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ANTIMICROBIAL POTENTIAL OF SOME XANTHONES FROM *SWERTIA CILIATA* BUCH. ET HAM.

M. ASIF SAEED[1*], ZAHEER-UD-DIN KHAN[2], M.RAFIQ FORD[3]

[1]Department of Pharmacy, University of the Punjab (Allama Iqbal Campus) Lahore 54000
[2]Department of Botany, Government College, Lahore
[3]Pakistan Medical Research Council, Fatima Jinnah Medical College, Lahore
PAKISTAN

Antimicrobial potentials of four xanthones isolated from Swertia ciliata, identified as swertianolin (1), norswertianin (2), bellidifolin (3) and swertianin (4) were investigated by measuring their zones of inhibition (ZI) and by evaluating their minimum inhibitory concentration (MIC). Filter paper disc diffusion method for ZI and Microdilution Broth Method for ascertaining MIC were used against five species of gram positive bacteria (Bacillus megaterium, B. subtilis, B. thuringiensis, Sarcina lutea and Staphylococcus aureus), four species of gram negative bacteria (Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris and Pseudomonas sp.) and eight species of fungi (Aspergillus niger, A. fumigatus, A. parasiticus, Candida albicans, C. stellatoidea, Monilia sitophilia, Penicillium digitation and Trichophyton tonsurance). Kanamycin as antibacterial and clotrimazole as antifungal agents were used as standard compounds. These four xanthones were isolated first time from this species and identified with the help of spectral evidences, after comparison with similar compounds. All the four compounds exhibited distinct antimicrobial potential against the types of bacteria and fungi used. Norswertianin (2) and bellidifolin (3) were the most potent antibacterial agents with least MIC. Swertianolin (1) was an intermediate and swertianin (4) was the least antibacterial agent when compared with kanamycin. On the other hand, bellidifolin (3) and swertianin (4) appeared to be more potent antifungal compounds than swertianolin (1) and norswertianin (3) when compared with clotrimazole.

Keywords:*Antimicrobial potnetial; Zones of inhibition; MIC; Xanthones; Swertia ciliata*

Introduction

Many species of genus *Swertia* (Gentianaceae) possess very high medicinal potentials. In China and India about 20 *Swertia* species are extensively used as traditional remedies for the treatment of chronic fever, anemia, asthma, liver disorders and as hypoglycemic agents (1), as an antimalarial (2), bitter tonic, laxative and febrifuge (3). *S. hookeri* was used in microbial infection and in hypertension (4), while *S. japonica* was widely used as antispasmodic and as hair tonic (2,5-7). These activities were attributed due to the presence of swertimarin in the plant (8). *S. randiensis* has also been shown to pos-sess CNS depres-sant (9) and antihepa-totoxic activities (10). Many species of genus *Swertia* including *S. ciliata* are fo-und as weeds in many areas of Pakistan during the harvesting sea-(11,12). This species is fully flourished son of crops. In spite of its extensive usage in folk medi-cine no attempt has been made to isolate, characterize and evaluate the possible ef-fects of it's chemical constituents. In the present communicaiton, we describe the antimicrobial potential of Swertia ciliata, followed by the fractionation to isolate and characterize its active compounds, whose antimicrobial potentials were evaluated by measuring their zones of inhibition (ZI) and by minimum inhibitory concentration (MIC).

Materials and Methods

General

Unless otherwise stated, all the chemicals used were of analytical grade. Concentrations

*Correspondence

were performed under reduced pressure at bath temperatures not exceeding 55°C. Melting points were uncorrected. UV spectra were measured on Hitachi-270-30 spectrophotometer in MeOH and IR spectra of the compounds were obtained as KBr disc on Pye-Unicam SP-8-400. ^{1}H-NMR spectra were acquired in DMSO-d_6 solvent at 300 MHz and ^{13}C NMR spectra at 75 MHz on Bruker AM-300 NMR spectrometers using tetramethylsilane as an internal reference. EI and FD mass spectra were recorded on a Varian MAT-312 double focusing mass spectrometer using the direct inlet method. FAB (positive), in glycerin, was carried out on JEOL JMS-110 spectrometer. Column chromatography was performed on silica gel 60 (70-230 mesh) and TLC was performed on silica gel F_{254} with 0.25 mm thicknesses. The spots were visualized either by exposure to UV light (365/254 nm) or with I_2 vapours or with cerric sulphate {$Ce(SO_4)_2$ in conc. H_2SO_4} spary.

