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A Novel Cyclohexane Carboxylic Acid Derivative from Black Turtle Bean (*Phaseolus vulgaris* L.)

Balkisu Abdulrahman [1,2], Fadime Aydogan [1,3], Fazila Zulfiqar [1], Jianping Zhao [1], Zulfiqar Ali [1*], Ikhlas A. Khan [1], Bala Muntari [4] and Bello Oluwasesan [5]

[1]*National Centre for Natural Product Research, School of Pharmacy, University of Mississippi, University, MS, USA*

[2]*Department of Biochemistry and Molecular Biology, Federal University Dutsin- Ma, Katsina, Nigeria*

[3]*Department of Pharmaceutical Botany, Faculty of Pharmacy, Dicle University, Diyarbakır, Turkey*

[4]*Department of Biochemistry, Faculty of Basic Medical science, Bayero University Kano, Nigeria*

[5]*Department of Chemistry, Faculty of Physical sciences, Federal University Dutsin- Ma, Katsina, Nigeria*

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Abstract: Black turtle bean is a variety of *Phaseolus vulgaris* L. in the legume family Fabaceae and is consumed as food worldwide. A targeted phytochemical investigation of the ethanolic extract of its seeds, focusing on constituents other than the lipophilic metabolites, resulted in the isolation and characterization of five compounds (**1**-**5**). Compound **1** (phasvulic acid), a previously undescribed cyclohexane carboxylic acid derivative, was characterized as (*Z*)-3-hydroxy-2-(5-hydroxypent-2-en-1-yl)cyclohexane-1-carboxylic acid based on spectral data including 1D and 2D NMR (^{1}H, ^{13}C, COSY, HSQC, and HMBC) and high-resolution electrospray ionization mass spectrometry (HR-ESI-MS). Other compounds were formerly described as dihydrophaseic acid (**2**), uridine (**3**), stigmasterol-3-*O*-*β*-D glucopyranoside (**4**), and *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (**5**).

Keywords: Black turtle bean; *Phaseolus vulgaris;* phasvulic acid; legume; Fabaceae.

1. Introduction

Black turtle bean is a variety of common bean belonging to the *Phaseolus vulgaris* L. species of the Fabaceae family and is one of the most important varieties of cultivated edible legumes like kidney, pea, white, yellow beans, etc. [1]. *P. vulgaris* seeds are an important dietary component widely

* Corresponding author: E-Mail: zulfiqar@olemiss.edu; Phone:001-662-9151005 *Fax:*001-662-9157989

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cultivated and consumed in Nigeria and other tropical and subtropical countries [2]. Black turtle beans, considered a functional food due to the presence of nutrients such as lectins and proteins, may exhibit the ability to prevent disease conditions like cardiovascular, diabetes, cancer, metabolic syndrome, and obesity [3–6]. Various biological activities for extracts of *P. vulgaris* seeds have been reported including antidepressant, antioxidant, anti-inflammatory, anti-sickling, antioxidative, and antidiabetic [3,7–9]. Beans have high nutritional value due to their protein content with peptides, lectins, and amino acids. Furthermore, flavonoids, phenolic acids, tannins, alkaloids, sapogenins, and lectins-type phytochemicals have been reported in *P. vulgaris* seeds [5,10–12]. The growing interest in the identification of naturally occurring nutritive and non-nutritive chemical components of cereals, legumes, and spices and their roles in preventing chronic diseases has led to investigation of *P. vulgaris* seeds [3,9,13]. The secondary metabolite profiles of beans differ by variety and environmental factors [14]. This study reports the isolation of five constituents from the ethanolic extract of black turtle beans including a previously undescribed compound.

2. Materials and Methods

2.1. General

Mass data were obtained using an Agilent Technologies 6200 series mass spectrometer. NMR data were recorded on BRUKER AU III 500 MHz NMR spectrometer. The specific rotation was measured on an AUTOPOL IV Automatic Polarimeter (Rudolph, Hackettstown, NJ, USA). UV spectra were recorded on a Varian Cary 50 Bio UV-visible spectrophotometer. IR spectra were recorded on an Agilent Technologies Carry 630 FTIR spectrometer. Column chromatography was performed using silica gel (32–63μ, Dynamic Adsorbents Inc, Norcross, GA, USA). Preparative thin-layer chromatography was carried out using a glass silica gel plate (20 cm × 20 cm, 500 μm) (Sorbent Tech., Norcross, GA, USA). Analytical TLC was done using silica gel F_{254} aluminum sheet (20 cm × 20 cm, 200 μm) (Sorbent Tech., Norcross, GA, USA). The spots were detected on TLC by heating after spraying with 0.5% vanillin (Sigma, St. Louis, MO, USA) solution in H_2SO_4–EtOH (5:95). Preparative high-performance liquid chromatography (HPLC) was carried out using JAIGEL GS-310 resin column (500 mm × 20 mm). Analytical grade solvents (Fisher Chemicals, Hampton, NH, USA) were used for extraction and purification.

