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Structure Elucidation and Analgesic Activity of Separated Active Compounds from the Methanolic Extract of *Solanum torvum*

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Methanolic extract of fruits of *Solanum torvum* Swartz was subjected for column chromatography taking ethyl acetate as mobile phase. The separated and purified chemical constituents and crude extract were tested for their analgesic activity by acetic acid induced writhing method. The structures of therapeutically active constituents were elucidated by spectral analysis and they were steroidal glycosides.

Key Words: Fruits, *Solanum torvum*, Analgesia, Glycosides.

INTRODUCTION

The fruits of *Solanum torvum* Swartz (Family: Solanaceae) are edible to the tribal people of Tripura, India. These are eaten as vegetable. From the mature fruits tribal people take out the seeds and cook a dish namely Gudak or simply fry. Methanolic extract of fruits were able to exhibit analgesic activity in mice, which was comparable to the standard drug aspirin[1]. The physico-chemical characterization, antifungal, CNS depressant activity, hypnotic activity and LD_{50} of the methanolic extract of the fruits of *S. torvum* are also reported[2,3]. Studies on leaves, whole plants, roots and fruits of *S. torvum*[4-7] were carried out by different research groups. The present investigation is designed to elucidate the structure of the analgesic active components.

EXPERIMENTAL

The fruits of *Solanum torvum* Swartz was collected from the local market Agartala, Tripura, India and was authenticated by the expert of Tribal Research Institute, Agartala, Tripura, India. Fruits were cleared from

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extraneous matters and were shade dried with occasional shifting of material to avoid any growth of fungi. Completely dried fruits were powdered and passed through sieve 40. Extraction was done by using Soxhlet apparatus in methanol. Liquid extract was collected for further works *i.e.* for separation, purification and Structure elucidation by spectral (IR, ^{1}H NMR, ^{13}C NMR, DEPT NMR, 2D COSY, LC-MS) analysis.

TLC plates (20 cm × 20 cm) and chamber (25 cm × 25 cm × 10 cm), column for chromatography purpose were of Borosil made. Systronic colorimeter was also used to determine maximum wave length. Melting points reported were determined by open capillaries method and are uncorrected. The IR spectra of the compounds were recorded in KBr pellet on Jasco FT/IR-5300 and expressed in cm^{-1}, the ^{1}H NMR and ^{13}C NMR in $CDCl_3$ and DMSO, respectively were recorded in Varian Mercury Plus 400 NMR spectrometer. The 2DCOSY and DEPT-NMR in DMSO-d_6 were also recorded in Bruker ACF-200. The LC-Mass spectra were observed on Shimadzu LCMS-2010A. HPLC conditions applied were as column: C18, mobile phase: methanol and water mixture (90:10), flow rate: 0.2 mL/min, detector: UV (254 nm), volume injected: 5 µL, sample dissolved in methanol, temperature: 25 ºC and probe: APCI (atmospheric pressure chemical ionization). The elemental analysis were also carried out in Perkin-Elmer 2400 series II CHNS/O Analyzer. Chemicals were used of analytical grade.

Isolation and characterization: Isolation of the active component was carried out by column chromatography technique, where ethyl acetate was taken as mobile phase and silica gel for column chromatography as stationary phase. Total 200 mL of extract volume was applied and left for 6 d for separation and collection purpose. Three numbers of bend were distinctly visualized and at first, let, bend for compound A was eluted and collected in a beaker and then for B (2nd bend) and at last for C (3rd bend) separately. For 1st bend total 42 mL (yellowish green colour) was collected, for 2nd 63 mL (yellowish red) and for 3rd 87 mL (wine red). Ultimately 8 mL extract was absorbed by stationary phase.

