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Secondary Metabolites from the Fruits of *Solanum torvum* SW

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Abstract
The phytochemicals such as steroids, terpenoids, saponins, tannins, alkaloids etc. were identified in *Solanum torvum* Sw. fruits. The iron and ascorbic acid content of the fruits were estimated. Free and bound fatty acids were quantified by GLC. Attempt has been taken for isolation and characterization of compounds from different extracts of *Solanum torvum* Sw. fruits by chromatographic and spectroscopic techniques. Methyl stearate, 3-*O*-acetyl-stigmasta-5,25-diene-2,3-diol have been isolated from dichloromethane extract using chromatographic technique which were characterized by spectroscopic techniques. 21,25-dimethylmelianodiol has been isolated from methanol extract of the fruits. The structure of the compound has been established by spectroscopic techniques.

Keywords: *Solanum torvum,* fruits, fatty acids, 3-*O*-acetyl-stigmasta-5,25-diene-2,3-diol, methyl stearate, 21,25-dimethylmelianodiol.

1. Introduction
Plants are useful and valuable to us. Human life cycle largely depends on plants. The fruits bark and leaves of a large number of plants are valuable as drugs. They are powdered and used as important ingredients in medicine. However, a very little is known about the chemical composition of these plant materials. Therefore, studies on isolation and characterization of the medicinally important compounds from them are very important for the well being of the human society. Solanaceae is one of the important families which comprised of thousands of species [1]. Among them, one of the important fruits of this family is *Solanum torvum* Sw. (locally known as Ban Begun, Gotha Begun, and Wild Eggplant). Pharmacological studies on this plant have demonstrated cytotoxic activities [2], antimicrobial [3, 4] and antiviral activity [5]. Plant parts are used as sedative, diuretic and digestive [6]. Fruits are often eaten as vegetables. Extracts of the fruits and leaves are said to be useful in cases of liver and spleen enlargement and in the treatment of cough [6]. The C-4 sulfated isoflavonoid, torvanol A, and the steroidal glycoside, torvoside H, together with the known glycoside, torvoside A, have been isolated from a methanol extract of *Solanum torvum* Sw. fruits [5]. Two novel C-22 steroidal lactone saponins, namely solanolactosides A, B and two new spirostanol glycosides, namely torvosides M, N were isolated from ethanol extract of aerial parts of Solanum torvum and their structures were characterized on the basis of spectroscopic analysis [7].Torvonin-B [8], Torvonin-A [9], from *Solanum torvum* Sw. have been isolated. Three unusual 22-β-*O*-23-hydroxy-(5α)-spirostanol glycosides, three novel 22-β-*O*-spirostanol oligoglycosides, torvosides J, K and L have been reported [10]. It appears from literature survey that further phytochemical investigations are necessary to explore more medicinally important compounds of this fruits (if any) and with the hope of identifying the medicinally important compounds and highlighting their potential based on structure activity relationship, this study has been undertaken. So this paper deals with the screenings of photochemical and isolation and characterization of three compounds from its dichloromethane and methanol extract along with the studies of fatty acids composition, iron and ascorbic acid content.

2. Materials and Methods
2.1 Sample collection
The fresh unripe fruits of *Solanvm torvum* Sw. were collected from Agailjhara, Barisal, Bangladesh. A voucher specimen was deposited in the Bangladesh National Herbarium (BNH) having ACCESSION NO is DACB 32892. The collected fresh unripe fruits were cleaned thoroughly and dried in open air and then at 45°C in an oven. The dried fruits material was

ground to powder by a grinder (Cyclotec, 200 meshes) and the powder was stored in an air tight container.

2.2 Phytochemical Screening
The phytochemical screening for the different constituents was carried out using standard procedures [11-15] to identify the phytochemical constituents. The presence of tannins, phlabotannins, saponins, flavonoids, steroids, terpenoids were observed.

