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CHEMICAL CONSTITUENTS OF *Physalis angulata* L. (FAMILY SOLANACEAE)

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ABSTRACT

This work describes the isolation and characterization of 3 compounds, including physalin B (1), physalin G (2) and quercetin 3-O-rutinoside (3) from the dichloromethane and n-butanol extracts of the aerial parts of the medicinal species Physalis angulata L. collected from Dong Thap province. The chemical structures were elucidated based on various spectra data (ESI-MS, 1D- and 2D-NMR spectroscopy) and by comparing with known spectral data. For the first time, the inhibition activity of physalin G on α-glucosidase enzyme was announced.

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1 INTRODUCTION

Physalis angulata L., a common plant in tropical regions, especially in Viet Nam, is used to treat some diseases such as skin diseases, infection, fever, hepatitis (Di Stasi *et al.*, 1989). There are some researches of pharmaceutical application of *P. angulata* L., over the world (Martinez *et al.*, 1998). This is not only a popular medicinal plant in Viet Nam, but also in some nations such as Brazil, Mexico, New Guinea,… (Mitsuhashi *et al.*, 1988; Salgado and Arana, 2013). In Viet Nam, *P. angulata* L. is used all aerial parts as an effective way to treat some hepatic diseases. According to the treatment experiences in some traditional health agencies, in Dong Thap province, it has recently made a proposal of hypoglycemia activity of diabetes type 2. However, in Viet Nam, *P. angulata* L. is just a plant used in traditional cure and there have not had any accurate scientific researches about its bioactivities as well as its chemical constituents. Therefore, making a clear discovery of pharmaceutics of this plant is an essential and meaningful work.

2 EXPERIMENTAL

2.1 Material

The aerial parts of *P. angulata* L. were collected in Chau Thanh district, Dong Thap province in October, 2014. The plant was identified at Biology Department of College of Natural Sciences, Can Tho University and compared to references (Loi, 2004). All collected parts of *P. angulata* L. were cleaned, spread into the room with the fan then heated into 60°C to dry, and ground to powder.

2.2 Method

Silica gel 60 (0.063-0.200 mm, Merck, Germany), Diaion HP-20 and Bondesil C-18 Merck, Germany) were used for column chromatography. TLC F_{254} plate (Merck, Germany) and TLC RP-18 F_{254} plate (Merck, Germany) were used for thin layer chromatography. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopy were recorded by a Bruker Avance 500 MHz spectrometer, ESI-MS was recorded with a VG 7070 Mass spectrometer operating at 70 eV. All spectroscopic methods were carried out at Institute of Chemistry, Vietnam Academy of Science and Technology.

The assay on α-glucosidase enzyme by multi diluted concentrations of the samples to confirm the IC_{50} value (µg/ml), was tested at the lab of Biological Experiment, Institute of Chemistry.

2.3 Experiment

2.3.1 Extraction and isolation

Dried powder of the aerial parts of the *P. angulata* L. (5 kg) were exhaustively extracted with methanol. The filtrated solution was concentrated *in vacuum* then suspended in water and partitioned with petroleum ether (PE), dichloromethane (DC), ethyl acetate (EtOAc), *n*-butanol (*n*-BuOH) and methanol/water (MeOH/H_2O), respectively. The partitioned solutions were evaporated to give five appropriate extracts: PE (80 g), DC (71 g), EtOAc (25 g), *n*-BuOH (60 g) and MeOH/H_2O (75 g).

The DC extract was subjected to silica gel column, eluted with PE – EtOAc gradients to afford compounds **1** and **2**.

