PHYTOCHEMICAL PROFILING OF *PREMNA ODORATA*: ISOLATION AND STRUCTURE OF TRITERPENOIDS AND FLAVONOIDS FROM SEEDS IN PHU YEN, VIETNAM

NGHIÊN CỨU HÓA THỰC VẬT *PREMNA ODORATA*: TÁCH CHIẾT VÀ CẤU TRÚC TRITERPENOID CÙNG FLAVONOID TỪ HẠT Ở PHÚ YÊN, VIỆT NAM

Nguyen Hoang Sa[1,*]

DOI: http://doi.org/10.57001/huih5804.2025.211

ABSTRACT

Phytochemical exploration of the seeds of *Premna odorata*, sourced from Phu Yen province, Vietnam, has yielded significant insights into its chemical composition. This investigation led to the isolation of four compounds: two triterpenoids, identified as arjunolic acid and ursolic acid, and two flavonoids, characterized as cirsiliol and luteolin. The structures of these isolates were meticulously determined through the application of advanced spectroscopic methods, including electrospray ionization mass spectrometry (ESI-MS) and nuclear magnetic resonance (NMR) spectroscopy. These findings represent a new contribution to the phytochemical profile of *P. odorata*, broadening the knowledge of its molecular diversity and underscoring its potential as a reservoir of biologically relevant natural products. This study enhances the foundation for further research into the therapeutic and ecological significance of this species within the domain of organic chemistry and natural products.

Keywords: *Premna odorata, arjunolic acid, ursolic acid, cirsiliol, luteolin.*

TÓM TẮT

Nghiên cứu hóa thực vật trên hạt của *Premna odorata* (Cách lá thơm), thu thập lần đầu tiên từ tỉnh Phú Yên, Việt Nam, đã mang lại những hiểu biết quan trọng về thành phần hóa học của loài này. Qua quá trình nghiên cứu, bốn hợp chất đã được phân lập, bao gồm hai triterpenoid là arjunolic acid và ursolic acid, cùng hai flavonoid là cirsiliol và luteolin. Cấu trúc của các hợp chất này được xác định chi sử dụng các kỹ thuật tiên tiến như phổ khối ion hóa phun mù điện tử (ESI-MS) và phổ cộng hưởng từ hạt nhân (NMR). Những phát hiện này đóng góp mới vào hồ sơ hóa thực vật của *P. odorata*, mở rộng hiểu biết về sự đa dạng về thành phần hóa học của loài và nhấn mạnh tiềm năng của nó như một nguồn cung cấp các hợp chất thiên nhiên có giá trị sinh học. Nghiên cứu này tạo tiền đề cho các công trình tiếp theo nhằm khám phá ý nghĩa về các hợp chất mang hoạt tính sinh học của loài cây này trong lĩnh vực hóa học hữu cơ và hợp chất thiên nhiên.

Từ khóa: *Premna odorata, arjunolic acid, ursolic acid, cirsiliol, luteolin.*

[1]University of Khanh Hoa, Vietnam
[*]Email: nhoangsa@gmail.com
Received: 20/02/2025
Revised: 22/5/2025
Accepted: 28/5/2025

1. INTRODUCTION

The genus *Premna* (Lamiaceae) encompasses a wide variety of species, notably *P. corymbosa*, *P. integrifolia*, and *P. odorata*, which flourish in the tropical and subtropical belts of the world. These species have attracted considerable scientific interest due to their longstanding roles in traditional medicine, underpinned by a rich repertoire of biological activities. Research has substantiated the therapeutic potential of *Premna* species, revealing a suite of properties that include

immunomodulatory, anti-atherosclerotic, antioxidant, anti-diabetic, anti-inflammatory, and antitumor effects [1-4]. Such diverse bioactivities position the genus as a valuable reservoir for the discovery of bioactive natural products.

P. odorata, indigenous to numerous tropical locales, is particularly esteemed for its ethnobotanical significance. In traditional medicinal practices, leaf decoctions of this plant have been utilized as febrifuges, diuretics, and carminatives, while also serving as remedies for ailments such as abdominal pain, vaginal irritation, dysentery, and coughs. This broad therapeutic application reflects the plant's integral role in folk medicine. Despite its prominence, the scientific exploration of *P. odorata* remains in its infancy. Early phytochemical studies have identified a range of active constituents, including flavonoids, phenylethanoids, iridoids, and acylated rhamnopyranosides [5-8], which are thought to contribute to its documented anti-inflammatory, anti-tuberculosis, and cytotoxic properties [9-12]. However, the limited scope of these investigations highlights a critical need for deeper inquiry into the plant's chemical composition and pharmacological potential.

