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ISOLATION AND CHARACTERIZATION OF SOME PHYTOCHEMICAL COMPOUNDS FROM THE METHANOLIC EXTRACT OF *SOLANUM TORVUM* SWARTZ FRUITS

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ABSTRACT: *Solanum torvum* Sw. belonging to the family Solanaceae is locally called as pea eggplant, sundai and cherry egg-plant in India. The fruits of *Solanum torvum* Sw. used to cure diseases of the diuretic, malaria, stomach aches, problems with the spleen, treat coughs, improve the eyesight, treats headaches which attribute many properties in Ayurveda, siddha, unani and local health traditions. The purpose of this study is to isolate and characterize the STL, STB and STD from the fruits of *Solanum torvum* Sw. The isolation was done using column chromatography using gradient elution with different mobile phase. Structural elucidation of the isolated compounds was established out on the basis of elemental analysis and spectroscopic evidence of IR, ^{1}H NMR, ^{13}C NMR spectra. The purification of the isolated compounds by HPLC and HPTLC was carried out. On the basis of the spectral data and chemical reactions, the compounds have been established as STL, STB, and STD are being reported from this plant for the first time.

INTRODUCTION: *Solanum torvum* Sw. belonging to the family *Solanaceae* and widely distributed in Pakistan, India, Malaysia, China and naturalized in South and Southeast Asia [1]. The fruits of *Solanum torvum* Sw. have antimicrobial [2], antiviral [3], cytotoxic [4], diuretic and digestive activity [5]. Fruits are often eaten as vegetables and useful in cases of liver and spleen enlargement and in the treatment of cough [6]. The major chemical constituents of *Solanum torvum* Sw. are steroids, steroidal saponins, terpenoids, steroid alkaloids, and phenols [7]. The previous study suggested that the steroidal glycoside, C-4 sulfated isoflavonoid, torvanol a, torvoside A and H have been isolated from the methanolic extract of fruits of *Solanum torvum* Sw [8].

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Two novel C-22 sterqoidal lactone saponins namely solanolactosides A, B and two new spirostanol glycosides namely torvosides M, N and torvonin a were isolated from the ethanol extract of aerial parts of *Solanum torvum* [9, 10]. Three novel 22-β-O-spirostanololigoglycosides, three unusual 22-β-O-23- hydroxy-(5α)-spirostanol glycosides, torvosides J, K and L have been reported [11]. Dry fruits contain minimum amount of chronogenic, neochronogenic, isochlorogenic, and caffeic acids.

After the fruit extraction of *Solanum xanthocarpum* two particular sterols one is carpesterol 12 (chemically the STD) and beta-sitosterol, sitosteryl glucoside, cycloartenol, cycloartanol, solamargine, β-solamargine, cam-pesterol, cholesterol, stigmasterol and stigmasteryl glucoside constituents were identified. However, researchers also found the occurrence of triterpenes [12]. It appears from the literature survey that further phytochemical investigations are necessary to explore more medicinally important compounds of this fruits of *Solanum torvum*, and with the hope of identifying

the medicinally important compounds and highlyghting their potential based on structure-activity relationship, this study has been undertaken. So, this paper deals with the isolation and characterrization of three important compounds STL, STB and STD from the methanol extract of fruits of *Solanum torvum* Sw.

MATERIALS AND METHOD:

Collection of Plant Material: The fruits of *Solanum torvum* Sw. were collected in the month of October from Trichy, Tamil Nadu, India. The plant was identified, and fruits of *Solanum torvum* Sw. were authenticated by Rev. Dr. S. John Britto SJ, Director, Rapinat herbarium, St. Joseph College, Tiruchirapalli, and Tamil Nadu for identifying the plants. The voucher specimen number PR001.

Chemicals and Reagents: All the chemicals, including solvents such as hexane, ethyl acetate, chloroform, methanol, anisaldehydesulphuric acid reagents (0.5 ml p-anisaldehyde in 50 ml glacial acetic acid and 1 ml conc. sulfuric acid. Heat to 105 °C until maximum visualization of spots) were of analytical grade and were procured from E. Merck, India. Libermann Burchard reagent (0.5 ml of sulphuric acid dissolved in 10 ml of acetic anhydride. Covered and kept in an ice bucket). All the chemicals used, including the solvents, were of analytical grade.

Preparation of Methanol Extracts: The fruits of *Solanum torvum* Sw. were washed in running water, cut into small pieces and then shade dried for a week at 35-40 ºC, after which it was grinded to a uniform powder of 40 mesh size. The methanol extracts were prepared by soaking 100 g each of the dried powder plant materials in 1 L of methanol using a Soxhlet extractor continuously for 10 h.

The extracts were filtered through Whatmann filter paper No. 42 (125 mm) to remove all impurities, including cellular materials and other constitutions that are insoluble in the extraction solvent. The entire extracts were concentrated to dryness using a rotary evaporator under reduced pressure.

The final dried samples were stored in labeled sterile bottles and kept at -20 ºC. The filtrate obtained was used as a sample solution for further isolation [13].

