

New cucurbitane type triterpenes from *Momordica foetida* Schumach. (Cucurbitaceae)

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ABSTRACT

Phytochemical studies on *Momordica foetida* led to the isolation and characterization of Momordin (23,24,25,26,27-pentanorcucurbitacin) (3), a new pentanor cucurbitane along with the known β -sitosterol 3-O- β -D-glucopyranoside (4), β -sitosterol (5) and kaempferol-7-O- β -D-glucopyranoside (6). In addition to those components, two previously unreported cucurbitane glucosides, namely 3 β -hydroxy-7-oxo-23(R)-cucurbita-5,24-diene-23-O- β -D-glucopyranoside (Momordiside A) (1) and 3 β -hydroxy-7 β -methoxy-23(R)-cucurbita-5,24-diene-23-O- β -D-glucopyranoside (Momordiside B) (2) were also obtained. The structures were elucidated by HR-ESI-MS and spectroscopic analysis including 1D- and 2D-NMR. Compounds 1 and 2 showed significant antioxidant potential in DPPH radical scavenging assay. In addition, compound 2 further revealed significant inhibitory potential against the enzyme urease *in vitro* as well as moderate activity against oral cancer cell line CAL-27.

1. Introduction

The genus *Momordica* (Cucurbitaceae) comprises about 60 species of annual, perennial climbers herbaceous or rarely small shrubs native of tropical and subtropical Africa, Asia and Australia. They grow in rain-forest, deciduous forests, bushlands, savannas, or grasslands (Hanno and Susanne, 2010). *Momordica foetida* Schumach. is a perennial herbaceous climbing vine native of tropical Africa (Mulholland et al., 1997). Synonym names are *M. cordifolia* E. Mey. ex Sond., *M. mannii* Hook. f. and *M. morkorra* A. Rich. (The Plant List, 2013). The leaf is used in traditional medicine for treatment of a wide range of diseases and disorders including stomach troubles, as an emetic, as a vermifuge, against smallpox, chickenpox, measles, body swellings, oedema and gout, as antidote for venomous stings such as bee stings and also as a pain-killer (Hakizamungu

et al., 1992; Froelich et al., 2007; Rosaria et al., 2013; Nantia Akono et al., 2018). Pharmacological studies have indicated antidiabetic, antilipogenic, antiplasmodial, antimalarial, antioxidant activities of crude extracts (Froelich et al., 2007; Rosaria et al., 2013; Nantia Akono et al., 2018), providing scientific support of its indigenous use. Phytochemical investigations led to the isolation of cucurbitane triterpenoids, polyphenolics, alkaloids and glycosides (Rosaria et al., 2013). The chemotaxonomic and ethnopharmacological significance of the genus *Momordica* sparked the investigation of the secondary metabolites of *M. foetida*. Herein we report the isolation of two previously unreported cucurbitane glycosides, named momordisides A (1) and B (2) along with momordin (3), a previously undescribed pentanortriterpene. In addition, three known secondary metabolites namely β -sitosterol 3-O- β -D-glucopyranoside, β -sitosterol and kaempferol-7-O- β -D-glucopyranoside were also isolated.

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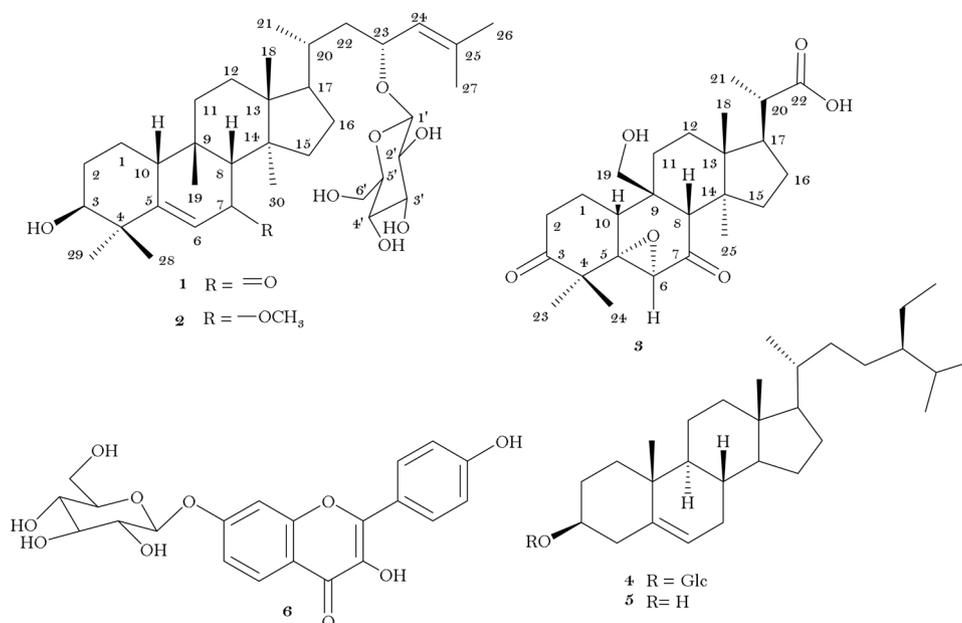


Fig. 1. Structures of compounds 1-6 isolated from *M. foetida*.