In Vietnam, where *P. odorata* is found across diverse ecological regions, including the central provinces, research efforts have been notably scarce. This gap is particularly evident when considering the plant's abundance and cultural relevance in the region. To bridge this knowledge divide, the current study examines the phytochemical profile of *P. odorata* sourced from Phu Yen province, Vietnam, with a specific emphasis on its seed system. The isolation and structural characterization of four compounds: two triterpenoids (compounds **1** and **2**) and two flavonoids (compounds **3** and **4**) were reported. These classes of natural products are renowned for their structural diversity and bioactivity, making them compelling targets for organic chemistry and natural products research. This investigation not only enriches the phytochemical understanding of *P. odorata* but also lays the groundwork for future studies aimed at harnessing its therapeutic potential within a scientifically rigorous framework.

2. MATERIALS AND METHODS

2.1. General Experimental Procedures

ESI-MS data were acquired using a Sciex X500RQTOF mass spectrometer (Sciex, Massachusetts, USA), employing electrospray ionization (ESI-MS) for molecular weight determination. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance III 500 spectrometer (Bruker, Fällanden, Switzerland), operating at 500MHz for ^{1}H-NMR and 125.72MHz for ^{13}C-NMR, with chemical shifts referenced to the residual solvent signals. Chromatographic separations were conducted using normal-phase silica gel 60 (particle size 0.040 - 0.063mm, Merck, Darmstadt, Germany), reversed-phase RP_{18} gel (30 - 50µm, Fuji Silysia Chemical Ltd., Kasugai, Japan), and Sephadex LH_{20} (Amersham Pharmacia Biotech, Tokyo, Japan) for gel filtration. Thin-layer chromatography (TLC) was performed on precoated silica gel $60F_{254}$ and RP_{18} F_{254S} plates (Merck, 0.25 or 0.50mm thickness), with visualization achieved by spraying with cerium(IV) sulfate or vaniline/sulfuric acid followed by heating.

2.2. Plant material

Seeds of *Premna odorata* were collected from the Deo Ca mountains in Phu Yen province, Vietnam, in 10/2021. Botanical identification was confirmed by Dr. Phan Duc Ngai, University of Khanh Hoa, and a voucher specimen (No. HS21.04) was deposited at the Laboratory of Drug Research and Discovery, Institute of Chemistry, Vietnam Academy of Science and Technology (VAST), Hanoi, Vietnam.

2.3. Experimental

Air-dried and finely powdered seeds of *P. odorata* (1.8kg) were subjected to sequential solvent extraction. Initially, the material was extracted with dichloromethane (CH_2Cl_2, 10L×3) at ambient temperature for 3 days. The solvent was evaporated under reduced pressure using a rotary evaporator, yielding a dark brown dichloromethane extract (28.2g). The remaining marc was subsequently extracted with ethyl acetate (EtOAc, 10L×3) under identical conditions, affording a dark brown EtOAc residue (17.1g) after solvent removal.

The EtOAc extract was analyzed by TLC using multiple solvent systems: hexanes:EtOAc (60:40), CH_2Cl_2:EtOAc (90:10), CH_2Cl_2:acetone (90:10), and hexanes: CH_2Cl_2:MeOH (50:45:5). The last system proved optimal for resolving the extract's components, with visualization via cerium(IV) sulfate revealing yellow-to-orange spots suggestive of flavonoids.

The EtOAc extract was then fractionated by silica gel column chromatography using a gradient elution of acetone in a hexanes:CH_2Cl_2:MeOH mixture (90:10:0 to 50:35:15), yielding six fractions (A-F). Fraction A (1.0g) underwent further purification via silica gel

chromatography with a CH_2Cl_2:MeOH gradient (100:1, 100:5, 100:20), followed by gel filtration on Sephadex LH_{20} (acetone-MeOH, 1:10), resulting in the isolation of compound **2** (17mg). Fraction C (1.2g) was separated by silica gel column chromatography using a CH2Cl2:MeOH gradient (100:1, 100:5, 100:20, 100:30, 100:50), producing four subfractions (A1-A4). Subfraction A3 (1.05g, eluted with 1.5% MeOH in CH_2Cl_2) was filtered through Sephadex LH_{20} (MeOH:CH_2Cl_2, 95:5) and further purified on an RP_{18} column with a MeOH:water gradient (100:5 to 100:50), affording compound **1** (12.0mg). Fraction D (1.51g) was chromatographed on silica gel with a hexane: CH_2Cl_2 gradient (8:2 to 1:1), yielding five subfractions (D.1-D.5). Subfraction D.3 (367mg) was subjected to RP_{18} chromatography with a MeOH:water gradient (100:5 to 100:50), followed by final purification on Sephadex LH_{20} MeOH:CH_2Cl_2, 95:5), resulting in the isolation of compound **3** (13.2mg) and compound **4** (16.3mg).

