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ISOLATION OF WEED DUAL TOPOPOISONS (I & II) FROM *SOLANUM MAURITIANUM* SCOP

M. V. N. L. Chaitanya[1*], S. P. Dhanabal [2], B. Duraiswamy[3], P. Dhamodaran[4], Sameer varma[5]

[1]Research Scholar, Department of Pharmacognosy and Phytopharmacy, JSS College of Pharmacy (Jagadguru Sri Shivarathreeshwara University, Mysore), Rockland's, Ooty-643001, Tamilnadu, India. Mobile: +917867912811, Email: chaitanya.phyto@gmail.com.

[2] Principal & Professor, JSS College of Pharmacy (Jagadguru Sri Shivarathreeshwara University, Mysore), Rockland's, Ooty-643001, Tamilnadu, India. Mobile: +919489044577, Email: spdhanabal@jsscpooty.org.

[3]Professor & Head , Department of Pharmacognosy & Phytopharmacy, JSS College of Pharmacy (Jagadguru Sri Shivarathreeshwara University, Mysore), Rockland's, Ooty-643001, Tamilnadu, India. Mobile : +919442083818, Email: bdurais@yahoo.com.

[4] Professor , Department of Pharmacognosy & Phytopharmacy, JSS College of Pharmacy (Jagadguru Sri Shivarathreeshwara University, Mysore), Rockland's, Ooty-643001, Tamilnadu, India. Mobile:+919443376349, Email: dhamsjss@hotmail.com.

[5]Research Scholar, Department of Pharmaceutical Biotechnology, JSS College of Pharmacy (Jagadguru Sri Shivarathreeshwara University, Mysore), Rockland's, Ooty-643001, Tamilnadu, India. Mobile : +918489784615 , Email: sameer.varma22@gmail.com.

Abstract:

The present research is focused on the phytochemical and biological investigation of Solanum mauritianum as a potent human dual topo I & II poisons. A good amount of secondary metabolites like alkaloids (163.10 µg/g Atropine equivalents), phenolics (119.94 ug/g µg/g Gallic acid equivalents), flavanoids (59.94 µg/g quercetin equivalents) and saponins (14.2 % w/w) were identified from this plant. First time, a novel saponin glycoside Tetrahydro-2-(hydroxymethyl)-6-(octadecahydro-2,7,10a-trimethyl-1propylchrysen-8-yloxy)-2H-Pyran-3,4,5-triol (CH1) was isolated from the chloroform fraction of the aerial parts of this plant using flash chromatography, a total ESI-MS spectra was carried out in negative mode. The saponins namely, Nocturnoside –b, Protodioscine, Paraquisoside –b, Solanine, Solasodine and Solasonine were identified based on the mass peaks. The isolated compound CH1 also showed good CTC50 values on A- 549 (53.7±1.7), MCF-7 (64.6±1.2) & HCT-116 (72.8±0.6) incompare to standard Quercetin. Based on docking results on topoisomerase I (1K4T, 3AL2) and topoisomerase II (1ZXN, 3QX3) showed the apoptosis is may be due to dual topo- poisoning activity. The isolated compound (CH-1) arrest the cell cycle at S Phase and showed dual human topopoison I & II activity at 200 µg/ml.

Key words: *Saponins, Flash chromatography, CTC50 Values , human dual topo I & II Poisons, saponins and Tetrahydro-2-(hydroxymethyl)-6-(octadecahydro-2,7,10a-trimethyl-1propylchrysen-8-yloxy)-2H-Pyran-3,4,5-triol (CH1).*

Corresponding Author:

Mr. M. V. N. L. Chaitanya,
Research Associate,
Department of Pharmacognosy and Phytopharmacy,
JSS College of Pharmacy (JSS University), Rockland's,
Ooty-643001, Tamilnadu, India.
Mobile: +917867912811,
Email: chaitanya.phyto@gmail.com.

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INTRODUCTION:

Saponins are the plant glycosides which form colloidal soapy solutions in water which can effect haemolysis of red blood cells at high dilution and can act as fish stupefying agents. The saponins may be existed as non-steroidal, steroidal and as steroidal amines which are also referred to as steroidal alkaloids. The current research in drug discovery is focused on the plant saponins as they having many pharmacological activities like molluscicidal, antimicrobial, antispasmodic, antidiabetic, anticancer, hypocholesterolemic, antioxidant, anticonvulsant, analgesic, anthelmintic, antitussive and cytotoxic activities [1].

Now a days, the scientists in cancer drug discovery are in keen interest towards plant saponins as many of them are having potent anticancer properties with different mechanisms, which is very important in treatment of different types of resistant cancers for example, certain types of saponins like Saponinum album (Spn) from Gypsophila paniculata L. synergizes with antitumour drugs and enhances the efficacy of chimeric toxins against tumours expressing human epidermal growth factor receptors in mice[2], saponins like gypenosides induced apoptosis in human hepatoma cells by Ca2+ Overload Mediated by endoplasmic-reticulum which is one of the important mechanism that the anticancer drugs should possess[3] and a novel anticancer saponin, Saikosaponin-d was isolated from Bupleurum falcatum L proved that the anticancer property is due to induction of autophagic cell death in apoptosis-defective cells and autophagy being a tumor suppressor mechanism got its clinical significance in treatment of various tpes of resistant cancers [4].

Most of this saponins are reported for their anticancer activity, hence this molecules are gaining importance in anticancer drug discovery [5]. However, In the past 5 years a relatively large interest on these saponins is increased in researchers as many of these saponins were reported as a potent cytotoxic agents and having enormous structural diversty. The cytotoxic effect of most of the reported saponins weres due to various mechanisms like apoptotic process in tumor cells, cell cycle arrestment, autophagic cell death stimulation, inhibiting of metastasis and cytoskeleton disintegration [6].

Cancer is defined as an abnormal growth of cells which tend to proliferate in an uncontrolled way and, in some cases, to metastasize (spread). It is not a single disease but it is a group of more than 100 different and distinctive diseases. Cancer can affect any tissue of the body and have many different forms in each body area and the cancers are named for the type of cell or organ in which they start. The frequency of a particular cancer may depend on gender. While skin cancer is the most common type of malignancy for both men and women, the second most common type in men is prostate cancer and in women, breast cancer. The role of diet and nutrition in carcinogenesis and cancer prevention has been an area of active research for decades. A holy grail has been the identification of nutritional supplements with cancer preventive properties. Such dietary factors would clearly have major public health implications, but unfortunately, investigations into supplementation of various vitamins, trace elements and other dietary constituents have generally yielded disappointing and even troubling results [7].

The main challenging task in any cancer treatment is its resistance, that's why all the globe is interested towards a discovery of a novel anticancer molecules which is possible only through plants. The potent anticancer drugs like camptothecin and salvicine which are role models in the cancer treatment proved that their anti-cancer mechanism is due to their capability of poisoning human topoisomerases I & II which are the important and latest targets for any potent anticancer drugs. So, the current trend in anticancer drug discovery is to find out novel topo-poisons which having the capability to drug the dual targets i.e., human topoisomerases I & II [8].

For over 40 years, natural products derived from plants have been proved as promising anticancer chemotherapeutic agents. However, the search for natural anticancer lead molecules has been continued in recent years also. The supply for these precious and promising anticancer natural lead molecules are in demand as it is possible to investigate the primary notion of structre- activity relationships and of its potential mechanism of action from these lead molecules[9].

In recent years, the current research is focused on the novel natural saponins as they proved as promising leads in the anticancer drug discovery. However, no attempt was made by researchers to prove their molecular mechanism and clinical efficacy of these lead molecules [10, 11, 12, 13 and 14].

Generally, weeds are considered as nuisances in the garden and enemies to the farmer, as there is a misconception that they are useless. Although many of the herbs used in Indian traditional medicine and tribal medicine are weeds, they are prohibited by agriculturists and field botanists ie Phyllanthus amarus L., Eclipta alba L., Centella asiatica (L.) etc.. Even though many of these weeds have high ethno-pharmacological importance, they are being destroyed and there is a lack of scientific knowledge and guidance. In the Nilgiris many medicinally valuable weeds like Achyranthes bidentata Blume., Artemisia nilagirica Clarke., Centella asiatica L., are

very prominent having good therapeutic values like diuretic, antimalarial and brain tonic.

The Niligiris district has an area of 2,452.50 km2. The district is basically a hilly region, situated at an elevation of 2000 to 2,600 masl. Almost the entire district lies in the Western Ghats of India. Its latitudinal and longitudinal dimensions being 130 km (Latitude: 10 - 38 WP 11-49N) by 185 km (Longitude: 76° E to 77.15° E) and it is a major hub for these weeds. Many of these weeds will grow naturally all over the season on the road sides and near to drainages. Based up on our field survey, we traced out nearly 50 prominent weeds belong to 26 different families.