Solvent was then allowed to be evaporated in air at room temperature and thus compounds were dried. The appearance of components B and C were solid and A appeared as paste. In the case of A, solvent was allowed to be evaporated for 10 d (where in case of B and C, solvent was fully evaporated by 5 d); even then A remained as paste. Therefore, it was taken for colorimetric measurement in 400-800 nm. For this purpose, *ca.* 0.1 g dissolved in 100 mL acetone and λ_{max} determined by taking acetone as blank. It was found that the λ_{max} = 667 nm, which was nothing but due to chlorophyll and pigment[8]. The analgesic activity[1] was observed for A, B and C. The solubility tests were carried out for component B and C, by taking *ca.* 0.1 g in 10 mL of available solvent and shaken without applying

heat. Component B was soluble in benzene and acetone; sparingly soluble in water, ethyl acetate and ether; not soluble in methanol and ethanol. Component C was soluble in methanol, ether, acetone, benzene and ethyl acetate; sparingly soluble in water and ethanol.

Further component A, B and C were subjected to TLC by taking butanol:acetic acid:water (B:A:W) = 4:1:3 as mobile phase and silica gel G as stationary phase. After observing the tailing effect in TLC plate for B, the mobile phase was changed by benzene and the R_f value for both the cases of mobile phase were calculated and presented in Table-1.

TABLE-1
R_f VALUE OBTAINED FROM TLC OF THE COMPONENTS SEPARATED BY COLUMN CHROMATOGRAPHY OF THE EXTRACT OF TENDER FRUITS OF *Solanum torvum* SWARTZ

Component	Solvent system (mobile phase)	R_f values
A	B:A:W = 4:1:3	0.75
	Benzene	0.17
B	B:A:W = 4:1:3	0.53
	Benzene	0.14
		0.29
C	B:A:W = 4:1:3	0.14
	Benzene	0.12

Steps for further purification of component B: Component B was treated with dil. (10 % v/v) glacial acetic acid (1 g/10 mL) and immediately filtered, washed with cold water repeatedly to remove acetic acid. It was found that yellowish part was removed and drained with acetic acid solution. Further residue (purified B) was subjected to TLC, taking benzene as mobile phase and found that the R_f value: 0.29. The analgesic activity[1] was also again checked for this compound and found same as earlier. The results obtained from analgesic tests are displayed in Table-2.

TABLE-2
ANALGESIC ACTIVITY OF THE FRACTIONS/COMPONENTS/ COMPOUNDS OBTAINED FROM THE EXTRACTS OF THE FRUITS OF *Solanum torvum*

Components	Average writhings	% Protection to the induced writhing (analgesic activity) ± SEM
Control	59.6	0.0
A	59.4	0.336 ± 2.56
B	43.0	27.85 ± 1.30
C	21.8	63.42 ± 1.35

Compound B and C were recrystallized from acetone and melting point was recorded as 190 and 108 ºC respectively. The colour of B was brown and that of C was brick red.

RESULTS AND DISCUSSION

From 1.5 kg raw materials (fruits of *Solanum torvum* Swartz), component B and C (recrystallized from acetone) were obtained 1.1 and 2.4 g, respectively. Analgesic activity of the components A, B and C were carried out and was found that component A had no analgesic activity, compound C had analgesic activity more than B and individual compound B and C had analgesic activity less than total extract (69.13 + 2.94:SEM)[1].

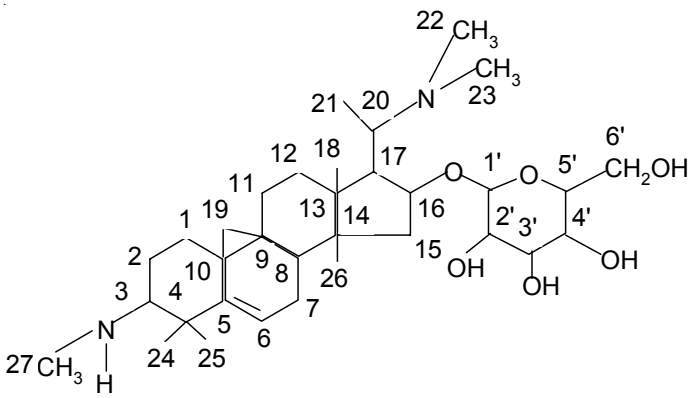
Structure elucidation of active compounds (two numbers: B and C) were carried out by following spectral analysis.

