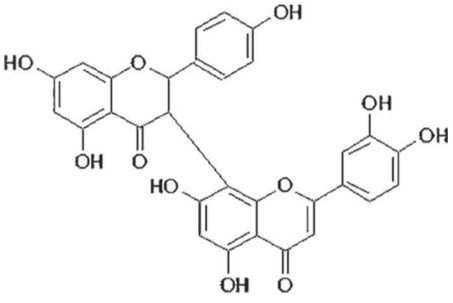
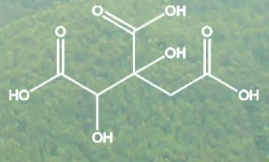
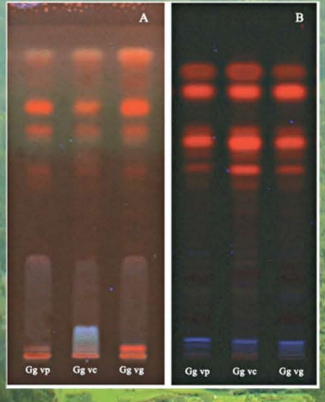
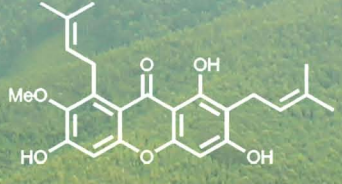
Diversity of *Garcinia* species in the Western Ghats: Phytochemical Perspective

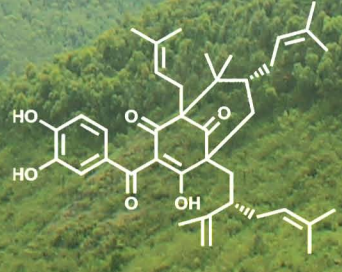
K. B. Rameshkumar











Jawaharlal Nehru Tropical Botanic Garden and Research Institute

Diversity of *Garcinia* species in the Western Ghats: Phytochemical Perspective

Editor

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Thiruvananthapuram

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Editor: K. B. Rameshkumar

Published by: Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Palode, Thiruvananthapuram 695 562, Kerala, India

ISBN No.: 978-81-924674-5-0

Printed at: Akshara Offset, Thiruvananthapuram- 695 035

Foreword

I am delighted to write a Foreword to the Book 'Diversity of *Garcinia* species in the Western Ghats: Phytochemical Perspective' edited by my student Dr. K. B. Rameshkumar who took *Garcinia imberti* as a subject for his doctoral studies. It gives me all the more pleasure and gratification to see that he continued with his studies on *Garcinia* species of the Western Ghats along with his students and colleagues. Unlike many other doctoral students, he kept alive his passion for the studies on *Garcinia* and the present book is the outcome of his dedicated efforts during the last one and a half decades. Pursuit of science is a passion and unravelling the subtleties of nature is an ecstasy which fulfils the inner urge for quest and discovery.

The genus *Garcinia* is important by virtue of their reputation in traditional medicines, established pharmacological activities, diversity in chemical structures and potential nutritional properties. Despite recent progress in phytochemical and pharmacological studies on *Garcinia* species world over, significant gaps still exist concerning the exploration of the vast data on phytochemical diversity of *Garcinia* species. The present book provides a comprehensive and updated report on different aspects including distribution, conservation, morphology, chemotaxonomy, molecular taxonomy and pharmacology of *Garcinia* plants, with emphasis on Western Ghats species. Its specific focus on the Phytochemistry of *Garcinia* species is a great contribution to the lesser known subject Phytochemistry, especially in India. The authors are experts in their relevant field of research, as revealed by the contents and the in-depth presentation of individual chapters. The compiled data may provide useful clues to promote further investigations for the development of new lead molecules and value added products from *Garcinia* species. Furthermore, the book will give basic information on possible conservation strategies for the Western Ghats *Garcinia* plants. I personally am privileged to present this elegant work on 'Phytochemistry of *Garcinia* species' before the scientific community.

Prof. Dr. V. George Ph.D., FRSC
Director
Amity Institute of Phytochemistry and Phytomedicine
Thiruvananthapuram

Preface

The plant kingdom represents an extraordinary reservoir of molecules, that can be beneficial to mankind in several ways and currently there is a worldwide interest in the use of natural products, particularly plant derived products. The Western Ghats, one among 36 global biodiversity hotspots, harbors one of the finest tropical forests in the world. A recent enumeration has identified nearly 7500 flowering plants in the Western Ghats, of which more than 1250 are endemic to the region. Literature review revealed that nearly 80% of the endemic flowering plants of the region are hitherto uninvestigated for their chemical constituents, bioactivities or potential utilities. *Garcinia* species are one among such least explored group of plants, represented by 9 species and 2 varieties in the Western Ghats, of which 7 species and 2 varieties are endemic to the region. The genus *Garcinia* is important as a source of edible fruits, edible fats like kokum butter, oleoresin and coloring agents, the much valued anti-obesity phytochemical hydroxycitric acid (HCA) and other bioactive compounds like biflavonoids and xanthones. Due to the diversity of natural products and the presence of high value compounds, several industrial sectors like pharmaceutical, nutraceutical, paint and food additives are centred around this potential group of trees. In south India, *G. gummi-gutta* and *G. indica* are cultivated for commercial extraction of a variety of products such as bioactive acids, nutraceuticals, fats and condiments.

Literature review reveals that out of the nearly 250 *Garcinia* species, 120 species have so far been investigated for their chemical constituents. *Garcinia* species are found to be rich sources of structurally diverse secondary metabolites such as xanthones, benzophenones and biflavonoids, in addition to flavonoids, biphenyls, phloroglucinols, depsidones and triterpenoids as minor constituents. Though the Western Ghats has a rich diversity of *Garcinia* species, only a few species are exploited sustainably for their potential utilities. The rich floristic wealth can be harvested profitably by taking advantage of the developments in phytochemical analytical techniques. Phytochemistry, being an interdisciplinary subject linked to different disciplines, the present book also includes recent research activities in the fields such as botany, pharmacology and plant biotechnology of the genus. It is expected that the effort will open new vistas of knowledge and prove to be an excellent exposition of current research efforts in India in the field of Phytochemistry.

K. B. Rameshkumar

Acknowledgements

First of all I would like to extend my profound thanks and sincere gratitude to my research guide, Dr. V. George, who introduced me to the fascinating world of plant chemistry. I am also indebted to the taxonomists of JNTBGRI for introducing me to the unexplored and fascinating world of tropical forest flora.

This book is indeed the result of the scholarly inputs from different experts and I would like to extend profound gratitude to all of the authors for their sincere efforts.

I also wish to acknowledge the assistance by the research students Mr. A. P. Anu Aravind and Mr. P. S. Shameer for their enduring effort during the preparation of the book.

This book is produced through the financial support of Kerala State Council for Science Technology and Environment (KSCSTE), SRS project entitled 'Biflavonoids from *Garcinia* species- Chemical, Molecular and Pharmacological Evaluation' (No. 008/SRSPS/2011/CSTE). The support of STP Division, KSCSTE and the advice and suggestions of the experts of SRS-GMW in successful completion of the project is also thankfully acknowledged here.

A special thanks to my family for their understanding and support during the time of producing this book.

K. B. Rameshkumar

Chapter 1

Diversity of *Garcinia* species in the Western Ghats

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Abstract
The Western Ghats, being one of the hotspots of biodiversity, support an enormous plant wealth. The genus *Garcinia* is an important component of the flora of the Western Ghats and is well known for their edible fruits and nutraceutical properties. The present chapter elaborates the diversity and distribution of *Garcinia* species in the Western Ghats. Conservation status of *Garcinia* species of the Western Ghats has also been revised. Field surveys, herbarium examinations and literature references revealed that there are 9 species and 2 varieties of the genus indigenous to the Western Ghats of which 7 species and 2 varieties are endemic to the region. The diversity of floral morphology, leaf morphology and fruit morphology were elaborated along with a dichotomous key to the Western Ghats species.

Key words: *Garcinia*, Clusiaceae, Western Ghats, Diversity, Conservation

Introduction

The dioecious genus *Garcinia* is the largest genus within the family Clusiaceae (formerly Guttiferae) and comprises nearly 250 species world over. *Garcinia* species are generally small or medium sized evergreen trees, (occasionally shrubs: *G. buchneri* Engl.), and are distributed in pantropical regions, with high species richness in South-East Asia (**Figure 1**). The centre of diversity of *Garcinia* species is the Malaysian region, with some species reaching India and the Micronesian islands and also extending to tropical Africa and the Neotropics (Rogers and Sweeney 2007, Stevens, 2007, Jones, 1980, Sharma *et al*., 2013, Nimanthika and Kaththriarchi 2010).

The genus name *Garcinia* honours the Dutch army doctor and naturalist Laurentius Garcin (1683-1752), who described the fruiting specimen of mangosteen collected from Moluccas, the Maluku islands, Indonesia (Garcin, 1733). This species was later named *Garcinia mangostana* by Linnaeus in 1753*,* which became the type species for the genus. The family Guttiferae was created by Jussieu (1789) based on the presence of the exudates secreted from cut stems and leaves. Thereafter, several monumental works such as that of Hooker (1875), Engler (1925), Robson (1961), Whitmore (1973) and Bamps (1978) reviewed the taxonomic status of *Garcinia* in different parts of the world. The first review of Indian *Garcinia* was in the 'Flora of British India', where Anderson describes 30 species in British India and including the pentamerous group also in section *Xanthochymus* (Anderson, 1874).

Maheshwari in 1964, describes 31 species as naturally distributed in India (Maheshwari, 1964). In Flora of India, Singh (1993) included 34 indigenous *Garcinia* species.

India is one among the 12 megadiversity nations of the world. The wide range of climatic and topographical features have resulted in a high level of ecosystem diversity encompassing forests, wetlands, grasslands, deserts, coastal and marine ecosystems, each with unique assemblage of species. The Western Ghats, a mountain range that runs nearly1,600 km, extends from the west coast of peninsular India from the river Tapti in north to Kanyakumari in south. It is perhaps the most important centers of biodiversity and floristic wealth in India. The region is a UNESCO World Heritage Site and also one among the 36 global biodiversity hot spots in the world. Among the 36 global biodiversity hotspots, Western Ghats occupies 5th position in the economic potential of its biological resources. Over 7,500 species of flowering plants, comprising about 27% of the Indian flora, were reported from the region, of which nearly 1250 are endemic to the region (Anonymous, 2014). Moreover, the Western Ghats is the centre of origin and diversity of a number of economically important plants and there exists a variety of wild relatives of important food and spice crops. The rich biodiversity of tropical forest is attributed to a constant amount of energy from the sun, abundant rain fall and year round warmth, which makes life more favourable than any other place on earth.

In India, the genus *Garcinia* is represented by 43 species and 5 varieties, of which 37 species and 4 varieties occur in wild, whereas the rest were introduced into cultivation (Anderson, 1874, Maheshwari, 1964, Singh, 1993, Srivastava, 1994, Mohanan *et al*., 1997, Sabu *et al*., 2013, Sarma *et al.,* 2016). Among the 37 indigenous *Garcinia* taxa, 16 species and 4 varieties are endemic to the country. In India, *Garcinia* species are distributed mainly in three phyto-geographical zones; North East India, the Western Ghats and Andaman and Nicobar Islands. North East India hosts 17 species, of which 2 species and 1 variety are endemic to the region. The Western Ghats hosts 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.

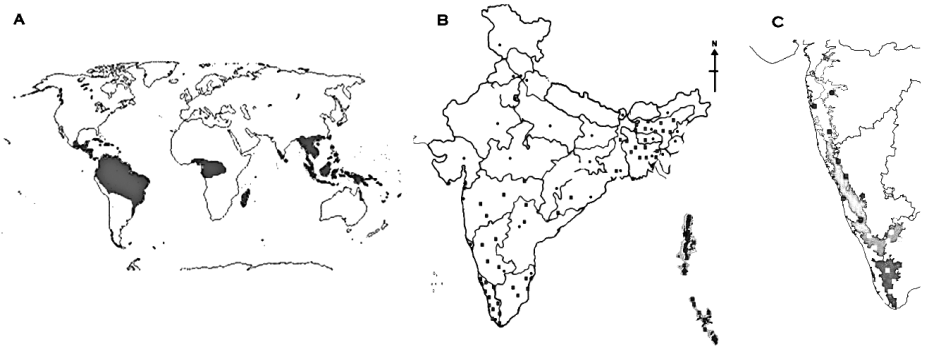

Figure 1. Distribution map of *Garcinia* species in the world (**A**), in India (**B**) and in the Western Ghats (**C**)

1. Distribution of *Garcinia* species in the Western Ghats

In the Western Ghats, most of the *Garcinia* species are distributed in semi evergreen to evergreen habitat, except *G. wightii* which is also found in riparian habitats. Altitude wise they are found from sea shore (*G. gummi-gutta* var. *gummi-gutta*) to high land up to 1500 m (*G. travancorica*). Recent checklist (Nayar *et al*., 2014) reported the natural occurrence of 10 species and 2 varieties of *Garcinia* in the Western Ghats region. However, field survey and detailed study of various flora revealed the presence of 9 species and 2 varieties as indigenous to the Western Ghats, of which 7 species and 2 varieties are endemic to the region (**Table 1**). *G. morella, G. talbotii* and *G. gummi-gutta* var. *gummi-gutta* are the most widely distributed species in the Western Ghats. Our study revealed that Agasthyamala Biosphere Reserve in the Western Ghats is the centre of maximum diversity of the genus, with 6 species of which three species viz., *G. travancorica, G. imberti* and *G. rubro-echinata* are endemic to the region (**Table 1**).

Among the nine species indigenous in the Western Ghats, *G. gummi-gutta* is an economically important and widely cultivated fruit crop in Southern Western Ghats, while *G. indica* is cultivated widely in Central Western Ghats region for their fruits. Besides, 6 introduced species (*G. cowa* Roxb. ex. DC., *G. hombroniana* Pierre, *G. xanthochymus* Hook. f. ex T. Anderson, *G. cymosa* (K. Schum.) I. M. Turner and P. F. Stevens, *G. intermedia* (Pittier) Hammel, *G. mangostana* L.) are also reported as cultivated in the Western Ghats region either as fruit plants or as ornamental plants. *Garcinia mangostana* L., source of the edible fruit mangosteen, is native to South East Asia and now cultivated throughout the Western Ghats for their delicious fruits. *G. hombroniana,* known as sea shore mangosteen, an allied species is getting popular in the Western Ghats region as source of edible fruits. The introduced tree *G. xanthochymus* is also getting popular as a fruit crop and avenue tree.

Garcinia echinocarpa Thw. (1854) was considered as a species distributed in South India and Sri Lanka, until Kostermans (1977) separated the South Indian taxon as a distinct species *viz*. *G. rubro-echinata*. Though later Singh (1993) reduced *G. echinocarpa* var. *monticola* as a synonym of *G. rubro-echinata,* detailed literature survey and examination of type specimens in the present study revealed that *G. rubro-echinata* is distinct from *G. echinocarpa* var. *monticola*.

Garcinia talbotii Raizada ex Santapau was considered as a species distributed in Western Ghats of India and was first reported from Gairsoppah Ghats, North Kanara, Karanataka (Raizada, 1960). This species is closely allied to *Garcinia spicata* Wight and Arn. which is native to Sri Lanka (1875). In most of the Indian Floras, *G. talbotii* has been misidentified as *G. spicata*, which is not naturally occurring in India. Thorough examination of literature, type specimens and live specimens from the Western Ghats, live specimen from AJCB Indian Botanic Garden Kolkata (*G. spicata, Herb.* Wallich 4838, Wight 138) and herbarium specimens housed at various Herbarium like MH, ASSAM, PBL, CAL, FRC, CALI, KFRI and KEW, it was found that *G. talbotii* is distinct from *G. spicata* by the milky exudation turning brownish after exposure, elliptic, ovate-oblong leaf, more number of lateral veins, fascicles or pseudo spikate male inflorescence, number of stamens and stigmatic lobes and globose fruit.

Table 1. *Garcinia* species in the Western Ghats: IUCN status and distribution

Sl. No.	*Garcinia* species	IUCN status	Distribution (altitude, meter)	Locality
1	*G. gummi-gutta* (L.) N. Robson var. *gummi-gutta* N. P. Singh	--	India, Sri Lanka (50- 900 m)	Throughout the evergreen-semi evergreen forests of the Western Ghats
	G. gummi-gutta var. *conicarpa* (Wight) N. P. Singh	--	Endemic to the Western Ghats (1350- 1950 m)	Kerala: Kadllar, Munnar, Rajamala, Chinnar (Idukki); Vellarimala (Kozhikode)
	G. gummi-gutta var. *papilla* (Wight) N. P. Singh	--	Endemic to the Western Ghats (800-1850 m)	Kerala: Wallakkad, Silent Valley (Palakkad) TamilNadu: Nilagiri Biosphere Reserve
2	*G. imberti* Bourd.	EN	Endemic to South Western Ghats (900-1200 m)	Kerala: Agasthyamala Biosphere Reserve (Thiruvananthapuram), Shankily, Shendaruni (Kollam).
3	*G. indica* (Thouars) Choisy	VU	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* (Gaertn.) Desr.	--	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* T. Sabu, N. Mohanan, Krishnaraj and Shareef	--	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* Kosterm.	VU	Endemic to South Western Ghats (800-1200 m)	Kerala: Ponmudi, Chemmunji Hills (Thiruvananthapuram). Tamil Nadu: Kalakkad Mundanthurai Tiger Reserve (Thirunelveli)
7	*G. talbotii* Raizada ex Santapau	--	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* Bedd.	VU	Endemic to South Western Ghats (950-1500 m)	Kerala: Athirumala, Chemmunjii (Thiruvananthapuram). Tamil Nadu: Kalakkad Mundanthuarai Tiger Reservae (Thirunelveli)
9	*G. wightii* T. Anderson	VU	Endemic to South Western Ghats (250-700 m)	Kerala: Vazhachal, Athirappally (Thrissur); Paniyeli-poru (Eranakulam)

VU- Vulnerable, EN- Endangered

According to Anderson (1874), Maheshwari (1964) and Singh (1993) *Garcinia xanthochymus* is distributed in the Western Ghats. However, detailed literature survey, herbarium references and field collections revealed that *G. xanthochymus* is naturally found only in the North East India and Andaman Nicobar Islands. *G. xanthochymus* is cultivated elsewhere in the Western Ghats for its delicious fruits. Most of the specimens identified in Indian Herbaria as *G. xanthochymus,* on close examination revealed to be distinct, which resembles to the new species *G. pushpangadaniana,* reported from Kadalar forest Division of Munnar, Southern Western Ghats of India (Sabu *et al*., 2013).

Garcinia gummi-gutta (L.) Robs. is an economically important fruit crop and a vital component of the forest flora of the Western Ghats. Three varieties of the species *viz*; *G. gummi-gutta* (L.) Robs. var. *gummi-gutta*, *G. gummi-gutta* var. *papilla* (Wight) N. P. Singh and *G. gummi-gutta* var. *conicarpa* (Wight) N. P. Singh are reported from India. Among the three varieties, var. *gummi-gutta* is the most common and economically important one, widely cultivated throughout the Western Ghats region, especially in Kerala, ranging from sea shore to high land and also found in the wild. The variety *conicarpa* and var. *papilla* are rare and distributed restrictedly in highlands of evergreen forest. The large fruit size, pulpy aril and more number of seeds (4-8) per fruit were the favorable features of var. *gummi-gutta* for its wide distribution and preference for cultivation over the other two varieties. The variety *conicarpa* was found morphologically distinct by the absence of leaf ligules and by the arrangement of stamens in convex torus head, in addition to the conical nature of fruits. We suggest reinstating the species status of *G. gummi-gutta* var. *conicarpa* to *G. conicarpa* based on the unique morphological characters.

2. Conservation status

Literature review revealed that *Garcinia* species in the Western Ghats have not been assessed critically for their distribution and conservation and a comprehensive revision on the conservation status of the *Garcinia* species appears to be vital.

G. travancorica, G. imberti and *G. rubro-echinata* are distributed strictly endemic to the forest regions of Agasthyamala Biosphere Reserve, at an altitude ranging from 800-1400 m. According to the guidelines of IUCN Red List and World Conservation Monitoring Centre (Moat, 2007), *G. imberti* Bourd. is an endangered tree species, while *G. travancorica* and *G. rubro-echinata* belongs to 'vulnerable' category. Our field surveys revealed that population size of *G. imberti* is rather larger than that of *G. travancorica* and *G. rubro-echinata*. The two varieties of *G. gummi-gutta*; var. *conicarpa* and var. *papilla* are also very rare in the evergreen forest of Southern Western Ghats, suggesting vulnerable status for these two varieties.

3. Taxonomy

The genus *Garcinia* is considered as a taxonomically difficult one due to the complexity in floral characteristics. While majority of *Garcinia* species are dioecious, a few species or races are reported as hermaphrodite (Dunthorn, 2004). *Garcinia* species generally display an unusual evolutionary plasticity and there are many unresolved phylogenic issues surrounding the genus. Among the different phylogenic analytical strategies, morphology in all its aspects, from micromorphology to embryology, palynology, seed, fruit, floral, stem and leaf

morphology, still remains to be the most indispensible tool. Several identification keys have been reported for *Garcinia* species across the globe based on morphological features of flower, fruit and leaf (Jones, 1980, Nimanthika and Kaththriarachchi, 2010).

3.1. Diversity in floral morphology

Male and female flowers are seen on different trees (dioecious) or rarely male or female and hermaphrodites flowers on the same tree (polygamodioecious) in *Garcinia* species. The basic inflorescence type of *Garcinia* is a simple cyme or few flowered (2 to 16) clusters in fascicles. Exceptions are in the case of *G. travancorica* with trichotomous cyme and *G. wightii* with solitary or rarely 2-3 flowers. Flowers of *Garcinia* are generally sessile except *G. talbotii* and *G. pushpangadaniana* with pedicellate (**Figure 2**). Flowers are solitary or in fascicles, terminal or axillary and variously coloured. Sepals and petals 4-5, stamens usually numerous, very variable in arrangement and structure, sometime with pistillode; ovary 1-12 loculed with a single apical ovule per locule, ovule 1 in each locule; stigma conspicuous and variously lobed, usually peltate. Characteristic differences in the floral architecture were observed even among closely related taxa of *Garcinia* (Pierre, 1883, Jones, 1980, Gustafsson *et al*., 2002, Sweeney, 2008).

Male flowers: Inflorescence of male flowers are observed both in terminal and axillary positions; axillary inflorescence being common. Species like *G. morella*, *G. pushpangadaniana, G. wightii* and *G. gummi-gutta* have flowers in axills, whereas in the case of *G. imberti, G. indica, G. rubro-echinata* and *G. talbotii*, flowers are found both in axillary and terminal position. *G. travancorica* flowers are found only terminal or sub-terminal.

The sepals are usually orbicular and green or yellowish in colour. Species like *G. pushpangadaniana* and *G. talbotii* have ciliate margins. The petals, however, have brighter colour, from yellow (*G. imberti, G. gummi-gutta, G. indica*) to white (*G. talbotii, G. wightii*), cream (*G. morella*), pink or red (*G. pushpangadaniana*), pale greenish (*G. travancorica*) and green (*G. rubro-echinata*). Petal of the male and female flowers of the same species are usually similar, but varies considerably among different species from ovate to oblong, or oblanceolate to obovate. The stamens are always united in a bundle at the centre of the flower. In the case of *G. gunmmi-gutta*, stamens were arranged usually on tetragonous receptacle and also as androphore. In *Garcinia*, pistillodes have a fungiform-shape, consisting of a cap and the shaft (or stipe), which is homologous to the stigma and ovary respectively. The pistillodes are small in diameter, varied from 1 mm for *G. gummi-gutta* to 5 mm for *G. rubro-echinata*. The stipe can be slender or ovoid and the margin of the cap may be crenate or lobed. However, pistillode is lacking in *G. talbotii* and *G. pushpangadaniana*.

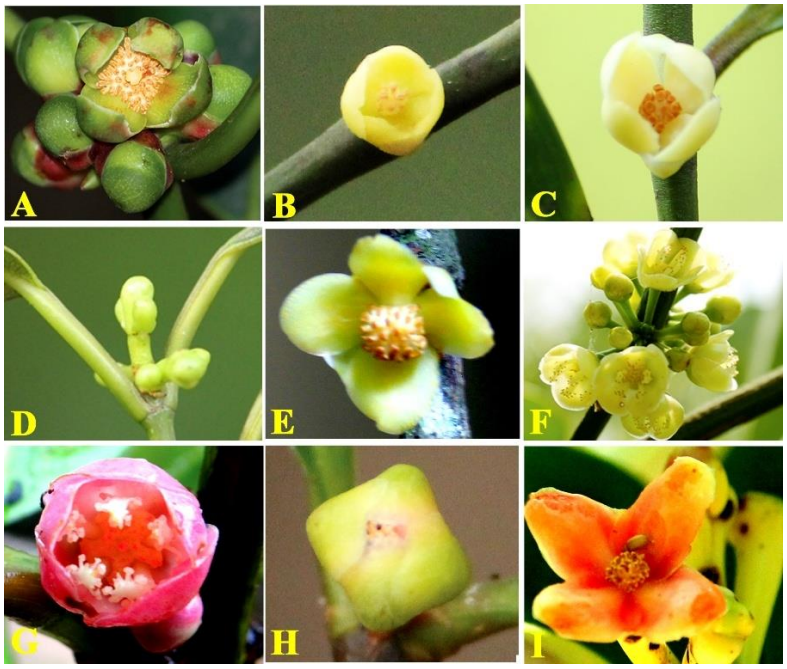

Figure 2. Male flowers of *Garcinia* species in the Western Ghats (**A.** *G. rubro-echinata*, **B.** *G. imberti*, **C.** *G. wightii*, **D.** *G. travancorica*, **E.** *G. morella*, **F.** *G. talbotii*, **G.** *G. pushpangadaniana*, **H.** *G. indica* and **I.** *G. gummi-gutta*)

Female flowers: Inflorescence of female flowers are usually terminal in position. *G. morella, G. pushpangadaniana, G. talbotii* and *G. wightii* have axillary flowers while *G. gummigutta* exhibit both axillary and terminal flowers (**Figure 3**). The female flowers are fewer compared to male flowers and in the case of *G. rubro-echinata, G. imberti* and *G. wightii*, the female flowers are strictly solitary. The female flowers have shorter, stouter pedicels and peduncles comparatively smaller than the male flowers. In general, the ovary in *Garcinia* is superior and very few species have constant locule numbers. Most of the *Garcinia* species have 4 or 5 locules (*G. morella, G. wightii, G. rubro-echinata* and *G. talbotii*) but rarely 1or 2 loculed (*G. imberti, G. travancorica*) and more than 5 loculed (*G. indica, G. gummi-gutta, G. pushpangadaniana*). Generally, ovary is globose to ovoid. Variation is also found in the shape of ovary, however, it has less taxonomic value and is not really an important character for species delimitation in *Garcinia.*

The stigma is usually sessile and wide variation exists. In most species the stigma is large and conspicuous, and in some species like *G. travancorica* and *G. imberti* the stigma is larger than the ovary. Lobes are slightly divided (*G. pushpangadaniana, G. talbotii, G. rubro-echinata* and *G. wightii*) to completely divided in to rays (*G. gummi-gutta, G. indica* and *G. morella*), whereas in some species stigma exists as broad convex disc (*G. travancorica and G. imberti*).

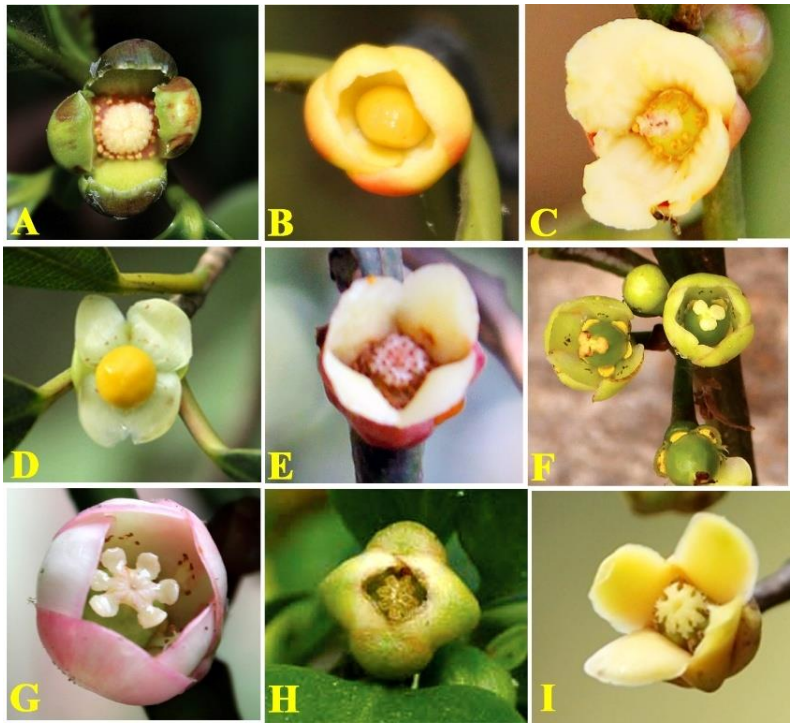

Figure 3. Female flowers of *Garcinia* species in the Western Ghats (**A.** *G. rubro-echinata*, **B.** *G. imberti*, **C.** *G. wightii*, **D.** *G. travancorica*, **E.** *G. morella*, **F.** *G. talbotii*, **G.** *G. pushpangadaniana*, **H.** *G. indica* and **I.** *G. gummi-gutta*)

3.2. Diversity in branching and bark exudates

Garcinia species were characterized by their monopodial branching form, where secondary shoots or branches arise behind the growing point but remain subsidiary to the main stem, which continues to grow indefinitely (Tootil, 1984). Hence *Garcinia* species usually exhibited horizontal spreading branching pattern. However, *G. gummi-gutta* var. *gummi-gutta* and *G. morella* showed pendulous drooping branchlets whereas *G. indica* showed crown shaped canopy ending with horizontal branchlets. *G. pushpangadaniana* has pyramidal crown with pendulous drooping branchlets.

Bark is usually grey to brown, inner bark is yellow or occasionally white. The stem and twigs produce yellow, white or cream exudates, known as 'Gamboge' (**Figure 4**). Gamboge is solidified resin and is sticky in nature and is also found in immature fruit rind and leaves in addition to stem bark. Gamboge is used as a pigment in paint and varnishes. The colour of the exudates varies from yellow to white and is a characteristic identification feature for *Garcinia* species. Species like *G. travancorica, G. morella, G. wightii* and *G. gummi-gutta* have yellow exudation. *G. pushpangadaniana, G. imberti, G. talbotii, G. indica* and *G. rubro-echinata* have white exudation. Gamboge of *G. morella* is widely used in the preparation of golden coloured water colours and spirit varnishes for metals and also for dyeing silk fabrics. A golden yellow coloured ink was prepared from the gamboge of *G. morella* for writing on black paper (Anonymous, 1950).

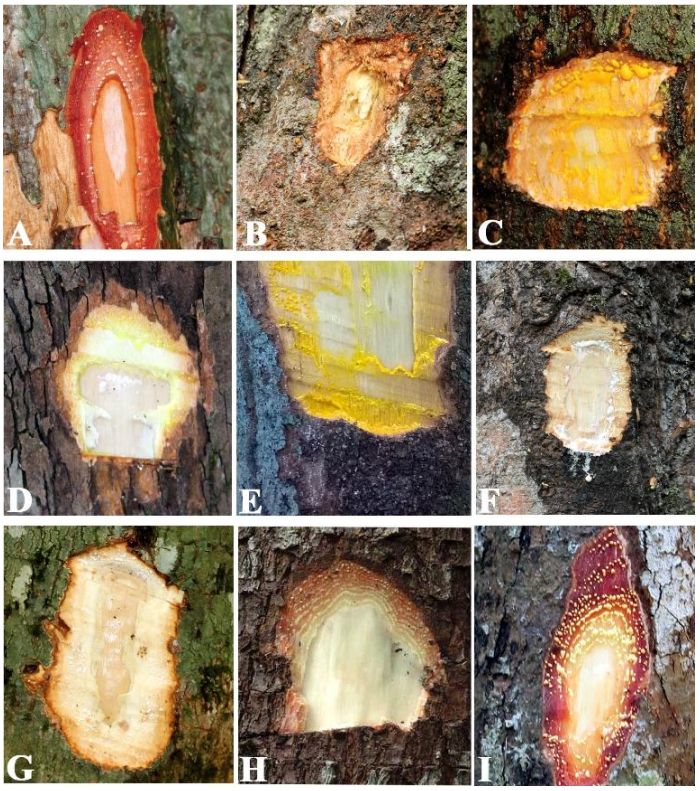

Figure 4. Stem bark exudates in *Garcinia* species in the Western Ghats (**A.** *G. rubro-echinata*, **B.** *G. imberti*, **C.** *G. wightii*, **D.** *G. travancorica*, **E.** *G. morella*, **F.** *G. talbotii*, **G.** *G. pushpangadaniana*, **H.** *G. indica* and **I.** *G. gummi-gutta*)

3.3. Diversity in leaf morphology

Leaves of *Garcinia* species are opposite, usually thick and characterized by the presence of a foveola (an excavation with an extension resembling a ligule) at the base of the petiole. Based on the arrangement of leaf lamina, the Western Ghats species can be classified into two groups, those possess lamina with conspicuous secondary veins and the group with inconspicuous secondary veins. Also, the arrangement of secondary veins falls into two patterns; loose and dense. *G. travancorica* and *G. rubro-echinata* exhibit loosely arranged secondary veins, while all other species showed densely arranged veins. Lamina size and nature of petiole were also distinguishing features. *G. pushpangadaniana* and *G. talbotii* have large leaves (>15 x 8 cm) with stout petiole. Coriaceous leaf texture was prominent in most of the *Garcinia* species except *G. imberti, G. wightii* and *G. indica* which possess subcoriaceous leaves. *G. talbotii* and *G. gummi-gutta* were the two species that showed maximum diversity in leaf shape (**Figure 5**).

Figure 5. Leaf morphology of *Garcinia* species in the Western Ghats (**A.** *G. rubro-echinata*, **B.** *G. imberti*, **C.** *G. wightii*, **D.** *G. travancorica*, **E.** *G. morella*, **F.** *G. talbotii*, **G.** *G. pushpangadaniana*, **H.** *G. indica* and **I.** *G. gummi-gutta*)

3.4. Diversity in fruit morphology

Relatively few investigations have been carried out on fruit and seed morphology of *Garcinia*. Fruits are fleshy to woody berry; seated on the usually persistent calyx. Seed 1-12, often flattened and enclosed in pulp. Regarding fruit size, *G. wightii* has the smallest (10-15 gm), while the largest is that of *G. pushpangadaniana,* weighing upto 750 gm. Most of the fruits are globose in shape except sub-globose to ellipsoid in *G. rubro-echinata*, oblong to sub-globose in *G. imberti* and *G. travancorica*. Texture of fruit surface is another distinguishing feature, where *G. imberti, G. travancorica, G. morella, G. wightii* and *G. indica* possess smooth fruit surface, grooved in *G. gummi-gutta*, warty nature in *G. pushpangadaniana* while the fruit surface of *G. rubro-echinata* is covered with broad sharp tubercles (**Figure 6**). Species like *G. imberti, G. wightii, G. travancorica*, *G. morella* and *G. indica* have pulpy aril while the aril of *G. pushpangadaniana* is crispy and that of

Figure 6. Fruit morphology of *Garcinia* species in the Western Ghats (**A.** *G. rubro-echinata*, **B.** *G. imberti*, **C.** *G. wightii*, **D.** *G. travancorica*, **E.** *G. morella*, **F.** *G. talbotii*, **G.** *G. pushpangadaniana*, **H.** *G. indica* and **I.** *G. gummi-gutta*)

G. rubro-echinata is fibrous. The seed shape was oblong in most of the *Garcinia* species, except plano-convex for *G. pushpangadania*, ovoid-reniform for *G. morella* and *G. gummi-gutta.* The fruit colour is a characteristic distinguishing feature which varies from yellowish green in *G. travancorica, G. imberti* and *G. talbotii*, brownish yellow in *G. pushpangadaniana,* yellow in *G. gummi-gutta,* red in *G. wightii* and *G. morella* and purple in *G. indica.*

4. Key to the *Garcinia* species of the Western Ghats

Vegetative morphological characters among the *Garcinia* species of the Western Ghats were evaluated systematically to construct an identification key, which will be a valuable tool for identification of the Western Ghats species in the field.

1a Fruit surface smooth……2
2a. Fruit less than 3 cm in diam. ……...3
3a Fruit with 2 loculed ovary, rarely one……..4
4a Leaf linear-oblong with distinct closely arranged parallel veins….....***G. travancorica***
4b Leaf oblanceolate indistinct veins…….....***G. imberti***
3b Fruit with more than 2 loculed ovary……...5
5a Leaves linear-lanceolate, fruit size of a small cherry, with pinkish

tinge……..…….***G. wightii***

5b Leaves elliptic, fruit globose or subglobose red……***G. morella***

2b Fruit more than 3 cm in diam……6

6a Stem cut showing white exudation, fruit with stalk, yellowish green in colour……***G. talbotii***

6b Stem cut showing yellow exudation, fruit without stalk, red or dark purple in colour……..…***G. indica***

1b Fruit surface rough…….……7

7a Fruit grooved……...8

8a Leaf ligule absent, fruit with 3-5 grooves, fruit conical……***G. gummi-gutta* var. *conicarpa***

8b Leaf ligule present, fruit with more than 6 grooves, fruit ovoid-oblong or globose…….9

9a Fruit with 6-8 grooves, fruit ovoid-oblong with elongated beak…….…….***G.gummi-gutta* var. *papilla***

9b Fruit with 6-10 grooves, fruit, globose……***G. gummi-gutta* var. *gummi-gutta***

7b Fruit warty or echinate……10

10a Leaves larger than 20 cm, thick coriaceous, indistinct veins, fruit warty, ca. 750 gm……***G. pushpangadaniana***

10b Leaves less than 20 cm, coriaceous, distinct closely parallel veins, fruit with echines, ca. 100 gm……...……..***G. rubro-echinata***

5. Western Ghats *Garcinia* species

5.1. *Garcinia gummi-gutta* (L.) N. Robson

Evergreen tree up to 20 m high; exudation pale yellow, sticky.

Leaves: Elliptic, obelliptic-ovate, 6-13 x 2.5-6 cm.

Male flowers: Tetramerous, 3-8 flowers on axillary fascicles, 1-1.7 x 1-1.2 cm, pedicel 7-12 mm long; sepals orbicular, margin membraneous with fimbril like projections; petals oblong, pale yellow or orange yellow, membraneous on margin; stamens in a globose head; rudimentary pistil absent or if present stigma discoid with 4 lobed cleft.

Female flowers: Tetramerous, solitary or 1-3 fascicle on terminal or axillary, 1.5-2 x 1.5 cm; staminodes 10-20; ovary 4-12 locular, *ca*. 1 mm long, ovule one in each locule, subglobose or ovoid, grooved, stigmatic rays spreading, free nearly to the base, margin crenate, tuberculate.

Fruits: Globose, 6-8 cm in diam., 6-10 grooved, yellow or orange yellow on ripening, pericarp very thick, fleshy.

Seeds: 6-8, ovoid, 2-3.3 x 0.7-0.9 mm, compressed, surrounded by white or red pulpy aril.

Field identification characters

i. Leaves elliptic, 6-13 cm long.
ii. Stigmatic lobes 6-10.
iii. Fruit deeply grooved, grooves 6-10.

Garcinia gummi-gutta* var. *papilla (Wight) N. P. Singh

Evergreen tree up to 15 m high; exudation yellow.

Leaves: Elliptic, 6-9 x 1.5-3cm.

Male flowers: Tetramerous, 3-5 flowers in axillary fascicles, ,1-1.5 x 1-1.2 cm; pedicels stout, 5-7 mm long; sepals ovate to oblong, margin membraneous ; petals oblong, brick red, margin membraneous; stamens in a globose androphore; rudimentary pistil rarely present.

Female flowers: Tetramerous, 1-3 flowers on solitary or fascicles, terminal or axillary, 1-1.2 x 7-10 mm; staminodes in a ring; ovary 6-8 locular, 1-ovule in each locule, subglobose, grooved, stigmatic rays 4-8.
Fruits: Subglobose, yellowish green, ca. 6 cm in diam., 4-8 grooved with a terminal mamilla, pericarp very thick, fleshy.
Seeds: 3-5, sub-triangular, 2-3 x 0.8-10 mm, enclosed in a thick mass of fibrous aril.
Field identification characters

i. Young shoot and margin of leaf shows reddish tinge.
ii. Fruit ovoid-oblong with 4-8 grooves and with terminal mamilla

Garcinia gummi-gutta* var. *conicarpa (Wight) N. P. Singh
Evergreen tree up to 15 m high; exudation yellow.
Leaves: Obovate-ovate, rarely oblong or broader beyond the middle, 6-10 x 4-8 cm.
Male flowers: Tetramerous, solitary or 2-5 flowered fascicles, axillary or terminal, 1-1.5 x 1-1.2 cm, pedicels stout, ca. 5 mm long; sepals ovate, margin membraneous with fimbril like projection; petals yellow, oblong-orbicular, slightly membraneous margin; stamens in a convex torus head; rudimentary pistil absent or present.
Female flowers: Tetramerous, solitary or 2-3 flowered fascicles, terminal or sub terminal, 1-1.5 x 1-3 cm, sessile; staminodes in a ring; ovary 3-5 locular, ovule one in each locule, ovoid, grooved, stigmatic rays 3-5.
Fruits: Usually conical, rarely ovoid, yellowish green, ca. 5 cm in diam., 3-5 grooves with a terminal mamilla, grooves, pericarp very thick, fleshy.
Seeds: 2-4, ovate-oblong, 2-3 x 0.8-10 mm, enclosed in a thin fibrous aril.
Due to the distinct morphological and chemical characteristics, it is suggested that species status may be reinstated for the variety *conicarpa* (Chapter 8).
Field identification characters

i. Absence of leaf ligule on petiole.
ii. Shape of leaf broader beyond the middle.
iii. Conical shape of fruit with 3-5 grooves.

5.2. *Garcinia imberti* Bourd.
Evergreen medium sized tree up to 20 m high; exudation white; branches horizontal spreading.
Leaves oblanceolate, 6-12 x 2-6 cm.
Male flowers: Tetramerous, 3-6 or 9 flowerered fascicles, or rarely cyme or paired, terminal 5-6 x 4-5 mm, sessile; sepals sub orbicular, membraneous; petals orbicular, pale yellow, membraneous; stamens in a central globose mass, pistil rudimentary.
Female flowers: Tetramerous, solitary, or rarely in pairs, terminal, 6-8 x 6 mm; ovary 2-loculed, globose, ovule one in each locule, stigma sessile, convex, capitate; staminodes many, united in a ring around the ovary.
Fruits: Sub-globose, greenish, 2.2-2.5 cm in diam., smooth
Seeds: 1-2, enclosed in a fibrous aril.
Field identification characters

i. Bark brown mottled with white.

ii. Leaves less than 12 cm long, oblanceolate with shortly caudate acuminate at apex.
iii. Berry sub-globose, usually 1-2 seeded fruit, crowned by capitated stigma.

5.3. *Garcinia indica* (Thouars) Choisy

Evergreen to semi-evergreen tree up to 15 m high; exudation milky; branches with conical crown or pendulous drooping.
Leaves: Lanceolate or obovate-oblong, 6-12 x 1.5-5 cm,
Male flowers: Tetramerous, 4-8 flowered fascicles, axillary or terminal, 5-9 x 5-8 mm, pedicel stout, ca. 4 mm long; sepals ovate-rotundate, membraneous; petals orbicular, creamy white, membraneous; stamens inserted on hemispheric, sub-quadrate torus; rudimentary pistil absent or if present as long as stamens.
Female flowers: Tetramerous, solitary, terminal, sub-sessile; ovary, subglobose, stigmas convex, 4-8 rayed, coronate, sessile.
Fruits: Spherical, orange-pink, deep purple when ripe, up to 4 cm in diam., pulp red, fleshy.
Seeds: 5-8, compressed.
Field identification characters

i. Branches with conical crown or pendulous drooping.
ii. Berries smooth, not grooved, deep purple when ripe.

5.4. *Garcinia morella* (Gaertn.) Desr.

Evergreen medium sized tree up to 18 m high; exudation deep yellow, sticky.
Leaves: Elliptic, ovate or obovate, 10-15 x 4-8 cm.
Male flowers: Tetramerous, ca. 3 flowered fascicles, axills of fallen leaves, 1-1.2 x 5-10 mm, sessile or short pedicel, 4-6 mm long; sepals orbicular or elliptic, membraneous; petals rotundate or orbicular, white to pink, membraneous; stamens in a central subglobose mass; rudimentary pistil absent.
Female flowers: Tetramerous, solitary, axillary, ca. 1 x ca. 0.5 cm, sessile; staminodes, connate at base into a ring around ovary; ovary 4-locular, sub-globose; stigma coronate, tubercled.
Fruits: Sub-globose or globose, yellow with reddish tinge, 2.5-3 x 2-3 cm, smooth
Seeds: Ovoid-reniform, 4, laterally compressed and dark brown.
Field identification characters

i. Petiole folding longitudinally above.
ii. Leaves with 8-12 pairs of lateral veins, midrib prominent below and margin revolute and wavy.
iii. Tubercled stigma.

5.5. *Garcinia pushpangadaniana* T. Sabu, N. Mohanan, Krishnaraj and Shareef

Evergreen to semi-evergreen medium sized tree up to 20 m high; bark exudation milky.
Leaves: Elliptic-oblong, 14-20 x 6-8 cm.
Male flowers: Pentamerous, ca. 2-10 flowered fascicles, axillary, 1-1.5 x.1cm, pedicel 7-10 mm long; sepals orbicular-sub-orbicular, margin ciliate; petals orbicular, pinkish pale greenish white, membraneous margin; stamen 5-phalangiate; rudimentary pistil present.

Female flowers: Pentamerous, ca. 2-8 flowered fascicles, axillary, 1-1.5 x 1-1.3 cm; staminodes arranged in 5-phalanges; ovary 6-8 loculed, 6 mm in diam., globose, stigma 6-8 lobed, oblong, stellate.
Fruits: Globose, pale yellowish brown, 13 x 11 cm, fleshy, without pulpy aril, irregularly ridged surface.
Seeds: 1-4, plano-convex, whitish yellow, up to ca. 2 cm long.
Field identification characters

i. Tree with pyramidal crown.
ii. Leaves 14-20 × 6-8 cm long, elliptic-oblong, thick coriaceous, lateral nerves 28-34 pairs.
iii. Large fruits (600-750g), globose and irregularly ridged on the surface.

5.6. *Garcinia rubro-echinata* Kosterm.
Evergreen tree up to 20 m tall; exudate brownish-white.
Leaves: Sub-obovate to broadly elliptic, 8-15 x 3-8 cm.
Male flowers Tetramerous, fascicled, axillary or terminal, pale green, 1.6-2 x 1.5 cm, sessile; sepal orbicular-obtuse, margin membraneous; petals sub-orbicular to oblong, pale green, membraneous; stamens in a tetragonous torus; pistil rudimentary.
Female flowers: Tetramerous, solitary, terminal, pale green, 1.8-2.5 x 1.5-1.8 cm, sessile; staminodes ca.22, connate in to a ring at base, disc present at intercalary position; ovary 3-4 locular, covered with numerous fleshy scales; stigmas peltate, irregularly lobed.
Fruits: Sub-globose or ellipsoid, dark red, 4-6 x 2.5-4 cm, covered with spines or broad tubercles.
Seeds: 1-3, oblong, up to 4cm long with scanty aril.
Field identification characters

i. Bark greenish white with yellow red or white mottles.
ii. Lamina usually obovate with numerous parallel lateral veins.
iii. Fruit covered with spines.

5.7. *Garcinia talbotii* Raizada ex Santapau
Evergreen tree up to 20 m tall; exudate white, turning brownish after exposure.
Leaves: Elliptic-ovate, oblong or ovate-oblong, 7.5-18 x 3-10 cm.
Male flowers: Pentamerous, fascicled, axillary or terminal, creamy-white, 1.8-2.3 cm long, pedicel, ca. 1 cm long; sepal orbicular, margin membraneous, rarely ciliate; petals orbicular-obovate, rarely sub-orbicular, creamy-white or greenish-yellow, margin membraneous; stamens in to 5 phalanges; rudimentary pistil absent.
Female flowers: Pentamerous, fascicled, axillary, creamy-white, 1.8-2.7 cm long, pedicel, ca. 1 cm long; staminodes in 5 delicate phalanges; ovary 3-locular, very rarely 4, globose, stigma peltate, 3 lobed.
Fruits: Globose, greeninsh-yellow on ripening, 4-6 x 3.8-5 cm, fleshy, rind surface shows an yellow resins.
Seeds: 1-3, oblong, ca. 3cm long with yellow pulpy aril.
Field identification characters

i. Exudation milky, turning brownish after exposure.

ii. Leaves usually ovate.
iii. Fruit greenish yellow, ripe fruit pulp sweet-scented, stigmatic lobe 3.

5.8. *Garcinia travancorica* Bedd.

Evergreen tree up to 15 m high; exudate yellow.
Leaves: Linear-oblong, 5.5-10 x 1-2 cm.
Male flowers: Tetramerous, trichotomous short cymes, terminal or sub terminal, 1.2-1.5 x 0.8-1 cm, pedicel short, ca. 2-3 mm long; sepals orbicular, margin membraneous; petals orbicular, creamy white, membraneous; stamens numerous in 4-tetragone masse; rudimentary pistil columnar, with a circular peltate stigma.
Female flowers: Tetramerous, solitary or paired, terminal or sub terminal, 1.3-1.5 x 8-1.2 cm; staminodes in 5-phalanges; ovary 1-2 locular, subglobose or pyriform; stigma 3-lobed and spreading.
Fruits: Ovoid-oblong, 2-3 x 1-2.5 cm, stigma persistent to fruit.
Seeds: Usually 1, rarely 2, ovoid, up to 2-2.5 x 0.7-1 cm.
Field identification characters

i. Leaves narrow oblong, less than 3 cm broad with secondary nerves closely parallel and horizontal.
ii. Male flowers trichotomous cyme.
iii. Female flowers with broad yellow stigma.

5.9. *Garcinia wightii* T. Anderson

Evergreen tree up to 15 m high; exudation deep yellow to orange yellow.
Leaves: Linear-lanceoalte, 6-14 x 1.5-3 cm.
Male flowers: Tetramerous, solitary or 2-3 together, sometimes numerous, axillary, 1-1.2 x 0.8-1 cm, sessile; sepals orbicular, margin membraneous; petals obovate, creamy white, membraneous; stamens in tetragons head.
Female flowers: Tetramerous, solitary, axillary, 1-1.5 x 5-7 mm, sessile; staminodes 4-phalanges; ovary 4-locular, globose; stigma 4-lobed.
Fruits: Sub-globose, rose with pinkish tinged, 1.2-1.5 x 0.9-1 cm, smooth, with persistent stigma and sepals.
Seeds: 4, up to ca. 9.5 x 4.5 mm long.
Field identification characters

1. Leaves less than 3 cm wide, linear-lanceolate tapering at both ends, secondary veins very oblique.
2. Fruit colour rose with pinkish tinge.

Conclusions

Garcinia species are important components of the flora of the Western Ghats and also an economically important group. Field surveys revealed that 9 species and 2 varieties are indigenous to the Western Ghats of which 7 species and 2 varieties are endemic to the region. Distribution, distinguished morphological features and conservation aspects of *Garcinia* species of the Western Ghats were discussed in detail. Agasthyamala forests in the Western Ghats region, with natural distribution of 6 *Garcinia* species, can be considered as the centre of diversity of *Garcinia* species in the Western Ghats.

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Chapter 2

Structural diversity of secondary metabolites in *Garcinia* species

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Abstract
Plants of the genus *Garcinia* produce structurally diverse secondary metabolites such as biflavonoids, xanthones, benzophenones, flavonoids, biphenyls, acyl phloroglucinols, depsidones and terpenoids. The rich diversity in chemical structures made the genus *Garcinia* attractive for the phytochemists. In addition, several industrial sectors such as cosmetic, food, pharmaceutics, neutraceutics and paints are centered around the genus. The genus is represented by more than 250 species, among which nearly 120 species were subjected to phytochemical investigation. A review of the structural diversity of secondary metabolites of *Garcinia* species revealed that xanthones are the important class of secondary metabolites, distributed in 74 *Garcinia* species, followed by benzophenones in 50 species and biflavonoids in 45 species. Biphenyls, acyl phloroglucinols, depsidones and flavonoids are some other interesting group of phenolic compounds in *Garcinia* species. The present chapter enlists the major phenolic compounds reported from *Garcinia* species.

Keywords: *Garcinia*, Secondary metabolites, Xanthones, Biflavonoids, Benzophenones

Introduction

Plants continue to be an important source of diverse chemical structures with broad utilities in several fields like medicines, cosmetics, food, neutraceutics and pesticides. Despite the availability of alternative synthetic substituents, there has been an increasing awareness worldwide towards the use of phytochemicals and other plant derived products. The ever increasing demand for phytochemicals can be attributed to their diverse and complex chemical structures that are difficult to replicate in the laboratory, greater number of chiral centres and increased steric complexity compared to synthetic compounds (Croteau *et al*., 2000, Hostettman and Marston**,** 2002).

The genus *Garcinia* is well known for the value added products such as essential oils, fats, resins and colouring materials. Gamboge, the yellow colouring pigment, is a well known product from *Garcinia* species. Fruits of some *Garcinia* species are rich source of red pigments in the plant kingdom. *Garcinia* fruits are the source for a natural diet ingredient (-) hydroxycitric acid (HCA), which is an anti-obesity compound (Hemshekhar, *et al*., 2011, Parthasarathy, *et al.,*2013).

Recently, *Garcinia* species have received considerable attention worldwide from scientific as well as industrial sectors and several novel structures, bioactivities and potential utilities have been reported. Several industrial sectors like pharmaceutical, nutraceutical,

paint and food additives were centred around this potential group of trees (Hemshekhar, *et al*., 2011, Magadula and Mbwambo 2014). In south India, *G. gummi-gutta* and *G. indica* were cultivated for commercial extraction of a variety of products such as bioactive acids, nutraceuticals, fats and condiments. In USA alone, mangosteen based beverages had a turnover of more than $200 million in 2008.

The genus *Garcinia* is represented by 250 species in the pantropical region, with high species richness in South East Asia. In India, 43 species and 5 varieties of the genus are reported, of which 37 species and 4 varieties occur in wild naturally, while the rest were introduced into cultivation. Nine *Garcinia* species were reported to occure naturally in the Western ghtas, of which 7 are endemic to the region (Sabu *et al*., 2013, Sarma *et al.* 2016). Of the nearly 250 species reported from world over, nearly 120 species were subjected to phytochemical investigation. Though several monographs and reviews on *Garcinia* species have appeared, a compilation of the phytochemistry of the *Garcinia* species has seldom been attempted (Obolskiy *et al*., 2009). Venkataraman (1973) has reviewed the chemistry of pigments from *Garcinia* species. A recent review on phytochemistry of *Garcinia* species in Africa revealed that out of the 80 *Garcinia* species reported in Africa, only 21 species have been investigated phytochemicaly (Magadula and Mbwambo 2014). Literature review revealed that out of the 9 *Garcinia* species reported from the Western Ghats, only 4 species have been studied in detail for their phytochemicals (Pandey *et al*., 2015, Anu Aravind *et al*., 2015).

