

Thành phần hóa học của cây lăn tăn

TÓM TẮT

Chi *Pilea* là chi lớn nhất của họ Urticaceae, bao gồm hơn 600 loài. *Pilea* được mô tả lần đầu tiên bởi nhóm tác giả Lindley (1821) và Weddell (1869) và có thể dễ dàng phân biệt với các chi khác trong họ Urticaceae bằng sự kết hợp của các lá mọc đối, các lá kèm trong cuống lá có dây chằng ở mỗi nách lá. Bảng phương pháp này 159 loài ^o ^{hiểu} của chi *Pilea* đã được định danh và phân thành 3 nhóm: Integifoliae, Heterophyllae và Dentatae. Từ cao chloroform và cao acetone của cây Lăn tăn (*Pilea microphylla*) đã phân lập được bảy hợp chất tinh khiết, bao gồm ergosterol (1), β -sitosterol (2), daucosterol (3), isoarborinyl acetate (4), 2',~~4'~~-dihydroxy-6'-methoxy-3',5'-dimethylchalcone (5), 3,5,7-trihydroxy-8-methoxyflavone (6) và kaempferol (7). Cấu trúc hóa học của các hợp chất được xác định dựa trên các phương pháp hóa lý hiện đại như phổ HR-ESI-MS, 1D và 2D-NMR và so sánh với tài liệu tham khảo. Tất cả bảy hợp chất này lần đầu tiên được cô lập từ chi *Pilea*.

Từ khóa: *Pilea microphylla* (L.), Urticaceae, steroid, triterpenoid, flavonoid.

Chemical constituents of *Pilea microphylla* (L.)

ABSTRACT

Pilea, the largest genus of the Urticaceae, included over 600 species. This genus, first described by Lindley (1821) and Weddell (1869), is easily distinguished from other Neotropical Urticaceae by the combination of opposite leaves and ligulate intrapetiolar stipules in each leaf axil. Based on the isomorphy and margin morphology of the leaf 159 species were recognized and classified into three groups: Integrifoliae, Heterophyllae, and Dentatae. Killip (1936) subdivided *Pilea* into 12 groups largely based on Weddell's (1856, 1869) studies. Most of the species are small herbs, many of which are facultatively epiphytic or epipetric. Phytochemical investigations of the chloroform and acetone extracts of the whole plant *Pilea microphylla* led to the isolation of seven pure compounds, including ergosterol (1), β -sitosterol (2), daucosterol (3), isoarborinyl acetate (4), 2',4'-dihydroxy-6'-methoxy-3',5'-dimethylchalcone (5), 3,5,7-trihydroxy-8-methoxyflavone (6), and kaempferol (7). Their chemical structures were elucidated by extensive HR-ESI-MS, 1D and 2D-NMR spectroscopic data analysis, and comparison with previously published ones. Seven compounds were reported for the first time from *Pilea* genus.

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Keywords: *Pilea microphylla* (L.), Urticaceae, steroids, triterpenoid, flavonoid.

1. INTRODUCTION

Pilea microphylla, a succulent herb or small shrub growing in heavy shade, does not produce fruit. This species can spread entirely depending on vegetative reproduction. According to Pacific Island Ecosystems at Risk (2010), *P. microphylla* is considered as a problematic weed affecting the tropical and subtropical environments worldwide^{1,2}. Zou *et al*³ reported the presence of some flavonoid glycosides in *P. microphylla*, quercetin 3-*O*-rutinoside, 3-*O*-caffeoylequinic acid, luteolin 7-*O*-glucoside, apigenin 7-*O*-rutinoside, apigenin 7-*O*- β -D-glucopyranoside and quercetin³. Chahardehi *et al*⁴ showed that some extracts of this plant possessed antioxidant and antimicrobial activities. This paper would like to present some secondary metabolites of this species.

2. MATERIALS AND METHODS

2.1. General experimental procedures

The HR-ESI-MS was recorded on an HR-ESI-MS MicrOTOF-Q mass spectrometer. The ¹H-NMR (500 MHz) and ¹³C-NMR (125 MHz) spectra were recorded on a Bruker Avance 500 spectrometer. Chemical shifts are expressed in ppm using a residual solvent signal as an internal reference (CDCl_3 δ_H 7.26, δ_C 77.2). Thin-layer chromatography (TLC) was carried out on precoated silica gel 60 F₂₅₄ or silica gel 60 RP-18

F254S (Merck) and the isolated compounds were visualized by spraying with 5% vanillin solution *co acid Sulfuric?* followed by heating. Gravity column chromatography was performed on silica gel 60 (0.040 mm \pm 0.063 mm, Himedia).