Isolation of STL, STB and STD by Column Chromatography Method: The condensed methanol extract of fruits (403 g) of the sample was subjected to column chromatography over TLC grade silica gel. Elution of the column first with n-hexane, an increasing amount of ethyl acetate in n-hexane and finally with methanol yielded a number of fractions. The preparation of solvent systems used to obtain STL (42 mg / 403 g) was n-hexane: Ethyl acetate (70:30) from fraction 8, STD (76 mg / 403 g) was Ethyl acetate: methanol (70:30 v\v) from fraction 13 and STB (58 mg / 403 g) Ethyl acetate: methanol (60:40 v\v) from fractions [13]. The compounds were detected on TLC plates by spraying with Libermann Burchard reagent and heated at 100 °C for 10 min [14-16].

Purification of Isolated Compounds by HPTLC and High-Performance Liquid Chromatography:

High-Performance Thin-layer Chromatography (HPTLC): The isolated pure compound was dissolved in appropriate solvents. 5 µl of isolated compounds (STL, STD, and STB) were applied to silica gel plates, Merck (Germany) 20 × 20 cm, 0.25 mm in thickness. Plates were developed using the solvent system n-Hexane: Ethyl acetate (7:3 v\v) for STL, Toluene: ethyl acetate: formic acid (7:3:1 v\v) for STD, and Toluene: ethyl acetate: glacial acetic acid (4:4:2) for STB. The separated zones were visualized with freshly prepared (anisaldehydesulphuric acid reagents) for STB and STD, (Libermann Burchard reagent) for STL and heated at 100 °C for 10 min. Chromatograms were then examined under daylight within 10 min [13, 17].

High-performance Liquid Chromatography (HPLC): The analytical HPLC system (Shimadzu) was equipped with a diode array detector, a 20 µl loop, 200 × 4.6 mm C18 column, methanol (HPLC grade, 0.2 mm filtered) used as a mobile phase. The isolated STL compound was separated using a mobile phase of methanol: water (70:30 v/v). The isolated STB compound was separated using a mobile phase of acetonitrile and water (95:5 v/v). The isolated STD compound was separated using a mobile phase of acetonitrile and water (80:20 v/v). The isolated compounds were separated at a flow rate of 1.0 ml/min, column temperature 30 °C. Injection volume was 40 µl, and detection carried out at 346 nm [14, 15, 18].

Structural Elucidation Study of Isolated Compound: Different spectroscopic methods including UV, FTIR, and ^{1H}NMR, ^{13}C NMR and GC-MS were used to elucidate the structure of isolated compounds.

The UV-visible spectrum of the isolated compounds in methanol was recorded using a Shimadzu 160 a UV-visible spectrophotometer. The Fourier Transform Infrared (FTIR) spectra were recorded with a nominal resolution of 4 cm^{-1} and a wavenumber range from 400 to 4000 cm^{-1} using the KBr pellet technique. ^{1}H and ^{13}C NMR spectra were acquired on Bruker WP 200 SY and AM 200 SY instruments (^{1}H, 200.13 MHz; ^{13}C, 50.32 MHz) using TMS as internal standard and $CDCL_3$ as solvent [14, 15, 19-21].

RESULTS AND DISCUSSION:

Structural Elucidation of Isolated Compounds: STL is an amorphous powder and was crystallized in a methanol-chloroform mixture. STL melting point 213 °C, which corresponds to the molecular formulae $C_{30}H_{50}O$. The **UV** λ_{max} value of compound STL was 355 nm **Fig. 1**.

