Chemical fingerprinting and cytotoxicity correlation of *Solanum americanum* leaves extracts via HPLC-DAD and LC-HRMS-based metabolomics

Pawan Kumar Goswami[1], Sonakshi Antal[1]*, Sandip Chatterjee[2] and Arvind Kumar Patel[2]

[1]SRM Modinagar College of Pharmacy, Faculty of Medicine and Health Sciences, SRM Institute of Science and Technology, NCR Campus, Delhi–Meerut Road, Modinagar, Ghaziabad 201204, Uttar Pradesh, India

[2]Narayan Institute of Pharmacy, Gopal Narayan Singh University, Jamuhar, Sasaram, Rohtas- 821305, Bihar, India

Email: sonakshiantal@gmail.com Orchid ID: 0009-0001-8563-2327

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ABSTRACT

A study was made to extract and characterize bioactive compounds from Solanum americanum aerial part (leaves) using a successive solvent extraction method, enriching polar compounds in the methanolic extract using TLC, UV, RP-HPLC, and LC-HRMS. Cytotoxicity (MTT, scratch assay) and hemolytic activity were evaluated to assess their anti-proliferative and wound-healing potential. HPLC-DAD analysis of S. americanum methanolic extract revealed two major polar compounds (65% and 14%) along with minor steroidal alkaloids and flavonoids. The extract exhibited saponin presence through persistent froth (2 cm, 15 min) and hemolysis at 100 µg/mL. LC-HRMS identified Tigogenin, Rhamnetin 3-O-glucoside, β2-Solamargine, and flavonoids. Cytotoxicity assays showed IC50 values of 97.6, 717.36, and 1075.1 µg/mL for the hexane extract, ACN fraction, and hexane residue on Caco-2 cells, while the methanolic extract showed minimal cytotoxicity. On B16F10 cells, IC50 values were 2841, 1051, and 544.2 µg/mL for the hexane extract, hexane residue, and EtOAc extract. In the scratch assay, the ACN fraction achieved full gap closure within 12 hours, while other extracts closed the gap in 24 hours, except for the methanolic extract, which inhibited cell migration.

Keywords: Cytotoxicity assay, HPLC-DAD, LC-HRMS**,** *Solanum americanum,* TLC, traditional medicine, wound healing

INTRODUCTION

Solanum americanum Mill. (family Solanaceae) is a therapeutically valuable plant extensively utilized in traditional medicine for the management of inflammation, infectious diseases, wound healing, and cancer-related ailments (Ukwubile, 2024**).** Despite its well-documented ethnomedicinal applications, the comprehensive understanding of its phytochemical diversity and the molecular mechanisms underpinning its pharmacological potential remains inadequate and poorly standardized (Thakur *et al.,* 2024; Sindhu *et al*., 2021). Previous ethnopharmacological studies suggest that related species of *S. americanum* is a rich source of structurally diverse bioactive metabolites such as alkaloids, glycosides, phenolics, and steroidal saponins, which are believed to contribute significantly to its cytotoxic, antimicrobial, and therapeutic effects (Paraschiv *et al.,* 2022; Imran *et al*., 2022). However, inconsistencies in harvesting conditions, extraction techniques, and the

absence of a validated chemical fingerprint have led to irreproducible outcomes and hindered systematic pharmacological evaluation (Sindhu *et al.,* 2021; Gaudêncio *et al.,* 2023). To address these challenges, the integration of high-performance liquid chromatography with diode-array detection (HPLC-DAD) and liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS) provides a comprehensive analytical strategy for detailed metabolite profiling, dereplication, and identification of bioactive compounds (Lima *et al.,* 2022; Pantharos *et al.,* 2022).

This aim of study uses HPLC-DAD and LC-HRMS-based metabolomics to metabolites profile aerial extracts of *Solanum americanum* and correlate their chemical composition with in vitro cytotoxicity. Reproducibility and phytochemical integrity will be optimized extraction and sample preparation. Accurate metabolite identification will be made possible by high-resolution chromatographic and mass spectral data, assisted by spectral libraries and in-silico fragmentation. Potency and selectivity will be evaluated by cytotoxicity against specific cancer and normal cell lines, and key bioactive metabolites will be identified by integrating chemical and biological data using multivariate tools (PCA, OPLS-DA). The creation of a standardized metabolomic fingerprint and chemical markers for quality assurance and additional pharmacological testing will be aided by these correlations.

MATERIALS AND METHODS

The plant sample was collected from the campus of NIPER-Hajipur, Export Promotion Industrial Park, Hajipur, Bihar, India. The plant voucher specimen was deposited in the herbarium of the Botanical Survey of India, Shillong-793003. The plant specimen was identified and assigned the accession number 99378. Aerial Part (Leaves) of *S. americanum* were collected and thoroughly cleaned to remove debris and impurities. The aerial part (leaves) of the plant were shade-dried for two weeks and subsequently pulverized into a fine powder. The powdered material was extracted successively with 100% hexane, followed by partitioning of the hexane extract with acetonitrile:hexane. Further extraction was carried out with 100% ethyl acetate, and finally with methanol:water (8:2). The methanolic extract was further fractionated into methanol and hexane fractions using a partitioning method. All extractions were performed using the cold maceration technique (Palermo, 2023). The obtained extracts were concentrated under reduced pressure using a rotary evaporator and stored at 4 °C until further use (Vakte and Nehete, 2025).

Thin Layer Chromatography (TLC) and UV Spectroscopic for the MeOH extract

TLC profiling of the methanolic extract of *S. americanum* was performed on silica gel coated aluminum plates. The extract was dissolved in methanol and developed using ethyl acetate:hexane (50:50). Compounds were visualized under UV light (254 nm), in a fluorescence chamber (365 nm), and under visible light. The extract was also analyzed using n-butanol:acetic acid: water (4:1:1) and visualized with 50% H□SO□ in ethanol; charring produced a black band indicating a steroidal nucleus (Borisov *et al*., 2021).

High-Performance Liquid Chromatography (HPLC)

10 mg/ml concentration of each of the extracts and fractions were prepared in methanol and vortex for 30 minutes then filtered through syringe filters (Nylon 0.45µm). The filtered solutions were used for the analysis**.**

RP-HPLC profiling of the extracts

RP-HPLC profiling of *S. americanum* leaf extracts and fractions was performed using an Agilent 1260 Infinity II system equipped with a C18 column (5 µm, 250 × 4.6 mm), autosampler, quaternary pump, and FLD, DAD, and RID detectors. The mobile phase consisted of HPLC-grade water (solvent A) and acetonitrile (solvent B) in gradient mode, with a flow rate of 0.1 mL/min and an

injection volume of 10 µL of 10 mg/mL sample solution. Detection was carried out at 210, 254, 320, and 360 nm (DAD), with the column maintained at 25 °C and a total run time of 60 minutes (Herqash *et al.,* 2024; Moustafa *et al.,* 2025).