2.2. Plant material

Pilea microphylla (L.) was collected in August 2018, at Bien Hoa city, Dong Nai province, Vietnam. The scientific name was authenticated by PhD. Dang Van Son, Institute of Tropical Biology, Southern Vietnam.

2.3. Extraction and isolation

The fresh whole plant (47.0 kg) was cleaned under running tap water, then air-dried and ground. The dried powder (3.1 kg) was macerated with methanol at room temperature. After filtration, the methanol solution was evaporated exhaustively at the reduced pressure yielding a dark-green residue (483.7 g). The methanol residue was subjected to silica gel solid phase extraction and eluted consecutively with *n*-hexane, chloroform, acetone, ethyl acetate. After evaporated at the reduced pressure of these extracted solutions, five extracts were obtained, including *n*-hexane (25.5 g), chloroform (40.7 g), ethyl acetate (36.9 g), acetone (71.9 g), and the remaining methanol residue (189.1 g).

The chloroform extract (40.7 g) was applied to silica gel column chromatography eluted with *n*-hexane : chloroform (stepwise, 9:1

to 0:10) to afford thirteen fractions (C1 \div C13). The fraction C5 (126.3 mg) was selected for further fractionation by silica gel column chromatography, eluting with *n*-hexane : chloroform (stepwise, 10:0 to 0:10) to obtain compound 4 (20 mg). Fraction C11 (4,850.4 mg) was silica gel column chromatography eluting with *n*-hexane : ethyl acetate (stepwise, 9.8:0.2 to 5:5) to obtain compound 2 (15 mg). Fraction C12 (3,664.5 mg) was selected for further fractionation by silica gel column chromatography using an isocratic mobile phase consisting of *n*-hexane : ethyl acetate (10:0 to 0:10) to obtain compound 1 (7.5 mg).

applied on

The acetone extract (7.5 g) was applied to silica gel column chromatography, eluted with solvent systems of *n*-hexane : ethyl acetate (10:0 to 0:10), then ethyl acetate : methanol (7:3 to 0:10) to afford eight fractions (A1 \div A8). The fraction A2 (168.2 mg) was silica gel column chromatography using *n*-hexane : ethyl acetate (8:2), then *n*-hexane : chloroform (8:2) and finally by *n*-hexane : acetone (9:1) to obtain compound 5 (5.0 mg). The fraction A5 (1150 mg) was silica gel column chromatography using *n*-hexane : ethyl acetate (6:4 to 0:10) then methanol 100% to obtain four subfractions (A5.1 \div A5.4). The A5.1 (39.1 mg) was applied to a silica gel column chromatography using *n*-hexane : chloroform (5:5 to 0:10), then *n*-hexane : acetone (9:1) to obtain compound 6 (5.3 mg). The same procedure was applied to A5.2 (222.6 mg), using *n*-hexane : chloroform (5:5 to 0:10), then chloroform : methanol (9:1) to obtain compound 7 (9.3 mg). Fraction A7 (408.5 mg) was silica gel column chromatography using chloroform : methanol (stepwise, 9:1 to 0:10) to obtain compound 3 (15 mg).

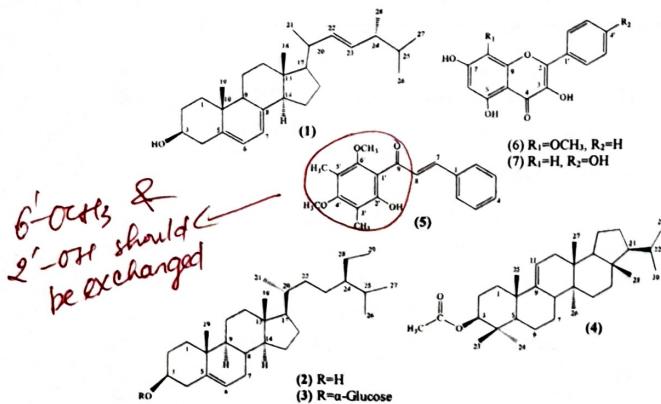


Figure 1. Structures of isolated compounds 1 \div 7

3. RESULTS

By using efficient separation techniques, the chemical investigation of the chloroform and acetone extracts of the whole plant of *Pilea microphylla* led to the isolation of seven compounds. Their chemical structures were elucidated by 1D and 2D NMR and HR-ESI-MS analysis. They were three steroids, ergosterol (1), β -sitosterol (2), daucosterol (3), one triterpenoid, isoarborinyl acetate (4) and three flavonoids, 2',4'-dihydroxy-6'-methoxy-3',5'-dimethylchalcone (5), 3,5,7-trihydroxy-8-methoxyflavone (6), and kaempferol (7).

