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PHYTOCHEMICAL ANALYSIS OF *CYRTANTHUS OBLIQUUS* BULBS FROM THE INFORMAL STREET MARKET OF KWAZULU-NATAL, SOUTH AFRICA

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Abstract

Background: *Cyrtanthus obliquus* is a medicinal plant commonly known by the locals in South Africa as *Umathunga*. It is used in traditional South African medicine for the treatment of scrofula, chronic coughs and headaches and is also used to prepare the popular decoction known as *Imbiza*.
Materials and methods: The secondary metabolites from *C. obliquus* bulbs were extracted using solvent extraction techniques, isolated using column chromatography and identified using spectroscopic techniques. The antioxidant potential of the isolated compounds was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and the ferric reducing antioxidant potential (FRAP) assay.
Results: The extracts yielded four homoisoflavonoids, 5,7-dihydroxy-6-methoxy-3-(4'-methoxybenzyl)chroman-4-one, 5,7-dihydroxy-3-(4'-hydroxybenzyl)chroman-4-one,5,7-dihydroxy-6-methoxy-3-(4'-methoxybenzylidene)chroman-4-one and 5,7-dihydroxy-3-(4'-hydroxybenzylidene)chroman-4-one. An unusual 3-β-glucopyranosyl-22,27-dihydroxy-lanosta-8-ene was also isolated from the bulbs. The antioxidant studies showed that 3-benzylchromanones have better activity than 3-benzylidenechromanones.
Conclusion: Four homoisoflavanones namely 5,7-dihydroxy-6-methoxy-3-(4'-methoxybenzyl)chroman-4-one; 5,7-dihydroxy-6-methoxy-3-(4'-hydroxybenzyl)chroman-4-one and two 5,7-dihydroxy-6-methoxy-3-(4'-methoxybenzylidene)chroman-4-one; 5,7-dihydroxy-3-(4'-hydroxybenzylidene)-chroman-4-one were obtained from *Cyrtanthus obliquus*.

Key words: Amaryllidaceae, homoisoflavonoids, medicinal plant, traditional medicine

Introduction

A large majority of the world's population utilises traditional medicine in one way or another (IARC, 2002). In South Africa, the biodiversity is quite high which lends itself to a large variety of medicinal plants that are sold commercially, both in conventional and informal markets in the country (Chen et al., 2004). Currently, about 80% of South Africans use traditional medicine to meet their primary healthcare needs (Gqaleni et al., 2007; Mbatha et al., 2012). More than 1000 medicinal plants are used for traditional medicine in the province of KwaZulu-Natal, South Africa, alone (Mander, 1998). *Cyrtanthus obliquus* of the plant family Amaryllidaceae, is one of the medicinal plants commonly purchased from the herbalists in South Africa. This evergreen and bulbous plant is known by the locals as *Umathunga*, and is used in African traditional medicine for the treatment of scrofula, chronic coughs and headaches (Du Plessis and Duncan, 1989; Hutchings et al., 1996; Watt et al., 1962). In South Africa, this plant is also used to prepare the popular decoction known as *Imbiza* (Ndhlala et al., 2009).

Other *Cyrtanthus* species have been used by Black South African women to treat pregnancy-related ailments and by traditional healers to treat patients suffering from cystitis, age-related dementia and leprosy (Herrera et al., 2001; Nair et al., 2011; Veale et al., 1992). Thus far, research has been conducted on the alkaloidal content of *C. obliquus* bulbs only (Brine et al., 2002). Previously, we reported on the antibacterial properties of the fruits and leaves of *Carissa macrocarpa* and the antioxidant activity of the fruits and bark of *Harpephyllum caffrum*, two South African medicinal plant species (Moodley et al., 2011; Moodley et al., 2014). In current study, a phytochemical investigation on the bulbs of the South African medicinal plant, *C. obliquus* was conducted using spectroscopic techniques to determine the other classes of compounds present in the plant. Additionally, the antioxidant activity of the isolated compounds was also evaluated.

