MORPHOLOGICAL AND BIOCHEMICAL DIVERSITY ASSESSMENT OF *Garcinia indica* (Thouars) Choisy GERMPLASM .

BY

RAVEENA KAPATIA

(2017-17-013)

THESIS

Submitted in partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE IN FORESTRY

FACULTY OF FORESTRY

KERALA AGRICULTURAL UNIVERSITY

DEPARTMENT OF FOREST BIOLOGY AND TREE IMPROVEMENT

COLLEGE OF FORESTRY

VELLANIKKARA, THRISSUR- 680 656

KERALA, INDIA

2019

DECLARATION

I hereby declare that the thesis entitled **"Morphological and biochemical diversity assessment of *Garcinia indica* (Thouars) Choisy germplasm"** is a bonafide record of research done by me during the course of research and that this thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

Vellanikkara
Date: 08/07/2019

Raveena Kapatia
(2017-17-013)

Dr. A.V. Santhoshkumar
Professor and Head
Department of Forest Biology and Tree Improvement
College of Forestry
Kerala Agricultural University
Vellanikkara, Thrissur, Kerala.

Date: 08/07/2019

CERTIFICATE

Certified that the thesis, entitled **"Morphological and biochemical diversity assessment of *Garcinia indica* (Thouars) Choisy germplasm"** is a record of research work done independently by **Raveena Kapatia (2017-17-013)** under my guidance and supervision and that it is not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

Vellanikkara

Dr. A.V.Santhoshkumar
Chairperson
Advisory Committee

CERTIFICATE

We, the undersigned members of the Advisory Committee of Ms. **Raveena Kapatia (2017-17-013)**, a candidate for the degree of Master of Science in Forestry, agree that this thesis entitled **"Morphological and biochemical diversity assessment of *Garcinia indica* Choisy (Thouars) germplasm"** may be submitted by her in partial fulfillment of the requirement for the degree.

Dr. A.V. Santhoshkumar
Professor and Head
Department of Forest Biology and Tree Improvement
College of Forestry
Kerala Agricultural University
Vellanikkara, Thrissur, Kerala.

Dr. S. Gopakumar
Professor
Department of Natural Resource Management
College of Forestry
Kerala Agricultural University
Vellanikkara, Thrissur, Kerala.

Dr. Jiji Joseph
Professor and Head
Department of Plant Genetics and Breeding
College of Horticulture
Kerala Agricultural University
Vellanikkara, Thrissur, Kerala.

Dr. Latha M.
Principal Scientist
NBPGR,
Vellanikkara, Thrissur, Kerala.

External Examiner
Rekha. K
Senior Scientist
RRII, Kottayam

Acknowledgement

I wish to place my sincere gratitude from the bottom of my heart to my major advisor Dr. A.V. Santhoshkumar, Professor and head , Department of Forest Biology and Tree Improvement, College of Forestry for his guidance, constant encouragement, suggestions, friendly approach and warm concern to me throughout the study period. He consistently allowed this thesis to be my own work, but steered me in the right direction whenever he thought I needed it.

I extend my cordial thanks to ***Dr. K. Vidyasagaran****, Dean, College of Forestry for extending the facilities available in the college for conducting the present study. I express my deep sense of gratitude to Kerala Agricultural University for the financial and technical support for pursuance of my research.*

I am extremely grateful to my advisory committee members ***Dr. Latha M.*** *Principal Scientist, NBPGR, Vellanikkara,* ***Dr. S. Gopakumar,*** *Professor , Natural Resource Management and* ***Dr. Jiji Joseph,*** *Professor and head, Department of Plant Genetics and Breeding for their constant encouragement and constructive suggestions throughout the study period and also for the critical evaluation of the manuscript.*

I owe my sincere thanks to NBPGR, Regional Station, Vellanikkara for permitting me to access kokum trees at NBPGR.

My sincere thanks to ***Dr.Berin Pathrose****, Assistant Professor, Department of Agricultural Entomology for giving me access to the laboratory and research facilities.*

Special thanks to ***Mr. Vishnu B.R*** *who had been with me throughout my statistical analysis.*

Special thanks to ***my classmates, seniors and juniors*** *for their valuable support and helping me throughout the study.*

Finally, I express my very profound gratitude to my parents and brother for providing me with unfailing support and continuous encouragement throughout the process of researching and writing this thesis. This accomplishment would not have been possible without them. Thank you.

Raveena Kapatia

Dedicated to my parents

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

INTRODUCTION

1. INTRODUCTION

The genus *Garcinia* (Family: Clusiaceae) consists of more than 200 species of small to medium sized trees distributed widely in southern parts of Thailand and Peninsular Malaysia to Indonesia, and in South East Asian region (Sharma *et al*., 1993). Out of the 200 species of *Garcinia* found in different parts of the world it is estimated that only 40 species produce edible fruits (Yapattanphum *et al.*, 2002). Some species are widely domesticated for fruits for eg. *Garcinia atrociridis* and *Garcinia hombroniana*, in Malaysia; *Garcinia indica* and *Garcinia cambogia* in India; *Garcinia multiflora* in Vietnam and *Garcinia pedunculata* in North East India and Bangladesh.

Garcinias are evergreen polygamous trees, shrubs, and herbs. It is estimated that around 35 species of the genus are found in India, some of which are endemic and known for their immense medicinal properties. *Garcinia indica* is native to the Western Ghats region of India. It is found throughout Konkan region, Goa, Karnataka, North Malabar, Coorg and Waynad as well as in West Bengal and Assam (Parle, 2013).

Garcinia indica (kokum) is a slender tree with drooping branches. It generally grows upto a height of 15-20 m. It has a dense canopy with dark green coloured leaves. Kokum grows well up to an elevation of about 800 m from.the mean sea level. Warm and humid tropical climate is suitable for its proper growth and development. It flourishes well in coastal areas that receive more than 250 cm of rainfall per annum with a temperature variation in the range of 15-35°C. It prefers alluvial soils with depth of 1.0 m and pH of 6.7 (Krishnamurthy, 1984). The monocrop orchards of kokum are not common. It is generally grown as an intercrop with coconut and arecanut or as roadside plantation (Malik *et al.*, 2005). *G. cambogia* (Malabar tamarind) plants are grown widely in the home gardens of Kerala. Kottayam and Ernakulam districts of Kerala are famous for the cultivation and export of Malabar tamarind (Abraham *et al*., 2006).

Flowering in kokum usually occurs during December–January and fruits are ready to harvest during April-May. Fruits of *Garcinia indica* are mainly spherical in shape and have smooth and shining surface. Fruits generally has 5-8 kidney shaped seeds embedded in the soft white coloured pulp. It is usually propagated by seeds or vegetative methods like air layering and by softwood grafting. Seeds have very less viability period and are required to be sown immediately after they are extracted from fruits (Pangsuban *et al.,* 2009).

Kokum is commonly not used as fresh fruit. Dried rind of *Garcinia indica* has a pleasant, tangy-sweet taste and is used as a spice in southern parts of the country for making curry. The fruit is anthelmintic and is useful against piles and dysentery. The fruit is also used as a heart tonic. Kokum has 4 percent sugar which is fermented to make good quality wine. Seeds give kokum butter that is used in chocolate and confectionary industry as well as pharmaceutical and cosmetic industry as surfactant. Kokum fruit is a potential source of hydroxy citric acid, anthocyanins and a polyisoprenylated benzophenone derivative, garcinol (Padhye *et al.,* 2009). Recently, hydroxyl citric acid has been found to be used as a potent metabolic regulator of obesity and lipid abnormalities in mammalian system. Kokum has a good amount of vitamins and minerals like potassium, manganese and magnesium, that help in controlling heart rate and blood pressure, which provide protection against stroke and coronary heart diseases. Its fruits are also used to make medicines to treat digestion related problems such as indigestion, flatulence, acidity and constipation.

In India, the production of kokum is estimated to be around 10,200 metric tonnes with productivity of 8.50 tons/ha. However, there is a continuous increase in its demand as it is evident by the domestic market trends and export scenario. (Nayak, 2010)

Variability study is the basic step of every tree improvement programme. Exploration of variability is a prerequisite for any breeding programme as well as its commercial exploitation. The present study is aimed to throw light upon the

variability existing in different accessions of kokum in the germplasm of ICAR-NBPGR, Regional Station,Thrissur.

The study was carried out in ICAR- NBPGR Regional Station, Thrissur with the objective of investigating following -

1. Variability in the general tree characters-

   Shape of the tree canopy, GBH, branching habit, height of the tree, height of first branch from the base, time of flowering.

2. Variability in leaf characters-

   Leaf length, leaf breadth, length to breadth ratio, leaf area, petiole length,

3. Variability in flower characters –

   Floral morphology, flower weight, number of flower per cluster, colour of petal, number of stamens per flower.

4. Variability in fruit characters-

   Fruit weight, fruits yield, rind weight, fruit apex, rind colour

5. Biochemical variability analysis-

   Organic acid, Primary metabolites (carbohydrate, reducing sugar, protein content, fat content and total soluble solids), Vitamin, Minerals (P, K, Na, Mg and Ca), secondary metabolites (total phenols and total flavanoids), analysis of physical (colour, aroma, pour point, melting point) and chemical properties (saponification value, acid value and iodine value) of seed kernel butter

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The review of literature with respect to the present study titled "Morphological and biochemical diversity assessment of *Garcinia indica* Choisy germplasm" is presented in this chapter.

2.1. *Garcinia* genus

Garcinia belongs to the family Clusiaceae (Syn: Guttiferae) native to Asia, Australia, America, and southern Africa. The centre of diversity of *Garcinia* species is the Malaysian region, with some species distributed widely in India and the Micronesian islands and also in tropical Africa and the Neotropics (Nimanthika and Kaththriarchi, 2010). The total number of species in the genus is highly disputed, various sources claim the number to be in between 50 to 300. The plants in this genus are called by various common names like mangosteens, sap trees, garcinias or monkey fruit. Many *Garcinia* species are threatened mainly due to habitat destruction, and *G. cadelliana* which was earlier found in South Andaman island is considered extinct .

In India, the genus *Garcinia* has around 43 species and 5 varieties, of which 37 species and 4 varieties occur in the wild, whereas the rest were introduced into cultivation (Sharma *et al*., 2015). Out of the 37 indigenous *Garcinia* taxa, 16 species and 4 varieties are endemic to India. In India, *Garcinia* species are distributed mainly in three phyto-geographical zones; North East India, Western Ghats and Andaman and Nicobar Islands. 17 species of *Garcinia* are found in North eastern part of India, of which 2 species and 1 variety are endemic to the region. The Western Ghats has 9 species and 2 varieties, of which 7 species and 2 varieties are endemic and the Andaman and Nicobar Islands hosts 15 taxa, of which 6 species and 1 variety are endemic to the region (Shameer *et al.*, 2016).

Table 1. *Garcinia* species in the Western Ghats

Sl. No.	Species	Distribution (altitude, meter)	Locality
1.	*G. gummi-gutta*	India, Sri Lanka (50- 900 m)	Throughout the evergreen-semi evergreen forests of the Western Ghats
2.	*G. imberti*	Endemic to South Western Ghats (900-1200 m)	Kerala: Agasthyamala Biosphere Reserve (Trivendrum), Shankily, Shendaruni (Kollam)
3.	*G. indica*	Endemic to India. the Western Ghats, North East India (50- 550 m)	Kerala: Badi Baduka, Thaliparamba; Maharashtra: Thungar Hill, North Kanara; Karnataka: Tinai Ghat. Assam: Karbi Anglong Dist.
4.	*G. Morella*	Indo-Malay, Sri Lanka (500- 1100 m)	Kerala: Chenathnair, Kuruva Island, Kambamala (Wayanad); Thamarassery, Vellarimala (Kozhikode); Silent Valley (Palakkad); Kodakkalthodu, Payampara (Thrissur); Pampa (Pathanamthitta); Pandimotta, Chemmunjii, Attayar (Thiruvananthapuram) Karnataka: Horanad Forests; Tamil Nadu: Anamalai Hills, Iyyerpadi, Kannikketyy. Assam: Pasighat, Rani Dawa bang
5.	*G. pushpangadaniana*	Endemic to the Western Ghats (850-1400 m)	Kerala: Kadalar, Pampadumchola, Munnar (Idukki); Wallakad of Silent Valley (Palakkad); Tamil Nadu: Anamalai Hills
6.	*G. rubro-echinata*	Endemic to South Western Ghats (800-1200 m)	Kerala: Ponmudi, Chemmunji Hills (Thiruvananthapuram). Tamil Nadu: Kalakkad Mundanthurai Tiger Reserve (Thirunelveli)
7.	*G. talbotii*	Endemic to the Western Ghats (100 -500 m)	Kerala: Uduma, Cheemani (Kasaragode); Vellarimala (Kozhikode); Vazhachal (Thrissur); Pampa, Pandarakayam (Pathanamthitta); Pandimotta, Rosemala (Thiruvananthapuram)
8.	*G. travancorica*	Endemic to South	Kerala: Athirumala, Chemmunjii

19

		Western Ghats (950-1500 m)	(Thiruvananthapuram). Tamil Nadu: Kalakkad Mundanthuarai Tiger Reservae (Thirunelveli)
9.	*G. wightii*	Endemic to South Western Ghats (250-700 m)	Kerala: Vazhachal, Athirappally (Thrissur); Paniyeli-poru (Eranakulam)

(Shameer *et al.*, 2016)

2.2. *Garcinia* indica: An overview

2.2.1. Distribution

In India *Garcinia indica* is widely distributed throughout the Western Ghats Maharashtra, Assam, Meghalaya and West Bengal. In the Western Ghats, it is mainly distributed along the coastal belt of Konkan region, Ratnagiri district of Maharashtra, Goa, Kasaragod region of Kerala and Uttara Kannada and Udupi Districts of Karnataka (Haldankar *et al.*, 2011). It is believed that kokum has a wide diversity in the Western Ghats due to its dioecious nature and cross pollination (Swami *et al.*, 2014)

G. indica flourishes well below an altitude of 800 m and requires warm and humid tropical climate. Coastal areas that receive more than 2500mm of rainfall are suited best for its growth and development. It prefers lateritic, alluvial soils with about 1.0 m depth and 6.7.pH (Nayak *et al.*, 2010). Kokum is mainly found in evergreen and semi-evergreen forests and in homegardens. In Western Ghats, kokum is cultivated as a rain-fed crop at a limited scale and is often mixed with other fruit trees.

2.2.2. Utilization

The dried rind of kokum fruit is used as a substitute for tamarind in food preparations because of its sweet-tangy taste (Jayaprakasha and Sakariah, 2002). The fruits are also used for manufacturing wine (Baliga *et al.*, 2011). It is used traditionally in herbal medicines to treat diarrhoea, dermatitis, digestion problems,

and rheumatic pains. It is also helpful in preventing hyper perspiration and also acts as an antihelmintic and cardiotonic. Kokum juice obtained from the rind is useful against piles, colic problems, dysentery and diarrhoea.

Several small and large scale industries like nutraceutical, food supplementary, beverage and cosmetics industries produce important value added products from kokum fruits (Swami *et al.,* 2014). Several products such as kokum syrup, kokum agal (kokum juice concentrate), kokum sharbat, kokum amsul (dried salted rind), kokum butter and kokum beverages are sold in the market. Kokum butter can be used as a substitute of cocoa in chocolate manufacturing because of its relatively high melting point (39 to 43°C). It has proved to improve the texture of chocolate without affecting its flavour. Kokum is also used in manufacturing soaps, candles lotions and ointments (Maheshwari and Reddy, 2005).

The fruit rind contains good amount of Hydroxycitric acid, which is in a great demand for its use in manufacturing anti-obesity drugs (Heymsfield *et al.*, 1998). It prevents the conversion of carbohydrates into fats by inhibiting ATP citrate lyase, which is an important enzyme in Kreb's cycle. Deore *et al.* (2011) has reported that aqueous extract from *G.indica* showed a significant reduction in gastric lesion (52.94%) whereas standard drug ranitidine showed inhibition at 58.26 percent in experimental rat.

2.3. Variability in the general tree characters

Chandran (1996) observed different canopy shapes viz., pyramidal and drooping, with height variability from 10-15m in kokum tree. Similar observations were also reported by Korikanthimath *et al.* (2008). Ravishankar and Sakthivel (2010) stated wide variability in canopy shape of trees (dome, round, pyramidal and conical) and branching patterns (drooping, spreading and erect) in Malabar tamarind (*G. gummi-gutta*). A wide variability in morphological characters within and between the *Garcinia* species such as *G. indica, G. xanthochymus, G. mangostana, ,G. morella* and *G. gummi-gutta* was

also reported by them. Khanvilkar (1984) observed the total canopy volume was in range of 25.91 to 64.79 m^3 among the different genotypes studied by him.

Jagtap *et al.* (2015) reported that the tree grows up to 10-18 meters with drooping branches. In a diversity analysis of kokum conducted in Goa by Devi *et al.*(2013), it was observed that out of total 268 trees studied 22 percent were conical in shape, 26 percent were pyramidal and spreading, 6 percent were spreading, 3 percent were pyramidical and drooping and only one percent were conical and medium spreading. This study also suggested that minimum one and maximum 42 primary branches arouse from the main trunk.

Bark of kokum tree is commonly grey to brown in colour and the inner bark is usually yellow or white. A yellow, white or cream exudate, known as 'Gamboge' is produced by twigs and stem (Shameer *et al.*, 2016).

2.4. Variability in leaf characters

Leaves of *Garcinia* species are usually opposite, thick and characterized by the presence of a foveola (an excavation with an extension resembling a ligule) at the base of the petiole. Coriaceous textured leaves are found in most of the *Garcinia* species except *G. imberti, G. wightii* and *G. indica* which possess subcoriaceous leaves (Devi *et al.*, 2013).

Parthasarathy and Nandakishore (2014) conducted morphological study on nine species of *Garcinia* and reported that *Garcinia* species are evergreen and having opposite decussate foliar arrangement. They have simple petiolate leaves. The petiols are small and appears to be sessile. Venation of the leaves was pinnate in all the cases.

Raysad (2016) conducted variability studies on 32 accessions of *Garcinia gummi-gutta* and stated that the leaf area varied from 22.07 cm^2 to 56.39 cm^2. Devi *et al.* (2013) reported that the kokum leaves are mainly lanceolate, few broadly lanceolate, narrowly lanceolate, some with obvate and ovate shapes. Leaf length was in the range of 6.24 -11.95 cm, whereas range of leaf width was 2.42 -

5.25 cm. Ratio between length and width ranged from 1.87- 4.12 cm. Petiole length varied from 0.60-1.37 cm. Sherly (1994) reported that colour of emerging leaves showed marked differences among male and hermaphrodites in *Garcinia cambogia*.

2.5. Time of flowering

Flowering period of kokum is reported to be from November to February (Jagtap *et al.*, 2015). Senthilkumar *et al.* (2014) reported that in coastal areas, kokum flowers in November -December while in the hilly regions it flowers in January-February. Devi *et al.* (2013) reported that mid season bearers showed flower initiation during 3rd and 4th week of November, while in late bearers flowering initiation occurred in 2nd week of December. The same study revealed that the span of flowering was from 3rd week of November to 4th week of January, whereas in late bearers the span was throughout December and January.

According to Tripathi *et al.* (2015) flowering in mangosteen starts in March- April while in *Garacinia xanthochymus,* it takes place usually in May-June.

2.6. Variability in flower characters

Kokum tree produces small and unisexual flowers; the male and female flowers are found on the same tree. The calyx is of four free sepals and corolla of four free petals (Chandran, 1996). Devi *et al.* (2013) in their study on genetic diversity of kokum in Goa, observed that male trees generally has flowers with long pedicels, fertile stamens but pistils are absent in this type. Female flowers are globose in shape with well developed ovary, and stigmatic surface with eight distinct lobes.

Study conducted by Thatte and Deobahar (2012) suggested that male flowers are around one cm long and pedicillate. Petals are usually yellow-red in colour and 4-5 in number. Typical female flower has four yellowish coloured petals and pistil of 1.5 -2.5 mm in diameter which is surrounded by staminodes

arranged in 4 tufts, while in bisexual flowers stamens are arranged in eight tufts. Rameshkumar (2017) in his study on diversity assessment of *Garcinia* species in the Western Ghats reported that female flowers in kokum were solitary, terminal, sub-sessile; ovary was subglobose with convex stigmas.

2.7. Variability in fruit characters

Fruit ripening in kokum occur in April to May (Jagtap *et al.*, 2015). They also stated that ripened kokum fruit is dark purple or red colored with 3-8 large seeds. The fruit is mainly spherical in shape with 2.5 to 3.0 cm diameter. Seeds are usually connected to the rind by tissue and embedded in an acidic pulp.

Sawant *et al.* (1999) evaluated 36 high yielding and early types in *G. indica* for fruiting and quality traits. They reported that highest width, circumference, weight and rind thickness were 4.15cm, 13.15 cm, 34.45 g and 4.45 mm respectively. Gawankar *et al.* (2001) also reported similar variation in length (3.2-4.28cm) and diameter (3.3-4.75) of kokum fruit. They also found that rind thickness and rind percentage of six accessions of kokum varied from 0.30 cm to 0.48 cm and 34.08 to 79.13 percent, respectively.

Hegde *et al.* (2010) conducted a study on evaluation of morphological and chemical variations in two species of *Garcinia.* Wide morphological variation for shape, colour and size was reported by them. Different shapes like round, flat, pear shaped with or without prominent navel fruits were found. Fruit colour also showed variations from dark red to crimson red and pure yellow fruits were also observed. Devi *et al.* (2013) suggested that the average length of the kokum fruits was 2.63 cm and average diameter was 2.97 cm.

2.7.1. Fruit weight

Mirgal *et al.* (2010) worked on characterization of fruit and seed traits of *Garcinia indica* and revealed that there was a significant variation for fruit characters among the selected individual trees. The fruit weight was in the range

of 14.7 to 71.82 gm with an average weight of 25.49 g. Similarly, the thickness of the fruit rind ranged from 0.20 to 0.38 cm and average rind weight was 0.26 g.

Devi *et al.* (2013) reported that mean value of individual fruit weight varied widely from 6.8 to 47.6 g. Krishnamurthy and Sampathu (1988) in their study reported that the weight was in range of 21-85 g. In an evaluation study by Shinde *et al.* (2001) on performance of grafts of promising types of kokum conducted in clonal orchards, the average fruit weight range was from 25.4 to 58.38 g.

2.7.2. Fruit yield

A fully mature tree yields about 300-400 kg of fruits. Average yield of kokum fruit in Goa was 8.5 t/ha in which rind contributed to 3.6t/ha, fresh seed 1.9t/ha and pulp 3.0t/ha (Devi *et al.*, 2013). Korikantthimath and Desai (2005) after conducting a survey concluded that homesteads usually have 4-10 kokum trees, and the fruits production in each household can range from 50 kg to more than 200 kg. Similarly

2.8. Organic acid

Hydroxy citric acid

HCA is an important organic acid present in leaves and fruit rinds of some species of *Garcinia*. Various studies have revealed that *Garcinia indica* have a high concentration of HCA in its rind and leaves. HCA is known for its importance in accelerating fat burning and inhibiting fatty acid synthesis. It through thermogenesis reduces the lipid such as cholesterol and triglycerides bv initiating fatty acid oxidation in lever. It slowly burns the fat without stimulation of central nervous system. HCA inhibits the activity of enzymes responsible for storing fat. It plays a role in mobilising the body fat and dissolves fat in the lever and also throughout the body. Thus, HCA helps in slower weight loss and supports natural appetite suppression mechanism of the body without altering ones food habits (Majeed, 1994). Other minor acids found in kokum are citric

acid, malic acid and ascorbic acid (Jena *et al.,* 2002). A method using HPLC has been developed to determine the amount of HCA and HCA-lactone in kokum leaves as well as rinds by Jayaprakash and Sakariah (2002).

