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SHORT COMMUNICATION

Diverse Glycosides from *Gardenia latifolia* with Antiviral Activity and Chemosystematic Significance

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Abstract

Several influenza pandemics have impacted our life, each with variable prevalence and severity. In a search for natural antivirals, further phytochemical investigation of *Gardenia latifolia* Aiton, Rubiaceae, was conducted. As a result, five structurally diverse glycosides were isolated, offering valuable chemotaxonomic data. Using the crystal violet technique, three isolates, canthoside C, (6*R*,7*S*,8*S*)-7α-[(β-D-glucopyranosyl) oxy] lyoniresinol, and ecdysanrosin A, were evaluated for their anti-influenza A (H1N1) activities. Based on previously reported anti-inflammatory activity of the guaiane class, we investigated the inhibitory effect of (1*R*,7*R*,8*S*,10*R*)-7,8,11-trihydroxy-guai-4-ene-3-one 8-*O*-β-D-glucopyranoside, a rare guaiane sesquiterpene glucoside, on inducible nitric oxide (NO) production by Griess assay. Regarding antiviral assay, canthoside C was the most active. It considerably inhibited H1N1 infectivity at an IC_{50} value of 10.93 µg/ml, showing a selectivity index (SI) of 12.88, compared with acyclovir as a standard. Besides, ecdysanrosin A displayed a moderate selective antiviral activity with an IC_{50} value of 28.03 µg/ml. Considering their low cytotoxicity on the host cells, canthoside C and ecdysanrosin A have additional merit as potential antiviral agents. Despite the claimed anti-inflammatory activity of guaianes, (1*R*,7*R*,8*S*,10*R*)-7,8,11-trihydroxy-guai-4-ene-3-one 8-*O*-β-D-glucopyranoside showed a limited anti-inflammatory activity.

Keywords Phenolic diglycosides · Cytopathic effect inhibition · Chromatographic separation · Spectral analysis · RAW 264.7 macrophages · Madin-Darby canine kidney cells

Introduction

Gardenia genus comprises about 140 species that are found in warm and tropical regions (Wong and Low 2011). The fruit of some species is edible in China; moreover, five species are listed in Flora of China and traditionally prescribed as sedative, diuretic, cholagogue, antipyretic, and anti-inflammatory drugs (Yu et al. 2011; Yin and Liu 2018). To date, a few reliable phytochemical studies on *Gardenia latifolia* Aiton, Rubiaceae, Indian boxwood, were conducted. Previous studies revealed the presence of terpenoids, steroids, flavonoids, phenolic acids, tannins, and saponins (Reddy et al. 1975, 2021). Our earlier study on *G. latifolia* reported cytotoxic phytochemicals (Selim et al. 2022). Moreover, a recent study has demonstrated a marked anti-hyperglycemic activity of *G. latifolia* extract in type-2 diabetic rats (Alshabi and Shaikh 2022). Thus, we aimed to expand the phytochemical knowledge regarding the polar fraction, as a part of the continuing search for biologically active phytoconstituents. In consequence, five compounds were isolated and identified, for the first time, from this species. The structural diversity of these isolated phytoconstituents introduces many activities to be explored. Among these activities, antiviral properties of two isolated phenolic diglycosides, canthoside C (**1**) and ecdysanrosin A (**4**), and the lignan **3** were evaluated against influenza A (H1N1) virus, for the first time. As statistically estimated, influenza pandemics emerge three to four times every century (Morens et al. 2010). During

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2009, swine flu pandemic, influenza A (H1N1), a current seasonal influenza virus, was a leading morbidity cause (Franco-Paredes et al. 2009). In this regard, *Gardenia jasminoides* fruit has proven to inhibit influenza A infection in MDCK cells and mice, through viral replication suppression (Guo et al. 2020). Depending on these data and seeking new antiviral agents, the present study aimed to investigate the antiviral properties of **1**, **3**, and **4** against H1N1. In addition, compound **2** was examined for its anti-inflammatory activity, justified by previous guaiane class biological properties.

