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Research Article

CHEMICAL CONSTITUENTS OF THE LIVERWORT *MARCHANTIA POLYMORPHA* GROWING IN VIETNAM

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ABSTRACT

*Marchantia polymorpha has been associated with many pharmaceutical applications in Asian countries, such as treating inflammation, liver disease, open wounds, boils, fractures, insect and snake bites, etc. This study investigated the phytochemical of M. polymorpha grown in Lam Dong Province. The acetone extract of M. polymorpha was applied to silica gel column chromatography to obtain five 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. Five compounds were elucidated as (Z)-dimethomorph (**1**), (E)-dimethomorph (**2**), methyl 3,4-dihydroxybenzoic acid (**3**), marchantin A (**4**), and quercetin (**5**).*

Keywords: dimethomorph; marchantin A; *Marchantia polymorpha;* quercetin

1. Introduction

Marchantia polymorpha L. is a liverwort belonging to the family Marchantiaceae (Lindl.). *M. polymorpha* is widely distributed in the western Himalayas and other mountainous regions of India (Gahtori et al., 2011). It is native to Vietnam, growing along rivers, streams, and wet lawns, mainly on rocks (Scott, 1987). This liverwort is well-known as medicinal source in many countries such as India, China, some countries in Europe, and Vietnam (Gahtori et al., 2011; Scott, 1987; Chandra et al., 2017). It is widely used as a folk medicine to treat inflammation, liver disease, open wounds, boils, fractures, insect and snake bites, or as a diuretic (Chandra et al., 2017). Previous studies of *M. polymorpha* have so far shown the presence of over 55 compounds (Asakawa et al., 1990; Fang et al., 2007, Jensen et al., 2012, Madubunyi et al., 1994; Markham et al., 1974; Mawjo et al., 1985; Qu et al., 2007; Asakawa et al., 1987), including bis-bibenzyl, sesquiterpenoids, flavonoids, phenolics, coumarins, and glycosides. The extracts of this liverwort showed antioxidant, antibacterial,

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tyrosinase, and α-glucosidase inhibitors in the treatment of type 2 diabetes, mitotic resistance on the breast cancer cell line MCF-7 (Gahtori & Chaturvedi, 2011), (Gökbulut et al., 2012; Mewari et al., 2008; Tran et al., 2020). However, chemical constituents of the Vietnam-native material are scarce. This paper described the isolation and structural elucidation of five compounds from *M. polymorpha* collected in Lam Dong province, Vietnam. Their chemical structures are determined as *(Z)*-dimethomorph (**1**), *(E)*-dimethomorph (**2)**, methyl 3,4-dihydroxybenzoic acid (**3**), marchantin A (**4**), and quercetin (**5**).

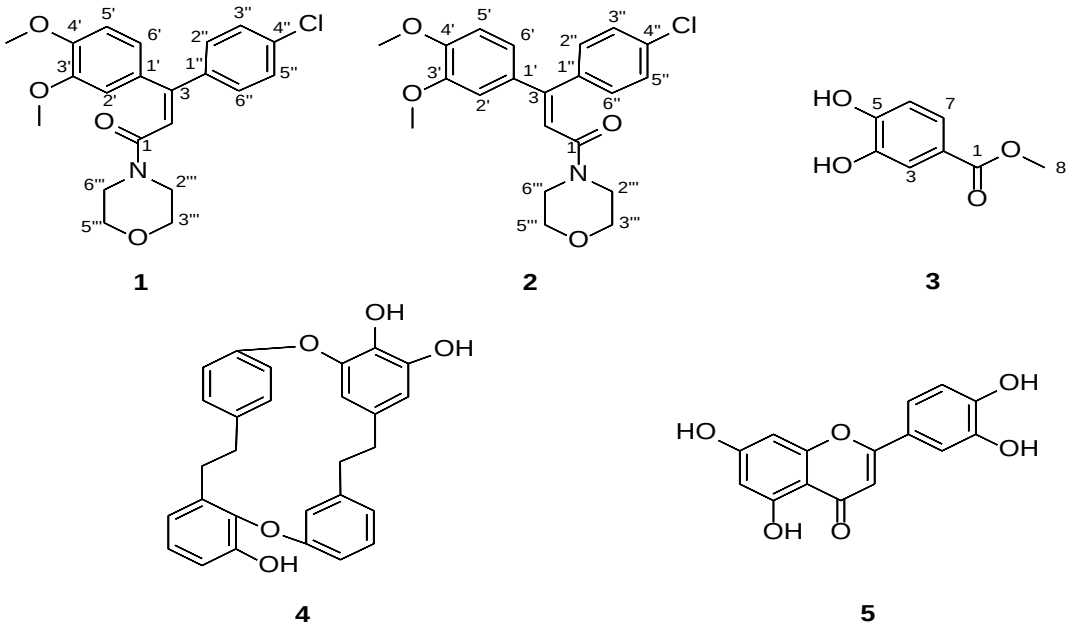

Figure 1. *Chemical structures of isolated compounds* ***1-5***

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). *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