Materials and Methods

General experimental procedure

Melting points were recorded on an Ernst Leitz Wetzer micro-hot stage melting point apparatus. Specific rotations were measured at room temperature in methanol (MeOH) on a PerkinElmer™, Model 341 Polarimeter with a 10 cm flow tube. Infrared (IR) spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR spectrometer with Universal ATR sampling accessory. Ultraviolet (UV) spectra were obtained on a UV-Vis-NIR Shimadzu UV-3600 Spectrophotometer in MeOH. All 1-D and 2-D NMR spectra were recorded in deuterated chloroform or deuterated MeOH at room temperature on a Bruker Avance^{III} 400 MHz NMR spectrometer. Liquid-chromatography-mass spectroscopy (LC-MS) was done on an Agilent LC/MSD Trap 1100 Series. High resolution-mass spectroscopy (HR-MS) was done on a Bruker Micro TOF-QII instrument. The UV Spectrophotometer Biochrom Libra S11 was used for antioxidant assays. Merck silica gel 60 (0.040-0.063 mm) was used for column chromatography and Merck silica gel 60, F254 aluminium sheets were utilized for thin layer chromatography (TLC). TLC plates were monitored by analysis under UV (254 nm and 366 nm) and visualized using anisaldehyde spray reagent (97: 2: 1(v/v) MeOH: conc. H_2SO_4: anisaldehyde).

Plant material

Fresh bulbs from *C. obliquus* were purchased at the Berea market in Durban and identified by a curator from the School of Biological and Conservation Sciences, UKZN, Westville and a voucher specimen (Mahlangeni, N1) was deposited in the ward herbarium at UKZN.

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Extraction and isolation

The plant material (2.04 kg) was cut into small pieces, air-dried overnight and then sequentially extracted with hexane, dichloromethane (DCM) and MeOH by continuous shaking on an orbital shaker for 48 h. The crude extracts where evaporated under reduced pressure then subjected to column chromatography and monitored by TLC. Hexane and DCM extracts were combined due to similar TLC profiles. Combined extracts were then separated with a hexane: ethyl acetate (EtOAc) step gradient system starting from 100% hexane to 90% EtOAc. Fractions with similar TLC profiles were combined and concentrated using the rotary evaporator. Compound **1** (71.5 mg) and compound **2** (45.7 mg) was obtained after separation with a hexane: EtOAc (80: 20, v/v) solvent system.

The crude MeOH extract was further fractionated in triplicate with 500 mL DCM then with 500 mL EtOAc for 48 h each. The resulting DCM and EtOAc fractions were concentrated then loaded into separate columns. The column containing the DCM fraction (9.48 g) was separated with a hexane: EtOAc step gradient starting from 100% hexane to 100% EtOAc. Compound **3** (3.9 mg) was obtained from a hexane: EtOAc (80: 20, v/v) solvent system. The EtOAc fraction (3.40 g) was separated by increasing the polarity of DCM with MeOH, starting with 100% DCM until a DCM: MeOH (70: 30, v/v)) solvent system was reached. Compound **5** (3.1 mg) was obtained as a white solid from a DCM: MeOH (80: 20, v/v) solvent system. Fractions 51-64 from this extract were combined and further purified using DCM: MeOH (98: 2, v/v) to yield compound **4** (4.3 mg).

Spectral data

5,7-dihydroxy-6-methoxy-3-(4'-methoxybenzyl)chroman-4-one **(1)** (71.5 mg): Yellow crystals; m.p. 90-92 ˚C; $[\alpha]^{20}$: -0.11˚ (*c* 0.10, MeOH); IR ν_{max} cm^{-1}: 3391, 2932, 1638, 1602, 1461, 1018; UV (MeOH) λ_{max} nm (log ε): 216 (4.20), 292 (3.84), 347 (3.44); ^{1}HNMR ($CDCl_3$, 400MHz) spectral data, see Table 1; ^{13}C-NMR ($CDCl_3$, 400MHz) spectral data, see Table 2; LC-MS (negative mode): *m/z* 329.0 $[M\text{-}H\text{-}H_2O]^-$; HREIMS: *m/z* 330.1286 $[M - H_2O]^-$, (Calc. for $C_{18}H_{18}O_6$).