Materials and Methods

The general experimental procedures are included in (Supporting Information, S1). The aerial parts of *Gardenia latifolia* Aiton, Rubiaceae, were collected in May 2017 from Aswan Botanical Garden, Aswan, Egypt (24° 05′ 26.95″ N 32° 53′ 57.91″ E). The plant was authenticated by Dr. Hafeez Rofaeel. A voucher specimen (292,002) has been deposited at the Herbarium of Flora and Phytotaxonomy Research, Horticultural Research Institute, Agricultural Research Center, Dokki (Cairo), Egypt. The 70% methanolic extract of the aerial parts was suspended in water then fractionated successively with hexane, DCM, and EtOAc. Part of the remaining dried aqueous residue (15 g) was further fractionated using Diaion HP-20 (500 g) and eluted with 100% distilled water then a gradient MeOH in H_2O mixtures till 100% MeOH to give seven subfractions (A1–A7). Subsequent chromatographic procedures on these subfractions afforded compounds (**1**–**5**) (Supporting Information, S2). The physicochemical and spectral analysis of the isolated compounds are also included (Supporting Information, S3 and Fig. S1–S17).

The *in vitro* antiviral activity of **1**, **3**, and **4** was evaluated against influenza A virus (H1N1), applying the cytopathic effect (CPE) inhibition–based protocol (Schmidtke et al. 2001), as described in (Supporting Information, S4). Concurrently with antiviral assay, the cytotoxic effects of **1**, **3**, and **4** were examined, via crystal violet method (Supporting Information, S5). The anti-inflammatory potential of **2** was measured by evaluating the inhibitory effect of this compound on NO production in LPS-activated RAW 264.7 macrophages. The detailed procedures of the assay are provided (Supporting Information, S6).

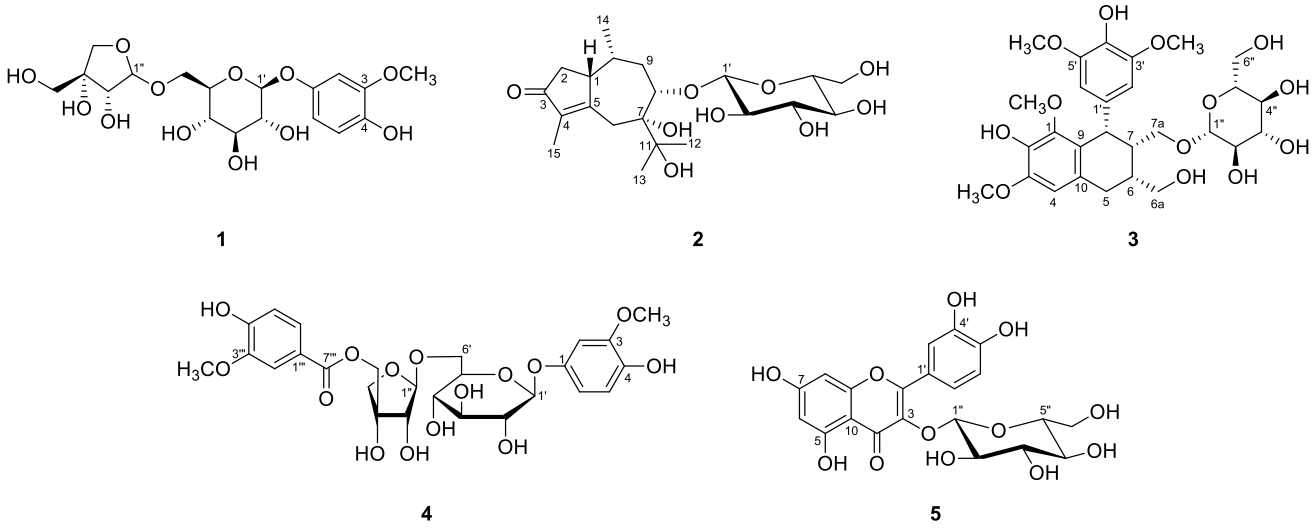
Results and Discussion

Five specialized metabolites belonging to different chemical classes were isolated after repeated chromatographic procedures on the polar extract of *G. latifolia*. The structures of these isolates were elucidated throughout spectral analysis (Fig. S1–S17), which were consistent with the corresponding literature. Compounds **1** and **4** are phenolic diglycosides. 4-Hydroxy-3-methoxyphenyl-6-*O*-β-D-apiofuranosyl-β-D-glucopyranoside (**1**), trivially named canthoside C, was isolated twice from family Rubiaceae (Kanchanapoom et al. 2002; Zhou et al. 2016). However, this is its first report from the genus *Gardenia*. Compound **4** is a hydroquinone diglycoside acyl ester, named ecdysanrosin A (Zhu et al. 2010). Interestingly, this is its first report from family Rubiaceae.

