ISSN 1859 - 4735

JOURNAL OF MEDICINAL MATERIALS

No. 2 - Vol. 30
3/2025

NATIONAL INSTITUTE OF MEDICINAL MATERIALS

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CHEMICAL CONSTITUENT OF THE LEAVESAND STEMS OF *PHARBATIS NIL*

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Received February 28th, 2025 Accepted March 12th, 2025

Summary

Chemical Constituent of the Leaves and Stems of *Pharbatis nil*

The phytochemical investigation of the leaves and stems of *Pharbatis nil* led to the isolation of nine compounds (**1–9**). The structures of these compounds were determined through 1D- and 2D-NMR spectroscopic analysis, MS, and comparison with published spectroscopic data. The isolated compounds include uracil (**1**), thymidine (**2**), ethyl α-L-arabinofuranoside (**3**), ethyl-β-D-galactofuranoside (**4**), vitexin (**5**), β-sitosterol (**6**), stigmast-4-en-3-one (**7**), stigmast-4-en-3,6-dione (**8**), and daucosterol (**9**). Compounds **2**, **4**, **5**, **7**, and **8** were reported for the first time in this species.

Keywords: *Pharbatis nil; Pyrimidine derivatives; Sugars; Flavonoid; Sterols.*

1. Introduction

Pharbitis nil (L.) Choisy, also known as *Ipomoea nil* (L.) Roth, belongs to the Convolvulaceae family. In Vietnam, it is commonly known as Bìm bìm biếc, with other local names including Bìm bìm lam and Bìm bìm khía. This species is found in China, Australia, and several Southeast Asian countries [1]. In Vietnam, it has been cultivated for about ten years and is now widely distributed across various provinces, including Lang Son, Hoa Binh, Hanoi, Hai Phong, Thanh Hoa, Ninh Binh, Dak Lak, Ninh Thuan, Binh Duong, and Ba Ria-Vung Tau [1]. According to traditional Vietnamese medicine, Pharbitidis Semen is used to treat ascites, abdominal pain caused by parasites, phlegm-related asthma, and constipation [2]. Phytochemical studies have identified various compound groups in *P. nil*, including terpenoids, phenylpropanoids, resin glycosides, lipids, xanthones, flavonoids, and sterols. Extracts and isolated compounds from this plant have been reported to exhibit laxative, nephroprotective, neuroprotective, anti-inflammatory, antioxidant, and antibacterial activities [3]. Additionally, research has explored its potential applications in immune-related diseases, inflammation, allergies, and laxative formulations [4],[5],[6]. Despite its traditional medicinal use and presence in some health products, no comprehensive studies on the chemical composition and biological activities of *P. nil* have been conducted in Vietnam. This study presents the isolation and structural identification of nine compounds from its leaves and stems.

2. Materials and methods

2.1. Plant material

The leaves and stems of *Pharbitis nil* (L.) Choisy were supplied by Traphaco Joint Stock Company. The plants were harvested in June 2023 from a GACP-WHO-certified cultivation site in Thach Dong commune, Thanh Thuy town, Thanh Thuy district, Phu Tho province, Vietnam. It was taxonomically identified by the Center of Medicinal Material Resources, National Institute of Medicinal Materials (NIMM). A voucher specimen (DV-010724) has been deposited at the Department of Analytical Chemistry and Standardization, NIMM.

2.2. General experimental procedures

NMR spectra were recorded on a Bruker 600 MHz NMR spectrometer (Karlsruhe, Germany), using TMS as the internal reference. ESI-MS spectra were recorded using an LC-MS/MS system with a DAD detector (Shimadzu, Japan). Column chromatography (CC) was carried out using *silica gel* (Merck, 0.040–0.063 mm) and RP-18 (Merck, 30–50 µm). Thin-layer chromatography (TLC) was performed on pre-coated *silica gel* 60F_{254} (Merck, 0.25 mm) and RP-18F_{254} (Merck, 0.25 mm) plates. Spot detection was conducted under ultraviolet (UV) light, followed by spraying with a 10% sulfuric acid solution in ethanol and heating.