Ergosterol (1): Colorless crystals. HR-ESI-MS (positive mode) *m/z* 397.3483 [M+H]⁺ (calcd. for C₂₈H₄₅O⁺, 397.3473). The ¹H and ¹³C-NMR (CDCl₃) see Table 1.

β -Sitosterol (2): White powder. ¹H-NMR data (CDCl₃) (*J* in Hertz): δ _H 3.55 (1H, *ddd*, 15.8, 11.0, 4.6, H-3), 5.38 (d, 5.2, H-6), 1.03 (3H, *s*, H-18), 0.70 (3H, *s*, H-19), 0.95 (3H, *d*, 6.6, H-21), 0.88 (3H, *d*, 7.5, H-26), 0.84 (3H, *d*, 6.9, H-27), 0.85 (3H, *s*, H-29). The ¹³C-NMR (CDCl₃): δ _C 37.3 (C-1), 31.7 (C-2), 71.8 (C-3), 42.3 (C-4, C-13), 140.8 (C-5), 121.7 (C-6), 31.9 (C-7, C-8), 50.2 (C-9), 36.1 (C-10, C-20), 21.1 (C-11), 39.8 (C-12), 56.8 (C-14), 26.2 (C-15), 28.2 (C-16), 56.1 (C-17), 11.9 (C-18), 18.8 (C-19, C-21), 34.0 (C-22), 24.3 (C-23), 45.9 (C-24), 29.2 (C-25), 19.4 (C-26), 19.8 (C-27), 23.1 (C-28), 12.0 (C-29).

H-29
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triplet

Daucosterol (3): White amorphous powder, HR-ESI-MS (positive mode) *m/z* 577.4498 [M+H]⁺ (calcd. for C₃₅H₆₀O⁺ + H, 577.4428). ¹H-NMR data (CDCl₃) (*J* in Hertz): δ _H 4.24 (*m*, H-3), 5.33 (*m*, H-6), 0.63 (3H, *s*, H-18), 0.91 (3H, *s*, H-19), 0.96 (3H, *d*, 6.4, H-21), 0.83 (3H, *d*, 6.8, H-26), 0.87 (3H, *d*, 7.4, H-27), 0.85 (3H, *m*, H-29), 5.01 (1H, *d*, 7.7, H-1'), 4.02 (1H, *t*, 8.1, H-2'), 3.89-3.96 (1H, *m*, H3', 4'), 4.24 (1H, *m*, H-5'), 4.37 (1H, *dd*, 11.7, 5.3, H-6'a), 4.52 (1H, *dd*, 11.8, 2.5, H-6'b). The ¹³C-NMR (CDCl₃): δ _C 37.5 (C-1), 30.8 (C-2), 79.1 (C-3), 39.9 (C-4), 141.5 (C-5), 122.4 (C-6), 32.6 (C-7), 32.7 (C-8), 51.0 (C-9), 38.0 (C-10), 21.8 (C-11), 40.5 (C-12), 43.0 (C-13), 57.4 (C-14), 25.0 (C-15), 29.1 (C-16), 56.8 (C-17), 12.5 (C-18), 20.0 (C-19), 36.9 (C-20), 19.6 (C-21), 34.8 (C-22), 27.0 (C-23), 46.6 (C-24), 30.0 (C-25), 19.8 (C-26), 20.5 (C-27), 24.0 (C-28), 12.7 (C-29), 103.1 (C-1'), 75.9 (C-2'), 79.0 (C-3'), 72.3 (C-4'), 78.7 (C-5'), 63.4 (C-6').

