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Three new phloroglucinol derivatives from the aerial parts of *Pogostemon auricularius* and their cytotoxic activity

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ABSTRACT

Pogostemonons A–C (**1**–**3**), three new phloroglucinol derivatives were isolated from the aerial parts of *Pogostemon auricularius* (L.) Hassk. Their chemical structures were established by 1D, 2D NMR and HRESIMS data. These compounds showed weak to moderate growth inhibitory activities against lung adenocarcinoma (LU-1), oral epidermoid carcinoma (KB), liver hepatocellular carcinoma (Hep-G2), colon adenocarcinoma (SW-480), and gastric adenocarcinoma (AGS) cell lines with IC_{50} values ranging from 39.80 ± 4.39 to 98.32 ± 8.94 μg/mL.

1. Introduction

Pogostemon auricularius (L.) Hassk. is an annual herb belonging to the Lamiaceae family and broadly distributed in India, Sri Lanka, Bangladesh, China and Southeast Asia (Wu and Raven, 1999; Phuong, 2000). The aerial parts of this plant have been used in traditional Asian folk medicine to treat diarrhea, snakebites, rheumatism, and reduce fever (Chi, 2000; Valkenburg and Bunyapraphatsara, 2001; Quattrocchi, 2012). In addition, its leaves are used to relieve stomach ache and urinary problems in children (Valkenburg and Bunyapraphatsara, 2001). Moreover, the extracts from *P. auricularius* have shown several biological activities such as microbial resistance, anti-inflammatory, thrombolytic activity, α-amylase inhibitor and anti-diarrhea (Nasir et al., 2015; Nur et al., 2015). However, the knowledge about the chemical composition of *P. auricularius* is modest. Previous phytochemical studies of this plant have led to the isolation of four diterpenes, including cleistanth-13,15-dien-19-oic acid (auricularic acid) (Prakash et al., 1987), 7-hydroxyclethylthio-13,15-diene-18-oic acid, 7-acetoxyclithiol-13,15-diene-18-oic acid, and (4*R*,5*S*,8*R*,9*S*,10*S*,14*R*)-cleistanth-12,15-diene-19-oic acid (Hussaini et al., 1993). Three new cytotoxic meroterpenoids pogostemins A–C have been found from *P. auricularius* by us (Nguyen et al., 2018), also the chemical composition of the essential oil of *P. auricularius* growing in Viet Nam has been studied (Satyal et al., 2018).

This study reported the extraction and isolation of three new compounds, phloroglucinol derivatives pogostemonons A–C (**1–3**) from the aerial parts of *P. auricularius*. The findings also include details about the chemical structure elucidation and cytotoxic activity of these compounds.

2. Results and discussion

2.1. Spectral and physical data of new isolated compounds

2.1.1. Pogostemonon A (1)

Compound **1** was isolated as a yellow powder. Its molecular formula was established as $C_{23}H_{28}O_7$ from the positive mode HRESIMS quasi-molecular ion at *m/z* 417.1906 $[M+H]^+$ (calcd. for $C_{23}H_{29}O_7$, 417.1913). In the ^{1}H-NMR spectrum, an olefin proton at δ_H 5.75 (1H, qq, J = 1.5, 7.0 Hz, H3′), and a sextet signal at δ_H 4.09 (1H, sex, J = 6.5, H-2″) were assigned. In the high field, the signals of six methyl groups were identified at δ_H 1.98 (3H, s, H-14), 1.93 (3H, s, H-15), 1.89 (3H, t-like, J = 1.5 Hz, H-1′), 1.79 (3H, dd-like, J = 1.0, 7.0 Hz, H-4′),

Abbreviations: AGS, gastric cancer; DMSO, dimethyl sulfoxide; Hep-G2, hepatoma cancer; KB, epidermoid carcinoma; LU-1, lung cancer; SRB, sulforhodamine B; SW-480, human colon adenocarcinoma

