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PHYTOCHEMICAL CONSTITUENTS OF THE BRANCHES AND LEAVES OF *ALANGIUM SALVIIFOLIUM* (L.f.) WANGERIN COLLECTED IN QUANG NGAI, VIETNAM

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Summary

Alangium salviifolium has been traditionally used for medicinal purposes, such as rheumatism, diabetes, digestive disorders, hypertension, and inflammatory conditions. In this study, a phytochemical investigation of the branches and leaves of *A. salviifolium* collected in Vietnam was carried out using successive solvent extraction, chromatographic separation, and spectroscopic analyses. Five compounds were isolated and structurally identified as alangiside (**1**), demethylalangiside (**2**), astragalin (**3**), betulinic acid (**4**), and 22-dehydroclerosterol 3-*O*-*β*-D-glucopyranoside (**5**). The structures were elucidated based on detailed spectroscopic data, including 1D- and 2D-NMR as well as ESI-MS, supported by comparison with published data. Notably, compound **5** was isolated and identified from *A. salviifolium* for the first time.

Keywords: *Alangium salviifolium;*Alangiside; *Astragalin; Betulinic acid; Phytosterol.*

1. Introduction

Alangium salviifolium (L.f.) Wangerin, belonging to the family Alangiaceae (sometimes classified under Cornaceae), is commonly known in Vietnam as "Quăng lông" and "Quăng gai" [1],[2]. This species is widely distributed in East and Southeast Asia, including Vietnam. In traditional medicine, *A. salviifolium* has been used as a medicinal plant for the treatment of various ailments such as rheumatism, diabetes, digestive disorders, hypertension, and inflammatory conditions [3],[4],[5]. The leaves and bark possess astringent properties and are traditionally used in the treatment of rheumatism, leprosy, syphilis, and asthma; they are also reported to be effective in the management of jaundice and gastric pain. Phytochemical investigations have demonstrated that different parts of *A. salviifolium* contain a wide range of secondary metabolites, including alkaloids, terpenoids, flavonoids, steroids, and phenolic compounds [5],[6],[7]. Modern pharmacological investigations have demonstrated that *A. salviifolium* possesses significant biological activities, including antidiabetic, antioxidant, anti-inflammatory, antibacterial, antifungal, enzyme inhibitory, and insecticidal effects [7],[8],[9],[10]. These findings highlight the considerable potential of this species for the development of pharmaceuticals and health-related products.

Despite its medicinal importance, research on *A. salviifolium* in Vietnam remains limited and has not yet fully reflected its therapeutic potential. In 2023, Dang T.L.T. et al. conducted a preliminary phytochemical screening using a modified Ciuley method, reporting major classes of compounds such as alkaloids, terpenoids, flavonoids, coumarins, reducing sugars, and essential oils in the roots of *A. salviifolium* [11]. However, detailed studies on the chemical constituents, particularly from other plant parts such as branches and leaves, remain scarce.

Moreover, the increasing exploitation of this plant for agricultural land use has led to a decline in its natural populations, raising concerns about the sustainability of this valuable medicinal resource [12]. Therefore, systematic research on the chemical constituents and biological activities of *A. salviifolium* is necessary to promote the rational utilization of this valuable medicinal resource and to provide a scientific basis for its conservation and sustainable development in Vietnam.

2. Experimental

2.1. Plant materials

The branches and leaves of *Alangium salviifolium* (L.f.) Wang were collected in Quang Ngai province, Vietnam, in July 2025, and taxonomically identified by Ms. Vu Thanh Thao, University of Medicine and Pharmacy at HCMC (UMPSTC) (voucher specimen N-25-DV-479). The collected samples were chopped into small pieces, shade-dried, further dried in an oven at 50 °C, ground into a coarse powder, and stored in sealed plastic bags under cool and dry conditions, protected from direct sunlight.