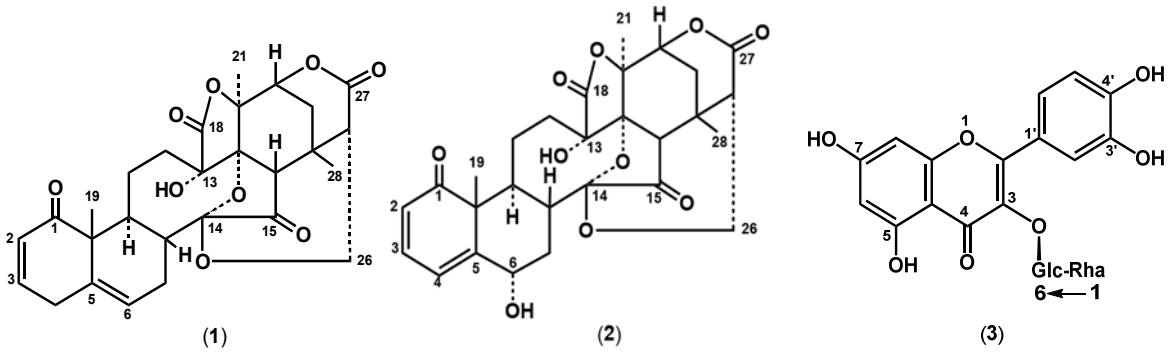
The *n*-BuOH extract was first chromatographed with Diaion HP-20 column, eluted with MeOH/H_2O gradients to give six fractions (F1-F6). The F4 fraction (3g) was then subjected to Bondesil C-18 column, eluted with MeOH/H_2O gradients to obtain compound **3.**

2.4 Identification

Physalin B (**1**): 2140 mg, colorless crystals, ^{1}H NMR (500 MHz, $CDCl_3$) ppm: 5.92 (1H, *dd*, J_1 = 2, J_2 = 10 Hz; H-2); 6.79 (1H, *m*; H-3); 5.58 (1H, *m*; H-6); 2.45 (1H, *d*, *J* = 4 Hz; H-8); 3.30 (1H, *dd*, J_1 = 22, J_2 = 2.5 Hz; H-9); 1.22 (3H, *s*; H-19); 1.98 (3H, *s*; H-21); 3.78 (1H, *dd*, J_1 = 13, J_2 = 1 Hz; H-26); 4.54 (1H, *m*; H-26`); 1.26 (3H, *s*; H-28); 4.13 (1H, *s*; OH-13). ^{13}C and DEPT NMR (125 MHz, $CDCl_3$) δ: 205.7 (C-1), 127.4 (C-2), 146.1 (C-3), 33.1 (C-4), 133.9 (C-5), 124.5 (C-6), 24.8 (C-7), 40.0 (C-8), 33.2 (C-9), 52.7 (C-10), 24.2 (C-11), 25.9 (C-12), 80.2 (C-13), 107.5 (C-14), 208.1 (C-15), 56.4 (C-16), 81.0 (C-17), 172.3 (C-18), 17.9 (C-19), 79.7 (C-20), 21.4 (C-21), 76.9 (C-22), 32.7 (C-23), 31.1 (C-24), 50.9 (C-25), 60.7 (C-26), 166.7 (C-27), 26.5 (C-28).

Physalin G (**2**): 1510 mg, colorless crystals, ^{1}H NMR (500 MHz, $CDCl_3$) ppm: 5.96 (1H, *d*, *J* = 9.5 Hz; H-2); 6.84 (1H, *dd*, J_1 = 9.5, J_2 = 5.5 Hz; H-3); 6.06 (1H, *d*, *J* = 5.5 Hz; H-4); 4.54 (3H, *m*; H-6, H-22, H-26); 1.53 (4H, *s*; H-7, H-19); 2.44 (1H, *m*; H-7`); 2.67 (1H, *m*; H-8); 2.93 (1H, *m*; H-9); 1.96 (3H, *s*; H-21); 2.2 (1H, *s*; H-16); 2.05 (2H, *m*; H-23); 2.44 (1H, *m*; H-25); 3.78 (1H, *dd*, $J_1$ = 13, $J_2$ = 1; H-26`); 1.27 (3H, *s*; H-28); 2.36 (1H, *s*; OH-6); 2.00 (1H, *s*; OH-13). ^{13}C and DEPT NMR (125 MHz, $CDCl_3$) δ: 208.0 (C-1), 125.6 (C-2), 139.4 (C-3), 119.7 (C-4), 129.0 (C-5), 71.1 (C-6), 31.1 (C-7), 38.3 (C-8), 35.2 (C-9), 54.2 (C-10), 25.1 (C-11), 25.9 (C-12), 79.8 (C-13), 107.1 (C-14), 211.2 (C-15), 56.0 (C-16), 80.4 (C-17), 172.0 (C-18), 21.4 (C-19), 81.0 (C-20), 21.5 (C-21), 77.0 (C-22), 33.0 (C-23), 30.9 (C-24), 50.9 (C-25), 60.9 (C-26), 166.6 (C-27), 26.6 (C-28).