Plant Materials

Whole plants of *Swertia ciliata* (1.40 kg) were collected from the waste and uncultivated areas of Hazara (i.e., the northern areas of Pakistan) in June/July 1997. These were authenticated by the herbarium staff, Department of Botany, Univer-sity of the Punjab, Lahore. The voucher speci-men was deposited in the Herbarium of Pharma-cognosy Section, Department of Pharmacy, Uni-versity of the Punjab Lahore for further reference. The plant material was air dried and pulverized.

Extraction

The ground material (900 g) was extracted in soxhlet apparatus successively with petroleum ether (40-60°), $CHCl_3$ and MeOH. Extraction was carried out with each solvent for 48 hours. Each extract was concentrated under reduced pressure.

Isolation of swertianolin (1)

The MeOH extract when allowed to stand for 48 hours, produced solid material (4.9 g) which on recrystallization from hot MeOH offered compound **1** (1.54 g). Pale yellow needles; m.p. 204°C. UV λ_{max} 254, 278 and 328 nm. IR bands: 3452 (OH), 3212 (C-H), 1658 (ketonic C=O), 1610, 1574 (C=C), 1493, 1308, 1278, 1164 (C-O) and 1080 cm^{-1}. ^{1}H-NMR (DMSO-d_6 300 MHz):δ 3.18-3.50 (m, sugar-H), 3.18 (1H, dt, *J*=5.2, 8.4 Hz, H-4'), 3.91 (3H, s, Ar-OMe), 4.83 (1H, d, *J*=7.9 Hz glu, H-1'; anomeric), 5.12 (1H, s, glu-OH at C-3'), 5.06 (1H, s, glu-OH at C-2'), 5.08 (1H, s, glu-OH at C-4'), 5.03 (1H s, glu-OH at C-6'), 6.41 (1H, d, J=2.4 Hz, H-2), 6.61 (1H, d, *J*=2.6 Hz, H-4), 7.11 (1H, d, *J*=8.7 Hz, H-7), 7.28 (1H, d, *J*=8.7 Hz, H-6) 10.07 (1H, s, OH at C-5), 13.06 (1H, s, OH at C-1). ^{13}C NMR, 75 MHz (Table 1). HRMS m/z: 274.0477 (mol. formula; $C_{14}H_{10}O_6$). FAB (pos.) m/z: 437 [M+1]. EIMS, m/z (rel. int.%): No[M]$^+$, 274 [M$^+$-162)] (100), 273 (16), 259 (10), 245 (34), 244 (10), 231 (10), 217(7), 203 (12), 152 (7), 137 (21), 123 (15), 60 (10). NOESY (H-H interactions): δ 4.82 (H-1'), -3.43 (H-2'), 3.36 (H-5'), 7.10 (H-7), 3.41 (H-2'), -3.23 (H-3'), 3.54 (H-6'), 4.82 (H-1'), 7.14 (H-7), 7.28 (H-6), 3.89 (H; OCH_3), -6.38 (H-2), 6.52 (H-4), 7.15 (H-7), -7.23 (H-6).

Isolation of norswertianin (2)

