TẠP CHÍ KHOA HỌC
TRƯỜNG ĐẠI HỌC SƯ PHẠM TP HỒ CHÍ MINH
Tập 19, Số 10 (2022): 1593-1598
Website: https://journal.hcmue.edu.vn
ISSN: 2734-9918

HO CHI MINH CITY UNIVERSITY OF EDUCATION
JOURNAL OF SCIENCE
Vol. 19, No. 10 (2022): 1593-1598
https://doi.org/10.54607/hcmue.js.19.10.3381(2022)

Research Article

STUDY ON CHEMICAL CONSTITUENTS OF THE LEAVES OF *DICRANOPTERIS LINEARIS* (BURM. F.) UNDERW.

Nguyen Thu Anh[1], Nguyen Thi Hoai Thu[2], Nguyen Ngoc Khanh Van[3], Duong Thuc Huy[1*]

[1]Ho Chi Minh City Universiry of Education, Vietnam

[2]University of Medicine and Pharmacy at HCMC, Vietnam

[3]Gia Dinh High School, Ho Chi Minh City, Vietnam

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

Received: March 03, 2022; Revised: April 06, 2022; Accepted: October 06, 2022

ABSTRACT

Dicranopteris linearis (Burm. F.) Underw. has many popular traditional uses in Asian countries, such as treating ulcers and boils, allergic symptoms, or respiratory troubles. This study investigated the phytochemical of D. linearis grown in Lam Dong Province. The leaf powder of D. linearis was used to prepare a crude extract. This extract was then applied to the liquid-liquid partition to give different polar fractions. The EA fraction was thus applied to silica gel column chromatography to obtain four compounds. Their chemical structures were elucidated by using Nuclear Magnetic Resonance spectroscopy, as well as by the comparison of their NMR data with reported ones. Four compounds consisted of three flavonols, isoquercetin ***(1)****, quercetin* ***(2)****, kaempferol* ***(3)****, and a sterol, stigmast-5,22-dien-3β-ol-3-O-β-D-glucopyranoside* ***(4)****.*

***Keywords*:** *Dicranopteris linearis,* isoquercetin; kaempferol; quercetin; stigmast-5-en-3β-ol-3-O-β-D-glucopyranoside

1. Introduction

Dicranopteris linearis (Burm. F.) Underw. is a common fern and belongs to the Gleicheniaceae family. They are distributed in Africa and Asia and grow widely in Vietnam, especially in low, hot, and dry mountainous areas (Chi, 2002). The *D. linearis* extracts showed antidiabetic, anticancer, antibacterial, antioxidant, analgesic, and anti-HIV activities (Li et al., 2006; Li et al., 2008; Chen et al., 2014; Ponnusamy et al., 2015; Zakaria et al., 2016; Duy et al., 2019). In particular, they are widely used as a folk medicine to treat fever (Malaysia), intestinal worms (Indochina), asthma, infertility in women (India), wound (Papua New Guinea) (Kamisan et al., 2014), cough, allergic, and respiratory disorders (Mymensingh) (Sarker et al., 2009).

Cite this article as: Nguyen Thu Anh, Nguyen Thi Hoai Thu, Nguyen Ngoc Khanh Van, & Duong Thuc Huy (2022). Study on chemical constituents of the leaves of *Dicranopteris linearis* (Burm. F.) Underw.. *Ho Chi Minh City University of Education Journal of Science, 19*(10), 1593-1598.

This study reported the isolation and structural elucidation of four compounds, including isoquercetin **(1)**, quercetin **(2)**, kaempferol **(3)**, and *β*-sitosterol 3-O-*β*-D-glucopyranoside **(4)** from the leaves of *D. linearis* collected in Lam Dong Province, Vietnam.

