A New Xanthone Glucopyranoside from *Swertia Ciliata*

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Summary: A new xanthone glucoside has been isolated from *Swertia ciliata* (G.Don) and its structure established as 1,8-dihydroxy-3-methoxyxanthone-7-O-glucopyranoside on the basis of spectral evidence, particularly 2D NMR. Six known compounds, oleanolic acid, 3β, 28-dihydroxyoleanane-3-palmitate, β-sitosterol, bellidifolin, swertianolin and norswertianin have also been isolated from the same specie

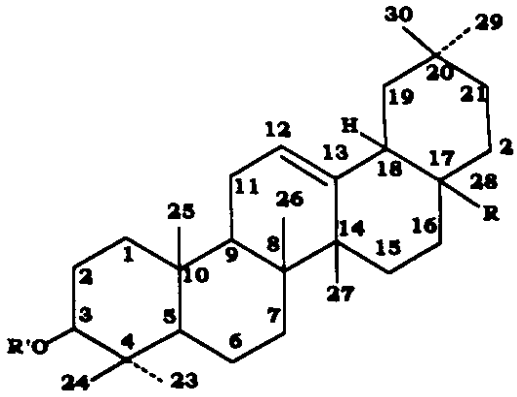
Introduction

The genus *Swertia*, possessing 170 species, is one of the 70 generae of family *Gentianaceae* [1]. A number of plants of this genus have been used in chinese traditional medicine for the treatment of hepatitis, cholecystitis, pneumonia, dysentery, scabies and spasm [2]. In India, *Swertia* herbs are extensively used as a traditional remedy for chronic fever, anaemia, asthma and liver disorders [3] as well as antimalarial [4], bitter tonic, laxative and febrifuges [5]. The hexane extract of *S. chirata* has been reported to possess significant hypoglycemic activity [2] which was traced to swerchirin [3]. Recent invegtigations [6] have shown that bellidifolin, a common xanthone of *Swertia* herbs, and its derivatives possesses a marked hypoglycemic activity when administered to rats. *Swertia hookeri* is used in microbial infections and hypertension [7]. *Swertia japonica* is being widely used as antispasmodic [8], antihepatotoxic [9] and as hair tonic. These effects being attributed to the presence of swertiamarin in the plant [10, 11]. Moreover *S. randaiensis* have shown to posses antihepatotoxic principles [12]. The plants of the genus *Swertia* a rich source of xanthonoids, flavonoids, irridoids and terpenoids [13]. The medicinal potential of *Swertiae*, prompted the chemical investigation of the locally available species viz *S. ciliata* and *S. thomsonii*. The *S. ciliata* is a perennial herb which is widely distributed in the northern areas of Pakistan. Inspite of its extensive usage in folk medicine, no attempt was made to characterize its chemical constituents. In our previous papers we have reported the isolation and identification of bellidifolin (**4**), swertianolin (**5**) and norswertianin (**6**) from *S.ciliata* [14] and 1,7-dihydroxy-3,8-dimethoxyxanthone and ursolic acid from *S. thomsonii* [15]. In this paper we describe the isolation and characterization of a new xanthone glucoside; 1,8-dihydroxy-3-methoxyxanthone-7-O-glucopyranoside (**7**) in addition to three known individuals viz: oleanolic acid (**1**), 3β, 28-dihydroxy oleanane-3-palmitate (**2**) and β-sitosterol (**3**) (flow sheet). The compound **2** was previously reported from *Medhuca butyracea* in 1968 [16] however its isolation from the genus *Swertia* is reported for the first time.

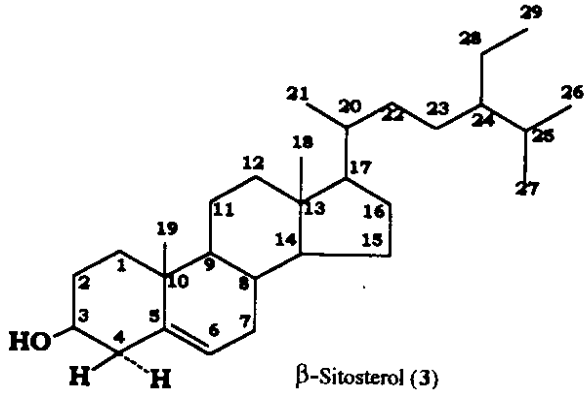
Results & Discussion

The methanol extract of *S. ciliata*, after filtration of settled material, was dried and subjected to column chromatography, eluting the column with chloroform/methanol (9:1) solvent system. After collection of the fraction containing **6**, elulion was continued and a fraction was collected which afforded a pale yellow compound (**7**), m.p. 266-268°C. The compond was purified by re-crystallization from methanol.

