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In vitro study on α-amylase inhibitory and α-glucosidase of a new stigmastane-type steroid saponin from the leaves of *Vernonia amygdalina*

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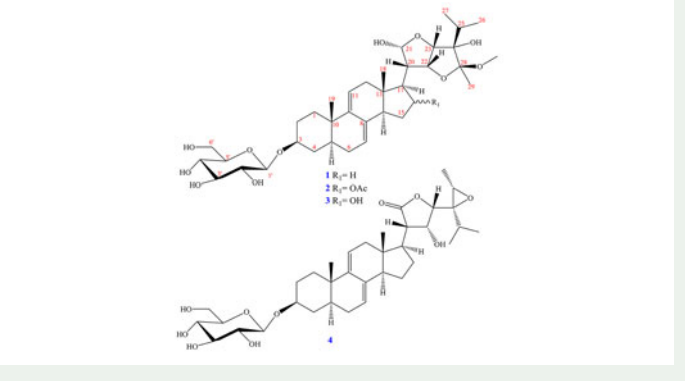
ABSTRACT

Using various chromatographic separations, four compounds, including one new steroid saponin named vernoamyoside E (**1**), were isolated from the leaves of the Vietnamese medicinal plant *Vernonia amygdalina* Delile (Asteraceae). Their structures were established by spectroscopic methods such as 1D- and 2D-NMR, HR-ESI-MS, and HPLC analysis. The inhibitory activities against α-glucosidase and α-amylase of the isolated compounds from *V. amygdalina* were reported for the first time. The results indicated that compound **1** significantly inhibited both against α-amylase and α-glucosidase activity.



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1. Introduction

Vernonia amygdalina Del. is a shrub or small tree that is mainly grown in tropical areas of Africa (Atangwho et al. 2012). In Africa, it can be used as a traditional treatment for diabetes, nausea, dermatitis, arthritis, ascariasis, stomached, anaemia, jaundice, pneumonia, fever, tonsillitis, schistosomiasis, malaria, amoeba, and intestinal upsets (Farombi and Owoeye 2011). The principal chemical components have shown that *V. amygdalina* contains steroids, polyphenolics, alkaloids, anthraquinones, lignans, flavonoids, and coumarins (Liu et al. 2019). Biological activities of extracts and isolated compounds from *V. amygdalina* have been reported, such as antibacterial, antifungal, antiparasite, antiviral, anticancer, anti-obesity, antioxidant, antihypertensive, and liver protective activity (Atangwho et al. 2012; Atangwho et al. 2013; Igile et al. 1994; Kupchan et al. 1969; Taiwo et al. 2009; Yeap et al. 2010). In continuation to study secondary metabolites and evaluation anti-diabetic compounds of Vietnamese medicinal plants, herein, we reported the isolation and structural elucidation of four steroid saponins including one new compound (**1**) from the leaves of *V. amygdalina*.

2. Results and discussion

In the present study, the methanol (MeOH) extract from the leaves of *V. amygdalina* were evaluated to against α-amylase and α-glucosidase activity. In this regard, the MeOH extract significantly inhibited both against α-amylase and α-glucosidase activity with IC_{50} 0.64 ± 0.12 mg/mL and 1.78 ± 0.31 mg/mL, respectively. Anti-diabetic guide fraction and purification led to isolation of four compounds including one new compound (**1**), and three known compounds (**2**–**4**) (Figure 1). The known compounds were identified vernoniacums B (**2**) (Ma et al. 2016), vernonioside B_1 (**3**) (Jisaka et al. 1992), and vernonioside B_2 (**4**) (Jisaka et al. 1993) by comparing their spectroscopic data ($[\alpha]_D^{20}$.1D, 2D NMR, MS) with reported values.