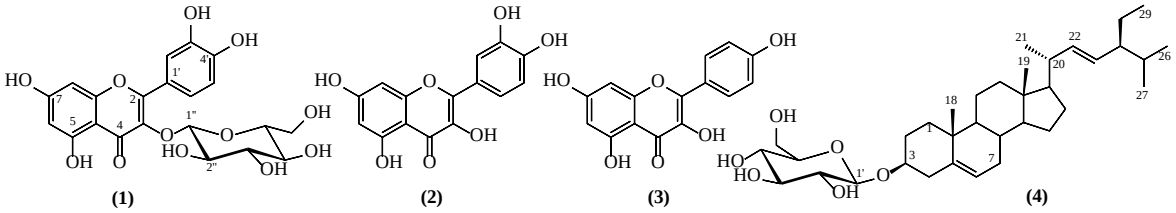

Figure 1. *Chemical structures of isolated compounds* ***1-4***

2. Experiment

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). *n*-Hexane, ethyl acetate (EtOAc), methanol (MeOH), and acetone were used to prepare extracts and to elute column chromatography and thin-layer chromatography. Thin-layer chromatography was carried out 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

The leaves of *D. linearis* (Burm. F.) Underw. were collected in Lam Dong Province, Vietnam, from January to March 2021. The specimen was identified by Prof. Tran Cong Luan, Tay Do University and deposited at the herbarium in the laboratory of the Faculty of Chemistry, Ho Chi Minh City University of Education, Vietnam.

2.3. Extraction and isolation

The leaf powder of *D. linearis* (5.0 kg) was macerated with methanol three times (3 x 30 L) at room temperature. The solvent was removed from the filtrated solution at reduced pressure to obtain a crude extract (280.0 g). This extract was successively applied to the liquid-liquid partition with increasing polarity of solvents: *n*-hexane, *n*-hexane: EtOAc (1:1, v/v), EtOAc to give H (27.44 g), HEA (34.79 g), and EA (23.00 g) extracts. The water-soluble layer was dried to obtain the MeOH extract (60.03 g).

The EA extract (23.0 g) was subjected to a silica gel column chromatography with a mobile phase of *n*-hexane-EtOAc-acetone (1:4:4, v/v/v) to give 13 fractions (EA1-EA13). Fraction EA2 (3.6 g) was subjected to a silica gel column chromatography with a gradient solvent of *n*-hexane-EtOAc-acetone (1:4:4, v/v/v) to yield seven subfractions (EA2.1-EA2.7). Subfraction EA2.2 (1.12 g) was rechromatographed on a silica gel eluting with *n*-hexane: EtOAc (1:1) to afford **1** (12.0 mg). Subfraction EA2.3 (0.93 g) was rechromatographed on a silica gel eluting with *n*-hexane: EtOAc: acetone (2:19:9, v/v/v) to afford **2** (40.0 mg). Subfraction EA2.4 (0.62 g) was rechromatographed on a silica gel eluting

with *n*-hexane: EtOAc: acetone (1:4:2, v/v/v) to afford **3** (16.0 mg). Subfraction EA4 (3.3 g) was chromatographed on a silica gel using *n*-hexane-EtOAc-acetone (1:4:4) to yield 12 subfractions (EA4.1-EA4.12). Subfraction EA4.6 (0.33 g) was chromatographed on a silica gel using *n*-hexane-EtOAc (1:3) as eluent to give **4** (8.3 mg).

