Phytochemical investigations on the leaves of the plant *Clerodendrum viscosum* Vent.

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Abstract
One steroidal compound (22*E*, 24*S*)-stigmasta-5, 22, 25-trien-3β-ol and a non-steroidal compound, clerodin have been isolated from the *n*-hexane extract of the leaves of the plant ***Clerodendrum viscosum*** Vent. The isolated compounds were characterized on the basis of their physical properties and spectroscopic data analysis.

Key words: Steroid, clerodin, fractional crystallization, chromatography, ***Clerodendrum viscosum*** Vent.

Introduction

Plants have been used as a source of medicine by traditional practitioners since ancient time. Very recently, the research on natural products chemistry has attracted the attention of chemists to isolate the biologically active compounds from the plants that can be used as a traditional medicine for the treatment of various types of diseases. The people of developing countries like ours cannot afford to use the expensive modern chemotherapeutic drugs due to the economic problems. But our country abounds with a vast majority of medicinal plants and herbs. But no serious systematic survey of the available medicinal plant resources appears to have been made in Bangladesh.

No doubt a number of chemists in our country are engaged in exploring the active principles of the various medicinal plants which are reported to be used in the traditional system of medicine. The study unfortunately is being carried out in an unplanned way. Most of the medicinal plants of Bangladesh grow widely in forest, jungles, hillocks and gardens. Many of these medicinal plants are being used by the Hekims, Vaidays, Kabirajes as successful medicine for the remedy of various types of diseases.

The plant ***Clerodendrum viscosum Vent*** belong to the family of ***Lamiaceae*** **(*Local name*: Bhat)** is an important medicinal plants of Bangladesh and widely distributed all over the country and in the other countries of the Indian subcontinent [1]. The plant has attracted the attention of chemists for its medicinal value. It has antimicrobial activity against gram positive bacteria ***Staphylococcus aureus*** [2]. The plants have been shown to possess antibacterial [2], antifungal [2], analgesic [5], antioxidant [6], anti-cancer [3] and anti-inflamatory activies [7]. The roots of the plant have been shown to contain significant amount of cytotoxicity [4,8] and anthelmantic activity [4,8].

Phytochemical investigations on the roots [4, 9-11], seeds [16], leaves [9,14-15, 17], aerial parts [17-18] and flowers [5, 11-13] have been reported to contain various types of compounds such as lupeol, β-sitosterol, stigmasterol, fumaric acid, caffeic acid esters, apigenin, saponins, oleic acid, stearic acid. The present research work has been undertaken to isolate more compounds from the leaves of the plant ***Clerodendrum viscosum*** Vent.

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Experimental section

Infrared (IR) spectra were recorded on a Shimadzu FTIR spectrometer as KBr pellet, ^{1}HNMR and ^{13}CNMR spectra were recorded on a Bruker WH 400 MHz NMR spectrometer in $CDCl_3$ at the Wazed Miah Science Research Center, Jahangirnagar University, Savar, Dhaka. Pre-coated TLC plates with silica gel 60 F_{254} (E. MERCK) on aluminum foils were used for the thin layer chromatographic (TLC).

Materials and Method

The leaves of the plant ***Clerodendrum viscosum*** Vent were collected from the adjoining areas of Al-Beruni Hall extension building, Jahangirnagar University, Savar, Dhaka. The collected leaves were dried under shade in the absence of sun light and powdered it properly. About 1. 0 Kg of the dried powdered leaves was successively extracted with *n*-hexane, ethyl acetate and ethanol (96%) at room temperature for about 72 hours. Each of the extraction process was repeated three more times to ensure the complete the extraction. The solvents were removed under reduced pressure below 45◦C temperature when an ***n-hexane extract*** (7.74 g), ***ethyl acetate extract*** (7.78 g) and ***ethanol extract*** (8.75g) were obtained.