It is possible that some of these weeds could provide an additional income for farmers. There is increasing evidence to support that weeds are relatively high in bioactive molecules, thus very important for new drug discovery. However, this current research is focused on the phytochemical and biological investigation on saponins isolated from weed Solanum mauritianum Scop (A very well distributed weed in the ooty or blue hills, The Niligiris district, Tamilnadu, India) as a cytotoxic agents[15,16].

Solanum mauritianum Scop also known as *Solanum auriculatum* is a small tree or shrub native to South America & India which is commonly known as woolly nightshade, ear-leaved nightshade (or "earleaf nightshade"), flannel weed, bugweed, tobacco weed, tobacco bush, wild tobacco and kerosene plant. he plant has a life of up to thirty years, and can grow up to 10 m (33 ft) tall. Its large oval leaves are grey-green in color and covered with felt-like hairs. The flower is purple with a yellow center. The plant can flower year round but fruiting occurs in late spring to early summer. It is tolerant of many soil types and quickly becomes established around plantations, forest margins, scrub and open land [17].

However, this plant is not explored phytochemically and biologicaly as an anticancer agent. Hence the current research is an attempt to prove that the secondary metabolites as an anticancer leads and an attempt was made to isolate the secondary metabolites using flash chromatography. The plant extracts, fractions and isolated compounds proved that they having significant antioxidant and cytotoxic activity on various cancer cell lines. The molecular docking studies proved that the cytotoxic activity is due to their dual inhibition activity of human topoisomerases I & II.

MATERIALS & METHODS:

Microscopical Authentication

The plant material (Whole plant) used in the present study was collected from Udhagamandalam in June 2013, The Niligiris district, Tamilnadu, India and was authenticated by the field taxonomist Dr. S. Rajan, Survey of Medicinal Plants and collection unit, Department of Ayush, Emarald and the voucher specimen was deposited at Department of Pharmacognosy and Phytopharmacy, JSS College of Pharmacy, Ooty (JSS University, Mysore) with identification no (JSSCP.PhD001/14). The Microscopical studies of the collected fresh material (leaf and stem) were carried out at Plant Anatomy Research Centre, Chennai, Tamilnadu, India. Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic units. For normal observations of bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against a dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard Anatomy books [18, 19 and 20].

Extract Preparation

The whole plant of Erigeron karvinkianus DC was collected and shade dried for 7 days. 1kg of dried, finely powdered material was triple macerated using 3 L of 70% v/v hydro-ethanol for 72 hrs. The obtained filtrate was subjected to vacuum distillation using Buchi Rotavap (R-210). The obtained extract was dried in a desiccator and the percentage yield was calculated on dried basis [21, 22].

Fractions Preparation

20g of above obtained extract was dissolved in 50 ml of double distilled water and subjected to liquid-liquid fractionation using different solvents like 200 ml of n-hexane, 500 ml of chloroform, and 500 ml of ethyl acetate. The obtained filtrate was subjected to vacuum distillation using Buchi Rotavap (R-210) and the percentage yield of obtaining fractions was calculated on dried basis [23,24,25].

Qualitative Phytochemical Screening

The prepared extract and fractions were subjected to a preliminary qualitative phytochemical screening following the methods based on standard protocols [26,27].

Total Saponin content estimation

The total saponin content was isolated from the 100g of crude drug using the gravimetric separation technique. Take 100g sample crude drug powder with 90% v/v methanol (500ml) by refluxing for half an hour. Extract the residue two more times by taking 500 ml methanol. Combine the methanol extract and distill off the solvent. Treat soft extract left after

distillation of alcohol, with petroleum ether 60-800c, 500 ml by refluxing for half an hour. Cool and remove the solvent by decantation. Now treat the same soft extract successively with chloroform 500 ml and ethyl acetate 500 ml and pour the solvents after cooling, keeping the soft extract in the same flask. Dissolve the soft extract (after three extractions cited above) in 500 ml of butanol. Filter and concentrate to 100 ml. add the above drop by drop with constant stirring to 500 ml with Acetone in order to precipitate the saponins. The precipitates are filtered, collected and dried to a constant weight at 105ºC [28,29].

TLC Finger printing of 2º metabolites present in different fractions

After phytochemical screening, the prepared fractions were subjected to thin layer chromatography analysis using standard methods described by wagner [30].

TLC Finger printing of fractions containing alkaloids

1 g of a substance is treated with 1 ml of 10% sodium carbonate and then extracted with 6 ml of chloroform and the chloroform residuals was subjected to thin layer chromatography and 10ul of sample was applied to aluminium precoated silica gel 60 GF 254 TLC plate using Ethyl acetate: Methanol: Water (100 : 13.5 : 10) and the elated alkaloid spots were identified under 254 & 366 NM in a TLC visualizer (CAMAG).

TLC Finger printing of fractions containing Cardiac glycosides

1 g of substance was treated with 3 ml of 50% ethanol, sonicated for 10 min and add 10 ml of 10% lead acetate solution , cool it and filter , then the solution was gently shake with 1.5 ml of dichloromethane , The dichloromethane fraction was dissolved in 0.1 ml of dichloromethane / isopropanol (3:2) and 10ul of sample was applied on aluminum precoated silica gel 60 GF 254 TLC plate using Ethyl acetate : Methanol : Water (100 : 13.5 : 10) and the eluted alkaloid spots were identified under 254 & 366 nm in a TLC Visulaiser (CAMAG).

TLC Finger printing of fractions containing flavanoids

5 ml (1mg/ml) of hydro-alcohol extract is concentrated to about 2 ml; to this add 1 ml of water and 10 ml of ethyl acetate is added. The ethyl acetate layer was concentrated and used for TLC analysis after defatting with 1 ml of petroleum ether. and 10ul of sample was applied on aluminum precoated silica gel 60 GF 254 TLC plate using Ethyl acetate :formic acid: glacial acetic acid : water (100:11:11:26) and the eluted alkaloid spots were identified under 254 & 366 nm in a TLC Visulaiser (CAMAG).

HPTLC Finger printing of fractions containing saponins

1g of the isolated fraction was dissolved in 5 ml of methanol and evaporated to about 1ml, mixed with 0.5 ml water and then extracted with 3 ml of n-Butanol. The n-Butanol layer was collected and evaporated used for TLC analysis by using 20 µl as sample was applied on aluminum precoated silica gel 60 GF 254 TLC plate using Chloroform : Methanol : Water (65 : 35 : 10) and the eluted saponin spots were identified under visible light & 366 nm in a TLC Visualiser (CAMAG).

LC-MS Finger Printing of Total Saponin Fraction (LC/ESI-Ms)

Shimadzu HPLC, Japan with system controller (SCL -10A), twin pump (LC-10AT VP), UV-Vis detector (SPD-10A VP) and rheodyne injector with 100 µl injection loop and the separation was done using lichrosorb micro bondapack C-18 Column (Merck). The data processing was done by using shimadzu HPLC Software class –VP (V5.03). The LC-MS experiment was carried out on a micromass quadro II triple quadrupole mass spectrometer. And the ESI capillary was set at 3.5 KV and the voltage was 40 V. The total positive mode spectrum and negative mode spectrum was carried out and the identity of saponins in fractions was done by the known mass values which was detected based on m+1 or M-1 ion peaks [31,32].

Isolation of Saponin glycoside (CH I) from acetone fraction using flash chromatography system

Isolera flash chromatography system having a touch screen display, which is a solvent-resistant, color LCD screen with a resolution of 800 x 600 pixels. It serves both as a display and as the system's input device via on-screen touch controls. A fraction collector, which collects fractions into a wide variety of collection racks and vessels. A pump module, which directs the liquid flow through the system. A default flow rate is specified for each cartridge but, if desired, the flow rate can be changed. If the flow rate is increased, the system will start the run at the default flow rate and then regulate towards the flow rate defined in the method. Note that the system regulates on both flow rate and pressure. If 90% of the maximum allowed pressure is reached before the defined flow rate, the flow rate at 90% pressure will be used. An internal detector, which provides the system with information on the light absorbance of the solvents and samples passing through the detector flow cell. The different fractions can be collected through an automated fraction collector based on the Rf the compound of interest can be identified and

pooled together after performing thin layer chromatographic analysis.

A novel method was developed for isolation of saponin glycoside using flash chromatography [33 – 34]. 5 g of 70%v/v hydro-alcohol extract was dissolved in 50 mL of water and sonicated for 25 minutes and filtered through whatman filter paper. The filtrate was partitioned with 180 mL of N-Butanol (3 x 60) mL and pooled together. The Pooled fractions were concentrated under vacuum using rotary evaporator (Buchi R-120), leaving a sticky mass. Brown color flakes were formed on addition of 0.5 mL of diethyl ether to the sticky mass.

The obtained 100 mg of flakes were subjected to flash chromatography, by directly applying on 10 g sample and was dried with air drier. A constant flow rate of 50 mL/ min of mobile phase N- Butanol : glacial acetic acid : water was used based on TLC profile. A total no of 70 fractions, each of 16 mL was collected, the fraction no 5 of 16 mL was collected and subjected to evaporation leaving 50 mg of glittery buff color flakes.

