Diversity of Indian Garcinia – A medicinally important spice crop in India

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Diversity of Indian *Garcinia* – a Medicinally Important Spice Crop in India

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Abstract

The genus *Garcinia* has over 200 species distributed in the tropics of the world. About 35 species occur in India, many of which are endemic and economically important, with immense medicinal properties. *Garcinia* is the source for a natural diet ingredient Hydroxy Citric Acid (HCA) which is an anti obesity compound. However lack of awareness, coupled with habitat destruction, is leading to genetic erosion of this forest resource and many species are threatened. Work done in India, mainly at Indian Institute of Spices Research, Calicut (IISR), on survey and collection of *Garcinia* genetic resources; development of GIS (Geographic Information System) prediction models for locating the probable new areas of diversity; biochemical and molecular characterization for estimating the genetic diversity; identifying genotypes with high HCA content; and developing micro propagation protocols for multiplying the unique endangered species for their conservation and utilization, was summarized in this paper.

INTRODUCTION

The genus *Garcinia* (Family: *Cluciaceae*) consists of over 200 species distributed in the tropics of the world, chiefly in Asia, Africa, and Polynesia. They are evergreen polygamous trees, shrubs and herbs. About 35 species are reported to exist in India, many of which are endemic and economically important, with immense medicinal properties (Roberts, 1984).

In India, species of *Garcinia* grow extensively in a semi-wild state, in the 'Konkan' region of Maharashtra, Goa, coastal areas of Karnataka and Kerala, evergreen forests of Assam, Khasi, Jantia hills, West Bengal and Gujarat. In 'Malabar' and 'Konkan' region of southern India they are used in garnishing curries and also as a replacement for tamarind. In North Eastern India, the sundried slices of the fruits are used for culinary purposes and as folk medicine. Some species, like *Garcinia cambogia*, *G. indica* and *G. cowa*, are cultivated in certain parts of India. *Garcinia pedunculata*, *G. kydia*, *G. cowa* and *G. lancifolia* are the most important species in North Eastern parts of India. Many species of *Garcinia* have fruit with edible arils and are eaten locally. The best-known species is the mangosteen (*G. mangostana*), which is now cultivated throughout Southeast Asia and other tropical countries. The seeds of *G. indica* fruits yield valuable edible fat known as 'kokum butter'. The fruits of *Garcinia* are a food source for several animals (CSIR, 1956). Most species in *Garcinia* are known for their gum resin which is used as purgative or cathartic. Fruits of some *Garcinia* species are also one of the richest sources of red pigments in the plant kingdom. Fruit and syrup of *G. indica* is very popular in 'Konkan' region and has antioxidant (Mishra et al., 2006) and antibacterial (Negi et al., 2008) activities. *Garcinia* also contains a high amount of Vitamin C and is used as a heart tonic.

Garcinia is the source for a natural diet ingredient (-) hydroxycitric acid. HCA, (1,2-dihydroxypropane-1,2,3-tricarboxylic acid) (Fig. 1) which is an anti-obesity compound present in the fruit rind and leaves of *Garcinia* and is known to inhibit lipid and fatty acid synthesis in living systems (Lewis and Neelakandan, 1965). HCA is also a

hypocholesterolamic agent (Lowenstein, 1971; Sullivan et al., 1972; Sullivan and Triscari, 1977). On a dry weight basis, HCA constitutes about 20-30% of the fruit.

Lack of knowledge, coupled with habitat destruction, is leading to genetic erosion of this forest resource and many species are threatened (Cheek, 2004). They need to be studied and conserved. Forecasting and management of diversity by GIS models are becoming increasingly important and a sensible approach in the study of forest resource. The GIS tools supported by GPS provide an efficient technology for compilation of survey data and for analysis, with the help of map or graph. Once the survey data are digitized and complied in a GIS software, it becomes very easy to compare and interpolate the attribute characters and infer the extent of diversity and distribution of a plant species (Umashankar et al., 2002) The species diversity, hotspots of species richness and genetic variability of populations of many plant species were studied by many authors (Utpala et al., 2006) with the help of GIS.