5,7-dihydroxy-6-methoxy-3-(4'-methoxybenzylidene)chroman-4-one **(2)** (45.7 mg): Orange crystals; m.p. 130-133 ˚C; $[\alpha]^{20}$: +0.01˚ (*c* 0.10, MeOH); IR ν_{max} cm^{-1} :3360, 293, 1636, 1600, 1513, 1460, 1019; UV (MeOH) λ_{max} nm (log ε): 215 (4.15), 360 (3.30); ^{1}H-NMR ($CDCl_3$, 400MHz) spectral data, see Table 1; ^{13}C-NMR ($CDCl_3$, 400MHz) spectral data, see Table 2; LC-MS (negative mode): *m/z*: 329.0 $[M\text{-}OH]^-$; HREIMS: *m/z* 328.1051 $[M\text{-}H_2O]^-$ (Calc. for $C_{18}H_{16}O_6$).

5,7-dihydroxy-6-methoxy-3-(4'-hydroxybenzyl)chroman-4-one **(3)**: Pale yellow needles (3.9 mg); m.p. 194-196 ˚C; $[\alpha]^{20}$: 0˚ (*c* 0.10, MeOH); IR ν_{max} cm^{-1}: 3288, 2945, 2831, 1693, 1586, 1450, 1019; UV (MeOH) λ_{max} nm (log ε): 216 (4.13), 362 (3.47); ^{1}H-NMR ($CDCl_3$, 400MHz) spectral data, see Table 1; ^{13}C-NMR ($CDCl_3$, 400MHz) spectral data, see Table 2; LC-MS (negative mode): *m/z*: 282.9 $[M\text{-}H\text{-}H_2O\text{-}CH_2OH]^-$; HREIMS: *m/z* 315.0961 $[M\text{-}H_2O]^-$ (Calc. for $C_{17}H_{15}O_6$).

5,7-dihydroxy-3-(4'-hydroxybenzylidene)chroman-4-one **(4)**: Yellow crystals (4.3 mg); m.p. 205-207 ˚C; $[\alpha]^{20}$: +0.01˚ (*c* 0.10, MeOH); IR ν_{max} cm^{-1}: 3211, 2942, 2831, 1673, 1587, 1450, 1022; UV (MeOH) λ_{max} nm (log ε): 213 (4.09), 292 (2.88), 362 (3.20); ^{1}H-NMR (MeOD, 400MHz) spectral data, see Table 1; ^{13}C-NMR (MeOD, 400MHz) spectral data, see Table 2; LC-MS (negative mode): *m/z* 282.9 $[M\text{-}H\text{-}H_2O]^-$; HR-MS: *m/z* 284.0830 $[M\text{-}H_2O]^-$ (Calc. for $C_{16}H_{12}O_5$).

3-β-glucopyranosyl-22,27-dihydroxy-lanosta-8-ene **(5)**: White solid (3.1 mg); m.p. 245-248 ˚C;: $[\alpha]^{20}$: +0.02˚ (*c* 0.10, MeOH); IR ν_{max} : 3325, 2943, 2832, 1658, 1449, 1410, 1108, 1019; UV (MeOH) λ_{max} nm (log ε): 213 (4.36), 262 (2.73); ^{1}H-NMR (400MHz, MeOD): δ 0.69 (3H, s, H-18), 0.92 (3H, s, H-35), 0.98 (2H, d, *J* = 10.17Hz, H-6), 0.98 (6H, s, H-31/32), 0.99 (3H, s, H-19), 1.18 (2H, d, *J* = 5.24 Hz, H-5/26), 1.21 (9H, s, H-28/29/33), 1.30 (8H, d, *J* = 14.49 Hz, H-1/2/15/16), 1.37 (4H, s, H-20/30), 1.46 (2H, d, *J* = 4.56 Hz, H-23), 1.57 (1H, d, *J* = 9.21 Hz, H-17), 1.90 (3H, s, H-21), 1.97 (6H, d, *J* = 5.52 Hz, H-7/12/25), 2.04 (2H, s, H-24), 2.06 (2H, s, H-11), 3.16 (1H, d, *J* = 4.72 Hz, H-2'), 3.18 (1H, d, *J* = 4.68 Hz, H-3'), 3.28 (1H, d, *J* = 4.68 Hz, H-4'), 3.29 (1H, d, *J* = 4.68 Hz, H-5'), 3.35 (1H, s, H-3), 3.37 (1H, d, $J_{34\alpha}$ = 11.25 Hz, H-34), 3.41 (1H, d, $J_{34\beta}$ = 11.25 Hz, H-34), 3.69 (1H, dd, $J_{6'\alpha}$ = 11.97, 5.72 Hz, H-6'), 3.88 (1H, dd, $J_{6'\beta}$ = 11.89, 2.12 Hz, H-6'), 4.23 (1H, d, *J* = 7.84 Hz, H-11), 4.91 (1H, s, H-22)**;** ^{13}C-NMR (400MHz, MeOD): δ 135.3 (C-9), 135.2 (C-8), 100.1 (C-1'), 80.7 (C-3), 77.8 (C-5'), 77.7 (C-27/3'), 74.7 (C-2'), 72.2 (C-22), 71.3 (C-4'), 64.7 (C-34), 62.3 (C-6'), 52.1 (C-5/26), 51.9 (C-17), 50.6 (C-14), 45.2 (C-13), 43.1 (C-4), 42.5 (C-23), 37.5 (C-10), 34.4 (C-20/30), 31.8 (C-1/16), 31.4 (C-2/15), 29.0 (C-24), 27.4 (C-7/12/25), 24.1 (C-35), 22.6 (C-28/29/33), 21.6 (C-11), 19.7 (C-19), 19.6 (C-31/32), 19.2 (C-6), 15.8 (C-18), 13.7 (C-21)**;** LC-MS (negative mode): *m/z*: 649.5 $[M\text{-}C_3H_7O]^-$.

