

### 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 2 $\alpha$ -hydroxy-3 $\beta$ -acetoxy-urs-12-en-28-oic acid and the steroid stigmaterol. 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-QqQLIT-MS/MS technique. Twenty two phenolic compounds comprising xanthenes ( $\alpha$ -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-QqQLIT-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 xanthenes, 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, xanthenes, 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 2 $\alpha$ -hydroxy, 3 $\beta$ -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 (Elfta *et al.*, 2009).

Compound **2** was eluted from the hexane extract using the solvent system hexane: chloroform (1:1) and was identified as 2 $\alpha$ -hydroxy-3 $\beta$ -acetoxy-urs-12-en-28-oic acid by analyzing the mass spectra, <sup>1</sup>H and <sup>13</sup>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- $\beta$  position and a free carboxylic acid group at C-17 are rare. The compound has been reported to possess polymerase  $\beta$ -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 (Pinkaw *et al.*, 2009).

**Stigmasterol (1):** Colourless crystals, mp: 160-162° C. R<sub>f</sub>: 0.48 (chloroform 100%). IR (KBr cm<sup>-1</sup>): 3435, 2961, 2937, 2889, 2864, 1461, 1382, 1368, 1061, 970cm<sup>-1</sup>. EI-MS (70 eV) m/z (%): 412 (M<sup>+</sup>, 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). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  0.84(3H,d, J= 6.6Hz, H-27);  $\delta$  0.81(3H,d, J= 7.2 Hz, H-26);  $\delta$  5.03 (1H, dd, J=15.1, 8.4, H-23);  $\delta$  5.14 (1H, dd, J=15.1, 8.4, H-22);  $\delta$  1.02(d, J= 6.6 Hz, H-21);  $\delta$  1.01 (s, H-19);  $\delta$  0.69 (s, H-18);  $\delta$  5.35(1H, d, J= 4.8 Hz, H-6);  $\delta$  3.52 (m, H-3). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): 12.0 (CH<sub>3</sub>), 12.2 (CH<sub>3</sub>), 19.0 (CH<sub>3</sub>), 19.4 (CH<sub>3</sub>), 21.1 (CH<sub>3</sub>), 21.1 (CH<sub>2</sub>), 21.2 (CH<sub>3</sub>), 24.3 (CH<sub>2</sub>), 25.4 (CH<sub>2</sub>), 28.9 (CH<sub>2</sub>), 31.6 (CH<sub>2</sub>), 31.9 (CH x 2), 36.5 (C), 37.2 (CH<sub>2</sub>), 39.7 (CH<sub>2</sub>), 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)

**2 $\alpha$ -Hydroxy-3 $\beta$ -acetoxy-urs-12-en-28-oic acid (2):** Colourless crystals, mp: 199-202° C. R<sub>f</sub>: 0.54 (hexane: chloroform: methanol, 5:4.5:0.5), [ $\alpha$ ]<sub>D</sub>: - 40.9 (c 0.10, MeOH), IR (KBr): 3450, 2970, 2931, 2873, 1720, 1693, 1458, 1373, 1245, 1049, 1029, 960, cm<sup>-1</sup>. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): 1.02 (1H, m, H-1 $\alpha$ , ax), 2.1 (1H, m, H-1 $\beta$ , 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). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): 16.3 (CH<sub>3</sub>), 16.7 (CH<sub>3</sub> x 2), 17.3 (CH<sub>3</sub>), 17.9 (CH<sub>2</sub>), 20.8 (CH<sub>3</sub> x 2), 22.9

(CH<sub>2</sub>), 23.1 (CH<sub>3</sub>), 23.7 (CH<sub>2</sub>), 27.6 (CH<sub>2</sub>), 28.2 (CH<sub>3</sub>), 30.3 (CH<sub>2</sub>), 32.4 (CH<sub>2</sub>), 36.3 (CH<sub>2</sub>), 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<sub>2</sub>), 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]<sup>+</sup> (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<sub>f</sub>: 0.62 (CHCl<sub>3</sub>: MeOH, 17.0: 3.0), [α]<sub>D</sub>: -59.9 (c 0.10, MeOH), IR (KBr): 3224, 1643, 1610, 1515, 1426, 1448, 1367, 1261, 1184, 1164, 839, cm<sup>-1</sup>; UV/Vis λ<sub>max</sub> (MeOH) nm: 341, 288, 277 and 227, <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): 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'') <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): 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