The present paper summarizes the work done in India, mainly at Indian Institute of Spices Research, Calicut (IISR), on survey and collection of *Garcinia* genetic resources in India, using prediction models for locating the probable new areas of diversity for *Garcinia*, biochemical and molecular characterization for estimating the genetic diversity, identifying genotypes with high HCA content and developing micro-propagation protocols for multiplying the unique endangered species for their conservation and utilization.

MATERIALS AND METHODS

Survey and Collection

Surveys were conducted in the forests of Western Ghats based on the floristic data and in the north eastern regions based on GIS predictions as well as floristic data. About 144 collections of *Garcinia* from Western Ghats region and 60 collections from north eastern region were made. The altitude of collection sites ranged from 50 to 1040 m a.s.l. The species identity was confirmed based on regional herbaria and with Floras of the region of Botanical Survey of India, Kolkata.

GIS Predictions

A systematic survey was conducted in the Western Ghats region of Kerala for collection of *Garcinia* species. Data on variables like altitude, rainfall and temperature were recorded during the collection surveys, along with longitude and latitude with the help of GPS. The records were plotted in the map with the help of DIVA GIS software along with collection sites of Kerala, Karnataka and Tamil Nadu. Bioclim model of DIVA GIS (Hijmans et al., 2003) for altitude, and rainfall suitable for the *Garcinia* was prepared. Bioclim summarizes the bioclimatic parameters for a list of sites to produce a species profile, these points can be supplied either as a list of coordinates or as a regular grid. Surveys were conducted in the predicted suitable areas for *Garcinia* in the North Eastern states of Meghalaya, Assam (Kamrup District of lower Assam and Jorhat and Sibsagar Districts of upper Assam) and Nagaland.

Micropropagation

The seeds and its cut segments were cultured on MS medium (Murashige and Skoog, 1962) supplemented with various concentrations of BAP (0, 1, 2, 2.5, 3, 4 mg/L). The media were supplemented with 30 g/L sucrose and solidified with 8 g/L agar after adjusting the pH to 5.7-5.8. After the shoot developed, the cultures were transferred to media containing BAP (1-4 mg/L) and NAA (1 and 2 mg/L). The cultures were first kept in dark for 72 hours. Then they were incubated at 22°C and were given a photoperiod of 16 hours with a light intensity of 2000 lux. Sub culturing was done in every 21 days.

Molecular Characterization

DNA was isolated from *Garcinia* leaves as well as dried fruit rinds using the

modified protocol of Doyle and Doyle (1990). About 30 random primers were screened and 12 random primers, which gave good polymorphism, were used in molecular profiling. NTSYS was used for diversity analysis.

Biochemical Characterization

1. Estimation of HCA Content. HCA content was estimated based on the methodology standardized at IISR using HPLC (Ashis et al., 2008).

2. Identification of Volatile Constituents and Fatty Acid from *Garcinia* spp. by Gas Chromatography (GC). Essential oil from four common *Garcinia* species was extracted by hydro distillation. The oil was further subjected to GC-MS profiling and corresponding constituents were identified by matching the mass spectral data with those stored in NIST and Wiley libraries. Similarly, the free fatty acids present in the seed kernel of *G. gummigutta* and *G. indica* were converted to fatty acid methyl esters (FAME) and were identified by GC-FID using authentic standards.

3. Extraction of Butter from the Seed Kernels. Fresh fruits were cut into halves and separated seeds were dried for 15-20 days. The dried seeds were rubbed on a hard surface with a wooden plank to separate the kernels from the seed coat. About 100 g of kernel paste was boiled in 500 ml of water for 4-6 h in an open pan. Later the pan was covered with cloth and butter was separated by decantation and yield of butter was noted.