Table 1: ^{13}C and ^1H NMR data for compound **1** and compound **4** (125 MHz and 500 MHz)

No.	1		4	
	δ_{H}, J	δ_{C}	δ_{H}, J	δ_{C}
1		38.5		35.7
2		32.2		24.2
3	3.63 (1H, <i>m</i>)	70.6	4.47 (1H, <i>dd</i> , 11.7, 4.1)	80.9
4		41.0		38.0
5		139.9		52.4
6	5.57 (1H, <i>dd</i> , 5.8, 2.6)	119.7		21.3
7	5.38 (1H, <i>dt</i> , 5.6, 2.8)	116.4		26.6
8		141.5		40.9
9		46.4		148.5
10		37.2		39.5
11		21.3	5.22 (1H, <i>d</i> , 6.2)	114.6
12		39.2		36.1
13		43.0		36.8
14		54.7		38.2
15		23.2		29.7
16		28.4		35.9
17		55.9		42.9
18	0.95 (3H, <i>s</i>)	12.2		52.1
19	0.63 (3H, <i>s</i>)	16.4		20.2
20		40.6		28.2
21	1.04 (3H, <i>d</i> , 6.7)	21.3		59.6
22	5.17 (1H, <i>dd</i> , 15.3, 7.7)	135.7		30.8
23	5.23 (1H, <i>dd</i> , 15.3, 7.1)	132.1	0.85 (3H, <i>s</i>)	28.2
24		43.0	0.87 (3H, <i>s</i>)	16.8
25	0.83 (3H, <i>d</i> , 7.6, 6.8) ^{coupling} 19.8	33.2	1.04 (3H, <i>s</i>)	22.2
26	0.83 (3H, <i>d</i> , 7.6, 6.8) ^{constant} 20.1		0.79 (3H, <i>s</i>)	17.0
27	0.92 (3H, <i>d</i> , 6.9) ^{value} 17.7		0.75 (3H, <i>s</i>)	15.3
28			0.74 (3H, <i>s</i>)	14.0
29			0.88 (3H, <i>s</i>) ^{should be doublet} 22.1	22.1
30			0.82 (3H, <i>d</i> , 6.5)	23.0
	$\text{H}_3\text{C}-\text{CO}$			21.3
	$\text{H}_3\text{C}-\text{CO}$		2.04 (3H, <i>s</i>)	171.0

Isoarborinyl acetate (**4**): Colorless powder. HR-ESI-MS (positive mode) m/z 469.4044 $[\text{M}+\text{H}]^+$ (calcd. for $\text{C}_{32}\text{H}_{53}\text{O}_2^+$, 469.4067). The ^1H and ^{13}C -NMR (CDCl_3) see Table 1.

2',4'-Dihydroxy-6'-methoxy-3',5'-dimethylchalcone (**5**): Pale yellow powder, HR-ESI-MS (positive mode) m/z 299.1291 $[\text{M}+\text{H}]^+$ (calcd. for $\text{C}_{18}\text{H}_{18}\text{O}_4^+$, 299.1283). The ^1H and ^{13}C -NMR (CDCl_3) see Table 2.

3,5,7-Trihydroxy-8-methoxyflavone (**6**): White powder, HR-ESI-MS (positive mode) m/z 301.0706 $[\text{M}+\text{H}]^+$ (calcd. for $\text{C}_{16}\text{H}_{12}\text{O}_6^+$, 301.0712). The ^1H and ^{13}C -NMR (CDCl_3) see Table 2.

Kaempferol (**7**): Yellow amorphous powder, HR-ESI-MS (positive mode) m/z 287.0515

$[\text{M}+\text{H}]^+$ (calcd. for $\text{C}_{15}\text{H}_{10}\text{O}_6^+$, 287.0555). The ^1H and ^{13}C -NMR (CDCl_3) see Table 2.

4. DISCUSSION

The chemical structures of seven isolated compounds were elucidated based on the analysis of HR-MS, 1D and 2D NMR spectroscopic spectra as well as the comparison of their data with those in the literature. The common compounds in plants such as β -sitosterol⁵ and daucosterol^{6,7}, after comparison of the data with the ones in the literature, their NMR ones were presented in the Part 2- Material and Methods, without discussion on their chemical elucidation.

Table 2: ^{13}C and ^1H NMR data for compound 5-7 (125 MHz and 500 MHz)

No.	5		6		7	
	δ_{H}, J	δ_{C}	δ_{H}, J	δ_{C}	δ_{H}, J	δ_{C}
1		135.6				
2	7.65 (dd, 7.6, 2.0)	128.9		145.1		148.1
3	7.41 (m)	129.1		136.7		137.2
4	7.41 (m)	130.3		175.8		177.4
5	7.41 (m)	126.0		155.6		162.5
6	7.65 (dd, 7.6, 2.0)	128.6	6.46 (s)	98.4	6.18 (d, 2.1)	99.3
7	7.84 (d, 15.7)	143.0		156.8		165.6
8	7.98 (d, 15.7)	127.0		127.1	6.40 (d, 2.1)	94.5
9		193.5		148.2		158.3
10				103.8		104.6
1'		106.7		130.9		123.8
2'		162.2	8.23 (t, 1.7)	127.7	8.09 (d, 8.9)	130.7
3'		109.0	7.54 (m)	128.9	6.91 (d, 8.9)	116.3
4'		159.3	7.50 (m)	130.5		160.5
5'		109.3	7.56 (m)	128.9	6.91 (d, 8.9)	116.3
6'		159.1	8.22 (dd, 1.9, 0.8)	127.7	8.09 (d, 8.9)	130.7
2'-OH	13.58 (s)	-				
3'-Me	2.16 (s)	8.4				
5'-Me	2.13 (s)	7.7				
6'-OMe	3.66 (s)	62.5				
8-OMe				4.05 (s)	62.1	