Antioxidant activity

Determination of DPPH free radical scavenging capacity

The DPPH free radical scavenging activity was determined according to the method described by Murthy et al. (2012) with some modifications. A volume of 150 µL of methanolic solution of the compound at varying concentrations (10-1000 µg mL^{-1}) was mixed with 2850 µL of the methanolic solution of DPPH (0.1 mM). An equal amount of MeOH and DPPH without sample served as a control. After 30 min of reaction at room temperature in the dark, the absorbance was measured at 517 nm against MeOH as a blank using a UV spectrophotometer. Ascorbic acid was used as a positive control. All procedures were performed in triplicate. The percentage free radical scavenging activity was calculated according to the following equation:

$$\%\ Inhibition = \left(\frac{Ac - As}{Ac} \right) x100$$

Where Ac = Absorbance of control and As = Absorbance of sample

The concentration providing 50% maximal inhibition (IC_{50}) was obtained by plotting a dose-response curve.

Determination of the reducing potential by the FRAP assay

The ferric reducing antioxidant potential (FRAP) assay was determined according to the method by Murthy et al. (2012) with some

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modifications. A 2.5 mL volume of different concentrations of the compounds (10-500 µg mL^{-1}) was mixed with 2.5 mL phosphate buffer solution (0.2 M, pH = 6.6) and 2.5 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$] in test tubes. The mixture was placed in a water bath at 50 ºC for 20 min. A volume of 2.5 mL of 10% trichloroacetic acid (TCA) was added to the mixture and mixed thoroughly. A volume of 2.5 mL of this mixture was then mixed with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ solution (0.1%) and allowed to stand for 10 min. The absorbance of the mixture was measured at 700 nm using a UV-VIS spectrophotometer. Ascorbic acid was used as a positive control. All procedures were performed in triplicate.

Results and Discussion

The ^{1}H-NMR spectrum of compound **1** showed resonances in the aromatic region at δ_H 6.84 (H-3′/5′, d, *J* = 8.52 Hz) and δ_H 7.11 (H-2′/6′, d, J = 8.52 Hz), each integrating to two protons. This was also observed for compound **2** at δ_H 6.87 (H-3′/5′, d, *J* = 9.70 Hz) and δ_H 7.80 (H-2′/6′, d, *J* = 8.80 Hz); compound **3** at δ_H 6.78 (H-3′/5′, d, *J* = 8.40 Hz) and δ_H 7.08 (H-2′/6′, d, *J* = 8.40 Hz) and compound **4** at δ_H 6.88 (H-3′/5′, d, *J* = 8.60 Hz) and δ_H 7.26 (H-2′/6′, d, *J* = 8.60 Hz) also integrating to two protons. This suggested the presence of a mono-substituted aromatic ring for each compound (Fig. 1).