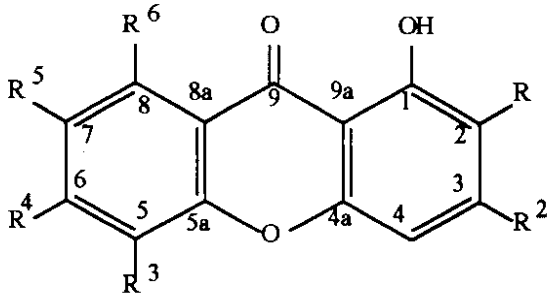
The **FAB (+)** of the compound showed the molecular ion peak at m/z 437 [M+1]. The **EIMS** of the compound exhibited molecular ion peak of aglycon at m/z 274 [M-162; sugar], as base paeak, deducing the molecular formula of aglycon as $C_{14}H_{10}O_6$ suggesting ten double bond equivalents in



(1) R^1 = H, R^2 = COOH
(2) R^1 = $CH_3(CH_2)_{14}CO$, R^2 = CH_2OH



β-Sitosterol (3)



	R^1	R^2	R^3	R^4	R^5	R^6
4	H	OMe	OH	H	H	OH
5	H	OMe	OH	H	H	OGlu
6	H	OH	H	H	OH	OH
7	H	OMe	H	H	OGlu	OH

the molecule (see experimental). A peak at m/z 260 ($C_{13}H_8O_6$) indicated the loss of methyl group. An ion fragment at m/z 245 ($C_{13}H_9O_5$) arose due to loss of CHO group from the molecule while a peak at m/z 231 ($C_{12}H_7O_5$) again indicated the loss of methyl group in addition to CHO group. UV spectra showed absorption maxima at 328,276 and 252nm, in methanol, indicated a xanthone chromophore [17,18]. There was no bathochromic shift by the addition of sodium hydroxide which indicated that C-3 and C-6 positions of xanthone nucleus possess no hydroxyl group(s). It is known that if hydroxyl group is present at C-3 or C-6 locations of xanthone nucleus then the addition of a few drops of NaOH or NaOAc, in ethanol, gives rise a bathochromic shift with increase in intensity (300-330 nm) in UV spectrum [17,18]. The **1R** spectrum showed bands at 3300 and 1654 cm^{-1} suggesting the presence of hydroxyl and carbonyl groups in the molecule respectively [19].

The tatraoxygenated pattern of the xanthone nucleus was revealed by its **^{1}H NMR** spectrum in which two *meta* coupled proton doublets, appeared at δ 6.36 and 6.57 (J=2.0 Hz), and two *ortho* coupled proton doublets, appeared at δ 7.13 and 7.27 (J = 8.8 Hz), as well as two singlets at δ 10.09 and 13.09 due to phenolic hydroxyl groups and a three proton singlet, appeared at δ 3.89, due to methoxyl group. Signals of a pair of *meta* coupled 1H doublets (J = 2.0 Hz) at δ 6.36 and 6.57 were assigned to the protons located at C-2 and C-4 carbon atoms whereas the signals of a pair of *ortho* coupled 1H doublets (J = 8.8 Hz) at δ 7.13 and 7.27 were assigned to protons attached at C-5 and C-6 carbon atoms respectively [20,21]. The down field values of hydroxyl protons at δ 13.09 and 10.09 indicated their chelation with carbonyl oxygen atom, thus assigning their attachments at C-1 and C-8 carbons of xanthone moiety. The methoxyl group (3H, singlet) was proposed to be attached at C-3 of xanthone nucleus in order to allow *meta* couplings between H-2 and H-4 protons. A doublet appeared at δ 4.82 (1H, J=7.6 Hz) was assigned to anomeric proton (H-1′) of sugar moiety whereas the four signals, 1H each, of sugar moiety appeared at δ 3.38, 3.31, 3.20 and 3.36 were assigned to H-2′, H-3′, H-4′ and H-5′ respectively, and the two signals appeared at δ 3.52 and 3.74 were assigned to methene protons (H-6′) of sugar residue. The bonding of glucose moiety was proposed to be at C-7 carbon of xanthone nucleus in order to allow *ortho* couplings between H-5 and H-6 protons. The **^{1}H NMR** of 7 was repeated in D_2O solvent and it was observed that the phenolic hydroxyl peaks at δ 13.09 (OH-1) and 10.09 (OH-8)

Table 1: ^{13}C NMR Spectral data (75 MHz) of compound 2 (δ ppm, $CDCl_3$)

C.No.	^{13}C value (BB)	C-State (DEPT)	C.No.	^{13}C value (BB)	C-State (DEPT)
1.	38.39	CH_2	24.	16.79	Me
2.	34.89	CH_2	25.	15.59	Me
3.	80.62	CH	26.	16.82	Me
4.	37.06	C	27.	25.93	Me
5.	55.39	CH	28.	69.72	CH_2OH
6.	18.33	CH_2	29.	33.18	Me
7.	32.65	CH_2	30.	23.64	Me
8.	39.93	C	31.	173.63	COO
9.	47.62	CH	32.	34.19	CH_2
10.	37.83	C	33.	31.94	CH_2
11.	23.60	CH_2	34.	29.70	CH_2
12.	122.38	CH	35.	29.60	CH_2
13.	144.31	C	36.	29.48	CH_2
14.	41.84	C	37.	29.36	CH_2
15.	25.65	CH_2	38.	29.22	CH_2
16.	22.16	CH_2	39.	29.21	CH_2
17.	36.94	C	40.	26.72	CH_2
18.	42.45	CH	41.	25.20	CH_2
19.	46.55	CH_2	42.	23.66	CH_2
20.	30.97	C	43.	22.69	CH_2
21.	23.81	CH_2	44.	18.79	CH_2
22.	31.09	CH_2	45.	17.74	CH_2
23.	28.12	Me	46.	14.04	Me