Vernoamyoside E (**1**), $[\alpha]_D^{20}$.+24.7 (*c* 0.15, MeOH), was obtained as a white amorphous powder. The molecular formula ($C_{36}H_{56}O_{11}$) of **1** was determined by HR-ESI-MS with a sodium molecular ion peak at *m/z* 687.3708 $[M + Na]^+$ (calcd for $C_{36}H_{56}NaO_{11}{}^+$, 687.3720). The NMR spectra (Supplementary material) are indicative of a steroid saponin with three *tert*-methyl proton signals at δ_H 0.57, 0.93, and 1.42 (each, 3H, s), two doublet methyl signals at δ_H 0.94 (3H, d, *J* = 7.0 Hz, H-26), 0.95 (3H, d, *J* = 7.0 Hz, H-27), a methoxy signal at δ_H 3.21 (3H, s, 28-OCH_3), and two doublet olefinic protons at δ_H 5.43 (1H, d, *J* = 5.0 Hz, H-7), 5.53 (1H, d, *J* = 5.5 Hz, H-11), as well as one anomeric proton signals at δ_H 4.42 (1H, d, *J* = 8.0 Hz, H-1′). The ^{13}C-NMR spectrum of **1** exhibited the signals of 36, of which 6 carbon signals were assigned to a sugar unit and the remaining 29 carbon signals were assigned to a steroid aglycon (one of the main constituents of *V. amygdalina*) on the basis 1D-2D-NMR. Analysis ^{13}C-NMR signals were indicated an isopropyl unit at δ_C 32.7 (C-25), 17.4 (C-26), 18.1 (C-27), three hydroxylated carbons at δ_C 81.0 (C-22), 91.3 (C-23), 83.2 (C-24), two hemiacetal carbons at δ_C 100.0 (C-21), 112.9 (C-28), a methoxy group at δ_C 48.3 (28-OCH_3), and a methyl group at δ_C 17.4 (C-29) by combined HSQC signals. The aglycon of **1** was identified as $\Delta^{7,9(11)}$ dienstigmastane glycoside previously reported from *V. amygdalina* by clear agreement of its ^{1}H and ^{13}C NMR data (Quasie et al. 2016). Comparison of the ^{1}H, and ^{13}C-NMR data of **1** with those

of vernoniosides B_1 (**3**) demonstrated that two compounds were almost identical in the same NMR solvent. The only difference between these two compounds was the replacement of the hydroxyl group at C-16 (δ_C 28.0 ppm). In addition, the NOESY spectrum revealed significant cross-peaks between H-20 and each proton at C-21, C-22, and C-23. H-22 and each proton at C-21, and C-23, H-23 and one of methyl (H-27) of the isopropyl group, and H-22 and the methoxy group showing that all of the hydrogens at C-20-C-23, and the isopropyl and the methoxy groups are β-orientated. Furthermore, absolute configurations of the sugar moieties were identified as β-D-glucopyranosyl by coupling constants of anomeric position δ_H 4.42 ($J = 8.0$, H-1′), and acid hydrolysis of **1** followed by TLC and HPLC analysis in comparison with the authentic samples (Supplementary data). Attachment of the monosaccharide chain at C-3 of the aglycon was confirmed by HMBC correlation of the anomeric proton H-3 (δ_H 3.71) with C-1′ (δ_C 102.6). Therefore, the structure of **1** was established as (22*R*,23*S*,24*R*,28*S*)-3β-glucosyl-28-methoxy-7,8,9,11-tetradehydro-21,24-dihydroxy-21,23:22,28-diepoxy-5α-stigmastane.

Diabetes mellitus is a chronic disease that affects millions of people worldwide. To date, much effort has been studied to identify effective with α-amylase, and α-glucosidase inhibitors from natural sources by isolation compounds develop functional food for use against diabetes (Fatmawati et al. 2011). Previously, we were investigated that isolated compounds which were exhibited potential treatment of diabetes from *Rosa rugose*, and *Ecklonia cava* (Park et al. 2018; Thao et al. 2014). Bitter leaf (*V. amygdalina*) is a popular vegetable commonly consumed and which has been employed in traditional medicine for the prevention and management of diabetes and hypertension (Saliu et al. 2012). In this study, the inhibitory effects of the MeOH extract, and isolated compounds against α-amylase and α-glucosidase were evaluated. As the obtained results, compound **1** exhibited potent inhibitory activity against α-glucosidase enzyme, more than positive control on both 100 and 500 μg/mL concentrations with values 50.17 ± 1,03 μg/mL and 60,03 ± 3,85 μg/mL, respectively. While, compounds **1**, **2**, and **3** showed significant inhibition against α-amylase enzyme with IC_{50} values of 102.23, 83.17, and 87.09 μg/mL, respectively when compared with that of a positive control, acarbose, 6.3 μg/mL.

All compounds (**1**–**3**) have shown significantly against both α-amylase and α-glucosidase. The most active compound **1**, not only indicated a more potent α-glucosidase inhibitory activity than acarbose (positive control) but also showed significant activity against α-amylase.

Given the potential against both α-amylase and α-glucosidase of *V. amygdalina* extracts, and isolated compounds, compound **1** might be potential candidate for further investigation on the molecular mechanisms of action on specific anti-diabetic targets.

