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# ISOLATION AND CHARACTERIZATION OF THREE NEW COUMARIN DERIVATIVES AND A STEROIDAL GLUCOSIDE FROM THE PLANT OF CUSCUTA REFLEXA

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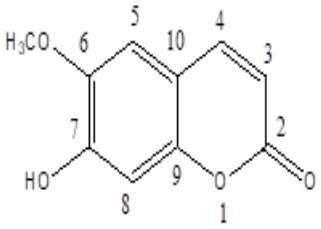
## ABSTRACT
*Cuscuta reflexa*, a parasitic plant, which is commonly known as Swarnalata in Bengali and dodder in English, is an important medicinal plant for its traditional uses and a valuable source of new drugs from *Cuscuta* genus and Convolvulaceae family. Compounds: 7-hydroxy-6-methoxycoumarin (**N-1**), 5, 6, 7-trimethoxycoumarin (**N-2**), 6,7-dimethoxy-5-hydroxycoumarin (**N-3**) and β-sitosteryl-β-D-glucopyranoside (**N-4**) were isolated from this plant for the first time. Among these, compounds **N-1** was isolated from crude chloroform extract, **N-2** & **N-3** were isolated from the ethyl acetate soluble part of methanol extract and **N-4** was isolated from residual methanol extract. By analyzing various spectroscopic data, the compounds' structures have been elucidated.

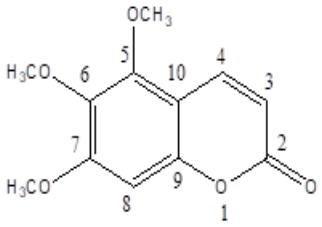
**KEYWORDS:** *Cuscuta reflexa*, Isolation, Structure elucidation, Spectroscopic methods.

## INTRODUCTION
Natural product chemistry has become one of the most interesting and finest areas of modern chemistry as it is continuously driving the isolation of new bioactive compounds from living organisms for the remedy of fatal diseases. Compounds isolated from nature possesses a great extent of biological profile and pharmacological potential than anything synthesized by man.[1]

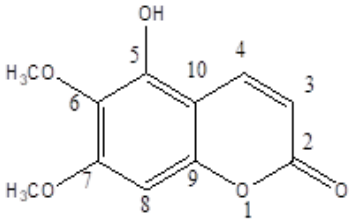
*Cuscuta reflexa*, a parasitic plant, belongs to the Convolvulaceae family and *Cuscuta* genus, which is commonly known as Swarnalata in Bengali[2] and dodder in English.[3] It is an important member of medicinal plants. Due to the important pharmaceutical value, it has attracted the chemist. *Cuscuta reflexa* showed hemodynamic, bradycardia, antiviral, antispasmodic, anticonvulsant, and antisteroidogenic activities.[4] The plant is also used as a blood purifier and to treat jaundice, urinary issues, muscle spasms, and cough. Both its heated paste and whole-plant paste are used to treat rheumatism and headaches, respectively.[5] *Cuscuta reflexa* seeds are used for the treatment of bilious disorders due to their anthelmintic activities.[6] The plant contains various pharmacological compound including flavonoids, coumarins, terpenoids, glucosides, alkaloids and steroids.[7,8] Literature survey showed a few phytochemical studies have been done on this plant so far. Our recent study on the whole plants of *Cuscuta reflexa* has led to the isolation of 7-hydroxy-6-methoxycoumarin (**N-1**), 5,6,7-trimethoxycoumarin (**N-2**), 6,7-dimethoxy-5-hydroxycoumarin (**N-3**) and β-sitosteryl-β-D-glucopyranoside (**N-4**) (Figure-1). For the first time from this plant, the compounds were isolated.