The petroleum ether extract after drying under reduced pressure, was chromatographed over silica gel column; elution with petroleum ether (40-60°) was followed by $CHCl_3$ and then $CHCl_3$/MeOH (9:1) mixture. Collection of fraction (2.8l) at this stage yielded compound **2** (1.27 g) after evaporting the solvent and recrystallizing from acetone/petroleum ether (1:4) mixture. Yellow needles; m.p. 335°C. UV λ_{max} 206, 234, 264 and 330 nm. IR bands: 3450 (OH), 3290 (C-H), 1658 (ketonic C=O), 1622, 1610, 1588 (C=C), 1470, 1280, 1144 (C-O), 829 and 806 cm^{-1}, ^{1}H NMR (DMSO-d_6, 300 MHz): δ 6.25 (1H, d, *J*=2.1 Hz, H-2), 6.38 (1H, d, *J*=2.1 Hz, H-4), 6.82 (1H, d, *J*=8.6 Hz, H-5), 7.24 (1H, d, *J*=8.7 Hz, H-6), 9.33 (1H, s, OH at C-3), 11.18 (1H, s, OH at C-7), 11.70 (1H, s, OH at C-1 or C-8), 11.91 (1H, s, OH at C-1 or C-8). ^{13}C NMR, 75 MHz (Table 1). HRMS. m/z: 260.0321 (mol. formula; $C_{13}H_8O_6$). EIMS, m/z (rel. int. %): 261 [M$^+$+1] (16), 260 [M$^+$], (100), 231 (13), 203 (14), 186 (9), 152 (8), 116 (20),79 (24), 69 (29).

Isolation of bellidifolin (3)

The $CHCl_3$ extract on standing for 48 hours, allowed some solid material to settle down (2.6 g), which on filtration and recrystallization from MeOH yielded compound **3** (1.41 g). Yellow needles, m.p. 263°C. UV λ_{max} 203, 256, 281, 330 and 392 nm. IR bands: 3452 (OH), 3301, 3252, 1656 (ketonic C=O), 1624, 1614, 1590 (C=C), 1510, 1394, 1284, 1180, 1156 (C-O), 990 cm^{-1}. ^1H NMR (DMSO-d_6, 300 MHz): δ 3.88 (3H, s, Ar-OMe), 6.42 (1H, d, *J*=2.6 Hz, H-2), 6.62 (1H, d, *J*=2.4 Hz, H-4), 6.62 (1H, d, *J*=8.8 Hz, H-7), 7.22 (1H, d, *J*= 8.8 Hz, H-6), 9.68 (1H, s, OH at C-5), 11.16 (1H, s, OH at C-1 or C-8), 11.90 (1H, s, OH at C-1 or C-8). ^{13}C NMR (Table 1.) HRMS m/z:274.0477 (mol. formula; $C_{14}H_{10}O_6$). FDMS m/z: 274 [M^+]. EIMS, m/z (rel. int. %): 275 [M^++1] (23), 274 [M^+], (100), 245 (18), 231 (16), 217 (9), 203 (8), 137 (8), 123 (16), 69 (13).

Isolation of swertianin (4)

The $CHCl_3$ extract after the removal of compound 3 was concentrated and chromatographed over silica gel column and eluted with $CHCl_3$/MeOH (80:20) solvent mixture. A fraction(1.3 l) was collected and after evaporating the solvent, the residue was recrystallized from MeOH to afford compound **4** (0.74 g). Yellow needles, m.p. 221ßC. UV λ_{max} 204, 239, 268, 326 and 392 nm. IR bands: 3454 (OH), 3322, 3212, 1668 (ketonic C=O), 1648, 1608, 1584 (C=C), 1512, 1474, 1328, 1284, 1218 (C-O), 1173, 1154, 1090, 1066, 968 cm^{-1}. ^1H NMR (DMSO-d_6, 300 MHz): δ3.88 (3H, s, Ar-OMe), 6.38 (1H, d, *J*=2.4 Hz, H-2), 6.54 (1H, d, *J*=2.4 Hz, H-4), 6.82 (1H, d, *J*=8.4 Hz, H-5), 7.28 (1H, d, *J*=8.6 Hz, H-6), 9.40 (1H, s, OH at C-7), 11.60 (1H, s, OH at C-1 or C-8), 11.84 (1H, s, OH at C-1 ar C-8). ^{13}C NMR (Table 1). HRMS. m/z: 274.0477 (mol. formula; $C_{14}H_{10}O_6$). FDMS m/z: 274 [M^+] EIMS, m/z (rel. int. %): 275 [M^++1] (20), 274 [M^+], (100), 245 (24), 231 (16), 136(8), 123 (15).

