Phytochemical constituents of the flowers of *Sarcococca coriacea* of Nepalese origin.

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Abstract:

From the flowers of Sarcococca *coriacea,* a triterpenoic acid, oleanolic acid, a pentahydric sugar alcohol, xylitol along with the mixture of steroidal glycosides, stigmasterol-3-O-β-D-glucopyranoside and β-sitosterol-3-O-β-D-glucopyranoside have been isolated by chromatographic technique. Their structures were established on the basis of IR, ^{1}H-NMR, ^{13}C-NMR, spectral data as well as melting point and Co-TLC comparison with the authentic samples.

Introduction:

Sarcococca coriacea (Buxaceae) is an ever green shrub which may grow up to a height of two meters. It is distributed from central to eastern parts of Nepal at an altitude[1] 600-1600 m. The flowers are white and creamy blossom in February and fruiting takes place in August. Local people called Fitifiya, Pipiree in Bhojpur District, Nepal. Plants of the genus *Sarcococca* are used by the local people as a useful drug for the treatment of various diseases like malaria[2], rheumatism[2], skin infection[2], in the folk system of medicine and also exhibit, antiulcer[3], antitumor[3], ganglion-blocking[3] anticholinesterase[4] and antibacterial[5,6] activities. Previous studies[7,8] on the aerial parts of *Sarcococca coriacea*(Hook f.) sweet (Buxaceae) have resulted in the isolation of many steroidal alkaloids and most of them are reported to have anticholinesterase activities. So far no report on chemical investigation on flowers of *Sarcococca* has been reported. This paper deals with the isolation and identification of the constituents like pentahydric sugar alcohol (pentitol)-xylitol, oleanolic acid along with the mixture of steroidal glycosides-stigmasterol -3-O-β-D-glucopyranoside and sitosterol-3-O-β-D-glucopyranoside. To the best of our knowledge the rare sugar alcohol, xylitol previously reported from *Bupleurum tenue*[16] have been reported for the first time from the genus *Sarcococca*.

Results and Discussion

The dried and powdered flower (56gm.) of *Sarcococca coriacea* was extracted with methanol and concentrated under reduced pressure to yield waxy residue(5.4gm). On repeated column chromatography of methanolic extract(5.3gm) gave compounds **Sc1**(76mg), **Sc2**(8mg) and **Sc3**(8mg).

Compound Sc1: The **Compound Sc1**, a white amorphous solid, mp 286^{0}C, was soluble in ethyl acetate, acetone and methanol. Comparing its IR spectra, mp, CoTLC with the authentic oleanolic acid, isolated earlier in our laboratory from the aerial parts of *S. coriacea*[9] and *Coriaria nepalensis*[10], the compound was identified to be oleanolic acid.**Compound Sc2**: **Compound Sc2** was isolated as an amorphous powder, mp. 265^{0}C (dec.).The compound responded positively to Liebermann Buchard and Molisch tests. The IR spectrum showed the absorption signal at 3400 cm^{-1} due to hydroxyl group, intense peaks at 2950 and 2860 cm^{-1} due to C-H stretch, a doublet of equal intensities at 1380 and 1370 cm^{-1} due to C-H bending vibration of isopropyl (gem dimethyl groups) and a peak at 1648 cm^{-1} due to double bond[11]. The intensity of two C-18 methyl proton peaks in its ^{1}H-NMR indicated that the compound Sc2 was a mixture of two sterols approximately in the ratio of 1: 0.75. The major component was found to have two