Table 2: ^{13}C NMR spectral data of compounds 4, 5, 6 and 7 (δ ppm, DMSO-d_6)

C.No.	4		5		6		7	
	^{13}C-value (BB)	C-State (DEPT)	^{13}C-value (BB)	C-State (DEPT)	^{13}C-value (BB)	C-State (DEPT)	^{13}C value (BB)	C-State
1	161.9	C	162.67	C	161.96	C	162.6	C
2	97.4	CH	97.10	CH	97.96	CH	97.1	CH
3	167.1	C	166.24	C	166.17	C	166.2	C
4	92.6	CH	92.14	CH	93.82	CH	92.1	CH
4a	157.4	C	156.37	C	157.59	C	156.3	C
5	137.3	C	140.95	C	105.71	CH	112.3	CH
5a	143.4	C	145.01	C	146.86	C	144.9	C
6	123.9	CH	121.08	CH	123.72	CH	121.0	CH
7	109.5	CH	112.43	CH	140.17	C	149.3	C
8	151.8	C	149.38	C	147.70	C	140.9	C
8a	107.5	C	111.89	C	107.20	C	111.8	C
9	184.0	C	180.99	C	183.69	C	181.0	C
9a	102.1	C	103.50	C	100.63	C	103.4	C
Ar-OMe	56.1	CH_3	56.00	CH_3	-		56.0	CH_3
1′	-		103.19	CH	-		103.0	CH
2′	-		73.49	CH	-		73.4	CH
3′	-		76.04	CH	-		76.0	CH
4′	-		69.80	CH	-		69.6	CH
5′	-		77.37	CH	-		77.3	CH
6′	-		60.86	CH_2	-		60.3	CH_2

and the glucosyl hydroxyl peaks at δ 5.07 (OH-2′), 5.10 (OH-3′), 5.08 (OH-4′), 5.14 (OH-5′) and 4.67 (OH-6′) disappeared due to proton-deuterium exchange (see experimental).

The ^{13}C NMR spectrum of compound 7 in DMSO-d_6, showed 20 carbon resonances (Table 2). The multiplicities determined by DEPT experiments [22,23] revealed that these resonances were due to one methyl, one methene, nine methines and nine quaternary carbon atoms. This included one methene and five methine carbon resonances due to glucosyl residue. Four aromatic methine resonances appeared at δ 97.1, 92.1, 112.3 and 121.0 were assigned to C-2, C-4, C-5 and C-6 carbon atoms respectively while the methyl carbon resonance at δ 56.0 was assigned to methoxyl group. The carbon resonances at δ 103.0, 73.4, 76.0, 69.6, 77.3 and 60.3 were assigned

Table 3.: COSY (H-H) spectral data of compound 7

^{1}H NMR, δ	Interactions in DMSO-d_6 Pure solvent, δ	Interactions in DMSO + D_2O, δ
6.36 (H-2)	6.57 (H-4)	6.57 (H-4)
6.57 (H-4)	6.36 (H-2)	6.36 (H-2)
7.27 (H-5)	7.13 (H-6)	7.13 (H-6)
7.13 (H-6)	7.27 (H-5)	7.27 (H-5)
4.82 (H-1')	3.38 (H-2')	3.38 (H-2')
3.38 (H-2')	4.82 (H-1'), 5.07 (OH-2')	4.82 (H-1'), 3.20(H-4')
3.31 (H-3')	3.20 (H-4'), 5.14 (OH-5')	3.20 (H-4')
3.20 (H-4')	3.36 (H-5'), 5.08 (OH-4')	3.31 (H-3'), 3.36(H-5')
3.36 (H-5')	4.82 (H-1'), 3.52 (H-6'), 5.07 (OH-2')	3.52 (H-6')
3.52 (H-6')	3.36 (H-5'), 3.74 (H-6')	3.36 (H-5'), 3.74 (H-6')
3.74 (H-6')	3.52 (H-6')	3.52 (H-6')
5.07 (OH-2')	3.38 (H-2')	-
5.10 (OH-3')	3.31 (H-3')	-
5.08 (OH-4')	3.20 (H-4')	-
5.14 (OH-5')	3.31 (H-3')	-
4.67 (OH-6')	3.52 (H-6'), 3.74 (H-6')	-

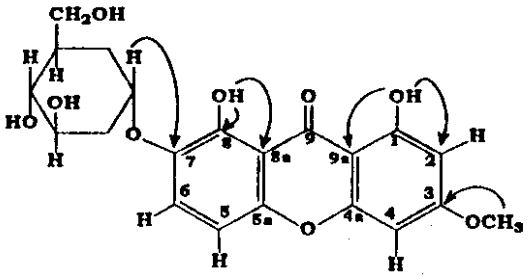
to glucoside carbons C-1'' to C-6' respectively [21]. All these assignments were made on the basis of comparison with other tetraoxygenated xanthonoids [21,24]. The hydroxyl group connectivities were proposed at δ 162.6 (C-1) and 140.9 (C-8) while methoxyl and glucosyl linkages at δ 166.2 (C-3) and 149.3 (C-7) respectively. The assignments were supported by HMQC and HMBC experiments, discussed below.

