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).

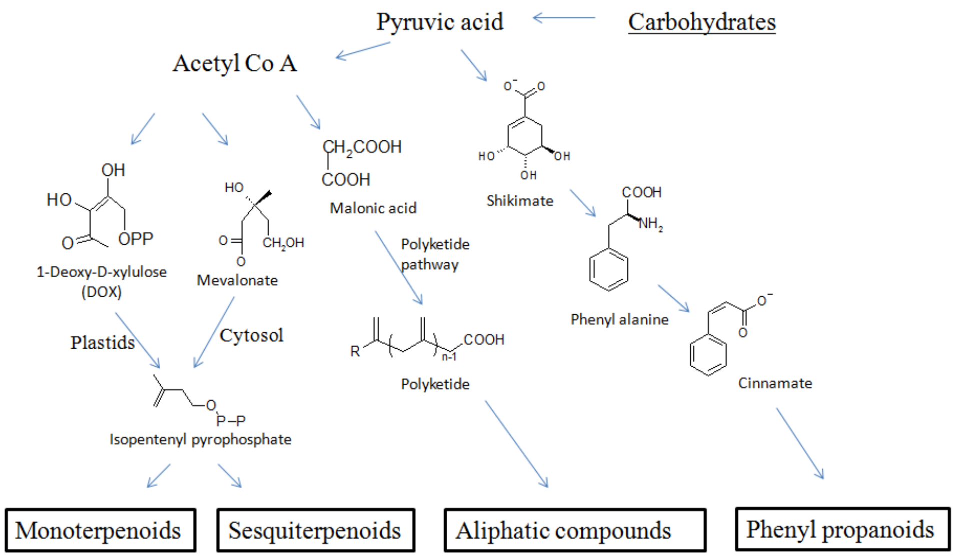

Figure 1. General biosynthetic pathways of different classes of volatile chemicals in *Garcinia* species

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).

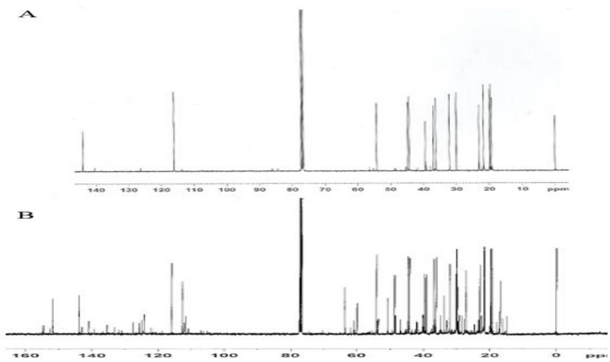

Figure 2. ^{13}C NMR of α-copaene (A) and *Garcinia talbotii* leaf essential oil (B).

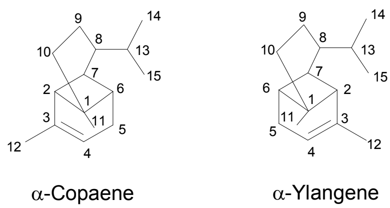

Figure 3. Structures of α-copaene and α-ylangene

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.

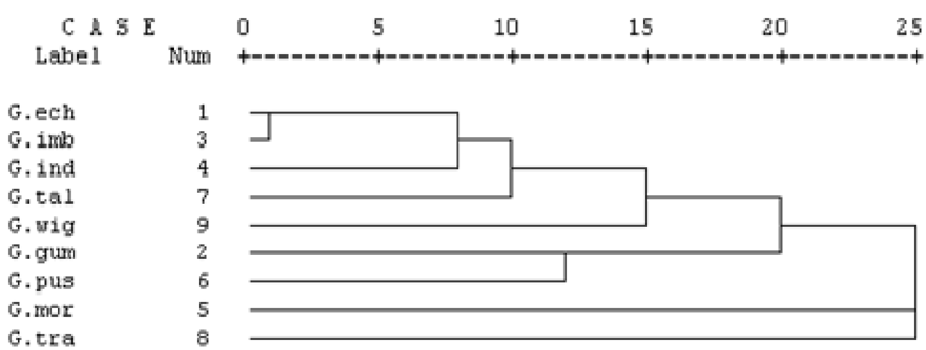

Figure 4. Dendrogram showing subgrouping of *Garcinia* species based on volatile chemical profile using between groups linkage (SPSS version 16.0)

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.

