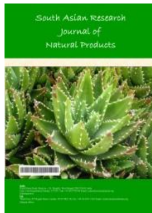

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Steroidal Saponins from *Solanum torvum* Swartz Collected in Dibombari, Cameroon

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Authors' contributions

This work was carried out in collaboration among all authors. Author ABFL designed the study, performed the extraction, fractionation and isolation of compounds. Author JLS worked on literature search, reviewed the study and wrote the final draft of manuscript. Authors JPLE and AAF wrote the protocol and the first draft of the manuscript. Author ML performed the biological studies. Author MSA conducted the NMR experiments. Authors JDW, NS, ENH and AFKW managed the analyses of the study, read and revised the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This work aimed to investigate the phytochemical constituents of Cameroonian species of *Solanum torvum* Swartz and to carry out. Antioxidant, enzyme inhibition (urease and glucosidase) and antibacterial activities of methanol crude extract and isolated compounds.

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Methodology: The stems of *Solanum torvum* were collected and extracted by maceration in methanol. The crude extract was subjected to repeated column chromatographic separation. Their structures were elucidated on the basis of spectral analysis of ESI-MS, 1D and 2D NMR.
The methanol crude extract and pure compounds were tested against *Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Salmonella typhi, Klebsiella pneumonia, Staphylococcus aureus*, *Streptococcus faecalis, Micrococcus sp.* and *Saccharomyces cerevisiae* using the method of disk diffusion. The radical scavenging (DPPH) and the enzyme inhibition (urease and glucosidase) were perfomed according to the standards methods
Results: One new compound neochlorogenin-6-*O*-*β*-*D*-xylopyranosyl-(1→3)-*α*-*D*-quinovopyranoside, together with eight known compounds including four steroidal derivatives, neochlorogenin-6-*O*-*β*-*L*-rhamnopyranosyl-(1→3)-*β*-D-quinovopyranoside, yamogenin-3-*O*-*β*-*D*-glucopyranosyl-(1→6)-*O*-*β*-*D*-glucopyranoside, diosgenin, chlorogenin; three phytosterols stigmasterol, *β*-sitosterol, *β*-sitosterol-3-*O*-*β*-*D*-glucopyranoside and one pentacyclic derivative, betullinic acid were isolated from the stems of *Solanum torvum*.. Diosgenin was isolated from *S. torvum* for the first time. All the tested compounds were found to be inactive while methanol crude extract showed moderate urease and significant glucosidase inhibition activities with IC_{50} = 61.2± 0.68 and 32.5± 0.87 μM respectively.
Conclusion: These results suggested that *Solanum torvum* might be used as an enzyme inhibition agent particularly for alpha glycosidase inhibition.

Keywords: Solanaceae; Solanum torvum; steroidal saponins; biological activities.

1. INTRODUCTION

Solanum torvum Swartz is a small shrub belonging to the Solanaceae family and is commonly called turkey berry. It is widely distributed in various parts of Cameroon. *Solanum torvum* Swartz is used in Africa folk medicine particularly in Cameroon, to cure numerous ailments. For instance, the fruits are used against cough, liver complaints and spleen [1]. The fruits and leaves are used in the treatment of pains, fever and have antioxidants properties, but also in decoction as a tonic and hemopoietic agents [2]. Furthermore, the plant is sedative, diuretic and the leaves are used as hemostats. Additionally, this plant is used as a poison antidote, wounds, dental caries and arterial hypertension [3]. Several phytochemical studies reported steroidal glycosides as main constituents of *Solanum torvum* Swartz [4-9] with an important range of biological activities including antioxidant [10], antibacterial [11], antiviral [6], analgesic, anti-inflammatory [12] and cytotoxic [7,8].

In the present study, we report the isolation and characterization of one new steroidal saponins, neochlorogenin -6-*O*-*β*-*D*-xylopyranosyl-(1→3)-*α*-*D*-quinovopyranoside (1) together with eight known compounds.