- **Isoquercetin** (**1**). Yellow powder. The ^{1}H–NMR data (500 MHz, DMSO-d_6, δ ppm, *J* in Hertz): 12.64 (1H, *s*, 5-OH), 10.87 (1H, *s*, 7-OH), 9.75 (1H, *s*, 3'-OH), 9.17 (1H, *s*, 4'-OH), 7.66 (1H, *brd*, 8.5 Hz, H-6'), 7.52 (1H, *brs*, H-2'), 6.81 (1H, *d*, 8.5 Hz, H-5'), 6.40 (1H, *brs*, H-8), 6.20 (1H, *brs*, H-6), 5.37 (1H, *d*, 8.0 Hz, H-1''), 5.29 (1H, *s*, 2''-OH), 5.14 (1H, *s*, 3''-OH), 4.87 (1H, *s*, 4''-OH), 4.44 (1H, *s*, 6''-OH), 3.28-3.56 (*m*, H-2'' to H-6''), 3.24 (1H, *m*, H-2''), 3.23 (1H, *m*, H-3''), and 3.08 (1H, *m*, H-5''). ^{13}C-NMR (125 MHz, DMSO-d_6): 60.0 (C-6''), 67.8 (C-4''), 71.0 (C-2''), 73.0 (C-3''), 75.7 (C-5''), 93.3 (C-8), 98.5 (C-6), 101.6 (C-1''), 103.7 (C-10), 115.0 (C-2'), 116.0 (C-5'), 120.9 (C-6'), 121.8 (C-1'), 133.3 (C-3), 144.6 (C-4'), 148.3 (C-3'), 156.1 (C-2), 161.1 (C-9), 164.0 (C-5), 165.2 (C-7), and 177.3 (C-4).
- **Quercetin (2)**. Yellow powder. The ^{1}H–NMR data (Acetone-d_6, δ ppm, *J* in Hertz): 12.49 (1H, *s*, 5-OH), 10.84 (1H, *s*, 7-OH), 9.69 (1H, *s*, 4'-OH), 9.35 (1H, *s*, 3'-OH), 8.09 (1H, *s*, 3-OH), 7.67 (1H, *d*, 2.5 Hz, H-2'), 7.54 (1H, *dd*, 8.5, 2.5 Hz, H-6'), 6.88 (1H, *d*, 8.5 Hz, H-5'), 6.40 (1H, *d*, 2.0 Hz, H-8), and 6.18 (1H, *d*, 2.0 Hz, H-6).
- **Kaempferol** (**3**). Yellow powder. The ^{1}H–NMR data (Acetone-d_6, δ ppm, *J* in Hertz): 8.16 (2H, *d*, 8.5 Hz, H-2', H-6'), 7.02 (2H, *d*, 8.5 Hz, H-3', H-5'), 6.54 (1H, *d*, 2.0 Hz, H-8), and 6.27 (1H, *d*, 2.0 Hz, H-6).
- ***Stigmast-5,22-dien-3β-ol-3-O-β-D-glucopyranoside*** **(4).** Yellow powder. The ^{1}H–NMR data (DMSO-d_6, δ ppm, *J* in Hertz): 5.33 (1H, *d*, 3.2 Hz, H-6), 4.84-4.88 (3H, 2'-OH, 3'-OH, 4'-OH), 4.42 (1H, *t*, 5.8 Hz, 6'-OH), 4.22 (1H, *d*, 7.8 Hz, H-1'), 3.93 (1H, *m*, H-3), 3.01-3.56 (6H, H-2', H-3', H-4', H-5', H-6'), 0.90 (3H, *d*, 6.4 Hz, H-21), 0.86 (3H, *s*, H-19), 0.82 (3H, *t*, 6.5 Hz, H-29), 0.81 (3H, *d*, 5.0 Hz, H-26), 0.79 (3H, *d*, 5.0 Hz, H-27), and 0.65 (3H, *s*, H-18). ^{13}C–NMR (125 MHz, DMSO-d_6): 12.2 (C-18), 12.3 (C-29), 19.1 (C-21), 19.4 (C-19), 19.6 (C-27), 20.2 (C-26), 21.1 (C-11), 23.1 (C-28), 24.3 (C-15), 25.9 (C-23), 28.3 (C-16), 29.2 (C-25), 29.7 (C-7), 31.9 (C-2), 31.9 (C-8), 33.8 (C-22), 36.0 (C-20), 36.7 (C-10), 37.3 (C-1), 38.8 (C-12), 40.0 (overlap, C-4), 42.4 (C-13), 45.6 (C-24), 50.1 (C-9), 55.9 (C-17), 56.7 (C-14), 61.6 (C-6'), 70.6 (C-4'), 74.0 (C-2'), 77.2 (C-3'), 77.3 (C-5'), 77.4 (C-3), 101.3 (C-1'), 121.7 (C-6), and 140.9 (C-5).

