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Research Article

SOME FLAVONOIDS THE LEAVES OF *COMBRETUM QUADRANGULARE* GROWING IN VIETNAM

Dao Thi Bich Ngoc, Nguyen Thi Huyen Dieu, Duong Thuc Huy*

Ho Chi Minh City Universty of Education, Vietnam

**Corresponding author: Duong Thuc Huy – Email: huydt@hcmue.edu.vn*

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ABSTRACT

*The leaves of Combretum quadrangulare Kurz. collected in Long An Province were chemically investigated using multiple chromatographic methods. Four compounds, 5-hydroxy-3,7,4'-trimethoxyflavone (**1**), ayanin (**2**), kamatakenin (**3**), and luteolin (**4**) were isolated and elucidated. Their chemical structures were elucidated by comparing their spectroscopic data with those in previous reports. Compounds **1** and **4** was obtained for the first time in Combretum quadrangulare.*

Keywords: *alpha-glucosidase; antibacterial activity; ayanin; Combretum quadrangulare* Kurz.; *kamatakenin*

1. Introduction

Combretum quadrangulare Kurz is widely used in folk medicine in Eastern Asia and is associated with various ethnopharmacological claims including hepatoprotective, antipyretic, analgesic, antidysenteric, and anthelmintic properties. In Vietnam, this plant was used as antihelmintic and antihepatitis agents (Adnyana, Tezuka, Banskota, et al. 2000; Banskota et al. 2003). Phytochemical data of *C. quadrangulare* reported the presence of numerous triterpenes (cycloartanes, ursanes, lupanes, and oleananes) and some flavonoids (Pettit et al. 1995; Banskota et al. 1998; Arjun H. Banskota et al. 2000; Arjun Hari Banskota, Tezuka, Tran, et al. 2000; Adnyana et al. 2001). Among different organs of the plant, leaves of *C. quadrangulare* had attracted the chemists to investigate. The crude MeOH extracts of the Vietnamese plant showed significant hepatoprotective effect, xanthine oxidase inhibition and cytotoxicity against several cancer cell lines (Arjun Hari Banskota, Tezuka, Tran, et al. 2000; Arjun Hari Banskota, Tezuka, Adnyana, et al. 2000; Adnyana, Tezuka, Awale, et al. 2000). This paper presented the isolations and structural

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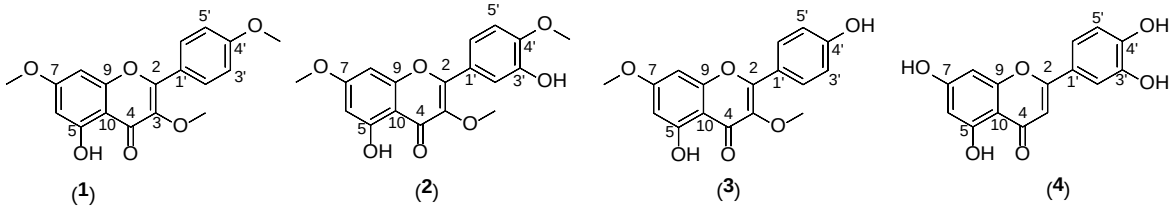
elucidation of four compounds, including 5-Hydroxy-3,7,4′-trimethoxyflavone (**1**), ayanin (**2**), kamatakenin (**3**), and luteolin (**4**), from *Combretum quadrangulare* Kurz leaves collected in Long An Province, Vietnam.



(**1**) (**2**) (**3**) (**4**)

Figure 1. *Chemical structures of isolated compounds* ***1-4***

2. Experimental

2.1. General experimental procedures

The NMR spectra were recorded on a Bruker Avance 500 spectrometer (500 MHz for ^{1}H–NMR and 125 MHz for ^{13}C–NMR) in acetone-d_6, and DMSO-d_6 solutions. Thin layer chromatography was carried out on on silica gel 60 (Merck, 40-63 μm) and spots were visualized by spraying with 10% H_2SO_4 solution, followed by heating.

2.2. Plant material

Leaves of *Combretum quadrangulare* were collected in Duc Hoa, Long An Province in March-April 2020. The plant was identified as *Combretum quandrangulare* Kurz by Dr. Tran Cong Luan, Tay Do University, Vietnam. A voucher specimen (No UE-002) was deposited in the herbarium of the Department of Organic Chemistry, Faculty of Chemistry, Ho Chi Minh University of Education, Ho Chi Minh City, Vietnam.

