ISSN 1859 - 4735

JOURNAL OF MEDICINAL MATERIALS

No. 5 - Vol. 30
9/2025

NATIONAL INSTITUTE OF MEDICINAL MATERIALS

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JOURNAL OF MEDICINAL MATERIALS (Print and Electronic Journals)

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(Pages 305 - 384)

Journal of Medicinal Materials, 2025, Vol. 30, No. 5 (313 - 319)

CHEMICAL CONSTITUENTS FROM THE SEEDS OF *ZIZIPHUS MAURITIANA* LAM. COLLECTED IN VIETNAM

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Received July 10th, 2025 Accepted September 22nd, 2025

Summary

Eight known compounds, including two triterpenoids: betulinic acid (**1**) and oleanolic acid (**2**), three flavonoid *C*-glycosides: spinosin (**3**), 6‴-feruloylspinosin (**4**), and isospinosin (**5**), two nucleosides: uridine (**6**) and adenosine (**7**), and one sugar: sucrose (**8**), were isolated from the seeds of *Ziziphus mauritiana* Lam. The structures of these compounds were identified by using extensive nuclear magnetic resonance spectroscopy (NMR) data, mass spectrometry (MS), and by comparing with published literature. These compounds were isolated to identify key chemical markers for evaluating the quality of this species in Vietnam.

Keywords: *Ziziphus mauritiana* Lam.*; Triterpenoids; Flavonoids; Nucleosides; Sugar.*

1. Introduction

Ziziphus mauritiana Lam., commonly known as Jujube Ber, Indian jujube, Malay apple, and Chinese apple, is a fruit tree belonging to the family Rhamnaceae [1]. It is mainly distributed in Asia, Africa, Australia, and the Mediterranean region [2]. The fruit is highly valued in rural areas for its rich nutritional content and is often referred to as the poor man's apple. Traditionally, it has been used to treat various diseases in Pakistan and other countries such as Afghanistan, Australia, India, and Iran [3],[4]. In Vietnam, *Z. mauritiana* has long been used as a traditional medicinal herb. Various parts of the plant have been employed in folk remedies: the leaves are used to treat diarrhea, wounds, abscesses, gonorrhea, liver disorders, asthma, and fever; the bark has been reported to exhibit cytotoxic effects against different cancer cell lines. Numerous studies have highlighted both the nutritional and pharmaceutical value of this species. Phytochemical investigations have identified a wide range of compounds in *Z. mauritiana* Lam., including proteins, amino acids, flavonoids, alkaloids, phenols, terpenoids, glycosides, and organic acids [5], which have been associated with biological activities such as anticancer, antimicrobial, antihyperglycemic, and antioxidant effects [6],[7],[8],[9]. In previous reports, flavonoids, saponins, triterpenoids, and other compounds, including spinosin, 6‴-feruloyl spinosin, 6‴-sinapoyl spinosin, jujubosides A–B, betulinic aldehyde, betulinic acid, ceanothic acid, frangufoline, *β*-sitosterol, daucosterol, daucosterol-6'-octadecanoate, sucrose, docosanoic acid, stearic acid, and palmitoleic acid, were isolated from the seeds of this species [5],[10],[11].

Despite its traditional medicinal use and inclusion in some health products, previous studies in Vietnam have not yet provided a comprehensive phytochemical profile of the seeds of *Z. mauritiana* Lam. The source of semen *Z. mauritiana* Lam. on the market is still unstable, easily confused with samples from China. Therefore, in this study, we report the isolation and structural elucidation of eight compounds from the seeds of *Z. mauritiana* Lam. collected in Vietnam, including betulinic acid (**1**), oleanolic acid (**2**), spinosin (**3**), 6‴-feruloylspinosin (**4**), isospinosin (**5**), uridine (**6**), adenosine (**7**), and sucrose (**8**) (Fig. 1).

2. Materials and methods

2.1. Plant materials

The seeds of *Ziziphus mauritiana* Lam. were collected in Van Giang, Hung Yen, Vietnam, in December 2024. The plant was identified by MSc. Phan Van Truong (National Institute of Medicinal Materials, NIMM). A voucher specimen was deposited in the Center of Medicinal Material Resources, NIMM (Hanoi, Vietnam).

