

Chemical Constituents and Anti-dengue Activity of the Metabolites from *Helicia Petelotii* Collected in Sapa, Vietnam

Le Quang Truong¹, Nguyen Thi Luu¹, Dang Lan Huong², Pham Phuc Huy²,
Nguyen Quoc Binh³, Nguyen Thi Thu Thuy⁴, Vu Thi Bich Hau⁴, Tran Van Loc¹,
Tran Van Sung¹, Myint Myint Khine⁵, Irina Smirnova⁶, Amandio Vincent Vieira⁷,
Tran Thi Phuong Thao^{1*}

¹Institute of Chemistry, Vietnam Academy of Science and Technology (VAST), Ha Noi, Vietnam

²VNU University of Science, Ha Noi, Vietnam

³Vietnam National Museum of Nature, VAST, Ha Noi, Vietnam

⁴National Institute of Hygiene and Epidemiology (NIHE), Ha Noi, Vietnam

⁵Yangon University, Yangon, Myanmar

⁶Institute of Chemistry, Ufa Scientific Research Center - Russian Academy of Sciences, Ufa, Russia

⁷Nutrition and Metabolism Research Laboratory, Simon Fraser University, Canada

*Corresponding author: ntuelam2010@gmail.com

Abstract:

From the leaves, twigs, and fruits of *Helicia petelotii* collected from the Hoang Lien mountain range in Sapa, Vietnam, six compounds were isolated including β -sitosterol (1), daucosterol (2), 3-O- $[\beta$ -D-(6'-nonadecanoate)glucopyranosyl]- β -sitosterol (3), β -arbutin (4), breynioside B (5), and glycerol monostearate (6). Among them, compounds 3 and 6 were isolated from the fruits, while compounds 1, 2, 4, and 5 were obtained from the leaves and twigs. Advanced spectroscopic methods such as NMR and HR-ESI-MS were applied to accomplish the structures identification of the above compounds. The anti-denge potential of compounds 1-6 were examined against two dengue virus serotypes, DENV-1 and DENV-2. Among them compounds 3 and 5 demonstrated inhibitory activity against DENV-2, with PNRT_{50} values of 18.25 μM and 159.43 μM , respectively, while the remaining compounds were inactive against all the virus serotypes. This is the first report of phytochemicals and antiviral properties of *Helicia petelotii* against Dengue virus.

Keywords: *Helicia petelotii*; phytochemical, anti-dengue activity, virus serotypes, PNRT_{50}

1. Introduction

Dengue fever is a vector-mediated acute viral disorder attributed to the Dengue virus, a member of the *Flavivirus* genus within the *Flaviviridae* family. *Aedes aegypti* and *Aedes albopictus* mosquito are the dominant vector species contributing to the epidemiological spread of the Dengue virus. The virus comprises four primary viral strains- DENV-1 to DENV-4, with an additional, but less common variant, DENV-5 [1–3]. The disease related-symptoms vary depending on the serotype involved, and in some cases, individuals may experience severe pathological repercussions, which can result in fatal consequences within 12 to 24 hours [2–3]. Dengue fever continues to pose a prominent concern in global healthcare and is classified as one of the most remarkable vector-driven viral outbreaks [4]. Annually, up to 400 million individuals contracted the dengue virus throughout the world, with approximately 100 million developing clinical illness and around 40,000 deaths attributed to the dengue

fever [3]. In Vietnam, the number of dengue cases continues to rise, with increasingly complex epidemiological patterns. In 2023, more than 172,000 dengue cases were reported in the country. Although this number represented a 54% decrease compared to 2022, the outbreak exhibited unpredictable trends [5]. According to the Ministry of Health, 114,900 cases were recorded nationwide in 2024 [6]. From the beginning of 2025 to date, despite the strong efforts in prevention and control, Vietnam Ministry of Health has reported nearly 30,000 dengue cases, including 5 fatalities [7]. The World Health Organization (WHO) has indicated the lack of a disease-specific antiviral regimen for addressing dengue fever [4]. At present, the only licensed vaccine-Dengvaxia-is approved for use in a limited number of countries and is effective only in children aged 9 years and older [8–9]. Vaccine development remains challenging due to the presence of four distinct serotypes (DENV-1 to DENV-4)

[8–9]. Moreover, individuals infected with one serotype may develop immunity to that specific type yet still be susceptible to reinfection with other serotypes in endemic regions [2]. Therefore, the discovery and development of effective antiviral agents against the Dengue virus are essential and urgent for public health.

In recent decades, scientists worldwide have been focusing on the search and screening of naturally derived compounds for the treatment of dengue fever that are less toxic, safe, and cost-effective. Several classes of compounds, including polysaccharides, flavonoids, alkaloids, polycyclic quinones, and phenolics, have been shown to display promising anti-dengue activity [10]. Some plant species have been studied and demonstrated antiviral potential against the Dengue virus, such as *Andrographis paniculata*, *Acorus calamus*, and *Cladogynos orientalis*, ...etc [10]. In Vietnam, our research group has conducted screening studies and identified anti-Dengue virus activity in several Vietnamese traditional medicinal plants [11]. Many of them exhibited notable antiviral potential, such as *Carica papaya* [12], *Elaeagnus latifolia* [13], and *Euphorbia cyathophora* [14]. The promising results from the above study provided a strong foundation for further research, aiming to identify the plants with effective antiviral activity against Dengue virus.

The genus *Helicia*, belonging to the family Proteaceae, comprises approximately 110 species, primarily shrubs and small trees [15]. These species occur naturally in the southwestern Pacific, northern and eastern Australia, and various regions of East and South Asia, including Indonesia, Malaysia, China, Taiwan, Japan, India, and the Philippines [16]. Many *Helicia* species are currently threatened and on the brink of extinction [17]. Chemical profiling of *Helicia* species has revealed the presence of phenolic compounds, terpenoid derivatives, flavonoid constituents etc., the groups of natural plant-based metabolites with potential anti-dengue virus activity, along with other bioactive constituents of pharmacological interest. [18–20]. *Helicia petelotii* Merr., commonly known in Vietnamese as "cheo thui," "quả hoa," or "mà sua", is a large tree species that can reach up to 10 meters in height. In Vietnam, this species is distributed on Mount Fansipan, Lao Cai Province, at elevations of approximately 1400 meters above sea level [15]. *H. petelotii* is currently facing the risk of extinction due to habitat destruction. To date, no reports on the chemical constituents or biological activities of *H. petelotii* have been published either in Vietnam or internationally. As a part of this investigation, we describe the extraction and identification of six constituents obtained from the twigs, leaves and fruits of *H. petelotii* collected from the Hoang Lien summit, Sa Pa, Lao Cai province. The isolated compounds included β -sitosterol (1), daucosterol (2), 3-O-[β -D-(6'-nonadecanoate)glucopyranosyl]- β -sitosterol

