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α-Glucosidase inhibitory constituents from *Chrozophora plicata*

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ABSTRACT

Five new secondary metabolites have been isolated from *Chrozophora plicata* including an acacetin derivative (**1**), three pyrrole alkaloids plicatanins A–C (**2**–**4**, resp.) and the bilactone plicatanone (**5**). Together with these compounds, the known compounds, β-sitosterol (**6**), methyl p-coumarate (**7**), 4-hydroxyphenylacetic acid (**8**), succinic acid (**9**), speranberculatine A (**10**), β-sitosterol-3-O-β-D-glucopyranoside (**11**) and apigenin-5-O-β-D-glucopyranoside (**12**) have also been isolated. The structures of isolates **1**–**12** were established by 1D (^{1}H, ^{13}C) and 2D NMR (HMQC, HMBC, COSY) spectroscopy and mass spectrometry (EIMS, HREIMS, FABMS, HRFABMS). The structure of plicatanin A (**3**) was further confirmed through single crystal X-ray technique. Compounds **1**–**12** were evaluated for their inhibitory activity against the enzyme yeast α-glucosidase. The compound **4** was found to be most potent with IC_{50} value 27.8 μM.

1. Introduction

The genus *Chrozophora* (Euphorbiaceae) comprises 11 species mostly shrubs and under shrubs distributed in Pakistan, India, West Africa and Mediterranean regions. One of these is *Chrozophora plicata* which grows in warmer climate and temperate regions (Chopra, 1988; Forster and Welzem, 1999). It possesses emetic, drastic and corrosive properties. Its seeds are used as cathartic (Gamble, 1967). The plant poisoning causes salivation, dyspnea, bloat, dullness, diarrhea, paresis of the hind limbs, recumbency and lateral deviation of the head and neck (Galal and Adam, 1988). Literature survey revealed the presence of diterpenoids (Mohamed et al., 1994), triterpenoids (Tanira et al., 1994), flavonoids (Hashim et al., 1990) and chromone glucosides (Agarwal and Singh, 1988) in different species of the genus *Chrozophora*, while hydrocarbons, cholesterol, stigmasterol, β-sitosterol, β-amyrin, squalene, octacosanol, hexacosanol and tetracosanol have previously been reported from *C. plicata* (Radwan et al., 2000). In the present investigation, the methanolic extract of the whole plant of *C. plicata* showed inhibitory activity against the yeast α-glucosidase which prompted us to carry out further phytochemical studies on this plant. Herein we report the isolation and structure elucidation of five new secondary metabolites **1–5** and seven known compounds **6–12**. All of these were found to inhibit the enzyme α-glucosidase with IC_{50} values ranging between 27.8 and 287.1 μM while compound **9** was inactive.

α-Glucosidase (EC 3.2.1.20) comprises a family of enzymes, hydrolases, located in the brush-border surface membrane of small intestinal cells. Its major function is to hydrolyze the glycosidic linkage and produce glucose and other monosaccharide (Hirsh et al., 1997; Chiba, 1997). α-Glucosidase inhibitors are used as oral anti-diabetic drugs for patients with type-2 diabetic mellitus. Postprandial hyperglycemia has a vital role in the development of type-2 diabetes and complications associated with disease such as nephropathy, neuropathy, micoangiopathy, macroangipathy (Baron, 1998; Bonora and Muggeo, 2001). The inhibitors of this enzyme can retard the liberation of glucose and delay glucose absorption, resulting in reduced postprandial hyperglycemia (Lebovitz, 1997; Puls et al., 1984). Therefore, inhibition of α-glucosidase is considered important in managing type-2 diabetes. Acarbose, voglibose and miglitol are commerical α-glucosidase inhibitors that are considered as first-line treatment for diabetic individuals with post-prandial hyperglycemia. α-Glucosidase from *Saccharomyces cereviciae* is used routinely preliminary *in vitro* studies in the assay because of the structural and functional similarities between the yeast (eukaryote) and mammalian enzyme. The objective of present study is to identify new metabolites which can inhibit yeast α-glucosidase and be considered as target molecule for future anti-diabetic drugs.