3. Results and discussion

Compound **1** was obtained as a yellow powder. The ^{1}H-NMR spectrum of **1** displayed signals of a flavanone skeleton. This spectrum showed a chelated hydroxyl proton signal at δ_H 12.64 (1H, *s*) of a 5-OH as normal, along with other hydroxy proton signals at δ_H 10.87 (1H, *s*, 7-OH), 9.75 (1H, *s*, 3'-OH), and 9.17 (1H, *s*, 4'-OH). At the higher magnetic field, it revealed a set of three aromatic protons at δ_H 7.66 (1H, *brd*, 8.5 Hz, H-6'), 7.52 (1H, *brs*, H-2'), and 6.81 (1H, *d*, 8.5 Hz, H-5'), identifying the presence of a 1,3,4-trisubstituted benzene

ring. The proton spectrum also showed a pair of broad-singlet aromatic protons with a small coupling constant at δ_H 6.40 (1H, *brs*, H-8) and 6.20 (1H, *brs*, H-6). These signals were characteristic markers for the flavonol backbone. On the other hand, the proton spectrum of **1** showed signals of a *β*-glucose unit, including an anomeric proton at δ_H 5.37 (1H, *d*, 8.0 Hz, H-1") and five oxymethines in the range δ_H 3.08-3.56. These corresponded to the presence of twenty-one carbon signals on the ^{13}C-NMR spectrum of a flavonol glucoside, including eight quaternary aromatic carbons, six aromatic methine carbons, an anomeric carbon (δ_C 101.6, C-1"), one carbonyl carbon (δ_C 177.34), four oxygenated methine carbons at δ_C 67.8 (C-4"), 71.0 (C-2"), 73.0 (C-3"), 75.7 (C-5") and an oxygenated methylene signal at δ_C 60.0 (C-6"). Additionally, in comparison of ^{1}H- and ^{13}C-NMR spectral data of compound **1** with those reported in the literature (Kazuma et al., 2003; Napolitano et al., 2012), compound **1** was assigned to be isoquercetin.

Compound **2** was obtained as a yellow powder. The ^{1}H-NMR spectrum of **2** displayed five hydroxy proton signals at δ_H 12.49 (1H, *s*, 5-OH), 10.86 (1H, *s*, 7'-OH), 9.69 (1H, *s*, 4'-OH), 9.35 (1H, *s*, 3'-OH), and 8.09 (1H, *s*, 3-OH). The 1,3,4–trisubstituted benzene ring in **2** was determined by the displaying of three proton signals at δ_H 7.67 (1H, *d*, 2.5 Hz, H-2'), 7.54 (1H, *dd*, 8.5 Hz, 2.5 Hz, H-6'), and 6.88 (1H, *d*, 8.5 Hz, H-5'). Moreover, its ^{1}H-NMR showed two *meta*-coupling proton signals at δ_H 6.40 (1H, *d*, 2.0 Hz, H-8) and 6.18 (1H, *d*, 2.0 Hz, H-6). All the above signals were characterized for a flavone skeleton. The good compatibility between its NMR data and those in the literature (Zhang et al., 2008) suggested the structure of **2** to be quercetin.

Compound **3** was obtained as a yellow powder. The comparison NMR data of **2** and **3** showed many similar signals of a flavone. However, the proton spectrum of **2** possessed signals at δ_H 8.16 (2H, *d*, 8.5 Hz, H-2′ and H-6′) and 7.02 (2H, *d*, 8.5 Hz, H-3′ and H-5′) of a *para*–disubstituted benzene ring, instead of a 1,3,4–trisubstituted benzene ring as in **2**. Based on the above analysis and the good correspondence of NMR data of **3** with those reported in the literature (Xiao et al., 2006), **3** were thus assigned as kaempferol.