***In-silico* molecular docking studies against Topo I & Topo II enzymes**

The molecular docking studies were carried out into the catalytic domain of Human topo isomerases I (PDB ID: 1K4T at 2.10 A˚, PDB ID: 3AL2 at 2.0 A0) and Human topo isomerases II (PDB ID: 1ZXN at 2.51 A˚, PDB ID : 3QX3 at 2.16 A˚) [33,34,35,36] . The docking studies were carried out between the selected isomerase proteins and energetically minimized chemical compounds using GLIDE Program (Grid Based Ligand Docking from Energetics, from Schrodinger 9.9/2014-3 suite) [37,38] . The Epik studies were carried out for the selected compounds using the same software in order to know their pKa values [39,40]. The ADMET studies were carried out using QikProp [41,42] . The binding free energy of inhibitors in the catalytic domain enzyme (1K4T) was calculated by Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) VSGB 2.0 method. The calculated inhibitor-enzyme complex was relaxed using the local optimization feature in Prime, version 4.1, Schrödinger 2014, and the energies of the system were calculated using the VSGB 2.0 (solvation model) method available in Schrödinger suite 2014 (Schrodinger 9.9/2014-3 suite) [43,44,45].

***In-vitro* antioxidant assay using DPPH and ABTS assay**

DPPH (1,1diphenyl-2-picryl hydrazyl) radical scavenging activity

1 ml of test solution added to 1 ml of DPPH in methanol (0.33%). After keeping for 30 minutes at 37˚C the absorbance at 518nm was measured using UV spectrophotometer. Corresponding blanks were taken. The absorbance of DPPH as control was measured at 518 nm. Lower absorbance of the reaction mixture indicated higher radical scavenging activity. DPPH accepts an electron to become a stable diamagnetic molecule. The methanolic solution of DPPH (violet colour) has got a strong UV absorbance at 518nm. The presence of a reducing agent in this methanolic solution pairs the odd electrons of DPPH radical and further the solution losses colour stoichiometrically and also the absorbance of the solution decreases at 518nm [46,47].

The scavenging effect (%) was measured using the following formula:

$$\textbf{ScavangingEffect(\%)} = \frac{\textbf{(Control absorbance – Test absorbance)} \times \textbf{100}}{\textbf{Control Absorbance}}$$

ABTS.$^{+}$ Assay

The ABTS.+ radical cation decolorization assay was performed to evaluate the radical scavenging ability of test drugs by the standard method50 – 51. ABTS radical cation (ABTS. +) was generated by adding 2.45 mM potassium persulfate to 7 mM ABTS and incubated in darkness at room temperature for 12–16 h. This stock solution of ABTS.+ was diluted with ethanol to give an absorbance of 0.70 (± 0.02) at 734 nm, which act as a positive control. 10 µl of test drug (prepared in ethanol/DMSO) will be mixed with 1.0 mL of diluted ABTS.+ solution and incubated at 30ºC for 30 Min. The absorbance value was measured at 734nm with UV–visible spectrophotometer. Trolox standard will also be prepared (in ethanol: 0–1.5 mM) to get the concentration response curve. The unit of trolox equivalent antioxidant activity (TEA) was defined as the concentration of Trolox having the equivalent antioxidant activity expressed as mmol/g of extracts [48,49].

***In-vitro* cyto-toxic studies using Sulphorhodamine B (SRB) assay**

Cell lines and culture medium

The cell cultures used in this research were procured from National Center for Cell Sciences, Pune, India. The cells were grown in Earls Minimal Essential Medium supplemented with 2 mmol L-glutamine, 10 % Fetal Bovine Serum, Pencillin (100 µg/ml) and amphotericin B (5µg/ml) and the cells were maintained at 370C in a humidified atmosphere with 5 % CO2 and subculture twice a week.

In-vitro Topo enzyme poisoning effect

In-vitro cyto-toxic studies were carried on the isolated compounds and fractions based up on the

standard protocol and compared with standard quercetin [50].

In-vitro wound healing studies

The Human Dermal Fibroblast cell lines (HDF) were grown in Dulbecco's modified eagle medium supplemented with 10% Fetal bovine serum (FBS) medium, then seed the cells into 24-well tissue culture plate at a density that after 24 h of growth and should reach ~70-80% confluence as a monolayer. The medium should not be changed, gently and slowly scratch the monolayer with a new 1 mL pipette tip across the center of the well. While scratching across the surface of the well, the long-axial of the tip should always be perpendicular to the bottom of the well. The resulting gap distance therefore equals to the outer diameter of the end of the tip. The gap distance can be adjusted by using different types of tips. Scratch a straight line in one direction. Scratch another straight line perpendicular to the first line to create a cross in each well. After scratching, gently wash the well twice with medium to remove the detached cells. Replenish the well with fresh medium and Grow cells for additional 48 h (or the time required if different cells are used). The cells were washed twice with 1x PBS, then fix the cells with 3.7% paraformaldehye for 30 min. The fixed cells were stained with 1% crystal violet in 2% ethanol for 30 min and photos were taken for the stained monolayer on a microscope (Kimberly MA et al.2015).

TLC Based Direct Bioautography against Methicillin Resistant Staphylococcus aureus (MCC 2408)

Ten µl (10 mg/ml) of each saponin fractions and 10 µl of (1mg/ml) of isolated compounds were loaded onto TLC plates in a narrow band and eluted using the developed mobile solvent systems. The developed plates were dried under a stream of fast moving air to remove traces of solvent on the plates. Overnight cultures grown on MH broth were used and the densities of bacterial organism used for S. aureus were approximately d 3 ×1012 cfu/ml, respectively. The prepared chromatograms were sprayed with the fungal or bacterial suspension until wet. This process was carried out in a biosafety Class II cabinet for bacteria. Thereafter, the plates were incubated overnight at 35°C and 100% relative humidity in the dark and then sprayed with a 2 mg/ml solution of p-iodonitrotetrazolium violet. White bands indicate where reduction of INT to the colored formazan did not take place due to the presence of compounds that inhibited the growth of tested organisms [52].

Topo drugging studies (Human Topoisomerases I & II)

Topoisomerase I & II inhibition assay

The compound -2 (Tetrahydro-2- (hydroxymethyl)-6-(octadecahydro-2,7,10a-trimethyl-1-propylchrysen-8-yloxy)-2HPyran- 3,4,5-triol (CH-2) and compound -4 (1E)-1-hydroxyprop-1-en-2-yl (2E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoate) was subjected to topo-isomerase- I&II inhibition assay.

Stock of the compound was prepared at a concentration of 1mg/mL in water. The Corresponding sub-stocks were prepared such that addition of 5ul of the stock gives a working concentration of 50, 100, 200 and 400µg/mL for Topo-I& 100,250& 400 µg/mL for Topo-II. The compounds (different concentrations) were incubated with 1unit of topo1 enzyme and 4 units of topo2 for 15 min at room temperature. To this 0.5ug of pBR329 was added and the volume was made up to 20ul with relaxation buffer and kept at 37 ^{0}C for 30 min and the relaxation was terminated by adding 5µl of loading buffer. The reaction was analysed in 0.8% agarose gel prepared and the gel was run at 120V for 2hrs, then the gel was stained with Ethidium Bromide solution for 1hr and destained with water overnight and photo was taken [53].

Cell cycle Analysis

Cell lines were washed with 1X PBS and trypsinised using 1X Trypsin. The Cells were gently suspended in DMEM Media and centrifuged at 3000rpm for 5 minutes at 4ºC. The supernatant was discarded and the pellet was resuspended in 1ml 1X PBS. Then, it is centrifuged at 3000rpm for 5 minutes at 4ºC. The supernatant was discarded. Add 1ml ice cold 1X PBS and centrifuged at 3000rpm for 5 minutes at 4ºC. The supernatant was discarded and 1ml of ice cold 70% ethanol was added through the sides of the tube. Incubate at 4ºC for 45 minutes. Add 1ml of ice cold 1X PBS and centrifuged at 3000rpm for 5 minutes at 4ºC. The supernatant was discarded. Add 1X PBS and centrifuged at 3000rpm for 5 minutes at 4ºC. The supernatant was discarded. Add 250µl of 0.4mg/ml RNase to each tube and incubated at 37ºC for 30 minutes. Add 10µl of 1mg/ml PI to each tube and incubate it in dark for 5 minutes. The samples are filtered through cell strainer in to FACS tube. The samples were analyzed in Flow cytometer (FACS Aria II).

RESULTS AND DISCUSSION:

Microscopical authentication

The leaf consists of a wide and thick midrib.

It consists of a short and thick adaxial hump and wide lobed abaxial part. It is 850 µm and the abaxial part is 1.15 µm wide. The midrib consists of a thin wavy epidermal layer of small squarish cells. Inner to the epidermis are two or three layers of collenchyma cells followed by a wide zone of large thin walled compact parenchymatous ground tissue. The vascular strand is single, shallow, wide are of bicollateral xylem and phloem. It consists of diffusely distributed angular, thick walled wide xylem elements. Several discrete nests of phloem elements occur both on the adaxial and abaxial sides of the xylem strand. No sclerenchyma cells are seen on the adaxial and abaxial phloem units.