Devi, *et al.* (2010) reported that HCA percentage in dried fruit rind varied from 19.32 percent to 37.39 percent. Out of all the accessions, 53 accessions recorded more than 30.74 percent HCA in rinds.

Antony *et al.* (1998) reported that the total acids in fruits ranged from 19-26 percent. Content of HCA present in the plant extract was found to be in range of 42-49 percent by using isocratic ellusion in RP-HPLC with 0.01M sulphuric acid as mobile phase with a flow rate of 1.0ml/min using UV detection at 210nm.

Niveditha (2013) estimated the HCA content in different morpho types of *Garcinia indica* and observed that Red, Green, Orange and Yellow morpho-type had 19.5, 20.35, 5.7 and 14.45 g/100 g of HCA in their rind. Whereas according to Parthasarthy and Nandkishore(2014) 7.43 percent of HCA was present in kokum fruit. *G. gummi-gutta, G. mangostana, G. xanthochymus, G. subelliptica, G. kydia, G. lanceaefolia* and *G. pedunculata* were having HCA content of 15.48, 0.26, 0.10, 1.16, 8.97, 1.93 and 1.33 g/100 g, respectively.

2.9. Primary metabolites

Primary metabolites are directly involved in the growth, development and reproduction of the plant and also serve as a source of energy.

2.9.1. Total carbohydrates

In a study conducted on various *Garcinia spp.* it was observed that the carbohydrates were the major metabolites in fruits of *Garcinia* followed by proteins. The study showed that the carbohydrate content ranged from 3.75 percent to 15.12 percent among various *Garcinia* species (Parthasarathy and Nandakishore, 2014). The average carbohydrate content in *G. indica* fruit was found to be 6.24 g/100 g.

Similarly Hande *et al.* (2014) opined that the carbohydrate content of fresh kokum rind was 3.52±0.07 percent and it increased upto 31.133±0.35 percent in tray dried kokum rind. Carbohydrates content in sun dried kokum rind was 3.52 ± 0.07 percent. However, some fruits like *Rubus ellipticus* (86.4g/100g) and *Rubus niveus* (85.35g/100g) have even higher amount of carbohydrate (Ahmad *et al.*, 2015)

2.9.2. Reducing sugar

A study done by Hande *et al.* (2014) showed that the percentage of reducing sugars in fresh kokum rind was 1.32 percent but on sun drying , solar drying and tray drying the percentage of reducing sugar obtained was 3.74, 3.49 and 5.88 percent respectively. It was stated that this increase in reducing sugar might be due to concentration of fruit flavors and calories during drying. Similar behaviour was reported during drying of grape by Maskan *et al.* (2002).

According to the study of Parthasarthy and Nandkishore (2014), the amount of reducing sugars in *G. indica* was 0.63g/100g and almost similar amount was observed in *G. gummi-gutta* and *G. Kydia i.e,* 0.51g/100g and 0.60g/100g respectively.

Gogoi (2015) noticed that concentration of reducing sugars was less in *Garcinia* species. This might be due to the very sour taste of the ripened fruits. Similar results were also reported by Miguez *et al.* (2004) for chestnut.

Niveditha (2013) in her study on four morphotypes revealed that significant variation was observed in amount of reducing sugar in fruit rind. Maximum amount of reducing sugar was recorded in green morpho type (8.9 %) while least in red morpho type (5.8 %). Fruits like *Artocarpus hirsutus* has a higher concentration of reducing sugar i.e, 11.94 percent (Thakur, 2013).

2.9.3. Total protein content

Hande *et al.* (2014) revealed that fresh kokum rind has 1.75 percent protein while on sun, solar and tray drying the percentage increases to 4.70

percent, 5.06 percent and 4.83 percent, respectively. According to Parthasarthy and Nandkishore (2014), almost similar concentration of protein is present in fruits of *G. Indica* (4.788g/100g) and *G. Kydia* (4.33g/100g). Whereas Krishnamurthy (1988) opined that 1.92 percent protein is found in kokum rind. Kokum leaves are reported to contain 2.3g of protein.

Tripathi, *et al.* (2015) opined that the protein content in kokum was 1.92%, while in *Garcinia gummi-gutta* it varied from 0.50 to 0.60 g/100g. In *Garcinia xanthochymus* and *Garcinia pedunculata*, protein content was estimated to be 5.01 percent and 3.16 percent, respectively (Sharma *et. al.,* 2015). Martinez *et al.*(2012) concluded that amount of protein in dry pulps of some of the most commonly cultivated fruits such as mango (8g/100g) and pineapple (4.0g/100g) are high. Raysad (2016) in her study conducted on diversity assessment of *Garcinia gummi-gutta,* has reported that the range of protein content in different accessions was 0.93 g/100 g to 1.29 g/100 g.

2.9.4. Crude fat content

Fats are considered as the second largest source of energy in living cells. Ramchandran (2014) reported that the percentage of fat in kokum rind was 1.4g/100g. Parthasarthy and Nandkishore (2014) observed very low concentration of crude fat in rind of *G. gummi-gutta* (0.34 g/100g), *G. xanthochymus* (0.41 g/100 g) and *G.kydia* (0.42 g/100 g).

A study conducted by Tripathi *et al.*(2015) showed that edible portion of mangosteen contained 0.1 to 0.6 g/100g of crude fat, whereas in kokum rind the amount was around 10 % of its dry weight. The same percentage of crude fat in kokum rind was noted by Swami *et al.* (2014). Furthermore, the study by Ahmad *et al.* (2015), showed that concentration of fat in *Rubus ellipticus* and *Rubus niveus* are 2.7 g/10 g and 1.1 g/100 g, respectively. According to Sharma *et al.,* (2015) the percentage of fat in *G. pedunculata* and *G.xanthochymus* was 0.44 percent and 7.57 percent, respectively. Study conducted by Martinez *et al.*(2002)

in cultivated fruits like mango, passion fruits and guava revealed that they have very low amount of fat.

2.9.5. Total soluble solids

Gogoi (2015) reported that the concentration of TSS of *Garcinia pedunculata* varied from 7.95°B to 7.22°B, for *Garcinia cowa* it was 7.95°B - 7.60°B and for *Garcinia lanceaefolia* it was 5.40°B-4.77°B. However, 11.73°B TSS was reported by Cavalcante *et al.* (2006) in kokum. Augustin and Azudin (1986) stated that the total soluble solids of mangosteen stored at 8 °C varied between 17.7 and 20.4 °Brix while in the same kind of study by Castro *et al.*(2012) it was found to be in the range from 15.2 to 19.1 °Brix.

2.10. Minerals

2.10.1. Phosphorus

Phosphorus is an essential component of nucleic acid and plays a very important role in metabolism of cells (Gopalan *et al.*, 1994). Parthasarthy and Nandkishore (2014) observed that different *Garcinia* species such as *G. gummi-gatta, G. indica, G. subelliptica* and *G. kydia* had almost similar concentration of phosphorus i.e, 5.34 mg/kg, 4.51 mg/kg, 5.43 mg/kg and 4.32 mg/kg, respectively. According to Tripathi *et al.* (2015) in edible portion of mangosteen it ranges from 0.02 to 12.0 mg/g. Study conducted by Morabandza *et al.* (2013) revealed that mesocarp of *Garcinia kola* contains approx. 59.32mg/100g of phosphorus. While, Ahmad *et al.*(2015) stated that wild fruits like *Rubus ellipticus* and *Rubus niveus* are rich in Phosphorus (1.26 mg/100g and 1.48 mg/100g, respectively).

2.10.2. Potassium

According to Parthasarthy and Nandkishore (2014) the potassium content in different *Garcinia* species had a significant difference e.g. *G. indica* (44.5 mg/100g), *G. mangosteena* (78.3 mg/100g), *G. gummi-gutta* (26.6 mg/100g) and *G. kydia* (38.7mg/100g). Morabandza *et al.* (2013) opined that *G. kola* contains

31.04 mg/100g of potassium in its mesocarp. Ahmad *et al.,* (2015) noted a high amount of K in *Rubus ellipticus* and *Rubus niveus i.e,* 680.16 mg/100g and 720mg/100g respectively. Mg and K were observed to be the most important minerals in *Garcinia* fruits. *G. mangostana* was richer in potassium (78.3 mg), magnesium (60.43 mg) and phosphorus (7.45 mg/kg) (Parthasarthy and Nandakishore, 2014).

Study conducted by Adeyeye and Ayejuyo (1994) on *Garcinia* showed that phosphorus (289 mg/kg) and calcium (41.5 mg/ kg) were present in greatest quantity in the hull while potassium (335 mg/ kg) and phosphorus (243 mg/kg) were the most abundant in the seed. However, these values differ from earlier reports by Dosunmu and Johnson (1995) who reported 499 and 720 mg/100 g for potassium and phosphorus, respectively, in the seed; 990 and 200 mg/ 100 g for potassium and calcium, respectively, in the hull. The K/Na ratio obtained for *Garcinia kola* seeds was more than twice that of *Garcinia kola* hulls while the reverse holds for Ca/Mg ratio.

2.10.3. Sodium

Parthasarthy and Nandkishore (2014) determined the sodium content of different species of *Garcinia* such as *G.indica* (1.55 mg/100g), *G. gummi-gatta* (2.88 mg/100g), *G. mangosteena* (2.58 mg/100 g) and *G. kydia* (2.54 mg/100g). High concentration of sodium was reported in *Ziziphus oenoplia* (26.15 mg/100 g) and *Mimusops elengi* (52.97 mg/100g) by Mahadkar *et al.*(2012). Raysad (2016) reported that the Na concentration in *G.gummi-gutta* was 41mg/100g to 98mg/100g. The study conducted by Jeeva (2009) reported that wild fruits such as *Prunus cerasoides* (3.79%) and *Eleagnus latifolia* (1.25%) are a good source of sodium.

2.10.4. Calcium

Calcium is believed to be an essential factor governing fruit storage quality (Lechaudel *et al.,* 2005). Different species of *Garcinia* were studied by

Parthasarthy and Nandkishore (2014) and it was observed that the calcium content in fruits of *G. indica* (13.21 mg/100g), *G.gummi-gutta* (12.61 mg/100g), *G. xanthochymus* (13.07 mg/100g), *G.subelliptica* (12.33mg/100g) and *G. kydia* (12.54 mg/g) was almost similar. Low value of Ca was reported in mesocarp of *G.kola* (4.30 mg/100 g) by Morabandza (2013). Ca content in edible portion of mangosteen varied from 0.01- 80 mg/100g (Tripathi *et al.*, 2015). Mahadkar *et al.* (2012) studied mineral composition of different wild fruits and observed that fruits like *Phyllanthus acidus* (163.22 mg/100g), *Solanuma torvum* (146.57 mg/100g) and *Ziziphus oenoplia* (94.76 mg/g) are good source of Ca.

2.10.5. Magnesium

Parthasarthy and Nandkishore (2014) observed that the Mg content in fruit rind of kokum was 33.45 mg/100. However, the content in other species such as *G. gummigutta, G. mangostana, G. xanthochymus* and *G. subelliptica* was 14.35 mg/100g, 60.43mg/100g, 30.62 mg/100g and 34.45 mg/100g, respectively. Morabandza (2013) reported lower level of Mg in *G. kola i.e,* 2.40 mg/100 g, while it was reported to be 2200 ppm by Adesuyi *et al.*(2011). Study done by Valvi and Rathod (2011) revealed that wild edible fruits of *Grewia tilifolia, Cordia dichotoma* and *Ficus racemosa* contain very high amount of Mg i.e, 402.2 mg/100g, 272.9 mg/100g and 123.6 mg/100g, respectively. Raysad (2016) reported the concentration to be 10 mg/100 g to 12.87 mg/100 g in *G. gummi-gutta* .

2.11. Secondary metabolites

2.11.1. Total phenols

Phenols in plants are involved in defence against UV radiation, pathogens and predators. They cause bitterness and astringency in fruits (Dai and Russel, 2010). Phenolic compounds present in plants also help in pollination by providing colour and fragrance, defense against pathogens and herbivores (Harborne, 2005). Parthasarthy and Nandkishore (2014) observed that the

phenolics content in *G.indica* was 5.01 g/100g. In the same study it was observed that other *Garcinia* species such as *G. gummi-gutta*, *G. mangostana*, *G. xanthochymus*, *G. subelliptica*, *G. kydia*, *G. lanceaefolia* and *G. pedunculata* had 3.26 g/100 g, 2.33 g/100 g, 4.43 g/100 g, 3.14 g/100 g, 4.32 g/100g, 3.03 g/100g and 1.36 g/100 g of phenolics, respectively.

Chowdhury (2014) analyzed total phenolic content of some species of *Garcinia* (*G. pedunculata, G. morella, G. lanceaefolia, G. xanthochymus and G. acuminate*). Total phenolic content *in vitro* were determined for water, methanol, ethanol, acetone ethyl acetate and petroleum ether extracts of leaves, flowers, stems, fruit and seeds. It was observed that phenolic compounds were present in high amount in water, methalonic and dichloromethane extracts, among which the concentration was highest in methanol stem extracts of *G. pedunculata* (76.30 mg GA/g).

Muthulakshmi (1998) stated that in *G.cambogia* total phenol content ranged from 265-380 mg/100 g. However, in mesocarp of *G. kola* it was found to be 68.33 mg/100 g (Moranandza *et al.*, 2013). Similarly, the amount of phenolics in *G. pedunculata* and *G. xanthochymus* was 9.44 mg GAE/g and 31.31 mg GAE/g (Sharma *et al.*, 2015).

Zadernowski *et al.* (2009) reported that the total content of phenolic acids, determined by GC-FID ranged from 265.7 ± 12.7 (aril) to 5027.7 ± 188.0 (peel) mg per kg of dry matter of sample in *Garcinia mangostana*. While, total phenolic content in *Garcinia talbotti* was estimated to be 2.609±0.130 % (Patil and Potdar., 2018).

Study by Parthasarathy and Nandakishore, (2016) on *G. indica* bark exudates showed its total phenol and xanthone content as 53.43 g/100 g and 32.42 g/100 g respectively, revealing it as a potential source of antioxidants.

2.11.2. Total flavanoids

An extensive LC-MS study on methanol extracts of *G. indica* leaves led to the identification of multiclass bioactive constituents belonging to organic acids, flavonoids, biflavonoids, benzophenones, xanthones, and terpenoids (Pandey *et al.*, 2015). Total flavanoids content of kokum rind was found to be 143.84 µg/g of extract as calculated as quercetine equivalent (Nagendra *et al.*, 2014). According to Sharma *et al.* (2015), its concentration in kokum was 137.27µg QE/g. He also stated that *G. pedunculata* and *G.xanthochymus* has 50.60 and 313 mg quercetin equivalent/g of extract respectively.

Karuppusamy *et al.*, (2011) revealed that wild edible fruits like *Rubus ellipticus* (86.4 mg QE/100g), *Grewia tiliaefolia* (47.1 QE/100g) and *Ziziphus rugosa* (41.1 mg QE/100g) are also good source of flavanoids. Patil and Potdar (2018) found that the total flavonoid content in *Garcinia talbotti* was 5.043±0252 mg/100 g in the methanolic extract.

Study conducted by Hutchin *et al.* (2014) reported that high content of flavanoids was present in custard apple (418.24 QE/100 g FW) and *Annona squamosa* (200.9 mg QE/100g FW)

2.12. Vitamins

According to Parthasarthy and Nandkishore (2014) the vitamins present in the detectable range are vitamins B1 (52µg/100g), B2 (320 µg/100g), B3 (63 µg/100g), B12 (12.06 µg/100g) and C (33.45 µg/100g) in kokum. Total vitamin content was observed to be 34.00 mg/100 g and ascorbic acid was found to be the major vitamin. The total vitamin content was highest in *G. mangostana* (61 mg/100g), followed by *G. pedunculata* (36 mg/100g). Except ascorbic acid, other vitamins showed only a small variation (<10%) among the species studied. Ascorbic acid varied in a range of 14%-60% in different *Garcinia* spp. However, *G. gummi-gutta* had 48 µg/100g of B1, 275 µg/100g of B2, 45 µg/100g of B3, 14.35 µg/100g of C and 8.75 µg/100g of B12 (Parthasarthy and Nandkishore, 2014). Tripathi *et al.*(2015) stated that mangosteen contains 34.45 mg/100 g

while, kokum contain 0.06 % of vitamin C. Study conducted by Raysad (2016) revealed that the vitamin C content in *G. gummi-gutta* ranged from 16.69 mg/100g to 27.76 mg/100g.

2.13. Physical properties of kokum butter

Lipids or fats are hydrophobic, hydrocarbon molecules. Fats are the storage form of energy and in plants found much abundant in seeds. Fats are the second largest energy source for living cells. *Garcinia* seed kernel contains 30-40 of fixed oil, in comparison to other vegetable seed fats like castor seed (50%), mustard (35%), sunflower (32%), ground nut kernel (42%), palm kernel (36%), and coconut (60%) (Jain *et al.,* 2005). High concentration of fixed oil indicates that *Garcinia* seeds are a rich source of fatty acids.

Parthasarthy and Nandkishore (2014) observed that yield of butter was higher in *G. gummi-gutta* (47%) in comparison to *G. indica* (29.33%), *G. xanthochymus* (25.71%) and *G. mangostana* (24%). They also observed that the colour of kokum butter was pale white while light brown and creamy yellow butter was obtained from *G. gummi-gutta* and *G. xanthochymus,* respectively.

2.13.1. Melting point

Kokum butter remains solid at room temperature and is almost as hard as cocoa butter, so can be used as a good substitute of cocoa butter. The melting point of kokum butter is high (about 40°C), hence it is used as an ingredient in chocolate manufacturing, to enhance the heat resistance property and hardness of the chocolate. It is helpful in preventing heat induced softening and loss of consistency of chocolates, mainly in hot climatic regions (Parthasarthy and Nandakishore, 2014).

Swami *et al.* (2014) concluded that melting point of kokum butter was in the range of 39.5 - 40°C. Ramachandran (2014) found the value to be 34-40°C. Similar studies conducted by Parthasarthy and Nandkishore (2014) revealed that

there was not much difference in melting point of *Garcinia spp.* such as *G. Mangostana* (37.9°C), *G. gummi-gutta* (39.4°C) *and G. xanthochymus* (38.2°C).

2.14. Chemical properties of butter

2.14.1. Acid value

Acid value determines the freshness and storage quality of an oil or fat. It is the measure of susceptibility and the extent of decomposition. Parthasarthy and Nandakishore (2014) conducted a study and found out that acid value of four *Garcinia spp. G. indica, G. gummi-gutta, G. xanthochymus* and *G. mangostana* were 4.9, 3.7, 4.8 and 4.5 mg NaOH/g of oil. The major fatty acids present were palmitic acid, linoleic acid, elaidic acid, oleic acid, stearic acid, arachidic acid and eicosenoic acid. *G. mangostana* was found to be highly rich in Palmitic acid (47%), while it was present in moderate amount in other species. Palmitic acid helps to recover some reproductive abnormalities and also control obesity (Scott *et al.*, 1988). It is opined that the diet enriched with palmitic acid is good for diabetes (Parthasarthy *et al.,* 2014).

High concentration of stearic acid was present in (30-40%) in *G. gummi-gutta, G. indica* and *G. xanthochymus;* while its percentage was less in *G. mangostana* (2.3%). Stearic acid is used in the manufacture of soaps, shampoo, shaving creams, detergents and other cosmetic products. After palmitic acid stearic acid is one of the most common saturated fatty acids found in the plants (Parthasarthy and Nandakishore, 2014). Butter rich in stearic acid remains solid at room temperature. Linoleic acid is also present in different *Garcinia* species in a range of 5-11 per cent linoleic. This is considered to be useful in losing lose body fat and preventing breast and colon cancer (Nirvair *et al.*, 2007).

Choppa *et al.,* (2015) stated that Malabar tamarind seed oil has higher acid value (5.04 mg KOH/g) than sunflower oil (3.09 KOH/g), but lower than olive oil (6.6 mg KOH/g).

2.14.2. Saponification value

Saponification number gives the information regarding the character of the fatty acid present in the fat. Fats with the high saponification number yield quite soluble soaps. The saponification value of olive oil is 187-196, for sunflower oil, it is 188-194, for ground nut it is 188-195, for mustard oil it is 169-176 and for sesame oil it is 188-195, while it is very high in coconut oil and ghee (251-263 and 220 respectively) (Parthasarthy and Nandakishore, 2014).

Saponification number of different *Garcinnia spp.* such as, *G. indica, G. gummi-gutta, G. xanthochymus* and *G. mangostana* was 200.2 , 187.9, 190.3 and 140.5 mg KOH/g of oil, respectively (Parthasarthy and Nandakishore, 2014).

According to Ramachandran (2014), saponification value of kokum butter varied from 187-193 mg KOH/g of oil. Raysad (2016) concluded that the value varied from 171.81 mg KOH/g to 189.34 mg KOH/g in *Garcinia gummi-gutta.*

2.14.3. Iodine value

Iodine value (IV) is a measure of the unsaturated nature of the fat. The iodine value between 25 and 50 is considered ideal. Iodine value helps in determining the tendency of fat to become rancid. The iodine value is very low in coconut oil (7.5- 10.5) and hence, it tends to get rancid easily (Parthasarthy and Nandakishore, 2014). IV of, *G. indica, G. gummi-gutta, G. xanthochymus* and *G. mangostana* were, 39.4, 50.2, 37.4 and 51.8, respectively (Parthasarthy and Nandakishore, 2014).

Study conducted by Ramachandran (2014) showed that the IV of kokum was 34 - 40 and similar result was obtained by Swami *et al.,* (2014) i.e, 37.4. Choppa *et al.* (2015) found out that IV in *G. gummi-gutta* (131.0g/100g) and sunflower oil (131g/100g oil) was almost similar.

MATERIALS AND METHODS

3. Materials and methods

The present study was conducted in trees maintained as a germplasm collection, in the orchard of NBPGR, Regional Station, Vellanikkara. The orchard is located on a well levelled land of laterite soil, that has a pH range of 5.0 to 5.5.

The accessions taken for the study along with the place of their collection and date of planting are given in Table 2.

3.1. Variability in the general tree characters

3.1.1. Shape of the tree canopy

Shapes of canopy were recorded through visual observation. Variation in canopy shape was recorded as conical, pyramidal and irregular.

3.1.2. Height of the tree (m)

Height of the trees was measured from the ground to the tip of the tree with the help of Blume Leiss Hypsometer and recorded in meter. The height of the first branch was measured with the help of measuring tape and recorded in meters.

3.1.3. GBH and branching habit

Girth was measured at 1.37m from the ground with the help of measuring tape in cm. Branching habit was carefully observed visually and noted down as drooping, horizontal and erect.