2.3. Extraction and isolation

The dried stems and leaves of *P. nil* (5 kg) were extracted four times with 70% ethanol at room temperature for two days each time. The combined extracts were filtered and evaporated under reduced pressure to remove the solvent, yielding a residue (373 g). This residue was suspended in hot water and subjected to liquid-liquid partitioning with solvents of increasing polarity, resulting in *n*-hexane (2.1 g), ethyl acetate (20.7 g), *n*-butanol (82.7 g), and aqueous (240.44 g) extracts.

The EtOAc extract (20 g) was separated using *silica gel* CC with an *n*-hexane–acetone gradient system (50:1 to 1:1, *v/v*) and 100% MeOH as eluents, yielding 12 fractions (E1–E12). Fraction E2 (260 mg) was fractionated by *silica gel* CC using an *n*-hexane–EtOAc gradient system (19:1 to 1:1, *v/v*), resulting in four fractions (E2.1–E2.4). Fraction E2.2 (102 mg) was further subjected to *silica gel* CC with an *n*-hexane–acetone gradient system (19:1 to 1:1, *v/v*), yielding four fractions (E2.2.1–E2.2.4). Fraction E2.2.2 (28.9 mg) was purified using Sephadex CC with MeOH elution, affording compound **7** (7 mg). Fraction E5 (166 mg) yielded a white precipitate, which was washed with MeOH to obtain compound **6** (102.5 mg). The filtrate, after solvent removal (63.5 mg), was purified by *silica gel* CC using an *n*-hexane–EtOAc system (4:1, *v/v*), affording compound **8** (11.9 mg). Similarly, fraction E11 (560 mg) also produced a white precipitate, which was washed with MeOH to yield compound **9** (26.4 mg).

The BuOH extract (80 g) was fractionated by *silica gel* CC using an EtOAc–MeOH–H_2O gradient system (90:5:5 to 70:25:5, *v/v/v*), yielding five fractions (B1–B5). Fraction B2 (1 g) was separated using *silica gel* CC with a DCM–MeOH–H_2O system (7:1:0.05, *v/v/v*), resulting in seven fractions (B2.1–B2.7). Fraction B2.2 (70 mg) was subjected to reversed-phase CC with a MeOH–H_2O system (1:3, *v/v*), yielding fraction B2.2.1. This fraction (28 mg) was further purified through Sephadex CC with a MeOH–H_2O system (1:2, *v/v*), followed by *silica gel* CC using a DCM–MeOH system (8:1, *v/v*), affording compound **3** (1.4 mg). Fraction B2.3 (200 mg) was fractionated by reversed-phase CC with a MeOH–H_2O system (1:3, *v/v*), yielding three fractions (B2.3.1–B2.3.3). Fraction B2.3.1 (48 mg) was sequentially purified by Sephadex CC with a MeOH–H_2O system (1:1, *v/v*), followed by *silica gel* CC using an EtOAc–MeOH system (30:1, *v/v*), affording compound **2** (4.5 mg). Similarly, fraction B2.3.3 (28 mg) was purified using Sephadex CC with a MeOH–H_2O system (1:1, *v/v*), yielding compound **4** (3.6 mg). Fraction B2.5 (100 mg) was further separated by *silica gel* CC using an EtOAc–MeOH system (12:1, *v/v*), yielding three fractions (B2.5.1–B2.5.3). Fraction B2.5.2 (18 mg) was purified by Sephadex CC with a MeOH–H_2O system (1:1, *v/v*), affording compound **5** (2 mg). Fraction B2.5.3 (28 mg) was sequentially purified using Sephadex CC with a MeOH–H_2O system (1:1, *v/v*), followed by *silica gel* CC using an EtOAc–MeOH system (30:1, *v/v*), yielding compound **1** (3 mg).

