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# Chemical constituents from *Oncocalyx glabratus* and their biological activities

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ABSTRACT

Chemical investigation of the aerial parts of *Oncocalyx glabratus* resulted in the isolation of three new flavan derivatives, 5,3′,4′-trihydroxyflavan 7-*O*-gallate (**1**), 5,4′-dihydroxyflavan 7-3′-*O*-digallate (**2**) and 5,3′-dihydroxyflavan 7-4′-*O*-digallate (**3**), named oncoglabrinol A, B and C, respectively, together with four known flavonols, (+)-catechin (**4**), (+)-catechin-7-*O*-gallate (**5**), catechin-7-4′-*O*-digallate (**6A**) and catechin-7-3′-*O*-digallate (**6B**). The structures of the compounds were established by 1D, 2D NMR and ESI-HRMS spectral analyses. The biological activity of the compounds was tested through a series of *in vitro* assays designed for determining cytotoxicity, antiviral activity against hepatitis B virus, and antidiabetic activity. All compounds were found non-toxic and showed moderate anti-HBV activity. Compounds **3** and **6** showed dual PPAR agonistic activity while others were not effective.

## 1. Introduction

*Oncocalyx glabratus* belongs to family *Loranthaceae* which is the largest family of flowering parasitic plants that includes about 70 genera and 1000 species (Calvin and Wilson, 2006). This family comprises epiphytic and hemiparasitic plants, most popularly known as mistletoe (Lorenzi, 2000). Mistletoes are known as “cure all” and have been found to have remedial effects for many health problems (Adodo, 2004), including diabetes (Obatomi et al., 1994).

*O. glabratus* is locally available in the Kingdom of Saudi Arabia (Collenette, 1999). A literature survey of the plant revealed antibacterial activity and phytochemical screening showed presence of reducing sugar, terpenoids, steroids, flavonoids and tannins (Waly et al., 2012).

The polyphenolic catechins such as epigallocatechin-3-gallate (EGCG), epicatechin-3-gallate (ECG), epigallocatechin (EGC), and epicatechin (EC), major constituents of green tea (*Camellia sinensis*), are reported to have antioxidative, anti-inflammatory, antitumor, antidiabetic and antibacterial activities *in vitro* and *in vivo* (Jin, 2013). Moreover, the antiviral efficacy of EGCG and ECG has been demonstrated against human immunodeficiency virus (HIV), human T-cell leukemia virus (HTLV), influenza virus, herpes simplex virus (HSV), rotavirus, adenovirus, Epstein–Barr virus (EBV), and hepatitis viruses (Jin, 2013).

We have recently reported hypoglycemic and antidiabetic activity of this plant (Ahmed et al., 2015). In the present study, we report on the isolation and structure elucidation of three new flavan derivatives, namely oncoglabrinol A (**1**), oncoglabrinol B (**2**) and oncoglabrinol C (**3**) (Fig. 1) from the active ethyl acetate extract of *O. glabratus*. In addition, this material also yielded four known compounds, (+)-catechin (**4**) (Nonaka et al., 1983), (+)-catechin-7-*O*-gallate (**5**) (Tanaka et al., 1983), catechin-7,4′-*O*-digallate (**6A**) and catechin-7,3′-*O*-digallate (**6B**) (El-Toumy and Mahdy, 2004). The cytotoxicity, antiviral activity against hepatitis B virus and antidabetic activity of the isolates is also reported.

## 2. Results and discussion

Compound **1** was obtained as yellowish color sticky material with the molecular formula $C_{22}H_{18}O_9$ determined by ESI-HRMS. The $^1$H and $^{13}$C NMR assignments of **1** (Table 1) were based on the COSY, HSQC, and HMBC spectra. The $^{13}$C NMR and DEPT spectra confirmed the presence of twenty-two carbons: twelve quaternary, two methylene and eight methine carbons. The $^1$H and $^{13}$C NMR spectra (Table 1) showed two aromatic signals [$\delta_H$ 6.68 (d, $J$ = 7.7 Hz, 1H, H-6′), 6.73 (d,

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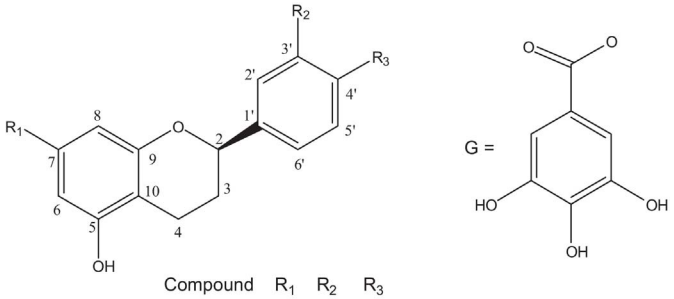


| Compound | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| **1** | G | OH | OH |
| **2** | G | G | OH |
| **3** | G | OH | G |