3.1.4. Time of flowering

Months during which flowering takes place in different accessions were recorded.

Plate 1. *Garcinia indica* germplasm maintained in NBPGR, Vellanikkara

3.2. Variability in leaf character

3.2.1. Leaf length (cm) and breadth (cm)

For each accession, 10 leaves were selected and their length and breadth was measured and the average was recorded in cm.

3.2.2. Length to breadth ratio

Length to breadth ratio was calculated for each leaf.

3.2.3. Leaf area

Ten leaves were collected randomly from each tree and area was calculated with the help of leaf area meter.

3.2.4. Petiole length (cm)

Petiole length of ten leaves of each tree was measured and the average was recorded.

3.3. Variability in flower characters

Flowers were selected randomly from different parts of the tree canopy of each tree and following observations were made.

3.3.1. Flower length and breadth

From each tree ten flowers were taken and their length and breadth were measured.

3.3.2. Pedicel length

Length of pedicel of ten flowers from each tree was measured and their mean was calculated.

Table 2: List of accessions used for the study

IC No.	Date of planting	Village	District	State
IC 136687-1	5/11/1989	Sonagu, Siddhapura	Mangalore	Karnataka
IC 136687-2	5/11/1989	Sonagu, Siddhapura	Mangalore	Karnataka
IC 136687-3	5/11/1989	Sonagu, Siddhapura	Mangalore	Karnataka
IC 136685-1	5/11/1989	Walpoi		Goa
IC 136682-2	5-10-1989		Sirsi	Karnataka
IC 552528-1	27/3/2007	Ganjagadde	Kodagu	Karnataka
IC 552528-3	27/3/2007			
IC 552526-2	22/3/2007	Kepu, Kuntrody	Dakshin Kanadda	Karnataka
IC 550572-1	2002			Karnataka
IC 342322-1	29/04/2002	Ramdurga Extn., Sagar		Karnataka
IC 342297-1	25/04/2002	Hireguthi	Uttar Kanna	Karnataka
IC 342303-2	27/04/2002	Kukkana Mane,Theretana halli,Kuluru Panchayat	Uttar Kannad	Karnataka
IC 342302-2	27/04/2002	Kukkana Mane,Theretana halli,Kuluru Panchayat	Uttar Kannad	Karnataka
IC 342329-3	30/4/2002	Thedimberi gutte	Udupi	Karnataka
IC 342319-3	28/04/2002	Kakkalli	Uttar Kannad	Karnataka
IC 342327-1	29/4/2002	Balur farm,Ripponpet	Shimoga	Karnataka

3.3.3. Flower weight

The weight of ten flowers from each tree was taken using an electronic balance and then their mean was calculated.

3.4. Variability in fruit characters

From each accession, 10 fruits were collected randomly and following observations were taken.

3.4.1. Fruit weight

Fresh Weight of ten fruits from each accession is measured with the help of electronic balance and their mean was calculated.

3.4.2. Fruits yield

Total number of fruits on each tree were counted and recorded.

3.4.3. Rind weight

Rind was separated from ten fruits of each accession individually and then average was calculated.

3.4.4. Fruit apex

Shape of apex of fruits from each tree was observed.

3.4.5. Rind colour

Colour was determined according to descriptor developed by NBPGR.

3.5.1. Estimation of HCA

High Performance Liquid Chromatography (HPLC) analysis (Jayaprakash and Sakariah, 2002):

Hydroxy citric acid was analyzed by reverse phase HPLC (Make: Shimadzu, LC-8A,) on a C18 column 4.6 X 250 mm (5µm). The HPLC conditions were: 210 nm wavelength, UV detector, 20µL sample loop and 1 ml/min flow rate. The mobile phase was isocratic, 8mM sulphuric acid and HPLC grade water. Potassium hydroxy citrate (Sigma: tribasic monohydrate) was used for preparing standard. The standard was prepared using HPLC grade water. The retention time of standard HCA was 1.84 min. Run time for each sample was 20 m.

Fruit collection

Drying

Dried rind

Grinding

Rind powder

Plate 2. Procedure followed for making dried rind powder of kokum fruit.

Procedure for preparation of mobile phase

To prepare 1 litre mobile phase 0.43 ml of 8mM sulphuric acid was added and made up to 1000 ml with triple distilled water. Mobile phase was prepared fresh on the day of HPLC, after preparing mobile phase it was degassed properly by using sonicating instrument. Mobile phase was used to wash the column for about half an hour. Then sample was injected into HPLC slot. Then the retention time was noted down and compared with standard solution retention time.

Preparation of pure HCA from potassium hydroxicitrate salt

HCA was prepared from Potassium hydroxy citrate tribasic monohydrate. 100 mg of it was suspended in a 50 ml beaker containing 5.0 ml distilled water and was treated with 500 mg of Dowex 50 [H+]. The mixture was stirred continuously using glass rod or magnetic stirrer for 10 min. After 10 min salt and resin settled at the bottom and only supernatants was decanted. This step was repeated 6-7 times till the resin came to neutral pH. The washing and the supernatants were combined and made up to 100 ml, mixed and filtered using Whatman's filter paper 1 and used for HPLC.

Procedure for Sample extraction

About one gram of dried sample powder was taken in a test tube and 100 ml of distilled water was added to it. It was then vortexed for 10-15 minutes and then filtered using whatman paper 4. Then 200 µl of filtrate was transferred to eppendorff tube and 400 µl of ethanl was added to it and then vorttexed for 1 min and kept in fridge overnight for incubation. Next day it was centrifuged at 5000 rpm for 10 min. and the supernatant was taken in another eppendorff tube. The supernatant was then dried using nitrogen gas and kept in refrigerators (4°C). On the day of HPLC 100 µl HPLC grade water was used to dissolve the dried sample and was used for injection to the HPLC.

3.5.2. Estimation of primary metabolites

3.5.2.1. Total carbohydrates

100mg of the sample was hydrolysed with 5.0 ml of 2.5 N HCl in a boiling water bath for three hours and cooled to room temperature. This was then neutralized with sodium carbonate until the effervescence ceased and volume was made upto 100 ml and centrifuged, the supernatant was collected and 0.2ml was taken for analysis in a test tube. Standards were prepared by taking 0.2-1.0 ml of the working standards. One ml of water served as a blank, volume was made up to one ml in all the tubes with distilled water. Then four ml of anthrone reagent was added to each test tube and kept for heating for eight minutes in a boiling water bath. Green colour was read at 630 nm after being cooled rapidly (Sadasivam and Manikam, 1996).

3.5.2.2. Reducing sugar

Five milliliters of Fehling's A and Fehling's B with 500ml of distilled water were taken in a flask. Dextrose (0.2% solution) was taken in a burette and titrated against hot Fehling's solution with Methylene blue indicator until the blue colour changed to brick red.

$$\text{Fehling's Factor} = \frac{\text{Titre Value} \times 2.5}{1000}$$

Two grams of sample was taken and crushed in a pestle and mortar with 10 ml of distilled water. To this two ml of 45% lead acetate solution was added and mixed for 10 min, then 10 ml of 22% potassium oxalate was added and mixed well for five minutes, it was then neutralized using 10% NaOH with phenolphthalein as an indicator, made up to 250 ml with distilled water, mixed and filtered. The filtrate was titrated against Fehling's solution A and B using methylene blue as indicator (Ranganna, 1991).

Reducing sugar (%) = $\frac{\text{Fehling's Factor} \times \text{Dilution} \times 100}{\text{Titre value} \times \text{Weight of the sample}}$

3.5.2.3. Total protein content

Lowery's method (Sadasivam and Manikam, 1996) was used to estimate total protein. The extraction of sample was done by using Tris buffer then to it, 5 ml alkaline copper solution was added and was then allowed to stand for 10 minutes. The mixture was then treated with Folin- Ciocalteau reagent and incubated at room temperature in dark for 30 minutes and blue colour was read at 660 nm in spectrophotometer. A standard calibration curve was plotted against standard bovine serum albumin.

3.6.2.4. Total fat content

Extraction of crude fat is done with the help of soxhlet apparatus for 6 hours, using petroleum ether as solvent. Then ether was evaporated on a water bath until no odour of ether remains. Percentage of crude fat is then calculated by using following formula (Sadasivam and Manikam, 1996).

Crude fat(%) = $\frac{\text{Wt. of fat (g) X 100}}{\text{Wt. of sample (g)}}$

3.6.2.5. Total soluble solid

It was determined with the help of hand refractometer.

3.6.3. Analysis of Vitamin C

It was done with help of the method described by Sadasivam and Manikam (1996). Stock standard solution was first prepared by dissolving 100 mg ascorbic acid in 100 ml of 4 % oxalic acid solution. From this working standard was then prepared by diluting 10ml of the stock solution to 100ml with 4 % oxalic acid. Five ml of the working standard solution was taken and to it 10 ml of oxalic acid was added, this was then titrated against 2,6-dichloro phenol

indophenols dye(V_1). End point was the appearance of pink colour that persists for a few minutes. The sample was extracted in 4% oxalic acid and made upto a known volume (100ml) and centrifuged. Ten ml of 4% oxalic was added to 5ml of supernatant and then it was titrated against the dye. The amount of ascorbic acid was estimated as following.

$$\text{Amount of Ascorbic acid mg/100g sample} = \frac{0.55\text{mg} \times V_2 \times 100\text{ml}}{V_2 \times \text{Wt. of the sample}} \times 100$$

3.6.4. Analysis of Minerals

3.6.4.1. Phosphorus

Phosphorus content was analysed colorimetrically (Jackson, 1973). To five ml of pre-digested aliquot, 10 ml of nitric acid vanamolybdate reagent was added and volume was made upto 50 ml with distilled water. After 10 minutes yellow colour was read on the spectrophotometer at 420 nm wavelength. A standard graph was prepared using serial dilution of P solution and finally the result was expressed in mg per 100g of sample.

3.6.4.2. Potassium and Sodium

Flame photometric method was used to estimate K and Na content (Jackson, 1973). One gram of sample was first digested with 9:4 mixture of nitric acid and perchloric acid and was made upto 50 ml. This was then read in flame photometer by using KCl as standard and NaCl for Na. The results were then expressed in mg per 100 g of sample.

3.6.4.3. Calcium and Magnesium

Atomic absorption spectrophotometric method was used to determine Ca and Mg content (Yash, 1998). One gram of the sample was pre-digested with 15ml of 9:4 mixture of nitric acid and made upto 50ml and used directly in atomic absorption spectrophotometer for the estimation of Ca and then expressed in mg per 100 g of the sample.

3.5.5. Analysis of secondary metabolites

3.6.5.1. Total phenols

Total phenols were estimated using Folin- Cocalteau method. One gram of sample was ground in 80% of ethanol and the mixture was centrifuged. After centrifugation residue was re extracted with ethanol. Then supernatant was dried by evaporation. Then After dilution the solution of each extract was mixed with Folin- Ciocalteu. The mixture was kept in room temperature for three minutes and then sodium carbonate solution was added. It was placed in a boiling water bath for two minutes. Absorbance was then measured at 650 nm against reagent as blank. A standard calibration curve was plotted against catechol. Result was expressed as mg of catechol equivalent (CE)/ 100g of fruit weight (Sadasivam and Manikam, 1996).

3.6.5.2. Total flavonoids

Flavonoids determination was done by the method of Bohm and Kocipai-Abyazan (1994). Ten grams of the plant sample was extracted repeatedly with 100 ml of 80% methanol at room temperature by centrifuging for 20 minutes each time. The whole solution was filtered through whatman filter paper No. 42 (125 mm). The filtrate was then transferred into a crucible and evaporated to dryness over a water bath and weight was then taken when it became constant.

3.8. Analysis of seed kernel butter

Kernels were dried and ground and then fat extraction was done with the help of soxhlet apparatus using petroleum ether as solvent. After completion of process the seed oil was separated from evaporating petroleum ether at 60°.

3.8.1. Physical properties of butter

3.8.1.1. Pour point

It was estimated by treating sample to the specific temperature for a minute duration and observe the physical change in sample (Parthasarthy and Nandkishore, 2014).

3.8.1.2. Melting point

It was estimated by method given Ranganna (1986). Butter was heated above melting point and dipped fine glass capillary tube was dipped into it to allow the sample to rise up the capillary tube. Fat was solidified for 24 hrs later the capillary tubes were attached to the bulb of a thermometer with a rubber and immersed in cold water. The temperature at which the fats started rising was noted.

3.8.1.3. Colour

Colour was measured with the help of spectrophotometer using CCl4 as blank at the wavelength of maximum absorption (510 nm) as mentioned by Raysad (2016).

3.8.2. Chemical properties of fats

3.8.2.1. Acid value

Five gram of oil was dissolved in 50 ml of neutral solvent and phenolphthalein was added as indicator. Then titrated against 0.1 N potassium hydroxide. It was shook until a pink colour which persists for 15 seconds is obtained.

$$\text{Acid value (mg KOH/g)} = \frac{\text{Titre value X Normamlity of KOH X 56.1}}{\text{Weight of the sample (g)}}$$

Extraction of seeds from pulp

Dried seed with seed coat

Dried seed without seed coat

Seed powder

Extraction of butter through soxhlet apparatus

Kokum butter

Plate 3. Procedure followed for extraction of kokum butter from seeds of different accessions of *G. indica*.

3.8.2.2. Saponification value

A method described by Sadasivam and Manikam (1996) was followed to determine the saponification value. A known amount of melted fat was taken in a flask and mixed with 50 ml of alcoholic KOH and refluxed by using an air condenser for 60 minutes. After that was cooled, one ml of phenolphthalein indicator was added and it was titrated against 0.5 N HCL until pink colour disappeared. 50 ml of alcoholic KOH was used as blank.

$$\text{Saponification value} = \frac{28.05 \times (\text{titre value of blank} - \text{titre value of sample})}{\text{Weight of the sample (g)}}$$

3.8.2.3. Iodine value

It was estimated by method given by Sadasivam and Manikam (1996). A known quantity of butter was melted and mixed with 10 ml CCl4 and 25 ml Wijs solution. It was stored in dark for 30 minuted and added with 15 ml of 10% potassium iodide solution. It was titrated with 0.1 Na2S2O3 solution using starch as an indicator.

$$\text{I.V} = \frac{(\text{Blank titre} - \text{sample titre}) \times \text{N of } Na_2S_2O_3 \times 12.66}{\text{Weight of sample (g)}}$$

3.9. Statistical analysis

3.9.1. Cluster analysis

The data of biochemical and morphological variations of different accessions was subjected to hierarchial clustering analysis. The cluster analysis was carried out using between link linkage as the clustering method and elucedian distance as the interval using the SPSS software.

3.9.2. Principal component analysis

The collected data on morphological and physical analysis was subjected to principle component analysis using SPSS software.

3.9.3. Path coefficient analysis

Path coefficient analysis was performed by using SPSS software.

RESULTS

4. RESULTS

The results obtained in the present investigation titled "Morphological and biochemical diversity assessment of *Garcinia indica* (Thouars) Choisy germplasm" are presented in this chapter.

4.1 Morphometric characters

Information regarding the morphometric characters of the selected accessions is given in Table 3.

The variation in height was in the range of 4.2 m (IC 552528-1) to 15.5 m (IC 136687-1). Average height was estimated to be 11.1 m and the CV being 26.4%. Three of the accessions had tree height almost similar to the average i.e, IC 136687-2 (11.5 m), IC 342322-1 (11.5 m) and IC 342297-1 (11.0 m).

Variation in the height of first branch from the base of the tree varied from 0.8 m (IC 552528-1) to 6.52 m (IC342322-1). The average and CV for height of first branch were 2.89 m and 50.9% respectively.

The GBH varied from 41 cm to 110 cm. IC 136685-1 had a significantly large girth than rest of the trees i.e, 110 cm. Trees with significantly smaller GBH were IC 552528-1 (51.5 cm), IC 552528-3 (50.2 cm), IC 342322-1 (50.6 cm) and IC 342297-1 (41.0 cm). Average GBH was 65.03 0cm, while the CV was 24.51%.

Trees did not show much variations in the shape of canopy, two had irregular shaped canopy, while rest were conical in shape. Similarly much variations were absent in branching habit. Two types of branching were observed i.e. drooping and horizontal. Only four trees had horizontal branching while rest had drooping branches.

4.2. Leaf characters

Information regarding the leaf characters of the 16 accessions is given in Table 4. IC342329-3 had significantly smaller leaf area i.e, 13.53cm ^{2} then the rest of the accessions . IC 552526-2 had the largest leaf area (26.24 cm ^{2}) followed by

IC 136682-2 (24.91 cm^2) and IC 550572-1 (24.43 cm^2). The average leaf area was 20.413 cm ^{2}, while CV was 16.2 percent. Correlation analysis showed a positive correlation between the size of leaf and number of fruits produced by different accessions. This can be explained as, the trees with larger leaf area showed greater photosynthetic activity by intercepting more light and hence showed higher productivity in terms of number of fruits. So leaf size can be considered as a factor while making selection for higher productivity.

Leaf length varied from 6.98 cm (IC 136687-1) to 10.72 cm (IC 552526-2) whereas, leaf breadth ranged from 2.91 cm (IC342327-1) to 4.24 cm (IC 550572-1). Average length and breadth of the leaf were 8.62 cm and 3.73 cm respectively, while the CV for leaf length and leaf breadth were estimated to be 9.8 percent and 9.9 percent respectively. Length to width ratio was maximum for IC342319-3 (2.91 cm) which made the leaves more narrow. IC 136687-1 and IC 552528-3 had relatively broad leave with leaf length to breadth ratio of 1.66 and 1.99 respectively.

The CV value (20.2%) indicated wide variations in petiole length. It varied from 0.75 cm (IC 552528-1) to 1.41 cm (IC 136682-2). The average prtiole length was estimated to be 1.007 cm.

4.3. Leaf emergence

Not much variations was observed in the emergence of the leaves. Pinkish flushes were observed in all the accessions which turned into green colour after an average of 11 days of emergence. It took an average of 29 days to complete leaf development.

4.4. Flower characters

Information regarding the flower characters of the selected accessions is given in Table 5.

Table 3: Morphometric characters of different accessions of *Garcinia indica*

Sl. No.	Accessions	Crown shape	Branching habit	GBH (cm)	Tree height (m)	Height of first branch (m)
1	IC 136687-1	Conical	Drooping	70.7	15.5	2.50
2	IC 136687-2	Conical	Drooping	60.1	11.5	2.12
3	IC 136687-3	Conical	Drooping	80.1	13.5	1.92
4	IC 136685-1	Conical	Drooping	110	14.5	2.64
5	IC 136682-2	Conical	Drooping	70.3	12.5	2.16
6	IC 552528-1	Irregular	Horizontal	51.5	4.2	0.80
7	IC 552528-3	Irregular	Drooping	50.2	7.9	1.25
8	IC 552526-2	Conical	Drooping	53.6	6.5	1.64
9	IC 550572-1	Conical	Drooping	70.7	13.7	3.54
10	IC 342322-1	Conical	Horizontal	50.6	11.5	6.52
11	IC 342297-1	Conical	Drooping	41.0	11	2.52
12	IC 342303-2	Conical	Drooping	70.2	12.5	4.92
13	IC 342302-2	Conical	Drooping	60.6	10.1	4.83
14	IC 342329-3	Conical	Horizontal	60.1	10.2	3.12
15	IC 342319-3	Conical	Drooping	70.5	12	2.91
16	IC 342327-1	Conical	Horizontal	70.3	10.6	2.88

Table 4: Leaf morphology of different accessions of *Garcinia indica*

Sl. No.	Accessions	Leaf area (cm^2)	Length (cm)	Width (cm)	Length to width ratio	Petiole (cm)
1	IC 136687-1	18.68	6.98	4.20	1.66	0.99
2	IC 136687-2	17.57	8.38	3.53	2.37	1.32
3	IC 136687-3	21.40	8.51	3.82	2.22	1.03
4	IC 136685-1	22.14	8.42	3.69	2.28	1.06
5	IC 136682-2	24.91	8.71	3.89	2.23	1.41
6	IC 552528-1	19.12	8.28	3.85	2.15	0.75
7	IC 552528-3	21.37	8.31	4.17	1.99	0.82
8	IC 552526-2	26.24	10.72	4.21	2.54	0.89
9	IC 550572-1	24.43	8.66	4.24	2.04	1.34
10	IC 342322-1	17.21	7.98	3.56	2.24	0.88
11	IC 342297-1	18.74	8.51	3.52	2.41	0.84
12	IC342303-2	18.03	8.60	3.62	2.37	0.89
13	IC342302-2	20.26	8.78	3.84	2.28	0.91
14	IC342329-3	13.53	8.62	3.22	2.67	0.86
15	IC342319-3	23.43	10.26	3.52	2.91	1.21
16	IC342327-1	19.56	8.24	2.91	2.83	0.92

Plate 4. Variability in leaf size among different accessions of *Garcinia indica*

Plate 5. Leaf development in *Garcinia indica*

Length of flower ranged from 5.31 mm (IC 552526-2) to 8.44 mm (IC 342297-1). Average flower length was 6.67cm, while CV was 14.22 percent. Flower diameter varied from 4.09 mm (IC 552528-1) to 6.67 mm (IC342303-2). The mean flower diameter was estimated to be 5.509 mm and the CV was 11.8 percent. Weight of the flower did not show much variation it ranged from 0.48 gm (IC342327-1) to 0.63 gm (IC 136687-3). The average flower weight was 0.55g, while the CV was 8.7 percent. IC 136687-2 had weight equal to the average weight of all the accessions i.e, 0.55 g. The pedicel length varied from 0.52 cm (IC 136682-2) to 2.24 cm (IC342329-3). The average pedicel length was found to be 0.90 cm, while CV was 48.5 percent.

Significant variation was observed in the flowering time. Six trees flowered in October, five in November, three in December and two in January. Not much colour variations were observed in petals, all the female flowers had yellowish petals. Female flowers were not present in clusters while male were present in 4-5 clusters. Female flowers lacked stamen while in males on an average 52 stamens were present.

4.5. Variability in fruit characters

Information regarding the fruit characters of the selected accessions is given in Table 6.