2.2. General experimental procedures

NMR spectra were recorded on Bruker 600 MHz FT-NMR spectrometers using tetramethylsilane (TMS) as the internal standard. Electrospray ionization mass spectrometry (ESI-MS) data were obtained with an Agilent 1260 Series Single Quadrupole LC/MS system (Agilent, USA). Column chromatography (CC) was performed on *silica gel* (Merck, Darmstadt, Germany; particle size 63–200 μm or 40–63 μm), reversed-phase YMC ODS-AA12S75 (75 μm), and Sephadex® LH-20 (GE Healthcare). High-Performance Liquid Chromatography (HPLC) was carried out on a preparative HPLC Shimadzu LC20-AP with a SunFire ® C_{18} OBD ™ Prep column 100 A^o (250 × 10 mm; 10 μm). Thin-layer chromatography (TLC) was conducted on DC-Alufolien 60 F_{254} pre-coated *silica gel* plates (Merck 1.05715), with visualization under UV light or after spraying with 10% H_2SO_4/EtOH followed by heating.

2.3. Extraction and isolation

The dried branches and leaves of *A. salviifolium* (7.6 kg) were extracted with 80% ethanol (50 L × 4 days) at room temperature. The combined ethanolic extracts were filtered and concentrated under reduced pressure to yield a crude extract (836 g). This extract was suspended in water and successively partitioned with *n*-hexane, dichloromethane (DCM), ethyl acetate (EtOAc), and *n*-butanol. Evaporation of the respective solvents under vacuum afforded the *n*-hexane (51.76 g), DCM (29.41 g), EtOAc (32.36 g), and *n*-butanol (165.37 g) soluble fractions.

The *n*-butanol fraction (70 g) was subjected to *silica gel* CC, eluted with an EtOAc–MeOH solvent system (100:0 → 0:100, v/v), to afford nine fractions (ASB1–ASB9). Subfraction ASB5 (6.0 g) was further separated on *silica gel* CC with EtOAc-MeOH (100:0 → 0:100, v/v) to afford thirteen fractions (ASB5.1–ASB5.13). Fraction ASB5.11 (2.09 g) was subjected to an RP-18 CC using a MeOH–H_2O gradient solvent system (1:4 → 1:0, v/v), yielding compound **1** (78.9 mg). Subfraction ASB8 (6.65 g) was further separated on *silica gel* CC with EtOAc–MeOH (100:0 → 0:100, v/v) to afford nine fractions (ASB8.1–ASB8.9). Fraction ASB8.7 (0.81 g) was purified by a Sephadex LH-20 CC, using methanol, to afford seven fractions (ASB8.7.1-ASB8.7.7). Subfraction ASB8.7.3 (0.386 g) was further purified by preparative HPLC (UV 254 nm) with a MeOH-H_2O solvent system (0-20 min: 50% MeOH, 20-25 min: 60% MeOH, 2.5 mL/min) to obtain compound **2** (13.2 mg, t_R = 15.3 min). Fraction ASB5.6 (0.30 g) was purified by a Sephadex LH-20 CC using methanol to yield compound **3** (10.6 mg).

The DCM fraction (29.41 g) was subjected to *silica gel* CC, eluted with a DCM–MeOH gradient solvent system (100:0 → 0:100, v/v) to yield nine fractions, ASD1–ASD9. Fraction ASD2 (1.18 g) was further separated by *silica gel* CC using *n*-hexane–ethyl acetate (50:1-1:1, v/v) as the eluent, affording 4 subfractions (ASD2.1–ASD2.4). Fraction ASD2 (300 mg) was further separated by RP-18 CC, eluted with acetone–H_2O (4:1-9:1, v/v), to afford subfraction ASD2.2.3 (95 mg). This fraction was further purified by *silica gel* CC using *n*-hexane–EtOAc (9:1, v/v) as the mobile phase to afford compound **4** (10.2 mg). Fraction ASD8 (1.13 g) was separated by *silica gel* CC using a DCM–MeOH gradient solvent

system (80:1 → 50:1 → 25:1 → 9:1, v/v), affording eight subfractions (ASD8.1–ASD8.8). Compound **5** (15.2 mg) was obtained by recrystallization in methanol from fraction ASD8.3 (200.5 mg).

