QUADRANGULARIC ACID E AND 5,7,3′-TRIHYDROXY-6,4′,5′-TRIMETHOXYFLAVONE ISOLATED FROM ETHYL ACETATE EXTRACT OF *GARDENIA PHILASTREI* IN BA RIA – VUNG TAU

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TÓM TẮT

QUADRANGULARIC ACID E VÀ 5,7,3′-TRIHYDROXY-6,4′,5′-TRIMETHOXYFLAVONE PHÂN LẬP TỪ CẶN CHIẾT ETHYL ACETATE CỦA LÁ CÂY LOÀI *GARDENIA PHILASTREI* TẠI BÀ RỊA – VŨNG TÀU

Chi dành dành (Gardenia) là một chi có khoảng 140 loài khác nhau thuộc họ cà phê (Rubiaceae) phân bố tại nhiều nơi trên thế giới. Hiện tại, loài dành dành Gardenia philastrei vẫn chưa được các nhà khoa học trong nước và trên thế giới quan tâm nghiên cứu. Trong nghiên cứu này, từ dịch chiết ethyl acetate của lá cây G. philastrei thu hái tại tỉnh Bà Rịa – Vũng Tàu đã phân lập được hai hợp chất cycloartane triterpene quadrangularic acid E và flavonoid 5,7,3′-trihydroxy-6,4′,5′-trimethoxyflavone. Cấu trúc hóa học của hai hợp chất được xác định thông qua phương pháp phổ NMR. Đây là lần đầu tiên hai hợp chất này được phát hiện ở loài G. philastrei. Các nghiên cứu trước đây cho thấy hai hợp chất thể hiện nhiều hoạt tính sinh học tiềm năng.

Keywords*: Flavonoid, Gardenia philastrei, quadrangularic acid E, 5,7,3′-trihydroxy-6,4′,5′-trimethoxyflavone, triterpenoid.*

1. INTRODUCTION

Gardenia philastrei belongs to the genus *Gardenia*, a member of Rubiaceae family consisting of approximately 250 flowering plant species, native to the tropical and subtropical regions of Africa, Asia, Madagascar and Pacific islands [1-2]. *G. philastrei* is mainly distributed in Vietnam and Cambodia [3]. Currently, the studies on this species are still limited, although numerous phytochemical and pharmacological research on several species in the genus have been conducted. Various potential activities were reported such as anti-inflammatory activity [4], neural protection activity [5] or blood glucose control in type 2 diabetes [6] and hepatoprotection or anti-depression [7] from *G. jasminoides*; nervous disorders treatment and anti-bacterial activity from

G. gummifera [8] or treatment of gastric diseases and infertility [9].

Because of precious applications in human health care, various studies on phytochemicals of Gardenia sp. species have been published. Various secondary metabolites were purified belonging to terpene, flavonoid, steroids, and other organic acids classes [7]. Some of them showed potential applications in pharmacy, for instance, crocins or geniposide for diabetic and antioxidant medicine therapy [10-11]. Due to the potentiality of *Gardenia* genus, the phytochemical constituent of *G. philastrei* was investigated. Herein, we report the isolation and structural identification of quadrangularic acid E **(1),** 5,7,3′-trihydroxy-6,4′,5′-trimethoxyflavone **(2)** from ethyl acetate extract of Vietnamese *G. philastrei* for the first time.

2. EXPERIMENT

2.1. General procedure

NMR spectra were recorded by a Bruker DRX 500 or Bruker AvanceNeo 600 MHz spectrometer (Germany) at the Institute of Chemistry, VAST. To calculate the chemical shift, tetramethyl silane was added as an internal reference. The coupling constants *(J)* were given in Hertz (Hz). Column chromatography (CC) was performed on silica gel 100 (63-200 μm) and Sephadex LH-20, (Merck, Ltd). Precoated silica gel 60 F_{254} (Merck, Ltd) was used for thin-layer chromatography (TLC). TLC plates were visualized with the vanillin sulfuric acid reagent in combination with heating.