2. Results and discussion

2.1. Isolation and structure elucidation

The air-dried whole plant and fruit of *M. foetida* were extracted separately with MeOH at room temperature. The whole plant extract (75.8 g) was subjected to column chromatography on silica gel yielding compounds 1, 2, 4, 5 and 6 (Fig. 1). The methanol extract of the fruit was suspended in water and extracted with hexane, ethyl acetate and *n*-butanol. The *n*-butanol extract (20.52 g) was subjected to column chromatography over silica gel to afford compound 3. The known compounds were characterized as β -sitosterol 3-O- β -D-glucopyranoside (4) (Huh et al., 2011; Cho et al., 2012; Lee et al., 2013), β -sitosterol (5) (Rubinstein et al., 1976; Lee et al., 2013) and kaempferol-7-O- β -D-glucopyranoside (6) (Froelich et al., 2007).

Momordiside A (1) was obtained as a white powder; $[\alpha]_D^{25} = +17.72$. The molecular formula was deduced as C₃₆H₅₈O₈, through HR-ESI-MS which showed an ion cluster at m/z [M+H]⁺ peak at m/z 619.4205 (calcd. for C₃₆H₅₈O₈Na. 641.4024) implying 8 degrees of unsaturation. The UV spectrum showed absorption bands at λ_{max} (log ϵ) 205 (0.724), 248 (1.259) nm suggesting the presence of an α , β -unsaturated ketone chromophore (Chang et al., 2008). The IR spectrum showed absorptions for hydroxyl group (3416 cm⁻¹), the conjugated ketone (1685 cm⁻¹), olefinic (1652 cm⁻¹) and conjugated olefin (1600 cm⁻¹) functionalities.

In the ¹H NMR spectra of 1 (Table 1) the aglycone showed typical signals for five tertiary methyl groups at δ_H 0.84, 0.92, 0.95, 1.15, 1.22 (3H each, s), one secondary methyl as doublet at δ_H 0.94 (3H, d, $J = 6.0$ Hz), two allylic methyls at 1.61, 1.66 (3H, s), two oxymethines at 3.60 (1H, m, H-3), 3.64 (1H, m H-23) and two olefinic protons at δ_H 5.33 (1H, t, H-24) and 6.03 (1H, s, H-5). The anomeric proton was observed as doublet at δ_H 4.66 (d, $J = 7.5$ Hz). The larger coupling constant allowed us to assign β and axial configuration of the-glucopyranosyl moiety.

The ¹³C (broadband and DEPT) and HSQC NMR spectra data of 1 (Table 1) showed 30 signals of triterpene moiety and a further six signals of a sugar unit including eight methyl (δ_C 13.8, 15.7, 17.9, 18.8, 25.4, 25.9, 28.2 and 28.5), seven methylene (δ_C 22.1, 28.0, 29.1, 29.6, 30.9, 32.2, 35.9), one oxymethylene (δ_C 63.1), four methine (δ_C 41.7, 42.2, 47.9 and 61.2), seven oxymethine (δ_C 68.9, 72.6, 72.9, 75.1, 77.4, 84.7, 102.6), five quaternary carbons (δ_C 36.9, 44.0, 47.1, 49.2); the

