Research Article **ISSN: 2349 – 7106**

Asian Journal of Research in Chemistry and Pharmaceutical Sciences

Journal home page: www.ajrcps.com

https://doi.org/10.36673/AJRCPS.2020.v08.i03.A35

CHEMICAL CONSTITUENTS ISOLATED FROM THE ROOTS OF *SPERMACOCE LATIFOLIA* AUBLET

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ABSTRACT

Spermacoce latifolia is one of the most important members of medicinal plant belonging to the family Rubiaceae and have a lot of pharmacological properties. A phytochemical study on the roots of the plant *Spermacoce latifolia* led to the isolation of β-amyrin (1) from the chloroform extract and7-hydroxy-6-methoxycoumarin (2), stigmasteryl-β-D-glucopyranoside (3) and sucrose (4) from the methanol extract. The structure of these compounds has been established on the basis of detailed spectroscopic data analysis including UV, IR, 1D and 2D NMR and Mass. All of these four compounds were isolated from this plant for the first time.

KEYWORDS

Spermacoce latifolia, Isolation, Structure and Spectroscopic techniques.

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INTRODUCTON

Nature has been a source of medicinal agents for thousands of years. Since the ancient time natural products obtained from medicinal plants are being used in this subcontinent for the treatment of various diseases[1]. Thus medicinal plants are called the backbone of traditional medicine. An impressive number of modern drugs have been isolated from natural sources, many of which based on their use in traditional medicine. *Spermacoce latifolia* is a medicinal plant belongs to the family Rubiaceae. The Rubiaceae family comprises one of the largest angiosperm families, with 650 genera[2] and approximately 13,000 species[3], which is distributed mainly not only in tropical and subtropical regions, but also reaching the temperate and cold regions of Europe and Northern Canada[4]. *Spermacoce latifolia*

Aubl. [Syn; *Borreria latifolia* (Aubl.) K. Schum.] is a herbaceous species native to South America and known as an exotic invasive plant in many tropical and subtropical countries of the world[5,6]. It is an annual or prostrate herb occurring throughout Bangladesh and locally known as Ghuiojhil Shak. It's common name in English is Broad leaved button weed or Oval leaf false button weed[7]. Various parts of the plant have been claimed to use for the ailments of various diseases. The root juice is very useful for treating malaria. Seeds have good medicated properties for treating diarrhea, dysentery, fever and dental problem[8]. The leaves of the plant are used as ophthalmic purposes, inflammation of eye and gums, fever, blindness, spleen complaints etc. and the leaf paste is used by the Tanchngya ethnic people in our country for treatment of boils[9]. Biological investigations revealed that the plant showed moderate to strong activity as antimicrobial, cytotoxic and as antioxidant[10,11]. Literature survey showed that phytochemical studies on the whole plant have led to isolate few iridoid glycosides, a diterpenoid, pentacyclictriterpene acids, anthraquinone and naphthoquinone[12-14]. Earlier, four compounds: stigmasterol, 3β-hydroxy-12-oleanan-28-oic acid, a dienone and phthalic acid have been isolated from the aerial parts[15] and three compounds: palmitic acid, an anthraquinone and a coumarin derivative have been isolated from the roots[16] of *Spermacoce latifolia* in our laboratory. As a continuation of our phytochemical study on the roots of *S. latifolia*, we herein report the isolation of four compounds including β-amyrin (1) from chloroform extract and 7-hydroxy-6-methoxycoumarin (2), stigmasteryl-β-D-glucopyranoside (3) and sucrose (4) from methanol extract of the same plant (Figure No.1). The compounds were isolated for the first time from this plant.

MATERIAL AND METHODS

Melting points were determined by thin disc method on a Thermo Scientific 00590Q Fisher-John's melting point apparatus. UV spectral data was recorded in chloroform on a UV-1800 Shimadzu UV-Vis spectrophotometer. IR spectra were recorded on a Shimadzu Prestige-21 FT-IR spectrometer as KBr disc. NMR spectra were recorded in $CDCl_3$ or CD_3OD using Bruker WH 400 MHz NMR spectrometer (AVANCE III HD, BRUKER). Mass spectra of the compounds were measured on Bruker, Micro TOF II APCI mass spectrometer. Separation by column chromatography was carried out using silica gel 40 (70-230 mesh, E. Merck). Thin layer chromatography was carried out on TLC plastic sheets pre-coated with silica gel 60 F_{254} (E. Merck).