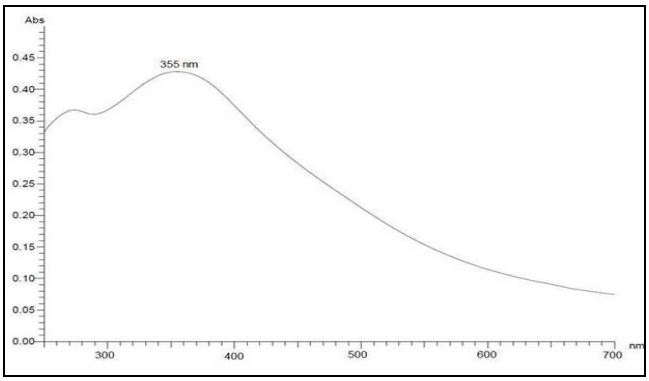

FIG. 1: UV SPECTRA OF THE ISOLATED COMPOUND STL

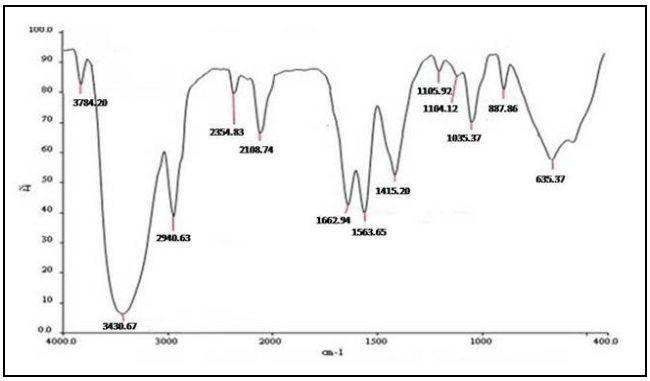

FIG. 2: FTIR SPECTRA OF THE ISOLATED COMPOUND STL

In the IR spectrum of isolated compounds **Fig. 2** a very broad peak at 3430.67 cm^{-1} (Hydrogen bonded OH Stretch), 2940.63 cm^{-1} and 2354.83 cm^{-1}(C-H Stretch in CH_2 and CH_3), 2108.74cm^{-1} (C≡C Stretch), 1662.94 cm^{-1} (C=C Symmetric stretch), 156.65 (C=C Asymmetric stretch), 1415.20 cm^{-1} (C-H deformation in CH_2 and CH_3), 1035.37 cm^{-1} (C-O Stretch of secondary alcohol), moderately intense band at 1105.92 cm^{-1} and 635.37 cm^{-1} were observed for the O-H bond vibration of hydroxyl group, 887.86 cm^{-1} (=C-H bending exocyclic CH_2). In the proton ^{1}H NMR spectra of STL **Fig. 3** showed δ7.21 and 7.19 (CDCL3 peak), δ 4.61 (1H, s, H29b), 4.50 (1H, s, H-29a), 3.15 (1H, dd, J = 3.4, 12.8 Hz, H-3), 2.10 (3H, s, H2/), 1.64 (3H, s, H-30), 1.09 (3H, s, H-25) 0.95 (3H, s, H-28), 0.89 (3H, s, H-23), 0.87 (3H, s, H-24), 0.83 (3H, s, H-26), 0.75 (3H, s, H-27).

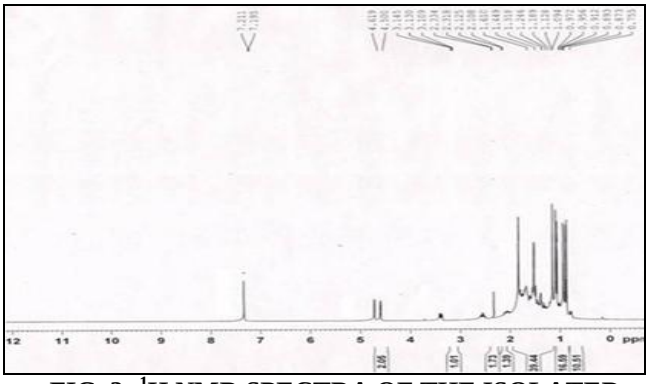

FIG. 3: ^{1}H NMR SPECTRA OF THE ISOLATED COMPOUND STL

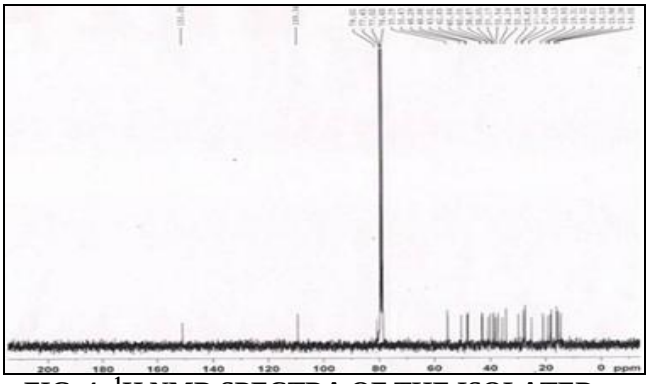

FIG. 4: ^{1}H NMR SPECTRA OF THE ISOLATED COMPOUND STL

In the carbon ^{13}C NMR spectra of STL **Fig. 4** showed δ_C: δ 37.17 (C-1), δ 20.93 (C-2), δ 79.02 (C-3), δ 38.05 (C-4), δ 55.29 (C-5), δ 18.32 (C-6), δ 27.73 (C-7), δ 38.87 (C-8), δ 50.43 (C-9), δ 34.29

(C-10), δ 19.31 (C-11), δ 20.93 (C-12), δ 35.56 (C-13), δ 40.01 (C-14), δ 25.13 (C-15), δ 29.83 (C-16), δ 40.86 (C-17), δ 48.29 (C-18), δ 48.02 (C-19), δ 151.01 (C-20), δ 27.73(C-21), δ 38.87 (C-22), δ 25.13 (C-23), δ 15.98 (C-24), δ 15.38 (C-25), δ 16.13 (C-26), δ 14.35 (C-27), δ 18.01 (C-28), δ 109.34 (C-29) and δ 18.32 (C-30). The previous studies suggested that the IR spectrum of isolated compounds showed characteristic absorption frequencies at 3784.20 and 1105.92 cm^{-1} typical of the O-H and C-O bond vibrations, respectively; the absorption observed at 887.86 cm^{-1} was due to an unsaturated out of plane C-H vibration; the C=C vibrations was shown around 1563.65 cm^{-1} as weakly intense band; stretching and bending vibrations due to methyl groups were represented by the bands at 2940.63 cm^{-1} and 1563.65 cm^{-1} and the signal at 1415.20 cm^{-1} was due to methylenic vibration. The ^{1}H NMR spectrum revealed the presence of seven tertiary methyl protons at δ 0.75, 0.76, 0.87, 0.95, 0.97, 1.09 and 1.61 (integrated for 3H-each). A sextet of one proton at δ 2.31 ascribable to 19β - H is characteristic of STL. The H-3 proton showed a multiplet at δ 3.14 while a pair of broad singlets at δ 4.50 and δ 4.62 (^{1}H, each) was indicative of olefinic protons at (H-29 a & b). These assignments are in good agreement for the structure of STL [22]. The structural assignment of R1 was further substantiated by the ^{13}C NMR experiments which showed seven methyl groups at [δc: 28.0 (C-23), 18.0 (C-28), 16.1 (C-25), 16.0 (C-26), 15.5 (C24), 14.8 (C-27) and 19.5 (C-30)]; the signals due to an exomethylene group at [δc: 109.3 (C-29) and 151.0 (C-20)]; ten methylene, five methine and five quaternary carbons are in good agreement for the structure of STL[23]. The deshielded singnal at δc 79.0 was due to C-3 with a hydroxyl group attached to it [24]. STB is white crystalline needles like substance with and melting point 136 °C which corresponds to the molecular formulae $C_{29}H_{50}O$. The UV λ_{max} value of compound STBwas 255 nm **Fig. 5**.

