REVIEW OF LITERATURE
Genus *Garcinia* belonging to the family Clusiaceae has approximately four hundred species having dioecious, evergreen trees growing in tropical parts of the world (Maheswari, 1964). In India, thirty-six species of *Garcinia* are reported and approximately thirty species are cultivated and produce edible fruits (Arora, 1998). Malaysia and Africa, with a large number of endemic species, appear to be the two main centers of development of the genus *Garcinia*. In India, eleven species occur in the southern Western Ghats, out of which six species are endemic, while sixteen species occur in the north-eastern India, out of which two are endemic, while sixteen species are found in Andaman and Nicobar Islands, out of which seven are endemic (Kundu, 2006).

### 3.1. ABOUT *Garcinia* Species

The genus *Garcinia* (Clusiaceae) is widespread in the paleotropics with about 400 species (Whitmore, 1973). The genus contains several important economic species such as *G. mangostana* (mangosteen), *G. indica*, *G. cowa*, and *G. gummi-gutta*. Preparations from *Garcinia* have been widely used by ethnobotanists and ethnomedicians as a preservative for seasoning and for medicinal purposes. Richards (1990a) 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 be high (*G. parviflora* - Ha et al., 1988, *G. mangostana* - Kaur et al., 1978, *G. stortechtini* - Thomas, 1997, *G. hombronia* - Richards, 1990b). Of the *Garcinia* species investigated, only *G. cantleyana* has been shown to lack apomixy.
Richards (1997) claims that hundreds of *Garcinia* species might be apomictic. In addition to reports of apomixy, Thomas (1997) reported that many *Garcinia* species have female biased sex ratios, especially in South East Asian forests. Michaels and Bazzaz (1986) have shown that in *Antennaria parlinia*, apomictic individuals have lower competitive abilities than sexual forms. Kaur et al. (1978) have suggested that due to reduced genetic vigor apomictic individuals might be at a competitive disadvantage in diverse species assemblages. Although thirty-one species of *Garcinia* occur in India (Maheshwari, 1974) there is little information on the breeding systems of any of these species.

In the genus *Garcinia*, conventionally the trees are propagated by seeds (Mathew et al., 1995). Seeds are reportedly shed at high moisture content levels, possess no dormancy, exhibit low viability and are short-lived as in *G. mangostana* (Gonzalez and Anoos, 1951, Morton, 1987) and *G. gummigutta* (Chacko and Pillai, 1997). *G. indica*, *G. gummigutta* and *G. tinctoria* are semi-wild species which bear large seeds with high moisture content at shedding. In none of the three *Garcinia* species there were well defined endosperm/cotyledons and embryonic axis or any structure remotely similar to it, indicating that it is not a true seed. Instead, a vascular strand is seen which is similar to that referred to as “embryonic axis” in *Garcinia Mangostana* (Teo, 1992).

Three economically important species out of 35 Indian *Garcinia* species, bearing edible and medicinally valued fruits namely *G. indica*, *G. gummigutta* and *G. tinctoria* were taken up for detailed studies on seed morphology and
germination characteristics. The absence of differentiated embryo, endosperm or embryonic axis indicated that the so-called "seed" is not a true seed. Germination characteristics of "seed" showed the clear-cut presence of polarity in all the three species. Regeneration of multiple seedlings from whole seeds and seed pieces further indicated the apomictic nature of seed. Based on seed characteristics and field examination of natural and raised populations, facultative agamospermy in *G. indica* and *G. cambogia* and obligate agamospermy in *G. xanthochymus* (*G. tinctoria*) is indicated (Malik et al., 2005).

Clonal multiplication of superior selected trees is desirable in economically important species. In *Garcinia* species, the same can be achieved either by softwood grafting using scion from the selected tree or by micropropagation using in vitro techniques. Softwood grafting, however, is a season and genotype dependent process requiring large space, trained manpower and large number of rootstocks. Micropropagation is best achieved by the use of vegetative parts from selected mature trees, as clonal progenies are generally desired. However, in most of the tropical tree species, in vitro establishment and multiplication is difficult (Drew, 2000). Apomictic nature of *Garcinia* seeds make them the ideal explants for in vitro establishment and multiplication of selected superior clones for obtaining true-to-type plants.

According to Sompong (2007), the nature of *Garcinia* differs from species to species. Some can grow under shade while the others survive under arid conditions or full sunlight. Among these species, mangosteen is very sensitive to unsuitable environments especially under drought, as mangosteen is a very slow


growing tree and seed germination is very poor. Studies on the morphology of these species are very rare. It is reported by Richards (1990a) that most or all *Garcinia* spp. are apomictic and the fruit develops without fertilization. Moreover, pollination has not been reported by any researchers except in *G. hombroniana* (Richards, 1990b). From this investigation, morphological characteristics are of great importance that helps to know the way for pollination. It has been stated by Sompong (2007) that each species of *Garcinia* has different floral morphology and pollen viability. Some have both male and female flowers while other has only male or female flowers. Data from this morphological observation and pollen viability investigation might be of great importance in crossing some species which are very close, e.g., mangosteen and phawa. In future, new characters of mangosteen, especially drought tolerance may be possible to obtain from these crossings.

*G. gummigutta* is common in evergreen and lower shola forests up to a height of 1000 m (Tissot *et al.*, 1994). Hegde *et al.*, (1998) reported that *G. gummigutta* trees have a dark, smooth bark with an average thickness of 5.3 mm. The trees grow to an average height of 18 m and attain diameters of up to 70 cm. *G. gummigutta* trees are dioecious, with a male to female sex ratio of 1:1 and trees of both the sexes usually commence flower production when they are about 14 cm in diameter. It was reported by Janson (1983) and Terborgh (1986) that unripe *G. gummigutta* fruits are green while the ripe fruit is a bright yellow and the pulp of *G. gummigutta* fruits is consumed by two primate species (*Presbytus entellus* and *Macaca radiata*) and two species of civets (*Paradoxorus*...
hermaphroditus and *P. jerdonii*). The seeds are consumed by two species of arboreal squirrels (*Ratufa indica* and *Funambulus palmarum*). Mathew (1995) and Chacko (1997) reported that *G. gummigutta* seeds are about 5 cm long and 2 cm wide and weigh about 1 g. Seeds lie dormant for 8 months which coincides with the amount of time between the cessation and onset of annual rainfall. Male and female flowers of *G. gummigutta* are remarkably similar in outward appearance, color, and size. Both flower types are usually red, but trees with yellow flowers are also observed. Male flowers occur in clusters of 15 to 20 flowers. Flowers have 4 petals and are about 12 mm wide and 11 mm long. Anthers are attached to a pistillode with a non-functional stigma. Pollen is easily picked up by wind when flowers are tapped lightly. The exine of the pollen is reticulated and 4 colporate (Tissot *et al.*, 1994). Female flowers occur singly or in clusters of up to 4 flowers. Female flowers are the same size as males, but they differ in the internal structure, with an enlarged stigmatic surface and no style. Pistillate flowers have rudimentary and nonfunctional staminodes. Neither male nor female flowers produce nectar.