Liquid chromatography-high-resolution mass spectrometry (LC-HRMS)

1 mg/ml concentration of each of the extracts and fractions was prepared in methanol and vortex for 30 minutes then filtered through syringe filters (Nylon 0.45 µm). The filtered solutions were used for the analysis. UHPLC–MS analysis was conducted using a Thermo Scientific Ultimate Dionex 3000 coupled with an Orbitrap Exploris 240 system. Data processing utilized Xcalibur, Chromeleon, Orbitrap Exploris Tune, and Compound Discoverer software. Separation was achieved on a Hypersil GOLD C18 column (100 × 2.1 mm, 1.9 µm) using a water–acetonitrile gradient containing 0.1% formic acid over 40 minutes, with detection at 210–320 nm. The system operated at 4 °C (autosampler) and 40 °C (column oven). The mass spectrometer used heated ESI in both positive and negative modes, with optimized gas flows and temperatures, acquiring data in full scan and ddMS² modes at 120,000 resolution across 100–2000 m/z (Wankhade *et al.*, 2024). The Orbitrap Exploris 240 operated with a HESI source using: spray voltage +3.5 kV / –2.5 kV, ion transfer tube 320 °C, heater 350 °C, sheath gas 35, auxiliary gas 10, sweep gas 1, and S-lens RF 70%. Instrument tuning was performed automatically before analysis. Compounds were qualitatively identified from accurate mass (<5 ppm), isotopic pattern, and MS/MS fragmentation in Xcalibur and Compound Discoverer. No external standards or quantification were used; therefore, all identifications are putative (Anzano *et al.*, 2025).

Anti-proliferative property

The anti-proliferative activity of the extracts was assessed on Caco-2 and B16F10 cell lines using the MTT assay (Nozhat *et al.*, 2022). Cells (10,000/well) were seeded in 96-well plates, incubated for 24 h, and treated with varying concentrations of *S. americanum* extracts for another 24 h. After exposure to 0.5 mg/mL MTT for 4 h, DMSO was added to dissolve formazan crystals, and absorbance was measured at 590 nm using a Synergy Biotek reader. Medium alone served as control (Chaudhry *et al.*, 2024).

In vitro scratch assay

B16F10 cells were seeded at 3 × 10□ cells/well in 24-well plates and grown to confluence. A scratch was made using a 200 µL pipette tip along a reference line, and wells were washed with PBS to remove detached cells. Cells were then incubated in FBS-free medium containing 50 µg of the MeOH extract at 37 °C. Scratch images were captured at 0, 6, 12, and 24 h using an inverted microscope (Zeiss), and gap closure due to cell migration was measured using Zen software. Wound closure at 0 h was set as 100%, and healing at 24 h was expressed relative to the initial wound (Roy *et al.*, 2023).

Hemolytic activity of *S. americanum* MEOH extract

Blood from a healthy O_2 male volunteer was collected in EDTA tubes, centrifuged at 800 × g for 15 min, and washed 3–4 times with PBS (pH 7.4). The packed RBCs were diluted with PBS and mixed with varying concentrations (25–1000 µg/mL) of *S. americanum* MeOH extract. After incubation at 37 °C for 3–4 h, samples were centrifuged at 8000 rpm for 10 min, and the supernatants were transferred to 96-well plates to measure absorbance at 540 nm (Soltani *et al.,* 2022).

RESULTS AND DISCUSSION

RP-HPLC profiling of fractions

HPLC profiling of Hexane, EtOAc, and Methanol extracts from the aerial parts (leaves) of *S. americanum* was performed

using an Agilent 1260 Infinity II system with Open Lab Chem Station software. Separation was carried out on a C18 column (250 × 4.6 mm, 5 µm) using a water–acetonitrile gradient: 60% B (0–40 min), 80% B (41–45 min), returning to 60% B (46–60 min). Detection was at 210, 254, 320, and 360 nm using a diode-array detector (DAD), with a 10 µl injection volume, 22 °C column temperature, 1 mL/min flow rate, and samples at 10 mg/ml. HPLC analysis revealed enrichment of certain phytocomponents during successive extraction and fractionation. The chromatograms compare Hexane, EtOAc, and MeOH extracts at 210 nm by RP-HPLC–DAD.

The chromatogram (Figure 1) shows enrichment of polar compounds in the EtOAc and MeOH extracts of *S. americanum*, with two major peaks at 2.471 and 2.700 min (65% and 14%). The methanolic extract tested positive in foam and hemolytic assays, and UV spectroscopy and LC-HRMS identified the major compound as Tigogenin, a steroidal saponin as depicted in Figure 13.

TLC profiling for MeOH extract

LC profiling of the MeOH extract of *S. americanum* using EtOAc:Hex (50:50) revealed five UV-active compounds (Rf 0–0.7), with the most polar retained in the methanol fraction, while non-polar compounds largely partitioned into the hexane fraction. Visualization was performed under UV (254 and 365 nm) and visible light. TLC Profiling of the methanolic extract of the areal part of *S. americanum* reviled the presence of 5 (C-1 to C-5) uv active and colored compounds in the methanolic crude extract with Rf values of 0.7, 0.6, 0.5, 0.23, and the last one Rf value of 0 which seems to be a very polar compound and a total of 5 uv active compound where observed in the hexane fraction with Rf values of 0.92, 0.7, 0.6, 0.5 and 0.23. Whereas in the methanol fraction, only one polar compound with Rf value 0 remained whereas other non-polar compounds were almost get fractionated into the hexane fraction shown in Figure 2.

HPLC-DAD profiling of enriched MeOH extract

HPLC-DAD fingerprinting of the MeOH crude and hexane fraction of *S. americanum* was performed using an Agilent 1260 Infinity II with Open Lab Chem Station, a C18 column (250 × 4.6 mm, 5 µm), and a H_2O–ACN gradient (10–85% B, 0–45 min, returning to 10% B by 60 min). Detection at 210, 254, 320, and 360 nm using a PDA detector, with 10 µL injection, 22 °C column temperature, 1 mL/min flow, and 1 mg/mL sample, revealed the major compounds in successive extracts. On comparisons of UV spectra which were recorded online during HPLC analysis of an enriched methanolic extract of aerial part of *S. americanum* at 210, 254, 330, 360 nm shown in Figure 3-6. At a detection wavelength of 210 nm, it was observed that peak 2 at 2.995 min is present in the highest amount with a % Area of 79.5625 and this compound was suspected to be some kind of saponin because of its uv spectrum which is similar to that of a standard saponin (Terki *et al.*, 2023). At a detection wavelength of 254 nm, less intensity of compounds were detected, upon comparing with the reported spectra of plant secondary metabolites peak no 7 and 8 where identify as flavonoids. Similarly, peak no Peak 5 at 18.062 min of 330 nm peak 1 at 18.063 min, and peak 2 at 21.088 min of 360 nm were also identified as flavonoids in comparison with the reported flavonoids spectra shown in figure 6.

HPLC- FLD profiling of enriched MeOH extract

HPLC-FLD profiling of the MeOH crude and hexane fraction of *S. americanum* was performed using an Agilent 1260 Infinity II with Open Lab Chem Station on a C18 column (250 × 4.6 mm, 5 µm) and a H_2O–ACN gradient (10–85% B, 0–45 min, returning to 10% B by 60 min). A fluorescence detector was used (Ex 280 nm,

Em 340 nm), with 7 µL injection, 22 °C column temperature, 1 ml/min flow rate, and 1 mg/ml sample.

HPLC–FLD analysis was performed using the methanolic extract and its fractions of the aerial part of *S.americanum* reviled the presence of the colored compound which further confirm the presence of flavonoid and other polyphenol compounds illustrated in Figure 7.

TLC of MeOH extract for identification of steroid nucleus

On TLC analysis of the methanolic extract and its fractions of the areal part of *S.americanum* using n-butanol: acetic acid**:** water (4:1:1) and 50% H_2SO_4 in ethanol was used as a visualizing agent a black colored band was observed which indicate the presence of steroid nucleus shown in Figure 8.

