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'''').

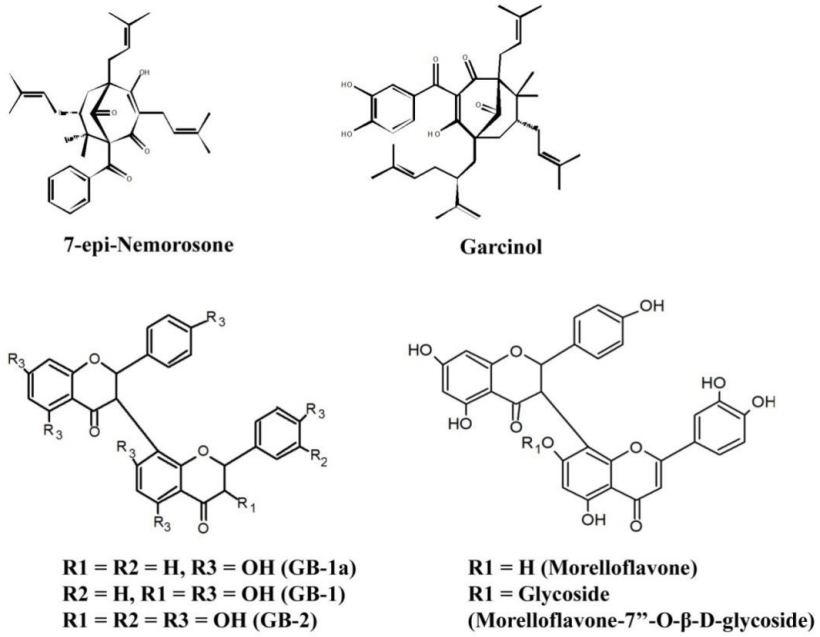

Figure 2. Structures of compounds 1 to 7

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.).

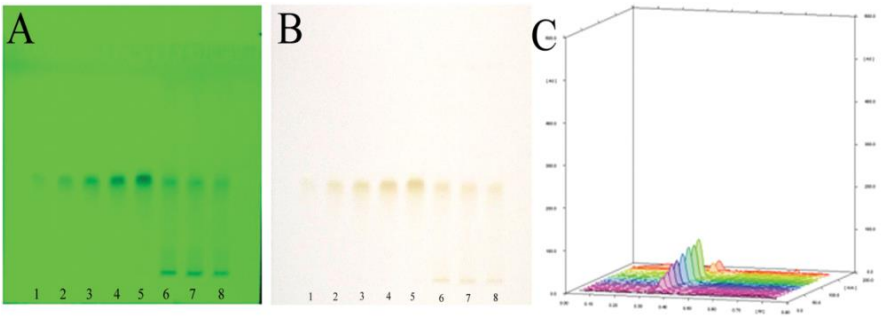

Figure 3. HPTLC densitogram of morelloflavone-7''-O-β-D-glycoside: A- UV (254 nm), B: Visible (580 nm), C: 3D Graph

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).

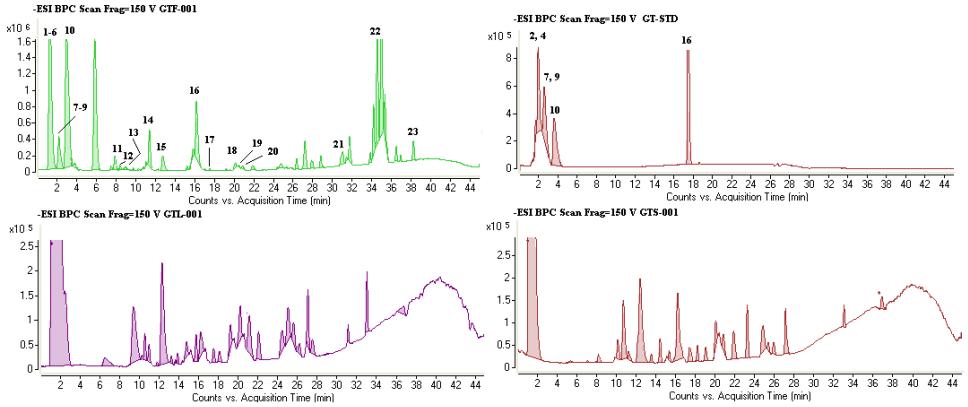

Figure 4. HPLC-QTOF-MS Base peak chromatograms of fruit, leaf, stem bark and mix reference standards of *G. travancorica.* (GTF; fruit, GT-STD; mix reference standards, GTL; leaf, GTS; stem bark)

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.