2.3 Extraction
The powder of *Solanum torvum* Sw. fruits were extracted with petroleum ether (b.p. 40–60 °C) followed by dichloromethane and methanol. All the extractives were filtered and concentrated in a rotary evaporator (Buchii) to a dry mass. The amount of different extracts was presented in Table 1.

2.4 Determination of water and ash content
The water and ash content of the dry powder of *Solanum torvum* Sw. fruits were determined using standard method [16] and the result are given in Table 2.

2.5 Determination of iron content
The iron content of *Solanum torvum* Sw. fruits was estimated [17] spectrophotometrically as the coloured complex by the reaction of Fe(II) and 1,10-phenanthroline. Hydroxylamine was used to reduce Fe(III) to Fe(II). The result is presented in Table 2.

2.6 Estimation of ascorbic acid
The total vitamin C (L-ascorbic acid and L-dehydroascorbic acid) content in fruit samples was determined by 2,4-dinitrophenylhydrazine method (DNPH) [18]. This method employs the coupling reaction of 2,4-dinitrophenylhydrazine with L-dehydroascorbic acid, followed by spectrophotometric determination (at 522 nm). L-ascorbic acid is converted into L-dehydroascorbic acid earlier by oxidation reaction with bromine water. Three determinations were done in this method and absorbances were recorded thrice and average value was taken for each sample. The result is presented in Table 2.

2.7 Analysis of fatty acids
The free fatty acids (FFA) and bound fatty acids (BFA) of the *Solanum torvum* Sw. fruits were isolated [19, 20] from petroleum ether (b.p. 40–60 °C) extract. The fatty acids of the plant were converted [19, 20] to their methyl esters and analyzed by GLC (Shimadzu 9A, Column BP-50, detector-FID, at 170 to 270 °C, rising temperature 4 °C/min for 30 minutes). The fatty acids present in the plant were identified and their relative percentages were determined by comparison with retention time of the standard samples. The result is presented in Table 3 and 4.

2.8 Isolation and characterization of compounds from dichloromethane extract
The dichloromethane extract subjected to TLC screening when it showed several spots in iodine chamber and vanillin-sulfuric acid spray on TLC plate. The dry mass (4.0 g) of dichloromethane extract was subjected to column chromatography over column grade silica gel (silica gel 60) using petroleum ether (b.p. 40–60 °C) as an eluting solvent with increasing percentage of dichloromethane and methanol. Each of the fractions was monitored by TLC and the fractions of similar behaviors were combined together and marked as F-1 to F-9. Among the fractions, the fraction F-3 and F-5 were analyzed by preparative thin layer Chromatography (PTLC). The purified fractions F-3 and F-5 were concentrated and allowed to stand for several days when the fraction F-3 yielded a white solid marked as A (9.8 mg) and the fraction F-5 yielded a white solid marked as B (10.7 mg).

2.9 Isolation and characterization of compounds from methanol extract
Water was added to the filtrate of methanol extract at a ratio 50:50 to precipitate the water insoluble part. The mixture was centrifuged and water insoluble part was collected and dried. TLC analysis of the water insoluble part showed several spots in iodine chamber and vanillin-sulfuric acid spray on TLC plate. The dry mass (3.0 g) was subjected to column chromatography over column grade silica gel (silica gel 60) using petroleum ether (b.p. 40-60 °C) as an eluting solvent with increasing percentage of dichloromethane and methanol. Each of the fractions was monitored by TLC and the fractions of similar behaviors were combined together and marked as F-1 to F-8. Among the fractions, fraction F-2 was found to be single. The fraction F-2 was concentrated and allowed to stand for several days when the fraction F-2 yielded a yellow crystalline solid which was marked as C (16 mg).

3. Results and Discussion
The fresh unripe fruits of *Solanum torvum* Sw. were collected from local market and dried. The dried fruits were grinded to powder. The powder materials were then extracted with distilled petroleum ether (b.p. 40–60 °C) followed by dichloromethane and methanol. The percentage of the extract was determined (Table 1). It showed that the percentage of methanol extract was the highest and petroleum ether extract was the lowest. It indicates the presence of lower proportion of fatty materials.