Compound 1: Pale yellow solid; ESI-MS: *m/z* = 111.10 $[M-H]^-$; ^{1}H-NMR (600 MHz, CD_3OD): δ_H 7.38 (1H, d, J = 7.8 Hz, H-4), 5.60 (1H, d, J = 7.8 Hz, H-5); ^{13}C-NMR (150 MHz, CD_3OD): δ_C 153.5 (C-2), 101.7 (C-4), 143.5 (C-5), 167.3 (C-6).

Compound 2: Pale yellow solid; ESI-MS: *m/z* = 243.60 $[M+H]^+$; ^{1}H-NMR (600 MHz, CD_3OD): δ_H 7.80 (1H, d, J = 1.2 Hz, H-4), 6.27 (1H, t, J = 7.2 Hz, H-1′), 2.23 (2H, m, H-2′), 4.39 (1H, m, H-3′), 3.90 (1H, m, H-4′), 3.78 (1H, dd, J = 3.0, 12.0 Hz, H-5′), 3.72 (1H, dd, J = 4.2, 12.0 Hz, H-5′), 1.87 (1H, d, J = 1.2 Hz, 5-CH_3); ^{13}C-NMR (150 MHz, CD_3OD): δ_C 152.4 (C-2), 138.2 (C-4), 111.5 (C-5), 166.4 (C-6), 88.8 (C-1′), 41.2 (C-2′), 72.2 (C-3′), 86.3 (C-4′), 62.9 (C-5′), 12.4 (5-CH_3).

Compound 3: White powder; ESI-MS: *m/z* = 177.10 $[M-H]^-$; ^{1}H-NMR (600 MHz, CD_3OD): δ_H 4.88 (1H, d, J = 1.8 Hz, H-1), 3.95 (1H, m, H-2), 3.84 (1H, m, H-3), 3.93 (1H, m, H-4), 3.65 (1H, dd, J = 5.4, 12.0 Hz, H-5), 3.50 (1H, m, H-5), 3.78 (2H, m, H-1′), 1.22 (3H, t, J = 7.2 Hz, H-2′); ^{13}C-NMR (150 MHz, CD_3OD): δ_C 109.3 (C-1), 83.6 (C-2), 78.7 (C-3), 85.2 (C-4), 63.0 (C-5), 64.3 (C-1′), 15.4 (C-2′).

Compound 4: White powder; ESI-MS: *m/z* = 207.15 $[M-H]^-$; ^{1}H-NMR (600 MHz, CD_3OD): δ_H 4.88 (1H, d, J = 2.4 Hz, H-1), 3.93 (1H, m, H-2), 4.01 (1H, dd, J = 4.2, 6.6 Hz, H-3), 3.93 (1H, m, H-4), 3.72 (1H, m, H-5), 3.63 (2H, m, H-6), 3.78 (1H, m, H-1′), 3.50 (1H, m, H-1′), 1.22 (3H, t, J = 7.2 Hz, H-2′); ^{13}C-NMR (150 MHz, CD_3OD): δ_C 109.2 (C-1), 83.4 (C-2), 78.7 (C-3), 84.2 (C-4), 72.5 (C-5), 64.5 (C-6), 64.3 (C-1′), 15.5 (C-2′).

Compound 5: Yellow powder; ESI-MS: *m/z* = 431.15 $[M-H]^-$; ^{1}H-NMR (600 MHz, DMSO-d_6): δ_H 6.78 (1H, s, H-3), 6.26 (1H, s, H-6), 8.02 (2H, d, J = 7.5 Hz, H-2′, H-6′), 6.89 (2H, d, J = 7.5 Hz, H-3′, H-5′), 4.69 (1H, d, J = 9.0 Hz, H-1″), 3.84 (1H, t, J = 9.0 Hz, H-2″), 3.27 (1H, m, H-3″), 3.37 (1H, m, H-4″), 3.23 (1H, m, H-5″), 3.75 (1H, m, H-6″), 3.52 (1H, m, H-6″), 13.2 (1H, s, 5-OH); ^{13}C-NMR (150 MHz, DMSO-d_6): δ_C 163.9 (C-2), 102.4 (C-3), 182.0