Arjunolic acid: $C_{30}H_{48}O_5$; white powder**,** mp = 327 - 328°C; ESI-MS *m/z* 511.34 $[M+Na]^+$

^{1}H-NMR (DMSO-d_6, 500MHz): δ_H 12.03 (s, 1H, carboxylic acid), 5.16 (s, 1H, H-12), 4.41(t, *J* = 1.5Hz, 1H, -O**H**), 4.22 (d, *J* = 3.6Hz, 1H, -O**H**), 4.16 (d, *J* = 3.0Hz, -O**H**), 3.48 (m, 1H, H-2); 3.29 (m, 1H) and 3.03 (m, 1H) H-23; 3.16 (d, *J* = 6.5Hz, 1H, H-3); 2.75 (dd, *J* = 13.5, 4.0Hz, H-18); 1.13 (s, 3H, H-27), 0.92 (s, 3H, H-25), 0.88 (s, 6H, H-29 and H-30), 0.71 (s, 3H, H-26), 0.55 (s, 3H, H-24).

^{13}C-NMR (DMSO-d_6, 125.72MHz): δ_C 178.63 (C-28), 143.95 (C-13), 121.51 (C-12), 75.48 (C-3), 67.42 (C-2), 63.87 (C-23), 47.07 (C-1), 46.71 (C-19), 45.99 (C-17), 45.68 (C-5), 45.45 (C-22), 42.52 (C-4), 41.40 (C-14), 40.80 (C-8), 40.05 (C-18), 38.89 (C-10), 37.39 (C-9), 32.86 (C-7), 32.11(C-21), 31.89 (C-16), 30.44 (C-20), 27.18 (C-15), 25.72 (C-27), 23.43 (C-11), 23.02 (C-29), 22.01 (C-30), 17.53 (C-6), 16.91 (C-25), 16.80 (C-26), 13.82 (C-24).

Ursolic acid: $C_{30}H_{48}O_3$; white powder, mp = 285 - 286°C, ESI-MS *m/z* 479.35 $[M+Na]^+$

^{1}H NMR (500MHz, methanol-d_4) δ_H 5.41 (t, *J* = 4.0Hz, 1H, H-12), 3.17 (dd, *J* = 7.5, 4.0Hz, 1H, H-3), 2.17 (d, *J* = 8.5Hz, 1H, H-18), 2.03 - 1.91 (m, 2H, H-16), 1.86 - 1.77 (m, 1H, H-11), 1.62 - 1.22 (m, 12H), 1.23 (s, 3H, H-27), 1.07 (s, 3H, H-26), 1.01 (s, 3H, H-23), 0.89 (s, 3H, H-30), 0.87 (s, 3H, H-25), 0.82 (s, 3H, H-29), 0.80 (s, 3H, H-24).

^{13}C-NMR (methanol-d_4, 125.72 MHz): δ_C 181.64 (C-28), 138.93 (C-13), 125.74 (C-12), 78.22 (C-3), 56.75 (C-5), 54.36 (C-18), 49.05 (C-9), 48.48 (C-17), 43.25 (C-14), 40.79 (C-8), 40.42 (C-20), 40.41 (C-19), 40.02 (C-1), 39.83 (C-4), 38.11 (C-22), 38.01 (C-10), 34.35 (C-7), 31.75 (C-21), 28.84 (C-23), 28.22 (C-2), 27.88 (C-15), 25.32 (C-16), 24.36 (C-11), 24.11 (C-30), 21.55 (C-27), 19.50 (C-6), 17.80 (C-26), 17.65 (C-29), 16.38 (C-24), 16.01 (C-25).

Cirsiliol: $C_{17}H_{14}O_7$, yellow amorphous powder, mp = 280 - 281°C, ESI-MS *m/z* 331.09 $[M+H]^+$

^{1}H-NMR (500MHz, DMSO-d_6), δ_H: 12.95 (s, 1 H, 5-OH), 7.46 (s, 1H, H-2′) overlap with 7.45 (s, 1H, H-6′), 6.92 (d, *J* = 8.7Hz, 1 H, H-5′), 6.88 (s, 1 H, H-8), 6.74 (s, 1 H, H-3), 3.93 (s, 3 H, 7-OC**H**$_3$), 3.75 (s, 3 H, 6-OC**H**$_3$).