Table 1: Amount of different solvent extracts of *Solanum torvum* Sw. fruits

Extract	Petroleum ether	Dichloromethane	Methanol
Amount (g /100 g on dry weight basis)	3.58	4.44	10.48

Phytochemical screening of the *Solanum torvum* Sw. fruits indicates that those contain tannins, phlobatannins, saponins, flavonoids, steroids, alkaloids and terpenoids. The medicinal importance of these plants may be explained on the basis of this finding.
Solanum torvum Sw. fruits contain a considerable amount of iron (Table 2) and these may be a source of dietary iron. The results indicated that fruits are good sources of vitamin C (Table-2). About 200 g of fruits might fulfill our daily requirement of ascorbic acid and hence their use as leafy vegetables may be encouraged to the local people. So, these fruits may play an important role in the nutrition of our health as a combined source of iron and vitamin C.

Table 2: Different nutrients content present in *Solanum torvum* Sw. fruits

Water content*	Ash content		Ascorbic acid content*	Iron content**
	Dry sample**	Fresh sample*		
71.7 ± 0.5	5.53 ± 0.2	1.54 ± 0.5	24.64 ± 0.8	4.78 ± 0.1

Note: Values are mean±sd, n=6; *g /100 g fresh fruits; **g /100 g dry fruits powder

3.1 Analysis of fatty acids

The free and bound fatty acids of *Solanum torvum* Sw. fruits were isolated and it was found that the amount of fatty acids existing in Free State was less than the fatty acids associated with lipids or esterified to other organic compounds (Table 3).

Table 3: Amount of petroleum ether extract, bound and free fatty acids isolated from *Solanum torvum* Sw. fruits

Amount (g/100 g of dry powder)			
Petroleum ether extract	**Bound fatty acids (BFA)**	**Free fatty acids (FFA)**	**Total fatty acids**
3.58	0.67	0.35	1.02

Table 4: The total amount and relative percentages of free fatty acids and bound fatty acids in *Solanum torvum* Sw. fruits

Relative percentages (%)	Saturated fatty acid							Unsaturated fatty acid	
	Caprylic	**Capric**	**Lauric**	**Myristic**	**Arachidic**	**Stearic**	**Behenic**	**Oleic**	**Lignoceric**
BFA	1.99	1.98	2.00	12.86	13.24	12.83	7.99	42.35	4.56
FFA	0.52	-	6.47	1.05	1.72	4.08	0.52	84.36	1.28

The analysis of free fatty acids (Table 4) showed that the fruits contain the highest proportion of oleic acid (84.36 %) and the lowest proportion of caprylic and behenic acid (0.52%). Analysis of bound fatty acids (Table-4) indicated that fruits contain the highest proportion of oleic acid (42.35%). In addition to this acid, arachidic (13.42%), stearic (12.83%), myristic (12.86%), behenic (7.99%) and lignoceric (4.56%) acids were found to be present in small proportion. Oleic acid is common in cottonseed oil, soybean, corn, linseed and coconut oil. The composition of free and bound fatty acids of *Solanum torvum* Sw. fruits indicates that fruits are rich in unsaturated fatty acid.

