



NOVEL NAPHTHALENE GLYCOSIDE DERIVATIVE FROM LEAVES OF *Cassia grandis* L.F

Ngo Quoc Luan¹, Vu Duy Khanh¹, Le Tien Dung², Mai Dinh Tri², Phan Nhat Minh², Nguyen Tan Phat², Nguyen Ngoc Hanh² and Nguyen Kim Phi Phung³

¹School of Education, Can Tho University, Vietnam

²Institute of Chemical Technology - Vietnam Academy of Science and Technology, Vietnam

³University of Natural Science of Ho Chi Minh city, Vietnam

ARTICLE INFO

Received date: 27/07/2015

Accepted date: 26/11/2015

KEYWORDS

Cassia grandis L.f,
casgranone C

ABSTRACT

Chemical investigation of *Cassia grandis* L.f in Mekong Delta-Vietnam to contribute to chemistry data of medicinal plant. The research has been going since 2011 and more than 40 natural compounds were reported. In this twelfth report, we describe the extraction with organic solvents and isolation by chromatography of a compound named casgranone C (I) from methanol extracts of leaves of *Cassia grandis* L.f. The chemical structure of it was determined by modern spectral methods as HRMS, 1D and 2D-NMR. This is a new natural compound firstly detected in over the world confirmed by SciFinder software.

Cited as: Luan, N.Q., Khanh, V.D., Dung, L.T., Tri, M.D., Minh, P.N., Phat, N.T., Hanh, N.N. and Phung, N.K.P., 2015. Novel naphthalene glycoside derivative from leaves of *Cassia grandis* L.f. Can Tho University Journal of Science. Vol 1: 10-13.

1 INTRODUCTION

Cassia grandis L.f was known in Vietnam for folk remedies such as back pain, snakebites, skin diseases and so on (Loi, 2004). Chemical investigation of *Cassia grandis* L.f has been reported recently. More than 30 natural compounds have been isolated from this plant (Luan *et al.*, 2012; 2013a-d; 2014a-d). In this paper we continue to report a new natural compound detected in the leaves of *Cassia grandis* L.f.

2 MATERIALS AND METHODS

2.1 Plant material

Leaves of *Cassia grandis* L.f were collected in Ca Mau Province in January, 2013. Voucher specimens have been identified by Dr. Dang Minh Quan, Can Tho University. After cleaning, the poor quality leaves were removed. Good materials were dried at 50°C to decrease humidity to 0-2 %, followed by crushing into fine powder.

2.2 General experimental procedures

2.2.1 Extraction and purification

Solid-liquid, liquid-liquid extractions were conducted by using solvents such as ethanol 96%, *n*-hexane, EtOAc, BuOH, MeOH. Solvent was evaporated under reduced pressure by using Buchi R-210 (Switzerland) rotary evaporator system.

Thin layer chromatography (TLC) was carried out on pre-coated silica gel 60F₂₅₄ (0.25 mm) aluminum sheet (Merck) and compounds were detected under UV (254/365 nm) fluorescence or spraying 10% H₂SO₄ solution in EtOH, followed by heating at 105°C for 1-2 min on electric stove.

For common phase column chromatography (CP-CC), silica gel 60 (0.040-0.063 mm, Merck) with increasing polarity solvent systems including *n*-hexane (H), EtOAc (E), CHCl₃ (C), MeOH (M) and H₂O (W) were used.

For reverse phase column chromatography (RP-CC), silica gel RP-18 (Merck) with decreasing polarity solvent systems including H₂O (W) and MeOH (M) were used. Sections with similar traces as indicated by TLC were collected into a fraction.

Compound purification was applied by re-crystallization more than once in pure solvents.

2.2.2 Structural elucidation and identification

Melting point (mp.) was recorded on a melting point meter (Electrothermal 9100, UK), using with capillary, uncorrected. ¹H-NMR, ¹³C-NMR, DEPT, HSQC, HMBC, COSY spectra were recorded on a Bruker AM500 FT-NMR spectrometer (USA, using CD₃OD and TMS). Mass spectrum (MS) was recorded on mass spectrometer (HP 1100 series, LC/MSD Trap, Agilent, USA). These used equipment are available at Vietnam Academy of Science and Technology.

2.3 Extraction and isolation

The dried leaves (2.9 kg) of *Cassia grandis* L.f were powdered and exhaustively extracted with ethanol 96% to gain ethanol extract (0.5 kg). The ethanol extract was consecutively distributed into *n*-hexane, chloroform, ethyl acetate and methanol.