Abraham *et al.* (2004) conducted a collaborative specific exploration and collection mission to collect the germplasm and to study the population size of male and female/hermaphrodite trees of Malabar tamarind (*Garcinia gummigutta*). A total of 56 accessions of Malabar tamarind were collected. Two collections of Malabar tamarind were found to be very specific because of the uncommon fruit colour, which is pinkish red. All the collected accessions are grown at National Bureau of Plant Genetic Resources (NBPGR) Regional
Station, Thrissur, India for characterization and conservation. Extensive range of
variability was found in fruit colour, shape, size and nature of branching and
canopy of trees. Characterization of thirteen fruit and five seed characters was
done for 51 accessions. The variability was found to be maximum for nipple
length (74.8%) and minimum for fruit girth (12.8).

*Garcinia indica* is an underexploited fruit species which is popularly known
in India as kokum, brindon or bhirand, or ansil, murgal, punarpulli. It belongs to
the family of Guttiferae and it is a slow-growing slender tree with drooping
branches growing to a height of 16–18 m (Krishnamurthy *et al.*, 1982). Krishnamurthy *et al.*, (1982) reported that kokum fruit is colored either dark
purple or red tinged with yellow and it contains 3–8 large seeds embedded in a
red acid pulp, in a regular pattern like orange segments in the white pulpy
material and the shape of the fruit varies from round to oval and it weighs around
21–85 g. The expected shelf life of fresh fruit is about one week. Bhat *et al*.
(1986) suggested that sun drying is a commonly used method to preserve the
fruit and it takes around 6–8 days for complete drying. The fruit has a pleasant
flavor and also sour taste.

Kore *et al.*, (2005) studied the fruit characters of 22 genotypes of *kokum*
(*G. indica*) and observations were recorded for fruit length, breadth, weight,
circumference, volume, fresh rind weight, dry rind weight, pulp weight, fresh seed
weight, dry seed weight, dry kernel weight, seed number and filled seed number.
The genotypes exhibited significant variation for all the fruit characters, and the
environmental coefficient of variation recorded for all the fruit characters was very
low The higher magnitudes of phenotypic and genotypic coefficient of variations indicated good amount of variation among the genotypes

Akanksha et al., (2006) reported that *Garcinia indica* is used for making several vegetarian and non-vegetarian 'curry' preparations, including the popular 'sol kadhi' and the fruits are steeped in sugar syrup to make 'amrut kokam', a healthy soft drink to relieve sunstroke, which is popular during summer. It is a traditional home remedy in case of flatulence, heat strokes and infections. Kirtikar and Basu (1984) reported that many therapeutic effects of the fruit have been described in traditional medicine based on Ayurveda. These include its usefulness as an infusion, in skin ailments such as rashes caused by allergies, treatment of burns, scalds and chaffed skin, to relieve sunstroke, remedy for dysentery and mucous diarrhoea, an appetizer and a good liver tonic, to improve appetite and to allay thirst. Patil et al. (2005) reported that kokum is grown in home gardens and cultivated at a limited scale as a rain fed crop. It is usually mixed with other fruit trees in the Western Ghats region, estimated to be grown in an area of 1200 ha with an annual production of 10,400 tonnes which constitutes the dried rind of the fruit is used mainly as an acidulent in cosmetic products and moisturizing and rind has got medicinal properties and used in the treatment of piles, dysentery, tumours and heart complaints.

*Garcinia indica* has wide culinary, pharmaceutical and industrial use

Culinary uses (Koppad and Shivanna, 2010)
• The extract/concentrate of this fruit is called Aagal in Konkai and Marathi, it is ready to use for preparation of Solkadhi when mixed with coconut milk
• The outer cover of fruit is dried in the sun to get Aamsol or Kokum. It is used as a slightly sour spice in recipes from Maharashtra that yields peculiar taste and dark colour
• It is preferred substitute for tamarind in curries and other dishes from Konkan. It is also used Konkani cuisine, in Gujarat, and some cuisines of South India
• Kokum makes an extremely cooling drink in summer, and its uses in cooking as a flavoring agent have chefs of the South and West swearing by it
• Kokum enhances coconut- based curries and when added to vegetables like bhendi, potatoes and lentils, bring a zesty tartarsh taste to the food
• G. indica butter is rich in healthy fats like stearic and oleic acid and can also be used as edible oil

Pharmaceutical uses

• G. indica has medicinal uses both as a digestive tonic, and to cope with paralysis
• For the digestive tonic, about half to one glass of curry is prepared from the fruits, with a little salt and sugar to be taken after meals
• For paralysis, the clean-chopped stem bark is finely powdered and is added to boiling water. After 2-3 minutes a lightly cooled decoction is used for washing the affected parts two to three times a day.

• Kokum juice is also effective against allergies due to bee bites and other insect bites and sun exposure related symptoms as well as acidity.

• The fruits contain rich amounts of anti-oxidants that bind with free radicals and prevent oxidative damage to body cells.

• *G. indica* also promotes cell regeneration and repair in addition to this, it helps in bringing down fever and allergic reaction.

• Its juice is especially popular during scorching summer months and it has a cooling effect on the body and shields the body against dehydration and sun stroke.

