Journal of Science and Technology – Engineering and Technology for Sustainable Development
Volume xx, Issue x, Month 000-000

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

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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-[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 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 serve 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)

p-ISSN 3093-3242
e-ISSN 3093-3579
https://doi.org/10.51316/jst.xxx.etsd.202x.xx.x.x
Received: Jul 30, 2025; Revised: Oct 13, 2025
Accepted: Oct 14, 2025; Online: Nov 20, 2025.

[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 "chẹo thui," "quắn hoa," or "mạ 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, Sapa, 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 reports 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 μM and 159.43 μM, 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. petetlotii* were collected on Mount Hoang Lien, Sapa, Laocai 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.

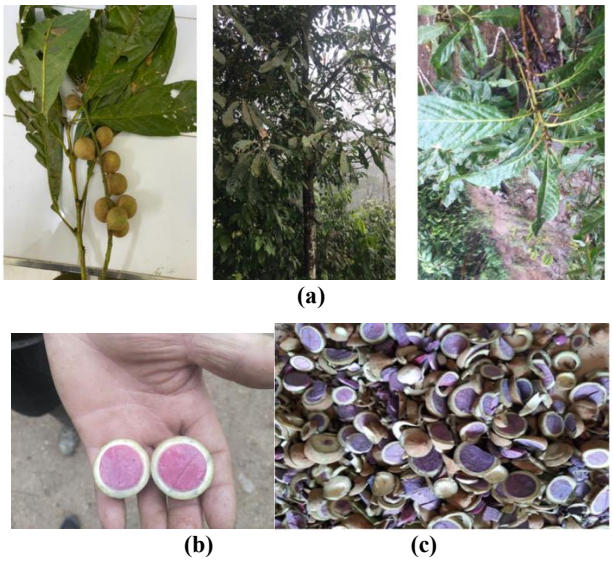

Fig. 1a. The twigs, leaves, and fruits of *H. petelotii* collected from Mount Hoang Lien, Sapa, Lao Cai Province, Vietnam.

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 (Untraviolet) 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 × 5 h × 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 CH_2Cl_2:*n*-hexane (95:5) to CH_2Cl_2 100%, and finally CH_2Cl_2: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 CH_2Cl_2:MeOH:H_2O 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 CH_2Cl_2: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 × 5 h × 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 × 5 h × 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 CH_2Cl_2 (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:CH_2Cl_2 (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), ^{1}H NMR (500 MHz, $CDCl_3$,), 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). ^{13}C NMR (125 MHz, $CDCl_3$), 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, ^{1}H NMR (600 MHz, CD_3OD and $CDCl_3$) 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). ^{13}C NMR (150 MHz, CD_3OD and $CDCl_3$), 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). ^{1}H 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). ^{1}H NMR and ^{13}C NMR of compound **4**: see Table 1.

Compound **5** (Breynioside B): white powder. ^{1}H 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 [25, 26]

Carbon Nr.	**4**		*β*- arbutin		**5**		Breynioside B	
	δ_H (ppm) (*J* = Hz), (CD_3OD, 600 MHz)	δ_C (ppm) (CD_3OD, 150 MHz)	δ_H (ppm) (*J* = Hz), (D_2O, 500 MHz)	δ_C (ppm) (D_2O, 125 MHz)	δ_H (ppm) (*J* = Hz), (CD_3OD, 600 MHz)	δ_C (ppm) (CD_3OD, 150 MHz)	δ_H (ppm) (*J* = 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 d (9.0)	119.4	6.69 d (9.0)	121.2	6.96 d (9.0)	119.5	6.96 d (9.0)	119.6
3,5	6.98 d (9.0)	116.6	6.96 d (9.0)	119.0	6.63 d (9.0)	116.5	6.63 d (9,0)	116.6
4	-	152.4	-	153.2	-	153.8	-	153.9
1'	4.74 d (7.2)	103.7	4.73 d (7.0)	104.1	4.74 d (7.2)	103.6	4.75 d (7.5)	103.7
2'	3.42 (4H, m)	75.0	3.36 – 3.45	75.8	3.43 – 3.49 (3H, m)	74.9	3.48 dd (9.0; 7.5)	74.9
3'		78.1		78.4		78.0	3.50 dd (9.0; 7.0)	78.2
4'		71.4		72.3		72.0	3.45 t (9.0)	72.1
5'		78.0		78.9	3.74 (1H, m)	75.5	3.74 ddd (9.0; 7.0; 2.0)	75.5
6'	3.91 (1H, m, H-6'a); 3.70 (1H, m, H-6'b)	62.6	3.88 d (7.0); 3.69 d (17.0)	63.4	4.69 dd (11.4; 1.8; H-6'a) 4.36 dd (11.4; 7.8; H-6'b)	65.0	4.70 dd (11.5; 2.0) 4,36 dd (11.5; 7.0)	65.1
1"	-	-	-	-	-	122.0	-	122.2
2", 6"	-	-	-	-	7.92 d (8.4)	132.9	7.92 d (9.0)	132.9
3", 5"	-	-	-	-	6.88 d (8.4)	116.2	6.88 d (9.0)	116.2
4"	-	-	-	-	-	163.8	-	163.6
7"	-	-	-	-	-	167.9	-	167.9