For compound C: The m.f. of compound **C** ($C_{33}H_{56}N_2O_6$) was deduced from elemental analysis and LCMS - APC I (+ve) mode mass spectrum by exhibiting a pseudomolecular ion at m/e 577 $(M+H)^+$. Its IR spectrum exhibited characteristic bands at 1614 cm^{-1} for unsaturation (C=C), at 1041 cm^{-1} for C-N aliphatic amine, at 1246 cm^{-1} for cyclic C-O, at 2928 cm^{-1} for cyclic -CH_2- and at 3400 cm^{-1} for hydroxy and -NH groups. The ^{1}H NMR spectrum showed a sugar derivative with an anomeric proton at δ 5.00 and a complex multiplet signal between δ 3 to 4 for the rest of the sugar moiety protons and the hydroxyl groups. The carbon resonances in ^{13}C NMR spectrum at δ 101.09, 61.25, 70.50, 73.23, 80.21 and 82.55 suggested the presence of an hexose moiety. Including this, the ^{13}C NMR spectrum displayed signals for 33 carbons, which were edited by DEPT experiments: 11 methines, 6 non-protonated carbons, 8 methylene and 8 methyl carbons. In which suggests that the compound may be a steroidal alkaloid. In the ^{1}H NMR spectrum in addition to the sugar protons the signals also contain 3 methyl group signals attached to the nitrogen atom. The signal at δ 5.27 is due to the unsaturated proton of the steroidal nucleus which shows cross peaks with the H-7 protons at δ 2.1 in the ^{1}H-^{1}H COSY spectrum. The signal at δ 0.80 is due to the methylene protons of the cyclopropane ring system complemented by the methylene signal at δ 14.0 in ^{13}C NMR spectrum. The presence of the hexose sugar was further confirmed by the appearance of a molecular ion peak at m/z 415. The detailed analysis of the ^{13}C NMR signals (Table-3) shows that the probable structure of the compound **C** is a steroidal compound with an attachment of a hexose moiety, which is also supported by its mass spectral fragments.

For compound B: Compound **B** is not a pure compound as separation could not be done through column chromatography, which is clearly under-

TABLE-3
^{13}C NMR SPECTRAL DATA OF COMPOUND **C**

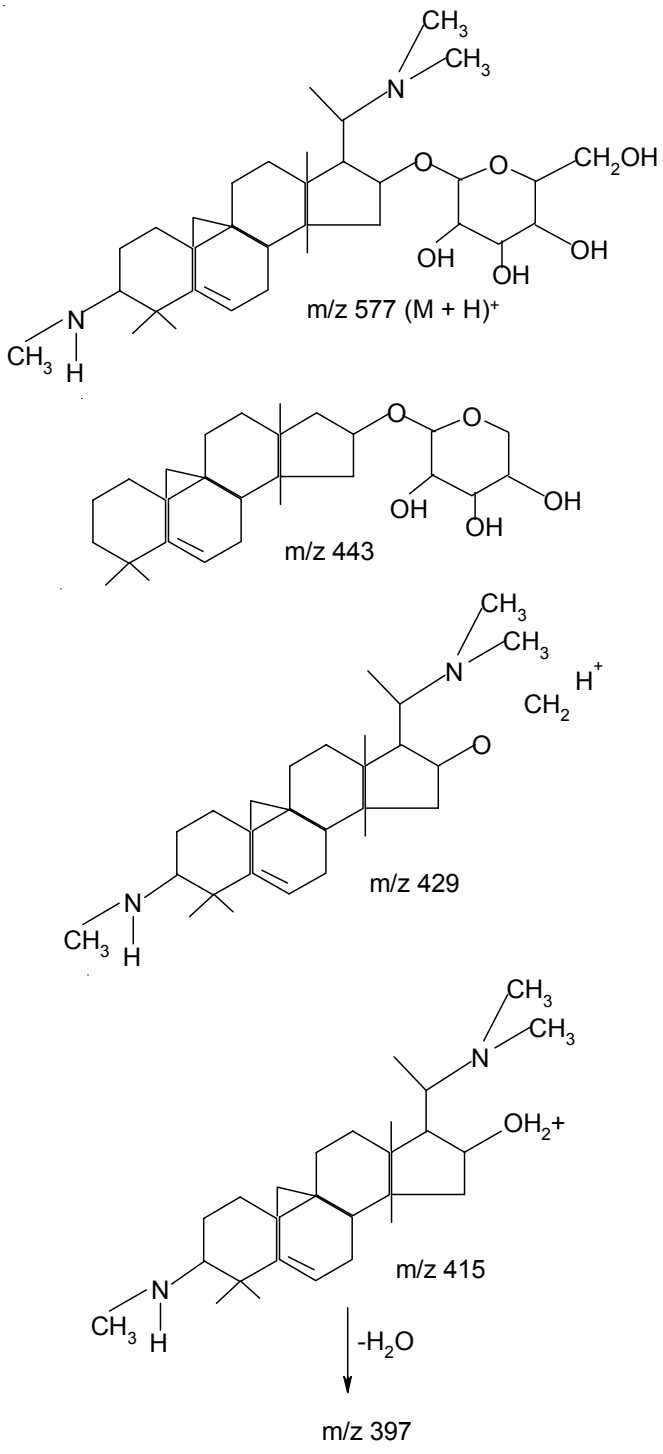
Carbon	Signal	Multiplicity	Carbon	Signal	Multiplicity
1	39.91	t	18	20.32	q
2	36.35	t	19	14.03	q
3	69.04	d	20	65.71	d
4	39.06	–	21	16.71	q
5	140.10	–	22	19.00	q
6	121.13	d	23	15.68	q
7	30.96	t	24	17.41	q
8	49.55	d	25	55.93	q
9	38.86	–	26	60.55	q
10	40.11	–	27	69.28	q
11	29.12	t	1’	101.09	d
12	36.78	t	2’	82.55	d
13	39.00	–	3’	80.21	d
14	39.61	–	4’	70.50	d
15	27.00	t	5’	73.23	d
16	76.45	d	6’	61.25	t
17	56.14	d	–	–	–