(3), β -arbutin (4), breynioside B (5), and glycerol monostearate (6). Among them, compounds 3 and 6 were isolated from the fruits, while compounds 1, 2, 4, and 5 were obtained from the twigs and leaves of *H. petelotii*. This study report the first documentation of these constituents from *H. petelotii*. All six compounds (1-6) were assessed for their inhibitory effect against DENV-1 and DENV-2 Dengue virus. The results demonstrated that compounds 3 and 5 exhibited antiviral activity against the DENV-2 strain, with $PNRT_{50}$ values of 18.25 μ M and 159.43 μ M, respectively, while the remaining compounds showed no activity against DENV-1. The other compounds did not display antiviral activity against the tested dengue virus serotypes.

2. Materials and Methods

2.1. Plant Material

The leaves, twigs and fruits of *H. petelotii* were collected on Mount Hoang Lien, Sapa, Lao Cai province, Vietnam in December 2023. Botanical identification of the plant was accomplished by Dr. Nguyen Quoc Binh from Vietnam National Museum of Nature, VAST. The authenticated botanical specimen (CT01) is stored in the Institute of Chemistry herbarium, VAST.

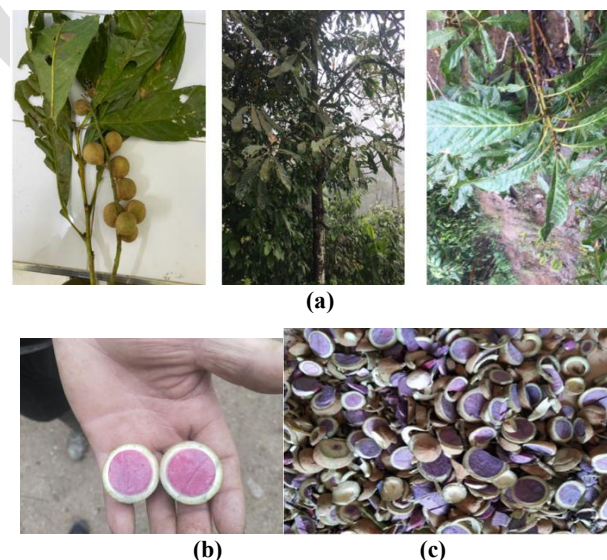


Fig. 1a. The twigs, leaves, and fruits of *H. petelotii* collected from Mount Hoang Lien, Sapa, Lao Cai Province, Vietnam.

Fig. 1b. The fresh fruits of *H. petelotii* displaying white fleshy mesocarp and pink-colored seeds.

Fig. 1c. The dried fruits of *H. petelotii*, showing a light yellow mesocarp and dark purple seeds.

2.2. General Experimental Procedures

Bruker Avance 500 and 600 MHz spectrometers (USA) were used to measure the NMR (Nuclear magnetic resonance) spectra. HR-ESI-MS (High-resolution electrospray ionization mass spectrometry) analyses were performed on an X500R QTOF mass spectrometer. Prior to utilization, the solvents *n*-hexane, dichloromethane, *n*-butanol, methanol, and ethanol (China) were purified by redistillation through a Vigreux column. Thin-layer chromatography (TLC) was employed on Merck silica gel 60 F254 plates, with spot visualization achieved under UV irradiation at 254 nm and post chromatographic derivatization using vanillin/sulfuric acid reagent. Column chromatographic separations were executed using silica gel (particle size 197–400 mesh, 0.040–0.063 mm), while size-exclusion chromatography was carried out on Sephadex LH-20 (Merck).

2.3. Extraction and Isolation

A total of 1550 g of dried twigs and leaves of *H. petelotii* were ground into powder and successively extracted with dichloromethane, methanol, and water at room temperature for 5 hours per solvent (2.5 L \times 5 h \times 3 times). The resulting extracts were concentrated to yield the dichloromethane (DCM) extract (CTL, 40.3 g), the methanol (MeOH) extract (CTLM, 140.2 g), and the aqueous extract (CTLN, 70.6 g). The dichloromethane extract CTL (40.3 g) was fractionated by chromatographic separation over silica gel using a stepwise gradient of *n*-hexane and acetone (ranging from 95:5 to 1:1, v/v) to afford 13 fractions (CTL1–CTL13). Fraction CTL7 yielded compound **1** (7.9 mg) as white needle-shaped crystals. The methanol extract CTLM (140.2 g) was fractionated using a dianion exchange resin column, resulting in three fractions (CTLM1–CTLM3). Fraction CTLM3 (22.3 g) was subsequently subjected to silica gel-mediated column separation employing a gradient elution system from CH₂Cl₂:*n*-hexane (95:5) to CH₂Cl₂ 100%, and finally CH₂Cl₂:MeOH (9:1) to yield five sub-fractions (CTLM3.1–CTLM3.5). Sub-fraction CTLM3.5 was subjected to additional purification using silica gel-packed column chromatography with saturated *n*-butanol as eluent, resulting in three subfractions (CTLM3.5.1–CTLM3.5.3). From subfraction CTLM3.5.3, compound **2** (20 mg), a white solid, was isolated. Fraction CTLM3.2 (2.65 g) was separated through sequential silica gel chromatographic procedures with CH₂Cl₂:MeOH:H₂O and saturated *n*-butanol as eluents, yielding three subfractions (CTLM3.2.1–CTLM3.2.3). Subfraction CTLM3.2.1 was additionally chromatography on Sephadex LH-20 column, using methanol as mobile phase to afford three fractions. From CTLM3.2.1.2.2, compound **4** (3.3 mg), a white powder, was obtained. Finally, fraction CTLM3.5 (1.2 g) underwent repeated silica gel chromatography with

saturated *n*-butanol and CH₂Cl₂:MeOH (9:1), completed with Sephadex LH-20 fractionation in methanol, resulting in the isolation of compound **5** (3.8 mg) as a white powder.