Table 1: ^{1}H-NMR data (compound **1**, **2**, **3** in $CDCl_3$ and **4** in MeOD) (400 MHz; δ_H: ppm; *J*:Hz)

Position	1	2	3	4
1′	-	-	-	-
2′, 6′	7.11, d (8.52)	7.80, d (8.80)	7.08, d (8.40)	7.26, d (8.60)
3′, 5′	6.84, d (8.52)	6.87, d (9.70)	6.78, d (8.40)	6.88, d (8.60)
4′	-	-	-	-
3	2.83, m	-	2.80, m	-
2	4.17 Hα, dd (7.10, 11.39); 4.33 Hβ, dd (4.10, 11.39)	4.96, d (1.04)	4.18 Hα, dd (7.16, 11.41); 4.35 Hβ, dd (4.20, 11.37)	5.32, d (1.72)
9	2.69 Hα, dd (10.39, 13.85); 3.15 Hβ, dd (4.32, 13.85)	6.88, s	2.69 Hα, dd (10.38, 13.85); 3.17 Hβ, dd (4.34, 13.83)	7.74, s
4	-	-	-	-
4a	-	-	-	-
8a	-	-	-	-
8	6.10, s	6.12, s	6.10, s	5.91, d (2.20)
7	-	-	-	-
6	-	-	-	5.84, d (1.76)
5	-	-	-	-
4′-OCH3	3.77, s	3.82, s	-	-
6-OCH3	3.81, s	3.85, s	3.83, s	-

Similarities with resonances between compounds **1** and **3** were observed at H-3, H-2 and H-9. The ^{13}C-NMR and DEPT spectra showed methylene

e resonances at C-9 and C-2, as expected, with the resonance at C-2 shifting more downfield due to the attachment of the oxygen (Table 2). The COSY and NOESY data confirmed the coupling of protons at H-3 and H-9 as well as H-2.

The ^{1}H-NMR and ^{13}C-NMR spectral data were in good agreement with reported homoisoflavanones 5,7-dihydroxy-6-methoxy-3-(4-methoxybenzyl)chroman-4-one (**1**) and 5,7-dihydroxy-6-methoxy-3-(4-hydroxybenzyl)chroman-4-one (**3**) (Adinolfi et al., 1985; Adinolfi et al., 1987) isolated for the first time from *C. obliquus*. Both compounds were unstable with compound **1** losing H_2O (*m/z* 330.1286) and compound **3** losing a proton (*m/z* 315.0961).

The ^{13}C-NMR spectrum for compound **2** showed strong resonances at δ_C 113.46 and δ_C 132.91 which, in the HSQC experiment, correlated to the protons at δ_H 6.87 (H-3′/5′) and δ_H 7.80 (H-2′/6′), respectively. The methoxy group singlet resonating at δ_H 3.82 was attributed to C-4′ in the B ring. The ^{1}H-NMR spectrum showed resonances at δ_H 6.88 (s, H-9) and δ_H 4.96 (d, H-2, *J* = 1.04 Hz) which integrated to two protons. The singlet at δ_H 6.88 (H-9) correlated to the carbon resonance at δ_C 141.09 (C-9) in the HSQC experiment and it correlated to the carbon resonance at δ_C 125.08 (C-3) in the HMBC experiment. The upfield shift of the carbonyl resonance at δ_C 187.02 (C-4) compared to compound **1** confirmed the 3, 9 moiety to be unsaturated. The data obtained for compound **2** compared well with literature values therefore it was identified as 5,7-dihydroxy-6-methoxy-3-(4-

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methoxybenzylidene)chroman-4-one (Adinolfi et al., 1989) which has been isolated for the first time in *C. obliquus*. The compound was seen to be unstable with a molecular ion peak at m/z 328.1051 due to loss of a H_2O molecule.