2. MATERIALS AND METHODS

2.1 Plant Material

The stems of *Solanum torvum* were collected in the coastal region of Cameroon, precisely in the locality of Dibombari in August 2018 with the geolocation of 4°06′49″N, 9°34′46″E. The whole plant was identified by the botanist Mr. Victor Nana and a voucher sample was deposited at the National Herbarium of Cameroon under reference number 44263 HNC.

2.2 Extraction and Isolation of Compounds

The air-dried powdered stem of *Solanum torvum* (3.75 kg) was macerated with methanol at room temperature for 72h. The solvent was removed using a rotary evaporator to afford crude extract (60.0 g). The crude extract was subjected to silica gel column chromatography and eluted with a gradient of EtOAc in *n*-hexane from 100:0 to 0:100 (v/v) to afford mainly 3 sub-fractions (A1–A3). Sub-fraction A1[200.0 mg, EtOAc – MeOH (3:1, v/v)], was further chromatographed on a silica gel column and eluted with an isocratic solvent system of EtOAc – MeOH (3:1, v/v) to obtain compound [1] (10.0 mg), compound 2 (10.0 mg) and compound 3 (8.0 mg). Purification of sub-fraction A2 [130.0 mg, *n*-hexane – EtOAc (3:2, v/v)], by the previous methodology led to compound 4 (10.0mg), compound 5 (13.0 mg), compound 6 (8.0 mg) and compound 7 (14.0

mg). While compound 8 (14.0mg), and compound 9 (10.0 mg), were obtained from A3 [95.0 mg, *n*-hexane – EtOAc (1:3, v/v)] by the same method.

2.3 Structural Identification

FTIR spectra were recorded on a JASCO 302-A spectrophotometer. ESI-MS were recorded on a Finnigan MAT 95 spectrometer (70 eV) with perfluorokerosene as reference substance for ESI-MS. The ^{1}H and ^{13}C NMR spectra were recorded on Bruker AMX 500 NMR spectrometer. Chemical shifts and coupling constants (*J*) were measured in Hz. Chromatographic separation was carried out on silica gel (70-230 mesh, Merck). Purity of compounds was checked by Thin layer chromatography (TLC) using Merck precoated aluminium foil (silica gel 60 F_{254}) and spots were detected using diluted sulfuric acid spray reagent followed by heating.

2.4 Determination of DPPH Radical Scavenging Activity

The free radical scavenging activity was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH) using the method described by Gulcin et al. [13].

2.5 Urease Assay and Inhibition

Reaction mixtures made as follows : 25 µL of enzyme (Jack bean Urease) solution and 55 µL of buffers containing 100 mM urea. These mixtures were incubated with 5 µL of test compounds (1 mM concentration) at 30°C for 15 min in 96-well plates [14]. The Urease activity was determined by measuring ammonia production using the indophenol method as described by Weatherburn [15].

2.6 Inhibition of Alpha-glucosidase

Alpha-glucosidase inhibition assay is based on the breakdown of a substrate to produce a colored product, followed by measuring the absorbance over a period of time [16-18].

2.7 Antibacterial Activity by Inhibition Method

Pure culture strains were obtained from the Department of Microbiology University of Karachi, Pakistan. The cultures were maintained on Nutrient and LB media. Gram negative bacteria: *Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Salmonella typhi, Klebsiella pneumonia, Staphylococcus aureus* and Gram-positive bacteria/Yeast: *Streptococcus faecalis, Micrococcus sp., Saccharomyces cerevisiae* were tested.

Antibacterial activity was determined by the method of disc diffusion [19-21].

3. RESULTS AND DISCUSSION

3.1 Phytochemical Study

The methanol extract of the stems of *Solanum torvum* Swartz was separated by repeated column chromatography on silica gel to afford one new derivative and eight known compounds. The structures of known compounds were identified by comparison of spectra data with published values and comparison with authentic samples as Neochlorogenine A (2) [22,23], Torvoside M (3) [7,23,24], Diosgenin (4) [23,24], Chlorogenin (5) [23,25,26], stigmasterol (6) [25], *β*-sitosterol (7) [25], and *β*-sitosterol-3-*O*-*β*-*D*-glucopyranoside (8) [25], betullinic acid (9) [26] respectively.