UV-spectroscopy of the MeOH extract

The UV–Vis spectra of *S. americanum* extracts showed strong absorption at 200–250 nm due to π–π* and n–π* transitions of phenolics and flavonoids, with methanolic extract and fraction showing higher intensity than the MeOH–Hex fraction, while diosgenin had a sharp band at 210–220 nm. Absorbance decreased toward 400 nm, indicating few visible-region chromophores Figure 10.

Test for saponin

Foam test: 0.5 g of the methanolic extract of the areal part of *S.americanum* was added to 10 ml of water and was shaken for mins till the formation of froth for about 2 cm and was allowed to stand and it was observed that the froth persists for more than 15 min which indicates the presence of a saponin compound in the extract illustrated in Figure 11.

Hemolytic test: The hemolytic test was performed by using PBS buffer as a negative control and 1 % Sodium dodecyl sulfate (1% SDS) as a positive control. And different concentrations of the methanolic extract of the areal part (leaves) of *S.americanum* were used ranging from 0-1000 µg/ ml and the degree of hemolysis was determined by measuring the optical densities (OD) at 540 nm (Figure 9). A hemolytic test of the enriched methanolic extract of the aerial part (leaves) of *S.americanum* indicates the present Saponin compound(s) as the principal phytoconstituent of the MeOH extract. It was observed that the extract showed hemolytic activity in a dose-dependent manner. At a concentration of 100 mg/ml, the tested extract started to show hemolytic activity but it cause a lesser extent of hemolysis of RBCs as compared to that of a standard (1% SDS).

LC-HRMS Profiling

LC-HRMS profiling methanolic extract of the aerial part (leaves) of *S.americanum* has led to the identification of a total of 30 compounds and reviled the presence of high concentrations of Steroidal Sapogenins, glycoalkaloids and a small amount of flavonoid and other polyphenolic compounds. LC-HRMS technique with ESI was employed to identify the principle wound healing components of the complex matrix of the tested plant extracts and fractions of *S.americanum.* These have led to the identification of a high abundance of Tigogenin a well-known anti-inflammatory and a very weak anti–proliferative activity compound. Hence, it is beneficial for wound healing activity depict in Table 1.

Cytotoxic activity

Cytotoxic activity of the extracts and fractions of the aerial part (leaves) of *S.americanum* were tested against Caco2 cell lines and B16F10 cell lines (colon cancer cell lines and Human skin cancer cell lines) were evaluated by MTT assay. Cytotoxic activity tested extracts were represented by IC_{50} values. All screened extracts and fractions show very weak cytotoxic activity against both cell lines. The MTT assay performed on the Caco2 Cell lines showed that there is no significant decrease in the viable cell number after being cultured with 0-200 µg of methanolic and hexane extract of S.

americanum for 48 h. whereas a slight decrease in the number of viable cells was observed in the hexane residue and ACN fraction with an increase in concentrations illustrated in Table 2 and Figure 12.

***In vitro* scratch assay**

B16F10 cells were seeded at a density of 30000 cells/well and grown for 24. A linear scratch was made in each well and incubated in a 3 mL FBS-free culture medium containing different ratios of the tested extracts and fractions 50µg/ ml at 37 °C. Images of each scratch were captured at 0, 6,12, and 24 h with a digital camera on an inverted microscope (Zeiss). The wound closure analysis was made from edge to edge using the zen software. For all treatments, the wound at time 0 was arbitrarily assigned as 100% and the percentage of wound healing at 24 h was compared to each cell treatment at the initial time as shown in Figure 14 and 15.

CONCLUSION

The study revealed that the enriched methanolic extract of leaves *Solanum americanum* contains major polar compounds and saponins, as confirmed by HPLC-DAD, froth formation, and hemolytic activity. LC-HRMS identified Tigogenin, Rhamnetin 3-O-glucoside, β2-Solamargine, 3-Methoxy-5,7,3′,4′-tetrahydroxy-flavone, and Isorhamnetin as key constituents. The methanolic extract showed low cytotoxicity against Caco-2 and B16F10 cells, while the hexane and EtOAc extracts exhibited moderate activity. In vitro scratch assays indicated that the ACN fraction promoted faster cell migration, whereas the methanolic extract and its combinations inhibited migration. Standard wound-healing reference agents were not included in the assay, therefore the wound-closure effects observed for the extracts could not be benchmarked against established positive controls. This limits the ability to compare the efficacy of the tested extracts with known wound-healing standards.

List of Abbreviations

ACN – Acetonitrile; **B** – Organic phase (Acetonitrile) in HPLC gradient;
BSI – Botanical Survey of India; **Caco-2** – Human colon adenocarcinoma cell line;
CCD – Charge-coupled device (microscope camera; if applicable);

DAD – Diode Array Detector; **ddMS²** – Data-dependent MS/MS;
DMSO – Dimethyl sulfoxide; **EDTA** – Ethylenediaminetetraacetic acid;
EtOAc – Ethyl acetate; **ESI** – Electrospray Ionization;;**FBS** – Fetal Bovine Serum;
FLD – Fluorescence Detector; **g** – Relative centrifugal force;
HESI – Heated Electrospray Ionization;
HPLC – High-Performance Liquid Chromatography;
HRMS – High-Resolution Mass Spectrometry;
IC_{50} – Half-maximal inhibitory concentration;
LC-HRMS – Liquid Chromatography–High-Resolution Mass Spectrometry;
MeOH – Methanol / Methanolic; **MeOH–Hex** – Methanol–Hexane fraction
MTT – 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide;
m/z – Mass-to-charge ratio; **PBS** – Phosphate-buffered saline;
PDA/DAD – Photodiode Array Detector / Diode Array Detector;
Rf – Retention factor (TLC); **RBCs** – Red Blood Cells; **rpm** – Revolutions per minute
RP-HPLC – Reverse-Phase High-Performance Liquid Chromatography;
SDS – Sodium dodecyl sulfate; **UHPLC** – Ultra-High-Performance Liquid Chromatography
UV–Vis – Ultraviolet–Visible spectroscopy

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES:

Anzano, A., Grauso, L., De Falco, B. and Lanzotti, V. 2025. Untargeted LC-HRMS metabolomics and chemometrics of *Aloe vera* across diverse geographical origins and cultivation practices. *Plants,* **14**(11):1685. https://doi.org/10.3390/plants14111685

Borisov, R., Kanateva, A. and Zhilyaev, D. 2021. Recent advances in combinations of TLC with MALDI and other desorption/ionization mass-spectrometry techniques. *Frontiers in Chemistry*, **9**: https://doi.org/10.3389/fchem.2021.771801

Chaudhry, G., Zeenia, Safdar, N., Begum, S., Akim, A.M., Sung, Y.Y. and Muhammad, T.S.T. 2024. Cytotoxicity assays for cancer drug screening: methodological insights and considerations for reliable assessment in drug discovery. *Brazilian Journal of Biology*, **84**:. https://doi.org/10.1590/1519-6984.284409

Gaudêncio, S.P., Bayram, E., Lukić Bilela, L., Cueto, M., Díaz-Marrero, A.R., Haznedaroglu, B.Z., Jimenez, C., Mandalakis, M., Pereira, F., Reyes, F. and Tasdemir, D. 2023. Advanced methods for natural products discovery: bioactivity screening, dereplication, metabolomics profiling, genomic sequencing, databases and informatic tools, and structure elucidation. *Marine drugs*, **21**(5):308. https://doi.org/10.3390/md21050308

