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CHEMICAL CONSTITUENTS OF THE AERIAL PARTS OF *ADENOSMA CAERULEUM* R. Br.

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Summary

Chemical Constituents of the Aerial Parts of *Adenosma caeruleum* R. Br.

Seven compounds including maltol (**1**), hydroquinone (**2**), apigenin (**3**), nevadensin (**4**), betulinic acid (**5**), β-sitosterol (**6**), and daucosterol (**7**), were isolated from the 80% EtOH extract of the aerial parts of *Adenosma caeruleum*. Their structures were identified using nuclear magnetic resonance spectroscopy (NMR) data, mass spectrometry (MS), and comparison with those of reported literatures. Of these, compounds **1**, **2**, **4**, and **7** were reported for the first time from arrial parts of *A. caeruleum*.

Keywords: *Adenosma caeruleum, Pyrone, Quinol, Flavones, Triterpenoid, Sterols.*

1. Introduction

The *Adenosma* genus comprises 26 reported species, primarily found in the subtropical and tropical regions of Asia, the Pacific Islands, and Oceania [1]. Among these species, seven species are utilized in folk healthcare systems as herbal medicines, supplements, and tonics [1]. One of the notable species is *Adenosma caeruleum* R. Br. (synonym: *Adenosma glutinosum* (L.) Druce), a perennial herb widely distributed across India, China, Laos, Cambodia, Thailand, Malaysia, and Indonesia. In Vietnam, it is commonly known as "Nhan tran" and is found in northern mountainous provinces. Traditionally *A. caeruleum* has been used for blood tonic, appetite stimulatation, postpartum rehabilitation, and jaundiced hepatitis [2]. The aerial parts of *A. caeruleum* have been reported to comprise a range of chemical compounds such as flavonoids, phenylpropanoids, phenols, terpenoids, and steroids [1],[3],[4] and exhibited various pharmacological properties including anti-cancer, anti-inflammatory, anti-bacterial, and antioxidant [5],[6],[7],[8]. In our ongoing investigations on natural products from Vietnamese medicinal plants, in the present study, we isolated and elucidated seven compounds from the aerial parts of *A. caeruleum*.

2. Materials and methods

2.1. Plant material

Samples of *Adenosma caeruleum* (Scrophulariaceae) were collected in Ngoc Hoi, Thanh Tri, Hanoi, Vietnam, in September 2022. The plant was identified by Assoc. Prof. Pham Thanh Huyen and Ms. Phan Van Truong (National Institute of Medicinal Materials, NIMM). A voucher specimen (DL-090922) was deposited in the Center of Medicinal Material Resources, NIMM (Hanoi, Vietnam).

2.2. General experimental procedures

NMR spectra were recorded on Bruker Avance Digital 600 MHz NMR spectrometers (Karlsruhe, Germany) using TMS as an internal standard, *J* in Hz, at 294 K. Electrospray ionization mass spectra (ESI-MS) were recorded from an LCMS-8045 Shimadzu system. *Silica gel* (Merck, 63-200 mm particle size) was used for column chromatography (CC). Thin-layer chromatography (TLC) was carried out with *silica gel* $60F_{254}$ and RP-$18F_{254}$ plates. Spots were detected under UV light or by spraying with 10% sulfuric acid in 96% ethanol (EtOH) and heating.

2.3. Extraction and isolation

The dried aerial parts of *A. caeruleum* (15.0 kg) were ground and extracted with 80% EtOH at room temperature (3 times, 3 days each time). The extracts were filtered, combined, and evaporated under reduced pressure, yielding a green residue (1950.0 g), which was suspended in water and successively partitioned with *n*-hexane and ethyl acetate (EtOAc). The partitioned extracts were combined and evaporated under reduced pressure to obtain the corresponding fractions: *n*-hexane extract (46.0 g), EtOAc extract (134.2 g), and water layer (1420.0 g).