A wide variation was observed in fruit weight. IC 136687-1 had significantly smaller fruits (16.83 g) when compared to the rest of the accessions. IC 136685-1 also had relatively smaller fruits (20.47g). IC 342319-3 had largest fruits (51.03 g) followed by IC342302-2 (50.91 g). The average fruit weight was 34.39g, while CV was 30.1 percent. Colour of the fruits was mostly red and purple. Like fruit weight, wide variation was noted in the rind weight also. Rind

Table 5: Flower characters of different accessions of *Garcinia indica*

Sl. No.	Accessions	Flower length (mm)	Flower diameter (mm)	Weight of flower (gm)	Pedicel length (cm)	Time of flowering
1	IC 136687-1	6.20	5.01	0.50	0.81	October
2	IC 136687-2	7.06	5.12	0.55	0.91	October
3	IC 136687-3	7.96	5.92	0.63	0.86	October
4	IC 136685-1	7.05	6.02	0.59	0.59	October
5	IC 136682-2	7.12	5.98	0.61	0.52	October
6	IC 552528-1	5.36	4.09	0.52	0.61	November
7	IC 552528-3	6.01	5.61	0.57	0.62	November
8	IC 552526-2	5.31	4.68	0.51	0.58	October
9	IC 550572-1	6.04	5.69	0.53	0.74	November
10	IC 342322-1	7.13	6.09	0.59	1.36	December
11	IC 342297-1	8.44	5.96	0.61	1.42	November
12	IC 342303-2	6.99	6.67	0.59	0.96	January
13	IC 342302-2	7.18	5.74	0.60	0.72	January
14	IC 342329-3	7.64	5.15	0.52	2.24	December
15	IC 342319-3	5.68	5.59	0.49	0.65	December
16	IC 342327-1	5.62	4.83	0.48	0.94	November

Table 6: Fruit characters of different accessions of *Garcinia indica*

Sl. No.	Accessions	Fruit weight (g)	Rind weight (g)	Number of fruits per tree	Fruit apex
1	IC 136687-1	16.83	6.33	220	Slightly projected
2	IC 136687-2	31.91	13.61	510	Sunken
3	IC 136687-3	35.05	14.9	750	Flat
4	IC 136685-1	20.47	11.4	870	Flat
5	IC 136682-2	29.52	12.73	715	Flat
6	IC 552528-1	40.67	15.40	545	Flat
7	IC 552528-3	39.97	18.06	530	Flat
8	IC 552526-2	43.81	17.95	670	Sunken
9	IC 550572-1	18.42	9.01	1050	Flat
10	IC 342322-1	30.75	13.40	610	Slightly sunken
11	IC 342297-1	42.41	15.60	540	Sunken
12	IC342303-2	30.19	13.51	465	Sunken
13	IC342302-2	50.91	19.22	650	Sunken
14	IC342329-3	31.08	14.12	310	Sunken
15	IC342319-3	51.03	18.85	450	Sunken
16	IC342327-1	37.33	15.18	850	Slightly sunken

Plate 6. Variability in fruits of different accessions of *Garcinia indica*

Plat 7. Kokum rind

Projected

Flat

Sunken

Plate 8. Variability in fruit apex of *Garcinia indica*

weight was minimum for IC 136687-1 (6.33 g) due to the small size of fruits while it was maximum for IC 342302-2 (19.22g). Average rind weight was 14.32g, while CV was 24.2 percent. Yield in terms of number of fruits ranged from 220 (IC 136687-1) to 1050 (IC 550572-1). Average fruit yield was found to be 608, while CV was 34.7 percent. Wide variations were observed in the fruit apex. Six trees had fruits with flat apex, six had sunken, two slightly sunken and one slightly projected

4.6. HCA

Information regarding the HCA concentration of the selected accessions is given in Table 7.

It was observed that HCA was present in large quantity in fruit rind, it ranged from 24.08 g/100g to 40.89 g/100g. The value was highest for IC 136687-2 followed by IC342302-2 and lowest for IC 136687-1. Average HCA content was found to be 36.49 g/100 g, while CV for it was 12.5 percent.

4.7. Primary metabolites

Information regarding the primary metabolites of the selected accessions is given in Table 8.

The high CV value (22.3%) indicated wide variations in carbohydrate content in kokum fruit. The minimum carbohydrate content was observed in IC 342322-1 (2.5g/100g) followed by IC342303-2 (4.0 g/100g) and IC342327-1 (4.8g/100g). The highest carbohydrate content was observed in IC 136687-2 (7.8 g/100g) followed by IC 136687-3 (7 g/100g) and IC342302-2 (6.6 g/100g). The average carbohydrate content was found to be 5.55 g/100 g.

Protein content was observed to be lowest in IC 552528-3 (4 g/100g) and the content was highest in IC 136687-2 (6.6 g/100g). The mean protein content was estimated to be 5.45 g/100g and the CV was 15.5 percent. The crude fat was observed to be in the range of 3.02 percent (IC 550572-1) to 5.35 percent (IC342302-2). The mean crude fat was 4.33 %, while the CV was 17 percent.

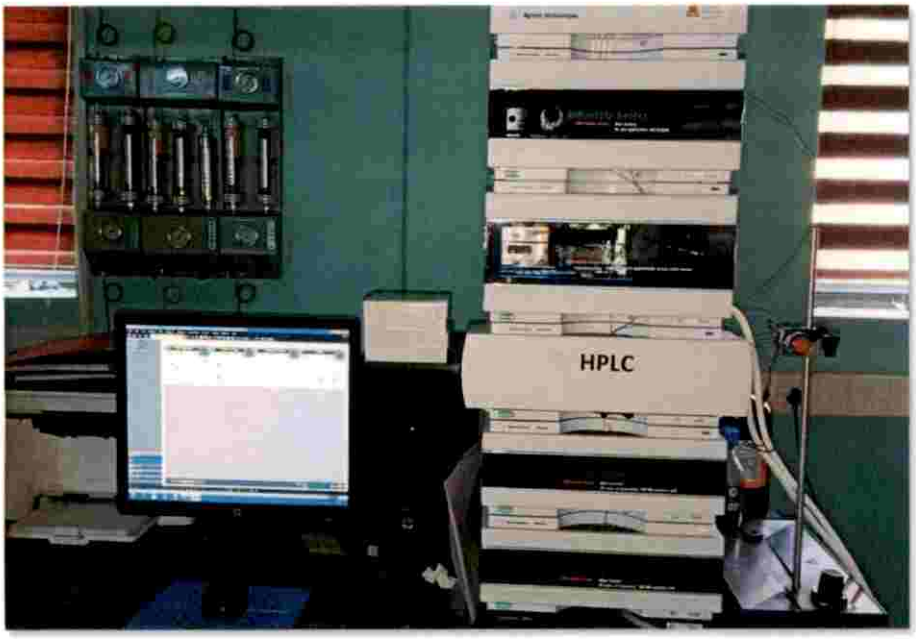

Plate 9. HPLC system

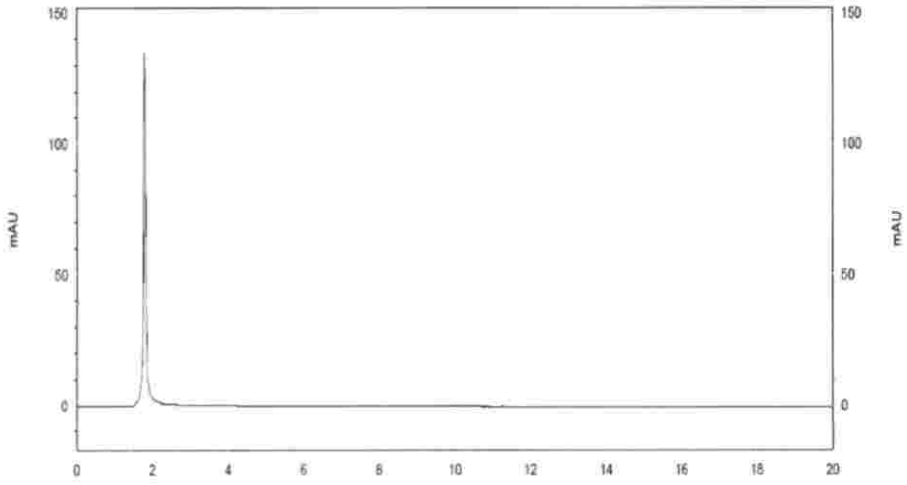

Fig. 1. HCA standard graph

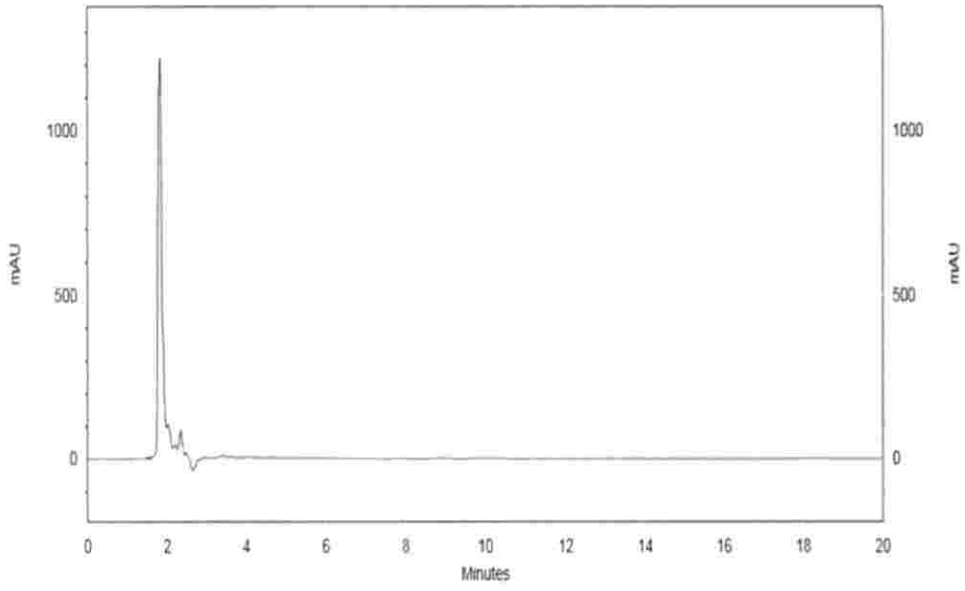

Fig.2. Sample chromatograph showing peak at 1.84 minutes

Table. 7: HCA content in different accessions of *Garcinia indica*

Sl. No.	Accessions	HCA (g/100g)
1	IC 136687-1	24.86
2	IC 136687-2	40.89
3	IC 136687-3	28.37
4	IC 136685-1	35.06
5	IC 136682-2	38.75
6	IC 552528-1	36.09
7	IC 552528-3	39.17
8	IC 552526-2	36.66
9	IC 550572-1	31.36
10	IC 342322-1	38.04
11	IC 342297-1	37.84
12	IC 342303-2	38.08
13	IC 342302-2	40.70
14	IC 342329-3	38.04
15	IC 342319-3	40.40
16	IC 342327-1	39.56

Table 8: Primary metabolites in different accessions of *Garcinia indica*

Sl. No.	Accessions	Carbohydrates (g/100g)	Proteins (g/100g)	Crude fat (%)	Reducing sugar (%)	Total soluble solids (%)
1	IC 136687-1	5.6	5.1	3.98	4.76	9.9
2	IC 136687-2	7.8	6.6	4.04	5.58	10.6
3	IC 136687-3	7.0	6.0	3.46	5.71	12.5
4	IC 136685-1	5.8	6.3	4.09	5.65	10.1
5	IC 136682-2	6.1	5.1	4.96	5.69	9.2
6	IC 552528-1	4.9	6.2	5.14	5.02	10.2
7	IC 552528-3	5.2	4.0	5.06	5.87	12.3
8	IC 552526-2	5.4	5.9	3.91	6.26	12.1
9	IC 550572-1	6.5	6.4	3.02	4.30	9.9
10	IC 342322-1	2.5	4.3	4.67	5.98	10.5
11	IC 342297-1	5.2	4.0	4.49	5.74	11.1
12	IC 342303-2	4.0	5.9	3.08	5.72	10.6
13	IC 342302-2	6.6	5.8	5.35	6.48	10.9
14	IC 342329-3	6.2	5.3	4.27	5.69	10.1
15	IC 342319-3	5.2	5.7	5.32	6.14	11.8
16	IC 342327-1	4.8	4.7	4.56	5.87	10.3

The reducing sugar was found maximum in IC342302-2 (6.48 %) and minimum in IC 550572-1 (4.3 %) and its average content in all trees was 5.65 %. Whereas, the total solube solids ranged from 9.2 percent (IC 136682-2) to 12.5 percent (IC 136687-3) with the CV of 8.9 percent and the average being 10.75 percent.

4.8. Secondary metabolites

Information regarding the secondary metabolites of the accessions is given in Table 9.

Variation in phenolic content was more than that of flavanoid content. The average phenolic content was found to be 796.87 mg/100 g. The maximum was observed in IC 550572-1 (1140 mg/100g) followed by IC 552528-1 (1120 mg/100 g), IC 342297-1 (1080 mg/100 g) and IC 552528-3 (1060 mg/100 g). The

minimum phenolic content was observed in IC 136687-1 (460 mg/100g). The CV value was estimated to be 29.1 percent.

The flavanoids content was maximum in IC 136687-2 (470 mg/100g), followed by IC 136682-2 (411 mg/100g) while the lowest was in IC342329-3 (238 mg/100g). The average flavanoids content was found to be 327.75 mg/100 g, while the CV was 17.9 percent.

4.9. Vitamin and minerals composition in fruit rind

Information regarding the Vitamin C and minerals composition of the selected accessions is given in Table 10 and 11 respectively.

Vitamin C content was found to be maximum in IC 136687-3 (58.17 mg/100g) followed by IC 342322-1 (50.90 mg/100g) and minimum in IC 342329-3 (21.81 mg/100g).The average content was 37.17 mg/100g, while the CV was 27.6%. IC 136687-1, IC 136685-1 and IC 552528-1 had near average value i.e, 36.36, 39.99 and 38.54, respectively.

Ca content varied from 4.56 mg/100g to 7.45 mg/100g. (IC 342322-1) had the least value (4.56 mg/100g), followed by IC342302-2 (5.91 mg/100g) and the maximum value was shown by IC 136687-2 (7.45 mg/100 g). Average content of Ca was estimated to be 6.39 mg/100g and the CV being 10.1 percent. The Na content showed a wide variation (CV being 49%). It ranged from 0.23 mg/100g (IC 342297-1) to 1.92 mg/100g (IC342327-1). The average Na content was 1.10 mg/100g. IC 136687-3, IC 136685-1 and IC 342322-1 had values almost similar to the average i.e, 1.25 mg/100g, 1.06 mg/100g and 1.25 mg/100g, respectively. The minimum content of K was observed in IC 342297-1 (24.65 mg/100g) followed by IC 550572-1 (25.16 mg/100g) while maximum was in IC 552526-2 (33.05 mg/100g) followed by IC 552528-3 (32.83 mg/100g). The variation in P content ranged from 2.09 mg/kg (IC 136687-1) to 4.85 mg/kg(IC 136682-2). Average content of P was 3.48 mg/kg and the CV was 24.4 percent. The concentration of Magnesium ranged from 27.98 mg/100 g (IC 136687-2) to

17.20 mg/100g (IC 136682-2). The average content of Mg was 22.74 mg/100 g, while the CV was found to be 19.3 percent.

4.10. Physiochemical properties of butter

Information regarding the Physiochemical properties of butter of the selected accessions is given in Table 12.

Maximum percentage of butter was obtained from IC342319-3 (35.69 %) followed by IC342302-2 (35.13%) and IC 136687-2 (33.81%). Minimum quantity of butter was obtained from IC 550572-1 (23.17%) followed by IC342327-1 (25.65%). And the average percentage of butter obtained was 30.60 percent. For nine accessions the percentage of butter obtained was more than the average value. The saponification value showed less variation. The saponification value was maximum for IC 552526-2 (194.94 mg of KOH/g) followed by IC342329-3 (193.54 mg of KOH/g). The value was minimum for IC 136687-2 (171.10 mg of KOH/g). The average saponification value was 183.98 mg of KOH/g, while the CV for it was just 4 percent. The Acid value ranged from 2.6 mg of KOH/g (IC342303-2, IC342327-1) to 3.4 mg of KOH/g (IC 136687-3). Three accessions showed the same acid value (2.8 mg of KOH/g) i.e, IC 552526-2, IC 342322-1 and IC 342297-1. The average acid value was 2.95 mg of KOH/g, while the CV was 8.4 percent. The Iodine value did not show much variation, it ranged from 36.41 (IC342329-3) to 40.56 (IC 552528-1). The average iodine value was 38.63 and the CV was 3.5 percent.

Table 9: Secondary metabolites in different accessions of *Garcinia indica*

Sl. No.	Accessions	Phenols (mg/100g)	Flavanoids (mg/100g)
1	IC 136687-1	460	288
2	IC 136687-2	990	470
3	IC 136687-3	840	320
4	IC 136685-1	980	342
5	IC 136682-2	640	411
6	IC 552528-1	1120	310
7	IC 552528-3	1060	321
8	IC 552526-2	680	298
9	IC 550572-1	1140	303
10	IC 342322-1	560	314
11	IC 342297-1	1080	299
12	IC 342303-2	660	260
13	IC 342302-2	540	350
14	IC 342329-3	720	238
15	IC 342319-3	740	410
16	IC 342327-1	540	310

Table 10: Vitamin C in fruits of different accessions of *Garcinia indica*

Sl. No.	Accessions	Vitamin C (mg/100g)
1	IC 136687-1	36.36
2	IC 136687-2	43.63
3	IC 136687-3	58.17
4	IC 136685-1	39.99
5	IC 136682-2	29.08
6	IC 552528-1	38.54
7	IC 552528-3	32.72
8	IC 552526-2	35.63
9	IC 550572-1	47.26
10	IC 342322-1	50.90
11	IC 342297-1	27.63
12	IC342303-2	44.35
13	IC342302-2	39.99
14	IC342329-3	21.81
15	IC342319-3	25.45
16	IC342327-1	23.27

Table 11: Minerals content of fruits in different accessions of *Garcinia indica*

Sl. No.	Accessions	Ca (mg/100g)	Na (mg/100g)	K (mg/100g)	P (mg/kg)	Mg (mg/100g)
1	IC 136687-1	6.69	0.62	28.94	2.09	21.12
2	IC 136687-2	7.45	0.66	30.16	3.39	27.98
3	IC 136687-3	6.68	1.25	31.62	4.19	25.94
4	IC 136685-1	7.11	1.06	30.25	4.78	27.19
5	IC 136682-2	6.46	0.91	29.16	4.85	17.20
6	IC 552528-1	5.92	0.75	30.56	3.10	21.56
7	IC 552528-3	6.31	1.68	32.83	4.17	22.51
8	IC 552526-2	6.55	1.91	33.05	3.16	25.61
9	IC 550572-1	6.41	1.56	25.16	3.42	26.32
10	IC 342322-1	4.56	1.25	25.23	3.41	11.62
11	IC 342297-1	6.35	0.23	24.65	4.61	22.02
12	IC 342303-2	6.22	1.36	31.54	2.63	27.26
13	IC 342302-2	5.91	0.38	28.49	3.14	20.65
14	IC 342329-3	6.01	0.58	30.52	2.10	18.05
15	IC 342319-3	6.70	1.62	25.68	3.22	23.64
16	IC 342327-1	7.01	1.92	31.62	3.49	25.21

Table 12: Physio- chemical properties of butter from different accessions of *G. indica*

Sl. No.	Accessions	Butter %	Melting range (°C)	Pour point (°C)	Colour (510nm)	Saponification value (mg KOH/g)	Acid value (mgKOH/g)	Iodine value
1	IC 136687-1	27.18	38-42	36	0.39	176.71	3.3	40.04
2	IC 136687-2	33.81	39-42	36	0.21	171.10	3.0	37.06
3	IC 136687-3	32.21	39-43	37	0.42	190.74	3.4	40.43
4	IC 136685-1	32.94	36-40	36	0.39	180.92	3.1	39.65
5	IC 136682-2	25.90	36-40	37	0.22	178.11	2.9	37.58
6	IC 552528-1	31.21	35-40	36	0.31	189.33	2.7	40.56
7	IC 552528-3	30.12	35-39	36	0.59	175.31	3.1	38.10
8	IC 552526-2	34.23	36-40	36	0.41	194.94	2.8	40.17
9	IC 550572-1	23.17	35-41	36	0.21	190.74	3.2	36.93
10	IC 342322-1	31.80	40-44	37	0.47	179.52	2.8	39.00
11	IC 342297-1	30.45	36-40	35	0.27	176.71	2.8	39.26
12	IC 342303-2	31.48	38-41	36	0.41	180.92	2.6	37.19
13	IC 342302-2	35.13	36-41	37	0.54	185.13	3.2	38.10
14	IC 342329-3	27.75	35-40	36	0.22	193.54	3.0	36.41
15	IC 342319-3	35.69	36-40	35	0.21	190.74	2.7	38.23
16	IC342327-1	25.65	36-40	36	0.41	189.33	2.6	39.39

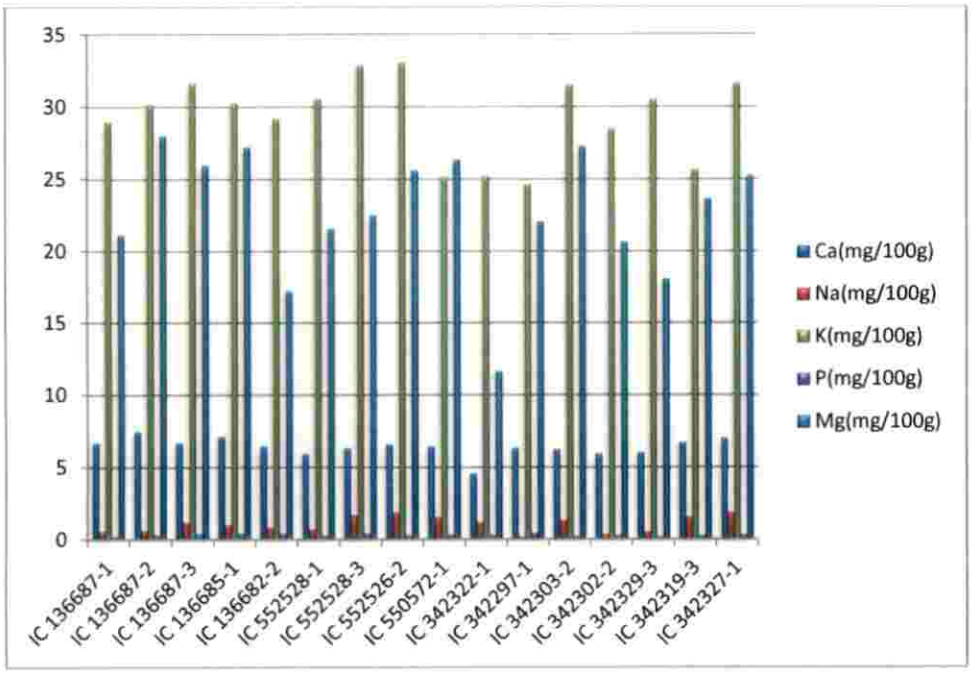

Fig. 3. Graph showing variability in minerals content of fruits in different accessions of *Garcinia indica*

Plate 10. Kokum butter

Table 13: Correlation between morphological characters of *Garcinia indica*

Traits	GBH	Tree ht.	Branch ht.	Leaf area	Leaf length	Leaf width	Leaf/width ratio	Petiole length	Flower length	Flower diameter	Flower Weight	Pedicel length	Fruit weight	Rind weight	No. of fruits
GBH	1														
Tree ht.	.630**	1													
Branch ht.	-.001	.355	1												
Leaf area	.254	.002	-.315	1											
Leaf length	-.073	-.352	-.131	.562*	1										
Leaf width	-.036	-0.28	-.259	.561	.055	1									
Leaf/width ratio	-.014	-.208	.089	-0.37	0.630**	-.734**	1								
Petiole length	.404	.542*	-0.47	.449	.132	.138	-.011	1							
Flower length	.037	.420	.295	-.420	-.280	-.265	-.045	.027	1						

Traits	GBH	Tree ht.	Branch ht.	Leaf area	Leaf length	Leaf width	Leaf/width ratio	Petiole length	Flower length	Flower diameter	Flower weight	Pedicel length	Fruit weight	Rind weight	No. of fruits
Flower diameter	.279	.597*	.574*	.034	-.060	-.019	-.066	.219	.612*	1					
Flower weight	.078	.228	.231	.001	-.174	.125	-.291	.031	.762**	-.721**	1				
Pedicel length	-.304	.042	.342	-.779**	-.201	-.557*	.284	-.326	.537*	.072	-.002	1			
Fruit weight	-.487	-.624**	-.098	.110	.588*	-.196	.541*	-.334	-.116	-.173	-.022	-.078	1		
Rind weight	-.395	-.646**	-.095	.131	.634**	-.203	.570*	-.342	-.093	-.082	.064	-.067	.953**	1	
Number of fruits	.379	.102	.035	.571*	.172	.077	.054	.342	-.095	.160	.198	-.390	-.137	-.035	1

Table 14:. Correlation between biochemical characters of *Garcinia indica*

Traits	Carbohy-drate	Protein	Crude fat	Reducin-g sugar	TSS	HCA	Vit C	Phenol	Flavan-oids	Ca	Na	K	P	Mg.
Carbohydrate	1													
Protein	.527**	1												
Crude fat	-.163	-.397	1											
Reducing sugar	-.168	-.256	.471	1										
TSS	.029	-.108	.048	.494	1									
HCA	-.154	-.163	.539*	.647**	.055	1								
Vit. C	.050	.405	-.499*	-.213	.171	-.426	1							
Phenol	.280	.206	-.116	-.396	.44	.011	.136	1						
Flavanoids	.418	.264	.358	.190	.000	-.367	.031	.112	1					
Ca	.655**	.401	-.244	-.134	.058	-.108	-.199	.209	.405	1				
Na	-.300	.003	-.193	.109	.369	.046	.038	--.066	-.063	.109	1			
K	.173	.174	-.126	.207	.261	.004	-.002	-.090	-.148	.343	.303	1		
P	.114	-.186	.164	.167	.120	.155	.073	.418	.404	.198	.053	-.090	1	
Mg	.464	.579*	-.514*	-.157	.275	-.122	.145	.381	.130	.796**	.298	.371	.080	1

4.11. 1. Correlation study on morphological characters

Correlation analysis of morphological characters is given in Table 13. It showed that there was a significant and positive relation between leaf area and leaf length, fruit weight and rind weight, flower weight and flower length, flower diameter and flower length, flower diameter and branch height and height and diameter. Significant negative correlation was observed between flower weight and flower diameter, fruit weight and tree height, pedicel length and leaf area and rind weight and tree height.