References

1. Adams RP. **2007**. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. Fourth edition. Allured Pub. Co., Carol Stream, IL.
2. De Buyck LF, De Pooter HL, Schamp NM, De Bruyn R, Zhang W, Budesinsky M and Motl O. **1989**. Terpenes from *Otacanthus coeruleus* Lindl.: Identification of β-copaen-4α-ol and a new criterion for discriminating between isomeric copaene and ylangene structures. *Flavour Fragr. J.*, 4, 53-57.
3. David EC. **1999**. Sesquiterpene Biosynthesis: Cyclisation Mechanisms. *In*: Comprehensive Natural Product Chemistry (Ed.). Derek Barton and Koji Nakanishi. Elsevier, Amsterdam, Vol.II, p.167.
4. Dool VH and Kratz PD. **1963**. A generalization of the retention index system including linear temperature programmed gas liquid partition chromatography. *J. Chromatogr.*, 11, 463-471.
5. Esau, K. **1965**. Plant Anatomy. 2nd ed. Wiley Eastern Limited, New Delhi.
6. Handa SS. **2008**. An overview of extraction techniques for medicinal and aromatic plants. *In*: Extraction Technologies for Medicinal and Aromatic Plants. (Eds.) Handa SS, Singh SP, Longo KG and Rakesh DD. International Centre for Science and High Technology, ICS-UNIDO, Trieste. pp. 21-52.
7. Hemshekhar M, Sunitha K, Santhosh MS, Devaraja S, Kemparaju K, Vishwanath BS, Niranjana SR and Girish KS. **2011**. An overview on genus *Garcinia*: Phytochemical and therapeutical aspects. *Phytochem. Rev.*, 10(3), 325-351.
8. Hiltunen R and Holm Y. **1999.** Basil: The Genus *Ocimum*. Harwood Academic Publishers, Amsterdam.
9. Huang M, Sanchez-Moreiras A M, Abel C, Sohrabi R, Lee S, Gershenzon J and Tholl D. **2012**. The major volatile organic compound emitted from *Arabidopsis thaliana* flowers,

the sesquiterpene (E)-b-caryophyllene, is a defense against a bacterial pathogen. *New Phytologist* 193: 997-1008.

10. Kubeczka KH and Formacek V. **2002**. Essential Oils Analysis by Capillary Gas Chromatography and Carbon-13 NMR Spectroscopy. John Wiley and Sons, Chichester.
11. Macleod JA and Pieris NM. **1982**. Volatile flavour components of Mangosteen, *Garcinia mangostana*. *Phytochemistry*, 21(1), 117-119.
12. Maheswari JK. **1964**. Taxonomic studies on Indian Guttiferae III. The genus *Garcinia* Linn. *Bull. Bot. Surv. India*, 6, 107-135.
13. Martins FT, Doriguetto AC, de Souza TC, de Souza KR, dos Santos MH, Moreira ME and Barbosa LC. **2008**. Composition and anti-inflammatory and antioxidant activities of the volatile oil from the fruit peel of *G.brasiliensis*. *Chem. Biodivers.*, 5(2), 251-258.
14. Nimanthika WJ, Kaththriarachchi HS. **2010**. Systematics of genus *Garcinia* L. (Clusiaceae) in Sri Lanka. New insights from vegetative morphology. *Journal of National Science Foundation,* 38, 29-44.
15. Nogueira PC, Bittrich V, Shepherd GJ, Lopes AV and Marsaioli AJ. **2001**. The ecological and taxonomic importance of flower volatiles of *Clusia* species (Guttiferae). *Phytochemistry*, 56(5), 443-452.
16. Onayade OA, Looman AMG, Scheffer JJC and Gbile ZO. **1998**. Lavender lactone and other volatile constituents of the oleoresin from seeds of *Garcinia kola* Heckel. *Flavor and Fragrance Journal,* 13(6), 409-412.
17. Parthasarathy U, Nirmal Babu K, Senthil Kumar R, Ashis GR, Mohan S and Parthasarathy VA. **2013**. Diversity of Indian *Garcinia* - A medicinally important spice crop in India. *Acta Hortic.,* 979, 467-476.
18. Pathirana PSK and Herat TR. **2004**. Comparative vegetative anatomical study of the genus *Garcinia* L. (Clusiaceae/ Guttiferae) in Sri Lanka. *Ceylon Journal* of *Science,* 32, 39-66.
19. Rameshkumar KB, Shiburaj S and George V. **2005**. Constituents and antibacterial activity of the stem bark oil of *Garcinia imberti. J. Trop. Med. Plants,* 6, 271-273.
20. Sabu T, Mohanan N, Krishnaraj MV, Shareef SM, Shameer PS and Roy PE **2013**. *Garcinia pushpangadaniana,* (Clusiaceae) a new species from southern Western Ghats, India. *Phytotaxa,* 116 (2), 51-56.
21. Schiestl FP, Ayasse M, Paulus HF, Löfstedt C, Hansson BS, Ibarra F and Francke W. **2000**. Sex pheromone mimicry in the early spider orchid (Ophrys sphegodes): Patterns of hydrocarbons as the key mechanism for pollination by sexual deception. *J. Comp. Physiol. A*, 186(6), 567-574.
22. Schofield EK **1968**. Petiole anatomy of the Guttiferae and related families. Mem. New York Bot. Gard. 18,1-55.
23. Sweeney PW. **2008**. Phylogeny and floral diversity in the genus *Garcinia* (Clusiaceae) and relatives. *Int. J. Plant Sci*., 169(9), 1288-1303.
24. Wang R, Peng S, Zeng R, Ding LW and Xu Z. **2009.** Cloning, expression and wounding induction of β-caryophyllene synthase gene from *Mikania micrantha* H.B.K. and allelopathic potential of β-caryophyllene. *Allelopathy Journal*, 24 (1), 35-44.
25. Waterman PG and Hussain RA. **1983**. Systematic significance of xanthones, benzophenones and biflavonoids in *Garcinia*. *Biochem. Syst. Ecol.,* 11(1), 21-28.