The fruits of *H. petelotii* were separated into distinct anatomical parts: mesocarp (pulp) and seeds (Fig. 1b and Fig. 1c). A total of 620 g of dried mesocarp powder was subjected to successive extraction at room temperature using dichloromethane and methanol (1.5 L \times 5 h \times 3 times), yielding 6.6 g of dichloromethane extract (TC), 30.4 g of methanol extract (TM), and 25.3 g of aqueous extract (TN). Separately, 480 g of dried seed powder was extracted using dichloromethane, methanol, and water under the same conditions (1 L \times 5 h \times 3 times), yielding DCM extract (HC, 6 g), MeOH extract (HM, 40 g), and 35.3 g of aqueous extract (HN). Thin-layer chromatography (TLC) analysis revealed similar chromatographic profiles between the dichloromethane extracts of the pulp (TC) and seeds (HC). Therefore, they were combined to obtain the extract CT1 (12.6 g). The combined extract CT1 was isolated through a silica gel column chromatography using increasing polarity mixtures of *n*-hexane and CH₂Cl₂ (95:5 to 1:1), affording 24 fractions (CT1.1–CT1.24). Sub-fraction CT1.20 (940.5 mg) was subsequently refined through a silica column using *n*-hexane:acetone as eluent (92:8 to 1:1), yielding 16 sub-fractions (CT1.20.1–CT1.20.16). Sub-fraction CT1.20.5 afforded compound **6** (6.9 mg) as a white powder. Sub-fraction CT1.20.11 (80.4 mg) underwent silica gel column fractionation using a stepwise elution with *n*-hexane:CH₂Cl₂ (99:1 to 1:1), yielding compound **3** (7.9 mg) as a white powder.

Compound **1** (β -sitosterol): white needle crystals (recrystallization in a mixture of *n*-hexane/DCM 1:1), ¹H NMR (500 MHz, CDCl₃), δ_H , *J* (ppm, Hz): 0.70 (s, 3H); 0.84 (d, *J* = 7.0, 3H); 0.85 (d, *J* = 7.0, 3H); 0.87 (t, *J* = 7.0, 3H); 1.00 (d, *J* = 6.5, 3H); 1.07 (s, 3H); 1.32 (m, 20H); 1.68 (m, 2H); 1.85 (m, 3H); 2.02 (m, 2H); 2.30 (m, 2H); 3.54 (m, 1H); 5.37 (m, 1H). ¹³C NMR (125 MHz, CDCl₃), δ_C (ppm): 11.8; 12.0; 18.8; 19.0; 19.4; 19.8; 21.1; 23.1; 24.3; 26.1; 28.2; 29.2; 31.6; 31.9; 31.9; 33.9; 36.1; 36.5; 37.2; 39.8; 42.3; 42.3; 45.8; 50.1; 56.1; 56.8; 71.8; 121.7; 140.7.

Compound **2** (Daucosterol): white powder, ¹H NMR (600 MHz, CD₃OD and CDCl₃) δ_H , *J* (ppm, Hz): 0.67 (3H, s); 0.80 (d, *J* = 6.0, 3H); 0.81 (d, *J* = 6.0, 3H); 0.83 (t, *J* = 6.6, 3H); 0.90 (d, *J* = 7.8, 3H); 0.99 (s, 3H); 1.55 (m, 28H); 2.25 (m, 1H); 2.37 (m, 1H); 3.28 (m, 1H); 3.33 (m, 1H); 3.38 (m, 1H); 3.56 (1H, m); 3.72 (dd, *J* = 14.4; 6.0, 1H); 3.82 (dd, *J* = 14.4; 3.6, 1H); 4.38 (t, *J* = 9.6, 1H); 5.34 (m, 1H). ¹³C NMR (150 MHz, CD₃OD and CDCl₃), δ_C (ppm): 12.0; 12.1; 18.9; 19.1; 19.5; 19.9; 21.3; 23.3; 24.5; 26.4; 28.5; 29.5; 29.9; 31.9; 32.2; 34.3; 36.4; 37.0; 37.6; 39.0; 40.1; 42.6; 46.2; 50.5; 56.4; 57.1; 62.2; 70.6; 73.9; 76.2; 76.9; 79.4; 101.5; 122.3; 140.7.

Compound **3** (3-*O*-[β -D-(6'-nonadecanoate) glucopyranosyl]- β -sitosterol): white powder. HR-ESI-MS:

m/z 875,7320 $[M+H_2O+H]^+$ (theoretical calculation for $C_{54}H_{99}O_8$ 875,7340). 1H NMR (600 MHz, $CDCl_3$), δ_H , J (ppm, Hz): 0.70 (s, H-18, 3H); 0.83 (d, $J = 6.6$, H-27, 3H); 0.84 (t, $J = 7.8$, H-29, 3H); 0.85 (d, $J = 6.6$, H-26, 3H); 0.90 (t, $J = 6.6$, H-19'', 3H); 0.94 (d, $J = 6.6$, H-21, 3H); 1.03 (s, H-19, 3H); 2.36 (t, $J = 7.2$, H-2'', 2H); 3.45 (m, H-2'-H-5', 4H); 3.59 (m, H-3, 1H); 4.28 (dd, $J = 12.0$; 2.4, H-6'a, 1H); 4.41 (d, $J = 7.8$, H-1', 1H); 4.49 (dd, $J = 12.0$; 4.8, H-6'b, 1H); 5.38 (m, H-6, 1H). ^{13}C NMR (150 MHz, $CDCl_3$), δ_C (ppm): 11.8 (C-18); 12.0 (C-29); 14.1 (C-19''); 18.8 (C-21); 19.0 (C-27); 19.3 (C-19); 19.8 (C-26); 21.1 (C-11); 22.7 (C-18''); 23.1 (C-28); 24.3 (C-15); 24.9 (C-3''); 28.2 (C-23); 29.1 (C-16); 29.3 (C-25); 29.3-29.7 (C-4''-C-17''); 31.9 (C-8); 31.9 (C-7); 32.0 (C-2); 33.9 (C-22); 34.2 (C-2''); 36.1 (C-20);

36.7 (C-10); 37.3 (C-1); 38.9 (C-12); 39.8 (C-4); 42.3 (C-13); 45.8 (C-24); 50.2 (C-9); 56.1 (C-17); 56.8 (C-14); 63.2 (C-6'); 70.1 (C-4'); 73.6 (C-2'); 74.0 (C-5'); 76.0 (C-3'); 79.5 (C-3); 101.2 (C-1'); 122.1 (C-6); 140.3 (C-5); 174.6 (C-1'').