^{13}C-NMR (DMSO-d_6, 125.72MHz): δ_C 164.27 (C-2), 102.65 (C-3), 182.07 (C-4), 152.06 (C-5), 131.87 (C-6), 158.55 (C-7), 91.42 (C-8), 152.56 (C-9), 105.03 (C-10), 121.36 (C-1′), 113.47 (C-2′), 145.81 (C-3′), 149.94 (C-4′), 115.97 (C-5′), 119.02 (C-6′), 59.99 (6-O**C**H$_3$), 56.39 (7-O**C**H$_3$).

Luteolin: $C_{15}H_{10}O_6$, yellowish powder, mp = 229-230°C, ESI-MS *m/z* 285.01 $[M-H]^-$

^{1}H-NMR (500MHz, DMSO-d_6), δ_H: 12.97 (s, 1 H, 5-OH), 7.41 (m, 1H, H-2′) overlap with 7.39 (m, 1H, H-6′), 6.88 (d, *J* = 8.5Hz, 1H, H-5′), 6.66 (s, 1H, H-3), 6.43 (d, *J* = 2.0Hz, 1H, H-8), 6.18 (d, *J* = 2.0Hz, 1H, H-6).

^{13}C-NMR (DMSO-d_6, 125.72MHz): δ_C 163.78 (C-2), 102.85 (C-3), 181.62 (C-4), 161.46 (C-5), 98.82 (C-6), 164.14 (C-7), 93.82 (C-8), 157.26 (C-9), 103.67 (C-10), 121.50 (C-1′), 113.35 (C-2′), 145.72 (C-3′), 149.69 (C-4′), 116.00 (C-5′), 118.96 (C-6′).

3. RESULTS AND DISCUSSION

Compound 1 was obtained as a white amorphous powder with a melting point of 327 -328°C. Its molecular formula was determined to be $C_{30}H_{48}O_5$ based on electrospray ionization mass spectrometry (ESI-MS), which revealed a prominent sodium adduct ion at *m/z* 511.34 $[M+Na]^+$. This molecular composition suggests a triterpene framework with multiple oxygenated functionalities.

The ^{13}C-NMR spectrum, recorded in DMSO-d_6 at 125.72MHz, along with DEPT analysis, provided detailed insight into the carbon skeleton of compound **1**. The spectrum indicated the presence of 30 carbon atoms, categorized as follows: seven methyl groups, nine methylene units (including one oxygenated carbon at δ_C 63.87), six methine carbons (comprising one olefinic carbon at δ_C 121.51 and two oxygenated carbons at δ_C 67.42 and 75.48), and eight quaternary carbons (including one carboxyl carbon at δ_C 178.63 and one alkenyl carbon at δ_C 143.95). These spectral features are

consistent with a highly functionalized triterpenoid structure.

The ^{1}H-NMR spectrum, acquired in DMSO-d_6 at 500MHz, further corroborated the structural assignment. Key signals included a singlet at δ_H 12.03 (1H), indicative of a carboxylic acid proton, and a broad singlet at δ_H 5.16 (1H), assigned to the olefinic H-12 proton. The presence of five methyl singlets at δ_H 0.55 (H-24), 0.71 (H-26), 0.92 (H-25), and 0.88 (H-29 and H-30, 6H combined) suggested a pentacyclic triterpene backbone with multiple tertiary methyl groups. Additionally, hydroxyl-bearing protons were observed at δ_H 4.41 (t, J = 1.5Hz), 4.22 (d, J = 3.6Hz), and 4.16 (d, J = 3.0Hz), reflecting the polyoxygenated nature of the molecule. The olean-12-ene skeleton was inferred from the characteristic chemical shifts of the olefinic carbons (C-12 at δ_C 121.51 and C-13 at δ_C 143.95) and the corresponding H-12 proton signal at δ_H 5.16, which are hallmarks of this triterpene class [13]. Further structural clarification came from the oxygenated methine protons at δ_H 3.48 (m, H-2) and δ_H 3.16 (d, J = 6.5Hz, H-3), alongside carbon resonances at δ_C 67.42 (C-2) and δ_C 75.48 (C-3), indicating hydroxyl substitutions at these positions. The presence of a hydroxymethyl group (–CH_2OH) was evidenced by two proton signals at δ_H 3.29 (m) and 3.03 (m), assigned to H-23, correlating with the oxygenated methylene carbon at δ_C 63.87 (C-23). The attachment of this –CH_2OH group to the quaternary C-4 (δ_C 42.52) was supported by the upfield shift of the adjacent methyl carbon C-24 (δ_C 13.82), a feature consistent with steric and electronic effects induced by the hydroxymethyl substituent.