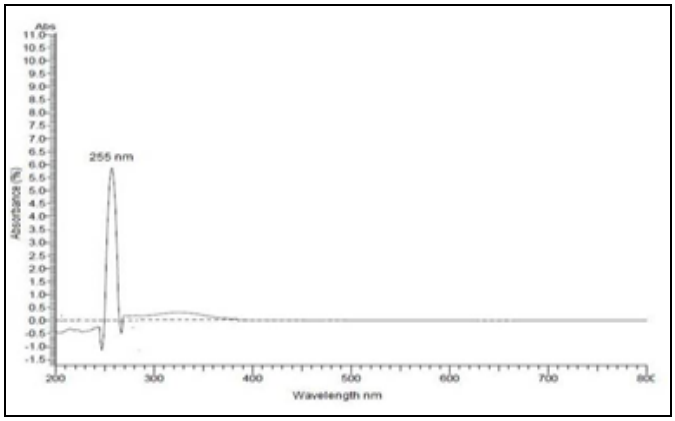

FIG. 5: UV SPECTRA OF THE ISOLATED COMPOUND STB

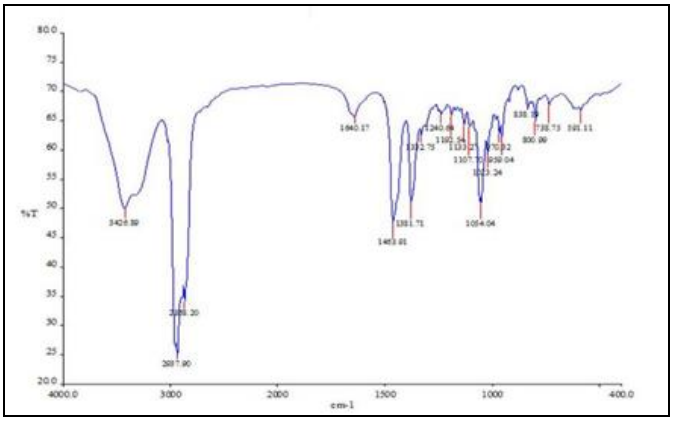

FIG. 6: FTIR SPECTRA OF THE ISOLATED COMPOUND ST

The IR absorption spectrum of isolated compounds showed absorption peaks at 3451.40 cm^{-1} (O-H stretching.); 2950.82 cm^{-1} (aliphatic C-H stretching); 1637.65 cm^{-1} (C=C absorption peak); other absorption peaks includes 1456.27cm^{-1} (CH2); 1376.21cm^{-1} (OH def), 1057.33 cm^{-1} (cycloalkane) and 809.13 cm^{-1} **Fig. 6**. In the ^{1}H NMR spectra of isolated compounds **Fig. 7** has given signals at δ 7.26 (s, O-H), 5.36 (m, 1H, H-6), 3.53 (tdd, 1H, H-3), 1.23 (s, 3H, H-19), 1.17 (s, 3H, H-18), 2.27 (1H, m, H-20), 1.03 (s, 3H, H-26), 1.00 (s, 3H, H-27), 0.91 (s, 3H, H-21), 0.89 (s, 3H, H-29) In the ^{13}C NMR spectrum of isolated compounds **Fig. 8** signal at 37.26(C-1), 31.90(C-2), 77.76 (C-3), 42.21 (C-4), 140.76 (C-5), 121.70 (C-6), 32.62 (C-7), 32.62 (C-8), 50.13 (C-9), 36.51 (C-10), 21.24 (C-11), 39.78 (C-12), 42.27(C-13), 56.87 (C-14), 26.07 (C-15), 28.26 (C-16), 56.06 (C-17), 36.16 (C-18), 19.41 (C-19), 33.94 (C-20), 26.07 (C-21), 45.82 (C-22), 23.07 (C-23), 12.27 (C-24), 29.15 (C-25), 19.84 (C-26), 19.41 (C-27), 19.00 (C-28), 11.99 (C-29). The previous study suggested that the STB is difficult to be obtained in pure state [25, 26]. This is the first time reported that STB isolated is 100% pure from the *Solanum torvum* Sw fruits. The physical and spectral analysis in the present study (FT-IR, ^{1}H-NMR, ^{13}C-NMR) shows a strong relevance to experimental data available in literature [27]. On subjection to IR spectroscopic analysis, absorptions bands appeared