Garcinia species are reported as rich depository of structurally diverse secondary metabolites such as xanthones, benzophenones and biflavonoids, in addition to flavonoids, biphenyls, acyl phloroglucinols, depsidones and triterpenoids as minor constituents. Volatile mono and sesqui terpenoids, and phenyl proapnoids were also reported from *Garcinia* species. Present chapter review the diversity of phytochemicals, especially the phenolic compounds, reported from *Garcinia* species worldover.

1. Xanthones

Xanthones, with two aromatic rings linked via carbonyl and ether linkages, are a group of secondary metabolites originated biosynthetically by condensation of acetate and shikimate derived moieties. Xanthones can be considered as regioselectively cyclized benzophenone derivatives.The mixed biogenetic origin of xanthone necessitates that the carbons be numbered according to biosynthetic convention (**Figure 1**). Carbons 1-4 were assigned to the acetate derived ring A, while the carbons 5-8 to the shikimate derived ring B (Gottlieb, 1968, Bennett and Lee, 1989).

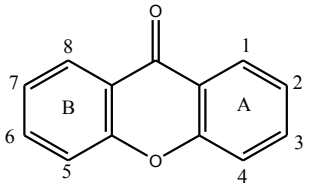


Figure 1. Numbering in typical xanthone structure

Xanthones are limited in distribution to a few plant families such as Clusiaceae, Gentianaceae, Moraceae and Polygalaceae and several reviews on xanthones have been published (Afsal and Al Hassan, 1980, Sultanbawa, 1980; Bennet and Lee, 1989, Peres *et al.,* 2000, Chantarasriwong *et al*., 2010, Anantachoke *et al.,* 2012). *Garcinia* species are important sources of xanthones and literature review revealed that 74 *Garcinia* species, comprising more than half of all the *Garcinia* species studied so far, were reported to contain xanthones (**Table 1**). Among different *Garcinia* species, *G. mangostana* has been studied extensively, and reported to conatin the highest number of xanthones followed by *G. cowa*.

The xanthones isolated can be classified into five major groups: simple oxygenated xanthones, prenylated xanthones, xanthone glycosides, xanthonolignoids, and miscellaneous xanthones (Mandal *et al.,*1992). Simple oxygenated xanthones are subdivided according to the degree of oxygenation into mono-, di-, tri-, tetra-, penta- and hexa-oxygenated xanthones. Isopentenyl and geranyl substituted xanthones are the common types in the genus *Garcinia.* The isopentenyl group may be modified by terminal cyclisation with ortho hydroxyl group to give a chromene system as in the case of jacareubin (Bennet and Lee, 1989). In some cases, the geranyl group may undergo cyclisation leading to structurally intriguing class of secondary metabolites known as caged xanthones, where C ring has been converted into an unusual 4-oxa-tricyclo[4.3.1.03,7]dec-8-en-2-one ring (caged) scaffold (Yang, *et al*., 2012). Caged xanthones like gambogic acid and morellin were mainly reported from the genus *Garcinia* (**Figure 2**). Some of the bixanthones reported from *Garcinia* species are bigarcinenone (*G. xanthochymus*), garcilivins (*G. livingstonei*), garciobioxanthone (*G. oblongifolia*) and griffipavixanthone (*G. griffithi*). Bennet and Lee (1989**)** have pointed that 1,3,5,6 tetraoxygenated xanthones were reported only from African *Garcinia* species and not from any of the Asian *Garcinia* species.

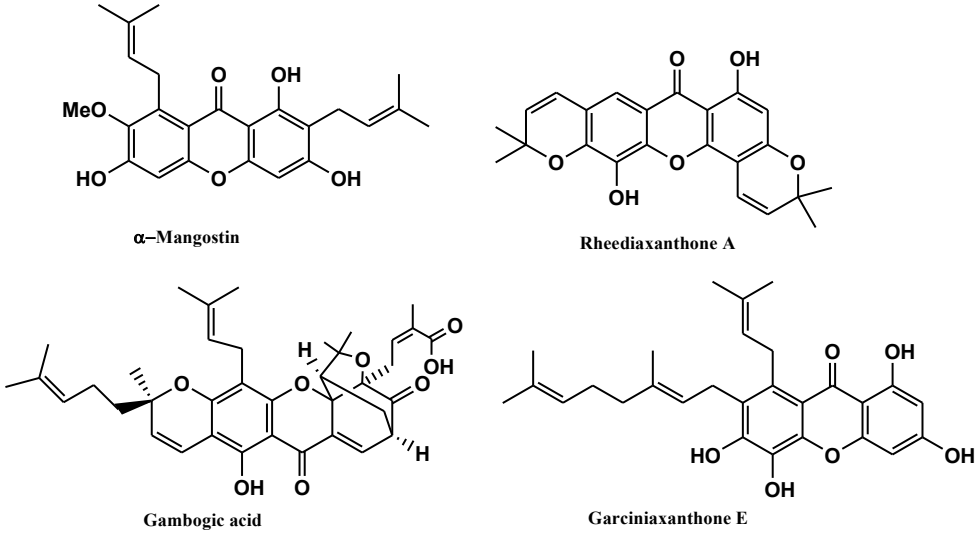


Figure 2. Prenylated xanthone (α-mangostin), xanthone with terminal cyclisation and ortho-hydroxyl group (rheediaxanthone), geranyl substituted xanthone (garciniaxanthone E) and caged xanthone (gambogic acid)

Though complex in structure, Yang, *et al.* (2012) reported the rapid characterization of caged xanthones in the resin of *G. hanburyi* using multiple mass spectrometric scanning modes. The hyphenated approach combining centrifugal partition chromatography (CPC), high-performance liquid chromatography (HPLC) with diode-array detection (DAD) and mass spectrometry (MS) was applied to the fractionation and purification of xanthones from *G. mangostana* fruits, where CPC efficiently separated the metabolites while the structural information was obtained from mass spectral data (Michel *et al*., 2012). A simple UV-Vis spectrophotometry method has been reported for the estimation of xanthones in *G. mangostana*, using α-mangostin, that has absorption maxima at 243.4 and 316.4 nm, as the reference compound (Aisha *et al*., 2013).

Xanthones are attributed with remarkable bioactivities such as antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory and cytotoxic to cancer cells (Chin *et al*., 2008; Peres *et al.,*2000). The xanthone α-mangostin, attributed with antioxidant and anticarcinogenic properties, is one of the active ingredients of nutritional supplements derived from mangosteen (*G. mangostana*) fruits (Gutierrez-Orozco and Failla, 2013). Most of the caged xanthones are reported with potential antitumor activity, with gambogic acid being the best representative and most studied member of this group of compounds (Han and Xu, 2009, Chantarasriwong *et al.*, 2010, Xu *et al.*, 2015). Desoxymorellin, morellic acid, gambogic acid, forbesione, hanburin, and dihydroisomorellin were reported to exhibit anti-HIV-1 activity (Reutrakul *et al.,* 2007). 7-O-methylgarcinone E, cowanin, cowanol, cowaxanthone, and β-mangostin were found to possess *in vitro* antimalarial activity against *Plasmodium falciparum* (Likhitwitayawuid *et al.,*1998). α- and β-Mangostins, and garcinone B exhibited strong inhibitory effect against *Mycobacterium tuberculosis*. Structure activity relationship (SAR) studies showed that tri- and tetra-oxygenated xanthones with di-C5 units or with a C5 and a modified C5 groups are essential for higher activities (Suksamrarn *et al.,* 2003).

Table 1. Xanthones reported from *Garcinia* species

Sl. No.	*Garcinia* species	Plant part	Xanthones	Reference
1	*G. afzelii*	Stem bark	Afzeliixanthones A and B	Waffo *et al*., 2006
2	*G. amplexicaulis*	Stem bark	Cudraxanthone G, 1,3,5-trihydroxy-4-prenylxanthone, nigrolineaxanthone F, and 1,3,7-trihydroxy-2-prenylxanthone	Lavaud *et al*, 2015
3	*G. assigu*	Stem bark	Assiguxanthone A and B, dulxanthone A-D, and latisxanthone A-D	Ito *et al*., 1997
4	*G. atroviridis*	Stem bark	Garcinexanthone G	Tan *et al*.,2016
5	*G. benthamiana*	Leaf	1,3,6,7-Tetrahydroxy xanthone	Amelia *et al*., 2015
		Stem bark	Benthamianone	See *et al*., 2016
6	*G. bracteata*	Leaf	Garcibracteatone, xerophenone C, 5-O-methylxanthone V_1, nemorosonol, and 10-O-methyl macluraxanthone	Thoison *et al*, 2005
		Bark	Neoisobractatins A and B, and bracteaxanthone I and II	Thoison *et al*, 2005

		Stem bark	1,4,5,6-Tetrahydroxy xanthone, bracteaxanthones III-VI, 1,4,6-trihydroxy-5-methoxy-7-prenylxanthone, 1,4,5,6-tetrahydroxy-7,8-di(3-methylbut-2-enyl)xanthone, 1,4,5,6-tetrahydroxy-7-prenylxanthone, 1,4,5-trihydroxyxanthone, 1,4-dihydroxy-5,6-dimethoxyxanthone, garciniaxanthone H, symphoxanthone, 1-O-methylsymphoxanthone, morusignin I, garcinexanthone B, 6-deoxyjacareubin, 1,3,5,6-tetrahydroxyxanthone, 1,3,6,7-tetrahydroxyxanthone, 1,5-dihydroxy-3-methoxyxanthone, 1,5-dihydroxy-3,8-dimethoxyxanthone, 1,7-dihydroxyxanthone, 1,2,5-trihydroxyxanthone, 2,6-dihy droxy-1,5-dimethoxyxanthone, 2,5-dihy droxy-1-methoxyxanthone, 1,2, 5-trihydroxy-6-methoxyxanthone, 12β-hydroxy- D-garcigerrin A, 3-hydroxy-1, 5-dimethoxyxanthone, garciniaxanthone E, 6-deoxyisojacareubin, and garciduol A	Niu *et al*, 2012
		Twig	Neobractatin	Na *et al*, 2010
7	*G. brasiliensis*	Epicarp	1,3,6,7-Tetrahydroxyxanthone	Gontijo *et al*, 2012
8	*G. buchananii*	Heartwood	Buchanaxanthone, 1,5,6-trihydroxyxanthone and 1,5-dihydroxyxanthone	Jackson *et al*., 1968a
9	*G. cantleyana*	Twig	1,3,6-Trihydroxy-5 -methoxy-7-(30-methyl-20-oxo-but-30-enyl)xanthone, 1,3,5-tri- hydroxyxanthone, 1,3,8-trihydroxyxanthone, 2,4,7-trihydroxy xanthone, and 1,3,5,7-tetrahydroxyx anthone	Jantan and Saputri, 2012
		Leaf and trunk bark	Cantleyanone A, 7-hydroxyforbesione, 4-(1,1-dimethylprop-2-enyl)-1,3,5,8-tetrahydroxy xanthone, and cantleyanones B-D	Shadid *et al*, 2007
10	*G. chapelieri*	Bark	Chapexanthone A and B	Rambeloson *et al*, 2014
11	*G. cochinchinensis*	Pericarp	Dulxanthone A, 1,3,5-trihydroxy-6-methoxy-7-(3-methylbut-2-enyl)xanthone, 1,3,5-trihy- droxy-13,13-dimethyl-2H-pyran[7,6-b]xanthen-9-one, and 1,3-dihydroxy-5,6-dimethoxy-7-(3-methyl- but-2-enyl)xanthone	Nguyen *et al*, 2011
12	*G. costata*	Branch	Costatin	Nuangnaowarat *et al*.,2010
13	*G. cowa*	Leaf	Cambogic acid and mangostin	Pandey *et al*., 2015
		Stem bark	Garciniacowol, garciniacowone, parvifoliol F, α-mangostin, β-mangostin , cowaxanthone, norcowanin, cowanin, cowanol, cowagarcinone B, cowagarcinone D, cowagarcinone E, fuscaxanthone A, fuscaxanthone C, 6-O-methylmangostanin, cowaxanthone D, and 1,7-dihydroxyxanthone	Siridechakorn *et al*, 2012
			2-(3-Methyl-2-butenyl)-1,5,6-trihydroxy-3-methoxy-4- (1,1-dimethyl-2-propenyl)-9H-xanthen-9-one, and rubraxanthone	Wahyuni *et al*, 2004

			7-O-Methyl garcinone E	Likhitwitayawuid *et al*.,1997
			Cowanin, cowanol, norcowanin, cowaxanthone, and 1,3,6-trihydroxy-7-methyl-2,5-bis(preny1) xanthone	Thongtheerapap *et al*., 1994
			1,3.6-Trihydroxy-7-methoxy-8-(3,7-dimethyl)-2,6-octadieny xanthone	Lee *et al*.,1977
		Fruit	Cowaxanthones A-E, l,6-dihydroxy-3,7-dimethoxy-2-(3-methyl-2- butenyl)xanthone, fuscaxanthone C, 7-O-methylgarcinone E, β-mangostin, mangostanin, 6-O-methyl mangostanin, α-mangostin, and cowaxanthone	Panthong *et al.*, 2006
			Garcicowanones A and B, 9-hydroxy calabaxanthone, β-mangostin, fuscaxanthone A, cowaxanthone D, cowanin, α-mangostin, cowagarcinone E, and rubraxanthone	Auranwiwat *et al.*, 2014
			Garciniacowones A-E, cowaxanthone,l 3-O-methylmangostenone D, garcinianone A, and garcinianone B	Sriyatep *et al.*, 2015
		Twig	Cowaxanthone F and 1,6-dihydroxyxanthone	Panthong *et al.*, 2009
			β-Mangostin, cowanol, cowanin, norcowanin and 3,6-di-O-methyl-γ-mangostin	Cheenpracha *et al*., 2011
		Flower	Garciniacowones D and E, mangostanin, 6-O-methylmangostanin, fuscaxanthone A, fuscaxanthone C, 7-O-methylgarcinone E, cowaxanthone D, α-mangostin, β-mangostin, 3,6-di-O-methyl-γ-mangostin, and rubraxanthone	Sriyatep *et al.,* 2015
		Root	Kaennacowanols A-C	Kaennakam *et al.,* 2015
		Leaf	Cowaxanthones G and H, 1,3,5-trihydroxy-6′,6′-dimethyl-2*H*-pyrano(2′,3′:6,7)xanthone, 1,5,6-trihydroxy-2-prenyl-6′,6′-dimethyl-2*H*-pyrano(2′,3′:3,4)xanthone, isojacareubin, guttiferone F, jacareubin, xanthone V1, isoprenylxanthone, garcinexanthone C, xanthone V1a, 1,3,5-trihydroxyxanthone, ugaxanthone, 1,5,6-trihydroxy-3-methoxyxanthone, 1,3,7-trihydroxy xanthone, and 1,4,5-trihydroxyxanthone	Xia *et al.,* 2015
		Latex	Cowagarcinone A-E, cowaxanthone, cowanin, cowanol, 1,3,6-trihydroxy-7-methoxy-2,5-bis(3-methyl-2- butenyl)xanthone, mangostinone, and fucaxanthone A	Mahabusarakam *et al.,* 2005
14	*G. cylindrocarpa*	Stem bark	Cylindroxanthones A-C	Sukandar *et al.,* 2016
15	*G. cuneifolia*	Stem bark	Cuneifolin	Ee *et al*., 2003
16	*G. densivenia*	Stem bark	Pyranojacareubin	Waterman and Crichton, 1980
17	*G. dulcis*	Leaf	Dulxanthone E	Kosela *et al.,* 1999
		Fruit	Dulcisxanthone A, l,6-dihydroxy-3,7-dimethoxy-2-	Deachathai *et*

			(3-methyl-2-butenyl)xanthone, cowaxanthone, cowanin, l,7-dihydroxy-3-methoxy-2-(3- methyl-2-butenyl)xanthone, 1,5,8-trihydroxy-3-methoxy-2-(3-methyl-2- butenyl) xanthone, BR-xanthone A, mangostin, 6,8,12-trihydroxy-7-(3-methyl-2-butenyl)- 2-methyl-2-(4-methyl-3-pentenyl)pyrano(20,30:7, 8)xan thone, garcinone D, mangostenol, tovophyllin A, and cratoxylone	*al.,* 2005
18	*G. echinocarpa*	Bark and wood	1,5-Dihydroxyxanthone and 1,3,6,7-tetrahydroxy xanthone	Bandaranayake *et al.,* 1975
		Leaf	Cambogic acid and mangostin acid	Pandey *et al.,* 2015
19	*G. edulis*	Root bark	1,4,6-Trihydroxy-3-methoxy-2-(3-methyl-2-butenyl)-5-(1,1- dimethyl-prop-2-enyl) xanthone and forbexanthone	Magadula, 2010
20	*G. esculenta*	Twig	1,3,5,7-Tetrahydroxy-8-isoprenylxanthone	Zhang *et al.,* 2015
21	*G. eugenifolia*	Twig	5,9-Dihydroxy-8-methoxy-2,2-dimethyl -7-(3-methylbut-2-enyl)pyrano[3,2-b]xanthen-6(2H)-one	Mian *et al.,* 2010
		Heart wood	Euxanthone, gentisin, 1,4,7-trihydroxy-3-methoxyxanthone, 1,5,6-trihydroxyxanthone, and 1,6,7-trihydroxyxanthone	Jackson *et al*., 1969
22	*G. forbesii*	Branch and twig	Forbexanthone, pyranojacareubin, and 1,3,7-trihydroxy-2-(3-methylbut-2-enyl)-xanthone	Harrison *et al.,*1993
23	*G. fusca*	Root	Fuscaxanthone I, β-mangostin, fuscaxanthone A, cowanin, cowaxanthone, α-mangostin, cowanol, isojacareubin, fuscaxanthone G, and 1,3,5,6-tetrahydroxyxanthone	Nontakham *et al.,* 2014
		Stem bark	Fuscaxanthone A-H, cowaxanthone, β-mangostin, cowanin, rubraxanthone, α-mangostin, cowanol, norcowanin, 7-O-methylgarcinone, and garbogiol	Ito *et al.,* 2003a
24	*G. gaudichaudii*	Bark	Gaudispirolactone	Wu *et al.,* 2001
			Gaudichaudiic acids F , G , H and I	Xu *et al.,* 2000
		Leaf	Gaudichaudiones A- H, gaudichaudiic acids A-E, morellic acid, and forbesione	Cao *et al.,* 1998
25	*G. griffithii*	Stem bark	1,5-Dihydroxy-3,6-dimethoxy-2,7-diprenylxanthone and 1,6- dihydroxyxanthone	Elfita *et al.,* 2009
			1,7-dihydroxyxanthone, 1,3,6,7-tetrahydroxyxanthone and 1,3,5,6-tetrahydroxy xanthone	Nguyen *et al.,* 2005
			Griffipavixanthone	Xu *et al*., 1998
		Leaf	1,3,5,6-Tetrahydroxy-7-(3-methylbut-2-enyl)xanthone and rubraxanthone	Alkadi *et al.,* 2013
26	*G. gummi-gutta* (*G. cambogia*)	Leaf	Cambogic acid and mangostin	Pandey *et al.,* 2015
		Root	Garbogiol	Iinuma *et al*., 1998 Semwal *et al.,* 2015
		Bark	Rheediaxanthone	Semwal *et al.,* 2015

		Fruit	Oxy-guttiferone K , oxy-guttiferone K2 and oxy-guttiferone Oxy-guttiferones M, K, K2 and I	Masullo *et al*, 2010 and Semwal et al., 2015
27	*G. hanburyi*	Resin	Garcinolic acid, 10α-ethoxy-9,10-dihydromorellic acid, and 10α-ethoxy-9,10-dihydrogambogenic acid	Deng *et al.,* 2012
			Gambogic aldehyde	Wang *et al.,* 2008
			Forbesione, isomorellic acid, morellic acid, R-30-hydroxygambogic acid, S-30-hydroxygambogic acid, isogambogenic acid, gambogenic acid, R-isogambogic acid, S-isogambogic acid, R-gambogic acid, S-gambogic acid, desoxymorellin, isogambogenin and isomorellinol	Zhou *et al.,* 2008a
			Forbesione, forbesionic acid, isoforbesionic acid, desoxygaudichaudione A, gaudichaudionol, isogaudichaudionol, epoxylgaudichaudione A, gaudichaudione A, isogaudichaudione A, gaudichaudionic acid, isogaudichaudionic acid, desoxymorellin, morellinol, isomorellinol, morellin isomorellin, morellic acid, isomorellic acid, desoxygambogenin, gambogeninol, isogambogeninol, gambogenin, isogambogenin, gambogenic acid, isogambogenic acid, dihydrodesoxygambogenin S-gambogic acid,R-gambogic acid, S-30-hydroxygambogic acid, R-30-hydroxygambogic acid, R tetrahydrogambogic acid, and hanburin R	Yang *et al.,* 2012
		Latex	Gambogin, morellin dimethyl acetal, isomoreollin B, moreollic acid, gambogenic acid, gambogenin, isogambogenin, desoxygambogenin, gambogenin dimethyl acetal, gambogellic acid, hanburin, gambogic acid, isomorellin, morellic acid, and desoxymorellin	Asano *et al.,* 1996
			Isogambogenic acid, desoxymorellinin, 10-methoxygambogenic acid, 10-methoxygambogic Acid, and 10-ethoxy gambogic acid	Feng *et al*., 2007
28	*G. hombroniana*	Leaf	Cambogic acid and mangostin	Pandey *et al.,* 2015
		Twig	Garcihombronones A-D	Klaiklay *et al.,* 2013
		Bark	1,3,6-Trihydroxy-7-methoxy-2,8-(3-methyl- 2-butenyl) xanthone	Jamila *et al.,* 2014
			1,3,6,7-Tetrahydroxy xanthone	Jamila *et al.,* 2014a
29	*G. indica*	Leaf	Cambogic acid and mangostin	Pandey *et al.,* 2015
30	*G. linii*	Root	1,5-Dihydroxy-6-methoxy xanthone and 1,7-dihydroxy-3-methoxy xanthone	Chen *et al*., 2006
31	*G. lancilimba*	Stem bark	1,5,6-Trihydroxy-6',6'-dimethyl-2H-pyrano(2',3':3,4)-2-(3-methylbut-2-enyl) xanthone	Yang *et al.,*2007

			and 1,6,7-trihydroxy-6',6'-dimethyl-2H-pyrano(2',3':3,2)-4-(3-methylbut-2-enyl) xanthone	
32	*G. lateriflora*	Stem bark	Isomoreollic acid, isogaudichaudiic acid, isogaudichaudiic acid E, 11,12-dihydro -12-hydroxy morellic acid, and isogaudichaudiic acid B	Ren *et al.,* 2010
33	*G. livingstonei*	Root bark	1,4,5-Trihydroxy-3-(3-methylbut-2-enyl)-9H-xanthen-9-one, 1,4,5-Trimetkoxy-3-(3-methylbut-2-enyl)-9H-xanthen-9-one, 3,4-dihydro-6,1l-dihydroxy-2,2-dimethyl-pyrano[3,2-c]-xan-then-7(2H)-one, 6,11-dihydroxy-2,2-dimethyl-pyrano [3,2-c] xanthen-7(2H)-one, and 6,l l-dihydroxy-3-methyl-3-(4-methylpent-3-enyL)- 3H,7H-pyrano[2,3-c] xanthen-7-one	Sordat-Diserens *et al.,* 1992a
			Garcilivin A-C	Sordat-Diserens *et al.,* 1992
34	*G. lucida*	Stem bark	1,2-Dihydroxy xanthone and 1-hydroxy-2-methoxy xanthone	Momo *et al*., 2011
35	*G. malaccensis*	Stem bark	α and β-Mangostins	Taher *et al.,* 2012
36	*G. mangostana*	Leaf	Cambogic acid and mangostin	Pandey *et al.,* 2015
			Gartanin	Sen *et al.,*1980
			1,5,8-Trihydroxy-3-methoxy-2[3- methyl-2-butenyl] xanthone, and 1,6-dlhydroxy-3- methoxy-2[3-methyl-2-butenyl]xanthone	Parveen and Khan,1988
		Pericarp	Mangostinone, α, β and γ-mangostins, gartanin, garcinone E, 1,5-dihydroxy-2-(3-methylbut- 2-enyl)-3-methoxy xanthone, and 1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-methoxyxanthone	Asai *et al.,*1995
			1,3,7-Trihydroxy-2-(3-methyl-2-butenyl)-8-(3-hydroxy-3-methylbutyl)-xanthone, 1,3,8-trihydroxy-2-(3-methyl-2-butenyl)-4-(3-hydroxy-3-methylbutanoyl)-xanthone, garcinones C and D, gartanin, xanthone I, and γ-mangostin	Xu *et al.,* 2014
			3-Hydroxy-6-methoxy-5'-isopropyl-4',5'-dihydrofuro [2',3' : 7, 8]-6",6"-dimethyl-4",5"-dihydropyrano[2",3" : 1,2]xanthone, and 1,6-dihydroxy-7-methoxy-8-(3-methylbut-3-enyl)-6',6'-dimethyl-4',5'-dihyd ropyrano[2'3'' : 3,2] xanthone	Zhao *et al.,* 2012
			Garcimangosxanthone A-C, α-mangostin, γ-mangostin, garcinone C and D, trapezifolixanthone, 8-deoxygartanin, gartanin, 2-(γ,γ-dimethylallyl)-1,7-dihydroxy-3-methoxyxanthone 1,5-dihydroxy-3-methoxy-2-prenylxanthone garcinone B, 9-hydroxycalabaxanthone, dulxanthone D and 1,3,7-trihydroxy-2-(3-methylbut-2-enyl)-xanthone and tevophyllin A	Zhang *et al.,* 2010a
			8-Hydroxycudraxanthone G, mangostingone [7-methoxy- 2-(3-methyl-2-butenyl)-8-(3-methyl-2-oxo-3-butenyl)-1,3,6-trihydroxyxanthone, cudraxanthone G, 8-deoxygartanin, garcimangosone B, garcinone D, garcinone E, gartanin, 1-	Jung *et al.,* 2006

			isomangostin, R-mangostin, γ-mangostin, mangostinone, smeathxanthone A, and tovophyllin A	
			Garcimangosxanthone F-G	Zhou *et al*., 2015
			Garcimangosxanthone D-E	Zhou *et al*., 2011
			1,3,6-Trihydroxy-2-(3-methylbut-2-enyl)-8-(3-formyloxy-3-methylbutyl)xanthone	Xu *et al*., 2016
			3-Isomangostin, 8-desoxygartanin, gartanin, α-mangostin, 9-hydroxycalabaxanthone, and β-mangostin	Ji *et al.,* 2007
			3-Isomangostin, 8-desoxygartanin, gartanin, α-mangostin, 9-hydroxycalabaxanthone, and β-mangostin	Ji *et al.,* 2007
			Mangostin, Gartanin, ϒ-Mangostin, β-mangostin, 3-isomangostin, 3-isomangostin hydrate and 1-isomangostin hydrate	Mahabusakaram *et al*., 1987
		Fruit	1,2-Dihydro-1,8,10-trihydroxy-2-(2-hydroxypropan-2-yl)-9-(3-methylbut-2-enyl)furo[3,2-a]xanthen-11-one, 6-deoxy-7-demethylmangostanin, 1,3,7-trihydroxy- 2,8-di-(3-methylbut-2-enyl)xanthone, mangostanin, and α-mangostin	Chin *et al.,* 2008
			3-Isomangostin, mangostanol, 8-deoxygartanin gartanin, α-mangostin, garcinone E, 9-hydroxy calabaxanthone, and γ-mangostin	Zarena and Sankar, 2009
			α-Mangostin, γ-mangotsin,gartanin,1-isomangostanin, garcinone E, and tilirosidea	Quan *et al.,* 2010
		Fruit hull	Garcinones A, B and C	Sen *et al.,* 1982
			Mangostenol, mangostenone A, mangostenone B, trapezifolixanthone, tovophyllin B, α and β-mangostins, garcinone B, mangostinone, and mangostanol	Suksamrarn *et al.,* 2002
			α and γ-Mangostin	Chen *et al.,* 2008
			BR-Xanthone A and B	Balasubramanian and Rajagopalan, 1988
			Mangostanol, α-mangostin, γ-mangostin, gartanin, 8-deoxygartnin, 5,9-dihydroxy-2,2-dimethyl-8-methoxy-7-(3- methylbut-2-enyl)-2H,6H-pyrano[3,2-b]xanthen-6-one, garcininone E, and 2-(γ,γ-dimethylallyl)-l,7-dihydroxy-3-methoxyxanthone	Chairungsrilerd *et al.,* 1996
			β-Mangostin, 9 hydroxy calabaxanthone, mangostanol, mangostenone F, allanxanthone E, α-mangostin, mangostingone, garcinone D, γ-mangostin, mangosenone G, cudraxanthone, 1,5,8-trihydroxy-3-methoxy-2-(3-methylbut-2-enyl)xanthone, 8- deoxygartanin, gartanin, smeathxanthone A, and 1,3,6-trihydroxy-7-	Ryu *et al.,* 2011

			methoxy-2-(3-methylbut-2-enyl)-8-(2-oxoethyl)-9H-xanthen-9-one	
			2,7-Di- 3-methylbut-2-enyl -1,3,8-trihydroxy-4-methyl xanthone and 2,8-di- 3-methylbut-2-enyl -7-carboxy-1,3-dihydroxyxanthone	Gopalakrishnan *et al.,* 2000
			1,3,6,7-Tetrahydroxy-2,8-(3- methyl-2-butenyl) xanthone and1,3,6-trihydroxy-7-methoxyl-2,8-(3-methyl-2- butenyl) xanthone	Yu *et al.,* 2007
			Garcimangosone A, garcimangosone B, and garcimangosone C	Huang *et al.,* 2001
			1,5-dihydroxy-2-(3-methyIbut-2-enyl)-3-methoxyxanthone and 1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-methoxyxanthone	Sen *et al.,*1981
			Mangostin, BR-xanthone A, gartanin, β-mangostin γ-mangostin, and garcinone D	Gopalakrishnan *et al.,* 1997
			Gartanin, 8-deoxygartanin, normangostin, α-mangostin, and β-mangostin	Govindachari *et al*., 1971
			Mangostin	Yates and Stout, 1958
		Seed case	β-Mangostin, 9-hydroxy calabaxanthone, mangostanone, α-mangostin, garcinone D, γ-mangostin, cudraxanthone, 8-deoxygartanin, gartanin, smeathxanthone A, and mangostenone F, G	Ryu *et al.,* 2010
		Heartwood	Mangoxanthone, dulxanthone D, 1,3,7-trihydroxy-2-meth- oxyxanthone, and 1,3,5-trihydroxy-13,13-dimethyl-2H-pyran[7,6-b]xanthen-9-one	Nguyen *et al.,* 2005
			α-Mangostin, β-mangostin, γ- mangostin, garciniafuran, 1-hydroxy-8-(2-hydroxy-3-methylbut-3-enyl)- 3,6,7-trimethoxy-2-(3-methylbut-2-enyl)-xanthone, 1,6-dihydroxy-2-(2-hydroxy-3-methylbut-3-enyl)- 3,7-dimethoxy-8-(3-methylbut-2-enyl)-xanthone, 1,6-dihydroxy-8-(2-hydroxy-3-methylbut-3-enyl)- 3,7-dimethoxy-2-(3-methylbut-2-enyl)-xanthone, 1-hydroxy-3,6,7-trimethoxy-2-(2-hydroxy-3- methylbut-3-enyl)-8-(3-methylbut-2-enyl)-xanthone, 1,3-dihydroxy-2-(2-hydroxy-3-methylbut-3-enyl)- 6,7-dimethoxy-8-(3-methylbut-2-enyl)-xanthone, mangostanin, (16E)-1,6-dihydroxy-8-(3-hydroxy-3-methylbut-1- enyl)-3,7-dimethoxy-2-(3-methylbut-2-enyl)-xanthone , 6-O-methylmangostanin, (16E)-1-hydroxy-3,6,7-trimethoxy-2-(3-methylbut- 2-enyl)-8-(3-hydroxy-3-methylbut-1-enyl)-xanthone, 1,6-dihydroxy-3,7-dimethoxy-2-(3-methylbut-2- enyl)-xanthone, 1-hydroxy-3,6,7-trimethoxy-2-(3-methylbut-2- enyl)-8-(2-oxo-3- methylbut-3-enyl)-xanthone, and 1-hydroxy-3,6,7-trimethoxy-2-(3-methylbut-2- enyl)-xanthone	Nilar and Harrison, 2002
		Aril	Mangostin, Calbaxanthone, Demethylcalbaxanthone, 2-(γ,γ-dimethylallyl)-l,7-dihydroxy-3- methoxyxanthone and 2,8-bis-(γ,γ-dimethylallyl)-l,3,7-trihydroxyxanthone	Mahabusakaram *et al*., 1987

		Aril and pericarp	1,7-Dihydroxy-3-methoxy- 2-(3-methylbut-2-enyl)xanthone, γ-mangostin, 8-deoxygartanin 1,3,7-trihydroxy-2,8-di- (3-methylbut-2-enyl)xanthone, 1,3,7-trihydroxy-2,8-di- (3-methylbut-2- enyl)xanthon gartanin, α-mangostin, and garcinon E	Wittenauer *et al.,* 2012
		Stem and root	2,6-Dihydroxy-8-methoxy-5-(3- methylbut-2-enyl)-xanthone	Ee *et al*., 2006
		Stem	Mangosharin , (2,6-dihydroxy-8-methoxy-5-(3-methylbut-2-enyl)-xanthone), α -mangostin, β-mangostin, garcinone D, 1,6-dihydroxy-3,7-dimethoxy-2-(3-methylbut-2-enyl)-xanthone, mangostanol and 5,9-dihydroxy-8-methoxy-2,2-dimethyl-7-(3-methylbut-2-enyl)-2H,6H-pyrano-[3,2-b]-xanthene-6-one	Ee *et al*., 2006
		Stem bark	Mangaxanthone B, mangostanin, and mangostenol	See *et al.,* 2014
			11-Hydroxy-3-O-methyl-1-isomangostin,11-hydroxy-1-isomangostin, 11α-mangostanin, 3-isomangostin, α-mangostin, β-mangostin, garcinone D , 9 hydroxy calabaxanthone, 8-deoxygartanin, gartanin, and cratoxyxanthone	Han *et al.,* 2009
		Root bark	β-Mangostin, α-mangostin, garcinone-D, mangostanol, and gartanin	Ee *et al*.,2006
			Mangostin and β-mangostin	Govindhachari *et al*., 1971
		Latex	Mangostin and β-mangostin	Govindachari *et al*., 1971
37	*G. merguensis*	Bark	Merguenone, 1,5-dihydroxy-60-methyl-60-(4-methyl-3-pentenyl)- pyrano(20,30:3,2)-xanthone, subelliptenone H, 8-deoxygartanin, rheediaxanthone A, morusignin G, 6-deoxyjacareubin, 1,3,5-trihy-droxy-4,8-di(3-methylbut-2-enyl)-xanthone, rheediachromenoxanthone, and 6-deoxyisojacareubin	Nguyen *et al.,* 2003
		Twig	Merguensinone and 1,5,6- trihydroxy-2-prenyl-60,60-dimethyl-2H-pyrano(20,30:3,4)xanthone	Trisuwan *et al.,* 2013
		Wood	5-Farnesyltoxyloxanthone B, α-mangostin, rubraxanthone, and isocowanol	Kijjoa *et al.,* 2008
38	*G. morella*	Seed	Morellin	Rao 1937, Rao and Natarajan 1950 and Kartha *et al*., 1963
		Pericarp	Morellin	Karanjgaokar *et al.,* 1967
		Leaf	Cambogic acid and mangostin	Pandey *et al.,* 2015
39	*G. nervosa*	Stem bark	Nervosaxanthone	Ampofo and Waterman, 1986
40	*G. nobilis*	Stem bark	Caroxanthone, 4-prenyl-2-(3,7-dimethyl-2,6-octadienyl)-1,3,5,8-tetrahydroxyxanthone, smeathxanthone A, gartanin, euxanthone, 8-hydroxycudraxanthone G, and morusignin I	Fouotsa *et al.,*2012

41	*G. nigrolineata*	Leaf	Nigrolineaxanthones J-S	Rukachaisirikul *et al.,* 2003
		Stem bark	Nigrolineaxanthones A–I, 1,3,5-trihydroxy-4-(3-hydroxy-3-methylbutyl)xanthone, 1,3,7-trihydroxy-2-(3-hydroxy- 3-methylbutyl)xanthone, 6-deoxyjacreubin, morusignin C, 1,5-dihydroxy-6',6'-dimethylpyrano[2',3':3,2] xanthone, and tovoxanthone	Rukachaisirikul *et al.,* 2003c
42	*G. nitida*	Stem bark	1,6-Dihydroxy-5-methoxy-6,6-dimethylpyrano[2',3':2,3]-xanthone, inophyllin B, osajaxanthone, 3-isomangostin, and rubraxanthone	Ee *et al.,* 2012
43	*G. nujiangensis*	Twig	Nujiangexanthones C-F, jacareubin, guttiferone F, cudratricusxanthone E, and garcihombronone B	Tang *et al.,* 2015
		Leaf	Nujiangexanthones A and B	Xia *et al.,* 2012
44	*G. oligantha*	Stem	Oliganthins A-D and gaudichaudione H	Gao *et al.,* 2012
		Leaf	Oliganthin H, I, K and L, oliganthic acids A-C, oliganthaxanthone A, oliganthaxanthone B, gaudichaudione H, and cantleyanone	Tang *et al*., 2016
		Stem bark	Macluraxanthone	Waterman and Crichton 1980b
45	*G. opaca*	Leaf	Macluraxanthone, 1,3,5-trihydroxy-6',6'-dimethylpyrano-(2,3':6,7)-4-(l,l-dimethylprop-2-enyl)xanthone, 1,3,5-trihydroxy-6′,6′-dimethylpyrano(2′,3′:6,7)-2-(3-methylbut-2- enyl)-4-(1,1- dimethylprop-2-enyl)xanthone, and 4″,5″-dihydro-1,5-dihydro-1,5-dihydroxy-6′,6′-dimethylpyrano(2′,3′:6,7)-2-(3- methylbut-2-enyl)-4″,4″,5″-trimethylfurano(2″,3″:3,4) xanthone	Goh *et al.,*1992
46	*G. paucinervis*	Leaf	Paucinervins H-J	Li *et al*., 2016a
47	*G. parvifolia*	Twig	Parvifolixanthones A-C	Rukachaisirikul *et al.,* 2006
		Bark	Parvixanthones A−I	Xu *et al*., 2001
			Parvixanthone A and rubraxanthone	Kardono *et al*., 2006
48	*G. pedunculata*	Bark	Pedunxanthones A–C, 1,5-dihydroxy-3- methoxy-6',6'-dimethyl-2H-pyrano(2',3':6,7)-4-(3-methylbut-2-enyl)xanthone, 1,5-dihydroxy-3-methoxy-4-(3-methylbut-2-enyl)xanthone, dulxanthone A, and garbogiol	Vo *et al.,* 2012
		Heartwood	1,3,5,7-tetrahydroxyxanthone and 1,3,6,7-tetrahydroxyxanthone	Rao *et al.,*1974
		Pericarp	Pedunxanthones D-F	Vo *et al.,* 2015
49	*G. penangiana*	Leaf	4-(1,1-Dimethylprop-2-enyl)-1,3,5,8-tetrahydroxyxanthone penangianaxanthone, cudratricusxanthone H, macluraxanthone C, and gerontoxanthone C	Jabit *et al.,* 2007
50	*G. polyantha*	Stem bark	Bangangxanthone A and B, 1,5-dihydroxyxanthone, and 2-hydroxy-1,7-dimethoxyxanth- one	Lannang *et al.,* 2005
			Isorheediaxanthone B	Ampofo and Waterman, 1986
			Polyanxanthone	Komguem *et al*., 2006

		Root bark	Garciniaxanthone I, smeathxanthone A, smeathxanthone B, and chefouxanthone	Lannang *et al.,* 2008
		Wood trunk	Polyanxanthone A, B, C, 1,3,5-trihydroxyxanthone, 1,5-dihydroxyxanthone, 1,3,6,7-tetrahydroxy xanthone, 1,6-dihydroxy-5-methoxy xanthone, and 1,3,5,6-tetrahydroxy xanthone	Louh *et al.,* 2008
51	*G. porrecta*	---	Porxanthone A and dulxanthone E-G	Kardono *et al*., 2006
52	*G. propinqua*	Twig	Doitunggarcinone C, dulxanthone B, 5-O-methylxanthone V1, 10-O-methylmacluraxanthone, macluraxanthone, gartanin, and morusignin J	Tantapakul *et al.,* 2012
		Root	Doitunggarcinone D	Meesakul *et al*., 2016
53	*G. pushpangadaniana*	Leaf	Cambogic acid and mangostin	Pandey *et al.,* 2015
54	*G. pyrifera*	Stem bark	Rubraxanthone, isocowanin, and isocowanol	Ampofo and Waterman, 1986
55	*G. quadrifaria*	Stem bark	1, 3, S-Trihydroxy-4, 8di(3, 3-dimethylallyl)xanthone	Waterman and Hussain, 1982
56	*G. rigida*	Leaf	Yahyaxanthone	Elya *et al.,*2008
			Musaxanthone and asmaxanthone	Elya *et al*., 2006a
57	*G. schomburgkiana*	Bark	6-O-Demethyloliverixanthone, schomburgxanthone, cowanin, cowanol, fuscaxanthones A and B, 3-isomangostin hydrate, and 1,7-dihydroxyxanthone	Vo *et al.,* 2012a
		Root	Schomburgxanthone A	Sukandar *et al.,* 2016a
		Branch	Euxanthone and gentisein	Meechai *et al*., 2016
58	*G. scortechinii*	Twig	Scortechinones A-C	Rukachaisirikul *et al.,* 2000a
		Fruit	Scortechinones Q-T, scortechinones U-X, scortechinones A-F, H, I, M, L, and P	Sukpondma *et al.,* 2005
		Latex	Scortechinones D-K	Rukachaisirikul *et al.,* 2003b
59	*G. smeathmannii*	Stem bark	Smeathxanthone A and B	Komguem *et al.,* 2005
			Cheffouxanthone, 1,5 dihy- droxyxanthone, 1,3,5-trihydroxyxanthone, bangang xanthone A, smeathxanthone B, and smeathxanthone A	Kuete *et al.,* 2007
			1,3,5,8-Tetrahydroxy-2-(3-methybut-2-enyl)-4-(3,7-dimethylocta-2,6-dienyl)xanthone, cheffouxanthone, smeathxanthone A, smeathxanthone B, and ananixanthone	Fouotsa *et al.,* 2015
		Root bark	Cheffouxanthone , smeathxanthones A, and smeathxanthones B	Lannang *et al.,* 2006
60	*G. speciosa*	Bark	α-Mangostin, cowanin and cowanol	Okudaira *et al.,* 2000
61	*G. spicata*	Leaf	Cambogic acid and mangostin	Pandey *et al.,*

				2015
62	*G. staudtii*	Stem bark	Rheediaxanthone-A	Waterman and Hussain, 1982
		Twig	Staudtiixanthones A-D, α-mangostin, 9 garcinone B, 9 demethylcalabaxanthone, gartanin, and xanthone V_1	Ngoupayo *et al.,* 2009
63	*G. subelliptica*	Heartwood	Garciniaxanthones A and B	Fukuyama *et al.,* 1991
		Wood	Garciniaxanthone C, 1,2,5-trihydroxyxanthone, 2,6-dihydroxy-1,5-dimethoxyxanthone, and 1,2-dihydroxy-5,6-dimethoxyxanthone	Minami *et al*., 1994
			2,5-Dihydroxy-1-methoxylxanthone, l-O-methylsymphoxanthone, garciniaxanthone E symphoxanthone, and subelliptenone A	Minami *et al.,* 1996
			1,6-O-Dimethylsymphoxanthone	Minami *et al.,* 1998
		Root bark	1,4,5,6-Tetrahydroxy-2-(1,1-dimethyl-2- propenyl)-7,8,-di-(3-methyl-2-butenyl)xanthone, and 1,2,5,6-tetrahydroxy-4-(1,l-dimethyl-2-propenyl)-7- (3-methyl-2-butenyl)xanthone, subelliptenones A and B	Iinuma *et al.,* 1994
			Subelliptenones C and subelliptenones D	Iinuma *et al.,*1995
			Subelliptenones H and subelliptenones I	Iinuma *et al*., 1995a
			Subelliptenones E and subelliptenones F	Iinuma *et al*., 1995b
64	*G. terpnophylla*	Timber and bark	1,5-Dihydroxyxanthone and mangostin	Bandaranayake *et al.,* 1975
65	*G. tetralata*	Stem bark	Garcinexanthone B, morellic acid acetate, toxyloxanthone A, 6,11-dihydroxy-2,2-dimethylpyrano[3,2-c]xanthen-7(2H)-one, and 1,4-dihydroxy-5,6-dimethoxy xanthone	Guo *et al.,* 2011
66	*G. tetrandra*	Stem bark	1,3-Dihydroxy,2′,2′-dimethyl pyrano (5′,6′,5,6) xanthone	Hartati *et al*., 2008
67	*G. urophylla*	Leaf	7-Hydroxydesoxymorellin, isocaledonixanthone D, gaudichudione H, 1,7-dihydroxy-3-methoxy-2-(3-methyl- 2-butenyl)xanthone, 1,5-dihydroxy-3-methoxy-2-(3-methyl-2butenyl)xanthone, and 1,3,7-trihydroxy-2-(3-methyl-2-butenyl)xanthone	Khalid *et al*., 2007
68	*G. vieillardii*	Stem bark	Vieillardiixanthones B and C, pancixanthones A, B, 1,6-dihydroxyxanthone, pyranojacareubin and 5,6-O-dimethyl-2-deprenylrheediaxanthone	Hay *et al.,* 2008
			1,6-Dihydroxyxanthone, pancixanthone A, isocudraniax- anthone B, isocudraniaxanthone A, 2-deprenyl rheediaxanthone B and 1,4,5-trihydroxyxanthone	Hay *et al.,* 2004
69	*G. vilersiana*	Bark	Globuxanthone, subelliptenone H, subelliptenone B, 12b-hydroxy-des-D-garcigerrin A, 1-*O*-methylglobuxanthone, and symphoxanthone	Nguyen *et al.,* 2000
70	*G. virgata*	Stem bark	Virgataxanthone A and B	Merza *et al.,* 2004
71	*G. yunnanensis*	Pericarp	Garciyunnanins A and B	Xu *et al.,* 2008

72	*G. wightii*	Leaf	Cambogic acid and mangostin	Pandey *et al.,* 2015
73	*G. xanthochymus*	Leaf	Cambogic acid and mangostin	Pandey *et al.,* 2015
		wood	1,4,5,6-Tetrahydroxy-7,8-di(3-methylbut-2-enyl)xanthone, 1,2,6-trihydroxy-5-methoxy-7-(3-methylbut-2-enyl)xanthone, and 12β- hydroxy- D-garcigerrin	Chanmahasathien *et al.,* 2003
		Bark	1,6-Dihydroxy-4,5-dimethoxyxanthone and 1,5,6-trihydroxy-7,8-di(3-methyl-2-butenyl)-60,60-dimethylpyrano(20,30:3,4) xanthone	Zhong *et al.,* 2007
			1,5,6- Trihydroxy-7-(3-methyl-2-butenyl)-8-(3-hydroxy-3-methylbutyl)furano(2′,3′:3,4) xanthone, 1,5,6-trihydroxy-7-(3-methyl-2-butenyl)- 8-(3-hydroxy-3-methylbutyl)–6′, 6′-dimethylpyrano (2′,3′:3,4) xanthone, 1,5,6-trihydroxy-7-(3- methyl-2-butenyl)-8-(3-hydroxy-3-methylbutyl)–5′-(1-hydroxy-1-methylethyl)-4′, 5′-dihydrofurano (2′,3′:3,4) xanthone, 1, 2, 5, 4′-tetrahydroxy-4-(1,1-dimethylallyl)-5′-(2-hydroxypropan-2-yl)-4′, 5′-dihydrofurano-(2′, 3′ : 6, 7)xanthone, 1, 3, 5, 6-tetrahydroxy-7-geranylxanthone, and 1, 4-dihydroxy-6′, 6′- dimethylpyrano (2′, 3′: 5, 6) xanthone	Chen *et al.,* 2010
			1,7-dihydroxyxanthone and 1,5-dihydroxyxanthone	Baslas and Kumar 1979
		Twig bark	1,4,6-Trihydroxy-5-methoxy-7-prenylxanthone, 1,4,5,6-tetrahydroxy-7-prenylxanthone, 1,2,5,6-tetrahydroxy-7-geranylxanthone, 1,4,5,6-tetrahydroxy-7,8-diprenylxanthone , 1,3,5,6-tetrahydroxy-4,7,8-triprenylxanthone , garciniaxanthone E, and 6-prenylapigenin	Han *et al*., 2007
74	*G. xipshuanbannaensis*	Twig	Bannaxanthone H, 1,3,5,6-tetrahydroxy-2-(3-methylbut-2-enyl)xanthone, bannaxanthone F, garcinone C, 1,3,6,7-tetrahydroxy-8-(3-methylbut-2-enyl)xanthone, bannaxanthone G, bannaxanthone B, γ-mangostin, garcinone E, bananxanthone E, allanxanthone C, bannaxanthone D, 1,3,5,6-tetrahydroxy-7-(3-methylbut-2-enyl)xanthone, xanthone V1a, and nigrolinexanthone V	Zhou *et al.,* 2008
			Bannaxanthones A-H, allanxanthone C, isojacareubin, garcinone C, and γ-mangostin	Han *et al.,* 2008

2. Benzophenones

Benzophenones are a series of compounds with phenol-carbonyl-phenol skeleton, synthesised through the mixed shikimic acid and acetate pathway, in which the acetate derived benzene ring is modified by intervention of prenyl groups. Biogenetically isoprenylated benzophenones are derived from maclurin which was regarded as a precursor for many xanthones in higher plants. Garciduols A-E, reported from *G. dulcis* possesses the novel benzophenone xanthone dimer skeletal structure, supporting the biosynthetic route that benzophenones are precursors of xanthones (Iinuma *et al.,*1996). Naturally occurring

benzophenones that consists of more than 300 members are reported with great structural diversity with oxidized and polyisoprenylated structures (Cuesta-Rubio *et al.,* 2005, Acuna *et al.,* 2009). The genus *Garcinia* and *Clusia* are the major source of natural benzophenones. Literature review revealed that out of 120 *Garcinia* species subjected to phytochemical investigation, 50 *Garcinia* species contain benzophenones (**Table 2**). Floral resins and latex of some of the Clusiaceae members are mainly constituted of benzophenones and can contain up to 70% of benzophenones (Cuesta-Rubio *et al.,* 2001).

Generally the benzophenones can be classified into simple polyisoprenylated benzophenones and complex bicyclo-[3.3.1]-nonane derivatives (**Figure 3, Figure 4**). Most of the benzophenones reported from the genus *Garcinia* are polyisoprenylated bezophenones, derived from maclurin. Karanjgoakar *et al.* in 1973 isolated xanthochymol, the first bicyclo-[3.3.1]-nonane benzophenone from the fruits of *G. xanthochymus* (Karanjgoakar *et al.,* 1973). Camboginol (garcinol) and cambogin (isogarcinol; xanthochymol) were two important benzophenones isolated from the latex of *G. gummi-gutta* in large quantities (37.0% and 5.5% respectively) (Rao *et al.,* 1980). Porto *et al* (2000) attempted a chemotaxonomical approach based on the distribution of benzophenones in the floral resins of *Clusia* members, where simple benzophenone derivatives and the bicyclo-[3.3.1]-nonane benzophenone structures demarcated the species.

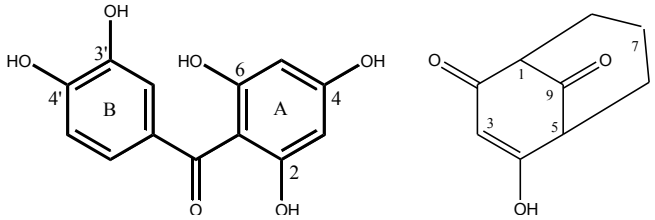


Figure 3. Typical benzophenone (maclurin) and bicyclo-[3.3.1]-nonane 2,4,9-trione structure

Recent developments in phytochemical analytical methods, especially the hyphenated LC-MS techniques, made tremendous contributions to the detection of secondary metabolites that are present in minute quantities in plants. The limit of detection for xanthochymol in *G. indica* fruit rinds was reported as 20 μg/mL by HPLC and the method was inadequate to detect or estimate xanthochymol present in minute quantity in other parts of the plant. Consequently, LC-ESI/MS/MS method has been developed for the detection and quantification of xanthochymol at ppb level in *Garcinia* species. In addition, the isomeric compound isoxanthochymol can be differentiated from xanthochymol by the fragment ions obtained through MS/MS (Chattopadhyay and Kumar, 2006). Powder X-ray diffraction (PXRD) technique has been reported as a non-destructive analytical tool for the detection of the anti-HIV benzophenones, 7-*epi*-clusianone and guttiferone in *G. brasiliensis* extracts by Martins, *et al*., (2011). The compounds were detected in plant powder by comparing the powder diffraction profile of raw plant powder with the reported single crystal profiles of marker compounds (Martins, *et al*., 2011).

Benzophenones have shown different biological properties especially activity against HIV-1 (Cuesta-Rubio *et al.,* 2005). Garcinol is an important polyisoprenylated benzophenone distributed in several *Garcinia* species and is one of the active ingredients of nutraceutical

products from *G. indica* and *G. cambogia*. The structural similarity with curcumin, with *β*-diketone moiety that shows keto enol tautomerism, make garcinol interesting for pharmacological screening studies (Padhye *et al*., 2009). The significant antioxidant activity of Kokum syrup, a delicious drink popular in northern Kerala and Konkan region, made from *G. indica* fruits, is attributed mainly to the presence of garcinol and anthocyanins (Mishra, *et al*., 2006). Guttiferones, another class of benzophenones isolated from *Garcinia* species such as *G. pyrifera* and *G. aristata* are of great interest in pharmaceutical research particularly due to the anti-HIV, trypanocidal and cytotoxic activities (Acuna *et al*., 2009).