Collection of the Plant Materials

The roots of matured *Spermacoce latifolia* plants were collected from Jahangirnagar University campus, Savar, Dhaka. The plant was identified by Bangladesh National Herbarium at Dhaka and a voucher specimen (specimen no. 37755) was deposited at the herbarium.

Extraction of the Roots of *S. Latifolia*

The roots were cleaned and cut into small pieces, and then the plant materials were air-dried and powdered by using a grinder machine. The dried plant materials (500g) were extracted with chloroform and methanol at room temperature. At first, the powdered plant materials were soaked in chloroform for 72 hours. The extraction solvent was collected by filtration and again fresh distilled solvent was added to the plant materials. The extraction process was repeated for three times and the combined filtrate was evaporated and dried completely by using a rotary evaporator at low temperature (40^oC) to get greenish crude chloroform extract (2.96g). The residual plant materials were then soaked in methanol and the extraction process was performed by the same way as for chloroform to get brownish crude methanol extract (13.5g).

Isolation of Compounds from Crude Extracts

The crude chloroform extract (2.96g) was subjected to column chromatography over silica gel and was eluted with pet. Ether, pet. ether-ethyl acetate, ethyl acetate and ethyl acetate-methanol in gradient manner. The collections were divided into 12 fractions according to their TLC behaviors. Fraction number 7(296mg) of the column was rechroma to graphed by using a small sized silica gel column

eluting with pet. Ether-ethyl acetate solvent system to get compound 1(5mg) as a pure form.

The crude methanol extract (13.5g) was triturated first with chloroform and ethyl acetate successively, and the residue which was only soluble in methanol (11.6g) was collected. The methanol soluble part was then subjected to a big sized column over silica gel and was eluted with chloroform-ethyl acetate, ethyl acetate, ethyl acetate-methanol and methanol in gradient manner. The collections from the column were divided into 8 fractions according to their TLC behaviors. Compound 2(21mg) was isolated as yellow crystal from fraction 3(256mg) by repeated column chromatography using chloroform-ethyl acetate solvent system as mobile phase. Fraction 5(195mg) of the above column was concentrated and allowed to stand overnight. A white precipitate was formed which was washed with few drops of chloroform and methanol to get compound 3(120mg) in its pure form. Colorless crystals were separated out from the concentrated solution of fraction 7(3.1g) of the same column and the crystals were purified by recrystallization method from methanol to get the compound 4(128mg).

SPECTROSCOPIC DATA OF THE ISOLATED COMPOUNDS

β-amyrin or 3β-Olean-12-en-3-ol (1)

Pale yellow solid (5mg); MP 189-191^{o}C;IR (KBr disc) υ 3419 (br. O-H), 2926, 2856, 1687 (C=C), 1458, 1386, 1253, 1139 (C-O) cm^{-1};[1] HNMR ($CDCl_3$) δ 5.26 (1H, m, H-12),3.19-3.23 (1H, m, H-3), 2.34 (1H, t, J = 7.6 Hz), 2.20 (1H, m), 1.83-2.02 (2H, m), 1.45-1.66 (8H, m), 1.20-1.39 (12H, unr. s), 1.08 (3H, s), 0.98 (3H, s), 0.92 (3H, s), 0.87 (3H, s), 0.86 (3H, s), 0.85 (3H, s), 0.78 (3H, s), 0.77 (3H, s); ^{13}CNMR ($CDCl_3$) δ 137.9 (C-13), 125.8 (C-12), 79.0 (C-3), 55.2, 47.9, 47.5, 42.0, 39.5, 39.0, 38.7, 38.6, 37.0, 36.7, 32.9, 30.6, 29.5, 29.2, 29.0, 28.1, 27.2, 24.7, 24.1, 23.5, 23.3, 21.1, 18.3, 17.0, 16.9, 15.5, 14.0; APCI Mass m/z 427.5268 $(M+H)^+$.

