PART - 3

Summary and Conclusion

References

Annexure -1

Annexure -2

Research Papers
SUMMARY AND CONCLUSION
SUMMARY AND CONCLUSION

Garcinia is one of the potential under exploited multipurpose crops and recently gained a lot of attention as a popular means of weight lose because of the presence of (-) hydroxy citric acid in its fruit. Out of the 35 species found in India, 17 are endemic out of which 7 are available in Western Ghats. The increase in the level of endemism from 50% to 60% is an important indication of the shrinking population of these species in Western Ghats region. Expanding market size and product range of Garcinia have been visualized in the near future, hence issues related to chemical composition, methods for quantification, diversity and distribution among Garcinia Spp assume a greater importance. There are distinct morphological variations exist among the species of Garcinia in Western Ghats viz. G gummigutta, G indica, G tinctoria and G cowa. It is essential to study the biochemical and molecular characters of these species to get an overall idea about the crop. Domestication and improvement of the species will be easy if the detailed profile is known.

COLLECTION OF Garcinia Spp.

Two main species of Garcinia such as G gummigutta, G indica were noticed in large number during the survey. The species G. tinctoria was found only in Coorg areas of Karnataka. At the time of collection only one huge estate of wild G gummigutta was noticed in Thirunelveli (8° 59' Lat, 77° 18' Long) located in southern part of Western Ghats region. The prepared
collection map based on longitude and latitude of collection sites indicates that the density of *Garcinia* Spp is more in the central region (14°58' Lat to 12°03' Lat) of Western Ghats than the rest of the area

**BIOCHEMICAL STUDIES IN *Garcinia* Spp.**

Conventional method for the quantification of (-) HCA from *Garcinia* extract is titration with phenolphthalein indicator and this method of assay of (-) HCA in *Garcinia* extracts has the limitation of interference by other organic acids present in the samples. However, the percentage of (-) HCA from the leaves, fresh and dry rinds of *Garcinia* Spp. were quantified exactly by the present HPLC method using sodium sulphate as mobile phase and found comparatively high in *G. gummigutta* and almost nil in *G. tinctoria*. It is also possible to record the exact percentage of (-) HCA in commercial samples by this HPLC method and can trace about any possible adulterations of market sample if any. A dendrogram (SPSS) was also prepared based on the leaf and fruit (-) HCA percentage to understand the distribution of (-) HCA with different geographical area. It was not found any locational influence but the species wise variation was specific in this dendrogram.

HPLC method with RI detector was introduced for the quantification of monosaccharide, mainly fructose and dextrose in *Garcinia* species. Interestingly, the monosaccharide dextrose was found only in rinds (fresh & dry) while fructose was more predominant in the leaves of this species. Quantification of primary metabolites by HPLC- RI, revealed the inter species
variation of monosaccharide in *Garcinia* Spp. It was also helpful to understand that *G. tinctoria* has a separate carbohydrate profile than the other three species.

There are no reports regarding the estimation and profiling of volatile oil in *Garcinia* species yet. The volatile oil yielded after hydro distillation from the leaves was found high in *G. indica* followed by *G. gummi-gutta* and *G. cowa*. However, *G. tinctoria* showed the least yield. Trans-caryophyllene and Gamma-Muurolene were found the common compounds present in all the species of *Garcinia*. The metabolites identified from *Garcinia* Spp. such as Alpha-humulene, trans-Caryophyllene, Gamma-Cadinene, Beta-selinene are used in fragrance industries. However, the volatile oil obtained by hydro distillation of *Garcinia* leaves did not show any characteristic piquant aroma though the constituents are exactly same as that of other spice oils. The reason may be the poor yield of volatile oil in *Garcinia* species compared with other spice. Here it is important to mention that though *Garcinia* is considered as a spice crop, it is used and consumed as fruits.

Fatty acids are valuable products because of their involvement in several aspects of human health. Market demand for most fatty acid is growing continually and current sources are considered insufficient for satisfying this demand. In this context the high percentage of Stearic and Palmitic acid present in the seed butter of *Garcinia indica* and *Garcinia gummi-gutta* requires special attention for the commercial exploitation of these crops in future.
Quantification of primary metabolites i.e., carbohydrates in *Garcinia tinctoria* was found high when compared with other three species. Interestingly, the percentage of (-) HCA was found high in other three species (*G. gummi-gutta, G. indica*, and *G. cowa*) and almost nil in *G. tinctoria*. It can be concluded that the formation of carbohydrate in *G. tinctoria* is high, where (-) HCA is absent and the carbohydrate formation by the other species is low when (-) HCA is present. A detailed future study with respect to plant metabolism in *G. gummi-gutta* and *G. tinctoria* can sort out the issue of medicinal importance of this crop which is being used for centuries without knowing its importance. Metabolites profiling are strong diagnostic tools to assess individual metabolic phenotypes in response to inter species diversity and ecophysiological adjustment. Here the performance of individual plant species (*G. tinctoria*) has highly variable effects on increasing plant diversity.