TLC examination of the *n*-hexane and ethyl acetate extracts were carried out in different solvent systems. Of these two extracts the *n*-hexane extract gave fairly well resolved spots on the TLC plates in various solvent systems such as in pet. ether and gradient mixtures of pet. ether-ethyl acetate, ethyl acetate and ethyl acetate-methanol. Best resolution on the TLC plates was obtained in 15% ethyl acetate in pet ether. There were five distinct spots on the TLC plates at R_f 0.91, 0.51, 0.43, 0.32 and 0.19 in 15% ethyl acetate in pet. ether along with a tailing from the base line. Therefore, the present research work was concentrated only on the *n*-hexane extract.

Isolation of compounds from the n-hexane extract

The ***n-hexane extract*** (3.138 gm) was subjected to column chromatographic separation. The column was eluted successively with pet. ether and a gradient mixtures of pet. ether-ethyl acetate, ethyl acetate, ethyl acetate-methanol and finally the column was washed down with methanol. A number of colored bands such as yellow, orange-red, light green, green, greenish black, brownish green and light green were observed during the elution of the column. The eluants were divided into total 23 fractions on the basis of their TLC behavior. Of these 23 fractions two of the fractions $\mathbf{F_{14}}$ (182 mg) and $\mathbf{F_{18}}$ (215mg) gave fairly well resolve spots on the TLC plates.

Study on fraction no. F_{14}

The ***fraction no F_{14}*** (182 mg) was a greenish colored solid material which was soluble in *n*-hexane, ethyl acetate and partially soluble in methanol. Fraction no. $\mathbf{F_{14}}$ showed a number of well resolved spots on the TLC plates at R_f 0.47 and 0.29 in 10% ethyl acetate in pet. ether with a tailing from the base line.

On repeated column chromatographic separation followed by fractional crystallization we have a TLC pure white color needle shape crystalline compound from the fraction no. $\mathbf{F_{14}}$ which was designated as ***compound 1*** (2.0 mg; mp. 115-157◦C).

Study on the fraction no F_{18}

Fraction no F_{18} (215 mg) was light greenish yellow colored solid substance. It was soluble in ethyl acetate, chloroform and partially soluble in methanol. TLC examination of this fraction showed a well resolved single spot on the TLC plates at R_f 0.44 in 30% ethyl acetate in pet.

ether and 0.45 in 2% methanol in dichloromethane with a small tailing from the base line. The TLC resolution in 2% methanol in dichloromethane was fairly better than 30% ethyl acetate in pet. ether.

On repeated column chromatographic separation we have a TLC pure white color crystalline compound from the fraction no. **F_{18}** which was designated as ***compound 2*** (10 mg, mp. 157-160◦C).

Results and discussion

The dried powdered leaves of the plant ***Clerodendrum viscosum*** Vent were successively extracted with *n*-hexane, ethyl acetate and ethanol (96%) at room temperature for 72 hours. The extraction process was repeated three more times to ensure the complete the extraction. The solvents were removed under reduced pressure below 45◦C temperature when an ***n*-hexane extract** (7.74 g), ***ethyl acetate extract*** (7.78 g) and ***ethanol extract*** (8.75g) were obtained. Of these three extracts the ***n-hexane extract*** gave fairly well resolved spots on the TLC plates in various solvent systems.

Therefore, the present research work was concentrated only on the ***n-hexane extract***. The *n*-hexane extract was divided into 23 fractions by column chromatography on the basis of their TLC behavior. Of these 23 fractions the fraction nos. **F_{14}** and **F_{18}** gave fairly well resolved spots on the TLC plates. Rest of the fractions was found to be a complicated mixture. Therefore, no further work was done on rest of the fractions.

Column chromatographic separation followed by fractional crystallization we have a TLC pure white color needle shape crystalline compound from the fraction no. **F_{14}** which was designated as ***compound 1*** (2.0 mg). On column chromatographic separation of the fraction no. **F_{18}** we have a TLC pure white color crystalline compound which was designated as ***compound 2*** (10.0 mg).

Characterization of the Compound 1

The ***compound 1***(2.0 mg) was a white color needle shape crystalline substance, melted at 155-157°C temperature. It was soluble in pet. ether, ethyl acetate, chloroform and dichloromethane. It gave single spot on the TLC plates at R_f 0.47 in 10% ethyl acetate in pet ether and R_f 0.48 in 2% methanol in dichloromethane without any tailing.