2.2. Plant Material

Black turtle beans of *P. vulgaris* L. were obtained at the Mangu local government area of Plateau State, Nigeria, and were authenticated (voucher # BUKHAN 61) at the Botany Department of Bayero University Kano, Nigeria.

2.3. Extraction and Isolation

Black turtle seeds (1.5 kg) were ground into a fine powder and extracted with 95% ethanol (4 L) at room temperature by soaking for 24 hours. The extraction process was repeated two more times. The crude extract (36.5 g) obtained by evaporating the solvent using a rotary evaporator was partitioned between methanol and hexane to remove the lipophilic portion. The methanol fraction yielded 22.3 g of extract which was subjected to column chromatography (CC) over silica gel (38 cm × 5 cm) using a mixture of solvents in the order of increasing polarity [EtOAc:CH_2Cl_2:MeOH:H_2O (15:8:2:0.5, 6.5 L), (15:8:4:1, 6 L), and (6:4:4:1, 3 L)] to afford 15 fractions (A-N). Fr. E was purified by repeated CC over silica gel followed by preparative thin-layer chromatography (PTLC) [silica gel plate, $CHCl_3$: MeOH (8:2)] to afford compounds **1** (4.5 mg) and **2** (3.5 mg). Fr. J was subjected to CC [silica gel (60 cm × 3 cm), EtOAc: CH_2Cl_2: MeOH: H_2O (15:8:4:1, 2L)] to yield seven subfractions (J1-J7). Compound **3** (4.3 mg) was purified from J5 by semi-preparative HPLC [Jaigel GS-310 column (500

mm × 20 mm), isocratic, 100 % MeOH, flow rate, 8 mL/min]. A mixture of compounds **4** and **5** (92.3 mg) was obtained from Fr. D as CH_2Cl_2 insoluble part.

2.4. Spectral Data

Phasvulic acid (***1***): White powder. $[\alpha]^{26}_D$ +25.0 (*c* 0.2, CH_3OH); IR ν_{max} cm^{-1}: 3308, 2924, 2854, 2361, 2340, 1729, 1572, 1407, 1084; UV (CH_3OH) λ_{max} nm (log ε): 216 (0.5); HR-ESI-MS: *m/z* 251.1247 $[M+Na]^+$ (calcd. for $C_{12}H_{20}O_4Na$, 251.1259). ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (125 MHz, CD_3OD): see Table 1.

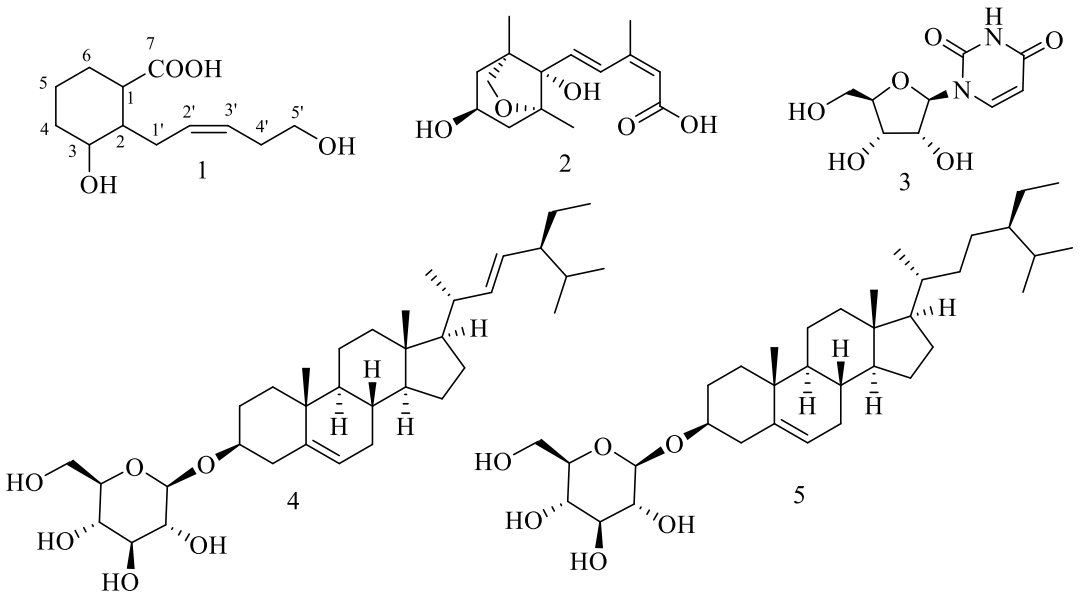

Figure 1. Chemical structures of compounds **1-5**

3. Results and Discussion

In this study, five compounds (**1-5**) (Figure 1) were isolated from the 95% EtOH extract of black turtle beans. Compound **1**, named phasvulic acid, was previously undescribed. Based on NMR and mass spectral data analysis along with a comparison to published data, the known compounds were identified as dihydrophaseic acid (**2**) [15]**,** uridine (**3**) [16], stigmasterol-3-*O*-*β*-D glucopyranoside (**4**), and *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (**5**) [17–19].