• It has a soothing and healing properties, it is also applied directly to wounds and infected areas on skin.

• The extract from the *G. indica* fruit are traditionally used to relieve gastric problems like acidity, flatulence, constipation and indigestion.

• It is known to strengthen the cardio-vascular system and stabilize liver function.

**Industrial use**

• *G. indica* seed contains 23-36 % oil, (Heymsfield et al., 1998) which remains solid at room temperature and is used in the preparations of confectionery medicines and cosmetics.
• Recently, industries have started extracting (-) HCA from the rind of the fruits

• *G. indica* seeds contain a high percentage of oil that freezes to form Kokum butter. Kokum butter is extensively used in the pharmaceutical and cosmetic industry as it works wonders on dry, chapped, sensitive, irritated or burnt skin.

• *G. indica* butter is rapidly gaining popularity over cocoa butter as an intensive skin moisturizer.

According to Ítalo Herbert *et al.*, (2006), Yellow mangosteen (*G. tinctoria*) tree can reach 20 m height, has erect trunk, brown-clear bark and pyramidal pantry, leaves in oval-oblong form, acute apex, rounded off base, salient ribbings in the two sides, yellow base, with 30 cm length and 10 cm width, white flowers with short pedicel and bloom period between November and February. The fruits are eatable with fruition between March and August, a spherical berry of green-yellowish colour at full maturity, with succulent pulp and lightly acidic.

*Garcinia cowa* Roxb. commonly known as “kydia”, is a small to medium sized tree found scattered in lowland, undulating areas and peat swamp forests. The fruits and leaves are used for the improvement of blood circulation and expectorant for the treatment of coughs and indigestion and as a laxative, while the root is used for fever relief (Poomipamorn and Kumkong, 1997). The bark of *G. cowa* has been used in Thai folk medicine as an antipyresis agent (Na Pattalung *et al.*, 1994). Previous investigation of the latex of *G. cowa* revealed the presence of antibacterial prenylated xanthones (Na Pattalung *et al.*, 1994).
However Subhadrabandhu (2001) stated that its horticultural potential has only been poorly developed In particular, its reproductive biology is still not well understood A deeper knowledge of this could reveal barriers to seed and fruit set, increase fruit production, accelerate breeding programs, and promote its conservation

3.2. BIOCHEMICAL IMPORTANCE OF *Garcinia* Species.

Jayaprakash *et al.*, (2006) has reported that the major and potent phytoconstituent in *Garcinia cambogia* or *G. gummigutta* is (-) hydroxycitric acid This principal acid has been found to suppress the fatty acid synthesis, lipogenesis, food intake, and promotes glycogenesis, gluconeogenesis and induced weight loss (-) HCA is found in the fruit rinds of certain species of *Garcinia* which include *G. cambogia*, *G. indica*, *G. cowa* and *G. atroviridis* (Lewis *et al.*, 1965) and Jena *et al.*, (2002) reported that the plant contains acids such as tartaric, citric and phosphoric acid The latex of *Garcinia cambogia* contains two polisoprenylated benzophenone derivatives, camboginol (I) and cambogin (II) Deshpande (1958) reported the presence of condensing enzyme in *Garcinia* leaves He reported that these enzymes are formed through the formation of citrate from acetyl phosphate and oxaloacetate in the presence of coenzyme A

Lewis (1969) reported that (-) HCA is susceptible to lactonization during evaporation and concentration In commercial samples of *G. cambogia* extracts, (-) HCA is present as its calcium salt for the reason of stability However free (-) HCA can be generated from *G. cambogia* extract samples by passing through an
aqueous solution of the calcium salt through a cation exchange resin. Majeed et al., (1998) have reported the preparation of potassium hydroxycitrate from *Garcinia* fruit. It involves the extraction of (-) HCA from *Garcinia* fruit using alkyl alcohol and the combined extract was treated with potassium hydroxy citrate. Balasubramanyam et al. (2000) have reported the preparation of a new soluble metal double salt of group IA and IIA of (-) HCA. It involves the aqueous extraction of (-) HCA and treating the extract with different metal hydroxides and metal chlorides to get a double metal salt. Tbnusaud et al. (2000) have reported the isolation of *Garcinia* acid from the fresh or dried rinds of the fruits of *G. cambogia, G. indica, and G. atroviridis* It involves four to five extractions of *Garcinia* fruits with boiling water for 20 hr. The combined extract was concentrated and treated with methanol to remove the pectin and filtered. The filtrate was treated with aqueous sodium hydroxide at 80°C to obtain sodium hydroxycitrate.

Hypolipidemic property of *Garcinia cambogia*, which reduces the peroxidative damage enhanced by ethanol, was reported by Mahendran and Shyamala Devi (2001). Sullivan (1984) studied the biological effect of (-)-HCA. He found that its effect starts from the inhibition of extramitochondrial cleavage of citrate to oxaloacetate and acetyl-CoA catalysed by ATP- citrate lyase. This 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. Citrate cleavage enzyme that is ATP- citrate
lyase catalyzes the extramitochondrial cleavage of citrate to oxaloacetate and acetyl-CoA. Watson et al. (1969) encountered the powerful inhibition of ATP-citrate lyase by (-) HCA with purified enzyme from rat liver. In that experiment, (-)HCA had a much greater affinity for the purified enzyme than the natural substrate, that is citrate. It was also found by Cheema-Dhadli et al., (1973) that both free (-) HCA and (-) HCA lactone was found to be a very effective inhibitor of citrate cleavage enzyme. Stallings et al. (1979) observed that of four isomers of HCA, only (-) HCA was the only potent inhibitor of ATP-citrate lyase. It was found to inhibit partially purified ATP-citrate lyase from human liver competitively with respect to citrate.

**Garcinia gummigutta extract** is found to increase the mucosal defensive mechanisms in stomach and thereby it is helpful as a protective agent against gastric ulcers (Koppad and Shivanna, 2010)

- **Garcinia** blocking lipogenesis or conversion of starches in fat therefore it prevents accumulation of more fats in body.
- It promotes the oxidation of lipids and spares carbohydrate. This action will result in lower blood levels of cholesterol and lipid.
- It contains significant amounts of Vitamin C and has been used as a heart tonic.
- Because of **Garcinia** excess glucose is mediated by gluconeogenesis pathway to form glycogen. Glycogen signals the brain satiety center that sufficient food has been taken. So glycogen production with this herb further suppresses appetite.
• It promotes thermogenesis process i.e. body's production of heat. This burns more calories and excess of stored fat.

