DISCUSSION
In India several species of *Garcinia* are reported to occur naturally in Southern Western Ghats, North-Eastern states and Andaman & Nicobar Islands. Many species are threatened due to habitat destruction and it is reported that *G. cadeliana* from south Andaman is almost extinct (Cheek, 2004). The lack of knowledge about the importance of crop coupled with habitat distraction is leading to genetic erosion and many species of *Garcinia* are threatened. Forecasting and diversity by GIS models are becoming increasingly important and sensible approach in the study of forest resources. In the present study four important species of *Garcinia* have been considered to study their overall characterization to understand the nutritional, medicinal importance of the crop as well as the locational influence in characters.

### 6.1 MORPHOLOGICAL VARIATIONS IN *Garcinia* species

*Garcinia* are understorey evergreen trees and shrubs with straight trunk, dense canopy and drooping branches with a pyramidal or conical shape. The typical features of *Garcinia* species include monopodial growth, a yellow exudates from stem, conaceous or leathery textured leaves. Most of the plants are known for their oil glands which contain yellow or brightly coloured resins (CSIR 1956). In the genus *Garcinia*, conventionally trees are propagated by seeds. Presence of agamospermy (seed apomixes) is known or suspected in at least ten species which have been further classified as obligate agamosperms (Richards, 1990a). Several of this species are wild...
and semi wild and very little information is known about their breeding biology. Seed longevity and seed storage behavior of many wild and semi domesticated species from the tropical regions of the world is still to be authenticated.

The leaves of *G. gummi-gutta, G. indica*, and *G. cowa* are dark green, elliptical shaped with an average length of 5-15 cm long and 2.5 - 15 cm wide where as in *G. tinctoria*, the leaves in oval-oblong form, acute apex, rounded off base, salient ribbings in the two sides, with 40 cm length and 20 cm width. *G. gummi-gutta* grows to an average height of 18 m and the largest *G. gummi-gutta* trees in closed canopy forest are around 60 cm diameter where *G. tinctoria* tree can reaches 22 m height, has erect trunk, brown-clear bark and pyramidal pantry.

The fruiting time of *G. gummi-gutta, G. tinctoria* and *G. cowa* are during June – July where in *G. indica*, it is in April- May. *G. gummi-gutta* fruits are spherical/round shaped with lemon yellow to bold yellow in colour. It has a prominent snout and is medium ridged. The fruits of *G. indica* are spherical in shape with sweetish acid taste. The colour of the fruit is either dark purple or red tinged with yellow and seeds are embedded in red acid pulp. The expected shelf life of a fresh fruit in *G. indica* is about one week. The fruits of *G. cowa* are dark green with 5 cm in diameter and will turn to yellow or orange when ripe. These fruits are eatable but not palatable. However, Subhadrabandhu (2001) stated that the horticultural potential of *G. cowa* has poorly been developed in particular and its reproductive biology is still not well authenticated.
understood A deeper knowledge of this could reveal barriers to seed and fruit set in this species. The fruits of *G. tinctoria* are unique shaped with hook like pointed projection and are too acidic to be eaten. *G. gummigutta* and *G. indica* have four or five seeds where in *G. cowa* the total number of seeds varies from 4 - 8. Interestingly the total number of seeds in *G. tinctoria* is 2- 4. It was reported by Abraham et al., (2010) that the seed germination pattern in all the three species (*G. gummigutta*, *G. indica* and *G. tinctoria*) was similar and peculiar, where at first instance the root and shoot sprouted from distal end of the seed in intact and cut form, indicating polarity. Longevity of *G. tinctoria* seeds was found minimum in comparison to *G. indica* and *G. gummigutta* (Malik et al., 2005). The fruit size, fruit colour and shape of the four *Garcinia* species are given in plate 1-4. From the above discussion it is clear that, the morphological features showing wide variability among the genus *Garcinia* and it is very important to know the morphological diversity to understand the genus completely.

6.2 COLLECTION OF *Garcinia* species

Western Ghats is one of the main centers of origin of *Garcinia* species. It has been identified as one of the 25 global biodiversity ‘hotspots’, and one of eight ‘hottest hotspots’ available today with a very high endemism (Myers, (1990), Myers et al., 2000). Seven species of *Garcinia* are endemic to this region of which four species (*G. gummigutta*, *G. indica*, *G. tinctoria*, *G. Morella*) are being commercially exploited (Seetharam, 2006).
About 90 accessions of *Garcinia* species were collected from 12 different locations of Western Ghats. The latitude of these collection spots ranged from $8^\circ 59'$ latitude to $17^\circ 76'$ latitude, which included Kerala, Tamilnadu, Karnataka, Goa and Maharashtra regions of India. Three main species of *Garcinia* viz. *G. gummigutta*, *G. indica* and *G. tinctoria* were noticed in large number during the survey. All the accessions used in the present study were collected from the natural forests of Western Ghats, India.

*G. gummigutta* grows on the humid slopes of the Western Ghats. The availability of *G. gummigutta* species decreases gradually from lower latitudinal to higher latitudinal area ($8^\circ 54'$ latitude – $12^\circ 44'$ latitude). Abraham *et al.*, (2004) also reported that the species *G. gummigutta* was found wild in the evergreen forest of South Western Ghats from South Karnataka extending southwards to Kerala and Tamilnadu. The wild and domesticated distribution of *G. indica* was seen in Konkan patches ($12^\circ 44'$ latitude – $17^\circ 76'$ latitude) of Western Ghats only. *G. indica* prefers partial shade and is more associated with fire protected secondary forests (Raju *et al.*, 2002). *G. indica* is also known as *kokum* in Hindi, *brindon* in Goa, *amsol*, *kokum*, *katambi*, *ratamba* in Marathi and Konkani, *murugal* in Karnataka. Different species of *Garcinias* are commonly known with a reference to ‘Gamboge’ as Indian Gamboge, Malabar Gamboge etc. The species *G. tinctoria* was found only in Coorg areas of Karnataka. It seems *G. tinctoria* is a high altitude loving crop. Utpala *et al.*, (2010) reported the presence of *G. tinctoria* at an altitude of 550 MSL to 650 MSL in North Eastern parts of India. Though *Garcinia* is a crop
used by the people of Kerala, Tamilnadu and Karnataka villagers, domestication of the crop is not a common practice. Villagers enjoy the fruits raw without knowing its medicinal value and there is no tendency to domesticate the plants. It was reported by Shrikanth et al., (2010) that the lesser regeneration of the population of *G. gummigutta*, *G. indica* and *G. tinctoria* is probably due to the high extraction of fruits. At the time of collection, a huge estate of wild *G. gummigutta* population was noticed in Thirunelveli (8°59' Lat, 77°18' Long) located in southern region of Western Ghats.

