant used a plant to treat more than one disease in the same category, it was considered as a single use-report (Treyvaud et al., 2005).

ii. Informant consensus factor (ICF)

The Informant consensus factor (ICF) was calculated for each category to identify the homogeneity of the informants on the reported cures for the group of diseases. The ICF was calculated as follows: number of use citations in each category (nur) minus the number of species used (n), and divided by the numbers of use citations in each category (nur) minus one (Subramanyam Ragupathy et al., 2008).

\[
ICF = \frac{n_{ur} - n}{n_{ur} - 1}
\]

ICF values are low (near 0) if plants are chosen randomly or if there is no exchange of information about their use among informants, and approach one (1) when there is a well-defined selection criterion in the community and/or if information is exchanged between informants (Musa S. Musa et al., 2011).
iii› **Use Value**

The use value, a quantitative method that demonstrates the relative importance of species known locally, was calculated (Luiz Rodrigo Saldanha Gazzaneo *et al.*, 2005).

\[ UV = \frac{\sum U_i}{n} \]

Where, \( UV \) = use value of a species, \( U_i \) is the number of use-reports cited by each informant for a given species and \( n \) refers to the total number of informants.

iv› **Fidelity level (FL)**

Because many plant species may be used in the same use category, it is interesting to determine the most preferred species used in treatment of particular ailment, which can be done with the FL (Auragh Singh *et al.*, 2012).

\[ FL(\%) = \frac{N_p}{N} \times 100 \]

Where, \( N_p \) is the number of use-reports cited for a given species for a particular ailment and \( N \) is the total number of use-reports cited for any given species. High FLs (near 100%) are obtained for plants for which almost all use reports refer to the same way of using it, whereas low FLs are obtained for plants that are used for many different purposes.

4.2 **Pharmacological study of *Momordica charantia* L.**

i› **Collection of plant material**

The leaves and fruits of *M. charantia* L. grown in black and red soils were collected in fresh bags from Balavanchi village of H. B. Halli taluk and brought to the laboratory. The collected plant materials were initially rinsed with distilled water to remove soil and other contaminants and dried on paper towel in laboratory at 37 ± 2°C for a week.
ii) Extraction of plant material by soxhlet apparatus

The leaves and fruits after drying in shade were ground in a grinding machine in the laboratory and then 25 g of this powdered plant material was weighed and extracted successively with methanol in soxhlet extractor for 48h. The methanol extracts were concentrated under reduced pressure and preserved in refrigerator in airtight bottle for further use.

iii) Test microorganisms

Three fungal cultures viz., Aspergillus niger, Aspergillus flavus and Trichopyton rubrum and three bacterial cultures viz., Bacillus subtilis, Escherichia coli and Staphylococcus aureus were used in the antimicrobial test. All these tested strains were obtained from the Department of Microbiology, Gulbarga University, Gulbarga. The fungal cultures were grown in potato dextrose broth at 28°C and maintained on potato dextrose agar slants at 4°C. Bacterial cultures were grown in nutrient broth (Hi-media, M002) at 37°C and maintained on nutrient agar slants at 4°C.

iv) Agar-well diffusion method

The assay was conducted by agar well diffusion method. About 15 to 20 ml of potato dextrose agar medium was poured in the sterilized petri dishes and allowed to solidify. Fungal lawn was prepared using 5 days old culture strain. The fungal strains were suspended in a saline solution (0.85% NaCl) and adjusted to a turbidity of 0.5 Mac Farland standards (108 CFU/ml). One ml of fungal strain was spread over the medium using a sterilized glass spreader. Using flamed sterile borer, 6 wells of 4 mm diameter were punctured in the culture medium.

Required concentration of diluted plant extracts (40mg/ml) were added to 4 wells. Leaf extract and fruit extract of black soil sample was added to well numbers 1
and 2 while, leaf extract and fruit extract of red soil sample was added to well numbers 3 and 4 respectively. Dimethylformamide (DMF) was taken in well number 5 as control and Ketoconazole in well number 6 as standard. The plates thus prepared were left for diffusion of extracts into media for one hour in the refrigerator and then incubated at 37°C. After incubation for 48h, the plates were observed for zones of inhibition. The diameter zone of inhibition was measured and expressed in millimeters. DMF was used as a negative control. The experiments were conducted in triplicates.

The same method was followed for testing antibacterial activity using nutrient agar medium incubated at 37°C for 18h. But in well number 6 Streptomycin was taken as standard instead of Ketoconazole. After incubation for 48h, the plates were observed for zones of inhibition.

**Determination of minimum inhibitory concentration (MIC)**

The Minimum inhibitory concentration (MIC) value was considered as the lowest extract concentration with no visible growth for each plant extract test pathogens. To measure the MIC values, various concentrations of the fruit extract of black soil sample i.e., 40, 20, 10, 5, 2.5, 1.25 and 0.62mg/ml was taken in well numbers 1, 2, 3, 4, 6, 7 and 8 respectively. In the 5th well control (DMF) and in the 9th well, standard (Ketoconazole/Streptomycin) was taken. After 2 days of incubation at 26 ±2°C for *T. rubrum*, *A.niger* and *A. flavus* and 18h of incubation at 36 ± 2°C for *B. subtilis*, *E. coli* and *S. aureus*, the plates were examined and the minimum inhibition concentrations were measured.