tertiary methyl signals at δ0.688, 0.954, three doublets (3H each) at δ0.878 (3H, d, J=6.5Hz), 0.928 (3H, d, J=6.5 Hz), 1.091 (3H, d, J=6.5 Hz), a triplet centered at 0.899 (3H, t, J=7.5 Hz) assignable to a primary methyl group. These characteristic signals suggest the steroidal skeleton. Further ^{1}H-NMR displayed a distorted triplet signal[12] at δ5.36 which is characteristic of Δ^5-sterols and two double doublets, which account for two olefinic protons, at δ5.234 (1H, dd, J=8.75Hz, 15.25 Hz), δ5.086 (1H, dd, J=8.25, 11.75 Hz). Furthermore the signals of two olefinic carbon atoms at δc121.961 and δc 140.97 undoubtedly proved the presence of endocyclic double bond[12] between C-5 and C-6 of the aglycone. These evidences are consistent with the 24ξ-ethylcholestan-5, 22-dien-3β-ol. However the signal due to αH-3 proton shifted downfield at δ4.00 (1H, m, αH-3) which was expected at δ3.505 for the Δ^5-sterols[13] indicating the presence of glucosyl moiety at C-3 position of the aglycone. It was further supported by the appearance of an anomeric carbon signal[14] at δc102.6 and that of C-3 shifted downfield[12] at δc78.67. It showed the linkage of the sugar moiety at C-3 of stigmasterol 3-O-β-D-glucopyranoside. The ^{1}H-NMR and ^{13}C-NMR spectral data (Table-I, II) were found identical with that of stigmasterol-3-O-β-D-glucopyranosyl isolated earlier in our lab from the aerial parts of *S. coriacea*[9]. Therefore the compound was confirmed as stigmasterol-3-O-β-D-glucopyranoside.

The ^{1}H-NMR of the minor component displayed methyl signals, distinctly different from those of the major component, at δ0.674 (3H, s), 0.954 (3H, s), 1.0025 (3H, d, J=6.5Hz). Besides these three methyl groups there are three more methyl groups are present as shown by its DEPT and ^{13}C-NMR spectrum. However the complete signals due to those methyl groups were not observed distinctly and appeared only as shoulders in the 1H-NMR spectrum because of their close δ values with those of the major component. The complementary signal at δ0.923 of 0.909 (observed) as required for the doublet centered at δ0.916 (3H, d, J=7.8 Hz) is masked by the signal at δ0.921.The signal at δ0.902 complementary of δ0.888 (observed), δ0.876 (observed) needed for the triplet centered at δ0.888 (3H, d, J=6.5Hz) is masked by 0.899. Both signals at δ0.882, δ0.868 needed for the doublet centered at δ0.875 (3H, d, J=7.0Hz) were masked by the signal at δ0.884 and δ0.871 respectively. ^{1}H-NMR and ^{13}C-NMR data of the compound were almost identical with those of the literature value[15] of 3-O- β-D-glucopyranosyl sitosterol (Table I, II). Thus, it leads us to conclude that the **Compound Sc2** was a mixture of stigmasterol-3-O-β-D-glucopyranoside (major component) and sitosterol-3-O- β-D-glucopyranoside (minor component). Comparison of ^{1}H-NMR and ^{13}C-NMR shift of **Compound Sc2** with that of reported glycosides are given in the table I and II respectively.

^{1}H-NMR chemical shift of **Compound Sc2** and reported data of. Stigmasterol-3-O-β-D- glucopyranoside[9](A) and β-Sitosterol-3-O-β-D- glucopyranoside[15](B) is given in Table-I.

Table:-I.

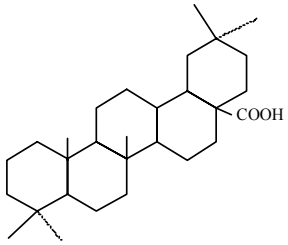
.S. No.	Compound Sc2 (pyridine-d_5, 500 MHz)	Reported data	
		Stigmasterol-3-O-β-D-glucopyranoside[9] (A) (pyridine-d_5, 500 MHz)	β-Sitosterol-3-O-β-D-glucopyranoside[15] (B) (pyridine-d_5, 400 MHz)
1.	0.674 (3H,s, H-18)		0.67 (3H, s)
2.	0.689 (3H,s,H−18)	0.688 (3H,s, H-18)	
3	0.875 (3H,d ,J=7.0Hz)		0.86 (3H,d,J=7.7Hz)
4.	0.8775 (3H,d,J=6.5,Hz,H-27)	0.878 (3H,d, J=6.4Hz,H-27)	
5.	0.888 (3H,t,J=7.0Hz)		0.90 (3H,t,J=7.0Hz)
6.	0.899 (3H,t,J=7.5Hz,H-29)	0.899 (3H,t,J=7.5Hz,H-29)	
7.	0.916 (3H,d,J=7.0Hz,H-2..)		
8.	0.928(3H,d, J=6.5Hz,H-26)	0.928 (3H,d, J=6.5Hz,H-26)	
9.	0.954(3H,s, H-19)	0.954(3H,s, H-19)	0.93(3H,s)
10.	1.00(3H,d, J=6.5Hz)		1.02(3H,d, J=6.5Hz)
11.	1.09(3H,d, J=6.5Hz,H-21)	1.09(3H,d, J=6.4Hz,H-21)	
12.	4.00(1H,m,αH-3)	4.317(1H,m, αH-3)	
13.	5.086(1H,dd,J=8.25, 11.75Hz).	5.078(dd, 1H, J=8.9, 15.1Hz, H-23).	
14.	5.234(1H,dd,J=8.75,15.25 Hz.,H-22)	5.233(dd, 1H, J=8.7, 15.1Hz, H-22).	
15.	5.36(br s,1H.H-6).	5.36(H-6).	5.36(1H,m,H-6).