2.2. General experimental procedures

NMR spectra were recorded on a Bruker 600 MHz spectrometer (Bruker, Karlsruhe, Germany) using tetramethylsilane (TMS) as the internal reference. ESI-MS spectra were obtained on an LC-MS/MS system equipped with a DAD detector (Shimadzu, Japan). Column chromatography (CC) was performed using *silica gel* (0.040–0.063 mm, Merck), YMC ODS-A (12 nm, S-75 μm, YMC Co., Ltd., Japan), MCI gel

CHP20P (75-150 μm, Mitsubishi Chemical, Japan), and Diaion HP-20 (75-150 μm, Mitsubishi Chemical, Japan). Thin-layer chromatography (TLC) was conducted on pre-coated *silica gel* 60 F_{254} (0.25 mm, Merck) and *silica gel* 60 RP-18 $F_{254}S$ (0.25 mm, Merck) plates. Spots were visualized under ultraviolet (UV) light, followed by spraying with 10% sulfuric acid in ethanol and heating.

2.3. Extraction and isolation

The dried seeds of *Z. mauritiana* (5 kg) were extracted by ultrasonication with methanol (MeOH) three times (3 h each). The combined extracts were filtered and concentrated under reduced pressure to afford a brown residue (500 g). This residue was suspended in hot water and successively partitioned with organic solvents of increasing polarity, yielding *n*-hexane (300 g), dichloromethane (35 g), ethyl acetate (15 g), and aqueous (100 g) extracts.

The *n*-hexane extract (300 g) was separated by *silica gel* CC using a gradient of *n*-hexane-acetone (1:0 → 1:1, v/v), yielding 13 fractions (H1-H13). Fraction H11 (25.0 g) was subjected to *silica gel* CC with a gradient of dichloromethane-acetone (10:1 → 1:1, v/v) to give 10 fractions (H11.1-H11.10). Fraction H11.3 (1.0 g) was purified on *silica gel* CC using dichloromethane-ethyl acetate (20:1, v/v) to afford compounds **1** (110.2 mg) and **2** (36.8 mg).

The aqueous extract (100 g) was initially fractionated by Diaion HP-20 CC using a gradient of ethanol-water (0%, 50%, and 96%), affording three fractions. Among these, the 50% ethanol fraction (WE50, 52.8 g) was separated by *silica gel* CC with dichloromethane-methanol (15:1 → 3:1, v/v), followed by 100% MeOH, yielding seven fractions (W1-W7). Fraction W7 (15.6 g) was subsequently chromatographed on *silica gel* CC using ethyl acetate-methanol-water (18/1/1 → 8/1/1, v/v/v), affording two fractions (W7.1 and W7.2). Fraction W7.1 (7.5 g) was subjected to MCI gel CC using methanol-water (1:1, v/v), yielding two fractions (W7.1.1 and W7.1.2) and compound **8** (48.5 mg). Fraction W7.1.1 (5.2 g) was further chromatographed on *silica gel* CC with ethyl acetate-methanol-water (8:1:1 → 8:2:1, v/v/v) to obtain fraction W7.1.1.1 (3.6 g), which was then subjected to RP-C_{18} CC using a methanol-water gradient (1:4 → 1:1, v/v), affording five fractions (W7.1.1.1.1-W7.1.1.1.5). From these, fraction W7.1.1.1.1 (450 mg) was purified on *silica gel* CC using ethyl acetate-methanol-water (7.5:2:1, v/v/v) to isolate compound **6** (25.5 mg). Compound **7** (24.7 mg) was obtained by purifying fraction W7.1.1.1.4 (70.8 mg) on RP-C_{18} CC using methanol-water (1:3, v/v). In addition, fraction W7.1.1.1.5 (216.6 mg) was subjected to RP-C_{18} CC with methanol-water (1:1.5, v/v) to afford compound **5** (15.2 mg). Fraction W7.1.2 (660 mg) was subjected to RP-C_{18} CC using methanol-water (1:1.2, v/v), affording compound **3** (112 mg). Meanwhile, fraction W7.2 (1.06 g) was separated by *silica gel* CC using ethyl acetate-methanol-water (18:2.5:1, v/v/v), yielding two subfractions (W7.2.1 and W7.2.2). Finally, fraction W7.2.2 (356.2 mg) was purified on *silica gel* CC using dichloromethane-methanol-water (6:1:0.1, v/v/v), affording compound **4** (30.6 mg).