at 3426 cm^{-1} that is characteristic of O-H stretching , 2868 cm^{-1} is due aliphatic or C-H stretching or (CH_3), 1540 cm^{-1} due to double (C=C) stretching, 1054 cm^{-1} due to (C-O). The absorption frequency at 738 cm^{-1} signifies cycloalkane. The out of plane C-H vibration of unsaturated part was observed at 591 cm^{-1}. These absorption frequencies resemble the absorption frequencies observed for STB as resembled data was already published [28]. The ^{1}H NMR spectrum (300MHz, CDCl3) of compound **Fig. 7** has revealed a one proton multiplet at δ 2.26, the position and multiplicity of which was indicative of ^{3}H of the steroid nucleus. The typical 6H of the steroidal skeleton was evident as a multiplet at δ 5.36 that integrated for one proton. The spectrum further revealed signals at δ 1.49 and δ 1.19 (3H each) assignable to two tertiary methyl group at C- 18 and C-19 respectively. The ^{1}HNMR spectrum showed two doublets centered at δ 0.90 (J = 6.7 Hz) and δ 0.91 (J = 6.7 Hz) which could be attributed to two methyl groups at C-26 and C -27 respectively. The doublet at δ 1.65 (J = 6.5Hz) was demonstrative of a methyl group at C-21. On the other hand, the triplet of three proton intensities at δ 0.86 could be assigned to the primary methyl group at C- 29. This compound is having six methyl, eleven methylene, and three quaternary carbons with a hydroxyl group. The above spectral features are in close agreement to those observed for STB according to (Manoharan *et al.,* 2005 and Escudero *et al.,* 1985) [29, 30]. The ^{13}C-NMR has shown recognizable signals 140.76 and 129.27 ppm, which are assigned C5 and C6 double bonds respectively. The value at 24.31 ppm corresponds to angular carbon atom (C19). Spectra show twenty-nine carbon signal, including six methyls, nine methylenes, eleven methane, and three quaternary carbons. The alkene carbons appeared at 140.76 and 129.27 ppm. In comparison, the standard data matched with the simulated data which supports the proposed structure of this compound as STB.STD is a white needle-like crystals and melting point 201-203 °C, which corresponds to the molecular formulae $C_{27}H_{42}O_3$. The UV λ_{max} value of compound STD was 212 nm **Fig. 9**.