Compound 1 was obtained as a yellow powder. In (+) ESI-MS, a quasi-molecular ion peak $[M+H]^+$ was observed at *m*/z 711.7 indicating that its molecular weight might be 710, with $C_{38}H_{62}O_{12}$ as the estimated molecular formula. Two more significant peaks appeared at 579.6 $[M+H-132]^+$ and 433.4 $[M+H-132-146]^+$ corresponding to the loses of a xylose unit and xylose-quinovose units respectively.

The ^{1}H NMR data (Table 1) exhibited signals for two tertiary methyl groups [δ_H 0.84 (s, H-18); 0.91 (s, H-19)], two secondary methyl groups [δ_H 1.03 (d, *J*=7Hz, H-21); 1.17 (d, *J*=7.1Hz, H-27)], two typical diastereotopic proton of an oxymethylene signal[δ_H 3.33 (m, H_b-26); 3.97 (m, H_a-26)] and two oxymethyne signals [δ_H 4.45 (m, H-16); 3.48 (m, H-3)], characteristic of a spirostanol derivative [22]. The signals of one more oxymethyne was observed at δ_H 3.40 (m) as those of two anomeric protons at δ_H 4.30 (d, *J* = 7.9 Hz) and 5.17 (d, *J* = 1.8Hz). The methyl at δ_H 1.33 (dd, *J* = 6.2; 1.9Hz) is attributable to the quinovose unit.

In the ^{13}C NMR spectrum of compound **1** (Table 1), the signals at δc 51.3 (C-5), 79.3 (C-6), 110.7 (C-22), 27.9 (C-25), 65.7 (C-26) and also of 30.4 (C-2), 70.4 (C-3) and 32.3 (C-4) allowed the identification of (25*S*)-5-spirostan-3,6-diol or neochlorogenin as the aglycon of (1) [23].

Table 1. ^1H NMR and ^{13}C NMR data for aglycon moiety of compound 1 (δ in ppm, *J* in Hz)

N°	1	
	δ_C	δ_H
1	37.9	1.05(m) ; 1.70(m)
2	30.4	1.43 (m) ; 1.78 (m)
3	70.4	3.48 (m)
4	32.3	2.02 (m) ; 2.40 (m)
5	51.3	1.20 (m)
6	79.3	3.40 (m)
7	40.1	0.98 (m) ; 2.20 (m)
8	33.6	1.36 (m) ; 1.87 (m)
9	54.4	0.74 (m)
10	36.6	-
11	20.7	1.34 (m) ; 1.56 (m)
12	40.5	1.20 (m) ; 1.78(m)
13	41.4	-
14	56.9	1.22 (m)
15	31.5	1.36 (m) ; 1.87 (m)
16	81.8	4.45 (m)
17	63.1	1.79 (m)
18	16.8	0.84 (s)
19	13.7	0.91 (s)
20	42.8	1.89 (m)
21	14.7	1.03 (d, *J* = 7Hz)
22	110.7	-
23	26.5	1.38 (m) ; 1.94 (m)
24	26.3	1.48 (m) ; 2.09 (m)
25	27.9	1.72 (m)
26	65.7	3.33(m) ; 3.97 (m)
27	16.2	1.12 (d, *J*=7.1Hz)

Table 2. ^1H NMR and ^{13}C NMR data of sugars moiety of compound 1 (δ in ppm, J in Hz)

N°	1	
	Quinovose	
	δ_C	δ_H
1'	104.8	4.30 (d, *J* = 7.9Hz)
2'	75.9	3.33 (d, *J* = 3.7Hz)
3'	84.2	3.45 (d, *J* = 2.5Hz)
4'	74.2	3.43 (m)
5'	72.6	3.38 (m)
6'	18.2	1.33 (d, *J* = 6.2 Hz)
	Xylose	
1"	102.4	5.17 (d, *J* = 1.8Hz)
2"	71.9	3.98 (d, *J* = 2.5HZ)
3"	77.2	3.31 (d, *J* = 3.6Hz)
4"	71.9	3.72 (dd, *J* = 9.5; 3.4Hz)
5"	69.7	4.02 (m)