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2. Results and discussion

Compound **1** was isolated as yellow amorphous powder. The HRFABMS (*+ve* mode) exhibited quasi molecular ion peak $[M+H]^+$ at *m/z* 739.2250 corresponding to the molecular $C_{37}H_{39}O_{16}$ with 19 degree of unsaturation. The IR spectrum displayed absorption bands for hydroxyl (3344 cm^{-1}), conjugated ester (1714 cm^{-1}), conjugated ketone (1687 cm^{-1}) and aromatic (1610–1525 cm^{-1}) functionalities, whereas UV spectrum showed absorption maxima at 265 and 351 nm indicating the presence of acacetin nucleus (Marin et al., 2001). The ^1H NMR spectrum of **1** (Table 1) displayed two pairs of *ortho*-coupled doublets [δ 8.05 (2H, d, J = 8.1 Hz), 7.51 (2H, d, J = 8.1 Hz) and 7.18 (2H, d, J = 8.4 Hz), 6.60 (2H, d, J = 8.4 Hz)] attributed to two *para*-substituted benzene rings. The first pair could be assigned to the ring B of acacetin nucleus, while the other was due to a *p*-coumaroyl moiety, which was further substantiated by the signals of an *E*-olefin at δ 7.56 (1H, d, J = 16.4 Hz) and 6.45 (1H, d, J = 16.4 Hz). The two *meta*-coupled doublets at δ 6.83 (1H, d, J = 1.2 Hz) and 6.47 (1H, d, J = 1.2 Hz) could be assigned to ring A and the olefinic singlet at δ 6.96 (1H, s) was due to H-3 of acacetin nucleus. The presence of two sugar moieties was evident by the presence of two anomeric signals at δ 5.26 (1H, d, J = 7.5 Hz) and 4.53 (1H, br s) along with their other oxygenated methines in the range of δ 5.05–3.14. The ^{13}C NMR spectrum of **1** (Table 1) was in full agreement with the above data as it displayed signals for acacetin nucleus (δ 182.2, 164.1, 162.7, 161.2, 157.1, 105.6, 103.9, 99.5, 94.9, 55.7), *p*-coumarate (δ 166.2, 159.8, 144.7, 130.3, 125.3, 115.8, 114.7) and two sugar moieties (δ 99.5, 71.2, 77.0, 67.7, 70.7, 65.8; 100.6, 70.4, 70.7, 72.0, 68.5, 17.9). The signals for sugar moieties corresponded to galactose and rhamnose (Fu et al., 2009; Wanjala and Majind, 1999; Yu et al., 1999). Acid hydrolysis provided a binary mixture of aglycones which could be separated and identified as acacetin and *p*-coumaric acid, respectively. The glycones could be separated through preparative thin layer chromatography (PTLC) using EtOAc–MeOH–H_2O–HOAc; 4:2:2:2 as developing solvent and subsequently identified as D-galactose and L-rhamnose through their sign of optical rotation and comparison of retention times of their trimethylsilyl (TMS) ethers with those of standards in gas chromatography (GC). The location of various moieties was accomplished with the help of HSQC and COSY correlations. The attachment of both sugar units were confirmed by HMBC interactions (Table 1) in which H-1″ (δ 5.26) showed correlation with C-7 (δ 162.7), H-1‴ (δ 4.53) correlated with C-6″ of the galactose unit which was confirmed by the downfield shift of C-6″ (δ 65.8). The downfield shift of H-3″ (δ 5.05) of the galactose unit and its HMBC correlation with the carbonyl carbon at δ 166.2 confirmed the attachment of *p*-coumarate moiety at C-3″. Based on these evidence the compound **1** was assigned the structure acacetin-7-*O*-β-D-[α-L-rhamnosyl(1→6)]3″-*p*-coumaroylgalactoside named as plicatanoside (Fig. 1).

Table 1
^1H and ^{13}C NMR data, HMBC and COSY correlations of **1** (CD_3OD; 500, 125 MHz).