2.3. Extraction and isolation

Dried leaves of *C. quadrangulare* (11 kg) were crushed and extracted with 10L of MeOH (three times) at 70°C for 8h. The filtrated solution was evaporated to dryness under reduced pressure to obtain a crude extract (118.4g). This crude was successively partitioned by *n*-hexane, *n*-hexane: EtOAc 1:1, EtOAc, to afford **H** (29.1 g), **HEA** (160.3 g), **EA** (30.0 g), and **MeOH** (12.0 g) extracts, respectively. Fraction **HEA** (160.3 g) was applied to silica gel column chromatography, using an isocratic mobile phase consisting of *n*-hexane: EtOAc: acetone (5:1:1) to obtain 9 fractions **P1** (4.95 g), **P2** (9.72 g), **P3** (6.94 g), **P4** (4.82 g), **P5** (5.69 g), **P6** (4.23 g), **P7** (3.2 g), **P8** (4.15 g), **P9** (3.9 g). Fraction **P2** (9.72 g) was subjected to silica gel column chromatography, using an isocratic mobile phase consisting of a *n*-hexane: EtOAc: acetone solvent system (5:1:1, v/v/v) to obtain fractions **T1** (1.8 g), **T2** (600.0 mg), **T3** (900.0 mg), **T4** (2.0 g), **T5** (1.1 g), **T6** (1.3 g).

Fraction **T1** (1.8 g) was submitted to CC using the solvent system *n*-hexane: $CHCl_3$: EtOAc: acetone (3:2:1:1, v/v/v/v) to obtain four fractions **T1.1** (310.0 mg), **T1.2** (560.0 mg), **T1.3** (230.0 mg) and **T1.4** (190.0 mg). Fraction **T1.2** was rechromatographed, eluted with the same solvent system to obtain compounds **2** (55.2 mg). Fraction **T5** (1.1 g) was

submitted to CC using the solvent system *n*-hexane: $CHCl_3$: EtOAc: acetone (3:2:2:2, v/v/v/v) to obtain three fractions **T5.1** (210.0 mg), **T5.2** (350.0 mg), and **T5.3** (150.0 mg). Fraction **T5.1** was rechromatographed, eluted with the same solvent system to obtain two compounds **1** (7.4 mg) and **3** (45.0 mg). Fraction **T5.2** was subjected to silica gel column chromatography, eluted with the solvent system *n*-hexane: $CHCl_3$: EtOAc: acetone (4:3:3:1, v/v/v/v) to afford compound **4** (3.8 mg).