The lamina is thick and bifacial. It is 90 µm thick. The adaxial epidermis consists of dilated circular or elliptical thin walled cells. The abaxial epidermis has thin and cylindrical cells. The palaside cells are single layered thin and have wide gaps inbetween the cells. The abaxial zone of spongy parenchyma includes about five layers of lobed cells and form air chambers. Both on the abaxial and adaxial surfacial surfaces of the lamina occur dense stellate epidermal trichomes. The trichomes has thick, short stack which consists of three or four vertical mass of cylindrical cells. At the apex of the stalk are situated circular readiating lateral trichomes. These trichomes appear star shaped in surface view and hence they are called stellate trichomes. The trichome is 100µm in height and the lateral trichomes are up to 350 µm long (Fig 1).

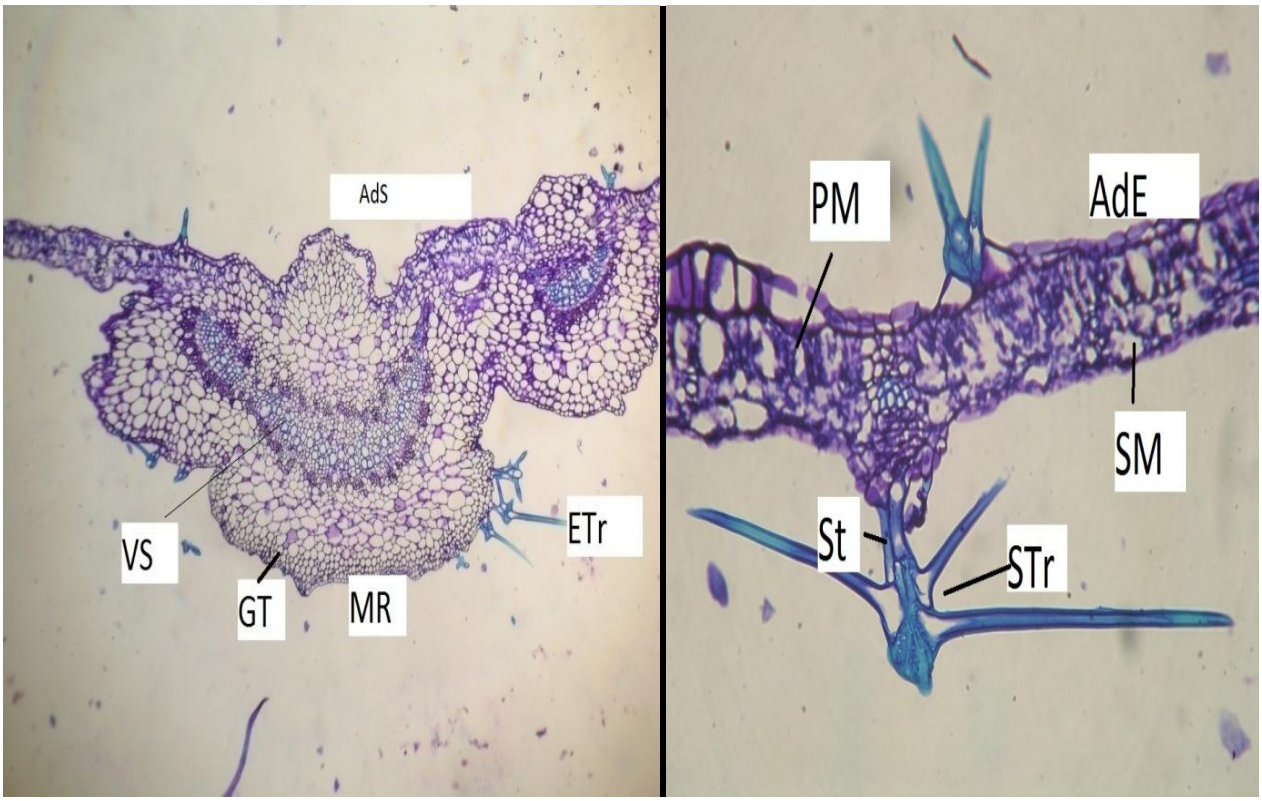


Fig 1:Transverse Section of *Solanum Mauritianum* SCOP leaf (AdS : Adaxial side, ETr: Epidermal trichomes, VS: Vascular strands, GT: Ground tissue, MR : Mid rib, VS: Vascular strand, AdE: Adaxial epidermis, PM: Palaside mesophyll, STr: Stellate trichome, SM : Spongy mesophyll and St: Stalk).

Qualitative Phytochemical Analysis

The extracts and the fractions were subjected to phytochemical screening and found to contain good content of alkaloids, cardiac glycosides, flavanoids, triterpenoids/steroids, saponins, phenolics, tannins, saponins, carbohydrates, cardiac gylcosides, saponins and coumarins and discussed below in the Table 1.

Table 1: Qualitative phytochemical analysis of Solanum Mauritianum SCOP extracts and fractions

S.NO	Name of the test	*Solanum Mauritianum* SCOP				
		ME	PF	CLF	ETF	AQF
1.	Test for Alkaloids	++	-	++	-	-
2.	Test for Cardiac glycosides	++	-	++	++	-
3.	Test for Flavanoids	++	-	-	++	-
4.	Test for Triterpenoids/ Steroids	++	++	++	++	++
5.	Test for Saponins	++	-	-	-	++
6.	Test for Napthoquinones	-	-	-	-	-
7.	Test for Phenolics	++	-	-	-	++
8.	Test for Carbohydrates	++	-	++	++	++
9.	Test for Coumarins	++	-	-	-	++
10.	Test for Tannins	++	-	-	-	++

(++ - Abundance, + - present and - -ve) (ME – 70% Hydro-ethanol mother extract, PF – Pet-ether fraction, CLF – Chloroform fraction, ETF – Ethyl acetate fraction, AQF – Aqueous fraction) **Table 2. TLC Finger printing analysis of 20 metabolites in various fractions**

TLC finger printing analysis of 2^0 metabolites present in various fractions

The various secondary metabolites was identified in different fractions of Solanum Mauritianum SCOP using thin layer chromatography analysis and the RF of the prominent spots were discussed below in Table 2 and in Fig. 2.

Table 2: TLC Finger printing analysis of 20 metabolites in various fractions

S.NO	Name of the fractions	R_F of alkaloids spots	R_F of cardiac glycosides spots	R_F of flavanoids spots	R_F of saponins spots
1.	Chloroform	0.23, 0.39, 0.46& 0.73	0.19,0.26, 0.28,0.33, 0.45,0.56, 0.69 & 0.71	-	-
2.	Ethyl acetate	-	0.35, 0.38, 0.64, 0.66, 0.82	0.38	-
3.	Total saponin	-	-	-	0.17, 0.22, 0.54, 0.56, 0.63, 0.67,0.74& 0.76

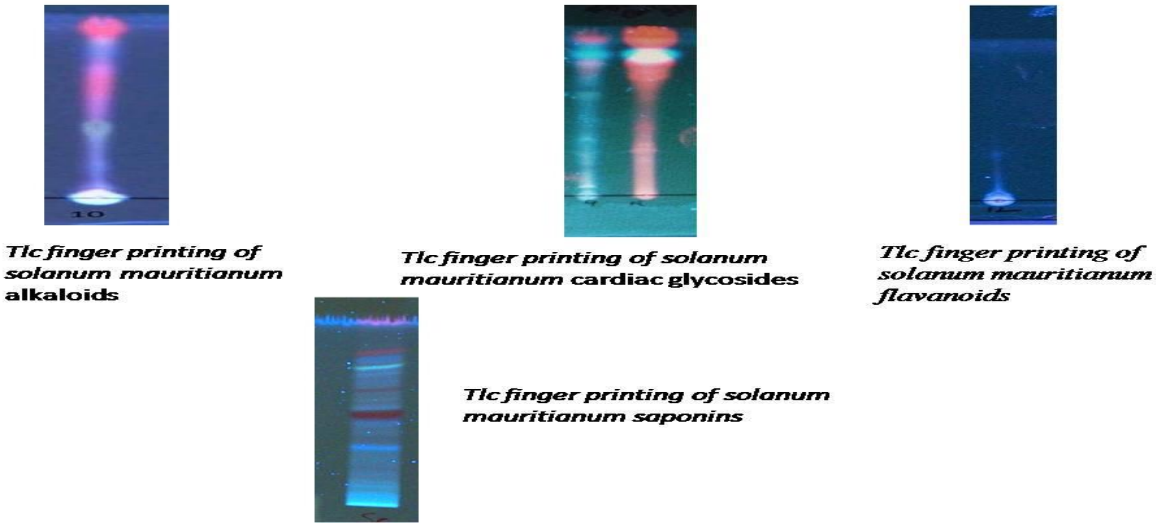


Fig 2: TLC Finger printing identification of secondary metabolites present in different *Solanum mauritianum* fractions

LC-MS Finger printing of total saponin fractions

he isolated total saponin fraction was subjected to LC-MS analysis and a total spectrum was run in both positive mode and negative mode. The saponin calenduloside E was identified as (M+H) peak in the positive mode scan and the saponin leiocarposide was identified as (M-2H) peak in the negative mode scan and the results were discussed in the below Table 3 and Fig 3.