Position	δ_H (J = Hz)	δ_C	HMBC (H→C)	COSY (H→H)
2	–	164.1	–	–
3	6.96, s	103.9	2,4,4a,1′	–
4	–	182.2	–	–
4a		105.6	–	–
5	–	161.2	–	–
6	6.47, d (1.2)	99.5	4a,5,7,8	H-6/H-8
7	–	162.7	–	–
8	6.83, d (1.2)	94.9	4a,6,7,8a	H-8/H-6
8a	–	157.1	–	–
1′	–	122.7	–	–
2′,6′	8.05, d (8.1)	128.6	2,1′,3′,4′,5′	H-2′,6′/H-3′,5′
3′,5′	7.51, d (8.1)	114.8	1′,2′,4′,6′	H-3′,5′/H-2′,6′
4′	–	162.3	–	–
1″	5.26, d (7.5)	99.5	7,2″,3″,5″	H-1″/H-2″
2″	3.44, m	71.2	1″,3″,4″	H-2″/H-1″,3″
3″	5.05, m	77.0	1″,2″,4″,5″	H-3″/H-2″,4″
4″	3.41, d (2.5)	67.7	2″,3″,5″,6″	H-4″/H-3″,5″
5″	3.78, m	70.7	1″,3″,4″,6″	H-5″/H-4″,6″
6″	3.80, 3.48, m	65.8	4″,5″,1‴	H-6″/H-5″
1‴	4.53 br s	100.6	6″,2‴,3‴,5‴	H-1‴/H-2‴
2‴	3.44, m	70.4	1‴,3‴,4‴	H-2‴/H-1‴,3‴
3‴	3.48, m	70.7	1‴,2‴,4‴,5‴	H-3‴/H-2‴,4‴
4‴	4.14, m	72.0	2‴,3‴,5‴,6‴,9‴′	H-4‴/H-3‴,5‴
5‴	3.39, m	68.5	1‴,3‴,4‴,6‴	H-5‴/H-4‴,6‴
6‴	1.05, d (6.5)	17.9	4‴,5‴	H-6‴/H-5‴
1‴′	–	125.3	–	–
2‴′,6‴′	7.18, d (8.4)	130.3	1‴′,3‴′,4‴′,5‴′,7‴′	H-2‴′,6‴′/H-3‴′,5‴′
3‴′,5‴′	6.60, d (8.4)	115.8	1‴′,2‴′,4‴′,6‴′	H-3‴′,5‴′/H-2‴′,6‴′
4‴′	–	159.8	–	–
7‴′	7.56, d (16.4)	144.7	1‴′,2‴′,6‴′,8‴′,9‴′	H-7‴′/H-8‴′
8‴′	6.45, d (16.4)	114.7	1‴′,7‴′,9‴′	H-8‴′/H-7‴′
9‴′	–	166.2	–	–
OCH_3	3.85, s	55.7	4′	–

Compound **2** was isolated as colorless amorphous solid. Its UV spectrum showed the absorption bands at 203, 231 and 270 nm and the IR spectrum displayed bands at 3295 (O–H) 2950 (C–H), 1715 (C=O) and 1595 (C=C) cm^{-1}. The HREIMS (*m/z* 157.0385) depicted the molecular formula as $C_6H_7NO_4$ with four DBE. The ^1H NMR spectrum (Table 2) showed two singlets at δ 3.77 and 2.94 whereas the ^{13}C NMR spectra (BB and DEPT) (Table 2) showed six signals for two methyl (δ 51.3, 26.9) and four quaternary carbons (δ 170.2, 165.1, 165.0, 156.8). The chemical shift of both the methyl groups indicated their attachment to oxygen and nitrogen, respectively. The above data was comparable with pyrrole alkaloids (Shi et al., 2000). The cumulative data led to the structure of **2** as N-methyl-4-hydroxy-3-methoxy-2,5-dioxopyrrole and named as plicatanin A.