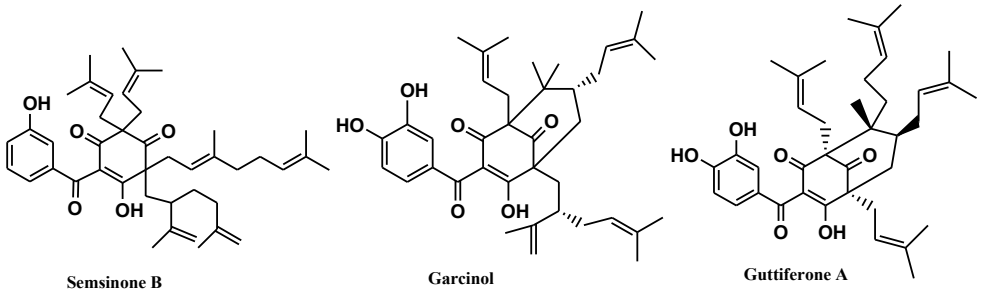


Figure 4. The simple benzophenone (semsinone B) and the bicyclo-[3.3.1]-nonane benzophenones (garcinol and guttiferone A)

Table 2. Benzophenones reported from *Garcinia* species

Sl. No.	*Garcinia* species	Plant part	Benzophenones	Reference
1	*G. achachairu*	Seed	Guttiferone A	Dal Molin *et al.,* 2012
2	*G. amplexicaulis*	Stem bark	Garcinal	Lavaud *et al.,* 2015
3	*G. aristata*	Fruit	Aristophenones A-B	Cuesta-Rubio *et al*., 2001
		Fruit	Guttiferone A, xanthochymol, and Guttiferone E	Acuna *et al.,* 2012,
4	*G. assigu*	Stem bark	Isogarcinol, garcinol ,18-0-methyl isogarcinol 18-0-methyl garcinol, and clusianone	Ito *et al.,* 2003
5	*G. benthami*	Stem bark	Benthaphenone	Nguyen *et al.,* 2011a
			Salimbenzophenone	Elya *et al*., 2006
6	*G. brasiliensis*	Fruit	7-epi-Clusianone and guttiferone A	Martins *et al.,* 2011
		Pericarp	7-epi-Clusianone, garciniaphenone, and guttiferone-A	Pereira *et al.,* 2010
		Leaf	7-epi-Clusianone	Santa-Cecília *et al.,* 2011
		Epicarp	7-epi-Clusianone and garciniaphenone	Derogis *et al.,* 2008
			7-epi-Clusianone	Castro *et al.,* 2015
7	*G. cantleyana*	Twig	2,6,3',5'-Tetrahydroxybenzophenone,	Jantan *et al.,*

			3,4,5,3',5'-pentahydroxybenzophenone, and 3,5,3',5'-tetrahydroxy-4-methoxybenzophenone	2012
8	*G. cochinchinensis*	Pericarp	Guttiferones Q-S and guttiferone I	Nguyen *et al.,* 2011
9	*G. cowa*	Leaf	Chamuangone	Sakunpak and Panichayupakarant, 2012
			Garcinol	Pandey *et al.,* 2015
10	*G. echinocarpa*	Leaf	Garcinol	Pandey *et al.,* 2015
11	*G. epunctata*	Stem bark	Epunctanone, 7-epiisogarcinol	Fotso *et al.,* 2014
12	*G. eugenifolia*	Root	(3,4-Dihydroxyphenyl),(3-hydroxy-5-methoxyphenyl) methanone, and (3- hydroxy-phenyl)3,4,5-trihydroxy phenyl) methanone	Joong *et al.,* 2012
		Stem bark	Eugeniaphenone	Hartati *et al*., 2008a
13	*G. griffithii*	Stem bark	Guttiferone I	Nguyen *et al.,* 2005
			Isoxanthochymol and guttiferone I	Elfita *et al.,* 2009
14	*G. gummi-gutta* (*G. cambogia*)	Fruit	Garcinol, guttiferones K, I, J, M and N Garcinol, guttiferones- K, I, J, M and N Guttiferone I, guttiferone N, guttiferone J, guttiferone K, and guttiferone M	Masullo *et al.,* 2008 Masullo *et al.,* 2010 Semwal *et al.,* 2015
		Latex	Cambogin (isogarcinol) and camboginol (garcinol)	Rao *et al.,*1980
		Leaf	Garcinol	Pandey *et al.,* 2015
		Bark	Guttiferone E and isogarcinol	Semwal *et al.,* 2015
15	*G. hombroniana*	Leaf	Garcinol	Pandey *et al.,* 2015
		Stem wood	Bronianone	Rao *et al.,* 1973 Ollis *et al*., 1969
		Fruits	Guttiferone A, xanthochymol, and guttiferone E	Acuna *et al.,* 2012
		Bark	2,3',4,5'-Tetrahydroxy-6-methoxybenzophenone, 2,3',4,4'-tetrahydroxy-6-methoxybenzophenone, and 2,3',4,6-tetrahydroxybenzophenone	Jamila *et al.,* 2014b
16	*G. huillensis*	Stem bark	Garcinol	Phongi *et al.,* 1987
17	*G. indica*	Leaf	Garcinol	Pandey *et al.,* 2015
		All parts	Xanthochymol and isoxanthochymol	Chattopadhyay *et al.,* 2006 Kumar *et al*., 2009.
		Fruit	Isogarcinol, garcinol, and 14-deoxyisogarcinol	Kaur *et al.,* 2012
18	*G. intermedia*	Fruit	Guttiferone A, xanthochymol, and	Acuna *et al.,*

			guttiferone E	2012
19	*G. kola*	Fruit	Guttiferone A, xanthochymol, kolanone, and guttiferone E	Acuna *et al.,* 2012 Waterman *et al.,* 1983
			Kolanone	Hussain *et al*., 1982
20	*G. livingstonei*	Fruit	Guttiferone A, xanthochymol, and guttiferone E	Acuna *et al.,* 2012
			Guttiferone A	Gustafson *et al*., 1992
21	*G. macrophylla*	Twigs	Guttiferone A and guttiferone G	Williams *et al.,* 2003
22	*G. maingayii*	Stem bark	Isoxanthochymol and camboginol	Hartati *et al*., 2007
23	*G. mangostana*	Leaf	Garcinol	Pandey *et al.,* 2015
		Heart wood	3',6-Dihydroxy-2,4,4'- trimethoxy benzophenone	Nguyen *et al.,* 2005
		Fruit	Guttiferone A, xanthochymol, and guttiferone E	Acuna *et al.,* 2012
		Fruit hull	2,4,6,7- Tetrahydroxyxanthone, 3,4,5,3'-tetrahydroxybenzophenone, and 2,4,6,3',5'-pentahydroxybenzophenone	Jiang *et al.,* 2010
			Garcimangosone D	Huang *et al.,* 2001
		Stem bark	Mangaphenone	See *et al.,* 2014
24	*G. mannii*	Stem bark	Xanthochymol	Crichton *et al.,* 1979
25	*G. morella*	Leaf	Garcinol	Pandey *et al.,* 2015
26	*G. multiflora*	Bark, stem	4,6,4'-Trihydroxy-2,3'-dimethoxy-3-prenylbenzophenone	Chiang *et al.,* 2003
		Fruit	13,14-Didehydoxyisogarcinol, garcimultiflorone A, garcimultiflorone B, 13-hydroxy garcimultiflorone B, and garcimultiflorone C	Chen *et al.,* 2009.
27	*G. myrtifolia*	Bark	Myrtiaphenone-A, B and vismiaphenone C	Spino *et al.,* 1995
28	*G. ovalifolia*	Leaf	Guttiferone E	Gustafson *et al.,* 1992
		Stem bark	Xanthochymol and isoxanthochymol	Waterman and Crichton, 1980b
		Fruit	Xanthochymol	Waterman *et al.,* 1980b
		Root	Epigarcinol and isogarcinol	Pieme *et al.,* 2015
29	*G. paucinervis*	Leaf	Paucinones A-D	Gao *et al.,* 2010
		Seed	Paucinones E–I	Li *et al*., 2016
30	*G. pedunculata*	Fruit	Pedunculol, garcinol, and cambogin	Sahu *et al.,*1989
		Heart	2,4,6,3',5'-Pentahydroxybenzophenone	Rao *et al.,* 1974

		wood		
31	*G. picrorrhiza*	Bark	Garcinopicobenzophenone and guttiferone F	Soemiati *et al*., 2006
32	*G. polyantha*	Stem bark	Xanthochymol and isoxanthochymol	Ampofo and Waterman, 1986
33	*G. propinqua*	Twig	Doitunggarcinones A and B	Tantapakul *et al.,* 2012
34	*G. pseudoguttifera*	Heart wood	Myrtiaphenone-A, myrtiaphenone-B, myrtiaphenone-C, and pseudoguttiaphenone-A	Ali *et al.,* 2000
35	*G. purpurea*	Pericarp	Xanthochymol, cambogin (isogarcinol), and camboginol (garcinol)	Matsumoto *et al.,* 2003 Iinuma *et al.,* 1996 Steller, 1995
36	*G. pushpangadaniana*	Leaf	Garcinol	Pandey *et al.,* 2015
37	*G. pyrifera*	Fruit	Guttiferone E and Xanthochymol	Roux *et al.,* 2000
38	*G. schomburgkiana*	Fruit	Schomburgkianones A-H	Le *et al*., 2016
39	*G. semseii*	Stem bark	Semsinones A-C	Magadula *et al.,* 2008
40	*G. smeathmannii*	Stem bark	Guttiferone I and isoxanthochymol	Kuete *et al.,* 2007
		Root bark	Guttiferone I and isoxanthochymol	Lannang *et al.,* 2006
41	*G. solomonensis*	Stem bark	Guttiferones O and P	Carrol *et al.,* 2009
42	*G. speciosa*	Trunk bark, stem	Garciosaphenone	Rukachaisirikul *et al.,* 2003a
43	*G. spicata*	Leaf	Garcinol	Pandey *et al.,* 2015
		Fruit	Guttiferone A, xanthochymol, and guttiferone E	Acuna *et al.,* 2012
44	*G. staudtii*	Stem bark	Xanthochymol	Waterman and Hussain, 1982
45	*G. subelliptica*	Fruits	Garcinialiptone A, garcinialiptone B, (−)-cycloxanthochymol, garcinialiptone C, garcinialiptone D, xanthochymol, isoxanthochymol, and cycloxanthochymol	Zhang *et al.,* 2010
		Wood	4′,6-dihydroxy-2,3′4-trimethoxybenzophenone	Minami *et al.,* 1994
46	*G. vieillardii*	Stem bark	Clusiachromene and 3-geranyl-2,4,6-trihydroxybenzophenone	Hay *et al.,* 2008
47	*G. virgata*	Stem bark	Guttiferone E, xanthochymol, and guttiferones I and J	Merza *et al*., 2006
48	*G. wightii*	Leaf	Garcinol	Pandey *et al.,* 2015
49	*G. xanthochymus*	Leaf	Garcinol	Pandey *et al.,* 2015
		Fruit	Guttiferone H and gambogenone	Bagget *et al.,* 2005

			Guttiferone A, xanthochymol, and guttiferone E	Acuna *et al.,* 2012
			Xanthochymol and garcinol	Jackson *et al.,* 2015
			Xanthochymol, Isoxanthochymol, and maclurin	Baslas and Kumar 1979
50	*G. xipshuanbannaensis*	Twig	Guttiferone E and xanthochymol	Han *et al*., 2008

3. Biflavonoids

Biflavonoids are a distinct class of naturally occurring flavonoid dimers linked by a C-C or C-O-C bond. The biogenesis of biflavonoids involves the radical pairing of two embryonic flavonoid units. The ring B and C of flavonoid units were formed through shikimic acid pathway, while ring A is formed through acetate pathway (**Figure 5**). Depending on the monomeric unit like flavones, flavanones, isoflavones, flavanols, chalcones, aurones and dihydrochalcones, different combinations of flavonoid dimers such as flavanone-flavone, flavones-flavone, flavone-flavonol are possible. Naturally occurring biflavonoids contains hydroxy or methoxy groups substituted at different positions leading to diverse array of biflavonoids (Mercader and Pomilio, 2012). Amentoflavones with the 3-8 linkage is considered as the primitive or basic form of biflavonoids in vascular plants.

The rapid growth in literature on biflavanoids led to various systems of naming and though systematic IUPAC and Locksley names exists, most of the biflavonoids are known by their vernacular names (Locksley, 1973). In Locksley system, for example, amentoflavone is named as I-4', II-4', I-5, II-5, I-7, II-7-hexahydroxy I-3', II-8 biflavone, while in IUPAC system, amentoflavone is named as 8-5-(5,7-dihydroxy-4-oxo-4H-chromen-2-il)-2-hydroxyphenyl-5,7-dihydroxy- 2-(4-hydroxy-phenyl)-chromen-4-on. Basic difference between the two systems is reference of structural skeleton, where Locksley use flavanoid structure, while IUPAC use chromen structure (Rahman *et al.,*2007).

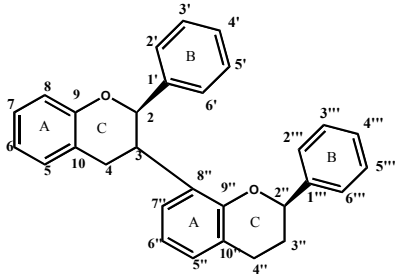


Figure 5. Numbering in typical biflavonoid structure

The distribution of biflavonoids is limited to some plant groups, especially in the primitive orders such as Bryales, Psilotales and Selaginellales, and sporadically in the angiosperms. According to Gieger and Quinn (1988), angiosperms lost the capacity to biosynthesis biflavonoids in the course of evolution, but was regained by a selected family. The genus *Garcinia* is a rich source of biflavonoids and out of the 120 *Garcinia* species studied for their secondary metabolites, biflavonoids were reported from 45 species (**Table 3**).

Majority of the naturally occurring biflavonoids contain C-C linked monomers and I(3)-II (8) linkage is the most prevalent inter-linkage in *Garcinia* biflavonoids (Yamaguchi *et al*., 2008). Biflavonoids reported from the *Garcinia* species with 3-8'' interflavonoid linkage can generally be divided into two subgroups; biflavonones made up of two flavanone units (GB type of biflavonoids) and those made up of one flavanone and one flavone subunits (morelloflavone and volkensiflavone) (**Figure 6**). Of the two types, biflavonones is the major type in *Garcinia* species whereas the co-occurrence of the two types of biflavonoids is rare (Waterman and Hussain, 1983). Morelloflavone, isolated from *G. morella* in 1967 is the first biflavonoid reported with a flavone and a flavonone unit (Karanjgaokar *et al*., 1967). Amentoflavone (5′,8″-biapigenin) is the common example for I(5′)-II(8) biflavonoid distributed in *Garcinia* species. It is interesting to note that the biflavonoid linkage has potential significance in systematic (Waterman and Husain, 1983).

Biflavonoids generally exist as rotamers and can be monitored by variable temperature NMR studies, where at room temperature the biflavonoids exhibit duplicate NMR signals, while at elevated temperature a single set of signals was obtained (Jamila, *et al*., 2014). Mass spectrometry is perhaps the most informative tool for structure elucidation of biflavonoids (Zhang *et al*., 2011). The most useful fragmentations in terms of structural identification are those involving the C-ring cleavage of biflavonoids. Fragmentation peaks for phloroglucinol (m/z 126), p-methoxy benzyl (m/z 138), p-hydroxy benzyl (m/z 124) and retro Diels Alder cleavage products are usually observed for biflavonoids.

A variety of biological activities like anti inflammatory, anti HIV, antifungal, anti tumor, hypocholesterolemic, and anti-plasmodial were attributed to biflavonoids (Gil *et al*., 1997, Lin *et al*., 1997 Yamaguchi *et al*., 2008, Pang *et al*., 2009). Of the different activities, antioxidant activity is of highly significant, where biflavonoids inhibits transition metal ions in free radical generating reactions by complexing and quenching the metal ions (Yamaguchi, *et al*., 2008).

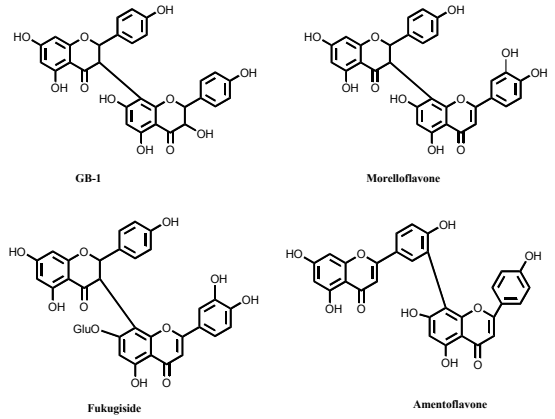


Figure 6. Structures of I(3)-II(8) linked biflavonones (GB1), flavanone-flavone (morelloflavone), flavanone-flavone glycoside (fukugiside) and I(5')-II(8) linked biflavone (amentoflavone)

Table 3. Biflavonoids reported from *Garcinia* species

Sl. No.	*Garcinia* species	Plant part	Biflavonoids	Reference
1	*G. atroviridis*	Stem bark	Garcineflavonol	Tan *et al.,* 2014
2	*G. bakeriana*	Leaf	4'"-O-Methyl-I3,II8-biapigenin, amentoflavone, 4'"-O-methylamentoflavone , 4'-O-methylcupressuflavone, GB-2a, volkensiflavone, 6"-(2-hydroxy-3-methyl-3-butenyl)-amentoflavone, I3,II8-biapigenin, and GB-1a	Al-Shagdari *et al.,* 2013
3	*G. brasiliensis*	Epicarp	Morelloflavone, morelloflavone-4‴-O-β-D-glycoside, and morelloflavone-7″-O-β-D-glycoside	Gontijo *et al.,* 2012
			Fukugetin	Castro *et al.,* 2015
		Branch, leaf	Procyanidin, fukugetin, amentoflavone, and podocarpusflavone	Arwa *et al*, 2015
4	*G. brevipedicellata*	Heart wood	Amentoflavone , 4‴-O-methyl amentoflavone, Robustaflavone, 4′-O-methyl robustaflavone, and tetrahinokiflavone	Abderamane *et al*., 2016
5	*G. buchananii*	Stem bark	GB-1, GB1a, GB-2 and GB-2a	Jackson *et al.,* 1968 and 1971
			(2R,3S,2″R,3″R)-Manniflavanone, (2R,3S,2″R,3″R)- isomanniflavanone, (2″R,3″R)-preussianone, (2R,3S,2″R,3″R)-GB-2 7″-O-β-D-glucopyranoside, and (2R,3S,2″R,3″R)-manniflavanone-7″-O-β-D-glucopyranoside	Stark *et al*., 2015
			(2R,3S,2″R,3″R)-Manniflavanone, (2R,3S,2″R,3″R)-GB-2 and (2R,3S,2″S)-buchananiflavanone	Stark *et al*., 2012
6	*G. conrauana*	Stem bark Heart wood	GB-1, GB1a, GB-2, morelloflavone, O-methyl fukugetin, and O-methyl fukugetin glycoside	Hussain and Waterman, 1982
7	*G. cornea*	Stem bark	Morelloflavone and fukugiside	Elfita *et al*., 2009
8	*G. cowa*	Branch	GB-2, morelloflavone, volkensiflavone, and fukugiside	Shen and Yang, 2007; Panthong *et al.,* 2009
		Fruit	Amentoflavone and morelloflavone	Shen and Yang, 2006
		Leaf	Fukugicide, amentoflavone, GB-1, and GB-2	Pandey *et al.,*2015
9	*G. cymosa*	Stem bark	Morelloflavone and morelloflavone-7"-*O*-β-D-glucoside	Elfita *et al.,* 2009
10	*G. densivenia*	Stem bark	Morelloflavone and O-methyl fukugetin	Waterman and Crichton, 1980a
11	*G. dulcis*	Leaf	Amentoflavone, fukugetin, volkensiflavone, and flavanone-(1-3:11-8)-chromone, 1-4' (flavanone- chromone)	Ansari *et al.,* 1976

			Dulcisbiflavonoid A	Saelee *et al.,* 2015
			Morelloflavone	Pinkaew *et al.,* 2009
		Branch	Podocarpusflavone A	Harrison *et al.,* 1994
		Fruit	Dulcisbiflavonoid A	Saelee *et al.,* 2015
12	*G. echinocarpa*	Timber, bark	Volkensiflavone, morelloflavone, and fukugetin	Bandaranayake *et al.,*1975
		Leaf	Fukugicide, GB-1 and amentoflavone	Pandey *et al.,* 2015
13	*G. eugeniifolia*	Heart wood	GB-1, GB-1a, GB-2 and GB-2a	Jackson *et al*., 1968 and 1969
14	*G. fusca*	Root	Vokensiflavone, fukugetin, fukugiside	Nontakham *et al.,* 2014
15	*G. gardneriana*	Leaf	Fukugetin and GB-2a	Castardo *et al.,* 2008
16	*G. gummi-gutta* (*G. cambogia*)	Leaf	Fukugicide, GB-1, and amentoflavone	Pandey *et al.,* 2015
		Heart wood, bark	Morelloflavone, dihydromorelloflavone and isomorellic acid	Venkataraman, 1973
17	*G. hombroniana*	Bark	Volkensiflavone, volkensiflavone-7-O-rhamnopyranoside, 4″-O-methyl-volkensiflavone, volkensiflavone-7-O-glucopyranoside, morelloflavone, 3″-O-methyl-morelloflavone, and morelloflavone-7-O-glucopyranoside	Jamila *et al.,* 2014
		Leaf	Fukugicide, GB-1, GB- 2, GB-1a, and amentoflavone	Pandey *et al.,* 2015
18	*G. indica*	Heartwood	Fukugetin and volkensiflavone	Cotterill *et al*., 1977
		Leaf	Fukugicide, GB-1, GB- 2, and amentoflavone	Pandey *et al.,* 2015
19	*G. intermedia*	Leaf	Podocarpusflavone A and amentoflavone	Abe *et al.,* 2004
20	*G. kola*	Stem bark	I-3', II-3, 3', II-4', I-5, II-5, I-7, II-7-Octahydroxy-1- 4'-methoxy-1-3, II-8-biflavanone, GB-1, and GB-2	Kabangu *et al.,* 1987
		Root	GB1, GB-2, kolaflavanone, manniflavanone, and garciniflavanone	Iwu *et al.,*1990
			GB 1	Han *et al*., 2005
		Seed	Amentoflavone, kolaflavone, GB-1, and GB-2	Iwu *et al.,*1982
			GB-1 and GB-2	Terashima *et al.,* 1997
			Kolaflavanone, GB-1, GB-1a, and GB-2	Kapadia *et al.,* 1994
			GB-1 and GB-2	Madubunyi, 1995

			Garcinianin	Terashima *et al*., 1995
			Kolaflavanone, GB-1, GB-1a, and GB-2	Tshibangu *et al*., 2016.
		Stem	Garcinianin, biflavanone GB-2a, (+) GB-1, (-) GB-1a, biapigenin,3-8'', and amentoflavone	Terashima *et al.,*1999, 1999a
21	*G. lateriflora*	Stem bark	Morelloflavone	Ren *et al.,* 2010
22	*G. linii*	Bark	Fukugetin, GB-1, GB- 2, GB-1a, and GB 2a	Konoshima *et al*., 1970
23	*G. livingstonii*	Heartwood, bark, leaf	Morelloflavone, BGH-III, amentoflavone podocarpusflavone A	Pelter *et al.,* 1971
		Fruit	Amentoflavone, 3,8″-biapigenin, volkensiflavone, morelloflavone and fukugiside	Yang *et al.,* 2010
		Root bark	Ent-naringeninyl-(I-3α, II-8)-4'-*O*-methylnaringenin	Mbwambo *et al.,* 2006
		Leaf	Amentoflavone and 4"-methoxy amentoflavone	Kaikabo *et al*., 2009
24	*G. madruno*	Leaf	Morelloflavone, volkensiflavone and amentoflavone	Osorio *et al.,* 2009
			7"-O-(6""-Acetyl) glucoside of morelloflavone, fukugiside, and spicataside	Osorio *et al.,* 2013
25	*G. mangostana*	Leaf	Fukugicide, GB-1, GB- 2, GB-1a, and amentoflavone	Pandey *et al.,* 2015
26	*G. mannii*	Stem bark	Manniflavanone, morelloflavone, and O-methyl fukugetin	Hussain *et al.,* 1982
			GB-1, GB-2, and manniflavanone	Crichton *et al.,* 1979
		Leaf	GB-1, GB-2, and manniflavanone	Hussain *et al.,* 1982
		Seed	GB-1, GB-2, and manniflavanone	Hussain *et al.,*1982
27	*G. merguensis*	Twig	GB-1a, GB-2a, (+)-morelloflavone, (+)-volkensiflavone, and amentoflavone	Trisuwan *et al.,* 2013
28	*G. morella*	Bark	Dihydromorelloflavone, morelloflavone-7''-β-glucoside, fukugetin, and fukugiside	Adawadkar *et al.,*1976
		Leaf	Fukugicide, GB-1, GB- 2 ,GB-1a, and amentoflavone	Pandey *et al.,* 2015
29	*G. multiflora*	Heartwood	(-)-GB-1a, (+)- GB-2a, (+) volkensiflavone, (+) morelloflavone, spicataside, fukugiside, xanthochymuside, 3, 8''-binaringenin-7''-O-β-glucoside, GB-1a, GB-2a, volkensiflavone, and morelloflavone	Chen *et al.,* 1975
			Fukugetin, fukugiside, GB-1a, GB-2a and GB-1a 7''-O-β-D-glucoside, and I-5, II-5, I-7, II-7, I-3', I-4', II-4'- heptahydroxy- [I-3,II-8]-flavanonyl- flavones	Lin *et al.,*1997

		Bark	Fukugetin, GB-1, GB- 2 ,GB-1a, GB-2a, and volkensiflavone	Konoshima *et al*., 1970
30	*G. nervosa*	Leaf	I-5, II-5, I-7, II-7, I-3', I-4', II-4'-Heptahydroxy- [I-3, II-8]- flavanonyl flavones and I-3, II-3, I-5, II-5, I-7, II-7, I-4', II-4'-octahydroxy [I-2', II-2'] biflavone	Babu *et al.,* 1988 Parveen *et al.,* 2004
31	*G. pedunculata*	Heart wood	GB-1a and volkensiflavone	Rao *et al.,* 1974
32	*G. prainiana*	Stem bark	Morelloflavone, O-methyl fukugetin, volkensiflavone, amentoflavone, and 4′″-methoxyamentoflavone	On *et al*., 2016
33	*G. preussii*	Leaf	Preussianone	Messi *et al.,* 2012
34	*G. pushpangadaniana*	Leaf	Fukugicide, GB-1, GB- 2 ,GB-1a, and amentoflavone	Pandey *et al.,* 2015
35	*G. quadrifaria*	Stembark Seed	Fukugetin and O-methyl fukugetin	Waterman and Hussain, 1982
36	*G. schomburgkiana*	Fruit	GB-1a, GB-2a, morelloflavone, and volkensiflavone	Le *et al*., 2016
37	*G. scortechinii*	Fruit	(+) Volkensiflavone and (+) morelloflavone	Sukpodma *et al.,* 2005
38	*G. spicata*	Leaf	GB-1, GB-1a, GB-2a, and fukugetin	Gunatilaka *et al.,* 1984
			Fukugicide, GB-1, GB- 2 ,GB-1a, and amentoflavone	Pandey *et al.,* 2015
		Bark	Fukugetin and 3-O-methyl fukugetin	Konoshima and Ikeshiro, 1969
			Fukugiside	Konoshima and Ikeshiro, 1970
			Volkensiflavone, spicataside, biflavonoid glycoside, GB-la, and GB-2a	Konoshima *et al.,* 1970a
39	*G. subelliptica*	Pericarp	Podocarpusflavone A	Iinuma *et al.,* 1996
		NSf	2R,3S-5,7,4',5",7",3'",4'"-Heptahydroxy flavanone[3-8"] flavone, and 5,7,4',5",7",3'",4'"-heptahydroxy[3-8"] biflavanone	Masuda *et al.,* 2005
40	*G. talboti*	Root	Talbotaflavone and morelloflavone	Joshi *et al*., 1970
41	*G. terpnophylla*	Timber, bark	GB-1a, GB1 and GB-2, 3''-3'''-4'-4'''-5-5''-7-7''-Octahydroxy-(3-8'') biflavanone, 3''-4'-4'''-5-5''-7-7''-heptahydroxy-(3-8'') biflavanone, and 4'-4'''-5-5''-7-7''-hexahydroxy -(3-8'') biflavanone	Bandaranayake *et al.,*1975
		Wood	3''-3'''-4'-4'''-5-5''-7-7''-Octahydroxy-(3-8'') biflavanone and 3''-4'-4'''-5-5''-7-7''-heptahydroxy-(3-8'') biflavanone	Bandaranayake *et al.,*1975
42	*G. thwaitesii*	Timber, bark	II-3, I-4', II-4', I-5, II-5,I-7,II-7- Heptahydroxy (I-3,II-8) biflavanone, I-4', Ii-4', I-5, II-5,I-7,II-7-hexahydroxy (I-3,II-8) biflavanone, II-3,II-3',I-4,II-4',I-5,II-5,I-7,II-7- octahydroxy (I-3,II-	Gunatilaka *et al.,* 1983

			8) biflavanone, I-4', II-3', II-4', I-5, II-5, I-7, II-7-heptahydroxy (I-3,II-8) biflavanone, I-4'-II-3'-II-4'-I-5-II -5-I-7-II-7-Heptahydroxy-(I-3-II-8) biflavanone, I-4'-II-4'-I-5-II-5-I-7 –II-7-hexahydroxy-(I-3-II-8) biflavanone, II-3-I-4'-II-4'-I-5-II-5-I-7-heptahydroxy-(I-3-II-8) biflavanone, II-3-II-3'-I-4'-II-4'-I -5-II-5-I-7-II-7-octahydroxy-(I-3-II-8) biflavanone, I-4'-II-3'-II-4'-I-5-II -5-I-7-II-7-Heptahydroxy-(I-3-II-8) biflavanone, I-4'-II-4'-I-5-II-5-I-7 –II-7-hexahydroxy-(I-3-II-8) biflavanone, and II-3-I-4'-II-4'-I-5-II- 5-I-7-II-7-heptahydroxy-(I-3-II-8) biflavanone	
43	*G. volkensii*	Heartwood	GB-1a, GB-2a, morelloflavone, and volkensiflavone	Herbin *et al.,* 1970
44	*G. wightii*	Leaf	Fukugicide, GB-1, GB- 2, GB-1a, and amentoflavone	Pandey *et al.,* 2015
45	*G. xanthochymus*	Leaf	Agathisflavone and 7-O-methyl amentoflavone	Parveen *et al.,* 1994
			Fukugicide, GB-1, GB- 2, GB-1a, and amentoflavone	Pandey *et al.,* 2015
		Fruit	Volkensiflavone, morelloflavone, GB-1, and GB-1a	Baslas and Kumar 1979
			3-8''- 3''-4'-4'''-5-5''-7''-Heptahydroxy biflavanone, 3-8''- 4'-4''-5-5''-7-7''-hexahydroxy biflavanone, fukugetin, and volkensiflavone	Baslas and Kumar 1981
		Leaf, root and fruit	GB-2a glucoside, GB-2a, and fukugetin	Li *et al.,*2014
		Wood, leaf	GB-1a, GB-2, volkensiflavone, fukugiside, xanthochymoside, and morelloflavone	Konoshima *et al.,* 1970

4. Depsidones

Depsidones comprise benzoic acid and phenol skeletons condensed at the *ortho*-positions through ester and ether linkages (**Figure 7**). This class of compounds is well known in *Garcinia* species (Ha *et al.,* 2012).

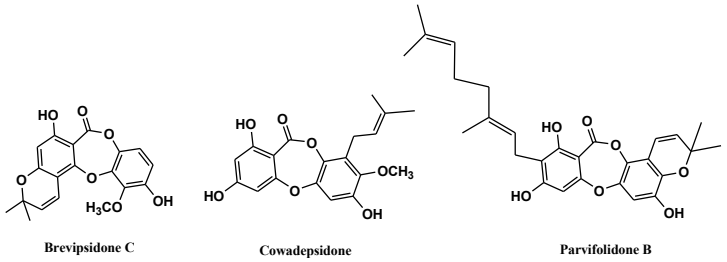


Figure 7. Structures of brevipsidone C (simple despidone), cowadespidone (monoprenylated despidone) and parvifolidone B (geranyl substituted despidone)

Table 4. Despidones reported from *Garcinia* species

Sl. No.	*Garcinia* species	Plant part	Depsidones	Reference
1	*G. assigu*	Stem bark	Garcinisidone A	Ito *et al*., 1997
2	*G. atroviridis*	Root	Atrovirisidone, Atrovirisidone B	Permana *et al*., 2001, 2005
3	*G. brevipedicellata*	Stem bark	Brevipsidones A-D	Ngoupayo *et al*., 2008
4	*G. buchananii*	Stem bark	Garcinisidone G	Stark *et al*., 2015a
5	*G. celebica*	Bark	Garcinisidone H	Bui *et al*., 2016
6	*G. cowa*	Twig	Cowadepsidone	Cheenpracha *et al*., 2011
7	*G. dulcis*	Stem bark	Garcinisidone A	Ito *et al*., 1997
8	*G. latissima*	Stem bark	Garcinisidone A	Ito *et al*., 1997
9	*G. neglecta*	Leaf	Garcinisidone B-F	Ito *et al*., 2001
10	*G. oliveri*	Bark	Oliveridepsidones A-D	Ha *et al*., 2012
11	*G. parvifolia*	Leaf	Garcidepsidone A, B, C, and D	Xu *et al*., 2000
			Garcidepsidone B	Rukachaisirikul *et al*., 2008
		Twig	Parvifolidones A, B	Rukachaisirikul *et al*., 2006
12	*G. puat*	Leaf	Garcinisidone B-F	Ito *et al*., 2001
13	*G. schomburgkiana*	Root	Schomburgdepsidones A, B	Sukandar *et al*., 2016a

5. Biphenyls

Biphenyls, reported as potential phytoalexins, are restricted to certain families and Clusiaceae is one among the families reported to contain biphenyls. Biphenyls are biosynthetically closely related to benzophenones and in a phylogenetic tree, biphenyl synthase (BIS) and benzophenone synthase (BPS) group together closely, indicating that they arise from a common ancestral gene. Biphenyl synthase (BIS) and benzophenone synthase (BPS) catalyze the formation of identical linear tetraketide intermediates from benzoyl-CoA (Beerhues and Liu, 2009).

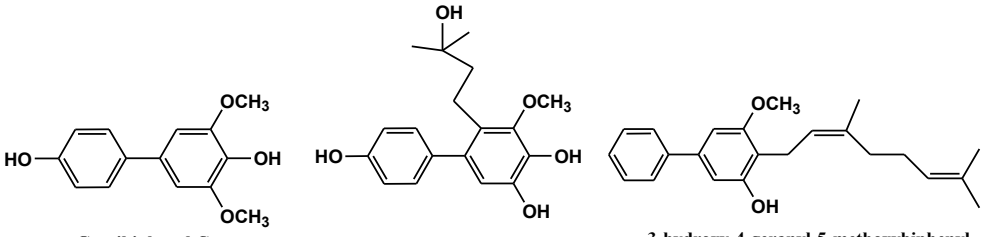


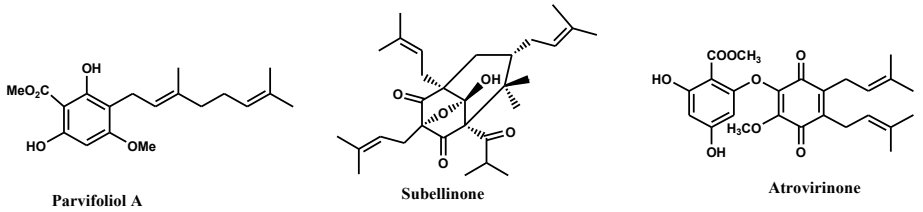
Figure 8. Structures of simple biphenyl (garcibiphenyl C), monoprenylated biphenyl (schomburgbiphenyl) and geranyl substituted biphenyl (3-hydroxy,4-geranyl,5-methoxy biphenyl)

Table 5. Biphenyls reported from *Garcinia* species

Sl. No.	*Garcinia* species	Plant part	Biphenyls	Reference
1	*G. bancana*	Twigs	[1,1'-Biphenyl]-2-(3-methyl-2-butenyl)-3-methoxy- 4,4',5,6-tetraol	Rukachaisirikul *et al*., 2005
2	*G. bracteata*	Twigs	Bractebiphenyls A-C, doitungbiphenyl A, doitungbiphenyl B, 2,2-dimethyl-3,5-dihydroxy-7-(4-hydroxyphenyl) chromane, oblongifoliagarcinine A, and schomburgbiphenyl	Li *et al*., 2015
3	*G. fucsa*	Root	Nigrolineabiphenyl B	Nontakham *et al*., 2014
4	*G. linii*	Root	Garcibiphenyl C, D and E	Chen *et al*., 2006
			Garcibiphenyl A and B	Chen *et al*., 2004
5	*G. mangostana*	Root bark	3-Hydroxy-4-geranyl-5-methoxybiphenyl	Dharmaratne *et al*., 2005
6	*G. multiflora*	Twig	Multiflorabiphenyls A and B	Xu *et al*., 2016a
		Leaf	Multiflorabiphenyls B-D	Fu *et al*., 2015
		Stem	Multiflorabiphenyls A-C	Gao *et al*., 2016
		Stem bark	Multiflorabiphenyls A	Jing *et al*., 2013
7	*G. nigrolineata*	Twig	Nigrolineabiphenyls A and B	Rukachaisirikul *et al.,* 2005a
8	*G. oblongifolia*	Leaf	Oblongifoliagarcinines A-D	Wu *et al*., 2008
9	*G. oligantha*	Stem	3-Methoxy-5-methoxycarbonyl-4-hydroxy biphenyl	Liu *et al*., 2015
10	*G. schomburkiana*	Wood	Schomburgbiphenyl	Mungmee *et al*.,2013
			Aucuparin, nigrolineabiphenyl B and Garcibiphenyl C	Mungmee *et al.,* 2012
		Stem	Schomburgbiphenyl A and B	Ito *et al*., 2013
11	*G. spp*	Twig	Doitungbiphenyls A and B	Siridechakorn *et al*., 2014
12	*G tetralata*	Twig	Tetralatabiphenyls A-C	Hu *et al*., 2016

6. Phloroglucinols

Phloroglucinols are an interesting group of phenolic compounds, based on a phloroglucinol or 1,3,5-benzenetriol skeleton. Phloroglucinols can be divided into subclasses such as acyl phloroglucinols, phloroglucinol glycosides and prenylated/geranylated phloroglucinols (Dakanali and Theodorakis, 2011). About 700 naturally occurring phloroglucinol compounds were reported, of which acylphloroglucinols (APGs) comprise the largest group of natural phloroglucinol compounds (Singh *et al*., 2010). Several *Garcinia* species have been reported to contain phloroglucinol derivatives (Zhou, *et al*., 2009). Benzophenones such as nemerosone and clusianone with close resemblance to phloroglucinol derivatives were also considered under the phloroglucinol category (Dakanali and Theodorakis, 2011).



Parvifoliol A Subellinone Atrovirinone

Figure 9. Structurs of monoprenylated phloroglucinol (parvifoliol A), polyprenylated phloroglucinol (subellinone) and phloroglucinic acid ester linked to a quinone moiety (atrovirinone)

Ultra performance liquid chromatography (UPLC) coupled with precursor ion discovery (PID) and tandem mass (MS/MS) scans has been reported as an efficient analytical tool for rapid screening of polycyclic polyprenylated acyl phloroglucinols from *Garcinia* species (Zhou, *et al*., 2009).

Phloroglucinol and its derivatives were reputed with biological activities such as antibacterial, cytotoxic, antiproliferative and antiangiogenic effects and have been widely used in medicine, cosmetics, pesticides, paints and dyes (Singh *et al*., 2010). The phloroglucinol Garsubellin A induces biosynthesis of acetylcholine, a neurotransmitter that at low concentrations can lead to Alzheimer's disease (Fukuyama *et al*., 1997).

Table 6. Phloroglucinols reported from *Garcinia* species

Sl. No.	*Garcinia* species	Plant part	Phloroglucinols	Reference
1	*G. atroviridis*	Root	Atrovirinone	Permana *et al*., 2001
2	*G. cowa*	Twig	Garcicowins A-D	Lin *et al*., 2010
3	*G. eugeniaefolia*	Stem bark	Enervosanone	Taher *et al*., 2007
4	*G. goudotiana*	Leaf	Goudotianone 1 and 2	Mahamodo *et al*., 2014
5	*G. multiflora*	Root	Garcinialone	Chien *et al*., 2008
6	*G. nujiangensis*	Leaf	Nujiangefolins A-C	Xia *et al*., 2012
7	*G. parvifolia*	Twig	Parvifoliols A-G	Rukachaisirikul *et al*., 2006
		Leaf	Parvifoliols B-E	Rukachaisirikul *et al*., 2008
8	*G. schomburgkiana*	Fruit	Oblongifolin C, garcicowin B, and garciyunnanin	Le *et al*., 2016
9	*G. subelliptica*	Heartwood	Garcinielliptone HF	Wu *et al*., 2008
			Garcinielliptone HA, HB, HC, HD, and HF	Lu *et al*., 2008
		Pericarp	Garcinielliptone FB	Wu *et al*., 2005
		Fruit	Garcinielliptone	Lin *et al*., 2005
		Wood	Subellinone	Fukuyama *et al*., 1993
			Garsubellins A	Fukuyama *et al*., 1997
			Garsubellins B-E	Fukuyama *et al*., 1998
			Cohulupone	Lin *et al*., 2010a
		Seed	Garcinielliptone A, B, C and D, and Garsubellins A	Weng *et al*., 2003
			Garcinielliptone K, L and M	Weng *et al*., 2004
			Garcinielliptone R	Lin *et al*., 2012
			Garcinielliptone P	Lin *et al*., 2010a
10	*G. verrucosa ssp orientalis*	Stem bark	Garcicosin	Rajaonarivelo *et al.,* 2009

7. Flavonoids

A variety of simple flavonoids such as quercetin, luteolin and apigenin were also reported from different *Garcinia* species.

Table 7. Flavonoids reported from *Garcinia* species

Sl. No.	*Garcinia* species	Plant part	Flavonoids	References
1	*G. andamanica*	Leaf	Scutellarein-7-diglucoside and sorbifolin-6-galactoside	Alam *et al*., 1986
			4′-Hydroxy wogonin 7-neohesperidoside	Alam *et al*., 1987
2	*G. bracteata*	Stem	Bracflavones A and B, quercetin, luteolin, apigenin, rhamnazin, and pilloin	Hu *et al*., 2014
			7-Methoxy-4',6-dihydroxy-8-isobutyryl-flavone	Li *et al*., 2015
		Twig	Bracteflavones A, bracteflavones, artocarmin D, 6-prenyl apigenin, cycloartocarpesin, and artochamin C	Yang *et al*., 2015
3	*G. brevipedicellata*	Stem bark	Pilloin	Ngoupayo *et al*., 2007
4	*G. celebica*	Stem bark	Epicatechin	Elfita *et al*., 2009
5	*G. conrauana*	Stem bark	Eriodictyol	Waterman and Chrichton, 1980
6	*G. cowa*	Stem	Quercetin	Shen *et al*., 2007
7	*G. dulcis*	Branch	3'-(3-Methyl-but-2-enyl) naringenin,	Harrison *et al*., 1994
		Ripe fruit	Dulcinoside, dulcisisoflavone, and dulcisflavan	Deachathai *et al*., 2005
8	*G. epunctata*	Stem bark	Taxifolin 6-C-glucoside	Mbafor *et al*., 1989
9	*G. eugenifolia*	Stem bark	Epicatechin	Taher *et al*., 2007
10	*G. gracilis*	Leaf	Apigenin-8-C-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside	Supasuteekul *et al*., 2016
11	*G. hombroniana*	Bark	3,3',4',5,5',7-Hexahydroxyflavone, 3,3',5,5',7-pentahydroxyflavanone, and 3,3',4',5,7-pentahydroxyflavone	Jamila *et al*., 2014
12	*G. kola*	Seed	Acacetin, apigenin-4'-5-7-trimethyl ether, and fisetin	Iwu and Igboko, 1982
			Naringin-7-rharmnoglucoseside	Okwu and Morah 2007
13	*G. livingstonei*	Seed	Eriodictyol	Srivastava and Sharma 1966
14	*G. mangostana*	Fruit hull	Taxifolin-3-o-α-L-rhamnoside	Huang *et al*., 2001
			Epicatechin	Yu *et al*., 2007
			Aromadendrin-8-C-glucopyranoside, and epicatechin	Abdallah *et al*., 2016
15	*G. multiflora*	Heart wood	Apigenin	Fa-Ching *et al*., 1975
16	*G. neglecta*	Leaf	Apigenin and narigenin	Ito *et al*., 2001
17	*G. nervosa*	Leaf	Nervosin, irigenin, and 7-methyl tectoirigenin	Ilyas *et al*., 1994
18	*G. parvifolia*	Leaf	Nigrolineaisoflavone A	Rukachaisirikul *et al*., 2008
19	*G. paucinervis*	Stem	Pauciisoflavone A	Hu *et al*., 2014
20	*G. purpurea*	Pericarp	Vitexin and apigenin-7-o-(6"-methyl ester)-glucuronide	Iinuma *et al*., 1996
21	*G. schomburgkiana*	Branch	Kaempferol, dihydrokaempferol and (-)-5,7,3′,5′-tetrahydroxyflavanone	Meechai *et al*., 2016
22	*G. vitiens*	Leaf	Vitexin	Parveen *et al*., 1994
23	*G.xipshuanbannaensis*	Fruit	Luteolin and 3᾽,5,7-4᾽-methoxy-flavone	Shen *et al*., 2006

Conclusions

Garcinia species are rich depository of structurally diverse secondary metabolites such as biflavonoids, prenylated and caged xanthones and polyisoprenylated benzophenones. Most of the *Garcinia* species are not yet explored for their chemical constituents or bioactivities. Literature survey revealed that, of the nearly 250 *Garcinia* species, less than 50% have been studied for their chemical constituents. Xanthones are the major class of phenolic compounds in *Garcinia* species, followed by benzophenones and biflavonoids. The chapter enlists the major phenolic compounds xanthones, benzophenones and biflavonoids, along with minor constituents biphenyls, despidones, phloroglucinols and simple flavonoids reported in *Garcinia* species world over.

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Chapter 3

Phytochemical investigation of the Western Ghats endemic species *Garcinia imberti* Bourd.

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Abstract

Phytochemical investigation of the stem bark of *Garcinia imberti*, a Western Ghats endemic species, resulted in the isolation and characterization of the biflavonoid morelloflavone, the triterpenoid 2α-hydroxy-3β-acetoxy-urs-12-en-28-oic acid and the steroid stigmasterol. The high content of morelloflavone (0.76% w/w) in the stem bark, estimated by HPTLC, projects the plant as a rich source of the bioactive biflavonoid. The major compound from the hexane extract of the leaves was isolated and characterized as the triterpenoid friedelin. HPTLC estimation showed high content of friedelin in the plant leaves (2.2% w/w). Quantitative screening of the phenolic compounds present in the leaf methanol extract of *G. imberti* was carried out using UHPLC-QqQ$_{LIT}$-MS/MS technique. Twenty two phenolic compounds comprising xanthones (α-mangostin and gambogic acid), biflavonoids (fukugiside, GB-2, GB-1, GB-1a, amentoflavone), benzophenone (garcinol), flavonoids (epicatechin, isoorientin, orientin, isovitexin, vitexin, kaempferol-3-O-rutinoside, luteolin, quercetin, apigenin, kaempferol) and phenolic acids (protocatechuic acid, caffeic acid, ferulic acid, vanillic acid) were identified and estimated in the leaves of the plant. The LC-MS study revealed the biflavonoid GB1 in abundance in the leaf methanol extract (22.1000 mg/g). The plant was also found as a rich source of essential oils and the volatile chemical studies revealed caryophyllene derivatives as the major constituents of the essential oils from leaf, bark and fruits.

Keywords: *Garcinia imberti,* Morelloflavone, GB1, Friedelin, Essential oil, Caryophyllene, UHPLC-QqQ$_{LIT}$-MS/MS

Introduction

Garcinia species have multiple applications in culinary, pharmaceutical and nutraceutical field. The genus has been the subject of elaborate phytochemical studies worldwide that revealed it as a rich source of diverse compounds such as xanthones, benzophenones, biflavanoids, flavonoids, acids, and lactones (Han *et al*., 2008). The phytochemicals reported from *Garcinia* species exhibited a wide range of pharmacological activities such as anti-microbial, anti-HIV, anti-diabetic, antioxidant and cytotoxic (Kim *et al*., 2008, Hemshekhar,

2011). The Western Ghats, one among the 36 global biodiversity hot spots, hosts 9 *Garcinia* species, of which 7 are endemic to the region (Maheswari, 1964, Sabu *et al*., 2013). Of the 9 *Garcinia* species distributed in the Western Ghats, *G. gummi gutta* and *G. indica* are cultivated widely and studied for their constituents and bioactivities. However, most of the endemic species are yet to be studied for their phytochemicals or potential utilities.

Garcinia imberti Bourd. is an evergreen tree, endemic to the Agastyamala forests of the Western Ghats (**Figure 1**). The species was originally described by T. F. Bourdillon in 1899 and rediscovered after nearly a century by Mohanan *et al* from the Agasthyamala Hills (Bourdillon, 1899, Mohanan, 1997). *G. imberti* is least investigated for their phytochemicals or bioactivities (Rameshkumar *et al*., 2005). Present chapter elaborates the phytochemical investigation of *G. imberti* and reports the presence of sesquiterpenoids, triterpenoids, steroids, flavonoids, biflavonoids, xanthones, benzophenones, and phenolic acids in the plant. Conventional phytochemical investigation techniques such as extraction, separation and characterization as well as modern rapid analytical techniques such as LC-MS and GC-MS were utilized for the phytochemical profiling.

Figure 1. *Garcinia imberti* twig with fruit

1. Phytochemical investigation of the stem bark of *Garcinia imberti*

The plant parts were collected from Chemmungi forest area of south Western Ghats, Thiruvananthapuram district, Kerala state, India and authenticated by Mr. M.S. Kiran Raj, JNTBGRI. A voucher specimen (TBGT No.40076) has been deposited at the JNTBGRI Herbarium (TBGT). IR spectra of the isolated compounds were taken on an ABB FTLA-2000 spectrometer, UV spectra using Shimadzu (1650 PC) UV-Visible spectrometer, NMR spectra using JEOL FT-NMR (300MHz) and Mass spectra using JEOL JMS-600 spectrometer.

Analyses of the hexane and methanol extracts of the stem bark of the plant resulted in the isolation and characterization of the steroid stigmasterol (**1**), the triterpenoid 2α-hydroxy, 3β-acetoxy urs-12-en, 28-oic acid (**2**) and the biflavonoid morelloflavone (**3**) (**Figure 2**). The isolated compounds were identified by detailed spectroscopic studies and comparison with literature data.

Compound **1** was isolated by column chromatography of the hexane extract of the stem bark. The compound was eluted in the solvent system hexane: chloroform (9:1) and identified as stigmasterol by comparison of the NMR and MS data (Conolly and Hill, 1994). The steroid is a common phytochemical distributed widely in the plant kingdom and has been isolated previously from several *Garcinia* species as well (Elfita *et al*., 2009).

Compound **2** was eluted from the hexane extract using the solvent system hexane: chloroform (1:1) and was identified as 2α-hydroxy-3β-acetoxy-urs-12-en-28-oic acid by analyzing the mass spectra, ^{1}H and ^{13}C NMR spectral data and comparison of the spectral data with those reported in the literature (Chaturvedula *et al.,* 2004). Despite the large number of literature reports of different urs-12-en triterpenoids with different possible substitutions and stereochemical orientations, the occurrence of ursolic acid derivatives with acetoxy group at 3-β position and a free carboxylic acid group at C-17 are rare. The compound has been reported to possess polymerase β-lyase activity (Chaturvedula *et al* 2004). The ursane triterpenoid has been isolated for the first time from Clusiaceae family.

Compound **3** was isolated by column chromatography of the stem bark methanol extract. The compound was eluted using hexane: EtOAc (7.5:2.5) and was identified as 3’’’,4’,4’’’,5,5’’,7,7’’-heptahydroxy-3(8’’)-flavonyl flavonone (morelloflavone) by comparison of the spectral data with those reported in the literature (Li *et al*., 2002). The biflavonoid morelloflavone, first reported from *Garcinia morella* by Karanjgaokar *et al.* is a common constituent among *Garcinia* species (Karanjgaokar *et al*., 1967). It is also the first biflavonoid reported with a flavone and a flavonone unit. Morelloflavone has been reported as anti inflammatory, anti HIV, anti fungal, anti tumor, hypocholesterolemic and anti plasmodial (Lin *et al*., 1997, Li *et al*., 2002, Pang *et al*., 2009, Ngouamegne *et al*., 2008). The biflavonoid also inhibits tyrosinase, the major enzyme responsible for skin melanization (Masuda *et al*., 2005) and prevents restenosis (Pinkaew *et al*., 2009).

Stigmasterol (**1**): Colourless crystals, mp: 160-162^{o} C. R_f: 0.48 (chloroform 100%). IR (KBr cm^{-1}): 3435, 2961, 2937, 2889, 2864, 1461, 1382, 1368, 1061, 970cm^{-1}. EI-MS (70 eV) m/z (%): 412 (M+, 70), 369 (8), 351 (13), 300 (28), 273 (17), 271 (28), 255 (30), 231 (10), 213 (20), 161 (19), 159 (22), 145 (42), 121 (26), 105 (36), 83 (60), 55 (100). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.84(3H,d, J= 6.6Hz, H-27); δ 0.81(3H,d, J= 7.2 Hz, H-26); δ 5.03 (1H, dd, J=15.1, 8.4, H-23); δ 5.14 (1H, dd, J=15.1, 8.4, H-22); δ 1.02(d, J= 6.6 Hz, H-21); δ 1.01 (s, H-19); δ 0.69 (s, H-18); δ 5.35(1H, d, J= 4.8 Hz, H-6); δ 3.52 (m, H-3). ^{13}C NMR (75 MHz, $CDCl_3$): 12.0 (CH_3), 12.2 (CH_3), 19.0 (CH_3), 19.4 (CH_3), 21.1 (CH_3), 21.1 (CH_2), 21.2 (CH_3), 24.3 (CH_2), 25.4 (CH_2), 28.9 (CH_2), 31.6 (CH_2), 31.9 (CH x 2), 36.5 (C), 37.2 (CH_2), 39.7 (CH_2), 40.5 (CH), 42.3 (C), 50.1 (CH), 51.2 (CH), 55.9 (CH), 56.9 (CH), 71.8 (CH), 121.7 (CH), 129.3 (CH), 138.3 (CH), 140.7 (C)

2α-Hydroxy-3β-acetoxy-urs-12-en-28-oic acid (**2**): Colourless crystals, mp: 199-202^{o} C. R_f: 0.54 (hexane: chloroform: methanol, 5:4.5:0.5), $[\alpha]_D$: – 40.9 (*c* 0.10, MeOH), IR (KBr): 3450, 2970, 2931, 2873, 1720, 1693, 1458, 1373, 1245, 1049, 1029, 960, cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): 1.02 (1H, m, H-1α, ax), 2.1 (1H, m, H-1β, eq), 3.76 (1H, m, H-2), 4.51 (1H, d, J= 9 Hz, H-3), 1.96 (2H, m, H-11), 5.23 (1H, m, H-12), 2.20 (1H, d, J=12Hz). ^{13}C NMR (75 MHz, $CDCl_3$): 16.3 (CH_3), 16.7 (CH_3 x 2), 17.3 (CH_3), 17.9 (CH_2), 20.8 (CH_3 x 2), 22.9

(CH_2), 23.1 (CH_3), 23.7 (CH_2), 27.6 (CH_2), 28.2 (CH_3), 30.3 (CH_2), 32.4 (CH_2), 36.3 (CH_2), 37.6 (C), 38.4 (CH), 38.6 (CH), 38.8 (C), 39.2 (C), 41.7 (C), 47.0 (CH), 47.1 (C), 47.3 (CH_2), 52.2 (CH), 54.6 (CH), 66.2 (CH), 84.3 (CH), 124.6 (CH), 138.0 (C), 171.4 (C), 179.5 (C). FAB-MS (pos.) m/z (%): 537 $[M+Na]^+$ (3), 514 (5), 469 (10), 455 (19), 437 (100), 262 (31), 248 (50), 203 (62), 189 (58), 133 (81).