Compound 1 (Alangiside): Brown amorphous solid. ESI-MS: *m/z* 506.05 $[M+H]^+$.^{1}H-NMR (600 MHz, CD_3OD), δ_H (ppm): 6.72 (1H, s, H-1), 6.72 (1H, s, H-4), 2.71 (1H, m, H_a-5), 2.79 (1H, m, H_b-5), 2.91 (1H, m, H_a-6), 4.75 (1H, m, H_b-6), 7.43 (1H, d, *J* = 2.4 Hz, H-9), 5.51 (1H, d, *J* = 1.8 Hz, H-11), 2.73 (1H, m, H-12), 3.22 (1H, overlap, H-12a), 4.75 (1H, overlap, H-13a), 2.33 (1H, m, H_a-13), 1.37 (1H, m, H_b-13), 5.55 (1H, m, H-14), 5.20 (1H, dd, *J* = 10.2, 1.8 Hz, H_a-15), 5.32 (1H, dd, *J* = 17.4, 1.8 Hz, H_b-15), 4.70 (1H, d, *J* = 7.8 Hz, H-1'), 3.21 (1H, dd, *J* = 9.0, 7.8 Hz, H-2'), 3.39 (1H, t, *J* = 9.0 Hz, H-3'), 3.29 (1H, m, H-4'), 3.32 (1H, m, H-5'), 3.71 (1H, dd, *J* = 12.0, 6.0 Hz, H_a-6'), 3.93 (1H, dd, *J* = 12.0, 2.4 Hz, H_b-6'), 3.85 (3H, s, 3-OCH_3). ^{13}C-NMR (150 MHz, CD_3OD), δ_C (ppm): 113.3 (C-1), 146.4 (C-2), 147.9 (C-3), 112.6 (C-4), 127.3 (C-4a), 29.5 (C-5), 40.9 (C-6), 165.9 (C-8), 109.3 (C-8a), 148.7 (C-9), 97.5 (C-11), 44.5 (C-12), 27.7 (C-12a), 35.0 (C-13a), 56.9 (C-13b), 130.2 (C-13), 133.9 (C-14), 120.4 (C-15), 99.6 (C-1'), 74.8 (C-2'), 77.9 (C-3'), 71.6 (C-4'), 78.3 (C-5'), 62.7 (C-6'), 56.4 (3-OCH_3).

Compound 2 (Demethylalangiside): Yellow oil. ESI-MS: *m/z* 492.20 $[M+H]^+$. ^{1}H-NMR (600 MHz, CD_3OD), δ_H (ppm): 6.65 (1H, s, H-1), 6.55 (1H, s, H-4), 2.70 (1H, m, H-5), 2.89 (1H, td, *J* = 12.6, 3.6 Hz, H_a-6), 4.65 (1H, dt, *J* = 12.6, 3.6 Hz, H_b-6), 7.40 (1H, d, *J* = 2.4 Hz, H-9), 5.49 (1H, d, *J* = 1.8 Hz, H-11), 2.60 (1H, ddd, *J* = 12.0, 6.6, 3.0 Hz, H-12), 3.19 (1H, m, H-12a), 1.36 (1H, *J* = 11.4 Hz, H_a-13), 2.29 (1H, td, *J* = 13.2, 3.6 Hz, H_b-13), 4.69 (1H, dd, *J* = 10.2, 3.6 Hz, H-13a), 5.52 (1H, dt, *J* = 17.4, 10.2 Hz, H-14), 5.20 (1H, dd, *J* = 10.2, 1.8 Hz, H_a-15), 5.29 (1H, dd, *J* = 16.8, 1.8 Hz, H_b-15), 4.69 (1H, d, *J* = 7.8 Hz, H-1'), 3.20 (1H, dd, *J* = 9.0, 7.8 Hz, H-2'), 3.39 (1H, t, *J* = 9.0 Hz, H-3'), 3.21 (1H, m, H-4'), 3.32 (1H, m, H-5'), 3.68 (1H, dd, *J* = 12.0, 5.4 Hz, H_a-6'), 3.90 (1H, dd, *J* = 12.0, 1.8 Hz, H_b-6'). ^{13}C-NMR (150 MHz, CD_3OD), δ_C (ppm): 113.3 (C-1), 145.3 (C-2), 145.3 (C-3), 116.0 (C-4), 127.4 (C-4a), 29.3 (C-5), 41.1 (C-6), 165.9 (C-8), 109.3 (C-8a), 148.7 (C-9), 97.5 (C-11), 44.5 (C-12), 27.7 (C-12a), 35.0 (C-13), 56.9 (C-13a), 129.0 (C-13b), 134.0 (C-14), 120.3 (C-15), 99.6 (C-1'), 74.8 (C-2'), 77.9 (C-3'), 71.6 (C-4'), 78.3 (C-5'), 62.7 (C-6').