downfield signals at δ_C 205.8 could be attributed to conjugated ketone carbonyl functionality while the olefinic carbons resonated at δ_C 174.1, 132.9, 126.0 and 123.5. Acid hydrolysis of 1 with 5% HCl/MeOH, provided the sugar residue which was identified as D-glucose by gas chromatography of its TMS ethers and the sign of its optical rotation. The sugar moiety was located at C-23 on the basis of the HMBC correlation between H-23 at δ_H 3.64 and the anomeric carbon at δ_C 102.6. The cumulative spectral data were similar to those reported for kuguaglycoside A (Chen et al., 2008), which has previously been reported from the roots of *Momordica charantia*. However, the notable difference was the presence of a carbonyl functionality at C-7 in momordiside A (1). This was further confirmed by the HMBC correlation depicted between the proton at δ_H 2.37 (s, H-8) and the carbon at δ_C 205.2 (C-7). The HMBC spectrum of 1 (Fig. 2) further showed correlations of H-3 (δ_H 3.60) with C-1 (δ_C 22.1), C-5 (δ_C 174.1) and C-29 (δ_C 28.5) confirming the location of hydroxyl group at C-3. The olefinic proton H-6 (δ_H 6.03) also exhibited HMBC correlations with C-4 (δ_C 44.0), C-5 (δ_C 174.1), C-8 (δ_C 61.2) and C-10 (δ_C 41.7). Correlations were also observed between H-8 (δ_H 2.37) and C-7 (δ_C 205.2), C-6 (δ_C 126.0), C-13 (δ_C 49.2), C-14 (δ_C 47.1), C-15 (δ_C 35.9), C-19 (δ_C 28.2), C-30 (δ_C 17.9). The olefinic proton H-24 (δ_H 5.33) also showed HMBC correlations with C-26 (δ_C 18.8) and C-27 (δ_C 25.9). The α -orientation of the glucose moiety at C-23 was determined by the cross-peaks between H-23 (δ_H 3.64), H-20 (δ_H 2.16), H-18 (δ_H 0.92) and H-19 (δ_H 0.95), respectively, in the NOESY spectrum of 1 (Fig. 3) (Liu et al., 2009). The structure of compound 1 was finally concluded as 3 β -hydroxy-7-oxo-23(R)-cucurbita-5,24-diene-23-O- β -D-glucopyranoside to which the trivial name momordiside A was given.

Compound 2 was isolated as a white powder; $[\alpha]_D^{25} = +31.46$. The molecular formula C₃₇H₆₂O₈ was deduced from the molecular ion peak at m/z 634.44320 in the HR-EI-MS (calcd for C₃₇H₆₂O₈ 634.4445). Acid hydrolysis furnished D-glucose which was identified as described for compound 1. The UV spectrum showed absorption bands at λ_{max} (log ϵ) 213 (0.0324) nm. The IR spectrum exhibited absorptions bands for hydroxyl (3414 cm⁻¹), C = C (1650 cm⁻¹) and methoxyl (1250 cm⁻¹) functionalities. The main difference between compounds 1 and 2 was replacement of the carbonyl group of compound 1 by a methoxyl moiety. This was confirmed by HMBC correlation of methoxyl protons at δ_H 3.49 with C-7 (δ_C 78.9) as well as correlations of H-8 (δ_H 2.05) with C-6 (δ_C 120.2) and C-7 (δ_C 78.9). Careful comparison of the ¹H and ¹³C NMR data of 2 (Table 1) with those of kuguaglycoside A (Chen

Table 1
 ^1H and ^{13}C NMR data of compounds 1–3 in CD_3OD .