Beynen and Geelen (1984) observed the inhibition of fatty acid synthesis from glucose by (-) HCA in isolated hepatocytes. He found that (-) HCA did not affect fatty acid synthesis from acetate rather (-) HCA was shown to stimulate fatty acid synthesis from acetate. The effect was observed both in the isolated rat liver and in the homogenates of this organ and was assumed to be due to an activation of acetyl-CoA carboxylase in analogy to other tricarboxylates (Barth et al., 1972, Greoglin et al., 1968 and Hashimoto et al., 1971). Moreover, the findings of Hackenschmidt et al. (1972) showed that (-) HCA is a positive effector of acetyl-CoA carboxylase and similarity of (-) HCA and citrate activation of enzyme activity was apparent in the time for maximal stimulation. It was concluded that (-) HCA acts as an inhibitor of lipogenesis only if cytoplasmic acetyl-CoA is produced by citrate cleavage enzyme reaction, but it will activate fatty acid synthesis wherever an alternate source of acetyl-CoA, for example, acetate is available. The increase in lipogenesis in brown adipose in response to glucose feeding was inhibited by (-) HCA (Sugdon et al., 1983). In starved rats, (-)-HCA did not inhibit the basal rates of brown fat lipogenesis and glucose utilization in response to insulin. As basal rates of lipogenesis were not inhibited by (-) HCA, it is suggested that acetate may be a lipogenic substrate for brown fat in starvation (Sugdon et al., 1982). Smith (1975) reported that rabbit adipose tissue has a low capacity for de novo fatty acid synthesis from glucose but a high capacity for synthesis from pyruvate and acetate. Experiments with (-) HCA have
demonstrated that citrate cleavage enzyme is an obligatory enzyme in lipogenesis from pyruvate and that the lipogenic system of rabbit adipose tissue resembles that of a ruminant in that it is adapted to utilize acetate rather than glucose.

Brunengraber et al. (1978) observed that in rat liver, the inhibition of fatty acid synthesis by (-) HCA was associated with an increase in the tissue content of glucose 6-phosphate and a diminution in glycolytic intermediates from fructose bisphosphate to phosphoenol pyruvate. Presumably the citrate content is elevated in cytoplasm in the presence of (-) HCA. This can be expected to result in a reduced activity of phosphofructokinase because citrate is well known to act as an inhibitor of this enzyme. It has also been seen that AMP contents drop in the presence of (-) HCA. This can also be expected to reduce the activity of phosphofructokinase, because AMP is an activator of this enzyme (Bloxham, 1995). The inhibition of phosphofructokinase by (-) HCA in rat hepatocytes has also been reported by McCune et al., (1989). It can be concluded that in rat liver, the inhibition of phosphofructokinase by (-) HCA leads to the accumulation of glucose 6-phosphate and fructose 6-phosphate and the decrease of glycolytic intermediates beyond fructose bisphosphate as the reaction catalyzed by phosphofructokinase in glycolysis is irreversible and controls the glycolysis.

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 and Rothacker, 1997) and results have been contradictory. Supporting evidence of human (-) HCA efficacy for
weight control is based largely on studies with small sample size (Come, 1993) studies that failed to include a placebo–treated group (Badmaev, 1995) and use of inaccurate measures of body change (Thome, 1996) To overcome limitations of the above studies and examine the effectiveness of (-) HCA for weight loss and fat mass reduction, Heymsfield (1998) designed their investigation with rigorous controlled trials Subjects were randomized to receive either active herbal compound (1500 mg of (-) HCA / day) or placebo Both groups were prescribed a high fiber, low–energy diet and the treatment period was 12 weeks Body weight was evaluated every week and fat mass was measured at weeks 0 and 12 On the basis of result obtained from these 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

Animal studies indicated that (-) HCA is no more toxic than citric acid, which is present in many foods in addition to being a normal intracellular compound Also (-) HCA is a component of a natural product, which has been used in Indian cuisine as well as medicinal purpose Clouatre and Rosenbaum (1994) pointed out that (-) HCA has extremely low levels of toxicity For example, recent 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 Because G cambogia has a long history of usage as a flavoring agent, preservative and herbal tonic and there are no reports of toxicity regarding traditional use of the Garcinia extract, it
is highly unlikely that there may be any possible negative effect that may occur due to excess intake. However, despite its inherent safety, (-) HCA like any other diet product, is not recommended for certain groups of people. (-) HCA has impacts on the body’s production of fatty acids and cholesterol, therefore it may directly influence the production of sterols, thus restricting the production of steroid hormones. As pregnancy is a time of extreme sensitivity to steroid hormones in human body, products containing (-) HCA should not be recommended during this period. Likewise women who are breast-feeding should also avoid (-) HCA. Although experience with fruit source of (-) HCA shows that they are not dangerous to young children, they are advised not to consume (-) HCA in large amounts for extended periods (Jena et al., 2002).