Test Organisms

The pure cultures of *Bacillus subtilis* ATCC 6633; *Escherichia coli* ATCC 8739; *Sarcina lutea* ATCC 9341 and *Staphylococcus aureus* ATCC 6536 were obtained from M/S Schazoo Laboratories, Lahore (Pakistan), while *Bacillus thuringiensis* HD-1 was procured from M/S Abbott Laboratories Karachi (Pakistan). However, *Bacillus megaterium, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas* sp., *Aspergillus niger* and *Candida stellatoidea* were acquired from our local culture collection, at the Microbiology Section, Department of Pharmacy, University of the Punjab, Lahore (Pakistan). *Aspergillus fumigatus, Trichophyton tonsurance* and *Candida albicans* were isolated from patients. *Aspergillus parasiticus* and *Penicillium digitatum* were isolated from plants. *Monilia sitophilia* was isolated from gram seeds.

Antimicrobial Activity

Antibacterial and antifungal activities were evaluated by measuring the zones of inhibition (ZI) and by determination of minimum inhibitory cencentration (MIC).

Filter paper disc diffusion method was used for measuring the zones of inhibition (13-18). The isolated compounds were tested against microorganisms mentioned above. 1 ml of each bacterial suspension was separately mixed with 14 ml of sterile molten N.A. medium in sterile petri dish (already labeled with bacterial or fungal name/compound under study) and 1 ml of fungus suspension was mixed with 14 ml of S.D.A. medium. The petri dish media after solidification was divided into four equal parts. Sterilized rounded filter papers (5.0 mm in diameter) soaked in the solution (20 mg/ml, w/v) of isolated compound, were placed in the respective position of the petri dishes with the help of sterile loop. Negative controlled plates received sterilized paper pieces only, while positive controlled plates received commercial kanamycin as antibacterial and commercial clotrimazole as antifungal agent under the similar conditions (13-18).

Minimum inhibitory concentration (MIC) of the active compounds was determined by the serial dilution method (19-22). The isolated compounds samples containing 200 mg/ml were dissolved in sterile distilled water (w/v). Testing was done in 1 ml of Muller-Hinton Broth (Difco) for bacteria (19-21) and in Sabouraud Liquid Medium for fungi, (22) both at pH 7.5, placed in each well of the petri dishes. Two-fold serial dilution technique was applied. The solution of

each compound at 10, 5, 2.5, 1.25,..... 0.156 mg/ml were prepared by diluting with the medium and placed in the wells. 1ml suspension of the microorganisms at 10^6 Cfu/ml concentrations were incubated to the two-fold diluted solution of the compounds. Distilled water-microorganisms and the pure media were used as negative controlled well, while kanamycin and clotrimazole were tested as positive control under the same conditions.

The petri dishes were then covered. The bacterial petri dishes were incubated at 37°C for 24 to 48 hours; while the fungi's petri dishes were incubated at 30°C for 72 hours. A few wet cotton-wool swabs were also placed in the incubation chamber to avoid evaporation. After this period, evaluation of the wells was performed.

Statistical Analysis

Zones of inhibition were measured by vernier calliper in mm. The zones produced by the isolated compounds were compared under the identical conditions with the zones of inhibition produced by commercial antibacterial (kanamycin) and antifungal (clotrimazole) agents. Mean diameter of zones of inhibition produced by the six replicates of crude extract and the isolated compounds against the microorganisms were calculated. Their effective ranges were also calculated by standard deviation and standard error (23).

In the second set of experiment, the last concentrations of the compounds in the wells, where no growth of the microorganisms was observed, were assessed as the minimum inhibitory concentraiton (MIC) of the compounds and expressed as mg/ml.

Results and Discussion

Four compounds from *Swertia ciliata* were isolated and purified by recrystallization and by chromatographic methods. These compounds were identified as xanthones.