Position	1		2		3	
	δ_c	δ_H (m; J in Hz)	δ_c	δ_H (m; J in Hz)	δ_c	δ_H (m; J in Hz)
1	22.1	1.76 (m) 1.80 (m)	22.3	1.75 (m) 1.81 (m)	24.1	1.74 (m) 2.17 (m)
2	29.6	1.76 (m) 2.09 (m)	30.0	1.69 (m) 1.94 (m)	39.0	2.61 (m) 2.95 (m)
3	77.4	3.60 (m)	77.5	-	211.3	-
4	44.0	-	42.4	-	50.3	-
5	174.1	-	149.4	-	70.8	-
6	126.0	6.03 (s)	120.2	5.78 (d, $J = 5$)	56.7	3.57 (s)
7	205.0	-	78.9	3.49 (m)	208.4	-
8	61.2	2.37 (s)	49.5	2.05 (s)	52.2	3.61 (d, $J = 4.5$)
9	36.9	-	35.2	-	40.1	-
10	41.7	2.82 (d, $J = 11$)	40.2	2.35(d, $J = 9.5$)	37.2	3.05 (dd, $J = 2.5, 2.5$)
11	29.1	2.08 (m) 2.18 (m)	27.9	1.54 (m) 1.79 (m)	26.2	1.55 (m) 1.61 (m)
12	28.0	1.54 (m) 1.75 (m)	33.7	1.48 (m) 1.69 (m)	29.7	1.54 (m) 1.74 (m)
13	49.2	-	48.6	-	45.7	-
14	47.1	-	47.5	-	49.0	-
15	35.9	1.01 (d, $J = 11$) 1.52 (m)	35.9	1.34 (m) 1.38 (m)	35.5	1.30 (m) 1.86 (m)
16	32.2	1.48 (m) 1.88 (m)	31.3	1.53 (m) 1.67 (m)	27.0	1.67 (m) 2.12 (m)
17	47.9	1.61 (m)	48.3	1.61 (m)	47.4	2.24 (m)
18	15.7	0.92 (s)	15.8	0.97 (s)	16.0	1.14 (s)
19	28.2	0.95 (s)	29.3	0.96 (s)	65.0	3.51 (d, $J = 10.5$) 4.13 (d, $J = 10.5$)
20	42.2	2.16 (m)	42.2	2.14 (m)	43.5	2.74 (m)
21	13.8	0.94 (s)	13.8	0.94 (s)	17.9	1.37 (d, $J = 10.5$)
22	30.9	1.61 (m) 1.80 (m)	29.1	2.09 (m) 2.21 (m)	179.1	-
23	84.7	3.64	84.8	3.66	15.1	1.1 (s)
24	123.5	5.33 (t)	123.6	5.33 (t)	25.3	1.43 (s)
25	132.9	-	132.8	-	20.9	1.02 (s)
26	25.9	1.66 (s)	25.9	1.67 (s)	-	-
27	18.8	1.61 (s)	17.9	1.62 (s)	-	-
28	25.4	1.22 (s)	25.9	1.17 (s)	-	-
29	28.5	1.15 (s)	28.7	1.03 (s)	-	-
30	17.9	0.84 (s)	18.8	0.72 (s)	-	-
Glucose						
1'	4.66 (d, $J = 7.5$)	102.6	4.67 (d, $J = 8.0$)	102.2		
2'	3.61 (m)	75.1	3.64 (m)	75.1		
3'	4.03 (m)	72.9	4.02 (m)	72.9		
4'	3.50 (dd, $J = 2.5, 2.5$)	68.9	3.51 (m)	69.0		
5'	3.27(d, $J = 2.5$)	72.6	3.27(d, $J = 3$)	72.6		
6'	3.67, 3.80 (d, $J = 10.0$)	63.1	3.67, 3.80(d, $J = 9.5$)	63.2		

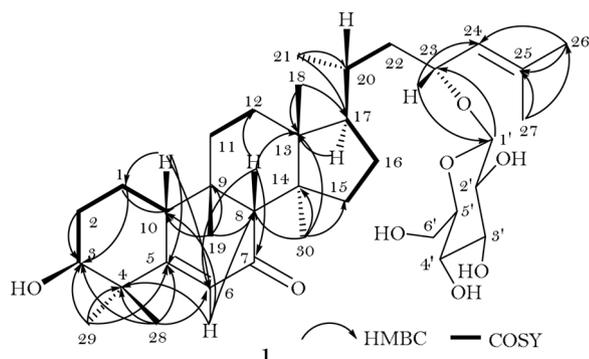


Fig. 2. Key HMBC and COSY correlations compound 1.

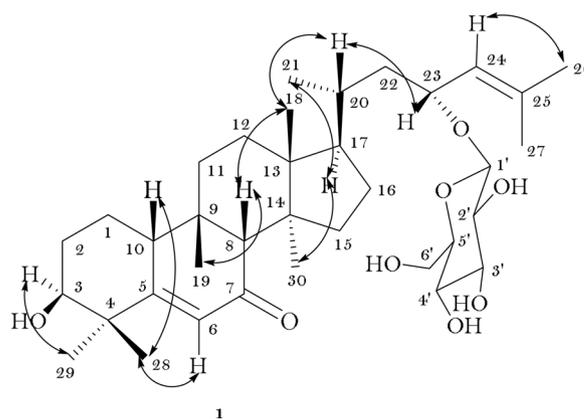


Fig. 3. Key NOESY correlations of compound 1.

et al., 2008) revealed that

compound 2 had similar structure except for the stereochemistry at C-23. The ROESY (Fig. 4) correlations between H-23 (δ_H 3.66), H-20 (δ_H 2.14), H-18 (δ_H 0.97) and H-19 (δ_H 0.96) revealed *R* configuration for C-23.