3.2 Characterization of compound A

The compound A was a white crystalline solid having R_f value 0.51 (over silica gel, Hexane: DCM =70:30 as the mobile phase). It was soluble in dichloromethane and its melting point was 37–39ºC. IR[21] (ν_{max}^{KBr}) cm^{-1}: 3429 (–OH stretching), 2918–2848 (aliphatic C–H stretching), 1735 (C=O stretching), 1465 and 1360 (–CH_2 and –CH_3 bending), 1175 and 1020 (C-O bending), 719 (–CH_2– bending). **^{1}H-NMR** (400 MHz, $CDCl_3$) δ ppm: 0.86 terminal methyl groups at C-18), 1.24 (broad peak, –C**H$_2$**– protons at C-4, C-5, C-6, C-7, C-8, C-9, C-10, C-11, C-12, C-13, C-14, C-15, C-16 and C-17), 1.59 (–C**H$_2$**–CH_2–CO–O– protons of C-3),2.28 (–C**H$_2$**–CO–O– protons of C-2), 3.65 (a band due to methyl proton of C**H$_3$**–O–CO–),ppm. **^{13}C-NMR** (150 MHz, $CDCl_3$) δ ppm: 174.0 (carboxylate carbon at C-1), 34.0 (at C-2), 24.0 (at C-3), 29.28 - 29.38 (C-4 to C-15 carbons of –CH_2– carbon), 32.2 (at C-16), 14.13 (terminal methyl carbon **C**H_3-CH_2- at C-18), 51.44 (methyl carbon **C**H_3–O–CO– at C-19) ppm. Finally ^{1}H and ^{13}C-NMR spectroscopic data of the compound A was compared with reported value[22] of ^{1}H and reported value[23] of ^{13}C-NMR data methyl stearate and hence the structure of the compound B was established as methyl stearate (methyl octadecanoate) (Fig 1).

3.3 Characterization of compound B

The compound B was a white crystalline solid having R_f value 0.75 (over silica gel, DCM: Methanol =98:02 as the mobile phase). It was soluble in dichloromethane and its melting point was 108–110ºC. IR[21] (ν_{max}^{KBr}) cm^{-1}: 3400 (–OH stretching), 3050 (sp^2 C–H stretching), 2900 (aliphatic C-H stretching), 1720 and 1610 (C=O and C=C stretching), 1470 and 1360 (–CH_2 and –CH_3 bending), 1240, 1120 and 1020 (C–O bending), 720 and 690 (–CH_2– bending and =CH– out of plane bending). ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 0.86 (m, –C**H$_3$**, C-29); 1.59 (br. s, –C**H$_3$**, C-27); ~1.00 (overlapped s and m, angular –C**H$_3$**, C-18 and C-19), 1.99 (s, –O–CO–C**H$_3$**, C-3), 4.51 (s, terminal –C**H$_3$**, C-26), 3.65 and 4.51 (m, C-2 and C-3), 5.32 (m, olefinic proton, C-6), 1.2-2.8 (m, d, t, s, different methylene and methene protons). Based on the analysis of IR and ^{1}H-NMR spectroscopic data of compound A and comparing to the published[24] data of ^{1}H-NMR spectrum of stigmasta-5,25-diene-3-ol (claresterol), the structure of the compound tentatively proposed as 3-*O*-acetyl-stigmasta-5,25-diene-2,3-diol (Fig 2).

3.4 Characterization of compound C

The compound C was a yellow crystalline solid having R_f value 0.35 (over silica gel, Hexane: DCM =60:40 as the mobile phase). It was soluble in dichloromethane. **IR[21]** (ν_{max}^{KBr}) cm^{-1}: 3429 (indicated the presence of –OH group), 2960 (for the presence of aliphatic C–H stretching of either –CH_3, –CH_2– or >CH– group), 1714 and 1641 (were suggestive the presence of stretching for C=O and C=C groups, respectively), 1463 and 1377 (due to the presence of bending of –CH_2 and –CH_3 groups), 1112 (was suggestive of bending of >C–O bond), **^{1}H-NMR** (400 MHz, $CDCl_3$) δ ppm: 5.19 (ascribable to olifenic proton at C-7), 0.87, 0.95, 0,98, 1.03, 1.08, 1.24 and 1.29 (for quaternary methyl groups at C-18, C-19, C-30, C-28, C-29, C-26 and C-27 respectively), 4.71 and 4.65 (for epoxy protons of C-21 and C-23), 3.40 and 3.50 (for OMe-25 and OMe-21). **^{13}C-NMR** (150 MHz, $CDCl_3$) δ ppm: 216.6, 145.80 and 117.0 (for C-3, C-8, and C-7) respectively. 49.2 and 54.8 (For two methoxyl group at C-25 and C-21), 22.9, 12.7, 28.0, 24.9, 21.5, 22.2 and 19.8 (were assigned to quaternary methyl groups of C-18, C-19, C-30, C-28, C-29, C-26 and C-27 respectively), 105.0 and 77.3 (for epoxy carbon of C-21 and C-23), 117.0 and 145.8 (for olifenic carbon at C-7 and C-8), 76.7 (for secondary alcoholic carbon >**C**H–OH at C-24).