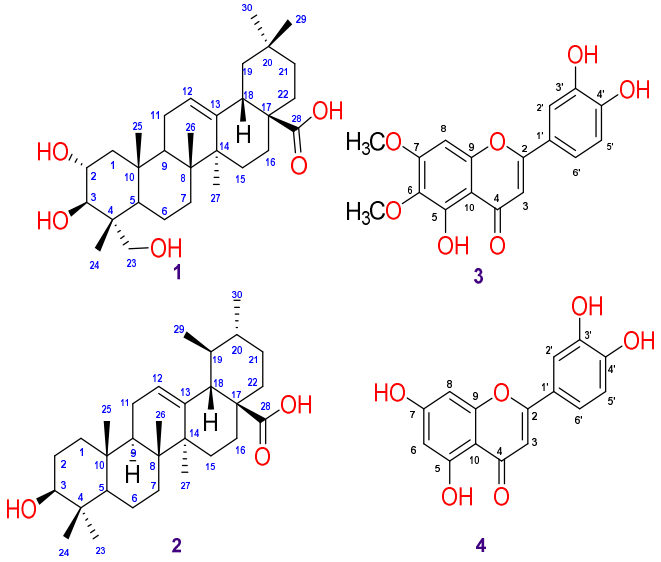

Figure 1. Triterpenoids (**1**-**2**) and flavonoids (**3**-**4**) isolated from the seeds of *Premna odorata*

Additional key resonances included the H-18 proton at δ_H 2.75 (dd, J = 13.5, 4.0Hz), linked to C-18 (δ_C 40.05), which is typical of the D/E ring junction in oleanane-type triterpenoids. The carboxyl group, assigned to C-28 (δ_C 178.63), aligned with the singlet at δ_H 12.03, reinforcing the structural integrity of the molecule. By integrating the ^{1}H-NMR and ^{13}C-NMR data and comparing them with literature values for arjunolic acid [13,14], compound **1** was unequivocally identified as arjunolic acid, a 2*α*,3*β*,23-trihydroxyolean-12-en-28-oic acid (structure depicted in Figure 1). This marks the first reported isolation of arjunolic acid from *P. odorata*, expanding the phytochemical profile of this species and highlighting its potential as a source of bioactive triterpenoids.

Compound 2 was obtained as a white powder with a melting point of 285 - 286°C. ESI-MS analysis revealed a sodium adduct ion at *m/z* 479.35 $[M+Na]^+$, consistent with the molecular formula $C_{30}H_{48}O_3$.

The ^{1}H-NMR spectrum, recorded in methanol-d_4 at 500MHz, provided critical insights into the proton environment of compound **2**. Seven distinct methyl singlets were observed at δ_H 1.01 (H-23), 0.80 (H-24), 0.87 (H-25), 1.07 (H-26), 1.23 (H-27), 0.82 (H-29), and 0.89 (H-30), each integrating to 3H, indicative of a highly methylated triterpene skeleton. An olefinic proton signal at δ_H 5.41 (t, J = 4.0Hz, 1H) was assigned to H-12, suggesting the presence of a double bond within the carbon framework. Additionally, an *axial* oxymethine proton at δ_H 3.17 (dd, J = 7.5, 4.0Hz, 1H) was attributed to H-3, pointing to a hydroxyl substitution at this position. Other notable signals included a doublet at δ_H 2.17 (d, J = 8.5Hz, 1H, H-18), characteristic of the ursane-type ring junction, and a series of overlapping multiplets between δ_H 1.22 - 2.03, representing the methylene and methine protons of the polycyclic core.

The ^{13}C-NMR spectrum, acquired in methanol-d_4 at 125.72MHz, resolved 30 carbon signals, fully accounting for the molecular formula. Key resonances included a carboxyl carbon at δ_C 181.64 (C-28), two olefinic carbons at δ_C 138.93 (C-13) and 125.74 (C-12), and an oxygenated methine carbon at δ_C 78.22 (C-3). The seven methyl carbons were identified at δ_C 28.84 (C-23), 16.38 (C-24), 16.01 (C-25), 17.80 (C-26), 21.55 (C-27), 17.65 (C-29), and 24.11 (C-30), corroborating the proton data. The remaining carbons were assigned to five methine and nine methylene units, consistent with the structural complexity of a pentacyclic triterpene. The chemical shift of C-18 (δ_C 54.36) and the corresponding H-18 proton

signal (δ_H 2.17) further supported the ursane-type configuration, distinguished by its unique D/E ring junction compared to oleanane analogs.