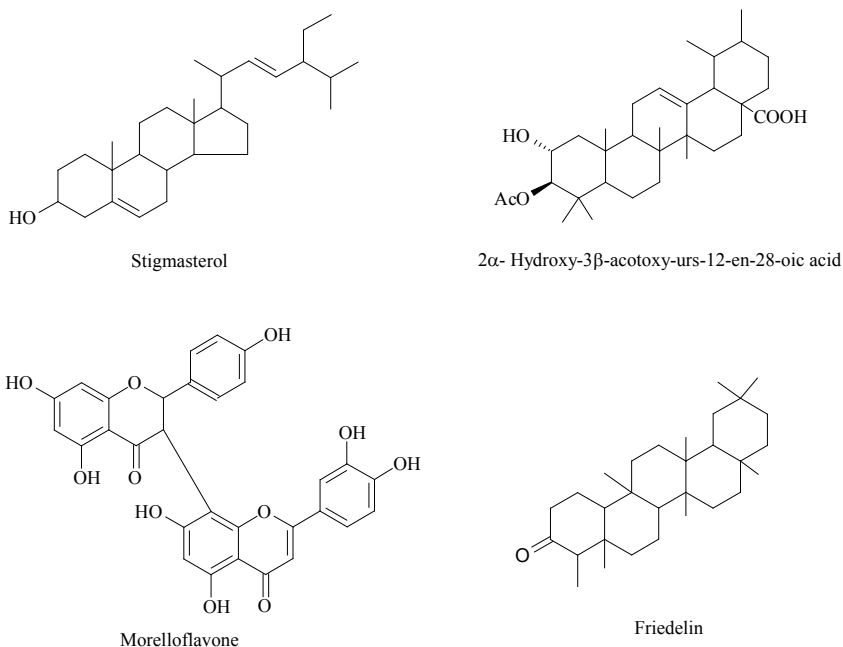
## 2. Phytochemical investigation of the leaves of *Garcinia imberti*

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 derivatives 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]<sup>+</sup> (8), 341 [M-Me]<sup>+</sup> (4), 302(14), 289 (7), 273[M-Me-H<sub>2</sub>O] + (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). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 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). <sup>13</sup>C NMR (500 MHz, CDCl<sub>3</sub>): 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.



**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  $\mu\text{L}$  of the extract was applied on the pre-coated silica gel plate 60F<sub>254</sub> (E. Merk, Germany) along with standard morelloflavone (1.0 to 2.5 $\mu\text{g}$  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  $0.76 \pm 0.09\%$  (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