^{13}C-NMR chemical shift of **Compound Sc2** and reported data of Stigmasterol-3-O-β-D glucopyranoside[9](A) and β-Sitosterol-3-O-β-D- glucopyranoside[15](B) is given in Table:-II.

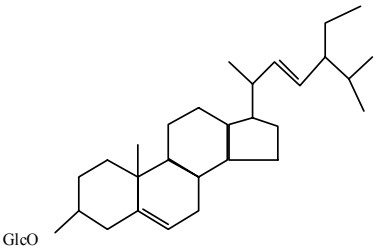
Table:-II.

S. No.	Compound Sc2 (pyridine-d_5, 125..65 MHz)		Reported data	
	δc	DEPT	Stigmasterol-3-O-β-D-glucopyranoside[9] (A) (pyridine-d_5, 75MHz)	β-Sitosterol-3-O-β-D-glucopyranoside[15] (B) (pyridine-d_5, 100.6 MHz)
1.	12.026	CH_3		11.99 (C-18), 12.12 (C-29).
2.	12.207	CH_3	12.19 (C-18)	
3.	12.560	CH_3	12.58 (C–29)	
4.	14.296	CH_3		
5.	19.059	CH_3		19.02(C-21)
6.	19.231	CH_3	19.23 (C-27)	.
7.	19.264	CH_3		19.22 (C-26).
8.	19.470	CH_3	19.47 (C-19)	19.43 (C-19)
9.	20.021	CH_3		20.00 (C-27).
10.	21.329	CH_3, CH_2	21.35 (C-26), 21.35(C-11).	21.28 (C-11).
11.	21.518	CH_3	21.52(C-21).	
12.	22.958	CH_2		
13.	23.459	CH_2		23.38(C-28).
14.	24.562	CH_2		24.5 (C-15)
15.	24.595	CH_2		
16.	25.738	CH_2		
17.	26.478	CH_2		26.35 (C-23)
18.	28.584	CH_2		28.55 (C-16)
19.	29.349	CH_2	29.37 (C-16)	
20.	29.538	CH		29.44 (C-25)
21.	29.629	CH_2		30.23(C-2)
22.	29.645	CH		
23.	30.32	CH_2	30.32 (C-2)	
24.	32.113	CH		32..05 (C-8)
25.	32.146	CH_2	32.22 (C-7)	32.17 (C-7).
26.	32.211	CH.		
27.	34.270	CH_2		34.19 (C-22)
28.	36.439	CH		36.39 (C-20)
29.	36.999	C	36.99 (C-10)	36.92 (C-10)
30.	37.542	CH_2	37.53 (C-1)	37.47 (C-1)
31.	39.401	CH_2		39.32 (C-12)
32.	39.886	CH_2	39.88 (C–12)	
33.	40.00	CH_2		39.94(C–4)
34.	40.815	CH		
35.	42.411	C	42.4 (C–13)	
36.	42.543	C		42.47 (C–13)
37.	46.113	CH		46.03 (C–24)
38.	50.415	CH		50.33 (C–9)
39.	51.470	CH		
40.	53.705	CH		
41.	56.140	CH		
42	56.312	CH		56.23 (C–17)
43.	56.888	CH		56.82(C–14)
44.	56.979	CH		