**Fig. 1.** Structure of new compounds **1–3**.

**Table 1**
$^{1}$H NMR (700 MHz) and $^{13}$C NMR (175 MHz) data of compound **1–3** in DMSO-$d_6$.

| No. | **1** | | **2** | | **3** | |
|---|---|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ (*J* in Hz) | $\delta_C$ | $\delta_H$ (*J* in Hz) | $\delta_C$ | $\delta_H$ (*J* in Hz) |
| 2 | 77.4 | 4.87 d (9.8) | 77.2 | 4.97 d (8.4) | 77.2 | 5.04 d (7.0) |
| 3 | 29.1 | 2.09 m, 1.88 m | 29.1 | 2.13 m, 1.97 m | 29.0 | 2.17 br s, 1.94 br s |
| 4 | 19.7 | 2.60 m | 19.6 | 2.63 m | 19.7 | 2.66 br s |
| 5 | 156.4 | – | 156.4 | – | 156.2 | – |
| 6 | 101.3 | 6.11 s | 101.4 | 6.19 s | 101.3 | 6.17 s |
| 7 | 150.0 | – | 150.0 | – | 150.1 | – |
| 8 | 100.9 | 6.18 s | 101.0 | 6.15 s | 101.1 | 6.21 s |
| 9 | 156.5 | – | 156.4 | – | 156.5 | – |
| 10 | 107.4 | – | 107.3 | – | 107.4 | – |
| 1′ | 132.8 | – | 132.6 | – | 140.2 | – |
| 2′ | 114.2 | 6.80 s | 121.8 | 7.15 s | 114.8 | 7.02 s |
| 3′ | 145.5 | – | 138.9 | – | 149.4 | – |
| 4′ | 145.4 | – | 149.2 | – | 138.7 | – |
| 5′ | 115.7 | 6.73 d (8.4) | 117.0 | 6.97 d (5.6) | 123.7 | 7.07 merge in 7.09–7.06 |
| 6′ | 117.7 | 6.68 d (7.7) | 124.9 | 7.16 br s | 117.2 | 6.89 br s |
| 7-*O*-galloyl | | | | | | |
| 1″ | 118.8 | – | 118.8 | – | 118.9 | – |
| 2″, 6″ | 109.5 | 7.05 s | 109.4 | 7.05 s | 109.4 | 7.09 s |
| 3″, 5″ | 146.2 | – | 146.2 | – | 146.2 | – |
| 4″ | 139.4 | – | 139.4 | – | 139.3 | – |
| 7″ | 164.7 | – | 164.6 | – | 164.7 | – |
| 3′-*O*-galloyl | | | | | | |
| 1‴ | | | 118.9 | – | | |
| 2‴, 6‴ | | | 109.6 | 7.09 s | | |
| 3‴, 5‴ | | | 146.1 | – | | |
| 4‴ | | | 139.5 | – | | |
| 7‴ | | | 164.9 | – | | |
| 4′-*O*-galloyl | | | | | | |
| 1⁗ | | | | | 118.8 | – |
| 2⁗, 6⁗ | | | | | 109.6 | 7.06 s |
| 3⁗, 5⁗ | | | | | 146.1 | – |
| 4⁗ | | | | | 139.6 | – |
| 7⁗ | | | | | 164.9 | – |
| OH-5 | | | | 9.76 s | | |
| OH-4′ | | | | 9.78 s | | |
| OHs | | | | 9.41 s, 9.12 s | | 9.79 s, 9.40 s, 9.13 s |