4. Conversion of Free Fatty Acid to Fatty Acid Methyl Esters (FAME). About 500 mg of butter was incubated (70°C) with 5 ml of 3 N of NaOH and methanol in the ratio 1:9 for 2 h. It was separated with 15 ml of hexane for removing steroids from free fatty acids. The lower fraction was taken from the separating funnel and it was acidified with 0.8 ml of 6 N HCl. The acidified fraction was further separated with 15 ml of hexane and the upper fraction was taken for treatment. The separated upper fraction was treated with methanol and HCl in the ratio 5:1 and incubated at 70°C for 5 h. Again it was separated with 15 ml of hexane and again the upper fraction was taken for further analysis. Thus the converted FAME was used for identification free fatty acids by using GC-FID.

5. Chromatographic Condition for Fatty Acid Standards and Samples. The carrier gas used was Nitrogen. The injection port was maintained at 240°C; the detector temperature was 240°C; the oven was programmed as follows; 150° C for 3 min and then increased to 200°C at the rate of 5°C/min at which the column was maintained for 4 min. The constituents of the hexane extract were identified by Perkin Elmer Gas chromatographic system with authentic standards of stearic, palmitic and arachidic acid methyl esters.

RESULTS AND DISCUSSION

Survey

The genus *Garcinia* consists of over 200 species distributed in the tropics of the world. Western Ghats region of peninsular India is one of the 18 Biodiversity Hotspots of the world. This region is considered a secondary centre of origin for *Garcinia.* About 35 species occur in India including some exotic introduced ones (Table 1). Of these, 7 are endemic to Western Ghats, 6 to Adaman and Nicobar Island and 6 to North East India (Rodrigues, 2001). *G. cambogia*, *G. indica* and *G. mangostana* are commercially cultivated in certain parts of India (Hooker, 1886; Gamble, 1925; Roberts, 1984). *G. morella* is also cultivated to a certain extent. *Garcinia pedunculata* and *Garcinia cowa* are the two most important species endemic in Northeastern parts of India. Both *G. cambogia* and *G. indica* occur in the western coast of India in a semi-wild state. Their habitat extends from Konkan southward to Travancore region of the Western Ghats and into the Shola forests of Nilgiris where it can reach an altitude of up to 2000 m above mean sea level. *G. cambogia* is very popular in Kerala where maximum diversity is seen. In some regions of Western Ghats near Thenmala large natural populations of *Garcinia* especially *G. combogia* exists. *Garcinia indica* or 'Kokum' is very common in the

'Konkan' region of the west coast of India from South Kanara onwards towards the north. The best suited regions for its growth are those having high humidity and an altitude of 400-100 m. However, the depletion of forest area, rampant destruction of the forest trees and undergrowth, and changes in the agro-ecological conditions all have led to a sharp decline in the populations of *Garcinia* and their related taxa.

Mapping of *Garcinia* Diversity Using GIS

Mapping of potential distribution of wild species of *Garcinia* of Western Ghats with the help of GIS techniques was done. Collection sites were plotted on map with the help of ArcGIS software (Fig. 2).

Surveys indicated that *Garcinia cambogia* is distributed throughout the Western Ghats, whereas *G. indica* is predominantly seen in the northern parts of Western Ghats. Other species located were *G. morella* in Siruvani forest of Palaghat district of Kerala and *G. xanthochymus* and *G. hombroniana* in the forests of South Kanara in Karnataka, indicating the poor distribution of these species. Out of the 12 species reported to exist in South India (Gamble, 1925) only five species, *G. cambogia*, *G. indica*, *G. hombroniana*, *G. xanthochymus* and *G. morella*, could be collected. This indicated that their distribution and population size is reduced to dangerous levels. Unless located and preserved, these species may quickly become endangered. The important species available in India are given in Table 1.

