Chapter - 6

Morpho-anatomical studies
6.1. INTRODUCTION AND REVIEW OF LITERATURE

The family Asteraceae consist of more than 1100 genera and about 20000 species, being the second largest family in the flowering plants. The only other family of comparable size is the Orchidaceae with about 30000 species (Cronquist 1981). *Artemisia* L. is the largest genus of the tribe Anthemideae and one of the largest genus of the family Asteraceae which comprises of about 500 species widely distributed in the Northern hemisphere, in Asia particularly Central and West Asia (Bremer and Humphries 1993). *Artemisia* is one of the important genus having a wide and different morphological features, and with a wide habitat. Hence their identification is complex (Noorbaksh, Ghahreman, and Attar 2008).

The present work concentrated on the Phytochemical and Molecular Systematics of *Artemisia* spp. namely *A. nilagirica* and *A. japonica*, which are reported from Kerala. But during the period of study, a plant which is morphologically similar to the juvenile plants of *Artemisia nilagirica* was found. The plant did not flower and hence proper identification was not possible. This plant which is considered to be *Artemisia* sp. was also taken for the study. The essential oil analysis of the plant samples exhibited considerable differences in the constitution. The major constituent in all seasons is thujone in *A. nilagirica*, whereas, it is camphor and caryophyllene oxide in unidentified *Artemisia* sp. The essential oil composition is distinct and is not matching perfectly with any other species. Artemisinin content was estimated by HPLC method (Kim et al. 2001) and was found to be higher (0.01%) compared to *A. nilagirica* (0.00465%-0.00965%) and *A. japonica* (0.00725-0.01%). DNA was extracted from the unidentified species of *Artemisia*. The BLAST hits favoured a 99% similarity with *A. argyi* and 97% similarity with *A. princeps* with ITS and trnH-psbA sequences respectively. The authenticated photographs (Fig. 66) sent by Dr. Filip Verloove of Botanic Garden of Meise (Belgium) for *A. princeps* and the herbarium images from Kew (Fig. 67) for *A. argyi* are not matching with the unidentified species. This necessitated us to conduct the morpho-anatomical studies of the *Artemisia* spp. showcasing a perfect mismatch to the title of the thesis, as well as the inseparability of classical and modern taxonomy.
distribution of foliar trichomes and revealed that foliar trichomes of the genus *Artemisia* are good taxonomic markers which can be used to resolve the taxonomic complexities.

Stover (1951) mentioned about the arrangement of vascular bundles in the stem and reported that, in Asteraceae, the interbundle tissue becomes thick walled and lignified. Cronquist (1981) is of the opinion that the stem of Asteraceae is sometimes showing well developed secondary growth, even when herbaceous and with initially separate vascular bundles. Konowalik and Kreitschitz (2012) studied the comparative morphological and anatomical analysis of two wormwood varieties, *Artemisia absinthium* var. absinthium and var. calcigena. Haghighi et al. "The applicability of morphological characters....." (2014) studied the applicability of morphological characters in the taxonomy of *Artemisia*. They concluded that phylogenetic analysis based on morphology is important in taxonomic application and taxa identification.

### 6.2. MATERIALS AND METHODS

The fresh samples of *Artemisia nilagirica*, *Artemisia japonica* and unidentified species of *Artemisia* were collected for taking hand sections. Morphological features of the plant samples were noted. Anatomy of stems, roots and leaves were observed after taking transverse sections. Sections were stained with safranin. Photographs of each hand section was taken with the help of Leica ATC2000 microscope and Nikon Coolpix E4500 high resolution digital camera. The images were transferred to the computer using Nikon View software, which stores all shooting data.
6.3. RESULTS AND DISCUSSION

Results of the morpho-anatomical study is summarised in Table 21.

6.3.1. Morphological features

The plant Artemisia nilagirica is a shrub, which is strongly aromatic and grows up to 5-6 ft in height with pinnatifid leaves (Plate 1. Fig. b). The stems and leaves are pubescent in nature. The leaves are tomentose with white hairs beneath. Flowering occurs almost throughout the year. Artemisia japonica is an inodorous herb, about 2 ft long, and the leaves are wedge shaped (Plate 2. Fig. b). Flowering occurs during July-December.