Structure of Compound C

stood from the spectral studies. Separation technique was unable to provide pure compounds as because components were present in a mixture form bearing a minute quantity individually and due to the structural similarities. Compound **B** is a mixture of total 2 (Two) components, where compound **C** (structure evaluated, here indicated as c) was also there along with b; but from the LC chromatogram data it could not be understood (only one peak at 254 nm: mAbs 0.615).

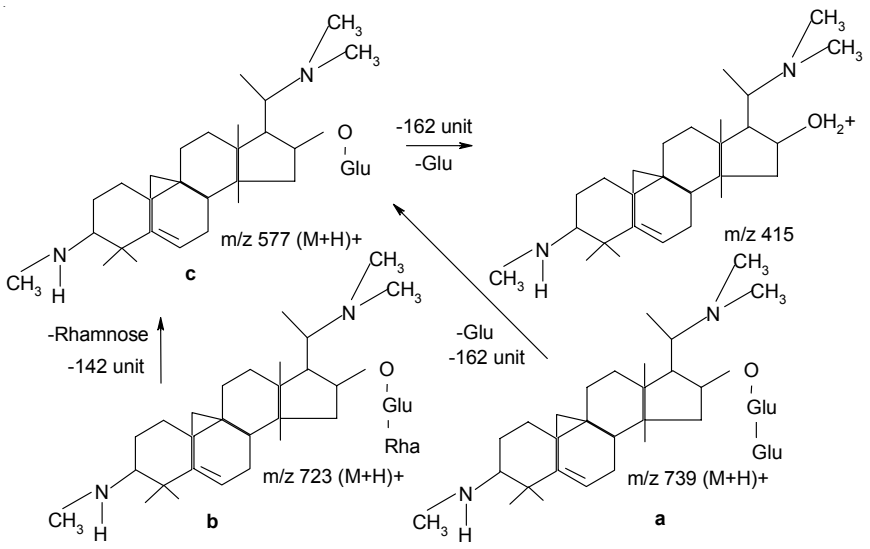
Mass spectral fragmentation of compound C:



The IR spectrum of compound B shows characteristic bands at 3408 cm^{-1} for hydroxyl group, 2918 cm^{-1} for cyclic -CH_2-, 1043 cm^{-1} for C-N aliphatic amine, 1161 cm^{-1} for C-O of cyclic 6-membered ring, 2851 cm^{-1} for N-CH_3, 1467 cm^{-1} for C-H deformation of -CH_3, 1741 cm^{-1} for carbonyl group and at 1643 cm^{-1} for unsaturation. The elemental analysis shows a high percentage of nitrogen and moreover the molecular mass seems to be even numbered (the $[M+H]^+$ ionic peaks in the mass spectrum are having odd numbers). So even number of nitrogen atoms (at least two) are expected to be present in the compound as per the 'nitrogen rule'.

