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Pharmacological activities of a novel phthalic acid ester and iridoid glycoside isolated from the root bark of *Anthocleista vogelii* Planch

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Abstract. In this study, a novel phthalic acid ester (**1**) and a known iridoid glycoside (**2**) were isolated from the root bark of *Anthocleista vogelii*. The structures of the novel compound and iridoid glycoside were elucidated on the basis of their chemical and spectral data (UV, FT-IR, EI-MS, 1D and 2D NMR) and found to be phthalic acid ester, 4-ethyl-6-propyl-4,5,6,7-tetrahydro-3H-2,8-benzodioxacycloundecine-1,9-dione (**1**) and sweroside (**2**). The compounds were evaluated for their *in vitro* inhibitory activities against pancreatic lipase, α-amylase and α-glucosidase, and *in vivo* laxative activity in rats. The metabolite phthalic acid ester (**1**) exhibited moderate inhibitory activity against pancreatic lipase (IC_{50} = 24.43 ± 0.096 µg/mL) and relatively good activity against α-glucosidase (IC_{50} = 10.28 ± 0.015 µg/mL). Sweroside (**2**) displayed weak activity against α-glucosidase (IC_{50} = 40.28 ± 0.063 µg/mL) but significantly ($p<0.05$) increased the feacal output of the treated animals compared to the normal and sodium picosulfate controls.

INTRODUCTION

Anthocleista vogelii Planch is a tree- and shrub-like plant of the genus *Anthocleista* in the Gentian family (Gentianaceae) and is native mainly to tropical Africa, Madagascar and the Comores (Leeuwenberg, 1973). *Anthocleista vogelii* is commonly called "cabbage tree" and is a medicinal plant widely used in West Africa. The plant is traditionally used to treat various diseases such as diabetes mellitus, constipation, hernia, malaria, hypertension, heamorrhoids, syphilis and stomach aches (Okorie, 1976; Olubomehin *et al.*, 2013). The plant also has other pharmacological activities such as being used a laxative, analgesic, antiulcerogenic, antiplasmodial, antimicrobial, hypoglycaemic, antiobesity, anti-inflammatory, antitrypanosomal and spasmogenic (Anyanwu *et al.*, 2015). A number of research on the medicinal plant stops at the pharmacological activities of the extracts or fractions. This study sought to isolate and characterize compounds from *A. vogelii* root bark and to investigate their ethnobotanical-related pharmacological activities. The antiobesity, antidiabetic and laxative activities of *A. vogelii* root bark were the pharmacological activities investigated in this study.

MATERIALS AND METHODS

Plant material

The roots of *A. vogelii* were collected from Umuekwune (5°18'04.8"N 7°02'19.0"E), Ngor-okpala, Imo State, Nigeria in January 2015. The plant was authenticated by Dr Ihuma, J.O., Department of Biological Sciences, Bingham University, Karu, Nigeria. The plant was allocated voucher number (GA134-7421) and specimens were deposited in Department of Biological Sciences of Bingham University.

Extraction and isolation

The root bark was peeled from the roots, sliced into very small pieces and dried at room temperature. The dried root bark was pulverized using a local engine grinder and 10 kg of *A. vogelii* powdered root bark was macerated in hexane (30 L x 3) at room temperature. It was sieved using a muslin cloth and the marc was macerated in 20 L methanol and water (80:20) repeatedly thrice in 3 days. The resulting filtrates were concentrated to a slurry using a rotary evaporator under reduced pressure at 40°C. The slurry was equally divided into two portions (P1 and P2). P1 was acidified by adding 2 M H_2SO_4 and extracted with chloroform (500 ml x 3), using a separating funnel. The chloroform portion was concentrated under reduced pressure and separated by column chromatography over silica gel eluted with n-hexane: EtOAc (100: 0, 90:10, 80:20, 70: 30) to give 22 fractions [1-2, 0 spots; 3-6, 3 spots; 7-14, 1 spot); 15-22, 3 spots]. Compound **1** was found in fractions 7-14 and was concentrated to give 3.9 g.