The position of the sugar residue in compound 1 was defined unambiguously to be at C-6 due to the ^3J correlation observed in the HMBC spectrum (Fig. 2) between the anomeric proton H-1' (δ_H 4.30) of the quinovose unit and the carbon of the aglycon at δ_C 79.3 (C-6). Also, a cross-peak due to the ^3J correlation between the anomeric proton H-1" of xylose at δ_H 5.17 (d, *J* = 1.8 Hz) and the carbon at δ_C 84.2 (C-3') indicated that xylose was the terminal saccharide unit linked to C-3' of the inner quinovose.

The coupling constants (*J* = 7.9 Hz > 7) and (*J* = 1.8 < 7 Hz) for the anomeric protons of quinovose and xylose units respectively suggested that quinovose unit has a *β*-configuration while xylose unit has an *α*-configuration. These NMR data were foundto be closed to those of neochlorogenin-6-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-quinovopyranoside described in the literature [24]. The main difference appeared in the relative configuration of the xylose anomeric proton. Thus, the structure of (1) was elucidated as neochlorogenin-6-*O*-*β*-D-xylopyranosyl-(1→3)-*α*-*D*-quinovopyranoside.

3.2 Biological Assays

Compounds 1, 3, 5 and the methanol crude extract were evaluated for their antioxidant activity, enzyme inhibition (urease and glucosidase) activity (Table 3) and antibacterial activity against seven terrestrial bacteria.

Methanol stems extract and compounds 1, 3, and 5 did not show any antioxidant activity. Methanol crude extract and compounds 1, 3, 5 showed a moderate Urease Inhibition Activity respectively with IC_{50} = 61.2 µM, 59.5 µM, 90.5 µM and 61.2 µM compared to Thiourea (IC_{50} = 22.4 µM). Only methanol crude extract showed significant alpha-Glucosidase Inhibition activity with IC_{50} = 32.5 µM compared to the positive control of DNJ (1-deoxynojirimycin) with an IC_{50} = 39 µM, while compounds 1, 3, 5 showed moderate alpha-Glycosidase Inhibition activity respectively with IC_{50} = 48.5 µM, 48.0 µM and IC_{50} = 48.10± 0.37 µM with the same positive control. Methanol crude extract and compounds 1, 3, 5 showed no antibacterial activity against all microorganisms tested.