Compound **1**: White needle-like crystals; ESI-MS: *m/z* = 455.50 $[M–H]^-$; ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H (ppm): 3.19 (1H, dd, *J* = 5.0, 11.0 Hz, H-3), 0.97 (3H, s, H-23), 0.75 (3H, s, H-24), 0.83 (3H, s, H-25), 0.94 (3H, s, H-26), 0.98 (3H, s, H-27), 4.61 (1H, s, H-29), 4.74 (1H, s, H-29), 1.69 (3H, s, H-30); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C (ppm): 38.8 (C-1), 27.4 (C-2), 79.0 (C-3), 38.9 (C-4), 55.4 (C-5), 18.3 (C-6), 34.4 (C-7), 40.7 (C-8), 50.6 (C-9), 37.2 (C-10), 20.9 (C-11), 25.6 (C-12), 38.4 (C-13), 42.5 (C-14), 30.6 (C-15), 32.2 (C-16), 56.3 (C-17), 49.3 (C-18), 46.9 (C-19), 150.4 (C-20), 29.7 (C-21), 37.0 (C-22), 28.0 (C-23), 15.4 (C-24), 16.1 (C-25), 16.0 (C-26), 14.7 (C-27), 179.5 (C-28), 109.7 (C-29), 19.4 (C-30).

Compound **2**: White powder; ESI-MS: *m/z* = 455.45 $[M–H]^-$; ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H (ppm): 3.22 (1H, dd, *J* = 4.2, 11.4 Hz, H-3), 5.28 (1H, t, *J* = 3.6 Hz, H-12), 0.99 (3H, s, H-23), 0.78 (3H, s, H-24), 0.92 (3H, s, H-25), 0.76 (3H, s, H-26), 1.14 (3H, s, H-27), 0.90 (3H, s, H-29), 0.93 (3H, s, H-30); ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C (ppm): 38.4 (C-1), 27.7 (C-2), 79.0 (C-3), 38.8 (C-4), 55.2 (C-5), 18.3 (C-6), 32.7 (C-7), 39.3 (C-8), 47.7 (C-9), 37.1 (C-10), 23.0 (C-11), 122.7 (C-12), 143.6 (C-13), 41.6 (C-14), 27.2 (C-15), 23.4 (C-16), 46.5 (C-17), 41.1 (C-18), 45.9 (C-19), 30.7 (C-20), 33.8 (C-21), 32.5 (C-22), 28.1 (C-23), 15.5 (C-24), 15.3 (C-25), 17.1 (C-26), 25.9 (C-27), 182.6 (C-28), 33.1 (C-29), 23.6 (C-30).

Compound **3**: Yellow powder; ESI-MS: *m/z* = 607.25 $[M–H]^-$; ^{1}H-NMR (600 MHz, DMSO-d_6) and ^{13}C-NMR (150 MHz, DMSO-d_6): see Table 1.

Compound **4**: Yellow powder; ESI-MS: *m/z* = 783.35 $[M–H]^-$; ^{1}H-NMR (600 MHz, DMSO-d_6)

and ^{13}C-NMR (150 MHz, DMSO-d_6): see Table 1.

Compound **5**: Yellow powder; ESI-MS: *m/z* = 607.30 $[M-H]^-$; ^{1}H-NMR (600 MHz, DMSO-d_6) and ^{13}C-NMR (150 MHz, DMSO-d_6): see Table 1.

Compound **6**: Yellow powder; ESI-MS: *m/z* = 243.15 $[M-H]^-$; ^{1}H-NMR (600 MHz, CD_3OD) δ_H (ppm): 5.72 (1H, d, *J* = 7.8 Hz, H-5), 8.01 (1H, d, *J* = 7.8 Hz, H-6), 5.91 (1H, d, *J* = 4.2 Hz, H-1′), 4.20 (1H, t, *J* = 4.8 Hz, H-2′), 4.17 (1H, t, *J* = 4.8 Hz, H-3′), 4.03 (1H, m, H-4′), 3.75 (1H, dd, *J* = 3.6, 12.0 Hz, H-5′), 3.85 (1H, dd, *J* = 2.4, 12.0 Hz, H-5′); ^{13}C-NMR (150 MHz, CD_3OD) δ_C (ppm): 152.5 (C-2), 166.2 (C-4), 102.7 (C-5), 142.7 (C-6), 90.7 (C-1′), 75.7 (C-2′), 71.3 (C-3′), 86.3 (C-4′), 62.3 (C-5′).