4.11.2. Correlation study on biochemical characters

Correlation matrix between biochemical characters is given in Table.14. It was observed that there was a positive and significant relation between carbohydrate and protein content, carbohydrate and Ca content, Protein and Mg content, crude fat and HCA, reducing sugar and HCA and CA and Mg while a significant and negative correlation was observed between crude fat and vitamin C and Mg and crude fat.

4.12. Cluster analysis

A hierarchical cluster analysis was carried out for the 16 accessions based on the Euclidian squared distance. The 16 accessions were grouped into six clusters. Character viz. GBH, height of the tree, height of first branch from the base, leaf length , leaf breadth, length to breadth ratio, leaf area, petiole length, flower weigh, fruit weight, fruits yield, flower length, flower width, pedicel length, rind weight, HCA, carbohydrate, reducing sugar, protein, crude fat, total soluble solids, vitamin C, phosphorus, potassium, sodium, calcium, magnesium, total phenols, total flavanoids, percentage of butter, pour point, melting point, saponification value, acid value and iodine value were considered and dendrogram was formulated.

Details of the six clusters are given in the Table 15. The accessions come in a cluster have similar morphological and biochemical characters where as it

differs between two clusters. From the table, the cluster III possess maximum number of accessions whereas the least number observed for the cluster V and VI respectively. So the accessions IC 550572-1 and IC 136687-1 possess difference in their morphological and biochemical characters with others.

Table 15:. Clusters of morphological and biochemical characters

Clusters					
I	**II**	**III**	**IV**	**V**	**VI**
IC 136687-2	IC 136687-3	IC 136682-2	IC 342329-3	IC 550572-1	IC 136687-1
IC 552528-1	IC 136685-1	IC 552526-2	IC 342319-3		
IC 552528-3		IC 342322-1			
IC 342297-1		IC 342303-2			
		IC 342302-2			
		IC 342327-1			

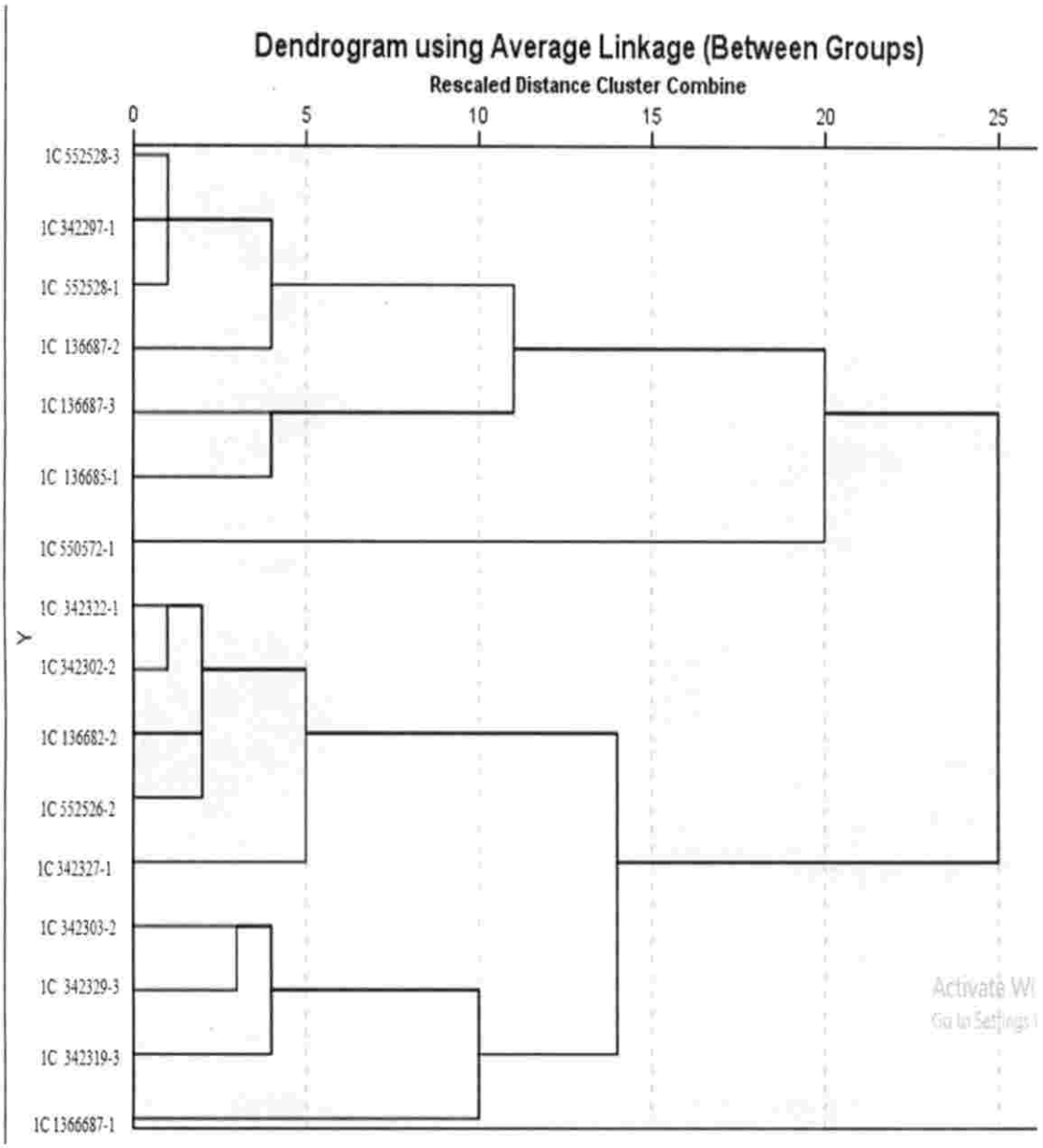

Fig. 4. Dendrogram on morphological and biochemical attributes *in G. indica*

Table 16: Table of inter and intra cluster distances

		Matrix showing inter and intra cluster distances				
	Cluster1	**Cluster2**	**Cluster3**	**Cluster4**	**Cluster5**	**Cluster6**
Cluster 1	**19862.72**					
Cluster 2	120229	**36100.48**				
Cluster 3	255009	146267.1	**46210.63**			
Cluster 4	154736.6	240867.7	126577.6	**50380.55**		
Cluster 5	286132.5	121185.5	460584.3	631422.5	**0**	
Cluster 6	472416.1	562616.1	235551.6	113783.3	1152011	**0**

The above table gives the inter and intra cluster distances. Intra cluster distances gives the average distance between the elements within a cluster whereas the distance between two clusters gives the inter cluster distances. The diagonal elements shows the intra cluster distances and the off diagonal elements shows the inter cluster distances. It is observed from the table that highest intra cluster distance shown by the IV the cluster and the highest inter cluster distance shown by clusters V and VI followed by clusters V and IV.

4.13. Path analysis for rind yield

In path analysis, the total correlation between an effect (Here rind yield) and each one of its causes (in this case the six quantitative traits such as flower length, flower diameter, flower weight, fruit weight, rind weight and number of fruits) is partitioned into a direct effects and a series of indirect effects. First a correlation study is carried out with rind yield and remaining six traits. The correlation coefficients of these six traits with the rind yield and the direct effects are compared. Based on these comparisons, it is possible to make further selections.

Table 17: Correlation analysis of rind yield with other traits.

	Flower length	Flower diameter	Flower weight	Fruit weight	Rind weight	Number of fruits	Rind yield
Flower length	1						
Flower diameter	0.61	1					
Flower weight	0.76	0.72	1				
Fruit weight	-0.12	-0.17	-0.02	1			
Rind weight	-0.09	-0.08	0.06	0.95	1		
Number of fruits	-0.09	0.16	0.20	-0.14	-0.03	1	
Rind yield	**-0.15**	**0.01**	**0.19**	**0.50**	**0.59**	**0.75**	**1**

The Table 17 shows the correlation analysis of rind yield with the remaining six traits. The number of fruit possess highest positive correlation with rind yield (0.75) followed by rind weight (0.59). The trait flower length showed a negative correlation with rind yield (-0.15).

Table 18: Path analysis with direct and indirect effects on rind yield of *G. indica*

	Flower length	Flower diameter	Flower weight	Fruit weight	Rind weight	Number of fruits
Flower length	**-0.05**	-0.07	0.11	-0.03	-0.03	-0.07
Flower diameter	-0.03	**-0.11**	0.10	-0.04	-0.03	0.12
Flower weight	-0.04	-0.08	**0.14**	-0.01	0.02	0.15
Fruit weight	0.01	0.02	0.00	**0.24**	0.34	-0.11
Rind weight	0.00	0.01	0.01	0.23	**0.36**	-0.02
Number of fruits	0.00	-0.02	0.03	-0.03	-0.01	**0.78**

The Table 18 shows the direct and indirect effects of the six quantitative traits with the rind yield. The diagonal elements show the direct effects. These values are used for the comparison studies. If the correlation coefficient between a trait and the effect is almost equal to its direct effect, then that means correlation explains the true relationship and a direct selection through this trait will be effective. If the correlation coefficient is positive but direct effect is negative or negligible, indirect effects seems to be the reason behind correlation. If both the correlation coefficient and direct effect is negative or negligible, that particular trait is discarded.

4.13.1. Direct effect on rind yield

A positive direct effect was exerted by the trait number of fruits (0.78) followed by rind weight (0.36), fruit weight (0.24) and flower weight (0.14). Since, the correlation coefficient of these four traits were positive and almost equal to its direct effects, it is advisable to go for direct selection. Whereas a low and negative direct effect was exerted by flower length (-0.05) followed by flower diameter (-0.11). Since, the correlation coefficient and direct effects of these two were negligible or negative, discard these traits.

4.13.2. Indirect effects on rind yield

4.13.2.1. Flower length

Negligible , positive and indirect effect was exerted by fruit weight (0.01) through flower length. While negligible and negative indirect effect was exerted by flower diameter (-0.04) and flower weight (-0.04) through flower length towards rind yield .

4.13.2.2. Flower diameter

It was estimated that the fruit weight (0.02) and rind weight (0.01) had negligible, positive and indirect effect through flower diameter towards rind yield while the flower weight had least indirect effect on rind yield (-0.08) through flower diameter.

4.13.2.3. Flower weight

Flower weight had negligible, positive and indirect effect was exerted by flower length (0.11) and flower diameter (0.10) through flower weight towards rind yield. While fruit weight (0.00) had least indirect effect on rind yield through flower weight.

4.13.2.4. Fruit weight

Rind weight (0.23) had low, positive and indirect effect through fruit weight on rind yield. While negligible, negative and indirect effect was exerted by flower length (-0.03), flower diameter (-0.04), flower weight (-0.01) and number of fruits (-0.03) on rind yield through fruit weight.

4.13.2.5. Rind weight

Fruit weight (0.34) and flower weight (0.36) had low, positive and indirect effect on rind yield through rind weight. While negligible, negative and indirect effect was exerted by flower length (-0.03), flower diameter (-0.03) and number of fruits (-0.01) on rind yield through rind weight.

4.13.2.6. Number of fruits

Low, positive and indirect effect was exerted by flower weight (0.15) and flower diameter (0.12) on rind yield through rind weight. While least indirect impact was exerted by flower length (-0.07).

Fig.5 Shows the path diagram. A path diagram is the diagrammatic representation of the path analysis. From the path diagram, the single headed arrow indicate the direct effects whereas the double-headed arrow shows the indirect effects. It will also shows the residual effects (in this case 0.05). If the residual effect is very close to zero, it is an indication that the causal variable (here traits) fully explains the system.

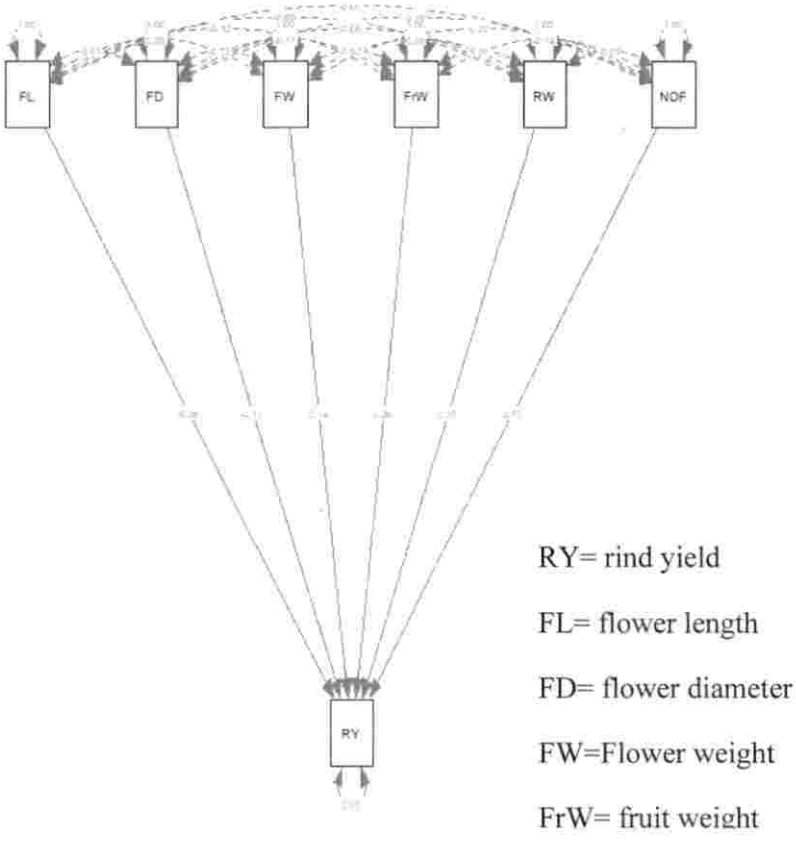

Fig.5: Path diagram showing direct and indirect effect on rind yield of *G. indica*

4.14. Principal component analysis (PCA)

4.14.1. Morphological features

PCA was conducted to emphasize the variations and bring out strong patterns in the data set of independent variables. Scree plot showed that four components had eigen value greater than one. These four components together accounted for 84.8% of the total variability. PC1 accounted for 27.8% of the total variability, which was mainly contributed positively by tree height, flower diameter, flower length and flower weight with a loading of 0.420, 0.291, 0.248 and 0.219 respectively. PC2 accounted for 23.0% of the total variability, which

was mainly contributed positively by pedicel length, flower length and branch height with a loading of 0.469, 0.314 and 0.239 respectively. PC3 accounted for 16.4% of the total variability, which was mainly contributed positively by leaf width and pedicel length with a loading of 0.164 and 0.047 respectively. PC4 accounted for 12.0% of the total variability, mainly contributed by leaf length to width ratio (0.432), GBH (0.325), tree height (0.209) and petiole length (0.247). A PCA biplot shows both PC scores of samples and loadings of variables. The further away these vectors are from a PC origin, the more influence they have on that PC. Loading plots also hint at how variables correlate with one another: a small angle implies positive correlation, a large one suggests negative correlation, and 90°angle indicates no correlation between two characteristics. The component loading biplot given in Fig.7 shows fruit weight and rind weight were closely related. Flower length and flower diameter also showed a close relation. On the other hand characters like pedicel length and leaf width, GBH and leaf length to width ratio showed negative association. On the basis of first two components of PC analysis grouping pattern was prepared. Sixteen accessions were classified into four groups. This was furnished in Fig. 6, which graphically represented relative positions of various accessions. It was observed that group IV had maximum and group III had minimum number of accessions under it.

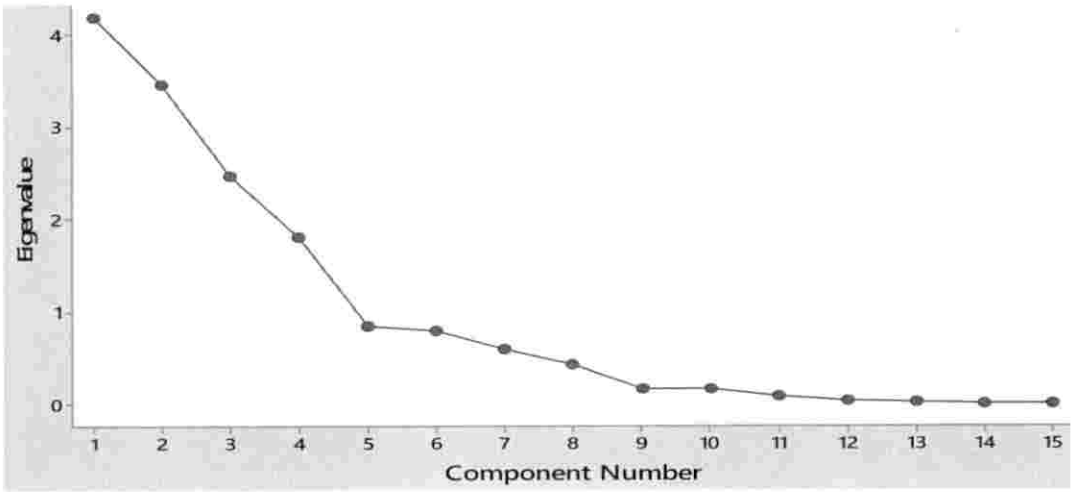

Fig. 6 Scree plot of morphological characters of *G. indica*

Table 19: Principal component analysis of morphological characters of *G. indica*

Variable	PC1	PC2	PC3	PC4
GBH	0.261	-0.202	-0.149	0.325
Tree height	0.420	-0.022	-0.127	0.209
Height of first branch	0.170	0.239	-0.218	0.035
Leaf area	-0.062	-0.481	-0.220	-0.067
Leaf length	-0.304	-0.162	-0.350	.086
Leaf width	0.065	-0.354	0.164	-0.436
Length/width ratio	-0.263	0.149	-0.339	0.432
Petiole length	0.198	-0.270	-0.186	0.247
Flower length	0.248	0.314	-0.243	-0.206
Flower diameter	0.291	0.092	-0.414	-0.186
flower weight	0.219	0.089	-0.322	-0.487
Pedicel length	0.035	0.469	0.047	0.140
Fruit weight	-0.404	0.081	-0.244	-0.169
Rind weight	-0.392	0.073	-0.302	-0.176
No. of fruits	0.076	-0.277	-0.282	0.081
Eigen value	4.175	3.447	2.463	1.798
Proportion	0.278	0.230	0.164	0.120
Cumulative	0.278	0.508	0.672	0.848

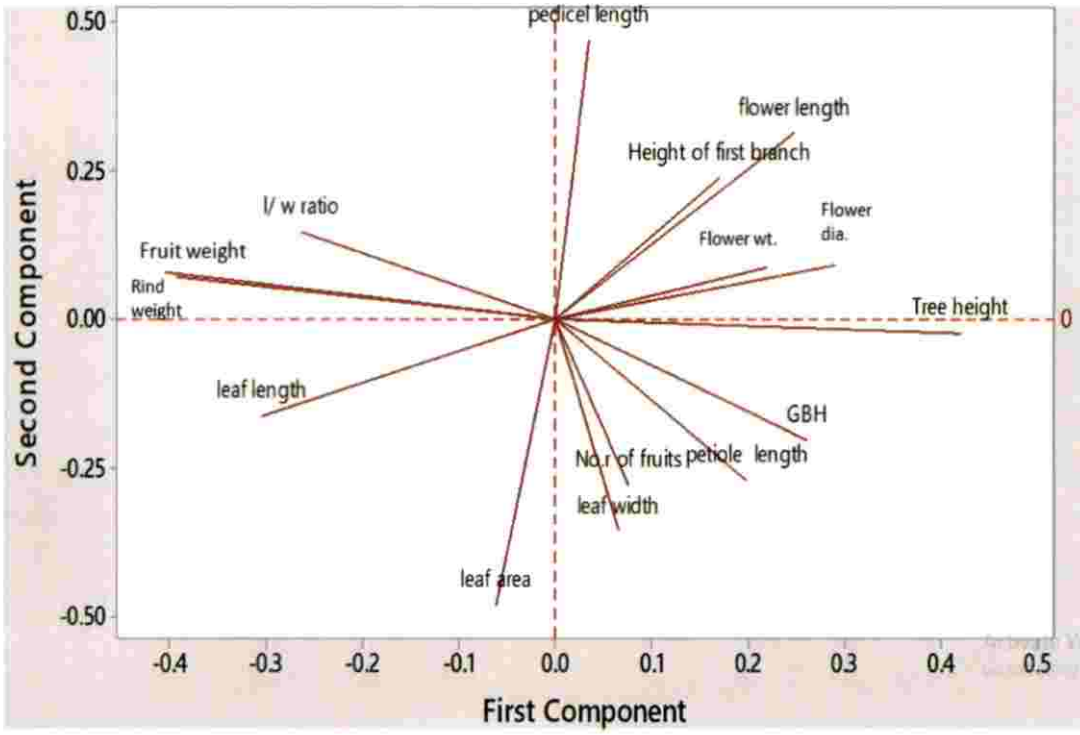

Fig.7 Loading plot of morphological characters of *G. indica.*

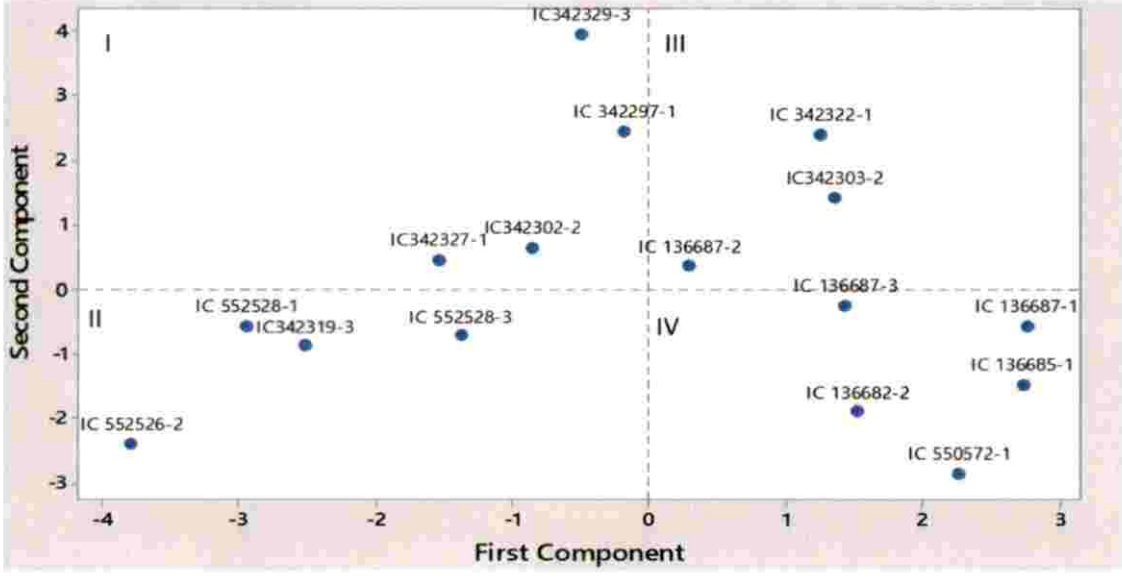

Fig. 8 Grouping of 16 accessions of *G. indica* based on morphological characters from first two components.