The longitude and latitude of each collection sites of *Garcinia* were recorded using GPS. The points in the form of latitude and longitude were used for the preparation of collection maps of *Garcinia* species in Western Ghats (Fig 1). The collection map shows that the density of *Garcinia* is more in the middle region of Western Ghats. The map indicates that Karwar and Sirsi forest of Western Ghats have the highest density with 10-12 accessions within a range of 10 kilometers. A GIS study conducted in this thesis shows that the altitude plays a major role for the distribution of the species. It was reported by Shrikanth et al., (2010) that the richness and density of *Garcinia* species were found more in the central and south central part of Western Ghats than the rest of the Western Ghats region.
The aim of the present study is to investigate the grouping of the *Garcinia* species on the basis of its biochemical and molecular data with the help of GIS to describe the geographic distribution of this species if any.

6.3 BIOCHEMICAL STUDIES IN *Garcinia* Spp.

6.3.1. Quantification of (-) Hydroxycitric acid from the leaves, fresh & dry rinds of *Garcinia* Spp.

*Garcinia* has gained a lot of attention of late as a popular means of weight loss because of the presence of (-) hydroxycitric acid / (-) HCA (1,2 dihydroxypropane-1,2,3-tricarboxylic acid) in fruit rinds and leaves (Varghese, 1996) Lowenstein (1971) found that (-) HCA strongly inhibited fatty acid synthesis in living system Sullivan (1984) studied the biological effect of (-) HCA and noticed that its effect starts from the inhibition of extra mitochondrial cleavage of citrate to oxaloacetate and acetyl-CoA catalyzed by ATP- citrate lyase (-) HCA limits the availability of acetyl-CoA units required for fatty acid synthesis and lipogenesis The inhibition of ATP citrate lyase by (-) HCA leads to less dietary carbohydrate utilization for the synthesis of fatty acids, resulting in more glycogen storage in the liver and muscles Watson *et al.*, (1969) encountered the powerful inhibition of ATP- citrate lyase by (-) HCA with purified enzyme from rat liver Thus a natural product of *Garcinia*, (-) HCA is working wonders in reducing weight and obesity

In later Heymsfield *et al.*, (1998) stated in their publication that although (-) HCA appears to be a promising experimental weight control
agent, studies in human are limited (Badmaev 1995, Thome, 1996, Rothacker, 1997) and results have been found contradictory. The evidence that supports human (-) HCA efficacy for weight control is based largely on studies with small sample size (Conie, 1993), studies that failed to include a placebo–treated group (Badmaev 1995) and use of inaccurate measures of body change (Thome, 1996). To overcome these three limitations and examine the effectiveness of (-) HCA for weight loss and fat mass reduction, Heymsfield et al., (1998) designed their investigation with rigorous controlled trials. On the basis of result obtained from their experiments Heymsfield et al., (1998) concluded that a prospective double-blind study failed to detect either weight loss or fat-mobilizing effects of (-) HCA beyond those of placebo.

The conventional method for the quantification of (-) HCA from *Garcinia* extract is titration with phenolphthalein indicator. The existing titration method of assay of (-) HCA in *Garcinia* extracts has the limitation of interference by other organic acids present in the samples. The acid–base titration method gives a slight higher percentage value for (-) HCA. In titration method it gives the total acidity of the sample. Bhabani et al., (2002) has reported the presence of oxalic and citric acids in minor quantity in the leaves and fruits of *Garcinia* species. However it is possible to record the exact amount of (-) HCA from the tissue with the help of HPLC-UV using Calcium salt of HCA as the standard.

The mobile phase used in the present liquid chromatography was 0.1 M sodium sulphate. C-18 reversed phase column was used for isocratic
elution The wavelength for the estimation and quantification was standardized at 203 nm The identity of (-) HCA was confirmed by spiking the retention time with the standard (Calcium salt of HCA) The relative retention time of (-) HCA standard was found around 4.52 minutes The values of (-) HCA were estimated corresponding to the area of (-) HCA in the chromatogram of the figure 3, 4 & 5 Along with (-) HCA standard, Fumaric acid also was used as an internal standard for calculation and showed a relative retention time of 10.1 minutes in HPLC pattern.

Of the four species studied, it is interesting that except *G. tinctoria*, the principal acid found in the fruit rinds and leaves was (-) HCA (Table 8, 9 & 10). *G. tinctoria* has different morphological features from the other three species also. The percentage of (-) HCA was found very high in *G. gummigutta* (5.046) and almost nil in *G. tinctoria*, indicating the specificity of percentage variation among these species. Jayaprakash *et al.* (2006) has reported that the major and potent phyto constituent in *G. gummigutta* is (-) hydroxycitric acid. When (-) HCA of the fruit rinds and leaves are compared, extraction percentage is always found higher in fruit rinds than that of leaf. Bhabani *et al.*, (2002) estimated the leaf and fruit (-) HCA of *G. cowa* using gradient elution and recorded 1.75% and 2.3% of (-) HCA respectively, while in the present study, HPLC method using mobile phase of 0.1M sodium sulphate recorded leaf (-) HCA as 1.60%, and fruit HCA as 2.98%.