Compound **7**: Yellow powder; ESI-MS: *m/z* = 268.10 $[M+H]^+$; ^{1}H-NMR (600 MHz, DMSO-d_6) δ_H (ppm): 8.14 (1H, s, H-2), 8.34 (1H, s, H-8), 5.88 (1H, d, *J* = 6.0 Hz, H-1′), 4.60 (1H, q, *J* = 6.0 Hz, H-2′), 4.14 (1H, q, *J* = 4.2 Hz, H-3′), 3.94 (1H, q, *J* = 3.0 Hz, H-4′), 3.56 (1H, m, H-5′), 3.67 (1H, m, H-5′), 7.31 (2H, br s, 6-NH_2), 5.44 (1H, d, *J* = 6.0 Hz, 2′-OH), 5.18 (1H, d, *J* = 4.8 Hz, 3′-OH), 5.41 (1H, t, *J* = 4.2 Hz, 5′-OH); ^{13}C-NMR (150 MHz, DMSO-d_6) δ_C (ppm): 152.4 (C-2), 149.1 (C-4), 119.4 (C-5), 156.2 (C-6), 139.9 (C-8), 87.9 (C-1′), 73.5 (C-2′), 70.7 (C-3′), 85.9 (C-4′), 61.7 (C-5′).

Compound **8**: White powder; ESI-MS: *m/z* = 341.20 $[M-H]^-$; ^{1}H-NMR (600 MHz, CD_3OD) δ_H (ppm): 3.64 (1H, d, *J* = 12.0 Hz, H-1), 3.68 (1H, d, *J* = 12.0 Hz, H-1), 4.12 (1H, d, *J* = 8.4 Hz, H-3), 4.05 (1H, t, *J* = 8.4 Hz, H-4), 3.79 (1H, m, H-5), 3.78 (2H, m, H-6), 5.42 (1H, d, *J* = 3.6 Hz, H-1′), 3.47 (1H, dd, *J* = 3.6, 9.0 Hz, H-2′), 3.85 (1H, m, H-3′), 3.39 (1H, d, *J* = 9.0 Hz, H-4′), 3.74 (1H, m, H-5′), 3.73 (1H, m, H-6′), 3.83 (1H, m, H-6′); ^{13}C-NMR (150 MHz, CD_3OD) δ_C (ppm): 64.1 (C-1), 105.3 (C-2), 79.4 (C-3), 75.7 (C-4), 83.7 (C-5), 63.4 (C-6), 93.6 (C-1′), 73.2 (C-2′), 74.4 (C-3′), 71.4 (C-4′), 74.6 (C-5′), 62.2 (C-6′).

Table 1. NMR data of compounds **3**–**5**

No.	3		4		5	
	δ_H[a,b] (*J* in Hz)	δ_C[a,c]	δ_H[a,b] (*J* in Hz)	δ_C[a,c]	δ_H[a,b] (*J* in Hz)	δ_C[a,c]
2	-	163.9/163.8	-	164.0/163.6	-	163.4
3	6.85 (s)/6.83 (s)	103.0/102.9	6.70 (s)/6.52 (s)	103.0/102.7	6.77 (s)	102.5
4	-	182.2/182.0	-	182.2/181.8	-	182.2
5	-	160.5/159.7	-	160.8/159.5	-	161.2
6	-	108.6	-	108.7	6.47 (s)	95.1
7	-	165.1	-	165.2/164.2	-	164.2
8	6.81 (s)/6.78 (s)	90.8/90.3	6.69 (s)/6.67 (s)	90.6/89.9	-	104.7
9	-	157.1/157.0	-	157.0/156.8	-	155.3
10	-	104.4/104.2	-	104.4/103.9	-	104.2
1′	-	120.9/120.5	-	120.9	-	121.5
2′, 6′	7.97 (d, 8.4)	128.5	7.82 (d, 9.0)/7.80 (d, 9.0)	128.5/128.4	8.02 (d, 9.0)	129.0
3′, 5′	6.93 (d, 8.4)	116.1	6.89 (d, 8.4)/6.84 (d, 8.4)	116.0/115.9	6.90 (d, 9.0)	115.9
4′	-	161.5	-	160.8	-	161.5
5-OH	13.63 (s)/13.51 (s)	-	13.63 (s)/13.50 (s)	-	13.3 (s)	-
7-OCH_3	3.90 (s)	56.5/56.1	3.91 (s)/3.86 (s)	56.4/56.1	3.87 (s)	56.6
1″	4.70 (d, 10.2)/4.67 (d, 10.2)	71.0/70.7	4.69 (d, 9.6)/4.68 (d, 9.6)	71.0/70.6	4.83 (d, 10.2)	71.4
2″	4.47 (m)/4.29 (m)	81.2/80.8	4.48 (t, 9.6)/4.23 (t, 9.6)	81.9/81.6	4.05 (t, 9.0)	80.9
3″	3.41 (m)	78.7/78.3	3.42 (m)	78.8/78.5	3.52 (m)	78.2
4″	3.16 (m)	70.5	3.16 (m)	70.3	3.44 (m)	70.1
5″	3.16 (m)	81.9/81.6	3.16 (m)	81.9	3.25 (m)	81.8
6″	3.69 (m); 3.39 (m)	61.5	3.69 (m); 3.37 (m)	61.5	3.55 (dd, 6.0, 12.0); 3.76 (dd, 3.0, 12.0)	60.9
1‴	4.18 (d, 7.8)/4.16 (d, 7.8)	105.4/105.2	4.28 (d, 7.8)/4.27 (d, 7.8)	105.7/105.2	3.86 (d, 7.8)	104.8
2‴	2.85 (m)/2.83 (m)	74.7/74.6	2.91 (m)	74.5/74.4	2.76 (t, 7.8)	74.5
3‴	3.05 (m)	76.4/76.3	3.10 (m)	76.3	2.91 (m)	76.1
4‴	3.01 (m)/2.96 (m)	69.5/69.2	3.05 (m)	68.9/68.7	2.91 (m)	69.4
5‴	2.76 (m)/2.55 (m)	76.7/76.4	3.00 (m)/2.93 (m)	73.4/73.2	2.43 (m)	76.1
6‴	3.16 (m); 2.96 (m)	60.6/60.1	3.97 (m); 3.81 (m) 3.89 (m); 3.61(m)	62.4/62.1	3.10 (m) 3.18 (m)	60.2
1‴′			-	125.1		