The methanol extracts (LM, 290.1 g) was subjected to CP-CC with E:M:W solvent systems (radient, 200:1:1 to 10:1:1) as eluent to give 6 fractions (LM1-LM6). The fraction LM2 (27.6 g) was taken CP-CC with E:M (radient, 50:1 to 10:1) as eluent to obtain 6 fractions (LM21-LM26). The fraction LM25 (0.8216 g) was continued with CP-CC (C:M, rradient, 10:1 to 3:1) to afford 5 fractions (LM251-LM255). The fraction LM253 (300 mg) was continued with CP-CC (C:M, rradient, 8:1 to 3:1) to give 5 fractions (LM2531-LM2535). The fraction LM2533 (114 mg) was continued with CP-CC (C:M, rradient, 12:1 to 3:1) to get 6 fractions (LM25331-LM25336). The fraction LM25334 (16.7 mg) was continued with RP-CC (M:W, 1:3) to afford 2 fractions (LM253341-LM253342). The fraction LM253341 (9.6 mg) was re-crystallized in MeOH to obtain compound **1** (7.1 mg).

2.4 Arranged 1D-NMR spectral data

¹H-NMR (MeOD, 500 MHz, δ_H ppm, *J* Hz): 6.94 (1H, *d*, *J*=2.0, H-5); 6.93 (1H, *d*, *J*=2.0, H-4); 6.73 (1H, *d*, *J*=2.0, H-7); 5.08 (1H, *d*, *J*=8.0, H-1'); 4.77 (1H, *d*, *J*=1.5, H-1''); 4.10 (1H, *dd*, *J*=1.5 and 11.0, H-6'a); 3.95 (1H, *dd*, *J*=1.5 and 3.5, H-2''); 3.36-3.75 (8H, *m*, 8 CH groups); 2.61 (1H, *s*, H-12);

2.30 (1H, *s*, H-13); 1.23 (1H, *d*, H-6'').

¹³C-NMR (MeOD, 125 MHz, δ_C ppm): 208.3 (C-11); 158.2 (C-6); 157.4 (C-8); 154.0 (C-1); 139.4 (C-10); 135.3 (C-3); 123.3 (C-2); 119.7 (C-4); 109.7 (C-9); 105.4 (C-7); 104.7 (C-5); 104.2 (C-1'); 102.3 (C-1''); 78.1 (C-3'); 77.5 (C-5'); 74.9 (C-2'); 74.1 (C-4''); 72.4 (C-3''); 72.1 (C-2''); 71.3 (C-4'); 69.9 (C-5''); 67.7 (C-6'); 32.6 (C-12); 20.3 (C-13); 17.9 (C-6'').

3 RESULTS AND DISCUSSION

Compound **1** was obtained as brown amorphous powder, mp. 165-166 °C, which produced a positive reaction to FeCl₃ reagent. The typical signals of proton and carbon in 1D-NMR showed that compound **1** could be a phenolic compound with two sugar moieties.

The ¹H-NMR spectrum of compound **1** revealed three aromatic protons which showed *m*-coupled doublet signals at δ_H 6.94, 6.93 and 6.73 ppm (each, *J*=2.0 Hz); two singlet signals of methyl groups at δ_H 2.61 and 2.30 ppm. In addition, there were also proton signals of a substituted rutinose in which two doublet signals at δ_H 5.08 (1H, *d*, *J*=8.0 Hz) and 4.77 (1H, *d*, *J*=1.5 Hz) were attributed of anomeric protons.

The ¹³C-NMR and DEPT spectra of compound **1** showed signals of total 25 carbons in which 10 carbons of a five substituted naphthalene; two carbons of an acetyl group (δ_C 32.6, 208.3 ppm, as confirmed by HMBC spectrum) and a methyl group (δ_C 20.3 ppm) directly connected to the benzene ring and 12 carbons of the rutinose moiety.

The molecular formula of compound **1** was speculated to be C₂₅H₃₂O₁₃ (calc. for 540.18) on the basis of the HR-MS (*m/z* 563.1747 [M+Na]⁺) and the above mentioned 1D-NMR data. Therefore, compound **1** gave the characteristic spectra possessing the similar substitution pattern of 5-hydroxydianellin (Fig. 1) in the literatures (Cichewicz, 2002; Dias, 2009).

The HSQC spectrum of compound **1** exhibited signals of three aromatic *m*-coupled doublet protons at δ_H 6.94, 6.93 and 6.73 ppm which in turn correlated with three aromatic methine carbons at δ_C 104.7, 119.7 and 105.4 ppm; proton at δ_H 2.61 ppm correlated with carbon at δ_C 32.6 ppm, proton at δ_H 2.30 ppm correlated with carbon at δ_C 20.3 ppm. Besides, protons and carbons of the rutinose moiety also correlated with each other.