Compound **3** was isolated as crystalline solid with molecular formula $C_{12}H_{12}N_2O_6$ being established by HREIMS. The ^1H NMR spectrum of **3** was similar to those of **2** whereas its ^{13}C NMR spectra (BB and DEPT) (Table 2) showed six signals comprising of two methyl (δ 60.4, 24.1) and four quaternary carbons (δ 171.9, 166.4, 158.5, 100.1). However, the number of carbons in HREIMS indicated **3** is a symmetrical dimer. The downfield shift of C-4 (δ 100.1) indicated the C–C linkage between C-3 and C-3′ (Shi et al., 2000). The substitution pattern was confirmed through HMBC correlations and found similar to that of **2**. The structure was further confirmed through single X-ray crystallography (Fig. 2). Based on these evidences the compound **3** could be identified as a symmetric bipyrrole alkaloid named as plicatanin B.

The HREIMS of compound **4** deduced its molecular formula as $C_{13}H_{16}N_2O_7$ (*m/z* 312.0970) whereas its UV and IR spectra were similar to those of **2** and **3**. The ^1H NMR (Table 2) showed five singlets at δ 4.06, 3.97, 3.80, 2.97 and 2.77, whereas, the ^{13}C NMR spectra (BB and DEPT) (Table 2) showed thirteen signals comprising five methyl (δ 60.3, 60.0, 54.1, 24.6, 24.0) and eight quaternary carbons (δ 172.5, 172.3, 171.3, 170.3, 168.8, 166.7, 101.8, 88.4). The reduction of one of the amide carbonyl was evident by appearance of a signal of an oxygenated quaternary carbon at δ 88.4. The above data indicated that **4** is the methoxyl derivative of speranberculatine A (Shi et al., 2000). The reduction of the amide carbonyl enabled the connectivity of two pyrrole rings through C-2 and C-3*?*, respectively. In HMBC experiment the three methoxyl signals (δ 4.06, 3.97, 3.80) showed HMBC correlations with downfield carbons at δ 168.8, 171.3, 170.3, respectively. The above data confirmed **4** as a bipyrrole alkaloid named as plicatanin C.

Compound **5** was isolated as colorless amorphous solid. Its IR spectrum showed the peaks at 2929 (C–H) and 1789 cm^{-1} (C=O). The molecular formula $C_8H_{10}O_4$ with four DBE was established by HREIMS, which exhibited molecular ion peak $[M]^+$ at *m/z* 170.0590.