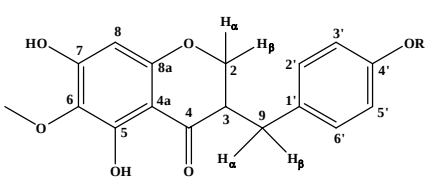
Table 2: ^{13}C-NMR data (compound **1**, **2**, **3** in $CDCl_3$ and **4** in MeOD) (400 MHz; δ_C: ppm)

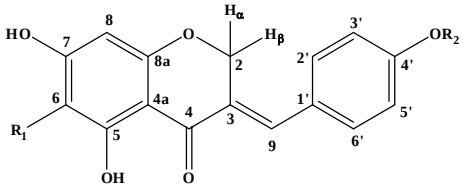
Position	1	2	3	4
1'	129.47	126.39	129.67	126.05
2', 6'	129.87	132.91	130.17	132.67
3', 5'	113.97	113.46	115.48	115.88
4'	158.22	161.05	154.35	159.77
3	46.50	125.08	46.65	127.44
2	69.12	75.50	69.31	67.68
9	31.81	141.09	31.81	137.15
4	197.38	187.02	197.60	185.53
4a	102.19	104.52	102.31	102.58
8a	153.01	152.88	152.88	165.40
8	95.86	96.17	95.77	96.39
7	157.84	157.37	157.61	167.43
6	127.22	127.22	127.15	95.02 (CH)
5	159.96	161.05	160.13	163.04
4'-OCH_3	55.71	55.43	-	-
6-OCH_3	61.36	61.78	61.39	-

Table 3: IC_{50} values of compounds **1**, **2**, **3** and **4** on the DPPH free radical.

Compound	IC_{50} Value (µg/mL)[a]
1	371.54
2	ns[b]
3	288.40
4	ns[b]
Ascorbic acid	27.54

[a] n=3 [b] ns: not significant





	R		R_1	R_2
Compound **1**:	CH_3	Compound **2**:	OCH_3	CH_3
Compound **3**:	H	Compound **4**:	H	H

Figure 1: Chemical structures of compounds **1-4**

The ^{1}H-NMR and ^{13}C-NMR spectra of compound **4** showed slight differences to that of compound **2**, in that there were no methoxy resonances. The ^{1}H-NMR spectrum showed a resonance at δ_H 5.84 (H-6, *J* = 1.76 Hz) that showed weak meta-coupling to the resonance at δ_H 5.91 (H-8, *J* = 2.20 Hz) in the COSY spectrum. The proton at C-6 in compound **4** replaced the methoxy group at C-6 in compound **2**. The methoxy group at C-4' in compound **2** was replaced by a hydroxy group at C-4' in compound **4** which was attached to the quaternary carbon resonance at δ_C 159.77 as confirmed by HMBC correlations. The data obtained for compound **4** compared well with literature values therefore it was identified as 5,7-dihydroxy-3-(4'-hydroxybenzylidene)chroman-4-one (Adinolfi et al., 1989) isolated for the first time in *C. obliquus*. The compound was seen to be unstable with a molecular ion peak at m/z 284.0830 due to loss of H_2O plus an extra proton.

The ^{1}H-NMR spectrum for compound **5** (Fig. 2) showed resonances at δ_H 0.69 (H-18), δ_H 0.92 (H-35), δ_H 0.98 (H-31/32), δ_H 0.99 (H-19), δ_H 1.21 (H-28/29/33) and δ_H 1.91 (H-21) which were all singlets. The protons resonating at δ_H 0.69, δ_H 0.92, δ_H 1.21 and δ_H 0.99 were seen to be attached to unsaturated carbons. The resonances at δ_H 0.98 (H-31/32) and δ_H 1.21 (H-28/29/33) integrated to six and nine protons, respectively therefore two carbons were resonating at δ_C 19.56 (C-31/32) and three where resonating at δ_C 22.60 (C-28/29/33). The HMBC spectrum showed that the proton resonance at δ_H 0.69 (H-18) correlated to the carbon resonances at δ_C 31.41 (C-2/15), δ_C 31.77 (C-1/16), δ_C 45.21 (C-13), δ_C 50.59 (C-14) and δ_C 51.88 (C-17), therefore it was positioned at C-18. The heavily substituted 2-methylheptane side chain was found to be hydroxylated at C-22 and C-27, which led to the shift in the resonance peaks at δ_C 42.50 (C-23) and δ_C 52.14 (C-26). The resonances at δ_C 135.21 (C-8) and δ_C 135.31 (C-9) confirmed a lanosterol skeleton with a substituted methylheptane side chain.