**MOLECULAR STUDIES IN Garcinia Spp.**

In order to study the inter species and intra species genetic relation, DNA isolation protocols are needed to be optimized in *Garcinia* species. Till no attempts have been made to apply the molecular techniques to screen the intra and inter specific variability in *Garcinia* Spp in India so far. This study is the first report of molecular characterization in *Garcinia* Spp available in Western Ghats. Optimum DNA isolation was possible with 4% CTAB (100 Mm Tris, 30 mM EDTA 1 4 M Nacl) followed by 15% PVP and 0.3% mercaptoethanol in leaves, but in fresh fruit rinds it was with 2% CTAB (100 Mm Tris, 30 mM EDTA 1 4 M Nacl) by 15% PVP and 0.3%
mercaptoethanol) Optimum concentrations of reagents to perform RAPD–PCR reaction in these species were also standardized. Among the 30 primers used for the analysis only 12 was able to produce polymorphic bands with *Garcinia* DNA and they were OPAA-11, OPAA-01, OPAB-11, OPAB-01, AP-20, AF-11, AO-12, PO-5, AV-03, W-15, AB-16, and BB-18.

The intra species polymorphism of *G gummigutta* and *G indica* accessions collected from Western Ghats were 98.2 and 100% respectively. Two monomorphic bands were found common among the species of *Garcinia* (bp 200, bp 480) with the primer AP – 20 and some more repetitions of the study is needed. The heterogeneity index of *G gummigutta* (0.81) and *G indica* (0.82) were also found very high, indicating the high molecular variability exists among *G gummigutta* and *G indica* accessions collected from different parts of Western Ghats. This will help the plant breeders to select the divergent plants for breeding programme and enabled conservation for genetic diversity in genus *Garcinia*. Because of this reason the more available species of *Garcinia* in Western Ghats, *G gummigutta* and *G indica* were taken in a detailed manner to study the distribution and diversity using GIS tools.

**MOLECULAR AND BIOCHEMICAL STUDIES AMONG Garcinia Spp. USING GIS TOOLS.**

NTSys-pc -UPGMA dendrograms of *G gummigutta* and *G indica* were prepared separately and these dendrograms were compared with the
BIOCLIM models of altitude, rainfall and temperature maps (20 km x 20 km grid) of *G. gummigutta* and *G. indica* prepared with the help of DIVA-GIS to study the impact of environmental factors associated with molecular grouping. The molecular distribution among *G. gummigutta* and *G. indica* was found similar. The accessions collected from its natural origin were grouped together. Those accessions collected from other parts of Western Ghats were grouped separately. It was clear from the GIS analysis that, though rainfall and temperature do not have much influence on molecular variation, the altitude plays a key role in genetic of genus *Garcina* in Western Ghats.

Though the influence of altitude was significant in dendrograms, there was not any significant impact in Shannons diversity index. The molecular and biochemical diversity observed in Shannons diversity map was not having any specific pattern. It showed high in Central part of Western Ghats (14° 56' to 12° 17'). But it is necessary to mention here that where the more number of collection (≤11) exist within a range of 10 km, the diversity index is very high, which reveals that to get a better information through GIS tools, large number of collections are required.

As this under exploited species is on the edge of its extinction, georeferencing and mapping the distribution patterns enables to understand the important domain of *Garcina* in Western Ghats. The present study will provide a baseline data for further analysis on exploration, conservation and collection of germplasm of wild crop relatives of *Garcina* also.
FUTURE RESEARCH FOCUS

- The present study of the important species of *Garcinia* in Western Ghats with GIS tool will generate a timely information for understanding the habitat distraction of this crop, which should control immediately by the concerned departments in India.

- The maps prepared show certain regions are having high concentration (10-12 Acc, within 10 km) of wild *Garcinia* species while other places are having a scanty distribution.

- The study highlights the identification and estimation of important nutritional and medicinaly important constituents in *Garcinia* spp. The quantification of these metabolites and molecular profiling are the first report of this type.

- With the help of Eco-crop model of DIVA-GIS, a prediction map of domain of *Garcinia* in India could be prepared with the help of present collection records. Some literature record tells that North Eastern Himalaya also have certain amount of *Garcinia* species, Eco-crop model may identify those in future.