2.2. Plant materials

Gardenia philastrei Pierre ex Pit. leaves were collected at Binh Chau-Phuoc Buu Nature Reserve, Ba Ria-Vung Tau province, Vietnam in May 2020. Samples were identified by botanist Le Van Son (VAST), A voucher specimen (GP2020-1) was deposited at the Center for High Technology Research and Development, VAST.

2.3. Extraction and isolation

The air-dried *G. philastrei* leaves (4.0 kg) were extracted with 95% methanol at room temperature 3 times (material: solvent ratio of 1:4 (w/w) by maceration method in combination with ultrasound supporting. The solvent was vacuum evaporated to give 792 g extract. The extract was dispersed in water and consecutively partitioned with n-hexane, dichloromethane (DCM), and ethyl acetate (EtOAc). The EtOAc extract (235 g) was fractioned to yield 8 fractions (Fr.1-Fr.8) by using silica gel chromatography with CH_2Cl_2–MeOH gradient solvent system (99:1 to 0:100) as an eluent.

Fr. 4 was fractionated on silica gel column with DCM/MeOH gradient solvent system (99:1 to 95:5) as an elution system to yield 8 sub-fractions Fr.4.1 to Fr.4.8. Compound **1** (9.3 mg) was obtained from Fr.4.6 fraction through Sephadex LH-20 chromatography eluting with 100% MeOH combined with preparative TLC eluting with DCM/MeOH/acetic acid 98:2: 0.01%.

The Fr.2 was partitioned on a silica gel column with *n*-hexane/EtOAc gradient (2:1 to 3:5) as a mobile phase to give Fr.2.1 to Fr.2.8 fractions. Fraction Fr.2.8 was separated on silica gel column with DCM/MeOH gradient (99:1 → 80:10) as elution to obtain 5 fractions F.r.2.8.1 →F.r.2.8.5. Compound **2** (11.5 mg) was obtained from F.r.2.8.1 fraction by Sephadex LH-20 column chromatography eluting with 100% MeOH combined with preparative TLC eluting with DCM/MeOH 90:10)

Quadrangularic acid E **(1):** White powder. ^{1}H-NMR (600 MHz, pyridine-d5) δ (ppm): 5.13 (m, H-3), 4.83 (s, H-31a), 4.81 (s, H-31b), 3.13 (m, H-2), 2.84 (dd, *J*= 12.0, 4.2 Hz, H-5), 2.53 (m, H-11), 1.71 (s, 3H-29), 1.27 (d, *J*= 3.5 Hz, H-19a), 1.04 (d, *J*= 5.5 Hz, 3H-26), 1,03 (d, *J*= 5.5 Hz, 3H-27), 0.99 (d, *J*= 3.5 Hz, H-19b), 0.97 (s, 3H-18), 0.89 (d, *J*= 7.5 Hz, H-21), 0,88 (s, 3H-30). ^{13}C-NMR (150 MHz, pyridine-d5) δ (ppm): 208.9 (C-1), 178.9 (C-28), 156.6 (C-24), 106.5 (C-31), 73.4 (C-3), 54.6 (C-4), 52.2 (C-17), 49.4 (C-14), 48.8 (C-2), 45.3 (C-13), 43.7 (C-8), 40.8 (C-5), 37.0 (C-10), 36.3 (C-20), 35.2 (C-22), 34.5 (C-15), 33.9 (C-25), 33.1 (C-12), 31.5 (C-23), 28.2 (C-19), 28.1 (C-9), 27.9 (C-7), 27.9 (C-11), 23.4 (C-16), 22.0 (C-6), 22.0 (C-27), 21.9 (C-26), 18.4 (C-18), 18.4 (C-30), 16. 9 (C-21), 10.5 (C-29).

