

Chapter 8

Diversity of Malabar Tamarind (*Garcinia gummi-gutta* (L.) N. Robson) in the Western Ghats- Morphological and phytochemical evaluation

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Abstract

Garcinia gummi-gutta (L.) Robs. (Clusiaceae) is an economically important fruit crop and the most widely distributed species in the Western Ghats of Kerala. The diversity of *G. gummi-gutta* in terms of morphological and chemical characters is discussed in this chapter. Three varieties of the species viz; *G. gummi-gutta* (L.) Robs. var. *gummi-gutta*, *G. gummi-gutta* var. *papilla* (Wight) N. P. Sing., and *G. gummi-gutta* var. *conicarpa* (Wight) N. P. Sing., are reported in India. The variety *conicarpa* is morphologically distinct by the absence of leaf ligules and by the arrangement of stamens in a convex torus head, in addition to the conical nature of fruits. The difference in morphological variation has been manifested in chemical constitution as well. Dendrogram based on leaf volatile chemical distribution of the three varieties revealed nearly 75% correlation between var. *gummi-gutta* and var. *papilla*, while variety *conicarpa* showed less than 20% similarity with the other two varieties. HPTLC analysis also showed distinct chemical profile for the variety *conicarpa*. The morphological and chemical variation of *G. gummi-gutta* var. *conicarpa* suggests species status for the variety. The diversity among cultivated accessions of var. *gummi-gutta* is also discussed in detail.

Keywords: *G. gummi-gutta* var. *gummi-gutta*, *G. gummi-gutta* var. *papilla*, *G. gummi-gutta* var. *conicarpa*, Leaf essential oils

Introduction

Garcinia species are an important component of the forest flora of the Western Ghats, with 9 species and 2 varieties, of which 7 species and 2 varieties are endemic to the region. *Garcinia gummi-gutta* (L.) Robs. the most widely distributed species among these, is also an economically important fruit crop of Kerala. The fruits are popularly known as *Malabar tamarind* or *Kudampuli* whose dried pericarp is used as a condiment and is used as an alternative of tamarind to impart a special flavour and taste to curries in Kerala (Anonymous, 1950). Also the fruits are commercially important as a rich source of the much valued anti-obesity phytochemical hydroxycitric acid and several industrial units are located in central Kerala for extracting the value added product from the fruits (Hemesekhar *et al.*, 2011).

Though three varieties are reported, literature review and herbarium specimen analysis revealed ambiguity in proper demarcation of the varieties. In this background, male

and female accessions of the varieties were collected from different parts of the Western Ghats and the present chapter elaborates the morphological features of the varieties along with comparison of chemical profile. Moreover, the diversity among the cultivated variety has also been evaluated critically.

1. Taxonomical history of the *Garcinia gummi-gutta*

Carl Linnaeus described the species *Cambogia gummi-gutta* L., in Gen. Pl., ed. 5: (1754) with a short description and Van Rheedee referred the material as ‘*Coddam-pulli*’ in *Hortus Malabaricus* (Van Rheedee, 1678). A combination nova was proposed for *Cambogia gummi-gutta* L. and *G. cambogia* (Gaertn.) Desr. (Desrous, 1792) by Robson as *G. gummi-gutta* (L.) N. Robson (Robson, 1968). Though Robert Wight proposed *Garcinia conicarpa* Wight [Wight, Icon. (Pl. Ind. Orient. t. 121. 1839 & Ill. Ind. Bot. 1.126. 1840, TYPE: Madras, Shevagherry hills, 1836, ex. Herb, Wight 142 (CAL)], the taxon was further treated by T. Anderson as a variety of *G. cambogia* (Gaertn.) Desr. var. *conicarpa* (Wight) T. Anderson (1874). Wight also collected another specimen from the evergreen forests of the Western Ghats and described the variety *papilla* (Wight, 1840) under *G. cambogia* (Desrous, 1792). Later N. P. Singh (Singh, 1993) proposed combination nova for these varieties as *G. gummi-gutta* var. *conicarpa* (Wight) N.P. Singh, and *G. gummi-gutta* var. *papilla* (Wight) N. P. Singh respectively.