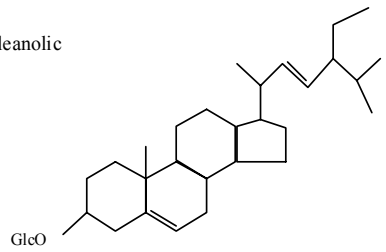
45.	62.918	CH_2	62.91(Glc-6')	62.80 (Glc-6')
46.	71.785	CH	71.77 (Glc-4')	71.64 (Glc-4')
47	75.404	CH	75.4 (Glc-2')	75.29 (Glc-2')
48.	78.160	CH		78.05 (Glc-5')
49.	78.530	CH	78.55 (Glc-5')	78.45 (Glc-3')
50.	78.670	CH	78.69 (C–3)	78.57 (C-3)
51.	102.639	CH	102.63 (Glc-1')	102.54 (Glc-1')
52.	121.961	CH	121.96 (C–6).	121.91 (C-6)
53.	129.528	CH	129.52 (C–23)	
54.	138.864	CH	138.23 (C-22)	
55.	140.978	C	140.97 (C-5)	140.88 (C-5).



Sc1: Oleanolic



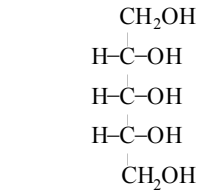
Sc2(minor): β-sitosterol-3-O-β-D-glucopyranoside



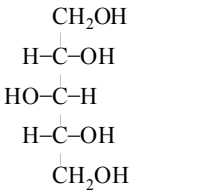
Sc2(major): Stigmasterol-3-O-β-D-glucopyranoside

Compound Sc3: The **Compound Sc3,** a crystalline compound, mp 100^0C, soluble in methanol, pyridine, insoluble in ethyl acetate, chloroform has R_f=0.293[1:3:0.05= methanol: ethyl acetate: acetic acid].

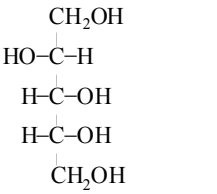
^{1}H-NMR displayed a triplet centered at δ4.825(1H, t, J=6.0 Hz.) and three multiplets at δ4.359-4.618 indicating the presence of CHOH and CH_2OH groups respectively.^{13}C-NMR exhibited distinctly five carbon signals attributable to carbinyl methylene and methine carbons. The signal exhibited at δ65.2 and 65.43 could be assigned for two carbinyl methylene carbons while the remaining three signals at δ72.352, 73.200 and 73.496 were due to the presence of three carbinyl methine carbons. Thus the compound was identified as one of the isomers of pentahydric alcohols (pentitols) namely meso isomers ribitol (adonitol), xylitol, optically active isomers DL-arabinitol (DL-arabitol) or DL-lyxitol.



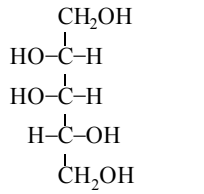
Ribitol (Adonitol)
mp[16,17]–102–4°C, 98–102°C



Xylitol
mp[16]–94.5–95°C



DL-Arabinitol (Arabitol)
mp[16]–D–form:103–4°C, L–form:101–2°C



D–Lyxitol (D–Arabitol)
L–Lyxitol (L–Arabitol)

^{13}C-NMR data of the compound when compared with those of the literature data[16] of xylitol, xylitol showed only three carbon signals at 100MHz. for five carbon atoms whereas the compound has displayed five distinct signals at 125.65 MHz. Signals at δ 66.8 and δ73.6 of xylitol showed the presence of two methylene and two methine carbon atoms. All those signals of xylitol were also observed in the ^{13}C-NMR spectrum of the **Compound Sc3** approximately at the same δ values along with two more signals at δ 65.122 and δ 73.200. These two additional signals have very close δ values with the signals at δ 66.8 which showed the presence of two methylene and the signal at δ73.6 which

showed the presence of two methine carbon atoms of xylitol. So it could be argued that the two signals at δ 66.8 and δ 73.6 which showed the presence of two methylene and two methine carbon atoms of the xylitol measured at 100 MHz have been resolved into two methylene at δ 65.122 and δ 65.426 and two methine at δ 73.200 and δ 73.496 signals respectively when measured at 125.65 MHz.(Table:-III).

Table:-III

^{13}C-NMR data(δc) of **Sc3** and reported data(δc) of xylitol.

s. no.	δc valus of the compound Sc3(125.65MHz.)	Reported data(δc)[16] of xylitol.(100 MHz.)
1.	65.122	66.8(CH_2OH).
2.	65.426	66.8(CH_2OH)
3.	72.352	72.9(CHOH)
4.	73.200	73.6(CHOH).
5.	73.496	73.6(CHOH).