No.	3		4		5	
	δ_H[a,b] (*J* in Hz)	δ_C[a,c]	δ_H[a,b] (*J* in Hz)	δ_C[a,c]	δ_H[a,b] (*J* in Hz)	δ_C[a,c]
2‴′			7.18 (br s)/7.05 (br s)	110.8/110.7		
3‴′			-	147.9		
4‴′			-	149.5		
5‴′			6.75 (d, 8.4)	115.7/115.4		
6‴′			6.93 (dd, 2.4, 8.4)/6.79 (dd, 2.4, 8.4)	123.2/123.0		
7‴′			7.22 (d, 16.2)/7.09 (d, 16.2)	144.8/144.7		
8‴′			6.24 (d, 16.2)/6.17 (d, 16.2)	114.0/113.5		
9‴′			-	166.3/166.2		
3‴′-OCH_3			3.82 (s)/3.81 (s)	55.6		

[a] DMSO-d_6, [b] 600 MHz, [c] 150 MHz.

3. Results and discussion

Compound **1** was isolated as white needle-like crystals. Its ESI-MS spectrum showed a deprotonated molecule's ion peak at *m/z* 455.50 $[M-H]^-$, consistent with the molecular formula $C_{30}H_{48}O_3$. The ^{1}H-NMR spectrum displayed a hydroxymethine proton at δ_H 3.19 (1H, dd, *J* = 5.0, 11.0 Hz, H-3), two exomethylene protons at δ_H 4.61 and 4.74 (each 1H, s, H-29), and six methyl signals at δ_H 0.97 (3H, s, H-23), 0.75 (3H, s, H-24), 0.83 (3H, s, H-25), 0.94 (3H, s, H-26), 0.98 (3H, s, H-27), and 1.69 (3H, s, H-30). The remaining signals appeared in the aliphatic region (δ_H 0.68-2.80). The ^{13}C-NMR spectrum showed thirty carbon resonances, including a hydroxymethine carbon at δ_C 79.0 (C-3), two exomethylene carbons at δ_C 150.4 (C-20) and 109.7 (C-29), six methyl carbons at δ_C 28.0, 19.4, 15.4, 16.1, 16.0, and 14.7, and a carboxyl carbon at δ_C 179.5 (C-28), supporting a lupane-type triterpenoid structure. The large coupling constant of H-3 (*J* = 11.0 Hz) confirmed the *β*-orientation of the 3-OH group [12]. Based on the above spectral analysis and comparison with the published data [13], compound **1** was determined to be betulinic acid.