7-Hydroxy-6-methoxycoumarin (2)

Yellow crystal (21mg); MP 203^{o}C; UV (Chloroform) λ_{max}340 and 295 nm; IR (KBr disk) υ 3336 (br. O-H), 3105 (aromatic C-H), 2989, 2850, 1703 (C=O), 1608 (aromatic C=C), 1566, 1510, 1435, 1375, 1290, 1263, 1139 (C-O) cm^{-1}; ^{1}HNMR ($CDCl_3$)δ7.62 (1H, d, J = 9.6 Hz, H-4), 6.93 (1H, s, H-8), 6.86 (1H, s, H-5), 6.29 (1H, d, J = 9.6 Hz, H-3), 3.97 (3H, s, -OCH_3 at C-6); ^{13}CNMR ($CDCl_3$)δ 161.4 (C-2), 150.2 (C-9), 149.7 (C-7), 144.0 (C-6), 143.3 (C-4), 113.3 (C-3), 111.5 (C-10), 107.5 (C-5), 103.2 (C-8), 56.4 (-OCH_3); APCI Mass m/z 193.0403 $(M+H)^+$.

Stigmasteryl-β-D-glucopyranoside (3)

White solid (120mg); MP 280^{o}C; IR (KBr disk) υ 3377 (br. O-H), 2931, 2866, 1462, 1367, 1253, 1166, 1024 (C-O) cm^{-1}; ^{1}HNMR($CDCl_3$)δ 5.31 (1H, unr. s, H-6), 5.08 (1H, dd, J = 4.4 Hz and 8.4 Hz, H-22 or H-23), 4.95 (1H, dd, J = 4.4 Hz and 7.6 Hz, H-22 or H-23), 4.35 (1H, d, J = 7.6 Hz, H-1′), 3.77-3.80 (1H, m), 3.68-3.72 (1H, m), 3.49-3.53 (2H, m), 3.15-3.24 (2H, m), 2.32-2.35 (1H, m), 2.17-2.23 (1H, m), 1.94 (2H, m), 1.79-1.86 (2H, m), 1.31-1.61 (14H, m), 1.20 (3H, s), 1.01-1.16 (4H, m), 0.95 (6H, unr. s), 0.87 (3H, s), 0.78 (6H, d, J = 3.2 Hz), 0.74 (3H, t, J = 4.8 Hz), 0.63 (3H, d, J = 7.2 Hz);^{13}CNMR ($CDCl_3$)δ 140.9, 138.2, 129.2, 122.1, 101.0 (C-1′), 79.1 (C-3), 76.2, 75.6, 73.4, 70.0, 61.8, 56.7, 56.6, 55,8, 51.1, 50.0, 45.7, 42.2, 42.1, 40.4, 39.6, 38.6, 37.1, 36.6, 31.8, 29.5, 28.8, 25.3, 24.2, 21.0, 20.9, 18.8, 12.1, 11.9, 11.7; APCI Mass m/z 395.4662 $[(M+H) - C_6H_{12}O_6]^+$.

Sucrose or α-D-Glucopyranosyl-(1→2′)-β-D-fructofuranoside (4)

Colorless crystal (128mg); MP 186^{o}C; IR (KBr disk) υ 3387 (br. O-H), 2939, 2895,1460, 1346, 1240, 1136, 1053 (C-O) cm^{-1}; ^{1}HNMR (CD_3OD)δ 5.40 (1H, d, J = 3.6 Hz, H-1), 4.12 (1H, d, J = 8.4 Hz, H-3′), 4.04 (1H, t, J=7.2 Hz, H-4), 3.70-3.84 (6H, m), 3.60-3.68 (2H, d, J = 5.2 Hz, H-1′), 3.36-3.46 (3H, m); ^{13}CNMR (CD_3OD)δ 103.9 (C-2′), 92.2 (C-1), 82.3 (C-5), 77.8 (C-3′), 74.2 (C-4), 73.2 (C-5′), 72.9 (C-3), 71.7 (C-2), 69.8 (C-4′), 62.5 (C-1′), 61.9 (C-6 or C-6′), 60.7 (C-6 or C-6′).