Compound 3 (Astragalin): Pale yellow solid. ESI-MS: *m/z* 449.25 $[M+H]^+$. ^{1}H-NMR (600 MHz, CD_3OD), δ_H (ppm): 6.23 (1H, d, *J* = 1.8 Hz, H-6), 6.43 (1H, d, *J* = 1.8 Hz, H-8), 8.07 (2H, d, *J* = 9.0 Hz, H-2', H-6'), 6.90 (2H, d, *J* = 9.0 Hz, H-3', H-5'), 5.26 (1H, d, *J* = 7.2 Hz, H1'), 3.20-3.47 (4H, m, H-2"-H-5"), 3.54 (1H, dd, *J* = 12.0, 5.4 Hz, H_a-6"), 3.70 (1H, dd, *J* = 12.0, 2.4 Hz, H_b-6"). ^{13}C-NMR (150 MHz, CD_3OD), δ_C (ppm): 158.6 (C-2), 135.5 (C-3), 179.6 (C-4), 163.1 (C-5), 99.9 (C-6), 166.0 (C-7), 94.8 (C-8), 159.1 (C-9), 105.8 (C-10), 122.8 (C-1'), 132.3 (C-2', C-6'), 116.1 (C-3', C-5'), 161.6 (C-4'), 104.1 (C-1"), 75.8 (C-2"), 78.1 (C-3"), 71.4 (C-4"), 78.4 (C-5"), 62.7 (C-6").

Compound 4 (Betulinic acid): White powder. ESI-MS: *m/z* 455.1 $[M-H]^-$.^{1}H-NMR (600 MHz, $CDCl_3$), δ_H (ppm): 3.00 (1H, m, H-3), 3.00 (1H, m, H-19), 0.94 (3H, s, H-23), 0.76 (3H, s, H-24), 0.83 (3H, s, H-25), 0.97 (3H, s, H-26), 0.98 (3H, s, H-27), 4.74 (1H, d, *J* = 1.8 Hz, H_a-29), 4.61 (1H, d, *J* = 1.8 Hz, H_b-29), 1.69 (3H, s, H-30). ^{13}C-NMR (150 MHz, $CDCl_3$), δ_C (ppm): 38.8 (C-1), 27.4 (C-2), 79.0 (C-3), 38.9 (C-4), 55.4 (C-5), 18.3 (C-6), 34.4 (C-7), 40.7 (C-8), 50.6 (C-9), 37.2 (C-10), 20.9 (C-11), 25.6 (C-12), 38.4 (C-13), 42.5 (C-14), 30.6 (C-15), 32.2 (C-16), 56.3 (C-17), 46.9 (C-18), 49.3 (C-19), 150.4 (C-20), 29.7 (C-21), 37.0 (C-22), 28.0 (C-23), 15.4 (C-24), 16.1 (C-25), 16.1 (C-26), 14.7 (C-27), 179.6 (C-28), 109.7 (C-29), 19.4 (C-30).

Compound 5 (22-Dehydroclerosterol 3-*O*-*β*-D-glucopyranoside): White amorphous solid.