In order to establish the exact locations of substituents on xanthone nucleus 2D NMR spectral studies (COSY, HMQC and HMBC) were carried out. The ^{1}H - ^{1}H interactions through bonds (**COSY**; Table 3) indicated that H-2 (δ 3.36) has interaction with H-4 (δ 6.57) and H-5 (δ 7.2) with H-6 (δ7.13) assigning their presence in ring A and C respectively. Similarly the glucosyl hydrogen atoms showed interactions with their respective neighboring hydrogens including hydroxyl protons. However the hydroxyl proton interactions were disappeared on addition of D_2O. These studies confirmed the C-H and O-H attachments in the compound and also indicated their locations.

The **HMQC** spectra [25] of compound 7 (Table 4) showed that C-2, C-4, C-5 and C-6 carbon atoms of xanthone moiety possesses hydrogen atoms while the others are quaternary carbon atoms. Similarly five carbon atoms of sugar moiety are methine and one methene in nature. This data supported the ^{1}H NMR, ^{13}C NMR and COSY assignments of this compound.

The **HMBC** spectral data [26] of compound 7 Table 4, on the other hand, showed that the hydrogen atoms of methoxyl group at δ 3.89 have long range interactions with the carbon at δ 166.2 (C-3) confirming its connectivity with C-3. This assignment was also supported by the *meta* coupling between H-2 and H-4 protons. Similarly the bonding of hydroxyl group at C-1 was achieved from the interaction of hydroxyl proton at δ 13.09 with the carbons at δ 162.6 (C-1), 97.1 (C-2) and 103.4 (C-9a), confirming its attachment at C-1. The bonding of second hydroxyl group with C-8 was achieved from its proton interaction at δ 10.09 with carbons at δ 140.9 (C-8) and 111.8 (C-8a). The linkage of glucosyl residue at C-7 carbon atom of xanthone nucleus was assigned on the basis of long range interaction of anomeric proton at δ 4.82 (H-1') with the carbon at δ 149.3 (C-7) (Table 4). These assignments supported the ^{1}H NMR, ^{13}C NMR and HMQC assignments of the compound discussed earlier.

Acid hydrolysis of 7 was carried out by heating with dilute sulphuric acid on steam bath for two hours [27]. The reaction mixture was diluted with water and the aglycon (xanthone moiety) was extracted with chloroform. Evaporation of solvent and re-crystallization of the residue from methanol gave pure compound. The compound was found identical with swertianin [15] by carrying out co-TLC and mixed TLC under different solvent systems as well as by carrying out its m.p. and mixed m.p. The aqueous hydrolyzate, on the other hand, was



1,8-Dihydroxy-3-methoxyxanthone-7-O-glucophyranoside (7)

Table 4. 2D NMR spectral data of compound 7 (^{1}H-^{13}C interactions)

^{1}H NMR, δ	Adjacent interacting carbon, δ (HMQC)	Neighboring interacting carbons, δ (HMBC)
4.82 (H-1')	103.0 (C-1')	149.3 (C-7)
3.38 (H-2')	73.4 (C-2')	-
3.31 (H-3')	76.0 (C-3')	-
3.20 (H-4')	69.6 (C-4')	-
3.36 (H-5')	77.3 (C-5')	-
3.52 (H-6')	60.9 (C-6')	-
3.74 (H-6')	60.9 (C-6')	-
3.89 (H; 3-OMe)	56.0 (C; 3-OMe)	166.2 (C-3)
6.36 (H-2)	97.1 (C-2)	162.6 (C-1), 166.2 (C-3), 92.1 (C-4), 103.4 (C-9a)
6.57 (H-4)	92.1 (C-4)	97.1 (C-2), 166.2 (C-3), 156.3 (C-4a), 103.4 (C-9a)
7.27 (H-5)	112.3 (C-5)	144.9 (C-5a), 149.3 (C-7), 140.9 (C-8)
7.13 (H-6)	121.0 (C-6)	149.3 (C-7), 140.9 (C-8), 111.8 (C-8a)
10.09 (H; 8-OH)	-	140.9 (C-8), 111.8 (C-8a)
13.09 (H; 1-OH)	-	162.6 (C-1), 97.1 (C-2), 103.4 (C-9a)

concentrated and subjected to paper chromatography using D-glucose as standard and applying n-BuOH/AcOH/H_2O (4:1:5) as solvent system. The spots were developed by spraying with ammonical silver nitrate reagent and heating at 120°C for two hours. Results have shown that the sugar moiety was identical with D-glucose. On the basis of these spectral and chemical studies the compound was assigned structure **7** and consequently 1,8-dihydroxy-3-methoxyxanthone-7-O-glucopyranoside (or 1,8-dihydroxy-6-methoxyxanthone-2-O-glucopyranoside) has not yet been encountered before in nature.