5,7,3′-trihydroxy-6,4′,5′-trimethoxyflavone **(2):** Yellow powder. ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 13.00 (s, H5-OH), 7.16 (d, *J*= 2.0 Hz, H-

2′), 6.96 (d, *J*= 2.0 Hz, H-6′), 6.56 (s, H-3), 6.49 (s, H-8), 4.05 (s, 3H-OMe), 3.99 (s, 3H-OMe), 3.95 (s, 3H-OMe). ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 182.9 (C-4), 163.7 (C-2), 155.1 (C-5), 153.2 (C-7), 152.5 (C-9), 152.1 (C-5′), 149.7 (C-3′), 138.6 (C-4′), 130.4 (C-6), 126.9 (C-1′), 106.7 (C-10), 105.8 (C-2′), 105.0 (C-6′), 102.5 (C-3), 93.4 (C-8), 61.2 (OMe), 60.9 (OMe), 56.2 (OMe).

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white powder. Spectroscopic data expressed the cycloartane triterpene characteristics of compound **1**. The ^{13}C-NMR spectral of compound **1** showed 31 carbon signal of triterpene backbone including a carbonyl at δ_C 208.94 (C-1), a carboxylic group at δ_C 178.9 (C-28), two sp^2 carbons δ_C 156.6 (C-24) and δ_C 106.5 (C-31), three quaternary carbon atoms, nine methylene sp^3 atoms, five methine sp^3 atoms and an oxymethine atoms at δ_C 73.4 (C-3). In addition, ^{13}C-NMR spectroscopic data showed the signals of six methyl carbon, including δ_C 21. 9 (C-26), δ_C 22.0 (C-27), δ_C 18.4 (C-18), δ_C 16. 9 (C-21), δ_C 10.5 (C-29), δ_C 18.4 (C-30).

Together with the aliphatic proton signals typical for the triterpene backbone, the ^{1}H-NMR spectrum showed two doublet protons δ_H 1.27 (d, *J*= 3.5 Hz, H-19a) and δ_H 0.99 (d, *J*= 3.5 Hz, H-19b) specific for cyclopropane ring in cycloartanes triterpene skeleton. Six methyl protons were also recorded including δ_H 1.71 (s, 3H-29), δ_H 1.04 (d, *J*= 5.5 Hz, 3H-26), δ_H 1.03 (d, *J*= 5.5 Hz, 3H-27), δ_H 0.97 (s, 3H-18), δ_H 0.89 (s, 3H-21) and δ_H 0.88 (s, 3H-30). Two methyl groups H-26 and H-27 appeared as doublets with a coupling constant *J*= 5.5 Hz, demonstrating the isopropyl structure in compound **1**. Signals of two olefinic protons at low-filed of ^{1}H-NMR with the chemical shifts at δ_H 4.83 (s, H-31a) and δ_H 4.81 (s, H-31b) presented for an exomethylene group at C-24/C-31 position. Proton signal at δ_H 5.13 (m, H-3) showed an oxymethine at C-3 position. From experimented data and comparison with the literature, compound **1** was determined to be quadrangularic acid E [12], a pentacyclic cycloartane triterpene with 1 carbonyl group attached at C-1, 1 carboxylic group at C-4 and 1 hydroxyl at C-3. Side-chain with exomethylene and isopropyl group was connected with the back-bone at C-17 position (Figure 1).

Although the research on quadrangularic acid E is still limited, Patoomratana *et al.* reported the inhibition ability of HIV of this cycloartane triterpene. The results showed that quadrangularic acid E significantly inhibited HIV reverse transcriptase with the inhibition efficiency of up to 80.1% and IC_{50} value of approximately 47.9 µg/ml. Quadrangularic acid E also inhibited the interaction of the virus with specific receptors on B cells with an EC_{50} at about 6.8 µg/ml [13].