Compound 3 was isolated as a colorless powder; $[\alpha]_D^{25} = -15.69$. It

gave brisk effervescence with dilute sodium bicarbonate revealing the presence of a free carboxylic group. Its positive HR-FABMS showed a pseudomolecular ion peak $[\text{M} + \text{H}]$ at m/z 433.2244, (calcd. for $\text{C}_{25}\text{H}_{36}\text{O}_6$, 433.2226) corresponding to the molecular formula $\text{C}_{25}\text{H}_{37}\text{O}_6$,

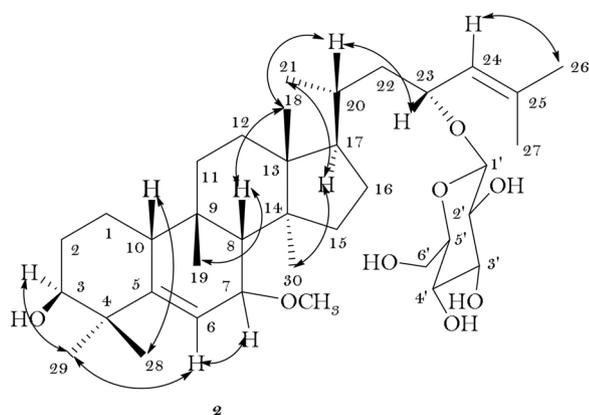


Fig. 4. Key ROESY correlations of compound 2.

which indicated eight degrees of unsaturation. The UV spectrum showed absorption bands at λ_{\max} ($\log \epsilon$) 230 (1.433). The IR spectrum displayed absorptions for hydroxyl group (3409 cm^{-1}), in addition to those of carbonyl and carboxylic groups (1708 cm^{-1}).

The ^1H NMR spectrum of 3 showed singlets for four tertiary methyls at δ_{H} 1.02, 1.10, 1.14, 1.43, doublet for a secondary methyl at δ_{H} 1.37 (d, $J = 7\text{ Hz}$), oxymethylene protons at δ_{H} 4.13 and 3.51 (d, $J = 10.5\text{ Hz}$, 1H each) and an oxymethine proton at δ_{H} 3.57 (s, 1H). The ^{13}C (broadband and DEPT) and HSQC NMR spectra data of 3 displayed 25 signals including five methyls (δ_{C} 15.1(C-23) 16.0(C-18), 17.9(C-21), 20.9(C-25) and 25.3(C-24)), an oxymethylene carbon (δ_{C} 65.1), oxymethine carbons (δ_{C} 56.7), seven methylenes (δ_{C} 24.1, 26.2, 27.0, 29.7, 35.5, 39.0, 65.1), five methines (δ_{C} 37.2, 43.5, 47.4, 52.2, 56.7) and eight quaternary carbons. The carbonyl carbons resonated at δ_{C} 179.1, 208.4, 211.3 while an oxygenated quaternary carbon appeared at δ_{C} 70.8. Its HMBC spectrum revealed correlations between H-1 (δ_{H} 2.17), C-3 (δ_{C} 211.3) and C-5 (δ_{C} 70.8); between H-2 (δ_{H} 2.61/2.94), C-3 (δ_{C} 211.3), C-10 (δ_{C} 37.2), H-23 (δ_{H} 1.10), H-24 (δ_{H} 1.43) with C-3 (δ_{C} 211.3), C-4 (δ_{C} 50.3), C-5 (δ_{C} 70.8) and C-6 (δ_{C} 56.7), providing evidence for the presence of a carbonyl group at C-3. Further HMBC correlations of H-6 (δ_{H} 3.57) with C-4 (δ_{C} 50.3), C-5 (δ_{C} 70.8), C-7 (δ_{C} 208.4); H-8 (δ_{H} 3.61) to C-6 (δ_{C} 56.7), C-7 (δ_{C} 208.4), C-19 (δ_{C} 65.1), C-9 (δ_{C} 40.1), C-10 (δ_{C} 37.2), C-14 (δ_{C} 49.0) enabled us to locate the carbonyl group at C-7, the oxymethine at C-6 and the oxygenated quaternary carbon at C-5. The NOESY correlations (Fig. 5) of H-8 to H-18, H-19; H-24; H-17 to H-25 established the stereochemistry of H-8 β , H-10 β , H-19 β , H-24 β and H-17 α . The β -orientation of H-6 was suggested by the correlations between H-6 and H-24. These data showed that compound 3 is virtually the same as kuguacin K (Chen et al., 2009) and differs only by the presence of a hydroxyl group instead of an

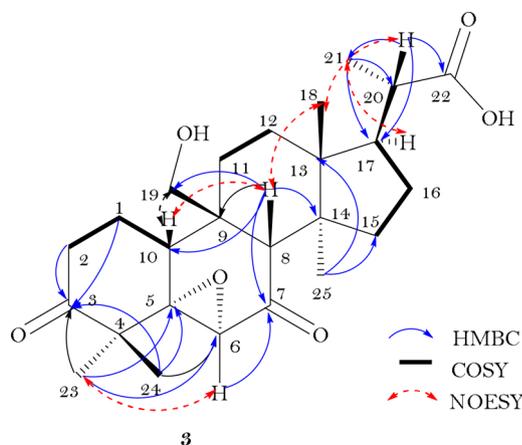


Fig. 5. Key HMBC, COSY and NOESY correlations of compound 3.