The EtOAc extract (100.0 g) was subjected to *silica gel* column chromatography (CC) using a gradient mixture of *n*-hexane - acetone (30:1 -

1:1, *v/v*) as eluents, resulting in nineteen fractions (E1 - E19). Compound **6** (48.2 mg) was isolated from fractions E6 and E7 by filtration of the crystalline substances. Compound **5** (1619.0 mg) was obtained by filtering and washing the crystalline substances of fractions E10 to E13 with dichloromethane. Fraction E15 (10.0 g) was subjected to *silica gel* CC with an isocratic mixture of dichloromethane - methanol (50:1 - 15:1, *v/v*), yielding nineteen subfractions (E15A - E15T). Compound **4** (33.0 mg) was isolated from the crystalline substances of fraction E15F (1.2 g) using dichloromethane. Compound **1** (21.0 mg) was obtained by combining the crystalline substances of fractions E15H and E15G. Compound **2** (1269.0 mg) was isolated from the crystalline substances of fraction E15P (2.1 g) using dichloromethane. Similarly, fraction E17 (13.0 g) was fractionated by *silica gel* CC using an isocratic mixture of dichloromethane - methanol (50:1 - 15:1, *v/v*), leading to sixteen subfractions (E17A - E17Q). Compound **3** (31.0 mg) was isolated from the crystalline substances of fraction E17K (0.9 g) using dichloromethane, and compound **7** (46.3 mg) was isolated from the crystalline substances of fractions E17N and E17O using methanol.

Compound **1** (maltol): White crystalline; ^{1}H-NMR (600 MHz, CD_3OD) δ_H: 2.36 (3H, s, 2-CH_3), 6.40 (1H, d, J = 6.0 Hz, H-5), 7.95 (1H, d, J = 6.0 Hz, H-6); ^{13}C-NMR (150 MHz, CD_3OD) δ_C: 14.2 (2-CH_3), 152.2 (C-2), 144.6 (C-3), 175.2 (C-4), 114.4 (C-5), 156.3 (C-6). ESI-MS: *m/z* 127.35 $[M+H]^+$.

Compound **2** (hydroquinone): Pale yellow crystalline; ^{1}H-NMR (600 MHz, CD_3OD) δ_H: 6.64 (4H, s, H-2, H-3, H-5, and H-6); ^{13}C-NMR (150 MHz, CD_3OD) δ_C: 151.2 (C-1 and C-4), 116.8 (C-2, C-3, C-5, and C-6).

Compound **3** (apigenin): Pale yellow powder. ^{1}H-NMR (600 MHz, DMSO) δ_H: 6.77 (1H, s, H-3), 6.19 (1H, d, J = 1.8 Hz, H-6), 6.48 (1H, d, J = 1.8 Hz, H-8), 7.92 (2H, d, J = 9.0 Hz, H-2' and H-6'), 6.93 (2H, d, J = 9.0 Hz, H-3' and H-5'), 12.95 (1H, s, 5-OH); ^{13}C-NMR (150 MHz, DMSO) δ_C: 163.7 (C-2), 102.8 (C-3), 181.7 (C-4), 161.4 (C-5), 98.8 (C-6), 164.1 (C-7), 93.9 (C-8), 157.3 (C-9), 103.7 (C-10), 121.2 (C-1'), 128.4 (C-2' and C-6'), 115.9 (C-3' and C-5'), 161.1 (C-4').

Compound **4** (nevadensin): Yellow powder. ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H: 6.58 (1H, s, H-3), 7.88 (2H, d, J = 9.0 Hz, H-2' and H-6'), 7.03 (2H, d, J = 9.0 Hz, H-3' and H-5'), 4.04 (3H, s, 6-OCH_3), 4.02 (3H, s, 8-OCH_3), 3.90 (3H, s, 4'-OCH_3), 12.77 (1H, s, 5-OH); ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C: 163.8 (C-2), 103.8 (C-3), 183.0 (C-4), 148.4 (C-5), 130.8 (C-6), 148.8 (C-7), 127.4 (C-8), 145.8 (C-9), 104.2 (C-10), 123.6 (C-1'), 128.1 (C-2' and C-6'), 114.7 (C-3' and C-5'), 162.7 (C-4'), 61.0 (6-OCH_3), 61.8 (8-OCH_3), 55.6 (4'-OCH_3). ESI-MS: *m/z* 343.30 $[M-H]^-$.