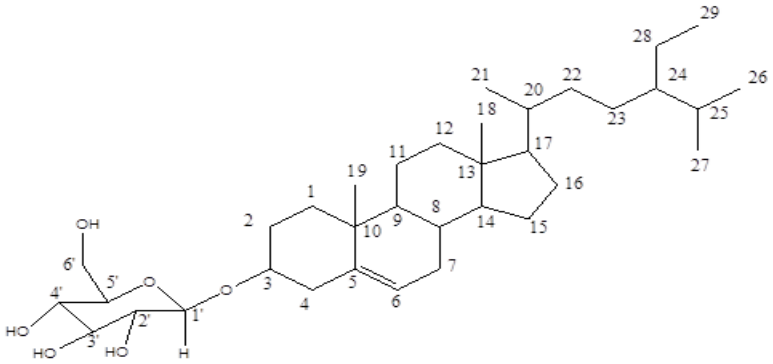

**7-Hydroxy-6-methoxycoumarin (N-1)**




**5, 6, 7- Trimethoxycoumarin (N-2)**



**6, 7-Dimethoxy-5-hydroxycoumarin (N-3)**



**β-Sitosteryl-β-D-glucopyranoside (N-4)**

**Figure 1: Isolated compound structures with numbering.**

## MATERIALS AND METHODS

On a Fisher-electrothermal John's melting point instrument, melting points were measured using the thin disc method. On a Shimadzu UV-Visible spectrophotometer, UV spectra were measured in methanol. On a Shimadzu FT-IR spectrometer, IR spectra were taken as thin films or KBr discs. $CDCl_3$, $CD_3OD$, or DMSO-$d_6$ NMR spectra were taken using a Bruker WH 400 MHz NMR spectrometer. Bruker, Micro TOF II APCI mass spectrometer was used to get the molecular mass of the compounds. Using silica gel 40, column chromatography was applied for separation (70-230 mesh, E. Merck). On TLC plastic sheets that had been previously coated with silica gel 60 $F_{254}$, TLC was performed (E. Merck).

### Collection and Identification of the plant

The parasitic plant, *Cuscuta reflexa* (Swarnalata), was collected from some areas of Dinajpur district, Bangladesh and identified by Bangladesh National Harbarium at Dhaka and a voucher specimen (specimen no. 43869) was deposited at the herbarium.

### Extraction of crude samples from the plant *cuscuta reflexa*

The plants were initially crushed into little pieces, dried under a shed, and then ground into a powder in a grinder machine. The dried powder (880 mg) was extracted successively with chloroform and methanol. Firstly, the powder material was soaked in $CHCl_3$ for 72 hours (3 days) at room temperature. The extracted solvent then separated using filtration method and solvent was further added to the residual plant material. The extraction procedure was repeated 3 times and the combined filtrates were evaporated using rotary evaporator to get a greenish dried crude chloroform extract (28.34 g). The same procedure was followed to get gummy brownish methanol extract (14.26 g).

### Isolation of compounds from crude extracts

After trituration with n-hexane, the residual chloroform extract (7.33 g) was subjected to column chromatographic separation using pet. ether, pet. ether-chloroform, chloroform, chloroform-ethyl acetate (EA), EA and EA-methanol with increasing polarity. According to their TLC behaviors, the collections were separated into 14 fractions, numbered C-1 to C-14. Fraction-6 was used to isolate compound **N-1**.

After trituration with ethyl acetate, we get crude ethyl acetate extract 1.67 g and residual crude methanol extract 12.59 g. The crude ethyl acetate extract was subjected to column chromatographic separation using pet. ether-chloroform, chloroform, chloroform-ethyl acetate (EA), EA, EA-methanol with increasing polarity. There were 15 fractions formed from the collections, E-1 to E-15 on the basis of their TLC behavior. Among these compounds **N-2** & **N-3** were isolated from fraction, E-5.

Finally, compound **N-4** was isolated from residual crude methanol extract by column chromatographic techniques.