Six known compunds (**1-6**) were islated from *S. ciliata* for the first time (flow sheet) and their sturctures were identified by comparision of the spectral and physical data. Of them compound **2** has been reported from the genus *Swertia* for the first time. Previously it was obtained from *Medhuca butyracea* [16].

Experimental

Plant material:

Swertia ciliata (whole plant), collected in the month of August from Skurdu, NWFP, Pakistan, was identified by Mr. Shahid Farooq, Plant Taxonomist, PCSIR, Peshawar, and a voucher specimen (No: 7920) of the plant was deposited in the Herbarium of PCSIR Peshawar.

Melting points were determined on electrothermal melting point apparatus and are uncorrected. UV spectra were recorded in ethanol on a Shimadzu UV-160 spectrophotometer. I.R. spectra were recorded in KBr on a Unicam SP 1000 instrument. HREIMS spectra were determined on a JEOL JMS-110 mass spectrometer. EIMS and FDMS spectra were recorded on Varian MAT 112 and 312 double focusing mass spectrometer. NMR spectra including DEPT and 2D experiments were recorded in $CDCl_3$ and d_6-DMSO using TMS as internal standard, on Bruker AM-400 and 500 instruments, operating at 400 and 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR. Silica gel used for column chromatography was kieselgel 60 (70-230 mesh; Merck) and for preparative TLC was Kieselgel 60 $PF_{254+366}$ (Merck). TLC for Rf were conducted on precoated silica gel F_{254} aluminum sheets (0.25 mm). The TLC chromatograms were visualized by UV light, exposure to I_2 vapour and spraying with 1% ceric sulphate in 1M H_2SO_4.

Isolation:

Air dried ground material of whole plant of *S. ciliata* (0.8 kg) was extracted with n-hexane, followed by extraction with $CHCl_3$ and MeOH and each extract was concentrated *in vacuo* (flow sheet). The hexane concentrate was allowed to stand for 48 hours. The separated solid material (10 g) was crystallized from MeOH (8 g) and re-crystallized from $CHCl_3$/Pet. ether (50:50) mixture which afforded **1** (5 g). The mother liquor after drying under vacuum (30 g) was chromatographed over silica gel column eluting with hexane, followed by benzene. A fraction (0.5 l) was collected, evaporated and crystallized from pet. ether which yielded **2** (0.03 g). Further elution with benzene and collection of fraction (3 l) yielded a mixture of two compounds which on preparative TLC with $CHCl_3$/Pet. ether (1:1) solvent mixture afforded **3** (0.5 g). The $CHCl_3$ extract, on standing for 48 hours, allowed some solid material to settle down (2 g), which on filtration and crystallization from MeOH yielded **4** (1 g). The extract of MeOH was also allowed to stand for 48 hours, allowing solid material (5 g) to settle down, which on crystallization from hot MeOH afforded **5** (1 g). The mother liquor was dried under vacuum (yielding 100 g) and chromatographed over silica gel column, eluting the column with n-hexane followed by $CHCl_3$ and then $CHCl_3$/MeOH (9:1) mixture. Collection of fraction (2 l) at this stage afforded **6** (0.5 g) when the solvent was removed. For purification the compound was dissolved in acetone (20 ml) and crystallized by adding pet.ether (70 ml). Elution of the column was continued with the same solvent system and a fraction (4 l) was collected. The solvent was removed and the residue was crystallized from MeOH that afforded **7** (0.28g).

Compound **1** *(Oleanolic acid)*

White crystals, mp 308-310° (lit. 310° [28]), TLC solvents: Benzene/MeOH (4:1), Rf: 0.62. UV λ_{max}^{MeOH} nm: 204. IR ν_{max}^{KBr} cm^{-1}: 3380 (alcoholic OH), 1690 (carboxylic C=O), 1460, 1380, 1025, 990. **^{1}H NMR** ($CDCl_3$; 400 MHz): δ 0.75 (3H, s, Me - 26), 0.77 (3H, s, Me - 24), 0.89 (3H, s, Me - 29), 0.91 (3H, s, Me - 30), 0.92 (3H, s, Me-25), 0.99 (3H, s, Me-23), 1.13 (3H, s, Me-27), 2.82 (IH, dd, J = 4.11, 13.6 Hz, H - 18), 3.20 (IH, dd, J = 6.7, 13 Hz, α H-3), 5.27 (IH, t, J = 3.56 Hz, H-12). **EIMS**, m/z (rel. int. %): 456 [M^+] (4) $C_{30}H_{48}O_3$, 248 (100), 207 (15), 203 (49), 189 (7), 133 (7), 69 (10).