The mass spectrum in full scan mode displayed a first approach to the compound structure, where the $[M+H]^+$ ion corresponds to the protonated molecule at m/z 577 and at m/z 415 is due to the loss of 162 units which indicated the presence of glucose moiety. It was identified as component **c**.

In addition to that, the peak at m/z 723 looses 146 unit (rhamonsyl moiety) to form the ionic peak at m/z 577, indicating that another glycosidic compound (**b**) is present. The aglycone part are being the same.



The presence of mixture of glycosides was further supported by the ^{13}C NMR spectrum. In addition to the signals of the aglycone (Table-4) of **c**, which forms the basic nucleus, it also exhibits other anomeric carbon signals at δ 100.62, 101.16, 102.95. This reveals the presence of more than one glycosidic linkages in this mixture. Apart from this it contains other signals in the unsaturated region at δ 120.42, 120.85, 121.19 which shows the presence of more than one but closely related compounds in this mixture. This was complimented by the bunch of signals between δ 69 to 87 for the

TABLE-4
^{13}C NMR SIGNAL FOR THE AGLYCONE

Carbon	Signal	DEPT	Carbon	Signal	DEPT
1	39.45	t	14	–	–
2	36.97	t	15	26.83	t
3	69.09	d	16	76.17	d
4	–	–	17	56.14	d
5	–	–	18	22.32	q
6	120.42	d	19	13.98	t
7	31.73	t	20	25.33	q
8	49.73	d	21	15.63	q
9	–	–	22	15.87	q
10	–	–	23	62.49	d
11	29.27	t	24	16.11	q
12	34.06	t	25	65.89	q
13	–	–	26	63.56	q
–	–	–	27	68.12	q

glycosidic carbons. The methyl group signals at δ 16.69 and 16.89 reveals the presence of rhamnose moiety in this mixture. There may be the appearance of another glycoside (**a**) in a very minute quantity *i.e.* just addition of one molecule of glucose with **c** (m/z 739 $[M+H]^+$), observed in both the LC-MS of **B** and **C**. The a after loosing 162 unit (glucose moiety) formed **c**. This is also supported by the anomeric carbon signals and signals in the unsaturated region. Some other m/z signals of B recorded as similar to C and indicates the further fragmentation of **c** takes place as mentioned in C. Similarly the CH and CH_2 signals are more in number indicating the presence of closely related compounds. The ^{13}C NMR was interpreted and edited with DEPT experiments. The ^{1}H NMR signals are in close agreement with the aglycone moiety along with sugar residues.

REFERENCES

1. R. Choudhury, M.D. Choudhury, B. De and S.B. Paul, *Asian J. Chem.*, **20**, 741 (2008).
2. M.D. Choudhury, R. Choudhury, B. De and S.B. Paul, *Biosc. Biotech. Res. Asia*, **4**, 145 (2007).
3. S.B. Paul, R. Choudhury, B. De and M.D. Choudhury, *Adv. Pharmacol. Toxicol.*, **8**, 13 (2007).
4. D. Arthan, P. Kittakooop, A. Esen and J. Svasti, *Phytochemistry*, **67**, 27 (2006).
5. C. Gousset, C. Cllonnier, K. Mulya, I. Mariska, G. Rotino, P. Besse, A. Servas and D. Sihachakr, *Plant Science (Oxford)*, **168**, 319 (2005).
6. H. Moriyama, T. Lizuka, M. Nagai, K. Hoshi, Y. Murata and A. Taniguchi, *Natural Medicines*, **57**, 133 (2003).
7. K.F. Chah, K.N. Muko and S.I. Oboegbulem, *Fitoterapia*, **71**, 187 (2000).
8. J.B. Harborne, In Phytochemical Methods, Chapman and Hall Ltd., London, p. 276 (1984).

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