Herqash, R.N., Fantoukh, O.I., Alqahtani, A.S., Shahat, A.A., Ahamad, S.R. and Alqahtani, A.M. 2024. GC-MS and RP-HPLC analysis reveals phytochemical compositions and antioxidant potential in *Solanum Schimperianum, Solanum Cordatum,* and *Solanum Nigrum* extracts from Saudi Arabia. *Egyptian Journal of Chemistry*, **67**(9):13-34. https://doi.org/10.21608/ejchem.2024.283184.9599

Imran, M., Majid, H., Khan, M.A. and Qadir, A. 2022. Phytochemical screening of *Solanum xanthocarpum* and its xanthine oxidase inhibitory activity. *Biological Sciences*, **2**(3): 311–323. https://doi.org/10.55006/biolsciences.2022.2308

Lima, G.S., Lima, N.M., Roque, J.V., de Aguiar, D.V., Oliveira, J.V., Dos Santos, G.F., Chaves, A.R. and Vaz, B.G. 2022. LC-HRMS/MS-based metabolomics approaches applied to the detection of antifungal compounds and a metabolic dynamic assessment of Orchidaceae. *Molecules*, ***27***(22):7937. https://doi.org/10.3390/molecules27227937

Moustafa, M., Aboelmaaty, W.S., Ebrahim, W., Mekky, R.H., Tammam, M.A., El-Demerdash, A. and Zaghloul, A.M. 2025. Chemical profiling of Lycium shawii via RP-HPLC-QTOF-MS and MS/MS: unveiling its in-vivo wound-healing potential supported by molecular docking investigations. *Fitoterapia*. https://doi.org/10.1016/j.fitote.2025.106749

Nozhat, Z., Khalaji, M.S., Hedayati, M. and Kia, S.K. 2022. Different methods for cell viability and proliferation assay: Essential tools in pharmaceutical studies. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)*, **22**(4):703-712. https://doi.org/10.2174/1871520621999201230202614

Palermo, A. 2023. Metabolomics-and systems-biology-guided discovery of metabolite lead compounds and druggable targets. *Drug Discovery Today*, **28**(2):103460. https://doi.org/10.1016/j.drudis.2022.103460

Pantharos, P., Sukcharoen, P., Phadungrakwittaya, R., Akarasereenont, P., Booranasubkajorn, S. and Lumlerdkij, N. 2022. Utilization of UPLC-PDA and GC–MS/MS coupled with metabolomics analysis to identify bioactive metabolites in medicinal turmeric at different ages for the quality assurance. *Phytomedicine,* **102**:154157.ht

tps://doi.org/10.1016/j.phymed.2022.154157

Paraschiv, M., Csiki, M., Diaconeasa, Z., Socaci, S., Balacescu, O., Rakosy-Tican, E. and Cruceriu, D. 2022. Phytochemical profile and selective cytotoxic activity of a *Solanum bulbocastanum* Dun. methanolic extract on breast cancer cells. *Plants*, **11**(23):3262. https://doi.org/10.3390/plants11233262

Roy, I., Magesh, K.T., Sathyakumar, M., Sivachandran, A., Purushothaman, D. and Aravindhan, R. 2023. Evaluation of wound healing property of the ethanolic extract of *Glycyrrhiza glabra* on vero cell lines using *in vitro* scratch assay test. *Journal of Pharmacy and Bio allied Sciences,* **15**(Suppl 1), pp.S630–S635. https://doi.org/10.4103/jpbs.jpbs_61_23

Sindhu, R.K., Kaur, P., Manshu, S., Goyal, A., Bala, R. and Sandhu, A. 2021.Phytochemicals: extraction, isolation methods, identification and therapeutic uses: a review. *Plant Arch*, **21**(1):174-184. https://doi.org/10.51470/PLANTARCHIVES.2021.v21.S1.032

Soltani, S., Zirah, S., Rebuffat, S., Couture, F., Boutin, Y., Biron, E., Subirade, M. and Fliss, I. 2022. Gastrointestinal stability and cytotoxicity of bacteriocins from Gram-positive and Gram-negative bacteria: a comparative in vitro study. *Frontiers in Microbiology*, **12**, https://doi.org/10.3389/fmicb.2021.780355

Terki, M., Benariba, N., Fekhikher, Z., Haci, I.A.E., Barek, S., Bensouici, C., Sara, A., Hanane, B., Radia, B.G., Belarbi, M. and Djaziri, R. 2023. Antimicrobial, antibiofilm and antioxidant activities of *Citrullus colocynthis* fruit extracts. *International Journal of Minor Fruits, Medicinal and Aromatic Plants,* **9**(1):1–13. https://doi.org/10.53552/ijmfmap.9.1.2023.1-13

Thakur, M., Verma, R., Kumar, D., Das, P.P., Dhalaria, R., Kumar, A., Kuca, K., Azizov, S. and Kumar, D. 2024. Revisiting the ethnomedicinal, ethnopharmacological, phytoconstituents and phytoremediation of the plant *Solanum viarum* Dunal. *Naunyn-Schmiedeberg's Archives of Pharmacology*, **397**(8):5513-5531. https://doi.org/10.1007/s00210-024-03034-6

Ukwubile, C.A. 2024. Phytochemical content and anti-inflammatory potential of *Solanum americanum Mill.* (Solanaceae) methanol leaf extract in Wistar rats. *International Journal of Complementary and Internal Medicine*, **6**(1):259–267. https://ijcimjournal.com/index.php/1/article/view/57

Vakte, S. and Nehete, J. 2025. Phytochemical and antioxidant studies on dried leaves of *Crotalaria gajureliana* Gholave, Madhav & Gosavi. *International Journal of Minor Fruits, Medicinal and Aromatic Plants*, **11 (1)**:196–203. https://doi.org/10.53552/ijmfmap.11.1.2025.196-203

Wankhade, S., Nikhil, P., Jain, R., Mishra, S., Kumarasamy, M. and Ramalingam, P., 2024. Forced degradation products of Olmesartan Medoxomil and their *in vitro* genotoxicity assessment by comet assay using HEK cells. *Chemistry Select*, **9**(17):e202400221. https://doi.org/10.1002/slct.20240022

Table 1: Compounds identified from different extracts and fraction of *Solanum americanum*

Name of compound	Calc. MW	m/z	RT [min]	Area (Max.)	Area: Hex-extract	Area: ACN fraction	Area: water Fraction raw	Area: Hex Residue	Area: MeOH.Crude	Area : EtOAc. extract
Auriculoside	450.2	0.9	116703.6	-	-	-	-	-	116703.6	82238.8
Gallic acid	170.0	171.0	4.5	82765.9	-	-	-	-	-	82765.9
Ellagic acid	302.0	301.0	9.1	12016515	12016515	-	-	-	-	-
Trifolin	448.1	449.1	10.6	10917114.0	1003970.0	170653.7	–	–	1342286.0	10917114.0
Kaempferol	286.0	287.1	10.6	5677112.0	271202.3	–	–	–	–	5677112.0
Isorhamnetin	316.1	317.1	11.6	46299942.0	–	–	1999180.0	–	46299942.0	–
Caffeic acid phenethyl ester	284.1	285.1	11.8	253306.2	–	–	–	–	–	253306.2
Kaempferitrin	624.2	625.2	12.1	19942695.0	–	–	4392411.0	–	19942695.0	300085.6
Solanigroside Y8	1376.6	1375.6	12.2	196337.2	–	–	–	–	196337.2	–
(+)-Lysergic acid	268.1	269.1	12.4	13576251.0	–	–	13576251.0	–	–	–
Nigroside A	1230.6	1211.6	12.6	4093779.0	–	–	–	–	4093779.0	–
Purapurine	883.5	884.5	12.7	1.3E+08	6638417.0	–	1.3E+08	–	24260792.0	–
Solanid-5-en-3-yl glycoside	867.5	868.5	13.0	34127571.0	1877561.0	–	34127571.0	–	26479652.0	390251.9
Rhamnetin 3-O-glucoside	478.1	477.1	13.2	1.3E+08	336812.3	4475284.0	7498602.0	317202.0	1.3E+08	35948618.0
7-O-Methoxyquercetrin	462.1	461.1	13.7	25483638.0	–	1335333.0	4988523.0	–	25483638.0	13389823.0
(3beta,22alpha,25R)-Spirosol-5-en-3-yl 4-O-(6-deoxy-alpha-L-	721.4	722.4	13.8	71691322	3147573	393044.9	71691322	–	70329456	6791673