3. Experimental

3.1. General experimental procedures

Optical rotations were determined on a JASCO P-2000 polarimeter (Hachioji, Tokyo, Japan). The high-resolution electrospray ionisation mass spectra (HR-ESI-MS) were obtained from a MicroQ-TOF III mass spectrometer (Bruker Daltonics, 255748

Germany). IR spectra were obtained on a Bruker TENSOR 37 FT-IR spectrometer. The NMR spectra were recorded on a Bruker Advance III HD 500 FT NMR spectrometers with TMS as an internal standard. HPLC was used with Kinetex C_{18} column (250 × 20.0 mm, 5 μm; Phenomenex, Torrance, CA). Column chromatography (CC) was performed on silica gel (Kiesel gel 60, 70–230 mesh and 230–400 mesh, Merck, Darmstadt, Germany), YMC*GEL (ODS-A, 12 nm S-150 μm, YMC Co., Ltd.) and Sephadex LH-20 (Sigma-Aldrich, USA) resins. TLC used pre-coated silica gel 60 F_{254} (1.05554.0001, Merck) and RP-18 F_{254S} plated (1.15685.0001, Merck), and compounds were visualized by spraying aqueous 10% H_2SO_4 and heating for 3–5 min.

3.2. Biological material

The leaves of *V. amygdalina* were collected at Phong Dien, Thua Thien Hue Province, Vietnam in August 2017, and were identified by Dr. Nguyen Quynh Nga (Institute of Medicinal Material, Vietnam). A voucher plant (MISR-2017-14) has been deposited at Mientrung Institute for Scientific Research, VAST, Vietnam.

3.3. Assay for α-glucosidase inhibition

The α-glucosidase enzyme inhibition assay was carry out according to similar the previously method (Luyen et al. 2019) and provide in supplementary data.

3.4. Assay for α-amylase inhibition

The α-amylase enzyme inhibitory activity was performed using the modification (Luyen et al. 2019) (Supplementary data).

3.5. Extraction and isolation

Air-dried leaves of *V. amygdalina* (1.2 kg) were extracted with 100% methanol (5 L × 3 times) under sonication at 50 °C for 4 h to yield a dark solid extract (200 g). This extract was suspended in water and successively partitioned with *n*-hexane, dichloromethane and ethyl acetate to give corresponding *n*-hexane (VAH, 52 g), dichloromethane (VAD, 42 g), ethyl acetate (VAE, 31 g) and water layer (VAW, 75 g). The VAE fraction (31 g) was subsequently chromatographed on a silica gel column eluting with dichloromethane/methanol (gradient from 100/1 – 0/1, v/v) to give five fractions (VAE1-VAE5). The VAE1 (1.2 g) fraction was further separated on a silica gel column eluting with dichloromethane/methanol/water (25/1/0.05, v/v/v) to give two smaller fractions (VAE1.1 and VAE1.2). Compound **1** (5 mg) was yielded from VAE1.1 (0.3 g) fraction by purify on a RP-18 column eluting with methanol/water (4/1, v/v). The VAE1.2 (0.2 g) fraction was further separated on a Sephadex LH-20 column eluting with methanol/water (1/1, v/v) to afford compound **3** (15 mg). The VAE2 (0.9 g) fraction was continuously chromatographed on a RP-18 column eluting with methanol/water (3/1, v/v) to yield compounds **2** (7 mg) and **4** (6 mg).