At Indian Institute of Spices Research (IISR), Calicut models to predict the availability of *Garcinia* species in North Eastern India were developed with the help of BIOCLIM Map of DIVA GIS. A good diversity of *Garcinia* could be collected from the states of Meghalaya, Lower and Upper Assam and Nagaland using these prediction models. These collection surveys indicated that the population size of *Garcinia* species in this region is very sparse, due to rare or no seed set, making these species vulnerable to extinction.

GIS Predictions

The precipitation and altitude maps of India (not presented) were prepared based on Bioclim model of DIVA GIS and using the base data of the collection sites of *Garcinia* from Western Ghats. The grid of the precipitation map indicated that most of the *Garcinia* collection sites in Western Ghats are located where the rainfall is between 1500 to 3000 mm and regions with similar rainfall pattern are located in parts of Orissa, Bengal, Assam, Meghalaya, Nagaland, Mizoram and Tripura.

Similarly, the altitude map of Western Ghats prepared as per Bioclim model of DIVA GIS shows similarity with the Himalayan Foothills in the North Eastern Region. The *Garcinia* collection sites of Western Ghats ranges from 0 to 1000 m a.s.l. Regions with similar altitude are present in the states of Bengal, Assam, Meghalaya, Nagaland, Mizoram and Tripura.

Thus, as observed from both these maps, it was predicted that sub Himalayan States of North Eastern India, viz: parts of Bengal, Assam, Meghalaya, Nagaland, Mizoram and Tripura with the same altitude and rainfall, are suitable for *Garcinia*.

Collection Surveys Based on Prediction Maps

Based on the above prediction three collection surveys were conducted to the North Eastern states like Meghalaya, Nagaland and Assam. Surveys indicated that the prediction models were correct and good diversity of *Garcinia* was observed from these regions and were collected. It was found that species of *Garcinia* occur in Meghalaya where the rainfall range is 2000 to 3000 mm and altitude is 100 to 600 m a.s.l. The species collected were *Garcinia kydia*, *G. cowa*, *G. xanthochymus* (Syn: *G. tintoria*). There is good diversity in leaf and fruit characters among species collected. The distribution of these species was in the forest margins bordering the human settlements and within the settlements themselves. It is presumed that these trees were preserved because of their edible fruits while cutting the forest when settlements were made.

The species occurring in Assam and Nagaland were *G. kydia*, *G. cowa*, *G. pedunculata*, *G. xanthochymus*, *G. lancifolia*. Another interesting but unidentified species found widely distributed is locally called "Mahi thekara".

A circular neighborhood map was constructed using the collection data from the surveys mentioned above utilizing DIAV GIS software with 10 km grid of North Eastern States. The *Garcinia* species richness map developed indicated that there are two hot spots for *Garcinia* diversity in North Eastern region, where 12-14 different species of *Garcinia* could be found within a 10 km radius. This prediction shows that 90°30' to 91°37' longitude and 25°17' to 25°54' latitude in the lower Assam and Meghalaya is one hot spot while between 94°11' to 95°10' and 26°23' to 27°38' in upper Assam including Nagaland is another hot spot for *Garcinia* diversity.

Similarly, the total data on *Garcinia* collections from Western Ghats and Himalayan foot hills region of North Eastern India, available at IISR, was used to identify domains or regions where *Garcinia* can grow in India. This Eco crop prediction map was prepared using 12 seasonal parameters of temperature and rainfall viz., annual mean temperature, mean monthly temperature, warmest month, coldest month, temperature annual range, mean temperature in wettest months and driest months, annual precipitation, precipitation of wettest months, driest months, precipitation seasonality, in wettest quarter and in driest quarter.