Marchantia polymorpha L. was collected in Lam Dong Province, Vietnam, from January to March 2021. The specimen was deposited at the herbarium in the laboratory of the Faculty of Chemistry, Ho Chi Minh City University of Education, Vietnam (UE-021). The scientific name was defined by Dr. Tram Nguyen Khanh Trinh, Faculty of Biology, Ho Chi Minh City University of Science.

2.3. Extraction and isolation

The dry powder of *M. polymorpha* (2 kg) was crushed and macerated with acetone (2 x 10 L) at room temperature every 24 hours. The solvent was removed from the filtrated solution at reduced pressure to obtain a crude acetone extract (181 g). This extract was successively applied to liquid-liquid partition with increasing polarity of solvents: *n*-hexane, *n*-hexane: EtOAc (1:1), respectively, to obtain **H** (51.9 g), **HEA** (48.3 g) and **Ac** (15.8 g) extracts, respectively. Extract HEA (48.3 g) was applied to silica gel column chromatography (CC), eluted with *n*-hexane: EtOAc (1:1) to afford eight fractions **HEA1**-**HEA8**. Fraction **HEA3.1** (3.4 g) was applied to silica gel CC, eluted with *n*-hexane: EtOAc-acetone (8:1:1) to afford eight fractions HEA3.1-10. Fraction HEA3.3 (120 mg) was applied to C-18-reverse phase CC, eluted with methanol:water (5:1) to afford compounds **1** (3.6 mg), **2** (4.1 mg), and **3** (11 mg). Fraction **HEA6** (8.7 g) was rechromatographed by silica gel CC, and eluted with $CHCl_3$ to afford four fractions **HEA6**.**1**-**4**. Purification **HEA6.4** by silica gel CC provided compound 4. Fraction **HEA8** (7.4 g) was subjected to silica gel CC, eluted with *n*-hexane: EtOAc-acetone (5:1:1) to provide five fractions **HEA8.1**-**5**. Washing **HEA8.5** (1.2 g) with acetone gave compound **5** (205 mg).

- ***(Z)*-Dimethomorph** (**1**). Yellow powder. The ^{1}H–NMR data (500 MHz, acetone-d_6) and ^{13}C-NMR (125 MHz, acetone-d_6) are shown in Table 1. HRESI-MS *m/z* 388.1322 $[M+H]^+$ (Calcd for $C_{21}H_{23}ClNO_4$: 388.1316).
- ***(E)*-Dimethomorph** (**2**). Yellow powder. The ^{1}H–NMR data (500 MHz, acetone-d_6) and ^{13}C-NMR (125 MHz, acetone-d_6) are shown in Table 1. HRESI-MS *m/z* 388.1322 $[M+H]^+$ (Calcd for $C_{21}H_{23}ClNO_4$: 388.1316).
- **Methyl 3,4-dihydroxybenzoic acid** (**3**). White powder. The ^{1}H–NMR data (Acetone-d_6, δ ppm, *J* in Hertz): 7,48 (1H, *d*, 2.0 Hz, H-3), 7.43 (1H, *dd*, 8.0 Hz, 2.0 Hz, H-7), 6.88 (1H, *d*, 8.0 Hz, H-6) and 3.79 (3H, s, H-8). ^{13}C-NMR (125 MHz, $CDCl_3$): 166.4 (C-1), 150.7 (C-5), 145.6 (C-4), 132.7 (C-6), 122.8 (C-2), 116.4 (C-3), 108.3 (C-7) and 51.7 (C-8).
- **Marchantin A (4).** Yellow powder. The ^{1}H-NMR data (500 MHz, $CDCl_3$, δ ppm, *J* in Hertz): 7.12 (1H, *dd*, 8.0 Hz, 7.5 Hz, H-11), 7.00 (1H, *dd*, 8.0 Hz, 1.5 Hz, H-10), 6.97 (1H, *t*, 7.8 Hz, H-13'), 6.91 (1H, *d*, 8.5 Hz, H-3), 6.91 (1H, *d*, 8.5 Hz, H-5), 6.85 (1H, *dd*, 8.0 Hz, 1.5 Hz, H-12), 6.57 (1H, *d*, 8.0 Hz, H-2), 6.57 (1H, *d*, 8.0 Hz, H-6), 6.57 (1H, *dd*, 2.5 Hz, 2.0 Hz, H-10'), 6.53 (1H, *dd*, 8.5 Hz, 2.0 Hz, H-12'), 6.46 (1H, *d*, 1.5 Hz, H-5'), 6.39 (1H, *brd*, 7.5 Hz, H-14'), 5.13 (1H, *d*, 1.5 Hz, H-3'), 2.97–3.01 (2H, *m*, H-7), 2.97-3.01 (2H, *m*, H-8), 2.78-2.80 (2H, *m*, H-7') and 2.72-2.74 (2H, *m*, H-8'). ^{13}C-NMR (125 MHz, $CDCl_3$): 156.8 (C-11'), 153.2 (C-1), 148.7 (C-13), 146.5 (C-2'), 144.3 (C-6'), 143.1 (C-9'), 139.7 (C-14), 139.1 (C-4), 136.2 (C-9), 132.5 (C-4'), 130.8 (C-1'), 129.6 (C-3), 129.6 (C-5), 128.9 (C-13'), 126.0 (C-11), 123.2 (C-14'), 121.9 (C-10), 121.2 (C-2), 121.2 (C-6), 115.5 (C-10'), 114.4 (C-12), 112.0 (C-12'), 109.3 (C-5'), 107.9 (C-3'), 35.5 (C-8'), 35.3 (C-7), 34.1 (C-7'), and 30.3 (C-8) (Nguyen et al., 2020).

