Chapter - 2

Materials and Methods - General
2.1. General description of the plants selected for the study

2.1.1. Artemisia nilagirica (C.B.Clarke) Pampan. (Plate 1, Fig.a-c)

Systematic Position

- **Division**: Phanerogamae
- **Class**: Dicotyledonae
- **Subclass**: Gamopetalae
- **Series**: Inferae
- **Order**: Asterales
- **Family**: Asteraceae
- **Genus**: Artemisia
- **Species**: nilagirica

_Artemisia nilagirica_ (C.B.Clarke) Pampan. (Syn. _A. vulgaris var. nilagirica_ C.B. Clarke) is endemic to India which is a tall aromatic shrub like herb, often forming thickets 6-8 ft high with branches as thick as the thumb, and leaves 7 inches long and broad; the leaves normally vary from very lobulate, like those of the garden chrysanthemum, to pinnatifidly 2-3 sect, and from green or hoary on both surfaces to thickly clothed with white or buff, tomentose beneath or on both the surfaces, heads too, vary greatly in number, disposition, size and form (Hooker 1882). The plant is popularly known as Makkipu, Masipattiri (Tamil) which is an aromatic, herbaceaous perennial plant, which grows upto 150 cm on nitrogenous soils in hilly districts in India and is native to Europe, Asia, Northern Africa, Alaska and North America. This plant is commonly called as ‘Indian wormwood’ and is widely used in the hilly areas of India as an insecticide (Bhattacharjee 2000).

2.1.2. Artemisia japonica Thunb. (Plate 2, Fig. a-c)

Systematic Position

- **Division**: Phanerogamae
- **Class**: Dicotyledonae
- **Subclass**: Gamopetalae
- **Series**: Inferae
- **Order**: Asterales
- **Family**: Asteraceae
- **Genus**: Artemisia
- **Species**: japonica
Artemisia japonica Thunb. (Syn. Artemisia parviflora Buch.-Ham. ex D. Don) is a member of the family Asteraceae (Compositae) and is distributed in India, Myanmar, Pakistan, Nepal, Bhutan, Afghanistan and Japan. It is a non aromatic shrubby perennial herb which is reported in the southern Kerala particularly in Munnar. This plant is used in medicine and essential oils are having anti-viral and anthelmintic properties (Rajeshkumar and Hosagoudar 2012). The plant has been used as a traditional medicine to treat fever and eczema (Kim 1998). In traditional medicine, various parts of Artemisia japonica (leaves, stem, seeds and fruits) have been widely used by tribal people for its wound healing properties, treatment of skin diseases, febrifuge, depurative properties, digestive and in ethnoveterinary medicine (Paramakrishnan et al. 2012). Antimalarial activity of A. japonica, A. maritima and A. nilagirica has also been studied (Valecha et al. 1994).

The plant is 1-3 ft. high, glabrous, stems erect or ascending stout panniculately branched plant. Leaves variable, 1-2 inches long, the lowest sometimes nearly 1 inch diameter with palmately spreading 3-5 fid lobes, the upper middle sometimes 1-2 pinnatifid with narrow lobes, but usually the lower and middle cauline are simply narrowly cuneate and acutely 3-5 fid at the broad end. All have generally a pair of stipule like narrow lobes at the base. Heads always pedicelled and greenish (Hooker 1882).

2.1.3. Artemisia sp. (Plate 3, Fig. a-c)

A short herb with height upto 1 ft, aromatic and is distributed in the hilly areas especially in Idukki district of Kerala with an elevation of 900-1200m and the plant often form thickets. The branches are about 2cm girth, stems leafy; leaves lobed laciniate or 1-2 pinnately partite, white tomentose beneath. Leaves upto 3.5 inches long and 2 inches broad. The leaves normally vary from pinnatifid 2-3 sects, lower surface thickly clothed, white or buff tomentose. Leaves irregularly serrate or lobulate, petioled with stipule like basal lobes, uppermost linear, lanceolate and entire.

2.2. Collection of plants

A. nilagirica were collected from the wild populations from Idukki (elevation of about 900-1200m), Wayanad (elevation of about 600-900m) and Palakkad (elevation of about 300-600m) (Srikumar and Suresh Kumar 2013) districts of Kerala. A. japonica which is seen in Munnar hills of Kerala was collected from wild populations. During the
collection, from Munnar areas of Idukki district, a plant with leaves which is morphologically similar to that of *Artemisia nilagirica* was seen. The plant is an aromatic perennial herb and had pinnatifid leaves. The leaves are pubescent and the plant is of short stature. The plant did not flower during the study period. So the plant was selected for study. The collected samples were authenticated and voucher specimens deposited in the herbarium of Department of Botany, Sacred Heart College, Thevara.

2.3. Analysis of essential oils

The methods of isolation of essential oil and GC-MS analysis are described in chapter 3.

2.4. Quantitative Estimation of Artemisinin

Artemisinin, a sesquiterpene lactone with an endoperoxide bridge is an effective antimalarial drug used in the treatment of malaria. Quantitative analysis of artemisinin in the selected samples of *Artemisia* spp. was done by HPLC analysis. The materials and methods of HPLC analysis of artemisinin are explained in the chapter 4.

2.5. DNA Barcoding

Methods of DNA Barcoding is given in detail in the chapter 5.

2.5.1. Phylogenetic analysis

The details of the methods adopted for phylogenetic analysis are given in chapter 5.

2.6. Morpho-anatomical studies

The methods to study morpho-anatomical characters are explained in chapter 6.
PLATE – 1

*Artemisia nilagirica* (C B. Clarke) Pampan.

a) Habit of *Artemisia nilagirica* (C.B. Clarke) Pampan.

b) Leaf morphology

c) Flowering shoot
PLATE – 2

*Artemisia japonica* Thunb.

a) Habit of *Artemisia japonica* Thunb.

b) Leaf morphology

c) Flowering shoot
PLATE - 3

Unidentified *Artemisia* sp.

a) Habit of *Artemisia* sp.
b) Leaf morphology
c) A single plant