4.14.2. Biochemical features

Scree plot (Fig. 9) showed that four components had eigen value greater than one. PC1 accounted for 25.7% of the total variability, which was mainly contributed positively by Mg(0.447), proteins(0.390), Ca(0.385) and carbohydrates (0.357). PC2 contributed for 18.5% of the total variability which is contributed positively by Vitamin C and proteins with a loading of 0.226 and 0.003 respectively, while rest of the factors contribute negatively to it. PC3 accounted for 14.3% of the total variability which is mainly contributed by Na, K, TSS and reducing sugars with a loading of 0.504, 0.425, 0.428 and 0.267, respectively. PC4 accounted for 10.5% of the total variability, which was mainly contributed by P(0.514), phenols(0.449), Vitamin C(0.440) and TSS (0.324). Component loading diagram (Fig. 10) showed that Mg, carbohydrates and Ca were positively related to each other, while factors like Vitamin C and crude fat were negatively related as they show wide angle between them. On the basis of first two components of PC analysis grouping pattern was prepared. Sixteen accessions were classified into four groups. This was furnished in Fig. 11 , which graphically represented relative positions of various accessions. It was observed that group II had maximum and group I had minimum number of accessions under it.

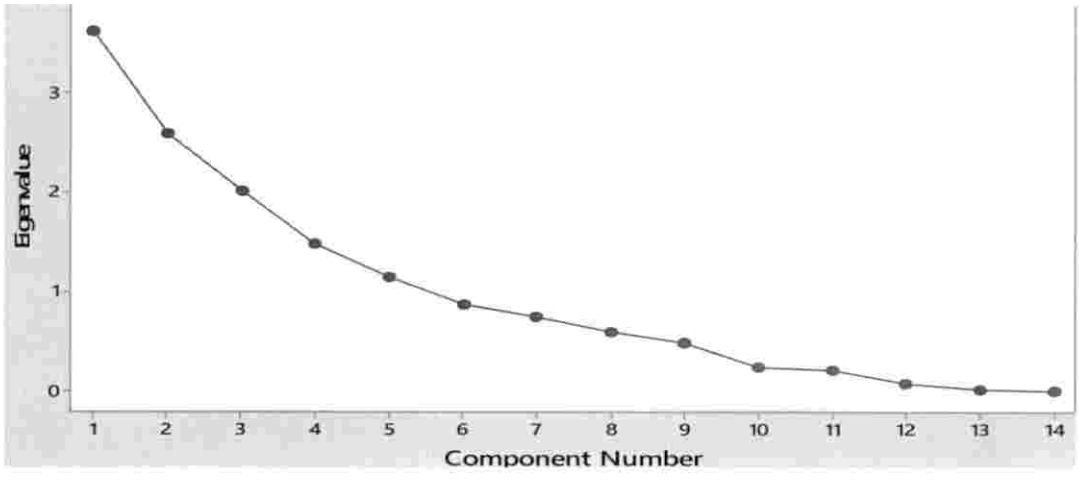

Fig. 9 Scree plot of biochemical characters of *G. indica*

Table 20: Principal component analysis of biochemical characters of *G. indica*

Variable	PC1	PC2	PC3	PC4
Carbohydrates	0.357	-0.195	-0.250	-0.194
Proteins	0.390	0.003	-0.067	-0.153
Crude fat	-0.347	-0.321	-0.186	-0.062
Reducing sugar	-0.252	-0.397	0.267	-0.034
TSS	0.036	-0.242	0.428	0.324
Vitamin C	0.205	0.226	0.070	0.440
Phenols	0.234	-0.093-	-0.209	0.449
Flavanoids	0.088	-0.419	-0.315	0.051
Ca	0.385	-0.283	-0.027	-0.249
Na	0.057	-0.083	0.504	0.156
K	0.155	-0.145	0.425	-0.275
P	0.033	-0.308	-0.165	0.514
Mg	0.447	-0.165	0.181	-0.033
HCA	-0.233	-0.421	-0.022	-0.063
Eigen value	3.598	2.584	2.001	1.469
Proportion	0.257	0.185	0.143	0.105
Cumulative	0.257	0.442	0.585	0.690

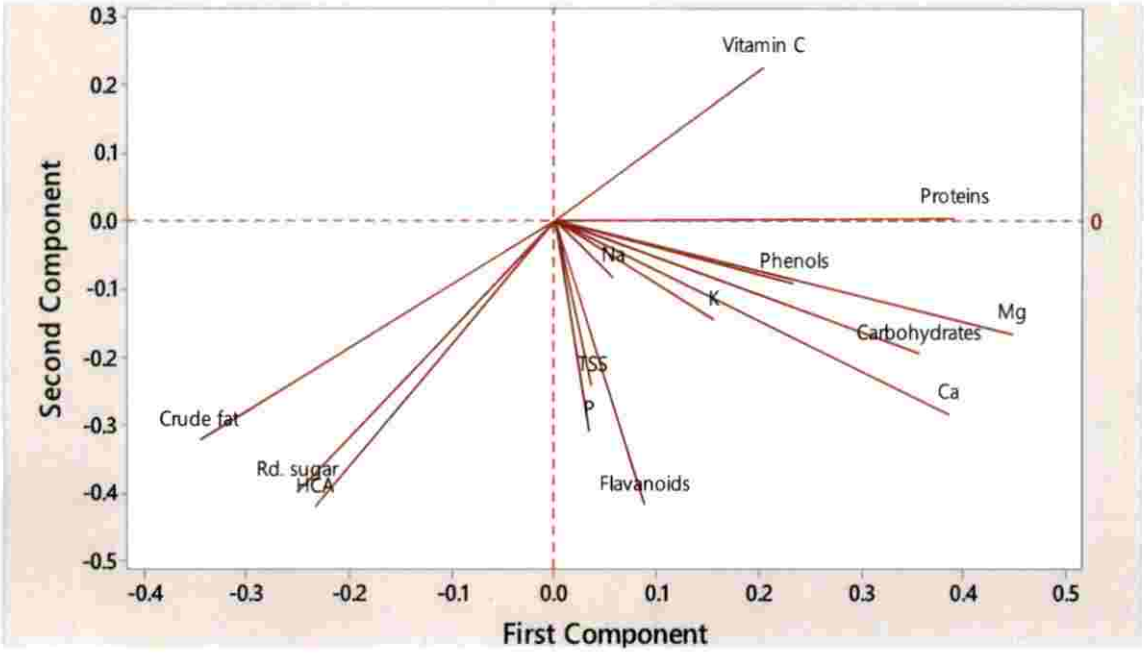

Fig. 10 Loading plot of biochemical characters of *G. indica.*

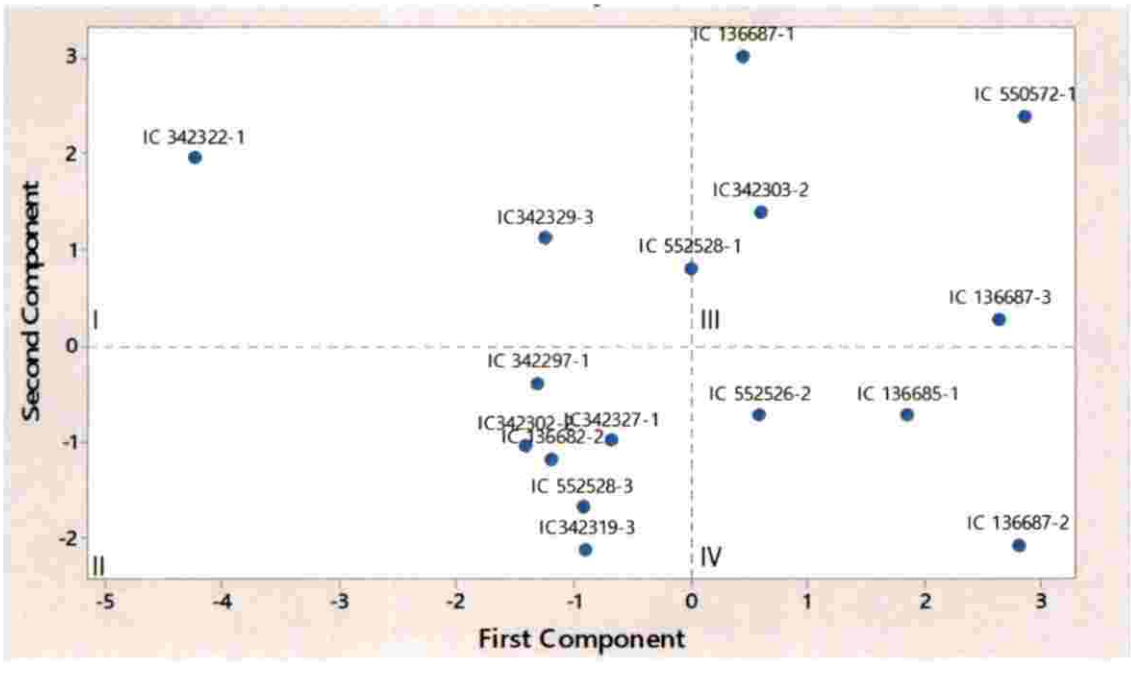

Fig. 11 Grouping of 16 accessions of *G. indica* based on biochemical characters from first two components.

4.15. Selection index

Selection index values were worked out for Rind yield (based on morphological characters) and HCA (based on biochemical characters) using principal component analysis. Principal component analysis was performed on all the morphological and biochemical analysis and the first principal component was taken as the index value for selection.

Table 21: Morphological characters for selection index

Variable	PC1	PC2
GBH	0.256	-0.349
Tree height	0.451	-0.178
Height of first branch	0.280	0.234
Leaf area	-0.079	-0.478
Length/width ratio	-0.144	0.165
Petiole length	0.199	-0.386
Flower length	0.394	0.295
Flower diameter	0.453	0.043
flower weight	0.355	0.101
Pedicel length	0.106	0.499
Fruit weight	-0.297	0.196

Rind Yield = 0.256 x GBH + 0.451 x Tree height + 0.28 x Height of first branch – 0.079 x leaf area -0.144 x length/width ratio + 0.199 x petiole length + 0.394 x flower length + 0.453 x flower diameter + 0.355 x flower weight + 0.106 x pedicel length – 0.297 x fruit weight.

Where Rind yield = rind weight x no. of fruits

Table 22: Biochemical characters for selection index

Variable	PC1	PC2
Carbohydrates	0.386	-0.113
Proteins	0.397	0.157
Crude fat	-0.294	-0.417
Reducing sugar	-0.182	-0.491
TSS	0.067	-0.338
Vitamin C	0.163	0.263
Phenols	0.254	-0.007
Flavanoids	0.160	-0.385
Ca	0.428	-0.221
Na	0.070	-0.118
K	0.178	-0.155
P	0.078	-0.353
Mg	0.477	-0.063

HCA = 0.386 x Carbohydrates + 0.397 x Proteins – 0.294 x Crude fat – 0.182 x Reducing sugars + 0.067 x TSS + 0.163 x Vitamin C + 0.254 x Phenols + 0.16 x Flavanoids + 0.428 x Ca + 0.07 x Na + 0.178 x K + 0.078 x P + 0.477 x Mg

DISCUSSION

5. Discussion

A study was conducted to find out variations in *G. indica* germplasm maintained at NBPGR Regional station, Thrissur, under the title 'Morphological and biochemical diversity assessment of *Garcinia indica* Choisy germplasm'. The characters taken for study included variability in general tree characters, flower, fruit, nutritional value and physio-chemical properties of butter. Results obtained under the present study have been discussed in this chapter.

5.1. Variability study

First important step for any tree improvement programme is to determine all the available variations within or between species. Evaluation and screening of different genotypes of tree species for morphological and biochemical characteristics is an important aspect of tree improvement programmes. The large variation for commercially important traits of fruits indicates that there are considerable opportunities for phenotypic selection to improve productivity. Before domestication of any plant genetic resource, it is important to determine the extent, cause and nature of variations present in natural population in order to use it efficiently in selection and breeding. This is because forest trees are generally genetically variable in order to survive, grow and reproduce under numerous environments (Antonovicks, 1971). Although *Garcinia indica* have a numerous use, there are scanty reports on exploration, identification and documentation of trees of intra-specific variation.

5.2. General tree characters

In nature there exists wide variations in general tree characters like height of tree, branch height, GBH, canopy shape etc. In case of timber species variations like large height and GBH are considered commercially important whereas, in case of fruit trees like kokum variations like large canopy and low branch height are considered more important.

Canopy is the outermost layer of the leaves formed by an individual tree or by the group of trees. In the present study, most of the trees which were studied had conical shapes. Younger trees like IC 552528-1 and IC 552528-3 had more irregular shape as crown was still not completely developed. Mainly two types of branching were observed i.e. drooping and horizontal. Only four trees had horizontal branching while rest had drooping branches. The difference in the Branching pattern might be due to their genetic makeup and response to soil and climatic conditions. The height variation was in the range of 4.2 m (IC 552528-1) to 15.5 m (IC 136687-1). Average height and CV being 11.106 m and 26.4%, respectively. Nine trees had height more than the average height. Difference in height is mainly due to the difference in their ages. IC 552528-1 (4.2 m), IC 552528-3 (7.9 m) and IC 552526-2 (6.5 m) had lesser height because they were planted in 2007 while the rest were planted in or before 2002. Moreover growth characters like height are more influenced by environment and hence, have low heritability. So selection based on height might not be the best choice.

Variation in the height of first branch from the base of the tree varied from 0.8 m (IC 552528-1) to 6.52 m (IC342322-1). In case of fruit trees like kokum, trees with lower height of branches allow easier harvesting of fruits, hence selection of such genotypes can prove to be beneficial.

Chandran (1996) has also reported different canopy shapes viz., drooping and pyramidal shape in kokum (*Garcinia indica*). Kokum has been described as a slender evergreen tree with drooping branches with a height of 10 to 20m and drooping branches by various other researchers too (Godbole and Das, 2000).

The GBH in the present study varied from 41cm (IC 342297-1) to 110 cm (IC 136685-1). IC 552528-1 (51.5 cm), IC 552528-3 (50.2 cm) and IC 552526-2 (53.6 cm) had significantly lesser GBH mainly due to their lesser age than the others.

Study similar to the present study was conducted by Raysad (2016) on *G. gummigatta* germplasm in NBPGR, Vellanikkara. She also reported wide

variations in general tree characters. The height variation was observed to be between 3 m to 19 m and GBH found to be in the range of 31 cm to 107 cm.

5.3. Leaf characters

The leaf area in a canopy is an important variable affecting light interception, and hence photosynthesis and carbohydrate production. It determines light interception and is an important parameter in determining plant productivity.

In the present study leaf area was in the range of 13.53cm ^{2} (IC342329-3) to 26.24 cm ^{2} (IC 552526-2) and the average leaf area was 20.413 cm^2. Leaf length varied from 6.98 cm (IC 136687-1) to 10.72 cm (IC 552526-2) whereas leaf breadth ranged from 2.91 cm (IC342327-1) to 4.24 cm (IC 550572-1). Average length and breadth of the leaf were 8.62 cm and 3.73 cm respectively. Length to width ratio ranged from 2.91 to 1.66. Various studies have proved that leaves with higher length-to-width ratios are relatively elongate and narrow and dissipate heat faster than leaves with smaller length to width ratios. Leaves with higher length-to-width ratios have more surface area per volume for heat loss than leaves with lower length to width ratios. In general, small, narrow leaves are well adapted to hot, dry, sunny environments. In the present study IC 342327-1, IC 342319-3 and IC 342329-3 were relatively narrower than the rest of the accessions.

The petiole length varied from 0.75 cm (IC 552528-1) to 1.41 cm (IC 136682-2) and the average being 1.007 cm. Petiole length strongly influences foliage clumping since increase in petiole length reduce the shading of basal lamina portions by the stem and adjacent leaves, leading to significantly increased light harvesting efficiency. Takenaka (1994) reported that a larger length: breadth ratio and longer petiole contributed to larger light capture per unit leaf area due to a reduced aggregation of leaf around the stem.

Variability study done by Devi *et al.* (2013) showed that leaf length was in the range of 6.24-11.95cm, whereas range of width was 2.42 -5.25cm. Ratio

between length and width ranged from 1.87- 4.12 cm. Petiole length varied from 0.60-1.37 cm.

5.4. Flower characters

It was observed that female flower had four yellowish coloured petals and pistil was surrounded by staminodes arranged in 4 tufts while in bisexual flowers stamens are arranged in eight tufts. Length of flower ranged from 5.31 mm (IC 552526-2) to 8.44 mm (IC 342297-1). Flower diameter ranged from 4.09 (IC 552528-1) to 6.67 mm (IC342303-2). CV value for flower length and flower diameter were almost similar i.e, 14 percent and 11 percent, respectively and statistical analysis showed a significant positive relation between flower length and flower diameter. Weight of the flower varied from 0.48 g (IC342327-1) to 0.63g (IC 136687-3). CV value indicate high variability in pedicel length i.e, 48.5 percent and the range being 0.52 cm (IC 136682-2) to 2.24 cm (IC342329-3).

Variability was observed in the flowering time of different accessions. Six trees flowered in October, five in November, three in December and two in January. Sawant *et al.* (1997) has reported flower bud appearance from 29th September to 8th October and flowering initiation from 30th October to 10th November in the different genotypes of kokum. While Senthilkumar *et al.*(2014) reported that in coastal areas kokum flowers in November-December while it flowers in January-February in the hilly regions. It was observed that male flowers were not present in clusters while male were present in 4-5 clusters. Female flowers lacked stamen while in males on an average 52 stamens were present. Similar observations were reported by Devi *et al.,* (2013).

5.5. Variability in fruit characters

The CV value of 30 percent indicates a wide variation in fruit weight which ranged from 16.83 gm (IC 136687-1) to 51.03 gm (IC 342319-3). This is somewhat similar to the observations recorded by Devi *et al.* (2012), who stated that average fruit weight of *G.indica* varied from 6.80 g to 47.60 g, whereas, the average fruit diameter varied widely from 1.80 to 5.15 cm. The average weight of

ripened fruit was 34.39 g. Gosavi (1998) reported that average weight of mature green kokum fruit was 40.34 g and that of ripe kokum fruit was 38.97 g. Sawant *et al.* (1999) evaluated 36 high yielding and early types *G. indica* for fruiting and quality traits. He reported that highest average width, average circumference, average weight and average rind thickness were 4.15cm, 13.15 cm, 34.45 g and 4.45 mm respectively.

In the present study rind weight varied from 6.33 g (IC 136687-1) to 19.22 gm (IC 342302-2). Khanvilkar (1984) reported that the fresh rind of ripened kokum fruits of 25 genotypes ranged from 12.93 g to 14.40 g which was more or less similar to the present study. Further, Joshi (1994) reported that the average weight of kokum rind was 14.78 g. Krishnamurthy *et al.* (1982) reported that the fresh rind of kokum was thick, red, dark purple in colour and constituted about 50 to 60 % of the whole fruit, whereas in the present study the average contribution of rind to the total fruit weight was 41.65 % of the whole fruit. Rind weight has huge importance in case of kokum fruit because of its various commercial uses, so the selection of genotypes with more rind yield can prove to be highly economic.

In the present study, in terms of number of fruits the yield ranged from 220 (IC 136687-1) to 1050 (IC 550572-1). Variations were also observed in the shape of the fruit apex. Six trees had fruits with flat apex, six had sunken, two slightly sunken while one had fruits with slightly projected apex. Colour of the fruits was mostly red and purple. Murlidharan (1970) reported that on an average kokum tree produced about 25 lbs per tree

A study on correlation was undertaken by Khanvilkar (1984) in kokum and it was observed that fruit length exhibited significant negative correlation with the yield. Whereas in the present study it was observed that there was no significant correlation between yield and fruit length.

5.6. HCA

Baliga *et al.* (2011) have reported that the kokum rind contains protein, pectin, tannin, sugar, fat, organic acid like (-) hydroxy citric acid, hydroxy citric acid lactone, anthocyanin, cyanidin-3-sambubioside and cyanidin-3-glucoside.

In the present study, it was observed that HCA was present in large quantity in fruit rind, it ranged from 24.08 g/100g to 40.89 g/100g . The value was highest for IC 136687-2 followed by IC342302-2 and lowest for IC 136687-1. Average HCA content was 36.49 g/100g. Study from Goa, Devi *et al.* (2010) have reported that HCA content in dried fruit rind of kokum varied from a minimum of 19.32 percent to a maximum of 37.39 percent. Among them, 53 accessions recorded more than 30 per cent (-) HCA in rinds. However, Hegde *et al.* (2010) have compared *Garcinia indica* and G. *gummi-gutta* for morphological and chemical traits and reported that the (-) HCA content in *G. indica* ranged from 7.0 to 13.0 percent. Nayak and Rastogi (2010) observed that the HCA is also present in leaves of *G. indica*. Jayaprakash and Sakariah (2002) have reported similar results indicating that the per cent HCA ranged from 10.3 to 12.7 percent in rinds and 4.1 to 4.6 percent in leaves. Jayaprakasha and Sakariah (2000) reported that HCA content in *G.indica* is tissue specific at the level of 2.5, 0.8, 3.0 and 20.1 percent in leaf, pulp, fresh fruit and dried rind respectively. Higher HCA concentration in the present study might be attributed to the genotypic interaction with the environment.

