

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, ¹H-NMR, ¹³C-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 altitude¹ 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 malaria², rheumatism², skin infection², in the folk system of medicine and also exhibit, antiulcer³, antitumor³, ganglion-blocking³ anticholinesterase⁴ 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 286⁰C, 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. 265⁰C (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 bond¹¹. The intensity of two C-18 methyl proton peaks in its ¹H-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 δ 0.688, 0.954, three doublets (3H each) at δ 0.878 (3H, d, $J=6.5$ Hz), 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 $^1\text{H-NMR}$ displayed a distorted triplet signal¹² at δ 5.36 which is characteristic of Δ^5 -sterols and two double doublets, which account for two olefinic protons, at δ 5.234 (1H, dd, $J=8.75$ Hz, 15.25 Hz), δ 5.086 (1H, dd, $J=8.25$, 11.75 Hz). Furthermore the signals of two olefinic carbon atoms at δ c121.961 and δ c 140.97 undoubtedly proved the presence of endocyclic double bond¹² between C-5 and C-6 of the aglycone. These evidences are consistent with the 24 ξ -ethylcholestan-5, 22-dien-3 β -ol. However the signal due to $\alpha\text{H-3}$ proton shifted downfield at δ 4.00 (1H, m, $\alpha\text{H-3}$) which was expected at δ 3.505 for the Δ^5 -sterols¹³ indicating the presence of glucosyl moiety at C-3 position of the aglycone. It was further supported by the appearance of an anomeric carbon signal¹⁴ at δ c102.6 and that of C-3 shifted downfield¹² at δ c78.67. It showed the linkage of the sugar moiety at C-3 of stigmasterol 3-O- β -D-glucopyranoside. The $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ 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 $^1\text{H-NMR}$ of the minor component displayed methyl signals, distinctly different from those of the major component, at δ 0.674 (3H, s), 0.954 (3H, s), 1.0025 (3H, d, $J=6.5$ Hz). Besides these three methyl groups there are three more methyl groups are present as shown by its DEPT and $^{13}\text{C-NMR}$ spectrum. However the complete signals due to those methyl groups were not observed distinctly and appeared only as shoulders in the $^1\text{H-NMR}$ spectrum because of their close δ values with those of the major component. The complementary signal at δ 0.923 of 0.909 (observed) as required for the doublet centered at δ 0.916 (3H, d, $J=7.8$ Hz) is masked by the signal at δ 0.921. The signal at δ 0.902 complementary of δ 0.888 (observed), δ 0.876 (observed) needed for the triplet centered at δ 0.888 (3H, d, $J=6.5$ Hz) is masked by 0.899. Both signals at δ 0.882, δ 0.868 needed for the doublet centered at δ 0.875 (3H, d, $J=7.0$ Hz) were masked by the signal at δ 0.884 and δ 0.871 respectively. $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ data of the compound were almost identical with those of the literature value¹⁵ 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 $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ shift of **Compound Sc2** with that of reported glycosides are given in the table I and II respectively.

$^1\text{H-NMR}$ chemical shift of **Compound Sc2** and reported data of. Stigmasterol-3-O- β -D- glucopyranoside⁹(A) and β -Sitosterol-3-O- β -D- glucopyranoside¹⁵(B) is given in Table-I.

Table:-I.