ESI-MS: *m/z* 573.65 $[M+H]^+$.^{1}H-NMR (600 MHz, DMSO-d_6), δ_H (ppm): 3.65 (1H, m, H-3), 5.32 (1H, br t, J = 1.8 Hz, H-6), 0.67 (3H, s, H-18), 0.96 (1H, s, H-19), 0.98 (3H, d, J = 6.6 Hz, H-21), 5.23 (1H, m, H-22), 5.17 (1H, m, H-23), 1.60 (3H, s, H-29), 4.82 (1H, d, J = 3.6 Hz, H_a-28), 4.83 (1H, d, J = 3.6 Hz, H_b-28), 0.78 (3H, t, J = 7.3 Hz, H-26), 4.21 (1H, d, J = 7.8 Hz, H-1′), 2.90 (1H, m, H-2′), 3.04 (1H, m, H-3′), 3.08 (1H, m, H-4′), 3.14 (1H, m, H-5′), 3.41 (1H, m, H_a-6′), 3.47 (1H, m, H_b-6′). ^{13}C-NMR (150 MHz, DMSO-d_6), δ_C (ppm): 36.8 (C-1), 31.3 (C-2), 76.9 (C-3), 38.3 (C-4), 140.4 (C-5), 121.1 (C-6), 29.2 (C-7), 31.4 (C-8), 49.6 (C-9), 36.2 (C-10), 20.6 (C-11), 38.3 (C-12), 41.7 (C-13), 56.2 (C-14), 23.8 (C-15), 28.2 (C-16), 55.3 (C-17), 11.8 (C-18), 18.8 (C-19), 39.9 (C-20), 19.0 (C-21), 136.7 (C-22), 129.5 (C-23), 51.2 (C-24), 147.6 (C-25), 109.9 (C-28), 20.5 (C-27), 25.2 (C-26), 11.9 (C-29), 100.8 (C-1′), 73.4 (C-2′), 76.7 (C-3′), 70.1 (C-4′), 76.7 (C-5′), 61.1 (C-6′).

3. Results and Discussion

Compound **1** was isolated as a brown amorphous solid. The molecular ion peak $[M+H]^+$ was observed at *m/z* 506.05 in the ESI-MS spectrum, consistent with the molecular formula $C_{25}H_{31}NO_{10}$. In the downfield region of the ^{1}H-NMR spectrum of compound **1**, signals corresponding to two aromatic protons were observed at δ_H 6.72 (2H, s, H-1 and H-4). In addition, the ^{1}H-NMR spectrum displayed a signal for an olefinic proton at δ_H 7.43 (1H, d, J = 2.4 Hz, H-9), indicating the presence of a trisubstituted double bond (>C=CH–), together with a doublet signal of an acetal proton at δ_H 5.51 (1H, d, J = 1.8 Hz, H-11). Furthermore, signals attributable to a vinyl group were observed at δ_H 5.55 (1H, m, H-14), 5.20 (1H, dd, J = 10.2, 1.8 Hz, H_a-15), and 5.32 (1H, dd, J = 17.4, 1.8 Hz, H_b-15).

In the upfield region, three methine protons were observed at δ_H 4.75 (1H, overlap, H-13a), 3.22 (1H, overlap, H-12a), and 2.73 (1H, m, H-12). Signals corresponding to three methylene groups were detected at δ_H 2.71 (1H, m, H_a-5), 2.79 (1H, m, H_b-5), 2.91 (1H, m, H_a-6), 4.75 (1H, m, H_b-6), 1.37 (1H, m, H_b-13), and 2.33 (1H, m, H_a-13). In addition, a singlet corresponding to a methoxy group was observed at δ_H 3.85 (3H, s, OCH_3), together with characteristic signals of a glucose moiety at δ_H 4.70 (1H, d, J = 7.8 Hz, H-1′), 3.21 (1H, dd, J = 9.0, 7.8 Hz, H-2′), 3.39 (1H, t, J = 9.0 Hz, H-3′), 3.29 (1H, m, H-4′), 3.32 (1H, m, H-5′), 3.71 (1H, dd, J = 12.0, 6.0 Hz, H_a-6′), and 3.93 (1H, dd, J = 12.0, 2.4 Hz, H_b-6′). The anomeric proton signal at δ_H 4.70 with a coupling constant of J = 7.8 Hz is characteristic of the β-configuration of a glucopyranosyl unit.

The ^{13}C-NMR spectrum of compound **1** revealed the presence of 25 carbon signals. Among them, six signals at δ_C 99.6 (C-1′), 74.8 (C-2′), 77.9 (C-3′), 71.6 (C-4′), 78.3 (C-5′), and 62.7 (C-6′) were attributed to a β- glucopyranosyl unit. The aromatic ring carbons appeared at δ_C 113.3 (C-1), 146.4 (C-2), 147.9 (C-3), 112.6 (C-4), 127.3 (C-4a), and 130.2 (C-13c), together with a carbonyl signal at δ_C 165.9 (C-8). Other signals observed at δ_C 109.3 (C-8a), 148.7 (C-9), and 97.5 (C-11) indicated the presence of a double bond at C-8a/C-9 and a carbon atom (C-11) bonded to two oxygen atoms. In addition, a methoxy signal appeared at δ_C 56.4 (3-OCH_3). The proton and carbon chemical shifts were assigned based on HSQC and HMBC spectra.