(-) HCA percentage of the dry fruit rind (market sample) of these four species was also quantified by the above HPLC method. Here also the
The high percentage value of (-) HCA in dry rind of *G. gummigutta* (16.28%) compared to fresh (5.046%) can be correlated to its drage. *G. gummigutta* is referred in the Malayalam vernacular as *kodampuli* or *kudumpuli* and are valued for their dried rind, which is used in Kerala, as a condiment for flavouring curries in place of tamarind or lime. The dried rind of *G. gummigutta* and *G. indica* are used in fish curries for imparting the unique delicate flavour (Muthulakshmi and Sarah 1999). In India, Kokum (*G. indica*) juice is highly preferred in summer season (-) HCA is found to be a potential dietary supplement for appetite control also. Several products of (-) HCA like Citrin, Citrmax, Garcinia spray, Garcinia puff, Garcinia soap etc. are available commercially in the market (Muhammed, et al., 1994). It is possible to record the exact percentage of (-) HCA in commercial sample by the above HPLC method and can trace about any possible adulterations if any (Asish et al., 2008).

In the present study the percentage of (-) HCA from the leaves, fresh and dry rinds of *Garcinia* spp. were quantified exactly by HPLC technique and found high in *G. gummigutta* and almost nil in *G. tinctoria*. The later studies in this thesis revealed that the percentage of primary metabolites (Total carbohydrate& phenol) was found high in *G. tinctoria*.

(-) HCA has impacts on body’s production of fatty acids and cholesterol, therefore it may directly influence the production of sterols, thus restricting the production of steroid hormones. Pregnancy is a time of extreme...
sensitivity to steroid hormones, during that time products containing (-) HCA should not be recommended. Likewise women who are breast-feeding should also avoid (-) HCA (Jena et al., 2002). Recently some of the (-) HCA product (ethylenediamine salt of HCA) was reported to cause testicular atrophy (shrinkage or non-development) in rats. (Saito et al., 2005) Although experience with fruit source of (-) HCA shows that they are not dangerous, young children and are not advised to consume (-) HCA in large amounts.

However, *G. gummigutta* has a long history of usage as a flavoring agent, preservative and herbal tonic. There are no reports of (-) HCA toxicity regarding traditional (Indian) use of the *Garcinia* extract. In addition to this, there are no reports of any possible negative effect that may occur due to excess intake also. Clouatre and Rosenbaum (1994) pointed out that (-) HCA has extremely low levels of toxicity. For example, oral toxicity studies pointed out that 5000 mg/kg of body weight of (-) HCA resulted in no visible symptoms of toxicity or deaths in laboratory animals. This is roughly equivalent to 350 g or 233 times the dosage of 1.5 g/day of (-) HCA that might be consumed by an average sized person. Preuss et al., (2004) has reported the superior bioavailability of a novel calcium-potassium salt of HCA derived from *Garcinia gummiguta* (HCA-SX, Super CitriMax). Acute oral, acute dermal, primary dermal irritation, primary eye irritation and 90-day chronic toxicity studies as well as Ames bacterial reverse mutation and mouse lymphoma tests, were assessed to determine the safety of HCA-SX. No remarkable toxicity results were detected, demonstrating the safety of HCA-
Furthermore, clinical studies to evaluate the safety and efficacy of HCA-SX over a period of eight weeks were conducted in 60 human volunteers. Here also no significant adverse effects were reported. These results demonstrate the safety, bioavailability and efficacy of HCA-SX in weight management.

### 6.3.1.1 Distribution of (-) HCA (%) in *Garcinia gummigutta* and *G. indica* with different geographical areas of Western Ghats, India.

A dendrogram (SPSS) was prepared based on the leaf and fruit (-) HCA percentage to understand the distribution of (-) HCA with different geographical area (Table 11, Fig-2). Main variation was observed with respect to species than with location. Four clusters were formed with the constructed dendrogram. All the *G. gummigutta* accessions from Kerala, Karnataka were grouped together and that of *G. indica* were clumped separately indicating no specific influence of location in (-)HCA percentage.

### 6.3.2. Identification and estimation of Sugars from *Garcinia* Spp. by HPLC method

A new HPLC method with RI detector was used for the quantification of monosaccharide mainly fructose and dextrose in *Garcinia* species. Spectrophotometric method, it can only possible to quantify the total amount of sugars in sample whereas in this HPLC with RI detector, it is possible to quantify the exact amount of each monosaccharide present in *Garcinia* species. Ping and Kalyan (1997) have also reported about an HPLC.
Discussion

Quantification of Monosaccharides from Glycoproteins In their gradient derivatization method, Monosaccharide derivatives were separated on a C-18 reversed-phase HPLC column (3.9 x 150 mm, 5 um and 4.6 x 75 mm, 3.5 um) using 1-butylamine-phosphoric acid-tetrahydrofuran mobile phase system. While in the present isocratic elution with mobile phase as water and acetonitrile in the ratio 75:25 was found simple than the earlier one.

There is not much reported work related to carbohydrate profiling in *Garcinia* species by HPLC-RI technique. Interestingly, the monosaccharide dextrose was found only in rinds (fresh & dry) while fructose was more predominant in the leaves of this species. This is very similar to Apricot, a fruit where dextrose is found higher than fructose in fruits (Bieleski and Redgwell, 1997). The retention time (RT) for the dextrose (standard) by this HPLC was found 7.9 minutes, which was reproducible in the sample also (Fig- 6 & 7). In dry rind, the percentage of dextrose ranged from 19-24 (%), while in fresh rinds it was 0.45-0.76%. The higher value of dextrose in dry fruit rind is because of the presence of moisture. The other monosaccharide fructose was found only in the leaves of this particular species. The relative retention time for fructose (standard) was found 3.73 minutes (Fig 8 & 9). The percentage value of fructose in leaves ranged from 0.73-1.2% (Table 12 & 13) with *G. tinctoria* showing the highest percentage. The percentage values of fructose and dextrose were almost similar in other three species.
Discussion

The quantification of this primary metabolite by HPLC-RI revealed the inter-species variation of monosaccharide in *Garcinia* Spp. It showed a clear indication that *G. tinctoria* has a separate carbohydrate profile than the other three species.

6.3.3 Identification of Volatile oil constituents in *Garcinia* Spp.

The volatile oil yielded after hydro distillation was found comparatively high in *G. indica* (0.1 ml in 100g) followed by *G. gummigutta* and *G. cowa* (0.08 ml in 100g). However, *G. tinctoria* showed the least yield of 0.06 ml in 100g (Fig-10, Table 14). There is no report regarding the estimation and profiling of volatile oil in these species yet.