IR (in KBr) of the compound 1

The IR spectrum of the ***compound 1*** showed a broad absorption band at 3410 cm^{-1} which is attributed to the -O–H stretching vibration. The absorption bands at 2937 and 2852cm^{-1} for the C–H asymmetric and symmetric stretching vibrations and the absorption bands at 1456, 1369 cm^{-1} for C–H bending vibrations, the absorption band at 1645 cm^{-1} is for the C=C bond stretching vibration.

^{1}HNMR (in $CDCl_3$) data of the ***compound 1*** and its corresponding reported ^{1}HNMR [19] data are given in the ***table 1***.

Table 1

Nos.	^{1}H NMR data (δ ppm)	Comment	Reported ^{1}HNMR data (δ ppm) [19]
H-3	3.55 (1H, m)	–C***H***–OH	3.53
H-6	5.37(1H,d)	–C=C***H***–	5.34
H-18	0.72 (3H, s)	Angular CH_3	0.68

H-19	1.03 (3H, s)	Angular CH_3	1.00
H-21	1.10 (3H, d)	Angular CH_3	1.01
H-22	5.23 (1H,dd, *J* =17.6 and 5.2 Hz)	–C***H***=CH–	5.17
H-23	5.23 (1H,dd, *J* =17.6 and 5.2 Hz)	–CH=C***H***–	5.24
H-24	2.45 (1H,m)	Tertiary hydrogen	-
H-26	4.72 (2H, s)	–C=C$\boldsymbol{H}_2$	4.70
H-27	1.67 (3H,s)	–C=C–C$\boldsymbol{H}_3$	1.68
H-28	Not clear	–CH(C$\boldsymbol{H}_2$)CH_3	-
H-29	0.86 (3H, t)	–CH_2–C$\boldsymbol{H}_3$	0.83

^{13}CNMR data and DEPT-135 (in $CDCl_3$) of the compound 1

The ^{13}CNMR and its corresponding DEPT-135 data are given in the following **table 2**.

Table 2

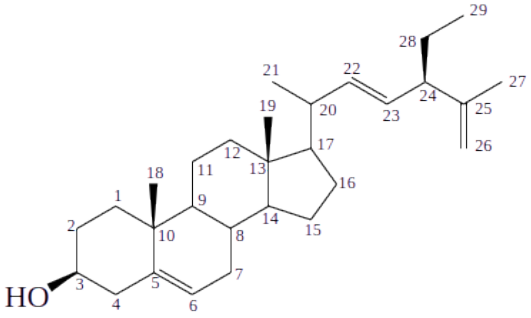
Nos.	^{13}C NMR (δ ppm)	DEPT-135	Comment
1	**31.91**	***Negative***	Methylene (CH_2) group
2	**37.27**	***Negative***	Methylene (CH_2) group
3	71.81	Positive	–***CH***–OH
4	**42.32**	***Negative***	Methylene (CH_2) group
5	140.77	No peak	Quaternary carbon attached to a double bond
6	121.69	Positive	–C=***CH***–
7	**31.68**	***Negative***	Methylene (CH_2) group
8	32.00	Positive	Tertiarycarbon (C_3***C–H***)
9	50.17	Positive	Tertiary carbon (C_3***C–H***)
10	36.53	No peak	Quaternary carbon (C_4***C*****)**
11	**21.08**	***Negative***	Methylene (CH_2) group
12	**39.69**	***Negative***	Methylene (CH_2) group
13	42.27	No peak	Quaternary carbon (C_4***C*****)**
14	56.86	Positive	Tertiary carbon (C_3***C–H***)
15	**25.72**	***Negative***	Methylene (CH_2) group
16	**28.71**	***Negative***	Methylene (CH_2) group
17	55.90	Positive	Tertiary carbon(C_3***C–H***)
18	12.06	Positive	Methyl group attached to quaternary carbon
19	19.40	Positive	Methyl group attached to quaternary carbon
20	40.18	Positive	Tertiary carbon(C_3***C–H***)