Compound 1 was isolated as colorless crystals. Its molecular formula was determined as $\text{C}_{28}\text{H}_{45}\text{O}$ through its molecular ion peak at m/z 397.3483 $[\text{M}+\text{H}]^+$ (calcd. for $\text{C}_{28}\text{H}_{45}\text{O}_3$ 397.3473). The ^1H -NMR data exhibited signals for six methyl protons δ_{H} 0.63 (s, H-19), 0.83 (d, $J = 6.5$ Hz, H-26), 0.83 (d, $J = 7.0$ Hz, H-27), 0.92 (d, $J = 7.0$ Hz, H-28), 0.95 (s, H-18) and 1.04 (d, $J = 6.5$ Hz, H-21), signals δ_{H} 5.56 (dd, $J = 5.8$, 2.6 Hz) and 5.38 (dd, $J = 5.6$, 2.8 Hz) diagnostic for olefin hydrogens H-6 and H-7, besides multiplet in δ_{H} 3.63 (H-3) indicate the presence of hydrogen linked to carbinolic carbon. Double bonds were observed at signal δ_{H} 5.19 (m) and 5.21 (m) relative to H-22 and H-23. The ^{13}C -NMR spectra revealed C28-sterol ergostane skeleton, including signals of six methyl carbons, seven methylene carbons, eleven methine carbons (two olefinic carbons, two aromatic carbons, one oxygenated methine carbon), and four quaternary carbons (two aromatic carbons) (Table 1). The good compatibility of its NMR and HR-ESI-MS data with those in the literature proposed that compound 1 was ergosterol.⁸

Compound 4 was obtained as colorless powder. Mass spectra exhibited a pseudo-molecular ion peak at m/z 469.4044 (calcd. for $\text{C}_{32}\text{H}_{53}\text{O}_2^+$, 469.4067), which corresponded with

$\text{C}_{32}\text{H}_{52}\text{O}_2$. The ^1H - and ^{13}C -NMR data of 4 disclosed 32 carbon signals including one acetyl ester group (δ_{H} 2.04, 3H, s; δ_{C} 21.3, 171.0, 3-COCH₃), one oxymethine (δ_{H} 4.47, dd, 11.7, 4.1 Hz; δ_{C} 80.9, C-3); one olefinic methine (δ_{H} 5.22, d, 6.2 Hz; δ_{C} 114.6, C-11); one olefinic quaternary carbon (δ_{C} 148.5, C-9); six quaternary methyls {(δ_{H} 0.85, 3H, s; δ_{C} 28.2, C-23); (0.87, 3H, s; 16.4, C-24); (1.04, 3H, s; 22.2, C-25); (0.79, 3H, s; 17.0, C-26); (0.75, 3H, s; 15.3, C-27); (0.74, 3H, s; 14.0, C-28)}; two tertiary methyls {(δ_{H} 0.88, 3H, d, 6.5 Hz; δ_{C} 22.1, C-29) and (0.82, 3H, d, 6.5 Hz; δ_{C} 23.0, C-30)} and 9 methylenes, 5 methines and 5 quarternary carbons in the high field zone. The presence of 32 signals on ^{13}C -NMR and the correlations observed on 1D and 2D spectra led to identification of compound 4 as isoarborinyl acetate, a hopane triterpene.⁵

Compound 5 was isolated as a pale yellow powder. The combination of analysis of ^1H - and ^{13}C -NMR data revealed that 5 contained a mono-substituted benzene ring {(δ_{H} 7.65, 2H, dd, 7.6, 2.0 Hz, H-2, H-6), (7.41, 3H, m, H-3, H-4, H-5); δ_{C} 135.6 (C1), 128.9 (C-2), 129.1 (C-3), 130.3 (C-4), 126.0 (C-5), 128.6 (C-6)}; a hexa-substituted benzene one { δ_{C} 106.7 (C1'), 162.2 (C-2'), 109.0 (C-3'), 159.3 (C-4'), 109.3 (C-5'), 159.1 (C-6')}; one conjugated ketone carbon (δ_{C} 193.5), two E-configuration olefinic carbons {(δ_{H} 7.84, 1H, d, 15.7 Hz, δ_{C} 143.0, C-7) and