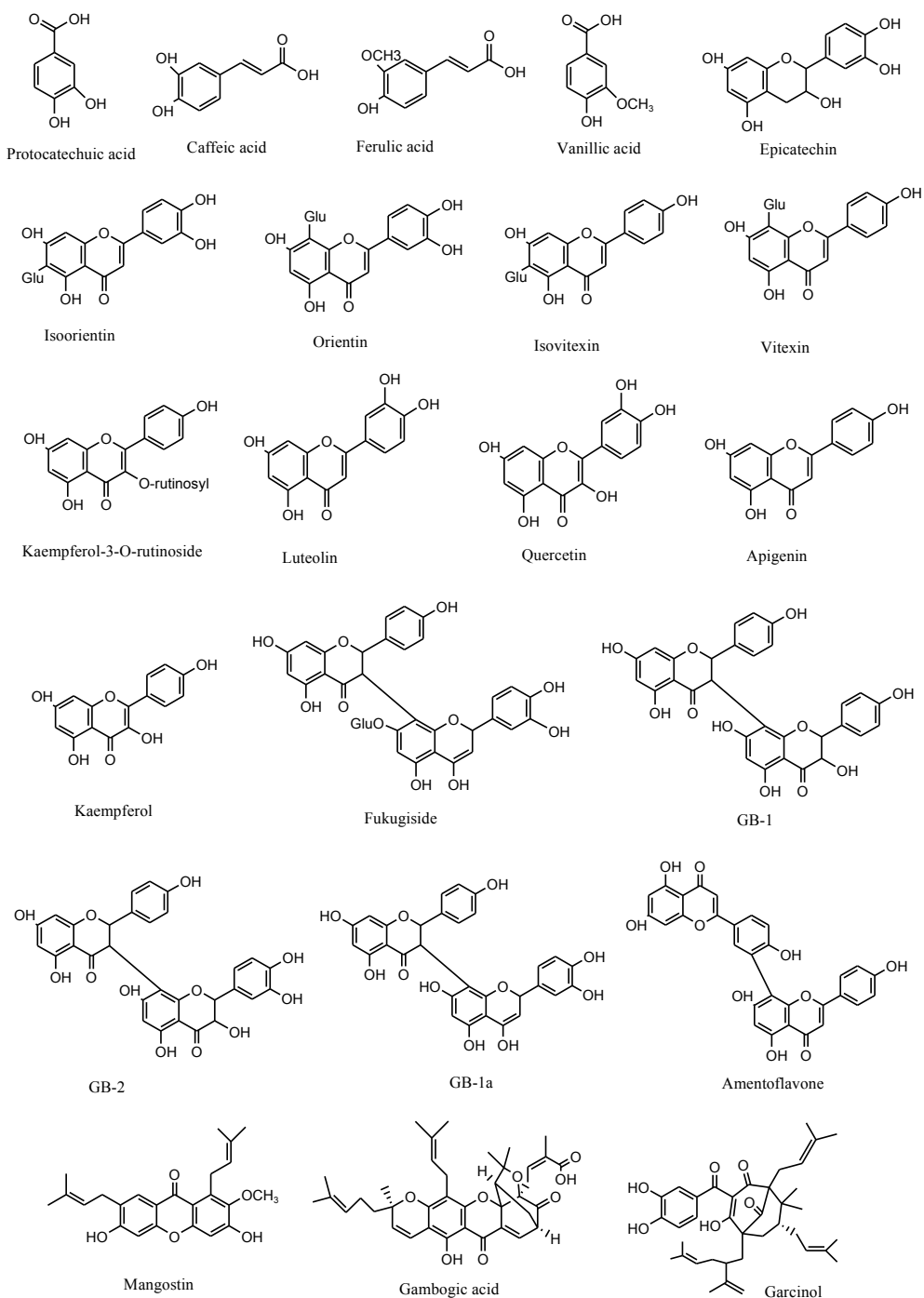
The dried, powdered leaf sample (2 g) was extracted with hexane using Soxhlet apparatus for 6h and made up to 100 ml using hexane. For estimation of friedelin in the leaf samples, the solvent system hexane-chloroform-ethylacetate (9:0.5:0.5) gave the best resolution. 3.0  $\mu\text{L}$  of the hexane extract was applied on the pre-coated silica gel plate 60F<sub>254</sub> (E. Merk, Germany). Standard friedelin at concentrations 0.5-4.0 $\mu\text{g}$  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  $2.2 \pm 0.5\%$  (w/w). The high content of friedelin proposes the plant as a novel source of the compound.

#### 4. UHPLC-QqQ<sub>LIT</sub>-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<sup>n</sup> and reported the distribution of acids, benzophenones, xanthenes, 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  $\mu$ L 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<sup>TM</sup> 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<sub>18</sub> column (50 mm  $\times$  2.1 mm id, 1.7 $\mu$ m) 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<sup>TM</sup> 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]<sup>-</sup>. 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).



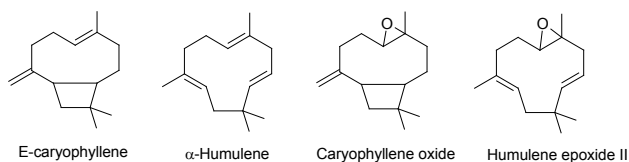
**Figure 3.** Structures of the 22 phenolic compounds detected in *Garcinia imberti* leaf methanol extract by UHPLC-QqQ<sub>LIT</sub>-MS/MS method