Table 3: LCMS identification of saponins in total saponin fraction

S.NO	Identified Saponin	Molecular Mass	Mass peak
1.	Nocturnoside B	1015	1014 (M-H)
2.	Protodioscine	1049	1046 (M-3H)
3.	Parquisoside – B	1193	1192 (M-H)
4.	Solasodine	413	411 (M-2H)
5.	Solasonine	884	884 (M Peak)

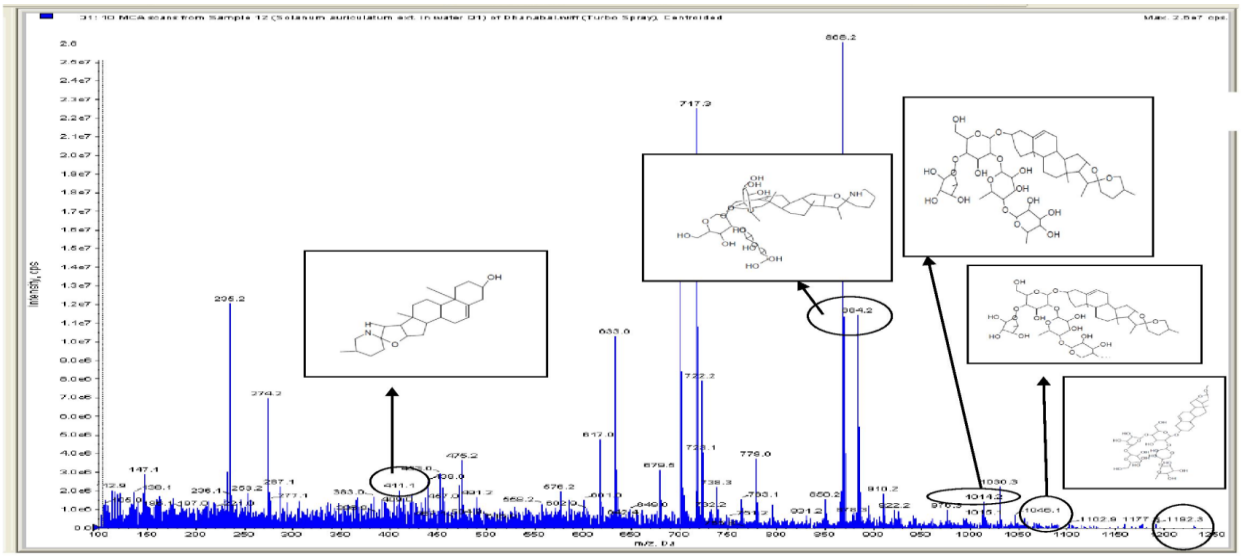


Fig 3: LC-MS ((LC/ESI-MS – ve Mode) Fingerprinting of *Solanum Mauritianum* SCOP total saponin fraction

Flash Chromatographic separation and characterization of isolated compounds

16 mL of fraction no.5 was evaporated using Rotavap (Buchi R 210) leaving a light brown mass and was precipitated with diethyl ether (0.2 mL). The precipitate was dried to form a white powder of 15 mg and was found to be having single solute at RF 0.72 using chloroform: Methanol (60:40) as a mobile phase and vanillin sulphuric acid as spraying agent . The isolated compounds were identified by phytochemical test, melting point, TLC analysis and structural elucidation were characterized by using IR, NMR & LC-MS Spectral studies (Fig 4, 5, 6, 7 & 8) & (Table 4).

Table 4: Characterization of isolated molecules

Compound name & Physical state	Phytochemical Test	Melting Range	R_F (cm)	Characterization			
				IR	^{1}H NMR	^{13}C NMR	LC-MS
Novel Saponin glycoside (CH1) Buffy crystalline powder soluble in DMSO & water	+ Ve For Molisch, legals test and foam test	212°c to 214°c	 **(0.72)**	3441(O-H str) 2922(C-H str) 2852(C-H str) 1535(C-C str) 1020 (C=O str	δ0.96(M) (CH3) δ1.06(Q) (CH3) δ1.11(D)(CH3) δ1.46(T) (CH)	δ 14.06 (CH3) δ 87.15 (CH) δ 74.9 (CH) δ 103 (CH) δ143.06 (C) δ 119.28(CH)	(M-1) Peak at –ve mode 507.36