The HMBC correlation between the anomeric proton δ_H 4.70 (H-1′) and C-11 (δ_C 97.5) indicated that the sugar moiety was attached to C-11. The position of the methoxy group was determined from the HMBC correlation between the proton signal at δ_H 3.85 (3H, s, 3-OCH_3) and the carbon signal at δ_C 147.9 (C-3).

Based on these spectroscopic data and comparison with literature data [13], compound **1** was identified as alangiside, a characteristic

monoterpenoid alkaloid glycoside of the genus *Alangium*. This compound was isolated from the leaves of *A. salviifolium*. Preliminary studies have shown that alangiside exhibits weak cytotoxic activity against several human cancer cell lines in *in vitro* conditions, including breast cancer MCF-7, prostate cancer PC-3, colon cancer HT-29, renal cancer 786-O, and leukemia HL-60 cell lines [14].

Compound **2** was obtained as a yellow oil. The ESI-MS spectrum exhibited a $[M+H]^+$ ion at *m/z* 492.20, consistent with the molecular formula $C_{24}H_{29}NO_{10}$. The analysis of the NMR spectroscopic data of compound **2** revealed a high degree of similarity to those of compound **1**, except for the absence of the methoxy group (δ_H 3.85, δ_C 56.4) at C-3 in the structure of compound **2**. This observation was supported by the upfield shift of C-3 from δ_C 147.9 in compound **1** to δ_C 145.3 in compound **2**. The remaining proton signals were largely consistent with those of compound **1**, including the olefinic proton at δ_H 7.40 (1H, d, J = 2.4 Hz, H-9), the anomeric proton of the glucose moiety at δ_H 4.69 (1H, d, J = 7.8 Hz, H-1'), 3.20 (1H, dd, J = 9.0, 7.8 Hz, H-2'), 3.39 (1H, t, J = 9.0 Hz, H-3'), 3.21 (1H, m, H-4'), 3.32 (1H, m, H-5'), 3.68 (1H, dd, J = 12.0, 5.4 Hz, H_a-6'), 3.90 (1H, dd, J = 12.0, 1.8 Hz, H_b-6'), and other characteristic proton signals appearing in the range of δ_H 5.49 (1H, d, J = 1.8 Hz, H-11), 2.60 (1H, ddd, J = 12.0, 6.6, 3.0 Hz, H-12), 3.19 (1H, m, H-12a), 1.36 (1H, t, J = 11.4 Hz, H_a-13), 2.29 (1H, td, J = 13.2, 3.6 Hz, H_b-13), 4.69 (1H, dd, J = 10.2, 3.6 Hz, H-13a), 5.52 (1H, dt, J =17.4, 10.2 Hz, H-14), 5.20 (1H, dd, J = 10.2, 1.8 Hz, H_a-15), and 5.29 (1H, dd, J = 16.8, 1.8 Hz, H_b-15).

Based on the analysis of the obtained ^{1}H-NMR and ^{13}C-NMR spectroscopic data in comparison with those of compound **1** and literature data [13], compound **2** was identified as demethylalangiside. This compound has been found in *A. salviifolium* [15]. Compound **3** was obtained as a pale yellow solid. The ESI-MS spectrum showed a molecular ion peak at *m/z* 449.25 $[M+H]^+$, consistent with the molecular formula $C_{21}H_{20}O_{11}$. The 1D-NMR spectra exhibited characteristic signals of a flavonoid glucoside. The ^{1}H-NMR spectrum of compound **3** displayed six aromatic proton signals, among which four signals formed an AA′BB′ coupling system at δ_H 8.07 (2H, d, J = 9.0 Hz, H-2′, H-6′) and 6.90 (2H, d, J = 9.0 Hz, H-3′, H-5′), corresponding to the protons of ring B. Two *meta*-coupled proton signals at δ_H 6.23 (1H, d, J = 1.8 Hz, H-6) and 6.43 (1H, d, J = 1.8 Hz, H-8) were assigned to ring A, which are characteristic of the kaempferol skeleton. In addition, in the upfield region of the ^{1}H-NMR spectrum, signals corresponding to the sugar moiety were observed, including an anomeric proton signal at δ_H 5.26 (1H, d, J = 7.2 Hz, H-1″), together with signals of hydroxymethine and hydroxymethylene protons in the range of δ_H 3.30–3.47 (m, H-2″–H-5″), and at δ_H 3.54 (1H, dd, J = 12.0, 5.4 Hz, H_a-6″) and 3.70 (1H, dd, J = 12.0, 2.4 Hz, H_b-6″), suggesting the presence of a *β*-glucopyranosyl unit.