Compound **1**(pale yellow needles with m.p. 204°C) was obtained after repeated crystallization from methanol. The FAB (+) showed molecular ion peak at m/z at 437 [M+1]. High resolution mass spectrum (HRMS) offered the molecular formula $C_{14}H_{10}O_6$ (274.0477), suggesting ten double bond equivalent in the molecule and a formula $C_{13}H_8O_6$ arose due to the loss of methyl group from the molecular ion fragment. The EIMS gave a base peak at m/z 274 and a peak at m/z 244 suggesting the loss of methoxy group from the molecular ion. The UV spectrum indicated values closely related to those proposed for swertianolin (24, 25). There was no bathochromic shift by addition of NaOH, suggesting that there was no hydroxyl group at C-3 and C-6 locations of xanthone nucleus (24). The IR bands at 3452 and 3212 cm^{-1} indicated the presence of chelating and free hydroxyl groups in the molecule respectively, whereas the band at 1658 cm^{-1} was obtained due to carbonyl group attached to C-C double bond at α-position. More over, the band at 1278 cm^{-1} suggesting ether linkage in the molecule. The ^1H NMR spectrum showed four sets of doublets. The two *meta* coupled doublets (J=2.4 Hz) at δ 6.41 and 6.61 were assigned to H-2 and H-4 respectively while the two *ortho* coupled doublets (J=8.7 Hz) at δ 7.11 and 7.28 were assigned to H-7 and H-6 respectively. A sharp singlet at δ 3.91 was assigned to methoxy protons. The two broad singlets at δ 10.07 and 13.06 were assigned to hydroxyl protons. Highly deshielded value (δ 13.06) suggested that one of the hydroxyl groups was chelated and thus associated with C-1 and C-8 location. The multiplet at δ 3.18-3.50 arose due to sugar proton couplings while a doublet at δ 4.83 (J=7.9 Hz) appeared due to the interaction of anomeric glucosyl proton (H-1'). The significantly low field signal of H-7 (δ 7.11) than the usual H-7 values of 1, 3, 5, 8-tetraoxygenated patterns (δ 4.83-5.03) (7,26,27) suggested O-glucoside linkage at C-8 and thus the

hydroxyl group at C-1. The methoxy group (δ 3.89) was assigned at C-3 to allow *meta* coupling between H-2 and H-4 protons. The ^{13}C NMR spectrum gave twenty carbon resonances. The multiplication detected by DEPT (28,29) revealed that these resonances corresponded to one methyl, nine methinens and nine quaternary carbon atoms (Table 1). The ^{13}C values of sugar moiety were compared (7) and found that they were corresponding to β-D- glucoside. The *beta* nature of the compound was however found from NOESY assignments that showed the interaction of H-1' with H-2' and H-5' while that of H-2' with H-1', H-3' and H-6'. Such interactions are only possible when the sugar fragment is of *beta* nature. All these evidences from spectral studies led to the conclusion that the structure of **1** was 1,5-dihydroxy-3-methoxy - xanthone - 8-O-β-glucopyranoside (swertianolin) (Fig.1).

Compound **2** (yellow needles with m.p. 335°C) was crystallized from aceto-ne/petroleum ether. The EIMS showed molecular ion peak at m/z 260 and a peak at m/z 231 arose due to the loss of CHO group. HRMS offered molecular formula $C_{13}H_8O_6$ (260.0321)

Table 1. ^{13}C NMR spectral data of Compounds 1-4 (δ ppm., DMSO-d_6)

C No	Compounds							
	Swertianolin (1)		Norswertianin(2)		Bellidifolin (3)		Swertianin (4)	
	^{13}C values (BB)	C-State DEPT	^{13}C values (BB)	C-State DEPT	^{13}C values (BB)	C-State DEPT	^{13}C Values (BB)	C-State DEPT
1	162.0	C	161.89	C	162.65	C	161.82	C
2	97.5	CH	97.94	CH	97.12	CH	97.08	CH
3	167.2	C	166.15	C	166.25	C	166.98	C
4	92.5	CH	93.84	CH	92.15	CH	92.64	CH
4a	157.2	C	157.61	C	156.38	C	157.64	C
5	137.2	C	105.72	CH	140.94	C	105.82	CH
5a	143.6	C	146.87	C	145.06	C	147.05	C
6	123.8	CH	123.74	CH	121.10	CH	124.07	CH
7	109.4	CH	140.18	C	112.42	CH	140.43	C
8	151.6	C	147.74	C	149.34	C	147.93	C
8a	107.4	C	107.21	C	111.90	C	107.28	C
9	185.0	C	183.67	C	180.89	C	184.15	C
9a	102.2	C	100.62	C	103.54	C	101.65	C
Ar-OMe	56.2	CH_3	---		56.00	CH_3	56.09	CH_3
1'	56.2		---		103.20	CH	---	
2'	---		---		73.47	CH	---	
3'	---		---		76.06	CH	---	
4'	---		---		69.82	CH	---	
5'	---		---		77.35	CH	---	
6'	---		---		60.84	CH_2	---	