2. Distribution and conservation status of the varieties of *Garcinia gummi-gutta*

The variety *gummi-gutta* is distributed widely in the evergreen forests of Western Ghats ranging, from 400 m to 900 m. It is fairly common and abundant in the forests of western Sri Lanka from sea level to 600 m and in Malaysia also. In Kerala, it is very popular in the Central Travancore areas, where maximum diversity is seen. Field studies revealed that the var. *gummi-gutta* is cultivated all over the low lands and mid lands of Kerala ranging from sea shore to the high lands up to 600 m. The other two varieties are restrictedly endemic to the Western Ghats. Variety *conicarpa* is a high altitude species (1350- 1950 m) distributed rarely in evergreen forests of South Western Ghats (**Table 1**). var. *papilla* is also very rare in the evergreen forests of Southern Western Ghats and found in an altitude of 800-1850 m. Samples of *G. gummi-gutta* var. *papilla* were collected from Silent Valley, Palakkad district and *G. gummi-gutta* var. *conicarpa* were collected from from Kadalar, Rajamala, Kottamala forest regions of Idukki district and Vellarimala, Chembra hills of Wayanad district. Though varieties *papilla* and *conicarpa* were not included in IUCN categories, we suggest both to be included in ‘endangered’ category, based on their restricted distribution within small scattered populations.

3. Morphological features of the varieties of *Garcinia gummi-gutta*

Critical evaluation of morphological characters through detailed qualitative and quantitative characters of male and female accessions of the varieties were carried out (**Table 1, Figure 1**). The demarcating morphological features noted for the varieties are lamina shape, presence of leaf ligule, pedicel length, stamen arrangement, fruit shape and number of fruit grooves in fruits. Based on the distinguishing morphological features of var. *conicarpa* such as absence of leaf ligules, lamina shape, arrangement of stamens in convex torus head, pedicel length, conical nature of fruits and the fibrous nature of arils, the variety *conicarpa* need to be reinstated as species *G. conicarpa*, early proposed by Wight.

G. gummi-gutta var. *gummi-gutta* *G. gummi-gutta* var. *conicarpa* *G. gummi-gutta* var. *papilla*



Figure 1. *G. gummi-gutta* varieties (A-C. Leaves, D-F. Male flowers, G-H. Female flowers, J-K. Fruits)

3.1. Key to *Garcinia gummi-gutta* varieties

- 1 Stamens 12-20, ovary 4-12 locular, stigmatic ray 6-10; berries 6-10 grooves.....**var. *gummi-gutta***
- 1 Stamens more than 20; ovary 3 – or 6- 8 locular, stigmatic rays 3 or 6-8; berries 3 or 6-8 grooves.....**2**
- 2 **2.a** Leaf ligule present; ovary 6-8 locular; stigmatic rays 4-8; berries ovoid-oblong, 4-8 grooved,**var. *papilla***
- 2 **2.b** Leaf ligule absent; ovary 3-5 locular; stigmatic rays 3-5; berries ovoid or conical, 3-5 grooved**var. *conicarpa***