Table 2

IC_{50} values of compounds 1 and 2 in Urease inhibition and antioxidant activity.

Compound	Antioxidant IC_{50} (μM)	Urease Inhibition IC_{50} (μM)
1	45.1 ± 0.49	Nil
2	59.2 ± 0.28	32.5 ± 0.45
BHA	44.2 ± 0.06	
Thiourea		21.6 ± 0.12

aldehyde at C-19. It was finally elucidated to 23,24,25,26,27-penta-norcucurbitacin to which we gave the trivial name momordin.

2.2. In vitro biological screening of isolated compounds

The new compounds isolated from *Momordica foetida* were evaluated for their therapeutic potential against various enzymes as well as antioxidant activity and antiproliferative activity against oral cancer cell line. The compound 1 showed significant antioxidant and urease inhibitory potential (Table 2) while 2 showed only promising antioxidant activity.

Compounds 1 and 2 were found to be cytotoxic against cancer cells at low doses i.e. IC_{50} 60.68 and 10.69, respectively (Table 3). These compounds were noncytotoxic against normal cells as the higher concentration. The activity of compound 1 was very near to the value of standard drug i.e. cisplatin.

3. Experimental

3.1. General experimental procedures

Column chromatography was carried out on silica gel (70-230 and 230-400 mesh, E. Merck, Darmstadt, Germany). Thin layer chromatography was performed on percolated 0.5 mm thick aluminium sheets (Merck Silica gel 60, F_{254}) and visualised by spraying with ceric sulphate solution followed by heating at 120°C on a hot plate. The mass spectra were recorded on a JEOL MS Route instrument and nuclear magnetic resonance (NMR) spectra were recorded on a Bruker DPX-400 instrument, ^1H and ^{13}C NMR probes operating at 500 and 125 MHz, respectively, with tetramethylsilane as an internal standard. Optical rotations were recorded on a JASCO P-2000, polarimeter (Japan). The UV spectra were measured on a HP 8451A diode array spectrophotometer.

3.2. Plant material

The whole plant and fruits of *Momordica foetida* were collected in August 2015 in Awae, in the Centre region of Cameroon and identified at the National Herbarium of Cameroon by comparison with a voucher specimen number 52290 HNC.

3.3. Extraction and isolation

The whole plant and fruits of *Momordica foetida* (Cucurbitaceae) were air dried and ground into fine powders. The powder from whole plant (2.25 kg) and fruits (1.42 kg) were macerated with methanol for

Table 3

IC_{50} values of compound 1 and 2 against cell lines NIH-3T3 and CAL-27.

Compound	NIH-3T3 IC_{50} (μM)	CAL-27 IC_{50} (μM)
1	82.92 ± 2.15	60.68 ± 0.31
2	18.45 ± 0.96	10.69 ± 1.36
Standard Drug: Cisplatin	$2.41\mu\text{M/ml}$	

72 h. The extracts were filtered and concentrated with the rotary evaporator to obtain crude extracts with 158.8 g and 123.25 g respectively. The crude extract of whole plant (80.8 g) was subjected to column chromatography over silica gel (230–400 mesh) and eluted with hexane, hexane/ EtOAc (7.5:2.5), hexane/ EtOAc (7:3), hexane/ EtOAc (1:1), EtOAc, EtOAc/ MeOH (9:1) and MeOH to afford seven fractions which were combined on the basis of their TLC profile into two batches F₁ (26.52 g) and F₂ (31.25 g), respectively. The fraction F₁ (26.52 g) was subjected to column chromatography over silica gel (230–400 mesh) and eluted with n-hexane/ EtOAc (15:5), to afford β -sitosterol (5). The batch F₂ (31.25 g) was also subjected to column chromatography with mixtures of n-hexane/ EtOAc in increasing order of polarity. The fraction which eluted with n-hexane/ EtOAc (12:8) afforded β -sitosterol-3-O- β -D-glucoside (4) (25.03 mg). Further elution with increasing ethyl acetate concentration afforded momordiside A (1) (56.01 mg) and momordiside B (2) (65.08 mg). The crude extract of fruits (100.85 g) was suspended in water and extracted successively with ethyl acetate and n-butanol to afford two major sub-fraction MFF1 (45.25 g) and MFF2 (23.83 g) respectively. MFF2 (23.83 g) was subjected to column chromatography over silica gel (230–400 mesh) with CH₂Cl₂ and mixtures of CH₂Cl₂/ MeOH in increasing order of polarity to provide compound 3 (8.52 mg) on elution with CH₂Cl₂/ MeOH (15:5).