mannopyranosyl)-beta-D-glucopyranoside (β2-Solamargine)										
15,23-Dihydroxyspirost-5-en-3-yl 4-O-hexopyranosylhexopyranoside	770.4	771.4	14.4	875490.9	-	-	-	-	875490.9	-
Naringenin	272.1	271.1	14.5	17362000	17362000	11467337	6982902	11121580	12132871	13172488
Luteolin	286	285	14.8	21826105					4741711	21826105
3-Methoxy-5,7,3',4'-tetrahydroxy-flavone	316.6	315.1	16.4	2.7E+08		5851486	-	1307617	50190139	2.7E+08
Solasodine	413.3	414.3	16.5	2.48E+09	1.55E+08	90640210	2.48E+09	-	30262862	71808329
alpha-chaconine	851.5	852.5	17.3	461312	-	-	461312	-	-	-
Tricin	330.1	329.1	17.7	74405.7	74405.7	-	-	-	-	-
Pedalitin	316	317.1	18.0	36916452	-	-	-	-	-	36916452
Solanigroside C	1110.5	1109.5	18.8	399643.6	-	-	399643.6	-	-	-
(3beta,5alpha,22R,25R)-26-(beta-D-Glucopyranosyloxy)-22-hydroxyfurostan-3-yl beta-D-glucopyranosyl-(1->2)-[beta-D-xylopyranosyl-(1->3)]-beta-D-glucopyranosyl-(1->4)-beta-D-galactopyranoside	1214.6	1215.7	20	82758.0	-	-	-	82758.0	-	-
Saikosaponin A	780.5	779.5	20.4	405853	-	-	405853	-	-	-
Tigogenin	416.3	417.3	20.8	4.04E+08	53751271	-	4.04E+08	-	3.14E+08	-
Uttronin A	1034.5	1033.5	20.9	123428.3	123428.3	-	-	-	-	-
(3beta,5alpha,17beta)-6-Oxocevan-3-yl beta-D-glucopyranoside	575.4	576.4	22	1826412	-	-	1826412	-	-	-

Table 2: Cell viability and cytotoxic activity of extracts and fractions of the aerial part of *S.americanum* were tested against Caco2 cell lines and B16F10 cell lines

Sl.no.	Compound	Caco2 IC_{50} (µg/ml)	B16F10 IC_{50} (µg/ml)
1.	Hex –extract	97.6	2841
2.	ACN- fraction	717.4	-
3.	Hex residue	1075.1	1051
4.	EtOAc extract	-	544.2
5.	MeOH –extract	No cell death	-

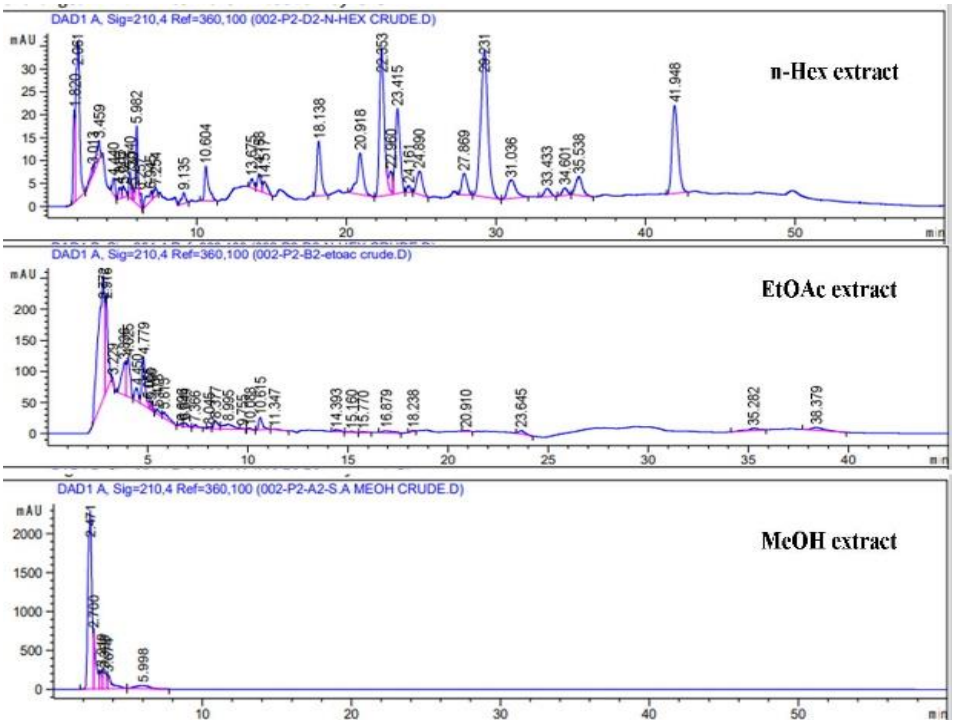


Figure 1: **Depict the comparison of the HPLC chromatogram of n-Hexane, ethyl acetate, and aerial parts (leaves) methanolic extracts of *S. americanum* (10mg/ml) at 210 nm.**

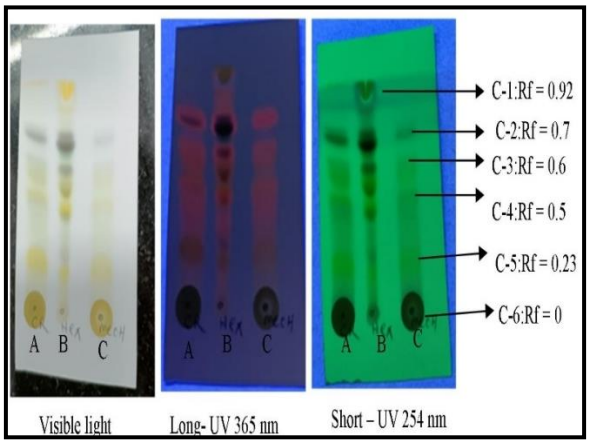


Figure 2: **TLC of *S. americanum* aerial parts (leaves)-A: crude methanolic extract, B: hexane fraction, C: MeOH fraction using EtOAc: Hex (50:50) and visualized under visible light, UV 254 nm, and UV 265 nm.**