The ^{13}C-NMR spectrum of compound **3** revealed 21 carbon signals, including 15 carbons attributable to the flavonol backbone, with a characteristic carbonyl carbon at δ_C 179.6 (C-4) and multiple aromatic carbons. The corresponding ^{13}C-NMR signals at δ_C 104.1 (C-1″), 75.8 (C-2″), 78.1 (C-3″), 71.4 (C-4″), 78.4 (C-5″), and 62.7 (C-6″) were consistent with a hexose, identified as a *β*-glucopyranosyl unit based on the large coupling constant of the anomeric proton.

The HMBC cross-peak between H-1″ (δ_H 5.26) and C-3 (δ_C135.5) confirmed that the *β*-glucopyranosyl unit was attached to the aglycone through an *O*-glycosidic linkage at C-3.

Based on the analysis of the obtained ^{1}H-NMR and ^{13}C-NMR spectroscopic data, together with comparison with literature data [16], compound **3** was identified as kaempferol 3-*O*-*β*-glucopyranoside (astragalin). This compound, isolated from the leaves of *A. salviifolium*, has been reported to possess anti-inflammatory, antioxidant, and anti-atopic dermatitis activities [17].

Compound **4** was isolated as a white powder, and its molecular formula was $C_{30}H_{48}O_3$ based on ESI-MS data (*m/z* 455.1 $[M–H]^-$). The NMR data indicated that compound **4** was a triterpenoid. The ^{1}H-NMR spectrum showed six singlet methyl groups in the upfield region, a characteristic feature of a lupane-type skeleton, at δ_H 0.94 (3H, s, H-23), 0.76 (3H, s, H-24), 0.83 (3H, s, H-25), 0.97 (3H, s, H-26), 0.98 (3H, s, H-27), and 1.69 (3H, s, H-30) and one oxymethine proton at δ_H 3.00 (1H, m, H-3). In addition, the presence of two doublets at δ_H 4.74 (1H, d, J = 1.8 Hz, H_a-29) and 4.61 (1H, d, J = 1.8 Hz, H_b-29) indicated an *exo*-methylene group. The ^{13}C-NMR spectrum displayed 30 carbon signals, including six methyl carbons [δ_C 28.0 (C-23), 15.4 (C-24), 16.1 (C-25 and C-26), 14.7 (C-27), and 19.4 (C-30)], eleven methylene carbons [δ_C 38.8 (C-1), 27.4 (C-2), 18.3 (C-6), 34.4 (C-7), 20.9 (C-11), 25.6 (C-12), 30.6 (C-15), 32.2 (C-16), 29.7 (C-21), 37.0 (C-22), and 109.7 (C-29)], six methine carbons [δ_C 79.0 (C-3), 55.4 (C-5), 50.6 (C-9), 38.4 (C-13), 46.9 (C-18), and 49.3 (C-19)], one carboxyl carbon [δ_C 179.6 (C-28)], and six quaternary carbons [δ_C 38.9 (C-4), 40.7 (C-8), 37.2 (C-10), 42.5 (C-14), 56.3 (C-17), and 150.4 (C-20)]. Based on the above spectroscopic evidence and comparison with literature data [18], compound **4** was identified as betulinic acid. Betulinic acid is a triterpenoid widely distributed in many plant species; the compound was isolated from the seeds of *A. lamarckii* Thwaites (syn. *A. salviifolium*) [19].