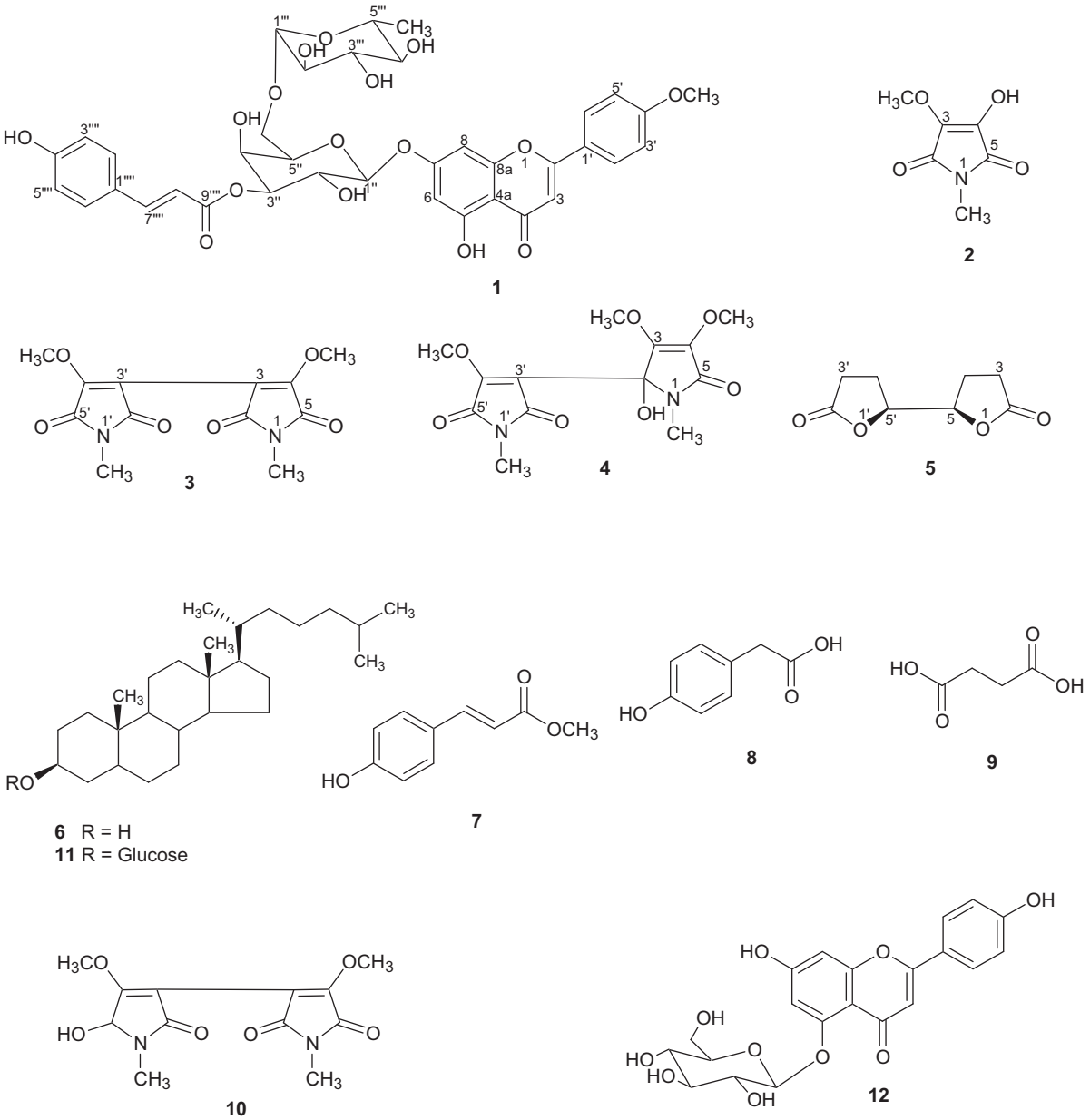

Fig. 1. Structures of compounds (**1–12**) isolated from *Chrozophora plicata*.

The ^{1}H NMR (Table 2) showed signal of an oxygenated methine at δ 5.11 (dd, J = 7.2, 1.3 Hz) and two saturated methylenes 2.45 (dt, J = 17.4, 9.0 Hz), 2.35 (m) and 2.18 (m). The ^{13}C NMR spectra (BB and DEPT) (Table 2) showed four signals for two methylene (δ 29.7, 29.2), one methine (δ 85.0) and a quaternary (δ 181.3) carbons. The COSY correlations between methylenes and oxymethine protons and HMBC interaction of oxymethine proton (δ 5.11) with carbonyl carbon (δ 181.3) as well as the number of carbons suggested the presence of two butanolide rings attached together through their oxymethine carbons. The bislactone **5** was named as plicatanone. Its melting point (104–105 °C) and optical rotation ($[\alpha]_D^{25}$ 0°) were in conformity to the *meso*-bis-γ-lactone which is synthetically known (Vekemans et al., 1990) but reported for the first time as a natural product.

2.1. α-Glucosidase inhibition of compounds *1–12*

The compounds **1–12** were evaluated for inhibitory activity against the enzyme yeast α-glucosidase and the results are shown in Table 3. All the isolates except **9** were inhibitors of α-glucosidase with IC_{50} values ranging between 27.8 and 287.1 μM. The compound **4** was found to be a potent inhibitor with IC_{50} value 27.8 μM.

3. Experimental

3.1. General experimental procedures

Column chromatography was carried out using silica gel of 70–230 and 230–400 mesh size. Aluminum sheets pre-coated with silica gel 60 F_{254} (20 cm × 20 cm, 0.2 mm thick; E-Merck) were used for TLC to check the purity of the compounds, which were either visualized under UV light (254 and 366 nm) or by spraying with ceric sulfate followed by heating. The UV spectra were recorded on a Hitachi UV-3200 spectrometer (λ_{max} in nm). IR spectra were recorded on Shimadzu IR-460 spectrophotometer (ν in cm^{-1}). EIMS, FABMS and HRFABMS spectra were recorded on JEOL JMS-HX 110 spectrometer with data system. The ^{1}H NMR

Table 2
^{1}H and ^{13}C NMR data of **2–5** (CD_3OD; 500, 125 MHz).