The unidentified Artemisia sp. which is collected during the study is a short herb, which is aromatic with pinnatifid leaves. The stems are leafy; leaves are tomentose in nature and they did not flower during the tenure of the study (Plate 3. Fig. b)

6.3.2. Anatomical features

6.3.2.1. Transverse Section of Stem (Plates 4,5)

The stem of A. nilagirica is almost circular in outline with shallow ridges and furrows. It consists of a thin continuous layer of epidermis. There are numerous hairs present on the epidermal cells (Plate 4 Fig. a and Plate 5 Fig. a) namely two-armed, uniseriate and wholly haired. The present study corroborates the earlier report by El-Sahhar, Nassar, and Farag (2011) regarding the fluted nature and hair patterns in the stem of A. vulgaris. Inner to the epidermis, especially along the ridges, there are three to four layers of thick walled cells. There are two to three layers of chlorenchymatous cells along the furrows. The pith and inner cortex region consists of parenchymatous tissue. The vascular bundles are arranged in the form of a ring. The vascular bundles are top shaped and thick. They are collateral and conjoint. The outer end of vascular bundles have a sclerenchymatous bundle cap.

The transverse section of the stem of A. japonica is with shallow ridges and furrows (Plate 4. Fig. b). The stem consists of a continuous layer of epidermis below which there are a few layers of thick walled cells. There are discrete vascular bundles arranged in a ring. The pith is parenchymatous. The vascular bundles are collateral and
conjoint with sclerenchymatous bundle cap. Parthasarathy (2011) reported similar type of anatomy for *A. japonica*.

The transverse section of unidentified *Artemisia* sp. stem is with ridges and furrows (plate 4. Fig. c). A single layered epidermis is present with limited number of hairs. Below that, there are parenchymatous cells. The central pith and inner cortex is parenchymatous. The vascular bundles are arranged in a ring. The vascular bundles are top shaped, conjoint and collateral with sclerenchymatous bundle cap.

### 6.3.2.2. Tranverse Section of Root (Plates 6, 7)

The root of *A. nilagirica* (Plate 6. Fig. a) consists of a parenchymatous cortex and an inner stelar portion which is demarcated by an endodermis. The central pith is occupied by the secondary xylem elements in which the primary exarch xylem elements are present. In secondary phloem, between the primary xylem elements, prominent phloem fibres are seen. The root of *A. japonica* (Plate 7. Fig. b) consists of large parenchymatous cortex and an inner stelar portion. An endodermis is present in between the cortex and the stele. Primary xylem is immersed in the secondary xylem elements. In the root T.S. of unidentified *Artemisia* sp. (Plate 6. Fig. c) a parenchymatous cortex is present. A distinct endodermis and pericycle are seen. Secondary thickening is low and secondary xylem elements are less compared to the other two species. The primary xylem elements are not clearly visible unlike the other two species. A prominent parenchymatous pith is obvious.

### 6.3.2.3. Cross Section of Leaf (Plates 8, 9)

In cross sectional view of the leaf of *A. nilagirica* (Plate 8. Fig. a) the adaxial region consists of an epidermal layer where numerous hairs are present. The vascular bundle of the midrib is single, with parallel rows of xylem elements and a wide abaxial arc of phloem elements. Chlorenchymatous mesophyll tissue is present.

The leaf of *A. japonica* is dorsiventral with one layered epidermis and chlorenchymatous mesophyll tissue (Plate 8, Fig. b). Thick outer cuticle is present. Trichomes are limited in number which are seen on abaxial surface of leaf. There is a
single, centrally placed vascular strand which consists of xylem and phloem elements. Secretory cavities are noticed in the midrib region.

The leaf of unidentified *Artemisia* sp. (Plate 8. Fig. c) consists of an outer thick cuticle and an inner one or two layers of thick walled cells. Mesophyll is chlorenchymatous. A single vascular bundle with xylem and phloem elements is seen. Sclerenchymatous cells are also found associated with the vascular bundle. Prominent secretory cavities are seen around the vascular bundle.

Fahn (1997) reported that in the leaves of *Artemisia* species, the palisade parenchyma is present on both sides of the leaf and only a small strip of spongy parenchyma is present in the central portion of lamina. The present study also reveals a similar result.

From the morpho-anatomical point of view, there exist considerable differences in the morphology and anatomy of stem, root and leaf of the unidentified *Artemisia* sp. with that of *A. nilagirica* and *A. japonica*. The current study reveals the complimentarity of classical and modern approaches in solving taxonomic problems.
Table 21. Comparison of the morpho-anatomical studies in selected *Artemisia* spp.