Table 1. Distinguishing characters of *Garcinia gummi-gutta* varieties

Sl. No.	Parameter	var. <i>gummi-gutta</i>	var. <i>papilla</i>	var. <i>conicarpa</i>
1	Branches	Parallel or pendulous drooping	Parallel	Parallel
2	Leaf shape	Elliptical-oblong or obovate	Elliptical	Obovate-ovate rarely oblong or broader beyond the middle
3	Length of petiole	1.5- 2 cm	1. 5 cm	> 1 cm
4	Leaf ligule	Present	Present	Absent
5	Length of Male flower pedicel	1.5-1.7 cm	0. 7 cm	>0.5 cm
6	Length of Female flower pedicel	4-6 mm	Ca.5 mm	sessile
7	Arrangement of Stamen	Globose head	Globose and androphore	Convex torus
8	Number of stamen / flower	12- 20	25 or more	Ca. 35
9	Rudimentary pistil	Present	Absent	Absent
10	Ovary	4-12 locular	6-8 locular	3-5 locular
11	Female flower position	Terminal or axillary	Terminal or axillary	Terminal or subterminal
12	No. of Stigmatic lobes	6-10	3- 8	3- 5
13	Staminodes	10-20	9-12	Ca. 20
14	Fruit shape	Globose	Sub globose	Ovoid- conical
15	Number of groove / Berries	6-10	3-8	4-5
16	Nature of Seed	Covered with pulpy aril	Covered with thick mass of fibrous aril	Covered with thin fibrous aril
17	Number of seeds	4-8	3-5	2-4
18	Seed shape	Ovoid	Sub triangular	Ovate- oblong
19	Flowering	Jan-Mar	Jan-Mar	Apr-Jun
20	Fruiting	Apr-Aug	Apr-Jul	Jul-Oct
21	Habit	Large tree	Large tree	Large tree
22	Habitat (wild)	Semi-evergreen to evergreen forests of Western Ghats at	Endemic to evergreen forests of Western Ghats in between	Endemic to Evergreen forests of Western Ghats in between
23	Cultivation status	Cultivated from sea shore to mid land and up to high land	Wild only	Wild only
24	Altitude (m)	50- 900 m	800-1850 m	1350- 1950 m
25	Distribution status	Common	Rare	Rare

3.2. Morphological diversity of *Garcinia gummi-gutta* var. *gummi-gutta*

Kerala seems to be the centre of diversity of cambogia and wide variations in the morphological characters are observed in the leaves, flowers, fruits and seeds of *Garcinia gummi-gutta* (Tharachand *et al.*, 2015, Abraham *et al.*, 2006). The diversity of var. *gummi-gutta* is more manifested among the cultivars, compared to the wild accessions.



Figure 2. Diversity of *Garcinia gummi-gutta* var. *gummi-gutta* fruits

Fruits of 18 accessions of var. *gummi-gutta*, cultivated in different parts of Kerala extending from coastal region to middle land, were collected and studied for assessing the variability in size and shape (**Table 2, Figure 2**). The large fruit size, pulpy aril and more number of seeds (4-8) per fruit were the favorable features of var. *gummi-gutta* supporting its wide distribution and preference for cultivation over the other two varieties. The processed pericarp of var. *gummi-gutta* is of great value for its delicate taste and flavour and the accessions were evaluated in terms of fruits size, rind thickness, acidity and yield. The average weight of fruits was 173 g. Previous studies on 13 fruit and five seed characters of 51 accessions of Malabar tamarind by Abraham *et al.*, (2006) reported that the variability was found to be maximum for nipple length (74.8%) and minimum for fruit girth (12.8%) and the average fruit weight was 161g.

Usually the branching pattern was horizontal, while pendulous drooping pattern has also been observed rarely. The average size of leaves was 7-12 x 3.5-5 cm while the leaf shape varied considerably from the typical elliptic to broad shapes. The apex and base of leaves were acute and rarely obtuse. The variation was also exhibited in flowers, fruits and seeds morphology. The fruit shape varied from globose, oblong and rarely to discoid shape. The thickness of fruit rind is a detrimental factor in food sector and the thickness varies from 6.25 mm to 16.03 mm among the selected accessions. The fresh weight of fruit was in the range 45.7-173.3 g. The number of grooves over the fruit surface also varied significantly from 5 to 11.