3.4. Acid hydrolysis

The solutions of 1 and 2 (3 mg) in methanol (4 mL) containing 1 N HCl (2 mL) were refluxed for 4 h. The mixture was concentrated, diluted with water, and extracted with ethyl acetate. The aqueous phase was concentrated to obtain the sugar residue which was identified to D-glucopyranose based on its optical rotation ($[\alpha]_D^{23} = 51.9$) the GC retention time of its Me₃Si ether (α anomer t_R 4.2, β anomer t_R 7.9) when compared with that of a standard sample.

3.5. Determination of DPPH radical scavenging activity

The free radical scavenging activity was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH) activity using a method previously described (Gulcin et al., 2005). The solution of DPPH of 0.3 mM was prepared in ethanol. Five microlitres of each sample of different concentration (62.5 μ g – 500 μ g) was mixed with 95 μ L of DPPH solution in ethanol. The mixture was dispersed in a 96 well plate and incubated at 37 °C for 30 min. The absorbance at 515 nm was measured by microtitre plate reader (Spectramax plus 384 Molecular Device, USA) and the percent radical scavenging activity was determined in comparison with the methanol treated control (Basar et al., 2015). BHA (Butylhydroxyanisole) was used as standard.

3.6. Urease inhibition assay

Reaction mixtures comprising 25 μ L of enzyme (Jack bean Urease) solution and 55 μ L of buffers containing 100 mM urea were incubated with 5 μ L of test compounds (1 mM concentration) at 30 °C for 15 min in 96-well plates (Mehta et al., 2003). Urease activity was determined by measuring ammonia production using the indophenol method as described by Weatherburn. Briefly, 45 μ L each of phenol reagent (1 % w/v phenol and 0.005 % w/v sodium nitroprusside) and 70 μ L of alkali reagent (0.5 % w/v NaOH and 0.1 % active chloride NaOCl) were added to each well. The increasing absorbance at 630 nm was measured after 50 min, using a microplate reader (Molecular Device, USA). All reactions were performed in triplicate in a final volume of 200 μ L. The results (change in absorbance per min) were processed by using SoftMax Pro software (Molecular Device, USA). Assays were performed at pH 8.2 (0.01 M K₂HPO₄·3H₂O, 1 mM EDTA and 0.01 M LiCl₂). Percentage inhibition was calculated from the formula $100 - (OD_{\text{testwell}} / OD_{\text{control}}) \times 100$, where OD stands for optical density. Thiourea was used as the standard inhibitor of urease.

3.7. Anticancer activity

The cytotoxic capability of compounds was evaluated on oral cancer (CAL-27) and normal mouse fibroblast (NIH-3T3) cells as described previously (Farooq et al., 2017). Cells were seeded at density 15,000 cells per well in 96 well microtiter plate and incubated overnight. After 24 hours of incubation, cells were treated with compounds at different concentrations in the range of 1.95 – 250 μ g and incubated for 48 h. After incubation, 10 μ L MTT dye was added in each well and incubated for further 4 h to evaluate metabolically active cells. The formazan crystals were then solubilized in DMSO and absorbance was measured at 570 nm. Percent inhibition of cells was calculated by using the following equation:

$$\text{cell proliferation inhibition (\%)} = 100 - \left[\frac{(\text{OD compound} - \text{OD Blank})}{(\text{OD Control} - \text{OD Blank})} \times 100 \right]$$

IC₅₀ of the compounds were calculated by using software (EZ-Fit Enzyme Kinetics; Perrella Scientific).

4. Conclusion

As a result of the phytochemical studies on the whole plant and fruits of *Momordica foetida*, three previously unreported cucurbitane triterpenes, have been isolated along with three known secondary metabolites and their biological activities have also been evaluated. Among these, compound 1 is found to be active against enzyme urease besides being significantly antioxidative and also showed anti-proliferative activity indicating its role as therapeutic against cancer and therefore suggested to conduct further pharmacological studies for its use in future for drug development.

Declaration of Competing Interest

None

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.phytol.2020.05.010>.