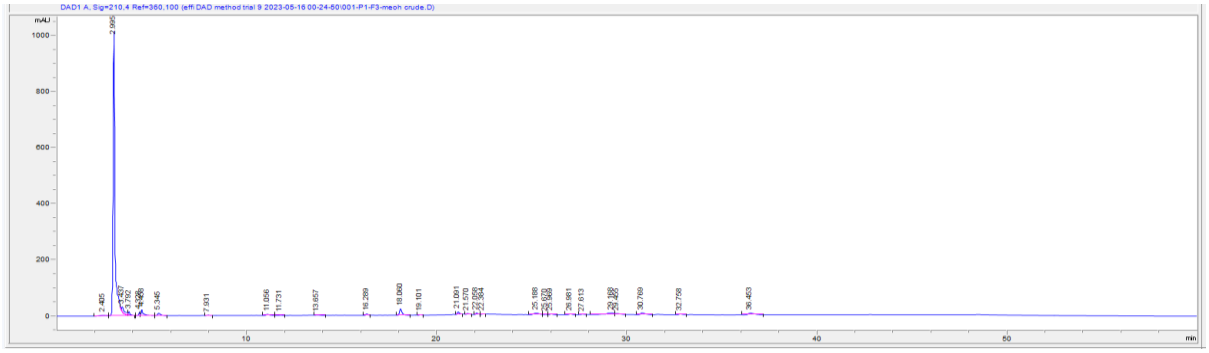


Figure 3: Depict the chromatogram and spectrum of the major compounds of MeOH crude of the aerial part (leaves) of *S.americanum* detected at 210 nm

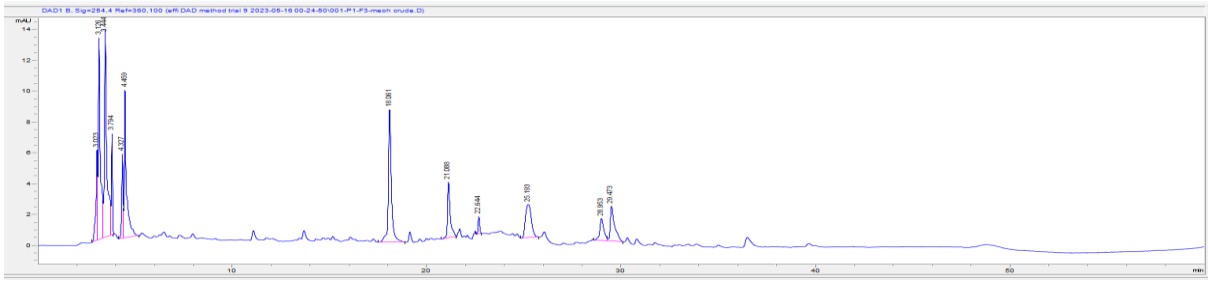
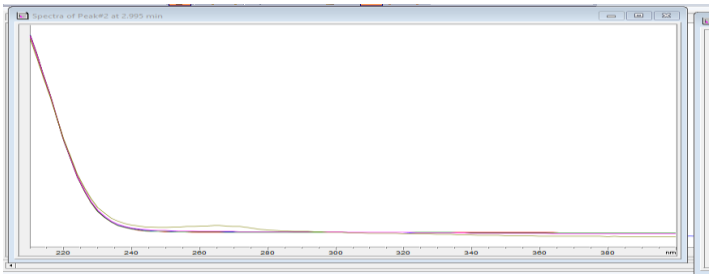


Figure 4: Depict the chromatogram and spectrum of the major compounds of MeOH crude of the aerial part (leaves) of *S.americanum* detect ed at 254 nm



Peak 2 at 2.995 min

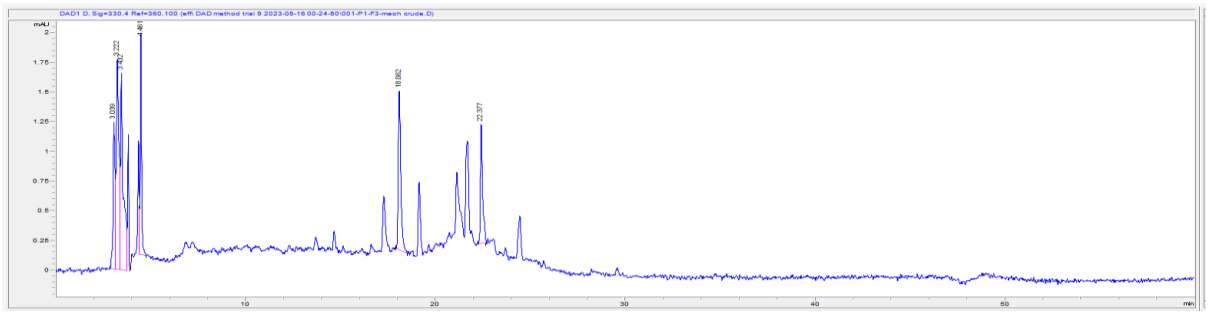
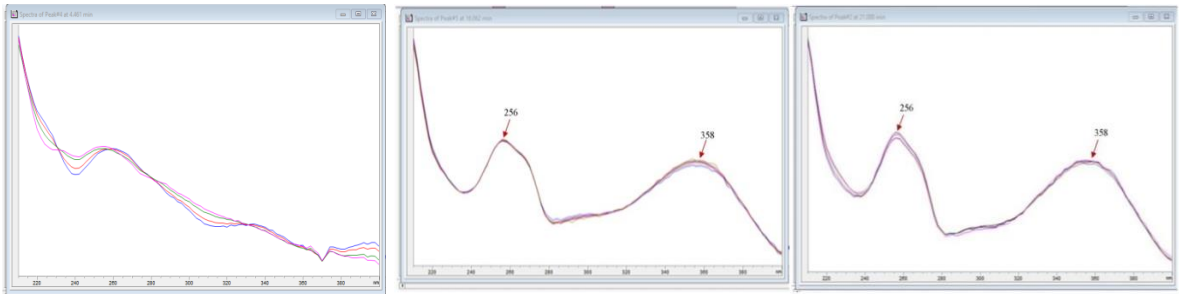


Figure 5: Depict the chromatogram and spectrum of the major compounds of MeOH extract of the aerial part (leaves) of *S.americanum* detected at 330 nm



Peak 1 at 2.344 min Peak 5 at 18.062 Peak 1 at 18.063

Figure 6: Depict the chromatogram and spectrum of the major compounds of MeOH extract of the aerial part (leaves) of *S.americanum* detected at 360 nm

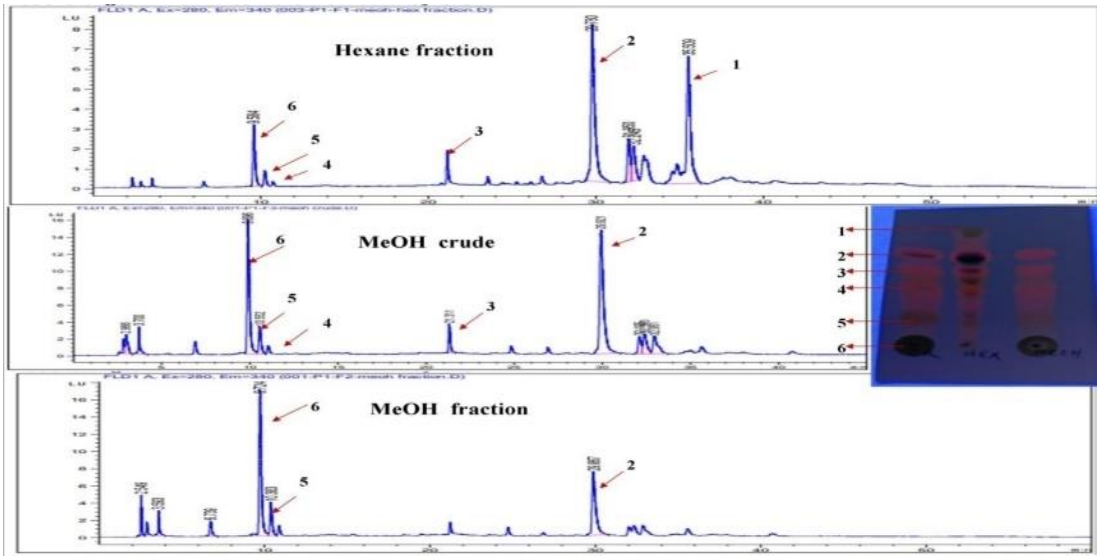


Figure 7: Depict the chromatogram of HPLC –FLD analysis of the methanolic extract and fractions of the aerial part (leaves) of *S. americanum*