Table 2. Morphological variation in *Garcinia gummi-gutta* var. *gummi-gutta*

Sl. No	Accession	Branching pattern	Leaf shape			Leaf size (cm)	Number of fruit grooves	Fruit rind thickness (mm)	Fruit shape	Fruit wt.	No. of seeds
			Lamina	Apex	Base						
1	Kotta	Horizontal spreading	Elliptic-Ovate	Acute	Acute	6-10 x 4-6	6-9	11.21	Globose, grooves splitted	52.16	2-4
2	Mezhuveli	Horizontal spreading	Elliptic	Acute	Acute	7-13x 4-6	7-9	10.31	Oblong	43.52	1-2
3	Karanikunnu (i)	Horizontal spreading	Elliptic-oblong	Acute-obtuse	Acute	6-10 x 3-4.5	7-8	11.71	Oblong, mamillae	89	5-7
4	Karanikunnu (ii)	Horizontal spreading	Elliptic-ovate	Acute	Obtuse	5-9 x 3-4	7-10	7.2	Oblong	45.68	2-4
5	Karannikunnu(iii)	Horizontal spreading	Elliptic	Acute	Acute	6-9 x 3.5-5	7-10	6.25	Globose-oblong	54.86	4-6
6	Ullanoor	Pyramidal drooping	Elliptic-broad elliptic	Obtusely acute	Acute	7-9 x 3-6	8	11.51	Globose, mamillate	85.28	7
7	Arammulla	Pyramidal drooping	Elliptic	Acute	Acute	6-10 x 3-4	8	13.8	Discoid	99.92	4
8	Kurianipally	Horizontal spreading	Elliptic	Acute	Acute	5-9 x 3.5-4	8	9.2	Oblong	58.42	5
9	Manipuzha	Horizontal spreading	Elliptic	Acute	Acute	6-10 x 3.5-5	8		Globose, grooves splitted	124.82	5
10	Pulikezh	Horizontal spreading	Elliptic	Acute	Acute	5.5-9 x 3.5-4.5	9	13.41	Globose-oblong with mamillae	46.98	7
11	Podiyadi	Horizontal spreading	Elliptic-broad elliptic	Acute	Acute	6-10 x 4-5	6		Globose-oblong with depressed	85.48	3
12	TBG. G.g - 1	Pyramidal drooping					6-8	16.03		198.8	4-5
13	TBG. G.g - 2	Horizontal spreading					6-9			148.94	4-6
14	Karimbam	Horizontal spreading	Elliptic	Acute	Acute		8-11		Globose	58.94	6-9
15	Calicut	Horizontal spreading					8-9		Globose, grooves splitted	66.24	7-8
16	Vaikom 1	Horizontal spreading					6-7		Globose, grooves splitted with mamillae	95.53	5-6
17	Vaikom - 2	Horizontal spreading					7-9		Globose, grooves splitted with mamillae		6-8
18	Wayanad	Horizontal spreading	Ovate-elliptic	Acute	Acute	6-9 x 3-5	5-6	12.7	Oblong		3-4

4. Chemotaxonomical studies of the varieties of *G. gummi-gutta*

The genus *Garcinia* is considered as a taxonomically difficult one due to the complexity and diversity in floral characteristics and differences in the floral architecture were observed even among closely related taxa of *Garcinia* (Sweeney, 2008, Nimanthika and Kaththriarachchi, 2010). Morphological characters are known to be affected by developmental and environmental factors and in the case of *Garcinia* species, an unusual evolutionary plasticity has been generally observed. Incorporation of biosystematics permits classifications using new descriptors and methods that yield more robust inter relations. Chemosystematic studies based on secondary metabolite profile has proven as an efficient supportive tool for plant systematics. The genus *Garcinia* is characterized by the presence of a large number of secondary metabolites such as xanthenes, benzophenones and biflavonoids, in addition to volatile secondary metabolites (Hemshkhar *et al.*, 2011). In the present study, volatile chemical profile as well as non volatile chemical profile was utilized for differentiating the three varieties.

4.1. Volatile chemical analysis of the varieties of *G. gummi-gutta*

Several attempts have been made to evaluate the phylogeny among Clusiaceae members through secondary metabolite profiling (Waterman and Hussain, 1983). Among the secondary metabolites, volatile chemicals can efficiently be utilized for chemotaxonomic purposes (Labra *et al.*, 2004). Most of the Clusiaceae members are known for their oil glands and secretory canals and volatile chemical profiles of several *Garcinia* species have been reported (Rameshkumar *et al.*, 2005, Martins *et al.*, 2008).