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

| Retention Time (min)  | Compound                  | Content (mg/g)<br>(mean $\pm$ SD, $n=3$ ) |
|-----------------------|---------------------------|---|
| <b>Phenolic acids</b> |                           |   |
| 1.43                  | Protocatechuic acid       | 0.9890 $\pm$ 0.002                        |
| 1.81                  | Caffeic acid              | 0.1420 $\pm$ 0.005                        |
| 2.47                  | Ferulic acid              | 0.5220 $\pm$ 0.001                        |
| 3.31                  | Vanillic acid             | 0.0008 $\pm$ 0.0002                       |
| <b>Flavonoids</b>     |                           |   |
| 1.79                  | Epicatechin               | 0.9240 $\pm$ 0.001                        |
| 1.91                  | Isoorientin               | 0.6070 $\pm$ 0.005                        |
| 2.04                  | Orientin                  | 0.5340 $\pm$ 0.004                        |
| 2.26                  | Isovitexin                | 1.4100 $\pm$ 0.029                        |
| 2.28                  | Vitexin                   | 1.1800 $\pm$ 0.015                        |
| 2.53                  | Kaempferol-3-O-rutinoside | 0.0637 $\pm$ 0.0005                       |
| 3.62                  | Luteolin                  | 0.1053 $\pm$ 0.0004                       |
| 3.63                  | Quercetin                 | 0.1920 $\pm$ 0.026                        |
| 4.04                  | Apigenin                  | 0.7010 $\pm$ 0.027                        |
| 4.14                  | Kaempferol                | 0.2820 $\pm$ 0.003                        |
| <b>Biflavonoids</b>   |                           |   |
| 3.56                  | Fukugiside                | 0.2910 $\pm$ 0.002                        |
| 3.57                  | GB-2                      | 0.3850 $\pm$ 0.012                        |
| 4.05                  | GB-1                      | 22.1000 $\pm$ 1.054                       |
| 4.46                  | GB-1a                     | 2.4700 $\pm$ 0.165                        |
| 4.52                  | Amentoflavone             | 0.0440 $\pm$ 0.003                        |
| <b>Xanthones</b>      |                           |   |
| 5.71                  | $\alpha$ -Mangostin       | 0.0056 $\pm$ 0.001                        |
| 6.19                  | Gambogic acid             | 2.8500 $\pm$ 0.032                        |
| <b>Benzophenone</b>   |                           |   |
| 6.50                  | Garcinol                  | 0.3290 $\pm$ 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  $\beta$ -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   |
|-----------------------------------|------|------|------|------|
| $\delta$ -Elemene                 | 1338 | 0.1  | --   | --   |
| $\alpha$ -Cubebene                | 1348 | 0.3  | --   | --   |
| $\alpha$ -Ylangene                | 1373 | 0.3  | --   | --   |
| $\alpha$ -Copaene                 | 1376 | 0.4  | --   | 0.1  |
| $\beta$ -Cubebene                 | 1387 | 0.3  | --   | --   |
| 2-epi- $\beta$ -funebrene         | 1415 | --   | --   | 6.7  |
| $\beta$ -Funebrene                | 1414 | --   | --   | 2.9  |
| $\beta$ -Caryophyllene            | 1419 | 38.1 | 41.4 | 1.8  |
| $\beta$ -Copaene                  | 1430 | 0.4  | --   | 3.7  |
| $\alpha$ -Humulene                | 1452 | 30.5 | 50.8 | 5.4  |
| Allo aromadendrene                | 1458 | 5.5  | --   | --   |
| $\alpha$ -Acoradiene              | 1464 | 0.3  | --   | --   |
| 9 epi E- Caryophyllene            | 1466 | --   | --   | 8.7  |
| $\beta$ -Acoradiene               | 1469 | 4.5  | --   | --   |
| cis $\beta$ -Guaiene              | 1492 | 0.1  | --   | --   |
| $\beta$ -Alaskene                 | 1498 | 2.5  | --   | --   |
| E- $\gamma$ -Bisabolene           | 1507 | 0.1  | --   | --   |
| $\delta$ -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  |
| $\gamma$ -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 xanthenes, polyisoprenylated benzophenones, biflavonoids, phenolic acids and flavonoids in leaf methanol extract of *G. imberti* using UHPLC-QqQLIT-MS/MS method.

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