Quercetin 3-*O*-rutinoside (**3**): 20 mg, yellow powder, ^{1}H NMR (500 MHz, DMSO-d_6) ppm: 6.19 (1H, *s*; H-6); 6.38 (1H, *s*; H-8); 6.84 (1H, *d*, *J* = 8 Hz; H-5'); 7.54 (2H, *m*; H-2', H-6'); 12,59 (1H, *s*; OH-5); 5,34 (1H, *d*, *J* = 6 Hz; H-1"); 5,27 (1H, *brs*; H-1'"); 0,99 (3H, *d*, *J* = 6 Hz; H-6'"). ^{13}C and DEPT NMR (125 MHz, DMSO-d_6) ppm: 156.4 (C-2), 133.3 (C-3), 177.4 (C-4), 161.2 (C-5), 98.7 (C-6), 164.2 (C-7), 93.6 (C-8), 156.6 (C-9), 103.9 (C-10), 121.2 (C-1'), 115.2 (C-2'), 144.8 (C-3'), 148.4 (C-4'), 116.3 (C-1'"), 70.0 (C-2'"), 70.6 (C-3'"), 71.9 (C-4'"), 68.2 (C-5'"), 17.7 (C-6'").



3 RESULTS AND DISCUSSION

3.1 Compounds

Compound **1** was obtained as colorless crystals. The ^{13}C and DEPT NMR spectra presented total 28 carbon resonances with three methyls, six methylenes, eight methines and eleven quaternary carbons. The characteristic carbon signals for carbonyl groups at δ 205.7 (C-1) and 208.1 (C-15), while the signals of carboxyl groups at δ 172.0 ppm (C-18) and 166.6 ppm (C-27) along with the appearance of characteristic CH signals of C-2, C-3, C-6 at 127.4, 146.1, 124.5 ppm; respectively. The ^{1}H NMR spectrum of **1** also displayed signals for three methyl groups at δ 1.22 (3H, *s*; H-19), 1.26 (3H, *s*; H-28), 1.98 (3H, *s*; H-21) and characteristic signals of a –OCH_2– system at δ 3.78 (1H, *dd*, J_1 = 13, J_2 = 1 Hz; H-26), 4.54 (1H, *m*; H-26'). Furthermore, the presence of a doublet at δ 5.58 (1H, *t*; H-6), 5.92 (1H, *dd*, J_1 = 2, J_2 = 10 Hz; H-2), 6.79 (1H, *m*; H-3) and the signals of hydroxyl proton at δ 4.13 (1H, *s*; OH). These data suggested the skeletal arrangement including the 2-en-1-one system. Therefore, compound **1** was identified as physalin B, in comparison with published data (Row *et al.*, 1980).

Compound **2** was obtained as colorless crystals, ESI - MS (positive) showed the pseudomolecular ion peak at *m/z* = 509 $[M - H_2O + H]^+$. The molecular formula was determined to be $C_{28}H_{30}O_{10}$ (calc. 526 amu). The ^{1}H NMR spectrum of **2** indicated the presence of three tertiary methyl groups at δ 1.27 (3H, *s*; H-28), 1.53 (3H, *s*; H-19), 1.96 (3H, *s*; H-21); a set of vinyl protons at δ 5.96 (1H, *d*, *J* = 9.5 Hz; H-2), 6.84 (1H, *dd*, J_1 = 9.5, J_2 = 5.5 Hz; H-3), 6.06 (1H, *d*, *J* = 5.5 Hz; H-4); the characteristic methylene oxide bridge –OCH_2CH– between C-14 and C-26 at δ 2.44 (1H, *m*; H-25), 3.78 (1H, *dd*, J_1 = 13, J_2 = 1; H-26'), 4.54 (1H, *m*; H-26) which commonly found in physalin B and related physalins. This structure modification was completely confirmed by analysis of the ^{13}C NMR signals, in which two ketone carbonyl signals for C-1, C-15 appeared at δ 208.0, 211.2 ppm; respectively, and the signals of two carbonyl groups at 172.0, 166.6 ppm; respectively. On the other hand, appearance of the characteristic CH signals of C-2, C-3, C-4 at 125.6, 139.4, 119.7 ppm; respectively was also observed. Therefore, compound **2** was identified as physalin G, in comparison with published data (Row *et al.*, 1980).