Compound **5** (betulinic acid): White powder; ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H: 3.18 (1H, dd, J = 5.0, 11.0 Hz, H-3), 3.00 (1H, m, H-19), 0.97 (3H, s, H-23), 0.76 (3H, s, H-24), 0.83 (3H, s, H-25), 0.94 (3H, s, H-26), 0.98 (3H, s, H-27), 4.61 (1H, s, H-28), 4.74 (1H, s, H-29), 1.69 (3H, s, H-30); ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C: 38.7 (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), 25.5 (C-12), 38.4 (C-13), 42.5 (C-14), 30.6 (C-15), 32.2 (C-16), 56.3 (C-17), 49.3 (C-18), 46.9 (C-19), 150.4 (C-20), 29.7 (C-21), 37.0 (C-22), 28.0 (C-23), 15.4 (C-24), 16.1 (C-25), 16.0 (C-26), 14.7 (C-27), 179.5 (C-28), 109.7 (C-29), 19.4 (C-30); ESI-MS: *m/z* 455.60 $[M-H]^-$.

Compound **6** (*β*-sitosterol): White powder.

Compound **7** (daucosterol): White powder.

3. Results and discussion

3.1. Extraction and isolation of compounds from the aerial parts of A. caeruleum

Seven compounds were isolated from the 80% ethanol extract of the aerial parts of *A. caeruleum* (Fig. 1). The structures of 7 compounds were elucidated by ESI-MS and NMR spectra and compared with standard compounds using TLC.

Compound **1** was obtained as a white crystalline. The molecular formula of **1** was suggested as $C_6H_6O_3$ based on a *pseudo*-molecular peak at *m/z* 127.35 $[M+H]^+$. The ^{1}H-NMR spectrum showed a pair of olefinic proton signals at δ_H 6.40 (1H, d, J = 6.0 Hz, H-5) and 7.95 (1H, d, J = 6.0 Hz, H-6), a methyl group at 2.36 (3H, s, 2-CH_3). The ^{13}C-NMR spectrum exhibited 6 carbon signals including four olefinic carbons [δ_C 152.2 (C-2), 144.6 (C-3), 114.4 (C-5), and 156.3 (C-6)], a carbonyl group [δ_C 175.2 (C-4)], and a methyl group [δ_C 14.2 (2-CH_3)]. Based on the data above and compared to the reference, the structure of **1** was elucidated as 3-hydroxyl-2-methylpyrone or maltol [9].

Compound **2** was isolated in the form of pale-yellow crystalline. The ^{1}H- and ^{13}C-NMR spectra displayed signals characteristic of an aromatic ring with two hydroxyl substituents at positions

C-1 and C-4 [δ_H 6.64 (4H, s, H-2, H-3, H-5, and H-6); δ_C 151.2 (C-1 and C-4), 116.8 (C-2, C-3, C-5, and C-6)]. Thus, the structure of **2** was identified as hydroquinone or quinol [10].

Compound **3** was obtained as a pale-yellow powder. The ^{1}H-NMR spectrum of the **3** showed four proton signals of AA'BB' system at δ_H 7.92 (2H, d, J = 9.0 Hz, H-2' and H-6') and 6.93 (2H, d, J = 9.0 Hz, H-3' and H-5'), two aromatic proton signals at δ_H 6.19 (1H, d, J = 1.8 Hz, H-6) and 6.48 (1H, d, J = 1.8 Hz, H-8) in the *meta* position, and a olefinic proton at δ_H 6.77 (1H, s, H-3). The ^{13}C-NMR spectrum exhibited 15 carbon signals including a carbonyl group (δ_C 181.7, C-4), 7 methine carbons [δ_C 102.8 (C-3), 98.8 (C-6), 93.9 (C-8), 128.4 (C-2' and C-6'), and 115.9 (C-3' and C-5')], and 7 non-protonated carbons [including 5 oxygenated carbons at δ_C 163.7 (C-2), 161.4 (C-5), 164.1 (C-7), 157.3 (C-9), and 161.1 (C-4') and 2 quaternary carbons at 103.7 (C-10) and 121.2 (C-1')]. Comparing NMR data of published reference [11], compound **3** was determined as apigenin.