Compound **4** (β -arbutin): white powder. HR-ESI-MS m/z 295.0782 $[M+Na]^+$ (theoretical calculation for $C_{12}H_{16}O_7Na$ 295.0794) and 273.0914 $[M+H]^+$ (calculated for $C_{12}H_{17}O_7$ 273.0974). 1H NMR and ^{13}C NMR of compound **4**: see Table 1.

Compound **5** (Breynioside B): white powder. 1H NMR and ^{13}C NMR of compound **5**: see Table 1.

Table 1: Comparison of the NMR data for compounds **4** and **5** with literature values

Carbon Nr.	4		β - arbutin [25]		5		Breynioside B [26]	
	δ_H (ppm) ($J = \text{Hz}$), (CD_3OD , 600 MHz)	δ_C (ppm) (CD_3OD , 150 MHz)	δ_H (ppm) ($J = \text{Hz}$), (D_2O , 500 MHz)	δ_C (ppm) (D_2O , 125 MHz)	δ_H (ppm) ($J = \text{Hz}$), (CD_3OD , 600 MHz)	δ_C (ppm) (CD_3OD , 150 MHz)	δ_H (ppm) ($J = \text{Hz}$), (CD_3OD , 500 MHz)	δ_C (ppm) (CD_3OD , 125 MHz)
1	-	153.8	-	154.1	-	152.2	-	152.3
2,6	6.71 <i>d</i> (9.0)	119.4	6.69 <i>d</i> (9.0)	121.2	6.96 <i>d</i> (9.0)	119.5	6.96 <i>d</i> (9.0)	119.6
3,5	6.98 <i>d</i> (9.0)	116.6	6.96 <i>d</i> (9.0)	119.0	6.63 <i>d</i> (9.0)	116.5	6.63 <i>d</i> (9.0)	116.6
4	-	152.4	-	153.2	-	153.8	-	153.9
1'	4.74 <i>d</i> (7.2)	103.7	4.73 <i>d</i> (7.0)	104.1	4.74 <i>d</i> (7.2)	103.6	4.75 <i>d</i> (7.5)	103.7
2'		75.0		75.8		74.9	3.48 <i>dd</i> (9.0; 7.5)	74.9
3'	3.42 (4H, <i>m</i>)	78.1	3.36 – 3.45	78.4	3.43 – 3.49 (3H, <i>m</i>)	78.0	3.50 <i>dd</i> (9.0; 7.0)	78.2
4'		71.4		72.3		72.0	3.45 <i>t</i> (9.0)	72.1
5'		78.0		78.9	3.74 (1H, <i>m</i>)	75.5	3.74 <i>ddd</i> (9.0; 7.0; 2.0)	75.5
6'	3.91 (1H, <i>m</i> , H-6'a); 3.70 (1H, <i>m</i> , H-6'b)	62.6	3.88 <i>d</i> (7.0); 3.69 <i>d</i> (17.0)	63.4	4.69 <i>dd</i> (11.4; 1.8; H-6'a); 4.36 <i>dd</i> (11.4; 7.8; H-6'b)	65.0	4.70 <i>dd</i> (11.5; 2.0); 4.36 <i>dd</i> (11.5; 7.0)	65.1
1''	-	-	-	-	-	122.0	-	122.2
2'', 6''	-	-	-	-	7.92 <i>d</i> (8.4)	132.9	7.92 <i>d</i> (9.0)	132.9
3'', 5''	-	-	-	-	6.88 <i>d</i> (8.4)	116.2	6.88 <i>d</i> (9.0)	116.2
4''	-	-	-	-	-	163.8	-	163.6
7''	-	-	-	-	-	167.9	-	167.9