P2 was extracted with water and partitioned with n-butanol (500 ml x 3) using a separating funnel. The aqueous portion was left aside and the n-butanol concentrate was subjected to column chromatography over silica gel eluted with CHCl3: EtOAc (100:0 to 0:100), CHCl3: EtOH (100:0 to 10: 90) and EtOAc: EtOH (100: 0 to 50:50) and gave many fractions [1-5, 3 spots; 4-5, 2 spots; 6-11, 0 spot; 12-41, 1 spot; 41-43, 0 spot]. Compound 2 was found in fraction 12-41 and con-centrated to give 7.2 g.

Compound **1**, was isolated as a pale yellow viscus oil: UV (MeOH) λ_{max} (log ε) 229 (1.395) nm; FTIR νmax 2927.7, 2958.6, 2860.2, 1728.1, 1461.9, 1380.9, 1276.8, 1124.4, 1072.3, 742.5 cm^{-1}; ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.69 (dd, 2H), 7.68 (d, 2H), 7.52 (d, 1H), 7.24 (d, 1H), 4.23 (d, 1H), 4.17 (d, 1H), 1.67 (m, 1H), 1.66 (m, 1H), 1.42 (d, 2H), 1.41 (d, 2H), 1.38 (m, 2H),1.31 (m, 2H), 1.29 (m, 6H), 1.23 (m, *J* = 14.1 Hz, 1H), 0.91 (t, 2H), 0.86 (t, 2H) and ^{13}C NMR (600 MHz, Chloroform-*d*) δ 167.82, 132.38, 130.93, 128.79, 68.12, 38.66, 30.30, 30.14, 29.71, 28.89, 23.68, 23.00, 14.11, 10.96; EIMS m/z 305.4 [M]+, establishing the molecular formula as $C_{18}H_{24}O_4$ (calcd for 304,172); elemental anal.C 71.03%, H 7.95%.

Sweroside **(2)** was a viscous pale yellow sweet smelling liquid: UV (MeOH) λ_{max} (log ε) 247 (2.35) nm; FTIR νmax 3417.6, 2914.2, 2061.8, 1693.4, 1616.2, 1406.0, 1359.7, 1317.3, 1272.9, 1203.5, 1153.4, 1026.1, 1001.0, 900.7, 827.4 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.47 (d, *J* = 1.0 Hz, 1H), 5.50 – 5.48 (m, 1H), 5.46 – 5.42 (m, 1H), 5.32 – 5.25 (m, 2H), 4.99 (d, *J* = 10.2 Hz, 1H), 4.95 (d, *J* = 9.4 Hz, 1H), 4.59 (d, *J* = 10.4 Hz, 1H), 4.50 – 4.48 (m, 2H), 4.36 (td, *J* = 7.1, 0.7 Hz, 2H), 3.68 (m, 1H), 3.40 (ddd, *J* = 12.3, 7.5, 6.7 Hz, 1H), 3.16 (dtt, *J* = 10.1, 6.8, 2.3 Hz, 1H), 3.14 (m, 3H), 3.03 – 2.98 (m, 1H), 2.66 (tdt, *J* = 7.0, 6.1, 1.0 Hz, 1H), 1.75 – 1.51 (m, 2H) and ^{13}C NMR (400 MHz, DMSO-d_6) δ 164.78, 151.49, 132.37, 120.36, 104.90, 98.14, 95.66, 77.35, 76.42, 73.16, 70.10, 67.76, 61.10, 41.57, 40.13, 26.82, 24.32; EIMS m/z 359.3 $[M]^+$; (calcd for $C_{16}H_{22}O_9$, 358.126); elemental anal.C 53.51 % , H 6.21 %.

Pancreatic lipase inhibitory activity

The pancreatic lipase inhibitory activity of the compounds was measured by methods established by Kim *et al.* (2009) with some modifications. The enzyme buffer was constituted by adding 10mM MOPS (morpholinepropanesulphonic acid) and 1mM EDTA pH 6.8, while the assay buffer was Tris buffer (100mM Tris-HC1 and 5mM $CaCl_2$, pH 7.0). In each well was added 164 µl of assay buffer, 6 µl pancreatic lipase solution (1 mg/ml), 20 µl of either the compounds at different concentration (0, 2.35, 4.69, 9.38,

18.75, 37.5, 75, 150, and 300 µg/ml) or orlistat (reference drug) and incubated for 10 min at 37°C. Then, 10 µl of substrate solution (10mM p-NPB (p-nitrophenyl butyrate) in assay buffer) was added and incubated for 15min at 37°C. The reaction was performed in triplicate and the absorbance was read at 405 nm using an ELISA reader. The inhibition of lipase activity by the plant compounds was calculated using the formula below:

$$\text{Lipase inhibition (\%)} = 100 - \left(\frac{B - b}{A - a}\right) 100$$

Where A = activity without inhibitor; a = negative control without inhibitor, B = activity with inhibitor, and b = negative control with inhibitor.