- **5-Hydroxy-3,7,4′-trimethoxyflavone** (**1**). Yellow needles. The ^{1}H-NMR data (500 MHz, Acetone-d_6, δ ppm, *J* in Hertz): 12.73 (1H, *s*), 8.11 (1H, *d*, *J*= 9.5 Hz, H-2', 6'), 7.12 (1H, *d*, *J*= 9.0 Hz, H-3', 5'), 6.67 (1H, *d*, *J*= 2.5 Hz, H-8), 6.33 (1H, *d*, *J*= 2.9 Hz, H-6), 3.93 (3H, *s*, 4'-OMe), 3.92 (3H, *s*, 3-OCH_3), 3.89 (3H, *s*, 7-OCH_3). ^{13}C-NMR (125 MHz, Acetone-d_6): 179.8 (C- 4), 166.8 (C- 7), 163.0 (C- 5/4'), 157.9 (C-2), 139.7 (C-3), 131.2 (C- 2'/6'), 123.8 (C-1'), 115.0 (C- 3'/5'), 106.7 (C-10), 98.6 (C- 6), 90.3 (C- 8), 60.4 (3-OCH_3), 56.7 (7-OCH_3), and 56.0 (4'- OCH_3) (Macedo et al. 2019).
- **Ayanin** (**2**). Yellow amorphous solid. The ^{1}H-NMR data (500 MHz, Acetone-d_6, δ ppm, *J* in Hertz): 12.75 (1H, *s*, 5-OH), 8.49 (1H, *s*, 5'-OH), 7.79 (1H, *d*, *J* = 2.1 Hz, H-6'), 7.72 – 7.69 (1H, *m*, H-2'), 7.01 (1H, *d*, *J* = 8.5 Hz, H-3'), 6.66 (1H, *d*, *J* = 2.3 Hz, H-8), 6.31 (1H, *d*, *J* = 2.1 Hz, H-6), 3.94 (3H, *s*, 7-OCH_3), 3.91 (3H, *s*, 4'-OCH_3), 3.90 (3H, *s*, 3-OCH_3). ^{13}C-NMR (125 MHz, Acetone-d_6): 179.6 (C- 4), 166.6 (C- 7), 162.9 (C- 5), 157.7 (C- 9), 156.9 (C- 2), 150.6 (C- 4'), 148.3 (C-3'), 139.5 (C- 3), 123.4 (C- 1'), 122.8 (C-6'), 116.1 (C- 2'), 112.7 (C-5'), 106.6 (C- 10), 98.5 (C- 6), 92.9 (C- 8), 60.2 (3-OCH_3), and 56.5 (7-OCH_3), 56.5 (4'-OCH_3) (Rahman et al. 2020).
- **Kamatakenin** (**3**). Yellow powder. The ^{1}H NMR data (500 Hz, DMSO-d_6, δ ppm, *J* in Hertz): δ_H 12.66 (1H, *s*, 5-OH), 7.98 (1H, *d*, *J*= 9.0 Hz, H-2', 6'), 6.95 (1H, *d*, *J*= 9.0 Hz, H-3',5'), 6.74 (1H, *d*, *J*= 2.5 Hz, H-8), 6.37 (1H, *d*, *J*= 2.0 Hz, H-6), 3.86 (3H, *s*, 3-OCH_3), 3.80 (3H, *s*, 7-OCH_3). ^{13}C NMR (125 Hz, DMSO-d_6) δ_C 178.1 (C- 4), 165.2 (C- 7), 161.0 (C- 5), 160.3 (C- 4'), 156.4 (C- 2), 156.0 (C- 9), 137.9 (C- 3), 130.2 (C- 2', 6'), 120.5 (C-1'), 115.7 (C- 3', 5'), 105.2 (C- 10), 97.8 (C- 6), 92.4 (C- 8), 59.8 (3-OCH_3), 56.1 (7-OCH_3) (Castillo et al. 2015).
- **Luteolin** (**4**). Light yellow powder. The ^{1}H–NMR data (500MHz, Acetone-d_6, δ ppm, *J* in Hertz): 12.98 (5-OH), 7.42 (1H, *dd*, *J* = 9.8 Hz, *J* = 2.0 Hz, H-6'), 7.39 (*s*, H-2'), 6.88 (1H, *d*, *J* = 8.4 Hz, H-5'), 6.65 (1H, *s*, H-3), 6.44 (1H, *d*, *J* = 1.6 Hz, H-8), 6.18 (1H, *d*, *J* = 2.0 Hz, H-6). ^{13}C-NMR (125 MHz, Acetone-d_6): 181.6 (C- 4), 164.8 (C- 7) 164.3 (C-2), 161.4 (C- 5) 157.3 (C- 9), 149.8 (C-4'), 145.8 (C-3'), 121.3 (C- 1'), 118.9 (C- 6'), 116.0

(C-5'), 113.3 (C- 2'), 103.6 (C- 10), 102.8 (C- 3), 98.4 (C- 6), 93.8 (C- 8) (Okamura et al. 1994).

3. Results and discussion

Compound **1** was obtained as yellow needles. The ^{1}H-NMR spectrum of **1** displayed characteristic signals of a flavanone skeleton: a hydrogen-bond hydroxyl group at δ 12.73 (1H, *s*) and two upfield *meta*–coupled aromatic protons at δ_H 6.33 (1H, *d*, 2.9, H-6) and 6.67 (1H, *d*, 2.5, H-8) in the A ring of common flavonoids. The *para*–disubstituted benzene ring (ring B) was determined by the presence of two doublet signals with a large coupling constant at δ_H 8.11 (2H, *d*, 9.5 Hz, H–2', H–6') and 7.12 (2H, *d*, 9.0 Hz, H–3', H–5'). The ^{13}C–NMR spectrum displayed 15 carbons comprising one carbonyl carbon at δ_C 179.8 (C–4) and 14 aromatic carbons in the range of 93.0 ppm to 166.8 ppm, and three methoxy groups (δ_C 60.4, 56.7, 56.0), strongly supporting the flavone scaffold. The good compatibility between its NMR data and those reported in the literature (Macedo et al. 2019) indicated the chemical structure of **1** to be 5-hydroxy-3,7,4′-trimethoxyflavone.