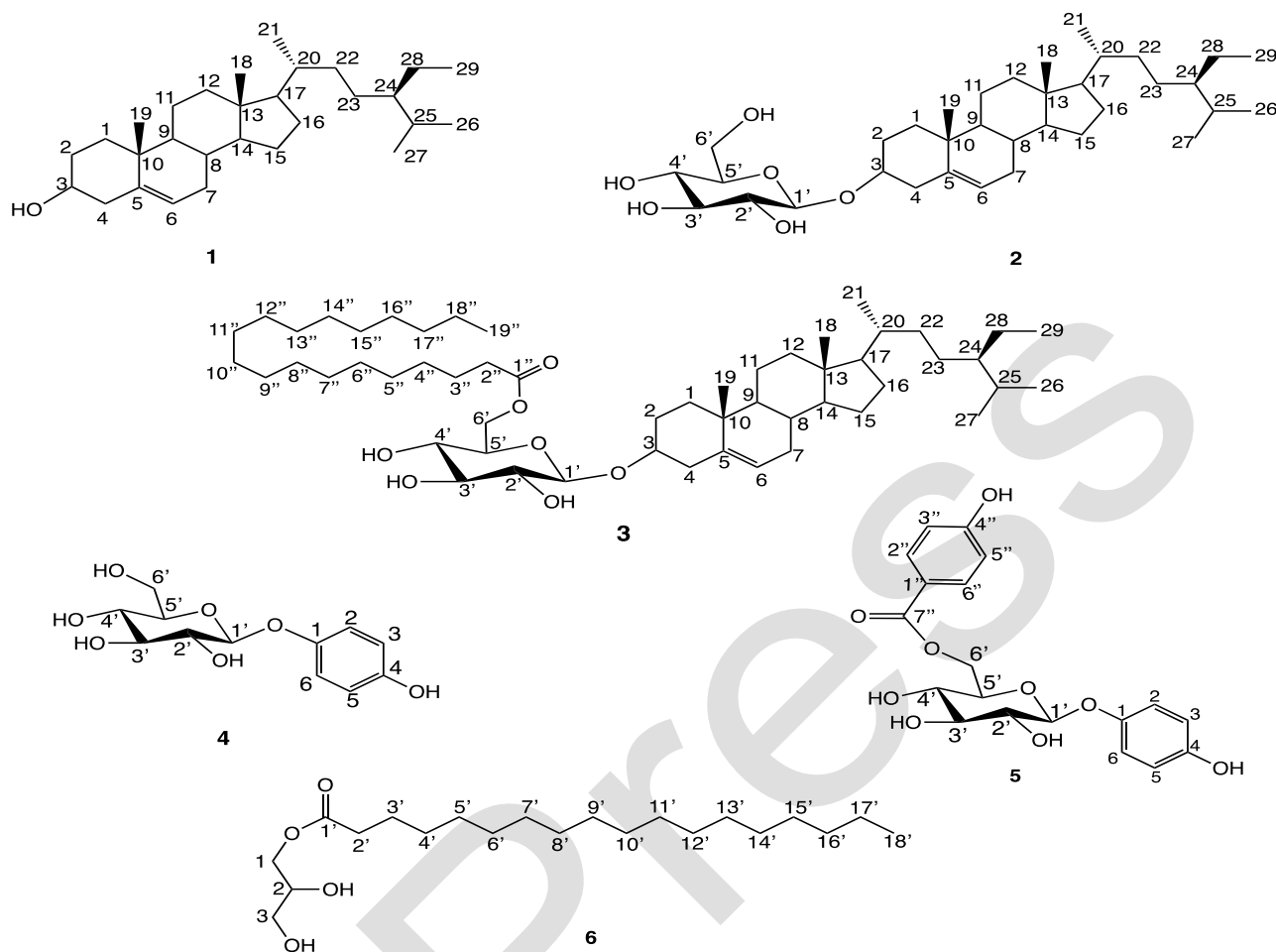


Fig. 2. The structure of the compounds (1-6) isolated from the leaves, twigs and fruits of *H. petelotii*

Compound **6** (Glycerol monostearate): white solid. HR-ESI-MS: m/z 359.3156 $[M+H]^+$ (theoretical calculation for $C_{21}H_{43}O_4$ 359.3161), m/z 381.2970 $[M+Na]^+$ (theoretical calculation for $C_{21}H_{42}O_4Na$ 381.2981). 1H NMR (600 MHz, $CDCl_3$), δ_H , J (ppm, Hz): 0.88 (t, $J = 7.2$, 3H); 1.26 (m, 28H); 1.63 (m, 2H); 2.36 (t, $J = 7.8$, 2H); 3.68 (m, 1H); 3.60 (m, 1H); 3.92 (m, 1H); 4.15 (dd, $J = 5.4$; 11.6, 1H); 4.21 (dd, $J = 4.2$, 1H); 11.6 (1H). ^{13}C NMR (150 MHz, $CDCl_3$), δ_C (ppm): 14.1; 22.7; 24.9; 29.1–29.7; 31.9; 34.1; 63.3; 65.2; 70.3; 174.3.

2.4. Anti-Dengue Activity Bioassay

The evaluation of anti-dengue activity was carried out at the National Institute of Hygiene and Epidemiology, Ministry of Health, employing the Plaque Reduction Neutralization Test (PRNT), an internationally standardized assay endorsed by the World Health Organization (WHO)- a recognized method for assessing Dengue virus (DENV) inhibitory activity [21]. Chemicals, Reagents, and Media: Culture medium: Cell lines were cultured under optimized conditions in Eagle's Minimum Essential Medium (EMEM; Nissui, Japan), with the addition of FBS-enriched culture medium (Fetal Bovine

Serum, Sigma-Aldrich, USA), and Dulbecco's Phosphate Buffered Saline (PBS; Gibco, USA); Dulbecco's Phosphate Buffered Saline (PBS, Gibco, USA); methyl cellulose-4000 (MC, Wako Pure Chemical Industry, Japan); crystal violet (Merck, Germany); formaldehyde (Sigma-Aldrich, USA). BHK21 cells were kindly provided by Nagasaki University, Japan. DENV-1 strain (VN/2017/D7709), DENV-2 strain (00St22A). 1 mL of cell culture medium containing BHK21 cells ($1.0 \times 10^4 - 2.0 \times 10^4$ cells/well) was added to individual chambers of 12-position culture plate. The plate was subjected to overnight incubation at $37^\circ C$, 5% CO_2 to reach a cell confluency of 70–90%. Each test sample was reconstituted in EMEM at a concentration of 500 $\mu g/mL$, and subsequently diluted in a base-2 logarithmic series using EMEM enriched with 2% FBS to reach final concentrations as low as 3.90 $\mu g/mL$. DENV was prepared at a working concentration of 2.5×10^3 PFU/mL using EMEM supplemented with 2% fetal bovine serum. Equal aliquots of the virus suspension and each concentration of test compound were thoroughly mixed and pre-treated at $37^\circ C$ for 60 minutes. Thereafter, existing culture media in

the wells were carefully removed, and 50 μ L of the virus-compound mixture was introduced into each designated well. The assay plates were then allowed to equilibrate at 37°C in a humidified atmosphere with 5% CO₂ for 1 hour, followed by further maintenance for 3–6 days under the same conditions. The formation of viral plaques (areas of cytopathic effect due to virus infection) was observed either under a microscope or by the naked eye. Characteristic plaques typically appear 3–6 days post-infection. Upon plaque emergence, the cellular layer was immobilized in 3.7% formalin for 60 minutes at standard laboratory temperature, and thereafter exposed to 0.25% crystal violet for at least one hour. After drying, the number of plaques was counted in each well. The lowest sample concentration capable of reducing plaque formation by 50% was defined as the PRNT₅₀ value (Plaque Reduction Neutralization Test). Virus-only wells were used as negative controls.

3. Results and Discussion

3.1. Structure Elucidation

Compound **1** was obtained as white needle-shaped crystals. Among the signals in the ¹H NMR spectrum, six methyl proton resonances were identified, including two singlets (δ_{H} 1.07 and 0.70), one triplet (δ_{H} 0.87, J = 7.0 Hz), and three doublets at δ_{H} 1.00 (J = 6.5 Hz), 0.87, and 0.85 (J = 7.0 Hz), respectively. An oxymethine proton was observed as a multiplet at δ_{H} 3.54 (1H), whereas the olefinic proton appeared at δ_{H} 5.37 (m, 1H) in the spectrum. Other proton signals overlapped in the range of δ_{H} 2.30–1.07. Signals for olefinic carbons at δ_{C} 140.7 and 121.7, as well as one hydroxylated methine carbon at δ_{C} 71.8, were discerned in the ¹³C NMR spectrum. Carbon resonances corresponding to six methyl groups appeared at δ_{C} 19.8; 19.4; 19.0; 18.8; 12.0; and 11.8, while the methine and methylene carbons were distributed across δ_{C} 56.8–21.1. The NMR data of compound **1** are consistent with the reported spectral data for β -sitosterol [22]. Accordingly, compound **1** was conclusively assigned as β -sitosterol, with its molecular composition established as C₂₉H₅₀O.