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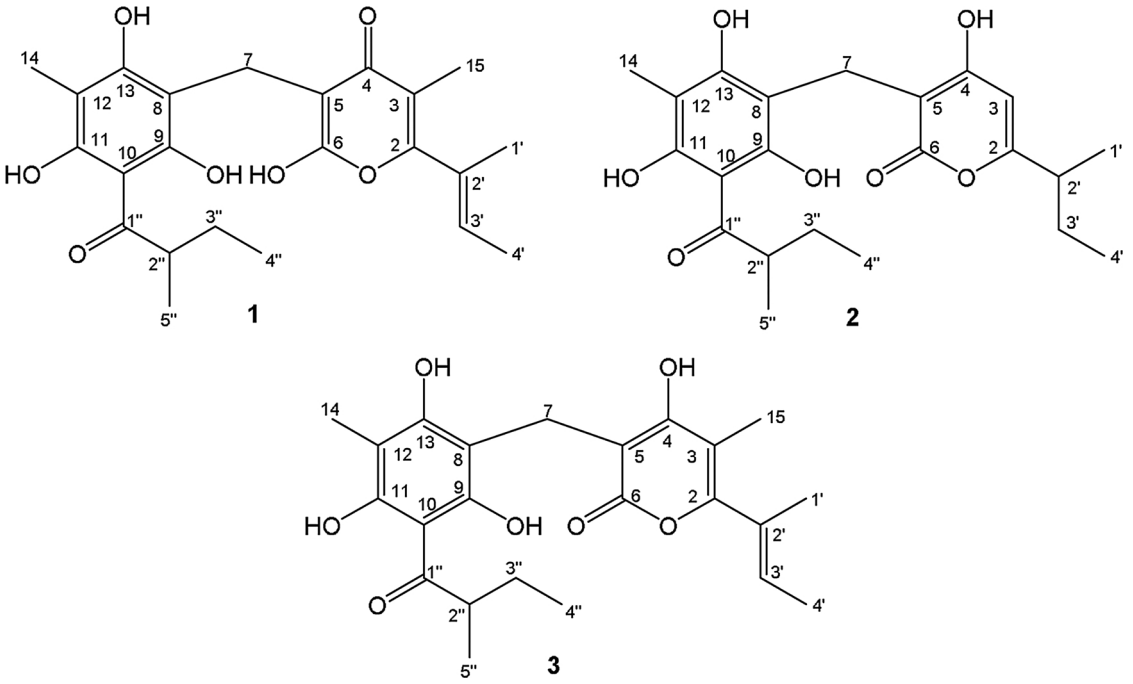

Fig. 1. Chemical structures of **1–3** from the aerial parts of *Pogostemon auricularius*.

1.15 (3H, d, J = 6.5 Hz, H-5″), and 0.92 (3H, t, J = 7.5 Hz, H-4″). In addition, the signal of one isolated methylene group was observed at δ_H 3.55 (2H, br s, H-7). Combination of the ^{13}C-NMR and HSQC spectra of **1** indicated that this compound contained 23 carbon atoms including 6 methyls, 2 methylenes, 2 methines groups and 13 non-protonated carbons. It is possible to predict the presence of some specific groups such as two carbonyl carbons at δ_C 212.4 (C-1″), and 180.7 (C-4), in addition to a methyl carbon that attached to the aromatic ring at δ_C 7.9 (C-14). Notably, the carbonyl signal at δ_C 180.7 (C-4) located toward high field in the ^{13}C-NMR spectrum suggested that the presence of enone system in **1**. Based on the analysis of the above spectrum data, **1** was proposed as a derivative of phloroglucinol (Ishiguro et al., 1987). In the HMBC spectrum, the correlations of H-4″ (δ_H 0.92)/H-5″ (δ_H 1.15) to C-2″ (δ_C 46.6)/C-3″ (δ_C 28.3), of H-5″ to C-1″ (δ_C 212.4) confirmed the presence of 1-oxo-2-methylbutyl group. The methyl group was located at C-12 of the benzene ring basing on the HMBC correlations between δ_H 1.98 (3H, H-14) with δ_C 162.7 (C-11), 104.7 (C-12), and 163.0 (C-13) (Fig. 2). Similarly, the HMBC correlations between H-1′ (δ_H 1.89)/H-4′ (δ_H 1.79) and C-2′ (δ_C 130.7)/C-3′ (δ_C 130.6) confirmed for the presence of the 1-methylprop-1-enyl fragment. Next, the configuration of C-2′/C-3′ double bond was identified by comparing the coupling constants values of H-1′, H-3′ and H-4′ in **1** with those of tiglic and angelic acids and their derivatives (Fraser, 1960; Joseph-Nathan et al., 1984). We notice that the $J_{H\text{-}1'/H\text{-}3'}$ value is larger than $J_{H\text{-}1'/H\text{-}4'}$ in the *E* configuration, and *vice versa*. The $J_{H\text{-}1'/H\text{-}3'}$,$J_{H\text{-}1'/H\text{-}4'}$ values of **1** were found as 1.5 and 1.0 Hz, respectively. Therefore, the *E* configuration was assigned for C-2′/C-3′ double bond of **1**. The 1-methylprop-1-enyl chain was further attached to C-2 by the observation of the HMBC correlations between H-1′ (δ_H 1.89)/H-3′ (δ_H 5.75) and C-2 (δ_C 160.0). The remain methyl singlet signal was located at C-3 based on interaction between δ_H 1.93 (3H, H-15) and C-2 (δ_C 160.0), C-3 (δ_C 113.9), and C-4 (δ_C 180.7) (Fig. 2). The linkage between phloroglucinol (A) and γ-pyrone (B) moieties was suggested through the methylene bridge (C(7) H_2). This suggestion was possibly deduced by the strong downfield shift of H-7 (δ_H 3.55) compared to normal methylene position (Bohlmann et al., 1980; Hänsel et al., 1980). Surprisingly, the signal of methylene protons (H-7) was very broad and exhibited the weakly correlations with C-5 (δ_C 101.7)/C-6 (δ_C 170.7) in the HMBC spectrum. This unusual phenomenon might be due to the slow rotation around the methylene bridge of two ring systems (Appendino et al., 2007). Consequently, compound **1** was elucidated as a new phloroglucinol derivative, and was named pogostemonon A.