(C-4), 161.1 (C-5), 98.1 (C-6), 162.6 (C-7), 104.6 (C-8), 156.0 (C-9), 103.9 (C-10), 121.6 (C-1′), 128.9 (C-2′, C-6′), 115.8 (C-3′, C-5′), 160.4 (C-4′), 73.3 (C-1″), 70.8 (C-2″), 78.6 (C-3″), 70.5 (C-4″), 81.8 (C-5″), 61.3. (C-6″).

Compound 6: White powder; ESI-MS: *m/z* = 413.00 $[M-H]^-$; ^{1}H-NMR (600 MHz, $CDCl_3$) and ^{13}C-NMR (150 MHz, $CDCl_3$): see Table 1.

Compound 7: White powder; ESI-MS: *m/z* = 413.35 $[M+H]^+$; ^{1}H-NMR (600 MHz, $CDCl_3$) and ^{13}C-NMR (150 MHz, $CDCl_3$): see Table 1.

Compound 8: White powder; ESI-MS: *m/z* = 427.15 $[M+H]^+$; ^{1}H-NMR (600 MHz, $CDCl_3$) and ^{13}C-NMR (150 MHz, $CDCl_3$): see Table 1.

Compound 9: White powder; ^{1}H-NMR (600 MHz, $CDCl_3$ & CD_3OD) and ^{13}C-NMR (150 MHz, $CDCl_3$ & CD_3OD): see Table 1.

3. Results and discussion

Nine compounds (**1–9**) were isolated from the 70% EtOH extract of *P. nil* using combined chromatographic experiments. Based on the spectroscopic analysis and comparison with the literature, the structures of isolated compounds were determined (Fig. 1).

Compound **1** was isolated as a pale yellow solid. The ESI-MS spectrum of **1** displayed a deprotonated molecular ion peak at *m/z* 111.10 $[M-H]^-$, consistent with the molecular formula $C_4H_4N_2O_2$. The ^{1}H-NMR spectrum of **1** exhibited signals corresponding to two vicinal olefinic protons at δ_H 7.38 (1H, d, J = 7.8 Hz, H-4) and δ_H 5.60 (1H, d, J = 7.8 Hz, H-5). The ^{13}C-NMR spectrum revealed the presence of four carbon signals, including two methine carbons [δ_C 101.7 (C-4) and 143.5 (C-5)] and two CONH carbons [δ_C 153.5 (C-2) and 167.3 (C-6)]. Based on these spectral data and comparison with the literature [7], **1** was identified as uracil.

Compound **2** was also isolated as a yellow solid, and its ESI-MS spectrum displayed a protonated molecular ion peak at *m/z* 243.60 $[M+H]^+$, consistent with the molecular formula $C_{10}H_{14}N_2O_5$. The ^{1}H-NMR spectrum of **2** exhibited a signal for one olefinic proton at δ_H 7.80 (1H, d, J = 1.2 Hz, H-4), one methyl group at δ_H 1.87 (1H, d, J = 1.2 Hz, 5-CH_3), one methylene group at δ_H 2.23 (1H, m), and signals for hydroxymethine and hydroxymethylene protons appearing in the 3.72–6.27 ppm range (5H). The ^{13}C-NMR spectrum revealed ten carbon signals, including five carbons of the thymine moiety [δ_C 152.4 (C-2), 138.2 (C-4), 111.5 (C-5), 166.4 (C-6), and 12.4 (5-CH_3)] and five carbons corresponding to the deoxyribose sugar unit [δ_C 88.8 (C-1′), 41.2 (C-2′), 72.2 (C-3′), 86.3 (C-4′), and 62.9 (C-5′)] [8]. The sugar moiety was determined to have a β-configuration based on a large coupling constant (J = 7.2 Hz) [9]. Based on these analyses, **2** was identified as thymidine [8].