Compound **2** was obtained as a white solid. The NMR spectroscopic features were consistent with those expected for a β -sitosterol derivative. The ¹³C-NMR spectrum revealed six additional carbon signals corresponding to a glucopyranose moiety. The presence of a β -anomeric proton at δ_{H} 4.38 (d, 1H, J = 9.6 Hz)/ δ_{C} 101.5 confirmed the presence of a β -glucopyranose unit. The signals corresponding to an oxymethylene group at δ_{H} 3.82 (1H, dd, J = 14.4, 3.6 Hz) and 3.72 (1H, dd, J = 14.4, 3.6 Hz)/ δ_{C} 62.2, along with four oxymethine carbons at δ_{C} 73.9; 76.9; 70.6 and 76.2, further supported the presence of a glucose moiety. The identity of compound **2** was substantiated through the close alignment of its NMR

spectral characteristics with those reported for daucosterol [23].

Compound **3** was obtained as a white powder. The HR-ESI-MS spectrum of compound **3**, showed a prominent ion at m/z 875.7320 [M+H₂O+H]⁺, in excellent agreement with the calculated mass (m/z 875.7340) for C₅₄H₉₈O₈. These findings defined its molecular formula as C₅₄H₉₆O₇. The ¹H and ¹³C NMR spectra indicated that compound **3** is a derivative of daucosterol. The ¹H-NMR spectrum showed signals corresponding to the aglycone moiety of daucosterol, a glucose unit, and a long chain alkyl group, with proton resonances in the alkyl region ranging from δ_{H} 0.90 to 2.36. Specifically, the terminal methyl group of the alkyl chain appeared at δ_{H} 0.90 (t, J = 6.6 Hz, H-19"), while methylene protons resonated between δ_{H} 1.03 and 2.36. The ¹³C-NMR spectrum supported these observations, showing 29 carbon signals for the aglycone moiety, 6 carbon signals for the glycosidic unit, including an anomeric carbon at δ_{C} 101.2, and glycosyl carbons resonating from δ_{C} 63.2 to 76.0. Notably, the C-6' carbon of the glucose unit was deshielded (δ_{C} 63.2) compared to that in daucosterol, indicating the esterification at this position. An ester carbonyl carbon signal appeared at δ_{C} 174. (C-1"), along with signals for the long chain fatty alkyl group at δ_{C} 34.2–14.1. Analysis of HR-ESI-MS, ¹H- and ¹³C-NMR data, combined with comparison to literature values [24], compound **3** was elucidated to be as 3-O-[β -D-(6'-nonadecanoate)glucopyranosyl]- β - sitosterol. This is the first report of this compound being isolated from *H. petelotii*.

Compound **4** was obtained as a white amorphous powder. High-resolution electrospray ionization mass spectrometry (HR-ESI-MS) revealed quasi-molecular ions at m/z 295.0782 [M+Na]⁺ (theoretical calculation for C₁₂H₁₆O₇Na: m/z 295.0794) and another at m/z 273.0914 [M + H]⁺ (theoretical calculation for C₁₂H₁₇O₇: m/z 273.0974), corresponding to the molecular formula C₁₂H₁₆O₇. ¹H NMR data indicated a 1,4-disubstituted aromatic ring with doublets at δ_{H} 6.98 and 6.71 (J = 9.0 Hz, 2H each). Additionally, signals from a glucose moiety were observed at δ_{H} 3.42 (m, H-2'-H-5', 4H), δ_{H} 3.91 and δ_{H} 3.70 (m, 1H each, H-6'a and H-6'b), along with an anomeric proton at δ_{H} 4.74 (1H, d, J = 7.2 Hz). The observed J -value (7.2 Hz) of the anomeric proton signal supports the assignment of a β -D-glucoside structure. Consistent with the ¹H-NMR data, the ¹³C-NMR spectrum revealed signals for six aromatic carbons at δ_{C} 153.8 (C-1), 152.4 (C-4), 119.4 (C-2, C-6), and 116.6 (C-3, C-5). Six signals corresponding to the glucopyranose ring were observed at δ_{C} 103.7 (C-1', anomeric carbon), 75.0 (C-2'), 78.0 (C-3', C-5'), 71.4 (C-4'), and 62.6 (C-6'), which are characteristic of a glucose unit. The identification of compound **4** as β -arbutin was supported

by the interpretation of combined spectral evidence and literature consistency [25].

Compound **5** was isolated as a white powder. Along with the typical resonances attributed to β -arbutin in the ^1H NMR spectrum, additional signals were detected, corresponding to a 1,4-disubstituted aromatic ring. Specifically, the aromatic protons appeared as two doublets at δ_{H} 7.92 and 6.88 (each 2H, d, $J = 8.4$ Hz), assigned to H-2"/H-6" and H-3"/H-5", respectively. The ^{13}C NMR spectrum was consistent with the ^1H NMR data, revealing a total of 19 carbon signals, including a carbonyl carbon of an ester group at δ_{C} 167.9 (C-7") and twelve aromatic carbon signals ranging from δ_{C} 116.2 to 163.6. Signals observed at δ_{C} 103.6, 74.9, 78.0, 72.0, 75.5, and 65.0 ppm were attributed to the carbon atoms of a β -D-glucopyranosyl unit, including the anomeric carbon at δ_{C} 103.6 (C-1'). This interpretation was further substantiated by spectroscopic data and verified through literature comparison [26]. The spectral characteristics of compound **5** were found to be in full agreement with those reported for breynioside B. This compound was previously isolated from *Rhizophora mucronata* Lam [27].