2.1.2. Pogostemonon B (2)

Compound **2** was obtained as a white amorphous powder. Its molecular formula was established as $C_{22}H_{28}O_7$ from the positive mode HRESIMS quasi-molecular ion at m/z 405.1909 $[M+H]^+$ (calcd. for $C_{22}H_{29}O_7$, 405.1913). The ^{1}H-NMR spectrum of **2** showed typical singlet signal of a tertiary methyl group [δ_H 2.01 (3H, s, H-14)], two secondary methyl groups [δ_H 1.23 (3H, d, J = 7.0 Hz, H-1′), 1.15 (3H, d, J = 6.5 Hz, H-5″)], two terminal methyl groups [δ_H 0.91, 0.92 (3H, each, J = 7.0 Hz, H-4′ and H-4″)] as well as one olefinic proton [δ_H 6.08 (1H, s, H-3)]. Analysis of the ^{13}C-NMR and HSQC spectra of **2** revealed 22 signals for five methyls, three methylenes, three methines, and eleven non-protonated carbons. The assignments of ^{1}H and ^{13}C-NMR data of **2** were completed with the aid of HSQC and HMBC (Fig. 2). These NMR data were well agreed with those of **1**, especially in the phloroglucinol (A) moiety. However, the ^{13}C chemical shifts of the pyrone ring of **2** [δ_C 169.5 (C-2), 102.2 (C-3), 170.0 (C-4), 103.0 (C-5), and 171.3 (C-6)] (numbering according to **2**) were similar to those of 6-(2-butyl)-3-ethyl-4-hydroxy-2-pyrone (germicidin) [δ_C 165.9 (C-2), 99.6 (C-3), 167.2 (C-4), 104.7 (C-5), and 167.5 (C-6)] isolated from the supernatant of submerged culture of *Streptomyces viridochromogenes* NRRL (Petersen et al., 1993) suggesting that **2** contained a α-pyrone ring. This was supported by the HMBC correlations from H-7 (δ_H 3.63) to C-4 (δ_C 170.0)/C-5 (δ_C 103.0)/C-6 (δ_C 171.3), from H-3 (δ_H 6.08, br s) to C-2 (δ_C 169.5)/C-5 but not C-6 (Fig. 2). Furthermore, the cross-peaks of H-1′ (δ_H 1.23)/H-4′ (δ_H 0.91) to C-2′ (δ_C 40.9)/C-3′ (δ_C 28.5), of H-1′/H-2′ (δ_H 2.54)/H-3′ (δ_H 1.58, 1.70) to C-2 in the HMBC spectra allowed the direct attachment of *sec*-butyl group to the α-pyrone ring at C-2 (numbering according to **2**). Thus, the structure of **2** was elucidated as shown in Fig. 1, and was named pogostemonon B.

2.1.3. Pogostemonon C (3)

Compound **3** was obtained as a white amorphous powder and its molecular formula was determined as $C_{23}H_{28}O_7$ from the NMR and HRESIMS data. The 1DNMR interpretation of **3** in combination with the aid of HSQC and HMBC showed that this compound is almost similar to