The organic acid present in *Garcinia* is responsible for the bacteriostatic effect of the pickling medium by lowering the pH. The organic acid present have been mistakenly identified as tartaric acid and citrate acids (Sreenivasan and Venkataraman 1959, Kuriyan and Pandya, 1931). The aqueous extract of the fruit showed two predominant acid spots on paper chromatograms run on different solvents, which were very near to tartaric and citric acids, but there was always a significant small difference in the Rf values in all solvent system. Analysis of the fruit juice by use of an ion exchange column as described by Palmer (1955) showed the largest peak to be the citric acid region, but the acids failed the cream of tartar test for tartaric acid and the pentabromoacetone test for citric acid (Lewis et al., 1964).
Lewis and Neelakantan (1965) have isolated the principal acid in the fruit rinds of *G. cambogia* and identified it as (-) HCA on the basis of chemical and spectroscopic studies. Identification and separation of the hydroxycitric acid on Whatman No 1 paper were performed using *n*-butanol/ acetic acid/ water (4:1:5) and *n*-proponol / formic acid/ water (4:1:5). The spots were identified with 5% metavanadate. Upon saponification of the acid mixture with excess alkali and passing it through a column of ion exchange resin, the elute showed only one lower spot (Rf =0.34) corresponding to the free (-) HCA. Moffeu et al., (1996) have developed a process for the aqueous extraction of (-) HCA from *Garcinia* rinds. The rind extract was loaded onto an anion exchange column for adsorption of (-) HCA, and it was eluted with sodium/potassium hydroxide for release of (-) HCA. The extract was passed through a cation exchange column to yield a free acid. Antony et al. (1998) reported that the presence of minor organic acids such as citric acid, tartaric acid and malic acid in *Garcinia* fruits and the lack of official methods for the assay of (-) HCA have created confusion among analyst. The existing method for the determination of (-) HCA content in *G. cambogia* extract involves acid –base titration, which gives the total acidity of the extract. However, in this method the concentration of (-) HCA and lactone cannot be estimated separately (AOAC, 1976)

Jayaprakasha and Sakanaaha (2000) have developed high performance liquid chromatography (HPLC) method for the detection of organic acids in the fruits of *G. cambogia*, commercial samples of *G. cambogia* extracts and leaves and rinds of *G. indica*. In this HPLC method, dilute extract can be quantified...
without concentration, drying and derivatization. According to them, the advantage is that the (-) HCA and its lactone can be quantified separately. Lowenstein and Brunengraber (1981) have estimated the (-) hydroxy citric acid content of the fruit of *G. cambogia* by gas chromatography (GC). Gas chromatographic estimation involves the conversion of acid to volatile silyl derivative. They used an OV-17 GC column (3m x 3mm). The column was run at 145° C using nitrogen as the carrier gas (40 ml/minute) with an injection port temperature of 250° C and a detector port temperature of 300° C. (-) HCA lactone is the major constituent of the extract and the recrystallized contains <0 5% of impurities. Silylation requires completely dried samples, but (-) HCA has a tendency for lactonization during drying because of the highly hygroscopic nature. So the free (-) HCA cannot be calculated in this method.

Kulkarni and Deodhar (2002) reported that *Garcinia indica* is a rich source of hydroxycitric acid (HCA) and found that female plants are the major source of, (-) hydroxycitric acid. They stated that due to the polygamodioecious nature of *G. indica*, the differentiation between male and female plants is detected only at the flowering stage (approx 12 yrs). Tissue culture methods offer scope for large scale propagation of female plants. They have standardized an efficient protocol for multiple shoots and callus to produce more (-) HCA. It is found that multiple shoots were obtained from immature seed explants on MS basal medium supplemented with NAA (2 69 μ M), BAP (8 9 μ M) and KN (0 93 μ M). Elongation of shoots was achieved on half MS medium supplemented with NAA (0 54 μ M), BAP (0 44 μ M) and KN (0 93 μ M). The shoots developed roots.
when they were treated with 4900 μ M IBA for 30 seconds and cultured on half MS basal medium. Multiple shoots and callus were obtained from explants derived from in vitro developed plantlets. The regenerated plantlets were successfully transferred to soil in pots. Multiple shoots and the callus cultures produced (-) HCA.

Kokum (G. Indica) contains about 10% malic acid and a little tartaric and citric acid. Composition of fresh kokum rind is as follows (Sampathu and Krishnamurthy, 1982): Moisture (80%), Protein (1.93%), Crude fibre (14.28%), Total ash (2.57%), Tannins (2.85%), pectin (5.82%), Starch (1%), Crude fat (10%), Pigment (2%), Ascorbic acid (0.64%), Acid (hydroxy citric acid) (22.80%), Carbohydrates (35%).

Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli decided to collect the information regarding Konkan Hatis and compared it with the earlier released variety ‘Konkan Amrta’. The rind chemical traits viz. moisture per cent, total soluble solids, reducing sugar (%), total sugar, acidity (%) and pH were estimated and are given in Table 1 & 2 as per the standard methods described in AOAC (1970).
Table 1 Comparison of flowering characters in 'Konkan Hatis' and 'Konkan Amruta'

<table>
<thead>
<tr>
<th>Sl.no</th>
<th>Characters</th>
<th>Konkan Amruta</th>
<th>Konkan Hatis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Time of Flower bud appearance</td>
<td>1st week of October</td>
<td>2nd week of November</td>
</tr>
<tr>
<td>2</td>
<td>Time of initiation of flowering</td>
<td>2nd week of November</td>
<td>2nd week of December</td>
</tr>
<tr>
<td>3</td>
<td>Fruit retention percent</td>
<td>24</td>
<td>22</td>
</tr>
<tr>
<td>4</td>
<td>Time of first harvesting</td>
<td>1st week of March</td>
<td>2nd week of April</td>
</tr>
<tr>
<td>5</td>
<td>Harvesting Period</td>
<td>March - April</td>
<td>April - May</td>
</tr>
<tr>
<td>6</td>
<td>Number of Pluckings</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>Time of completion of harvest</td>
<td>4th week of April</td>
<td>1st week of June</td>
</tr>
</tbody>
</table>

Table 2 Chemical analysis of fruit rind of ‘Konkan Hatis’ compared with ‘Konkan Amruta’ (Nagwekar et al., 2010)

<table>
<thead>
<tr>
<th>Sl.no</th>
<th>Characters</th>
<th>Konkan Amruta</th>
<th>Konkan Hatis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Moisture (%)</td>
<td>81.72</td>
<td>81.70</td>
</tr>
<tr>
<td>2</td>
<td>TSS</td>
<td>9.08</td>
<td>9.20</td>
</tr>
<tr>
<td>3</td>
<td>Reducing sugar (%)</td>
<td>2.41</td>
<td>2.40</td>
</tr>
<tr>
<td>4</td>
<td>Total Sugar</td>
<td>4.52</td>
<td>4.10</td>
</tr>
<tr>
<td>5</td>
<td>Acidity (%)</td>
<td>5.12</td>
<td>5.10</td>
</tr>
<tr>
<td>6</td>
<td>pH</td>
<td>1.81</td>
<td>1.80</td>
</tr>
</tbody>
</table>