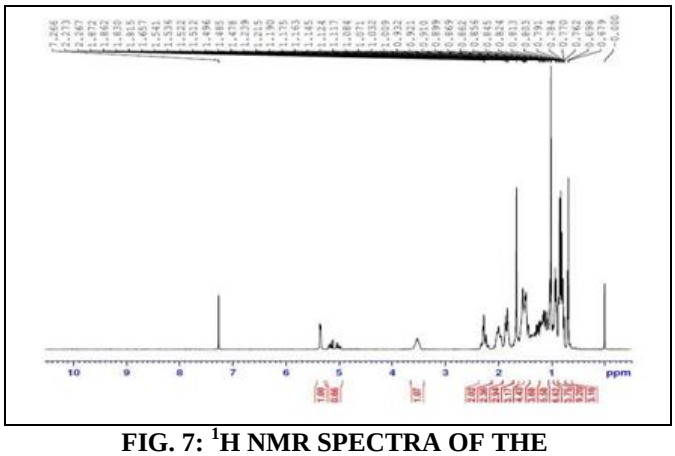

FIG. 7: ^{1}H NMR SPECTRA OF THE ISOLATED COMPOUND STB

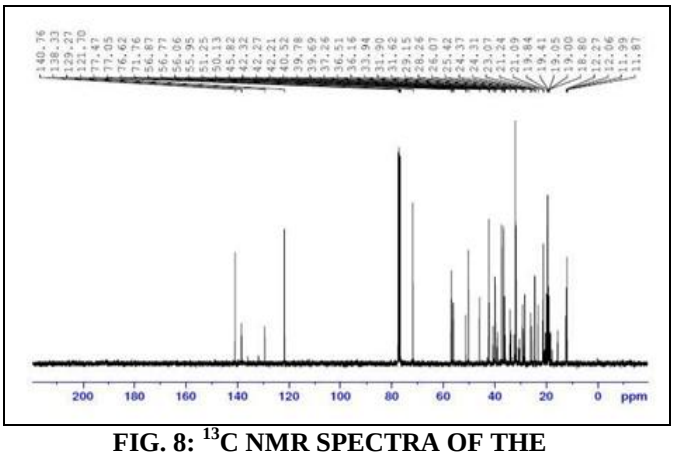

FIG. 8: ^{13}C NMR SPECTRA OF THE ISOLATED COMPOUND STB

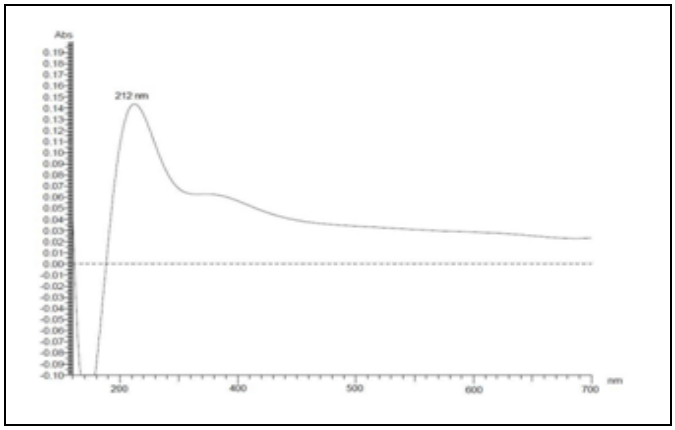

FIG. 9: UV SPECTRA OF THE ISOLATED COMPOUND STD

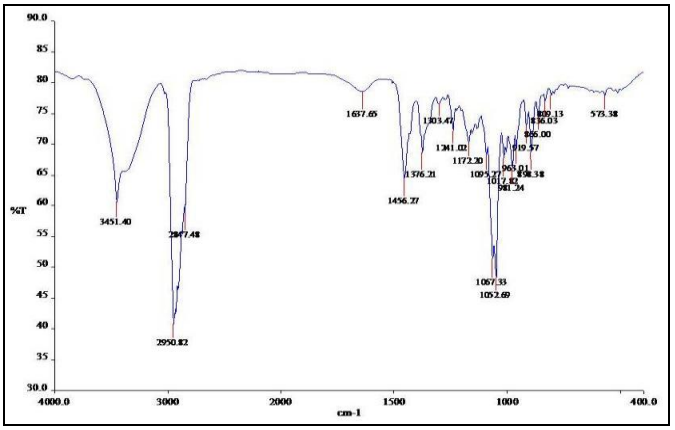

FIG. 10: FTIR SPECTRA OF THE ISOLATED COMPOUND STD

In the IR spectrum of isolated compounds **Fig. 10** a very broad peak at 3451.40 cm^{-1} (OH), 2950.52 cm^{-1} (sp3-H), 1637.65 cm^{-1} (C=C), 1456 cm^{-1} (methylene vibration), 1376.21 cm^{-1} (sp10 H), 1057.33 cm^{-1} (C-O), 919 cm^{-1}(spiroketal ring and 25 R configuration). In the ^{1}H NMR spectra of isolated compounds **Fig. 11** has given signals at 0.76 (s, C-18 methyl), 0.78 (d, J = 6.2 Hz; C-27 methyl), 0.82 (J = 7.1 Hz;C-21 methyl), 1.03 (s, C-19 methyl), 3.30 (t,J = 10.6 Hz; C-26a-H), 3.40 (d, dd, J = 10.5 Hz and J approx. 4Hz; C-26,B-H), 3.41 (broad, C-3a-H), 3.40 (q, J=7.lHz; C-16H), 5.31 (broad d,J = 5.3Hz; C-6H). In the ^{13}C NMR spectrum of isolated compounds **Fig. 12** signal at 37.31 (C1), 31.88 (C2), 70.79 (C3), 42.39 (C4), 141.51 (C5), 120.50 (C6), 31.31 (C7), 31.47 (C8), 50.28(C9), 36.56 (C10), 20.71(C11), 39.61(C12), 41.48 (C13), 56.42 (C14), 31.64 (C15), 80.57 (C16), 62.58 (C17), 16.56(C18), 18.91 (C19), 40.10 (C20), 14.13 (C21), 108.68 (C22), 31.57 (C23), 28.71 (C24), 30.20 (C25), 66.32 (C26), 16.56 (C27). The previous study suggested that the ^{1}HNMR (300 MHz, CDCl,) spectrum suggested the presence of two secondary methyl groups at 0.78 (d, J=6.2Hz) and 1.0 1 (d, J=6.1 Hz), two tertiary methyl groups as singlet at 1.03 and an olefinic proton appearing at 5.31 as doublet (J=5.1 *Hz).* A comparison of the carbon shifts for 1 with those of STD [31, 32] the assignment of all the carbon shifts and elucidation of the structure and stereochemistry of isolated compound as STD. The spectrum (75 MHz, CDCl3) of isolated compound showed the presence of signals appearing at 141.5, 120.5 are attributed to C-5 and C-6 respectively, the observed close similarity of the shifts for C-23, C-24, C-25, C-26 and C-27 in compound with those in STD are diagnostic of the equatorial orientation of the C-25 methyl in the 22 a-0-spirostane skeleton and thus settled the 22R and 25R configuration of compound.