Compound **3** was obtained as yellow powder. The ^{1}H NMR shows a signal at 12.59 ppm (1H, *s*; OH-5) which refers to the hydroxyl proton at C-5 having a hydrogen bond with the oxygen of the C-4 carbonyl group. The spectra also indicate the existence of two carbohydrate moieties: β-glucose and rhamnose *via* the multiple signals from 3.00 ppm to 3.80 ppm and the two anomeric proton signals at 5.34 ppm (1H, *d*, *J* = 6 Hz; H-1") and 5.27 ppm (1H, *brs*; H-1"') referring to β-glucose and rhamnose, respectively; along with the proton signal of the methyl group in the rhamnose moiety at 0.99 ppm (3H, *d*, *J* = 6 Hz; H-6"'). The ^{13}C and DEPT NMR present 27 carbon signals, including ten quaternaries, fifteen tertiary, one secondary and one primary carbons. Six out of ten quaternary carbons was specific for the flavonol skeleton (C-2, C-3, C-4, C-9, C-10 and C-1'); four quaternary carbon signals left suggested the presence of four hydroxyl substituents in the ring A and ring B of the flavonol structure. The presence of β-glucose moiety was proven by the anomeric carbon signal at 101.2 ppm, along with the –CHOH signals from 65.0 ppm to 77.0 ppm and a secondary carbon signal at 67.0 ppm while the signals that illustrated for the presence of rhamnose moiety are the anomeric carbon signal at 100.7 ppm, the signals between 65.0 ppm and 77.0 ppm and a primary carbon signal at 17.7 ppm. In comparison with myricetin 3-*O*-neohesperidoside (Ismail and Alam, 2001), the chemical shift of C-6" was downfield shifted at 67.0 ppm, demonstrating that the glucose moiety

was glycosylated by the rhamnopyranosyl unit at position of C-6" to form a rutinosyl group. Beside that, the chemical shift of C-3 was upfield shifted at 133.3 ppm in comparison with quercetin (Zhang *et al.*, 2006), inferring that the glycosylation of the flavonol by the rutinosyl group took place at 3-hydroxyl. Both ^{1}H NMR and ^{13}C NMR spectra of compound **3** are very similar to the published spectral data of rutin (Olszewska *et al.,* 2005). Therefore, the structure of compound **3** was demonstrated as quercetin 3-*O*-rutinoside.

3.2 The inhibition activity on α-glucosidase enzyme

The results of the α-glucosidase inhibitory activity indicated that compound **2** reached the IC_{50} value at concentration of 218.1 µg/ml while the control Arcabose attained the IC_{50} value at concentration of 183.4 µg/ml. Thus, compound **2** showed the enzyme inhibition potential with 84.1% efficiency compared to the commercial drug Acarbose®. Based on these results, the study concluded that physalin G or *P. angulata* L. is potential drug for diabetes treatment along with its earlier detected pharmaceutical properties.

4 CONCLUSION

From the aerial parts of the medical species *Physalis angulata* L., collected from Dong Thap provine in 2014, three compounds were isolated. Their chemical structures were identified as physalin B, physalin G and quercetin 3-*O*-rutinoside based on the spectra analysis. The two compounds – physalin B and physalin G – are the endemic compounds of the species *Physalis angulata* L. and the last one is first isolated from this material. The results of bioactivity test suggest that physalin G has potential in treatment of diabetes. This study contributed new result to the phytochemistry of the speciese *Physalis angulata* L. and the genus *Physalis.*

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