Pos	**2**		**3**		**4**		**5**	
	δ_H (J = Hz)	δ_C	δ_H (J = Hz)	δ_C	δ_H (J = Hz)	δ_C	δ_H (J = Hz)	δ_C
1	–	–	–	–	–	–	–	–
2	–	170.2	–	166.4	–	88.4	–	181.3
3	–	165.0	–	100.1	–	170.3	2.35, m	
2.18, m	29.7							
4	–	156.8	–	158.5	–	171.3	2.45, dt (17.4, 9.0)	29.2
5	–	165.1	–	171.9	–	172.3	5.11, dd (7.2, 1.3)	85.0
1′	–	–	–	–	–	–	–	–
2′	–	–	–	166.4		166.7	–	181.3
3′	–	–	–	100.1		101.8	2.35, m	
2.18, m	29.7							
4′	–	–	–	158.5		168.8	2.45, dt (17.4, 9.0)	29.2
5′	–	–	–	171.9		172.5	5.11, dd (7.2, 1.3)	85.0
$-NCH_3$	2.94, s	26.9	2.98, s	24.1	2.77, s	24.0	–	–
$-NCH_3$	–	–	–	–	2.97, s	24.6	–	–
$-OCH_3$	3.77, s	51.3	4.09, s	60.4	3.80, s	54.1	–	–
$-OCH_3$	–	–	–	–	3.97, s	60.3	–	–
$-OCH_3$	–	–	–	–	4.06, s	60.0	–	–

spectra were recorded on Bruker AMX-400 MHz instruments using TMS as an internal reference. The chemical shift values are reported in ppm (δ) units and the scalar coupling constants (J) are in Hz. The ^{13}C NMR spectra were recorded at 100 MHz on the same instrument. The 2D NMR data was also measured on the same instrument operating at 400 MHz.

3.2. Plant material

The fresh whole plant of the *C. plicata* (20 kg) was collected from Cholistan Desert (District Bahawalpur) in April 2008 and was identified by Dr. Muhammad Arshad (Late), Plant Taxonomist, Cholistan Institute of Desert Studies (CIDS), The Islamia University of Bahawalpur, Pakistan where a voucher specimen is deposited (CP-313/08).

3.3. Extraction and isolation

The shade dried, ground whole plant of the *C. plicata* (20 kg) was extracted with MeOH (3 × 50 L). The combined methanolic extract (550 g) was partitioned between water, *n*-hexane (90 g), dichloromethane (95 g), ethyl acetate (55 g), *n*-butanol (145 g) and water soluble (165 g) fractions. The dichloromethane (DCM) soluble fraction (95 g) was subjected to column chromatography over silica gel eluting with *n*-hexane, *n*-hexane:DCM, DCM, DCM:methanol and methanol in increasing order of polarity to obtain ten fractions (A–J). The fraction A (15 g) obtained from *n*-hexane–DCM (8.5:1.5) was chromatographed over silica gel eluting with gradient of *n*-hexane–DCM (8.2:1.8) to furnish **5** (15 mg) from the head and β-sitosterol (2.5 g) from the tail fractions. The fraction B (5 g) obtained at *n*-hexane–DCM (7.5:2.5) showed three spots on TLC which on further purification using CC and elution with same solvent system afforded 4-hydroxybenzaldehyde (33 mg) from the head, methyl *p*-coumarate (68 mg) from the middle and scopoletin (60 mg) from the tail fractions, respectively. The fraction C (50 mg) obtained from *n*-hexane–DCM (7:3) afforded (22 mg). The fraction D (1.7 g) obtained from *n*-hexane–DCM (6.5:3.5) was a binary mixture which on repetition of CC resulted in the isolation of 4-hydroxyphenylacetic acid (34 mg) and succinic acid (76 mg), respectively. The fraction E (1.5 g) obtained from *n*-hexane–DCM (4:6) showed three spots on TLC which on further purification by CC afforded **2** (29 mg) from the head, **4** (17 mg) from the middle and speranberculatine A (32 mg) from the tail fractions, respectively. The fraction G (2.5 g) obtained