Alpha amylase inhibitory activity

The α-amylase inhibitory activity of compounds was carried out in a 96-well microtitre plate based on the starch-iodine test according to Xiao *et al.* (2006). Exactly 25 µl of assay buffer, 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride), 20 µl of soluble starch (1%, w/v) and 20 µl of plant extracts/acarbose (0, 7.81, 15.63, 31.25, 62.5, 125, 250, 500, 1000 µg/ml) were incubated at 37°C for 5 min. Acarbose was used as a positive control at a concentration of 6 mg/ml. Then 15 µl of amylase solution (6 mg/ml) was placed into each reaction well and incubated for 15 min at 37°C. Thereafter, 20 µl of 1M HCl was added to stop the enzymatic reaction, and 100 µl of iodine reagent (5 mM I_2 and 5 mM KI) was added. The change in colour was noted and the absorbance was measured at 620 nm on a microplate reader. A dark-blue colour indicated the presence of starch (and a very active inhibitor); a yellow colour indicated the absence of starch (and inhibitor) while a brownish colour indicated partially degraded starch (and active/ partially active inhibitor) in the reaction mixture. The calculation of percentage inhibition of α-amylase activity was done as follows:

$$\text{\% Inhibition} = \left(\frac{\text{Abs of test}}{\text{Abs of control}}\right) 100$$

Alpha-glucosidase inhibitory activity

Alpha-glucosidase inhibition was measured using adaptations of the procedures of Johnson *et al.* (2013) and Kwon *et al.* (2008). To a 96-well plate in an orderly manner, 50 µL of compound/acarbose, 50 µL positive control, or 50 µL reagent blank were added to columns. Then, 100 µL of a 1.0U/ml α-glucosidase solution (in 0.1 M sodium phosphate buffer, pH 6.9) was added. The plate was incubated at 25°C for 10 min. Then, 50 µL of a 5 mM p-nitrophenyl-α-D-glucopyranoside solution (in 0.1 M sodium phosphate buffer, pH 6.9) was added into each well. The mixtures were incubated for 5 min at 25°C. The absorbance was recorded at 405nm before and after incubation. The percentage inhibition was calculated relative to the diabetes drug, acarbose, as the positive control, and to the negative control, which had 50 µL of buffer solution in place of the compound. The formula for calculation of percentage inhibition of α-glucosidase activity:

$$\text{\% Inhibition} = \left(\frac{\Delta A_{\text{Control}} - \Delta A_{\text{Extract}}}{\Delta A_{\text{Control}}}\right) 100$$

***Laxative activity* in vivo**

The compounds from *A. vogelii* were tested for laxative activity using the method reported by Capasso *et al.* (1986). Thirty-six male Sprague-Dawley rats (210 ± 10 g) selected at the Animal House of Pharmacy Department, COMSATS Institute of Information Technology, Abbottabad, Pakistan were placed into 6 groups (n=6). The rats were food fasted for 12 hours with water available *ad libitum* before the experiment. The rats in Group 1 (normal control) received only normal saline, Group 2 (standard drug) received 25 mg/kg b.w sodium picosulfate; Groups 3 and 4 received 25 and 50 mg/kg b.w of compound **1,** respectively; Groups 5 and 6 received 25 and 50 mg/kg b.w of compound **2,** respectively, and administration was oral using normal saline as the vehicle. The rats were placed in cages suitable for collection of faeces after administration, and faeces were collected and weighed at the 8th hour. Animal handling during the procedures were according to European Community

guidelines (EEC Directive of 1986; 86/609/EEC) and Research Ethics Committee of Pharmacy Department, CIIT, Abbottabad which gave the ethical approval number (PHM-0024/EC/M-4-5.15).