RESULTS AND DISCUSSION

The powdered roots of the plant *Spermacoce latifolia* were extracted successively with chloroform and methanol at room temperature yielded 2.96g and 13.5g of crude extracts,

respectively. The compound β-amyrin (1) was isolated and purified from the chloroform extract using repeated column chromatography. The compounds: 7-hydroxy-6-methoxycoumarin (2), stigmasteryl-β-D-glucopyranoside (3) and sucrose (4) have been isolated in pure form from the methanol extract using trituration, column chromatography and crystallization methods. The structures of these compounds were elucidated using different spectroscopic techniques.

Compound (1) was a pale yellow solid substance. The mass spectrum of the compound showed a molecular ion peak at m/z 427 $(M+H)^+$ which is corresponding to the molecular formula $C_{30}H_{50}O$. The IR spectrum of the compound showed a broad absorption band at 3419 cm^{-1} indicating the presence of hydroxyl group. The absorption bands at 1687 and 1139cm^{-1} due to C=C and alcoholic C-O stretching vibrations, respectively. The ^1H NMR spectrum showed one proton multiplet at δ 3.19-3.23 indicating the presence of methine proton of >CH-OH group in the compound at position-3. The peak at δ 5.26 (1H, m) represented the presence of olefinic proton at C-12. This was further supported by the two peaks in the^{13}C NMR spectral data at δ 137.9 and 125.8 for two olefinic carbons. The presence of eight methyl groups in the structure was clearly indicated by the peaks in the region at δ 0.77 to 1.08. The other methine and methylene protons present in the molecule could be assigned by the peaks in the region at δ 1.20 to 2.34. The presence of 30 carbons in the compound was indicated by 30 peaks available in the ^{13}C NMR spectrum. The peak at δ 79.0 in the same spectrum indicated the carbon at position-3 which is attached to the hydroxyl group. Above spectral data suggested that the compound is a triterpenoid containing one hydroxyl, one double bond and eight methyl groups. Based on all spectral data, literature values[17] and melting point (189-191^oC) of the compound, it was confirmed that compound (1) is β-amyrinor 3β-olean-12-en-3-ol.

Compound (2) was a yellow crystalline substance with mp 203^oC. The mass spectrum of the compound showed a molecular ion peak at m/z 193 $(M+H)^+$ which is corresponding to the molecular formula $C_{10}H_8O_4$. The UV spectrum showed absorption bands at λ_{max}340 and 295 nm which indicated the presence of conjugation and chromophoric groups in the molecule. The IR spectrum of the compound showed a broad absorption band at 3336cm^{-1} confirming the presence of hydroxyl group in the compound and absorption band at 3105cm^{-1} confirms the aromatic C-H stretching vibrations. A sharp band at 1703cm^{-1} indicated the presence of C=O stretching vibrations. Absorption bands at 1608, 1566 and 1510cm^{-1} indicated the presence of aromatic C=C stretching vibrations and bands at 1290, 1263 and 1139 cm^{-1} due to C-O stretching vibrations. The ^1H NMR spectrum showed two 1H doublets at δ 7.62 and 6.29 with same coupling constant J=9.6 Hz specified the presence of two *ortho* protons attached to C-3 and C-4, respectively. The position of these adjacent protons again confirmed by the ^1H-^1H correlation indicated in the COSY spectrum. Two 1H singlets at δ 6.93 and 6.86 could be assigned for two aromatic protons attached to C-8 and C-5, respectively. One 3H singlet at δ 3.97 indicated the presence of one methoxy group attached to C-6.The ^{13}C NMR spectrum showed 10 signals which clearly indicated the presence of 10 carbons in the molecule. The carbonyl carbon of cyclic ester group indicated by the characteristic peak at δ 161.4 and three carbons of the aromatic ring those are attached to oxygen might be assigned by the signals at δ 150.2 (C-9), 149.7 (C-7) and 144.0 (C-6). Two signals at δ 107.5 and 103.2 attributed to C-5 and C-8, respectively and one peak at δ 56.4 attributed to the carbon of methoxy group. All the above analysis of spectral data strongly suggested that the compound (2) is a coumarin derivative containing one hydroxy and one methoxy group. The structure of the compound including position of the substituent groups was finally assigned by the ^1H-^{13}C direct correlations available in the HSQC spectrum and ^1H-^{13}C long range correlations indicated in the HMBC spectrum. Based on all the spectroscopic data, literature values[18,19] and melting point of the compound, it was confirmed that the compound (2) is 7-hydroxy-6-methoxycoumarin.