Micropropagation

Since the traditional method of propagation of *Garcinia* has some limitations an efficient method for micropropagation of 3 endemic species of *Garcinia* – *G. indica*, *G. tinctoria* and *G. gummigutta* – was developed. MS medium supplemented with 2.5 mg/L 6 benzyl amino purine (BAP) gave best response and induced multiple shoot initiation. Root initiation took place in MS medium with or without α Naphthalene Acetic Acid (NAA) but root elongation was faster in MS medium supplemented with 2 mg/L of NAA. Species differences in in vitro response were observed. In vivo seed germination studies were also conducted to understand the seed germination pattern in the three species. This technology can be used for multiplication of elite genotypes and conservation of *Garcinia* species.

Most of the earlier studies pertaining to in vitro culture of genus *Garcinia* have been conducted in *G. mangostana* using seed and leaf explants. Kulkarni and Deodhar (2002) used immature seeds, young leaves, apical and axillary buds for in vitro establishment of *G. indica*, while Deshpande et al. (1999) and Mathew et al. (2001) conducted preliminary studies on in vitro establishment of kokum apical buds. Murthy and Patil (2010) had reported that in *G. tinctoria* seed explants developed an average of 11.1 multiple shoots on the medium supplemented with 3 mg BAP/L.

Molecular Characterization

RAPD profiling of *Garcinia* sp. was done for better understanding of inter-specific genetic diversity. The markers could successfully distinguished different species of the genus. Rao et al. (2003) studied both intra- and inter-species relationship among six *Garcinia* species, namely *G. indica*, *G. cambogia*, *G. cowa*, *G. mangostana*, *G. xanthochymus* and *G. hombroniana*, using RAPD polymorphism. The study indicated high molecular diversity within *G. cambogia*. Utpala et al. (unpublished) studied RAPD polymorphism in 33 accessions of *Garcinia* species collected from different areas of Western Ghats. The dendrogram clearly separated the collections of the 3 main species studied, *G. gummigatta*, *G. indica* and *G. xanthochymus* and suggested high amount of diversity within the collections of the same species. Similar study was also conducted on *Garcinia* collections from North Eastern India using RAPD. High molecular diversity was observed with the heterogeneity index within species ranging from 0.81 to 0.82 in four species, namely *G. gummigutta*, *G. indica*, *G. cowa* and *G. xynthochymus*.

Biochemical Characterization

1. Hydroxy Citric Acid (HCA). Variability of hydroxy citric acid and lycopene contents were analyzed in *Garcinia* species. Lycopene content did not show much variation. HCA content was estimated and given in Table 2.

The study revealed that HCA content was highest in leaf, fresh and dry fruit samples of *G. gummigutta* followed by *G. indica* and low in *G. pedunculata.* HCA could not be detected from leaf, fresh and dry fruit samples of *Garcinia xanthochymus*. in the Western Ghat collections as well as in N.E. samples. Another important thing to mention is that N.E India's species are all different from Western Ghat's except *Garcinia xanthochymus*.

2. Estimation and Identification Volatile Oil Constituents in *Garcinia* spp. Volatile oil content was estimated by hydro distillation of dried leaves of *Garcinia* species. The volatile oils were further subjected to gas chromatography-mass spectrum (GC-MS) for the identification of various secondary metabolites. GC-MS profiling of different *Garcinia* species recorded indicates that *G. cowa* has a total of 7 compounds, *G. indica* has 12 compounds, *G. tinctotoria* has 9 compounds and *G. gummigutta* has 8 important volatile compounds. Trans-Caryophyllene and Gamma-Muurolene are generally found in all. Alpha-Humulene, Gamma-Gurjunene and Gamma-Cadinene are common in all the three species except in *G. cowa*. Caryophyllene is one of the chemical compounds that contribute to the spiciness of black pepper. Recently, 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 mainly used in fragrance industry.