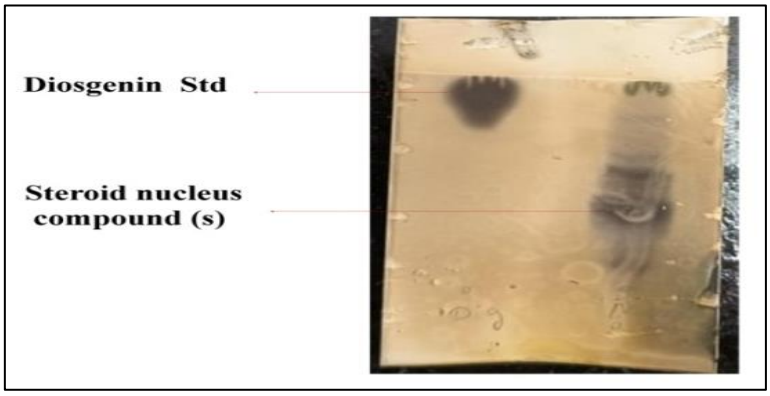


Figure 8: Depict TLC of Diosgenin std vs the methanolic extract. 50% H_2SO_4 in ethanol was used as a visualising agent then charred. a black-coloured band was observed which indicates the presence of steroid nucleus.

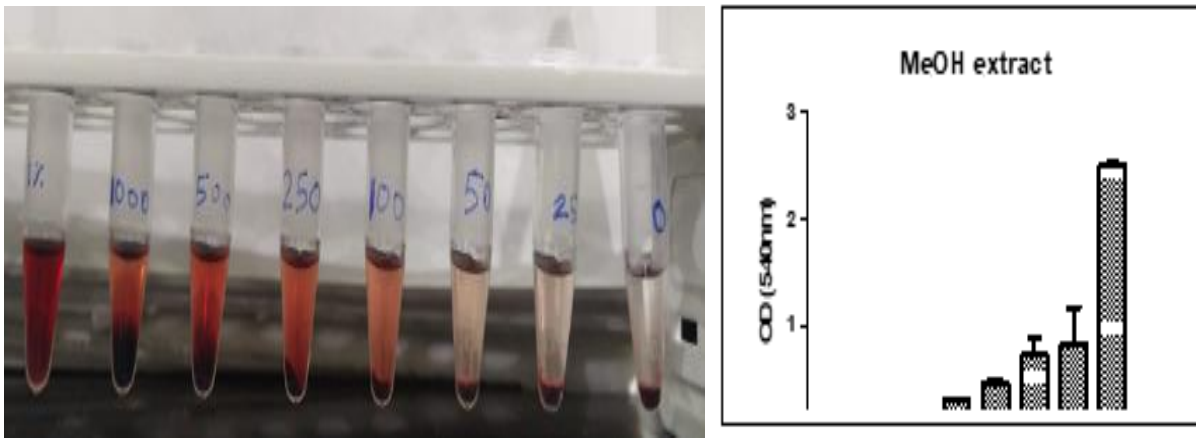


Figure 9: Depict the RBCs hemolysis on treatment with different concentrations 0, 25, 50, 100, 250, 500, 1000 ug/ml of the methanolic extract of the aerial part (leaves) of *S.americanum* and 1% SDS was taken as a positive control.

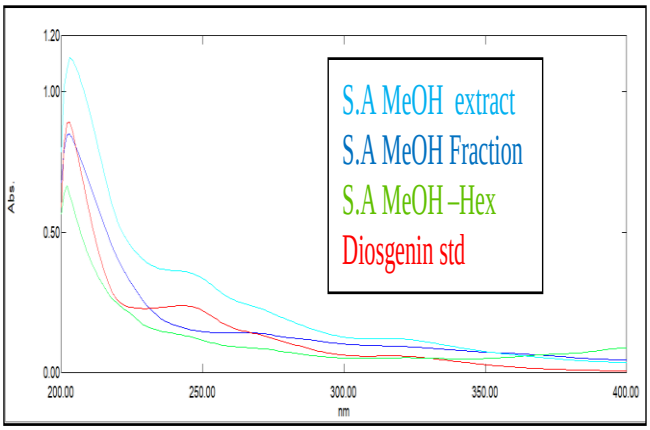


Figure 10: Depict the comparison UV spectrum of standard Diosgenin, Methanolic extract, hexane fraction and methanol residue.

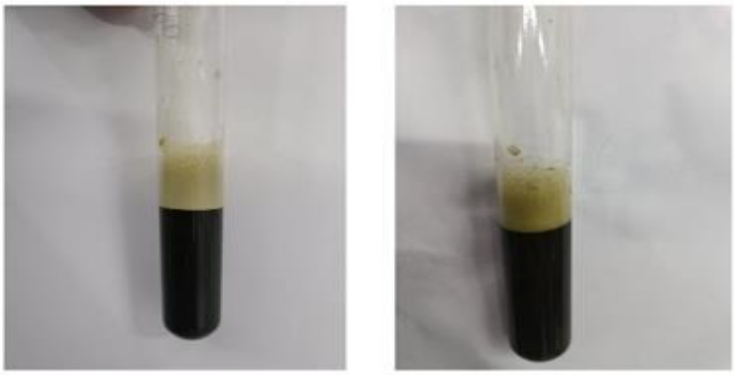


Figure 11: Depict the formation of froth on shaking the methanolic extract with H_2O