Statistical analysis

The results of the experiments were presented as the Mean ± S.E.M. The One Way ANOVA followed by Tukey's Multiple Range Test was used to analyze the statistical significance of difference in parameters amongst groups using GraphPad Prism 5. The significant level was taken as $P<0.05$.

RESULTS

Structural elucidation of isolated compounds

Compound **1**: The FT-IR data ν_{max} : 2927.7, 2958.6, 2860.2 are C-H stretching vibrations, 1728.1 cm^{-1} C=O of cyclic ester, 1276.8, 1124.4 and 1072.3 cm^{-1} C-O of cyclic ester, 1579.6 and 1461.9 cm^{-1} C=C of aromatic, 742.5, 702.0 and 651.9 cm^{-1} of an ortho disubstituted aromatic. The ^{1}H NMR spectrum of compound **1** showed four aromatic protons peaking at δH 7.69 (dd, 2H), 7.68 (d, 2H), 7.52 (d, 1H) and 7.24 (d, 1H) ppm, six methylene protons at δH 4.23 (d, 1H), 4.17 (d, 1H), 1.41 (d, 2H), 1.38 (m, 2H),1.31 (m, 2H), 1.29 (m, 6H) ppm, two methine protons at δH 1.67 (m, 1H), and 1.66 (m, 1H) ppm and two methyl group at δH 0.91 (t, 2H) and 0.86 (t, 2H) ppm. Investigation of the ^{13}C NMR spectrum reveals 18 carbon signals; corresponding to two methyl, six methylene, two methine, and six aromatic carbons on the DEPT-90 and -135 sub-spectra.

Analysis of the HSQC spectrum revealed a direct correlation between the aromatic protons at 7.52 ppm (H-14)and 7.68 ppm (H-12) with the carbon signals at 130.93 ppm (H-14) and 128.79 ppm (H-12), methine proton 4.17 ppm (H-7) and carbon at 68.12 ppm (H-7). The key COSY crosspeaks revealed a correlation between H-7 (4.23 ppm) and the methine proton H-6 (1.67 ppm) and methylene proton H-18 (1.42 ppm). The absence of further correlation by H-2 may be due to the chemical environment that was surrounded by an oxo group and quaternary carbons. The position of quaternary carbons was confirmed by the crosspeaks between H-14 (7.52 ppm) and H-7 (4.23 ppm) with C-1 and C-9 (167.82 ppm) placing the two quaternary carbons bond from the aromatic carbons C-11 and C-11 (132.38 ppm) HMBC spectrum. In the NOESY spectrum, there was spatial correlations between H-3 (4.66 ppm) with the methyl proton H-22 (1.35 ppm), methylene proton H-18 (1.35) and H-19 (1.35 ppm). Figure 1 reveals COSY, HMBC and NOESY correlations, while the structure of the isolated novel phthalic acid ester, 4-ethyl-6-propyl-4,5,6,7-tetrahydro-3H-2,8-benzodioxacycloundecine-1,9-dione (1) is shown in Figure 2.

Sweroside **(2)**: The FT-IR data ν_{max}; 3417.6 O-H and 1026.1 C–O of the sugar moiety, 2914.2 and 2061.8 C–H of alkane, 1693.4 C = O of ester absorption moved to lower wavelength due to conjugation, 1616.2 C = C of olefin cm^{-1}. ^{1}H-NMR (DMSO-

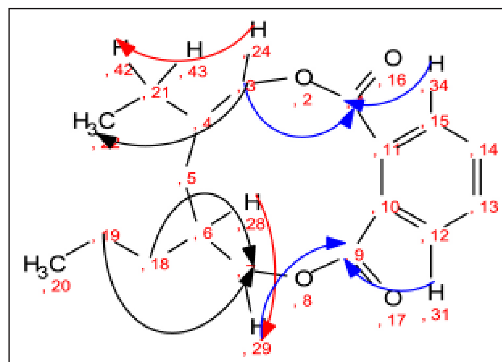

Figure 1. COSY (red), HMBC (blue) and NOESY (dotted black arrows) correlations.