*J* = 8.4 Hz, 1H, H-5′); $\delta_C$ 117.7 (C-6′), 115.7 (C-5′)], showing COSY correlation suggested that the two methine carbons were adjacent to each other. The signals resonating at [$\delta_H$ 6.80 (s, 1 H, H-2′); $\delta_C$ 114.2 (C-2′)] and the three quaternary carbons at $\delta_C$ 132.8 (C-1′), 145.5 (C-3′) and 145.4 (C-4′) suggesting the B ring of flavan. The down field shift of C3′ ($\delta_C$ 145.5) and C4′ (145.4) suggested the presence of hydroxyl group (Ahmed et al., 2014). Other signals at $\delta_H$ 1.88 (m, 1H, Ha-3), 2.09 (m, 1H, Hb-3), $\delta_C$ 29.1 (C-3); $\delta_H$ 2.60 (m, 2H, H-4), $\delta_C$ 19.7 (C-4) and 4.87 (d, *J* = 9.8 Hz, 1H, H-2); $\delta_C$ 77.4 (C-2) suggested the occurrence of sets of two methylene groups and one oxygenated methine moiety in ring C (Li et al., 2006). Additionally Ha-3 was showing COSY correlations with the geminal Hb-3 and both Ha/b-3 showing COSY correlations with vicinal H-2 and $H_2$-4. Furthermore the $^{1}$H NMR spectrum displayed two singlets at $\delta$ 6.11 (1H, s, H-6) and 6.18 (1H, s, H-8), which were consistent with a 5,7-dioxygenated A ring of flavan (Al-Taweel et al., 2012). The down field chemical shift of C-5 ($\delta_C$ 156.4) suggested the presence of OH group at C-5 position in ring A (Lee et al., 1992). Two methine aromatic carbons [$\delta_H$ 7.05 (s, 2 H); $\delta_C$ 109.5, 2 C] and five quaternary carbons ($\delta_C$ 139.4, 146.2 × 2, 118.8 and 164.7) indicating the presence of galloyl moiety (Al-Taweel et al., 2012). Up field shift of C-7 ($\delta_C$ 150.0) carbon as well as downfield shift of C-6 ($\delta_C$ 101.3), C-8 ($\delta_C$ 100.9) and C-10 ($\delta_C$ 107.4) and comparison of the data with 7-*O*-galloyl catechin (Tanaka et al., 1983), 5-*O*-galloyl catechin (Al-Taweel et al., 2012) and 7-*O*-galloyltricetiflavan (Li et al., 2006) in conformity of the attachment of galloyl group at C-7 position. On comparison of the NMR spectra, compound **1** was found closely similar to that of 7-*O*-galloyl tricetiflavan (Li et al., 2006) except that in **1**, OH-5′ was absent, thus presenting compound **1** as a new compound, named as oncoglabrinol A.

Compound **2** was obtained as colorless sticky material and its molecular formula, $C_{29}H_{22}O_{13}$ was determined by ESI-HRMS. The $^{1}$H and $^{13}$C NMR spectra of compound **2** directed a close structural similarity with that of **1** except one additional signal in $^{1}$H NMR at $\delta_H$ 7.09 (s) and five carbon signals at $\delta_C$ 118.9, 109.6, 146.1 and 139.5, 164.9 in $^{13}$C NMR and difference in chemical shifts of the B-ring of **2** as compared to that of **1** indicating the presence of one more galloyl moiety. The position of galloyl moiety at C-3′ was established by the up field shift of C-3′ ($\delta_C$ 138.9). Also due to the ester linkage at C-3′ position, the C-2′ appeared downfield at $\delta_C$ 121.8 which was in consistent with the previously reported data (El-Toumy and Mahdy, 2004). Compound **2** is also representing a new compound and named as oncoglabrinol B.

Compound **3** was obtained as colorless sticky material with the molecular formula, $C_{29}H_{22}O_{13}$ as determined by ESI-HRMS. The $^{1}$H and $^{13}$C NMR spectra of **3** have a very close structural similarity with that of **2** except the position of galloyl moiety at C-4′ in **3** instead of at C-3′ as in **2**. Due to this C-1′, C-2′, C-5′ and C-6′ appeared at $\delta_C$ 140.2, 114.8, 123.7 and 117.2, respectively. This was also demonstrated by the down field shift of C3′ ($\delta_C$ 149.4) which was in agreement with earlier described data having close structure similarities (El-Toumy and Mahdy, 2004). In this series compound **3** is also new and named as oncoglabrinol C. The configuration of C-2 for compounds **1–3** was defined as *β*, by comparing optical rotation values with that of 7-*O*-galloyltricetifavan and 7,4′-di-*O*-galloyltricetifavan (Li et al., 2006).

During the course of isolation of the above compounds, ethyl acetate extract of *O. glabratus* also yielded four known compounds. These compounds were identified by comparison of their physical and spectroscopic data with those reported in the literature. Upon subjecting compounds **1–6** to anti-HBV activity on cultured HepG2.2.15 cells by MTT assay at a concentration range of 6.25–100 μg/mL, all of them were non-cytotoxic and inhibited expression of HBsAg level at 100 μg/mL but not at 50 μg/mL. Nevertheless, all the compounds had mild antiviral activities (31–37% inhibition), compared to the standard drug lamivudine (Fig. 3). Although these compounds showed the anti-HBV activity at lower efficacy, further structural modifications may enhance their antiviral potential.

The ethyl acetate fraction of *O. glab*ratus was found to show agonistic activity toward both PPARα and PPARγ. The activation of PPARγ (5.35 fold) was stronger than the activation of PPARα (2.6 fold) at 100 μg/mL. Among the six compounds isolated from this fraction, two compounds **3** and **6** showed activation of both PPARα and PPARγ with a fold induction of 2 or higher at 50 μg/mL while others were inactive. As shown in Table 2 the effect was more pronounced on