By comparison of ^{1}H-NMR spectral data with the literature[25] value, Compound C was identified as 21,25-dimethylmelianodiol [(21,23-epoxy-24-hydroxy-21,25-methoxy) tirucalla-7-en-3-one] (Fig 3).

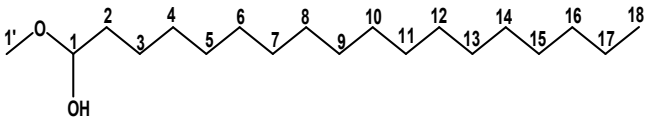

Fig 1: Structure of compound a (methyl stearate)

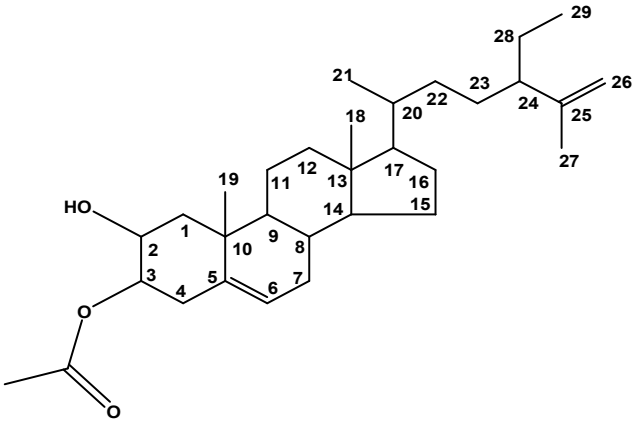

Fig 2: Structure of compound B (3-*O*-acetyl-stigmasta-5,25-diene-2,3-diol)

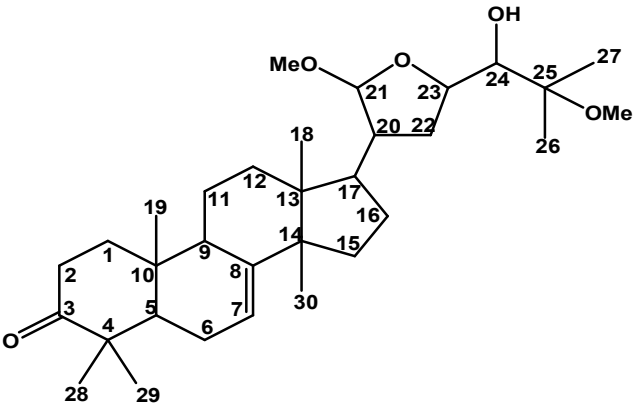

Fig 3: Structure of compound C (21,25-dimethylmelianodiol)

Although 21,25-dimethylmelianodiol is a known natural product, but from literature survey this is the first report of its occurrence from the species *Solanum torvum* Sw. This compound has weak antibacterial property[25].

4. Conclusion

From all these analytical data it can be concluded that as a vegetable the fruits of *Solanum torvum* Sw. may serve nutritional aspects in food in addition to its medicinal effect. From the results of preliminary phytochemical investigation, it appears that the fruit contains some biologically important compounds.

5. Acknowledgement

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