Compound (3) was a white solid substance with mp 280ºC. APCI mass spectrum of the compound showed a fragment ion at m/z 395 $[(M+H)]^+$-180] which indicated the molecular weight of the compound is 574 and the molecular formula is $C_{35}H_{58}O_6$. The IR spectrum of the compound showed a broad absorption band at 3377cm^{-1} indicated the presence of O-H stretching vibrations. The ^{1}H NMR spectrum showed singlet at δ 5.31 which is for olefinic proton at C-6. Two protons showed by two sets of doublet of doublet at δ 5.08 and 4.95 are situated at C-22 and C-23. The doublet at δ 4.35 with coupling constant 7.6 Hz indicated the anomeric proton in the pyranose ring at C-1′. The coupling constant value (J = 7.6 Hz) confirmed the configuration of the glycosidic linkage which is β. The signals in the region at δ 3.15 to 3.80 were assigned for the protons in the sugar moiety. The six methyl groups present in the structure indicated by the peaks at δ 1.20 (s), 0.87 (s), 0.78 (6H, d), 0.74 (t) and 0.63 (d). The ^{13}C NMR spectrum of the compound showed four signals in the olefinic region at δ 140.5, 138.2, 129.2 and 122.1 clearly indicated the carbons at C-5, C-22, C-23 and C-6, respectively of the steroid moiety. The characteristic signal at δ 101.0 clearly indicated the anomeric carbon, C-1′ of the glucose unit. The six signals at δ 79.1, 76.2, 75.6, 73.4, 70.0 and 61.8 in the ^{13}C NMR spectrum were found for five other carbons of glucose unit and the carbon at position-3 those are directly attached to the oxygen atom. The signals present in the region at δ 11.7 to 56.7 indicated all the other carbons available in the steroid moiety. The presence of 35 carbons in the molecule was clearly indicated by the 35 peaks available in the ^{13}C NMR spectrum which suggested that the compound is a steroidal glycoside. Based on all the spectroscopic data, literature values[20] and melting point, it was confirmed that the compound (3) is Stigmasteryl-β-D-glucopyranoside.

The compound (4) was a colorless crystal. The IR spectrum of the compound showed broad absorption band at 3387cm^{-1} confirms the presence of O-H stretching vibrations. The absorption bands at 1240, 1136 and 1053cm^{-1} indicated the C-O stretching vibrations. The ^{1}H NMR spectrum showed doublet for one proton at δ 5.40 with coupling constant 3.6 Hz indicated the anomeric proton attached with C-1 and the coupling constant of this anomeric proton confirmed that the configuration of glycosidic linkage for pyranose ring is α. The doublet at δ 4.12 for one proton indicated the proton at C-3′ and triplet at δ 4.04 for one proton indicated the proton at C-4. The two proton doublet at δ 3.60-3.68 indicated the methylene protons attached with C-1′. The ^{1}H-^{13}C direct correlations were confirmed by the HSQC spectrum. Other signals in the region at δ 3.36 to 3.84 represent the rest of the protons in the carbohydrate moiety. The ^{13}C NMR spectrum clearly showed 12 signals for 12 carbon atoms. The signals at δ 103.9 indicated the carbon at 2′ position which is attached with glycosidic linkage from furanose ring by β-configuration. The three methylene carbons showed their signals at δ 62.5, 61.9 and 60.7 which was confirmed by DEPT-135 spectrum. These three methylene groups significantly indicated that the disaccharide might be sucrose. The compound also gave positive chemical test (Molisch test) for carbohydrates. All the above analysis revealed that the isolated compound is a carbohydrate with 12 carbons. The structure of the compound was finally assigned by the ^{1}H-^{13}C direct correlations available in the HSQC spectrum and ^{1}H-^{13}C long range correlations indicated in the HMBC spectrum. Based on all spectroscopic data, literature values[21] and melting point (186ºC) of the compound, it was confirmed that the compound (4) is sucrose or α-D-Glucopyranosyl-(1→2′)-β-D-fructofuranoside.