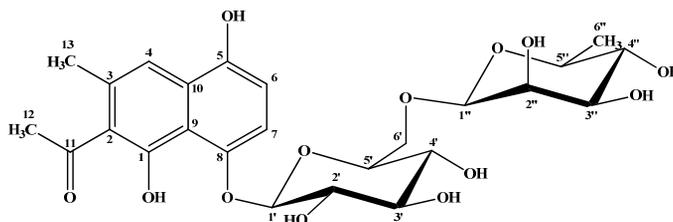


Fig. 1: Chemical structure of 5-hydroxydianellin

Ten aromatic carbons included 3 carbons of aromatic methine (determined by HSQC); 3 aromatic oxygenated carbons (including 1 carbon at δ_C 157.4 ppm connected to -O-glc-rha, 2 carbons δ_C 154.0 and 158.2 ppm connected to hydroxyl); 4 non-

oxygenated quaternary carbons (1 carbon δ_C 135.3 ppm connected to methyl group, 1 carbon δ_C 123.3 ppm connected to the acetyl group, 2 carbons δ_C 109.7 and 139.4 ppm were at the cutting between two benzene rings).

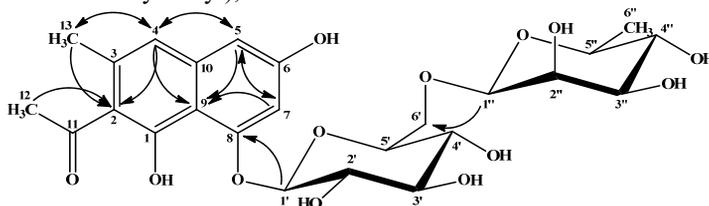


Fig. 2: Selected HMBC correlations used to determine the structure of compound 1

According to the HMBC spectrum of compound **1** (Figure. 2), the oxygenated carbon at δ_C 154.0 ppm with less correlations to protons was attributed to C-1. Correlation of the proton at δ_H 2.61 ppm and the carbon at δ_C 123.3 ppm proved that the acetyl group was linked via this carbon. Similarly, correlation of the proton at δ_H 2.30 ppm and the carbons at δ_C 135.3, 123.3 and 119.7 ppm, and the proton at δ_H 6.93 ppm with the carbons at δ_C 123.3 ppm indicated that the aromatic methyl group connected to the carbon at δ_C 135.3 and the carbon at δ_C 119.7 ppm was at *meta* position of the carbon at δ_C 123.3 ppm. Thus, the carbon at δ_C 123.3 ppm was assigned to C-2, the carbon at δ_C 135.3 ppm was assigned to C-3 and the carbon at δ_C 119.7 ppm was assigned to C-4. The *meta*-coupled proton at δ_H 6.94 ppm correlated to C-4 and H-4 (δ_H 6.93 ppm) correlated to the carbons at δ_C 104.7 ppm, so the proton at δ_H 6.94 ppm was assigned to H-5 and the carbons at δ_C 104.7 ppm was C-5. Similarly, the *meta*-coupled proton at δ_H 6.73 ppm correlated to C-5 and H-5 (δ_H 6.94 ppm) correlated to the carbon at δ_C 105.4 ppm, so the proton at δ_H 6.73 ppm was assigned to H-7 and the carbons at δ_C 105.4 ppm was C-7. The oxygenated carbon at δ_C 158.2 ppm which showed correlation with H-5, H-7 was C-6.

The glucopyranosyl anomeric proton at δ_H 5.08 ppm correlated to the oxygenated carbon at δ_C 157.4 ppm, which indicated that the glucopyranosyl was linked at this carbon (C-8, δ_C 157.4 ppm) of the naphthalene aglycone. The aromatic quaternary carbon at δ_C 109.7 ppm showed correlation with H-7 was C-9, so the aromatic quaternary carbon at δ_C 139.4 ppm was C-10.

In addition, the signal of the rhamnopyranose anomeric proton at δ_H 4.77 ppm correlated to the glucose methylene carbon at δ_C 67.7 ppm indicated that the rhamnopyranose was linked at C-6' of the glucopyranosyl. The other carbons and protons of the rutinose moiety were determined based on comparison with spectral data of 5-hydroxydianellin and the HMBC spectrum of compound **1**.

The important difference between $^1\text{H-NMR}$ spectrum of compound **1** and 5-hydroxydianellin was the *meta*-coupling ($J=2.0$ Hz) signals of H-5, H-7 (in compound **1**) and the *ortho*-coupling ($J=8.0$ Hz) signals of H-6, H-7 (in 5-hydroxydianellin), so the structure of compound **1** was identified as casgranon C (Fig. 3).