(-) Morelloflavone (**3**): Yellow solid from acetone/methanol, mp: 210° C (decomposing), R_f: 0.62 ($CHCl_3$: MeOH, 17.0: 3.0), $[\alpha]_D$: – 59.9 (c 0.10, MeOH), IR (KBr): 3224, 1643, 1610, 1515, 1426, 1448, 1367, 1261, 1184, 1164, 839, $cm^{-1,}$ UV/Vis λ_{max} (MeOH) nm: 341, 288, 277 and 227, ^1H NMR (300 MHz, $CDCl_3$): 5.76 (1H, d, J=12 Hz, H-2), 4.77 (1H, d, J=12 Hz, H-3), 12.94 (1H, s, 5-OH), 5.96 (1H, s, H-6), 6.35 (1H, s, H-3''), 6.26 (1H, s, H-6'') ^{13}C NMR (75 MHz, $CDCl_3$): 48.9, 81.3, 95.7, 96.6, 99.1, 100.8, 101.9, 102.7, 103.7, 113.6, 114.8, 116.5, 119.3, 121.6, 128.6, 128.7, 128.9, 146.3, 150.0, 155.7, 157.4, 161.1, 161.9, 163.3, 163.9, 164.3, 166.9, 182.1, 196.5

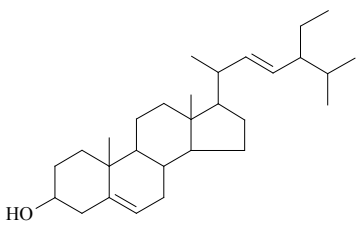
2. Phytochemical investigation of the leaves of *Garcinia imberti*

Compound **4** was isolated from the hexane extract of the leaves and identified as the triterpenoid friedelin by comparison of the spectral data with those reported in the literature (Antonisamy *et al*., 2011). Friedelin has been reported in different *Garcinia* species as well (Magadula, 2010, Jantan and Saputri, 2012). Friedelin and its derivates have anti-cancer, analgesic, anti-inflammatory, anti-bacterial, antioxidant, hepatoprotective, vascularizing activities and have potential to be used in pharmaceuticals or functional foods for the treatment or prevention of cardiovascular and cerebrovascular diseases and tumours (Moiteiro *et al*., 2006, Antonisamy *et al*., 2011, Sunil *et al*., 2013,).

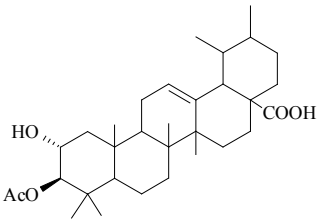
Friedelin (4): White solid, m.p. 242-246°C. MS *m/z* (rel. int.): 449 $[M+Na]^+$ (8), 341 $[M-Me]^+$ (4), 302(14), 289 (7), 273[M-Me-H_20] + (24), 246 (16), 231 (16), 205 (24), 191 (20), 163 (24), 149 (22), 125 (62), 123 (64), 109 (66), 95 (84), 81 (68), 69 (100). ^1H NMR ($CDCl_3$, 500 MHz) δ: 1.96 (1 H, m, H-1a), 1.71 (1 H, m, *J* = 10.1, H-1b), 2.37 (1 H, dd, *J* = 10, 3.5 and 4 Hz, H-2a), 2.26(1H,M,H-2b), 1.219-1.698(m, H3-H22), 0.86(3H, d, J=6.1Hz, Me-23), 0.70(3H,s, Me-24), 0 .84(3H,s,Me-25), 0.93(3H,s,Me-26), 1.03(3H,s,Me-27), 1.16(3H,s,Me-28), 0.98(3H, s ,H-29), 0.98(3H,s,H-30). ^{13}C NMR (500 MHz, $CDCl_3$): δ 22.3 (C-1), 41.5 (C-2), 213.3 (C-3), 58.2 (C-4), 42.2 (C-5), 41.3 (C- 6), 18.2 (C-7), 53.1 (C-8), 37.4 (C-9), 59.5 (C-10), 35.6 (C-11), 32.4 (C-12), 38.3 (C-13), 39.7 (C-14), 30.5 (C- 15), 36.0 (C-16), 30.0 (C-17), 42.8 (C-18), 35.3 (C-19), 28.2 (C-20), 32.8 (C-21), 29.6 (C-22), 6.8 (C-23), 14.7 (C-24), 18.2 (C-25), 18.7 (C-26), 20.3 (C-27), 32.1 (C- 28), 31.8 (C-29), 35.0 (C-30)

3. HPTLC estimation of the major compounds in *Garcinia imberti*

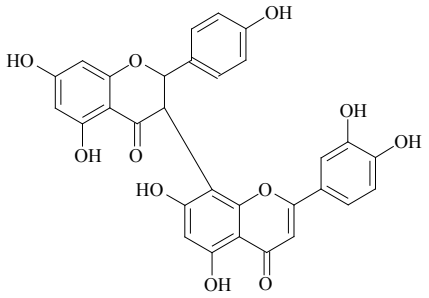
Among the different analytical techniques, HPTLC has emerged as a widely applied technique for qualitative and quantitative purposes in natural product analysis and the method has successfully been explored for the estimation of bioactive compounds from plant sources (Reich and Schibli, 2006, Aravind *et al*., 2008). In the present study, the HPTLC estimations were carried out using Camag HPTLC system (Switzerland) equipped with LinomatV sample applicator and Camag TLC scanner 3.



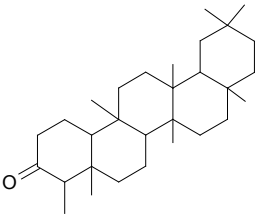
Stigmasterol



2α- Hydroxy-3β-acotoxy-urs-12-en-28-oic acid



Morelloflavone



Friedelin

Figure 2. Structures of compounds 1 to 4

3.1. HPTLC estimation of morelloflavone in *G. imberti* stem bark

The dried, powdered stem bark (5 g) was extracted with methanol using Soxhlet apparatus for 6h and made up to 200 ml using methanol. Morelloflavone isolated from the plant was used as the standard compound. 1.5 µL of the extract was applied on the pre-coated silica gel plate $60F_{254}$ (E. Merk, Germany) along with standard morelloflavone (1.0 to 2.5µg gave linear response). The separation was carried out in twin trough chamber using the solvent system chloroform: methanol (17:3) as mobile phase. Quantitation was carried out in absorbance mode at 254 nm. The percentage content of morelloflavone was found 0.76 ±0.09 % (w/w) in the stem bark. The plant can be considered as a new natural source of the bioactive biflavonoid morelloflavone.

3.2. HPTLC estimation of friedelin in *G. imberti* leaves

The dried, powdered leaf sample (2 g) was extracted with hexane using Soxhlet apparatus for 6h and made up to 100 ml using hexane. For estimation of friedelin in the leaf samples, the solvent system hexane-chlorofom-ethylacetate (9:0.5:0.5) gave the best resolution. 3.0 µL of the hexane extract was applied on the pre-coated silica gel plate $60F_{254}$ (E. Merk, Germany). Standard friedelin at concentrations 0.5-4.0µg gave linear response with regression equation $y=257x+356.8$ and the regression (r^2) 0.949 indicated a good linear relationship between peak area and concentration of the analyte. The specificity of the developed method was confirmed by close R_f values of standard friedelin (0.31). The content of friedelin was 2.2 ±0.5% (w/w). The high content of friedelin proposes the plant as a novel source of the compound.

4. UHPLC-QqQ$_{LIT}$-MS/MS analysis of *G. imberti* leaf methanol extract

Liquid chromatography-mass spectrometry using different combination of separation, ionisation and mass analysing techniques have proven as an efficient tool for the qualitative as well as quantitative characterization of phytochemicals (Wu *et al*., 2013). The hyphenated analytical technique provided extremely powerful tools for natural product researchers that offered both the separation and characterization in single run. Several *Garcinia* species have been studied by various LC-MS techniques like LC-ESI-MS, UPLC-Q-TOF-MS and HPLC-DAD-MS^n and reported the distribution of acids, benzophenones, xanthones, biflavonoids and acylphloroglucinols (Acuna *et al*, 2012, Ji *et al*, 2007; Zhou *et al*, 2010).

In the present study, the dried leaf powder (2g) was defatted with hexane and extracted with methanol using Soxhlet apparatus. The methanol extract (1mg/ml) was diluted with acetonitrile and spiked with internal standard curcumin (20 ng/mL final working concentration) and 4 µL aliquot was injected into the UHPLC-MS/MS system for analysis. A mixed standard stock solution (1 mg/mL) of the selected analytes were also prepared and diluted with acetonitrile to get final concentrations of 0.1 to 300 ng/mL, along with internal standard curcumin (20 ng/mL). The separation was achieved on Waters Acquity UPLC^{TM} system (Waters, Milford, MA, USA) equipped with binary solvent manager, sample manager, column oven and photodiode array detector (PAD). The chromatographic separation of selected analytes was carried out on an Acquity UPLC BEH C_{18} column (50 mm × 2.1 mm id, 1.7µm) at a column temperature of 25°C. Analysis was done with gradient elution of 0.1% formic acid in water (A) and acetonitrile (B) as mobile phase at a flow rate of 0.3 mL/min. The 7.5 min UPLC gradient elution program was as follows: 0-0.70 min, 5-15% B; 0.7-2.5 min, 15-23% B; 2.5-2.8 min, 23-33% B, 2.8-4.0 min, 33-40% B; 4.0-4.8 min, 40-95% B; 4.8-6.8 min, 95-95% B; 6.8-7.5 min, 95-5% B; equilibration time 1.5 min. The LC was interfaced with hybrid linear ion trap triple-quadrupole mass spectrometer (API 4000 QTRAP™ MS/MS system from AB Sciex, Concord, ON, Canada) equipped with an electrospray (Turbo V) ion source. AB Sciex Analyst software version 1.5.1 was used to control the LC-MS/MS system and for data acquisition and processing. Precursor ion scan was used for the screening and MRM acquisition mode for quantification of the analytes. All the analytes with internal standard (IS) were detected in negative electrospray ionization and mass spectra were recorded in the range of *m/z* 100-1000 at a cycle time of 9s with a step size of 0.1 Da. Nitrogen was used as the nebulizer, heater, and curtain gas as well as the collision activated dissociation gas (CAD). The optimized mass spectrometric source parameters were; ion spray voltage set at -4200 V, curtain gas, nebulizer gas (GS1) and heater gas (GS2) were set at 20psi and source temperature was set at 550°C. The compound dependent MRM parameters: DP, EP, CE and CXP were optimized for each investigated analyte by injecting the individual standard solution into the mass spectrometer to achieve the most abundant, specific and stable MRM transition.

The MS spectra generated for all the compounds by ESI-MS in the negative ion mode gave the deprotonated molecule $[M-H]^-$. The structures were further identified through characteristic fragment ions. The detected compounds and their quantities were shown in **Table 1** and **Figure 3**. Among the 22 phenolic compounds, content of the biflavonoid GB-1 was the highest (22.1000 mg/g) in the leaf extract of *G. imberti*, followed by the xanthone gambogic acid (2.8500 mg/g) and the biflavonoid GB-1a (2.4700 mg/g).

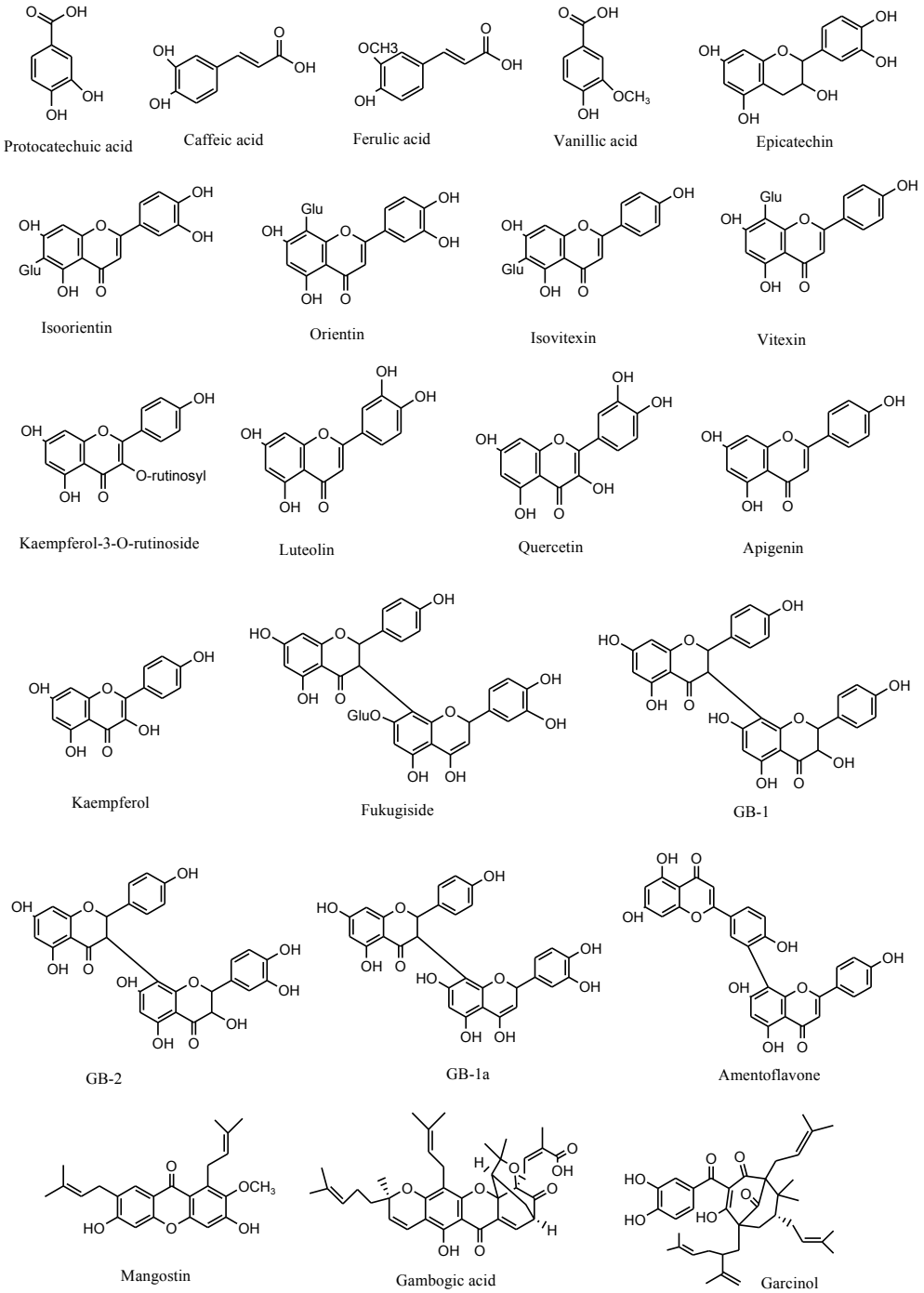


Figure 3. Structures of the 22 phenolic compounds detected in *Garcinia imberti* leaf methanol extract by UHPLC-QqQ$_{LIT}$-MS/MS method

Table 1. The content (mg/g) of 22 phenolic compounds in the leaf extract of *Garcinia imberti*

Retention Time (min)	Compound	Content (mg/g) (mean ± SD, *n*=3)
	Phenolic acids	
1.43	Protocatechuic acid	0.9890 ± 0.002
1.81	Caffeic acid	0.1420 ± 0.005
2.47	Ferulic acid	0.5220 ± 0.001
3.31	Vanillic acid	0.0008 ± 0.0002
	Flavonoids	
1.79	Epicatechin	0.9240 ± 0.001
1.91	Isoorientin	0.6070 ± 0.005
2.04	Orientin	0.5340 ± 0.004
2.26	Isovitexin	1.4100 ± 0.029
2.28	Vitexin	1.1800 ± 0.015
2.53	Kaempferol-3-O-rutinoside	0.0637 ± 0.0005
3.62	Luteolin	0.1053 ± 0.0004
3.63	Quercetin	0.1920 ± 0.026
4.04	Apigenin	0.7010 ± 0.027
4.14	Kaempferol	0.2820 ± 0.003
	Biflavonoids	
3.56	Fukugiside	0.2910 ± 0.002
3.57	GB-2	0.3850 ± 0.012
4.05	GB-1	22.1000 ± 1.054
4.46	GB-1a	2.4700 ± 0.165
4.52	Amentoflavone	0.0440 ± 0.003
	Xanthones	
5.71	α-Mangostin	0.0056 ± 0.001
6.19	Gambogic acid	2.8500 ± 0.032
	Benzophenone	
6.50	Garcinol	0.3290 ± 0.011

5. Volatile chemical profile of *Garcinia imberti*

Hydrodistillation of the stem bark, leaves and fruits revealed *G. imberti* as a rich source of essential oil. The oil yield was 0.62 % v/w for stem bark, 0.32% for leaf and 1.50% for fruits. A total of 25 volatile compounds were detected by GC-MS analysis of the essential oils (**Table 2**). The major constituents were humulene and β-caryophyllene in stem bark and leaf oil, while caryophyllene oxide and humulene epoxide were the major constituents in fruit oil (**Figure 4**). The caryophyllene derivatives such as humulene, caryophyllene and their oxides are biosynthetically derived from the common humulyl intermediate (Cane, 1999). The plant can be considered as a rich source of the caryophyllene compounds. It will be interesting to study the chemical ecological aspects of the high content of caryophyllene compounds in the species.

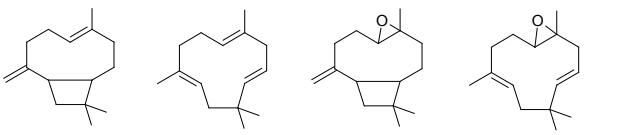


Figure 4. Structures of major volatile chemicals of *Garcinia imberti*

Table 2. Volatile chemical profiles of *Garcinia imberti* leaf, stem bark and fruits

Compound	RRI	LF	SB	FR
δ-Elemene	1338	0.1	--	--
α-Cubebene	1348	0.3	--	--
α-Ylangene	1373	0.3	--	--
α-Copaene	1376	0.4	--	0.1
β-Cubebene	1387	0.3	--	--
2-epi-β-funebrene	1415	--	--	6.7
β-Funebrene	1414	--	--	2.9
β-Caryophyllene	1419	38.1	41.4	1.8
β-Copaene	1430	0.4		3.7
α-Humulene	1452	30.5	50.8	5.4
Allo aromadendrene	1458	5.5		
α-Acoradiene	1464	0.3	--	--
9 epi E- Caryophyllene	1466	--	--	8.7
β-Acoradiene	1469	4.5	--	--
cis β-Guaiene	1492	0.1	--	--
β-Alaskene	1498	2.5	--	--
E-γ-Bisabolene	1507	0.1	--	--
δ-Amorphene	1511	0.4	--	--
Germacrene B	1559	0.3	--	--
Caryophyllene oxide	1582	0.3	2.3	33.2
Humulene epoxide II	1608	--	1.4	21.3
1,10-di epi Cubenol	1618	0.1	--	--
Caryophylla-4(12),8(13) diene	1639	--	--	2.0
Cubenol	1645	0.1	--	--
14- Hydroxy 9-epi-E-caryophyllene	1668	--	--	1.5
γ-Costol	1688	--	--	3.3
Total %		84.6	95.9	90.6
Sesquiterpene- hydrocarbons		84.1	92.2	29.3
Sesquiterpene-oxygenated		0.5	3.7	61.3
Total sesquiterpenoids		84.6	97.9	92.6

RRI: Relative retention index calculated on HP-5 column

Conclusions

Garcinia species were studied worldwide for the variety of interesting secondary metabolites and the present study revealed the Western Ghats endemic species *G. imberti* as a rich source of the bioactive biflavonoids morelloflavone and GB-1, along with the triterpenoid friedelin. The species is also a rich source of the volatile caryophyllene compounds. The chapter also elaborates a comprehensive quantitative analysis of multi class bioactive constituents including prenylated xanthones, polyisoprenylated benzophenones, biflavonoids, phenolic acids and flavonoids in leaf methanol extract of *G. imberti* using UHPLC-QqQ$_{LIT}$-MS/MS method.

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Chapter 4

Phytochemical Investigation of the Western Ghats endemic species *Garcinia travancorica* Bedd.

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Abstract
The leaves of *Garcinia travancorica*, an endemic species to the Western Ghats of south India, yielded the polyisoprenylated benzophenones, 7-epi-nemorosone and garcinol along with the biflavonoids GB-1a, GB-1, GB-2, morelloflavone and morelloflavone-7-O-β-D-glycoside (fukugiside). *G. travancorica* leaves were found as a rich source of the biflavonoid glycoside morelloflavone-7"-O-β-D-glycoside (7.12% dry wt) through a validated HPTLC estimation method. Qualitative screening of multiclass secondary metabolites present in the fruits, leaves and stem bark methanol extracts of *G. travancorica* using HPLC-QTOF-MS analysis resulted in the identification of 23 compounds including two acids (hydroxycitric acid and hydroxycitric acid lactone), eight biflavonoids (morelloflavone, GB-1, GB-1a, GB-2, GB-2a, fukugiside, xanthochymusside and GB-1a glucoside), nine xanthones (α-mangostin, γ-mangostin, 1,5-dihydroxy-3-methoxyxanthone, garciniaxanthone E, 4-(1,1-dimethylprop-2-enyl)-1,3,5,8-tetrahydroxy-xanthone, garcinone A, garcinone B, garcinone C and polyanxanthone C) and four polyisoprenylated benzophenones (gambogenone, aristophenone A, garcinol and garciyunnanin A). *G. travancorica* was also found as a rich source of essential oils and the aliphatic hydrocarbon n-undecane was the major volatile compound in leaf, stem bark and fruit.

Keywords: *Garcinia travancorica*, fukugiside, n-Undecane, Essential oil, Biflavonoids, Xanthones, Benzophenones, HPLC-QTOF-MS

Introduction

Garcinia species, with its rich diversity of biologically active compounds such as biflavonoids, xanthones, benzophenones and acids, received considerable attention worldwide from scientific as well as industrial sectors (Hemshekhar *et al*., 2011). Xanthones, biflavonoids and benzophenones from different *Garcinia* species were reported to possess remarkable levels of bioactivities against various ailments (Carvalho-Silva *et al*. 2012; Osorio *et al*. 2013). Among the different phenolic compounds reported from *Garcinia* species, the biological activities of biflavonoids are diverse, including anticancer, antibacterial, antifungal, antiviral, anti-inflammatory, analgesic, antioxidant, vasorelaxant and anticlotting. The mechanisms of activity of biflavonoids have also been elaborated in most of the cases

(Kim *et al*. 2008). *Garcinia travancorica* is a rare and endemic species, distributed in the evergreen forests of Agastyamala region of southern Western Ghats of India, where scattered populations were seen at altitude 1000-1300m (Mohanan and Sivadasan, 2002) (**Figure 1**). The species is least investigated for their phytochemicals (Anuaravind *et al*., 2016) and the present chapter reports the secondary metabolite profile of *G. travancorica*.

Figure 1. *Garcinia travancorica* twig with flower and fruit

1. Phytochemical investigation of the leaves of *G. travancorica*

Fresh leaves were collected from Chemunji forest area, part of the Agasthyamala forest region of South Western Ghats, Thiruvananthapuram district, Kerala, India and a voucher specimen (No. 66417) was deposited at the JNTBGRI Herbarium (TBGT).

UV spectra were recorded on a Shimadzu spectrophotometer -UV 1800, Japan. IR spectra were taken with Alpha FT-IR, Bruker Optics. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker-Avance 400 MHz FT-NMR spectrometer operating at 400 MHz for ^{1}H NMR and 100MHz for ^{13}C NMR. The chemical shifts were expressed as δ (ppm, parts per million) referring to internal standard, tetramethyl- silane (Me_4Si). Mass spectra were recorded using JEOL JMS 600 H mass spectrometer.

The polyisoprenylated benzophenones, 7-epi-nemorosone (**1**) and garcinol (**2**) were isolated from the hexane extract by column chromatography. Structures of these compounds were confirmed by UV, IR and NMR spectroscopic data, together with comparison of literature data (Rao *et al*. 1980; Padhye *et al*. 2009; de Castro *et al*. 2011). The bioactive benzophenone garcinol, also known as camboginol, was reported from different *Garcinia* species and showed antiglycation, antioxidant and free radical scavenging activities (Sahu *et al*. 1989; Rastogi & Mehrotra 1990; Yamaguchi *et al*. 2000; de Souza Marques *et al*. 2012).

The biflavonoids, namely GB-1a (**3**), GB-1 (**4**), GB-2 (**5**), morelloflavone (**6**) and morelloflavone-7”-O-β-D-glycoside or fukugiside (**7**) were isolated from the methanol extract by column chromatography (**Figure 2**). Structures of these compounds were elucidated by NMR, MS and comparison with the literature spectroscopic data (Kapadia *et al*.

1994; Elfita *et al*. 2009). The (3->8") linked biflavonoids isolated from *G. travancorica* can be generally divided into two groups; those made up of flavone and flavanone subunits and those made up of two flavanone units. GB-1a, GB-1 and GB-2 were biflavanones, while morelloflavone and morelloflavone-7"-O-β-D-glycoside were flavanone-flavone type biflavonoids. Of the two types, biflavononones were the dominant type in different *Garcinia* species, while the co-occurrence of the two types of biflavonoids is rare (Waterman and Hussain 1983).

7-epi-Nemorosone (1): Yellow liquid; TLC: Hexane-ethylacetate (9:1), R_f = 0.76; UV (CH_3Cl, 0.1%) λmax/nm: 281, 265. HRMS m/z-501.3018 $(M-H)^-$ for $C_{33}H_{41}O_4$ (calcd. 501.3005); MS^n experiment *m/z*-501.3, 432.2, 417.2, 363.2, 309.1, 242.0, 145.0. ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): δ 2.09 (H-6a, m); 2.11 (H-6b, m); 1.52 (H-7, m); 7.55 (H-12, dd, J= 7.6 and 1); 7.38 (H-13, t, J = 7.6); 7.39 (H-14, t, J= 7.6); 7.37 (H- 15, t, J = 7.6); 7.54 (H-16, d, J = 7.6); 2.72 (H-17a, overlapped); 2.72 (H-17b, overlapped); 5.01 (H-18, m); 1.70 (3H, s,CH_3 = 20); 1.70 (3H, s,CH_3 = 21); 2.54 (H-22a, m); 2.55 (H- 22b, m); 5.04 (H-23, m); 1.54 (3H, s, CH_3-25); 1.99 (H-27a, m); 2.16 (H-27b, m); 4.90 (H-28, m); 1.60 (3H, overlapped, CH_3-30); 1.64 (3H, overlapped, CH_3-31); 1.51 (3H, s, CH_3-32); 1.25 (3H, s, CH_3-33). ^{13}C NMR (100 MHz, δ ppm): δ 73.0 (C1); 192.6 (C2); 120.4 (C3); 193.9 (C4); 64.6 (C5); 41.5 (C6); 47.6 (C7); 48.6 (C8); 207.5 (C9); 197.5 (C10); 137.3 (C-11); 128.9 (C-12), 127.8 (C-13); 132.5 (C-14); 127.7 (C-15); 128.8 (C-16); 23.7 (C-17); 120.4 (C-18); 134.5 (C-19); 17.9 (C-20); 25.8 (C-21); 30.2 (C-22); 119.9 (C-23); 133.3 (C- 24); 18.1 (C-25); 25.6 (C-26); 29.7 (C-27); 123.3 (C-28); 132.5 (C- 29); 18.1 (C-30); 26.1 (C-31); 26.7 (C-32); 23.7 (C-33).

Garcinol (2): Pale yellow crystal; TLC solvent system: hexane-chloroform (7:3); R_f = 0.27; UV (CH_3Cl, 0.1%) λmax (nm) 306, 244. IR 3200-3500, 1727, 1562 cm^{-1}, HR-MS *m/z*: 603.3681 $(M+H)^+$ for $C_{38}H_{51}O_6$ (calcd. 603.3686); MS^n experiment *m/z*: 603.3, 467.2, 411.1, 343.1, 287.0, 233.0, 177.0, 137.1, 95.0; ^1H NMR (400 MHz, CD_3OD): δ 7.05, 6.71, 6.69 (d; J=8 Hz, aromatic protons) 4.9 1.58 1.68 (isopropylidine groups) 4.51 (isopropenyl group), 1.68 (Me), 0.97 and 1.17 (methyl groups) to 1.4 to 2.7 (methylene and methane). ^{13}C NMR spectrum of garcinol showed the presence of three methine carbons of trisubstituted olefinic groups at δ 124.4, 124.6 and 122.6 and at δ 112.0 for a terminal methylene carbon. Other assignments were δ 206.2 (C-9, C=O), 194.0 (C-2, C=O), 195.1 (C-4, C-OH), 199.0 (C-15, C=O); 131.5 (C-12, CMe_2), 132.3 (C-34, CMe_2), 134.0 (C-26, CMe_2); 149.8 (C-28, C (Me) $=CH_2$), δ 116.6 (C-17, Ar-CH), 149.8 (C-20, Ar-CH), 122.5 (C-21, Ar-CH); 145.2 (C-18, Ar-C-OH), 132.5 (C-19, Ar-C-OH); 126.3 (C-16, Ar-C-C=O)**;** 116.9 (C-3), 68.6 (C-1), 48.8 (C-8), 47.9 (C-7), 59.9 (C-5), 43.0 (C-6, 23); 26.8, 27.4, 32.9, 37.4, 43.0 (5 CH_2); 18.1, 18.3, 18.7, 25.9, 26.3 (6 Me, C=CMe); 23.3 (C(Me)=CH_2); 17.6 and 26.7 (ring CMe_2).

GB-1a (3): Yellow crystalline solid; TLC solvent system: Hexane-ethyl acetate (3:7); R_f = 0.37; UV (CH_3OH, 0.1%) λmax/nm: 289, 207. IR: 3227, 1598, 1515, 1158, 1084, 830 cm^{-1}. HR-MS *m/z*: 543.1264 $(M+H)^+$ for $C_{30}H_{23}O_{10}$ (calcd. 543.1291); MS^n experiment *m/z*: 541.1, 447.0, 415.0, 389.1, 179.3. ^1H NMR (CD_3OD, 400 MHz, δ-ppm): δ 5.42 (1H, d, J=11.2 Hz, H-2), 5.2 (1H, d, J=12 Hz, H-3), 5.91 (1H, d, J= 2 Hz, H-6), 5.72 (1H, d, J=2 Hz, H-8), 7.05

(2H, d, J=8.4 Hz, H-2',6'), 6.61 (2H, d, J=8.4 Hz, H-3',5'), 5.32 (1H, d, J=12 Hz, H-2''), 2.67 (2H, m, H-3''), 5.76 (1H, s, H-6''), 7.07 (2H, d, J=8.4 Hz, H-2''',6'''), 6.62 (2H, d, J=8.4 Hz, H-3''',5'''). ^{13}C NMR: δ 80.5 (C-2), 48.4 (C-3), 197.0 (C-4), 163.0 (C-5), 96.6 (C-6), 164.8 (C-7), 96.2 (C-8), 165.6 (C-9), 103.2 (C-10), 129.0 (C-1'), 127.9 (C-2'/6'), 115.7 (C-3'/5'), 158.7 (C-4'), 83.7 (C-2''), 44.0 (C-3''), 197.0 (C-4''), 164.8 (C-5''), 97.3 (C-6''), 168 (C-7''), 102.3 (C-8), 165.6 (C-9), 102.3 (C-10''), 83.7 (C-1'''), 129.8 (C-2'''/6'''), 116.3 (C-3'''/5'''), 158.7 (C-4''').

GB-1 (4): Yellow crystalline solid; TLC solvent system: Hexane-ethyl acetate (3:7); R_f = 0.48; UV (CH_3OH, 0.1%) λmax/nm: 290, 211. IR: 3200, 1595, 1515, 1155, 1083, 828 cm^{-1}. HR-MS *m/z*: 559.1221 $[M + H]^+$ for $C_{30}H_{23}O_{11}$ (Calcd. 559.1240) and 581.1043 $[M + Na]^+$; MS^n experiment $(M - H)^-$ *m/z*: 557.1, 431.0, 285.0. ^{1}H NMR (CD_3OD, 400 MHz, δ-ppm): δ 5.66 (1H, d, J=12 Hz, H-2), 3.31 (1H, s, H-3), 5.90 (1H, d, J=2 Hz, H-6), 5.97 (1H, m, H-8), 7.15 (2H, d, J=8 Hz, H-2',6'), 6.61 (2H, d, J=8 Hz, H-3',5'), 4.50 (1H, m, H-2''), 4.07 (2H, m, H-3''), 6.04 (1H, s, H-6''), 7.17 (2H, d, J=8 Hz, H-2''',6'''), 6.67 (2H, m, H-3''',5'''). ^{13}C NMR (100 MHz, δ-ppm): δ 79.5 (C-2), 49.1 (C-3), 196.0 (C-4), 164.9 (C-5), 97.2 (C-6), 165.1 (C-7), 98.4 (C-8), 105.7 (C-9), 103.2 (C-10), 129.4 (C-1'), 124.0 (C-2'/6'), 115.7 (C-3'/5'), 158.7 (C-4'), 82.8 (C-2''), 71.0 (C-3''), 196.0 (C-4''), 165.7 (C-5''), 98.9 (C-6''), 165.8 (C-7''), 102.0 (C-8''), 168.8 (C-9''), 103.3 (C-10''), 129.9 (C-1'''), 129.9 (C-2'''/6'''), 116.1 (C-3'''/5'''), 158.7 (C-4''').

GB-2 (5): Yellow crystalline solid; TLC solvent system: Hexane-ethyl acetate (3:7); R_f = 0.62; UV (CH_3OH, 0.1%) λmax/nm: 291, 207. IR: 3226, 1736, 1633, 1516, 1159, 1083, 830 cm^{-1}. HR-MS *m/z*: 575.1175 $(M + H)^+$ for $C_{30}H_{23}O_{12}$ (cald. 575.1189) and 597.0993 (M + Na) $^+$; MS^n experiment $(M-H)^-$ *m/z*: 573.1, 447.8, 447.0, 268.6. ^{1}H NMR (DMSO-d_6, 400 MHz, δ-ppm): δ 5.35 (1H, d, J=12 Hz, H-2), 4.48 (1H, d, J=12 Hz, H-3), 5.89 (1H, d, J=2 Hz, H-6), 5.77 (1H, d, J=2, H-8), 7.11 (2H, d, J=2 Hz, H-2',6'), 6.65 (2H, d, J=8 Hz, H-3',5'), 12.14 (1H, s, Chelated OH), 4.67 (1H, d, J=12, H-2''), 3.97 (2H, d, J=11, H-3''), 5.93 (1H, s, H-6''), 6.85 (1H, s, H-2'''), 6.81 (2H, d, J=8, H-5'''), 6.79 (1H, d, J=8, H-6'''), 11.7 (1H, s, Chelated OH). ^{13}C NMR (100 MHz, δ-ppm): δ 79.1 (C-2), 47.0 (C-3), 196.4 (C-4), 160.1 (C-5), 94.9 (C-6), 160.7 (C-7), 96.0 (C-8), 162.7 (C-9), 100.9 (C-10), 127.8 (C-1'), 128.0 (C-2'/6'), 115.3 (C-3'/5'), 157.7 (C-4'), 82.7 (C-2''), 71.9 (C-3''), 197.5 (C-4''), 162.0(C-5''), 96.0 (C-6''), 166.3 (C-7''), 101.2 (C-8''), 163.5 (C-9''), 106.0 (C-10''), 127.8 (C-1'''), 118.4 (C-2'''/5'''), 144.9 (C-3'''), 145.0 (C-4'''), 128.2 (C-6''').

Morelloflavone (6): Yellow crystalline solid; TLC solvent system: Ethyl acetate (100%); R_f = 0.47; UV (CH_3OH, 0.1%) λmax/nm: 376, 288. IR: 3348, 1557, 1410, 1269, 1167, 619 cm^{-1}. ^{1}H NMR (CD_3OD, 400 MHz, δ-ppm): δ 5.35 (1H, d, J=12 Hz, H-2), 4.48 (1H, d, J=12 Hz, H-3), 5.89 (1H, d, J=2 Hz, H-6), 5.77 (1H, d, J=2, H-8), 7.11 (2H, d, J=2 Hz, H-2',6'), 6.65 (2H, d, J=8 Hz, H-3',5'), 4.67 (1H, d, J=12, H-2''), 3.97 (2H, d, J=11, H-3''), 5.93 (1H, s, H-6''), 6.85 (1H, s, H-2'''), 6.81 (2H, d, J=8, H-5'''), 6.79 (1H, d, J=8, H-6'''). ^{13}C NMR (100 MHz, δ-ppm): δ 80.9 (C-2), 49.9 (C-3), 196.3 (C-4), 163.7 (C-5), 96.2 (C-6), 166.4 (C-7), 95.2 (C-8), 162.1 (C-9), 101.5 (C-10), 128.0 (C-1'), 128.4 (C-2'), 114.4 (C-3'), 157.2 (C-4'), 114.4 (C-5'), 128.4 (C-6'), 162.8 (C-2''), 102.4 (C-3''), 179.5 (C-4''), 159.7 (C-5''), 97.9

(C-6''), 161.3 (C-7''), 100.0 (C-8''), 154.0 (C-9''), 103.0 (C-10''), 121.6 (C-1'''), 114.6 (C-2'''), 145 (C-3'''), 147.6 (C-4'''), 116.2 (C-5'''), 120.3 (C-6''').

Morelloflavone-7''-O-β-D-glycoside (7): Yellow crystalline solid; TLC solvent system: Ethyl acetate-methanol (8:2); R_f = 0.57; α_D^{29} + 46.49 (c. 1% CH_3OH), UV (CH_3OH, 0.1%) λmax/nm: 377, 288. IR: 3252, 1738, 1593, 1364, 1069, 1083, 824 cm^{-1}. HR-MS *m/z*: 717.1446 $(M-H)^-$ for $C_{36}H_{31}O_{16}$ (calcd. 717.1461); MS^n experiment $(M-H)^-$ *m/z*: 717.1, 555.0, 403.55. ^1H NMR (DMSO-d_6, 400 MHz, δ-ppm): δ 5.80 (1H, d, J=12 Hz, H-2), 4.91 (1H, d, J=12 Hz, H-3), 5.94 (1H, d, J=4.6 Hz, H-6), 5.96 (1H, d, J=4, H-8), 7.17 (2H, d, J=8.4 Hz, H-2',6'), 6.53 (2H, d, J=8.4 Hz, H-3',5'), 12.65 (1OH, s, OH-5) 6.47 (1H, s, H-3''), 6.73 (2H, s, H-3''), 7.25 (1H, s, H-2'''), 6.93(1H, d, J=8.4, H-5'''), 7.59 (1H, d, J=8, H-6'''), 5.15 (1H, d, J=8, H-1''''), 3.3-3.8 (5H, m, H-2'''',3'''',4'''',5'''',6''''), 12.08 (1OH, s, OH-5''). ^{13}C NMR (100 MHz, δ-ppm): δ 82.5 (C-2), 50.7 (C-3), 195.0 (C-4), 164.5 (C-5), 96.5 (C-6), 165.7 (C-7), 97.7 (C-8), 167.0 (C-9), 103.5 (C-10), 130.3 (C-1'), 129.6 (C-2'/6'), 115.5 (C-3'/5'), 158.0 (C-4'), 165.8 (C-2''), 103.5 (C-3''), 182.0 (C-4''), 162.0 (C-5''), 100.0 (C-6''), 161.2 (C-7''), 103.5 (C-8''), 155.0 (C-9''), 106.4 (C-10''), 123.7(C-1'''), 114.9 (C-2'''), 146.0 (C-3'''), 152.5 (C-4'''), 114.9 (C-5'''), 120.6 (C-6'''), 101.6 (C-1''''), 76.1 (C-2''''), 77.5 (C-3''''), 69.6 (C-4''''), 79.1 (C-5''''), 60.9 (C-6'''').

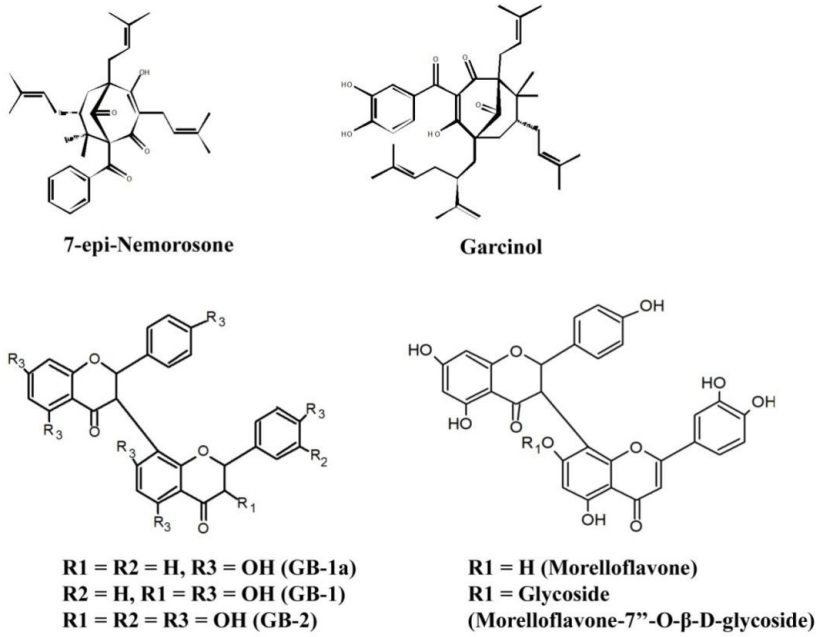

Figure 2. Structures of compounds 1 to 7

1.2. GC-MS analysis of low polar fraction of hexane extract

Column chromatographic separation of hexane extract of the leaves of *G. travancorica* using 100% hexane yielded a waxy white semi-solid. TLC of the fraction in reverse phase plates using 100% methanol as the solvent system revealed that the fraction was mixture of several

compounds with very close R_f values. GC-MS analysis revealed n-heptacosane ($C_{27}H_{56}$), a saturated hydrocarbon, as the major constituent of the waxy solid isolated from the leaves of *G. travancorica*.

The role of hydrocarbons is to prevent desiccation and to act as agents in chemical communications. n-Heptacosane is found in the epi-cuticular wax layer of different insects and is the major male courtship pheromeone of *Colias eurytheme* (Sappington and Taylor, 1990). It has been reported that the cuticular hydrocarbons in social insects signal the reproductive status of an individual and n-heptacosane has been identified as the major hydrocarbon on the wax coat of the mated queen of the ants *Ectatomma tuberculatum* (Hora *et al*., 2008).

2. HPTLC estimation of GB-2 and morelloflavone-7''-O-β-D-glycoside

HPTLC estimation of the biflavonoids, GB-2 and morelloflavone-7''-O-β-D-glycoside in the leaves of *G. travancorica* were carried out using CAMAG HPTLC system, using the mobile phase of 70% ethyl acetate in hexane (v/v). GB-2 gave R_f value of 0.30 and chromatogram of the compound was recorded at 288 nm. Standard GB-2 in the range 0.2 to 1.0 µg per band showed good linear response with correlation coefficient 0.983. The content of GB-2 was 0.91% (dry wt.).

Morelloflavone-7''-O-β-D-glycoside in the leaves was estimated using ethylacetate-methanol-formic acid (80:17.5:2.5 v/v) solvent system (R_f value 0.35). Development of the plates in this mobile phase resulted in sharp, symmetric and well resolved peaks (**Figure 3**). The HPTLC chromatogram of the compound was recorded in the visible range at 580 nm. Peak area and concentration were subjected to linear regression analysis to calculate the calibration equation and correlation coefficients. Morelloflavone-7''-O-β-D-glycoside in the range 0.5 to 1.5 µg per band gave linear response and the correlation coefficient 0.982 indicated a good linear relationship between peak area and concentration of standard. The content of morelloflavone-7''-O-β-D-glycoside was 7.12% (dry wt.).

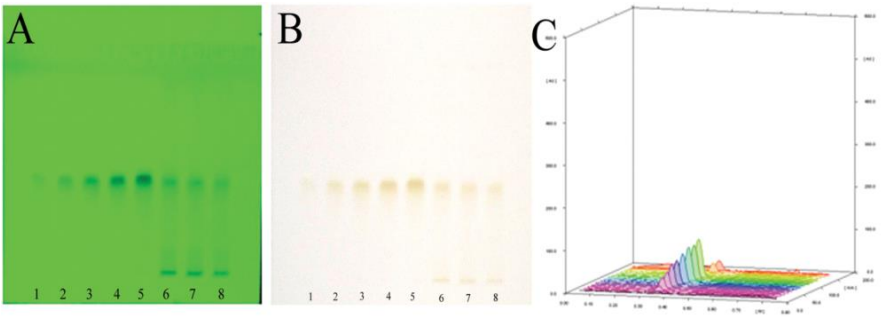

Figure 3. HPTLC densitogram of morelloflavone-7''-O-β-D-glycoside: A- UV (254 nm), B: Visible (580 nm), C: 3D Graph

3. HPLC-QTOF-MS Analysis of *G. travancorica* leaves, stem bark and fruits

Isolation, purification and structural elucidation of compounds, using conventional methods, from complex mixtures of natural origin are quite expensive in terms of time consumption

and labour (Shu, 1998; Konishi *et al*., 2007). The introduction of hyphenated analytical techniques provided natural product researchers extremely powerful tools that provided both the separation and characterisation in single run (Phonde and Magdum, 2015). Among the different hyphenated analytical techniques, liquid chromatography-mass spectrometric techniques became an important tool in phytochemical analysis for the rapid identification of secondary metabolites (Rosenberg, 2003). LC-MS is a powerful technique for identifying nontarget components where LC fractionate complex extracts with good resolution, sensitivity and reproducibility and MS techniques generate mass spectra with greater accuracy and precision (Shen *et al*., 2005; Konishi *et al*., 2007). *G. travancorica* fruits, leaves and stem bark were subjected to HPLC-QTOF-MS analysis for the identification of secondary metabolites present.

LC-MS analysis was carried out using Agilent 1200 HPLC (Agilent technologies, USA) coupled with an Agilent 6520 QTOF-MS/MS system via an electrospray ionisation interface (ESI). Agilent 1200 HPLC system consists of thermo stated column compartment (G1316C) and diode-array detector (G1315D). The HPLC separation was carried out on a Supelco Ascentis Express C18 column (10 cm × 2.1 mm, 2.7 µm) operated at 25°C. The mobile phase, consisted of 0.1 % formic acid aqueous solution (A) and acetonitrile (B), was delivered at a flow rate of 0.3 mL/min under the gradient program: 0-30 % (B) from 0 min to 5 min, 30-55 % (B) from 5 min to 10 min, 55-60 % (B) from 10 min to 15 min, 60-70 % (B) from 15 min to 20 min, 70-80 % (B) from 20 min to 25 min, 80-85 % (B) from 25 min to 30 min, 85-95 % (B) from 30 min to 40 min, and return to initial condition over 5 min. The sample injection volume was 5 µl.

In the ESI source, nitrogen was used as drying and collision gas. The heated capillary temperature was set at 320°C and nebulizer pressure at 40 psi. The drying gas flow rate was 10 lit/min. VCap, fragmentor, skimmer and octapole RF peak voltages were set at 3500V, 150V, 65V and 750V respectively in the ion source. Detection was carried out in negative ion mode within a mass range of m/z 100-1500 and resolving power above 15000 (FWHM). The data analyses were performed using Mass Hunter software version B.04.00 build 4.0.479.0 (Agilent Technology, USA).

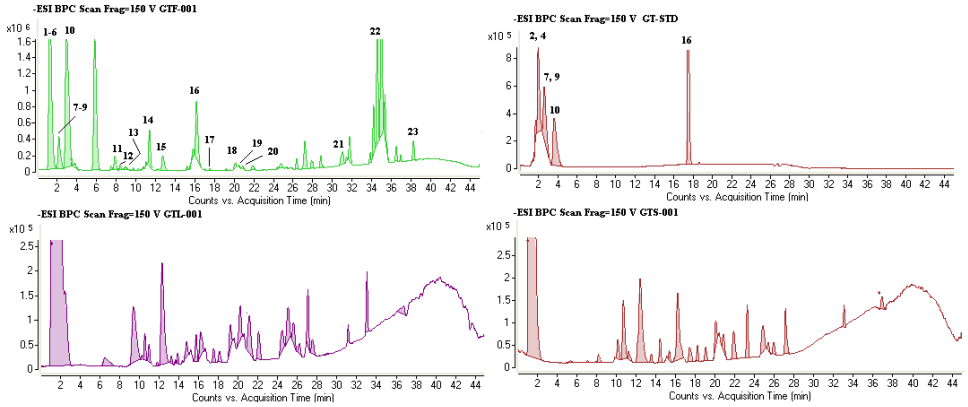

Figure 4. HPLC-QTOF-MS Base peak chromatograms of fruit, leaf, stem bark and mix reference standards of *G. travancorica.* (GTF; fruit, GT-STD; mix reference standards, GTL; leaf, GTS; stem bark)

A total of 23 compounds were identified by comparing retention times, MS spectra with available standards (hydroxycitric acid, fukugiside, α-mangostin, GB-1a, GB-1 and GB-2), HRMS of $(M-H)^-$ and fragmentation patterns (**Table1, Figure 4, Figure 5**). The proposed HPLC-QTOF-MS/MS method for the qualitative analysis is rapid, sensitive and efficient for simultaneous determination of acids, prenylated xanthones, benzophenoes and biflavonoids present in the plant species.

Hydroxycitric acid and its derivative hydroxycitric acid lactone (garcinia acid) were the two acids identified in fruits, leaves and stem bark of *G. travancorica*. Hydroxycitric acid is an antiobesity agent and the distribution of the compound is reported from many *Garcinia* species including *G. indica*, *G. cambogia*, *G. atrovirdis* and *G. cowa*. (Majeed *et al*., 1994; Kumar *et al*., 2013).

Morelloflavone, GB-1a, GB-1, GB-2 and GB-2a were the biflavonoids and fukugiside (morelloflavone-7''-O-β-D-glycoside), xanthochymusside, GB-1a glucoside were the biflavonoid glycosides identified from the plant. These compounds were distributed in all the plant parts studied.

Xanthones identified from the fruits were α-mangostin, γ-mangostin, 1,5-dihydroxy-3-methoxyxanthone, 4-(1, 1 – dimethylprop – 2 – enyl) -1, 3, 5, 8 – tetrahydroxy - xanthone, garciniaxanthone E, garcinone A, garcinone B, garcinone C and polyanxanthone C, while γ-mangostin and garcinone A were the xanthones identified from the leaves. γ-Mangostin, garcinone A, 1,5-dihydroxy-3-methoxy xanthone, garcinone B and garcinone C were present in the stem bark. Xanthones were especially noted for their potential antitumour and chemopreventive abilities along with other biological activities such as antibacterial, antifungal, antiviral, antioxidant and anti-inflammatory (Chin and Kinghorn 2008; Peres *et al*. 2000).

The benzophenones identified from the fruits were gambogenone, aristophenone A, garcinol and garciyunnanin A. Aristophenone A and garcinol were present in the leaves, while none of the benzophenones were detected in the stem bark of *G. travancorica*. Garciyunnanin A with 3-monohydroxy benzophenone skeleton is rarely distributed in *Garcinia* species (Xu *et al*., 2008). Most of the benzophenones reported from *Garcinia* species were polyisoprenylated structural group and exhibited wide spectrum of biological activities like antifungal, anti-HIV, antimicrobial, antioxidant, antiviral and cytotoxic (Kumar *et al*., 2007; Williams *et al*., 2003; Diaz-Carballo *et al*., 2012).

The study reports the chemical finger printing of *G. travancorica* leaves, stem bark and fruits using the hyphenated MS techniques. HPLC-QTOF-MS method was optimized and established for selective, reliable and simultaneous determination of 23 multiclass chemical constituents including acids, benzophenones, biflavonoids and xanthones present in the plant species.

Table 1. Identification of compounds from *Garcinia travancorica* by HPLC-QTOF-MS analysis

Sl. No.	RT (min)	Molecular Formula	HRMS, m/z, calc.	[M-H]- Obs.	Error (Δppm)	Compound	Fruit	Leaf	Stem bark
1	1.1	$C_6H_6O_7$	189.0041	189.0042	-0.55	Hydroxycitric acid lactone	P	P	P
2	1.2	$C_{36}H_{30}O_{16}$	717.1461	717.1468	-0.92	Fukugiside	P	P	P
3	1.3	$C_{36}H_{32}O_{17}$	735.1567	735.1564	0.32	Xanthochymusside	P	P	P
4	1.5	$C_6H_8O_8$	207.0146	207.0147	-0.32	Hydroxycitric acid	P	P	P
5	1.5	$C_{30}H_{22}O_{11}$	557.1089	557.1090	-0.12	GB-2a	P	P	P
6	1.8	$C_{30}H_{20}O_{11}$	555.0933	555.0933	0.1	Morelloflavone	P	N	P
7	2.1	$C_{3O}H_{22}O_{12}$	573.1038	573.1039	-0.15	GB-2	P	P	P
8	2.3	$C_{36}H_{32}O_{15}$	703.1668	703.1666	0.44	GB-1a glucoside	P	P	P
9	2.5	$C_{30}H_{22}O_{11}$	557.1089	557.1090	-0.15	GB-1	P	P	P
10	5.5	$C_{3O}H_{22}O_{10}$	541.1140	541.1143	0.52	GB-1a	P	P	P
11	7	$C_{24}H_{26}0_6$	409.1657	409.1663	-1.16	α-Mangostin	P	N	N
12	8	$C_{14}H_{10}O_5$	257.0455	257.0451	1.62	1,5-Dihydroxy-3-methoxyxanthone	P	N	P
13	8.3	$C_{18}H_{16}O_6$	327.0874	327.0876	-0.59	4-(1,1-Dimethylprop-2-enyl)-1,3,5,8-tetrahydroxy-xanthone	P	N	N
14	11.2	$C_{27}H_{32}O6$	451.2126	451.2130	-0.95	Gambogenone	P	N	N
15	13.4	$C_{23}H_{26}O_7$	413.1606	413.1605	0.39	Garcinone C	P	N	P
16	16	$C_{23}H_{24}O_6$	395.1500	395.1502	-0.6	γ-Mangostin	P	P	P
17	17.9	$C_{28}H_{32}O_6$	463.2126	463.2128	-1.15	Garciniaxanthone E	P	N	N
18	19.9	$C_{23}H_{22}0_6$	393.1344	393.1345	*-0.45*	Garcinone B	P	N	P
19	20.4	$C_{23}H_{24}0_5$	379.1551	379.1553	-0.46	Garcinone A	P	P	P
20	20.7	$C_{33}H_{42}O_6$	533.2909	533.2901	1.49	Aristophenone A	P	P	N
21	30.5	$C_{28}H_{32}O_4$	431.2228	431.2235	-1.72	Polyanxanthone C	P	N	N
22	35.1	$C_{38}H_{50}O_6$	601.3535	601.3539	-0.69	Garcinol	P	P	N
23	38.4	$C_{38}H_{50}O_5$	585.3585	585.3582	0.6	Garciyunnanin A	P	N	N

P: present, N: not present

4. Volatile chemical profile of *Garcinia travancorica*

Hydrodistillation revealed *G. travancorica* as rich source of essential oils with yield of 0.70%, 0.60% and 1.50% v/w respectively for leaf, stem bark and fruit. In total, 23 components were identified from the oils (**Table 2**). Fifteen components comprising 96.1% of the leaf oil were identified. The major components in the leaf oil were n-undecane (44.0%) followed by α-copaene (15.8%) and δ-amorphene (7.0%). Fifteen components comprising 95.0% of the stem bark oil were identified and n-undecane (39.0%) was the major constituent followed by β-alaskene (9.4%) and α-himachalene (6.4%). Fourteen components comprising 92.9% of fruit essential oil were identified where n-undecane (58.2%) was the major volatile constituent, followed by α-copaene (8.2%) and γ-cadinene (6.7%). α-Copaene and α-himachalene were the common sesquiterpene constituents in the oils.