suggesting ten double bond equivalents in the molecule. The UV spectrum showed absorption maxima identical with that proposed for norswertinin (30,31). The IR bands showed that the molecule possesses hydroxyl group (free OH at 3290 cm^{-1} and chelated at 3450 cm^{-1}), carbonyl group (1658, 1622 cm^{-1}) attached to C-C double bond at α-position and an ether linkage (1280 cm^{-1}). The ^1H NMR gave four sets of doublets and four broad singlets. The two doublets at δ 6.25 and 6.38 were *meta* coupled (*J*=2.1 Hz) and were assigned to H-2 and H-4 respectively, while the other two doublets at δ 6.82 and 7.24 were *ortho* coupled (*J*=8.6) and were assigned to H-5 and H-6 respectively (31). Four singlets were assigned to hydroxyl protons. Among them the hydroxyl group at δ 11.70 and 11.91 assigned C-1 and C-8 connectivities respectively because of their high down field delta values (chelated). The hydroxyl proton at 11.18 was linked at C-7 as it was deshielded due to hydroxyl group at C-8, while the OH at δ 9.33 was assigned at C-3 of the xanthone nucleus that allowed *meta* coupling between H-2 and H-4. The ^{13}C NMR gave thirteen carbon resonances. The DEPT experiments suggested that these signals were due to the four methines and nine quaternary carbon atoms (Table 1). The assignments were made on the basis of comparison with 1, 3, 7, 8-tetraoxygenated patterns(7). The studies resulted that C-1, C-3, C-7 and C-8 carbon atoms had hydroxyl oxygen connectivities because of their quaternary nature and relatively down field delta values. Moreover, the high down field delta value of C-7 (δ 140.18) than that of methybellidifolin (δ 105.3), bellidifolin (**3**; δ 109.4) and swertianolin (**1** δ 112.4) (7) that possessed C-H connectivities, further confirmed the hydroxyl oxygen linkage at C-7. All these assignments led to the conclusion that the structure of **2** was 1,3,7,8-tetrahydroxyxanthone (norswertianin) (Fig. 1).

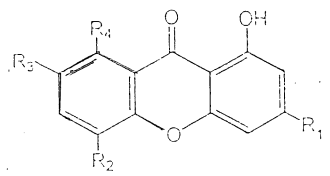
Compound **3** (yellow needles with m. p. 263°C) was crystallized from MeOH. The EIMS showed molecular ion peak at m/z 274 which was further confirmed by FDMS. A peak at m/z 245 arose due to the loss of CHO group from the molecule. HRMS offered molecular formula $C_{14}H_{10}O_6$ (274.0477) suggesting ten double bond equivalents in the molecule. The UV spectrum gave absorption maxima identical with that proposed for bellidifolin (7). The IR bands showed the presence of hydroxyl group (free OH at 3252 cm^{-1} and chelated at 3452 cm^{-1}), carbonyl group (1656 cm^{-1}) and ether linkage (1284 cm^{-1}) in the molecule. The ^1H NMR spectrum displayed four sets of doublets and four singlets. Two doublets at δ 6.42 and 6.62 were *meta* coupled (*J*=2.4 Hz) and assigned to H-2 and H-4 respectively while the other two sets at δ 7.22 and 6.62 were *ortho* coupled (*J*=8.8 Hz) and assigned to H-5 and H-7 respectively (7). A sharp singlet at δ 3.88 was assigned to methoxy protons and three broad singlets appeared at δ 9.68, 11.16 and 11.90 were assigned to three hydroxyl protons. The ^{13}C NMR of the compound indicated fourteen carbon resonances and DEPT studies had shown that they corresponded to one methyl, four methines and nine quaternary carbon atoms (Table 1). These assignments were made on the basis of comparison (7). Furthermore, the delta value of C-5 was found in the down field (δ 140.94) than the corresponding values of swertianin (**4**; δ 105.83) and norswertianin (**2**; δ 105.72) suggesting hydroxyl oxygen connectivity. All these assignments made, led to the conclusion that the struc-ture of **3** was 1, 5, 8-tetrahydroxy-3-methoxyxanthone (bellidifolin) (Fig. 1).