The ^{1}H-NMR spectrum showed resonances at δ_H 3.16 (H-2'), δ_H 3.18 (H-3'), δ_H 3.28 (H-4'), δ_H 3.29 (H-5'), δ_H 3.69 (H-6'α), δ_H 3.88 (H-6'β) and δ_H 4.23 (H-1'). The HSQC spectrum showed that δ_H 3.69 (1Hα, dd, *J* = 5.72, 11.97 Hz) and δ_H 3.88 (1Hβ, dd, *J* = 2.12, 11.89 Hz) were attached to the same carbon at δ_C 62.32 (C-6'). The HSQC spectrum showed that the proton resonances at δ_H 3.16, δ_H 3.29 and δ_H 4.23 correlated to carbon resonances at δ_C 74.69 (C-2'), δ_C 77.82 (C-5') and δ_C 100.07 (C-1'), respectively.

The COSY experiment showed the coupling of protons at δ_H 3.16 (H-2') with δ_H 4.23 (H-1') and protons at δ_H 3.88 (Hβ-6') with δ_H 3.69 (Hα-6'). This led to the assumption of the presence of a glycosidic linkage at the C-3 position. There was weak coupling of the protons on the glucoside as seen from the weak HMBC correlations further up-field, therefore correlations where minimal. The structural deduction of the entire molecule was found from the different experiments performed. The data for compound **5** was compared to NMR data for β-sitosterol glycoside (Akhtar et al., 2010) and many similarities especially at the glycosidic linkage were found. The melting point of the compound was 245-248 ºC which was close to that of β-sitosterol glycoside (252-253ºC) (Akhtar et al., 2010). This confirms the proposed structure of compound **5** which has molecular formula $C_{41}H_{72}O_9$ with a molecular mass of 708.5 g mol^{-1}. Compound **5** was therefore identified as 3-β-glucopyranosyl-22,27-dihydroxy-lanosta-8-ene, isolated for the first time in *C. obliquus*.