The combined NMR data strongly suggested an urs-12-ene skeleton, characterized by the Δ^{12-13} double bond (C-12 and C-13) and a hydroxyl group at C-3, as evidenced by the oxymethine carbon (δ_C 78.22) and its proton (δ_H 3.17). The carboxyl functionality at C-28 (δ_C 181.64) is a hallmark of ursolic acid, distinguishing it from related triterpenoids with differing oxygenation patterns. Comparison of these spectral features with established literature data [15, 16] confirmed the identity of compound **2** as ursolic acid (3*β*-hydroxy-urs-12-en-28-oic acid), a widely recognized bioactive triterpenoid known for its pharmacological properties.

Compound 3 was isolated as a yellow amorphous powder, and its molecular composition was established as $C_{17}H_{14}O_7$ through ESI-MS, which displayed a protonated molecular ion at *m/z* 331.09 $[M+H]^+$. This formula suggests a flavonoid framework with a moderate degree of oxygenation, consistent with the structural features commonly observed in this class of natural products. The ^{13}C-NMR spectrum revealed 17 distinct carbon signals, providing a comprehensive view of the molecule's carbon skeleton. These signals were categorized as follows: one carbonyl carbon at δ_C 182.07, indicative of the characteristic flavone C-4 ketone, fourteen sp^2-hybridized carbons forming the aromatic and conjugated systems, and two oxygenated sp^3 carbons at δ_C 59.99 and 56.39, suggestive of methoxy substituents. The predominance of sp^2 carbons aligns with the planar, aromatic nature of flavonoids, while the presence of oxygenated sp^3 carbons hints at *O*-methylation, a common modification in flavone derivatives. The ^{1}H-NMR spectrum further elucidated the proton environment of compound **3**. A singlet at δ_H 6.88 (1H) was assigned to the H-3 proton of the flavone C-ring, a typical feature of this scaffold due to its isolated position. An ABX spin system was observed in the aromatic region, with signals at δ_H 7.46 (1H), 6.92 (1H), and 7.45 (1H), corresponding to the protons of a trisubstituted B-ring, likely at positions H-2′, H-5′, and H-6′, respectively. This pattern is consistent with a 3′,4′-dihydroxy substitution, a frequent motif in flavonoids. Additionally, two singlets at δ_H 3.93 (3H) and 3.75 (3H) were attributed to two methoxy (-OC**H**$_3$) groups, corroborating the ^{13}C-NMR data and indicating *O*-methylation at two distinct positions on the flavone skeleton. The combined spectroscopic evidence points to a flavone structure bearing two methoxy groups and three hydroxyl functionalities. The placement of the methoxy groups at C-6 and C-7, alongside hydroxyl groups at C-5, C-3′, and C-4′, was inferred from the close alignment of the ^{1}H and ^{13}C-NMR data with those reported for 6,7-dimethoxy-5,3′,4′-trihydroxyflavone, a known compound in the literature [17]. Consequently, compound **3** was identified as cirsiliol, a naturally occurring flavone recognized for its polyoxygenated profile and potential bioactivity.

Compound 4 was isolated as a yellowish powder with a melting point of 229 - 230°C. ESI-MS analysis revealed a deprotonated molecular ion at *m/z* 285.01 $[M-H]^-$, confirming the molecular formula $C_{15}H_{10}O_6$. This composition is characteristic of a flavonoid with multiple hydroxyl groups, aligning with the structural motifs prevalent in flavone derivatives. The ^{13}C-NMR spectrum displayed 15 carbon resonances, fully accounting for the molecular framework. These were categorized as one carbonyl carbon at δ_C 181.62 (C-4), typical of the flavone ketone, and fourteen sp^2-hybridized carbons, encompassing the aromatic A- and B-rings and the heterocyclic C-ring. Notable carbon signals included δ_C 163.78 (C-2), 102.85 (C-3), and oxygenated aromatic carbons at δ_C 161.46 (C-5), 164.14 (C-7), 157.26 (C-9), 145.72 (C-3′), and 149.69 (C-4′), suggesting a polyhydroxy substitution pattern across the flavone skeleton.