Compound **2** *(3β, 28-Dihydroxy oleanane-3-palmitate)*

White needles; mp 108-110°. TLC solvents: $CHCl_3$/pet. ether (9:1). **UV** λ_{max}^{MeOH} nm: 201, 278. IR ν_{max}^{KBr} cm^{-1}:: 3425 (O-H), 1705 (C=O), 1245, 1060 (OH). **^{1}H NMR** ($CDCl_3$; 500 MHz): δ 0.851 (3H, m, Me-46) 0.855 (3H, s, Me-23), 0.86 (3H, s, Me-26), 0.865 (3H, s, Me-29), 0.87 (3H, s, Me-30), 0.92 (3H, s, Me-24), 0.94 (3H, s, Me-25), 1.15 (3H, s, Me-27), 1.17(2H,m,CH_2-15), 1.20-1.35(m,13 palmitate-CH_2 and CH_2-7 and CH_2-16) 1.96 (1H, dd, J=4.5, 13.7Hz, H-18), 2.27 (1H, td, J=6.65Hz, H-2), 2.285 (1H, td, J=6.65Hz, H-2), 3.20 (1H, d, J=10.95Hz, geminal, H-28), 3.53 (1H, d, J=10.95Hz, geminal, H-28), 4.48 (1H, dd, J=5.45, 10.5Hz, H_{ax}-3), 5.177 (1H, t, J=3.65, H - 12). **HRMS** m/z (mol. formula): 680.6128 ($C_{46}H_{80}O_3$). **EIMS** m/z (rel. int%): 680 [M]$^+$ (2), 662 (M-H_2O) (6), 445 (4), 425 (9), 393 (6), 234 (34), 216 (13), 203 (100), 95 (7), 69 (8), 43 (12). **MS, linked Scan** m/z (rel. int %): 680 (100) → D: 663 (12), 648 (7), 425 (7), 393 (8), 234 (11), 203 (3); 425 (100) → P: 680 (2); 234 (100) → P: 425 (63), 680 (100). (Where D and P denotes daughter and parent peaks). **^{13}C NMR**: See Table 1.

Compound **3** *(β-Sitosterol)*

White crystals, mp 139-140° (lit. 140° [29]), TLC solvents: $CHCl_3$/ Benzene (8:2), Rf: 0.50. UV λ_{max}^{MeOH} nm : 201, 221. IR ν_{max}^{KBr} cm^{-1}: . 3390 (OH), 2940 (CH), 1460 (C=C), 1380, 1050. **^{1}H NMR** ($CDCl_3$; 500 MHz): δ 0.66 (3H, s, Me-18), 0.79 (3H, d, J=6.8 Hz, Me - 27), 0.82 (3H, d, J=6.8 Hz, Me - 26), 0.83 (3H, t, J=7.5 Hz, Me - 29), 0.91 (3 H, d, J=6.5 Hz, Me - 21), 0.99 (3H, s, Me - 19), 2.23 (1H, ddd, J=2.5, 4.9, 11.1 Hz, H-2), 2.28 (1H, ddd, J=2.1, 4.9, 12.9 Hz H - 2, geminal), 3.50 (1H, dddd, J=4.35, 4.35, 9.6, 9.6 Hz, βH-3), 5.33 (1H, dd, J=2.25, 3.0 Hz, H-6). **EIMS**, m/z (rel. int. %): 414 [M^+] (100) ($C_{29}H_{50}O$), 396 (28), 381 (18), 329 (26), 303 (33), 255 (30), 213 (31), 159 (34), 145 (44), 107 (60), 95 (55), 81 (58), 55 (78).

Compound **4** *(Bellidifolin)*

Yellow needles, mp 263° (lit. 263°C [21]), TLC solvents: $CHCl_3$/Benzene (8:2),Rf: 0.18; $CHCl_3$/Pet. etter (8:2), Rf: 0.07 and Benzene/MeOH (4:1), Rf: 0.62. UV λ_{max}^{MeOH} nm : 203, 254, 279, 333, 390. IR ν_{max}^{KBr} cm^{-1}: : 3450 (OH), 3300, 3250, 1655 (C=O), 1625, 1610, 1690 (C=C), 1500, 1395,

1205, 1180, 1155, 990. **^{1}H NMR** (DMSO-d_6; 500 MHz): δ 3.89 (3H, s, Ar-OMe), 6.38 (IH, d, J=2.3 Hz, H-2), 6.59 (IH, d, J=2.3 Hz, H-4), 6.64 (IH, d, J=8.8 Hz, H-7), 7.25 (IH, d, J=8.8 Hz, H-6), 9.64 (IH, s, OH at C-5), 11.05 (IH, s, OH at C-1 or C-8, chelated), 11.89 (IH, s, OH at C-1 or C-8; chelated). **HRMS** m/z (mol. formula): 274.0477 ($C_{14}H_{10}O_6$). **FDMS** m/z: 274 [M]$^+$. **EIMS** m/z (rel. int %): 275 [M$^+$+1], (15), 274 [M$^+$], (100), 245 (14), 231 (11), 217 (2), 203 (3), 137 (5), 123 (6), 69 (6). **^{13}C NMR**, 125 MHz: See Table 2.