Compound **6**, obtained as a white amorphous solid, was characterized by HR-ESI-MS, which exhibited protonated and sodiated adduct peaks at m/z 359.3156 and 381.2970, respectively. These mass values corresponded precisely with the calculated values for $\text{C}_{21}\text{H}_{42}\text{O}_4$, thus confirming its molecular formula. Further structural elucidation was achieved by NMR analysis. Signals in the ^1H NMR spectrum at δ_{H} 4.21, 4.15, 3.92, 3.60, and 3.68 were attributed to protons of a glycerol backbone. The ^{13}C NMR spectrum showed a signal at δ_{C} 174.3 (C-1', COO), a range of methylene signals between δ_{C} 22.7–34.1, and a terminal methyl at δ_{C} 14.1. Additionally, the proton signals observed at δ_{H} 1.26, 1.63, 2.36, and 0.88 were consistent with those of a saturated aliphatic chain. Taken together with literature comparison [28], these spectroscopic features supported the identification of compound **6** as glycerol monostearate, marking the first report of this compound from *H. petelotii*.

3.2. Antidengue Activity

Compounds **1–6** were evaluated for their antiviral activity against the dengue virus on two serotypes: DENV-1 and DENV-2. The testing results demonstrated that compounds **3** and **5** possess virus-inhibiting activities against DENV-2 serotype, with PNRT_{50} values of 18.25 μM and 159.43 μM , respectively. These compounds did not display activity against the DENV-1 serotype. DENV-2 is considered to be the most virulent dengue virus serotype due to its association with increased infection rates and progression to dengue hemorrhagic fever and dengue shock syndrome [3]. The remaining compounds (**1**, **2**, **4**, and **6**) showed no antiviral activity against either DENV-1 or DENV-2. Theoretical calculations using molecular docking need to be

conducted to elucidate the binding mechanisms and active sites of the bioactive compounds **3** and **5** against dengue virus target proteins.

4. Conclusion

From the twigs, leaves, and fruits of *H. petelotii*, six compounds were isolated and structurally identified. This work presents for the first time the isolation of these compounds from *H. petelotii* and its anti-dengue properties. The antiviral evaluation revealed that compounds **3** and **5** exhibited inhibitory activity against the DENV-2 serotype of dengue virus, a strain known for its high virulence and association with dengue hemorrhagic fever and dengue shock syndrome.

Acknowledgments The authors sincerely express their appreciation for the financial support by the Ministry of Science and Technology of Vietnam (E-ASIA program between Vietnam, Russia, and Myanmar, Project Nr.: NĐT/e-Asia/23/05).

References

- [1] V. H. Ferreira-de-Lima and T. N. Lima-Camara, Natural vertical transmission of dengue virus in *Aedes aegypti* and *Aedes albopictus*: a system review, *Parasit. Vectors*. vol. 11, pp. 1–8, 2018, Art. no. 77.
<https://doi.org/10.1186/s13071-018-2643-9>
- [2] S. O. T. Bello, A. S. A. Tapsoba, A. A. Zoure, Y. J. R. Bassole, W.-L.-S. K. Yogo, P. Bado, O. F. Kouta, F. Tassemedo, T. M. Zohoncon, F. W. Djigma, A. Diabate, J. Simporé, Molecular characterization of the four serotypes (DENV-1, DENV-2, DENV-3 and DENV-4) of dengue virus circulating in Ouagadougou, Burkina Faso, *Open Journal Epidemiology*, vol. 14, no. 4, pp. 565–578, 2024.
<https://doi.org/10.4236/ojepi.2024.144040>
- [3] S. Melebari, A. Hafiz, H. A. Natto, M. O. Elamin, N. A. Jalal, A. Hakim, S. Rushan, O. Fallatah, K. Alzabeei, F. Malibari, H. Mashat, A. Alsaadi, A. Alhakam, A. Hadidi, G. S. Alkhalidi, A. Alkhyami, A. Alqarni, A. Alzahrani, M. Alghamdi, A. Siddiqi, and A. Khogeer, Estimation and characterization of dengue serotypes in patients presenting with dengue fever at Makkah hospitals, *Tropical Medicine Infectious Disease*, vol. 10, iss. 1, pp. 27, Jan. 2025.
<https://doi.org/10.3390/tropicalmed10010027>
- [4] World Health Organization- WHO: Dengue and severe dengue.. [Online]. Available: https://www.who.int/health-topics/dengue-and-severe-dengue#tab=tab_1
- [5] Vietnam News Agency: 2023: The whole country recorded more than 172,000 cases of dengue fever, Jan. 8, 2024. [Online]. Available: <https://vnanet.vn/vi/graphic/suc-khoe-7/nam-2023-ca-nuoc-ghi-nhan-hon-172000-ca-mac-sot-xuat-huyet-7201775.html>
- [6] Ministry of health portal: The whole country has recorded 114,900 cases of dengue fever, with patients still hospitalized in serious condition, Nov. 23, 2024. [Online]. Available: https://moh.gov.vn/hoat-dong-cua-dia-phuong/ghi-nhan-asset_publisher/gHb1a8vOQDuS/content/ca-nuoc-a-114-900-ca-mac-sot-xuat-huyet-van-co-benh-nhan-vao-vien-trong-tinh-trang-nang?inheritRedirect=false