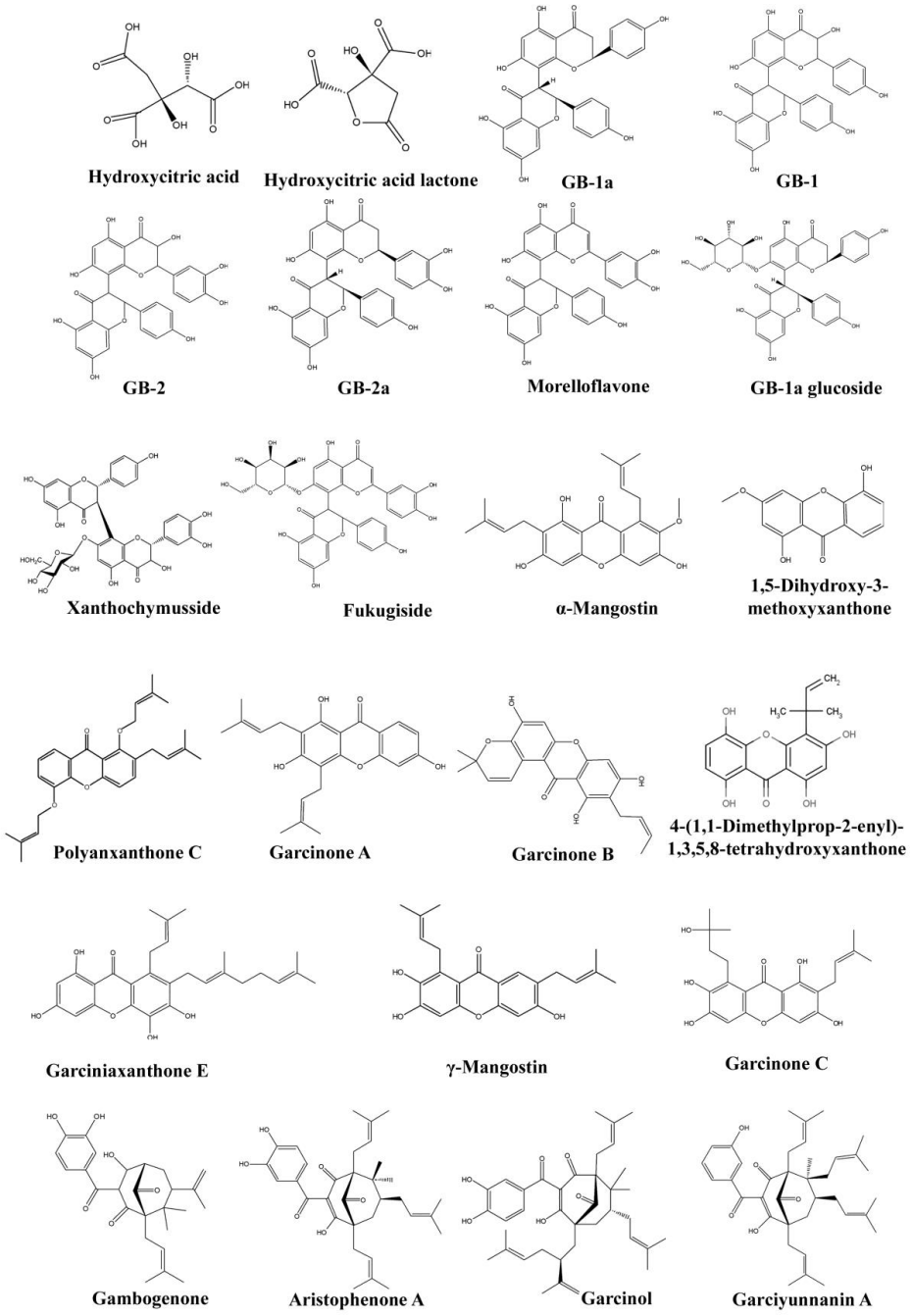

Figure 5. Structures of compounds identified from *Garcinia travancorica* by HPLC-QTOF-MS/MS analysis

Identification of the major compound n-undecane was further confirmed by the presence of their characteristic ^{13}C NMR signals in the ^{13}C NMR spectra of the oil (Formacek and Kubeczka, 2002) (**Table 3, Figure 6, Figure 7).** High content of the hydrocarbon n-undecane, with gasoline type odour, may possibly contribute to the characteristic smell of the plant. n-Undecane predominantly present in all the three oil samples. High quantity of n-undecane in the plant parts may play a key role in pollination as the compound was reported to possess pheromone type character which attracts the flies, moths and ants (Schiestl, 2000).

Table 2. Composition of the leaf, stem bark and fruit essential oils of *Garcinia travancorica*

Compound	RRI	Leaf	Stem Bark	Fruit
Z-β-Ocimene	1037	ng	2.6	ng
n-Undecane	1100	40.1	39.0	58.2
α-Ylangene	1373	1.0	ng	1.4
α-Copaene	1374	15.8	4.1	8.2
β-Funebrene	1414	3.3	-	1.8
β-Caryophyllene	1419	4.0	-	1.2
α-Funebrene	1402	-	3.9	
α-Trans bergamotene	1434	1.8	7.4	1.0
α-Himachalene	1449	3.1	6.4	1.9
Amorpha-4,11-diene	1451	2.2	4.1	1.5
α-Humulene	1452	0.1		
Cis cadina-1(6),4- diene	1461	2.4	2.9	-
Trans cadina-1(6),4- diene	1476	1.0	-	-
β-Acoradiene	1469	-	3.4	
ar-Curcumene	1481	-	2.3	1.6
γ-Himachalene	1482	2.3	-	-
β-Alaskene	1498	3.8	9.4	2.7
Epizonarene	1501	-	4.0	-
γ-Cadinene	1513	-	-	6.7
β-Bisabolene	1505	-	1.2	-
δ−Amorphene	1512	7.0	-	-
β-Curcumene	1514	-	4.3	-
δ-Cadinene	1522	4.5	-	4.2
1-Epi-cubenol	1627	-	-	2.5
Total identified		**92.4**	**95.0**	**92.9**
Monoterpene hydrocarbons (%)		ng	2.6%	ng
Oxygenated monoterpenes (%)		-	-	-
Sesquiterpene hydrocarbons (%)		52.1%	53.4%	34.7%
Oxygenated sesquiterpenes (%)		-	-	-
Aliphatic hydrocarbons		40.1%	39.0%	58.2%

ng: Negligible (<0.1%); RRI: Relative retention index calculated on HP-5 column

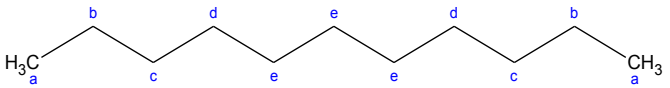

Figure 6. Structure of n-undecane

Table 3. NMR spectroscopic data of n- undecane ($CDCl_3$, δ in ppm)

Carbon Atom	δC	δH
a	14.13	0.90
b	22.71	1.30
c	31.94	1.26
d	29.63	0.90
e	29.67	0.90

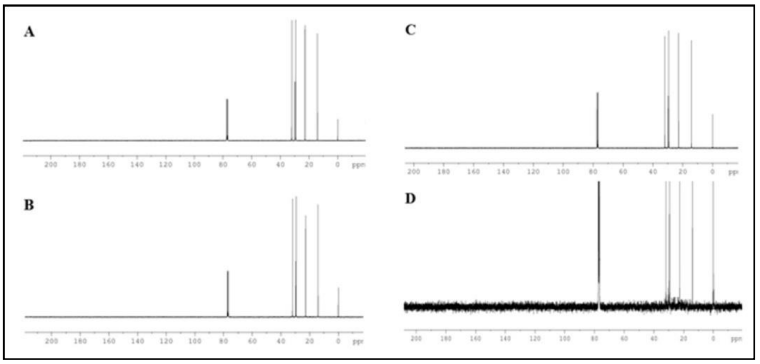

Figure 7. ^{13}C NMR of essential oils and n- undecane: **A**- Leaf oil, **B**- Stem bark oil, **C**-Fruit oil and **D**- n-undecane

Conclusions

Seven phenolic compounds including two polyisoprenylated benzophenones and five biflavonoids were isolated and characterised from *G. travancorica* leaves. The study highlights the plant as a rich source of the biflavonoid morelloflavone-7"-O-β-D-glycoside. HPLC-QTOF-MS method was optimized and established for selective, reliable and simultaneous determination of 23 multiclass chemical constituents including two acids, four benzophenones, seven biflavonoids and nine xanthones from *G. travancorica* fruits, leaves and stem bark. The essential oil composition of the leaves, stem bark and fruit of *G. travancorica* revealed the plant as a rich source of essential oils and the oils were predominated by the presence of aliphatic hydrocarbon n- undecane.

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Chapter 5

Leaf volatile chemical profiles of *Garcinia* species in the Western Ghats

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Abstract

The volatile chemical profiles of nine *Garcinia* species occurring naturally in the Western Ghats (*G. gummi-gutta, G. imberti, G. indica, G. morella, G. pushpangadaniana, G. rubro-echinata, G. talbotii, G. travancorica* and *G. wightii*) were studied for the first time. The leaf volatile chemicals were isolated by hydrodistillation and analyzed by GC-FID, GC-MS and ^{13}C NMR. The oil yield varied from 0.75 %v/w (*G. travancorica*) to 0.01 %v/w (*G. pushpangadaniana*). A total of 99 volatile compounds were identified, of which sesquiterpenoids derived from the mevalonic acid pathway were the predominant class of compounds distributed in all the *Garcinia* species. The sesquiterpene hydrocarbon α-copaene, which is present in all the *Garcinia* species studied, can be considered as the marker compound for the genus. In addition, specific marker compounds were also determined for the *Garcinia* species studied. The distribution of volatile compounds was analyzed by statistical methods and differentiation of the species was done by cluster analysis. Comparison with morphological classification revealed that the volatile chemical profiles were not related to the taxonomic classification of the genus, but rather to ecological interactions.

Keywords: *Garcinia,* Leaf essential oil, GC-MS, Chemotaxonomy, α-Copaene

Introduction

Garcinia species are an important component of the forest flora of the Western Ghats and some of the species are economically important as well. Nine *Garcinia* species were distributed wildly in the Western Ghats region, of which 7 species are endemic to the region (Table 1) (Maheswari, 1964, Sabu *et al*., 2013**)**. The genus *Garcinia* is well reputed as a source of valuable non wood forest products such as fats, oils, resins and colouring materials. Fruits of some *Garcinia* species are rich source of red pigments in the plant kingdom. Camboge, the yellow colouring pigment, is a well known product from *Garcinia* species. Recently, *Garcinia* species have received considerable attention worldwide from the scientific as well as industrial sectors due to the report of several bioactive structures such as biflavonoids, xanthones and benzophenones (Hemshekhar *et al*., 2011). In south India, *G. gummi-gutta* and *G. indica* were cultivated for commercial extraction of a variety of value added products such as bioactive acids, nutraceuticals, fats and condiments (Parthasarathy *et al*., 2013).

Although most of the species of the family Clusiaceae are known for their oil glands and secretary canals, literature review revealed that the reports on essential oils from *Garcinia* species are rare (Macleod and Pieris, 1982, Onayade, *et al*., 1998, Rameshkumar *et al*., 2005, Martins *et al*., 2008). Essential oils are complex mixtures of steam volatile chemical compounds, isolated generally by hydrodistillation of crude plant material. Essential oils occur in specialized secretary structures such as resin canals, lysigenous cavities, epidemic cells, glandular hairs, schizogenous passages, modified parenchymal cells or in oil tubes called vittae, in different plant parts such as buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood and bark (Handa, 2008). Majority of the volatile chemical constituents belong to the structural types terpenoids and phenylpropanoids, synthesized through the mevalonic acid pathway and shikimic acid pathway respectively. Different secondary metabolites present in these complex mixtures play diverse role in plants as antimicrobial, insecticidal and also as attractors of pollinating agents.

The present chapter discusses the volatile chemical profiles of *Garcinia* species of the Western Ghats and explores the possibility of evaluating species relationships through chemotaxonomy and to identify marker compounds for *Garcinia* species. Possible chemical ecological interactions were also discussed in the chapter.

1. Essential oil yield of *Garcinia* species

Fresh leaves of 9 *Garcinia* species, collected from different parts of the Western Ghats, were hydrodistilled using Clevenger type apparatus for 3h each. Comparison of essential oil yield (**Table 1**) revealed that *G. travancorica* possess maximum oil content (0.75%v/w), while *G. pushpangadaniana* possess the least oil content (0.01%v/w). *G. imberti* can also be considered as a rich source of essential oil (0.70%v/w). It is interesting to note that the three endemic *Garcinia* trees to Agasthyamala forests *viz*; *G. travancorica G. imberti* and *G. rubro-echinata* that occur at high altitudes possess high oil yield. However, the altitude is not a detrimental factor in essential oil yield, as evident from **Table 1**.

Table 1. Essential oil yield of fresh leaves of *Garcinia* species from the Western Ghats

Sl. No.	*Garcinia* species	Herbarium No.	Location, District	Altitude	Essential oil yield (%v/w)
1	*G. gummi-gutta*	66446	Vaikom, Kottayam	50 m	0.07
2	*G. imberti*	66416	Agastyamala forests, Thiruvananthapuram	994 m	0.70
3	*G. indica*	66423	Thaliparampa, Kannur	75 m	0.03
4	*G. morella*	66418	Agastyamala forests, Thiruvananthapuram	650 m	0.45
5	*G. pushpangadaniana*	66421	Kadalar, Munnar, Idukki	1401 m	0.01
6	*G. rubro-echinata*	66419	Agastyamala forests, Thiruvananthapuram	1074 m	0.33
7	*G. talbotii*	72622	Pampa, Pathanamthitta	224 m	0.50
8	*G. travancorica*	66417	Agastyamala forests, Thiruvananthapuram	1168 m	0.75
9	*G. wightii*	50987	Athirapally Vazhachal, Thrissur	149 m	0.03

The present observation on oil yield warrants detailed study on the distribution and nature of the secretary structures of *Garcinia* species in the Western Ghats (Esau 1965 and Schofield 1968). In a previous study, among the 10 Sri Lankan *Garcinia* species, *G. morella* and *G. spicata* stand out from the rest of the Sri Lankan *Garcinia* taxa on the basis that secretary spaces were observed in the palisade tissue rather than in the spongy tissue of lamina (Pathirana, 2004).

2. Analysis of essential oils

The essential oils were analyzed by GC-FID, GC-MS and ^{13}C NMR. GC-FID analyses were carried on a Shimadzu GC-2010 Plus Gas Chromatograph (Shimadzu, Japan), fitted with an Rxi-5 Sil MS capillary column (5% phenyl and 95% dimethyl polysiloxane, 30 m x 0.25 mm, 0.25 µm film thickness, Restek USA). 1 µL of the diluted oil in diethyl ether (1:50 dilution) were injected in both GC-FID and GC-MS under splitless condition. GC operation conditions: injector temperature, 270ºC; oven temperature programme, 60-250ºC (3ºC/min); hold time 2 min. at 250ºC; carrier gas, N_2 at 3 mL/min; detector temperature 270ºC. Relative percentages of cinnamaldehyde were obtained from the peak area percent report of volatiles from GC-FID data.

GC-MS analysis was done on a Hewlett Packard 6890 Gas Chromatograph fitted with an HP-5 (5% phenyl 95% dimethyl polysiloxane, 30 m x 0.32 mm, 0.25 µm film thickness) capillary column, coupled with a Model 5973 mass detector. GC-MS operation conditions: injector temperature, 220ºC; transfer line, 240ºC; oven temperature programme, 60-250ºC (3ºC/min); carrier gas, He at 1.4 mL/min. Mass spectra: Electron Impact (EI+) mode, 70 eV with a mass range of 40 to 450 m/z; ion source temperature, 240ºC. Relative retention indices (RRIs) of the constituents in HP-5 column were determined using standard C6-C30 hydrocarbons (Aldrich Chemical Company, USA) (Dool and Kratz, 1963). Individual components were identified by Wiley 275.L and NIST 05.L database matching, Co-GC with authentic standards, comparison of retention indices and comparison of mass spectra of constituents with published data (Adams, 2007). ^{13}C NMR was also used for confirmation of structures. A total of 99 compounds were identified from the essential oils of 9 *Garcinia* species (**Table 2**).

Table 2. Composition of the essential oils of the leaves of 9 *Garcinia* species in the Western Ghats

Compound	RI_{lit}	*G. gg*	*G. im*	*G. in*	*G. mr*	*G. ps*	*G. re*	*G. tl*	*G. tr*	*G. wg*
Myrcene	988				0.1					
Z-β-Ocimene	1032					0.2				
E-β-Ocimene	1044	1.1								
Terpinolene	1086	0.2								
Linalool	1095					1.8				
n-Undecane	1100								40.1	
Terpineol	1186					0.4				
Ascaridiole	1234				0.1					
Geraniol	1249					0.4				
δ-Elemene	1338		0.1		1.1	0.3	0.4			2.4
α-Cubebene	1348	0.4	0.3	1.2		0.7		0.7		
Cyclosativene	1371	1.3								
α-Ylangene	1373		0.3			0.8			1.0	

α-Bourbonene	1374			4.1						
α-Copaene	1376	30.2	0.4	1.2	1.3	3.1	0.2	27.0	15.8	1.7
β-Panasinsene	1381	1.3								
β-Bourbonene	1387					6.8		0.1		
β-Cubebene	1387		0.3			0.4				
β-Elemene	1390									0.9
α-Gurjunene	1409	0.3								3.1
β-Funebrene	1414								3.3	
β-Caryophyllene	1419	5.7	38.1	18.6	0.1	11.4	37.9	30.4	4.0	19.0
β-Copaene	1430	1.3	0.4	1.6	49.4			0.1		
α-trans Bergamotene	1434						0.8		1.8	
β-Gurjunene	1433				0.1			2.2		1.2
γ-Elemene	1434	2.1				0.4				
α-Guaiene	1437	0.3			0.1					
Aromadendrene	1439			0.5	2.8	1.1		1.6		6.8
cis- Muurola- 3,5-diene	1448	0.8								
α-Himachalene	1451								3.1	
Amorpha 4, 11- diene	1451	0.4							2.2	
α-Humulene	1452	1.8	30.5	17.6	18.5	3.2	40.6	10.7	0.1	4.6
Allo aromadendrene	1458		5.5		0.1					2.9
cis Cadina-1(6)-4-diene	1461	0.9				1.4		0.1	2.4	
α-Acoradiene	1464		0.3					0.1		
9 epi E-Caryophyllene	1466									0.5
β-Acoradiene	1469		4.5							
4,5-di epi-Aristalochene	1471			0.6						
γ-Gurjunene	1475							3.1		
trans Cadina-1 (6), 4-diene	1476	0.9							1.0	
γ-Muurolene	1478	4.3		5.9		11.7	7.2	3.8		
Amorpha- 4,7(11) – diene	1480	0.5								
γ- Himachalene	1482								2.3	1.1
α-Amorphene	1483							1.3		
β-Selinene	1489	1.1		12.3		0.6				
cis β-Guaiene	1492		0.1							
δ-Selinene	1492					0.9				
γ-Amorphene	1495					2.6				
α-Selinene	1498	1.5		18.2						
β-Alaskene	1498		2.5						3.8	
Bicyclogermacrene	1500						3.6			22.6
α-Muurolene	1500	1.5				3.7				
β-Bisabolene	1505						0.5			
E-γ-Bisabolene	1507		0.1							
Germacrene A	1508	0.6								

α-Bulnesene	1509						0.2			
δ-Amorphene	1511		0.4		0.5	1.2	0.3		7.0	
γ-Cadinene	1513	3.4		4.6		12.4				0.5
7 epi α-Selinene	1520									1.9
δ-Cadinene	1522	32.4		5.3		13.1			4.5	
trans Cadina 1,4-diene	1533	0.7		0.8		1.0		0.1		
Cadina-1(2),4-diene	1535							0.9		
α-Cadinene	1537	0.5		0.7		1.4		0.1		
Cadala-1(10),3,8-triene	1540							0.3		
α-Calacorene	1544	0.5		0.5		1.2				
Selina-3,7(11) diene	1545				0.2					
Elemol	1548			0.3						
Germacrene B	1559	0.3	0.3		0.8	0.4				
E-Nerolidol	1561					0.4				
Maaliol	1566						0.2			2.0
Caryophyllenyl alcohol	1570			0.9						
Epiglobulol	1576							0.2		
Spathulenol	1577				0.1					1.9
Caryophyllene oxide	1582		0.3		6.7	0.8		2.6		
Globulol	1590				1.9		0.7	0.1		6.0
Viridiflorol	1592						0.1			5.5
3,7-Cyclo undecadiene 1-ol, 1,5,5,8-tetramethyl	1584			1.4						
Cubeban-11-ol	1595				0.1			0.1		
Widdrol	1599				0.1					
Rosifoliol	1600				1.2		0.5			
Humulene epoxide II	1608				0.7			0.5		
Junenol	1618						0.2			
1,10-di epi Cubenol	1618		0.1					1.2		
α-Corocalene	1622					0.2				
1-epi-Cubenol	1627					1.5	0.1	0.1		
Muurola-4,10 (14)-diene-1-β-ol	1630									1.0
γ-Eudesmol	1630			0.3						
cis-Cadina-4-en-7-ol	1635					0.9				
Caryophylla-4(12),8(13) diene	1639							0.1		
epi-α-Muurolol	1640			0.3			0.4			
α-Muurolol	1644	0.4				0.5		0.2		
Cubenol	1645	0.2	0.1					0.8		
α-Cadinol	1652					0.9	0.3	0.1		
Selin-11-en-4α-ol	1659									0.5
14- Hydroxy (Z)-caryophyllene	1666							0.5		
14- Hydroxy 9-epi-E-caryophyllene	1668							0.1		

Germacra-4(15),5,10 (14) trien-1-α-ol	1685									0.7
δ-Cedren-13-ol	1688									0.7
Amorpha 4,9-dien-2-ol	1700									0.2
Total %		96.9	84.6	96.9	86.0	87.8	94.2	89.2	92.4	87.7
Total No (99)		30	19	21	21	34	18	30	15	23
Monoterpenoids		1.3	Nil	Nil	0.2	2.8	Nil	Nil	Nil	Nil
Sesquiterpene-hydrocarbons		95.0	84.1	93.7	75.0	79.8	91.7	82.6	52.3	69.2
Sesquiterpene-oxygenated		0.6	0.5	3.2	10.8	5.2	2.5	6.6	Nil	18.5
Total sesquiterpenoids		95.6	84.6	96.9	85.8	85.0	94.2	89.2	52.3	87.7
Aliphatic compounds			Nil	Nil	Nil	Nil	Nil	Nil	40.1	Nil

G.gg-*G. gummi-gutta*; G.im-*G. imberti*, G.in-*G. indica*; G.mr-*G. morella*; G.ps-*G. pushpangadaniana*; G.re-*G. rubro-echinata*; G.tl-*G. talbotii*; G.wg-*G.wightii*; G.tr-*G.travancorica;* RRI: Relative retention index calculated on HP-5 column.

The ubiquitous sesquiterpene hydrocarbons β-caryophyllene and the isomeric compound α-humulene were present in all the *Garcinia* species. The maximum content of β-caryophyllene was in *G. imberti* (38.1%), followed by *G. rubro-echinata* (37.9%), *G. talbotii* (30.4%), *G. wightii* (19.0%), *G. indica* (18.6%) and *G. pushpangadaniana* (11.4%). Except in *G. rubro-echinata* and *G. morella,* β-caryophyllene was in higher amount compared to α-humulene. α-Humulene was present in significant quantity in *G. rubro-echinata* (40.6%), *G. imberti* (30.5%), *G. indica* (17.6%), *G. morella* (18.5%) and *G. talbotii* (10.7%).

α-Copaene was the major compound in *G. gummi-gutta* (30.2%), *G. talbotii* (27.0%) and *G. travancorica* (15.8%). β-Copaene was the major compound in *G. morella* (49.4%). α-Selinene and β-selinene were present in significant quantity in *G. indica* (18.2 and 12.3% respectively). δ-Cadinene (13.1%), γ-cadinene (12.4%) and γ-muurolene (11.7%) were predominant in *G. pushpangadaniana.* Bicyclogermacrene (22.6%) was characteristically present in significant quantity in *G. wightii*.

Though petrochemicals are the raw materials for synthetic perfumery chemicals, natural isolates from plant sources are preferred over synthetics in many aspects and discovery of novel sources of natural aroma chemicals has a detrimental role in flavor and fragrance industries. *Garcinia* species of the Western Ghats can be considered as a rich source of volatile chemicals such as caryophyllene, humulene and undecane.

3. Biosynthetic pathways of volatile chemicals in *Garcinia* species

Three distinct chemical groups viz; monoterpenoids, sesquiterpenoids and aliphatic hydrocarbons could be characterized in the volatile chemicals of *Garcinia* species. An evaluation of the biosynthetic pathways of the volatile chemicals revealed that sesquiterpenoids derived from mevalonic acid pathway were the predominant volatile chemicals (**Figure 1**) (David, 1999).

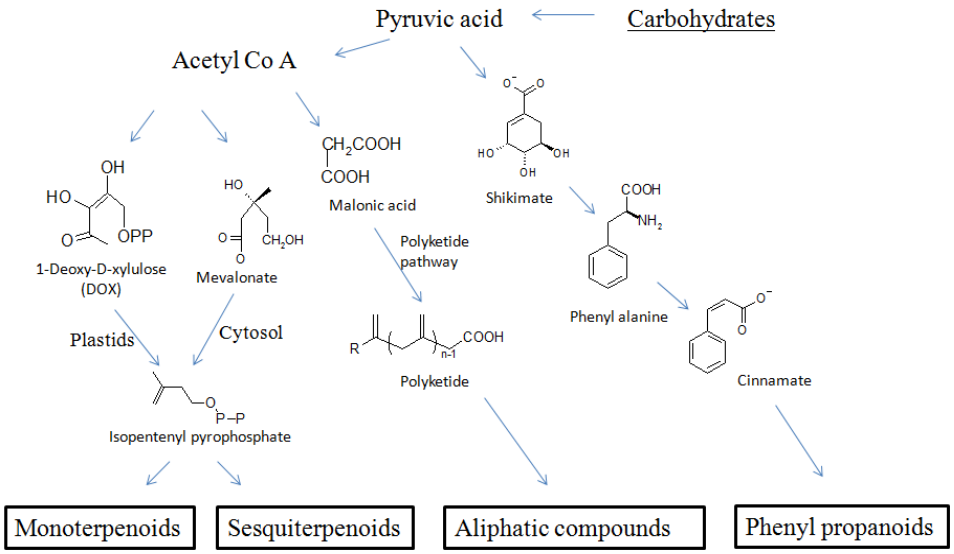

Figure 1. General biosynthetic pathways of different classes of volatile chemicals in *Garcinia* species

4. α-Copaene- The volatile chemical marker compound for the genus *Garcinia*

Biosynthesis of essential oil and volatile chemicals is a genetically determined attribution and it is possible to trace common progenies for volatile chemicals in related taxa. The volatile chemical profile analysis suggested that the sesquiterpene hydrocarbon α-copaene, can be considered as chemotaxonomic marker compound for the *Garcinia* species in the Western Ghats. Though β-caryophyllene and α-humulene were present in all the *Garcinia* species studied, the compounds are ubiquitous in most of the aromatic plants. The characteristic compound α-copaene with an unusual tricyclic decane ring system, that is present in all the *Garcinia* species studied, has been selected as the marker compound for the genus. The structure of α-copaene was unambiguously identified through ^{13}C NMR spectroscopic studies. ^{13}C NMR has now been evolved as a reliable tool for identification of volatile constituents in crude essential oils, where the Identification by ^{13}C NMR was carried out by comparison of the ^{13}C NMR signals of the total oil to the ^{13}C NMR signals for pure compounds compiled in our laboratory and available in the literature (Kubeczka and Formacek, 2002). The major compounds can unambiguously be identified by ^{13}C NMR taking into account the number of identified carbons, the number of overlapped signals and the difference of chemical shift of each resonance in the mixture and in the reference spectra. Further, α-copaene was isolated from the plants and the structure was confirmed through ^{13}C NMR studies of the isolated compound (**Figure 2**). α-Copaene exists as 2 isomeric forms, α-copaene and α-ylangene with different properties (**Figure 3**). α-Copaene, has been reported to be attractive to the Mediterranean fruit fly *Ceratitis capitata*, a highly destructive pest to several crops, while the attractive property of its isomeric form α-ylangene has not been confirmed in the fields. Through GC-MS it is quite difficult to differentiate the isomeric forms due to their close similarity in mass fragmentation pattern as well as close RRI values and α-copaene reported from various sources through GC-MS analysis might be a

mixture of α-copaene and α-ylangene. The structure of α-copaene was unambiguously differentiated from its stereoisomeric form α-ylangene by ^{13}C NMR. The ^{13}C chemical shifts of C-2 and C-6 of α-copaene showed striking differences of nearly 11 ppm from that of α-ylangene, enabling their differentiation through ^{13}C NMR (Buyck *et al*., 1989).

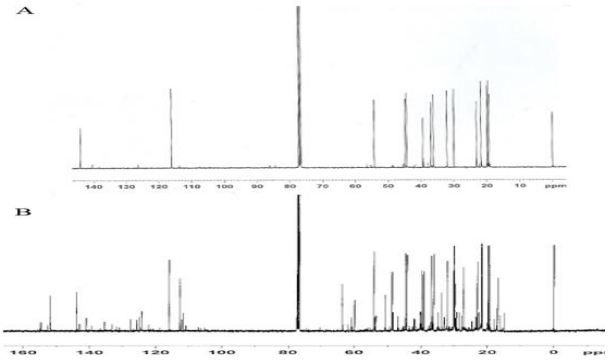

Figure 2. ^{13}C NMR of α-copaene (A) and *Garcinia talbotii* leaf essential oil (B).

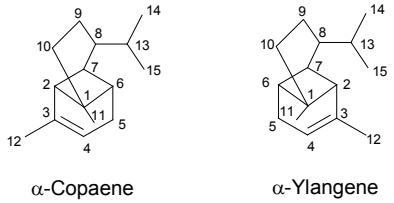

Figure 3. Structures of α-copaene and α-ylangene

5. Chemotaxonomic marker compounds for *Garcinia* species

Among the different volatile chemicals detected from *Garcinia* species, chemotaxonomic marker compounds were identified based on their uniqueness in the species. The marker compound may not be the major compound present in the species, but the uniqueness in chemical structure and biosynthetic pathway along with their presence in the species make the compound marker for the species. The consistency of the compound has been confirmed by analyzing at least 4 different accessions from different bio-geographical locations. The aliphatic compound n-undecane was exclusively present in *G. travancorica* and was also the major compound in the species. Other marker compounds identified were δ-Cadinene (*G. gummi-gutta*), β-caryophyllene (*G. imberti*), α-selinene (*G. indica*), β-copaene (*G. morella*), β-bourbonene (*G. pushpangadaniana*), α-copaene (*G. talbotii*) and bicyclogermcrene (*G. wightii*).

6. Chemotaxonomy of *Garcinia* species based on volatile chemical profile

The systematics of *Garcinia* species primarily depends on analysis of reproductive morphological features and the genus is often considered as a taxonomically difficult group due to the dioceous nature of plants and strict seasonality in flowering and fruiting

(Nimanthika and Kaththriarachchi, 2010). Combined multidisciplinary analysis of various tools such as vegetative and reproductive morphology, anatomy, molecular as well as chemotaxonomy will yield more robust phylogeny of this group. A comprehensive study on the vegetative anatomy has been carried out to assess the phylogenetic relationships of the genus *Garcinia* (Pathirana, 2004). Molecular analysis has also been reported as effective in such phylogenetic studies (Sweeney, 2008). The use of distribution patterns of secondary metabolites is well established as a major tool for characterize, classify and describe taxa. The vast information of secondary metabolites can also be utilized for investigating population structures, species and phyletic relationships and evolutionary status. The genus *Garcinia* is characterized by the presence of a large number of secondary metabolites with diverse structural features such as xanthones, benzophenones, biflavonoids and terpenoids. Several attempts have been made to evaluate the phylogeny among Clusiaceae members through secondary metabolite profiling (Waterman and Hussain, 1983, Nogueira *et al*., 2001). Volatile chemicals can efficiently be utilized for chemotaxonomic purposes. Though environmental factors affect the chemical composition of the essential oils, these changes particularly influence the accumulation of essential oil, as terpenoids and phenyl propanoids are generally under strict genetic control (Hiltunen and Holm 1999).

The relative percentages of all the 99 components of the essential oils were taken as variables and submitted to cluster analysis to sub group *Garcinia* species using SPSS 16.0 software (SPSS Inc, USA). The derived dendrogram depicts the grouping based on their chemical compositions.

Similarity and cladistic analyses performed statistically based on the distribution of volatile chemicals delimited the Western Ghats *Garcinia* species in the dendrogram (**Figure 4, Table 3**). Among the 9 *Garcinia* species, *G. travancorica* was isolated from other species. The aliphatic hydrocarbon n-undecane derived from polyketide pathway was the major constituent of the leaf oil of *G. travancorica*, while in all other species, the major constituents were sesquiterpenoids derived from mevalonic acid pathway. *G. morella* was also distinct from other species by the high content of β-copaene. *G. rubro-echinata* and *G. imberti* were close to each other by the presence of β-caryophyllene and α-humulene as the major compounds in both the species.

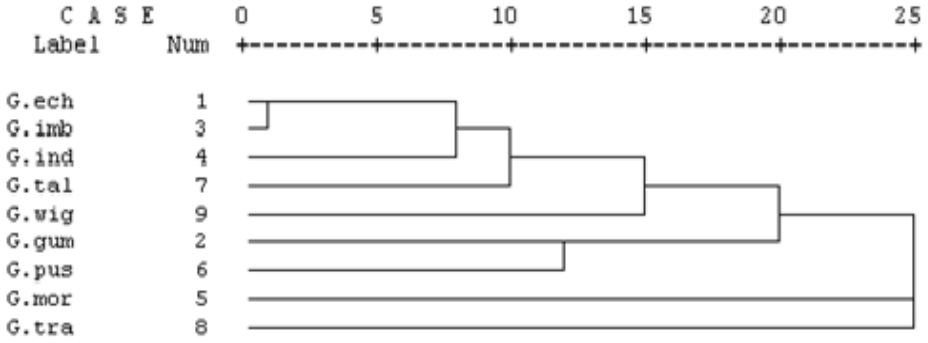

Figure 4. Dendrogram showing subgrouping of *Garcinia* species based on volatile chemical profile using between groups linkage (SPSS version 16.0)

Table 3. Similarity matrix between nine *Garcinia* species of the Western Ghats

Case	Correlation between vectors of values								
	1:1	2:2	3:3	4:4	5:5	6:6	7:7	8:8	9:9
1:1	1.0000	0.0959	0.9664	0.7287	0.2322	0.4093	0.6665	0.0311	0.5284
2:2	0.0959	1.0000	0.0943	0.2244	0.0233	0.5505	0.5101	0.2897	0.0620
3:3	0.9664	0.0943	1.0000	0.7041	0.2024	0.3792	0.7017	0.0451	0.5316
4:4	0.7287	0.2244	0.7041	1.0000	0.1822	0.4642	0.5117	0.0197	0.3368
5:5	0.2322	0.0233	0.2024	0.1822	1.0000	-0.0011	0.0853	-0.0230	0.0289
6:6	0.4093	0.5505	0.3792	0.4642	-0.0011	1.0000	0.4203	0.0781	0.2236
7:7	0.6665	0.5101	0.7017	0.5117	0.0853	0.4203	1.0000	0.2565	0.4688
8:8	0.0311	0.2897	0.0451	0.0197	-0.0230	0.0781	0.2565	1.0000	0.0181
9:9	0.5284	0.0620	0.5316	0.3368	0.0289	0.2236	0.4688	0.0181	1.0000

Comparison with morphological classification (Chapter 1) revealed that the composition of the leaf volatiles was not related to the taxonomic position of different *Garcinia* species. *G. pushpangadaniana* and *G. talbotii* are morphologically very similar with stamens in 5 phalanges and 5 set of sepals and petals and are placed as a separate clad in morphological classification. However, the volatile chemical composition was quite different in both the species, placing them in distant clads (**Figure 4**). Dendrograms based on end use related traits, such as oil composition, may be of practical interest related to ecological interactions, but do not necessarily correlate with taxonomy. Chemometric studies of the chemical composition of the floral volatiles of 16 species of the genus *Clusia* (family: Clusiaceae) revealed the composition was in part, but not always related to the taxonomic position of the genus, but to a minor extent to the type of pollinators visiting the flower (Nogueira *et al*., 2001). In the present study, it would be interesting to correlate the environmental and ecological factors to the leaf volatile profile, rather than the taxonomic positions based on morphological classifications.

7. Chemical ecology of the volatile chemicals of *Garcinia* species

Chemical ecology is an active, interdisciplinary field between chemistry and biology, dealing with the role of chemical compounds in interactions between organisms. Volatile organic compounds (VOCs) are important in chemical ecology and in plants, VOCs have important role in reproduction, by attracting and orienting pollinators and also as defense against feeding by ants, beetles and other insects (Huang *et al*., 2012). The present study of volatile organic compounds of *Garcinia* species revealed some interesting observations that can be related to chemical ecology.

High quantity of n-undecane with gasoline type odour may play a key role in pollination of *G. travancorica*, as the compound was reported to possess pheromone type character which attracts the flies, moths and ants (Schiestl, 2000). n-Undecane is the major pheromone found in Dufour's gland of the ant *Camponotus obscuripes* (Formicinae), while formic acid was the major component in the poison gland. When the ants sensed formic acid, they eluded the source of the odor; however, they aggressively approached odor of n-undecane. The mutualism in any possible ant-plant interaction need to be studied on a chemical ecological basis.

The sesquiterpene E-caryophyllene, a major volatile compound in several *Garcinia* species has been reported as a defence compound against herbivores and pathogens (Huang *et*

al., 2012). E-Caryophyllene is an important volatile sesquiterpene of plants that may serve as allelochemical to influence the neighboring plant growth or as an indirect defence to attract natural herbivore enemies (Wang *et al*., 2009). E-Caryophyllene is the major volatile organic compound in *G. imberti* and it is interesting to note that the diversity of other species in and around populations of *G. imberti* is much less, indicating possible allelopathic effect of the compound. E-caryophyllene has been reported as emitted from plants in response to herbivore attack. The compound has been reported as a semiochemical that attracts Asian lady beetle, *Harmonia axyridis* Pallas, a natural predator to aphids, the sap sucking plant lice.

Conclusions

The genus *Garcinia* is an important component of the forest flora of the Western Ghats and also an economically important group of plants. Even though 9 *Garcinia* species were distributed in the Western Ghats, none of them were previously investigated for their leaf volatile chemical constituents. Present study reports *Garcinia* species as a rich depository of essential oils. The chemotaxonomic relationships found in this study were not related to the taxonomic position of the genus based on morphological features. The volatile chemicals were rather evolved based on environmental and ecological interactions and the information may be useful in unraveling ecological interactions of *Garcinia* species.

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Chapter 6

Rapid estimation of bioactive constituents of *Garcinia* species in the Western Ghats using UHPLC-MS/MS Method

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Abstract
Species of the genus *Garcinia* (Family: Clusiaceae) are traditionally used in the preparation of food and as herbal supplements. Organic acids, prenylated xanthones, polyisoprenylated benzophenones and biflavonoids are the major medicinally active constituents present in different parts of *Garcinia* plants. Though the Western Ghats has a rich diversity of *Garcinia* species, only a few species have been exploited for their potential utilities. The rich floristic wealth can be harnessed profitably by exploiting the advances in phytochemical analytical techniques. Also, the establishment of an efficient analytical methodology for detection and estimation of the medicinally active constituents is crucial for quality assessment of derived herbal products from the *Garcinia* species. The present chapter provides an overview of different LC-MS analytical techniques used for quality control of *Garcinia* species. Further, detection and estimation of multi-class bioactive constituents in the leaf extracts of nine *Garcinia* species in the Western Ghats were reported using a validated UHPLC-ESI- QTOF-MS/MS method. Among the twenty six multi-class bioactive constituents analysed, biflavonoids and organic acids were the major class of compounds detected in *Garcinia* species. Acid content was high in the two economically important and widely distributed species, *G. gummi-gutta* and *G. indica,* while the biflavonoid content was highest in *G. travancorica* followed by *G. talbotii.*

Keywords: *Garcinia* species, Western Ghats, Quality control, UHPLC-ESI-QTOF-MS/MS

Introduction

The genus *Garcinia* belonging to the family Clusiaceae comprises more than 250 species of tropical trees and shrubs, indigenous to Asia, Southern Africa and Polynesia (Ritthiwigrom *et al*., 2013). About 37 species of *Garcinia* are distributed in the evergreen forest of the Western Ghats, Gujarat, Andaman and Nicobar Islands and the North Eastern region of India (Hemshekhar *et al*., 2011, Sarma *et al*., 2016). The fruits of several species of *Garcinia* are edible and used as spice in traditional Indian cuisines. Different plant parts of *Garcinia* species, mostly fruit, fruit rind, leaves and bark have been used worldwide as traditional medicine in the treatment of various ailments such as obesity, inflammation, microbial infection, abdominal pain, dysentery, diarrhea, infected wound, leucorrhea, chronic ulcer, gonorrhea, oxidative stress and cancer (Hemshekhar *et al*., 2011; Ritthiwigrom *et al.*, 2013).

Numerous pharmacological activities such as anticancer, antiobesity, diuretic, anti-inflammatory, antibacterial, antiviral, antifungal, anti-HIV, antidepressant and antioxidant have been reported for the *Garcinia* species (Han *et al*., 2006; Padhye *et al*., 2009; Ritthiwigrom *et al*., 2013; Xu *et al*., 2010). The antiobesity effect of *Garcinia* has been exploited commercially and several herbal supplements are available in the market.

Previous chemical investigations on the leaves, bark and fruits of *Garcinia* species have shown that the major constituents included biologically active biflavonoids, xanthones, benzophenones and organic acids and the minor constituents were terpenoids, steroids, flavonoids and phenolic acids (Hemshekhar *et al*., 2011; Ritthiwigrom *et al*, 2013). As the genus *Garcinia* has received much attention from pharmaceutical industries due to its extensive use in herbal dietary supplements, the quality control of its extracts in terms of bioactive constituents is essential to guarantee clinical efficacy and safety. Therefore, it is important to simultaneously monitor the bioactive constituents for their quality control and also explore the best suited species in terms of active constituents.

In recent years, numerous research groups reported analytical methods, using various chromatographic conditions and spectophotometric technologies, to develop quick and accurate analytical approaches for the identification, structural characterization and determination of chemical constituents of *Garcinia* species (Acuna *et al*., 2012; Aisha *et al*., 2012; Bharate *et al*., 2014; Chattopadhyay and Kumar, 2006, 2007; Jayaprakasha and Sakariah, 2000; Jena *et al*., 2002; Ji *et al*., 2007; Kumar *et al*., 2013, 2009; Li *et al*., 2008; Wittenauer *et al*., 2012; Zhou *et al*., 2010; Zhou *et al*., 2009; Zhou *et al*., 2008a, 2008b; Zadernowski *et al*., 2009).

Quantitative analysis of the major bioactive constituents of *Garcinia* is essential for quality control. Untill now only a few constituents (camboginol, garcinol, xanthochymol and isoxanthochymol) have been quantitatively determined by LC-MS/MS methods in *G. combogia* and *G. indica* (Chattopadhyay and Kumar, 2006, 2007; Bharate *et al*., 2014; Kumar *et al*., 2009). However, many species of *Garcinia* native to the Western Ghats of India are still unexplored in terms of their active chemical constituents. The main emphasis of the present chapter is the application of a validated UHPLC-ESI-MS/MS method for the rapid detection of multi-class bioactive constituents in the leaf extracts of nine *Garcinia* species distributed naturally in the Western Ghats of south India.

1. Bioactive chemical constituents from *Garcinia* species

The genus *Garcinia* is a rich source of organic acids, prenylated xanthones, polyisoprenylated benzophenones, biflavonoids, triterpenoids, phenolic acids and flavonoids which are also biologically active constituents (Xu *et al*., 2010; Hemshekhar *et al*., 2011; Ritthiwigrom *et al*, 2013). Garcinol, a polyisoprenylated benzophenone isolated from *Garcinia* species is a potent bioactive compound possessing antioxidant, anti-bacterial, anti-inflammatory, anticancer, anti-HIV and antiulcer activities (Hemshekhar *et al*., 2011; Padhye *et al*., 2009). The prenylated xanthones, gambogic acid and α-mangostin isolated from *Garcinia* species were found to have antioxidant, antibiotic, antitumor, anti-inflammatory and anticarcinogenic properties (Han *et al*., 2006; Ritthiwigrom *et al*, 2013; Xu *et al*., 2010). Hydroxycitric acid (HCA), a potential antiobesity and hypocholesterolaemic agent is present in fruits and leaves of *Garcinia* species and used as an ingredient in popular dietary supplements for weight loss

(Jena *et al*., 2002; Padhye *et al*., 2009). Biflavonoids, triterpenoids, flavonoids and phenolic acids found in *Garcinia* are also responsible for various pharmacological activities (Baggett *et al*., 2005; Hemshekhar *et al*., 2011; Ritthiwigrom *et al*, 2013).

2. Analytical methods used for quality control of *Garcinia* species

Several analytical methods, including high-performance liquid chromatography coupled to photodiode array detection/diode array detection (HPLC-PDA/DAD) and gas chromatography coupled to mass spectrometry (GC-MS) were used to evaluate the quality of *Garcinia* species (Acuna *et al*., 2012; Aisha *et al*., 2012; Jayaprakasha and Sakariah, 2000; Jena *et al*., 2002; Ji *et al*., 2007; Kumar *et al*., 2013; Li *et al*., 2008; Zadernowski *et al*., 2009). Most of the previous researchers have developed HPLC-PDA/DAD methods focusing on the simultaneous determination of only few classes of compounds in one or two *Garcinia* species except the work by Acuna *et al*. (2012).

Jena *et al*., and Jayaprakasha and Sakariah have developed HPLC-UV methods for the determination of organic acids (HCA, HCA lactone, oxalic acid, citric acid, tartaric acid and malic acid) in leaves, fruits, and dried rinds of *G. cowa* and commercial samples of *G. combogia* respectively. Kumar *et al*. have simultaneously determined the organic acid (HCA lactone) and xanthones (isoxanthochymol and xanthochymol) in leaves, seeds, fruit rinds and stem bark of *G. indica* by HPLC-PDA method. The xanthones were also determined by Aisha *et al*, Ji *et al* and Li *et al*. using HPLC-PDA method in the fruit rinds of *G. mangostana* and in the commercial samples of *G. hanburyi.*

Acuna *et al*. has developed an HPLC-PDA method for simultaneous detection and quantification of three benzophenones (guttiferone A, guttiferone E, and xanthochymol) and four biflavonoids amentoflavone, fukugiside, fukugetin, and volkensiflavone) in eight *Garcinia* species including seven edible fruits, *G. aristata*, *G. hombroniana* , *G. intermedia*, *G. livingstonei*, *G. mangostana*, *G. spicata*, and *G. xanthochymus* and the wood of *G. kola.* These analyses have shown that *G. spicata* contained all the seven phytoconstituents and the highest amounts of guttiferone E and xanthochymol was found in fruits of *G. spicata* and *G. xanthochymus*.

A GC-MS method was also applied for the identification of ten phenolic acids in various parts (peel, aril and rind) of the mangosteen fruit (*G. mangostana*) by Zadernowski *et al*. Quantification of the identified phenolic acids was carried out by GC coupled to flame ionization detection (FID) which showed protocatechuic acid as the major phenolic acid in the peel and rind, whereas *p*-hydroxybenzoic acid was the predominant phenolic acid in the aril.

The main drawbacks of the reported methods are low sensitivity, low resolution, and long analysis time with large solvent consumption and the need of derivatization in some cases. These drawbacks could be surmounted by using a more sensitive, selective and validated liquid chromatography tandem mass spectrometry (LC-MS/MS) method. Literature review revealed that there are a few reports on the development of LC-QTOF-MS/MS methods for the identification and characterization of xanthones and polyprenylated acylphloroglucinols in *Garcinia* species (Wittenauer *et al*., 2012; Zhou *et al*., 2010, 2009, 2008a, 2008b). The analytical techniques used for detection and estimation of bioactive constituents in *Garcinia* species are summarized in **Table 1.**

Table 1. Analytical techniques used for detection and determination of bioactive constituents in *Garcinia* species

Garcinia species	Plant part used	Sample preparation	Analytical method used	Stationary phase	Mobile phase, flow rate (mL/min)	Class of compound analyzed	Reference
G. buchananii	Leaf, root and stem	Methanol extraction	UPLC-ESI-TOF MS	Waters BEH C_{18} column (2 × 150 mm, 1.7 µm)	0.1% HCO_2H in H_2O and MeCN (0.1% HCO_2H), FR: 0.4	Flavonoids, biflavonoids, xanthones, benzophenones	Stark *et al.*, 2015
G. indica	Fruit	Methanol-water and dichloromethane-methanol extraction	LC-ESI-MS/MS	Chromolith Performance RP18 column (50 mm × 4.6 mm)	1% FA in water and acetonitrile, FR: 0.7	Polyisoprenylated benzophenones	Bharate *et al.*, 2014
G. indica	Fruit rind, stem bark, seed and leaves	Methanol extraction	HPLC-PDA	Waters Sunfire C_{18} column (150 mm × 4.6 mm id, 5 µm)	Acetonitrile - water (90:10, v/v) and methanol - acetic acid (99.5:0.5, v/v), FR: 0.5-0.8	Organic acids and polyisoprenylated benzophenones	Kumar *et al.*, 2013
G. mangostana	Fruit	Methanol, ethanol and toluene extraction	HPLC-DAD	RP Nucleosil C_{18} column (250 mm × 4.6 mm id, 5 µm)	0.1% H_3PO_4 in water and acetonitrile, FR: 1.0	Xanthones	Aisha *et al.*, 2012
G. aristata, G. hombroniana, G. intermedia, G. livingstonei, G. mangostana, G. spicata, *G. xanthochymus* and *G. kola*	Fruit and wood	Methanol extraction	HPLC-PDA	Phenomenex Synergi Hydro RP-18 column (250 mm × 2 mm id, 4 µm)	10 mM ammonium acetate buffer and acetonitrile, FR: 0.2	Benzophenones and biflavonoids	Acuna *et al.*, 2012
G. mangostana	Fruit	Methylene chloride extraction	HPLC–DAD–MS^n	Zorbax Eclipse XDB column (50 mm × 4.6 mm)	2% acetic acid in water and 0.5% acetic acid in acetonitrile, FR: 0.6	Xanthones	Wittenauer *et al.*, 2012
G. xanthochymus, G. oblongifolia, G. lancilimba, G. xipshuangbannaensis, G. cowa, G. subelliptica, G. paucinervis, G. multiflora, G. yunnanensis and *G. esculenta*	Fruit, twig, bark and leaf	Methanol extraction	UHPLC-ESI-QTOF-MS/MS	Waters Acquity BEH C_8 column (100 × 2.1 mm id., 1.7 µm)	0.1% FA in 80/20 water/methanol and 0.1% FA in acetonitrile, FR: 0.6	Polycyclic polyprenylated acylphloroglucinols	Zhou *et al.*, 2010
G. combogia and *G. indica*	Fruit rind, seed and	Methanol extraction	HPLC-PDA and LC-MS	Spheri-5 RP-8, Brownlee, Perkin-Elmer C_8column	Acetonitrile: water (80:20) and 1% acetic acid-	Polyisoprenylated benzophenones	Kumar *et al.*, 2009

	stem bark			(100 × 2.1 mm id, 5 µm)	methanol, FR: 0.45-0.8		
G. xanthochymus, G. oblongifolia, G. lancilimba, G. xipshuangbannaensis, G. cowa, G. subelliptica, G. paucinervis, G. multiflora, G. yunnanensis and *G. esculenta,*	Twig	Acetonitrile extraction	UHPLC-ESI-QTOF-MS/MS	Waters Acquity BEH C_{18} column (100 mm × 2.1 mm id, 1.7 µm)	0.1% FA in water and 0.1% FA in acetonitrile, FR: 0.6	Polycyclic polyprenylated acylphloroglucinols	Zhou *et al.*, 2009
G. mangostana	Fruit	Aqueous 80% (v/v) methanol extraction	GC-MS	SPB-1 silica-fused capillary column (30 m × 0.25 mm id, 0.25 µm)	Helium, FR: 28 cm^3/min	Phenolic acids	Zadernowski *et al.*, 2009
G. hanburyi	Commercial samples	Acetonitrile extraction	HPLC-PDA	SunFire C_8 column (2.1 mm × 150 mm id, 3.5µm)	Acetonitrile–methanol–0.3% aqueous TFA (35.5:33.5:31, v/v/v), FR: 0.22	Xanthones	Li *et al.*, 2008
G. hanburyi	Resin	Acetonitrile extraction	UHPLC-ESI-OTOF-MS^3	Waters Acquity BEH C_8 column (100 mm × 2.1 mm id, 1.7 µm)	0.1% FA in water and 0.1% FA in acetonitrile, FR: 0.3	Caged xanthones,	Zhou *et al.*, 2008a
G. xipshuangbannaensis	Twig	Methanol extraction	HPLC-ESI-OTOF-MS^3	Waters Acquity BEH C_{18} column (100 × 2.1 mm id., 1.7 µm)	0.1% FA in water and 0.1% FA in acetonitrile, FR: 0.3	Polyprenylated xanthones	Zhou *et al.*, 2008b
G. mangostana	Commercial samples (pericarp)	Acetone extraction	HPLC-PDA	Phenomenex Luna C_{18} column (150 mm × 3.00 mm id, 5 µm)	0.1% TFA in water and 0.1% TFA in methanol, FR: 0.5	Xanthones	Ji *et al.*, 2007
G. cambogia and *G. indica*	Fruit rind, seed and stem bark	Methanol extraction	LC-ESI-MS/MS	Brownlee RP-18 column (100 mm × 2.1 mm id, 5 µm)	Acetonitrile: water (9:1 v/v) and 0.5% acetic acid in methanol, FR: 0.4	Polyisoprenylated benzophenones	Chattopadhyay and Kumar, 2006, 2007;
G. cowa	Leaves, fruits and dried rinds	Water extraction and ethanol treatment	HPLC-UV	Zorbax C_{18} (Hewlett-Packard) analytical column (25 cm × 4.6 mm id, 5 µm)	Methanol and 0.01 M phosphoric acid, FR: 0.7	Organic acids,	Jena *et al.*, 2002
G. cambogia	Commercial samples	8 mM sulfuric acid treatment and water extraction	HPLC-UV	Waters µ -Bondapack^{TM} C_{18} column (300 mm × 3.9 mm)	6 m*M* sulfuric acid, FR: 1.0	Organic acids	Jayaprakasha and Sakariah, 2000

FA; formic acid, TFA; trifluoroacetic acid, FR; flow rate

3. UHPLC-MS/MS analysis of *Garcinia* species in the Western Ghats

A sensitive and efficient UHPLC-ESI-MS/MS method has been developed and validated in the MRM mode for rapid detection and determination of twenty six multi-class bioactive constituents in the leaf extracts of nine *Garcinia* species, viz. *G. rubro-echinata, G. gummi-gutta* (L.) Robs. (Syn. *G. cambogia* Desr.)*, G. imberti, G. indica, G. morella*, *G. pushpangadaniana, G. talbotii, G. travancorica* and *G. wightii*. The sample leaves were collected from various locations of Kerala, India and the sample code, specimen voucher number and collection location are shown in **Table 2.**

Table 2. Sample code, specimen voucher number and collection location of *Garcinia* species from Western Ghats, Kerala, India

Sl. No.	*Garcinia* species	Sample code	Voucher specimen number	Collection location
1	*G. rubro-echinata*	*G. re*	66419	Chemungi, Thiruvananthapuram
2	*G. gummi-gutta*	*G. gg*	66446	Palode, Thiruvananthapuram
3	*G. indica*	*G. in*	66423	Talipparamba, Kannur
4	*G. morella*	*G. mr*	66418	Chemungi, Thiruvananthapuram
5	*G. pushpangadaniana*	*G. ps*	66421	Kadalar, Idukki
6	*G. talbotii*	*G. tl*	50985	Palode, Thiruvananthapuram
7	*G. wightii*	*G. wg*	50987	Athirappilly, Thrissur
8	*G. imberti*	*G. im*	66416	Chemungi, Thiruvananthapuram
9	*G. travancorica*	*G. tr*	66417	Chemungi, Thiruvananthapuram

Methanolic extracts of the leaves were quantitatively analyzed by Waters Acquity UPLC^{TM} system (Waters, Milford, MA, USA) hyphenated with hybrid linear ion trap triple-quadrupole mass spectrometer (API 4000 QTRAP™ MS/MS system from AB Sciex, Concord, ON, Canada) using electrospray (Turbo V) ion source. Chromatographic separation of analytes was carried out on an Aquity UPLC BEH C_{18} column (50 mm × 2.1 mm id, 1.7 µm) using gradient elution of 0.1% formic acid in water and acetonitrile within 7.5 min. The targeted analytes in the samples were unambiguously identified using authentic standards based on their MS spectral data and diagnostic fragmentations (Pandey *et al*., 2015). Structures of targeted analytes are shown in **Figure 1.** The developed analytical method was validated as per International Conference on Harmonization (ICH, Q2R1) guidelines (Pandey *et al*., 2015).