### Spectroscopic data of the isolated compounds

**7-hydroxy-6-methoxy coumarin (N-1)**

Pale yellow crystal (4.5 mg); mp 204°C; UV ($CHCl_3$) $\lambda_{max}$ 340, 295 nm; IR (KBr disk) υ 3340 (O-H), 3068

(aromatic $C_{sp2}$-H), 2989, 2870 ($C_{sp3}$-H), 1705 (C=O), 1608 (aromatic C=C), 1567, 1498, 1376, 1291, 1262, 1140 (C-O str.) cm-$^{1}$; $^{1}$H NMR Spectrum ($CDCl_3$) δ 7.62 (1H, d, J = 9.6 Hz, H-4), 6.94 (1H, singlet, H-8), 6.86 (1H, singlet, H-5), 6.29 (1H, doublet, J = 9.6 Hz, H-3), 6.16 (1H, br. singlet, -OH), 3.98 (3H, singlet, -$OCH_3$ at C-6); $^{13}$C NMR Spectrum ($CDCl_3$) δ 161.3, (C-2), 150.3 (C-9), 149.8 (C-7), 143.9 (C-6), 143.2 (C-4), 113.4 (C-3), 111.6 (C-10), 107.4 (C-5), 103.3 (C-8), 56.3 (-$OCH_3$); APCI Mass Spectrum: m/z 193 $(M+H)^+$

**5, 6, 7- Trimethoxy coumarin (N-2)**

Reddish solid substance (6.5 mg); mp 118°C; UV ($CHCl_3$) $\lambda_{max}$ 316; IR (KBr disk) υ 3076 (aromatic $C_{sp2}$-H), 2945, 2853, 1741 (C=O), 1613 (aromatic C=C), 1465, 1381, 1262, 1139, 1110 (C-O) cm-$^{1}$; $^{1}$H NMR Spectrum ($CDCl_3$) δ 7.96 (1H, doublet, J = 9.6, H-4), 6.64 (1H, singlet, H-8), 6.24 (1H, doublet, J =9.6 Hz, H-3), 4.04 (3H, singlet, -$OCH_3$ at C-5), 3.94 (3H, singlet, -$OCH_3$ at C-7), 3.87 (3H, singlet, -$OCH_3$ at C-6); $^{13}$C NMR Spectrum ($CDCl_3$) δ 161.2 (C-2), 157.3 (C-7), 151.4 (C-9), 149.4 (C-5), 138.8 (C-4), 138.2 (C-6), 112.6 (C-3), 107.3 (C-10), 95.6 (C-8), 61.7 (-$OCH_3$ at C-5), 61.2 (-$OCH_3$ at C-6), 56.3 (-$OCH_3$ at C-7); APCI Mass Spectrum: m/z 237 $(M+H)^+$

**6, 7-Dimethoxy-5-hydroxy coumarin (N-3)**

Pale yellow crystal (3.2 mg); mp 185°C; UV($CHCl_3$) $\lambda_{max}$ 317; IR (KBr disk) υ 3321 (O-H), 3074, 2952, 2929, 1701 (C=O), 1623 (aromatic C=C), 1559, 1469, 1197, 1145, 1119 (C-O) cm-$^{1}$; $^{1}$H NMR Spectrum ($CDCl_3$) δ7.98 (1H, doublet, J = 9.6 Hz, H-4), 6.47 (1H, singlet, H-8), 6.26 (1H, br. singlet, -OH), 6.23 (1H, doublet, J = 9.6 Hz, H-3), 3.94 (3H, singlet, -$OCH_3$ at C-7), 3.92 (3H, singlet, -$OCH_3$ at C-6); $^{13}$C NMR Spectrum ($CDCl_3$) δ161.2 (C-2), 155.5 (C-7), 151.7 (C-9), 145.6 (C-5), 138.5 (C-4), 131.5 (C-6), 111.8 (C-3), 102.5 (C-10), 92.4 (C-8), 61.3 (-$OCH_3$ at C-6), 56.2 (-$OCH_3$ at C-7); APCI Mass Spectrum: m/z 223 $(M+H)^+$

