**SHORT COMMUNICATION**

**Phytochemical analysis and antioxidant potential of the leaves of *Garcinia travancorica* Bedd.**

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Phytochemical analysis of the leaves of *Garcinia travancorica*, a hitherto uninvestigated endemic species to the Western Ghats of south India, resulted in isolation and characterisation of the polyisoprenylated benzophenones 7-epi-nemorosone (1) and garcinol (2) along with biflavonoids GB-1a (3), GB-1 (4), GB-2 (5), morelloflavone (6) and morelloflavone-7'''-O-β-D-glycoside or fukugiside (7). The compounds were identified using various spectroscopic techniques, mainly through NMR and MS. The methanol extract and the biflavonoids 3, 4, 5 and 7 showed potential in vitro antioxidant activities. The IC$_{50}$ value of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of compound 7 was 8.34 ± 2.12 μg/mL, comparable to that of standard ascorbic acid (3.2 ± 0.50 μg/mL). In the superoxide radical scavenging assay, compound 7 gave IC$_{50}$ value of 6.95 ± 1.33 μg/mL close to standard ascorbic acid with IC$_{50}$ value of 5.8 ± 0.25 μg/mL. Validated HPTLC estimation revealed *G. travancorica* as a rich source of morelloflavone-7'''-O-β-D-glycoside (7.12% dry wt. leaves).

**Keywords:** *Garcinia travancorica*; fukugiside; garcinol; HPTLC; antioxidant

1. Introduction

The genus *Garcinia* (Family: Clusiaceae) comprises nearly 250 tree species, and South East Asia, the Indian subcontinent and tropical Africa are centres of diversity of *Garcinia* species (Waterman and Hussain 1983). In India, the genus is represented by 37 species of which 6 species are endemic to the Western Ghats (Maheswari 1964; Sabu et al. 2013). The genus *Garcinia* is a rich repository of biologically active phenolic compounds such as flavonoids, biflavonoids, prenylated xanthones, benzophenones and proanthocyanins (Waterman and Hussain 1983). Xanthones, biflavonoids and benzophenones from different *Garcina* species were reported to possess remarkable levels of...
bioactivities against various ailments, and the antioxidant activity of biflavonoids and benzophenones have great significance because these antioxidants can reduce the incidence of degenerative diseases including cancer, heart disease, inflammation, arthritis and immune system decline (Gordon 1996; 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, and the cellular mechanisms of activity have also been elaborated in most of the cases (Kim et al. 2008). The genus has been subjected to considerable amount of phytochemical investigation and the literature searches showed that nearly 90 *Garcinia* species have so far been subjected to detailed phytochemical investigation.

*Garcinia travancorica* is a locally endemic and threatened plant species of the southern end of Western Ghats of south India (Mohanan and Sivadasan 2002). This paper reports the isolation and characterisation of chemical constituents from *G. travancorica* leaves, antioxidant activity studies of the leaf methanol extract and isolated compounds and HPTLC estimation of the major constituents. This is the first report of the chemical constituents and antioxidant activity studies of *G. travancorica*.

### 2. Results and discussion

#### 2.1. Compounds from *G. travancorica* leaves

*G. travancorica* leaves were extracted with hexane followed by methanol. The hexane extract on column chromatographic separation yielded two polyisoprenylated benzophenones, namely 7-epi-nemorosone (1) and garcinol (2). Structures of these compounds were elucidated by UV, IR and NMR spectroscopic data, together with comparison of literature data (Rama Rao et al. 1980; Subhash et al. 2009; Ishida et al. 2011). The structures were further confirmed through HR-MS and fragmentation pattern of the compounds based on LC–MS/MS analysis (Supplementary Figures S1 and S2). Garcinol, also known as camboginol, was reported from different *Garcinia* species such as *G. gummi-gutta*, *G. indica*, *G. pedunculata* and *G. achachairu* and showed antiglycation, antioxidant and free radical scavenging activities (Sahu et al. 1989; Rastogi & Mehrotra 1990; Yamaguchi et al. 2000; Marques et al. 2012). The methanol extract on column chromatographic separation yielded five biflavonoids, namely GB-1a (3), GB-1 (4), GB-2 (5), morelloflavone (6) and morelloflavone-7″-O-β-D-glycoside or fukugiside (7). Structures of these compounds were confirmed by NMR, MS and comparison with the literature spectroscopic data (Kapadia et al. 1994; Elfta et al. 2009) (Figure 1). $^{13}$C NMR signals of compound 7 were similar to $^{13}$C NMR signals obtained for compound 6; however, additional signals for a glucose residue were also present. $^1$H NMR spectrum further showed multiplets at $\delta$ 3.3 to 3.9, characteristic of a glucose residue and a doublet at 4.7 ($J = 7.2$ Hz), assigned to anomic hydrogen (H-1″″) of glucosyl moiety. 2D NMR spectra of compound 7 showed long range correlation of the signal at $\delta$161.2 (C-7″) with anomic proton H-1″″ at $\delta$ 4.7, confirming the position of the glycoside linkage. The biflavonoids isolated from *G. travancorica* can be divided into two subgroups, those made up of one flavone and one flavanone subunit and those made up of two flavanone units. GB-1a (3), GB-1 (4) and GB-2 (5) were (3 → 8″) linked biflavonanes, while morelloflavone (6) and morelloflavone-7″-O-β-D-glycoside (7) were flavanone-(3 → 8″)-flavone type biflavonoids. Of the two types, biflavonones were the dominant type in different *Garcinia* species, whereas the co-occurrence of the two types of biflavonoids is rare (Waterman and Hussain 1983).