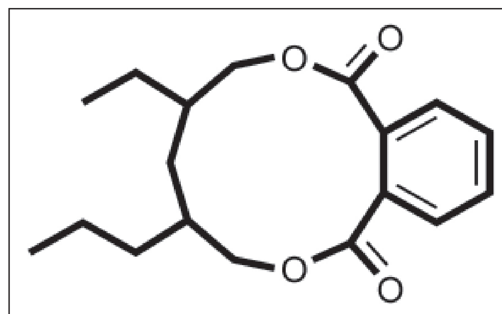

Figure 2. Structure of novel phthalic acid ester (**1**) isolated from the root bark of *A. vogelii*.

d_6) spectrum of compound **2** exhibited ten methine protons at δH 2.69, 3.27, 3.27, 3.31, 3.44, 3.65, 3.76, 4.05, 4.67, 5.26, 5.31, 5.53, 5.54, and 7.58 ppm and four methylene protons at δH 1.72 , 3.68, 4.35, 5.24 ppm. The ^{13}C-NMR spectrum of compound **2** showed 16 signals; 10 carbon signals attributed to the aglycone part and 6 signals to the sugar moiety. The DEPT-90 and -135 sub-spectra confirm the proton signals with four methylene and fourteen methine carbons. The ^{1}H-NMR spectrum showed a downfield doublet signal at δ 7.58 d, J = 7.4 Hz, indicating an oxyolefinic proton of the secoiridoids (Duke, 1990; Lim, 2014). Proton signal at δ 4.35,d, J = 6.5 was assigned to the methylene of C-12 by comparison with the data (Prasad and Sati, 2012; Aberham *et al.*, 2011). Also the two doublets at δ 5.50, *d*, J = 8.7, 9.4 Hz and δ 5.24, *d*, J = 9.4, 1.9 Hz that were assigned to two protons of a methylene group.

The HSQC data indicated a direct correlation between 7.46 ,d, J = 2.4, 5.50 ,d, J = 8.7 and 9.4 to C-7 (151.4 ppm), C-11 (132.3 ppm), respectively, while the postion of the olefinic bonds was confirmed by COSY data showing a correlation at H-7 (δH 7.46) and H-1 (δH 3.13) whereas the position of olefinic methylene hydrogen is verified by correction at H-12 (δH 4.35 ppm) and H-1 (δH 3.13 ppm). The HMBC data shows that the anomeric carbon is located at C-6 by the correlation between C-6(104.8 ppm) and H-2' (δH 4.94 ppm). In addition, the sugar moiety includes resonances of an anomeric proton signal at δ 4.94, d, J = 8.0 Hz, together with five proton signals for the remaining β-D-glucopyranoside protons. The NOESY spectrum are strongly in agreement with those reported for sweroside (Prasad *et al.*, 2007; De Oliveira *et al.*, 2015). The structure of compound 2 (sweroside) isolated from the root bark of *A. vogelii* Planch is shown on Figure 3.

Lipase, α-amylase and α-glucosidase inhibitory activities of isolated compounds

The results of lipase, α-amylase and α-glucosidase inhibitory activities of the compounds are presented on Table 1. The phthalic acid ester (**1**) had inhibitory activities against pancreatic lipase with IC_{50} values of 24.43 ± 0.096 µg/mL but showed no activity against α-amylase. Sweroside (**2**) was not active against pancreatic lipase and α-amylase. The phthalic acid ester (**1**) had good inhibitory activity against α-glucosidase (IC_{50} value of 10.28 ± 0.015 µg/mL), while sweroside (**2**) was moderately active against α-glucosidase (IC_{50} = 40.28 ± 0.063 µg/mL).

Laxative activity of isolated compounds

In Figure 4, the results revealed that sweroside (**2**) significantly ($p < 0.05$) increased the faeces output compared to the normal and sodium picosulfate controls, but phthalic acid ester (**1**) had no significant changes when compared to the normal control, although, it was significantly ($p < 0.05$) decreased compared to the sodium picosulfate control.

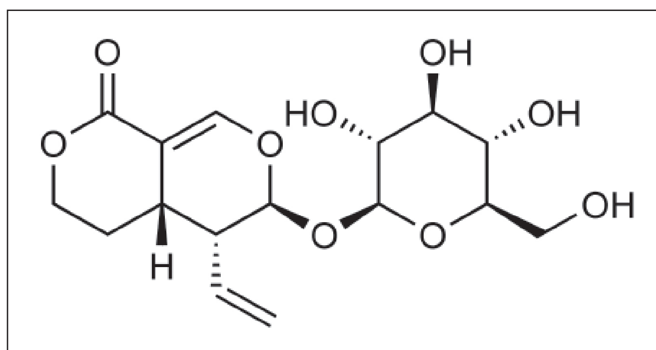

Figure 3. Structure of sweroside (**2**) isolated from the root bark of *A. vogelii* Planch.

