***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 $C_8$column | 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*.

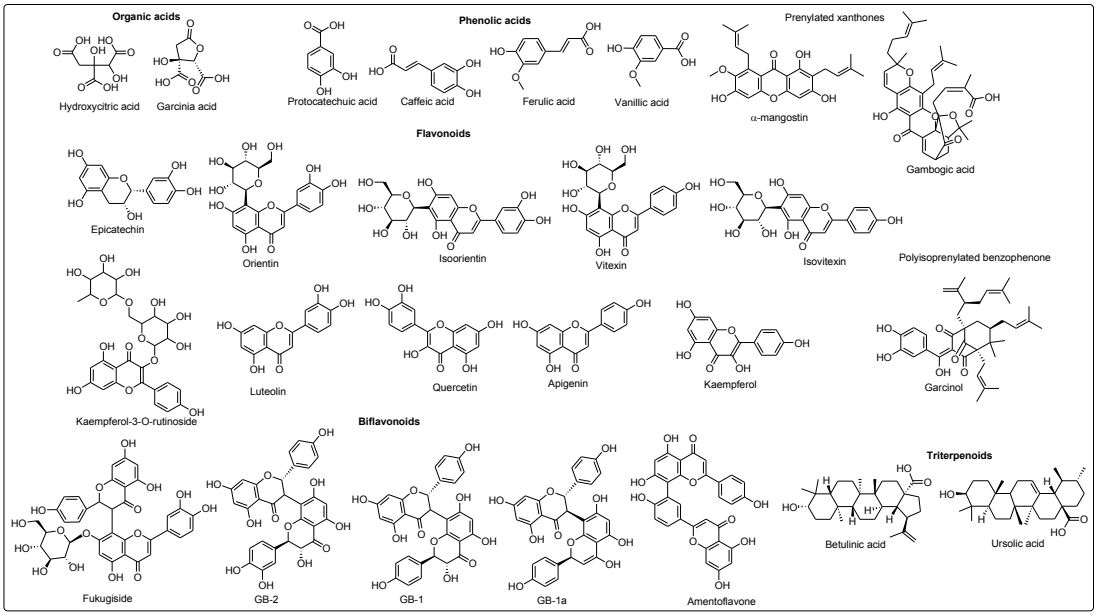


**Figure 1.** Structures of targeted analytes

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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