Compound 5 (Swertianolin)

Pale yellow needles, mp 204° (lit. 203°C [21]). TLC solvents: $CHCl_3$/ MeOH Re: 0.26 (6:1), Rf: 0.26 and Benzene/MeOH (4:1), Rf: 0.34. UV λ_{max}^{MeOH} nm : 253, 276, 326. IR ν_{max}^{KBr} cm^{-1}: :3450 (OH), 3300, 1655 (C=O), 1610, 1575 (C=C), 1495, 1310, 1280, 1160, 1080. **^{1}H NMR** (DMSO-d_6; 300 MHz): δ 3.16 - 3.51 (m, sugar - H), 3.17 (IH, dt, J = 5.2, 8.9 Hz, H-4'), 3.87 (3H, s, Ar-OMe), 4.79 (IH, d, J = 7.5 Hz, glu H-1'; anomeric), 5.084 (IH, s, glu-OH at C-3'), 5.044. (IH, s, glu-OH at C-2'), 5.035 (IH, s, glu-OH at C-4'), 5.026 (IH, s, glu-OH at C-6'), 6.35 (IH, d, J = 2.3 Hz, H-2), 6.56 (IH, d, J = 2.3 Hz, H-4), 7.11 (IH, d, J = 8.9 Hz, H-7), 7.25 (IH, d, J = 8.9 Hz, H-6), 10.05 (IH, s, OH at C-5), 13.06 (IH, s, OH at C-1, Chelated). **HRMS**, m/z (mol. formula): 274.0477 (C_{14} H_{10} O_6). **FAB (pos.)** m/z: 437 [M$^+$+1]. **EIMS**, m/z (rel. int. %): No [M]$^+$, 274 [M$^+$-162] (100), 273 (16), 259 (5), 245 (26), 244(7), 231 (20), 217 (5), 203 (7), 152 (5), 137 (9), 123 (10), 60 (11). **^{13}C NMR**, 75 MHz: See Table 2.

Acid Hydrolysis [27]:

Swertianolin (20 mg) was hydrolyzed with sulfuric acid (10%, 7 ml) for 2 hours on a steam bath, diluted with water (8 ml) and extracted with chloroform (20 ml). Evaporation of chloroform and crystallization of the residue from the methanol gave bellidifolin (4), compared by mixed melting point, Co-TLC and mixed TLC. The aqueous hydrolysate was subjected to paper chromatography (Whatman No.50) using n-BuOH/AcOH/H_2O (4:1:5) solvent system as the developer and was found to be D-glucose by comparison with authentic sample. $AgNO_3$/NH_4OH spraying reagent was used for developing the spots. The reagent was prepared by mixing $AgNO_3$ solution (O.1N) and NH_4OH solution (5N) in the ratio of 1:5. The spots were visualized by heating at 110°C for 2 hours.

Compound 6 (Norswertianin)

Yellow needles, mp 336°C (lit. 335°C [30]). TLC solvents: $CHCl_3$/MeOH (16:1), Rf: 0.34 and benzene/MeOH (4:1), Rf: 0.47. UV λ_{max}^{MeOH} nm : 237.5, 266, 334. IR ν_{max}^{KBr} cm^{-1}: : 3450 (OH), 3290, 1660 (C=O), 1630, 1610, 1585 (C=C), 1470, 1280, 1145, 825, 805. **^{1}H NMR** (DMSO-d_6; 300 MHz): δ 6.21 (IH, d, J = 2.1 Hz, H-2), 6.35 (IH, d, J=2.1 Hz, H-4), 6.86 (IH, d, J=8.9 Hz, H-5), 7.27 (IH, d, J=8.9 Hz, H-6), 9.33 (IH, s, OH at C-3), 11.17 (IH, s, OH at C-7), 11.70 (IH, s, OH at C-1 or C-8), 11.88 (IH, s, OH at C-1 or C-8). **HRMS**, m/z (mol. formula): 260.0321 ($C_{13}H_8O_6$). **EIMS**, m/z (rel. int. %): 261 [M$^+$+1] (15), 260 [M$^+$] (100), 232 (9), 203 (3),186 (5), 152 (3), 116 (13), 79 (8), 69 (19) **^{13}C NMR**, 75 MHz: See Table 2.