Sunil and Satyanshu (2006) described about a sensitive liquid chromatography/electrospray ionization tandem mass spectrometric (LC/ESI-MS/MS) method for the identification and quantification of two polyisoprenylated benzophenones xanthochymol and isoxanthochymol in the extracts of the fruit rinds, stem bark, seed pericarps and leaves of Garcinia indica and in the fruit.
rinds of *Garcinia cambogia* They found that the separation of xanthochymol and isoxanthochymol was achieved on a RP-18 column using the solvent system consisting of a mixture of acetonitrile–water (9:1) and methanol–acetic acid (99.5:0.5) as a mobile phase at a flow rate of 0.4 ml/min A multiple reaction monitoring (MRM) method was developed for quantification of xanthochymol and isoxanthochymol in the above extracts of *Garcinia* species. On the basis of signal to noise ratio of three the limits of detection in MRM mode for xanthochymol and isoxanthochymol were 1.0 ng/ml and 0.5 ng/ml respectively The method was validated in terms of linearity, accuracy and precision for 6 days The method developed was found to be useful for identification and quantification of xanthochymol and isoxanthochymol in the extracts of the fruit rinds, stem bark, seed pericarps and leaves of *G. indica* and in the fruit rinds of *G. cambogia*

Chetan *et al.*, (2010) studied the isolation and characterization of anthocyanins present in *Garcinia indica* (popularly known as kokum), which is a potential source of a natural food colorant He reported that kokum was found to contain a very high concentration of anthocyanins (2.4 g / 100 g of kokum fruit), compared to other natural sources Acid hydrolysis ascertained that this anthocyanin consisted of a single aglycone, i.e., cyanidin. HPLC, mass and NMR spectroscopy analyses confirmed that the pigment essentially contains two anthocyanins, which were identified as cyanidin 3-glucoside and cyanidin 3-sambubioside Also Akanksha *et al* (2006) reported that their studies using biochemical assays pertaining to different levels of antioxidant action showed that various preparations of kokam have significant antioxidant activity
Analysis of fatty acid composition and storage lipid content of *Garcinia indica* during seed development was studied by Jaiyanth *et al.*, (2003) and found that stearate content was high at the early stages followed by the progressive increase to 60% of the total fatty acids during development. When 14C-acetate was used as a precursor, it was preferentially incorporated into stearate that in turn, was esterified to triacylglycerol. It was also found that the kinetics of incorporation of radioactive stearate into diacylglycerol and triacylglycerol was about two fold higher than that of palmitate during various stages of seed development. Pulse-chase experiments with 14C-acetate provided evidence that phosphatidylcholine is involved in donating stearate and oleate for triacylglycerol biosynthesis. When assays were performed for acyltransferase activities in the microsomal membrane fraction with palmitoyl, stearoyl and oleoyl-CoAs, glycerol-3-phosphate acyltransferase and diacylglycerol acyltransferase showed preference for stearoyl-CoA, whereas lysophosphatidic acid acyltransferase had a preference for oleoyl-CoA. These results indicate that stearic acid preferring triacylglycerol biosynthetic machinery exists in the *G indica* seeds. These data demonstrate that *G indica* seeds are a desirable source of acyltransferases for engineering a high stearic acid phenotype in temperate oilseeds.

Fumio *et al* (2000) studied about Garcinol, a novel polysoprenylated benzophenone derivative, from *Garcinia indica* fruit rind. They had studied its antioxidative activity, chelating activity, free radical scavenging activity, and anti-glycation activity. Garcinol exhibited moderate antioxidative activity in the micellar linoleic acid peroxidation system and also exhibited chelating activity at almost
the same level as citrate. It also showed nearly 3 times greater DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity than \( \alpha \)-tocopherol by weight in aqueous ethanol solution. In a phenazine methosulfate/NADH–nitroblue tetrazolium system, garcinol exhibited superoxide anion scavenging activity and suppressed protein glycation in a bovine serum albumin/fructose system. Thus, garcinol might be beneficial as a potent antioxidant and a glycation inhibitor under specified conditions.

Italo Herber (2006) has studied the physico-chemical characterization of *G. tinctoria* fruits. Six samples of 25 fruits were harvested in yellow mangosteen plants of the Active Germoplasm Bank of São Paulo State University and characterized by evaluation of length and width, weight, percentage and number of seeds per fruit, peel and pulp percentage, soluble solid (SS), titratable acidity (TA), vitamin C and SS/TA rate. He found that yellow mangosteen fruit is an intermediate vitamin C source with an average content 120.33 mg/100g of fresh fruit and has good technological quality.

Wide variability in morphological and fruit characters were observed within and between the *Garcinia* species introduced at CHES, chettalli (3100 feet msl, \( 11^\circ 15' \) and \( 20^\circ 50' \) N, \( 72^\circ 25' \) and \( 76^\circ 14' \) E) during 1998-1999 and maintained in the field gene bank. A wide range of variation was observed among the four accessions in respect of both physical and chemical constituents of *G. indica* (Kokum) fruits. The mean fruit weight ranged from 25.5 g in Kokum-1 to Kokum-4 rind, being an important part for extraction of juice, higher mean rind weight was in Kokum-3 (17.5 g) while Kokum-5 had the minimum rind weight of 9.0 g.
Higher Total Soluble Solids (TSS) and ascorbic acid content were recorded in the pulp than in the rind whereas, titrable acidity was higher in fruit than in pulp (Ravishankar and Sakthivel, 2010).

Kanda et al. (2006) have reported that they have isolated tetraoxygenated xanthones, cowaxanthones A–E, together with 10 previously reported tetraoxygenated xanthones, from the crude hexane extract of the fruits of *Garcinia cowa*. Cowaxanthone B has previously been reported as synthetic xanthone. They have elucidated their structures by analysis of spectroscopic data, especially by 1D and 2D NMR.