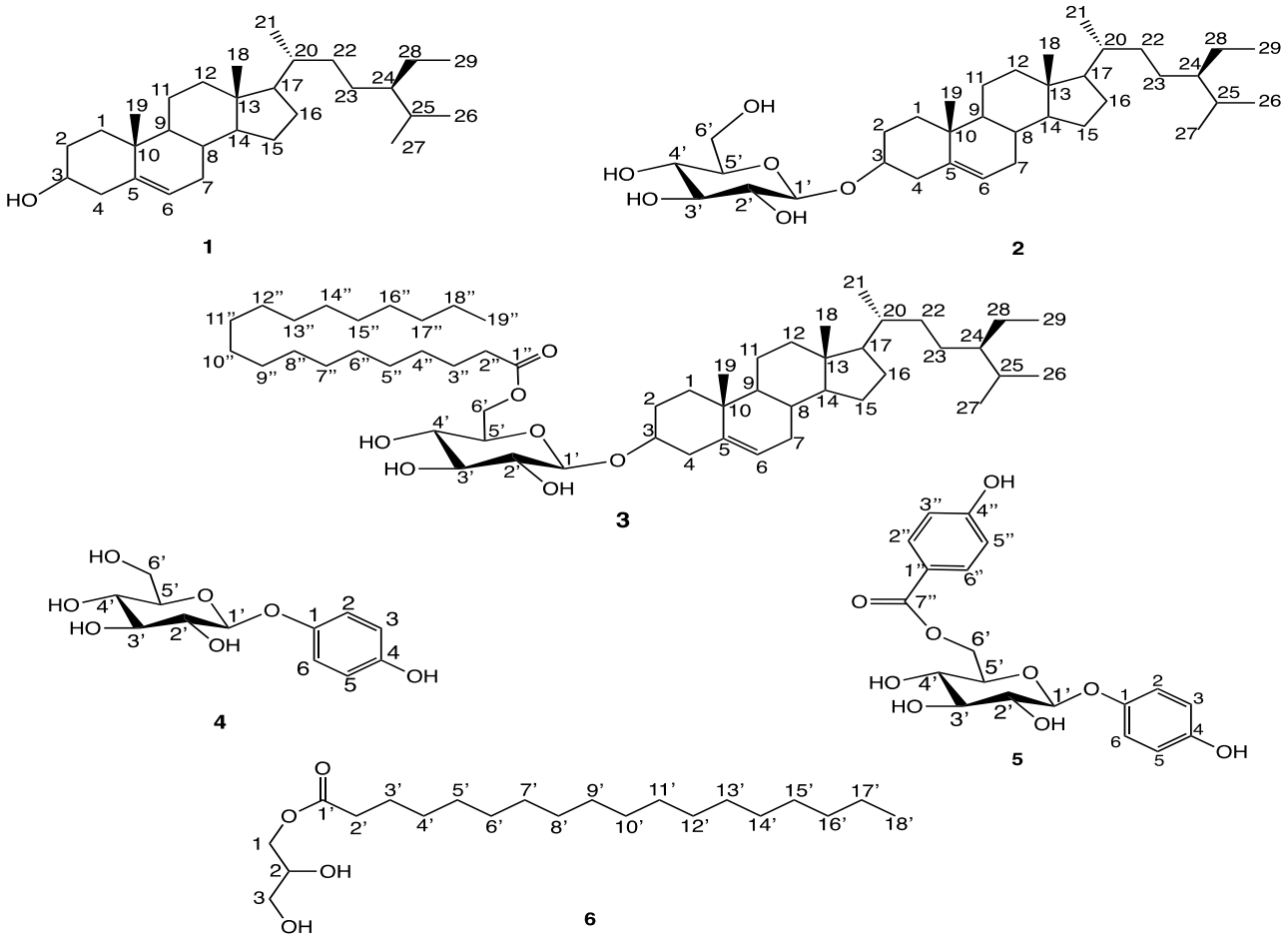

Fig. 2. The structure of the compounds (**1-6)** isolated from the leaves, twigs and fruits of *H. petelotii*.

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°C, 5% CO_2 to reach a cell confluency of 70–90%. Each test sample was reconstituted in EMEM at a concentration of 500 µg/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 µg/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 °C for 60 minutes. Thereafter, existing culture media in the wells were carefully removed, and 50 µL 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% CO_2 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 $PRNT_{50}$ 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 ^{1}H 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 ^{13}C 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 $C_{29}H_{50}O$.

Compound **2** was obtained as a white solid. The NMR spectroscopic features were consistent with those expected for a *β*-sitosterol derivative. The ^{13}CNMR 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 $[M+H_2O+H]^+$, in excellent agreement with the calculated mass (*m/z* 875.7340) for $C_{54}H_{99}O_8$. These findings defined its molecular formula as $C_{54}H_{96}O_7$. The ^{1}H and ^{13}C NMR spectra indicated that compound **3** is a derivative of daucosterol. The ^{1}HNMR 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 ^{13}C 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, ^{1}H and ^{13}CNMR 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 $C_{12}H_{16}O_7Na$: *m/z* 295.0794) and another at *m/z* 273.0914 $[M+H]^+$ (theoretical calculation for $C_{12}H_{17}O_7$: *m/z* 273.0974), corresponding to the molecular formula $C_{12}H_{16}O_7$. ^{1}H 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 ^{1}HNMR data, the ^{13}CNMR 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 ^{1}H 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 ^{1}H 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 $C_{21}H_{42}O_4$, thus confirming its molecular formula. Further structural elucidation was achieved by NMR analysis. Signals in the ^{1}H 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).

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