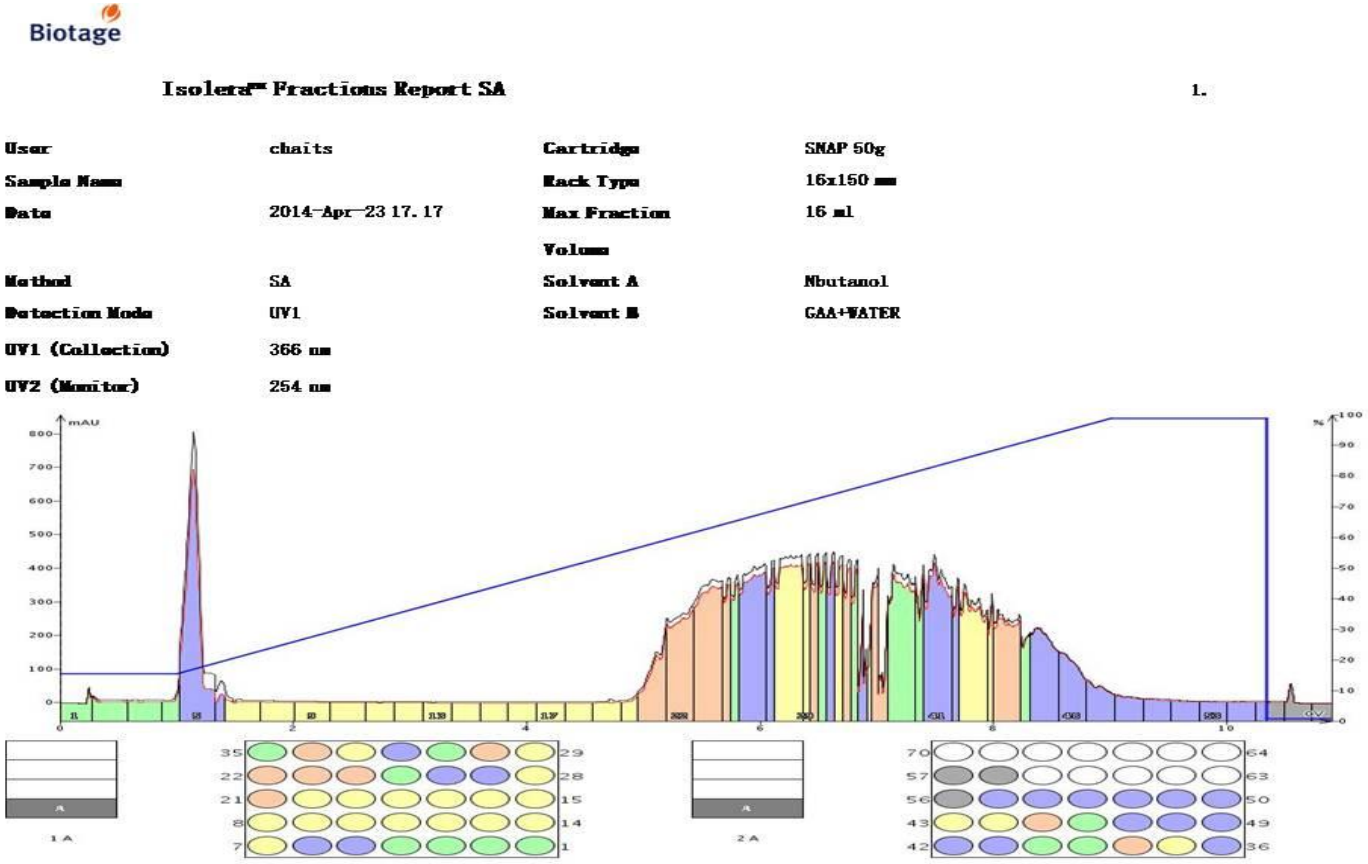


Fig 4: Flash chromatogram report of *Solanum Mauritianum* SCOP fractions

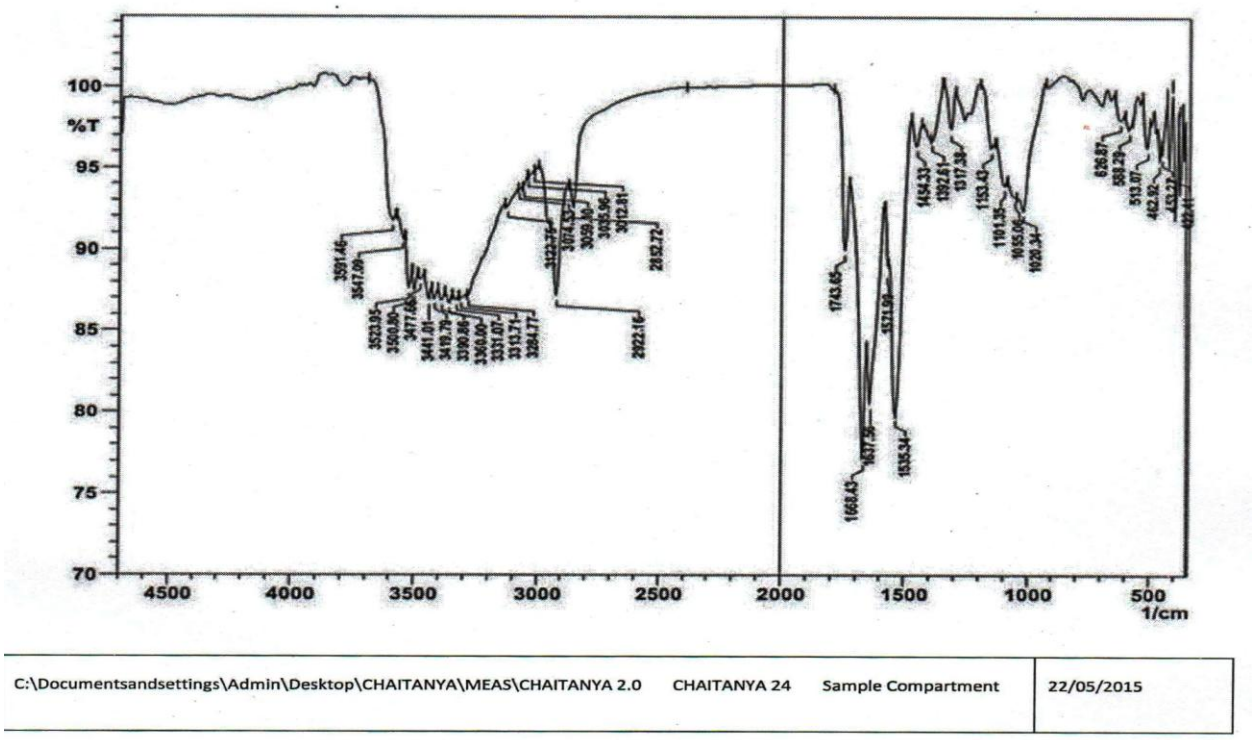


Fig 5: IR spectra of isolated compound (CH 1)

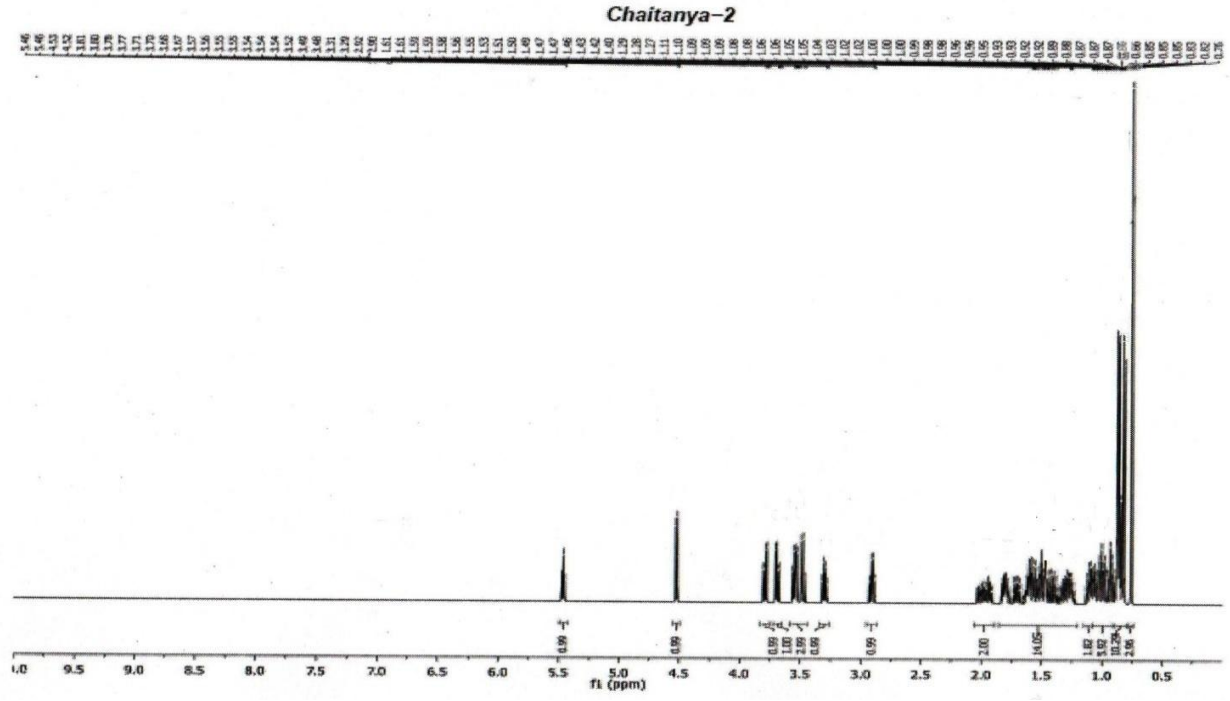


Fig 6: 1 H- NMR spectra of isolated compound (CH 1)

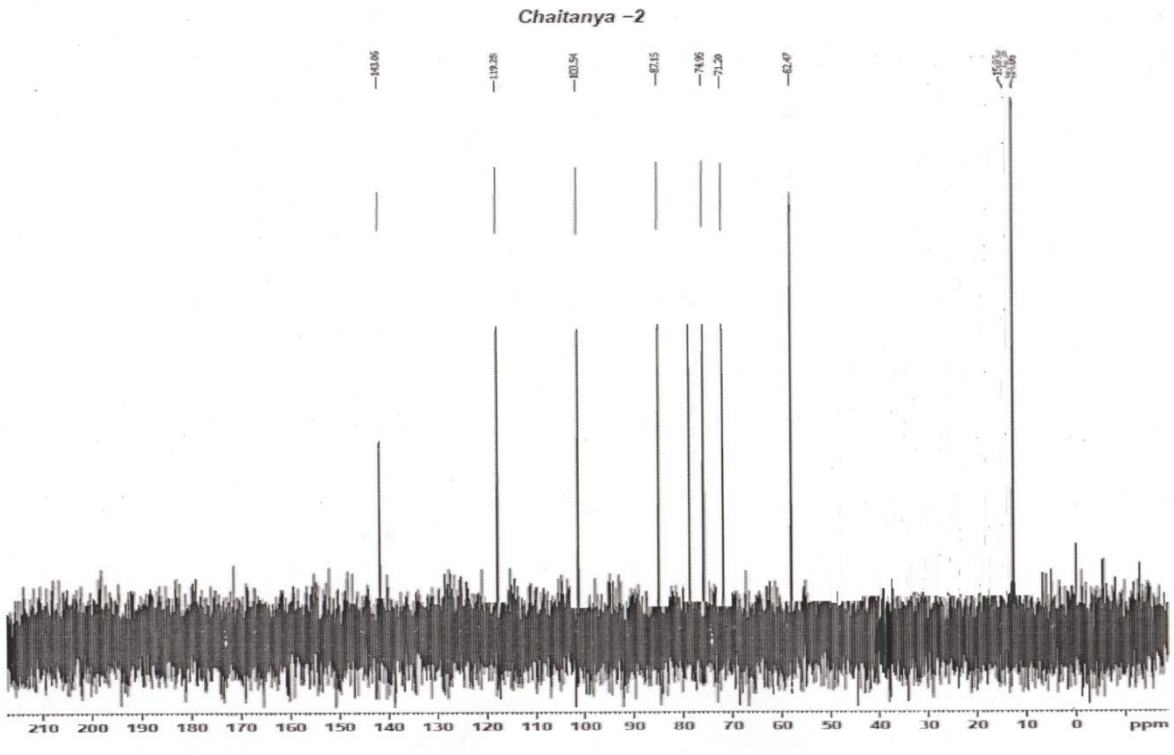


Fig 7: 13C - NMR spectra of isolated compound (CH 1)