In the present work, volatile chemical profiles of the leaves of the female accessions of the three varieties were studied using GC-MS analysis of the essential oils. The essential oils were isolated from fresh leaves by hydrodistillation for 3h using Clevenger type apparatus. The oils were analyzed by gas chromatography methods. GC-FID analysis was carried out on a Varian CP-3800 gas chromatograph equipped with a flame ionization detector (FID) and a CP Sil 8CB fused silica capillary column (30 m × 0.32 mm, film thickness- 0.25 μm). The GC-MS analysis was done on a Hewlett Packard 6890 gas chromatograph fitted with a cross-linked 5% phenyl methyl siloxane HP-5 MS capillary column (30 m × 0.32 mm, film thickness- 0.25 μm) coupled with a 5973 series selective mass detector. The constituents were identified by retention indices calculated using homologues of n-alkanes (C₈-C₂₂) (Dool and Kratz 1963), comparing mass spectra with published data (Adams, 2007) and by mass spectra library search (Wiley 275 and NIST). Similarities among the varieties were studied by hierarchical clustering based on the volatile chemical distribution, using SPSS (ver.16.0).

Thirty eight compounds were identified in the leaf essential oils of 3 varieties and sesquiterpenoids were the predominant compounds (**Table 3**). Comparison of the volatile chemical profile revealed that the variety *conicarpa* possess distinct chemical profile. While α-copaene was the major compound in varieties *gummi-gutta* (30.2) and *papilla* (24.3), var. *conicarpa* possess only 1.5% α-copaene. The content of β-caryophyllene was higher in var. *conicarpa* (18.1) compared to varieties *gummi-gutta* (5.7%) and *papilla* (8.4). Major component of var. *conicarpa* was γ-cadinene (46.2%), which is present in less quantity in varieties *gummi-gutta* (3.4%) and *papilla* (3.4%).

Table 3. Distribution of leaf volatile chemicals in *Garcinia gummi-gutta* varieties

Compound	RI	Gg.vg. F1	Gg.vp. F1	Gg.vc. F1
E- β -Ocimene	1044	1.1	–	–
Terpinolene	1086	0.2	–	–
α -Cubebene	1348	0.4	0.3	–
Cyclosativene	1369	1.3	1.1	1.3
α -Copaene	1374	30.2	24.3	1.5
β -Panasinsene	1382	1.3	0.6	0.1
α -Gurjunene	1409	0.3	–	0.1
β - Caryophyllene	1417	5.7	8.4	18.1
β -Copaene	1430	1.3	1.1	–
γ -Elemene	1434	2.1	1.3	–
α -Guaiene	1437	0.3	–	2.3
cis- Muurolo- 3,5- diene	1448	0.8	–	0.1
Amorpha- 4,11 – diene	1449	0.4	–	7.1
α -Humulene	1452	1.8	0.9	3.7
cis- Cadina-1(6),4- diene	1461	0.9	–	0.7
trans- Cadina- 1(6),4 - diene	1475	0.9	–	–
γ - Muurolene	1478	4.3	6.3	–
Amorpha- 4,7(11) –diene	1480	0.5	0.1	–
β -Selinene	1489	1.1	12.3	–
δ -Selinene	1492	–	1.5	0.7
trans- Muurolo- 4,(14)5 - diene	1493	–	–	1.2
α - Selinene	1498	1.5	13.9	–
α - Muurolene	1500	1.5	2.5	–
Germarene A	1509	0.6	–	–
γ - Cadinene	1513	3.4	3.4	46.2
7- epi- α - Selinene	1520	–	–	1.9
δ - Cadinene	1522	32.4	10.6	10.0
Zonarene	1525	–	0.8	–
trans- Cadina 1,4 diene	1533	0.7	0.5	0.1
α - Cadinene	1537	0.5	0.6	0.5
α - Calacorene	1544	0.5	0.8	1.0
Germarene B	1559	0.3	–	–
Caryophyllenyl alcohol	1570	–	–	0.9
1-epi-Cubenol	1627	–	–	–
α - Muuralol	–	0.4	0.2	–
Cubenol	1645	0.2	–	–
n- Hexadecanol	1874	–	–	0.1
n- Octadecanol	2077	–	–	0.1
Total identified (%)	–	96.9	91.5	97.7
Total identified (No.)	–	30	21	21
Monoterpenoids	–	1.3	nil	nil
Sesquiterpene- hydrocarbons	–	95.0	91.3	96.8
Sesquiterpene-oxygenated	–	0.6	0.2	0.9
Total sesquiterpenoids	–	95.6	91.5	97.7