Compound **4** was isolated as a yellow powder. The molecular formula of **4** was suggested as $C_{18}H_{16}O_7$ based on a *pseudo*-molecular ion peak at *m/z* 343.30 $[M-H]^-$ and NMR spectra. The ^{1}H-NMR spectrum showed two pairs of proton signals of an AA'BB' system [δ_H 7.88 (2H, d, J = 9.0 Hz, H-2' and H-6') and 7.03 (2H, d, J = 9.0 Hz, H-3' and H-5')], an olefinic proton at δ_H 6.58 (1H, s, H-3), and three methoxy groups [δ_H 4.04 (3H, s, 6-OCH_3), 4.02 (3H, s, 8-OCH_3), and 3.90 (3H, s, 4'-OCH_3)]. The ^{13}C-NMR spectrum showed signals of 18 carbons, in which the carbon signal at δ_C 183.0 (C-4) was specific for the carboxyl group, two olefinic carbons [δ_C 163.8 (C-2) and 103.8 (C-3)], three methoxy carbons [δ_C 61.0 (6-OCH_3), 61.8 (8-OCH_3), and 55.6 (4'-OCH_3)], and 12 aromatic carbons. These spectra suggested that **4** is very similar to **3**, except for the additional three methoxy groups and the absence of two aromatic protons. The positions of the methoxy groups were determined through HMBC correlations. Specifically, the HMBC correlations between the protons at δ_H 12.77 (5-OH) and 4.04 (OCH_3) and the carbon at δ_C 130.8 (C-6) confirmed the location of this methoxy group at C-6. The position of the second methoxy group was identified at C-4' through HMBC correlations between the protons at δ_H 7.88 (H-2' and H-6'), 7.03 (H-3' and H-5'), and 3.90 (OCH_3) with the carbon at δ_C 162.7 (C-4'). The remaining methoxy group was determined to be at C-8 through the HMBC correlation between the proton at δ_H 4.02 (OCH_3) and the carbon at δ_C 127.4 (C-8) (Fig. 2). According to this evidence and published literature [11], compound **4** was identified as nevadensin.

Compound **5** was obtained as a white powder. Its molecular formula was suggested as $C_{30}H_{48}O_3$ based on a *pseudo*-molecular ion peak at *m/z* 455.60 $[M-H]^-$ and NMR spectra. The ^{1}H-NMR spectrum of **5** showed the signals of lupane-type triterpenoid skeleton with signals of a hydroxymethin proton [δ_H 3.18 (1H, dd, J = 5.0, 11.0 Hz, H-3)], two olefinic protons [δ_H 4.61 (1H, s, H-28), 4.74 (1H, s, H-29)], six methyl protons [δ_H 0.97 (3H, s, H-23), 0.76 (3H, s, H-24), 0.83 (3H, s, H-25), 0.94 (3H, s, H-26), 0.98 (3H, s, H-27), 4.61 (1H, s, H-29), 4.74 (1H, s, H-29), and 1.69 (3H, s, H-30)]. The ^{13}C-NMR and DEPT spectra exhibited 30 carbon signals including an oxygenated methine [δ_C 79.0 (C-3)], a carboxyl group [δ_C 179.5 (C-28)], two olefinic carbons [δ_C 150.4 (C-20) and 109.7 (C-29)], 10 methylene groups, 5 methine groups, and 5 quaternary carbons. Based on the above spectral data and compared with those of references [1], the structure of compound **5** was identified as betulinic acid.