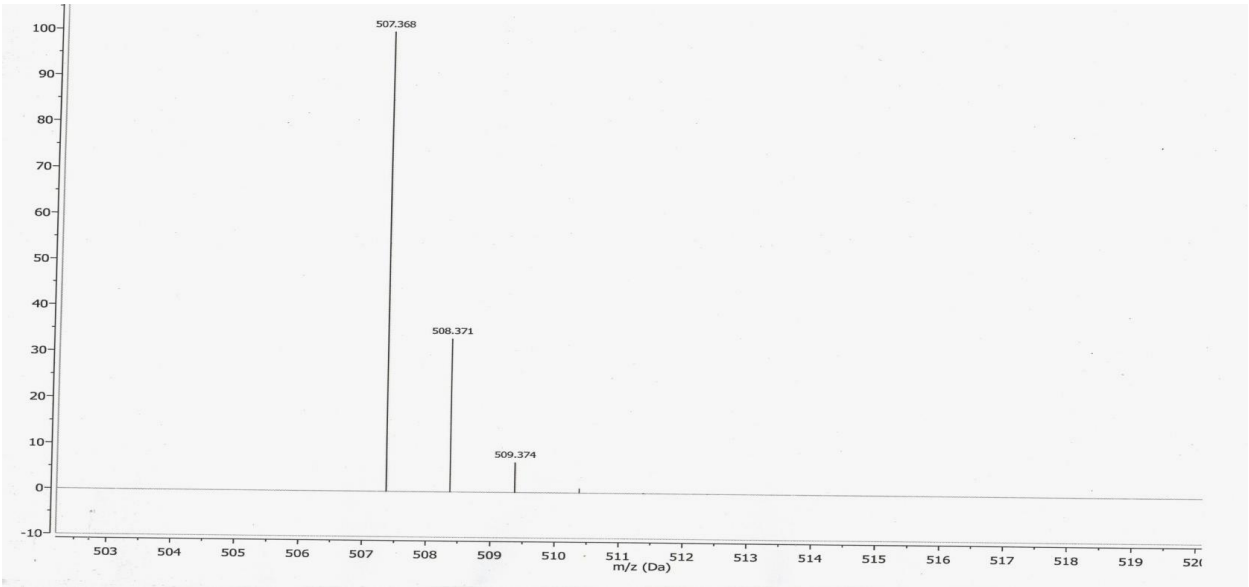


Fig 8: LC-MS spectra of isolated compound (CH 1)

***In-silico* molecular docking studies**

In-silico studies were carried in order to know the mechanism of action of the isolated compounds as anticancer agents in comparison to standard quericitin. All these compounds showed good g-scores against topo enzymes I & II. All these compounds are having good binding capacity and good ADMET profile. The results were discussed in the table 5.

Table 5: G-Scores from the glide XP docking run of standards& isolated compounds with 1K4T & 3AL2 (TOP-1), 1ZXN & 3QX3 (TOP-II).

S.NO	Name of the Compound	Topo-I enzyme Targets							
		1K4T				3AL2			
		G-Score	H-bond	Penalties	Epik-stael	G-score	H-bond	Penalties	Epik-stael
1.	Std quercetin	-6.7	-2.65	0	0.01	-4.95	-2.44	0	0.01
2.	Std Camptothecin	-4.49	-1.29	1	0	-3.84	-5.14	0	0
3.	CH1 (Saponin glycoside)	-4.23	-1.69	1	0	-2.94	-0.69	0	0
4.	Nocturnoside - B	-7.88	-5.18	0	0.01	-7.51	-4.88	0	0.01
5.	Protodioscine	-8.62	-6.31	0	0	-10.9	-6.92	0	0
6.	Parquisoside-B	-8.6	-5.35	0	0	-9.25	-5.56	0	0
7.	Solasodine	-2.2	-0.7	0	0.01	-1.98	0	1	0.01
8.	Solasonine	-8.04	-4.89	0	0.01	-8.11	-5.35	0	0.01
		Topo- II enzyme Targets							
		1ZXN				3QX3			
S.NO	Name of the Compound	G-Score	H-bond	Penalties	Epik-stael	G-Score	H-bond	Penalties	Epik-stael
1.	Std querceitin	-9.57	-4.65	0	0.01	-7.24	-2.29	0	0.01
2.	Std Salvicine	-7.05	-2.31	0	0	-6.90	-0.48	0	0
3.	CH1 (Saponin glycoside)	-7.15	-4.29	0	0	-	-	-	-
4.	Nocturnoside - B	-	-	-	-	-10.38	-4.87	0	0
5.	Protodioscine	-	-	-	-	-12.38	-5.95	0	0
6.	Parquisoside-B	-	-	-	-	-12.55	-5.76	0	0
7.	Solasodine	-3.95	-0.48	0	0.01	-	-	0	0.01
8.	Solasonine	-	-	-	-	-9.87	-3.78	0	0
		Quick PROP 3.4 predictions of ADMET for the compounds							
S.NO	Name of the Compound	CNS	Mol wt	Donor HB	Acceptor HB	QPlog HERG	Human oral Absorption	Rule of 5	Rule of 3
1.	Std quercetin	-2	302	4	5.25	-4.96	2	0	1
2.	Std Salvicine	-2	330	2	6.45	-4.80	3	0	0
3.	CH1 (Saponin glycoside)	-2	508	4	10.2	-4.73	3	1	1
4.	Nocturnoside - B	-2	1015	10	32.1	-5.94	1	3	2
5.	Protodioscine	-2	1049	13	35.5	-6.3	1	3	2
6.	Parquisoside-B	-2	1193	14	42.3	-6.8	1	3	2
7.	Solasodine	-1	413	2	3.45	-4.82	3	1	1
8.	Solasonine	-2	884	10	27.25	-6.28	1	3	2
S.NO	Name of the Compound	Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) with 1K4T (Topo-I) Enzyme Target							
1.	Std camptothecin	-91.5039							
2.	Std quercetin	-79.6387							
3.	Std Salvicine	-98.8745							
4.	CH1 (Saponin glycoside)	-128.7							
5.	Nocturnoside - B	-161.97							
6.	Protodioscine	-107.4							
7.	Parquisoside-B	-125.9							
8.	Solasodine	-89.15							
9.	Solasonine	-164.2							

CNS: predicted central nervous system activity on a -2 (inactive) to +2 (active) scales, Mol. wt: Molecular weight of the molecule, Donor HB: Estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution, AcceptHB: Estimated number of hydrogen bonds that would be accepted by the solute to water molecules in an aqueous solution, QP Log Her G- Rule of 5: Number of violations of Lipinski's rule of five & Rule of 3Number of violations of Jorgensen's's rule of three.

***In-vitro* antioxidant and anticancer studies**

In-vitro antioxidant studies using DPPH and SRB (Sulphorhodamine B) assays, In-vitro anticancer studies on Vero, A-549, MCF-7 & HCT-116 using SRB (Sulphorhodamine B) method were carried out using extracts, different fractions & isolated compounds in comparison to standard quericitin. The total saponin fraction and isolated compound (CH-1) showed good antioxidant and anticancer activities (Table 6). The anticancer activity of these compounds is may be due to inhibition of human topo isomerase enzymes I & II which is proved by molecular docking studies as discussed in Table 6.

Table 6: In-vitro antioxidant & Cytotoxic Screening of Total Saponin Fractions & Isolated compounds

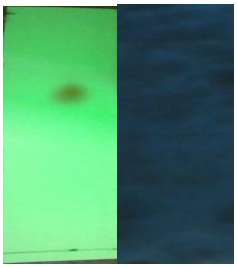
	Name of the Compound	Antioxidant assay IC_{50} Values (µg/ ml)		SRB assay CTC_{50} Values (µg/ ml ± SEM)				
				Cell lines				
		DPPH	ABTS	Concentration (µg/ml)	Vero	A-549	MCF-7	HCT-116
1.	*Solanum auriculatum* (Total saponin fraction)	101.7	55.0	1000 500 250 125 62.5	851.8±1.01	288.9±1.97	190±1.322	169.5±1.60
2.	Isolated Compound (CH1)	26.3	12.8	1000 500 250 125 62.5 31.25	641.3±0.88	53.78±1.78	64.666±1.2	72.833±0.60
3.	Std Quericitin	6.94	7.90	1000 500 250 125 62.5	390.6±0.53	155.3±0	36.25±0.14	20.0± 0.12

***In-vitro* wound healing studies**

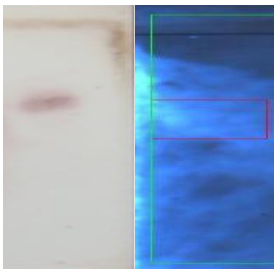
The isolated compound (CH-1) showed good wound healing activity at 26.5 µg/ml on HDF cell lines and the results showed in the Table 7. TLC Based Direct Bioautography against Methicillin Resistant *Staphylococcus aureus* (MCC 2408). The isolated compound (CH-1) showed good anti *staphylococcus* activity (Fig 9).