It has been reported by Gang Xu et al. (2008) that a bioassay and Ultraperformance liquid chromatography/photodiode array/mass spectrometry (UPLC/PDA/MS) guided isolation of the apoptosis-inducing active metabolites on HeLa-C3 cells from the pericarp of *Garcinia yunnanensis* (Guttiferae) yielded five active compounds, including the new garciyunnannins A (1) and B (2). The structures of the compounds were elucidated by comprehensive nuclear magnetic resonance and mass spectrometry analysis. Garciyunnannin B (2), featured with a natural tetracyclic xanthone skeleton derived from a polysoprenylated benzophenone, is structurally interesting since it can be seen as an evidence of the previously described cyclization of garcinol by 2,2-diphenyl-1-picrylhydrazyl (DPPH). Garciyunnannin A (1) contains a 3-monohydroxy benzophenone skeleton, which is rarely found in *Garcinia* species. Both new compounds induce HeLa-C3 cells into apoptosis after 72 h of incubation at 15 μM. It is noteworthy that oblongifolin C (4), the major constituent...
of this plant, has proved to be the most active one among the isolates for inducing apoptotic cell death in cervical cancer derived HeLa-C3 sensor cells

3.3. MOLECULAR STUDIES IN *Garcinia* Spp.

Molecular markers have proved to be invaluable for understanding the genetic make-up of agricultural crops. Molecular markers take advantage of technologies that allow scientists and plant breeders to observe genetic differences between two or more individuals. Genetic markers are seen as morphological differences. Morphological differences have been used since the turn of the 20th century to build genetic maps (Paterson *et al.*, 1991). Molecular markers differ from genetic markers in several ways: (i) molecular markers usually occur in greater numbers, (ii) molecular markers can be distinguished without relying on complete development of the plant, that is, tissue from a plant may be analyzed rather than waiting for the plant to exhibit some morphological feature, and (iii) does not alter the expression of a molecular marker ( Tanksley, 1983). In general, molecular markers are commonly used to examine genetic diversity, systematics and phylogeny. They are used in combination with other markers to construct genetic maps and are used in linkage studies. An understanding of genetic diversity and its phylogeny among cultivated plant accession significantly influence on the quality increase and it also improves the management of germplasm conservation (Roldan *et al.*, 2001).

A series of techniques and genetic markers have been developed to estimate genetic diversity, but no single technique is universally ideal, each
available technique exhibits both strengths and weaknesses. The discovery of PCR has brought about a new class of DNA markers and they are constantly being modified to enhance their utility and to bring about automation in the process of genome analysis. Of the many molecular marker techniques available today, polymerase chain reaction (PCR)-based approaches are in demand because of their simplicity and requirement for only small quantities of sample DNA. Three widely-used PCR-based markers are RAPDs, SSRs or microsatellites, and AFLPs. RAPD markers are very quick and easy to develop (because of the arbitrary sequence of the primers) but lack reproducibility. AFLP has medium reproducibility but is labour intensive and has high operational and development costs. Microsatellites are specific and highly polymorphous, but they require knowledge of the genomic sequence to design specific primers and, thus, are limited primarily to economically important species (Karp et al., 1996).

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. Randomly amplified polymorphic DNA (RAPD) utilizes the polymerase chain reaction (PCR). Polymorphic markers are generated using single primers which are usually ten base pairs long (Williams et al., 1990). RAPDs have been used in *Garcinia* research to study genetic diversity, linkage and to provide additional molecular markers for mapping.

Wacharee et al. (2006) has studied the genetic diversity of 22 accessions of *Garcinia*. The study was conducted using tools such as peroxidase, RAPD...
Among the 15 isozymes tested, only peroxidase produced reproducible, polymorphic bands with polymorphism information content (PIC) of 0.79. A total of eight bands were generated forming three fingerprint patterns distinct for *G. mangostana*, *G. bmucao*, *G. kydia* and *G. lateriflora*. No bands were observed for *G. hvmgstonei* and *G. xanthochymus*. The three RAPD primers showed high PIC of 0.92 (OPB-04), 0.78 (OPB-06) and 0.91 (OPB-07). For GSSAP markers, two sets of primers based on the conserved regions of acyl-ACP thioesterase (ACYL-ACP), and chalcone synthase (CHALCS) had relative PICs of 0.75 and 0.89 for ACYL-ACP and CHALCS, respectively. The high PICs indicate the capability of these techniques to quantify genetic diversity in *Garcinia* species. The dendrograms using UPGMA-SAHN cluster analysis based on peroxidase, RAPD and GSS amplification polymorphism showed that *Garcinia* species clustered into five groups at mean similarity coefficient 0.54. Group I consisted of all 17 *G. mangostana* accessions and was further classified into three subgroups (Ia, Ib and Ic). Group II composed of *G. kydia* and *G. lateriflora* showed a genetic similarity of 0.94. *G. livingstonei*, *G. xanthochymus* and *G. bmucao* were unique in their groups. This study showed that the *G. mangostana* accessions analyzed had low genetic variation and that the different species can be clearly distinguished by combined peroxidase, RAPD and gene sequence specific amplification polymorphism.

Tools such as isoenzyme and AFLP marker on 13 accessions of mangosteen and their close relatives were used by Sobir *et al.* (2009).
of isoenzyme marker using four enzyme systems produced 25 bands and 88% out of them were polymorphic and elucidate genetic variability at similarity level ranged between 0.38-0.89. AFLP markers with three primer system produced 220 polymorphic bands and revealed genetic variability at similarity level ranged between 0.38-0.89 successfully produced high polymorphism bands and elucidates genetic variability at similarity coefficient ranged between 0.21-0.77. Both markers exhibited similar clustering pattern, and grouped successfully G. mangostana accessions in one clustering group. Furthermore, G. malaccensis and G. porrecta consistently showed closer genetic relationship to G. mangostana clustering group in both markers, in comparison to G. hombroniana, which implies the assumption they may be the progenitor of G. mangostana, and should be reviewed with more accurate data.