#### 2.2. HPTLC estimation

HPTLC estimation of two compounds, GB-2 and morelloflavone-7″-O-β-D-glycoside, was carried out using HPTLC system (CAMAG, Switzerland). The HPTLC method using the mobile
phase of 70% ethyl acetate in hexane (v/v) could precisely estimate the compound GB-2 ($R_f$ value of 0.30). Standard GB-2 in the range 0.2–1.0 μg per band gave linear response with regression equation $Y = 2346x$. The correlation coefficient 0.983 indicated a good linear relationship between peak area and concentration of the standard. The solvent system ethyl acetate–methanol–formic acid (80.0:17.5:2.5 v/v) gave better resolution of morelloflavone-7''-O-β-D-glycoside with $R_f$ value of 0.35 (Supplementary Figure S3). Standard morelloflavone-7''-O-β-D-glycoside in the range 0.5–1.5 μg per band gave linear response with regression equation $Y = 687.2x$. The correlation coefficient 0.982 indicated a good linear relationship between peak area and concentration of the standard. GB-2 and morelloflavone-7''-O-β-D-glycoside were present in 0.91% and 7.12% of dry weight leaf powder, respectively. The HPTLC method presented can successfully separate the biflavonoids in the leaf extracts of *G. travancorica*. The method validation data showed that the developed HPTLC method is sensitive, selective and can be used as a tool for routine analysis of the compound.

2.3. Antioxidant activity

*G. travancorica* leaf methanol extract and isolated compounds showed promising level of antioxidant activity against various free radicals in vitro. High quantity of phenolics (435.53 ± 23.85 mg/g extract) and flavonoids (143.4 ± 11.60 mg/g of extract) present in the
leaves showed a direct correlation with its antioxidant potential. The antioxidant activities of GB-1a, GB-1, GB-2, morelloflavone-7‴-O-β-D-glycoside along with G. travancorica leaf and control ascorbic acid are summarised in Table 1. The isolated compounds and extract showed remarkable level of activities against DDPH radicals and superoxide radicals (Supplementary Figure S4). The activity was reported as IC\textsubscript{50} value, the concentration of the sample required to scavenge 50% of radicals. Experiments were done in triplicate and the results are expressed as mean value with standard deviation. Compound 7 gave comparable activity with standard ascorbic acid. The IC\textsubscript{50} value of the DPPH radical scavenging activity of compound 7 was 8.34 ± 2.12 µg/mL, while that of standard ascorbic acid was 3.2 ± 0.50 µg/mL. In the superoxide radical scavenging assay, compound 7 gave IC\textsubscript{50} value of 6.95 ± 1.33 µg/mL close to standard ascorbic acid with IC\textsubscript{50} value of 5.8 ± 0.25 µg/mL. In reducing power assay, the activity of major constituent morelloflavone-7‴-O-β-D-glycoside was very close to that of the standard ascorbic acid (Supplementary Figure S5). The antioxidant activities of the glycosylated flavonoids are usually weaker than aglycones but bioavailability is sometimes enhanced by a glucose moiety (Ratty and Das 1988). Hydroxyl groups at the B ring is the most determinant structural feature for scavenging activity of flavonoids and the remarkable activity of compound 7 may be due to the 3‴,4‴-dihydroxyl unit present in the B ring (Bors et al. 1990).

### 3. Conclusions

This is the first report of the chemical constituents and antioxidant evaluation of G. travancorica. Seven constituents that include two polyisoprenylated benzophenones and five biflavonoids were isolated and characterised from G. travancorica leaves. The biflavonoids isolated, especially morelloflavone-7‴-O-β-D-glycoside, showed remarkable level of free radical scavenging activity in different in vitro assays. The study also highlights the plant as a rich source of the biflavonoid morelloflavone-7‴-O-β-D-glycoside by a validated HPTLC estimation method.

### Supplementary material

Experimental details are available online, along with Figures S1–S5.

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### Disclosure statement

No potential conflict of interest was reported by the authors.
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