Compound **5** was obtained as a white amorphous solid with a molecular formula of $C_{35}H_{56}O_6$, as indicated by ESI-MS spectral data (*m/z* 573.65 $[M+H]^+$). The 1D-NMR spectra of **5** showed characteristic signals of a sterol glycoside. The ^{1}H-NMR spectrum displayed characteristic signals of a steroidal skeleton, including two tertiary methyl groups at δ_H 0.67 (3H, s, H-18) and 0.96 (3H, s, H-19), one secondary methyl group at δ_H 0.98 (3H, m, J = 6.6 Hz, H-21), and one primary methyl group at δ_H 0.78 (3H, t, J = 7.3 Hz, H-26). An oxymethine proton signal at δ_H 3.65 (1H, m, H-3) indicated a hydroxylated methine group. An olefinic proton at δ_H 5.32 (1H, br t, J = 1.8 Hz, H-6) suggested the presence of a double bond in the steroid nucleus. Additionally, two *trans*-olefinic protons at δ_H 5.23 (1H, m, H-22) and 5.17 (1H, m, H-23) indicated a *trans*-double bond in the side chain. Signals attributable to an isopropenyl fragment were also observed at δ_H 1.60 (3H, s, H-29) and 4.82 (1H, d, J = 3.6 Hz, H_a-28), 4.83 (1H, d, J = 3.6 Hz, H_b-28), supporting a modified sterol side chain. Importantly, the presence of an anomeric proton signal at δ_H 4.21 (1H, d, J = 7.8 Hz, H-1′) indicated a sugar moiety. The ^{13}C-NMR data showed six additional carbon signals attributable to a hexose unit. The chemical shifts of these carbons were consistent with a glucopyranosyl unit at δ_C 100.8 (C-1′), 73.5 (C-2′), 76.7 (C-3′), 70.1 (C-4′), 76.8 (C-5′), and 61.1 (C-6′); the large coupling constant (J = 7.8 Hz) confirmed a *β*-glycosidic configuration. Furthermore, the downfield chemical shift of C-3 (δ_C 76.9) indicated glycosylation at this position, confirming that the glucose moiety was attached to C-3 of the sterol nucleus. Based on the above spectroscopic evidence and comparison with reported data [20], compound **5** was identified as 22-dehydroclerosterol 3-*O*-*β*-D-glucopyranoside. This compound was reported from *A. salviifolium* for the first time.

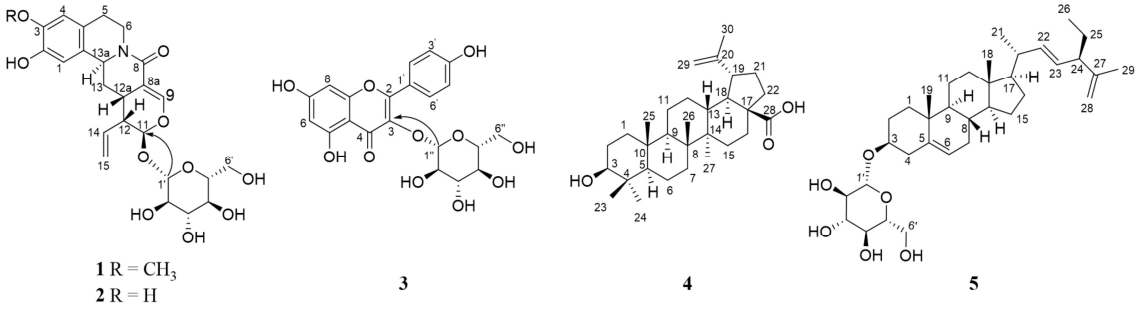


Fig 1. Chemical structures and key HMBC correlations (→) of compounds **1-5**

4. Conclusions

By using various chromatographic techniques, five compounds were isolated from the *n*-butanol and dichloromethane fractions of the branches and leaves of *A. salviifolium*. The isolated substances were identified, including two alkaloid glycosides: alangiside (**1**) and demethylalangiside (**2**); one flavonoid: astragalin (**3**); one triterpenoid: betulinic acid (**4**); and one sterol glycoside: 22-dehydroclerosterol 3-*O*-*β*-D-glucopyranoside (**5**). Notably, compound **5** was isolated and identified from *A. salviifolium* for the first time. The structures of these compounds were confirmed through analysis of their spectral data in comparison with previously published data.

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