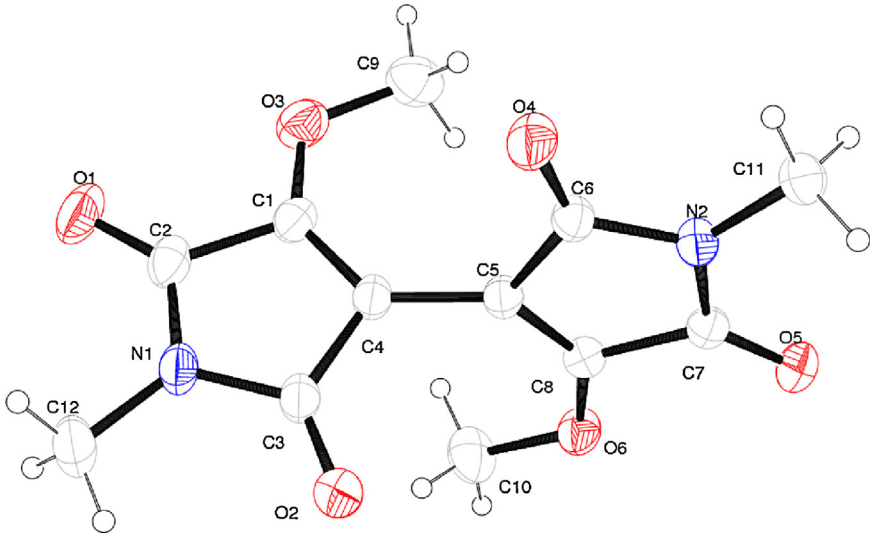

Fig. 2. An ORTEP diagram of **3**.

Table 3
α-Glucosidase inhibition of compounds **1–12**.

Compound[a]	Percentage inhibition (%) at 0.5 mM	IC_{50} (μM)
1	90.23 ± 1.75	111.23 ± 0.65
2	89.17 ± 1.75	202.3 ± 0.33
3	89.95 ± 2.35	178.62 ± 0.78
4	96.54 ± 0.96	27.85 ± 0.75
5	40.47 ± 0.85	>500
6	42.37 ± 0.04	277.7 ± 0.003
7	99.59 ± 0.05	54.15 ± 0.005
8	98.12 ± 0.24	27.42 ± 0.15
9	–	–
10	95.14 ± 0.96	57.15 ± 0.44
11	87.15 ± 0.09	258.71 ± 0.07
12	85.27 ± 0.74	287.12 ± 0.75
Acarbose	92.23 ± 0.14	38.25 ± 0.12

[a] All compounds were prepared in methanol.
All the measurements were done in triplicate and statistical analysis was performed by Microsoft Excel 2003. Results are presented as mean ± sem.

from DCM:methanol (9.9:0.1) was chromatographed and eluted with DCM:methanol (9.8:0.2) to provide β-sitosterol-3-*O*-β-D-glucopyranoside (75 mg). The fraction H (1.5 g) obtained from DCM:methanol (9.5:0.5) on further purification by CC using the same solvent system yielded apigenin-5-*O*-β-D-glucopyranoside (55 mg). The fraction I (1.5 g) obtained from DCM:methanol (9:1) was chromatographed using same solvent system to furnish **1** (25 mg).

*3.3.1. Plicatanoside {acacetin-7-O-β-D-[α-L-rhamnosyl(1→6)]3″-p-coumaroylgalactoside (**1**)}*

Yellow amorphous solid (25 mg); UV (MeOH) nm: 265 (3.7), 351 (4.1); IR (KBr): 3344, 1714, 1687, 1610–1525 cm^{-1}; ^1H and ^{13}C NMR: see Table 1; HRFABMS: *m/z* 739.2250 $[M+H]^+$, calcd. for $C_{37}H_{39}O_{16}$, 739.2238.