Compound **4** (also yellow needles with m.p. 221°C) was crystallized from MeOH. The EIMS showed molecular ion peak at m/z 274 which was further confirmed by FDMS. HRMS offered molecular

formula $C_{14}H_{10}O_6$ (274.0477) suggesting ten double bond equivalents in the molecule. The UV spectrum indicated the presence of xanthone nucleus and there was no change in the spectrum after the addition of NaOH, thus suggesting that there is no hydroxyl group at C-3 and C-6 locations (24). The IR bands indicated the presence of hydroxyl group (3454 cm^{-1}) and carbonyl group (1668 cm^{-1}) at C-C double bond in the molecule. The ^1H NMR displayed four doublets and four singlets. Two doublets appeared at δ 6.38 and 6.54 were *meta* coupled (J=2.4 Hz) and were assigned to H-2 and H-4 respectively, while the other two sets were *ortho* coupled (J=8.6 Hz) and were assigned to H-5 and H-6 protons respectively (31). A sharp proton singlet, at δ 3.88 was assigned to methoxy group while three, one proton singlets at δ 9.40, 11.60 and 11.84 were assigned to three hydroxyl protons, the former value of which was proposed to be free while the latter two were chelated. The ^{13}C NMR spectrum showed fourteen carbon resonances corresponding to one methyl, four methines and nine quaternary carbon atoms (Table 1). The high down field delta value of C-7 (δ 140.43) than that of the compounds **1** and **3** (δ 109.40 and 112.42) and similarity with compound **2** (δ 140.18) indicated the link of hydroxyl group at this carbon atom. All these evidences from spectral studies led to the conclusion that the structure of **4** was 1, 7, 8 - trihydroxy - 3 - methoxy - xanthone (swertianin) (Fig.1).

The antimicrobial activities of these compounds have been evaluated by famous antimicrobial parameters by measuring the zones of inhibition (ZI) and by calculating their minimum inhibitory concentrations (MIC) and comparing with the MIC of the standard antibacterial and antifungal agents under the similar coditions. The ZI and MIC of these compounds have also been outlined in Table 2 and 3.

For comparing the antimicrobial properties of these compounds, a number of antibacterial (such as amoxicillin, ampicillin, chloramphenicol, clarithromycin, erythromycin, haloprogin and kanamycin) and antifungal compounds (such



1. R_1 = OMe, R_2 = OH, R_3 = H, R_4 = OGlu
2. R_1 = R_2 = R_4 = OH, R_3 = H
3. R_1 = OMe, R_2 = R_4 = OH, R_3 = H
4. R_1 = OMe, R_2 = H, R_3 = R_4 = H

Fig.1.Structures of the compounds isolated from *Swertia ciliata*

as benzoic acid, canesten, clotrimazole, gentian violet, griseofulvin, magenta paint, nystatin and tineafax) were used as standard medicines against such microorganisms. The ZI and MIC data produced by these standard compounds have not been displayed here, but it was observed that all these standard compounds indicated well marked antimicrobial activity against the microorganisms used and could be comparable with the similar parameters of *S. ciliata's* compounds. One of the standard antibacterial compounds "kanamycin" and antifungal standard compound "clotrimazole" that gave well defined ZI and MIC, were further used for the comparison with the isolated compounds.

The results indicated that all the four isolated compounds of *S. ciliata* were effective against the microorganisms used. The highest antibacterial activity exhibited by all these four compounds and also by the standard kanamycin were against *Bacillus megaterium, Escherichia*