21	20.22	Positive	Methyl group attached to tertiary carbon
22	137.20	Positive	–***CH***=CH–
23	130.05	Positive	–CH=***CH***–
24	52.00	Positive	Tertiary carbon(C_3***C–H***)
25	148.63	No peak	Quaternary carbon attached to a double bond
26	**109.52**	***Negative***	–C=***CH***$_2$
27	20.80	Positive	Methyl group attached to quaternary carbon
28	**24.33**	***Negative***	Methylene (CH_2) group
29	12.13	Positive	Methyl group attached to secondary carbon

The physical property and the above IR, ^{1}HNMR ^{13}CNMR and DEPT-135 data can be nicely accommodated if the ***compound* 1** is (22*E*, 24*S*)-stigmasta-5, 22, 25-trien-3β-ol.

Literature survey reveals that the ***compound* 1** is a known compound that has already been isolated from the ***methanol extract*** of the whole plant of ***Clerodendrum viscosum*** Vent [19-20].

In the present research work the ***compound 1*** has been isolated from the *n*-hexane extract of the leaves of the plant ***Clerodendrum viscosum Vent*** but not from the whole plant. Therefore, the present research work reveals that the ***compound 1*** also present in the leaves of the plant. In the present research work to isolate the ***compound 1*** a different extraction route and solvent system have been used. The isolated ***compound 1*** has been characterized on the basis of the IR, ^{1}HNMR, ^{13}CNMR and DEPT-135 spectral data analysis.



Compound 1

Characterization of the Compound 2

The ***Compound 2*** (10 mg) was a white color crystalline material, melted at 158-160°C. It was soluble in dichloromethane, chloroform and ethyl acetate. It gave a well resolve single spot on the TLC plates at R_f 0.45 in 2% methanol in dichloromethane and R_f 0.44 in 30% ethyl acetate in pet ether.

IR (in KBr) of the compound 2

The IR spectrum of the compound showed absorption bands at 2985, 2964, 2943 and 1463, 1390, 1361cm^{-1} for the aliphatic C–H stretching and bending vibrations respectively. The strong absorption bands at 1739 cm^{-1} for the C=O stretching vibrations of the carbonyl groups

of the ester function, 1618 (C=C stretch), 1286, 1238, 1141, 1093 and 1018 cm^{-1} (C–O stretch).

^{1}HNMR (in $CDCl_3$) data of the ***compound 2*** and its corresponding reported ^{1}H NMR [21] data are given in the **table 3**.

Table 3

Nos	^{1}H NMR data (δ ppm)	Reported ^{1}H NMR data (δ ppm) [21]	Comment
1	1.49 (2H, m, unresolved)	1.91, 142	Methylene (CH_2) group.
2	1.60 (2H, m, unresolved)	1.60	Methylene (CH_2) group.
3	1.05 (2H, m)	1.05, 2.13	Methylene (CH_2) group.
4	No peak	No peak	Quaternary carbon (C_4***C***).
5	No peak	No peak	Quaternary carbon (C_4***C***).
6	4.71 (1H, dd)	4.71	Tertiary carbon (C_3C–H).
7	1.48 (1H, m), 1.67 (1H, m)	1.48,1.67	Methylene (CH_2) group.
8	1.48 (1H, m)	1.48	Tertiary carbon atom attached with a methyl (CH_3) group and a methylene (CH_2) group.
9	No peak	No peak	Quaternary (C_4***C***).
10	1.60 (1H, t, unresolved)	1.68	Tertiary carbon attached to methylene (CH_2) group.
11	4.05 (1H, dd)	4.05	Tertiary carbon attached to the methylene (CH_2) group.with non-equivalent hydrogen atoms.
12	1.75 (1H, d, unresolved), 1.67 (1H, d, unresolved)	1.76, 1.67	Methylene (CH_2) group with non-equivalent protons.
13	3.58 (1H, m, unresolved)	3.59	Tertiary carbon (C_3**C–*H***).
14	4.83 (1H, t)	4.84	–O–CH=C***H***–CH–
15	6.48 (1H, d)	6.49	–O–C***H***=CH–
16	6.03 (1H, d).	6.04	–O–C***H***–O–
17	0.86 (3H, d).	0.86	Methyl (CH_3) group attached to tertiary carbon.
18	3.00 (1H, bs), 2.23 (1H, d)	3.01, 2.24	Methylene (CH_2) group with non-equivalent hydrogen atoms.
19	4.91 (1H, d), 4.40 (1H, d).	4.92, 4.40	Methylene (CH_2) group with non-equivalent hydrogen atoms.
20	0.99 (3H, s)	0.99	Methyl (CH_3) group attached to quaternary carbon atom.
21	No peak	No peak	Carbonyl group (>C=O).
22	1.97 (3H, s)	1.97	Methyl (CH_3) group attached to carbonyl carbon atom.
23	No peak	No peak	Carbonyl group (>C=O).
24	2.12 (3H, s)	2.13	Methyl (CH_3) group attached to carbonyl carbon atom.