The UHPLC-ESI-MS/MS analysis showed significant chemical variation among the nine *Garcinia* species (**Table 3**). Among the twenty six multi-class bioactive constituents, organic acids were the major class of compounds in *G. rubro-echinata, G. gummi-gutta* and *G. indica*. Hydoxycitric acid lactone or garcinia acid was the major constituent in the leaf extract of *G. rubro-echinata, G. gummi-gutta,* and *G. indica.* The acid content was highest in *G. gummi-gutta* (308.0 mg/g) while *G. talbotii* possess the least acid content (7.0 mg/g). Literature survey indicated that *G. gummi-gutta* and *G. indica* are incorporated into many pharmaceutical preparations and marketed as popular weight loss products due to the higher amount of hydoxycitric acid and garcinia acid in their fruit extracts (Jena *et al*., 2002; Padhye *et al*., 2009). Our findings suggested that the leaf extracts of *G. gummi-gutta* and *G. indica* might be a suitable source for swapping fruit extract due to the presence of higher level of organic acids (308 mg/g, 276 mg/g and 265 mg/g, respectively) (Jena *et al*., 2002).

Table 3. Contents (mg/g) of twenty six investigated bioactive constituents in the leaf extracts of nine *Garcinia* species distributed in the Western Ghats

Analytes (mg/g)	*G. gg*	*G. in*	*G. re*	*G. mr*	*G. ps*	*G. tl*	*G. wg*	*G. im*	*G. tr*
Organic acids									
Hydroxycitric acid	95.0	120.0	1.75	3.55	3.18	1.2	2.32	0.9930	1.6600
Garcinia acid	213.0	156.0	26.4	6.46	9.01	5.83	6.61	7.3800	9.4500
Phenolic acids									
Protocatechuic acid	0.427	0.407	0.67	10.7	0.294	0.341	1.00	0.9890	2.1700
Caffeic acid	0.379	0.578	0.622	0.595	0.263	0.34	0.413	0.1420	1.4200
Ferulic acid	0.094	0.123	0.121	0.191	0.1	0.117	0.078	0.5220	0.0403
Vanillic acid	0.0003	0.099	0.0285	0.001	nd	0.107	0.0005	0.0008	0.0222
Flavonoids									
Epicatechin	0.132	0.219	2.55	0.218	1.34	0.199	0.191	0.9240	0.1190
Isoorientin	0.441	0.626	0.297	1.32	0.343	1.02	0.409	0.6070	0.4340
Orientin	0.004	0.147	0.065	2.21	0.011	0.614	0.064	0.5340	0.1260
Isovitexin	1.47	3.03	1.81	3.55	1.67	3.38	1.79	1.4100	2.1000
Vitexin	1.19	2.86	1.37	2.16	1.24	1.59	1.57	1.1800	1.6400
Kaempferol-3-O-rutinoside	0.022	0.033	0.011	0.006	0.006	0.007	0.011	0.0637	0.2657
Luteolin	0.008	0.059	0.478	0.588	0.066	0.042	0.701	0.1053	0.0830
Quercetin	0.148	0.126	0.188	0.238	0.147	0.077	0.276	0.1920	0.6030
Apigenin	0.416	0.614	0.659	0.724	1.11	0.687	0.485	0.7010	1.4600
Kaempferol	0.246	0.253	0.237	0.289	0.287	0.281	0.274	0.2820	0.2320
Biflavonoids									
Fukugiside	0.066	0.075	0.020	nd	1.21	52.10	0.141	0.2910	35.3000
GB-2	bdl	0.338	bdl	6.14	2.077	28.3	0.683	0.3850	17.1333
GB-1	0.215	0.231	0.219	399	279	25.8	46.4	22.1000	72.0000
GB-1 a	bdl	bdl	bdl	22.1	13.4	6.24	2.143	2.4700	3.9000
Amentoflavone	0.309	0.309	2.98	2.51	3.06	1.443	0.046	0.0440	0.0467
Xanthones									
Mangostin	0.002	0.017	0.002	0.085	0.024	0.002	0.008	0.0056	0.0015
Gambogic acid	2.79	2.86	2.78	1.79	2.80	2.89	2.87	2.8500	2.7800
Benzophenones									
Garcinol	0.593	0.383	0.37	0.318	0.284	0.262	0.267	0.3290	0.2900
Triterpenoids									
Ursolic acid	0.742	0.73	0.915	1.25	1.35	0.92	0.757	1.4700	2.6200
Betulinic acid	2.44	1.37	1.55	1.83	1.64	3.75	1.19	1.3200	2.6500

G.re-*G. rubro-echinata*; G.gg-*G. gummi-gutta*; G.in-*G. indica*; G.mr-*G. morella*; G.ps-*G. pushpangadaniana*; G.tl-*G. talbotii*; G.wg-*G.wightii*; G.im-*G.imberti*, G.tr-*G.travancorica*; nd- not detected; bdl- below detection level (Pandey *et al*., 2015)

Biflavonoids were the major class of compounds in in *G. imberti, G. morella, G. pushpangadaniana, G. talbotii, G. travancorica* and *G. wightii*. The biflavonoid content was highest in *G. morella*, followed by *G. pushpangadania*. Among the five biflavonoids screened, GB-1 and GB-1a were the major ones distributed in the *Garcinia* species. Garcinia biflavonoid, GB-1 was the major constituent in the leaf extract of *G. morella, G. pushpangadaniana* and *G. wightii*. Fukugiside, GB-2 and GB-1were the major components in the leaf extracts of *G.talbotii*.

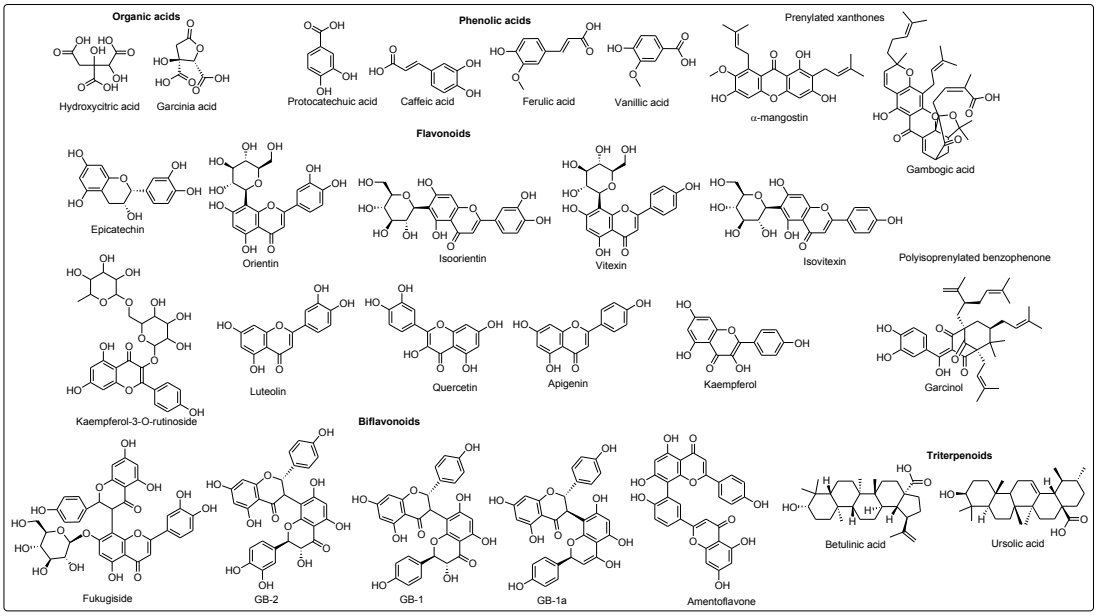

Figure 1. Structures of targeted analytes

Among the nine *Garcinia* species studied, *G. rubro-echinata, G. gummi-gutta,* and *G. indica* were distinct by high content of acids compared to other species. Among the 4 biflavonoids screened, only amentoflavone possess I(5′)-II(8) biflavonoid linkage, whereas the other 3 biflavonoids were with I(3)-II(8) linkage, the most prevalent interflavonoid linkage reported in *Garcinia* biflavonoids. It is interesting to note that the three species *G. rubro-echinata, G. gummi-gutta,* and *G. indica* were also distinct with regard to the biflavonoid distribution, where amentoflavone was present in higher quantity in the three species compared to the common I(3)-II(8) biflavonoids.

Conclusions

The developments in the field of analytical technologies improved fingerprinting authentication and quantitative determination of medicinally active constituents from plants and their commercial products. The selectivity and specificity in phytochemical analysis have increased significantly through hyphenation of chromatographic separation and mass spectrometry detection as in the case of LC-MS. Twenty six multi-class bioactive constituents in the leaf extracts of nine *Garcinia* species of the Western Ghats were detected and estimated through the UHPLC-MS/MS analysis. The UHPLC system combined with mass spectrometry detection in MRM acquisition mode enables significant reductions in separation time, solvent consumption and ensures excellent selectivity and sensitivity for quantitative analyses in shorter duration. In *G. rubro-echinata, G. gummi-gutta* and *G. indica*, organic acids were present in higher level, while in *other Garcinia* species (*G. morella, G. pushpangadaniana, G. talbotii* and *G. wightii*, *G. imberti* and *G. travancorica*) biflavonoids were the major class of compounds.

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Chapter 7

Morphological, chemical and molecular taxonomy of a new *Garcinia* species- *Garcinia pushpangadaniana* Sabu *et al.*

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Abstract

The genus *Garcinia* is an important component of the forest flora of the Western Ghats, and the region hosts a wide diversity with several taxa, including ones yet to be described. The genus is considered as a taxonomically difficult one due to the complexity and diversity in floral characteristics. The present chapter describes the biosystematics of a new *Garcinia* species, *G. pushpangadaniana*, described from the Western Ghats, using chemosystematics and molecular systematics. The HPTLC profile and volatile chemical profiles of the leaves supported the species status and allied nature to *G. xanthochymus* and *G. talbotii*. Molecular taxonomy using the chloroplast coding region *mat*K could demarcate the new taxon as a distinct species, closely allied to the species *G. xanthochymus* and *G. talbotii.*

Keywords: *Garcinia pushpangadaniana*, *Garcinia xanthochymus, Garcinia talbotii,* Chemotaxonomy, Molecular taxonomy

Introduction

The forests of the Western Ghats, with nearly 7500 flowering plants, is a rich repository of plant wealth with several new species having been discovered from the region (Nayar *et al*., 2014). The region hosts wild relatives of many important spice crops and food crops and also is the centre of origin and diversity of several such plant groups. The genus *Garcinia* is an economically important group of plants distributed in the tropical regions of the world. The Western Ghats is a centre of diversity of *Garcinia* species in India. Out of the 37 *Garcinia* species distributed in India, 7 are endemic to the Western Ghats.

The genus *Garcinia* is considered as a taxonomically difficult one due to the complexity and diversity in floral characteristics with many unresolved phylogenic issues surrounding the genus. Characteristic differences in the floral architecture were observed even among closely related taxa of *Garcinia* (Gustafsson *et al*., 2002, Sweeney, 2008). 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 and the classification of *Garcinia* species and its phylogeny solely depending on morphological characters proved to be more uncertain. The incorporation of biosystematics in such taxonomically difficult groups will allow classifications using new descriptors and methods that yield more robust inter relations.

Biosystematics 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 xanthones, benzophenones and biflavonoids, in addition to volatile secondary metabolites (Hemsekhar *et al*., 2011). 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). Though the volatile chemical profile reflects the evolutionary history, it is more indicative of the ecological conditions (Nogueira *et al*., 2001).

In the last decade, new valuable tools based on DNA analysis were made available for taxonomic studies (Winfield, 2003; Labra *et al*., 2004). The use of DNA genotyping has been instrumental in solving controversial taxon attributions by comparing genotypes independently from phenotypes. DNA genotyping offers the unique capacity to classify accessions regardless of environmental condition and plant growth stage.

A new taxon of the genus *Garcinia* has been collected from the forests of the Western Ghats. In the present chapter, the efficiency of chemotaxonomy and molecular taxonomy to support the species status of the new *Garcinia* taxon has been evaluated.

1. Morphological studies

The new taxon *Garcinia pushpangadaniana* T. Sabu, N. Mohanan, Krishnaraj, & Shareef (Holotype *TBGT 72601*) was collected from Kadalar forests, Idukki district, Kerala (**Figure 1**). Detailed evaluation of the vegetative and reproductive morphological features revealed the new taxon has distant relation to *G. xanthochymus* and *G. talbotii* with pentamerous flowers and the absence of rudimentary pistils in male flowers (**Table 1**). *G. xanthochymus* Hook. f. ex T. Anderson is an indigenous tree in Indo-Malay region, and its distribution in India is extended to the evergreen to semi-evergreen forests (100-1000m) of North East India and Andaman Nicobar Islands. *G. talbotii* Raizada ex. Santapau is an endemic species to the evergreen to semi-evergreen forests (100-350 m) of the Western Ghats. However, the prominent morphological differences in shape of leaf, pedicel length of male and female flowers, nature of staminodes, number of stigma, ovary and seeds, features in fruits and seeds qualify the new taxa to be a distinct species. The demarcating feature of the new taxon is the large fruits that weigh upto 750 g, with irregular ridges on the fruit surface.

Table 1. Characteristic morphological features of the new taxon in comparison with *G. talbotii* and *G. xanthochymus*

Plant part	*G. talbotii*	*G. xanthochymus*	*G. pushpangadaniana*
Leaf	Ovate, elliptic-oblong or lanceolate. Emarginate or acute at apex 9-22 x 4-8 cm.	Linear- oblong or oblong-lanceolate. Acute or acuminate at apex 12-35 x 4-10 cm.	Elliptic- oblong. Acute or obtuse at apex 14-20 x 6-8 cm.
Flower	Fascicled or pseudo spikes Stamens 8-10 in each of 5 long clawed, spathulate bundles. Stigma 3-4 lobed, peltate Ovary 3-4 locular.	Fascicled Stamens 15-20 in 5 phalanges bundles of 3-5 each. Stigma 5 lobed, oblong Ovary 5 locular	Fascicled Stamens 12-15 in phalanges Stigma 6-8 lobed, oblong Ovary 6-8 locular.
Fruit	Broadly oblong, smooth Up to 4 cm diam. Weight: Upto 45g	Subglobose, smooth. *ca*. 6.5 cm diam Weight: Upto 55 g	Irregular ridges on the surface, *ca*. 12 x 11 cm diam. Weight: Upto 750g
Seeds	Oblong 1-3, up to 2.5 cm	Oblong 1-4, up to 3.5 x 1.8 cm	Plano convex 2-6, ca. 2 x 1 cm
Latex	White or yellowish white	Milky white or pale green turning yellow	Milky white

Figure 1. *G. pushpangadaniana* **A.** Habit, **B**. Stem bark, **C**. Leaf, **D**. Male flower, **E**. Female flower, **F**. Seed and **G**. Fruit

Dichotomous key prepared for *G. pushpangadaniana* and related species

Stamens in 5 phalanges; sepals and petals 5 rarely 4
Leaves more than thrice as long as abroad, over 30 cm long; berry with distinct mamilla or beak…….....*G. xanthochymus*
Leaves less than twice as long as broad, less than 20 cm long; berry without distinct mamilla or beak
Fruit large, without any pulp, irregularly ridged on the surface, seeds planoconvex…….....*G. pushpangadaniana*
Male flowers fascicled or pseudo spikes stigmatic lobes 3- 4…… *G. talbotii*

2. Chemotaxonomy of the new species

The use of distribution patterns of secondary metabolites is well established as a major tool for characterize, classify and describe taxa. The vast information of secondary metabolites can also be utilized for investigating population structures, species and phyletic relationships and evolutionary status. The genus *Garcinia* is characterized by the presence of a large number of secondary metabolites with diverse structural features such as xanthones,

benzophenones, flavonoids, biflavonoids and terpenoids and the vast data on secondary metabolites has been utilised successfully to demarcate species (Waterman and Hussain, 1983).

2.1. Chemotaxonomy based on HPTLC profiles

The versatile and cost effective analytical tool HPTLC allows us to analyze up to 20 plants in a single analytical run and the phytochemical profile can yield valuable information on plant identity. The HPTLC profile can be utilized as very detailed differentiating fingerprints of different species, often closely related species that would otherwise be impossible to distinguish from each other physically (Reich and Schibli, 2007).

In the present study, the leaf methanol extracts were analysed using Camag HPTLC system, using silica gel HPTLC plates (Kieselgel 60 F 254, 20 cm × 20 cm, 0.2 mm thickness, Merck, Germany). The extracts were spotted by means of Camag Linomat V fitted with a Hamilton microlitre syringe. The plates were developed using chloroform: methanol (17:3) in the CAMAG twin-trough glass chamber, previously saturated with the solvent for 30 minutes. The mobile phase compositions were chosen after testing different solvent systems of varying polarity. The flavonoid profile was obtained on exposure of the plate to NH_3 vapour.

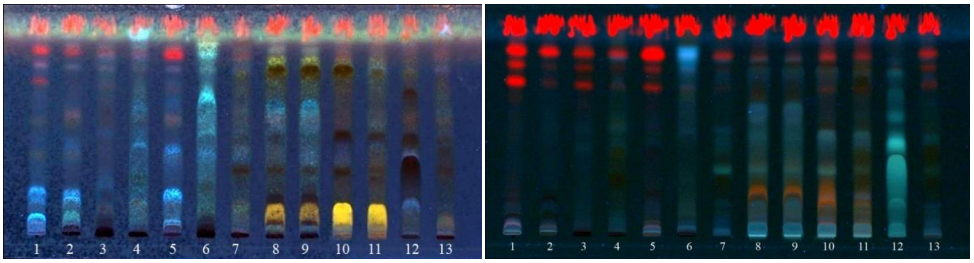

Figure 2. HPTLC profile of the leaf methanol extract along with 11 other *Garcinia* species **A**. 366 nm after exposure to NH_3. **B**. 366 nm after derivatisation (1. *G. gummi-gutta;* 2. *G. cowa,* 3. *G. rubro-echinata,* 4. *G. imberti,* 5. *G. indica,* 6. *G. mangostana,* 7. *G. morella,* **8. *G. pushpangadaniana* (Ist acc.), 9. *G. pushpangadaniana* (IInd acc.),** 10. *G. talbotii,* 11. *G. xanthochymus,* 12. *G. travancrica,* 13. *G. wightii*)

Biflavonoids, xanthones and benzophenones are the major phenolic compounds present in *Garcinia* species and the HPTLC of the methanol extracts represents the phenolic profile, especially the biflavonoids that shows intense fluorescence under exposure to NH_3 vapour. The secondary metabolite profile revealed that *G. xanthochymus, G. talbotii* and the new taxon comes under the same group and the presence of characteristic spots to the new taxon supports its species status (**Figure 2**).

2.2. Chemotaxonomy based on leaf volatile chemical profiles

Standardized descriptors based on volatile oil constituents have been proposed as an efficient tool for differentiation of plants. However, the use of volatile oil constituents for species differentiation is limited by the fact that several environmental factors may influence the plant chemical composition (Labra *et al*., 2004 and Grayer *et al*., 1996).

Volatile chemical profiles of the leaves 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. The 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 (30m × 0.32mm, film thickness- 0.25µm). 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 (30m × 0.32mm, film thickness- 0.25µm) coupled with a 5973 series selective mass detector. The constituents were identified by retention indices calculated using homologues of n-alkanes (C_8-C_{22}) (Dool and Kratz 1963), comparing mass spectra with published data (Adams, 2007) and by mass spectra library search (Wiley 275 and NIST).

Gas chromatography- mass spectrometry (GC-MS) studies of the leaf essential oils resulted in the identification of 58 volatile compounds in all the three species (**Table 2**). The major volatile constituents of all the three species, the sesquiterpenoids, were derived from trans, trans farnesyl pyrophosphate (FPP), through mevalonic acid pathway, pointing to the allied nature of the species. However, in the new taxon compared to other species, monoterpenoids (2.8%) biosynthesized through trans geranyl pyrophosphate (GPP) were also present, while in *G. xanthochymus,* diterpenoids (4.4%) biosynthesized through trans geranyl geranyl pyrophosphate (GGPP) were exclusively present. The presence of monoterpenoids formed from a distinct biosynthetic pathway support the species status for the new taxon, as elucidated through morphological studies. The presence of more complicated diterpenoids ($C_{20}H_{32}$) in *G. xanthochymus* compared to the simple monoterpenoids ($C_{10}H_{16}$) and sesquiterpenoids ($C_{15}H_{24}$) suggests that *G. xanthochymus* is more evolved in the group.

Table 2. Essential oil composition of the leaves of *Garcinia pushpangadaniana, Garcinia xanthochymus* and *Garcinia talbotii*

Compound	RRI	*G. xan* (%)	*G. pus* (%)	*G. tal* (%)
Z-β-Ocimene	1032	--	0.2	--
Linalool	1095	--	1.8	--
Terpineol	1186	--	0.4	--
Geraniol	1249	--	0.4	--
δ-Elemene	1338	0.3	0.3	--
α-Cubebene	1348	0.9	0.7	0.7
Cyclosativene	1371	0.4	--	--
α-Ylangene	1373	--	0.8	--
α-Copaene	1376	13.0	3.1	27.0
β-Bourbonene	1387	3.2	6.8	0.1
β-Cubebene	1387	--	0.4	--
β-Elemene	1390	4.6	--	--
β-Caryophyllene	1419	17.0	11.4	30.4
β-Copaene	1430	1.6	--	0.1
β-Gurjunene	1433	--	--	2.2
γ-Elemene	1434	0.1	0.4	--
Aromadendrene	1439	0.3	1.1	1.6
α-Humulene	1452	6.6	3.2	10.7
cis-Cadina-1(6)-4-diene	1461	--	1.4	0.1
α-Acoradiene	1464	--	--	0.1
γ-Gurjunene	1475	1.2	--	3.1
γ-Muurolene	1478	12.5	11.7	3.8

Amorpha-4,7 (11)-diene	1479	0.1	--	--
α-Amorphene	1483	--	--	1.3
β-Selinene	1489	0.1	0.6	--
δ-Selinene	1492	3.2	0.9	--
trans-Muurola-4(14)-5-diene	1493	9.0	--	--
γ-Amorphene	1495	--	2.6	--
α-Muurolene	1500	1.2	3.7	--
δ-Amorphene	1511	--	1.2	--
γ-Cadinene	1513	2.7	12.4	--
δ-Cadinene	1522	4.6	13.1	--
trans Cadina 1,4-diene	1533	0.1	1.0	0.1
Cadina-1(2),4-diene	1535	--	--	0.9
α-Cadinene	1537	0.4	1.4	0.1
Cadala-1(10),3,8-triene	1540	--	--	0.3
α-Calacorene	1544	0.3	1.2	--
Germacrene B	1559	0.5	0.4	--
Nerolidol	1561	--	0.4	--
Epiglobulol	1576	--	--	0.2
Spathulenol	1577	0.1	--	--
Caryophyllene oxide	1582	2.3	0.8	2.6
Globulol	1590	--	--	0.1
Cubeban-11-ol	1595	--	--	0.1
Humulene epoxide II	1608	0.4	--	0.5
1,10-di epi Cubenol	1618	--	--	1.2
α-Corocalane	1622	--	0.2	--
1-epi-Cubenol	1627	0.1	1.5	0.1
cis-Cadina-4-en-7-ol	1635		0.9	--
allo Aromadendrene epoxide	1639	0.4	--	--
Caryophylla-4(12),8(13)-diene	1639	--	--	0.1
α-Muurolol	1644	--	0.5	0.2
Cubenol	1645	0.1	--	0.8
α-Cadinol	1652	0.5	0.9	0.1
Cis-calamenen-10-ol	1660	0.1	--	--
14-Hydroxy 9-epi-Z-caryophyllene	1666	--	--	0.5
14-Hydroxy 9-epi-E-caryophyllene	1668	--	--	0.1
3E-Cembrene A	1947	4.4	--	--
Total (%)		92.3	87.8	89.2
Monoterpenoids		Nil	2.8	Nil
Sesquiterpene- Hydrocarbons		83.9	79.8	82.6
Sesquiterpene-Oxygenated		4	5.2	6.6
Diterpenoids		4.4	Nil	Nil

RRI: Relative retention index calculated on HP-5 column

Similarity and cladistic analyses performed statistically based on the distribution of 58 volatile chemicals using SPSS software (ver.16.0) showed *G. pushpangadaniana* distinct from other two species (**Figure 3, Table 3**). The species is more related to *G. xanthochymus* 62%), compared to *G. talbotii* (39%).

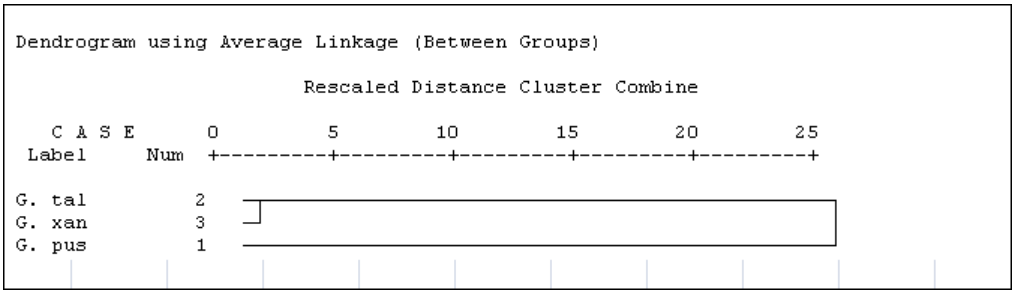

Figure 3. Dendrogram based on essential oil constituents of the leaves of *Garcinia pushpangadaniana, Garcinia xanthochyma* and *Garcinia talbotii.*

Table 3. Similarity matrix between three *Garcinia* species of the Western Ghats based on volatile chemical profile.

Case	Correlation between vectors of values		
	1:1	2:2	3:3
1:1	1.000	.391	.622
2:2	.391	1.000	.794
3:3	.622	.794	1.000

3. Molecular taxonomy

Molecular taxonomic approaches may be defined as DNA based methods that permit an exact and rapid method of distinguishing specimens based on their variation in genetic composition. Molecular markers are a direct assay of hereditary material and unlike morphological markers, molecular markers are not prone to environmental influences and can complement data from descriptors such as morphological characters (Mba and Tohme, 2005). Molecular systematics has become a major tool used in conservation biology for describing biodiversity, discriminating among taxa and establishing likely paths of evolution through phylogenetic analysis (Avise, 1989; Soltis *et al*., 1999).

In the present study, Genomic DNA was isolated from young leaves using DNeasy plant DNA isolation kit (Qiagen). The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems). The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v 5.6. The phylogenetic analyses of 28 accessions of 10 *Garcinia* species were done using *matK* with *Clusia criuva* of Clusiaceae family as the out group member (ncbi-TNS:SK08071206). The analysis involved 28 nucleotide sequences. In the present study, *G. pushpangadaniana, G. talbotii* and *G. xanthochymus* comes under separate clad, in congruence with the morphological and chemical classifications. The dendrogram clearly delimits the species status of *G. pushpangadaniana* and is more allied to *G. talbotii* (**Figure 3**).

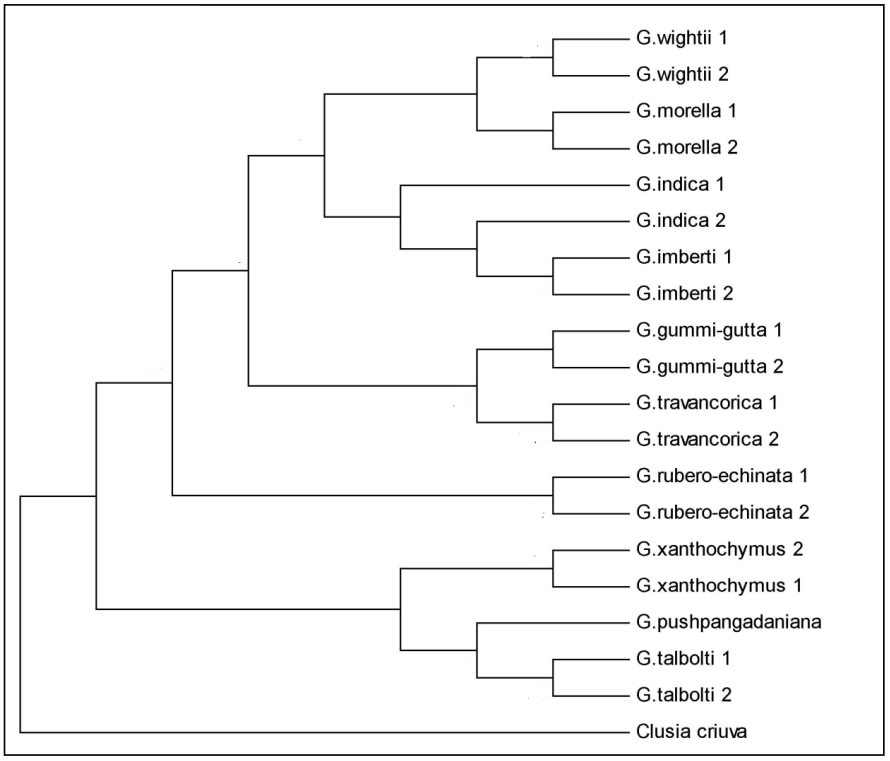

Figure 4. Phylogram based on *matK* loci of 28 accessions of 10 *Garcinia* species and the out group *Clusia criuva*

Conclusions

The HPTLC profile as well as the biosynthetic evaluation of the volatile terpenoids supported the species status for the new taxon. The molecular phylogeny also points to its proximity to *G. talbotii* and *G. xanthochymus* as elucidated through morphological evaluation. The present study highlights the importance of combined analysis of morphological traits, chemical profiles and genetic diversity that represents the optimal approach to assign species status to a new taxon.

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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. gummigutta* 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 Rheede referred the material as '*Coddam-pulli'* in *Hortus Malabaricus* (Van Rheede, 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 wildly 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.

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.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. *gummi-gutta*	var. *papilla*	var. *conicarpa*
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-oblanceolate	Acute-obtuse	Acute	6-10 x 3-4.5	7-8	11.71	Oblong, mamillae	89	5-7
4	Karanikkunu (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, mammillate	85.28	7
7	Arammulla	Pyramidal drooping	Elliptic	Acute	Acute	6-10 x 3-4	8	13.8	Discoid	99.92	4
8	Kurianippally	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 xanthones, benzophenones and biflavonoids, in addition to volatile secondary metabolites (Hemshekhar *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 secretary 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 (C_8-C_{22}) (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- Muurola- 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- Muurola- 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**).

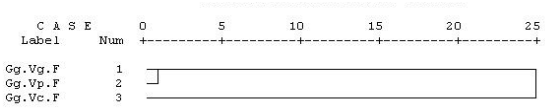

Figure 3. Dendrogram based on distribution of volatile compounds in the leaves of *Garcinia gummi-gutta* varieties

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**).

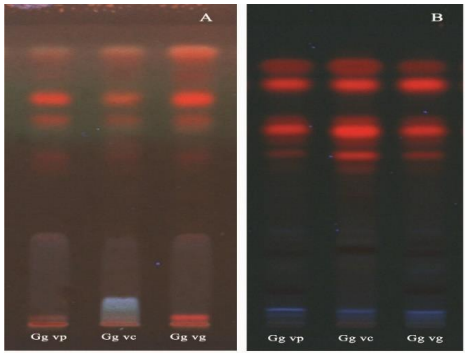

Figure 4. HPTLC profiles of *Garcinia gummi-gutta* varieties.
A. Leaf hexane extract; **B**. Leaf methanol extract

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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Chapter 9

Phytochemicals and bioactivities of *Garcinia indica* (Thouars) Choisy- A review

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Abstract

Garcinia indica is well known as a fruit tree of culinary, pharmaceutical, nutraceutical and industrial significance in south India, especially in the Konkan region. The fruit juice is much appreciated as a health drink while the dried fruit rind is used as a spice and condiment. The fat extracted from *G. indica* seeds is known as *kokum butter* and is used in foods, cosmetics and medicines. Stearic acid and oleic acid are the major fatty acids in kokum butter, while the fruit rind contains hydroxy citric acid, the much valued anti-obesity agent. The major class of secondary metabolites reported from different parts of the species are benzophenones, biflavonoids, xanthones and anthocyanin pigments. The fruit rind is a rich source of the benzophenone garcinol, attributed with potential bioactivities, especially antioxidant and cytotoxic. Cyanidin-3-glucoside and cyanidin-3-sambubioside were identified as the major red pigments in the fruit rind. The present review gives an overview of the phytochemical and pharmacological aspects of *G. indica*.

Keywords: *Garcinia indica,* Kokum, Anthocyanins, Garcinol, Isogarcinol

Introduction

Garcinia indica (Thouars) Choisy (Family: Clusiaceae) is one of the important indigenous *Garcinia* species grown in the Western Ghats of India. *Garcinia indica* (Kokum) is a slender, tropical evergreen tree that grows up to 15 m height. The branches are drooping, leaves ovate or oblong lanceolate, dark green above and pale beneath, stem bark thin lined, with pale yellow coloured exudates, and fruits globose or round, purple coloured when ripe, about 4 cm in diameter with 5-8 seeds. Flowering was observed during November-February and fruiting season was during April-June (Singh, 1993). *G. indica* is generally known as 'kokum tree', 'wild mangosteen' or 'goa butter tree' (Watt, 1890; Baliga *et al*., 2011).The species is well known for its food, medicinal and commercial values. The National Medicinal Plant Board (NMPB) has identified *G. indica* as an important plant for promotion and development. The present chapter gives a review on the distribution, traditional uses, pharmacological activities and phytochemical constituents of *G. indica*.

Figure 1.*Garcinia indica* twig and fruits

1. Distribution and conservation status

Garcinia indica is widely distributed along the Western Ghats of India and also found in the forests of Assam, Meghalaya and West Bengal. In the Western Ghats, the tree is mainly found along the costal belt of Konkan region of Ratnagiri district of Maharashtra, Goa, Uttara Kannada, Udupi and Dakshina Kannada Districts of Karnataka and Kasaragod area of Kerala. It thrives well below an altitude of 800m and at coastal areas (Braganza *et al.*, 2012; Nayak *et al.*, 2010). A wide diversity has been observed for kokum trees in the Western Ghats due to the dioecious nature and cross pollination (Swami *et al.*, 2014; Joseph and Murthy, 2015). The study conducted on 268 accessions of *G. indica* from different parts of the State of Goa, showed that the sugar level varied from 1.9 to 22.4^{o}Brix, while the total acid in fresh fruit rind was in the range 1.2 to 11.2 % (Braganza *et al*., 2012). *G. indica* is under vulnerable status as categorised by IUCN. Western Ghats Kokum Foundation (WGKF) is an organisation which promotes cultivation and works on conservation of *G. indica* in India.

2. Traditional uses of *Garcinia indica*

G. indica has got multifarious uses and finds various applications among the local population. The dried fruit rind of *G. indica* impart a sweet-tangy taste to food and is widely used as flavouring agent in food preparations as substitute for tamarind (Anonymous. 1956**;** Jayaprakasha and Sakariah, 2002). The fruits are also used as a substitute for grapes in wine making (Baliga *et al*., 2011). The fruit rind has also been utilized as a pink and purple food colouring agent (Kaur *et al*., 2012). Kokum drinks, made from the fruits of *G. indica*, served as a welcome drink in Goa during summer seasons. Konkani people of Goa and Maharashtra make *bhirindi saar*, a soup using kokum juice and also *kokum kadi* by mixing kokum juice and coconut milk, both used as after-meal drink to relieve any gastric problems (Menezes, 2001). Dried fruit rinds and syrup can be found as reserve in every house hold of Konkan region. Kokum butter is another important product obtained from the seeds of *G. indica*,

which is an important ingredient in cosmetic products like lip balms, lotions and soaps (Baliga *et al*., 2011).

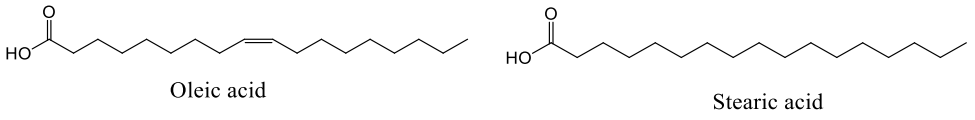
Traditionally, kokum is used in herbal medicines to treat diarrhoea, inflammatory ailments, dermatitis, bowel problems, rheumatic pains and to prevent hyper perspiration. Fruits are used as antihelmintic and cardiotonic. Kokum juice from the rind is used against piles, colic problems, dysentery and diarrhoea (Baliga *et al*., 2011; Watt, 1890). Decoction of fruit rinds are traditionally used against diabetes. Kokum butter is used traditionally to heal wounds, fissures in hands and is supposed to restore elasticity of skin and used as a moisturiser (Jeyarani and Reddy, 1999; Padhye *et al.*, 2009). Leaves of *G. indica* are used to treat skin ulcers, dyspepsia and hyperplasia.

3. Value added products from *Garcinia indica* fruits

With an estimated annual production of 10,200 tonnes of fruits (yield is 8.5 t/ha), the species is important for several industrial sectors such as nutraceutical, food supplementary, beverage and cosmetics (Braganza *et al.*, 2012; Swami *et al.,* 2014).. Several consumer products such as Kokum syrup, Kokum Agal (Kokum juice concentrate), Kokum sarbat*,* Kokum solkdhi, Kokum amsul (dried salted rind), Kokum butter and Kokum beverages are available in the market based on kokum fruits, rinds and kokum fat. Rinds are dried and stored, which can be used to prepare reconstitutable drinks during off season (Baliga *et al.*, 2011). It is also marketed as a spice in the local markets of Goa. Fresh rinds are added during wine making process, which gives the wine a pinkish appearance and a tingling taste. Kokum butter, because of its fatty acid content is used in soap and face creams (Padhye *et al.*, 2009). Kokum butter can be used as an ingredient in chocolate and due to the relatively high melting point (mp. 39 to 43°C), kokum butter prevents the chocolate from melting and can be used for preparing heat resistant chocolates (Maheshwari and Reddy, 2005; Jeyarani and Reddy, 1999). Kokum butter is sold as egg shaped lumps, used as edible fat and as a substitute of ghee in Goa.

3. Phytochemistry of *Garcinia indica*

The seed kernels of *G. indica* contains hard and brittle fat (mp. 39 to 43°C) up to 45 % yield, which is commercially known as 'kokum butter'. Kokum butter contains about 30% of fat content. Extensive studies have been carried out on the fatty acid composition of kokum butter and kokum fat was found to be rich in stearic acid ($C_{17}H_{35}COOH$) and oleic acid ($C_{17}H_{33}COOH$) (Krishnamurthy *et al*., 1982, Jeyarani and Reddy, 1999). Quantitative analysis of kokum butter revealed that in addition to fatty acids, it contains glycerides such as oleodistearin and stearodiolein (Lipp and Anklam, 1998). Seed oil is a source of palmitic acid, stearic acid, oleic acid and linoleic acid. Reports show that seed oil of *G. indica*, because of high content of fatty acid methyl esters, can be used as biofuel or can be mixed with other fuels to enhance its efficiency (Hosamani *et al.*, 2009).



Oleic acid Stearic acid

Figure 2. Structures of stearic acid and oleic acid

The fruit juice of *G. indica* is very acidic with a pH 1.5 to 2.0 and contains large amounts of acids. Major portion of organic acids in kokum is hydroxycitric acid (HCA) (1, 2 dihydroxypropane-1, 2, 3-tricarboxylic acid). Rinds contain about 20-30% of (-)-HCA on dry basis (Swami *et al*., 2014). HCA is an anti-obesity agent, attributed with reduced food intake, increased energy expenditure, suppression of fatty acid synthesis and an enhancement of glycogen synthesis in liver (Jena *et al*., 2002). Among the different *Garcinia* fruits, *G. gummi-gutta* possesses the highest HCA content, followed by *G. indica*. However, in a recent study, Pandey *et al* (2015) reported that among the 11 *Garcinia* species leaves analysed, HCA content was highest in *G. indica* leaves, 120mg/g leaf methanol extract, while in *G. gummi-gutta*, the HCA content was 95 mg/g. The total acid content (HCA and HCA lactone) was however higher in *G. gummi-gutta* leaves (308mg/g), compared to *G. indica* leaves (276 mg/g). Besides HCA, kokum juice contains malic acid, citric acid and tartaric acid (Parthasarathy *et al*., 2012).

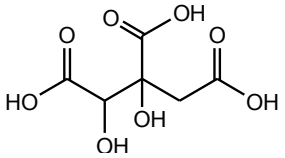


Figure 3. Structure of hydroxycitric acid

Table 1. Phytochemicals reported from *Garcinia indica*

Plant part	Compound	References
Leaves	D- Leucine	Cotterill and Scheinmann1977
	isogarcinol, xanthochymol, isoxanthochymol,	Chattopadhyay *et al.,*2006; Kumar *et al*., 2013
	HCA and HCA lactone	Jayaprakasha and Sakariah2002
	Cambogic acid, mangostin, garcinol, fukugicide, GB-1, GB- 2 and amentoflavone	Pandey *et al.,*2015
Fruits and fruit rinds	(-) HCA, HCA lactone	Cotterill and Scheinmann1977; Jayaprakasha and Sakariah 2002; Padhye *et al.,* 2009
	Garcinol, isogarcinol, citric acid, oxalic acid, xanthochymol, isoxanthochymol	Yamaguchi *et al*., 2000; Chattopadhyay *et al.,*2006; Padhye *et al*., 2009; Nayak *et al*., 2010; Kaur *et al*., 2012; Kumar *et al*., 2013; Bhagwat *et al*., 2014
	Anthocyanin, glucose, xylose, cyanidin-3-glucoside, cyanidin-3-sambubioside and 14-deoxyisogarcinol.	Nayak *et al*., 2010
	Polyprenylated acylphloroglucinol derivative	Kaur *et al*., 2012
Bark	Euxanthone (1,7-dihydroxy xanthone), volkensiflavone and morelloflavone	Cotterill and Scheinmann1977
	Xanthochymol, isoxanthochymol and camboginol	Chattopadhyay *et al.,*2006; Kumar *et al*., 2009
Seed pericarps and Seed oil	Isoxanthochymol, camboginol, palmitic acid, stearic acid, oleic acid and linoleic acid	Kumar *et al*., 2009; Hosamani *et al*., 2009

The major secondary metabolites reported from *G. indica* are polyisoprenylated benzophenones, xanthones and biflavonoids. Garcinol (camboginol), isogarcinol (xanthochymol) and isoxanthochymol are the major benzophenone derivatives isolated from *G. indica* fruits, dry rinds and leaves (Yamaguchi *et al*., 2000; Kumar *et al*, 2009; Kumar *et al*., 2013; Kaur *et al*., 2012, Chattopadhyay *et al.,* 2006; Pandey *et al.,*2015). Garcinol is

crystallized out as yellow needles (1.5%) from the hexane extract of the fruit rind, while its isomeric form isogarcinol is colourless. A simple reverse-phase high-performance liquid chromatography-electrospray ionization mass spectrometric method (ESI-MS) for the identification and quantification of the two isomeric benzophenones, isoxanthochymol and camboginol in the extracts of the stem bark, seeds and seed pericarps of *Garcinia indica* have been reported by Kumar *et al.* (2009). Two new compounds, 14-deoxyisogarcinol and a polyprenylated acylphloroglucinol derivative were isolated from *G. indica* fruits by Kaur *et al*., (2012). Xanthones and biflavanoids were also detected from *G. indica* (Cotterill and Scheinmann, 1977). An extensive LC-MS study on methanol extracts of *G. indica* leaves led to the identification of multiclass bioactive constituents belonging to organic acids, phenolic acids, flavonoids, biflavonoids, xanthones, benzophenones and terpenoids (Pandey *et al*., 2015).

The fruit rind of G. *indica* has been utilized as a pink and purple food coloring agent and the rind contains 2 to 3 % of water soluble red colour pigments. The major colouring compounds are the anthocyanin pigments cyanidin-3-glucoside and cyanidin-3-sambubioside which are usually present in the ratio of 4:1 (Nayak *et al.,* 2010).The variation in colour shades of kokum fruits can be attributed to the variation in substitution of hydroxyl and methoxyl groups to the anthocyanin structural skeletons. Anthocyanins are the major antioxidant constituents in *G. indica* and the 3' and 4'-OH in B-ring determine radical scavenging capacity with a saturated 2, 3- double bond. Major phytochemicals isolated from *G. indica* and their structures are given in **Table 1 and Figure 1.**

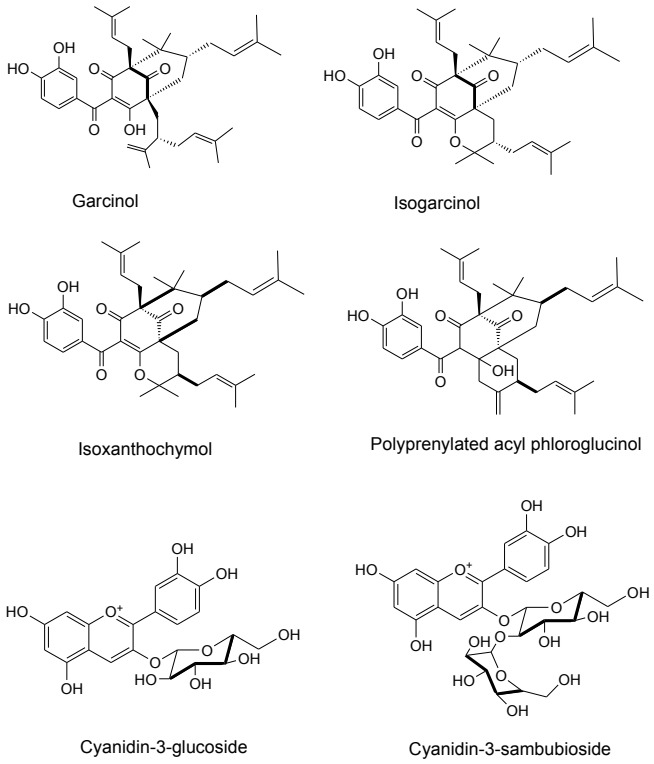


Figure 1. Characteristic compounds reported from *Garcinia indica*

4. Bioactivites of *Garcinia indica*

Extracts as well as compounds isolated from *G. indica* have been studied extensively for various bioactivities like antioxidant, antibacterial, antifungal, antiobesity, antidiabetic, gastroprotective and anticancer activities. The pharmacological studies validated the traditional uses of the plant in various ailments. Benzophenones, anthocyanins and organic acids are the major bioactive constituents reported in *G. indica*.

Among the different bioactivities reported, antioxidant properties are perhaps the most important activity for *G. indica* (Krishnamurthy and Sampathu 1988**;** Mishra *et al*., 2006). Choloroform extracts of *G. indica* fruit rinds exhibited excellent antioxidant activities in β-carotene-linoleate and DPPH assays (Tamilselvi *et al.*, 2003). Aqueous extracts of *G. indica* fruits available in markets acts as very good antioxidants as evident from their DPPH and lipid peroxidation assays. Aqueous extracts of kokum inhibit ascorbate-Fe^{2+} induced lipid peroxidation in rat liver mitochondrial fractions (Mishra *et al*, 2006). Organic acids like citric acid and malic acid from *G. indica* also acts as good antioxidants (Swami *et al*, 2014). A recent study on *G. indica* bark exudates showed its total phenol and xanthone content as 53.43 g/100g and 32.42 g/100g respectively, revealing it as a potential source of antioxidants (Parthasarathy and Nandakishore, 2016).

Kokum rind extracts showed antifungal effects against *Candida albicans, Penicillium* sp. and *Aspergillus flavus*. Also the extract showed inhibitory activity against '3T3' mouse fibroblasts (Mishra *et al*, 2006; Varalakshmi *et al.*, 2010; Tamilselvi *et al.*, 2003). Aqueous and methanol extracts of *G. indica* leaves and fruit rinds showed antibacterial activity against *Salmonella* sp (Pasha *et al.*, 2009). Methanol extracts of kokum fruits acted as an effective neuroprotective agent for striatal dopaminergic neurons in 6-OHDA lesioned rat model of Parkinsons disease (Antala *et al.*, 2012). Aqueous fruit rind extract of the kokum exhibited antidiabetic activity in streptozotocin-induced hyperglycemic rats (Kirana and Srinivasan, 2010). However, lyophilized aqueous-methanol extracts in water of *G. indica* fruit rinds showed a dose dependant genotoxicity in mice (Das *et al.,* 2016).

The major anthocyanin in *G. indica* fruits, cyanidin-3-glucoside decreased the number of non-malignant and malignant skin tumours in the two staged skin carcinogenesis and also caused a dose-dependent inhibitory effect on the migration and invasion of metastatic A549 human lung carcinoma cells (Ding *et al*., 2006, Chen *et al*., 2006). It was found effective in blocking accumulation of intracellular ROS and neurofilament protein expression and was effective against bipolar disorder by reducing ethanol-mediated activation of GSK3β. (Chen *et al.*, 2009). The biological activities of garcinol, the major polyisoprenylated benzophenone isolated from *G. indica* and (-) hydroxy citric acid, the major acid in *G. indica* fruits were dealt in detail in Chapter 10.

Conclusions

Recently, *Garcinia* species have received considerable attention worldwide from scientific as well as industrial sectors and several novel structures, bioactivities and potential utilities have been reported. In USA alone, mangosteen containing beverages had a turnover of more than $200 million in 2008. Kokum can be considered as a functional food that provide in addition to nutritional components, other physiological benefits as well. The consumption of high value products of kokum have increased tremendously due to the awareness of the potential health benefits associated with the diverse bioactive constituents in the plant. The review also

highlights the potential for developing *G. indica* as an economic crop to derive value added products with scientific validation.

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Chapter 10

Phytochemicals and bioactivities of *Garcinia gummi- gutta* (L.) N. Robson- A review

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Abstract

Among the different *Garcinia* species, *G. gummi-gutta* is the most widely distributed *Garcinia* species in Kerala, south India. The fruit is used as culinary spice, preservatives and also as a source of several nutraceutical products. The phytochemical analysis of *G. gummi-gutta* revealed the presence of several bioactive molecules such as xanthones, benzophenones and organic acids. The fruit contains 10% to 30% (-) hydroxycitric acid (HCA), a well known hypo-lipidemic agent and an important constituent of food supplement for weight management. The species is a rich source of the bioactive benzophenones camboginol (garcinol) and cambogin (isogarcinol). The present review summarises the traditional uses, phytochemicals and pharmacological activities of *G. gummi-gutta*.

Keywords: *Garcinia gummi-gutta,* Hydroxy citric acid, Benzophenones, Camboginol, Cambogin

Introduction

Garcinia is the largest genus of the Clusiaceae family comprising nearly 250 species. *Garcinia gummi-gutta* (L.) Roxb. (Syn.: *Garcinia cambogia* (Gaertn.) Desr; Common name: Malabar tamarind), is one of the most important members of the Clusiaceae family (**Figure 1**). It is a small or medium sized tree up to 12 m tall with dark green and shining leaves. The leaves are elliptic obovate, 2-5 inch long and 1-3 inch broad. Fruits are ovoid, 2 inches in diameter, yellow when ripe, with 6-8 grooves; seeds 6-8 surrounded by succulent aril (Singh, 1993). The aril and the fleshy covering encasing the seed is edible when ripe. The differentiation between male and female trees is known only at the flowering stage which takes approximately 7 to 9 years (Kalia *et al*., 2012). *G. gummi-gutta* is a common species found in the Western Ghats, from the Konkan southwards to Travancore eastwards. The species has now been introduced elsewhere in the subtropical region of Asia including China, Malaysia and the Philippines (Chuah *et al*., 2013). The present chapter reviews the traditional uses, pharmacological activities and phytochemicals of *G. gummi-gutta.*

Figure 1.*Garcinia gummi-gutta* twig with fruits

1. Traditional uses

G. gummi-gutta is traditionally used as a condiment for flavouring curries and as a fish preservative. The traditionally smoke dried fruit rind of *G. gummi-gutta*, known as 'Malabar tamarind' was used for "Colombo curing" of fish, where the pickling was done in brine along with the smoke dried rinds of *G. gummi-gutta* (Sreenivasan and Venkataraman 1959; Lewis and Neelakantan, 1965). The species yield an yellow, adhesive gum resin similar to gamboge from *G. morella*, but of inferior quality and insoluble in water. The seeds yield an oil, which is used in medicine (Watt, 1890). The wood is grey, cross grained, shining, hard and can be used in furniture making (Watt, 1890). The dried rind was used for polishing gold and silver and also used as a substitute for acetic and formic acids in the coagulation of rubber latex (Anonymous, 1956).