**β-sitosteryl-β-D-glucopyranoside (N-4)**

White solid crystal (16.5 mg); mp 290-292°C; IR (KBr disk) υ 3407 (br. O-H), 2959, 2868, 1462, 1429, 1379, 1198, 1023 (C-O) cm-$^{1}$; $^{1}$H NMR Spectrum ($CDCl_3$+ $CD_3OD$) δ 5.31 (1H, unresolved singlet, H-6), 4.35 (1H, doublet, J = 7.6 Hz, H-1′), 3.79 (1H, multiplet, H-6′), 3.69 (1H, double doublet, J = 4.0 & 12.0 Hz, H-6′), 3.48-3.55 (1H, multiplet, H-3), 3.38 (2H, doublet, J = 4.8 Hz, H-4′ & H-5′), 3.31 (1H, singlet), 3.18-3.22 (2H, multiplet, H-2′ & H-3′), 2.34 (1H, multiplet), 2.20 (1H, triplet, J = 12 Hz), 1.79-1.93 (6H, multiplet), 1.34- 1.60 (10H, m), 1.15-1.29 (7H, multiplet), 1.00-1.10 (7H, multiplet), 0.94 (3H, singlet, H-19), 0.85 (3H, doublet, J = 5.6 Hz, H-21), 0.73-0.77 (9H, multiplet, H-26, 27, 29), 0.61 (3H, singlet, H-18); $^{13}$C NMR Spectrum ($CDCl_3$+ $CD_3OD$) δ 140.2 (C-5), 122.0 (C-6), 101.0 (C-1′), 79.1 (C-3), 76.1 (C-5′), 75.5 (C-2′), 73.4 (C-3′), 70.1 (C-4′), 61.7 (C-6′), 56.6, 55.9, 50.0, 45.7, 42.2, 39.6, 38.6, 37.1, 36.6, 36.0, 33.8, 31.8, 31.7, 29.5, 29.0, 28.1, 25.9, 24.1, 22.9, 20.9, 19.6, 19.1, 18.8, 18.6, 11.8, 11.7; APCI Mass Spectrum m/z 397 [(M+H) - $C_6H_{12}O_6$] + ≡ $[577-180]^+$

**RESULS AND DISCUSSION**

Successive extraction of dried whole plant *cuscuta reflexa* with chloroform and methanol at room temperature yielded 28.34 g and 14.26 g of crude extract, respectively. Fractionation and isolation works has been done and four new compounds were isolated. Using spectroscopic methods, the structures of the compounds were established.

A molecular ion peak at m/z 193 $(M+H)^+$ corresponds to the chemical formula $C_{10}H_8O_4$ exhibited in the mass spectra of the compound **N-1**. The existence of conjugation and chromophoric groups in the molecule was confirmed by absorption peaks at $\lambda_{max}$ 340 and 295 nm in the UV spectra of **N-1**. From IR spectroscopic data, the existence of a -OH group in the molecule was confirmed by an absorption band at 3340 cm-$^{1}$ which was also further supported by the absorption bands at 1291, 1262 and 1140 cm-$^{1}$ due to C-O stretching vibration. The compound also showed a sharp peak at 1705 cm-$^{1}$ which indicated the existence of C=O stretching vibrations. Again, the bands at 3068 cm-$^{1}$ due to aromatic $C_{sp2}$-H stretching vibrations and at 1608 & 1567 cm-$^{1}$ confirms the existence of aromatic C=C bond stretching vibrations. From $^{1}$H NMR spectrum, two protons doublet at δ 7.61 and 6.29 with J = 9.6 Hz indicated the existence of two ortho protons at C-3 and C-4, respectively which was further confirmed by the $^{1}$H-$^{1}$H correlation showed in the COSY spectrum. Two 1H singlet at δ 6.94 and 6.86 indicated the existence of two aromatic protons at C-8 and C-5, respectively. One proton br. singlet at δ 6.16 confirmed the existence of phenolic –OH group at C-7. One three protons singlet at δ 3.98 indicated the existence of methoxy (-$OCH_3$) group at C-6. The 10 signals for 10 carbon atoms were clearly visible in the $^{13}$C NMR spectra. The value at δ 161.3 indicated the carbonyl carbon of cyclic ester group and three aromatic carbons designated by the signals at δ 150.3 (C-9), 149.8 (C-7) and 143.9 (C-6) are attached to oxygen. Again, the signals at δ 107.5 and 103.2 indicated the C-5 and C-8, respectively and the value at δ 56.4 indicated the carbon of methoxy (-$OCH_3$) group. All spectral data analysis, it is suggested that the compound **N-1** is a coumarin derivative containing one hydroxyl (-OH) and one methoxy (-$OCH_3$) group. From $^{1}$H-$^{13}$C direct correlations found in the HSQC spectrum and the $^{1}$H-$^{13}$C long range correlations found in the HMBC spectrum were finally used to establish the positions of the substituent groups and the structure of the compound. Based on all the spectroscopic data, literature value[9,10] and melting point of the compound, it was established that the compound, **N-1** is 7-hydroxy-6-methoxy coumarin.