References

- Chen, Jian-Chao, Wu-Qing, Liu, Lu, Lu., Ming-Hua, Qiu, Yong-Tang, Zheng, Liu-Meng, Yang, Xian-Min, Zhang, Lin, Zhou, Zhong-Rong, Li, 2009. Kuguacins F–S, cucurbitane triterpenoids from *Momordica charantia*. *Phytochemistry* 70, 133–140. <https://doi.org/10.1016/j.phytochem.2008.10.011>.
- Chen, Jian-Chao, Lu, Lu., Zhang, Xian-Ming, Lin, Zhou, Zhong-Rong, Li, Ming-Hua, Qiu, 2008. eight new cucurbitane glycosides, kuguaglycosides A–H, from the root of *Momordica charantia* L. *Helv. Chim. Acta* 91, 920–929. <https://doi.org/10.1002/hlca.200890097>.
- Chang, Chi-I, Chen, Chiy-Rong, Liao, Yun-Wen, Cheng, Hsueh-Ling, Chen, Yo-Chia, Chou, Chang-Hung, 2008. Cucurbitane-type triterpenoids from the Stems of *Momordica charantia*. *J. Nat. Prod.* 71, 1327–1330. <https://doi.org/10.1021/np070532u>.
- Cho, E.J., Choi, J.Y., Lee, K.H., Lee, S., 2012. Isolation of antibacterial compounds from *Parasenecio pseudotaimingasa*. *Hortic., Environ., and Biote.* 53, 561–564. <https://doi.org/10.1007/s13580-012-0040-4>.
- Mulholland, Dulcie A., Sewram, Vikash, Osborne, Roy, Pegel, Karl H., Connolly, Joseph D., 1997. Cucurbitane triterpenoids from the leaves of *Momordica foetida*. *Phytochemistry* 45 (2), 391–395. [https://doi.org/10.1016/S0031-9422\(96\)00814-X](https://doi.org/10.1016/S0031-9422(96)00814-X).
- Farooq, U., Naz, S., Zehra, B., Khan, A., Ali, S.A., Ahmed, A., Sarwar, R., Bukhari, S.M., Rauf, A., Ahmad, I., Mabkhot, Y.N., 2017. Isolation and characterization of three new anti-proliferative Sesquiterpenes from *Polygonum barbatum* and their mechanism via apoptotic pathway. *BMC Cancer* 17 (694), 01–13. <https://doi.org/10.1186/s12885-017-3667-9>.
- Froelich, S., Onegi, B., Kakooko, A., Siems, K., Schubert, C., Jenett-Siems, K., 2007. Plants traditionally used against malaria: phytochemical and pharmacological investigation

- of *Momordica foetida*. Braz J. Pharmacogn 17 (1), 01–07. <https://doi.org/10.1590/S0102-695X2007000100002>.
- Hakizamungu, E., Vanpuyvelde, L., Wery, M., 1992. Screening of Rwandese medicinal plants for anti-trichomonas activity. J. Ethnopharmacol. 36, 143–146. [https://doi.org/10.1016/0378-8741\(92\)90014-I](https://doi.org/10.1016/0378-8741(92)90014-I).
- Huh, G.W., Park, J.H., Shrestha, S., Lee, Y.H., Ahn, E.M., Kang, H.C., Baek, N.I., 2011. Sterols from *Lindera glauca* Blume stem wood. J. Appl. Biol. Chem. 54, 309–312. <https://doi.org/10.3839/jabc.2011.050>.
- Liu, Jie-Qing, Chen, Jian-Chao, Wang, Cui-Fang, Qiu, Ming-Hua, 2009. New Cucurbitane Triterpenoids and Steroidal Glycoside from *Momordica charantia*. Molecules 14, 4804–4813. <https://doi.org/10.3390/molecules14124804>.
- Lee, J.M., Lee, D.G., Lee, K.H., Cho, S.H., Nam, K.W., Lee, S., 2013. Isolation and identification of phytochemical constituents from the fruits of *Acanthopanax senticosus*. Afr. J. Pharm. Pharmacol. 7, 294–301. <https://doi.org/10.5897/AJPP12.898>.
- Nantia Akono, Edouard, Soh, Desire, Aphrodite, Choumessi T., Miriam, Ngum N.N., Nkwenti, Chi H.A., Augustave, Kenfack, 2018. *In vitro* antioxidant property of the methanol extracts of the whole plant and fruit of *Momordica foetida* (Cucurbitaceae). The Pharmaceutical and Chemical Journal 5 (6), 117–125. www.tpcj.org.
- Rubinstein, I., Goad, L.J., Clague, A.D.H., Mulheim, L., 1976. The 220 MHz NMR spectra of phytosterols. Phytochemistry 15, 195–200. [https://doi.org/10.1016/S0031-9422\(00\)89083-4](https://doi.org/10.1016/S0031-9422(00)89083-4).
- Hanno, Schaefer, Susanne, Renner S., 2010. A three-genome phylogeny of *Momordica* (Cucurbitaceae) suggests seven returns from dioecy to monoecy and recent long-distance dispersal to Asia. Mol Phylogenet Evol. 54 (2), 553–560. <https://doi.org/10.1016/j.ympev.2009.08.006>.