Though the tree has been mentioned in the 17th century treatise of medicinal plants, *Hortus Malabaricus*, the species is not part of the Ayurvedic medicine of ancient India (Manilal, 2003). However, it was widely reputed in the folk herbal healing practices and has been used traditionally for the treatment of edema, delayed menstruation, ulcers, open sores, hemorrhoids, fever, rheumatism, and also against intestinal parasites (Majeed *et al*., 1994, Semwal, *et al*., 2015). The astringent properties of the rind make it an indispensible ingredient in gargles for weak gums, bowel complaints, constipation, diarrhoea and dysentery. The plant is used in veterinary medicine, for mouth diseases in livestock.

2. Phytochemicals reported from *G. gummi-gutta*

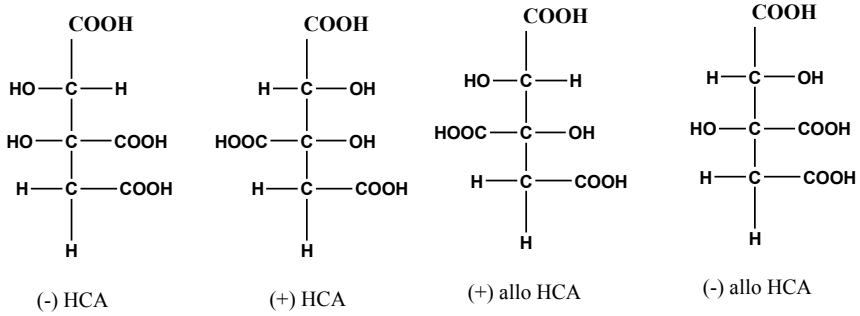
Though *G. gummi-gutta* is an economically important species, widely cultivated in south India, only a few reports are available in literature on the phytochemistry of the plant (**Table 1**). The fruit is well known for the acidic nature and the chemistry and analytical techniques of hydroxycitric acid, the major organic acid in *G. gummi-gutta,* has been dealt with detail in literature (Jena *et al*., 2002). Benzophenones are the major secondary metabolites in *G. gummi-gutta*, followed by xanthones and biflavonoids.

2.1. Organic Acids

Organic acids are of great significance in plants as intermediates in the metabolic processes and are directly involved in growth and maturation of fruits. The organic acids play a key role in fruit flavour and taste. Most of the *Garcinia* fruits are well known for their sour taste and high acidity, and of the different acids reported from *Garcinia* fruits, (−)-hydroxycitric acid (HCA) is the important one, being an anti-obesity agent and a chiral molecule of wide utility in chiral synthesis (Jena, *et al*., 2002). Malic acid, ascorbic acid, tartaric, oxalic acid and citric acids are also present to a lesser extent in *Garcinia* fruits.

Hydroxy citric acid: Hydroxycitric acid (HCA) is the major organic acid occurring in the fruits of *G. gummi-gutta*. The acid and its lactone were mistakenly identified as citric acid and tartaric acid, however, the acids failed to give positive result for pentabromacetone test for citric acid and cream of tartar test for tartaric acid (Sreenivasan and Venkataraman 1959, Lewis *et al*., 1964). HCA has been first reported from nature by Lewis and Neelakantan in 1965 from the fruit rinds of *G. gummi-gutta* (Lewis and Neelakantan, 1965). HCA (1,2 dihydroxypropane-1,2,3- tricarboxylic acid) has four isomeric forms, since it contains two asymmetric carbons: (-)-HCA, (+)-HCA, (+)-allo-HCA and (-)-allo-HCA (**Figure 2**). (2S, 3S) Hydroxycitric acid is the major acid from the fruit rinds of *G. gummi-gutta*. The fruit contains 10% to 30% (-)HCA which can be isolated in the free form, as a mineral salt or as a lactone. An HPLC analysis showed 4.1-4.6% (-)-HCA in the leaves while 10.3-12.7% in the fruits of *G. indica* (Jayaprakasha and Sakariah, 2002).

The leaves also contain HCA and a recent LC-MS screening revealed that among 13 *Garcinia* species, *G. gummi-gutta* contains the highest quantity of acids (308mg/g leaf methanol extract) and the HCA content was 95mg/g (Pandey *et al*., 2015). HCA is available in the market in the form of various salts such as calcium, magnesium and potassium as well as their mixtures (Yamada *et al*., 2007). Citrin is the trade name given to the calcium salt of hydroxy citric acid. HCA lactone or garcinia lactone was also reported from the fruit. Other organic acids such as tartaric acid, citric acid and malic acid also have been reported as minor constituents. It also contains 1.5% phosphoric acid as calcium triphosphate.



(-) HCA (+) HCA (+) allo HCA (-) allo HCA

Figure 2. Isomeric forms of hydroxycitric acid

Though citric acid is a common acid in plants, hydroxy citric acid is distributed in limited plant species such as the flowers of *Hibiscus subdariffa* and *H. rosasinensis*. However, the stereochemistry of HCA from *Hibiscus* species is (+) allo form and is different from that of

Garcinia (Lewis *et al*., 1964). Microbial strains such as *Streptomyces* sp. U121 and *Bacillus megaterium* G45C also produces HCA in trace amounts (Yamada *et al*., 2007). Hydroxycitric acid has also been synthesized from citric acid, through dehydration to form aconitic acid, which forms hydroxycitric acid via oxidation (Chen et al., 2001).

Paper chromatographic method (solvent system: n-butanol: acetic acid:water (BAW) in the ratio (4:1:5) separates and detects hydroxy citric acid, along with its lactone, on Whatman No.1 paper using bromophenol blue as spray reagent. The acid content of the fruits can be estimated by titrating against 0.1 N sodium hydroxide using phenolphthalein as indicator. However, in this method the concentrations of (-)-HCA and lactone cannot be estimated separately (Jayaprakasha and Sakariah, 1998). HCA can be estimated spectrophotometrically by the formation of reddish orange color complex between HCA and sodium meta vanadate (Antony *et al*., 1999). Quantification of HCA was also possible through HPLC analysis of aqueous solution, where (-)-HCA and its lactone can be quantified separately (Majeed *et al*., 1994, Jayaprakasha and Sakariah, 1998, 2000, 2002). The acid can also be detected and estimated using gas chromatography of the trimethyl derivative (Lowenstein and Brunengraber, 1981). In a recent report, UHPLC-QqQLIT–MS/MS method has been applied for the validated estimation of HCA and lactone separately in leaf samples of different *Garcinia* species (Pandey *et al*., 2015).

The fatty acid content of *G. gummi-gutta* seeds were 46.5%, and the major fatty acid was stearic acid (30.6%), followed by oleic acid (26.2%), linoleic acid (11.4%), elaidic acid (9.5%), palmitic acid (6.3%) and arachidic acid (5.4%) (See chapter 12 for further details).

The amino acid profile of *G. gummi-gutta* fruits was also reported. The amount of total free amino acids was determined to be less than 60 mg in 100 g of *G. gummi-gutta* fruit. The amino acids such as arginine, asparagine, glutamine, threonine, glycine, proline, γ-amino butyric acid, leucine, isoleucine, ornithine and lysine were detected in the fruits (Carratu *et al*., 2008).

Volatile chemical profiling of the leaves of *G. gummi-gutta* revealed sesquiterpenoids as the major class of volatile compounds and α-copaene has been reported as the major compound (30.2%) (*refer chapter 5 for details*).

2.2. Benzophenones

Rama Rao *et al.* in the late 1970's, isolated the benzophenones camboginol (garcinol) and cambogin (isogarcinol; xanthochymol) from the latex of *G. gummi-gutta* in large quantities (37.0% and 5.5% respectively) (Rao *et al.,* 1973). Camboginol (m.p. 132°C) was obtained in 37% yield from the latex of *G. gummi-gutta* by a simple crystallisation from pet-ether. Silica gel column chromatography of the remaining residue using hexane as the eluting solvent gave cambogin (Rao *et al.,* 1973). Cambogin has identical chemical and spectral properties as isoxanthochymol but having exactly opposite specific rotation, clearly indicating the compound as an enantiomer of isoxanthochymol. Later Iinuma, *et al* has also isolated garcinol and isogarcinol from the barks of *G. gummi-gutta* (Iinuma, *et al*., 1998). Phytochemical investigation of the fruits of *G. gummi-gutta* resulted in the isolation and characterisation of the benzophenones garcinol and guttiferones I, J, K, M, N (Masullo *et al*., 2008, 2010). In a recent report, the content of garcinol was highest in *G. gummi-gutta* (0.593mg/g) leaf methanol extract among the 13 *Garcinia* species screened (Pandey *et al*., 2015).

2.3. Xanthones

The xanthones garbogiol and rheediaxanthone A were isolated from the barks and roots of *G. gummi-gutta* (Iinuma, *et al*., 1998). Oxy-guttiferones M, K2, I and K were isolated from the fruits of *G. gummi-gutta* (Masullo *et al*., 2008, 2010). Oxy-guttiferones are tetracyclic xanthones derived from the oxidation of the corresponding polyisoprenylated benzophenones.

2.4. Biflavonoids

In a recent report, the biflavonoids fukugicide, GB-1 and amentoflavone were reported from *G. gummi-gutta* leaf extracts through a validated LC-MS analysis (Pandey *et al*., 2015). However, the biflavonoid content was lowest in *G. gummi-gutta* among all the screened *Garcinia* species. The phenolic acid and flavonoids were also lower compared to other *Garcinia* species (Pandey *et al*., 2015).

The major secondary metabolites benzophenones, xanthones and biflavonoids reported from the species are listed in **Table 1**.

Table 1. Phytochemicals reported from *Garcinia gummi-gutta*

Plant Part	Compounds	References
Leaf	Cambogic acid, mangostin, garcinol, fukugicide, GB-1 and amentoflavone	Pandey *et al.,*2015
Heart wood	Morelloflavone, dihydromorelloflavone, isomorellic acid	Venkataraman, 1973
Bark	Rheediaxanthone, guttiferone E and isogarcinol	Iinuma *et al*.,1998
Latex	Cambogin (isogarcinol) and camboginol (garcinol)	Rao *et al.,*1973
Root	Garbogiol	Iinuma *et al*.,1998
	Morelloflavone, dihydromorelloflavone, isomorellic acid	Venkataraman, 1973
Fruit	Guttiferones - K, I, J, M and N; oxy-guttiferones M, K, K2 and I	Masullo *et al*, 2008,2010

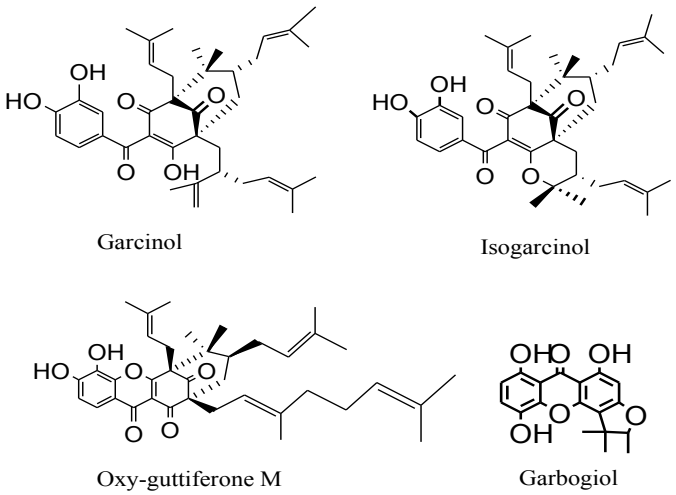


Figure 3. Structures of some secondary metabolites isolated from *G. gummi-gutta*

3. Biological activities reported for *Garcinia gummi-gutta*

3.1. Bioactivities of *G. gummi-gutta* crude extracts: The crude extract and isolated constituents from *G. gummi-gutta* exerted wide spectra of biological activities such as anthelmintic, anticholinesterase, diuretic, antifungal, gastroprotective and hepatoprotective activities in various *in vitro* and *in vivo* models (Semwal*, et al*., 2015). *G. gummi-gutta* also showed effect on reproductive system, lipid peroxidation, blood viscosity, haematology and plasma biochemistry (Semwal *et al*., 2015). G. *gummi-gutta* extract has shown significant antidiabetic property by efficiently improving glucose metabolism and displaying leptin like activity (Hayamizu *et al*., 2003). Remarkable antibacterial effect has been observed for various extracts of *G. gummi-gutta* (Jacob *et al*., 2015, Rani and Lawerence, 2015, Maridass *et al*., 2010). Different extracts of *G. gummi-gutta* fruits have shown good antioxidant property in various *in vitro* assays such as DPPH, hydroxyl radical, ferric reducing and lipid peroxidation (Jacob *et al*., 2015, Ranjani *et al*., 2014, Shivakumar *et al*., 2013, Subhashini *et al*., 2011). *G. gummi-gutta* extracts showed significant anti inflammatory activity in various experimental systems. In TNBS-induced colitis rats, the extract showed significant anti inflammatory activity and it could be related to a reduction in DNA damage in isolated colonocytes, observed with the comet assay. The extract also improved the macroscopic damage and caused substantial reductions in MPO activity, COX-2 and iNOS expression. It was also observed that treatment using *Garcinia* extract reduced PGE2 and IL-1β colonic levels. The leaves of *G. gummi-gutta* showed significant anti-inflammatory activity, especially against carrageenan induced paw oedema in rats and also exhibited moderate *in vitro* anti-inflammatory action in hRBC membrane stabilization method (Prasanth *et al*., 2013). Several compounds such as garcinol, guttiferone K and guttiferone M isolated from *G. gummi-gutta* also posses anti-inflammatory activity (Semwal *et al*., 2015). *G. gummi-gutta* decreases the acidity and increase the mucosal defence in the gastric areas, thereby it can be used as an anti ulcerogenic agent (Mahendran *et al*., 2002). The oral administration of a fruit extract of *G. gummi-gutta* at doses of 1000 mg/kg BW/day for 5, 10 or 15 days exerted protective effects against indomethacin-induced damage of the gastric mucosa in rats. *G. gummi-gutta* fruit extract showed anti-tumour activity against the cell viability in the murine neuroblastoma cell line (Neuro-2A cells) (Mazzio and Soliman, 2009). Garcinol, the major secondary metabolite in *G. gummi-gutta* was effectively used against different cancer types such as breast cancer, Burkitt lymphoma, colon cancer, esophageal cancer, hepatocellular carcinoma, HeLa cells, kidney cancer, leukemia, lung cancer, medulloblastoma, multiple myeloma, pancreatic cancer, prostate cancer and tongue cancer (Saadat and Gupta, 2012).

3.2. Antiobesity property of hydroxyl citric acid (HCA): (−)-HCA is one of the important supplements for anti-obesity and weight management (Chuah *et al*., 2013). The inhibition of faty acid synthesis *in vivo* by HCA was first reported by Lowenstein *et al*., in 1971. (-)-HCA at 1 mmole per kg of body weight inhibited fatty acid synthesis by about 75% (Lowenstein *et al*., 1981). Sullivan *et al*., reoprted that fatty acid and cholesterol synthesis were blocked significantly by HCA and also that rats fed with HCA tended to eat less compared to the control animals (Sullivan *et al*., 1974). They have also reported that HCA lowered body fat levels with no loss of body protein in test animals (Sullivan *et al*., 1974). Followed by these

observations, there has been a plethora of experiments on different models to test the anti obesity activity of HCA (Majeed *et al*., 1994).

HCA exhibited antiobesity activity by inhibiting the ATP-citrate lyase, a catalyst for the conversion process of citrate to acetyl-coenzyme A, the building block for fatty acid and cholesterol synthesis (Tharachand *et al*., 2013, Downs *et al*., 2005). In human trails HCA significantly improved blood lipid profiles by reducing total cholesterol, LDL and triglycerides levels significantly (Preuss *et al*., 2005). HCA promotes weight loss in humans without causing any stimulation in the central nervous system and produce only short term anorexia and does not carry the risk of being addictive (Majeed *et al*., 1994, Downs *et al*., 2005). HCA also regulated the serotonin levels related to satiety and decreased lipogenesis.

Garcinia extracts and HCA have widely been used for obesity and weight control treatments and the long term continuous consumption demands systematic toxicity evaluation and a number of reports about the toxicity of *G. gummi-gutta* fruits and supplements are available in literature (Majeed *et al*., 1994). However, the potential contributions of HCA as a weight loss agent in humans were controversial, especially regarding the long term benefits and when the randomized, placebo-controlled clinical trials were counted (Heymsfield *et al*., 1998; Marquez *et al*., 2012). Also, some clinical studies reported various toxic effects such as toxicity towards spermatogenesis and hepatotoxicity (Kim *et al*., 2013). However, scientific evidence based on structure, mechanism of action and long history of the use of *Garcinia* had shown 'no observed adverse effect level' (NOAEL) at levels up to 2800 mg/day and suggests that HCA is safe for use (Chuah *et al*., 2012, 2013).

3.3: Biological activities of garcinol: Garcinol, the major polyisoprenylated benzophenone isolated from *G. indica* exhibits potential antioxidant activity by scavenging DPPH radicals, hydroxyl radicals, suppressing superoxide anion, effective against peroxynitrite-induced lipid peroxidation and inhibiting xanthine oxidase activity. The strong antioxidant activity of garcinol is attributed to the presence of both the phenolic hydroxy groups and β-diketone moiety that shows keto enol tautomerism as in the case of curcumin (Padhye *et al*., 2009). Garcinol plays an important role in the treatment of gastric ulcers caused by the hydroxyl radical or by a chronic infection with *Helicobacter pylori* as evident from its antiulcer activity in rats induced by indomethacin and acts as a good antioxidant when administered orally (Yamaguchi *et al.,* 2000; Kolodziejczyk *et al.*, 2009). It shows antibiotic activity against methicillin-resistant *Staphylococcus aureus* comparable to that of vancomycin and also proven to exhibit several anticancer activities. Garcinol is also able to suppress colonic aberrant crypt foci (ACF) formation in rats and inhibits topoisomerases I and II at concentrations comparable to that of etoposide. Garcinol decreases the cell viability, increases cell death and apoptosis in human leukemia HL-60 cells, HT-29 cells, HeLa cells and colon cancer cells (Pan *et al.*, 2001; Balasubramanyam *et al.*, 2004). 4-NQO induced oral carcinogenesis in rats and Nic-induced human breast cancer (MDA-MB-231) cell proliferation were suppressed by garcinol (Yoshida *et al.*, 2005; Chen *et al.*, 2011). Earlier studies showed that garcinol acts as a neuroprotective agent by inhibiting the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in lipopolysaccharide activated macrophages (LPS) and blocks activation of eukaryotic transcription factor NF-κB induced by LPS (Liao *et al.*, 2004). It has been established that the phenolic hydroxyl groups as well as β-diketone moiety, that shows keto-enoltautomerism as in the case of curcumin, is

important for the biological activities of garcinol. The isoprenyl chain consists of hydrophobic sites, is also important for binding to biological targets (Padhye *et al*., 2009).

In addition, other secondary metabolites isolated from *G. gummi-gutta* also showed various biological activities. Xanthones reported from *G. gummi-gutta* shows activities such as vasodilatory, antimalarial, antiviral activity, human leukemia, cytotoxic activity, α-glucosidase activity, CNS activity and platelet activating factor (PAF). Guttiferones and polyisoprenylated benzophenones reported from *G. gummi-gutta* have shown interesting biological properties such as leishmanicidal, anticancer, antifungal, antiproteolytic, cytotoxicity, apoptotic, cytoprotection against HIV-1 *in vitro* and inhibited the binding activity of a-liver X receptor (LXRa) but is less effective against b-receptor (LXRb).

Conclusions

G. gummi-gutta is a common fruit plant of the Western Ghats, attributed with a wide range of applications ranging from food, medicines and nutraceutics. The fruit rind of *G. gummi-gutta* is the major source of (−)-hydroxycitric acid (HCA). In addition, secondary metabolites such as xanthones, benzophenones, organic and amino acids were also reported from this plant. The potential beneficial effects include antioxidant, antihelmenthic, antidiabetic, antimicrobial, antiobesity and hyperlipidaemic properties. Reports on the toxicity and observations during clinical trials suggest that *G. gummi-gutta* is safe for human consumption.

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Chapter 11

Gamboge- The bark exudate from *Garcinia* species

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Abstract

Garcinia bark exudates, known as gamboges, has been used as a pigment in Indian murals and European water colour-paintings. It has also been used for dyeing clothes and for colouring wood, metal and leather. Gamboge has several uses in traditional medicinal systems, especially as a purgative and also externally used for treating infected wounds. The major sources of gamboges are *Garcinia hanburyi* and *Garcinia morella*. Gamboge contains 70% to 80% yellow resin and 15% to 25% water soluble gum and the remaining portion is composed of esters, hydrocarbons, wax and ash. The characteristic bioactive compounds in gamboges were identified as caged xanthones, such as gambogic acid and morellin, that possess potential anticancer properties. This chapter provides a detailed account on history, distribution, chemistry and uses of gamboge.

Keywords: Gamboge, *Garcinia hanburyi, Garcinia morella,* Caged xanthones, Gambogic acid, Morellin

Introduction

Recently there has been an increased demand for plant derived natural products, mainly due to the safety concerns of the synthetic pigments, colouring agents and other additives that are essential ingredients in several industrial sectors such as cloth dyeing, food and nutraceutical. Among the different plant products, exudates are in high demand now, due to the low toxicity, abundant availability, biocompatibility, biodegradability and inertness compared to synthetic alternatives.

Gamboge, also known as camboge, is the exudate from the bark of *Garcinia* species. *Garcinia* species are perhaps known all over the world in ancient times by this value added product. The dried exudates are used as a pigment in Indian murals and European water-paintings and dyeing clothes and also for colouring wood, metal and leather. Though primarily gamboge was used as a colouring agent, several traditional medicinal uses were also attributed to the exudate. Recent phytochemical investigations showed the bark exudates as rich source of bioactive secondary metabolites such as caged xanthones. The present chapter summarises the history, traditional uses and phytochemistry of gamboge.

1. History of gamboge as a natural colouring agent

Plant exudates were used by ancient civilisations world over for various purposes and the usage can be traced back to about 3000 BC, where the Egyptian civilization used gum Arabica, the exudates from *Acacia*. The word gamboge comes from *Gambogia*, the Latin

word for Cambodia. Gamboge was used from ancient times to dye the clothes and also to make a transparent yellow varnish for the coloring of wood, metals and leather. The pigment was made more usable by mixing with other yellow pigments such as lemon yellow or alumnia. The color of gamboge is a deep tone of saffron, and gamboge is recognised as a distinct colour (Maerz and Paul, 1930). When used as a water colour, it gives a bright transparent golden yellow colour and is not a true pigment. In ancient India, gamboge had an important place among artists, herbalists and spiritual communities. The earliest evidence of the use of gamboge comes from artefacts of eighth century from East Asia, where the yellow colour is presumed to be derived from gamboge. *Garcinia* exudates were used to dye the robes of Buddhist monks (Lewington *et al.*, 1990). Gamboge was first brought to Europe, in 1603, by Admiral Van Neck, and used as a transparent oil color by Flemish painters (Chantarasriwong *et al*., 2010). John Smith in '*The Art of Painting in Oyl'*, published in 1701, describes a method for preparing the colour. The botanic artist William Hooker created the pigment 'Hooker's Green' that gives a special green to colouring leaves by mixing Green Malachite or Prussian blue and gamboge (Winter *et al.*, 1997). One can assume that since the gamboge faded so rapidly relative to iron blue, trees in some old artworks have become blue. A tradition of mural paintings in Kerala, south India, following the sixteenth century techniques, uses the exudates of *G. morella*, locally known as Eravikkara in Malayalam in combination with the leaves of *Indigofera tinctoria* to get different shades of green (Nayar *et al*., 1999). Jean Baptiste Perrin in his work on Brownian movement used a colloidal suspension of gamboge particles to investigate the phenomenon and derive a value for the Avogadro number in 1926 (Chantarasriwong *et al*., 2010).

2. Traditional medicinal uses of gamboge

The exudates from different *Garcinia* species were used therapeutically in traditional medicine, especially as emetics and cathartics (Majeed *et al*., 1994). Gamboge obtained from *Garcinia hanburyi* is used externally for infected wound and for pain and oedema in traditional Thai medicine. It has cathartic activity and is used in veterinary medicine as a drastic purgative. Gamboge is a laxative in doses of 10-15 cgm., produces abundant evacuations with violent colicky pains in doses of 30-50 cgm. It can cause vomiting, nausea and griping in high doses. It is also used as a vermifuge. It is usually combined with other purgatives such as aloe or calomel, to strengthen their effect. It is used in traditional medicine for the treatment of ulcers, skin infection, appetite suppression and to lower blood pressure (Panda, 2005). The resin of *G. morella* has purgative action and was mainly applied for intestinal complaints. The cathartic property of the exudate was made use for expelling tapeworms from the intestine. However, large doses are toxic, leading to gastro enteritis.

3. Extraction of gamboge

Gamboge is generally extracted by tapping of *Garcinia* species. The plant tissues of the Clusiaceae members were characterized by the presence of latex channels and different shades of yellow were reported for the exudates from *Garcinia* species (Nogueira *et al*., 2001). Generally trees of ten years old are tapped by making spiral incisions in the bark and traditionally collected in bamboo containers. The hard and brittle lumps of the solidified raw gamboge are dark yellow in color, which when pulverized, turns into a bright yellow powder. This powder is mixed with a variety of binders to make paints and varnishes.

4. Major sources of gamboge

The major sources of gamboge were *G. hanburyi* (Cambodia and Thailand), *G. morella* (India and Sri Lanka), and *G. elliptica* and *G. heterandra* (Myanmar). The chief trade supply was obtained from Siam in the form of cylindrical pieces or sticks and until recently, the gum resin of Siam was referred to *Garcinia cochin-sinensis* and that of Ceylon to *Hebradendron cambogioides*, while that of Southern India was supposed to be the produce of *Garcinia pictorial* (Watt,1890; Utpala and Nandakishore, 2016).

True gamboge of use in arts and medicine in India derives mainly from the gum resin of *G. morella* (**Figure 1**). The tree is distributed in Indo-Malay and Sri Lanka. All parts of the plant yield a thick yellow exudate.

Figure 1. *Garcinia morella* twig, seeds and bark

Table 1. Distribution of gamboge in different *Garcinia* species

Sl. No.	*Garcinia* species	Remarks
	G. anomala Planci. & Trian.	Gamboge is inferior in quality.
	G. cornea Linn.	Gamboge is inferior in quality.
	G. cowa Roxb.	Gamboges is inferior in quality, with paler colour than that of *G. morella* and is insoluble in water. Bark is used to extract a light yellow colour for colouring of the cloth for the garments of Buddist monks.
	G. eugeniaefolia Wall.	The exudate a green varnish
	G. gummi-gutta (L.) N. Robson	The tree yields a yellow, insoluble, very adhesive gum, which is valueless as a pigment on account of its insolubility in water
	G. hanburyi Hook. f.	Exudates is known as Siam gamboge and is used as a purgative and externally used for infected wounds in Thai traditional medicine.
	G. heterandra Wall.	This tree yields a superior kind of gamboge, so similar to the Gamboge of commerce. It readily forms an emulsion with water. Burmese priests occasionally use this gamboges to dye their robes and the Karens to dye their thread. The gum resin is occasionally employed as a medicine by Burman native practitioners.
	G. indica	The exudate is sparingly soluble in water, but it became

		insoluble when dried.
	G. mangostana Linn.	This species exudes gamboge of inferior quality
	G. morella Desrouss	This species produces the true gamboge of medicine and of the arts.
	G. speciosa Wall.	It yields an inferior gamboge.
	G. stipulata T. And.	The tree and fruit yield a yellow gum, but not used as gamboge.
	G. succifolia Kurz	The species yield inferior quality gamboge at very little yield.
	G. travancorica Beddome	Every portion of the tree yields an abundance of bright yellow gamboge.
	G. wightii T. Anderson	The gamboge of this species is very soluble and yields a good pigment.
	G. xanthochymus Hook. f.	This species yields a large quantity of inferior gamboge both from the stem and the fruit rind which is extensively used as a cotton dye in Assam. The exudate contains a larger proportion of gum than that derived from other species. The exudates are sparingly soluble in water, but it became insoluble when dried.

Figure 2 shows the exudates from 25 *Garcinia* species distributed in India. The colour of the exudates varies from white to different shades of yellow.

5. Chemistry of gamboge

Gamboge, being a well known commercial commodity of historical importance, had been a subject of intensive analytical investigation (Chantarasriwong *et al*., 2010; Utpala and Nandakishore 2016). Venkataraman (1973) has reviewed the chemistry of pigments from *Garcinia* species.

Exudates are a complex mixture of organic compounds that ooze out of plants through pores, or wounds. Gamboge is odorless but slightly acidic (Nayar *et al*., 1999). Exudates consist largely of gum, resin or latex, depending on the tree species. The exudates from *Garcinia* species are generally yellow translucent and sometimes white to reddish, which get solidified when exposed to air.

The resin portion of the exudates was separated through partition with ethyl acetate. The remaining aqueous portion represents gum content of the exudate. Gamboge contains about 70% to 80% yellow resin, 15% to 25% water soluble gum, and the remaining portion is composed of esters, hydrocarbons, wax and ash. In a recent report, *G. gummi-gutta* exudates contains 68% resin, while *G. indica* contains 60% resin followed by *G. xanthochyma* (40%) (Parthasarathy and Nandakishore, 2016**).** The brittle resin is deep orange colour in thin layers and when it is fine powdered, its colour is gamboge yellow. Gamboge resin is insoluble in water, but soluble in alcohol. It dissolves in a solution of caustic potash, forming a dark red liquid which gets precipitated by acids and lime water, and some metallic salts like lead, brown by protosulphate of iron and green by the nitrate of copper. The precipitates formed with the metallic salts are regarded as gambogiates of the respective metals, as they consist of the resin and the oxide of the metal.

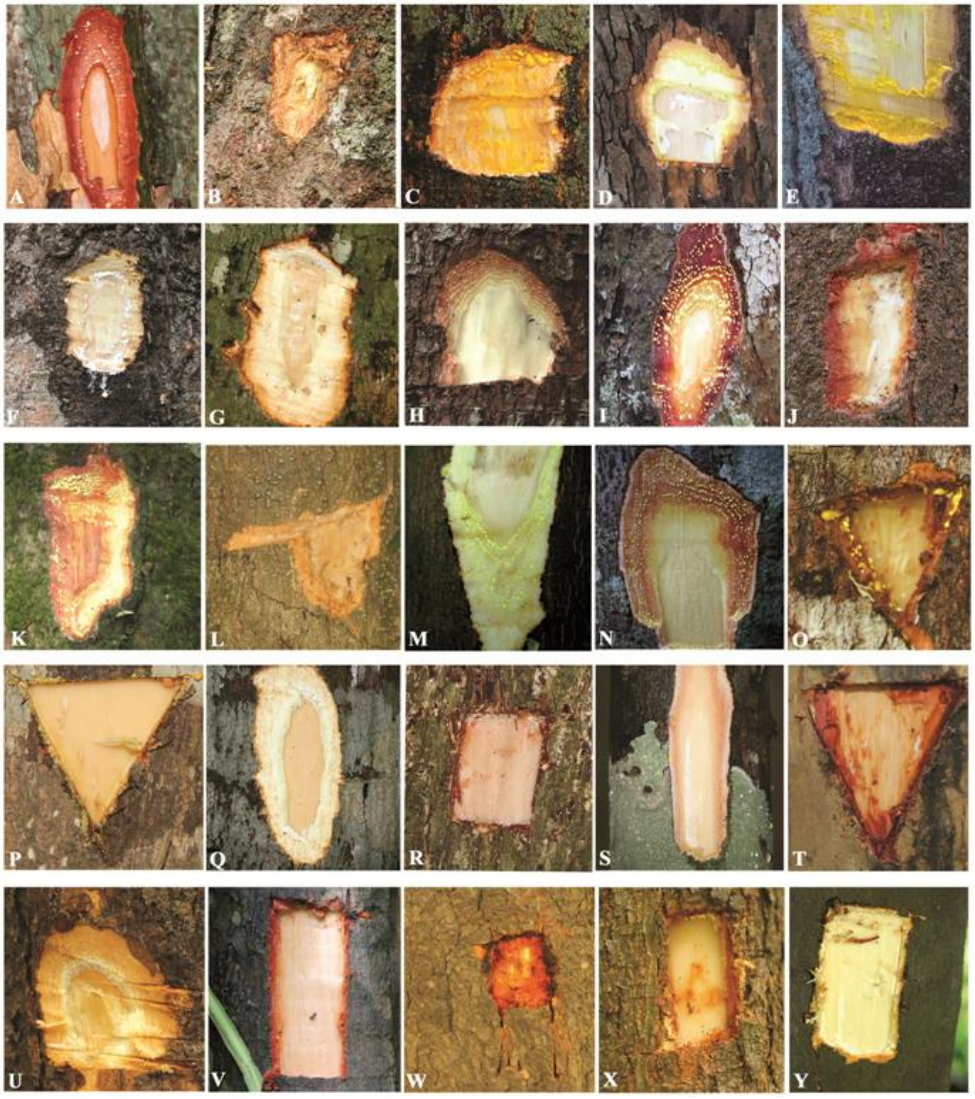
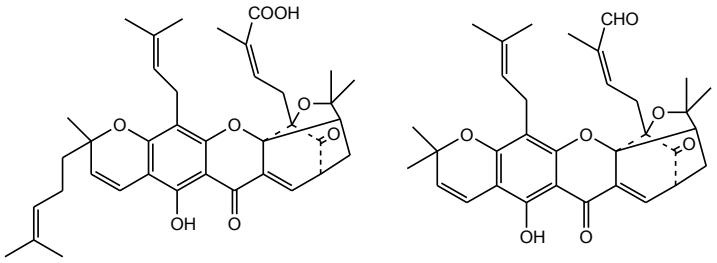


Figure 2. *Garcinia* bark exudates (**A.** *G. rubro-echinata*, **B.** *G. imberti*, **C.** *G. wightii*, **D.** *G. travancorica*, **E.** *G. morella*, **F.** *G. talbotii*, **G.** *G. pushpangadaniana*, **H.** *G. indica,* **I.** *G. gummi-gutta* var. *gummi-gutta,* **J.** *G. gummi-gutta* var. *papilla,* **K.** *G. gummi-gutta* var. *conicarpa,* **L.** *G. andamanica,* **M.** *G. assamica,* **N.** *G. anomala,* **O.** *G. cowa,* **P.** *G. dhanikariensis,* **Q.** *G. dulcis,* **R.** *G. hombroniana,* **S.** *G. kydia,* **T.** *G. speciosa,* **U.** *G. xanthochymus,* **V.** *G. cornea,* **W.** *G. livingstonei,* **X.** *G. mangostana,* **Y.** *G. spicata*)

(-) Gambogic acid has been identified as the principal pigment of gamboge derived from *Garcinia hanburyi*, while related investigations of the seeds and the resin of *Garcinia morella* led to the isolation of (-) morellin (**Figure 3**) (Rao, 1937; Lang and Katz, 1949; Yates *et al*., 1963). Both of the compounds belong to an interesting group of complex compounds known as caged xanthones, with unique 4-oxatricyclo [4.3.1.0] dec-2-one ring system. Gambogic acid occurs in nature as a mixture of epimers at the C2 center (C2R and

C2S) that can be separated by modern chromatographic and analytical techniques (Han *et al*., 2006). C2S Gambogic acid is also known as epigambogiac acid. *Garcinia hanburyi* has been reported as a rich source of such cytotoxic caged xanthones (Reutrakul *et al*., 2007). Many of such caged xanthones have been shown to possess anticancer and antitumor properties.



Gambogic acid Morellin

Figure 3. Structure of gambogic acid and morellin

6. Pharmacological activities of gamboge

The caged xanthone gambogic acid has been a centre of attraction for the pharmacological researchers as evident from the ever increasing number of publications over the compound (Chantarasriwong *et al*., 2010). The toxicity of gamboge was also noted early onwards and several accounts warn against licking brushes containing gamboge. Gambogic acid has been identified as a potent anti-tumor agent that inhibited cancer cell growth *in vitro* and *in vivo* with minimal toxicity to normal cells, in its pre-clinical trials (Kasibhatla *et al*., 2005). The unique caged xanthone structure is the basis of gambogic acid induced anti-cancer effects. Gambogic acid induced apoptosis has been reported in many cancer cell types including leukemia, cervical cancer, cholangio carcinoma, hepatoma, breast cancer, gastric cancer, glioblastoma and osteosarcoma (Zhao *et al*., 2004; Yu *et al*., 2006; Yang *et al*., 2007; Wang and Chen, 2012). Gambogic acid inhibits cell proliferation in multidrug-resistant cancer cells. It has also prevented cancer metastasis and angiogenesis, and has finished phase II clinical trials in China (Wang *et al*., 2011). The potent anticancer activity of gambogic acid is mainly attributed to its activation of the impaired apoptotic pathways in cancerous cells via down-regulation of telomerase (Guo *et al*., 2006). Morellin and gambogic acid have been reported as potential antibacterial compounds and exhibited high *in vitro* specific growth inhibitory effects on Gram-positive bacteria (Rao and Natarajan, 1950; Chantarasriwong *et al*., 2010).

Conclusions

Gamboge, the dried exudate from several *Garcinia* species, was used as a pigment in water paintings, dyeing cloths and also for coloring wood, metals and leather. Alternative products obtained from renewable sources, are getting prominence and the potential of gamboge as a natural substitute for colouring material is highly appreciated. Though historically known as source of coloring pigments, gamboge is now reputed as source of a new family of natural products, known as caged xanthones. The remarkable chemical structure, biosynthesis, biology and medicinal potential of the caged xanthones open up a new window to the potential utility of gamboge.

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Chapter 12

Nutrient properties of important *Garcinia* fruits of India

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Abstract

The importance of natural products is increasing day by day as the safety of synthetic alternatives has generated lots of controversial questions. *Garcinia* species are an important group of plants, being used for different purposes, especially as fruit crops, source of edible oils and fats, and nutraceuticals in different parts of the world. The nutraceutical property of a fruit is determined by the metabolites like carbohydrates, proteins, vitamins and minerals and also the secondary metabolites such as phenols and flavonoids. The food and nutritive values of *Garcinia* species have attracted significant scientific attention and the present chapter is an attempt to review the nutrient properties of important *Garcinia* fruits in India.

Keywords: *Garcinia* fruits, Nutrient properties, Minerals, Vitamins, Phenolics

Introduction

Plants and fruits are nature's wonderful gift to mankind; indeed, the edible fruits are life enhancing medicines packed with vitamins, minerals, antioxidants and many phyto-nutrients. They are an absolute feast to our sight, not just because of their color and flavor but for their unique nutrition profile that help to keep human body healthy. There are plenty of underutilized fruit crops which possess immense nutraceutical value. The underutilized species are restricted to the geographical place of their availability but not explored properly for their constitution or utility (Gruere *et al.,* 2006). Majority of them produce fruits which are rich sources of carbohydrates, proteins, fats, vitamins and minerals than the conventional fruits (Krishnamurthy and Sarala, 2011). *Garcinia* is one such underutilized group of fruit bearing plants.

Many species of *Garcinia* have fruits with edible arils and are eaten locally. Fresh and dry *Garcinia* fruit rinds (exocarp) are used as spice, condiment and garnish in several cuisines to impart an acidic flavour to the food and to enhance shelf life (Utpala *et al.,* 2010). *Garcinia* species such as *G. cowa*, *G. kydia*, *G. cowa, G. lanceaefolia, G. mangostana, G. atroviridis* and *G. prainiana* were cultivated for their fruits world over. The best known species is the mangosteen (*G. mangostana*), also known as the 'queen of tropical fruits', which is now cultivated throughout Southeast Asia and other tropical countries. In Travancore, Malabar and Konkan region of south India, the fruits of *G. cambogia* and *G. indica* are used in garnishing curries and also as a substitute for tamarind. Fruit and syrup of *G. indica* is very popular in 'Konkan' region as a refreshing and rejuvenating drink. *Garcinia pedunculata*, *G. kydia*, *G. cowa* and *G. lanceaefolia* are the most important species in North Eastern parts of India, where the sundried slices of the fruits were used for culinary purposes and as folk medicine.

The seeds of *Garcinia* species yield oil that can be used as edible oil as well as illuminating fuels. *Garcinia* butter is obtained from the seeds and used mainly as an edible fat. The seed of *G. indica* fruits yield valuable edible fat known as 'kokum butter' and is popular in south India. Refined and deodorized fat from *Garcinia* seeds are generally white or creamy in colour and compares favorably with high class hydrogenated fat. *Garcinia* fats are rich in stearic acid and are considered nutritive, demulcent, astringent and emollient. The use and preparation of *Garcinia* butter is still under exploited. *Garcinia* species have been considered recently to have ample medicinal importance as well (Korikanthimath and Desai, 2005; Utpala and Nandakishore, 2014).

Garcinia species are abundant in the Western Ghats and in the North Eastern Himalayas. *G. indica* and *G. gummi-gutta* are the most common fruit species of the Western Ghats while *G. pedunculata, G. lanceaefolia* and *G. kydia* are the common fruit species of North Eastern foot hills of Himalayas. *G. xanthochymus* and *G. mangostana* are available in both the ecosystems. The nutraceutical property of a fruit is determined by the metabolites like carbohydrates, proteins, vitamins and minerals present in it and their relative amount. The secondary metabolites such as phenols and flavonoids also contribute significantly to the medicinal utility. The present chapter elaborates the nutritional constituents of important *Garcinia* species in India.

1. Primary metabolites of *Garcinia* fruits

Primary metabolites are directly involved in the growth and development of the plant and also serve as source of energy. The concentration of primary metabolites such as sugars, proteins and crude fats of the *Garcinia* fruits are given in **Table 1**. Carbohydrates were the major metabolites present in *Garcinia* fruits followed by proteins. Carbohydrates are the major nutrients in fruits. They are the primary energy source of the cell and the simplest biomolecules that are synthesized naturally. Reducing sugars are the simplest carbohydrate molecules having free aldehyde or ketone group and can reduce metal ions to lower oxidation state. Reducing sugars like glucose and fructose are the sweetness principles of a fruit. Carbohydrate content showed a great variation among various *Garcinia* species; from 3.75 % to 15.12 %. Total proteins ranged from 1.82 % to 4.93 %. The percentage of reducing sugars is less in comparison to the other organic acids present. This may be the reason of very sour taste of the fruits even when they are ripened. The palatability of *G. mongostana* was due to the high content of reducing sugars (1.28 %). *G. indica* showed a higher amount of total proteins (4.78 %), while total carbohydrates and crude fats were higher in *G. mangostana*. This indicates that *G. mangostana* provides more calories than other *Garcinia* species. Crude fats were very nominal in all the *Garcinia* fruits, showing only very small variation among them.

2. Mineral composition of *Garcinia* fruits

Minerals do not provide energy, but play a major role in metabolism and functioning of cells and are required in small amounts for human health. The mineral composition of the fruit rinds of *Garcinia* species is given in **Table 2**.

Table 1. Primary metabolite composition of *Garcinia* fruits (Utpala and Nandakishore, 2014)

Garcinia species	Total carbohydrates (g/100g)	Reducing sugars (g/100g)	Total proteins (g/100g)	Crude fats (g/100g)
G. gummi-gutta	7.11	0.51	3.25	0.34
G. indica	6.24	0.63	4.78	0.12
G. mangostana	15.72	1.28	1.82	0.49
G. xanthochymus	4.12	0.98	4.01	0.41
G. subelliptica	4.82	0.71	3.76	0.15
G. kydia	9.07	0.6	4.33	0.42
G. lanceaefolia	5.85	0.65	3.45	0.13
G. pedunculata	7.93	0.95	4.93	0.20

G. mangostana (163.6 mg/100g) was richer in total minerals followed by *G. indica* (109.3 mg/100g). Potassium, calcium and magnesium showed a great variation (CV% being 27.5, 40.6 and 20.87 respectively) among the species while amount of sodium, iron and phosphorus were almost similar.

Table 2. Mineral compositions of *Garcinia* fruits (Utpala and Nandakishore, 2014)

Garcinia species	Sodium (mg/100g)	Potassium (mg/100g)	Calcium (mg/100g)	Magnesium (mg/100g)	Iron (mg/100g)	Phosphorus (mg/kg)
G. gummi-gutta	2.88	26.6	12.67	14.35	9.00	5.34
G. indica	1.55	44.5	13.21	33.45	12.06	4.51
G. mangostana	2.58	78.3	5.82	60.43	9.02	7.45
G. xanthochymus	2.06	28.4	13.07	30.62	10.82	3.48
G. subelliptica	1.52	43.3	12.33	34.45	9.00	5.43
G. kydia	2.54	38.7	12.54	25.25	10.00	4.32
G. lanceaefolia	1.35	52.3	12.54	30.23	9.00	3.64
G. pedunculata	2.48	27.3	13.21	35.43	10.12	4.32

Magnesium and potassium were found to be the predominant minerals in *Garcinia* fruits. *G. mangostana* is richer in potassium (78.3 mg), magnesium (60.43 mg) and phosphorus (7.45 mg/kg) (Utpala and Nandakishore, 2014). Potassium, calcium and magnesium are present in good percentage in fruit rind tissues, and make *Garcinia* an important medicinal fruit. Calcium is the major component of bones and teeth and is essential for muscular function and blood clotting (Decupyre, 2014). Other than potassium, *Garcinia* has a mineral content similar to major fruits like apple, grapes, peaches or banana (Decupyre, 2014). Magnesium, phosphorus and iron contents were also higher in *Garcinia* than the commonly consumed fruits.

3. Vitamin composition of *Garcinia* fruits

Vitamins are organic compounds that play a major role in regulation of enzymes, cell signals and metabolic pathways. The vitamins present in the detectable range were vitamins B1, B2, B3, B12 and C. Vitamin A, E and D could not be detected in *Garcinia* fruit extracts. The composition of vitamins in the fruits of *Garcinia* species are given in **Table 3**. Ascorbic acid was found to be the major vitamin in *Garcinia* fruits. The total vitamin content was highest in

G. mangostana (61 mg/100 g), followed by *G. pedunculata* (36 mg/100 g). Except ascorbic acid, other vitamins showed only small variation (<10%) among the species studied. Ascorbic acid was in a range of 14.0% to 60.0%. 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 delayed healing and scurvy. Ascorbic acid works as a preservative to prevent rancidity, acts as a dough conditioner in baking and prevents enzymatic browning. Riboflavin (vitamin B2) is another water soluble vitamin. As it is also not synthesized in the body or being stored, it is essential to eat foods rich in riboflavin every day. Riboflavin helps body cells use fat, protein and carbohydrates from foods to produce energy.

Table 3. Vitamin composition of *Garcinia* fruits (Utpala and Nandakishore, 2014)

Garcinia species	Thiamine (B1) (µg/100g)	Riboflavin (B2) (µg/100g)	Niacin (B3) (µg/100g)	Ascorbic acid (C) (mg/100g)	Vitamin B12 (µg/100g)	Total vitamin (mg/100g)
G. gummi-gutta	48	275	45	14.35	8.75	14.75
G. indica	52	320	63	33.45	12.06	34.00
G. mangostana	50	300	60	60.43	9.52	61.05
G. xanthochymus	37	250	50	30.62	10.76	30.97
G. subelliptica	50	281	45	34.45	9.03	34.94
G. kydia	47	267	50	25.25	10.15	25.82
G. lanceaefolia	52	283	45	30.23	8.02	30.62
G. pedunculata	49	276	47	35.43	8.12	35.81

4. Organic acids composition of *Garcinia* fruits

Organic acids are of great significance in plants. As intermediates in the metabolic processes of the fruit, acids are directly involved in growth and maturation. Fruit juices have a low pH, because they contain high levels of organic acids (James, 1985, Jena *et al*., 2002). The organic acids detected in the *Garcinia* fruits studied were (-) hydroxycitric acid (HCA), malic acid, citric acid, tartaric acid and acetic acid. The retention factor (R_f) values of standard acids were found to be oxalic acid (0.14), tartaric acid (0.21), malic acid (0.45), citric acid (0.38), hydroxycitric acid (0.24) and acetic acid (0.60) (Utpala and Nandakishore, 2014). The total acid content of *Garcinia* fruits and the percentage compositions of various organic acids present in the *Garcinia* acid extracts are given in **Table 4**. The total acidity of the fruits varied significantly from 4.39 % (*G. mangostana*) to 27.3 % (*G. kydia*). A very high variability in concentration was observed for HCA and malic acid.

G. kydia was the most acidic (27.3 %) followed by *G. gummi-gutta* (23.81 %). The anti-obesity compound HCA was highest in *G. gummi-gutta* (15.48 %), followed by *G. kydia* (8.97 %). *Garcinia* species and *Hibiscus sabdariffa* are the only abundant natural sources of HCA (Yamada *et al*., 2007). HCA was found to be the major organic acid in the Western Ghats species namely *G. gummi-gutta* and *G. indica* whereas in other species, malic acid was the predominant organic acid. During extensive animal studies, HCA has been proven to effectively curb appetite, suppress food intake, increase the rates of hepatic glycogen synthesis, reduce fatty acid synthesis and lipogenesis and decrease body-weight gain. Other organic acids were detected as minor compounds. *G. xanthochymus* had a total acid content

of 10.95 % of which citric acid was the major acid component (8.0 %). HCA was absent in *G. xanthochymus*. In case of *G. mangostana*, the percentages of organic acids were very low and HCA could not be detected.

Table 4. Total acidity and major organic acids present in *Garcinia* fruits (Utpala and Nandakishore, 2014)

Garcinia species	Total acidity (%)	HCA (%)	Malic acid (%)	Oxalic acid (%)	Citric acid (%)	Tartaric acid (%)	Acetic acid (%)
G. gummi-gutta	23.81	15.48	4.62	0.18	0.62	0.11	0.07
G. indica	14.11	7.43	2.67	0.63	0.79	0.51	0.31
G. mangostana	4.39	0.26	0.54	0.73	1.42	1.66	0.26
G. xanthochymus	10.95	0.10	0.73	0.37	8.00	0.20	0.04
G. subelliptica	9.76	1.16	4.87	0.92	0.81	1.18	1.32
G. kydia	27.30	8.97	13.42	0.60	1.35	1.80	0.23
G. lanceaefolia	15.17	1.93	10.02	1.70	1.45	0.23	0.14
G. pedunculata	12.92	1.33	8.95	0.51	1.30	0.12	trace

The organic acids play a key role in food products because of their influence on organoleptic properties. Besides, they also provide the sour flavour to the product and also act as antimicrobial agent for enhancing shelf life (Lillian *et al*., 2013). The total content of organic acids in a food affects the product's acidity, whereas the levels of a specific organic acid can directly influence the flavor and taste of the drink. Malic acid and citric acids are α-hydroxy acids reported to have functions like enhancing salivation, gastric secretion and exfoliation and are therefore important constituents of food and cosmetic formulations (Fiume, 2001). Citric acid also acts as food preservative and acidifying agent. The higher carbohydrate content and low acid content explains the sweeter taste of *G. mangostana* compared to other *Garcinia* fruits.

5. Phenolic compounds and antioxidant activities of *Garcinia* fruits

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 were attributed with antioxidant properties (Harborne, 2005). Phenolic compounds also facilitate pollination through colour and fragrance, defense against pathogens and prevent fruits consumed by herbivores (Harborne, 2005). In *Garcinia*, xanthones, biflavonoids and benzophenones were reported to be the major phenolic compounds (Aisha *et al.,* 2012).

The total phenolic contents (**Table 5**) were recorded to be highest in *G. indica* (5.01%), followed by *G. xanthochymus* (4.43%) and *G. kydia* (4.32%). The xanthone content was highest in *G. xanthochymus* (2.66 %) and was least in *G. indica* (0.9 %). The relative percentage of xanthones to the total phenolics was highest in *G. gummi-gutta*, *G. xanthochymus* and *G. subelliptica* (60.0%) and lowest in *G. indica* (20.0%).

Table 5. Total phenol, xanthone content and antioxidant activity of *Garcinia* fruits (Utpala and Nandakishore, 2014)

Garcinia species	Total phenolics (g/100g)	Total xanthones (g/100g)	DPPH activity IC_{50} (µg/ml)
G. gummi-gutta	3.26	1.96	38.39
G. indica	5.01	0.91	42.66
G. mangostana	2.33	1.30	39.42
G. xanthochymus	4.43	2.66	35.75
G. subelliptica	3.14	1.88	48.12
G. kydia	4.32	2.19	40.50
G. lanceaefolia	3.03	1.22	43.16
G. pedunculata	2.43	1.36	47.84
Ascorbic acid	-	-	10.25

As most of the *Garcinia* fruits are sour, they are consumed only as processed food or through formulations. The most commonly used forms are syrups, juices and dried rinds boiled along with other food ingredients. Hence the antioxidant activity of aqueous extract of fruits were also determined (**Table 5**). Piyawan *et al*. (2005) reported that antioxidant activity of *G. mangostana* is of moderate, close to that of orange, grapes, and papaya, while other tropical fruits such as mango, litchi and guava have higher antioxidant activities (IC_{50} ranging from 1.10 to 9.60), compared to *Garcinia* fruits.

6. Biochemistry of *Garcinia* seed butter

Lipids or fats are hydrocarbon molecules, but are hydrophobic. In plants, fats are the storage form of energy and found much abundant in seeds. Fats are the second largest energy source for living cells (Jain *et al*., 2005). *Garcinia* seed kernel contains (30-40%) fixed oil, in comparison to other vegetable seed fats like castor seed (50%), ground nut kernel (42%), mustard (35%), palm kernel (36%), sunflower (32%), sesame (50%) and coconut (60%). High yield of fixed oil indicates that *Garcinia* seeds can be utilized as a rich source of fatty acids. The physical properties of the seed fats of four *Garcinia* species showed that the yield of fatty oil is high in *G. gummi-gutta* (47%) while in *G. indica* and in *G. xanthochymus* it was around 30% and in case of *G. mangostana* it was less, around 24% (**Table 6**).

Table 6. Physical properties of *Garcinia* seed butter (Utpala and Nandakishore, 2014)

Parameters	*G. gummi-gutta*	*G. indica*	*G. xanthochymus*	*G. mangostana*
Total fat content (%)	46.54	29.33	25.71	24.20
Colour of fat	Light brown	Pale white	Creamy-yellow	Creamy-yellow
State at room temperature	Solid	Solid	Solid	Solid
Melting point (ºC)	39.4	40.3	38.2	37.9

Garcinia butter is solid at room temperature and is quite hard, almost as hard as cocoa butter, and is a good substitute in the recipes for cocoa butter. The melting point of *Garcinia* seed butter is high (about 40°C), hence it can be used along with cocoa butter to increase 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 (Utpala *et al*.,

2012). Acid value and percentage free fatty acids represent the freshness and storage quality of an oil or fat. It is the measure of susceptibility and the extent of decomposition. The acid value of the four species of *Garcinia* varies from 3.7 to 4.5; which shows the butter is good for the consumption. Free fatty acid content is commonly called the free acidity percent and lesser the free fatty acid content, better is the fat. Other than *G. indica* oil, all are having very low acid value (**Table 7**). Saponification number gives the information concerning 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). For *Garcinia* fats, the value ranged from 140 to 200. Iodine value is a measure of the unsaturated nature of the fat. The iodine value preferably should be 25-50. In different *Garcinia* seed butters, iodine value varies from 37-51(**Table 7**). Iodine value allows predicting the tendency of fat to become rancid. In coconut oil, the iodine value is very low (7.5- 10.5) and hence shows a high tendency to get rancid easily.