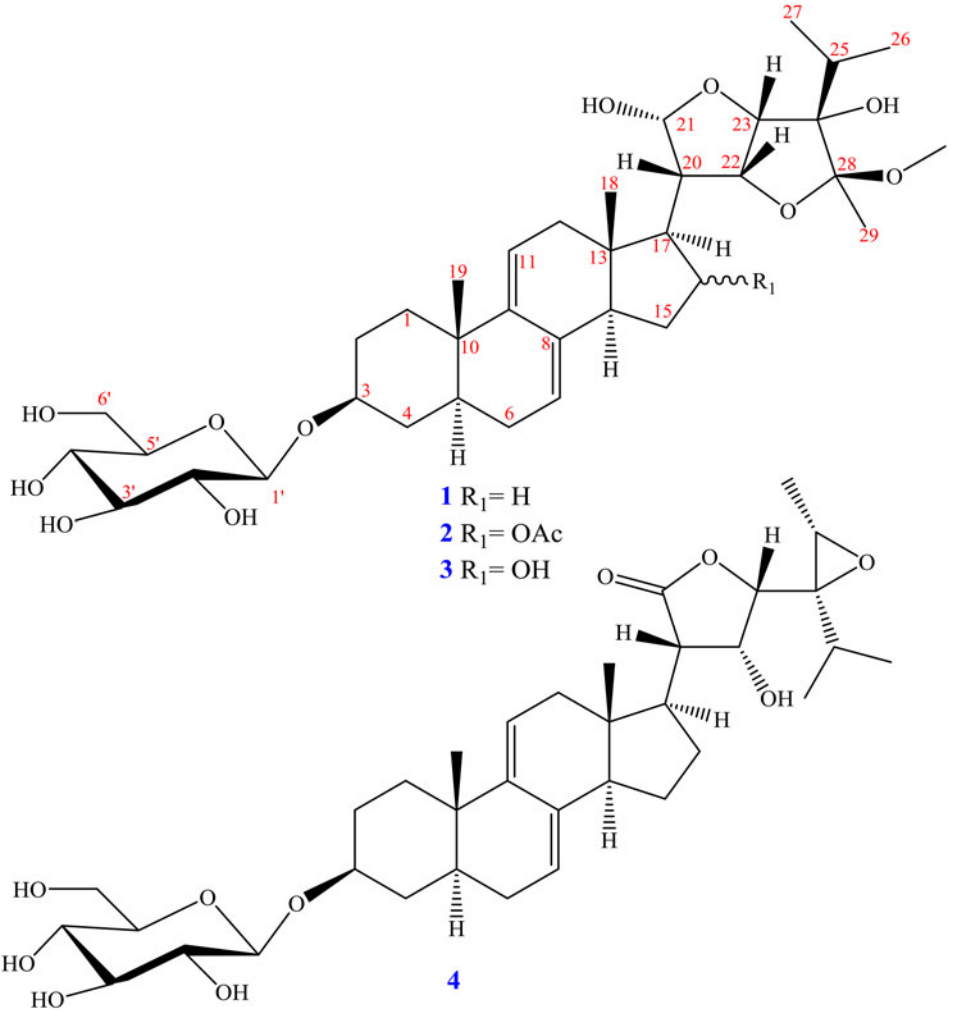

Figure 1. The structures of compounds **1**–**4.**

3.5.1. Vernoamyoside E *(1)*

White amorphous powder, $[\alpha]_D^{20}$. +24.7 (*c* 0.15, MeOH); IR (KBr) ν_{max} 3424, 2940, 1598, 1448, 1380; ^{1}H-NMR (MeOD-d_4, 500 MHz): δ_H 1.34 (1H, m, H_a-1), 1.99 (1H, m, H_b-1), 1.93 (1H, m, H_a-2), 3.71 (1H, m, H_b-2), 3.71 (1H, m, H-3), 1.32 (1H, m, H_a-4), 1.88 (1H, m, H_b-4), 1.41 (1H, m, H-5), 1.61 (1H, m, H_a-6), 1.97 (1H, m, H_b-6), 5.43 (1H, d, J = 5.0 Hz, H-7), 5.53 (1H, d, J = 5.5 Hz, H-11), 2.04 (1H, m, H_a-12), 2.22 (1H, m, H_b-12), 2.26 (1H, m, H-14), 1.49 (1H, m, H_a-15), 1.86 (1H, m, H_b-15), 1.53 (1H, m, H_a-16), 2.18 (1H, m, H_b-16), 2.08 (1H, m, H-17), 0.57 (3H, s, H-18), 0.93 (3H, s, H-19), 1.89 (1H, m, H-20), 5.43 (1H, d, J = 5.0 Hz, H-21), 4.26 (1H, t, J = 5.5 Hz, H-22), 4.45 (1H, d, J = 5.5 Hz, H-23), 2.20 (1H, m, H-25), 0.94 (3H, d, J = 7.0 Hz, H-26), 0.95 (3H, d, J = 7.0 Hz, H-27), 1.42 (3H, s, H-29), 4.42 (1H, d, J = 8.0, H-1′), 3.18 (1H, m, H-2′), 3.29 (1H, ddd, J = 2.5, 5.0, 9.5 Hz, H-3′), 3.32 (1H, m, H-4′), 3.39 (1H, t, J = 9.0 Hz, H-5′), 3.70 (1H, dd, J = 5.5, 12.0 Hz, H_a-6′), 3.87 (1H, J = 2.5, 12.0 Hz, H_b-6′) 3.21 (3H, s, OCH_3-28); ^{13}C-NMR (MeOD-d_4, 125 MHz): δ_C 35.8 (C-1), 30.8 (C-2), 78.9 (C-3), 34.8 (C-4), 40.1 (C-5), 30.3 (C-6), 121.4 (C-7), 137.2 (C-8), 144.8

(C-9), 36.8 (C-10), 119.5 (C-11), 41.9 (C-12), 42.7 (C-13), 51.9 (C-14), 24.2 (C-15), 28.0 (C-16), 45.4 (C-17), 12.9 (C-18), 19.8 (C-19), 50.2 (C-20), 100.0 (C-21), 81.0 (C-22), 91.3 (C-23), 83.2 (C-24), 32.7 (C-25), 17.4 (C-26), 18.1 (C-27), 112.9 (C-28), 17.4 (C-29), 102.6 (C-1′), 74.8 (C-2′), 77.4 (C-3′), 71.4 (C-4′), 77.7 (C-5′), 62.6 (C-6′), 48.3 (OCH_3-28); HR-ESI-MS *m/z* 687.3708 $[M + Na]^+$ (calcd for $C_{36}H_{56}NaO_{11}^+$, 687.3720).

Conflict of interest

The authors have declared that there is no conflict of interest.

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