Compound **3** was obtained as a white powder. Its ESI-MS spectrum displayed a deprotonated molecular ion peak at *m/z* 177.10 $[M-H]^-$, consistent with the molecular formula $C_7H_{14}O_5$. The ^{1}H-NMR spectrum revealed signals corresponding to eight hydroxymethine protons in the δ_H 3.50–4.88 range, including an anomeric proton at δ_H 4.88 (1H, d, J = 1.8 Hz, H-1). Additionally, a signal for a methyl group was observed at δ_H 1.22. The ^{13}C-NMR spectrum was consistent with the ^{1}H-NMR data, showing four oxymethine carbons at δ_C 109.3 (C-1), 83.6 (C-2), 78.7 (C-3), and 85.2 (C-4). Furthermore, two oxymethylene carbons were observed at δ_C 64.3 (C-1′) and 63.0 (C-5), along with a methyl group at δ_C 15.4. The presence of a triplet methyl signal [δ_H 1.22 (3H, t, J = 7.2 Hz)] and a hydroxymethylene signal [δ_H 3.78 (2H, m), δ_C 64.3] indicated the existence of an ethyl group. Spectral analysis suggested that **3** contains a furanose sugar moiety and an ethyl group, with data matching previously reported spectra of ethyl α-L-arabinofuranoside [10].

Compound **4** was isolated as a white powder. Its ESI-MS spectrum revealed a deprotonated molecular ion peak at *m/z* 207.15 $[M-H]^-$, which corresponds to the molecular formula $C_8H_{16}O_6$. The ^{1}H-NMR spectrum exhibited signals corresponding to nine hydroxymethine protons in the δ_H 3.50–4.88 range, including a characteristic anomeric proton signal at δ_H 4.88 (1H, d, J = 2.4 Hz, H-1). Additionally, signals at δ_H 1.22 confirmed the presence of a methyl group. The ^{13}C-NMR spectrum was consistent with the ^{1}H-NMR data, showing five oxymethine carbons at δ_C 109.2 (C-1), 83.4 (C-2), 78.7 (C-3), 84.2 (C-4), and 72.5 (C-5). Additionally, two oxymethylene carbons were observed at δ_C 64.3 (C-1′) and 64.5 (C-6), along with a methyl group at δ_C 15.5. The triplet methyl signal [δ_H 1.22 (3H, t, J = 7.2 Hz)] and the hydroxymethylene signal [δ_H 3.78 (1H, m), 3.50 (1H, m), δ_C 64.3] indicated the presence of an ethyl group. The spectrum of **4** closely resembled that of **3**, except for the presence of an additional hydroxymethine group at δ_C 72.5. The obtained spectral data matched those previously reported for ethyl-β-D-galactofuranoside [11].

Compound **5** was isolated as a pale yellow powder. Its ESI-MS spectrum exhibited a deprotonated molecular ion peak at *m/z* 431.15