The ^{1}H-NMR spectrum provided a detailed proton profile. A prominent singlet at δ_H 12.97 (1H) was assigned to the 5-OH proton, a feature stabilized by intramolecular hydrogen bonding with the C-4 carbonyl, a hallmark of 5-hydroxyflavones. The B-ring exhibited an ABX system with overlapping multiplets at δ_H 7.41 (1H, H-2′) and 7.39 (1H, H-6′), alongside a doublet at δ_H 6.88 (J = 8.5Hz, 1H, H-5′), indicative of a 3′,4′-dihydroxy catechol unit. A singlet at δ_H 6.66 (1H, H-3) confirmed the C-ring's olefinic proton, while two *meta*-coupled doublets at δ_H 6.43 (J = 2.0Hz, 1H, H-8) and 6.18 (J = 2.0Hz, 1H, H-6) reflected the A-ring's 5,7-dihydroxy substitution. The small *J*-value (2.0 Hz) is characteristic of *meta*-positioned protons, reinforcing the structural assignment. The spectroscopic data collectively delineate a flavone bearing four hydroxyl groups at C-5, C-7, C-3′, and C-4′. The ^{1}H and ^{13}C NMR profiles of compound **4** exhibited strong concordance with those reported for 3′,4′,5,7-tetrahydroxyflavone [18], a well-characterized natural flavonoid. Consequently, compound **4** was identified as luteolin, a flavone widely

recognized for its antioxidant, anti-inflammatory, and chemopreventive properties.

This study isolated four bioactive compounds from *Premna odorata* seeds. Ursolic acid (**2**) is a common triterpenoid across many plant species, while cirsiliol (**3**) is a widespread flavonoid. Luteolin (**4**) has been previously reported from *P. odorata* [19]. Notably, arjunolic acid (**1**) is reported from this species for the first time, enhancing the understanding of its chemical diversity. Future research should explore the anti-tuberculosis and anti-inflammatory activities of these compounds, given *P. odorata*'s traditional use in treating tuberculosis and inflammation. Arjunolic acid's cardioprotective and hepatoprotective properties warrant testing in relevant disease models. Ursolic acid's potential against breast cancer merits further investigation. Cirsiliol and luteolin, with their antioxidant and anti-inflammatory capabilities, could be evaluated for disorders linked to oxidative stress.

4. CONCLUSION

This study advances the phytochemical understanding of *P. odorata* by isolating and characterizing four bioactive compounds from its seeds, collected in Phu Yen, Vietnam: triterpenoids arjunolic acid (**1**) and ursolic acid (**2**), and flavonoids cirsiliol (**3**) and luteolin (**4**). Employing ESI-MS and NMR, we confirmed their structures, with arjunolic acid's first isolation from this species marking a new contribution. These findings highlight *P. odorata*'s chemical diversity and potential as a source of pharmacologically significant natural products, laying a foundation for future therapeutic and biosynthetic research in natural products chemistry.

FUNDING

This research is funded by University of Khanh Hoa under grant number "KHTN-24.02".

CONFLICT OF INTEREST

The authors disclosed no potential conflicts of interest.

ACKNOWLEDGEMENTS

The author thank Dr. Phan Duc Ngai of the University of Khanh Hoa for identifying the plant samples.

REFERENCES

[1]. Elmaidomy A.H., Mohammed R., Owis A.I., Hetta M.H., Aboul Magd A.M., Siddique A., Abdelmohsen U.R., Rateb M.E., El Sayed K.A., Hassan H.M., "Triple-negative breast cancer suppressive activities, antioxidants and pharmacophore model of new acylated rhamnopyranoses from Premna odorata," *RSC Advances*, 10, 10584-10598, 2020.

[2]. Hien T.T., Mai N.T., Tam B.T., Hien N.T., Cuong C.V.L., Anh H.L.T., "Glycosides isolated from the aerial parts of premna integrifolia growing in Thai Binh," *Vietnam Journal of Science and Technology*, 57(4), 420–427, 2019.

[3]. Kabra A., Kabra R., Baghel U.S., "Premna Species: A Review," *Journal of Biological and chemical Chronicles*, 1(1), 55-59, 2015.

[4]. Prashant Y.M., "Premna integrifolia L.: A review of its biodiversity, traditional uses and phytochemistry," *Ancient Science of Life*, 35(1), 4-11, 2015.

[5]. Otsuka H., Kubo N., Yamasaki K., Padolina W.G., "Premnosides A-D: Diacyl 6-O-α-Lrhamnopyranosylcatalpols from Premna odorata," *Phytochemistry*, 28, 3063–3067, 1989..

[6]. Otsuka H., Kubo N., Yamasaki K., Padolina W.G., "Two iridoid glycoside caffeoyl esters from Premna odorata," *Phytochemistry*, 28, 513-515, 1989.