- [7] Government e-newspaper: Dengue fever no longer occurs in cycles, Jun. 14, 2025. [Online]. Available: <https://baohinhphu.vn/benh-sot-xuat-huyet-khong-con-xay-ra-theo-chu-ky-10225061416374119.htm>
- [8] R. Shukla, V. Ramasamy, R. K. Shanmugam, R. Ahuja, and N. Khanna, Antibody-dependent enhancement: a challenge for developing a safe dengue vaccine, *Frontiers in Cellular Infection Microbiology*, vol. 10, pp. 572681, 2010. <https://doi.org/10.3389/fcimb.2020.572681>
- [9] J. Pintado Silva, A. Fernandez-Sesma, Challenges on the development of a dengue vaccine: a comprehensive review of the state of the art, *Journal of General Virology*, vol. 104, iss. 3, pp. 001831, Mar. 2023. <https://doi.org/10.1099/jgv.0.001831>
- [10] B. Rehman, A. Ahmed, S. Khan, N. Saleem, F. Naseer, and S. Ahma, Exploring plant-based dengue therapeutics: from laboratory to clinic, *Tropical Diseases, Travel Medicine and Vaccines*, vol. 10, pp. 1–19, Nov. 2024, Art. no. 23. <https://doi.org/10.1186/s40794-024-00232-1>
- [11] T. P. T. Tran, Q. C. Nguyen, N. A. Ho, T. L. Nguyen, T. B. H. Vu, T. T. T. Nguyen, V. C. Tran, T. A. Nguyen, T. Q. Bui B., D. C. To, T. Q. Phan, T. T. Nguyen, V. S. Tran, and T. A. N. Nguyen, Anti-dengue screening on several Vietnamese medicinal plants: experimental evidences and computational analyses, *Chemistry & Biodiversity*, vol. 19, iss. 7, pp. e202101026, Jul. 2022. <https://doi.org/10.1002/cbdv.202101026>
- [12] T. P. T. Tran, D. T. Nguyen, N. T. Pham, C. V. Tran, A. N. Ho, H. B. T. Vu, and T. T. T. Nguyen, Phytochemistry of the unpolar extract of *Carica papaya* leaves and its antidengue activity, *Vietnam Journal of Chemistry*, vol. 60, iss. 6, pp. 777–783, Dec. 2022. <https://doi.org/10.1002/vjch.202200049>
- [13] T. P. T. Tran, L. T. Nguyen, C. L. Nguyen, H. B. T. Vu, and T. T. T. Nguyen, The first phytochemical study of *Elaeagnus latifolia* in Vietnam, *Vietnam Journal of Chemistry*, vol. 59, iss. 3, pp. 376–382, Jun. 2021. <https://doi.org/10.1002/vjch.202000204>
- [14] D. T. Nguyen, N. T. Pham, L. T. Nguyen, A. T. Nguyen, H. B. T. Vu, T. T. T. Nguyen, A. N. Ho, H. D. Pham, and T. P. T. Tran, Phytochemical study of *Euphorbia cyathophora* collected in Dan Phuong, Hanoi and its antidengue activity against DENV1-4 virus serotypes, *Vietnam Journal of Chemistry*, vol. 61, iss. 3, pp. 372–378, Jun. 2023. <https://doi.org/10.1002/vjch.202300123>
- [15] Pham Hoang Ho, Vietnamese plants, Youth Publishing House – Ho Chi Minh city, 1999, pp.16.
- [16] H. Weston Peter and P. Barker Nigel, A new suprageneric classification of the Proteaceae, with an annotated checklist of genera, *Telopea* (Syd), vol. 11, iss. 3, pp. 314–344, 2006. <https://doi.org/10.7751/telopea20065733>
- [17] IUCN Red List of Threatened Species. [Online]. Available: <https://www.iucnredlist.org>
- [18] P. C. Lalawmpuii, C. Malsawmtluangi, R. Vanlalruata, and B. B. Kakoti, Evaluation of anti-inflammatory activity of *Helicia nilagirica* Bedd on cotton pellet-induced granuloma in rats, *International Journal of Pharmacy and Pharmaceutical Sciences*, vol. 8, iss. 7, pp. 455–456, 2016.
- [19] L. Tlau, L. Lalawmpuii, P. B. Lalthanpuii, V. Ralte, C. Lalnunfela, K. Lalchhandama, Study of the mizo medicinal plant, *Helicia excelsa* on its phytochemical components, antioxidant property and antibacterial activity, *Indian Journal of Science and Technology*, vol. 16, iss. Special issue 1, pp. 10–18, 2023. <https://doi.org/10.17485/IJST/v16sp1.msc2>
- [20] G. C. Jagetia and J. Zoremsiami, Anticancer activity of *Helicia nilagirica* bedd in mice transplanted with Dalton's lymphoma, *IJCAM*, vol. 11, iss. 2, pp. 209–220, Apr. 2018. <https://doi.org/10.15406/ijcam.2018.11.00380>
- [21] J. T. Roehrig, J. Hombach, and A. D. T. Barrett, Guidelines for plaque-reduction neutralization testing of human antibodies to dengue viruses, *Viral Immunology*, vol. 21, no. 2, pp. 123–132, 2008. <https://doi.org/10.1089/vim.2008.0007>
- [22] N. Kamal, C. Clements, A. I. Gray, R. A. Edrada-Ebel, Anti-infective activities of secondary metabolites from *Vitex pinnata*, *Journal of Applied Pharmaceutical Science*, vol. 6, iss. 1, pp. 102–106, Jan. 2016. <https://doi.org/10.7324/JAPS.2016.600117>
- [23] H. V. Do, H. T. Nguyen, T. T. T. Nguyen, N. T. Dang, B. H. T. Dao, A. H. T. Nguyen, H. L. T. Duong, T. H. Nguyen, Study on chemical constituents from the roots of *Panax bipinnatifidius* seem, Collected in Sapa, Laocai, *VNU Journal of Science*, vol. 33, no. 2, pp. 50–55, Dec. 2017. <https://doi.org/10.25073/2588-1132/vnumps.4079>
- [24] N. Sultana and A. J. Afolayan, A novel daucosterol derivative and antibacterial activity of compounds from *Arctotis arctotoides*, *Natural Product Research*, vol. 21, iss. 10, pp. 889–896, Aug. 2007. <https://doi.org/10.1080/14786410601129606>
- [25] C. Avonto, Y. -H. Wang, B. Avula, M. Wang, D. Rua, and I. A. Khan, Comparative studies on the chemical and enzymatic stability of alpha- and beta-arbutin, *International Journal of Cosmetic Science*, vol. 38, iss. 2, pp. 187–193, Sep. 2015. <https://doi.org/10.1111/ics.12275>
- [26] K. T. P. Linh, N. H. Quan, N. V. Chien, N. Q. Trung, V. H. Thong, N. V. Tuyen, and N. P. Thao, Secondary metabolites from the stem barks of *rhizophora mucronata* lamk, *VJST*, vol. 58, no. 6, pp. 653–664, 2020. <https://doi.org/10.15625/2525-2518/0/0/14783>
- [27] D. T. A. Nghia, H. T. N. H Hanh, L. T. Anh, L. T. H. Van, and H. V. Duc, Phenolic compounds and carotenoids from the leaves of *gymnosporia chevalieri* Tard, *Hue Journal of Medicine and Pharmacy*, vol. 15, iss. 2, pp. 170–179, May 2025. <https://doi.org/10.34071/jmp.2025.2.24>
- [28] N. O. V. Sonntag, Glycerolysis of fats and methyl esters – status, review and critique, *Journal of the American Oil Chemists' Society* (JAOCS), vol. 59, iss. 10, pp.795–802A, Oct. 1982. <http://doi.org/10.1007/BF02634442>