Essential oil from the leaves of four *Garcinia* species showed 7 to 12 (<1%) major components in GC-MS analysis (Table 15). Trans-caryophyllene and Gamma- Muurolene were found the common compounds present in all the species of *Garcinia*. Total Percentage of Trans caryophyllene was found high in *G. gummigutta* (16.18) followed by *G. tinctoria* (14.15). Caryophyllene is one of the chemical compound that contribute to the aroma of black pepper (*Piper nigrum*). Recently, in a study conducted by Jurg Gertsch *et al.*, (2008) from the Swiss Federal Institute of Technology found that, beta-caryophyllene was shown to selectively bind to the cannabinoid receptor type-2 and to exert significant cannabimimetic anti-inflammatory effects in mice. May be of this reason this spice crop *Garcinia* is used as a medicinal plant.

It is also interesting to note that some components are present in high percentages in some *Garcinia* species not in all, like Alpha – humulene (11%)
in *G. indica*, trans Caryophyllene (14.35%) in *G. tinctoria*, Gamma-Cadinene (23.44%) in *G. gummigutta* and Beta-selinene in *G. cowa* (13.28%) Secondary metabolites such as beta-bourbonene, Alpha-humulene, Gamma-Gurjunene and Gamma-Cadinene were present in all the species except *G. cowa*. These secondary metabolites are used mainly in fragrance industry. The figure representing the GC-MS profiling of *Garcinia* species are given in Fig 11-14.

The compounds which showed maximum similarity percentage (≥90%) with NIST and Wiley libraries only was taken and others which showed lesser similarity percentage was not taken for consideration. The essential oil constituents in *Asian Hypericum* L (one of clusiaceae family member) was also reported Beta-selinene, Gamma-Muurolene and Trans Caryophyllene as the major components (Demirci *et al.*, 2006).

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 (Utpala *et al.*, 2008), Gopalakrishnan *et al.*, 1993 and Zachariah & Parthasarathy, 2008). The reason may be the poor yield in *Garcinia* species comparing with other spice volatile oils.

6.3.4 Identification of free fatty acids from the seed kernel of *Garcinia gummigutta* and *G. indica* by GC-FID.

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 acid present in the seed butter of *Garcinia* species requires special attention.

The seed butter extracted from the seed kernel of *G. indica* and *G. gummigutta* were greasy to feel with whitish yellow in colour with a greasy texture and bland oily taste. The butter yielded around 20-30%. The butter is considered nutritive, demulcent, astringent and emollient. It is suitable for pharmaceutical purpose as ointments and suppositories. *Garcinia* butter is also considered as a specific remedy for diarrhea and dysentery. It is now used as cosmetics and medicines known as *Virkshamala* in *Ayurveda* (Subash Chandran, 1996).

The Free fatty acids present in the seed butter of these two species of *Garcinia* were converted to fatty acid methyl esters (FAME) and were identified by GC- FID with authentic standards. The relative retention time of Palmitate, Stearate and Arachidic esters were identified as 1.88, 3.09 and 5.59 respectively (Fig 15, 16 & 17). Among the identified fatty acids, the percentage of stearic acid was found higher in both the species followed by Palmitic acid (Table 16). A limited number of tree species, viz *Butyrospermum paradoxum* (shea), *Garcinia indica* (kokum), *Mangifera indica* (mango), *Shorea robusta* (sal), *Shorea stenoptera* (borneo tallow) and *Vateria indica* (dhupa) have been reported to accumulate more than 30% stearate in their seed oil (Salunke *et al.*, 1992). It was reported by Jayanth *et al.*, (2003) that analysis of fatty acids in *G. indica* at different developmental stages revealed that stearic acid is the major fatty acid and its concentration...
Discussion

In addition to stearic and palmitic acid, a small percentage of Arachidic acid was also found in both the species. Stearic acid is useful as an ingredient in making candles, plastics, dietary supplements, oil pastels, cosmetics, and for softening rubber. It is used to harden soaps, particularly those made with vegetable oil. Stearic acid is also used as a parting compound when making plaster castings from a plaster piece mold or waste mold and when making the mold from a shellacked clay original (Wootthikanokkhan and Tunjongnawi, 2002). In fireworks, stearic acid is often used to coat metal powders such as aluminum and iron (Tsenga et al., 1999). It is used with zinc as zinc stearate for cards to deliver smooth fanning motion. Stearic acid is one of the most commonly used lubricants during injection molding and pressing of ceramic powders. Stearic acid serves as an epilame (barrier film) treatment, applied to precision mechanical components to modify the surface properties to reduce the spreading (or creep) of subsequently applied lubricant films (Hunter, 2010). Recently, a long-acting anti-psychotic medication, paliperidone palmitate used in the treatment of schizophrenia has been synthesized using the oily palmitate ester (French et al., 2002). Andrea and Scott (1988) reported that total plasma cholesterol decreased by an average of 14% during consumption of high-stearic-acid diet. NLM - HSDB database record says, palmitic acid is nonirritating and nonsensitizing on skin.
Obesity is associated with insulin resistance and some reproductive abnormalities were suppressed by palmitic acid and stearic acid remarkably (Yi-Ming et al. 2001)

Comparative study of fatty acid profiling in these two major economical important species of Garcia followed by the high yield of butter and very high percentage of stearic and palmitic acid reveals the important economical utilization of this under exploited crops. Jaiyanth et al., (2003) has reported that G indica seeds can be used as a desirable source of acyltransferases for engineering a high stearic acid phenotype in temperate oilseeds.