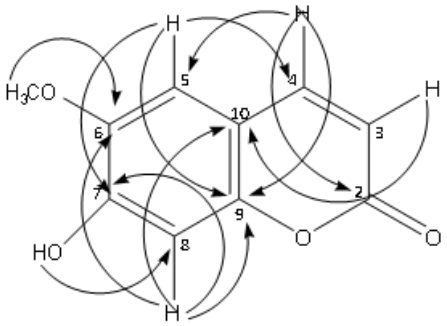



**Fig. 2: Selected $^{1}H$-$^{13}C$ long range correlations in the HMBC spectrum of the compound N-1.**

A molecular ion peak at m/z 237 $(M+H)^+$ corresponds to the chemical formula $C_{12}H_{12}O_5$ exhibited in the mass spectra of the compound **N-2**. The presence of conjugation and chromophoric groups in the molecule was confirmed by absorption peaks at $\lambda_{max}$ 316 nm in the UV spectra of **N-2**. From IR spectroscopic data, the absorption bands at 3076 $cm^{-1}$ due to aromatic $C_{sp2}$-H stretching vibrations and at 2945 and 2853 $cm^{-1}$ due to saturated C-H stretching vibrations. The sharp band at 1741 $cm^{-1}$ in the IR spectrum indicated the existence of C=O stretching vibration. The bands at 1613 $cm^{-1}$ for aromatic C=C stretching vibrations and at 1465 and 1381 $cm^{-1}$ due to saturated C-H bending vibrations. The compound also showed absorption bands at 1262, 1139 and 1110 $cm^{-1}$ for C-O stretching vibrations. From $^{1}H$ NMR spectrum, two 1H doublet at δ 7.96 and 6.24 with J = 9.6 Hz indicated the existence of two ortho protons at C-3 and C-4, respectively which was further confirmed by the $^{1}H$-$^{1}H$ correlation showed in the COSY spectrum. One proton singlet at δ 6.64 might be attributed to an aromatic proton attached to C-8. Three 3H singlets at δ 4.04, 3.94 and 3.87 must be due to the protons of three methoxy groups attached to C-5, C-7 and C-6, respectively. The $^{13}C$ NMR spectrum showed 12 signals for 12 carbons. The value at δ 161.2 indicated the carbonyl carbon of cyclic ester group and four carbons designated by the signals at δ 157.2 (C-7), 151.5 (C-9), 149.3 (C-5) and 138.1 (C-6) are attached to oxygen. One peak at δ 95.5 assigned by C-8 and three peaks at δ 61.8, 61.2 and 56.3 attributed to the carbons of three methoxy groups. All spectral data analysis, it is suggested that the compound **N-2** is a coumarin derivative containing three methoxy groups. From $^{1}H$-$^{13}C$ direct correlations found in the HSQC spectrum and the $^{1}H$-$^{13}C$ long range correlations found in the HMBC spectrum were finally used to establish the positions of the substituent groups and the structure of the compound. Based on all the spectroscopic data, literature value[9] and melting point of the compound, it was confirmed that the compound, **N-2** is 5,6,7-Trimethoxy coumarin.