3. Identification of Free Fatty Acids from the Seeds of *G .gummigutta* and *G. indica* by GC-FID. The main economical importance *G. indica* is associated with the rich content of 'kokum butter' present in the seed kernel. Free fatty acids present in *G. gummigutta* and *G. indica* seed kernels were converted to fatty acid methyl esters (FAME) and were subjected to GC- FID. The free fatty acids in butter were identified by spiking their retention time with authentic standards. Here in both cases stearic acid was found as the more common (28-40%) fatty acid in *G. gummigutta* and *G. indica* samples. Jaiyanth et al. (2003) reported that analysis of fatty acids in *G. indica* at different developmental stages revealed that stearic acid is the major fatty acid and its concentration increases during maturation (42 to 60%). Reddy and Prabhakar (1994) also reported the presence of stearic (50-60%) and palmitic (10-20%) acid in *G. indica* butter. Andrea and Scott (1988) reported plasma total cholesterol decreased by an average of 14% during consumption of the high stearic-acid diet. Obesity is associated with insulin resistance and some reproductive abnormalities. Palmitic acid and stearic acid, markedly suppressed these abnormalities (Ming et al., 2001). Thus *Garcina* species are the new sources of these useful fatty acid methyl esters.

CONCLUSION

The genus *Garcinia* is represented by about 35 species in India, many of which are endemic and economically important with immense medicinal properties. Lack of awareness, coupled with habitat destruction, is leading to genetic erosion of this forest resource and many species are threatened. In order to collect this valuable genetic resource, GIS prediction and species richness models were prepared for locating the probable areas of diversity and the collections were made accordingly. However, a few species could not be collected due to decline in their population and distribution. The collections were studied for their morphological, biochemical and molecular diversity and high variation were noted for all the characters studied. Micro-propagation protocols, developed for multiplying the unique endangered species for their conservation and utilization, were summarized in this paper. The content of the natural diet ingredient hydroxycitric acid varied in different collections of species.

Interest in *Garcinia* is a recent one. Though a good collection of *Garcinia* germplasm was maintained, many regions, especially North Eastern region, Andaman and

Nicobar Islands and parts of Karnataka, need to be extensively surveyed. The correct identities of many species also need to be authenticated. Identification of ecological niches and preservation of these will help in maintaining the fast dwindling genetic resources of *Garcinia*, especially the less important or lesser known species. Identification of high yielding females with high HCA content and other useful compounds/secondary metabolites with neutraceutical value must be given top priority.

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Tables

Table 1. Different species available in India (Krishnamoorthy et al., 2007).

Species	Distribution
Garcinia andamanica King	Andaman Islands
G. anomala Planc.	Khasi Hills
G. atroviridis Griff.	North Eastern districts of Assam
G. cornea L.	Bengal
G. cowa Roxb. (*G. kydia* Roxb)	Eastern parts of India, Assam, Bihar, Bengal, Orissa, Andaman and Meghalaya
G. dulcis (Roxb.) Kurz	Introduced into India from Malaysia
G. echinocarpa Thw.	Tirunelveli forests
G. cambogia (Gaetn.) Desr. (*G. gummi-gutta* (L). Robsmall)	Western Ghats, Maharashtra, Goa, Karnataka, Kerala, Shola forests of Nilgiris.
G. hanburyii Hook.	South India
G. hombroniana Pierre	Nicobar Island
G. imbertti Bourd	South India
G. indica Choicy	South India, Konkan region
G. lanceaefolia Roxb.	Assam, Khasi Hills
G. livingstonei T. Anders.	Introduced to India form East Africa
G. malabarica Talbot	South India
G. mangostana L.	Introduced to South India
G. microstigma Kurz	Andaman Islands
G. morella Desr.	Assam, Khasi Hills, Western Ghats
G. paniculata Roxb.	Foot hills of Himalayas, Assam, Khasi Hills
G. pedunculata Roxb.	Assam, Manipur
G. speciosa Wall.	Andaman islands
G. spitcata Hook. [*G. ovalifolia* Hook. f.]	Western Ghats form Konkan Southwards
G. stipulate T. Anders.	Eastern Himalayas
G. succifolia Kurz	South India
G. travancorica Beddome	Western Ghats
G. wightii T. Anders.	South Indian Forests
G. xanthochymus Hook. [*G. tinctoria* Wight *G. pictorius* Roxb.]	Eastern Himalayas (Assam and Meghalaya) Western Ghats, and Andaman islands

Table 2. HCA content from leaf, fresh and dry fruit samples of *Garcinia* species common in India.