6.3.5. Variation of Carbohydrate profiling and Total phenols in Garcia species

Garcinia species are widely used as a source of edible fruits. It is used extensively in culinary purposes in India, China, Srilanka and Thailand also Young leaves of G cowa are used as a food additive in many Thai dishes Young shoots and mature fruit of G tinctoria are eaten as vegetables and edible fruit in many South East Asian countrnes (Yapwattanaphun, et al., 2006)

The percentage of Total carbohydrate, starch & reducing sugar (carbohydrate profile) in G tinctoria was entirely different from other three species (Table 17, 18 &19) G gummingutta, G indica & G cowa did not show any observable variation in carbohydrate profile The percentage values
found were in good agreement with the reported work by Sampathu and Krishnamorty (1982) The higher percentage values of Carbohydrate profile in *G. tinctoria* indicating the nutritional importance and the reason of using it as a fruit in many South East Asian countries Interestingly *G. tinctoria* have different morphological features also Amit et al., (2010) has reported the preparation of a wine by fermentation of *Garcinia tinctoria* must (rind) using *Saccharomyces cerevisiae* They found that the wine had higher amount of residual sugars contributing to the calorific value The aldehydes and esters content in the final wine were 0.034% and 0.26%, respectively There was reduction of citric acid and oxalic acid (antinutritional factor) and synthesis of aspartic acid and glutamic acid It was also found that there was an increase in total phenolics (0.039% gallic acid equivalent) and reducing power on fermentation

The total phenol percentage in *G. tinctoria* was also found very high (2.45%) when compared with other three species (Table 20) It was reported by Yu Chen et al., (2010) that *G. tinctoria* has been widely used in traditional Chinese medicine for expelling worms and removing food toxins In addition to this they also reported that bioassay guided fractionation from *G. tinctoria* led to the isolation of six new xanthone (phenolic compound) derivatives indicating the medicinal impact of this particular species

Among the four *Garcinia* species accounts for consideration, the percentage of primary metabolites (Total carbohydrate, starch and reducing sugar) in *G. tinctoria* was very high when compared with other three species
of *Garcinia* Interestingly the percentage of (-) HCA was found high in other three species ( *G. gummigutta, G. indica* and *G. cowa*) and almost nil in *G. tinctoria*. The biological action of (-) HCA is believed to carry out the inhibition of ATP citrate lyase that leads to less dietary carbohydrate utilization for the synthesis of fatty acids, resulting in more glycogen storage in the liver and muscles. Based on the present study it can be concluded that the utilization of carbohydrate in *G. tinctoria* is high, where (-) HCA is absent and the carbohydrate utilization by the plant is low when (-) HCA is present. A detailed future study with respect to plant metabolism in *G. gummigutta* and *G. tinctoria* can sort out the issue with respect to the biological action of (-) HCA.

A study conducted by Scherling et al., (2010) demonstrates that metabolite profiling is a strong diagnostic tool to assess individual metabolic phenotypes in response to inter species diversity and ecophysiological adjustment. Here also the performance of individual plant species (*G. tinctoria*) has highly variable effects on increasing plant diversity.

6.4 MOLECULAR STUDIES IN *Garcinia* Spp,

Genetic studies will give a direct knowledge regarding the gene variations exist within and between the species. Since the biochemical composition of this species varies considerably the genetic variation is also expected. In order to study the inter species and intra species genetic
relation, DNA isolation protocols are needed to be optimized in *Garcinia* species

6.4.1. Standardisation of DNA isolation and RAPD protocols in *Garcinia* species

**Discussion**

RAPD-PCR technique is successfully used in determining genetic diversity in various plants from Western Ghats such as *Dendrocalamus strictus* and *Bambusa bambos* (Biradar *et al.*, 2005) ground parrot (Chan *et al.*, 2008) and *Humboldtia brunosis* (Borges *et al.*, 2010) along with *Urginea indica* (Sathyanarayana *et al.*, 2008), *Gmelina arborea* (Shankar *et al.*, 2009), *Sweet Sorghum* (Kacahapur *et al.*, 2009), *Allium stracheyi* (Ranjan *et al.*, 2010) RAPD is the most widely used technique as it requires low quantities of DNA, it is a fast and easy assay and it does not require any sequence data for primer design (Ercisi *et al.*, 2007) But up till, no attempts have been made to apply the molecular techniques to screen the intraspecific variability in *Garcinia* Spp in India so far This study is the first report of molecular characterization in *Garcinia* Spp

DNA extraction for woody plants is difficult due to the presence of contaminants such as polyphenols, polysaccharides and other secondary metabolites The problems encountered include degradation of DNA due to endonucleases, co-isolation of highly viscous polysaccharides, polyphenols, which are powerful oxidizing agents and these all factors can reduce the yield and purity of extracted DNA (Khanuja *et al.*, 1999) But up till now no attempts
have been made to apply the molecular techniques to screen the inter and intra specific variability in *Garcinia* species. A modified CTAB protocol for the extraction of DNA from *Garcinia* species was developed from the existing method that was originally made for other plants (Doyle and Doyle, 1987, Lodhi et al., 1994.) In addition to this, the protocol optimizes the various conditions for RAPD – PCR such as annealing temperature, DNA concentration and the amount of Taq polymerase also.

Optimum DNA isolation was possible with 4% CTAB (100 mM Tris, 30 mM EDTA 1 4 M NaCl) followed by 1 5% 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 1 5% PVP and 0 3% mercaptoethanol. The quality ratio (260/280) of all the isolated DNA samples from leaf and rind showed a value in between 1.62 to 1.82. The quantity of DNA isolated from fresh leaves were showed a value of 13-25 ng by using with 4% CTAB buffer while in fruit rinds (2% CTAB) the yield was 9.2-18.2 ng (Table 21 & 22). DNA yielded from young leaf tissue of *Garcinia* species were high compared to that of fresh fruit rind. For better quality and quantity of DNA, the choice of appropriate tissue must be an important factor. The genomic DNA isolated from the leaves and rinds are given in figure 18 & 19.

In case of fresh rinds the isolation was found better with 2% CTAB buffer. CTAB is poor with denaturing the proteins but should be more amenable to plant material which contains more polysaccharide (Edwards et
Hence in case of leaf sample by using 4% CTAB, the yield and quality was better but by using the same buffer (4% CTAB) for fruit rinds the quality was low because of more protein precipitation. Better yield was noticed with 30 mM EDTA than that of 10mM, 20 mM EDTA. The optimum concentration of EDTA was found to be 30mM for this Garcinia species. Use of chilled isopropanol for DNA precipitation improved the yield. The addition of 5M NaCl helped in precipitation of DNA. It is also reported that the addition of high concentration of NaCl increased the solubility of polysaccharides and effectively decreasing co-precipitation of the polysaccharides (Fang et al., 1992). The freer the DNA is from contaminants, the easier it is to resuspend the pellet.