Compound **4** was obtained as a yellow powder. The ^{1}H-NMR spectrum of compound **4** displayed an olefin proton signal at δ_H 5.33 (1H, *d*, 3.2 Hz, H-6), an oxymethine group at δ_H 3.93 (1H, *m*, H-3), six methyl proton signals including two tertiary methyl groups resonating at δ_H 0.86 (3H, *s*, CH_3-19) and 0.65 (3H, *s*, CH_3-18); three secondary methyl groups resonating at δ_H 0.90 (3H, *d*, 6.4 Hz, CH_3-21), 0.81 (3H, *d*, 5.0 Hz, CH_3-26), and 0.79 (3H, *d*, 5.0 Hz, CH_3-27); and one primary methyl group resonating at δ_H 0.82 (3H, *t*, 6.5 Hz, CH_3-29). These were the characteristics of a stigmastane-type steroid (Cayme & Ragasa, 2004). The ^{1}H-NMR spectrum also revealed an anomeric proton signal at δ_H 4.22 (1H, *d*, 7.8 Hz, H-1′), and the oxygenated methylene and methines in the range 3.01-3.56 ppm of H-1′ to H-6′), which was typical of a glucose moiety. These corresponded to the ^{13}C-NMR spectrum exhibiting 35 carbons, consisting of 1 quaternary olefinic carbon (δ_C 140.9), an

olefin methine carbon (δ_C 121.7), one hydroxymethyl carbon (δ_C 77.4) at C-3 and signals of a sugar unit comprising 1 anomeric carbon (δ_C 101.3, C-1'), 4 oxygenated methine carbons (δ_C 70.6, 74.0, 77.2, 77.3), and an oxygenated methylene carbon (61.6, C-6'). The ^{1}H and ^{13}C-NMR spectral features of **4** resembled those of stigmast-5,22-dien-3*β*-ol-3-*O*-*β*-D-glucopyranoside (Farabi et al., 2017), which was common phytosterol. Therefore, **4** was determined as stigmast-5-en-3*β*-ol-3-*O*-*β*-D-glucopyranoside. This compound was isolated for the first time from leaves of *D. linearis*.

4. Conclusions

From the leaves of *D. linearis* in Lam Dong Province, four compounds, including three flavonols isoquercetin (**1**), quercetin (**2**), and kaempferol (**3**), and one sterol stigmast-5,22-dien-3*β*-ol-3-*O*-*β*-D-glucopyranoside (**4**) were isolated. Their chemical structures were determined by using NMR spectroscopic method as well as comparison with the literature. Further studies on this species are in progress.

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

REFERENCES

Cayme, J. M., & Ragasa, C. (2004). Structure elucidation of stigmasterol and β-sitosterol from *Sesbania grandiflora* Linn Pers. and β-carotene from *Heliotropium indicum* Linn. by NMR spectroscopy. *Kimika*, *20*(1), 5-12.

Chen, J., Chen, J., & Gao, K. (2014). Chemical constituents and biological activities of *Dicranopteris linearis*. *Chem Nat Compd*, *49*(6), 970-972.

Farabi, K., Harneti, D., Nurlelasari, Maharani, R., Hidayat, A. T., Supratman, U., Awang, K., & Shiono, Y. (2017). Cytotoxic steroids from the bark of *Aglaia argentea* (Meliaceae). *CMU J Nat Sci.*, *16*(4), 293-306.

Kazuma, K., Noda, N., & Suzuki, M. (2003). Malonylated flavonol glycosides from the petals of *Clitoria ternate*. *Phytochemistry*, *62*(2), 229-237.

Le, Q. D., Nguyen, M. C., & Nguyen, P. T. (2016). Screening α-amylase and α-glucosidase inhibitor activities of traditional medicinal plants in diabetes treatment. *Vietnam Agric Fish*, *22*, 139-147.

Li, X., Cheng, X., Yang, L., Wang, R., & Zheng, Y. (2006). Dichotomains A and B: two new highly oxygenated phenolic derivatives from *Dicranopteris dichotoma*. *Org Lett*, *38*(3), 679-682.

Li, X., Tu, L., Zhao, Y., Peng, L., Xu, G., Cheng, X., & Zhao, Q. 2008. Terpenoids from two *Dicranopteris* Species. *Helv Chim Acta*. *91*, 856-861.