5.7. Primary metabolites

Primary metabolites are directly involved in the growth and development of the plant. Primary metabolites serve as the source of energy. Plants use carbohydrates as a source of energy to carry out normal functions such as growth, movement and metabolism. Carbohydrate content showed a great variation among the genotypes; from 2.5g/100g to 7.8 g/100g. Average carbohydrate content was 5.55 g/100g and the CV was 22.3 percent. This can be supported by the results (5.67%) obtained by Parthasarthy and Nandkishor (2014). This value was

however higher than the observations made by Handle *et al.* (2014), who reported it to be 3.52±0.07 percent.

The collection of proteins in a cell determines its health and function. Proteins are responsible for various tasks like product manufacture, waste cleanup and routine maintenance. Proteins also receive signals from outside the cell and mobilize intracellular response. Present study showed that the protein content in kokum varied from 4 g/100g (IC 552528-3) to 6.6 g/100g (IC 136687-2). The mean protein content was 5.45 g/100g. However, a lower percentage of protein was reported by Tripathi, *et al.*, (2015) i.e. 1.92%. This difference in the two studies might be attributed to the environmental and genetic variations.

The crude fat was observed to be in the range of 3.02 % (IC 550572-1) to 5.35 % (IC342302-2). The mean crude fat was 4.33 %. Lower concentration of crude fat (1.4g/100g) was observed by Ramchandran (2014). The reducing sugar was found maximum in IC342302-2 (6.48 %) and minimum in IC 550572-1 (4.3 %) and its average content in all trees was 5.65 %. Similar results were obtained by Dalvi (1998), who reported that the reducing and total sugars in ripe kokum fruits were 5.17 per cent and 10.75 per cent respectively. Joshi (1994) also reported almost similar amount of reducing sugar in kokum rind (5.44%) . Reducing sugars are responsible for the sweet taste in a fruit. The percentage of reducing sugars in kokum is less in comparison to the organic acids. This may be the reason for very sour taste of the fruits even when they are ripe.

The total soluble solids ranged from 9.2 % (IC 136682-2) to 12.5% (IC 136687-3), and the average was 10.75 %. Nair (1986) reported that the T.S.S. of kokum fruits ranged between 11.04 per cent to 16.44 per cent in the course of fruit growth and development. Joshi (1994) stated that the T.S.S. content of mature kokum fruit was 12.5 % and that of ripe fruit was 16.45 %. The increase was probably due to accumulation of sugars in the fruit due to hydrolysis of starch. Nagwekar *et al.* (2010) have stated that the rind of 'Konkan Hatis', a commercial variety of kokum , recorded 9.20% total soluble solids and 4.10% reducing sugar.

5.8. Secondary metabolites

Phenolic compounds are a class of secondary metabolites attributed with several bioactivities, especially antioxidant properties. Antioxidant activity of a substance is the ability of a molecule to eliminate or to neutralize a free radical. Several phytochemicals such as curcumin, tocopherol, catechin, xanthones and anthocyanins are attributed with antioxidant properties (Harborne, 2005). Phenolic compounds also facilitate pollination through colour and fragrance, defence against pathogens and prevent fruits consumed by herbivores (Harborne, 2005).

In the present study the average phenolic content in kokum was estimated to be 796.87 mg/100g. The maximum was observed in IC 342297-1 (1080 mg/100g), followed by IC 552528-3 (1060 mg/100g) and the minimum was in IC 136687-1 (460 mg/100g). However, a higher concentration (5.01%) of total phenolics was found in kokum by Parthasarthy and Nandkishora (2014). The variation might be due to influence of solvent used to extract phenolics. The study conducted by Garcia-Sala *et al.* (2010) proved that the solvent used to extract the compound has some influence on the amount of phenolics. However, variation observed during present study among different accessions was due to genetic or environmental difference, since same solvent (ethanol) was used for all the accessions.

Flavanoids are considered to be responsible for colour, aroma of flowers and fruit to attract pollinators consequently fruit dispersion; help in seed, spore germination, growth and development of seedling. Flavonoids have roles against frost hardiness, drought resistance and may play a functional role in plant heat acclimatization and freezing tolerance. In the present study flavanoids content in kokum was found to be in the range 470 mg/100g to 238 mg/100g. The average flavanoids content was found to be 327.75 mg/100g and the CV being 17.9%. These values were higher when compared to the flavanoids content (137.27 µg/g) observed in kokum by Sharma *et al.*, (2015). Variations in flavanoid content

might be due to various factors such type of soil, microclimatic conditions, geographic location, site, age and vegetative stage of plants.

5.9. Vitamin and minerals composition in fruit rind

Ascorbic acid, known as vitamin C, is a water soluble vitamin, not synthesized in the body, but must get through foods or supplements. It is an important antioxidant and its deficiency causes scurvy and delayed healing. Ascorbic acid works as a preservative to prevent rancidity, acts as a dough conditioner in baking and prevents enzymatic browning. In the present study of diversity assessment in kokum, large CV value (27.6%) indicates that large variability was present in vitamin C content. It was found to be maximum in IC 136687-3 (58.17 mg/100g) followed by IC 342322-1 (50.90 mg/100g) and minimum in IC 342329-3 (21.81 mg/100g). The average content was estimated to be 37.17 mg/100g. This is somewhat similar to the Vitamin C content (33.45mg/100g) estimated by Parthasarthy and Nandkishore (2014). However lower content was observed by Joshi (1994) i.e. 13.11 mg/100 gm.

Plants have inherent variability of mineral content which affects their physiology and consequently the herbivorous insects feeding on them (Varanda and Primavesi, 2005). The study reveals that Potassium and Magnesium are present in good percentage in fruit rind tissue which makes it an important medicinal fruit. Other than K, *Garcinia* species have mineral content similar to major fruits like apple, grapes, peaches or banana .

Calcium is responsible for holding together the cell walls of plants in the form of calcium pectate. It also plays a role in activating certain enzymes and to send signals that coordinate certain cellular activities. It was observed that Ca content varied from 4.56 mg/100 g (IC 342322-1) to 7.45 mg/100 g (IC 136687-2). The average Ca content (6.39 mg/100 g) was however lower than what was reported (13.07 mg/100 g) by Parthasarthy and Nandkishore (2014).

Sodium plays a role in maintaining osmotic pressure of the plant body and also involved in regulating acid- base balance (Soetan *et al.,* 2010). The Na

content showed the maximum variation among different accessions with CV of 49%. The Na content between different accessions ranged from 0.23 mg/100g (IC 342297-1) to 1.92 mg/100g(IC342327-1). The average was estimated to be 1.1 mg/100g, which is almost similar to the observation made by Parthasarthy and Nandkishore (2014) (1.55 mg/100g).

Potassium plays an important role in photosynthesis by regulating the opening and closing of stomata, and therefore regulates CO_2 uptake. Among all the minerals estimated K showed the least variability (CV being 9.5 %). The minimum content of K was observed in IC 342297-1 (24.65 mg/100g) while maximum was in IC 552526-2 (33.05 mg/100g). The average content of K (29.3 mg/100g) in present study was lower than estimation made in kokum by Parthasarthy and Nandkishore (2014) (44.5 mg/100g) but almost similar to the observation made by Raysad (2017) in *G. gummigatta.*

Phosphorus is a component of the complex nucleic acid structure of plants, which regulates protein synthesis. The variation in P content ranged from 2.09 mg/kg (IC 136687-1) to 4.85 mg/kg (IC 136682-2). The average was estimated to be 3.48 mg/kg, which was more or less similar to the observation made by Parthasarthy and Nandkishore (2014) i.e., 4.51 mg/kg.

The concentration of Magnesium ranged from 27.98 mg/100g (IC 136687-2) to 17.20 mg/100g (IC 136682-2) and the average being 22.74 mg/100g. This value was lower than the value (44.5 mg/100g) stated by Parthasarthy and Nandkishore (2014) in kokum but was significantly higher than the value observed by Morabandza (2013) in *G. kola* (2.40 mg/100g).

Raysad (2016) has also reported wide variations in Ca (105.44 mg/100 g to 212.25 mg/100 g), Na (41 mg/100 g to 98 mg/100 g), K (115.8 mg/100 g to 227.1 mg/100 g mg/100 g), P (20.4 mg/100 g to 52.4 mg/100 g) and Mg (10 mg/100 g to 12.87 mg/100 g) content of *G. gummigatta.*.

5.10. Physiochemical properties of butter

Percentage of butter was obtained was maximum from IC342319-3 (35.69 %) and minimum from IC 550572-1 (23.17 %) and the average percentage was 30.60 percent. However a higher percentage was reported by Niveditha (2013) i.e. 42.47 percent to 58.94 percent. According to her maximum seed oil content was observed in orange morpho (58.94 % in dry seed), followed by that of red morpho (58.57 % in dry seed), while yellow and green morpho had least 47.57 percent and 42.47 percent, respectively. According to Muralidharan (1970), kokum fat obtained from seeds varied from 23 to 26 percent. Similarly, Bhosale (1975) reported 25.41 per cent oil in air dried kokum seeds. Manjunatha *et al.* (2007) have also reported significant differences in butter content among different germplasm of *Garcinia indica*. However the range of seed oil varied from 23.73 percent to 12.25 percent, which is lesser than reported in the present study.

The saponification value varied from 194.94 mg of KOH/g (IC 552526-2) to 171.10 mg of KOH/g (IC 136687-2) which makes them suitable for making soaps. Thus the high saponification value indicates the presence of lower fatty acids with low molecular weight. Parthasarthy and Nandkishore (2014) reported saponification valueto be 200.2 mg of KOH/g.

While the Acid value ranged from 2.6 mg of KOH/g (IC342303-2, IC342327-1) to 3.4 mg of KOH/g (IC 136687-3) which shows that the butter is good for consumption. However, a higher value was observed by Parthasarthy and Nandkishore (2014) i.e. 4.9 mg NaOH/g of oil.

The Iodine value showed the variations in the range of 36.41 (IC342329-3) to 40.56 (IC 552528-1). Iodine value allows predicting the tendency of fat to become rancid, its value should preferably be 25-50. Parthasarthy and Nandkishora (2014) reported that IV of kokum butter was 39.4 which was similar to the accessions IC 550572-1(39.00), IC 342322-1(39.26) and IC342327-1(39.39).

5.11. Correlation between morphological characters of *Garcinia indica*

Correlation matrix between different morphological characters showed a significant and positive relation between leaf area and leaf length, fruit weight and rind weight, flower weight and flower length, flower diameter and flower length, flower diameter and branch height and height and diameter. And a significant negative correlation was observed between flower weight and flower diameter, fruit weight and tree height, pedicel length and leaf area and rind weight and tree height.

5.12. Path analysis for rind yield

A positive direct effect was exerted by the trait number of fruits (0.78) followed by rind weight (0.36), fruit weight (0.24) and flower weight (0.14). Since, the correlation coefficient of these four traits were positive and almost equal to its direct effects, it is advisable to go for direct selection. Whereas a low and negative direct effect was exerted by flower length (-0.05) followed by flower diameter (-0.11).

5.13. Cluster analysis

Sixteen trees were grouped into six clusters (Table 15) using hierarchial eucledeian cluster analysis. It helped to identify the most distant accessions and most closely placed ones for breeding experiment to obtain hybrid vigour. The cluster III possess maximum number of accessions whereas the least number observed for the cluster V and VI respectively. So the IC 550572-1 and IC 136687-1 differ in their morphological and biochemical characters than others.

5.14. Principal component analysis (PCA)

Principal component analysis showed that in case of morphological characters, four components that had eigen value greater then one together accounted for 84.8% of the total variability. PC1 accouned for 27.8% of the total variability, which was mainly contributed positively by tree height, flower diameter, flower length and flower weight. PC2 accounted for 23.0% of the total

variability, which was mainly contributed positively by pedicel length, flower length and branch height.

In case of biochemical characters PC1 accounted for 25.7% of the total variability, which was mainly contributed positively by Mg, proteins, Ca and carbohydrates. PC2 contributed for 18.5% of the total variability which is contributed positively by Vitamin C and proteins, while rest of the factors contribute negatively to it.

Greater variability in the base population ensures more success in tree improvement programme. The primary objective of germplasm conservation is to collect and preserve the genetic variability in indigenous collection of a species to make it available for present and future generations. In the present study general tree characters like height, GBH and branch height showed wide variability while characters like canopy shape and branching habit showed narrow variability. Fruit characters like fruit weight, rind weight and fruit yield in terms of number showed large variability, however low variability was observed in colour of fruits. Less variability was observed in leaf and flower characters. Primary metabolites like carbohydrates, proteins and crude fat showed considerable variability, while it was observed to be comparatively low in TSS and reducing sugar. Vitamin C and phenolics content showed wide variability, while it was estimated to be lesser in HCA and flavanoid content. In case of minerals high variability was observed in P and Na while rest of the minerals (Ca, K and Mg) showed narrow variability. Variability in physio-chemical characters of butter was estimated to be low.

It can be concluded from the results that a considerable morphological and biochemical variations exist in *Garcinia indica* germplasm, NBPGR, Vellanikkara. And for commercial characters that showed less variability e.g. HCA, more variability can be introduced. These results could be useful in efficient management of *G.indica* germplasm for the conservation and optimal utilization.

SUMMARY

6. Summary

The selection of superior strain from seedling population is one of the easiest and the best method of crop improvement. The characters showing wide range of variation have more scope for their improvement. The present study was conducted to determine the morphological and biochemical variations in 16 accessions of *Garcinia indica,* which are maintained as germplasm in ICAR NBPGR Regional Station, Thrissur. The salient features of the study are summarised below:

1. The variation in height of trees was in the range of 4.2 m to 15.5 m. Variation in the height of first branch from the base of the tree varied from 0.8 m to 6.52 m. While the GBH varied from 41 cm to 110 cm .
2. Most of the trees had conical shaped canopy with drooping or horizontal branches.
3. Area of the leaf was in the range of 13.53 cm^2 to 26.24 cm^2 . Leaf length varied from 6.98 cm to 10.72 cm whereas leaf breadth ranged from 2.91 cm to 4.24 cm . The petiole length varied from 0.75 cm to 1.41 cm.
4. Not much variations was observed in the emergence of the leaves. Pinkish flushes were observed in all the accessions which turned into green colour after 11 days of emergence.
5. The flowers had yellowish green petals to 8.44 mm. Flower diameter ranged from 4.09 to 6.67 mm. Weight of the flower varied from 0.48gm to 0.63gm and the pedicel length varied from 0.52 cm to 2.24 cm.
6. Variations were also observed in flowering time. Six trees flowered in October, five in November, three in December and two in January. Female flowers were not present in clusters while male were present in 4-5 clusters.
7. A wide variation was observed in fruit weight which ranged from 16.83 gm to 51.03 gm. Rind weight varied from 6.33 g to 19.22 g.
8. Rind weight has huge importance in case of kokum fruit because rind has various commercial uses, so the selection of genotypes with more rind

yield can prove to be highly economic. Yield ranged from 220 to 1050 fruits. Six trees had fruits with flat apex, six had sunken, two slightly sunken and one slightly projected. Colour of the fruits was mostly red and purple.

9. It was observed that HCA was present in large quantity in fruit rind, it ranged from 24.08 g/100g to 40.89 g/100g. Average HCA content was 36.49 g/100g. Higher HCA concentration in the present study might be attributed to the genotypic interaction with the environment.
10. Study of primary metabolites revealed that carbohydrate content was in the range of 2.5g/100g-7.8 g/100g. Variations in protein content ranged from 4 g/100g to 6.6 g/100g. The crude fat was observed to be in the range of 3.02 percent to 5.35 percent. The reducing sugar was found to be between 6.48 percent and 4.3 percent. Whereas the total soluble solids ranged from 9.2 percent to 12.5 percent .
11. Study of secondary metabolites showed that the phenolic content was in the range of 1080 mg/100g to 460 mg/100g while flavanoids content varied from 470 mg/100g 238 mg/100g.
12. Variation in Vitamin C content was found to be in between 58.17 mg/100g and 21.81 mg/100g .The average content was 37.17 mg/100g.
13. The study revealed that K and Mg are present in good percentage in fruit rind tissue which makes it an important medicinal fruit. Ca content varied from 4.56 mg/100g to 7.45 mg/100g. While the Na content between different accessions ranged from 0.23 mg/100g and 1.92 mg/100g. The concentration of K was observed between 24.65 -33.05 mg/100g. The variation in P content ranged from 2.09 mg/kg to 4.85 mg/kg. The concentration of Magnesium ranged from 27.98mg/100g to 17.20 mg/100g. Average content of Ca, Na, K, P and Mg were 37.17 mg/100g, 6.39 mg/100g, 1.10 mg/100g, 29.34 mg/100g, 3.48 mg/kg and 22.74 mg/100g respectively.
14. Percentage of butter was obtained from dried seed was in the range of 35.69 percent to 23.17 percent and the average percentage was 30.60

percent. The saponification value varied from 194.94 mg of KOH/g (IC 552526-2) to 171.10 mg of KOH/g. While the Acid value ranged from 2.6 mg of KOH/g to 3.4 mg of KOH/g. The Iodine value showed the variations in the range of 36.41 to 40.56 .The average saponification value, acid value and Iodine value were 183.98 mg of KOH/g, 2.95 mg of KOH/g and 38.63 respectively.

15. Path analysis showed that flower length and flower diameter had negligible effect on rind yield where as flower weight, fruit weight , rind weight and number of fruits had significant effect on rind yield
16. Correlation matrix between different morphological characters showed a significant and positive relation between leaf area and leaf length, fruit weight and rind weight, flower weight and flower length, flower diameter and flower length, and height and diameter. And a significant negative correlation was observed between flower weight and flower diameter, fruit weight and tree height, pedicel length and leaf area and rind weight and tree height
17. Correlation matrix between biochemical characters showed that there was a positive and significant relation between carbohydrate and protein content, carbohydrate and Ca content, Protein and Mg content, crude fat and HCA, reducing sugar and HCA and CA and Mg while a significant and negative correlation was observed between crude fat and vitamin C and Mg and crude fat.
18. A hierarchical cluster analysis was carried out for the 16 accessions based on the Euclidian squared distance. The 16 accessions were grouped into six clusters. The cluster III possess maximum number of accessions whereas the least number observed for the cluster V and VI respectively.
19. Principal component analysis showed that in case of morphological characters, four components that had eigen value greater then one together accounted for 84.8% of the total variability. PC1 accouned for 27.8% of the total variability, which was mainly contributed positively by tree height, flower diameter, flower length and flower weight. PC2 accounted

for 23.0% of the total variability, which was mainly contributed positively by pedicel length, flower length and branch height.

20. In case of biochemical characters PC1 accounted for 25.7% of the total variability, which was mainly contributed positively by Mg, proteins, Ca and carbohydrates. PC2 contributed for 18.5% of the total variability which is contributed positively by Vitamin C and proteins, while rest of the factors contribute negatively to it.
21. It can be concluded from the results that considerable morphological and biochemical variations exist in *Garcinia indica* germplasm, NBPGR Regional Station, Thrissur. For commercial characters that showed less variability e.g. HCA, more variability can be introduced.
22. These results could be useful in efficient management of *G.indica* germplasm for the conservation and optimal utilization.

174602

KERALA AGRICULTURAL UNIVERSITY
CENTRAL LIBRARY
P.O. (P.O.), THRISSUR

7. Reference

Abraham, Z., Malik, K., Rao, G., Narayanan, S.L., Biju, S. 2006. Collection and Characterisation of Malabar Tamarind [*Garcinia cambogia* (Gaertn.) Desr.]. *Genet Resour Crop Evol.* 53(2): 401-406.

Adesuyi, A.O., Elumm, I.K., Adaramola, F.B. and Nwokocha, A.G.M. 2012. Nutritional and phytochemical screening of *Garcinia kola*. *Advance J. Food Sci. and Technol.* 4(1): 9-14.

Adeyeye, E.I. and Ayejuyo, O.O. 1994. Chemical composition of *Cola acuminata* and *Garcinia kola* seeds grown in Nigeria. *Int. J. Food Sci. Nutr.* 45(4): 223-230.

Ahmad, M., Masood, S., Sultana, S., Hadda, T.B., Bader, A. and Zafar, M. 2015. Antioxidant and nutraceutical value of wild medicinal *Rubus* berries. *Pakistan J. Pharma. Sci.* 28(1): 241-247.

Antonovicks, J. 1971. The effect of heterogenous environment on the genetics of natural population. *American Sci.* 59: 593-595.

Antony, P.D., Josna, P. and Shankarnarayan, M.L. 1998. Quantitative analysis of hydroxycitric acid and hydroxycitric acid lactone in *Garcinia* fruit and *Garcinia* product. *J. Food. Sci. Technol.* 35: 399-402.

Augustin, M.A. and Azudim, M.N. 1986. Storage of mangosteen (*Garcinia mangostina* L.). *ASEAN Food J.* 2 (2): 78-80.

Baliga, M.S., Bhat, H.P., Pai, R.J., Boloor, R. and Princy, L.P. 2011. The chemistry and medicinal uses of the underutilized Indian fruit tree *Garcinia indica* Choisy (kokum): A review. *Food Res. Int.* 44:1790-1799.

Bhosale,S.B. 1975. Amino acid composition of Kokurn. *Khadi gramyodog.* 22(2): 100-102.

Bohm, B.A. and Kocipai, A.R. 1994. Flavonoid and condensed tannins from the leaves of *Vaccinum raticulation* and *Vaccinum calcyimium*. *Pacific Sci.* *48*(3): 458-463.

Castro, M.F.P.P.M., Anjos, V.D.D.A., Rezende, A.C.B., Benato, E.A. and Valentini, S.R.D.T., 2012. Postharvest technologies for mangosteen (*Garcinia mangostana* L.) conservation. *Food Sci. and Technol.* 32(4): 668-672.

Cavalcante, I.H.L., Jesus, N.D. and Martins, A.B.G. 2006. Physical and chemical characterization of yellow mangosteen. *Frutic. Jaboticabal.* 28(4): 325-327.

Chandran, M.D.S. 1996. The Kokum Tree. *Resonance*, 1: 86–89.

Choppa, T., Selvaraj, C.I. and Zachariah, A. 2015. Evaluation and characterization of Malabar tamarind (*Garcinia cambogia*) seed oil. *J. Food Sci Technol.* 52(9): 5906-5913.

Chowdhury, T. 2014. Comparison of total phenolic content of some selected indigenous *Garcinia* species found in Assam. *Int. J. Pharm. Pharmc. Sci.* 6(2): 598-601.

Dai, J. And Russel. J. 2010. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Mumper Mol.* 15: 7313-7352.

Dalvi, R.B. 1998. Studies on carbonation of fruit based beverages. M.Sc.(Agri.) Hort., thesis, Dr. B.S.K.K.V., Dapoli, Ratnagiri, M.S.