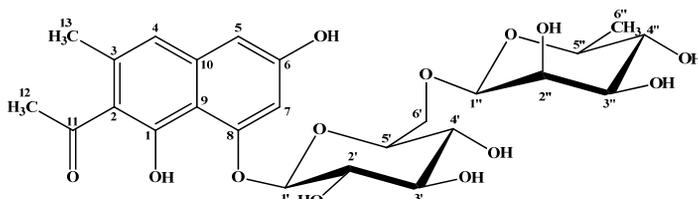


Fig. 3: Chemical structure of casgranone C

The chemical structure of casgranone C was checked by SciFinder software dated 07-04-2014 which showed that this was a new structural product all over the world being firstly isolated from plant in general.

4 CONCLUSION

From methanol extracts of leaves of *Cassia grandis* L.f collected in Ca Mau Province, a novel structural compound named casgranone C was detected. This is the first report about this compound being isolated and characterized from *Cassia grandis* L.f.

Acknowledgment: The authors acknowledge financial support from Can Tho University, thanks Dr. Dang Minh Quan for plant identification.

REFERENCES

- Cichewicz, R.H., Lim, K.C., McKerrow, J.H., Naira, M.G., 2002. Kwanzoquinones A-G and other constituents of *emerocallis fulva* 'Kwanzo' roots and their activity against the human pathogenic trematode *Schistosoma mansoni*. *Tetrahedron* 58: 8597-8606.
- Dias, D.A., Silva, C.A., Urban, S., 2009. Naphthalene Aglycones and Glycosides from the Australian Medicinal Plant, *Dianella callicarpa*. *Planta. Med.* 75: 1442-1447.
- Loi, D.T., 2004. Medicinal plants and remedies in Vietnam. Vietnam Medical Publisher, 591 (in Vietnamese).
- Luan, N.Q., Phong, D.H., Thuy, T.T.T., Tri, M.D., Dung, L.T., Trinh, P.T.N., Hanh, N.N., 2012. Flavonoid compounds from leaves of *Cassia grandis* L.. *Journal of Science and Technology, Vietnam Academy of Science and Technology* 50(3A): 296-301 (in Vietnamese).
- Luan, N.Q., Minh, N.K.K., Trang, N.T.T., Thuan, T.C., Tri, M.D., Dung, L.T., Hanh, N.N., 2013a. Chemical investigation of ethyl acetate extract from the stem bark of *Cassia grandis* L.f. *Vietnam Journal of Chemistry, Vietnam Academy of Science and Technology* 51(6ABC): 135-140 (in Vietnamese).
- Luan, N.Q., Khanh, V.D, Minh, N.K.K., Dung, L.T., Hanh, N.N., 2013b. Isolation natural compounds from leaves and stem bark of *Cassia grandis* L.f. *Journal of Analytical Sciences, Vietnam Analytical Sciences Society* 18(4): 101-108 (in Vietnamese).
- Luan, N.Q., Huy, L.D., Vinh, D.H., Minh, N.K.K., Hanh, N.N., 2013c. Isolation and identification of two compounds from the fruit of *Cassia grandis* L.f. *Journal of Science, Can Tho University* 26: 30-34 (in Vietnamese).
- Luan, N.Q., Tien, H.M, Khanh, V.D, Dung, L.T, Hanh, N.N., 2013d. Three flavonoid compounds from leaves of *Cassia grandis* L.f. *Journal of Science, Can Tho University* 29a: 111-116 (in Vietnamese).
- Luan, N.Q., Ril, L.V, Vinh, D.H, Minh, N.K.K., Hanh, N.N., 2014a. Three compounds from ethyl acetate extract of the fruit of *Cassia grandis* L.f. *Journal of Science, Can Tho University* 30a: 15-19 (in Vietnamese).
- Luan, N.Q., Yen, H.T.P., Nhan, N.T.H., Minh, N.K.K., Hanh, N.N., 2014b. New xanthone derivative from the root of *Cassia grandis* L.f. *Journal of Science, Can Tho University* 31a: 72-75 (in Vietnamese)
- Luan, N.Q., Kiet, N.V., Minh, N.K.K., Phat, N.T., Trung, P.V., Dung, L.T., Hanh, N.N., 2014c. Natural compounds from the fruit of *Cassia grandis* L.f. *Journal of Science and Technology, Vietnam Academy of Science and Technology* 52(5A): 174-179 (in Vietnamese).
- Luan, N.Q., Yen, H.T.P., Minh, N.K.K., Trung, P.V., Minh, P.N., Phat, N.T., Tri, M.D., Dung, L.T., Hanh, N.N., 2014d. Natural compounds from the root of *Cassia grandis* L.f. *Vietnam Journal of Chemistry, Vietnam Academy of Science and Technology* 52(5A): 646-650.