Compound **2** was obtained as a white powder. The ESI-MS spectrum exhibited a deprotonated molecule's ion peak at *m/z* 455.45 $[M-H]^-$, corresponding to the molecular formula $C_{30}H_{48}O_3$. In the ^{1}H-NMR spectrum, a hydroxymethine signal was observed at δ_H 3.22 (1H, dd, *J* = 4.2, 11.4 Hz, H-3), along with an olefinic proton at δ_H 5.28 (1H, t, *J* = 3.6 Hz, H-12). Additionally, seven methyl singlets appeared at δ_H 0.99, 0.78, 0.92, 0.76, 1.14, 0.90, and 0.93. The ^{13}C-NMR spectrum showed thirty carbon signals comprising five methine (CH), ten methylene (CH_2), seven methyl (CH_3), and seven quaternary (C) carbons. A downfield resonance at δ_C 182.5 indicated the presence of a carboxylic acid moiety. These spectroscopic characteristics indicated that compound **2** possesses an oleanane-type triterpenoid skeleton. Moreover, the large coupling constant of H-3 (*J* = 11.4 Hz) confirmed the *β*-axial orientation of the 3-OH group [12]. Comparison with spectroscopic data reported in the literature [14] allowed the identification of compound **2** as oleanolic acid.

Compound **3** was obtained as a yellow powder. Its ESI-MS spectrum showed a deprotonated molecule's ion peak at *m/z* 607.25 $[M-H]^-$, which was consistent with the molecular formula $C_{28}H_{32}O_{15}$. The NMR data indicated that **3** was a flavonoid glycoside. The signals in the NMR spectra appeared in pairs, which can be explained by restricted rotation around the C(6)-C(1″) bond due to the presence of a 7-OCH_3 group, and were recorded at room temperature (303 K). This is a characteristic feature of 6-*C*-glycosylflavones [15]. The ^{1}H-NMR spectrum displayed hydroxyl proton signals at δ_H 13.63/13.51 (s), a methoxy proton at δ_H 3.90, two pairs of singlet aromatic protons at δ_H 6.85/6.83 and 6.81/6.78, and a pair of *C*-*β*-glucopyranoside moieties with anomeric proton signals at δ_H 4.70/4.67 (d, *J* = 10.2 Hz). Additionally, a pair of anomeric proton signals for *O*-*β*-glucopyranoside units appeared at δ_H 4.18/4.16 (d, *J* = 7.8 Hz). The ^{13}C-NMR spectrum showed signals for one methoxy group at δ_C 56.5/56.1, 15 carbons belonging to the aglycone, and 12 carbons attributed to the *C*-*β*-glucopyranoside and *O*-*β*-glucopyranoside units. HMBC correlations between the anomeric protons at δ_H 4.18/4.16 and C-2″ (δ_C 81.2/80.8), as well as between H-2″ (δ_H 4.47/4.29) and C-1‴ (δ_C 105.4/105.2), C-3″ (δ_C 78.7/78.3), and C-4″ (δ_C 70.5), confirmed the presence of two *β*-glucopyranosyl moieties linked through the C-2″ position. The interactions between the other anomeric protons at δ_H 4.70/4.67 and C-5 (δ_C

160.5/159.7), C-6 (δ_C 108.6), and C-7 (δ_C 165.1) confirmed that the disaccharide moiety was attached to the aglycone through a (C1″) – *C*-(C6) bond. Furthermore, the location of the methoxy group was identified by the HMBC correlation between the methoxy proton (δ_H 3.90) and C-7 (δ_C 165.1). From the above analysis, together with data reported previously [10], the structure of **3** was elucidated as spinosin.

Compound **4** was isolated as a yellow powder. Its molecular formula was determined to be $C_{38}H_{40}O_{18}$ based on the ESI-MS spectrum, which showed a deprotonated molecule's ion peak at *m/z* 783.35 $[M-H]^-$. The ^{1}H- and ^{13}C-NMR data were similar to those of compound **3**, except for the presence of signals assigned to a feruloyl group, including protons of an ABX system at δ_H 7.18/7.05 (br s), 6.75 (d, J = 8.4 Hz), and 6.93/6.79 (dd, J = 2.4, 8.4 Hz), a *trans* double bond at δ_H 7.22/7.09 (d, J = 16.2 Hz) and 6.24/6.17 (d, J = 16.2 Hz), and a methoxy group at δ_H 3.82/3.81 (s). The ^{13}C-NMR spectrum further confirmed the presence of the feruloyl moiety through the characteristic carbon signals at δ_C 125.1, 110.8/110.7, 147.9, 149.5, 115.7/115.4, 123.2/123.0, 144.8/144.7, 114.0/113.5, 166.3/166.2, and 55.6. Notably, the downfield shift at C-6‴ (+1.8 and +2.0 ppm) and the upfield shift at C-5‴ (-3.3/-3.2 ppm) indicated that the feruloyl group was attached at the C-6‴ position. NMR analysis and agreement with the reported data [10] confirmed compound **4** as 6‴-feruloylspinosin.