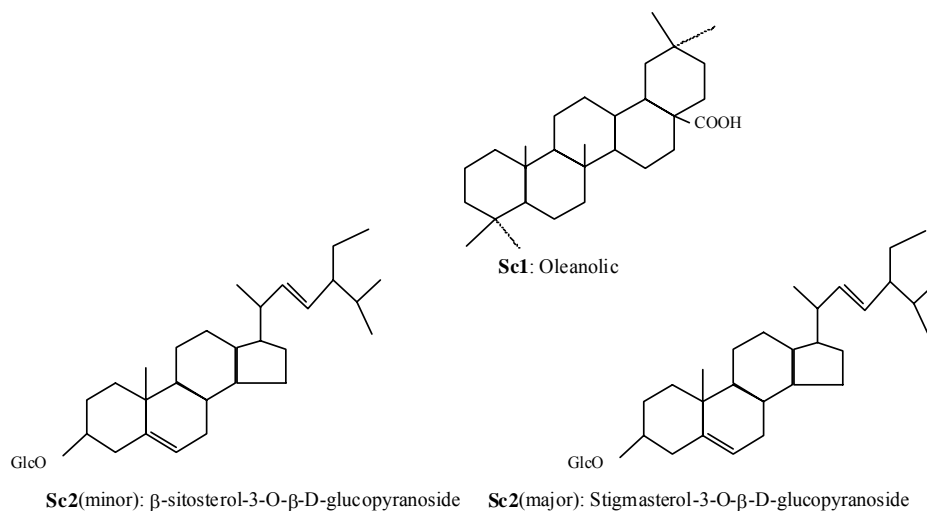
.S. No.	Compound Sc2 (pyridine-d ₅ , 500 MHz)	Reported data	
		Stigmasterol-3-O- β -D-glucopyranoside ⁹ (A) (pyridine-d ₅ , 500 MHz)	β -Sitosterol-3-O- β -D-glucopyranoside ¹⁵ (B) (pyridine-d ₅ , 400 MHz)
1.	0.674 (3H,s, H-18)		0.67 (3H, s)
2.	0.689 (3H,s,H-18)	0.688 (3H,s, H-18)	
3.	0.875 (3H,d , $J=7.0$ Hz)		0.86 (3H,d, $J=7.7$ Hz)
4.	0.8775 (3H,d, $J=6.5$,Hz,H-27)	0.878 (3H,d, $J=6.4$ Hz,H-27)	
5.	0.888 (3H,t, $J=7.0$ Hz)		0.90 (3H,t, $J=7.0$ Hz)
6.	0.899 (3H,t, $J=7.5$ Hz,H-29)	0.899 (3H,t, $J=7.5$ Hz,H-29)	
7.	0.916 (3H,d, $J=7.0$ Hz,H-2..)		
8.	0.928(3H,d, $J=6.5$ Hz,H-26)	0.928 (3H,d, $J=6.5$ Hz,H-26)	
9.	0.954(3H,s, H-19)	0.954(3H,s, H-19)	0.93(3H,s)
10.	1.00(3H,d, $J=6.5$ Hz)		1.02(3H,d, $J=6.5$ Hz)
11.	1.09(3H,d, $J=6.5$ Hz,H-21)	1.09(3H,d, $J=6.4$ Hz,H-21)	
12.	4.00(1H,m, $\alpha\text{H-3}$)	4.317(1H,m, $\alpha\text{H-3}$)	
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).

¹³C-NMR chemical shift of **Compound Sc2** and reported data of Stigmasterol-3-O-β-D glucopyranoside⁹(A) and β-Sitosterol-3-O-β-D- glucopyranoside¹⁵(B) is given in Table:-II.

Table:-II.

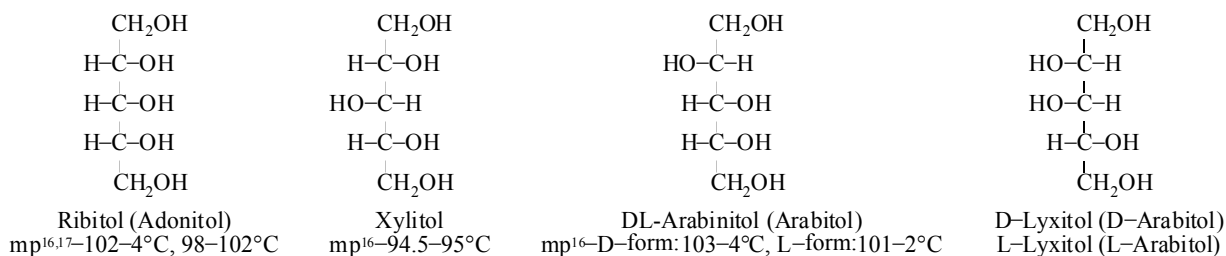
S. No.	Compound Sc2 (pyridine-d ₅ , 125.65 MHz)		Reported data	
	δc	DEPT	Stigmasterol-3-O-β-D-glucopyranoside ⁹ (A) (pyridine-d ₅ , 75MHz)	β-Sitosterol-3-O-β-D-glucopyranoside ¹⁵ (B) (pyridine-d ₅ , 100.6 MHz)
1.	12.026	CH ₃		11.99 (C-18), 12.12 (C-29).
2.	12.207	CH ₃	12.19 (C-18)	
3.	12.560	CH ₃	12.58 (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).



Compound Sc3: The **Compound Sc3**, a crystalline compound, mp 100⁰C, soluble in methanol, pyridine, insoluble in ethyl acetate, chloroform has $R_f=0.293$ [1:3:0.05= methanol: ethyl acetate: acetic acid].

¹H-NMR displayed a triplet centered at $\delta 4.825$ (1H, t, J=6.0 Hz.) and three multiplets at $\delta 4.359-4.618$ indicating the presence of CHOH and CH₂OH groups respectively. ¹³C-NMR exhibited distinctly five carbon signals attributable to carbinyl methylene and methine carbons. The signal exhibited at $\delta 65.2$ and $\delta 65.43$ could be assigned for two carbinyl methylene carbons while the remaining three signals at $\delta 72.352$, $\delta 73.200$ and $\delta 73.496$ 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.