- **Quercetin (5)**. Yellow powder. The ^{1}H–NMR data were consistent with those reported by Zhang et al. (2014).

3. Results and discussion

Compound **1** was obtained as a yellow powder. The ^{1}H-NMR spectrum showed the presence of a 1,2,4-trisubstituted benzyl ring with three aromatic protons at δ_H 6.93 (1H, *d*, 8.5 Hz, H-5'), 6.99 (1H, *d*, 2.0 Hz, H-2'), and 6.77 (1H, *dd*, 2.0, 8.5 Hz, H-6') and a 1,4-disubstituted benzyl ring [δ_H 7.44 (1H, *d*, 8.5 Hz, H-2"/6") and 7.27 (1H, *d*, 8.5 Hz, H-3"/5")]. In higher-field, the ^{1}H-NMR spectrum showed the presence of one olefinic proton at δ_H 6.42, two methoxy groups at δ_H 3.82 and 3.77, and four methylene groups in the range 3.22-3.42 ppm. In the so-called A-ring, HMBC correlations of proton H-6' to C-4' (δ_C 151.2), of H-2' to C-3' (δ_C 149.4) and C-4', of the methoxy at 3.77 to C-3' and the methoxy at 3.82 to C-4' indicated the presence of two methoxy groups at C-3' and C-4'. The HMBC spectrum showed the correlations of H-2" and H-6' to a quaternary carbon at δ_C 146.2 indicating the connection of both aromatic protons at C-3 and identifying the presence of a 1,2,4-trisubstituted benzene ring at C-3. HMBC correlations of H-2 (δ_H 6.42) to a carbonyl carbon at δ_C 166.9 (C-1) and C-3 indicated the position of this proton. The methylene groups at δ_H 3.22 and 3.35 gave HMBC correlation to C-1, and they showed HMBC correlations with each other, indicating that both methylene groups attached to a nitrogen atom of an amide group. HMBC correlations of the other methylene groups at δ_H 3.40 and 3.42 to C-2'" and C-6'". Moreover, these groups showed HMBC correlations, indicating the ether linkage between them. This spectroscopic feature indicated a morpholine ring connected to C-1. The final position C-4" was defined to attach a chlorine atom due to HRESI mass data. Comparing ^{1}H- and ^{13}C-NMR spectral data of compound **1** with those reported in the literature (Lu et al. 2018), compound **1** was assigned to be *Z*-dimethomorph.

Compound **2** was an isomer of **1** based on its HRESI mass data. NMR data of **2** were identical to those of **1,** with differences in the chemical shift of CH-2 and the morpholine moiety. This indicated that **2** has a different configuration of the double bond at C-2 and C-3. Accordingly, **2** was elucidated as *E*-dimethomorph.