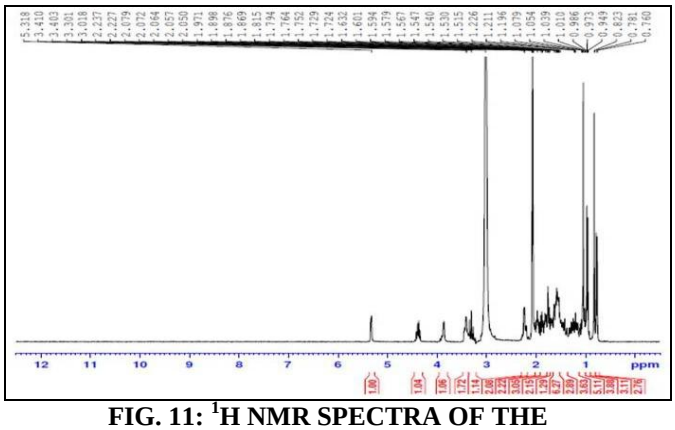

FIG. 11: ^{1}H NMR SPECTRA OF THE ISOLATED COMPOUND STD

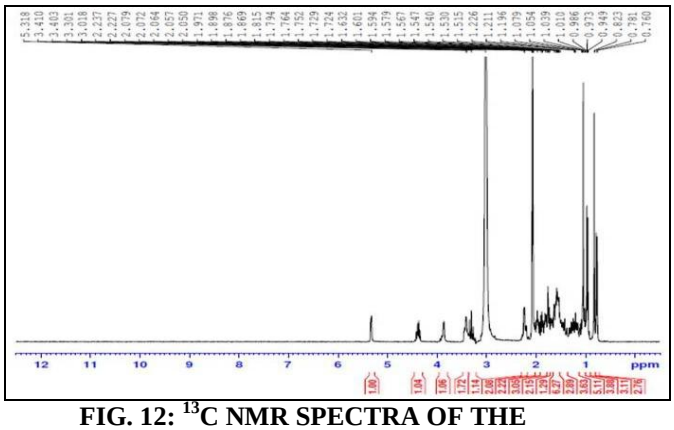

FIG. 12: ^{13}C NMR SPECTRA OF THE ISOLATED COMPOUND STD

Purification of Isolated Compounds by HPTLC and HPLC: HPTLC fingerprint patterns have been therefore evolved to check the purity of isolated compound STL, STB, and STD from methanolic extract of fruits of *Solanum torvum*. The R_f value of standard STL 0.54 was matched with the R_f value of isolated compound was about 0.54 was shown in peak **Fig. 13**.

The Retention time of STL isolated from the methanolic extract of sample was about 3.743 was shown by HPLC peak **Fig. 14**. The R_f value of standard STB 0.54 was matched with the R_f value of isolated compound STB was about 0.54 was shown in peak **Fig. 15**. The Retention time of STB isolated from the methanolic extract of the sample was about 8.230 was shown by HPLC peak **Fig. 16**.