Napolitano, J. G., Lankin, D. C., Chen, S. N., & Pauli, G. F. (2012). Complete ^{1}H-NMR spectral analysis of ten chemical markers of *Ginkgo biloba*. *Magn Reson Chem*, *50*(8), 569-575.

Ponnusamy, Y., Chear, N. J., Ramanathan, S., & Lai, C. (2015). Polyphenols rich fraction of *Dicranopteris linearis* promotes fi broblast cell migration and proliferation in *vitro*. *J Ethnopharmacol, 168,* 305-314.

Sarker, S. K., & Hossain, A. E. (2009). Pteridophytes of greater Mymensingh district of Bangladesh used as vegetables and medicines. *Bangladesh J Plant Taxon*, *16*(1), 47-56.

Vo, V. C. (2002). *Dictionary of medical plants in Vietnam.* Med Publshing House, 1083-1084.

Xiao, Z. P., Wu, H. K., Wu, T., Shi, H., Hang, B., & Aisa. H. A. (2006). Kaempferol and quercetin flavonoids from *Rosa rugosa*. *Chem Nat Compd, 42*(6), 736-737.

Zakaria, Z. A., Mohamed, A. M., Jamil, N. S. M., Somchit, M. N., Zuraini, A., Rofiee, M. S., Arifah, A. K., & Sulaiman, M. R. (2011). In *vitro* cytotoxic and antioxidant properties of the aqueous, chloroform and methanol extracts of *Dicranopteris linearis* leaves. *African J Biotechnol. 10*(2), 273-282.

Zhang, Y., Wang, D., Yang, L., Zhou, D., & Zhang, J. (2014). Purification and characterization of flavonoids from the leaves of *Zanthoxylum bungeanum* and correlation between their structure and antioxidant activity. *PloS One, 9*(8), e105725.

NGHIÊN CỨU THÀNH PHẦN HÓA HỌC CỦA LÁ CÂY RÁNG TÂY SƠN *DICRANOPTERIS LINEARIS* (BURM. F.) UNDERW.

Nguyễn Thu Anh[1], Nguyễn Thị Hoài Thu[2], Nguyễn Ngọc Khánh Vân[3], Dương Thúc Huy[1*]

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

[2]Trường Đại học Y Dược Thành phố Hồ Chí Minh, Việt Nam

[3]Trường THPT Gia Định, Thành phố Hồ Chí Minh, Việt Nam

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

Ngày nhận bài: 03-3-2022; ngày nhận bài sửa: 06-4-2022; ngày duyệt đăng: 06-10-2022

TÓM TẮT

*Dicranopteris linearis (*Burm. F.*) Underw. được ứng dụng rộng rãi trong y học cổ truyền ở các nước châu Á với các công dụng như điều trị loét, nhọt, các triệu chứng dị ứng và rối loạn hô hấp… Nghiên cứu này được thực hiện nhằm khảo sát hóa thực vật của lá cây Dicranopteris linearis mọc ở Lâm Đồng. Bột lá khô Dicranopteris linearis được điều chế thành cao thô. Sau đó, thực hiện chiết lỏng – lỏng cao thô thu được các cao có độ phân cực khác nhau. Cao EA được tiến hành sắc kí cột để cô lập bốn hợp chất. Cấu trúc hóa học của các hợp chất được xác định bằng các phương pháp phổ nghiệm kết hợp so sánh với tài liệu tham khảo. Bốn hợp chất trên bao gồm ba hợp chất flavonols, isoquercetin **(1)**, quercetin **(2)** và kaempferol **(3)** và một hợp chất sterol, stigmast-5,22-dien-3β-ol-3-O-β-D-glucopyranoside **(4)**. Hợp chất **4** lần đầu tiên được biết có hiện diện trong lá cây Dicranopteris linearis.*

Từ khóa: *Dicranopteris linearis;*isoquercetin; kaempferol; quercetin; stigmast-5-en-3β-ol-3-O-β-D-glucopyranoside