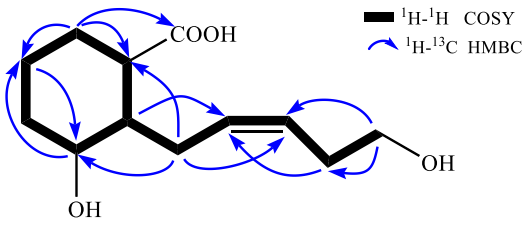

Figure 2. ^1H-^1H COSY and key HMBC correlations of compound **1**

3.1. Structure Elucidation

Compound **1** was isolated as a white powder. Its molecular formula was assigned as $C_{12}H_{20}O_4$, suggesting three degrees of unsaturation, based on ^{13}C NMR data (Table 1) and $[M+Na]^+$ ion peak at *m/z* 251.1247 (calcd. for $C_{12}H_{20}O_4Na$, 251.1259) in the HR-ESI-MS. The IR spectra showed the absorptions for hydroxy groups (3308 cm^{-1}) and carboxylic acid (1729 cm^{-1}). The ^{13}C NMR spectrum of **1** displayed twelve resonances corresponding to five methines, six methylenes (one being oxygenated), and a carbonyl. The NMR (^1H and ^{13}C) spectra of **1** exhibited characteristic resonances for two olefin methines [δ_H/δ_C 5.44 (1H, *dt*, *J*=11.0, 7.2 Hz, H-2')/131.1 (C-2') and 5.60 (1H, *dt*, *J*=11.0, 7.2 Hz, H-3') /127.7 (C-3′)], an oxymethine [δ_H/δ_C 3.82 (1H, *dt*, *J*=9.2, 5.2 Hz, H-3)/78.8 (C-3)], an oxymethylene [δ_H/δ_C 3.55 (2H, *t*, *J*=6.7 Hz, H_2-5')/62.8 (C-5')], and carbonyl of carboxylic acid [δ_C 182.6 (C-7)]. The coupling constant value (11.0 Hz) observed for olefin protons is typical for their *cis* configuration [20]. In the ^1H–^1H COSY spectrum, a part of the spin-spin system [$H_{a,b}$-1'↔H-2'↔H-3'↔H_2-4'↔H_2-5'] and HMBC correlations of H_2-5' (δ_H 3.55) to C-4' (δ_C 31.9), C-3' (δ_C 127.7) and of H_2-4' (δ_H 2.32) to C-2' (δ_C 131.1), C-3' (δ_C 127.7), C-5' (δ_C 62.8) and of H-2' (δ_H 5.44) to C-3', C-1' (δ_C 30.9) confirmed the presence of 5'-hydroxy-pent-2'-enyl allylic chain in the molecule (Figure 2). The HMBC correlations of H_2-1' with the oxymethine carbon (C-3, δ_C 78.8) and methine carbon (C-1, δ_C 42.1) determined the location of 5'-hydroxy-pent-2'-enyl allylic chain at C-2 and oxygenation at C-3 in the cyclohexane ring. The ^1H–^1H COSY spectrum showed the extension of spin-spin system from $H_{a,b}$-1' to H-2↔H-3↔H_2-4↔H_2-5↔H_2-6↔H-2 confirming the placement of cyclohexane ring protons. The HMBC correlations of H_2-6 with carbonyl carbon revealed the position of carboxyl group at C-1. Therefore, compound **1** was determined to be (*Z*)-3-hydroxy-2-(5-hydroxypent-2-en-1-yl)cyclohexane-1-carboxylic acid and was named *phasvulic acid.*

Table 1. NMR data for compound **1** (CD_3OD, δ in ppm)

Position	**δ_H (int., mult., *J* in Hz)**	**δ_C**
1	1.93 (1H, *m*)	42.1
2	1.49 (1H, *m*)	54.8
3	3.82 (1H, *dt*, *J* =9.2, 5.2 Hz)	78.8
4	1.76 (1H, *m*)	34.4
	1.57 (1H, *m*)	
5	1.84 (1H, *m*)	30.4
	1.48 (1H, *m*)	
6	2.43 (1H, *dd*, *J*=13.8, 5.0 Hz)	44.9
	2.11(1H, *m*)	
7	-	182.6
1′	1.82 (1H, *m*)	30.9
	1.48 (1H, *m*)	
2′	5.44 (1H, *dt*, *J*=11.0, 7.2 Hz)	131.1
3′	5.60 (1H, *dt*, *J*=11.0, 7.2 Hz)	127.7
4′	2.32 (2H, *dt*, *J*=7.3, 6.7 Hz)	31.9
5′	3.55 (2H, *t*, *J*=6.7 Hz)	62.8

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Supporting Information

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ORCID

Balkisu Abdulrahman: 0000-0001-9633-5953
Fadime Aydogan: 0000-0001-7810-3067
Fazila Zulfiqar: 0000-0001-7463-8742
Jianping Zhao: 0000-0002-4984-5931
Zulfiqar Ali: 0000-0003-3902-5152
Ikhlas A. Khan: 0000-0001-5464-4643
Bala Muntari: 0000-0002-5174-2181
Bello Oluwasesan: 0000-0003-1431-7319

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