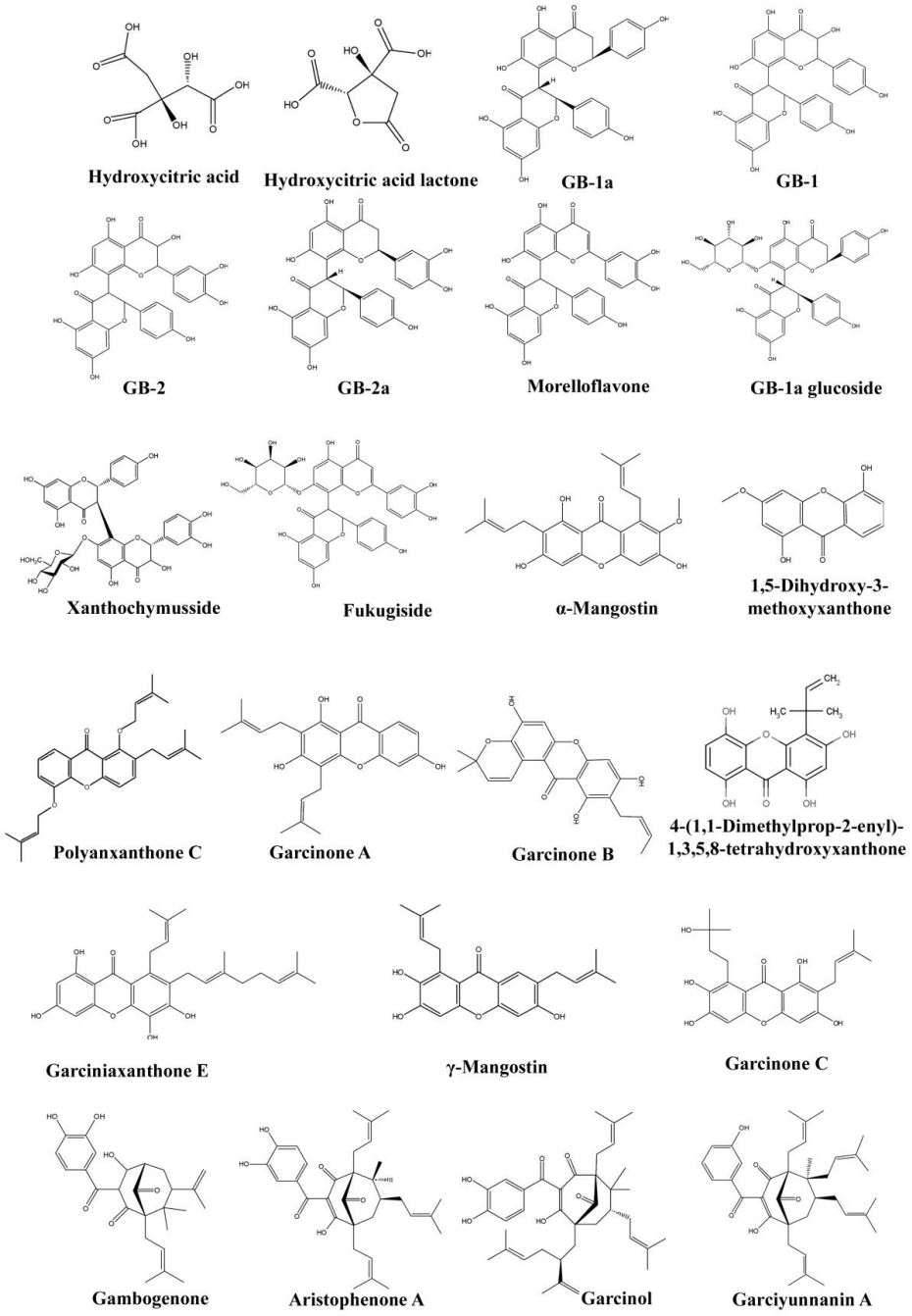

Figure 5. Structures of compounds identified from *Garcinia travancorica* by HPLC-QTOF-MS/MS analysis

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

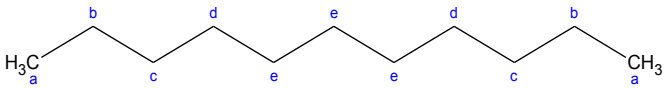

Figure 6. Structure of n-undecane

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

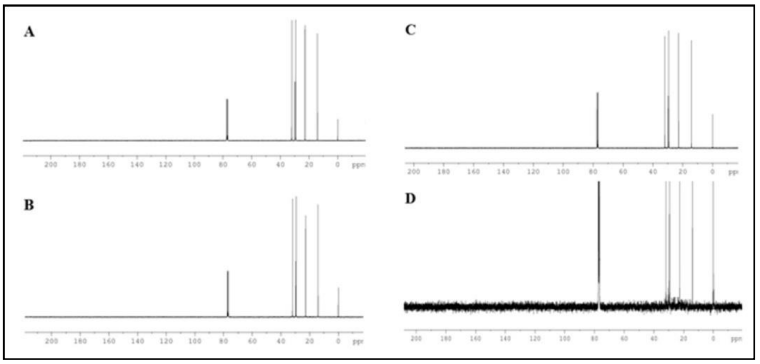

Figure 7. ^{13}C NMR of essential oils and n- undecane: **A**- Leaf oil, **B**- Stem bark oil, **C**-Fruit oil and **D**- n-undecane

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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