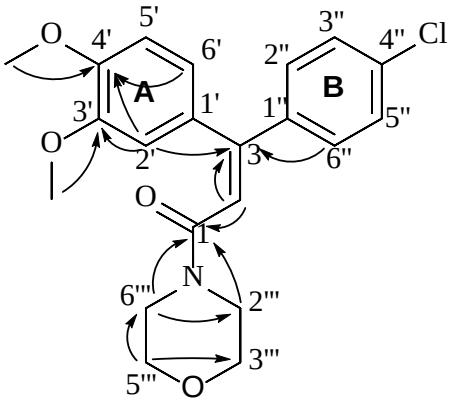

Figure 2. *Selected HMBC correlations of* ***1***

Table 1. *NMR data of **1** and **2** in acetone-d_6*

No	1		2	
	δ_H, (multi., *J* in Hz)	δ_C	δ_H, (multi., *J* in Hz)	δ_C
1	-	166.9	-	167.2
2	6.42 (1H, s)	112.3	6.29 (1H, s)	114.1
3	-	146.2	-	145.5
1'	-	126.7	-	131.9
2'	6.99 (1H, d, *J* = 2.0 Hz)	112.4	6.88 (1H, d, *J* = 2.0 Hz)	112.3
3'	-	149.4	-	150.1
4'	-	151.2	-	150.8
5'	6.93 (1H, d, *J* = 8.5 Hz)	121.6	6.98 (1H, d, *J* = 8.0 Hz)	123.2
6'	6.77 (1H, dd, J_1 = 8.5 Hz, J_2 = 2.5 Hz)	121.9	6.76 (1H, dd, J_1 = 8.0 Hz, J_2 = 2.0 Hz)	123.2
1''	-	138.8	-	141.1
2''	7.44 (1H, d, *J* = 8.5 Hz)	132.2	7.40 (1H, d, *J* = 8.5 Hz)	130.6
3''	7.27 (1H, d, *J* = 8.5 Hz)	129.0	7.37 (1H, d, *J* = 8.5 Hz)	129.3
4''	-	134.5	-	134.6
5''	7.27 (1H, d, *J* = 8.5 Hz)	129.0	7.37 (1H, d, *J* = 8.5 Hz)	129.3
6''	7.44 (1H, d, *J* = 8.5 Hz)	132.2	7.40 (1H, d, *J* = 8.5 Hz)	130.6
2'''	3.22 (2H, m)	42.0	3.10 (2H, m)	47.3
3'''	3.40 (2H, m)	66.9	3.42 (2H, m)	67.0
5'''	3.42 (2H, m)	67.3	3.45 (2H, m)	66.9
6'''	3.35 (2H, m)	47.3	3.32 (2H, m)	42.0
3'-OMe	3.77 (3H, s)	56.1	3.73 (3H, s)	56.2
4'-OMe	3.82 (3H, s)	56.0	3.84 (3H, s)	56.1

4. Conclusions

From the liverwort *M. polymorpha* growing in Lam Dong Province, five compounds were isolated and elucidated, including (*Z*)-dimethomorph (**1**), (*E*)-dimethomorph (**2)**, methyl 3,4-dihydroxybenzoic acid (**3**), marchantin A (**4**), and quercetin (**5**). Their chemical structures were determined by using NMR spectroscopic method as well as by comparison with the literature. Further studies on this species are in progress.

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

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NGHIÊN CỨU THÀNH PHẦN HÓA HỌC CỦA ĐỊA TIỄN *MARCHANTIA POLYMORPHA* SINH TRƯỞNG Ở VIỆT NAM

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TÓM TẮT

*Marchantia polymorpha có nhiều ứng dụng trong y học như kháng viêm, các bệnh lí về gan, các vết thương hở, vết thương rắn và côn trùng cắn… Nghiên cứu này được thực hiện nhằm khảo sát hóa thực vật của loài địa tiễn M. polymorpha mọc ở Lâm Đồng. Cao acetone của M. polymorpha được tiến hành sắc kí cột để cô lập năm 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. Năm hợp chất trên bao gồm (Z)-dimethomorph (**1**), (E)-dimethomorph (**2**), methyl 3,4-dihydroxybenzoic acid (**3**), marchantin A (**4**), và quercetin (**5**).*

Từ khóa: dimethomorph; marchantin A; *Marchantia polymorpha;* quercetin