¹³C-NMR data of the compound when compared with those of the literature data¹⁶ 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 $\delta 66.8$ and $\delta 73.6$ of xylitol showed the presence of two methylene and two methine carbon atoms. All those signals of xylitol were also observed in the ¹³C-NMR spectrum of the **Compound Sc3** approximately at the same δ values along with two more signals at $\delta 65.122$ and $\delta 73.200$. These two additional signals have very close δ values with the signals at $\delta 66.8$ which showed the presence of two methylene and the signal at $\delta 73.6$ 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 value of the compound Sc3 (125.65MHz.)	Reported data(δc) ¹⁶ of xylitol.(100 MHz.)
1.	65.122	66.8(CH ₂ OH).
2.	65.426	66.8(CH ₂ OH)
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}-102-4⁰C,98-102⁰C and L-arabitol (mp¹⁶ 101-2⁰C)

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 occurring in Nepal¹.

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} .¹HNMR and ^{13}C NMR 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 286⁰C (lit^{7,8} mp=258⁰C, 288⁰C), soluble in ethylacetate, methanol, acetone etc. R_f = 0.55(glass) [ethyl acetate: hexane=2:3]

IR spectrum:

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

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.265⁰C (dec)

¹H-NMR (500.00MHz, C₅D₅N):-See table-I.

¹³C-NMR (125.65MHz, C₅D₅N):-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]

¹H-NMR (500.00MHz, C₅D₅N):-

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

¹³C-NMR (125.65MHz, C₅D₅N)

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

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References:

1. H. Hara, A. O. Chater and L. H. J. Williams, *An Enumeration of the Flowering plants of Nepal*, A joint project of the British Museum (Natural History) London and University of Tokyo, Trustee of the British Museum (Natural History) Mansell Book Binders Limited, London.,1982, Vol. **3**, pp. 200.
2. G. A. Cordell "*Introduction to Alkaloids*" Wiley Interscience, New York.1981, 907
3. M. Qiu, R. Nie, and Z. Li, *Yunnan Zhiwu Yanjiu*, 1994, **16**, pp 286-300.
4. Atta-ur-Rahman, Zaheer-ul-Haq, A. Khalid, S. Anjum, M. R. Khan and M. I. Choudhary, *Helvetica Chimica Acta*, 2002, Vol **85**, pp 678-88.
5. U. L. B. Jayasinghe, M. Nadeem, Atta-ur-Rahman, M. I. Choudhary; H. D. Ratnayake and Z. Amtul, *Natural Product Letters*,1998, Vol. **12** (2), pp 103-109.
6. Atta-ur-Rahman, S. Anjum, A. Farooq, M. R. Khan, J. Parveen and M. I. Choudhary, *Journal of Natural Products*, 1998, Vol. **61**, pp 202-206.
7. S. K. Kalauni, M. I. Chaudhary, F. Shaheen, M. D. Manandhar, Atta-ur-Rahman, M. B. Gewali and A. Khalid, *Journal of Natural Products*, 2001, **64**, pp 842-844.
8. S. K. Kalauni, M. I. Chaudhary, A. Khalid, M. D. Manandhar, F. Shaheen, Atta-ur-Rahman and M. B. Gewali, *Chemical & Pharmaceutical Bulletin*, 2002, **50(11)**, 1423-1426.
9. A. Poudel, N. P. Rai, M. D. Manandhar, M. I. Chaudhary, K. Masuda and Atta-ur-Rahman, *ACGC Chemical Research Communication*, 2003, Vol.**16**, pp 19-27
10. N. P. Rai, M. D. Manandhar, R. D. Mckelvy and W. Krause, *Journal of Institute of Science and Technology*, 2004, Vol.**13**, pp 31-39.
11. Y. H. Zhang, J. K. Cheng, L. Yang and D. L. Cheng, *Journal of the Chinese Chemical Society*, 2002, Vol. **49**, No **1**, pp 117-124.

12. V. U. Ahmad, R. Aliya; S. Perveen and M. Shameel, *Phytochemistry*, 1992, Vol. **31**, No.4, pp 1429-1431.
13. V. K. Garg and W. R. Nes, *Phytochemistry*, 1984, Vol **23**, No **12**, pp 2925-2929.
14. F. H. Reginatto, C. Kauffmann, J. Schripsema, D. Guillaume, G. Gosmann and E. P. Schenkel., *Journal of the Brazilian Chemical Society*, 2001, Vol. **12**. No.1, pp 32-36.
15. D. -L. Cheng and X. -P. Cao, *Phytochemistry*, 1992, Vol. **31**, No. **4**, pp. 1317-1320.
16. K. S. Khetwal, N. Mani and N. Pant, *Indian Journal of Chemistry*, Vol. **39B**, June 2000, pp 448-450
17. D. Holland and J. F. Stoddart, *Journal of Chemical Society Perkin Trans I*, 1983, pp 1553-1571.

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