Table 7. Chemical properties of *Garcinia* seed butter (Utpala and Nandakishore, 2014)

Chemical properties	*G. gummi-gutta*	*G. indica*	*G. xanthochymus*	*G. mangostana*
Acid value (mg NaOH/g of oil)	3.7	4.9	4.8	4.5
Saponification number (mg KOH/g of oil)	187.9	200.2	190.3	140.5
Iodine value	50.2	39.4	37.4	51.8
Free acids (%)	1.42	5.64	2.82	2.21

The fatty acid profile presented in the **Table 8** shows that *Garcinia* butter has 7 important fatty acids with various percentages in different species. The major fatty acids present were palmitic acid, stearic acid, elaidic acid, oleic acid, linoleic acid, arachidic acid and eicosenoic acid. Palmitic acid is present in very high yield (47%) in *G. mangostana,* while it is moderate in other species. Palmitic acid is an ionic surfactant, which has a pleasing sensation to the body. It is thus mainly used to produce soaps, cosmetics and releasing agents. Palmitic acid is the commonest saturated fatty acid in the plants and animal lipids. Kokum butter from *G. indica* is popular in skin care products because of its ability to soften skin and heal ulcerations and fissures of the lips, hands and soles of feet. Palmitic acid helps to control obesity and also helps to recover some reproductive abnormalities (Scott *et al*., 1988). It is reported that the diet enriched with palmitic acid is good for diabetes (Utpala *et al*., 2012). Stearic acid is present in very high concentration (30-40%) in *G. gummi-gutta, G. indica* and *G. xanthochymus*; while its percentage is less in *G. mangostana* (2.3%) Stearic acid is commonly used in the manufacture of soaps, detergents, shampoo, shaving creams and other cosmetic products. It is one of the most common saturated fatty acids found in the nature following palmitic acid (Utpala and Nandakishore, 2014). Butter rich in stearic acid is solid at room temperature. It is also used in many food products because it remains stable at high temperatures. It is commonly used in margarine and other spreads. *Garcinia* fats could be taken as good source of stearic acid as well. A few plants which have stearic acid more than 30% in its seed oil are *Butyrospermum paradoxum* (shea), *Shorea robusta* (sal) and *Vateria*

indica (dhupa). It is reported that the total plasma cholesterol is decreased by an average of 14% during the consumption of high stearic acid diet (Andrea and Scott, 1988). Oleic acid also present in a good percentage in all the four species of *Garcinia* (26-35%). High oleic acid makes the butter less susceptible to spoilage, so could be useful in food preservation. Oleic acid may hinder the progression of adrenoleuko dystrophy, a fatal disease that affects the brain and adrenal glands and also may be responsible for the hypotensive effects of olive oil (Teres *et al*., 2008). Linoleic acid is another important acid which is present in a moderate percentage (5-11%) in different *Garcinia* species. The use may include, helping to lose body fat and possibly preventing colon or breast cancer (Nirvair *et al*., 2007). It is a strong antioxidant with benefits such as lowering high cholesterol and controlling weight. Arachidic acid (1-8%) is a saturated fatty acid and a minor constituent of peanut oil (1.1-1.7%) and corn oil (3%). Arachidic acid is used for the production of detergents, photographic materials and lubricants. The food rich with arachidonic acid is attributed with anti-inflammatory properties (Adama *et al*., 2003).

Table 8. Fatty acid profile of *Garcinia* species (Utpala and Nandakishore, 2014)

Fatty acid	Saturated/ unsaturated	*G. gummi-gutta* (%)	*G. indica* (%)	*G. xanthochymus* (%)	*G. mangostana* (%)
Palmitic acid	saturated	6.31	3.25	3.05	47.20
Stearic acid	saturated	30.61	45.33	44.53	2.31
Elaidic acid	unsaturated	9.54	3.00	1.51	- -
Oleic acid	unsaturated	26.23	34.42	35.33	34.02
Linoleic acid	unsaturated	11.38	5.25	4.82	1.32
Arachidic acid	saturated	5.41	1.20	1.00	8.04
Eicosenoic acid	unsaturated	- -	2.25	1.01	0.51
Other fatty acids		10.52	5.30	8.75	6.61

Conclusions

The awareness towards natural options in every walk of life created a new thrust for the plant based products that involve food additives, nutracueticals, cosmetic ingredients and herbal medicines. Herbal Technology (HT) is emerging as a promising field of modern science for India. The rich floristic wealth of our region offers several underutilized plants that can be used as source of gum, resins, fats, oils, condiments and nutraceutics. *Garcinia* is one among such underutilized tropical forest tree that accounts to the economy of the ethnic community associated. Pharmacological works are in progress in different parts of the world to use the products from *Garcinia* fruits as anti obesity, anti cancer and to solve other digestive problems The vitamins, minerals, micro-nutrients, pigments and phenolic compounds of major *Garcinia* fruits in India were reviewed in the chapter and the fruits are having very high nutraceutical values.

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Chapter 13

Antioxidant and antibacterial activities of *Garcinia* species in the Western Ghats

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Abstract

Garcinia species are reputed for the diversity of phenolic compounds such as biflavonoids, xanthones and benzophenones that can act as antioxidants. In the present study, various *in vitro* methods were used to investigate the antioxidant properties of nine *Garcinia* species in the Western Ghats. DPPH radical scavenging activity of *G. talbotii* was higher (IC_{50}: 2.8±0.6 µg/mL) compared to standard compound ascorbic acid (IC_{50}: 3.2±0.5 µg/mL), while *G. pushpangadaniana* showed the highest superoxide radical scavenging activity (IC_{50}:16.75±0.99µg/mL) and reducing activity. The potential antioxidant activities of the *Garcinia* species were in corroboration with the high phenolic and flavonoid contents present in these species. The antibacterial activities of the leaf methanol extracts were however negligible or nil, except against the Gram positive strain, *Bacillus subtilis.*

Keywords: Antioxidant, Antibacterial, *Garcinia* species, DPPH, Superoxide radical, Reducing power, *Bacillus subtilis*

Introduction

Oxygen is an indispensable element for life and is necessary for aerobic respiration in animals. However, reactive oxygen species (ROS) such as superoxide anion radicals (O_2^-), hydroxyl radicals (OH^-) and non-free radical species such as hydrogen peroxide (H_2O_2) and singlet oxygen, that are continuously produced during the normal metabolism of oxygen, are harmful to biological systems. Healthy humans can detoxify or eliminate these free radicals by enzymes such as superoxide dismutase, catalase, and peroxidase (Gulcin, 2006; Terashima *et al*., 2010). If the oxidative damage is beyond the capacity of the natural repair mechanisms of the cells, it may trigger several chronic diseases (Franco, 2008).

The consumption of diets which are rich in antioxidants can protect the human body from oxidative stress and associated diseases induced by endogenous and exogenous factors (Morganti, 2009). These health effects have been partially attributed to the presence of phenolic compounds in plants (Guo *et al*., 2011). *Garcinia* species are known to be rich in phenolic compounds such as flavonoids, phenolic acids, xanthones, biflavonoids and benzophenones. There are many compounds reported from the genus *Garcinia* with higher free radical scavenging activities compared to known standards. Griffipavixanthone, a prenylated xanthone isolated from *Garcinia virgata* was reported to possess promising antioxidant activity with lower EC_{50} value compared to the references BHA and α-tocopherol

(Merza *et al*., 2004). The phloroglucinol parvifoliol E from *Garcinia parvifolia* showed remarkable antioxidant acivity compared to standard BHT (Rukachaisirikul *et al*., 2006). 1,3,5,7-Tetrahydroxyxanthone exhibited strong antioxidant activity comparable to the reference molecule probucol (Jantan *et al*., 2012). α-Mangostin is a common xanthone reported from different *Garcinia* species, that exhibited stronger antioxidant activity than α-tocopherol in ferric thiocyanate (FTC) assay (Taher *et al*., 2012). Biflavonoids are dimers of two flavonoids, limited in distribution to some genus. This interesting group of compounds was reported from different *Garcinia* species and many of them exhibited remarkable antioxidant activities. The flavanone-(3-8")-flavone biflavonoid morelloflavone displayed considerable antioxidant activity and was more potent than quercetin (Osorio *et al*., 2013). 1,3,6-trihydroxy-7-methoxy-2,8-(3-methyl-2-butenyl) xanthone isolated from *Garcinia hombroniana* exhibited stronger antioxidant activity than the standard compounds trolox, gallic acid and ascorbic acid (Jamila *et al*, 2014). *Garcina* species were reported to possess remarkable level of activities against different diseases and the antioxidant activities of phenolic compounds from the genus have a major role in the mechanism of bioactivities.

Recently, a wide range of plants have been screened for antimicrobial property, because of the increased microbial resistance and harmful side effects of existing antimicrobial agents (Djeussi *et al*., 2013). *Garcinia* species have also been a subject of antimicrobial screening and potential activities have been reported for extracts and isolated compounds from several *Garcinia* species (Negi *et al*., 2008; Policegoudra, 2012; Fouotsa *et al*., 2013; Semwal *et al*., 2015).

Although the *Garcinia* species are gaining much attention worldwide due to their potential bioactivities, the *Garcinia* species in the Western Ghats are least investigated for their bioactivities. The present chapter elaborates the antioxidant and antibacterial activities of the leaf methanol extracts of nine *Garcinia* species (*G. gummi-gutta, G. imberti, G. indica, G. Morella, G. pushpangadaniana, G. rubro-echinata, G. talbotii, G. travancorica and G. wightii*) from the Western Ghats.

1. *In vitro* antioxidant activity of *Garcinia* species in the Western Ghats

Antioxidants act by several mechanisms and it is difficult to predict the full spectrum of activity in a single assay. In the present study, *in vitro* methods such as DPPH scavenging assay, superoxide radical scavenging assay and reducing power assay were used to evaluate the antioxidant property of *Garcinia* leaf methanol extracts.

DPPH scavenging activity: Among free radical scavenging methods, DPPH method is more rapid, simple and inexpensive in comparison to other test models. DPPH (2, 2-diphenyl-1-picrylhydrazyl (α,α-diphenyl-β-picrylhydrazyl) is a stable free radical that has an absorbance maximum in the visible region (517 nm). On accepting hydrogen from a donor, DPPH solutions lose the characteristic deep purple colour (Villano *et al*., 2007). The free radical scavenging activities of tested compounds are expressed as IC_{50} value, the concentration of the compound required to decrease the absorbance of DPPH solution by 50%.

Reducing power assay: In this method, antioxidant compound forms a coloured complex withpotassium ferricyanide, trichloro acetic acid and ferric chloride, which is measured at 700 nm. Increase in absorbance ofthe reaction mixture indicates the reducing power of the samples (Jayaprakash *et al*., 2008).

Superoxide radical scavenging assay: Superoxide anion radical is a weak oxidant that generates powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute significantly to oxidative stress. In the PMS/NADH-NBT system, the superoxide anion derived from dissolved oxygen and PMS/NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm thus indicates the consumption of superoxide anion in the reaction mixture. The superoxide anion scavenging activity was measured as described by Robak and Gryglewski (1988).

Total phenolic and flavonoid contents: Phenolic compounds consist of diverse group of secondary metabolites such as flavonoids, anthocyanins, coumarins, xanthones, benzophenones and phenolic acids, and possess ideal structural features for free radical scavenging activity. Antioxidative properties of phenolic compounds are due to different mechanisms such as scavenging of free radicals, chelation of metal ions like iron and copper, and inhibition of enzymes responsible for free radical generation (Benavente-Garcia, 1997; Rice-Evans *et al*., 1997). The phenol content was determined by Folin-Ciocateu reagent method (McDonald *et al*., 2001). The content of flavonoids was determined by aluminum chloride colourimetric method (Chang *et al*., 2002).

Leaf methanolic extrcacts of9 *Garcinia* species from the Western Ghats (*G. gummi-gutta, G. imberti, G. indica, G. morella, G. pushpangadaniana, G. rubro-echinata, G. talbotii, G. travancorica* and *G. wightii*) were subjected to antioxidant evaluation using different *in vitro* methods. Most of the species showed remarkable levels of antioxidant activities using *in vitro* models like DPPH radical scavenging assay, reducing power assay and super oxide radical scavenging assay (**Table 1**). Among the species studied *G. talbotii* (IC_{50}2.8±0.6 µg/mL), *G. rubro-echinata* (IC_{50}6.5±0.8 µg/mL), *G. imberti* (IC_{50}9.0±1.2 µg/mL), and *G. wighti* (IC_{50}16.0±2.0 µg/mL) showed a promising level of DPPH radical scavenging activity compared to standard ascorbic acid with IC_{50} value of 3.2±0.5 µg/mL. IC_{50} of *G. talbotii* leaf methanol extract against DPPH radical was higher than that of standard ascorbic acid.

Superoxide radical scavenging activity revealed a moderate level of activity compared to the standard ascorbic acid (IC_{50} value of 5.8±0.25 µg/mL). Among the species studied, *G. pushpangadaniana* showed highest activity with IC_{50} value of 16.75±0.99 µg/mL and *G. indica* showed the minimal level of activity with IC_{50} value of 196.96±14.16 µg/mL. Superoxide radical scavenging activity of the extracts were not correlated to the phenolic or flavonoid contents.

Table 1. Phenolic and flavonoid contents and antioxidant activities of *Garcinia* leaf extracts

Sl. No.	*Garcinia* species	Total phenolics (mg/g)	Total flavonoids (mg/g)	DPPH IC_{50} (µg/mL)	Superoxide IC_{50} (µg/mL)
1	*G. gummi-gutta*	97.45±7.28	17.2±2.83	128±2	86.2±2.62
2	*G. imberti*	273.6±9.6	108±7.82	9±1.2	40.3±1.12
3	*G. indica*	46.67±15.08	11.1±1.84	558.3±18.65	196.96±14.16
4	*G. morella*	177.57±18.86	53.8±5.37	104±3.35	86.5±7.92
5	*G. pushpangadaniana*	884.6±83.51	197.3±9.47	9.04±0.83	16.75±0.99
6	*G. rubro-echinata*	392.85±7.28	48.05±2.19	6.5±0.8	27.2±0.42
7	*G. talbotii*	342.9±5.80	55.56±2.31	2.8±0.6	30.4±1.13
8	*G. travancorica*	435.53±23.85	143.4±11.60	18.9±1.8	53.2±3.09
9	*G. wightii*	239.3±24.18	239.0±26.87	16±2	27.6±0.7
10	Ascorbic acid	-	-	3.2±0.5	5.8±0.25

Leaf methanolic extracts of the *Garcinia* species studied showed varying levels of activity in reducing power assays (**Table 2, Figure 1**). The *Garcinia* species that contain higher amount of phenolics, especially *G. pushpangadaniana*, *G. rubro-echinata* and *G. talbotii* showed remarkable activity in reducing power assay, whereas *G. gummi-gutta, G. indica and G. wightii* showed only moderate levels of activities.

Table 2. Reducing power assayof *Garcinia* species leaf extracts at different concentrations-Absorbance at 700 nm

Garcinia species	20 (µg/mL)	40 (µg/mL)	60 (µg/mL)	80 (µg/mL)	100 (µg/mL)
G. gummi-gutta	0.026	0.045	0.082	0.103	0.122
G. rubro-echinata	0.026	0.308	0.503	0.669	0.858
G. imberti	0.011	0.172	0.39	0.55	0.678
G. indica	0.034	0.054	0.068	0.08	0.09
G. morella	0.051	0.133	0.196	0.255	0.295
G. pushpangadani	0.231	0.45	0.623	0.833	1.083
G. talbotii	0.185	0.347	0.5	0.681	0.721
G. travancorica	0.094	0.209	0.301	0.408	0.526
G. wightii	0.018	0.034	0.117	0.239	0.303

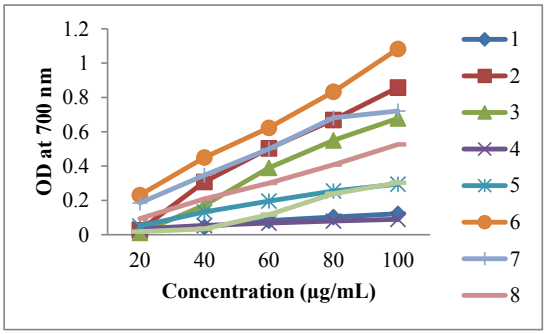

Figure 1. Reducing power assay of *Garcinia* leaf extracts (1- *G. gummi-gutta*, 2- *G. rubro-echinata*, 3- *G. imberti*, 4- *G. indica*, 5- *G. morella*, 6- *G. pushpangadaniana*, 7- *G. talbotii*, 8- *G. travancorica*, 9- *G. wightii*)

2. Antibacterial activity of *Garcinia* leaf methanol extracts

The plant extracts were dissolved in DMSO was used for the assay. The Kirby-Bauer method was used for antimicrobial susceptibility testing (Cappucino and Sherman1999). Briefly, the Mueller Hinton Broth (MHB) containing specific organisms were incubated at 37°C until it achieved the 0.5 McFarland standards (~1.5 x 10^8 CFU/ml). The dried surface of the Mueller-Hinton agar plate is inoculated by streaking the swab over the entire sterile agar surface. The discs impregnated with the extracts were placed on Mueller Hinton agar and incubated at 37^0 for 16-18 hours. After incubation, the diameters of the zones of complete inhibition were measured, including the diameter of the disc.

Table 3. Antibacterial activity (zone of inhibition in mm) of *Garcinia* leaf methanol extracts and standard kanamycin sulphate

Garcinia species	Conc. (µg/disc)	*P. vulgaris*	*E. faecalis*	*S. marscenes*	*P. aeruginosa*	*S. typhi*	*B. subtilis*	*S. mutants*
G. cowa	100	Nil	Nil	Nil	Nil	Nil	9.0	Nil
	500	Nil	Nil	Nil	Nil	Nil	9.5	Nil
	1000	Nil	Nil	Nil	Nil	Nil	10	Nil
G. rubro-echinata	100	Nil	Nil	Nil	Nil	Nil	6.5	Nil
	500	Nil	Nil	Nil	Nil	Nil	7.5	Nil
	1000	Nil	Nil	7.5	Nil	Nil	9.0	7.5
G. gummi-gutta	100	Nil	Nil	Nil	Nil	Nil	7.5	Nil
	500	Nil	Nil	Nil	Nil	Nil	8.0	Nil
	1000	Nil	Nil	Nil	Nil	Nil	8.5	Nil
	500	Nil	Nil	Nil	Nil	Nil	9.5	7.0
	1000	Nil	Nil	Nil	Nil	Nil	10.5	8.0
G. imberti	100	Nil	Nil	Nil	Nil	Nil	7.0	Nil
	500	Nil	Nil	Nil	Nil	Nil	9.0	Nil
	1000	Nil	Nil	Nil	Nil	Nil	10.5	Nil
G. indica	100	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	500	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	1000	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	500	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	1000	Nil	Nil	Nil	Nil	Nil	Nil	Nil
G. morella	100	Nil	Nil	Nil	Nil	Nil	6.5	Nil
	500	Nil	Nil	Nil	Nil	Nil	8.0	7.0
	1000	Nil	Nil	Nil	Nil	Nil	9.5	8.0
G. pushpangadaniana	100	Nil	Nil	Nil	Nil	Nil	7.0	6.5
	500	Nil	Nil	Nil	Nil	Nil	9.5	7.5
	1000	Nil	Nil	Nil	Nil	Nil	12.5	10.0
G. talbotii	100	Nil	Nil	Nil	Nil	Nil	10	9.0
	500	Nil	Nil	Nil	Nil	Nil	12	10.0
	1000	Nil	Nil	Nil	Nil	Nil	13.5	13.0
G. travancorica	100	Nil	Nil	Nil	Nil	Nil	8.0	Nil
	500	Nil	Nil	Nil	Nil	Nil	10.0	Nil
	1000	Nil	Nil	Nil	Nil	Nil	11.0	Nil
G. wightii	100	Nil	Nil	Nil	Nil	Nil	9.0	Nil
	500	Nil	Nil	Nil	Nil	Nil	11.0	6.5
	1000	Nil	Nil	Nil	Nil	Nil	12.0	8.0
	500	Nil	Nil	Nil	Nil	Nil	9.0	Nil
	1000	Nil	Nil	Nil	Nil	Nil	10.0	9.0
Kanamycin sulphate	30	20.0	22.0	25.0	20.0	27.0	28.0	25.0

In most of the cases, the extracts were inactive against the tested strains of bacteria (**Table 3**). Remarkable observation was the moderate activity against the gram positive *Bacillus subtilis* for all the extracts except *G. indica.* It is interesting to note that previous reports also reveal the activity of *Garcinia* extracts and compounds against Gram positive strains, especially *Bacillus subtilis* (Rao and Natarajan, 1950, Negi *et al*., 2008; Semwal *et al*., 2015).

The antimicrobial activities of *Garcinia* leaf methanol extracts against food pathogens such as *Escherichia coli, Bacillus cereus, Staphylococcus aureus, Salmonella enteric* ser.*typhi,* and *Vibrio cholera* were also screened (**Table 4**). The MIC values were determined by modified broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) (2009). Briefly, 200µl of Mueller Hinton Broth (MHB) was placed into each to wells of 96 well microplate. The plant extracts were dissolved in DMSO, and diluted to the required concentration. 1% of bacterial cell suspension was inoculated in MHB containing plant extracts and incubated at 37^0 C for 16 hours. *Garcinia* leaf methanol extracts were active against the Gram positive strains screened; *Bacillus cereus* and *Staphylococcus aureus*.

Table 4. Antibacterial activity (MIC in µg/ml) of *Garcinia* leaf methanol extracts against food pathogens

Garcinia species	*Escherichia coli* MTCC 441	*Bacillus cereus* MTCC430	*Staphylococcus aureus* MTCC7443	*Salmonella enterica* ser. *typhi* MTCC733	*Vibrio cholera* MTCC *3906*
G. pushpangadhania	Nil	100µg/ml	Nil	Nil	Nil
G. rubro-echinata	Nil	100µg/ml	100µg/ml	Nil	Nil
G. imberti	Nil	Nil	Nil	Nil	Nil
G. travancorica	Nil	Nil	Nil	Nil	Nil
G. talboti	Nil	Nil	Nil	Nil	Nil
G. morella	Nil	200µg/ml	500µg/ml	Nil	Nil
G.wightii	Nil	100µg/ml	200µg/ml	Nil	Nil
G. gummi-gutta	Nil	Nil	Nil	Nil	Nil

Conclusions

Leaf methanol extracts of nine *Garcinia* species from the Western Ghats exhibited remarkable *in vitro* antioxidant activity against various free radicals. The potential antioxidant activities were in corroboration with the high phenolic and flavonoid contents. Antioxidant activity is directly correlated to several curing mechanisms and the present study highlights the potential of *Garcinia* species as targets for future drug development. However, the antibacterial activities of the leaf methanol extracts were nil or negligible against the tested strains, except for *Bacillus subtilis.*

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Chapter 14

Antioxidant and cytotoxic activities of Fukugiside- The major biflavonoid from *Garcinia travancorica* Bedd.

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Abstract

Garcinia species are well known as source of complex molecules with diverse biological activities, especially antioxidant and anticancer activities. The present chapter elaborates the *in vitro* antioxidant activity of *Garcinia travancoria* extract and isolated compounds. The biflavonoid fukugiside has been identified as the active compound with significant free radical scavenging activities in DPPH (IC_{50}: 8.34 µg/mL), superoxide(IC_{50}: 6.95 µg/mL), and reducing power assays. Cytotoxicity studies of the biflavonoid fukugiside revealed a dose dependent cancer cell growth inhibition in A431 and HeLa cells. The antiproliferative effect appears to be due to the ability of fukugiside to induce S-phase arrest and apoptotic cell death. In HeLa cells, fukugiside reduced the expression of MAPKp38 by 26.1% compared to untreated control.

Keywords: *Garcinia travancoria,* Biflavonoid, Fukugiside, Antioxidant, Cytotoxicity

Introduction

Cancer, the uncontrolled division of abnormal cells in the body, still remains a threat to humankind. Surgery, chemotherapy, and radiation are the widely practised treatment methods to combat cancer (Tannock, 1998). Besides being expensive, most chemotherapeutic and radiation treatments suffer from adverse side effects. The situation warrants effective therapeutic approaches, and encourages researchers to depend more on medicinal plants that produce new and novel chemotherapeutics (Sheldon *et al.*, 1997; Reed and Pellecchia, 2005). Over 60% of the clinically used anticancer drugs are of natural origin and most of them are derived from higher plants. Vinblastine, vincristine, etoposide, teniposide, taxol, taxotere, topotecan, and irinotecan are examples for plant derived chemotherapeutics approved for use in cancer therapy (Lee, 1999).

Oxidative stress is perhaps a major cause for several diseases including cancer, and the chemical components of medicinal plants possessing antioxidant properties can protect the human body from oxidative stress and associated diseases (Guo *et al*., 2011, Nema *et al*., 2013). Phenolic compounds belonging to xanthones, biflavonoids and phloroglucnols present in *Garcinia* species were reported as potential antioxidant compounds (Merza *et al*., 2004; Rukachaisirikul *et al*., 2006; Jantan *et al*., 2012; Taher *et al*., 2012; Osorio *et al*., 2013; Jamila *et al*, 2014).

A number of extracts and isolated compounds from *Garcinia* species were reported to exhibit remarkable cytotoxic activity against different cancer cell lines. Polyisoprenylated benzophenones are perhaps the most promising group of secondary

metabolites in *Garcinia* species attributed with anticancer properties. The anticancer benzophenone garcinol induces apoptosis through the activation of caspases (Pan *et al*., 2001). Gambogic acid, the active component in gamboge, has potent cytotoxic activities against human hepatoma, gastric carcinoma, and lung cancer (Guo *et al*., 2004; Wang *et al*., 2008; Wu *et al*., 2004). Guttiferones, another group of polyisoprenylated benzophenones isolated from *Garcinia* species exhibited strong cytotoxic activity against different human cancer cell lines (Nguyen *et al*., 2011). Xanthones are another group of secondary metabolites from *Garcinia* species attributed with anticancer properties. Penangianaxanthone, cudratricusxanthone H, macluraxanthone C, and gerontoxanthone C from *G. penangiana* exhibited strong cytotoxic activity against three cell lines, MCF-7, NCI-H460) and DU-145 (Jabit *et al*., 2007). The xanthones bannaxanthone D, garcinone E and γ-mangostin inhibit cancer cell growth and promote cancer cell death in HeLa cells and the activity was more potent than clinically used anticancer drugs, camptothecin and etoposide (Han *et al*., 2008). Yahyaxanthone form *G. rigida* showed *in vitro* cytotoxic activity to L1210 murine leukemia cell lines (Elya *et al*., 2008). α-Mangostin, γ-mangostin, and 8-deoxy gartanin exerted strong growth inhibition in human melanoma SK-MEL-28 cell line (Wang *et al*., 2011). Gaudichaudione H, a xanthone from *G. oligantha* has potent apoptosis-inducing effect and cell growth inhibition effect on HeLa-C3 cells (Gao *et al*., 2012). 1,4,5,6-Tetrahydroxy-7,8-di(3-methylbut-2-enyl)xanthone, globuxanthone and garciniaxanthone E exhibited moderate activities against human leukaemic HL-60 cell line *in vitro* (Niu *et al*., 2012). Cowanin and fuscaxanthone B from *G. schomburgkiana* exhibited remarkable cytotoxicity towards HeLa cells (Vo *et al*., 2012). Xanthones from *G. cantleyana* such as 7-hydroxyforbesione, cantleyanone B, cantleyanone C, and deoxygaudichaudione A exhibited strong activity against the cell-lines, MDA-MB-231, MCF-7, CaOV-3, and HeLa cells (Shadid *et al*., 2007).

G. travancorica is a Western Ghats endemic tree species and the phytochemical studies of the plant showed the biflavonoid glycoside fukugiside as the major constituent (AnuAravind *et al*., 2016). The present chapter evaluates the antioxidant and cytotoxic activity of fukugiside isolated from *G. travancorica*.

1. Antioxidant activities of *G. travancorica* leaf methanol extract and isolated compounds

The isolated biflavonoids GB-1a, GB-1, GB-2 and morelloflavone-7''-O-β-D-glycoside (**Figure 1**), and leaf methanol extract (GTL) were studied for their antioxidant activities by various *in vitro* free radical scavenging assays**.** The activities were measured as percentage, calculated using the formula % scavenging = $[(A_{control}-A_{sample})/A_{control}]$ x 100 and reported as IC_{50} value; the concentration of sample required to scavenge 50% of radicals. Experiments were done in triplicate and the results were expressed as mean value with standard deviation.

The *in vitro* antioxidant activities of the extract and isolated biflavonoids against DPPH and superoxide radicals are shown in **Table 1**. High quantity of phenolics (435.53±23.85 mg/g extract) and flavonoids (143.4±11.60 mg/g of extract) present in the leaves showed a direct correlation with its antioxidant potential. The IC_{50} value of DPPH radical scavenging activity of morelloflavone-7''-O-β-D-glycoside was 8.34±2.12 µg/ml, comparable to that of standard ascorbic acid (3.2±0.50 µg/ml). In superoxide radical scavenging assay also, the compound showed comparable activity (IC_{50} 6.95±1.33 µg/ml), close to standard ascorbic acid (IC_{50} value of 5.8±0.25 µg/ml). In reducing power assay, the

activity of the compound was very close to that of standard ascorbic acid (**Figure 3**). Though the antioxidant activity of glycosylated flavonoids is usually weaker than the corresponding aglycones, bioavailability is generally enhanced by the presence of glucose moiety (Ratty and Das 1988). The potential antioxidant activity of morelloflavone-7''-O-β-D-glycoside can be attributed to 3", 4"- dihydroxy unit present in the B ring. The B ring hydroxyl configuration is the most significant determinant of scavenging activity of flavonoids (Bors *et al*, 1990).

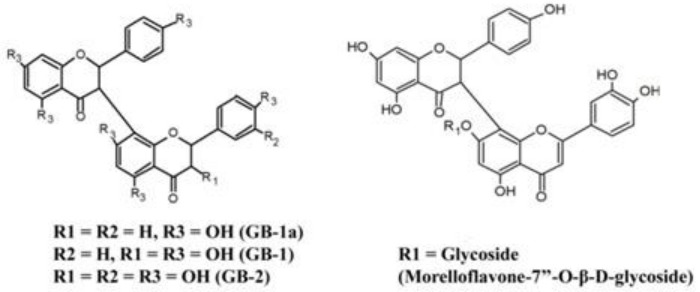

Figure 1. Structures of the biflavonoids GB-1a, GB-1, GB-2, and morelloflavone-7''-O-β-D-glycoside

Table 1. *In vitro* radical scavenging asays (DPPH and superoxide radical) of *G. travancorica* leaf methanol extract and isolated compounds

Extract/ compound	DPPH IC_{50} value (µg/mL)	Superoxide IC_{50} value (µg/mL)
G. trav. Lf MeOH extract	18.9±1.80	53.2±3.09
GB-1a	31.98±1.14	42.13±0.51
GB-1	22.31±2.33	37.52±2.10
GB-2	11.93±0.58	23.31±1.60
Morelloflavone-7''-O-β-D-glycoside	8.34±2.12	6.95±1.33
Ascorbic acid	3.2±0.50	5.8±0.25

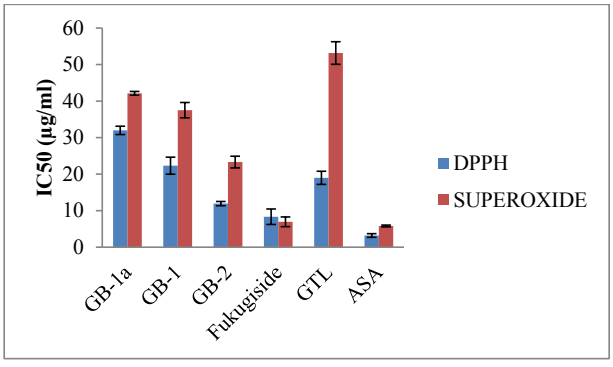

Figure 2. IC_{50} values of DPPH and superoxide radicals scavenging assay (GB-1a, GB-1, GB-2, Fukugiside, **GTL**- *G. travancorica* leaf methanol extract, **ASA**- standard ascorbic acid)

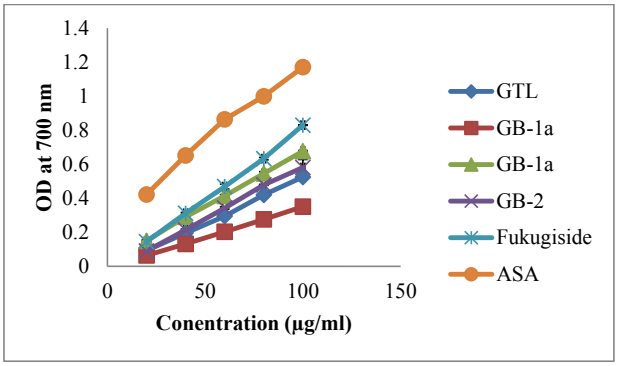

Figure 3. Reducing power assay (GB-1a, GB-1, GB-2, Fukugiside, **GTL**- *G. travancorica* leaf methanol extract, **ASA**- standard ascorbic acid)

2. Growth inhibitory effect of Fukugiside on cancer cell lines A431, HeLa, HT29 and normal cell line WRL68 cells

MTT assay was performed by seeding ~5000 cells per well in a 96 well plate and treating them under sub confluent conditions, with different concentrations of fukugiside such as 1 µg/mL, 10 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL and 150 µg/mL respectively. The experiment was performed in batches with respect to the incubation time as 48 hrs. MTT assay is widely used in the *in vitro* evaluation of the biosafety of plant extracts and compounds. This colorimetric assay is based on the capacity of mitochondrial succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble, coloured formazan product which is measured spectrophotometrically at 570 nm. Reduction of the dye MTT occurs only in metabolically active cells and the level of activity is a measure of the viability of the cells.

The study done on A431 and HeLa cells showed that fukugiside exhibited a concentration dependent cytotoxicity to both the cell lines. The cells were incubated with varying doses of fukugiside (1µg, 10 µg, 50 µg and 100 µg and 150 µg) and MTT assay was performed. Fukugiside inhibited the proliferation of human epidermal cancer cell line A431 and cervical cancer cell line HeLa in a dose dependent manner. Fukugiside exhibited significant cell death in A431 cell line with LD_{50} value of 150 µg/mL. Severe morphological changes were observed in HeLa cells treated with fukugiside under phase contrast microscope. Comparatively higher activity was exhibited by fukugiside against HeLa cells with LD_{50} value of 82.80 µg/mL compared with untreated control (**Figure 4**). The study done on normal liver cell line WRL68 and colorectal cancer cell line HT-29 cells treated with varying doses of fukugiside (1µg, 10 µg, 50 µg and 100 µg and 150 µg) did not exhibit any toxicity to the cells. From the results indicate that the compound exhibited toxicity to cancer cell lines A431 and HeLa in a dose dependent manner and no toxicity was observed against normal cell line WRL68.

Acridine orange/ethidium bromide (AO/EB) staining is used to visualize nuclear morphology and apoptotic body formation that are characteristic of apoptosis. Acridine orange is an important dye that will stain both live and dead cells, whereas ethidium bromide stain only those cells that have lost their membrane integrity (Jayadev *et al*., 2004).

Table 2. Cell viability in Fukugiside treated A431 and HeLa cells by MTT assay

Test material		% Cell viability	
		A431	HeLa
Control (0.01% DMSO)		100	100
Fukugiside (µg/mL)	1	110±3.6	85.85±0.19
	10	125±4.3	64.86±3.79
	50	86±3.4	56.50±2.46
	100	64±3.6	43.55±0.52
	150	49±3.4	39.88±.67

Values are mean±SD of three separate determinations. Cells were incubated at 37° C for 48 hrs in DMEM media in CO_2 incubator

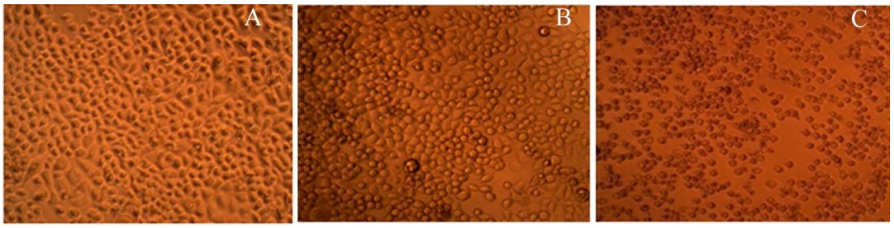

Figure 4. HeLa cells treated with fukugiside under phase contrast microscope: (A) HeLa cells treated with DMSO (0.01%); (B) DLA cells treated with fukugiside (50 µg/mL); (C) HeLa cells treated with fukugiside (150 µg/mL)

To corroborate that apoptosis has been induced by fukugiside, HeLa cells were analysed in the presence of acridine orange and ethidium bromide staining (AO/EB staining). Five concentrations of fukugiside used in MTT assay (1µg, 10 µg, 50 µg and 100 µg and 150 µg) were chosen for this experiment. HeLa cells cultured in complete media and stained with AO/EB (**Figure 5**) were used as control.

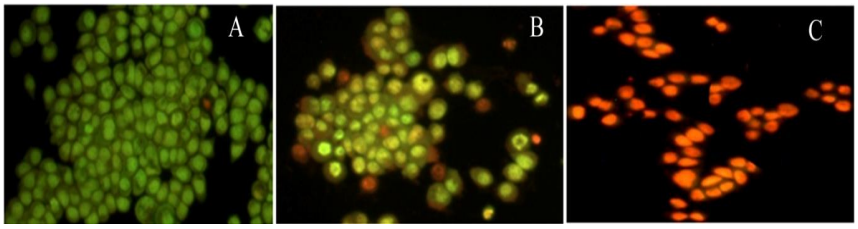

Figure 5. HeLa cells stained with acridine orange-ethidium bromide under fluorescent microscope: (A) HeLa cells treated with DMSO (0.01%) appeared in green color (live), (B) DLA cells treated with fukugiside (50 µg/mL) appeared in slight yellowish (early apoptotic cells), (C) HeLa cells treated with fukugiside (150 µg/mL) appeared in yellowish red (dead cells)

Figure 5 shows that the fukugiside at tested doses induced apoptosis after 48 hours incubation. Cells stained green represent viable cells (**Figure 5A**), whereas yellow staining represented early apoptotic cells (**Figure 5B**) and yellow to reddish orange staining represents late apoptotic cells (**Figure 5C**). As shown in **Figure 5**, HeLa cells treated with 150 µg/mL of fukugiside showed changes in cellular morphology, including chromatin

condensation and membrane blebbing. Stronger apoptosis signal was induced in HeLa cells with higher concentrations of fukugiside.

Effect of fukugiside on cell cycle distribution by flow cytometry

Considering that fukugiside decreased cell proliferation and induced cell death as evident from MTT assay and apoptotic induction by staining experiments, the effect of this molecule on cell cycle distribution was analysed by flow cytometry. Flow cytometric analysis was carried out on HeLa cells treated with 100 µg/mL of fukugiside for 48 hrs. In HeLa cells, 100 µg/mL of fukugiside induced accumulation of cells in S phase concurrently to a significant decrease in G0/G1 cells (**Figure 6**).

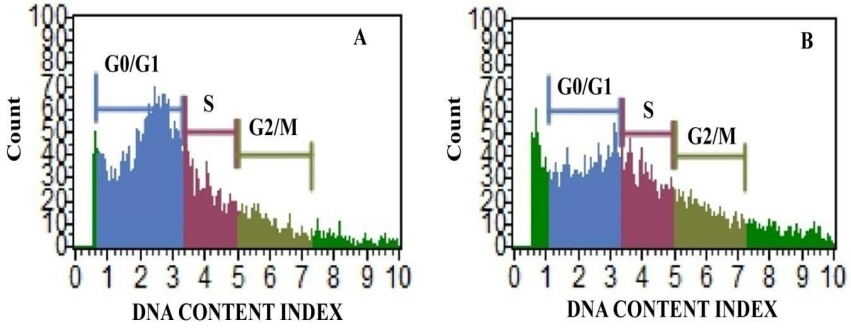

Figure 6. Comparison of DNA content in control (0.01% DMSO) and fukugiside (100 µg/mL) treated HeLa cells by flow cytometry

Deregulation of cell cycle is one of the critical events that drive cancer cells into uncontrolled proliferation (Evan and Vousden, 2001). Molecular changes, including the over expression of cyclins and CDKs and the loss of CDK inhibitors and tumor suppressor proteins resulting from gene mutations or epigenetic inactivation, are frequently detected in tumor cells (Sherr, 1996; Malumbres and Barbacid, 2001). Because of the important roles of cell cycle deregulation in tumorigenesis and tumor progression, molecules involved in cell cycle regulation also serve as potential targets for therapeutic intervention in cancers. Modulation of p21, and MAPK/ERK pathway can have a potent role in inhibiting cells at S phase. In the present study, addition of the compound fukugiside induced significant change in cell proliferation and the cells were found to be arrested in S phase compared to untreated control. The results were comparable with previous reports regarding inhibtion of MCF 7 cells by resveratrol and other flavonoid compounds in S phase (Joe *et al*., 2002).

Effect of fukugiside on the expression of MAPK p38 in HeLa cells

In continuation with the studies on cell cycle deregulation seen in S phase by fukugiside, the effects of fukugiside on the level of MAPK p38 in HeLa cells were examined. A series of time course experiments were conducted to analyse the expression of Erk in HeLa cells treated with fukugiside, where DMSO served as control. Reverse transcription polymerase chain reaction (RT-PCR) followed by agarose gel electrophoresis demonstrated that the expression levels of MAPK was decreased after 48 hrs of treatment with fukugiside. The intensity of the bands were analysed by ImageJ analyser and the results revealed that,

treatment with fukugiside lead to inhibition of MAPK expression by 26.15 % compared to untreated control (**Figure 6**).

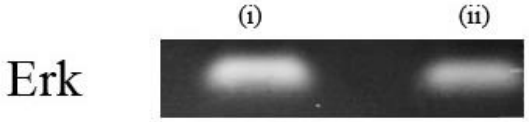

Figure 6. Intensity of MAPK p38 expression in agarose gel electrophoresis; **(i)** control, **(ii)** fukugiside

Conclusions

Garcinia species are well known for the diversity of secondary metabolites and potential bioactivities. The biflavonoid fukugiside has been identified as the major antioxidant component in *G. travancorica* through *in vitro* free radical scavenging assays and reducing power assay. Further, the antitumor properties of the molecule in different human cancer cell lines were also checked. Fukugiside caused a dose dependent cancer cell growth inhibition in A431 and HeLa cells, and the antiproliferative effect appears to be due to its ability to induce S-phase arrest and apoptotic cell death. In HeLa cells, fukugiside down regulated the MAPK p38 expression compared with untreated control. The study highlights fukugiside as a potential candidate for drug development.

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Chapter 15

Molecular Characterization of *Garcinia* species in the Western Ghats

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Abstract

The genus *Garcinia* L. (Family: Clusiaceae) is an important component of the forest flora of the Western Ghats with 9 species, of which 7 are endemic to the region. Systematics of the genus *Garcinia* is primarily based on morphological data, especially reproductive morphology and the genus is considered as a taxonomically difficult one due to the complexity and diversity in floral characteristics. Molecular tools are getting more acceptances as a convenient tool in the phylogenic studies of such taxonomically difficult groups. Molecular markers are potential in portraying the genetic relationship between plant groups and DNA based molecular taxonomic approaches give an exact and rapid method of distinguishing specimens based on their interspecies variation. In the present study, the genetic profile of 9 *Garcinia* species, *G. gummi-gutta, G. rubro-echinata, G. imberti, G. indica, G. morella, G. talbotii, G. pushpangadaniana, G. travancorica* and *G. wightii* distributed naturally in the Western Ghats of south India, were analyzed for better understanding of interspecific genetic diversity. Molecular profiling using the chloroplast coding region *mat*K could successfully demark different species of the genus *Garcinia*.

Keywords: *Garcinia* species, Western Ghats, Molecular taxonomy, *mat*K

Introduction

Systematics of the genus *Garcinia* is primarily based on reproductive morphology. However, the field identification of *Garcinias* is challenging due to the presence of unisexual flowers and strict seasonality in flowering and fruiting. The morphological assessment and variability studies of *Garcinia* species demonstrated that the morphological variants are enormous within the species with characters always overlapped within and between populations and the genus is often treated as a taxonomically difficult group (Nimanthika and Kaththriarachchi, 2010). Combined approaches based on morphological, molecular and chemical analyses are getting more acceptances in the phylogenic studies of such taxonomically difficult groups (Labra *et al*., 2004). While classical phylogenetic approach relies on morphological characteristics of an organism, in molecular phylogeny, the relationships among organisms were studied by comparing nucleotide sequences of RNA and DNA and sequences of amino acids of a protein. Dissimilarities among the sequences indicate genetic divergence as a result of molecular evolution during the course of time. Molecular markers are a direct assay of hereditary material and unlike morphological markers, molecular markers are not prone to

environmental influences and can complement data from descriptors such as morphological characters (Patwardhan, 2014; Mba and Tohme, 2005). Further, by comparing homologous molecules from different organisms it is possible to establish their degree of similarity, thereby establishing or revealing a hierarchy of relationship through a phylogenetic tree.

Many plant phylogenetic studies are based on chloroplast DNA (cpDNA). In plants, cpDNA is smallest as compared to mitochondria or nuclear genome. It is assumed to be conserved in its evolution in terms of nucleotide substitution with very little rearrangements which permits the molecule to be used in resolving phylogenetic relationships especially at deep levels of evolution. Selection of a gene of sufficient length and appropriate substitution rate is a crucial step and currently used cpDNA genes include rbcL, ndhF, rpl16, matK, atpB and many more.

In *Garcinia*, preliminary molecular phylogenetic work has been started by Rismita-Sari (2000) to test Jones (1980) classifications of *Garcinia* into 14 sections based mainly on male flower characters. Gustafsson *et al*. discussed the phylogenetic status of the Clusiaceae members in detail using chloroplast gene Rbcl and the study supported morphological based classifications (Gustafsson *et al*., 2002). The phylogenetic relationship among mangosteen and several wild relative species were analyzed by comparing sequences of the ITS region of nuclear ribosomal DNA. Both parsimonious and NJ analysis revealed that mangosteen is closely related to *G. malaccensis* (Chinawat and Subhadrabandu, 2004). Results from phylogenetic analyses utilizing chloroplast and nuclear DNA markers agree with morphology in support of the unification of all of *Rheedia* L. and part of *Ochrocarpos* Thouars with *Garcinia* (Sweeney, 2008). Genetic diversity based on morphological and Inter Simple Sequence Repeats (ISSR) of 19 accessions of mangosteen and their close relatives revealed that *G. malaccensis* and *G. celebia* were the ancestors for mangosteen (Sulassih *et al*. 2013).

Rao (2003) studied both intra and inter species relationship among six *Garcinia* species namely *G. indica*, *G. cambogia (G. gummi-gutta)*, *G. cowa*, *G. mangostana*, *G. xanthochymus* and *G. hombroniana*, using RAPD polymorphism. RAPD markers could successfully distinguish different species of the genus *Garcinia*. The study indicated high molecular diversity within *G. cambogia* (Rao, 2003). Parthasarathy *et al*. studied RAPD polymorphism in 33 accessions of *Garcinia* species collected from different areas of Western Ghats (Parthasarathy *et al*., 2013). The dendrogram clearly separated the collections of the 3 main species studied, *G. gummi-gutta*, *G. indica* and *G. xanthochymus,* and suggested high amount of diversity within the collections of the same species. Similar study was also conducted on *Garcinia* collections from North East India using RAPD. High molecular diversity was observed with the heterogeneity index within species ranging from 0.81 to 0.82 in four species, namely *G. gummi-gutta*, *G. indica*, *G. cowa* and *G. xanthochymus* (Parthasarathy *et al*., 2013).

Though Western Ghats is a centre of diversity of *Garcinia* species, a comprehensive study on the molecular profiles of *Garcinia* species of the region including the rare and endemic species has rarely been attempted. Present chapter discusses the molecular characterization of *Garcinia* species naturally occurring in the Western Ghats region, using chloroplast coding region *mat*K.

1. Genomic DNA isolation and sequencing

Genomic DNA was isolated from young leaves using DNeasy plant DNA isolation kit (Qiagen). PCR amplification reactions were carried out in a 20 µl reaction volume which contained 1X PCR buffer (150mM Tris HCl, pH-8; 500mM KCl), 0.2mM each dNTPs, 2.5mM $MgCl_2$, 20ng DNA, 1 unit of Ampli Taq Gold DNA polymerase enzyme, 0.1 mg/ml BSA and 4% DMSO, 5pM of forward and reverse primers (Table:01). The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) with an initial denaturation of 95° C for 5.00 min. followed by 40 cycles of 48° C for 0.40 min, 72° C for 1.00 min and 72° C for 5.00 min., followed by 4°C. PCR amplification (**Figure 1**) was followed by sequencing using the BigDye Terminator v 3.1. The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v 5.6.

Table1. Primers used for the molecular study of *Garcinia* species

Target	Primer Name	Direction	Sequence (5' → 3')	Reference
*mat*K	matK-390F	Forward	CGATCTATTCATTCAATATTTC	CBOL Plant Working Group (http://www.barcoding.si.edu /pdf/informationonbarcodeloci.pdf
	matK-1326R	Reverse	TCTAGCACACGAAAGTCGAAGT	

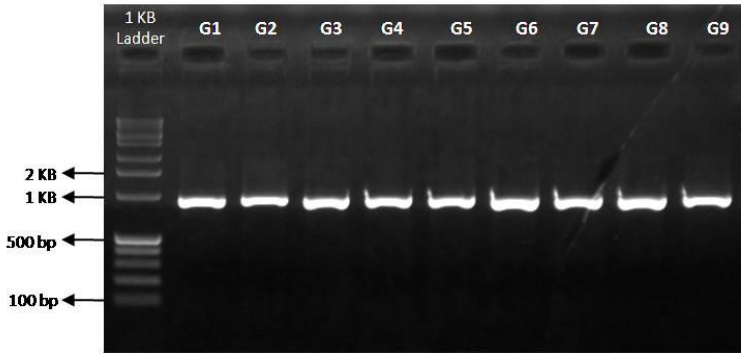

Figure 1. PCR products- *mat*K region of nine *Garcinia* species

2. Sequence analysis

The phylogenetic analyses of 9 *Garcinia* species distributed naturally in the Western Ghats were done using *MatK* with *Clusia criuva* of Clusiaceae family as the out group member (ncbi-TNS:SK08071206). The evolutionary history was inferred using Neighbor-Joining method as elaborated by Saitou and Nei (1987). The optimal tree with the sum of branch length 0.093 is shown in **Figure 2**. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used, to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980) and are in the units of the number of base substitutions per site. All positions containing gaps and

missing data were eliminated. There were a total of 802 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura *et al.,* 2011).

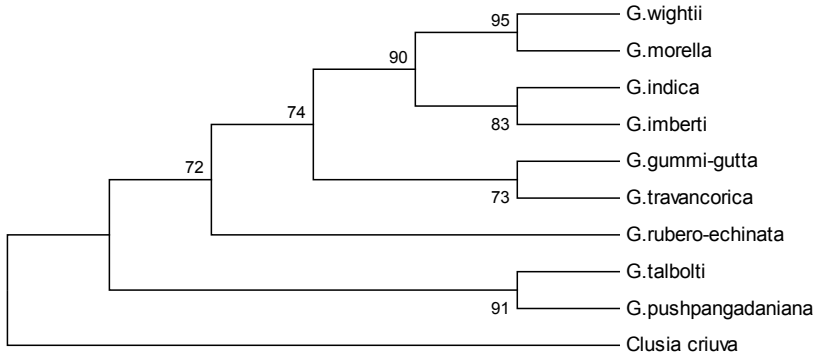

Figure 2. NJ- Phylogram based on *matK* loci of 9 species of *Garcinia* and the out group *Clusia criuva.*

All the accessions of the *Garcinia* species were clustered together in the NJ phylogram based on *matK* loci and the phylogram distinctly delimit all the 9 species and were also clearly differentiated from the out group *Clusia criuva*. In the first clad the accessions of *G. morella* and *G. wightii* were clustered together with a bootstrap value of 95%. The second clad includes *G. indica* and *G. imberti* and showed sister relationship with 83% bootstrap support. The third clad includes two sub clusters with *G. travancorica* in one cluster and *G. gummi-gutta* in the second cluster with bootstrap value of 73%. The fourth cluster is purely monophyletic with *G. rubro-echinata.* The fifth cluster includes *G. talbotii* and a recently published species *G. pushpangadaniana* with bootstrap value of 91%.

Generally, the classical morphology based classification and molecular analysis based classification complement each other since morphology of an organism is the manifestation of its genome, proteome and transcriptome profiles. The results of the current molecular study are in part congruent with the classification based on morphological features (Chapter 1). The species status of *G. pushpangadaniana* is confirmed and also its allied nature to *G. talbotii* (Sabu *et al*., 2013). *G. pushpangadaniana* and *G. talbotii* were morphologically distinct from other species by the characteristic features of stamens in 5 phalanges and 5 numbered sepals and petals. *G. morella* and *G. wightii* that showed as a separate clad in molecular phylogeny were allied and distinct from other species based on sessile fruits and 4 lobed stigma. *G. rubro-echinata* also stands distinct based on morphological features with echinate fruits and supports the monophyletic nature of *G. rubro-echinata* in the molecular phylogram. Combined multidisciplinary analysis of vegetative and reproductive morphology, along with molecular taxonomy yield more robust phylogeny which could be used for studies of phytogeography and evolutionary radiation of the *Garcinia* species.

Conclusions

The genus *Garcinia* is one of the taxa with poorly resolved phylogenetic relationships. Although widely practised even now, traditional morphology based systems of classification can have some limitations while systematics based on molecular markers can complement the traditional morphology based method for phylogenetic studies. Further, the genetic profile of

the *Garcinia* species of the Western Ghats can be used to solve the taxonomic enigmas and for analyzing the phylogeny of the group. The present work shows that the *Garcinia* species can be distinctly identified by the phylogram based on *matK* loci of the *Garcinia* species and molecular profiling has been successfully used to resolve species circumscriptions and identification of *Garcinia* species in the Western Ghats.

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Diversity of *Garcinia* species in the Western Ghats: Phytochemical Perspective

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Recently, *Garcinia* species have received considerable attention worldwide from scientific as well as industrial sectors and several novel structures, bioactivities and potential utilities have been reported for *Garcinia* species. Though phytochemical investigation of *Garcinia* species is progressing in a fast pace worldover as indicated by ever increasing publications and patents around the genus, the phytochemistry of *Garcinia* species in India has least been explored. The book focuses on the phytochemical aspects of *Garcinia* species of the Western Ghats, and also elaborates the morphology, genetics, pharmacology and nutritional aspects as well. The wealth of information provided can be linked to boarder zones between chemistry and biology as in the case of chemical ecology, chemogenomics and phylogenic studies. The work also highlights the importance of the floristic wealth of the Western Ghats and the need to harvest the hidden treasure through wise selection and skillful application of different phytochemical methods.

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