Compound **2** was obtained as yellow amorphous solid. The NMR data of **2** was identical with those of **1**. The difference is in the chemical structure of the B-ring with the presence of an additional hydroxyl group at C-3'. The 1,2,4-trisubsituted benzene ring was replaced for the 1,4-disubsituted benzene ring in **1**. This was supported by the presence of three aromatic protons at δ_H 7.79 (1H, *d*, *J* = 2.1 Hz, H-6'), 7.72 – 7.69 (1H, *m*, H-2'), 7.01 (1H, *d*, *J* = 8.5 Hz, H-3'). The NMR data of **2** were consistent with those reported in the literature (Rahman, A. 2020), thus, the chemical structure of **2** was determined as ayanin.

The NMR data of compound **3** was identical with those of **1**, except for the disappearance of one methyl group at C-4'. The good compatibility between its NMR data and those in the literature (Castillo et al. 2015) determined the structure of **3** to be kamatakenin**.**

Compound **4** was obtained as yellow amorphous solid. The ^{1}H-NMR spectrum of **4** displayed characteristic signals of a flavanone skeleton: a hydrogen-bond hydroxyl group at δ 12.98 (1H, *s*, 5-OH) and two upfield *meta*–coupled aromatic protons at δ_H 6.44 (1H, *d*, *J* = 1.6 Hz, H-8) and 6.18 (1H, *d*, *J* = 2.0 Hz, H-6) in the A ring of common flavonoids. The 1,2,4-trisubsituted benzene ring was determined by the aromatic protons at δ_H 7.42 (1H, *dd*, *J* = 9.8 Hz, *J* = 2.0 Hz, H-6'), 7.39 (*s*, H-2'), and 6.88 (1H, *d*, *J* = 8.4 Hz, H-5'). Moreover, ^{1}H NMR spectrum also showed the presence of the olefinic proton at δ_H 6.65 (1H, *s*, H-3). The NMR data of **4** were consistent with those of luteolin reported in the literature (Okamura et al. 1994), thus, the chemical structure of **4** was determined as luteolin.

4. Conclusions

From the leaves of *C. quadrangulare* collected in Long An Province, four compounds, including four flavonols 5-Hydroxy-3,7,4′-trimethoxyflavone (**1**), ayanin (**2**), kamatakenin (**3**), and luteolin (**4**) were isolated. Their chemical structures were determined by using NMR spectroscopic method as well as comparison with the literature. Compounds **1** and **4,** to the best of our knowledge, were isolated from *C. quadrangulare* for the first time. Further studies on this species are on the progress.

❖ ***Conflict of Interest:*** *Authors have no conflict of interest to declare.*

REFERENCES

Adnyana, I. K., Tezuka, Y., Awale, S., Banskota, A. H., Tran, K. Q., & Kadota, S. (2000). Quadranosides VI - XI, six new triterpene glucosides from the seeds of *Combretum quadrangulare*. *Chem Pharm Bull*, *48*(8),1114-1120.

Adnyana, I. K., Tezuka, Y., Banskota, A. H., Tran, K. Q., & Kadota, S. (2000). Hepatoprotective constituents of the seeds of *Combretum quadrangulare*. *Biol Pharm Bull*, *23*(11),1328-1332.

Adnyana, I. K., Tezuka, Y., Banskota, A. H., Tran, K. Q., & Kadota, S. (2001). Three new triterpenes from the seeds of *Combretum quadrangulare* and their hepatoprotective activity. *J Nat Prod*, *64*(3), 360-363.

Banskota Arjun Hari, Tezuka, Y., Adnyana, K. I., Xiong, Q., Hase, K., Tran, K. Q., Tanaka, K., Saiki, I., & Kadota, S. 2000. Hepatoprotective effect of *Combretum quadrangulare* and its constituents. *Biol Pharm Bull*, *23*(4), 456-460.