Compound 7 (1,8-Dihydroxy-3-methoxyxanthone-7-O-glucopyranoside)

Pale yellow needles, mp:266-268°C; TLC solvents: $CHCl_3$/ MeOH (5:1), Rf:0.34 and Benzene/MeOH (4:1), Rf: 0.37. UV λ_{max}^{MeOH} nm :252, 276, 328. IR ν_{max}^{KBr} cm^{-1}: :3300 (OH), 3100, 1654 (C=O), 1650, 1560 (C=C), 1360, 1160, 1079. **^{1}H NMR (DMSO-d_6; 400 MH$_z$)**: δ 3.20 (1H, dd, J=9.6 Hz, H-4'), 3.31 (1H, dd, J=9.1 & 8.6 Hz, H-3'), 3.36 (1H, ddd, J=9.6, 6.1 & 1.9 Hz, H-5'), 3.38 (1H, dd, J=9.7 & 7.6 Hz, H-2'), 3.52 (1H, dd, J=12.0 & 6.1 Hz, H-6'), 3.74 (1H, dd, J=12.0 & 1.9 Hz, H-6'), 4.82 (1H, d, J=7.6 Hz, H-1', Anomeric-H), 5.076 (1H, s, glu- OH), 5.08 (1H, s, glu-OH), 5.10 (1H, s, glu-OH), 5.14 (1H, d, J=4.8 Hz, glu-OH), 3.89 (3H, s, 3-OMe), 6.36 (1H, d, J=2.0 Hz, H-2), 6.57 (1H, d, J=2.0 Hz, H-4), 7.13 (1H, d, J=8.8 Hz, H-6), 7.27 (1H, d, J=8.8 Hz, H-5), 10.09 (1H, s, 8-OH), 13.09 (1H, s, 1-OH). **^{1}H NMR (DMSO-d_6/D_2O)**: δ 3.20 (1H, dd, J=9.6 & 8.6 Hz; H-4'), 3.31 (1H, dd, J=9.1 & 8.6 Hz, H-3'), 3.36 (1H, ddd, J=9.6, 6.1 & 1.9 Hz, H-5'), 3.38 (1H, dd, J= 9.7 & 7.6 Hz, H-2'), 3.52 (1H, dd, J=12.0 & 6.1Hz, H-6'), 3.74 (1H, dd, J=12.0 & 1.9 Hz, H-6'), 4.82 (1H, d, J=7.6 Hz, H-1'), 3.89 (3H, s, 3-OMe), 6.36 (1H, d, J=2.0 Hz, H-2), 6.57 (1H, d, J=2.0 Hz, H-4), 7.13 (1H,

Flow sheet for isolation of compounds from *S. ciliata*

Swertia ciliata

(Whole plant; 0.8 kg)

(Solvent Extn.)

n-Hexane

(Standing/Filtern)

Solid (white)
(10g)

(Crystn.)

Oleanolic acid
(1)
Yield: 5.0g
m.p.: 308-10 °C
M: 456
(C_{30} H_{48} O_3)

Filterate

(CC; Benzene)

Oleanane ester (2)
Yield: 0.03g
m.p.: 108-10 °C
M: 680.6128
(C_{46} H_{80} O_3)

ß-Sitosterol (3)
Yield: 0.5g
m.p.: 139-40 °C
M: 414
(C_{29} H_{50} O)

Chloroform

(Standing)

Solid (Yellow)
(2.0 g)

(Crystn.)

Bellidifolin (4)
Yield: 1.0g
m.p.: 263 °C
M: 274.0477
(C_{14} H_{10} O_6)

MeOH

(Standing/Filtern)

Solid (Yellow)

(Crystn)

Swertianolin (5)
Yield: 1.0g
m.p.: 204°C
FAB(+): 437
(C_{20} H_{20} O_{11})

Filterate

(CC; $CHCl_3$/ MeOH)

Norswertianin
(6)
Yield: 0.5g
m.p.: 336 °C
M: 260.0321
(C_{13} H_8 O_6)

1,8-Dihydroxy-3-methoxyxanthone -7-0-glucoside (7)
Yield: 0.28g
m.p.: 266-68°C
FAB(+): 437
(C_{20} H_{20} O_{11})

d, J=8.8 Hz, H-6), 7.27 (1H, d, J=8.8 Hz, H-5). **FAB (pos), m/z : 437 $[M+1]^+$ EIMS, m/z (rel. int. %)**: 275 (16.8), 274 (100) [M-162] ($C_{14}H_{10}O_6$), 273 (8.3), 260 (3) ($C_{13}H_9O_6$), 243 (3), 245 (12.2) ($C_{13}H_9O_5$), 231 (18.4), 217 (3.4), 203 (6.4), 152 (5.7), 137 (5.1), 123 (9.2), 79 (5.2), 73 (9.8), 69 (6.9), 60 (15.2), 57 (8.3), 51.2 (3.3). **COSY (H-H interactions):** See Table 3. **^{13}C NMR:** See Table 2. **HMQC & HMBC:** See Table 4.

Acid Hydrolysis [27]:

Compound 7 (10 mg) was hydrolyzed with H_2SO_4 (10%, 2ml) at 100°C on steam bath for 2 hours, diluted with water (4 ml) and extracted with $CHCl_3$ (15 ml). Evaporation of the solvent and crystallization of the residue from MeOH gave swertianin [4,30] compared by mp, mmp, Co-TLC and mixed TLC. The presence of glucose moiety in aqueous hydrolyzate was detected by performing Co-paper chromatography with authentic sample using n-BuOH/AcOH/H_2O (4:1:5) as solvent system and spraying with ammonical silver nitrate reagent (pl.see under comp. 5).

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