[M-H]⁻, corresponding to the molecular formula $C_{21}H_{20}O_{10}$. NMR spectral data confirmed that **5** is a flavone *C*-glucoside. The ^{1}H-NMR spectrum of **5** exhibited characteristic flavone signals, including a singlet for H-6 in ring A [δ_H 6.78 (1H, s)], two *ortho* doublets in ring B [δ_H 8.02 (2H, d, J = 7.5 Hz) and 6.89 (2H, d, J = 7.5 Hz)], and a singlet for H-3 in ring C [δ_H 6.27 (1H, s)]. A downfield signal at δ_H 13.2 indicated a hydrogen-bonded 5-OH group. Additionally, the spectrum also revealed signals corresponding to a glucose moiety linked to the aglycone via a *β-C*-glycoside bond, evidenced by the anomeric proton at δ_H 4.69 (1H, d, J = 9.0 Hz, H-1″) and other glucose signals at δ_H 3.84 (1H, t, J = 9.0 Hz, H-2″), 3.27 (1H, m, H-3″), 3.37 (1H, m, H-4″), 3.23 (1H, m, H-5″), 3.75 (1H, m, H-6″), and 3.52 (1H, m, H-6″). The ^{13}C-NMR spectrum revealed 21 carbon signals, with 15 carbons attributed to the aglycone, which was identified as apigenin [12], and six carbons corresponding to the glucopyranosyl moiety [δ_C 73.3 (C-1″), 70.8 (C-2″), 78.6 (C-3″), 70.5 (C-4″), 81.8 (C-5″), and 61.3 (C-6″)]. The glucose unit was assigned to the C-8 position based on HMBC correlations between H-1″ (δ_H 4.69) and C-9 (δ_C 156.0), as well as characteristic chemical shift changes at C-7 (-1.7 ppm), C-8 (+10.6 ppm), and C-9 (-1.3 ppm) compared to apigenin [13]. Based on these spectral data and literature comparison [14], **5** was identified as apigenin 8-*C*-*β*-D-glucopyranoside, also known as vitexin.

Table 1. NMR data of compounds **6**–**9**

No.	6		7		8		9	
	δ_H[a,b]	δ_C[a,c]	δ_H[a,b]	δ_C[a,c]	δ_H[a,b]	δ_C[a,c]	δ_H[d,b]	δ_C[d,c]
1		37.3		35.7		35.6		37.2
2		31.9		32.1		34.0		29.7
3	3.52 (1H, m)	71.8		199.6		199.4	3.59 (1H, m)	79.5
4		42.3	5.72 (1H, brs)	123.8	6.17 (1H, brs)	125.5		39.1
5		140.8		171.7		161.1		140.9
6	5.35 (1H, m)	121.7		33.0		202.3	5.37 (1H, m)	122.5
7		31.7		34.0		46.8		32.4
8		31.9		35.7		34.2		32.4
9		50.2		53.9		51.0		50.8
10		36.2		38.6		39.8		37.2
11		21.1		21.1		20.9		21.5
12		39.8		39.7		39.2		40.3
13		42.3		42.4		42.6		42.8
14		56.8		56.1		56.6		57.3
15		24.3		24.2		24.0		24.7
16		28.2		28.2		28.0		28.7
17		56.1		55.9		55.9		56.6
18	0.68 (3H, s)	12.0	0.71 (3H, s)	12.0	0.73 (3H, s)	12.0	0.70 (3H, s)	12.2
19	1.01 (3H, s)	19.4	1.18 (3H, s)	17.4	1.17 (3H, s)	17.5	1.03 (3H, s)	19.3
20		36.5		36.1		36.0		36.6
21	0.92 (3H, d, 6.6)	19.1	0.92 (3H, d, 6.6)	19.1	0.93 (3H, d, 6.6)	19.0	0.93 (3H, d, 6.6)	19.3
22		34.0		34.0		33.9		34.4
23		26.1		26.1		26.1		26.6
24		45.9		45.9		45.8		46.4
25		29.2		29.2		29.2		29.7
26	0.81 (3H, d, 6.6)	19.8	0.81 (3H, d, 6.6)	19.8	0.81 (3H, d, 6.6)	19.8	0.82 (3H, d, 6.6)	19.6
27	0,83 (3H, d, 6.6)	18.8	0.83 (3H, d, 6.6)	18.7	0.83 (3H, d, 6.6)	18.7	0.84 (3H, d, 6.6)	19.1
28		23.1		23.1		23.1		23.5
29	0.85 (3H, t, 7.2)	11.9	0.85 (3H, t, 7.2)	12.0	0.85 (3H, t, 7.2)	11.9	0.85 (3H, t, 7.2)	12.2
1′							4.41 (1H, d, 7.8)	101.6
2′							3.22 (1H, t, 7.8)	74.1
3′							3.29–3.43 (3H, m)	77.1
4′								70.8
5′								76.6
6′							3.73 (1H, dd, 4.2, 11.5) 3.86 (1H, d, 11.5)	62.3

[a] $CDCl_3$, [b] 600 MHz, [c] 150 MHz, [d] $CDCl_3$ &CD_3OD.