Deore, A.B., Sapakal, V.D., Dashputre, N.L. and Naikwade, N.S. 2011. Antiulcer activity of *Garcinia indica* fruit rinds. *J. Appl. Pharma. Sci.*1 (5): 151-154

Devi, S.P., Ramachandradu, K., Thangam, M., and Singh, S.P. 2010. Assesment of Kokum (*Garcinia indica*) diversity for Hydroxy Citric acid and Fat in Goa using DIVA- Geographical Information System (DIVA – GIS). In: Vasudeva, R., Janagoudar, B.S., Reddy, B.M.C., Sthapit, B. and Singh, H.P. (eds.). *Garcinia Genetic Resources: Linking Diversity, Livelihood and*

Management, Proceedings of national symposium, College of Forestry, Sirsi, Karnatka, pp.60- 64

Devi, S.P., Thangam, M., Ramchandrudu, K., Kumar, A.J. and Singh, N.P. 2013. Genetic diversity of Kokum (*Garcinia indica*) in Goa-Tree and fruit characters. Technical Bulletin No. 33, ICAR (RC), Goa.

Dosunmu, M.I. and Johnson, E.C. 1995. Chemical evaluation of the nutritive value and changes in ascorbic acid content during storage of the fruit of 'bitter kola' (*Garcinia kola*). *Food Chem.* 54: 67–71.

Garcia-Sala, P., Morales-Soto, A., Segura-Carretero, A. and Fernandez-Gutierrez. 2010. A phenolic compound extraction systems for fruit and vegetable samples, *Molecules,* 15(2): 8813-8826.

Gawankar, M.S., Shingre, D.V., Dalvi, M.B. and Jamadagni, B.M. 2001. Variability for morphophysical properties of fruits in kokum. In: Proceedings of First national seminar on kokum. RFRS, Vengurle, Maharashtra, India, pp.1-3.

Godbole, A. and Das, S.K. 2000. Flora of Maharashtra State In: Singh, N.P. and Karthikeyan .(eds) *Dicotvledons.* Published by Director Botanical Survey of India. Barboune Road, Calcutta. pp. 273-283.

Gogoi, B. 2015. Morpho-biochemical characterization of *Garcinia* species of Assam. M.Sc. (Agri) thesis, Assam Agricultural University, Jorhat, 79p.

Gopalan, C., Ramashastri, B.V., Balasubramaniam, S.C., Deosthale, Y.G. and Pant, K.C. 1994. *Nutritive Value of Indian Foods, National Institute of Nutrition.* Indian Council of Medical Research, Hyderabad.

Gosavi, R.C. 1998. Studies on drying and utilization of dried kokum (*Garcinia indica* Choisy) and Kdronda (*Carissa carandus*) fruits. MSc.(Agri.) Hort., thesis, Dr. B.S.K.K.V., Dapoli, Ratnagiri.

Haldankar, P.M., Khandekar, R.G. and Haldavanekar, P.C. 2011. Genetic and varietal diversity and its conservation—Tree spices. In Souvenir and Abstracts, National symposium on spices and aromatic crops (SYMSAC VI): Exploiting spices production potential of the Deccan region. Indian Society for Spices, Kozhikode, pp. 58-62.

Hande, A.R., Swami, S.B., Thakor, N.J. 2014. Effect of drying methods and packaging materials on quality parameters of stored kokum rind. *Int. J. Agric & Biol. Eng.* 7(4): 114－126

Harborne, J.B. 2005. Phenolic Compounds. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Springer, 3:40-43.

Hegde, L., Kuruvinshetty, M. S., Mahesh Padnad. and Thimmaraju, R., 2010, Evaluation of morphological and chemical variation in two endangered medicinal tree species of Garcinia. In: Vasudeva, R., Janagoudar, B.S., Reddy, B.M.C., Sthapit, B. and Singh, H.P (eds.). *Garcinia Genetic Resources: linking diversity, livelihood and Management,* Proceedings of national symposium, College of Forestry, Sirsi, Karnatka, pp.74-78.

Heymsfield, S.B., Allison, D.B., Vasselli, J.R., Pietobelli, D.G. and Nunthe, C. 1998. *Garcinia cambogia* (Hydroxycitric acid) as a potential anti-obesity agent. A randomized control trial. *J. Am. Med. Assoc.* 280: 1596-1600.

Hutchin, V.M.M., Mota, I.E., Glory, L.C., Vazquez, E.O., Vargas, M.L., Anncona, D.B. and Dutch, E.S. 2014. Determination of some physic-chemical characteristics, bioactive compounds and antioxidant activity of tropical fruits from Yucatan, Mexico. *Food Chem.* 152 (1): 508-551.

Jackson, M.L. 1973. *Soil Chemicaal Analysis.* Prentice Hall of India (Pvt.) Limited New Delhi, 299p.

Jagtap, P., Bhise, K. and Prakya, V. 2015. A Phytopharmacological Review on *Garcinia indica. Int. J. Herb. Med.* 3(4): 02-07.

Jain, J.L., Sunjay, J. and Nitin, J. 2005. Fundamentals of Biochemistry. S Chand and Co. Ltd, New Delhi.1:11-13.

Jayaprakasha, G.K. and Sakariah, K.K. 2000. Determination of Hydroxycitric acid in commercial samples of *Garcinia combogia* extract by liquid chromatography with ultraviolet detection. *J. Liquid Chromatography and Related Technologies*. 23 (6): 915–923.

Jayaprakasha, G.K. and Sakariah, K.K. 2002. Determination of organic acids in leaves and rinds of *Garcinia indica* (Desr.), *J. Pharm. Biomed. Anal*. 28: 379-384.

Jeeva, S. 2009. Horticultural potential of wild edible fruits used by the Khasi tribes of Meghalya. *J. Hortic. For*. 1(9): 182-192.

Jena, B.S., Jayaprakasha, G.K., Singh, R.P. and Sakariah, K.K. 2002. Chemistry and biochemistry of hydroxycitric acid from *Garcinia*. *J. Agric. Food Chem*. 50: 10-22

Joshi, A.B. 1994. Studies on some aspects of post harvest handling and processing of Kokum fruits. M.Sc. (Agri.) thesis, Konkan Krishi Vidyapeeth, Dapoli, Dist. Ratnagiri (M.S).

Karuppusamy, S., Muthuraja, G. and Rajasekaran, K.M. 2011. Antioxidant activity of selected lesser known edible fruits from Western ghats of India. *Indian J. Nat Products Resour*. 2(2): 174-178.

Khanvilkar, S.V. 1984. Evaluation of seedlings types in Kokum (*Garcnia indica* Choisy). M.Sc. (Agri.), thesis, Dr. B.S.K.K.V., Dapoli, Ratnagiri.

Korikanthimath, V.S., Devi, S.P. and Desai, A.R. 2008. Kokum (*Garcinia indica*)-a horticultural crop with great potential. In: Chadha, K.L., Singh,A.K. and Patel,V.B. (eds.) *Recent initiatives in Horticulture*, pp.393-399.

Korikanthimath, V.S. and A.R. Desai. 2005. Status of Kokum (*Garcinia indica* Choisy) in Goa. In: Preview of 2nd national seminar on kokum. Goa University, Goa, India. pp. 7-16.

Krishnamurthy, N. 1984. Chemical and technological studies on coloring matters from natural sources for use in foods. Ph. D. thesis, Mysore University, Mysore, Karnataka, India.

Krishnamurthy, N. and Sampathu, S.R. 1988. Antioxidant principles of kokum rind. *J. Food Sci. Technol.* 25 (1): 44–45.

Krishnamurthy, N., Lewis, Y.S. and Ravindranath, B. 1982. Chemical constituents of Kokum fruit rind. *J. Food Sci.Technol.*, 19 : 97-100.

Lechanudel, M., Joas, J., Caro, Y., Genard, M., and Jannoyer, M. 2005. Leaf: fruit ratio and irrigation supply affect seasonal changes in minerals, organic acids and sugars of mango fruit. *J. Sci. Food Agric.* 85: 251-260.

Mahadkar, S., Valvi, S., and Rathod, V. 2012. Nutritional assessment of some selected wild edible plants as a good source of mineral. *Asian J. Plant Sci. Res.* 2(4): 468-472.

Maheshwari, B and Reddy, S Y. 2005. Application of kokum (*Garcinia indica*) fat as cocoa butter improver in chocolate. *J. Sci. Food Agric.* 85: 135-140.

Majeed,M (1994). *Citrin- A revolutionary, herbal approach to weight management*. New Editions Publishing Co., Burlingame, pp. 8-14.

Malik, S. K., Chaudhury, R. and Abraham, Z. 2005. Seed morphology and germination characteristic in three *Garcinia* species. *Seed. Sci. Technol.*33(3): 595-604

Manjunatha, G.O., Patil, S.K., Shivanna, H. And Rathod, S.R. 2010. Standardisation of propagation techniques in *Garcinia indica* Choisy. In: Vasudeva, R., Janagoudar, B.S., Reddy, B.M.C., Sthapit, B. and Singh, H.P. (eds.) *Garcinia Genetic Resources: Linking Diversity, Livelihood and*

Management, Proceedings of national symposium, College of Forestry, Sirsi, Karnatka, pp. 122-123.

Martinez, R. Torres, P., Meneses, A.M., Figueroa, J.G., Alvarez, J.A. and Martos, M.V. 2012. Chemical, technological and invitro antioxidant properties of mango, guava, pineapple and passion fruit dietry fibre concentrate. *Food Chemistry*. 135 (3): 1520-1526.

Maskan, A., Kaya, S. and Maskan, M. 2002. Hot air and sun drying of grape leather (pestil). *J. Food Eng.* 54: 81–88.

Miguez, B.M., Dela, M.M.J. and Garcia, Q.J. 2004. Determination of sugars in varieties of chestnut fruits from Galicia (Spain). *J. Food Comp. Anol.* 17:63-67.

Mirgal, A.B., Rane, A.D., Rajesh, P. Gunaga., Narkhede, S. and Bhave, S.G. 2010. Characterization of fruit and seed traits of *Garcinia indica* Choisy. In: Vasudeva, R., Janagoudar, B.S., Reddy, B.M.C., Sthapit,B. and Singh, H.P (eds.) *Garcinia Genetic Resources: linking diversity, livelihood and Management,* Proceedings of national symposium, College of Forestry, Sirsi, Karnatka, pp. 96-98.

Morabandza, C.J., Ongoka R.P., Matini L., Epa, C., Nkounkou, L.C. and Abena, A.A. 2013. Chemical composition of the mesocarp of *Garcinia kola* fruit. *Res. J. Recent Sci.* 2(1): 53-58.

Muralidharan, H.G. 1970. Mysore margal (*Garcinia indica*) and some species sketch. *Indian oil and soap J.* 36(5): 137-142.

Muthulakshmi, T. 1998. Variability analysis in *Garcinia cambogia* (Malabar Tamarind). M.Sc. (Hort.) thesis, Kerala Agricultural University, Vellanikkara, Thrissur.

Nagendra, K., Kusum, R. And Ramchandran, H.D. 2014. Evaluationn of phytochemical screening and in vitro anti oxidant activities of total

phenolics and flavanoids contents from fruits of *Garcinia indica*. *Int J. Innovative Pharma Sci Res*. 3(1): 1-8.

Nagwekar, D. D., Sawant, V. S., Desai, V. S., Haldonkar, P. M., Rangwala, A. D. and Jadhav, B. B. 2010. "Kokam Hatis"- a bold type kokam (*Garcinia indica* Choisy) variety. National Symposium on Garcinia Genetic Resources: linking diversity, livelihood and management (Eds.) Vasudeva, R., Janagoudar, B.S., Reddy, B.M.C., Bhuwon, S. and Sing, H.P. College of Forestry, Sirsi, pp.143-147.

Nair, C.R. 1986. Studies on maturity indices and post harvest technology of Kokum (*Garcini indica* Choisy). M.Sc. (Agri.) Hort., thesis, Dr. B.S.K.K.V., Dapoli, Ratnagiri, M.S.

Nayak, CA., Rastogi, N.K. and Raghavarao, K.S.MS. 2010. Bioactive constituents present in *Garcinia indica* Choisy and its potential food applications: A review. *Int. J. Food Prop.*13: 441-453.

Nimanthika, W.J. and Kaththriarachchi, ,H.S. 2010. Systematics of genus *Garcinia* L. (Clusiaceae) in Sri Lanka. New insights from vegetative morphology. *J. Natl Sci. Foundation,* 38: 29-44.

Nirvair, S.K., Neil, E.H. and Kent, L.E. 2007. Conjugated linoleic acid isomers and cancer. *J. Nutri.* 137(12): 2599-2607.

Niveditha, M. 2013. Morphological and chemical characterization of different fruit morpho types in *Garcinia indica* Choisy. M.Sc.(Forestry) thesis, College of Forestry, Sirsi, pp. 11-12.

Padhye, S., Ahmad, A., Oswal, N and Sarkar, F.H. 2009. Emerging role of Garcinol, the antioxidant chalcone from *Garcinia indica* Choisy and its synthetic analogs. *J. Hematol Oncol.* 2(38): 1-13.

Pandey, R., Chandra, C., Brijeshkumar, Srivastva, M., AnuAravind, A.P., Shameer, P.S. and Rameshkumar, K.B. 2015. Simultaneous determination

of multi-class bioactive constituents for quality assessment of *Garcinia* species using UHPLC-QqQLIT-MS/MS. *Ind. Crop. Prod.* 77: 861-872

Pangsuban, S., Bamroongrugsa, N., Kanchanapoom K. and Nualsri, C. 2009. Facultative apomixes in *Garcinia atroviridis* (Clusiaceae) and effects of different pollination regimes on reproductive success. *Trop. Life Sci. Res.* 20 (2): 89-108.

Parle, M. and Dhamija, I. 2013. Golden benefits of drinking Kokamcola. *Int Res J. Pharmacy.* 4(5): 5-9.

Parthasarthy, U. and Nandakishore, O.P. 2014. A study on nutrient and medicinal compositions of selected Indian *Garcinia* spp. *Curr. Bioactive Compounds.* 10(1):1-7.

Parthasarathy, U and Nandakishore, O.P. 2016. *Garcinia* bark exudates- an important phytochemical source. *Curr. Sci.* 110: 1617-1619.

Patil, A.M. and Potdar, G.G. 2018. Estimation of phenolic and flavonoids content and antioxidant activity of *Garcinia talbotti* Raiz ex Sant. *Int. J. Bot. Stud.* 3: 68-70.

Patil, S., Shirol, A.M. and Kattimani, K.N. 2009. Variability studies in physico-chemical parameters in kokum (*Garcinia indica* Choicy) for syrup preparation. *Karnataka J. Agric. Sci.* 22(1): 244-245.

Ramchandran, H.D. 2014. Plant profile, phytochemistry and pharmacology of *Garcinia indica:* A review. *Int. J. Pharma. Sci. Rev. Res.* 27(2): 376-381.

Rameshkumar, K.B. 2017. Diversity of *Garcinia* species in the Western Ghats: Phytochemical Perspective. In: Rameshkumar, K.B.(ed.) *Jawaharlal Nehru Tropical Botanic Garden and Research Institute Thiruvananthapuram.* 14p.

Ravishankar, H. and Sakthivel,T. 2010, *Garcinia* diversity appraisal, conservation and utilization in Kodagu region of Karnataka. National Symposium on *Garcinia* genetic resources: linking diversity, livelihood and management.

(eds.) Vasudeva, R.., Janagoudar, B.S., Reddy, B.M.C., Sthapit, B. and Singh, H.P. College of Forestry, Sirsi. pp. 32-33.

Ranganna, S. 1986. *Manual of Fruits and Vegetable Products*. Tata McGraw Hill Publishing Ltd., New Delhi, 594p.

Ranganna, S. 1991. *Handbook of analysis and quality control for fruit and vegetable products.* Third edition Tata Mcgraw hill publishing Col. Ltd. New Delhi.

Raysad, K.S. 2016. Morpho-Physiological diversity assessment of *Garcinia gummigatta* (L.) Robs. Germplasm collection. M.Sc. (Forestry) thesis, Kerala Agricultural University, Thrissur, 105p.

Sadasivam, S. and Manickam, A. 1996. *Biochemical Methods* (2nd Ed.). New age International Limited, Publisher, New Delhi.

Sawant, D. S., Haldankar, P. M., Nagwekar, D. D. and Rajput, J. C., 1999, screening of kokam (*Garcinia indica* Choisy) genotypes. *Indian J. Areca. Spice Med Plants.* 1 (2): 55-59.

Scott, G., Florentin, L., Nix, D. and Whelan, M.F. 1988. Comparison of monounsaturated fatty acids and carbohydrates for reducing the raised levels of plasma cholesterol in man. *Am. J. Clin. Nutr.*, 47: 965-969.

Senthilkumar, R., Tripathi, P.C., Sankar,V and Karunakaran,G. 2014. Kokum (*Garcinia indica*) : A potential fruits of Western Ghats. Technical Bulletin. ICAR-IIHR, Central Horticultural Experiment Station, Chettalli, Kodagu, Karnataka, 6p.

Shameer, P.S., Rameshkumar, K.B. and Mohanan, N. 2016. Diversity of Garcinia species in the Western Ghats. *Diversity of Garcinia species in the Western Ghats: Phytochemical Perspective.* Jawaharlal Nehru Tropical Botanic Garden and Research Institute Palode, Akshara Offset Press Thiruvananthapuram, India, pp.01-18.

174602

Sharma, B.D., Sanjappa, M. and Balakrishnan, N.P. 1993. Flora of India. *Botanical Survey of India, Calcutta*. 3: 98-131.

Sharma, P.B., Handique, P.J. and Devi, H.S. 2015. Antioxidant properties, physio-chemical characteristics and proximate composition of five wild fruits of Manipur, India. *J. Food Sci. Technol.* 52(2): 894-902.

Sherly, R. 1994. Growth, flowering, fruit set and fruit development in kodumpuli (*Garcinia cambogia*). M.Sc.(Hort.) thesis, Kerala Agriculture University, Vellanikkara, Thrissur.

Shinde, A.K, Godse, S.K., Waghmare, G.M. and Patil, B.P. 2001. Performance of kokum grafts and seedlings during initial years. In: Proceedings of First NationalSeminar on Kokum. RFRS, Vengurle, Maharashtra, India. pp.37-38.

Soetan, K.O., Olaiya, C.O. and Oyewole, O.E. 2010. The importance of mineral elements for humans, domestic animals and plants: A review. *Afr. J. Food Sci.* 4(5): 200-222.

Swami, S.B., Thakor, N.J. and Patil, S.C. 2014. Kokum (*Garcinia indica*) and its many functional components as related to the human health: A review. *J. Food Res. Technol.* 2: 130-142.

Takenaka, A. 1994. Effects of leaf blade narrowness and petiole length on the light capture efficiency of a shoot. *Ecological Research*, 9(2): 109-114.

Thakur, N.S. 2013. Phenology and fruit characterization of *Artocarpus hirsutus* in two altitudinal zones of Thrissur district. M.Sc. (Forestry) thesis, Kerala Agricultural University, Vellanikkara.

Thatte, K.S. and Deodhar, M.A., 2012. Study of flowering behavior and sex determination in *Garcinia indica* (Thomas-Du Pettite) Choisy by means of molecular markers. *Biotechnology*, 11(4): 232-237.

KERALA AGRICULTURAL UNIVERSITY CENTRAL LIBRARY P.O. (P.O.), THRISSUR

119

Tripathi, P.C., Karunakaran,V., Sankar, V., and Senthilkumar, R. 2015. *Garcinia* in India- An introduction. In: Senthilkumar, R., Sankar, V. and Tripathi, P.C. (eds), *Workshop on Garcinia Species*. Souvenier & Abstracts, CHES, Chettalli, Kodagu,Karnatka, pp. 1-6.

Valvi, S.R. and Rathod, V.S. 2011. Mineral composition of some wild edible fruits from Kolhapur district. *Int. J. Appl. Biol. Pharmac. Technol.*2(1): 392-395.

Varanda, E.M. and Primavesi, A.C. 2005. Effect of the inherent variation in the mineral concentration of alfalfa cultivars on aphid populations, *Bragantia*, 64(2):.233-239.

Yapattanphum, C., Subhadrabandhu, S., Sugiura, A., Yonemori, K. and Utsunomiya, N. 2002. Utilization of some *Garcinia* species in Thailand. *Acta Hort.* 575(2): 563-570.

Yash, P.K. 1998. *Handbook of Methods for Plant Analysis*. Taylor and Francis group,LLC. pp. 57-60.

Zadernowski, R., Czaplicki, S. and Naczk, M. 2009. Phenolic acid profiles of mangosteen fruits (*Garcinia mangostana). Food Chemistry*, 112(3):685-68.

MORPHOLOGICAL AND BIOCHEMICAL DIVERSITY ASSESSMENT OF *Garcinia indica* (Thouars) Choisy GERMPLASM .

BY

RAVEENA KAPATIA

(2017-17-013)

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE IN FORESTRY

FACULTY OF FORESTRY

KERALA AGRICULTURAL UNIVERSITY

DEPARTMENT OF FOREST BIOLOGY AND TREE IMPROVEMENT

COLLEGE OF FORESTRY

VELLANIKKARA, THRISSUR- 680 656

KERALA, INDIA

2019

ABSTRACT

A study on 'Morphological and biochemical diversity assessment of *Garcinia indica* (Thouars) Choisy germplasm' was conducted to find out variations in *G. indica* germplasm maintained at NBPGR Regional station, Thrissur. The study attempted to evaluate variability in general tree characters, flower, fruit, biochemical characters and physio-chemical properties of butter.

It was observed that large variability was present in morphometric characters like height, GBH and height of first branch, whereas characters like canopy shape and branching habit did not show much variations. In comparison to the general tree characters, leaf and flower characters showed less variability. Among all the leaf characters studied, petiole length showed largest variation (CV=20.2 percent). In case of flower characters, maximum variability was observed in pedicel length. Variability was also observed in the timing of flowering, which ranged from October to January.

Variability study on fruit characters like fruit weight, rind weight and number of fruits showed significant variations. Yield ranged from 220 to 1050 fruits per tree. Fruit colour did not show much variations, mainly being dark purple and red.

Primary metabolites like carbohydrates, proteins and crude fat showed considerable variability, while it was observed to be comparatively low in TSS and reducing sugar. Variability was also observed in secondary metabolites like phenols (CV = 29.1 percent) and flavanoids (CV = 17.9 percent). It was observed that Hydroxy citric acid was present in large quantity in fruit rind, average being 36.5 g/100g. The variability study on minerals like Ca, Na, Mg, K and P revealed that K was present in the largest quantity, followed by Mg. Maximum variability was observed in P content (CV=24.4 percent) while minimum in K content (CV =9.5 percent). The average vitamin C content was estimated to be 37.17 mg/100g and CV being 27.6 percent.

Variability study on physio- chemical properties of kokum butter showed that on an average 30.55 percent of butter (CV=12 percent) was obtained from the seeds. Less variability was present in the melting and pour point of butter. Chemical properties of butter like saponification value, acid value and iodine value also showed less variability.

Path analysis was conducted and it was observed that a positive direct effect was exerted by the number of fruits, rind weight, fruit weight and flower weight on rind yield. The 16 accessions were grouped into six clusters through

cluster analysis. Cluster III possessed maximum number of accessions whereas the least number was observed for the cluster V and VI.

It can be concluded from the results that a considerable morphological and biochemical variations exist in *Garcinia indica* germplasm, NBPGR Regional Station, Thrissur. These results could be useful in efficient management of *G.indica* germplasm for breeding, conservation and optimal utilization.

174602

KERALA AGRICULTURAL UNIVERSITY
CENTRAL LIBRARY
P.O. (P.O.), THRISSUR