Table 1. Calculated IC_{50} values of lipase, α-amylase and α-glucosidase inhibitory activities

Compounds	Pancreatic lipase IC_{50} (µg/mL)	α-amylase IC_{50} (µg/mL)	α-glucosidase IC_{50} (µg/mL)
1	24.43 ± 0.096	–	10.28 ± 0.015
2	–	–	40.28 ± 0.063
Orlistat	0.068 ± 0.001	NA	NA
Acarbose	NA	41.10 ± 0.031	2.59 ± 0.001

Compound 1- phthalic acid ester and compound 2- sweroside. Values are mean ± SEM, n = 3. NA- not applicable; (–) compounds did not inhibit 50 % of enzymes.

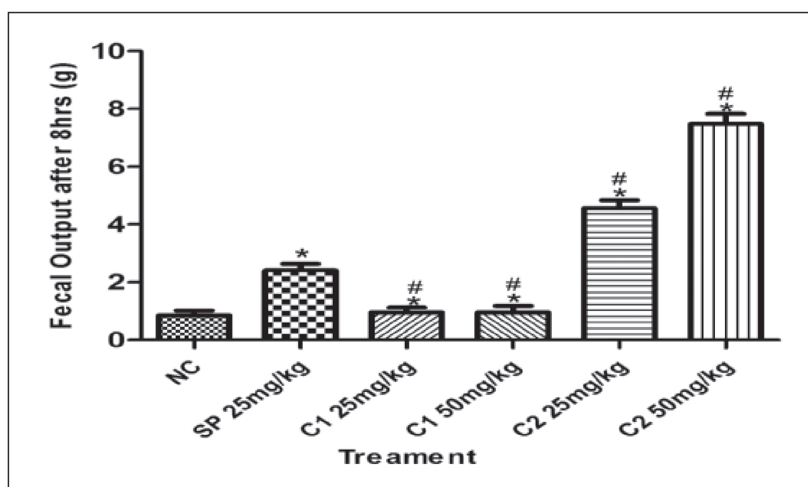

Figure 4. Fecal output of rats after 8 h of administration of compounds.
NC- normal control, SP- sodium picosulfate, C1- phthalic acid ester and C2- sweroside. Values are expressed as means ± SEM, * significantly different ($p < 0.05$) from normal control, and # significantly different ($p < 0.05$) from sodium picosulfate.

DISCUSSION

This is the first report of the novel phthalic acid ester, 4-ethyl-6-propyl-4,5,6,7-tetrahydro-3H-2,8-benzodioxacycloundecine-1,9-dione (**1**) isolated from *A. vogelii.* Although, it is not established that plants produce phthalates it is highly unlikely that all phthalic acid esters found in plants or other organisms are due to contaminated soil/ water and/or impurities introduced from solvents/plastics used during isolation process (Albro *et al.*, 1981; Graham, 1973; Namikoshi *et al.*, 2006). Several phthalates have been reported to have been isolated from plants and other organisms (Miyoshi *et al.*, 1974; Uddin *et al.*, 2013). Some phthalic acid esters from plant sources have displayed desirable biological activities such as the antiviral activity of 2”-(methoxycarbonyl)-5”-methylpentyl 2’-methylhexyl phthalate (Uddin *et al.*, 2013) and bis(2-methylheptyl) phthalate (Ramesh-thangam and Ramasamy, 2007).