<table>
<thead>
<tr>
<th>Features</th>
<th>Plant Species</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Morphological characters</strong></td>
<td><em>A. nilagirica</em></td>
</tr>
<tr>
<td>Habit</td>
<td>Shrub- 5-6 ft</td>
</tr>
<tr>
<td>Flowering period</td>
<td>October-June</td>
</tr>
<tr>
<td>Odour</td>
<td>Aromatic</td>
</tr>
</tbody>
</table>

**Leaf morphology**

**Anatomical features**

<table>
<thead>
<tr>
<th></th>
<th>Highly fluted</th>
<th>Fluted</th>
<th>Fluted</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stem</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Numerous hairs can be seen</td>
<td>Absence of hairs</td>
<td>Limited number of hairs</td>
<td></td>
</tr>
<tr>
<td><strong>Root</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The central pith is occupied by secondary xylem elements.</td>
<td>The central pith is occupied by secondary xylem elements.</td>
<td>Prominent parenchymatous pith is seen in the centre.</td>
<td></td>
</tr>
<tr>
<td>Primary xylem is exarch.</td>
<td>Primary xylem is exarch</td>
<td>Primary xylem is not clearly seen.</td>
<td></td>
</tr>
<tr>
<td>Secondary thickening present</td>
<td>Secondary thickening present</td>
<td>Secondary thickening is low with less secondary xylem elements.</td>
<td></td>
</tr>
<tr>
<td>Prominent phloem fibres can be seen.</td>
<td>Prominent phloem fibres cannot be seen.</td>
<td>Prominent phloem fibres cannot be seen.</td>
<td></td>
</tr>
<tr>
<td><strong>Leaf</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Numerous epidermal hairs are present</td>
<td>Epidermal hairs are absent</td>
<td>Epidermal hairs are limited in number</td>
<td></td>
</tr>
<tr>
<td>Chlorenchymatous mesophyll tissue can be seen</td>
<td>Chlorenchymatous mesophyll tissue can be seen</td>
<td>Chlorenchymatous mesophyll tissue can be seen</td>
<td></td>
</tr>
<tr>
<td>Secretory cavities cannot be seen</td>
<td>Secretory cavities can be seen</td>
<td>Numerous secretory cavities can be seen</td>
<td></td>
</tr>
<tr>
<td>Sclerenchymatous cells cannot be seen</td>
<td>Sclerenchymatous cells cannot be seen</td>
<td>Sclerenchymatous cells are seen associated with the vascular bundle.</td>
<td></td>
</tr>
</tbody>
</table>
PLATE 4
T.S. OF STEM- GROUND PLAN

Sbc - Sclerenchymatous bundle cap
Fu - Furow
Ri - Ridge
Vb - Vascular bundle
Pi - Pith
Tr - Trichome

a. *A. nilagirica*
b. *A. japonica*
c. *Artemisia* sp.
PLATE 5
T.S. OF STEM- A PORTION ENLARGED

Sbc - Sclerenchymatous bundle cap
P - Phloem
X - Xylem
Ch - Chlorenchyma
Ep - Epidermis
Twc - Thick walled cell
Pi - Pith
Tr - Trichome

a. A. nilagirica
b. A. japonica
c. Artemisia sp.
PLATE 6
T.S. OF ROOT- GROUND PLAN

Sp - Secondary phloem
Sx - Secondary xylem
Pf - Phloem fibre
E - Endodermis
C - Cortex

a. A. nilagirica
b. A. japonica
c. Artemisia sp.
PLATE 7
T.S. OF ROOT- A PORTION ENLARGED

Sp - Secondary phloem
Sx - Secondary xylem
Px - Primary xylem
Pf - Phloem fibre
E - Endodermis
Pe - Pericycle
C - Cortex
Rp - Ray parenchyma

a. A. nilagirica
b. A. japonica
c. Artemisia sp.
PLATE 8

LEAF- T.S AND MORPHOLOGY

Vb - Vascular Bundle
Ep - Epidermis
Tatr - Two armed trichome
Utr - Uniseriate trichome
Whtr- Wholly haired trichome
Cu - Cuticle

a. A. nilagirica - C.S of leaf
b. A. nilagirica - whole leaf
c. A. japonica - C.S of leaf
d. A. japonica - whole leaf
e. Artemisia sp. - C.S of leaf
f. Artemisia sp. - whole leaf

Phytochemical and Molecular Systematics of Artemisia spp.
PLATE 9

T.S OF LEAF- A PORTION ENLARGED

Cu - Cuticle
Ep - Epidermis
X - Xylem
P - Phloem
Sc - Secretory cavity
Tr - Trichome
Twc - Thick walled cell

a. A. nilagirica
b. A. japonica
c. Artemisia sp.