RRI: Relative retention index calculated on HP-5 column.

Dendrogram based on distribution of volatile compounds (SPSS) in the leaves of the varieties revealed 75% similarity between var. *gummi-gutta* and var. *papilla*, while var. *conicarpa* showed only 20% similarity with the other two varieties (Table 4, Figure 3).

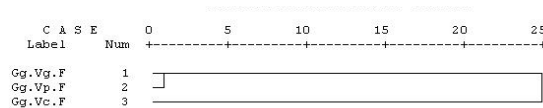


Figure 3. Dendrogram based on distribution of volatile compounds in the leaves of *Garcinia gummi-gutta* varieties

Table 4. Proximity matrix between varieties

Sample	Gg. vg	Gg. vp	Gg. vc
Gg. vg	1.000	.750	.209
Gg. vp	.750	1.000	.173
Gg. vc	.209	.173	1.000

4.2. HPTLC analysis of the varieties of *G. gummi-gutta*

The non volatile chemical profiles of the varieties were studied through HPTLC method. 5 g each of the dried leaf powders were extracted with hexane, followed by methanol in a Soxhlet apparatus for 4 h each. The HPTLC profile of the hexane and methanol extracts were studied using CAMAG HPTLC using the solvent system hexane: ethyl acetate (7:3) for hexane extracts and ethyl acetate: methanol: water (10: 1.7: 1.3) for methanol extract. The developed plates were visualized under UV light, both in long and short wavelengths. The spray reagent used for hexane extract was anisaldehyde-sulphuric acid, while 10% ethanolic KOH and 10% ethanolic phosphomolybdic acid were used as spraying reagents for methanol extracts.

HPTLC profiles of both the hexane and methanol extracts revealed characteristic differences for var. *conicarpa* compared to var. *gummi-gutta* and var. *papilla* (**Figure 4**).

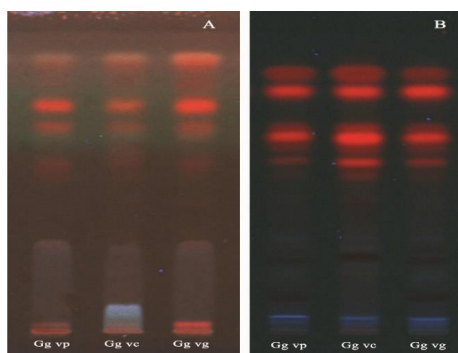


Figure 4. HPTLC profiles of *Garcinia gummi-gutta* varieties.

A. Leaf hexane extract; B. Leaf methanol extract

Conclusions

The chapter provides a comprehensive account on the distribution and diversity of *G. gummi-gutta* in the Western Ghats, combining morphological and phytochemical features. Among the three varieties, var. *papilla*, and var. *conicarpa* are rare and distributed only in the highlands of forests. The diversity of *G. gummi-gutta* var. *gummi-gutta* was more manifested among the cultivars. Evaluation of the morphological and chemical diversity of *G. gummi-gutta* varieties revealed distinct morphological and chemical characteristics for *G. gummi-gutta* var. *conicarpa*, which needs reinstating it as the distinct species, *G. conicarpa* done by Wight. The study supports the hypothesis that the southern Western Ghats is the centre of origin and diversity of *Garcinia gummi-gutta*.

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