To get consistent, reproducible and sharp amplicons, RAPD reaction parameters such as amount of DNA, amount of Taq polymerase, MgCl₂ and annealing temperatures are needed to be optimized. The effect of various concentrations of Taq polymerase, DNA template and MgCl₂ on PCR amplification were noticed for better amplification. In the present RAPD analysis, 50 ng of DNA sample was used in 25μl reaction was found to give distinct polymorphic bands. Different concentration (0.4 – 2 mM) of MgCl₂ were used for amplification; however, it was found that 0.7 mM MgCl₂ was found better in these species. Since Mg²⁺ complexes the single nucleotide in PCR reaction and Mg-nucleotide complex are the substrate for DNA polymerase, the concentration of Mg²⁺ influences the productivity and fidelity of polymerase reaction. (Henegru et al., 1997)
Discussion along with 1U Taq polymerase were used for this profiling with an annealing at 43°C for 1 minute was found to be better than with 37°C. In general, RAPD reactions are carried out at low annealing temperature 34 to 38°C. But there are reports where temperature as high as 50°C is used (Fernandez, 2002). For *G. mangostana* annealing temperature required was 41°C (Sompong, 2004). The concentration of primer was found to be better with 10 (P moles).

About 30 primers were screened for RAPD analysis with *Garcinia* DNA. 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. These primers were utilized in a detailed manner to study the molecular distribution of *Garcinia* species from different geographical areas of Western Ghats.

6.4.2. Molecular profiling and Heterogeneity index in *Garcinia gummigutta* and *G. indica* accessions.

In the present RAPD-PCR analysis, 112 clear DNA bands were produced with 12 random primers in two fruiting species of *Garcinia* named *G. gummigutta* and *G. indica*. Of the 112 band produced, 110 were found polymorphic among the screened genotypes of *G. gummigutta* accounting for 98.2%, where in *G. indica* it was 100%. It was reported by Priya et al., (2010) that in their RAPD-PCR analysis with 19 primers produced 96.18%
polymorphism in *G. indica* genotypes Arunrut and Kedsirin (2002) also reported that in *Garcinia atroviridis*, sixty-three out of ninety-nine clear DNA bands exhibited as polymorphic bands (63.64%).

Here the size of the amplified DNA products ranged from 3214 bp to 110 bp. The number of bands resulted from each primer varied between 7-13 bands or at average 9.5 bands for each primer. Among the 12 primers, the particular primer OPAA-01 was found good with maximum number of polymorphic DNA bands (13) followed by OPAB-11 (11). Only one band (monomeric) was found common among the genus *Garcinia* and that particular monomeric band (bp 200) was noticed with the primer AP – 20. The primers AV-03 and AP -20 was found successful to produce DNA bands (857 bp, 480 bp) for species specific identity in *G. gummigutta*.

The primers such as W-15 (bp 400), AO-12 (bp 1500), AB-16 (bp 1111) and OPAA-01 (bp 843) were successful in producing identical products (DNA bands) in almost all the accessions of *G. gummigutta* collected from different geographical areas of Western Ghats. In case of *G. indica*, the primers such as AV-03 (bp 583), AB-16 (bp 642), OPAA-01 (bp 1090), W-15 (bp 638) and AF-11 (bp 1000) were found successful.

This is the first report of molecular profiling in *G. gummigutta* and *G. indica* accessions of Western Ghats region. The heterogeneity (molecular variability) index of *G. gummigutta* (0.81) and *G. indica* (0.82) were also found very high. This represents that there is high molecular variability exist among *G. gummigutta* and *G. indica* accessions collected from different areas.
geographical regions of Western Ghats. In the present study all the used 12
primers (OPAA-11, OPAA-01, OPAB-11, OPAB-01, AP-20, AF-11, AO-12,
PO-5, AV-03, W-15, AB-16, BB-18) were found successful to resolve
molecular diversity in this species. It has been reported by Arunrut and
Kedsirim (2002) that the primers such as AA11, OPC05 and AB11 revealed
correlation between genetic difference in *Garcinia atroviridis*. Carl *et al*
(2004) also conducted a study with the help of Randomly Amplified DNA
Fingerprinting (RAF) to survey genetic relationships between 37 accessions
of the tropical fruit *G. mangostana* (mangosteen) and among 11 accessions
from eight other *Garcinia* species. Although mangosteen is believed to
reproduce exclusively through apomixis, their results showed that
considerable genetic diversity exists within *G. mangostana* and between
other *Garcinia* species.

Richards (1990) reported that most reports of apomixy in the
paleotropics have been restricted to the families Clusiaceae and
Dipterocarpaceae. The incidence of apomixy in *Garcinia* has been shown to
(1997) claims that hundreds of *Garcinia* species might be apomictic including
*G. gummigutta* and *G. indica*. Kaur *et al* (1978) suggested that due to
reduced genetic vigor apomictic individuals might be at a competitive
disadvantage in diverse species assemblages. But in the present study a
Wide molecular variability was observed within the collected accessions of *G. gummigutta* and *G. indica* from Western Ghats.

High genetic diversity as represented by heterogeneity is not common for *Garcinia* spp as an apomictic obligate, this might due to several factors such as accumulation of natural mutation or may be due to repeated hybridization in ploidy developmental processes (Carman, 2001) Thomas (1997), and Carl *et al.*, (2003) reported that many *Garcinia* species may have female biased sex ratios and considerable genetic diversity is exist among the species. High molecular variation among *Garcinia* species is a genetic potential to obtain high potential genotypes for specific purpose, which could be done through selection approach among superior trees in the field (Sobir and Poerwanto, 2007).