These evidences suggested the structure of the compound as xylitol. However the mp of the compound is much more close to the melting points of ribitol (mp[16,17]-102-4^{0}C,98-102^{0}C and L-arabitol (mp[16] 101-2^{0}C).

To the best of our knowledge the presence of petahydric alcohol have been reported for the first time from the plant belonging to Buxaceae *which comprises several genera including only two genera* Buxus *and* Sarcococca *have been reported occuring in Nepal*[1].

Experimental:

Melting points were determined in Sunvik electrical mp apparatus and are not corrected. IR spectra were recorded in KBr disc and absorption peaks were expressed in cm^{-1}.^{1}HNMR and ^{13}CNMR were recorded in 500MHz and 125.65 MHz spectrophotometer respectively using TMS as internal reference and deuterated pyridine was used as solvent. Chemical shift values were expressed in δ values. Silica gel (160-200 mesh) for column chromatography and precoated TLC plates from EMERCK-FRG were used for R_f values.

Plant materials:

Flowers remained dried on the tree were collected from different places of Kathmandu valley namely Dakshinkali, Godavari and mainly from Goldhunga in the month of November.

Extraction and isolation:

The dried and powdered flower (56gm) was extracted with methanol (5x100ml) by percolation method to afford waxy residue (5.4) after evaporation under reduced pressure. The extract (5.3gm) was then subjected to column (60x4.2cm) chromatography. The column was eluted with hexane, ethyl acetate, and methanol solvent systems in the order of increasing polarities. The column chromatography operation lead to the isolation of three compounds designated as **Sc1** (76 mg), **Sc2** (8mg) and **Sc3** (8mg). All those compounds were isolated for the first time from the flower of *Sarcococca coriaceae*. To the best of our knowledge compound **Sc3** was isolated for the first time from the genus *Sarcococca*.

Sc1(Oleanolic acid):- Green colored solution obtained by eluting with 10%ethyl acetate in hexane were mixed and then evaporated under reduced pressure yielded greenish white compound. It was filtered, washed with cold ethyl acetate. The compound was recrystallized from ethyl acetate which afforded 76 mg white amorphous compound designated as **Sc1**, mp 286^{0}C (lit[7,8] mp=258^{0}C, 288^{0}C), soluble in ethylacetate , methanol, acetone etc. R_f = 0.55(glass) [ethyl acetate: hexane=2:3]

IR spectrum:

$V_{max\ (KBr)}$ 3433.93cm^{-1} (OH), 2943.52 cm^{-1} (C-H), 1693 cm^{-1}(C=O), 1385 cm^{-1}, 1352 cm^{-1}.

Sc2(Stigmasterol glucoside+Sitosterol glucoside):-Similar fractions eluted with ethyl acetate were mixed together and then concentrated under reduced pressure .Black gummy

residue obtained by adding hexane to the concentrated solution was removed first. The yellow colored solution so obtained was further treated with hexane so that all the yellow colored gummy substances were precipitated out. The colorless solution on treating with more hexane gave white compound. It was filtered, washed then recrystallized from methanol to give white amorphous compound **Sc2** (8mg).The compound was soluble in pyridine, partially in methanol and chloroform, soluble in mixture of methanol and chloroform. R_f=0.54[MeOH: EtoAc=1:4], mp.265^{0}C (dec)

^{1}H-NMR (500.00MHz, C_5D_5N):-See table-I.

^{13}C-NMR (125.65MHz, C_5D_5N):-See table-II.

Sc3 (Pentitol):-Similar fractions eluted with ethyl acetate were mixed, concentrated under reduced pressure. The turbid solution so obtained was left for 24 hours at room temperature, gave white compound. The compound was washed with cold ethyl acetate then dissolved in methanol. The methanol solution when treated with ethyl acetate gave turbid solution. It was allowed to cool in freeze to afford colorless crystal of **Sc3** (8mg). The compound was soluble in methanol, insoluble in ethyl acetate and chloroform. R_f=0.292[1:3:0.05= methanol: ethyl acetate: acetic acid]

^{1}H-NMR (500.00MHz, C_5D_5N):-

δ4.25(1H, t, J=6.0Hz.), multiplets peaks between 4.359-4.841.

^{13}C-NMR (125.65MHz, C_5D_5N)

δc: 65.122, 65.426, 72.352, 73.200, and 73.496

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