(7.98, 1H, *d*, 15.7 Hz, δ_c 127.0, C-8}); one methoxy group (δ_H 3.66, 3H, *s*; δ_c 62.5, 6'-OCH₃) and two methyl groups {(δ_H 2.13, 3H, *s*; δ_c 7.7, 5'-CH₃) and (2.16, 3H, *s*; 8.4, 3'-CH₃)}. The positions of these substituents were supported by keys of HMBC correlation (Figure 2). The chemical structure of **5** whose molecular formula of C₁₈H₁₈O₄ was proved by the pseudomolecular ion peak at *m/z* 299.1291 [M+H]⁺ (calcd. for C₁₈H₁₈O₄ + H, 299.1283) in the HR-ESI-MS spectrum. Therefore, **5** was 2',4'-dihydroxy-6'-methoxy-3',5'-dimethylchalcone.^{6,7}

Compound **6** was isolated as white powder. Its molecular formula was determined as C₁₆H₁₂O₆ through its pseudomolecular ion peak at *m/z* 301.0706 [M+H]⁺ (calcd. for C₁₆H₁₂O₆ + H, 301.0712) in the HR-ESI-MS spectrum. The combination of analysis of HR-MS, ¹H- and ¹³C-NMR data revealed that **6** was a flavonoid with a mono-substituted B ring {(δ_H 8.23, 2H, *m*, H-2', H-6'), 7.50–7.54 (3H, *m*, H-3', H-4', H-5'); δ_c 130.9 (C-1'), 127.7 (C-2'), 128.9 (C-3'), 130.5 (C-4'), 128.9 (C-5'), 127.7 (C-6')}; a penta-substituted A ring {(δ_H 6.46, *s*, H-6); δ_c 155.6 (C-5), 98.4 (C-6), 156.8 (C-7), 127.1 (C-8), 148.2 (C-9), 103.8 (C-10)}; three carbons of the C ring { δ_c 145.1 (C-2), 136.7 (C-3), 175.8 (C-4)}, and a methoxy group (δ_H 4.05, 3H, *s*; δ_c 62.1, 8-OCH₃). The positions of these substituents were supported by keys of HMBC correlation (Figure 2). The comparison of these data with the 3,5,7-trihydroxy-8-methoxyflavone showed the similarity⁹. Therefore, the chemical structure of **6** was elucidated as shown.

Compound **7** was obtained as a yellow amorphous powder. Its molecular formula was determined as C₁₅H₁₀O₆ through its pseudomolecular ion peak at *m/z* 287.0515 [M+H]⁺ (calcd. for C₁₅H₁₀O₆ + H, 287.0555). The combined analysis of HR-MS, 1D and 2D-NMR data (Tables 2 and Figure 2) as well as the comparison of its data with the ones in the literature¹⁰ showed that compound **7** was kaempferol.

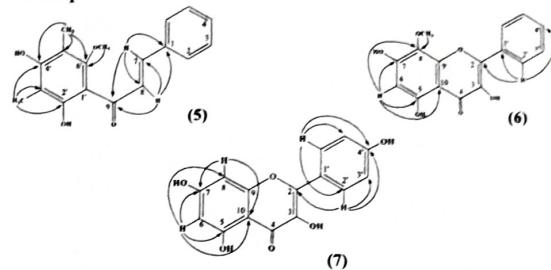


Figure 2. Keys HMBC correlation of **5** + **7**

5. CONCLUSION

From the chloroform and acetone extracts of the *Pilea microphylla* (L.), collected at Bien Hoa city, Dong Nai province, Vietnam, using various chromatographic methods, seven compounds were isolated. They were ergosterol (**1**), β -sitosterol (**2**), daucosterol (**3**), isoarborinyl acetate (**4**), 2',4'-dihydroxy-6'-methoxy-3',5'-dimethylchalcone (**5**), 3,5,7-trihydroxy-8-methoxyflavone (**6**), and kaempferol (**7**). Although these compounds were already known in other species, this is the first time they were reported in *Pilea microphylla*.

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Should be abbreviated