Compound **2**, (1*R*,7*R*,8*S*,10*R*)-7,8,11-trihydroxy-guai-4-ene-3-one 8-*O*-β-D-glucopyranoside, is a guaiane-type sesquiterpene glucoside that has been isolated once from the closely related species *G. jasminoides* (Machida et al. 2000). Yu and co-workers identified two closely related compounds from the same species (Yu et al. 2011). Consequently, this implies the importance of this scaffold as a chemotaxonomic marker for the genus *Gardenia*, even though most guaiane-type sesquiterpenes are widely distributed in the Asteraceae family, especially among the Anthemideae, Cichorieae, Inulae, and Eupatorieae tribes (Fraga 2012).

Compound **3**, (6*R*,7*S*,8*S*)-7α-[(β-D-glucopyranosyl) oxy] lyoniresinol, is an aryltetralin lignan, previously isolated from several families such as Lauraceae (Zeng et al. 2014) and Meliaceae (Fang et al. 2010). However, this is its first report from family Rubiaceae.

Compound **5**, isoquercitrin, possesses several biological activities (Valentová et al. 2014). It was recently detected through LC-MS-MS analysis of *G. latifolia* fruits (Reddy et al. 2021). According to a study led by Kim et al. (2010), isoquercitrin can block influenza A infection through inhibition of viral replication, in MDCK cells and mice. Moreover, isoquercitrin is an anti-inflammatory compound, acting by various mechanisms of action (Lee et al. 2008; Soberón et al. 2010; Kim et al. 2014). Lee et al. (2008) have found that isoquercetrin suppresses nitric oxide synthase expression and reduces nitrite production, in stimulated rat peritoneal macrophages. Since previous studies have proven the antiviral and anti-inflammatory activities for **5**, it was excluded from our investigation.



Compounds **1**, **3**, and **4** were chosen to be tested for anti-influenza A activities. As shown in Table 1, the phenolic diglycosides (**1** and **4**) exhibited moderate antiviral activities with IC_{50} values of 10.93 and 28.03 µg/ml, respectively, compared with acyclovir as a positive control. It is interesting that the present study investigates the antiviral activities for this chemical class for the first time. Compound **1**, canthoside C, showed low cytotoxicity on the MDCK host cells with a CC_{50} value of 140.8 µg/ml and a SI of 12.88. Likewise, ecdysanrosin A (**4**) demonstrated a selective antiviral activity with a low cellular toxicity (SI of 13). Such low cytotoxic effects of compounds **1** and **4**, on the normal cells, reinforce their therapeutic values as antivirals. In contrast, compound **3** showed a low antiviral activity and a nonselective cytotoxicity. The investigated compounds **1** and **4** showed high CC_{50} values, as shown in Table 1, implying their low cytotoxic effects on uninfected MDCK host cells, and excluding their nonspecific activities. On the other hand, **3** exhibited a relatively high cytotoxic effect, with a low SI of 3.5.

Numerous guaiane-type sesquiterpenes have proven to possess anti-inflammatory activity, through inhibition of nitric oxide (NO) production cells (Cheng et al. 2020; Liu et al. 2020). Therefore, we aimed to investigate the probable activity of our related compound **2**. Despite these previous studies, weak anti-inflammatory activity was observed for **2**. It induced only 20.3% inhibition of LPS-inducible NO production at as high a concentration as 100 µM.

Table 1 Cytotoxic effects and anti-influenza A (H1N1) activities of compounds **1**, **3**, and **4** in MDCK cells

Sample	CC_{50} (µg/ml)	IC_{50} (µg/ml)	SI
1	140.80	10.93	12.88
3	99.50	28.53	3.5
4	365.97	28.03	13
Acyclovir	124	6.131	20.22

Conclusion

Five diverse glycosides were isolated from the polar extract of *G. latifolia* aerial parts. Canthoside C (**1**) demonstrated a significant antiviral activity (IC_{50} value 10.93 µg/ml and SI 12.88) against human influenza A virus (H1N1), with a low cytotoxicity on the host MDCK cells. In addition, ecdysanrosin A (**4**) displayed a moderate antiviral activity with an IC_{50} value of 28.03 µg/ml and SI of 13. Therefore, these phenolic diglycosides have potential anti-influenza activity that warrant further *in vivo* studies to be verified.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s43450-022-00335-w.

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Author Contribution SMM and MAMA conducted the phytochemical investigation, analyzed the spectral data, and wrote the manuscript. SAR supervised the project, provided funding support, and proofread the manuscript. All of the authors approved the final version of the manuscript.

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