According to Tanksley *et al.* (1989) molecular markers based on differences in DNA sequences between individuals generally detect more polymorphism than morphological and protein markers. It has lead to monitor the DNA sequence variation in and among the species and also helpful in development of genetic fingerprints for every genotype. Genetic diversity among the parents is important in tree crops also, because a cross involving genetically diverse parents is likely to produce high heterozygous as well as heterotic effect. It is generally realized that more diverse the parents, more is the chance of pronounced heterotic effects. In genus *Garcinia* asexual propagation is very well possible. So, once a genotype with high biochemical, molecular variability is found, it could well be perpetuated by resorting to well.
established asexual method of propogation. This will help the plant breeders to select the divergent plants for breeding programme and enabled conservation for genetic diversity in genus *Garcinia*. Ercisli et al. (2007) reported the successful use of RAPD markers to detect polymorphism and environmental relationship among *Punica granatum*.

### 6.4.3. Distribution of genus *Garcinia* based on RAPD analysis

NTSys-pc - UPGMA dendrogram were constructed based on RAPD -PCR (Fig – 21) in *Garcinia* spp. In dendrogram, all the six accessions of *G. gummigutta* species from Central Kerala were grouped together in first cluster (Acc 4 – 9). Except one accession from Northern Kerala (Acc 13) all the remaining *G. gummigutta* accessions (Acc 10 – 15) from Karnataka and Goa regions were clumped together in the second cluster.

In case of *G. indica*, the accessions from Northern Karnataka, Vanahally (14°48' Lat) and Hablegadu (14°56' Lat)) were segregated and formed a separate cluster. The accessions (Acc 22 to Acc 26) from Goa, Maharashtra and Kerala (Out side Karnataka) were grouped separately. Interestingly the 3 accessions of *G. tinctoria* and one of *G. cowa* were clumped separately (Fig – 21).

It is natural that there will be species wise variation in DNA profiling. But here in dendrogram, the species *Garcinia gummigutta* and *G. indica* were clumped as according to its geographical origin. Because of this reason the more available species of *Garcinia* in Western Ghats, *G. gummigutta* and *G
Discussion

Indica were taken in a detailed manner to study the distribution and geographical diversity using GIS tools in the next content.

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

6.5.1. Distribution of Garcinia gummigutta and G. indica species based on RAPD analysis 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) of G. gummigutta and G. indica prepared with the help of DIVA-GIS to study the impact of environmental parameters associated with molecular grouping. The relation with respect to microenvironmental parameters and molecular distribution in G. gummigutta and G. indica are noted below.

6.5.1.1 Distribution of Garcinia gummigutta species based on RAPD analysis using GIS tools

Three clusters were formed in the constructed UPGMA dendrogram of G. gummigutta (Fig 22) The accessions from Calicut (11° 15 lat, Northern Kerala), Thirunelveli (8° 59 lat, Tamilnadu) and Appangala (12° 26 lat, Karnataka) were clumped in first group. All the six G. gummigutta accessions from Central Travancore (8° 55’ Lat to 10° 59’ Lat) of Kerala were grouped in the second cluster. The accessions from Karnataka (12° 25’ Lat to 14° 38’ Lat) and Goa (15° 29 Lat) were separated in the third and final cluster.
Discussion

Though the temperature, rainfall data were found almost identical in first cluster, the alititudinal data was entirely different (Calicut, 0 - 50 MSL, Appangala, 550- 600 MSL, Thirunelveli, and 150 – 200 MSL) in these three locations (Table 26) Of the 12 primers used, the primer OPAB-11 was found more specific to this cluster.

All the G. gummigutta accessions emanating from Karnataka and Goa were grouped in the third and final cluster The Acc 13 from northern Kerala (Lat 12°02') in this group was found geographically very near to Karnataka area of Western Ghats Among the 12 primers, the primer AF-11 was found more specific to this cluster, producing four DNA bands with the cluster members Though the temperature and rain fall were identical, the altitude was not found similar among the accessions The accessions clumped in this cluster were from high altitudinal (50 – 650 MSL) area

Interestingly the G. gummigutta accessions from Central Kerala were grouped in the second cluster All 12 primers used in the study were found successful among the accessions in this cluster The temperature (26° C-28° C), rainfall (2500 mm -3000 mm) and altitudinal (150 msl -200 msl) data corresponding to the geographical area among the accessions (Cluster 2) were found identical The accessions from Ranni (09° 22' Lat ) and Alapuzha (09° 30' Lat) of Central Travancore were found almost identical genotypes in this group Ramesh and Pascal (1997) reported that G. gummigutta originated from central Travancore areas and Central Kerala seems to be one of the main centers for G. gummigutta with maximum distribution
The percentage of polymorphism among *G. gummigutta* accessions was found 98.2. Though there was a wide molecular variability among the accessions, the accessions from its natural origin (Central Kerala, 8° 55' Lat - 10° 34' lat, Cluster, 2) were clumped together and those collected from other parts of Western Ghats were grouped separately. The altitudinal data of the accessions in Cluster 1 & 3 were not found identical and showed 50 MSL to 650 MSL, where in Cluster 2, it was found identical among the accessions (0-150 MSL) indicating the influence of altitude in, *G. gummigutta* diversity and reveals the importance of GIS for its identification. It was reported that the best suited regions for the growth of *G. gummigutta* are those having high humidity and an altitude of 150 MSL - 400 MSL (CSIR 1956) very similar to the environmental conditions of Central Travancore area of Kerala. As the species *G. gummigutta* is available in such a vast altitudinal diversity in Western Ghats, it will be a good reason to connect altitude with intra species diversity. Inasi et al., (2010) reported the D² analysis of 8 characters in 120 genotypes of *G. gummigutta* and reported the presence of high genetic diversity among the genotypes in this species from different altitudinal area.

### 6.5.1.2. Distribution of *Garcinia indica* species based on RAPD analysis using GIS tools

The heterogeneity index of *G. indica* (0.81) was found higher than that of *G. gummigutta* and the percentage of polymorphism among *G. indica* accessions was found 100%. Except the accessions from Maharashtra, Goa and Kerala, most of the *G. indica* accessions were collected from central and
northern part of Karnataka (NK) region. The molecular distribution among *G. indica* was found too similar to *G. gummigutta* that, the accessions from natural origin of these two species were grouped together and those accessions collected from other regions grouped separately.