Compound **6** was obtained as a white powder. Its ESI-MS spectrum displayed a deprotonated

molecular ion peak at *m/z* 413.00 $[M-H]^-$, which corresponds to the molecular formula $C_{29}H_{50}O$. The ^{1}H-NMR spectrum of **6** displayed an olefinic proton signal at δ_H 5.35 (1H, m, H-6) and a hydroxymethine proton at δ_H 3.52 (1H, m, H-3). Six methyl groups were observed, including two singlets at δ_H 0.68 (3H, s, H-18) and 1.00 (3H, s, H-19), three doublets at δ_H 0.92 (3H, d, J = 6.6 Hz, H-21), 0.81 (3H, d, J = 6.6 Hz, H-26), and 0.83 (3H, d, J = 6.6 Hz, H-27), and one triplet at δ_H 0.85 (3H, t, J = 7.2 Hz, H-29). The ^{13}C-NMR spectrum revealed 29 carbon signals, including one hydroxymethine carbon [δ_C 71.8 (C-3)], two olefinic carbons [δ_C 121.7 (C-6) and 140.8 (C-5)], and six methyl carbons [δ_C 11.9 (C-29), 12.0 (C-18), 18.8 (C-27), 19.1 (C-21), 19.4 (C-19), and 19.8 (C-26)]. The remaining 20 carbons were assigned to quaternary (C), methine (CH), and methylene (CH_2) groups. The NMR data indicated that **6** is a sterol compound. By comparing its spectral data with reported compounds [15], **6** was identified as *β*-sitosterol.

Compound **7**, obtained as a white powder, exhibited a protonated molecular ion peak at *m/z* 413.35 $[M+H]^+$ in its ESI-MS spectrum, corresponding to the molecular formula $C_{29}H_{48}O$. NMR data showed similarities to **6**, evidenced by an olefinic proton at δ_H 5.72 (1H, brs, H-4) and six methyl groups. These included two singlets at δ_H 0.71 (3H, s, H-18) and 1.18 (3H, s, H-19), three doublets at δ_H 0.92 (3H, d, J = 6.6 Hz, H-21), 0.81 (3H, d, J = 6.6 Hz, H-26), and 0.83 (3H, d, J = 6.6 Hz, H-27), along with one triplet at δ_H 0.85 (3H, t, J = 7.2 Hz, H-29). These spectral characteristics suggested that **7** also belongs to the sterol group. However, the ^{13}C-NMR spectrum of **7** revealed a ketone carbon signal at δ_C 199.6 (C-3), replacing the hydroxymethine group in **6**. Additionally, **7** showed absorption at 254 nm, indicating the presence of a conjugated double bond. Significant chemical shift changes at C-3 (+127.8 ppm), C-4 (+81.5 ppm), C-5 (+30.9 ppm), and C-6 (-88.7 ppm) suggested that C-3 was oxidized to a ketone and a conjugated double bond had formed in ring A. Based on these spectral data and reference data [16], **7** was identified as stigmast-4-en-3-one.

Compound **8** was obtained as a white powder. Its ESI-MS spectrum showed a protonated molecular ion peak at *m/z* 427.15 $[M+H]^+$, consistent with the molecular formula $C_{29}H_{46}O_2$. The spectral data of **8** were similar to those of **7**, except for the presence of a ketone group at δ_C 202.3 (C-6). Additionally, the quaternary carbon at C-5 shifted upfield by 10.6 ppm, likely due to the influence of the ketone group, suggesting its position at C-6. Based on these data, **8** was identified as stigmast-4-en-3,6-dione [16].