Compound **2** was obtained as a yellow powder. The ^{1}H-NMR spectrum of compound **2** shows four signals specific for aromatic protons in the low-field region from 6.0 to 7.5 ppm, of which two protons at δ_H 7.16 (d, *J*= 2.0 Hz, H-2′) and δ_H 6.96 (d, *J*= 2.0 Hz, H-6′) showed meta coupling. In addition, a proton signal at δ_H 13.01 (s, H-5OH) and three methoxy signals were also recorded: δ_H 4.05 (s, OMe); δ_H 3.95 (s, OMe) and δ_H 3.99 (s, OMe).

The ^{13}C-NMR data showed signals for eighteen carbon atoms, which included a carbonyl carbon signal δ_C 182.9 (C-4); three methoxy carbon signals δC 61.2, δ_C 60.9, δ_C 56.2, and fourteen aromatic carbon signals. ^{1}H- and ^{13}C-NMR spectra indicated that compound **2** is a flavonoid with a 15-carbon structural backbone. The appearance of both proton δ_H 13.0 and a carbonyl group at δ_C 182.9 demonstrated the hydroxy group attached at C-5 and C=O at C-4 positions [14].

Two meta-type coupling protons δ_H 7.16 (d, *J*= 2.0 Hz, H-2′) and δ_H 6.96 (d, *J*= 2.0 Hz, H-6′) belonged to the B-ring. On the other hand, proton signals at δ_H 6.56 (s; H-3) and δ_H 6.49 (s, H-8) demonstrated the flavone structure of compound **2**. Based on experimental data combined with published reports, compound **2** was determined to be 5,7,3'-trihydroxy-6,4',5'-trimethoxy flavone (Figure 1) [15]. Seo *et al.*, 2003 showed that 5,7,3′-trihydroxy-6,4′,5′-trimethoxyflavone (**2)** expressed anti-cancer ability mediated via inhibition on farnesyl:protein transferase (FPtase) enzyme [16].

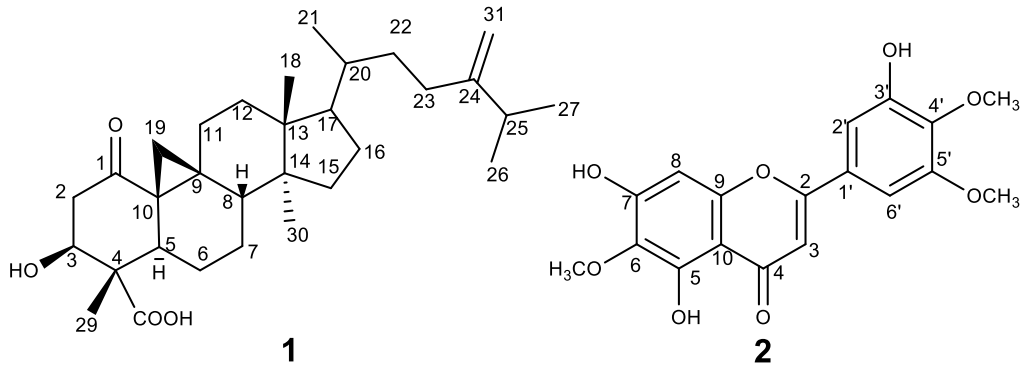

*Figure 1. Structure of compounds **1** and **2***

4. CONCLUSION

In the present study, two compounds quadrangularic acid E **(1)** and 5,7,3′-trihydroxy-6,4′,5′-trimethoxyflavone **(2)** were isolated by combined chromatography and preparative methods. This is the first isolation of these compounds in the species *G. philastrei*. Their structures were determined with spectroscopic methods such as ^{1}H-NMR and ^{13}C-NMR spectroscopy, along with compared with published data. According to previous studies, quadrangularic acid E **(1)** showed anti-HIV activity, meanwhile 5,7,3′-trihydroxy-6,4′,5′-trimethoxyflavone (**2)** expressed anti-cancer activity. Our study advocated further research on the application of *G. philastrei* in drug development programs

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