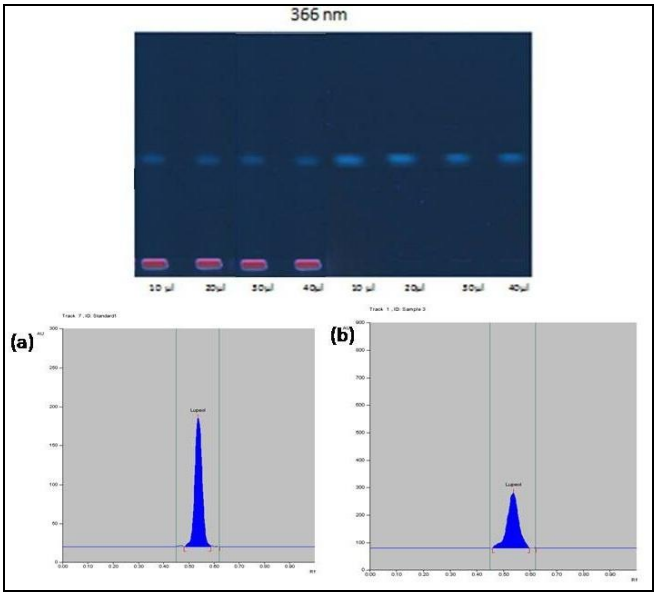

FIG. 13: HPTLC SPECTRA FOR ISOLATED STL

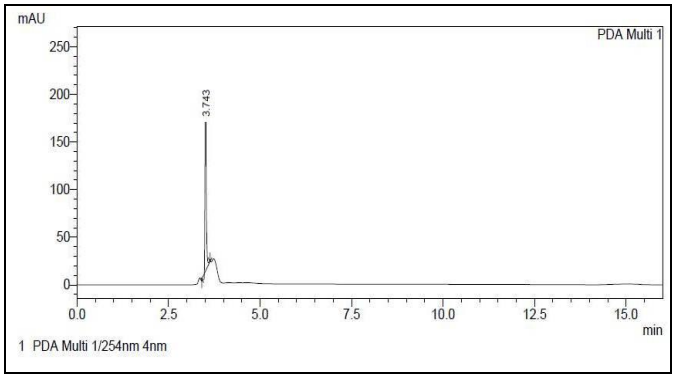

FIG. 14: HPLC SPECTRA FOR ISOLATED STL

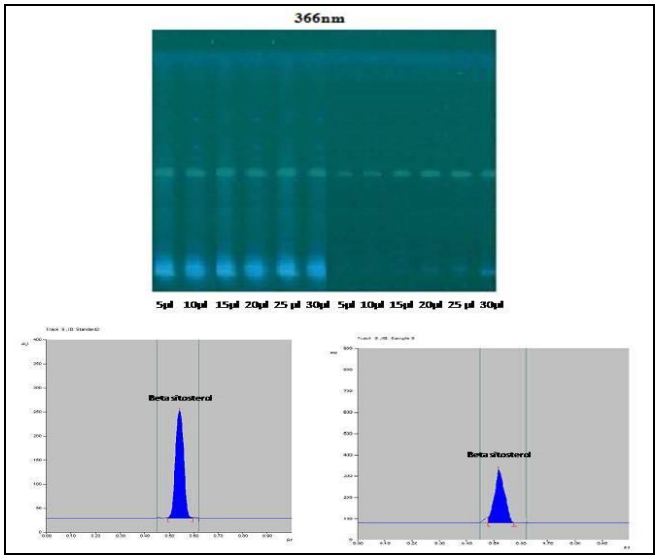

FIG. 15: HPTLC SPECTRA FOR ISOLATED STB

The R_f value of standard STD 0.77 was matched with the R_f value of isolated compound STD was about 0.77 was shown in peak **Fig. 17**. The retention time of STD isolated from the methanolic extract of the sample was about 2.295 was shown by HPLC peak **Fig. 18**. A previous study suggested that the STL, oleanolic acid, urolic acid, beta sitoterol, campesterol, ergosterol active compounds were detected in the fruit, stem, leaf and root of the *Solanum xanthocarpum* by using HPTLC [33].

β- sitosterol and Stigmasterol isolated from the aqueous extract of fruits of *Solanum xanthocarpum* these compounds may be responsible for immunomodulatory activity [34]. The fruits of *Solanum xanthocarpum* are reported to contain several steroidal alkaloids like Solana-carpine, solanacarpidine, solancarpine, solasodine, sola-sonine and solamargine. Other constituents like caffeic acid, coumarins like aesculetin and aesculin, steroids carpesterol, STD, campesterol, daucosterol and triterpenes like cycloartanol and cycloartenol are also reported from the fruits. So, in this paper, we report the isolation and characterization of bioactive principles, namely STL, STB and STD, from methanol extracts of fruits of *Solanum torvum*.

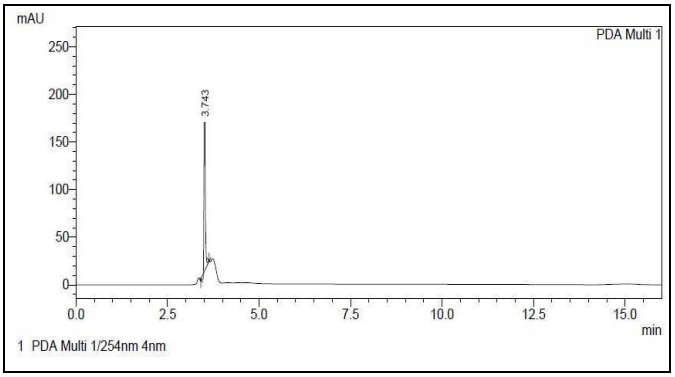

FIG. 16: HPLC SPECTRA FOR ISOLATED STB

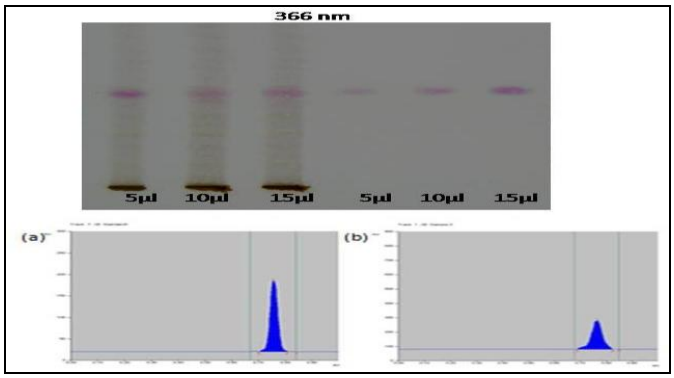

FIG. 17: HPTLC SPECTRA FOR ISOLATED STD

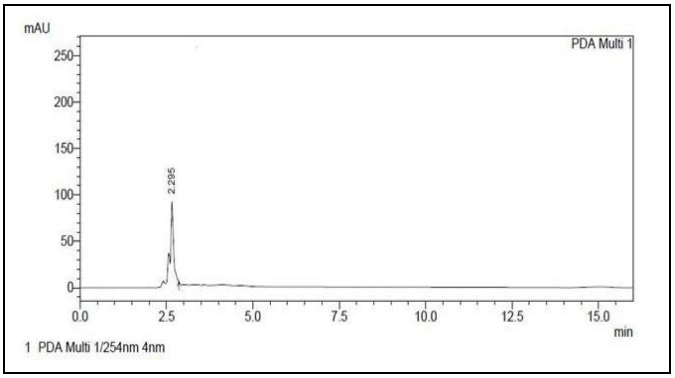

FIG. 18: HPLC SPECTRA FOR ISOLATED STD

CONCLUSION: From all these analytical data, it can reveal that the isolation and identification of compound STL, STB and STD from fruits of *Solanum torvum* Sw. was the first ever to be reported. So we can conclude that as a vegetable of the fruits of *Solanum torvum* Sw. may serve good nutritional aspects in food and in-addition it provides medicinal values because, from the results of the phytochemical investigation, it appears that the fruit contains many biologically important phytochemical compounds. However, further pharmacological studies would be followed for the efficacy and individual activity of isolated phytochemical compounds.

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