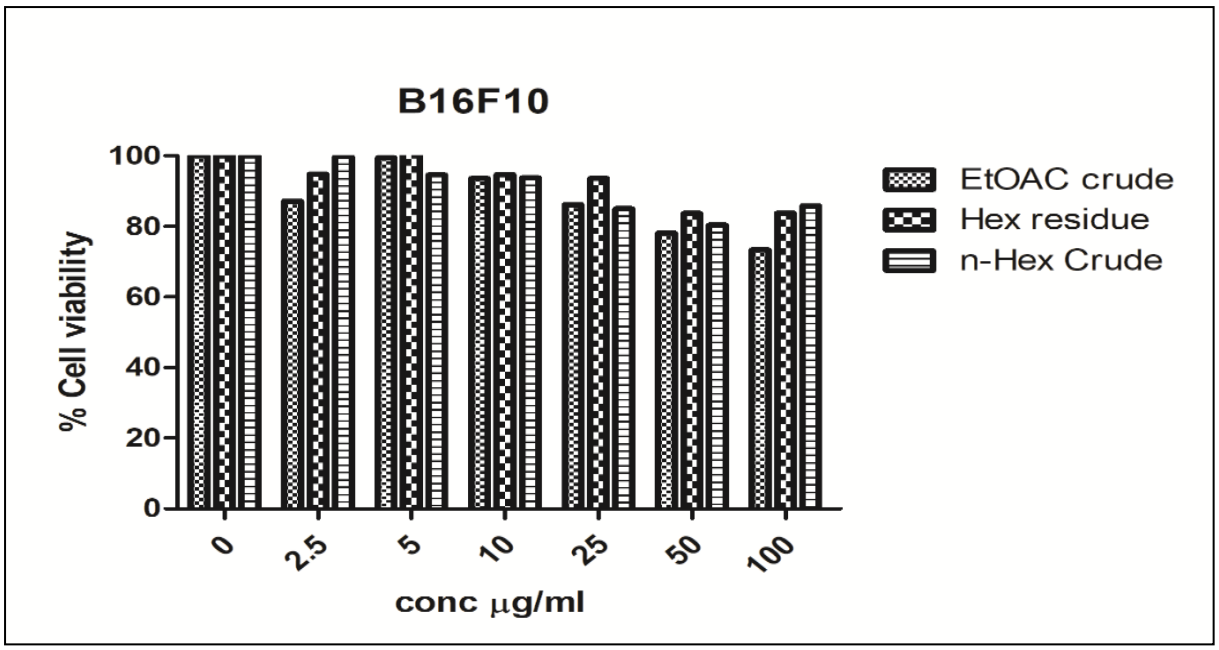


Figure 12: **The MTT assay performed on the B16F10 Cell lines showed that there is no significant decrease in the viable cell number after being cultured with 0-200 µg of EtOAC Crude and hexane extract of *S. americanum* for 48 h.**

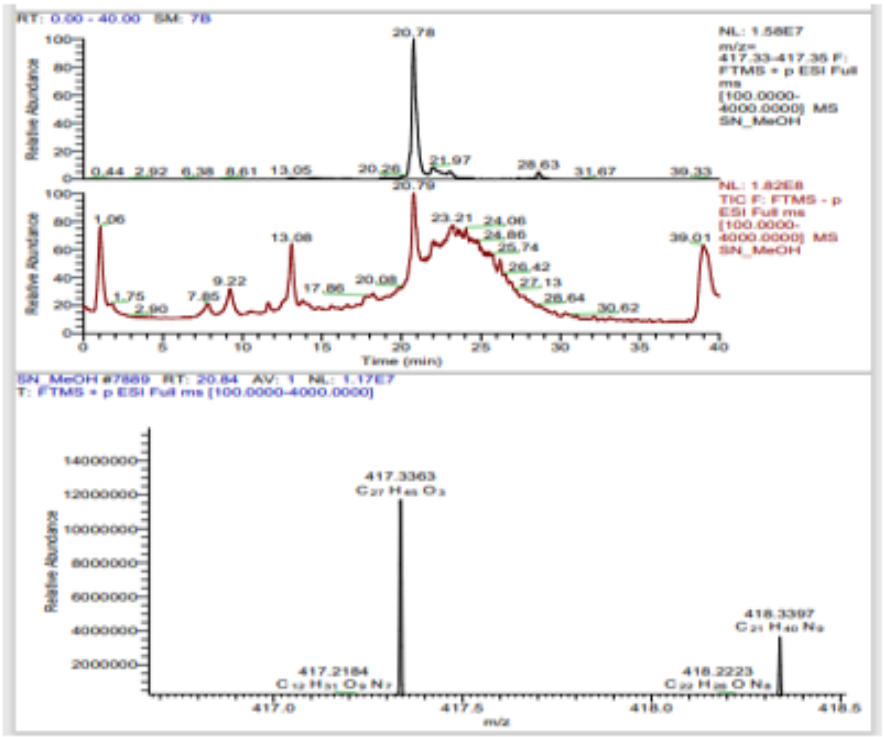


Figure 13: Extracted mass spectrum of Tigogenin in methanolic extract of aerial part of *S.americanum*

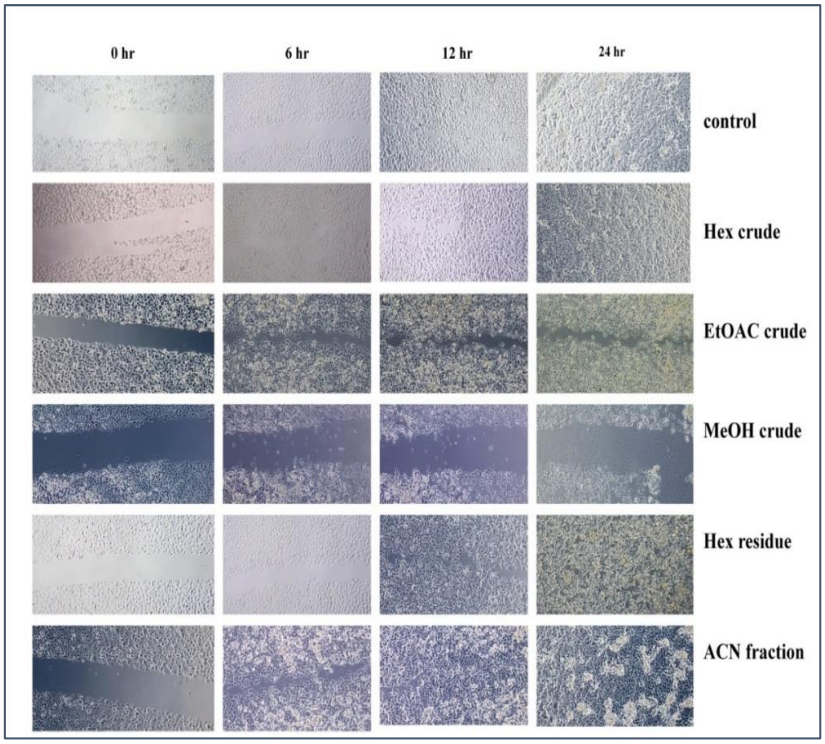


Figure 14: Time-lapse images under a Zeiss Inverted microscope showing in Vitro B16F10 cell migration after Treatment with different polar & Nonpolar solvents extract and fraction of *S. americanum*

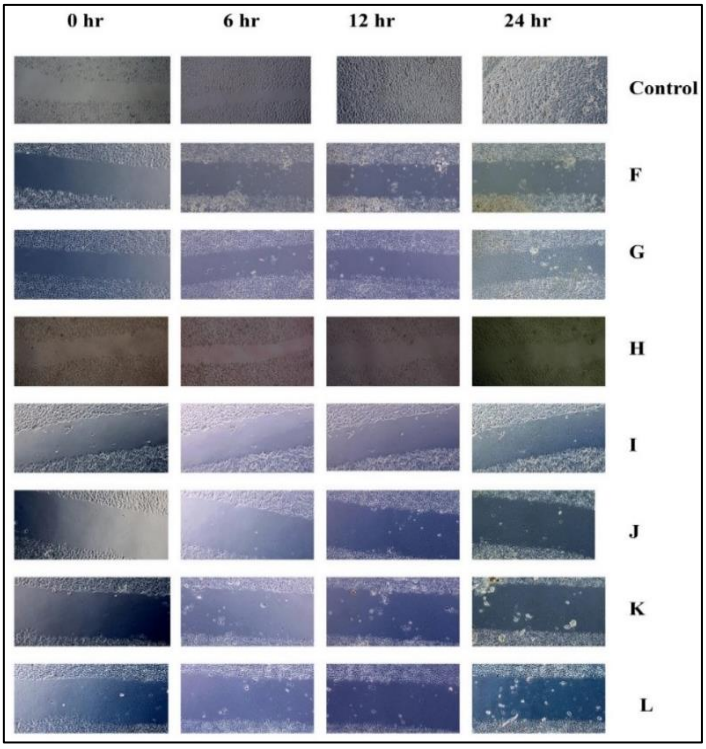


Figure 15: Time-lapse images of B16F10 cell migration under Zeiss microscope after treatment with various *S. americanum* extract combinations: F–L (ratios 1:1 to 1:1:1