Table 2. Antibaterial activity of *Swertia ciliata* compounds

Test organisms	Compounds									
	Swertiano-lin(1)		Norswertia-nin(2)		Bellidifolin (3)		Swertianin (4)		Kanamycin	
	ZI	MIC	ZI	MIC	ZI	MIC	ZI	MIC	ZI	MIC
GRAM (+) BACTERIA										
1. *Bacillus megaterim*	25	0.62	30	0.15	28	0.31	26	0.62	32	0.15
2. *Bacillus subtilis*	19	5.0	28	0.31	24	1.25	15	5.0	20	0.31
3. *Bacillus thuringiensis*	18	5.0	24	0.62	18	5.0	5	10.0	19	0.62
4. *Sarcina lutea*	14	2.5	21	1.25	16	5.0	8	5.0	18	0.31
5. *Staphylococcus aureus*	13	2.5	20	5.0	19	5.0	10	10.0	11	5.0
GRAM(-) BACTERIA										
6. *Escherichia coli*	21	10.0	19	5.0	11	5.0	10	10.0	14	5.0
7. *Klebsiella pneumoniae*	18	0.62	18	0.62	13	2.5	8	5.0	21	1.25
8. *Proteus vulgaris*	16	5.0	22	1.25	15	2.5	5	2.5	20	1.25
9. *Pseudomonas sp.*	20	5.0	21	1.25	14	1.25	6	10.0	10	2.5

Where: Compound concentration=20 mg/ml
Kanamycin concentration= 1 mg/ml
ZI=Mean zone of inhibition of six replicates in mm

Table 3. Antifungal activity of *Swertia ciliata* compounds

Test organisms	Compounds									
	Swertiano-lin(1)		Norswertia-nin(2)		Bellidifolin (3)		Swertianin (4)		Kanamycin	
	ZI	MIC	ZI	MIC	ZI	MIC	ZI	MIC	ZI	MIC
1. *Aspergillus niger*	24	0.62	21	2.50	32	0.62	28	0.31	24	0.62
2. *A. fumigatus*	26	1.25	25	1.25	35	0.15	26	0.62	20	0.62
3. *A. parasiticcus*	27	0.62	22	2.50	31	0.15	30	0.15	28	0.31
4. *Candida albicans*	20	2.50	24	2.50	30	0.15	20	1.25	27	0.31
5. *Candida stellatoidea*	15	2.50	25	2.50	32	0.15	22	1.25	29	0.31
6. *Monilia sitophilia*	23	1.25	28	0.62	30	0.31	25	0.62	28	0.31
7. *Penicillium digitation*	21	2.50	26	0.62	29	0.25	21	0.62	25	0.62
8. *Trichlophton tousurance*	31	0.31	25	0.31	31	0.31	30	0.15	32	0.15

Where: Compound concentration=20 mg/ml
Clotrimazole concentration= 1 mg/ml
ZI=Mean zone of inhibition of six replicates in mm
MIC=Minimum inhibitory concentration in mg/ml

coli and *Klebsiella pneumoniae* (Table 2). On the other hand, the results presented in Table 3 indicated that the growths of all the fungi species were also markedly affected by the *S. ciliata's* compounds when compared with the standard antifungal clotrimazole. The highest antifungal activity of these compounds and also the standard antifungal clotrimazole were against *Trichophyton tonsurance* (Table 3).

All the bacteria and fungi species discussed in this investigation often cause hospital infections and gained resistance in a very short time against antibacterial and antifungal medicines. The fact that the concentration ranges of the phytochemical compounds isolated from *S. ciliata* (about 20 mg/ml against both bacteria and fungi used) could be an important clinical aspect as regard the microbiological investigations are concerned.

Conclusion

We have thus concluded from this investigation that *Swertia ciliata* from our local source contained closely related xanthones, which have potent antimicrobial potentials against a number of gram positive bacteria, gram negative bacteria and fungi. Four of these xanthones were isolated and identified with the help of spectral data. All the isolated xanthones exhibited distinct antimicrobial potential against nine species of fungi. Among these four compounds, norswertianin (**2**) and bellidifolin (**3**) were the most potent antibacterial agents with largest ZI and least MIC. Swertianolin (**1**) was an intermediate and swertianin (**4**) was the least antibacterial agent when compared with kanamycin. On the other hand, bellidifolin (**3**) and swertianin (**4**) appeared to be more potent antifungal compounds than swertianolin (**1**) and norswertianin (**3**) when compared with clotrimazole. Further work is imperative to amplify this property through the preparation of their derivatives, which would possibility lead to the structure-activity relationship of such important antimicrobial compounds from our natural sources.

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