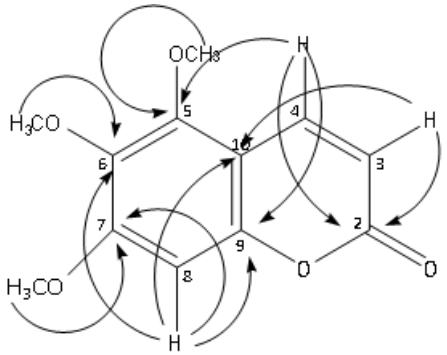



**Fig. 3: Selected $^{1}H$-$^{13}C$ long range correlations in the HMBC spectrum of the compound N-2.**

A molecular ion peak at m/z 223 $(M+H)^+$ corresponds to the chemical formula $C_{11}H_{10}O_5$ exhibited in the mass spectra of the compound **N-3**. The presence of conjugation and chromophoric groups in the molecule was confirmed by absorption peaks at $\lambda_{max}$ 317 nm in the UV spectra of **N-3**. From IR spectroscopic data, the existence of a hydroxyl (-OH) group in the molecule was confirmed by an absorption band at 3321 $cm^{-1}$ which was further supported by the absorption bands 1197, 1145 and 1119 $cm^{-1}$ due to C-O stretching vibrations. The absorption bands at 3074 $cm^{-1}$ due to aromatic $C_{sp2}$-H stretching and at 2952 & 2929 $cm^{-1}$ due to saturated C-H stretching vibrations were found. The sharp band at 1701 $cm^{-1}$ in the IR spectrum indicated the existence of C=O stretching vibrations. Absorption bands at 1623 and 1559 $cm^{-1}$ for aromatic C=C stretching vibrations and at 1469 $cm^{-1}$ due to saturated C-H bending vibrations. The $^{1}H$ NMR spectrum showed two protons doublet at δ 7.98 and 6.23 with J = 9.6 Hz indicated the existence of two ortho protons at C-3 and C-4, respectively which was further confirmed by the $^{1}H$-$^{1}H$ correlation showed in the COSY spectrum. One proton singlet at δ 6.47 might be attributed to an aromatic proton attached to C-8. One proton br. singlet at δ 6.26 confirmed the existence of

phenolic –OH group attached to C-5. Two 3H singlets at δ 3.94 and 3.92 confirmed the existence of two methoxy groups at C-7 and C-6, respectively. 11 signals for 11 carbons were visible in the $^{13}C$ NMR spectrum. The value at δ 161.3 indicated the carbonyl carbon of cyclic ester group and four carbons designated by the signals at δ 155.5 (C-7), 151.7 (C-9), 145.6 (C-5) and 131.5 (C-6) are attached to oxygen. The value at δ 92.4 indicated the aromatic carbon of C-8 and the two peaks at δ 61.3 and 56.2 attributed to the carbons of two methoxy (-$OCH_3$) groups. From $^{1}H$-$^{13}C$ direct correlations found in the HSQC spectrum and the $^{1}H$-$^{13}C$ long range correlations found in the HMBC spectrum were finally used to establish the positions of the substituent groups and the structure of the compound. Based on all spectroscopic data, literature value[9] and melting point of the compound, it was confirmed that the compound, **N-3** is 6,7-Dimethoxy-5-hydroxy coumarin.