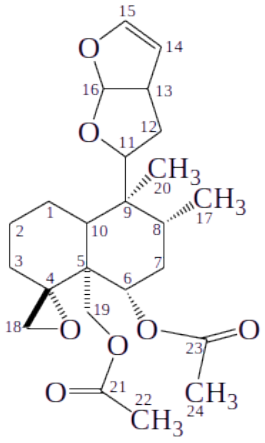
^{13}CNMR, corresponding reported ^{13}CNMR [21] data and ***DEPT-135 (in $CDCl_3$)*** of the ***compound 2*** are given in the **table 4**.

Table 4

Carbon nos.	^{13}C NMR data (δ ppm)	Reported ^{13}CNMR data (δ ppm) [21]	DEPT-135	Comment
1	**25.02**	**25.03**	***Negative***	Methylene (CH_2) group.
2	**22.27**	**22.26**	***Negative***	Methylene (CH_2) group.
3	**32.76**	**32.74**	***Negative***	Methylene (CH_2) group.
4	65.04	65.06	No peak	Quaternary carbon (C_4***C***).
5	45.59	45.56	No peak	Quaternary carbon (C_4***C***).
6	71.99	71.96	Positive	Tertiary carbon (C_3C–H).
7	**33.42**	**33.38**	***Negative***	Methylene (CH_2).
8	36.20	36.23	Positive	Tertiary carbon atom.
9	40.08	40.05	No peak	Quaternary (C_4***C***).
10	48.68	48.64	Positive	Tertiary carbon atom.
11	84.61	84.63	Positive	Tertiary carbon atom.
12	**31.28**	**31.24**	***Negative***	Methylene (CH_2) group.
13	46.04	46.04	Positive	Tertiary carbon (C_3**C**–H).
14	101.93	101.96	Positive	–O–CH=***CH***–CH–
15	146.89	146.89	Positive	–O–C***H***=CH–
16	107.73	107.71	Positive	–O–C***H***–O–
17	16.44	16.44	Positive	Methyl (CH_3) group.
18	**48.47**	**48.47**	***Negative***	Methylene (CH_2) group.
19	**61.76**	**61.74**	***Negative***	Methylene (CH_2) group.
20	14.13	14.18	Positive	Methyl (CH_3) group.
21	170.13	170.19	No peak	Carbonyl group (>C=O).
22	21.20	21.24	Positive	Methyl (CH_3) group.
23	170.91	170.98	No peak	Carbonyl group (>C=O).
24	21.23	21.28	Positive	Methyl (CH_3) group.

The physical property and the above IR, ^{1}HNMR ^{13}CNMR and DEPT-135 data can be nicely accommodated if the ***compound 2*** is ***clerodin***.

Literature survey reveals that the ***compound 2*** is a known compound that has already been isolated from the ***methanol*** and ***hexane*** extracts of the leaves of the plant of ***Clerodendrum viscosum*** Vent [21-22] and has been characterized on the basis of the ^{1}HNMR and ^{13}CNMR data analysis [21]. In the present research work the compound has also been isolated from the ***n-hexane extract*** of the leaves of the plant ***Clerodendrum viscosum*** Vent and characterized on the basis of the IR, ^{1}HNMR, ^{13}CNMR and DEPT-135 spectral data analysis.



Compound 2

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