Compound **5** was also obtained as a yellow powder. Its ESI-MS spectrum showed a deprotonated molecule's ion peak at *m/z* 607.30 $[M-H]^-$, corresponding to the molecular formula $C_{28}H_{32}O_{15}$. The NMR data were similar to those of compound **3**, showing the presence of a rhamnocitrin aglycone and two glucose moieties connected by a (C-1‴)-O-(C-2″) glycosidic linkage. However, the NMR signals of compound **5** did not appear as paired sets like those of compound **3**, indicating a difference in the attachment position of the sugar moiety to the aglycone. HMBC correlations between the anomeric proton at δ_H 4.83 (H-1″) and C-7 (δ_C 164.2), C-8 (δ_C 104.7), and C-9 (δ_C 155.3), along with correlations between H-6 (δ_H 6.47) and carbons C-5 (δ_C 161.2), C-7, C-8, confirmed that the sugar moiety was linked to the aglycone through a (C1″)-*C*-(C8) bond. Spectroscopic evidence consistent with that in the literature [16] led to the establishment of compound **5** as isospinosin.

Compound **6** was obtained as a yellow powder. Its ESI-MS spectrum displayed a deprotonated molecule's ion peak at *m/z* 243.15 $[M-H]^-$, consistent with the molecular formula $C_9H_{12}N_2O_6$. The ^{1}H-NMR spectrum showed signals for two adjacent olefinic protons at δ_H 8.01 (1H, d, J = 7.8 Hz, H-6) and 5.72 (1H, d, J = 7.8 Hz, H-5). In addition, signals corresponding to hydroxymethine and hydroxymethylene protons were observed in the range of δ_H 3.75-5.91 (6H). The ^{13}C-NMR spectrum displayed nine carbon signals, including four carbons assigned to a uracil moiety [δ_C 152.5 (C-2), 166.2 (C-4), 102.7 (C-5), and 142.7 (C-6)] and five signals attributed to a ribose unit [δ_C 90.7 (C-1′), 75.7 (C-2′), 71.3 (C-3′), 86.3 (C-4′), and 62.3 (C-5′)]. The configuration of the sugar moiety was identified to be β based on the large coupling constant (J = 7.8 Hz) of the anomeric proton. Thus, compound **6** was confirmed to be uridine [16].

Compound **7**, obtained as a yellow powder, showed a protonated molecule's ion peak at *m/z* 268.10 $[M+H]^+$ in the ESI-MS spectrum, which was consistent with the molecular formula $C_{10}H_{13}N_5O_4$. NMR data indicated that compound **7** is a nucleoside, composed of a purine base and a ribose unit similar to that of compound **6**. The base was elucidated as adenine, as evidenced by two olefinic proton signals at δ_H 8.14 (1H, s, H-2) and 8.34 (1H, s, H-8), together with five carbon signals at δ_C 152.4 (C-2), 149.1 (C-4), 119.4 (C-5), 156.2 (C-6), and 139.9 (C-8). The spectral characteristics were in good agreement with the reported values [17], enabling the identification of compound **7** as adenosine.

Compound **8** was isolated as a yellow powder. Its molecular formula was determined to be $C_{12}H_{22}O_{11}$ based on ESI-MS data (*m/z* 341.20 $[M-H]^-$). The ^{1}H-NMR and ^{13}C-NMR data confirmed that compound **8** was a disaccharide. The anomeric proton signal at δ_H 5.42 (1H, d, J = 3.6 Hz), along with the carbon signal at δ_C 93.6, indicated the presence of an α-glucopyranosyl moiety. The remaining anomeric carbon resonated at δ_C 105.3 without a corresponding proton signal, which was characteristic of a β-fructofuranosyl moiety involved in a glycosidic linkage at the C-2 position [18]. The doublet signals at δ_H 3.64 and 3.68 (J = 12.0 Hz), together with the carbon signal at δ_C 64.1, supported the presence of a methylene group at C-1 of the fructose unit. Additional proton signals in the range δ_H 3.39-4.12 and carbon signals between δ_C 62.2-83.7 were assigned to hydroxymethylene and hydroxymethine groups of both sugar units. Furthermore, the HMBC spectrum displayed a correlation between H-1′$_{Glc}$ (δ_H 5.42) and C-2$_{Fru}$ (δ_C 105.3). Comparison of NMR spectroscopic data of

8 with the previously reported values [18] supported the elucidation of compound **8** as sucrose.