Species	HCA content(%)		
	Leaf	Fresh fruit	Dry fruit
G. cowa (Western Ghats)	1.5	4.2	8.34
G. cowa (Kuji thekara-N.E India)	0.89	3.67	5.5
G. pedunculata	1.08	2.1	3.8
G. lancifolia (Rupohi thekara)	2.45	3.0-4.5	4.0 -5.8
G. oxyphylla (Mahi thekara)	0.06	0.01-.25	0.01-1
G. xanthochynus (tinctoria)	Nil	Nil	Nil
G. gummigutta (kodum pully)	3.95	5.43	16.28
G. indica (Kokum)	3.53	3.5	10.35

Figures

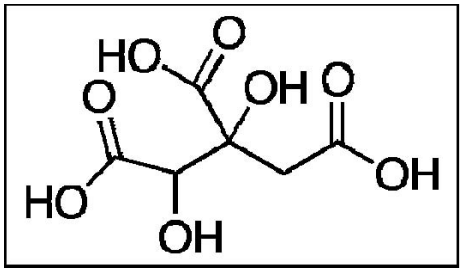

Fig. 1. Chemical structure of HCA.

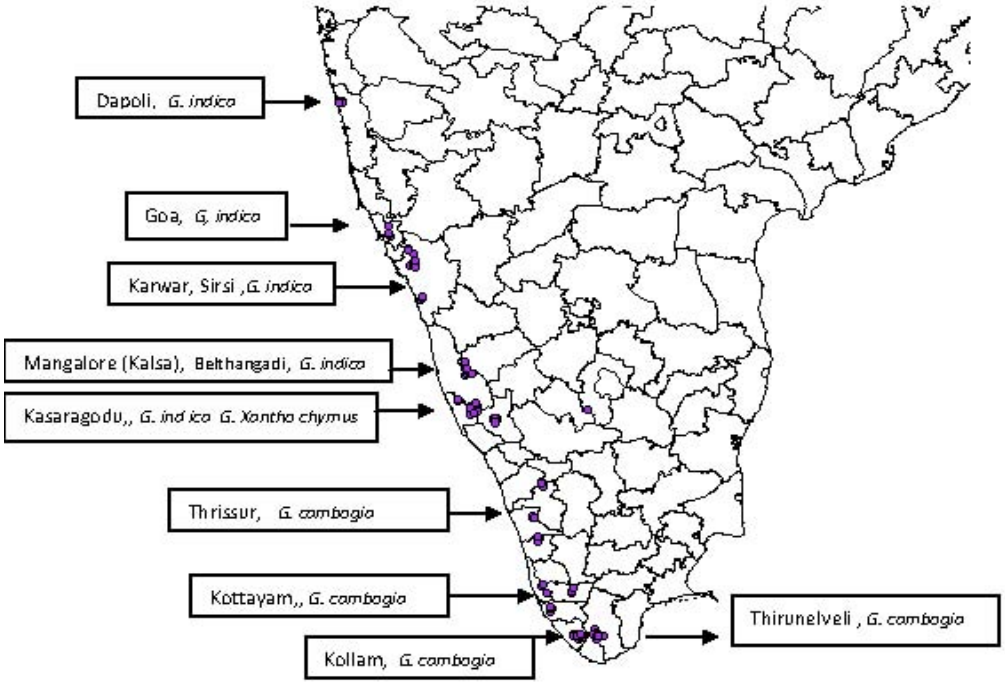

Fig. 2. The collection sites of Western Ghats.