The novel molecular marker technique Randomly Amplified DNA Fingerprinting (RAF) was studied by Carl et al. (2004). The aim of the study was 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, results showed that considerable genetic diversity exists within G. mangostana and between other Garcinia species. Among the 37 G. mangostana accessions examined, nine different genotypes were identified which clustered into three distinct groups based on correspondence analysis (reciprocal averaging). For 26 (70%) of the accessions no marker variation was detected over 530 loci screened. A further eight (22%) accessions exhibited very low
levels of variation (0.2–1%) suggesting at least one well conserved mangosteen genotype. The remaining three accessions (8%) showed extensive variation (22–31%) compared with the majority of accessions. The three mangosteen groups were 63–70% dissimilar to the other *Garcinia* species investigated.

Sompong *et al.* (2000) studied the genetic analysis in soma clones of Mangosteen (*Garcinia mangostana*) using RAPD markers. RAPD markers were employed to assess the genetic variations of 20 meristematic nodular callus lines and 8 soma clones from a single regenerated mangosteen leaf. The best conditions resulting in amplification of DNA template was with annealing temperature of 41°C. The technique can be used to rapidly point out genetic similarities or dissimilarities in micropropagation.

Sixty-seven RAPD primers were screened and 11 primers were selected to determine genetic variation in *Garcinia atroviridis*. Sixty-three out of ninety-nine clear DNA bands exhibited polymorphic bands (63.64%). Only three primers (AA11, OPC05 and AB11) revealed correlation between genetic difference and plant location. In this study conducted by Arunrut and Kedsirin (2003) none of 67 RAPD primers was sex specific in *G. atroviridis*.

Gutman *et al.* (2001) used RAPD markers to fingerprint 29 genotypes of *Cerus* species and examined their genetic diversity. The result showed that *C. peruvianus* has only a limited genetic base and that further improvement of this crop may require the introduction of additional germplasm into breeding.
3.4. ROLE OF GIS IN DIVERSITY STUDIES

Geographic Information System (GIS) is a computer based tool for mapping and analyzing geographic phenomenon that exists, and event that occur, on the earth. A Geographical Information System (GIS) integrates geographic information with a database management system to facilitate the management, analysis and display of geo-referenced or spatial data (Araújo & Guisan, 2006).

The mountains along the west coast of peninsular India, the Western Ghats, constitute one of the unique biological regions of the world. Rapidly occurring land-cover and land-use change in the Western Ghats has serious implications for the biodiversity of the region. Recent developments such as Geographic Information Systems (GIS) allow the use of a landscape ecology and spatial analysis approach to the problem of deforestation and biodiversity conservation in the Western Ghats (Peters et al, 1990, Shukla et al, 2002).

For developing prediction models, the geographical data of the collection sites of *Garcinia* of the Western Ghats were plotted in digitalized map with the help of Longitude and Latitude, annual average rain fall, temperature and altitude. Based on these environmental factors BIOCLIM map of DIVA-GIS (Hijmans et al, 2003) for the suitable sites of *Garcinia* were predicted. Applications of this type of approach include analyses of land-cover, estimation.
of deforestation rates and rates of forest fragmentation, change in distribution of biodiversity, biomass estimation and gap analysis of the effectiveness of the protected area network in conserving areas of importance for biodiversity conservation (Shairy and Kamaljit, 1997)

*P. nigrum* collected from the Western Ghats of Karnataka and Kerala were studied for the leaf volatile oil with the help of gas chromatography and the results are plotted in a map with the help of Arc-GIS software to understand the influence of location. Though 7–15 compounds were detected from volatile oils in different accessions, maximum variability was observed with respect to β-caryophyllene and nerolidol in the leaf oil of *P. nigrum* and the influence of location on these components is significant (Utpala *et al.*, 2008)

A case study conducted by Mathieu *et al.* (2009), tested the predictive accuracies of five consensus methods, namely Weighted Average (WA), Mean(All), Median(All), Median(PCA) and Best, for 28 threatened plant species. The mean AUC values varied between 0.697 (classification tree analysis) and 0.813 (random forest) for the single-models, and from 0.757 to 0.850 for the consensus methods. WA and Mean (All) consensus methods provided significantly more robust predictions than all the single-models and the other consensus methods. They concluded that consensus methods based on average function algorithms may increase significantly the accuracy of species distribution forecasts, and thus they showed considerable promise for different conservation of biological and bio geographical applications.
Fifteen qualitative morphological characters of 16 wild *piper* species of southern India were studied by Utpala et al., (2006) and plotted for the hierarchical clustering, using SPSS software. The cluster groups were compared using BIOCLIM model of DIVA-GIS to identify the areas or 'niches', where *Piper* species occur predominantly. A grid of 50 - 50 km cells and a circular neighborhood with a radius of 50 km to assign points to grid cells was used to map species richness and species diversity. The highest richness grid was found to have 15–16 species, while the highest diversity value was found to be 1.8 to 3.

Using a GIS based approach, a new map of African vascular plant species richness was presented by Mutke et al. (2001). The phytodiversity dataset comprises metadata on the floras of 450 geographical units in Africa and Madagascar. 151 of these were selected as suitable to analyze correlation of species richness with different environmental parameters in a GIS and to generate the map of African phytodiversity. Best correlation with species richness were found for annual sum of NDVI, number of dry months and water balance. In their study they found the centers of high species richness in Africa are the Capensis, Eastern Madagascar, the coastal areas of SE Nigeria, Cameroon, Equatorial Guinea and Gabon, as well as the Drakensberg mountains, and the East African Mountains.

Aruna et al. (2001) studied Geographic Information System (GIS) to map areas where *Anopheles dirus* is likely to be found. This species is found only in deep-forested areas where manual surveys are very difficult. Being a forest-based species, thematic maps of forest cover, altitude, rainfall and temperature...
were prepared. Overlaying and integration of thematic maps were done using Arc/Info NT and analysis by Arc/view 3.1 (GIS ESRI) software. The results were validated through reported distribution and were found correct. The technique can cover vast and inaccessible areas, fast and easily duplicable in other parts of the world.

An integrated geographical information system (GIS) was developed in northern China. The GIS was composed of a regional geographical database and collection of spatial models. The regional geographical database contained data sets from terrestrial-based sources including thematic maps and statistical records, and data obtained through remote sensing. A series of spatial models was developed by experts in various disciplines such as soil erosion, economics, rural development, and land management. These models were then implemented into the GIS and a series of thematic maps were produced (Zhou, 1998).