Table 7: *In-vitro* sratch assay on HDF cell lines

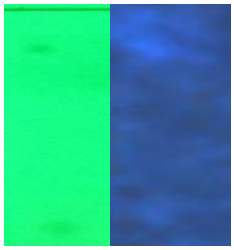
Time Interval	Compound (CH-1) 26.5 µg/ml
0 hr	
1 hr	
48 hr	



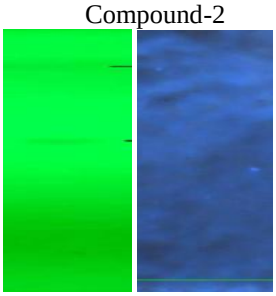
Compound-1



Compound-2



Compound-3



Compound-4

Fig 9: TLC based Bio-autography of isolated weed molecules

Human Dual topopoisioning activity

The isolated compound (CH-1) showed the isolated compound -2 and compound -4 showed Topo- isomerase I &II poisoning activity at concentration of 400 µg/ml in compare to standard camptothecin (50 µM) showed that these compounds are active at very high dose (Fig 10 11).

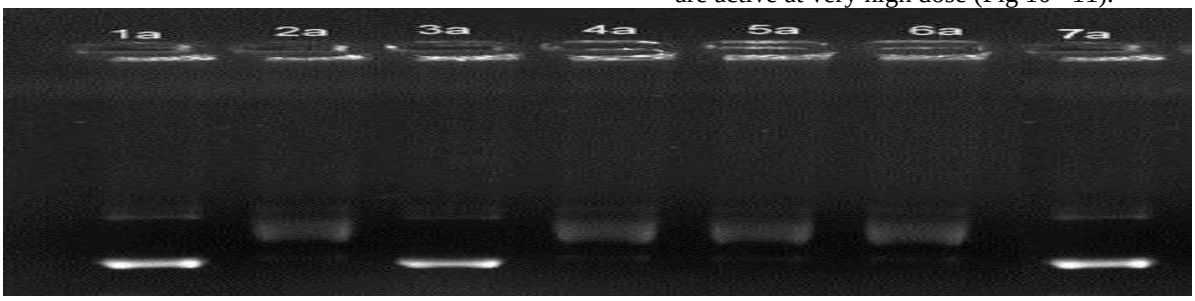


Fig 10: Topoisomerase- 1 inhibition assay of isolated weed molecules (Compound-2 & Compound-4)

Lane 1 (1a) : Undigested plasmid pBR329(shows supercoiled, nicked circular)
Lane 2 (2a): pBR 329 + Topoisomerase I (1 Unit) (Shows that the plasmid has been relaxed and the supercoiled form is lost)
Lane 3 (3a): pBR329 + Topo I + Camptothecin (50uM) – Positive control Camptothecin inhibits the action of Topo I and so the plasmid is as seen in the native form
Lane 4 (4a) : pBR329 + Topo I + 50ug/ml of compound 2 & 4
Lane 5 (5a): pBR329 + Topo I + 100ug/ml of Compound 2 & 4
Lane 6 (6a): pBR329 + Topo I + 200ug/ml of compound 2 & 4
Lane 7 (7a): pBR329 + Topo I + 400ug/ml of compound 2 & 4
Lane (4-6a) shows the plasmid to be relaxed, as there is no effect of the test compound on the action of Topoisomerase I.
Lane (7a) shows that compounds (2 &4) inhibiting the action of Topo I and so the plasmid is seen in the native form, but in very high concentration in compare to standard camptothecin, hence the compounds have to take for extensive studies for modification of structure. However these compounds can be a lead sources to develop novel topopoisons.

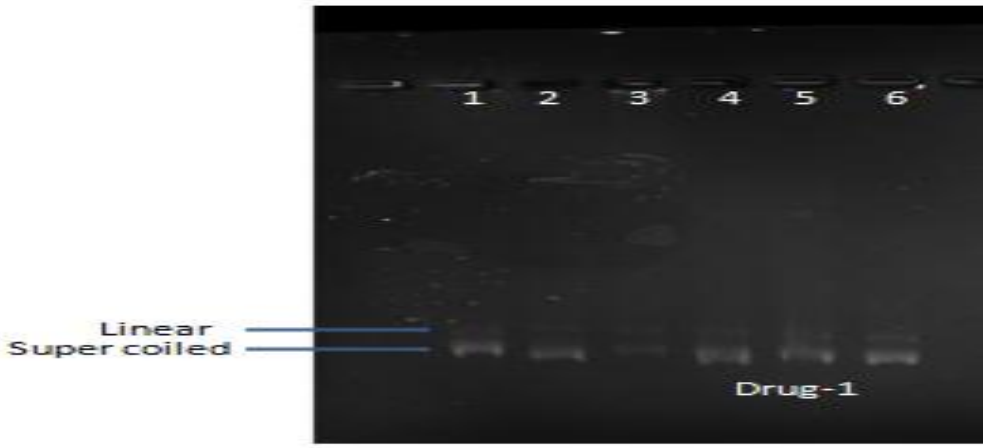


Fig 11: Topoisomerase- II inhibition assay of isolated weed molecules (Compound-2 & Compound-4)

Lane 1 : Undigested plasmid pBR329(shows supercoiled, nicked circular)
Lane 2 : pBR 329 + Topoisomerase II (4 Units)
Lane 3 : pBR329 + Topo II + Etoposide (600uM) – Positive control Etoposide inhibits the action of Topo II and so the plasmid is as seen in the native form
Lane 4 : pBR329 + Topo II + 100ug/ml of compound (Lysis started)
Lane 5 : pBR329 + Topo II + 250ug/ml of Compound (moderate lysis)
Lane 6 : pBR329 + Topo II + 400ug/ml of compound(moderate lysis)

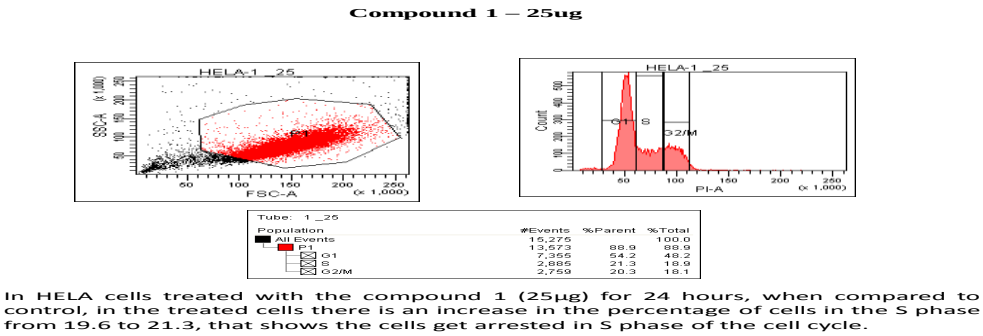


Fig 12: Cell cycle analysis of compounds 2

Cell Cycle Analysis

HELA cells treated with the isolated molecule II (25µg) for 24 hours, shows the cells get arrested in S phase of the cell cycle (Fig 12).

The selected plant *Solanum Mauritianum* SCOP is predominantly distributed unexplored weed of the Niligiris region. The selected plant has richest sources of phenolics, saponins , glycosides & Alkaloids. Hence the plant is selected for the phytochemical isolation of novel anticancer leads. The total saponin fraction which is isolated from this plant possess good antioxidant and cytotoxic activity. Based on the chemotaxonmic significance of saponins. The total saponin fraction isolated was subjected to LC-MS analysis. Based on the mass profiling, it is found that the total saponin fractions contains many important saponins, out of which few known saponins and saponin alkaloids structures were traced out .

First time a novel compound (CH-1) was isolated from this plant by using a flash chromatography technique which is a simple, economical and fastest method. The developed method can be used by natural product scientists for the isolation of saponins. Based on the docking scores, the isolated compound CH1 and identified saponins (Nocturnoside – B, Protodioscine, Parquisoside-B, Solasodine & Solasonine) present in the total saponin fraction proved that they are very good dual human topo poisons I & II which is an essential criteria on

which the current anticancer drug discovery is dependent on. The ADMET studies proved that these compounds are safe and Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) studies prove that these molecules having good binding surface areas against target enzyme. The isolated compound (CH-1) showed dual topo-poison activity at higher concentration and the cell cycle arrest at S-Phase on HeLA cell lines at 25 µg/ml.

CONCLUSION:

Based on the biological screening and molecular docking studies, the isolated and identified saponins proved that they are very good dual human topoisomerases I & II. The cytotoxic activity of the plant is may be due to this dual topo poison activity that may lead to cell apoptosis. As there is a current demand for these novel dual human topo poisons, the selected weed may be having a richest source of many secondary metabolites which can be a good dual human topo poisons I & II. The isolated saponins can be a good lead molecules in anticancer drug discovery. However, further in-depth studies have to be carried out on these identified molecules and the plant in order to prove its clinical significance as good anticancer drugs or plant and the work is in progress in our research laboratory. In fact this plant is explored phytochemically and biologically by our team and found that this plant and molecules may fill the lacuna in the cancer drug discovery which may came out with novel human dual topo poisons. The isolated molecules can serve as lead molecules towards anticancer drug discovery. The current research work may give a platform for the discovery of novel dual human topo I & II isomerases,where there is a current demand in the anticancer research. However the structre of the molecule have to be taken for extensive studies inorder to increase the clinical efficacy of the isolated molecule

COMPETING INTERESTS:

The authors declare that they have no competing interests.

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