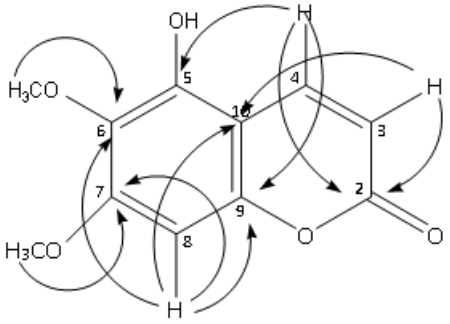



**Fig. 4: Selected $^{1}H$-$^{13}C$ long range correlations in the HMBC spectrum of the compound N-3.**

A molecular ion peak at m/z 397 $(M+H)^+$ corresponds to the chemical formula $C_{35}H_{60}O_6$ exhibited in the mass spectra of the compound **N-4** and obtained $C_{29}H_{48}$ after the elimination of glucose moiety ($C_6H_{12}O_6$) from the molecule. From IR spectroscopic data, a broad absorption band at 3407 $cm^{-1}$ indicating the existence of hydroxyl group (-OH) in the molecule which was further supported by the absorption bands at 1198 & 1023 $cm^{-1}$ due to C-O stretching vibration. The bands at 2959 & 2868 $cm^{-1}$ due to the saturated $C_{sp3}$-H stretching vibrations. In $^{1}H$ NMR spectrum, one peak at δ 5.30 (1H, unr. s) confirmed the presence of one olefinic proton at C-6 which was further supported by the peak at δ 140.1 & 122.0 for two olefinic carbons indicating that the compound contained one double bond in $^{13}C$ spectrum. The existence of six methyl groups clearly indicated by the peaks at 0.61 to 0.94, where signals at δ 0.94 (3H, s), 0.85 (3H, d), 0.73-0.77 (9H, m) and 0.61 (3H, s) were designated by H-19, H-21, H-26, H-27, H-29 and H-18, respectively. The peaks in the range from δ 1.0 to 2.34 could be used to identify the other methine and methylene protons that are present in the molecule. The protons of the glucose unit appeared in the region at δ 3.18 to 4.34 where one doublet at δ 4.34 (J = 7.6 Hz) was assigned by H-1′ and two peaks at δ 3.79 & 3.69 were assigned by two H-6′ protons. The other four protons of the glucose unit indicated by two overlapped signals at δ 3.38 and 3.18-3.22 indicating two pairs of protons due to their very close chemical shift value. The anomeric proton at δ 4.34 with coupling constant 7.6 Hz which is attached to C-1′ (δ 101.0) indicated by HSQC spectrum confirmed the presence of axial– axial coupling and this clearly suggested about the β- configuration of the glucose unit. The proton at C-1′ showed long range correlation with C-3 of β-sitosterol in the HMBC spectrum confirmed the position of the attachment between the two moieties. The $^{13}C$ NMR spectrum showed that the compound contained 35 signals for 35 carbon atoms. The glucose unit contained six carbons of which anomeric carbon C-1′ appeared at δ 101.0 and methylene carbon C-6′ appeared at δ 61.8. The other four carbons appeared at δ 79.1, 75.6, 73.4 and 70.0. All the above analysis of spectral data revealed that the isolated compound **N-4** was a steroidal glucoside. From $^{1}H$-$^{13}C$ direct correlations found in the HSQC spectrum and the $^{1}H$-$^{13}C$ long range correlations found in the HMBC spectrum were finally used to establish the positions of the substituent groups and the structure of the compound. Based on all the spectroscopic data, literature value[11,12] and melting point of the compound (290-292°C), it was discovered that the compound, **N-4** is β-sitosteryl-β-D-glucopyranoside.

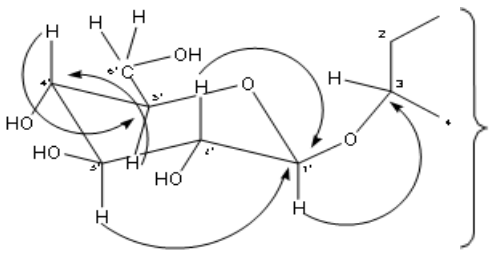



**Fig. 5: Selected $^{1}H$-$^{13}C$ long range correlations in the HMBC spectrum of the compound N-4.**

## CONCLUSION

From literature survey it was seen that, the plant *cuscuta reflexa* is a great source of coumarins. In this article, four new compounds have been isolated and characterized from this plant. In the future, we believe it will be possible to do more detailed phytochemical and biological research on this plant.

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