The results of screening phthalic acid ester (**1**) and sweroside (**2**) against lipase, α-amylase and α-glucosidase are shown in Table 1. Our search for antiobesity and antidiabetic active compounds from *A. vogelii* prompted the screening of isolated compounds for inhibitory activities against pancreatic lipase, α-amylase and α-glucosidase enzymes. The abnormal accumulation of body fat is referred to as obesity. Obesity, which facilitates the development of diabetes mellitus and other

diseases (Mohamed *et al.*, 2014), could be managed by the use of pancreatic lipase inhibitors. Pancreatic lipase hydrolyses triacylglycerols to monoacylglycerols and fatty acids facilitating the absorption of dietary triacylglycerol. However, the inhibition of pancreatic lipase decreases the absorption of dietary triacylglycerol and fat accumulation in the body thus preventing obesity. On the other hand, inhibiting α-amylase, a carbohydrate hydrolysing enzyme is a valid mode of action for some obesity drugs (Melnikova and Wages, 2006).

The phthalic acid ester (**1**) had inhibitory activities against pancreatic lipase but showed no activity against α-amylase (Table 1). Dietary fat is not directly absorbed by the intestine unless by the action of pancreatic lipase, and as such inhibitors of pancreatic lipases are proposed to function as antiobesity agents. Thus, the phthalic acid ester (**1**) from *A. vogelii* should be explored as an antiobesity agent for its activity against pancreatic lipase.

The isolated compounds were evaluated for antidiabetic properties by determining their inhibitory activity against α-amylase and α-glucosidase enzymes (Table 1). Shai *et al.* (2010) had reported that the inhibition of α-amylase and α-glucosidase is a therapeutic approach for antidiabetic agents as both enzymes act to decrease glucose production and absorption in the gastro-intestinal tract. The chronic elevation of blood glucose is a characteristic of diabetes mellitus type 2, and inhibitors of α-amylase and α-glucosidase (e.g. acarbose and miglitol) decrease the post-prandial elevation of blood glucose. Many medicinal plant crude extracts have α-amylase and α-glucosidase inhibitory activity (Bhandari *et al.*, 2008). However, possible bioactive principles responsible for these activities were not yet known.

The phthalic acid ester (**1**) had good inhibitory activity against α-glucosidase, whereas sweroside (**2**) was moderately active against α-glucosidase, nevertheless both compounds showed no inhibitory activity against α-amylase (Table 1). Although, a previous study has shown that aqueous methanol extract of *A. vogelii* possessed α-amylase inhibitory activity (Olubomehin *et al.*, 2013), the results of this study suggests that the α-amylase inhibitory activity might not be linked to either of the isolated compounds. However, the possibility of the use of phthalic acid ester (**1**) and sweroside (**2**) or their derivatives as an antidiabetic agent could be further researched because of their α-glucosidase inhibitory activity.

Sweroside (**2**) significantly increased the faeces output compared to the normal and sodium picosulfate controls. Thus, sweroside (**2**) acted as a potent laxative facilitating the excretion of faeces from the animals and observations showed that the faeces were brown, mushy and non-uniform in texture. Laxatives are known to loosen feces and escalate bowel movement thereby producing soft, mushy and/or watery feces. A previous study had reported the laxative activity of the methanolic extract of *A. vogelii* in rats (Anyanwu *et al.*, 2018). Thus, sweroside (**2**) might be the active laxative ingredient in *A. vogelii* that made it popular among traditional healers in managing constipation.

CONCLUSION

In this study, a novel phthalic acid ester (**1**) and sweroside (**2**) were isolated from *A. vogelii* root bark extract. The phthalic acid ester (**1**) revealed inhibitory activity against pancreatic lipase and α-glucosidase, thus suggesting its potential use for the management of obesity and diabetes and should further be explored. The increased faecal output of animals stimulated by sweroside has revealed an unreported biological activity of sweroside and suggests it as the main bioactive compound in *A. vogelii* responsible for its laxative activity.

Supplementary material

The copies of the spectra could be obtained by request from the corresponding author.

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Authorship

Nisar-ur-Rahman, J. Iqbal, K. Rauf and E.C. Onyeneke designed and supervised the entire experiments. The enzyme activity studies was done by S.A. Ejaz, S. Zaib, and G.O. Anyanwu. The isolation and spectroscopic analysis of compounds was handled by G.O. Anyanwu and Sabi-ur-Rehman. G.O. Anyanwu also performed the laxative studies on animals. Interpretation of spectroscopic data and structure elucidation of the compounds was executed by G.O. Anyanwu, B. Okoli and S.J. Modise. All authors participated in the writing, editing and approval of the manuscript.

Conflict of Interest

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

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