*3.3.2. Plicatanin A (**2**)*

Colorless amorphous solid (29 mg); UV (MeOH) nm: 203 (3.1), 231 (4.15), 270 (3.09); IR (KBr): 3295, 2950, 1715, 1595 cm^{-1}; ^1H and ^{13}C NMR: see Table 3; HREIMS: *m/z* 157.0385 $[M]^+$, calcd. for $C_6H_7NO_4$, 157.0375.

*3.3.3. Plicatanin B (**3**)*

Crystalline solid (22 mg); UV (MeOH) nm: 204 (3.3), 231 (3.5), 274 (3.9); IR (KBr): 3300, 2955, 1717, 1615–1499 cm^{-1}; ^1H and ^{13}C NMR: see Table 3; HREIMS: *m/z* 280.0705 $[M]^+$, calcd. for $C_{12}H_{12}N_2O_6$, 280.0705.

*3.3.4. Plicatanin C (**4**)*

Colorless amorphous solid (17 mg); UV (MeOH) nm: 208 (3.6), 235 (4.1), 280 (3.9); IR (KBr): 3302, 2960, 1719, 1614–1495 cm^{-1}; ^1H and ^{13}C NMR: see Table 3; HREIMS: *m/z* 312.0970 $[M]^+$, calcd. for $C_{13}H_{16}N_2O_7$, 312.0957.

*3.3.5. Plicatanone (**5**)*

Colorless amorphous solid (15 mg); IR (KBr): 2929, 1789 cm^{-1}; ^1H and ^{13}C NMR: see Table 3; HREIMS: *m/z* 170.0590 $[M]^+$, calcd. for $C_8H_{10}O_4$, 170.0579.

3.4. Acid hydrolysis

A solution of compound **1** (8 mg) in MeOH (5 ml) containing 1 N HCl (4 ml) was refluxed for 4 h, concentrated under reduced pressure, and diluted with H_2O (8 ml). The aglycones were extracted with EtOAc (3 × 15 ml). The aqueous phases were concentrated under reduced pressure and subjected to preparative thin layer chromatography using solvent system (EtOAc–MeOH–H_2O–HOAc; 4:2:2:2) and the sugars were identified as D-galactose and L-rhamnose by the sign of their optical rotation ($[\alpha]_D^{20}$ + 80.1) and ($[\alpha]_D^{20}$ + 8.1), respectively. It was also confirmed by comparison of the retention time of their TMS ethers in GC (D-galactose α-anomer 3.0 min, β-anomer 5.2 min and L-rhamnose 8.6 min) with the standards.

3.5. α-Glucosidase inhibition assay

The α-glucosidase (E.C. 3.2.1.20, Cat. No. 5003-1KU Type I lyophilized from *Saccharomyces cereviciae* from Sigma Inc.) inhibition assay was performed with slight modifications as done by Pierre *et al.* (1978). Total volume of 100 μL reaction mixture contained 70 μL 50 mM phosphate buffer, pH 6.8, 10 μL (0.5 mM) test compound, followed by the addition of 10 μL (0.0234 units, Sigma Inc.) enzyme. The contents were mixed, preincubated for 10 min at 37 °C and pre-read at 400 nm. The reaction was initiated by the addition of 10 μL of 0.5 mM substrate, *p*-nitrophenyl-α-D-glucopyranoside (Cat. No. N1377, Sigma Inc.). After 30 min of incubation at 37 °C, absorbance of the yellow color produced due to the formation of *p*-nitrophenol was measured at 400 nm using Synergy HT (BioTek, USA) using 96-well microplate reader. Acarbose (Cat. No. A8980 Sigma Inc.) was used as positive control. The percent inhibition was calculated by the following equation

$$\text{Inhibition}(\%) = \frac{\text{abs of control} - \text{abs of test}}{\text{abs of control}} \times 100$$

IC_{50} values were calculated using EZ-Fit Enzyme Kinetics Software (Perrella Scientific Inc. Amherst, USA).

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