Chapter - 7
Summary and conclusion
Plants are the gifts of Nature. They provide us with all the basic needs to sustain lives. Many of the plants are medicinal and cure us with their therapeutic properties. These medicinal properties may be due to the secondary metabolites present in the plant. *Artemisia* L. is a genus of small herbs and shrubs which belong to the large family of Compositae that constitute about 500 species distributed in Northern temperate regions. About 45 species are reported in India and two from Kerala, namely *Artemisia nilagirica* and *Artemisia japonica*. The most famous species of *Artemisia* is *Artemisia annua* which is the source of artemisinin, the antimalarial drug.

The present study was designed to analyse the essential oil composition of the *Artemisia* spp. in Kerala, their artemisinin content estimation and to study their molecular aspects. During the collection of plant samples, a plant which is morphologically similar but of short stature to that of *A. nilagirica* was observed. The plant did not flower during the study period. So the plant, which is morphologically very close to *Artemisia nilagirica* was also taken for the study.

The plants were collected from different locations in different seasons. *A. nilagirica* was collected from Munnar, Palakkad and Wayanad in post-monsoon, pre-monsoon and monsoon periods. The essential oil was yellowish in colour, yield varies from 1-1.8% and was dominated by oxygenated monoterpenes. GC-MS analysis of the essential oil of the shade dried leaves yielded different classes of compounds namely sesquiterpene hydrocarbons, oxygenated sesquiterpenes, monoterpane hydrocarbons, oxygenated monoterpenes and others. Irrespective of the growing conditions, *A. nilagirica* have its major constituent of essential oil as oxygenated monoterpane-thujone and β-thujone. The concentration of thujone varies from 4.5-58.1% and β-thujone from 22.8-49.5%. The highest concentration of thujone is observed from Munnar in the post-monsoon period where the concentration is 58.1%. The highest concentration of β-Thujone is seen from Wayanad in the pre-monsoon, where the concentration is 53.3%.

The essential oil of *Artemisia japonica* collected from Munnar hills of Idukki district in post-monsoon, pre-monsoon and monsoon seasons had yellow colour and the essential oil yield was 0.1-0.3% . The oil was dominated by sesquiterpene hydrocarbons. The major constituent was caryophyllene oxide in post-monsoon (10.5%) and pre-
monsoon (9.9%). Spathulenol was present throughout the year with the concentration in post-monsoon 6.7%, pre-monsoon 6.7% and monsoon 10.7%.

The essential oil yield of unidentified sp. of *Artemisia* was 1-1.2% and is blue in colour. The essential oil is dominated by sesquiterpene hydrocarbons with camphor as the major constituent (24.5%) and was present only in the post-monsoon period. Pre-monsoon and monsoon periods had caryophyllene oxide as the major compound with concentration 19% and 9.9% respectively.

The essential oil analysis of the plant samples showed variations in the constituents in different locations and seasons. Statistical analysis was done using Multivariate Statistical Package. The Principal Component Analysis and Cluster analysis of the essential oil constituents showed that unidentified *Artemisia* sp. stands distinct from *Artemisia nilagirica* and *Artemisia japonica*.

Artemisinin is a sesquiterpene lactone with an endoperoxide bridge, which possess antimalarial activity. From the estimation of artemisinin by HPLC, it was observed that artemisinin is present in the selected *Artemisia* spp., but the concentration is low. The concentration of artemisinin ranges from 0.0040-0.01% in the selected *Artemisia* spp. In *Artemisia nilagirica*, the concentration ranges from 0.00465-0.00965% in different agroclimatic conditions. The concentration of artemisinin in *Artemisia japonica* was in the range of 0.007-0.01% and in the unidentified species, it was 0.01% in all the growing seasons. Hence, the concentration of artemisinin showed variations with seasons and plant species.

DNA barcoding is a support for taxonomic identification which uses a short genomic region that is unanimously present in target lineages and has sufficient variation in the sequence to differentiate species. The present study focussed on the use of five barcode regions namely rbcL, matK, ITS, ITS2 and trnH-psbA. All the barcodes produced good quality sequences. The character sites variation were determined by Multiple Sequence Alignment and the variations in the nucleotides were in the order ITS>ITS2>trnH-psbA>matK>rbcL.

The results of the BLAST analysis showed that the plant which is of short stature is *Artemisia* sp. as it shows maximum identification similarity with the genus *Artemisia* deposited in the GenBank. The pairwise alignment showed maximum variations with ITS2 region.
Dendrogram constructed by UPGMA method using ITS2 gene showed *Artemisia* sp. in a distinct clade, which is adjacent to *A. nilagirica* from three locations. A combination of rbcL, matK, ITS, ITS2 and trnH-psbA also showed similar results. Phylogenetic analysis of the *Artemisia* spp. were done using the ITS2 sequences representing the five sections of the genus *Artemisia* namely *Artemisia*, *Dracunculus*, *Absinthium*, *Seriphidium* and *Tridentatae* using the data retrieved from the GenBank. The results of the analysis separates the five sections of the genus *Artemisia*, and the unidentified *Artemisia* sp. was placed in the section *Artemisia* next to *A. princeps*. The results also showed *A. nilagirica-Palakkad* as a sister clade to the section *Tridentatae*. It was found that *A. nilagirica* have a polyphyletic nature.

Morpho - anatomical studies were done inorder to compare the morphology and the anatomical features of *A. nilagirica* and *A. japonica* and the unidentified species of *Artemisia*. The plant *Artemisia nilagirica* is a tall shrub, which is strongly aromatic and grows upto 5-6 ft in height with pinnatifid leaves. 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 serrate. Flowering occurs during July- December. *Artemisia* sp. is a short herb, which is aromatic with pinnatifid leaves. The stems are leafy, leaves tomentose in nature. The plant did not flower during the study period. The anatomical features include the presence of numerous trichomes in the stem and leaf sections of *A. nilagirica*, whereas their number was found lesser in *A. japonica* and *Artemisia* sp. The presence of prominent phloem fibres was seen in root anatomy of *A. nilagirica*, whereas, it was not seen in *A. japonica* and unidentified *Artemisia* sp. Reduced secondary thickening was seen in unidentified *Artemisia* sp.

From the phytochemical and molecular point of view, *Artemisia* sp. which is short in stature, stands distinct from the studied *Artemisia* spp. Though it shows some similar morphological features with that of *A. nilagirica* like similarity in leaf morphology and aroma, it appears distinct in phytochemical and molecular aspects. Phytochemical and molecular studies of a plant is an aid for the classical taxonomic methods, when the identification becomes difficult when the flowers or fruits are lacking.
Thus the phytochemical, molecular and morpho-anatomical studies in *Artemisia* sp. showed that this may be a new taxon of *Artemisia*, with variations in morpho-anatomy, phytochemical and molecular aspects. It could be an introduced plant which failed to flower in the new plane. It may be a vegetatively fixed form or a new taxon evolved through some process which failed to flower but could be successfully propagated by vegetative means. It could be concluded that the genus *Artemisia* which is represented by around 500 species which span in five different sections *Artemisia, Dracunculus, Absinthium, Seriphidium and Tridentate* may represent a taxon with high evolutionary rate.