Compound **9** was obtained as a white powder. Its ^{1}H-NMR data were similar to those of **6**, showing an olefinic proton at δ_H 5.37 (1H, m, H-6), a hydroxymethine proton at δ_H 3.59 (1H, m, H-3), and six methyl groups at δ_H 0.70 (3H, s, H-18), 1.03 (3H, s, H-19), 0.93 (3H, d, J = 6.6 Hz, H-21), 0.82 (3H, d, J = 6.6 Hz, H-26), 0.84 (3H, d, J = 6.6 Hz, H-27), and 0.85 (3H, t, J = 7.2 Hz, H-29). However, **9** also exhibited an anomeric proton signal of a *β*-glucopyranosyl moiety at δ_H 4.41 (1H, d, J = 7.8 Hz, H-1′), along with hydroxymethine and hydroxymethylene signals in the δ_H 3.22–3.88 range. The ^{13}C-NMR spectrum showed 35 carbon signals, including two in the double-bond region (a quaternary carbon at δ_C 140.9 and a methine carbon at δ_C 122.5), six oxymethine carbons (δ_C 70.8 – 101.6), and one oxymethylene carbon at δ_C 62.3. Based on these spectral data, **9** was identified as a steroid glycoside. Comparison with literature data [17] confirmed that **9** is *β*-sitosterol-3-*O*-*β*-D-glucoside, also known as daucosterol.

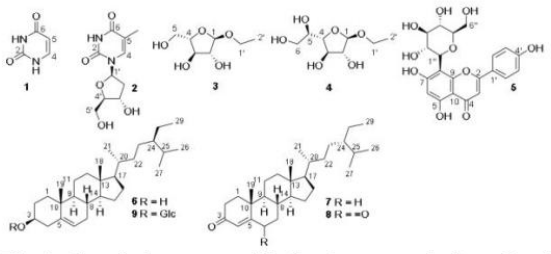

Fig. 1. Chemical structure of isolated compounds from *P. nil*

The isolated compounds are widely distributed in nature and have been studied for various biological activities. Among them, uracil (**1**) serves as an important scaffold in drug design, known for its broad range of biological activities. Its derivatives exhibit antiviral and antitumor properties, along with insecticidal, bactericidal, and herbicidal potential [18]. Similarly, vitexin (**5**) has garnered attention due to its strong antioxidant, anti-inflammatory, analgesic, and neuroprotective effects [19]. In addition to these bioactive compounds, *β*-sitosterol (**6**), a common phytosterol in the plant kingdom, plays a crucial role in stabilizing cell membranes. It has been reported to possess various therapeutic effects, including anti-inflammatory, immunomodulatory, cholesterol-lowering, anti-rheumatoid arthritis, and anti-androgenetic alopecia properties [20].

Likewise, daucosterol (**9**) has been extensively studied and exhibits significant pharmacological activities, such as antioxidant, antidiabetic, hypolipidemic, anti-inflammatory, immunomodulatory, neuroprotective, and anticancer effects [21].

4. Conclusion

In this study, we conducted a chemical analysis of the leaves and stems of *P. nil*, resulting in the isolation and identification of two pyrimidine derivatives (**1** and **2**), two sugars (**3** and **4**), one flavonoid (**5**), and four sterols (**6**–**9**). Notably, compounds **2**, **4**, **5**, **7**, and **8** were identified in this species for the first time. These findings contribute to expanding the chemical profile of *P. nil* and provide valuable data for future research on this species.

Acknowledgments: *This study was partially supported by the collaborative project between Traphaco Joint Stock Company and the National Institute of Medicinal Materials (NIMM), Vietnam, titled "Study on chemical composition and some biological activities, development of a standardized extraction process and standardization of Morning Glory (seeds), Semen Pharbitidis", under the contract code 09-2023/HĐNCKH-BB dated May 26, 2023.*

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