The isolated compounds are widely distributed in nature and have been studied for various biological activities. Two triterpenoids, betulinic acid and oleanolic acid, have been reported to exhibit remarkable effects, including anti-tumor, anti-HIV, anti-inflammatory, antibacterial, and antimalarial activities [17]. Notably, betulinic acid shows potent anti-tumor effects [19], making it a potential therapeutic candidate for cancer treatment. Through complex and multifactorial mechanisms, oleanolic acid exerts beneficial effects against dyslipidemia, diabetes, and metabolic syndrome [20].

In this study, we obtained three flavonoid *C*-glycosides: spinosin, isospinosin, and 6‴-feruloylspinosin. Their reported pharmacological effects include sedative activity [21], cognitive enhancement [22],[23],[24], melanin synthesis inhibition [25], and antioxidant activity [26], which are consistent with the main biological activities of *Semen Ziziphi mauritiana*. Additionally, spinosin and 6‴-feruloylspinosin protect cardiomyocytes against acute myocardial ischemia-reperfusion injury, likely *via* GSK3β inhibition. This leads to enhanced autophagy and activation of the PGC-1α/Nrf2/HO-1 pathway [27]. 6‴-Feruloylspinosin also shows potential in mitigating Alzheimer's disease (AD) progression by delaying cellular senescence, reducing paralysis incidence, improving heat stress resistance and motility, and promoting autophagy through the autophagy-lysosome pathway in GMC101 [28].

Among the constituents of *Semen Ziziphi mauritiana*, spinosin was found in significant amount. This compound plays an important role in the treatment of psychiatric disorders, including potentiation of pentobarbital-induced sleep via serotonergic mechanisms [21], and enhancement of non-rapid eye movement sleep in mice [29], which corresponds to the primary pharmacological actions of this species. These findings suggest that spinosin is a key marker of *Semen Ziziphi mauritiana* and should be considered for quality evaluation of this medicinal material.

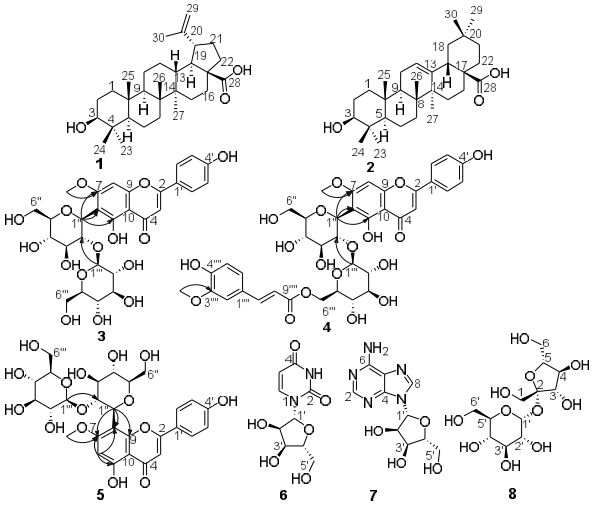

Fig. 1. Chemical structures and key HMBC correlations (→) of isolated compounds from *Z. mauritiana*

4. Conclusion

In summary, from the seeds of *Z. mauritiana* Lam., eight compounds were isolated, and their structures were elucidated by the analysis of their spectroscopic data (ESI-MS, ^{1}H-NMR,^{13}C-NMR, HMBC, and HSQC). These compounds included betulinic acid (**1**), oleanolic acid (**2**), spinosin (**3**), 6‴-feruloylspinosin (**4**), isospinosin (**5**), uridine (**6**), adenosine (**7**), and sucrose (**8**). Additionally, it's proposed to select spinosin (**3**) as a key marker. These findings contribute to expanding the chemical profile of *Z. mauritiana* Lam. and provide valuable data for future research on this species.

Acknowledgments: *This work was supported by the National Institute of Medicinal Materials – Ministry of Health (Vietnam) on the project (Contract No. 08/2024/HĐ-NVCV-HPTTC) Isolation and development of HPLC method for the quantitative determination of the main component in Semen Ziziphi mauritiamae.*

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