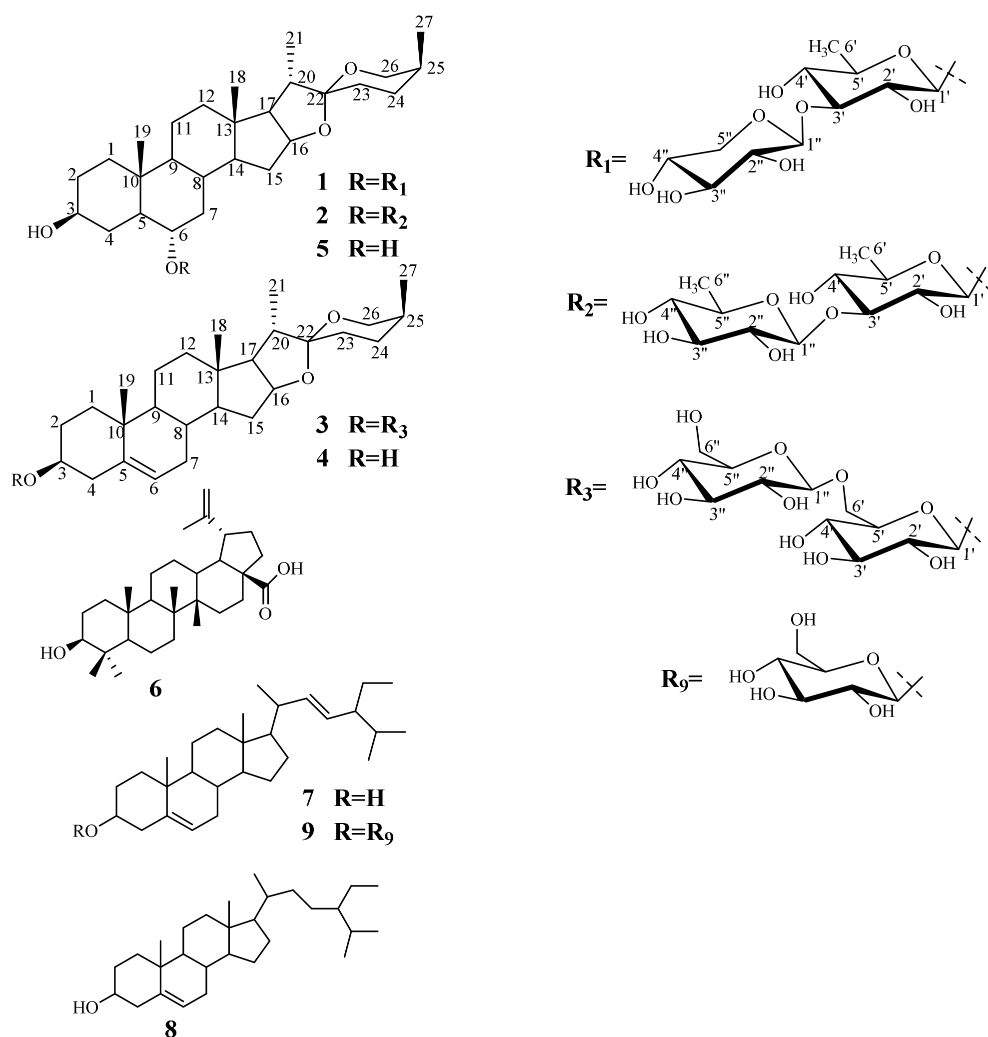

Fig. 1. Chemical structures of isolated compounds 1–9

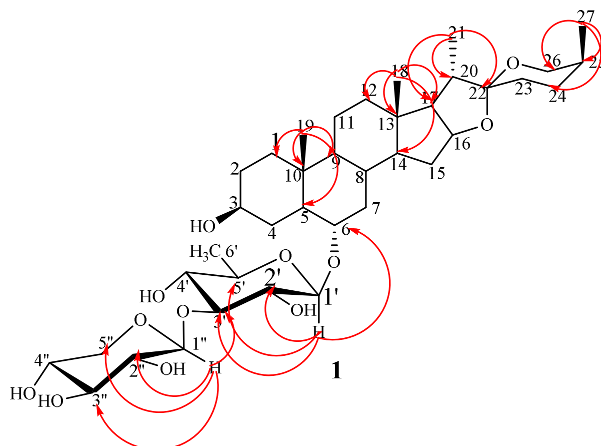

Fig. 2. HMBC correlations of compound 1

Table 3. Antioxidant activity and enzyme inhibition activities of compounds 1, 3, 5 and methanol crude extract

Extract / Compounds	IC_{50} ± SEM (uM)		
	Antioxidant activity	**Urease inhibition**	**Glycosidase inhibition**
Stems	-	61.20± 0.68	32.50± 0.87
1	-	59.50± 0.24	48.50± 0.44
3	-	90.50± 0.32	48.00± 0.11
5	-	61.20± 0.67	48.10± 0.37
BHA	44.20 ± 0.24	-	-
Thiourea	-	22.40 ± 0.24	-
DNJ (1-deoxynojirimycin)	-	-	39.00 ± 0.71

4. CONCLUSION

Phytochemical study of the stem of *Solanum torvum* gives one new compound together with eight know compounds in accordance with the chemotaxonomy in this plant family. Methanol crude extract and compounds 1, 3 and 5 exhibiting moderate enzyme inhibition activity. The biological results of our present study suggest that this plant can be used as an enzyme inhibition agent particularly for alpha glycosidase inhibition n. Since *Solanum torvum* is a medicinal plant, for optimization of its use, it may be appropriate to undergo more biological assays.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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