[7]. Otsuka H., Kashima N., Hayashi T., Kubo N., Yamasaki K., Padolina W.G., "Premnaodorosides A, B and C, iridoid glucoside diesters of an acyclic monoterpenediol from leaves of Premna odorata," *Phytochemistry,* 31, 3129-3133, 1992.

[8]. Elmaidomy A.H., Mohyeldin M.M., Ibrahim M.M., Hassan H.M., Amin E., Rateb M.E., Hetta M.H., El Sayed. K.A., " Acylated iridoids and rhamnopyranoses from Premna odorata (Lamiaceae) as novel mesenchymal-epithelial transition factor receptor inhibitors for the control of breast cancer," *Phytotherapy Research*, 31, 1546-1556, 2017.

[9]. El-Mudomy A.H., Hassan H.M., Amin E., Mohamed W.A., Hetta M.H., "Chemical composition and in vivo anti-inflammatory activity of the lipid extract from Premna odorata Blanco cultivated in Egypt," *World Journal of Pharmacy and Pharmaceutical Sciences*, 5, 129-135, 2015.

[10]. Tantengco O.A.G., Jacinto S.D., "Cytotoxic activity of crude extracts and fractions from Premna odorata (Blanco), Artocarpus camansi (Blanco) and Gliricidia sepium (Jacq.) against selected human cancer cell lines," *Asian Pacific Journal of Tropical Biomedicine*, 5, 1037-1041, 2015.

[11]. Elmaidomy A.H., Hassan H.M., Amin E., Mohamed W., Hetta M.H., "Premna odorata volatile oil as a new mycobacterium tuberculosis growth inhibitor for the control of tuberculosis disease," European Journal of Medicinal Plants, 21, 1-11, 2017.

[12]. Waleed A., Samah S., Mona F., Abeer H., Hossam M., Elham A., Mona H., "Immunomodulatory effect of Premna odorata volatile oils in Mycobacterium tuberculosis by inhibiting TLR4/NF-κB pathway," Journal of Herbmed Pharmacology, 8, 1-7, 2019.

[13]. Mahato S.B., Kundu,A.P., "^{13}C NMR spectra of pentacyclic triterpenoids: a compilation and some salient features," *Phytochemistry*, 37(6), 1517-1575, 1994.

[14]. Sai Ramesh A., Godwin Christopher J., Radhika R., Setty C.R., Thankamani V., "Isolation, characterisation and cytotoxicity study of arjunolic

acid from Terminalia arjuna," *Natural Product Research*, 26(16), 1549-1552, 2012.

[15]. Michael Azael Ludeña Huaman, Ana Luz Tupa Quispe, Reneé Isabel Huamán Quispe, Carlos Alberto Serrano Flores, Juana Robles Caycho, "A simple method to obtain ursolic acid," *Results in Chemistry*, 3, 100144, 2021.

[16]. Werner Seebacher, Nebojsa Simic, Robert Weis, Robert Saf, and Olaf Kunert, "Complete assignments of ^{1}H and ^{13}C NMR resonances of oleanolic acid, 18α-oleanolic acid, ursolic acid and their 11-oxo derivatives," *Magnetic Resonance in Chemistry*, 41, 636-638, 2003.

[17]. Marder M, Viola H, Wasowski C, Wolfman C, Waterman PG, Medina JH, Paladini AC, "Cirsiliol and caffeic acid ethyl ester, isolated from Salvia guaranitica, are competitive ligands for the central benzodiazepine receptors," *Phytomedicine*, 3(1), 29-31, 1996.

[18]. Doan Tran Duy Cuong, Ha Tuan Dat, Nguyen Trung Duan, Pham Dinh Thuong, Nguyen Tan Phat, Mai Dinh Tri, Dang Van Son, Nguyen Thi Hoa, Pham Nguyen Kim Tuyen, Nguyen Kim Phi Phung, "Isolation and characterization of six flavonoids from the leaves of Sterculia foetida Linn," *Vietnam Journal of Chemistry*, 57(4), 438-442, 2019.

[19]. Elmaidomy A.H., Mohammed R., Hassan H.M., Owis A.I., Rateb M.E., Khanfar M.A., Krischke M., Mueller M.J., Abdelmohsen U.R., "Metabolomic Profiling and Cytotoxic Tetrahydrofurofuran Lignans Investigations from Premna odorata Blanco," *Metabolites*, 9(10), 223, 2019.

THÔNG TIN TÁC GIẢ

Nguyễn Hoàng Sa

Trường Đại học Khánh Hòa