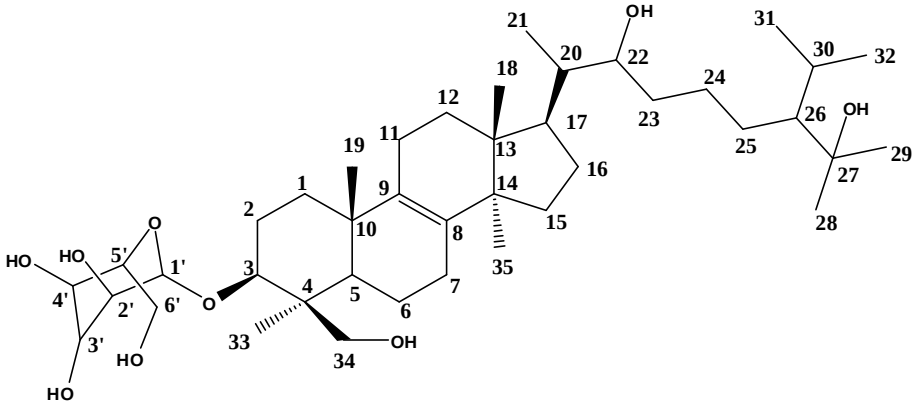


Figure 2: Chemical structure of compound **5**

http://dx.doi.org/10.4314/ajtcam.v12i5.5

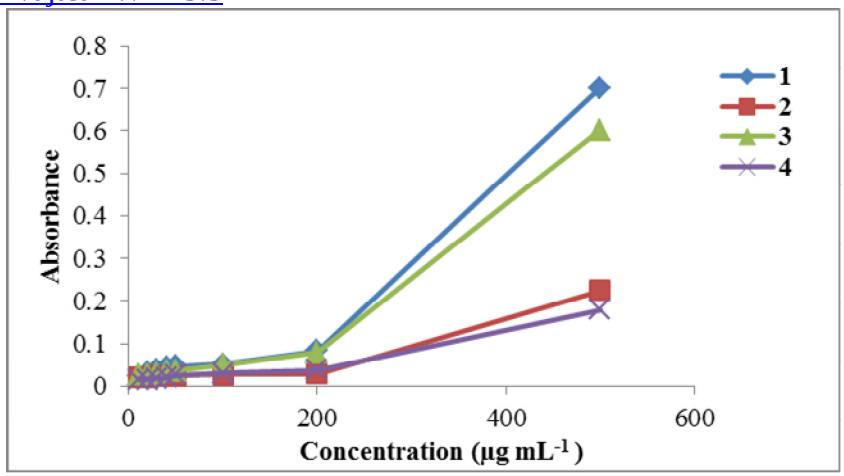


Figure 3: Comparison of the ferric reducing/ antioxidant power of compounds **1-4**

Compounds **2** and **4** were observed to be inactive when tested for antioxidant activity by the DPPH free radical scavenging assay (Table 3). The IC_{50} value of the 3-benzylidenechromanones was found to exceed the concentration range; this was also evident in previous studies (Nakamura et al., 2003; Mohamad et al., 2004). However, compounds **1** and **3** were observed to show significant activity compared to the 3-benzylidenechromanones though lower than ascorbic acid (Table 3). Both compounds possess the C_3-C_9 saturated moiety where the hydrogen may be easily donated to the DPPH radical to form DPPH-H.

The FRAP assay measures the Fe^{3+} to Fe^{2+} reduction in the presence of an antioxidant (Gupta et al., 2009). High absorbance of the reaction mixture is related to greater reducing power which indicates strong antioxidant activity. A similar trend was also exhibited by the FRAP assay where the activities of compounds were in decreasing order of Compound **1** > **3** > **2** > **4** (Fig. 3 and 4).

Conclusion

Four homoisoflavanones namely 5,7-dihydroxy-6-methoxy-3-(4'-methoxybenzyl)chroman-4-one; 5,7-dihydroxy-6-methoxy-3-(4'-hydroxybenzyl)chroman-4-one and two 5,7-dihydroxy-6-methoxy-3-(4'-methoxybenzylidene)chroman-4-one; 5,7-dihydroxy-3-(4'-hydroxybenzylidene)-chroman-4-one were obtained from *Cyrtanthus obliquus*. An unusual 3-β-glucopyranosyl-22,27-dihydroxy-lanosta-8-ene was also isolated from the bulbs. Thus, this study shows that the plant is not only rich in alkaloids, but also flavonoids, particularly homoisoflavanones. The antioxidant potential of the homoisoflavanones indicate that 3-benzylchromanones have better antioxidant activity than 3-benzylidenechromanones.

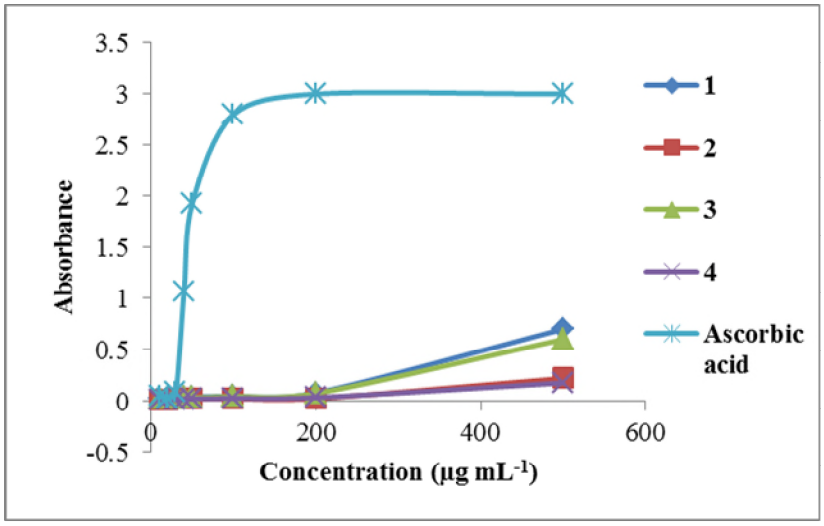


Figure 4: Ferric reducing/antioxidant power of compounds **1-4** and ascorbic acid standard, as measured by the FRAP assay

http://dx.doi.org/10.4314/ajtcam.v12i5.5

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