The three accessions from Northern Karnataka (NK) district (Lat. 13°18′ - 14°56′, Karnataka) were grouped together in the first cluster (Fig-23). Here the Acc. 17 (14° 56′ Lat) & Acc 18 (14° 48′) were from very near geographical area and showing very close similarity in the dendrogram also. The temperature (24 - 26° C), rainfall (1500 mm - 3000 mm) and altitudinal data (600 - 650 MSL) were also found similar in these locations. It was reported by Singh and Gadgil, (1996), Patil et al., (2010) that Uttar Karnataka (NK) is the northern most costal districts of Karnataka and the species *G. indica* is believed to be originated in this region. Interestingly in the present analysis, the accessions belonging to this area were clumped together in a separate block.

*G. indica* accessions from Goa, Maharashtra and Kerala (outside Karnataka accessions) were grouped in the third cluster indicating the locational influence associated with environmental factors on molecular segregation in *G. indica* also. The micro environmental parameters such as temperature, rainfall and altitude of this geographical area were not found identical that the altitude varied from 50 MSL to 400 MSL. Some of the accessions (Acc 27, Acc 28, Acc 29) from Central part of Karnataka were
clumped separately (cluster 4) Here also the altitude was not found similar among the collected accessions

The molecular variability among the accessions in genus *Garcinia* (*G. indica* & *G. gummigutta*) followed by GIS analysis shows that the altitude has a key role in *Garcinia* distribution and diversity. It was reported by Kushalappa *et al.*, (2010) that the species richness and diversity of *Garcinia* species decreased nonsignificantly with increase in elevation from above mean sea level.

Utpala *et al.*, 2008 studied the leaf volatile oil components in *Piper nigrum* of Western Ghats and its influence on geographical area with the help of Arc-GIS software. They also mentioned the influence of microenvironmental parameters on biochemical diversity in this species.

6.5.2 Latitudinal Diversity based on the total number of RAPD-PCR bands in *Garcinia* Spp.

The most popular diversity index is *Shannon’s diversity index* (SHDI) based on information theory (Shannon and Weaver 1949). SHDI are widely used to analyze diversity among bird species, bacterial diversity etc. (Kricher, 1972, Lupwayi *et al.*, 1998). The molecular diversity of *Garcinia* species were analyzed using SHDI with a simple and neighborhood plotting with a grid of 50 km x 50 km and 10 km x 10 km. This was done by mapping the total number of RAPD-DNA bands (molecular) with the corresponding
Discussion

geographical area of Garcmia collection spots in Western Ghats using DIVA-GIS. The prepared maps are given in plate 8 & 9.

In simple grid of 50 km X 50 km, the area from Kalsa (13° 02' Lat) to Belthangadi (13° 15' Lat) of central part of Western Ghats represented high molecular diversity with an index of 0.879 – 2.00. Similarly diversity was found moderately high (0.659 – 0.879) in areas of Goa (15° 33'), Sirsi (14° 58'), Appangala (12° 26') and Kasargodu (12° 17') regions of Western Ghats. Similarly it was found high in southern (Alapuzha, 9° 22' and Kollam, 8° 55') part of Western Ghats region also.

In another plotting of SHDI (neighborhood, grid of 10 km X 10 km) also a definite molecular diversity was not seen among the accessions. The central and south Central part of Western Ghats region starting from 14° 56' (Sirsi) to 10° 3' 1' (Attapadi) were showed the maximum diversity. For better understanding of the diversity of this species, separate grids for fruit and leaf (-) HCA were prepared.

6.5.2.1. Latitudinal Diversity based on fruit & leaf (-) HCA in Garcmia Spp.

Based on the percentages of (-) HCA (fruit and leaf), four Shannon diversity map were prepared (simple and neighborhood) to evaluate Shannon diversity index of (-) HCA content in rinds and leaves of Garcmia accessions collected from different parts of Western Ghats. Separate grids (Simple&
neighborhood) were prepared for leaf and fruit (-) HCA to get a better idea about the diversity index in different geographical regions of Western Ghats.

The regions from Kalsa (13° 02' Lat) to Kasargodu (12°17' Lat) indicated the maximum diversity index of 1.1-2.0 in simple grid of 50 km X 50 km in fruit (-) HCA (Plate 10). The diversity index of (-) HCA was showed around 0.55 to 0.832 in northern (15°30' - 14°59') and southern regions (8°55' Lat - 9°22' lat) of Western Ghats. Further confirmation of this result, a neighborhood with a grid of 10 km X 10 km were prepared (plate11). Here also the geographical area extending from the same latitude of 14°10' Lat (Ravan katta) to 12°03' Lat (Taliparamba) were showed a highest diversity index of 1.84 – 3.00 was in good agreement with the simple grid model of (-) fruit HCA. Interestingly the simple and neighbourhood grids of leaf (-) HCA for Shannon diversity index were also found similar to fruit (-) HCA. Here also the maximum diversity index (1.1-2.0) of leaf (-) HCA was found in Central part of Western Ghats (13°15' Lat to 13°02' Lat) (Plate 12) than the rest of the region.

It is interesting to note that though the influence of altitude was significant in dendrograms, there was not any significant impact with SHDI from the above prepared grids. The molecular and biochemical diversity observed here was not found in continuous manner and showed high in Central part of Western Ghats (14° 56' to 12° 17') than the rest of the Western Ghats regions. It may due to high collection (10 Acc -12 Acc,
within a range of 10 km) in Central part of Western Ghats (Karwar, 14° 59' Lat, Sirsi, 14° 37', Kalsa, 12° 59') than the rest of the region indicating that to enable GIS analysis, the data is an important factor and optimum number say 10-12 collection within 10 km may give better result for the analysis. In addition to this the concentration of variability was also found high among the accessions in Central part of Western Ghats. It can concluded that though the diversity was not continuous it was found high in Central regions (14° 56' Lat to 12° 17' Lat) of Western Ghats. It was reported by Shrikant et al., (2010) that in their GIS analysis, the diversity of genus *Garcinia* were found more in the Central part of Western Ghats than the rest of the region.