Banskota, A. H., Tezuka, Y., Phung, L. K., Tran, K. Q., Saiki, I., Miwa, Y., Taga, T., & Kadota, S. (1998). Cytotoxic cycloartane-type triterpenes from *Combretum quadrangulare*. *Bioorganic Med Chem Lett*, *8*(24), 3519-3524.

Banskota, A. H., Tezuka, Y., Tran, K. Q., Tanaka, K., Saiki, I., & Kadota, S. (2000). Thirteen novel cycloartane-type triterpenes from *Combretum quadrangulare*. *J Nat Prod*, *63*(1), 57-64.

Banskota, A. H., Tezuka, Y., Tran, K. Q., Tanaka, K., Saiki, I., & Kadota, S. (2000). Methyl quadrangularates A – D and related triterpenes from *Combretum quadrangulare*. *Chem Pharm Bull*, *48*(4), 496-504.

Banskota, A. H., Tezuka, Y., Tran, K. Q., & Kadota, S. (2003). Chemical constituents and biological activities of Vietnamese medicinal plants. *Curr Top Med Chem*, *3*(2),

227-248.

Castillo, Q. A., Triana, J., Eiroa, J. L., Padrón, J. M., Plata, G. B., Abel-Santos, E. V., Báez, L. A., Rodríguez, D. C., Jiménez, M. A., & Pérez-Pujols, M. F. (2015). Flavonoids from *Eupatorium illitum* and their antiproliferative activities. *Pharmacogn J, 7*(3).

Macedo, I., da Silva, J. H., da Silva, P. T., Cruz, B. G., do Vale, J. P. C., Dos Santos, H. S., Bandeira, P. N., de Souza, E. B., Xavier, M. R., & Coutinho, H. D. M. (2019). Structural and microbiological characterization of 5-hydroxy-3, 7, 4′-trimethoxyflavone: A flavonoid isolated from *Vitex* gardneriana Schauer leaves. *Microb Drug Resist. 25*(3), 434-438.

Okamura, N., Haraguchi, H., Hashimoto, K., & Yagi, A. (1994) Flavonoids in *Rosmarinus officinalis* leaves. *Phytochemistry, 37*(5), 1463-1466.

Pettit, G. R., Singh, S. B., Boyd, M. R., Hamel, E., Pettit, R. K., Schmidt, J. M., & Hogan, F. (1995). Additions and Corrections: Antineoplastic Agents. 291. Isolation and synthesis of combretastatins A-4, A-5, and A-6. *Journal of Medicinal Chemistry, 38*(10), 1666-1672.

Rahman, A., Mahardika, I., Saputri, R. D., Tjahjandarie, T. S., & Tanjung, M. (2020). Isolasi, karakterisasi, dan uji aktivitas antioksidan senyawa turunan kuersetin dari kulit batang melicope quercifolia. *J Sains dan Kesehat, 2*(4), 413-417.

MỘT SỐ FLAVONOID PHÂN LẬP TỪ LÁ CÂY TRÂM BẦU *COMBRETUM QUADRANGULARE* SINH TRƯỞNG Ở VIỆT NAM

Đào Thị Bích Ngọc, Nguyễn Thị Huyền Diệu, Dương Thúc Huy*

Trường Đại học Sư phạm Thành phố Hồ Chí Minh, Việt Nam

**Tác giả liên hệ: Dương Thúc Huy – Email: huydt@hcmue.edu.vn*

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TÓM TẮT

Nghiên cứu được thực hiện trên lá cây Trâm *bầu Combretum quadrangulare Kurz thu hoạch ở tỉnh Long An bằng các phương pháp sắc kí khác nhau. Bốn hợp chất 5-Hydroxy-3,7,4'-trimethoxyflavone (**1**), ayanin (**2**), kamatakenin (**3**), and luteolin (**4**) được cô lập và xác định cấu trúc hóa học. Cấu trúc hóa học của các hợp chất được xác định bằng các phương pháp phổ nghiệm đồng thời so sánh với các dữ liệu phổ đã được công bố. Hai hợp chất **1** và **4** lần đầu tiên được biết có hiện diện trong cây Trâm bầu.*

Từ khóa: *alpha-glucosidase; antibacterial activity; ayanin; Combretum quadrangulare* Kurz.; *kamatakenin*