Compound **6**, obtained as a white powder, was subjected to TLC and compared with β-sitosterol. The TLC analysis revealed that both compounds did not absorb UV light at 254 and 365 nm, and exhibited a color reaction after spraying with 10% H_2SO_4 reagent in 96% EtOH and subsequent heating. Furthermore, both **6** and β-sitosterol displayed an identical R_f value of 0.6 when using the solvent system *n*-hexane - acetone (5:1, *v/v*). Based on these results, it was concluded that **6** was β-sitosterol.

Compound **7**, isolated as a white powder, was analyzed using TLC and compared with daucosterol. The TLC analysis indicated that both compounds did not absorb UV light at 254 and 365 nm, but displayed a purple color reaction upon treatment with 10% H_2SO_4 reagent in 96% EtOH and heating. Additionally, both **7** and daucosterol exhibited an R_f value of 0.6 when using the solvent system dichloromethane - methanol (10:1, *v/v*). These findings led to the conclusion that the structure of **7** was daucosterol.

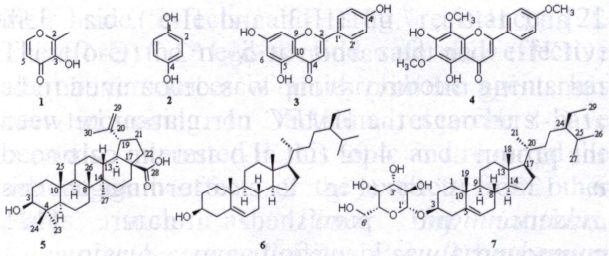

Fig. 1. Chemical structures of compounds (**1-7**) isolated the aerial parts of *A. caeruleum*

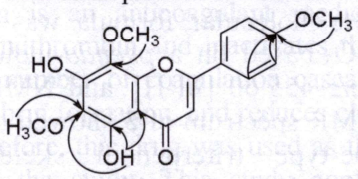

Fig. 2. Key HMBC correlations (→) of compound **4**

Seven compounds, including a pyrone (**1**), a quinol (**2**), two flavones (**3-4**), a lupane-type triterpenoid (**5**), and two sterols (**6-7**) were isolated and structurally identified from the aerial parts of *A. caeruleum*. These findings are in accordance with previous reports on the chemical composition of *A. caeruleum*. Notably, compounds **1**, **2**, **4**, and **7** were reported for the first time in this plant species.

Some of the isolated compounds have been extensively studied for their diverse biological activities. For instance, betulinic acid showed anti-inflammatory, antibacterial, antiviral, antidiabetic, antimalarial, anti-HIV, and antitumor properties [12]. Apigenin exhibits cytostatic and cytotoxic effects on various cancer cells, and it also protects atherogenesis, hypertension, cardiac hypertrophy, ischemia/reperfusion-induced heart injury, and autoimmune myocarditis. Moreover, apigenin protects against chemicals- and ischemia/reperfusion-induced liver injury, inhibits asthma, bleomycin-induced pulmonary fibrosis, abnormal behavior, and oxygen and glucose deprivation/reperfusion-induced neural cell apoptosis. It also improves conditions like pancreatitis, type 2 diabetes and its complications, osteoporosis, and collagen-induced arthritis [13]. Nevadensin, on the other hand, showed a wide range of significant biological activities, including hypotensive, anti-tubercular, antimicrobial, anti-inflammatory, antitumor, and anti-cancer effects [14]. Additionally, sterols have also been found to exhibit various biological activities [15],[16].

4. Conclusion

In this study, seven compounds, maltol (**1**), hydroquinone (**2**), apigenin (**3**), nevadensin (**4**), betulinic acid (**5**), β-sitosterol (**6**), and daucosterol (**7**), were isolated from the 80% EtOH extract of *A. caeruleum*. Among them, compounds **1**, **2**, **4**, and **7** were first reported in this plant.

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