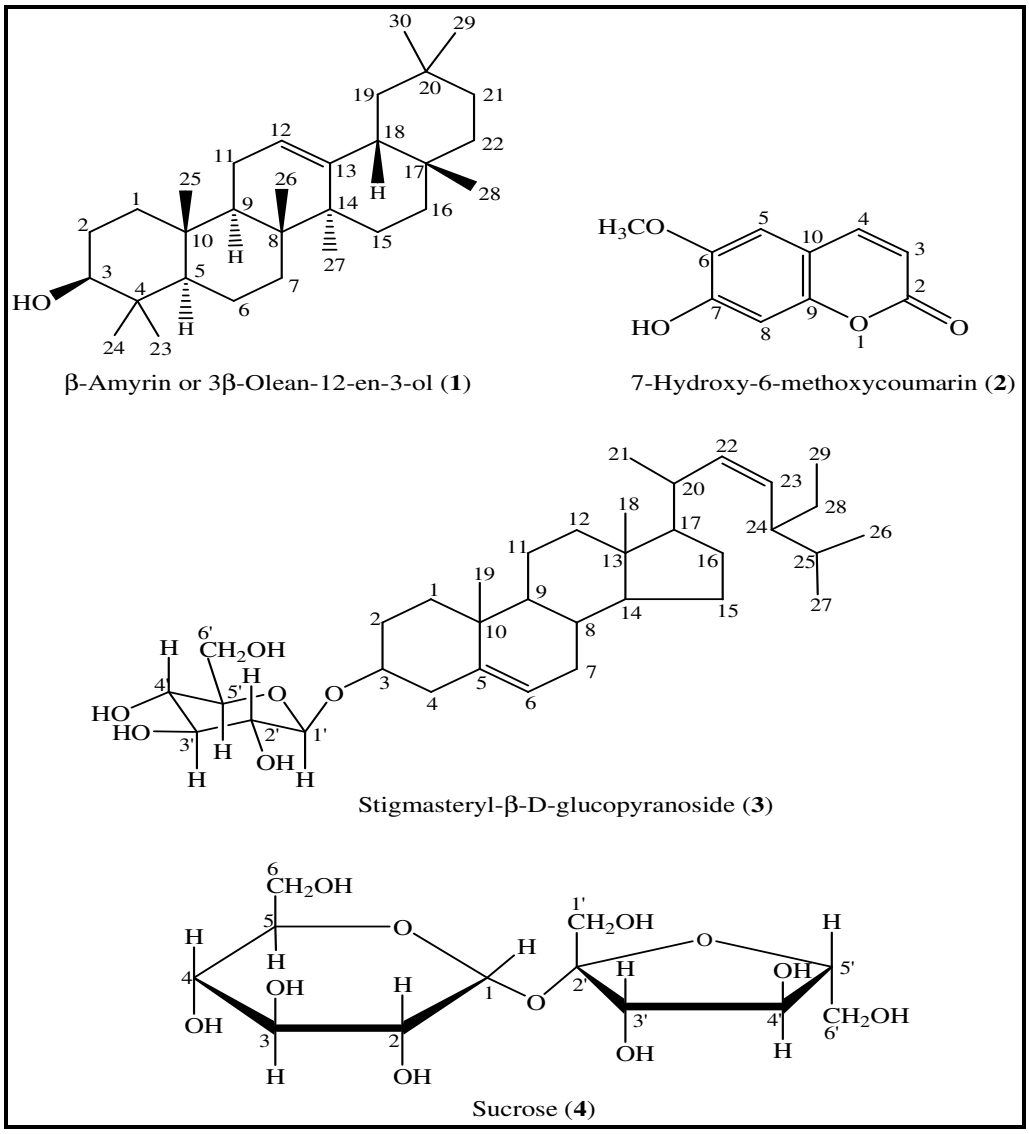

Figure No.1: Structures of the isolated compounds with numbering

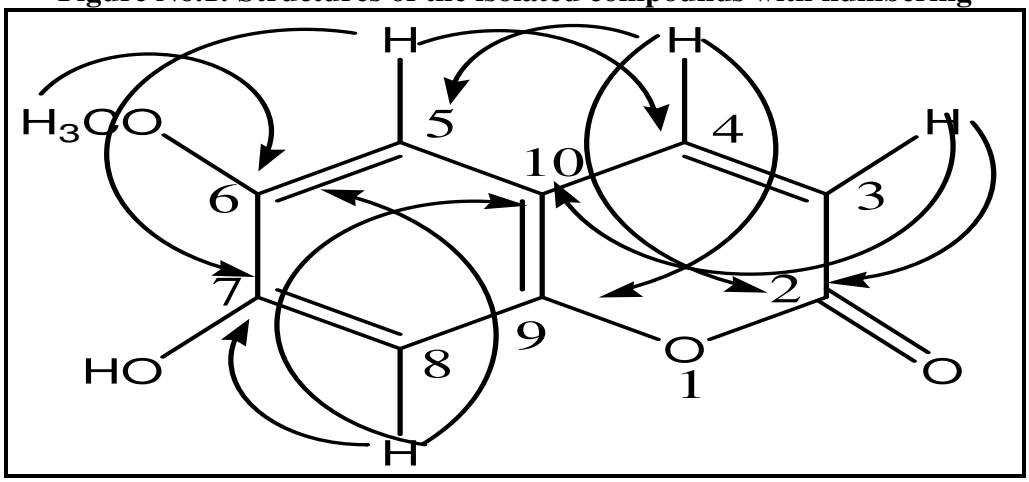

Figure No.2: Selected^{1}H-^{13}C long range correlations in the HMBC spectrum of the compound (2)

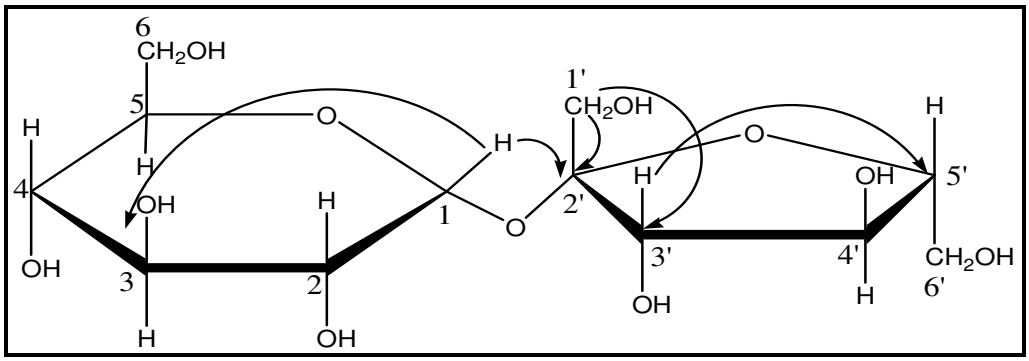

Figure No.3: Selected ^{1}H-^{13}C long range correlations in the HMBC spectrum of the compound (4)

CONCLUSION

Literature survey showed that not much phytochemical studies have done on *Spermacoce latifolia*, especially on the roots of the plant. The isolation and characterization of four compounds from the roots of the plant are reported here as a new sources of compounds. Moreover, it is believed that there is a great scope to do more phytochemical and biological studies on this plant material to isolate as well as elucidate structure of new bioactive compounds in future.

ACKNOWLEDGEMENT

We are thankful to Wazed Miah Science Research Centre, Jahangirnagar University, Savar, Dhaka for recording IR and NMR spectra of the compounds and grateful to the Department of Chemistry, Tokyo Metropolitan University, Japan to get APCI mass spectra of the isolated compounds.

CONFLICT OF INTEREST

We declare that we have no conflict of interest

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