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# Phytochemical Evaluation, Molecular Docking, ADMET Properties and Anticancer Activity of Ethanolic Extract of *Solanum trilobatum* Leaf Extract

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In this study, the anticancer potential of ethanolic extract of *Solanum trilobatum* leaf, which contained high quantities of alkaloids, terpenoids, steroids and flavonoids, was investigated. The GC-MS analysis detected 30 phytocompounds in the ethanolic extract and according to the Pubchem database, 10 of these phytocompounds have significant biological activity. When using AutoDock Vina, docking investigations were conducted using the cancer cell line proteins from the colon (5FGK) and cervical (4J96), respectively. ADMET predictions were made *via* SwissADME and the pkCSM online webserver. According to the molecular docking data, the phytocompound stigmasterol has better anticancer activity than reference drugs. Molecular docking studies found that stigmasterol had a higher docking score (-10.8 kcal/mol for 5FGK and -8.0 kcal/mol for 4J96) and more binding interaction than camptothecin. Silica gel column chromatography was used to isolate stigmasterol, which was afterwards characterized by IR, PMR, CMR, mass spectroscopy and elemental analysis. MTT tests comparing stigmasterol revealed the moderate efficacy ($IC_{50}$ = 83.14 µg/mL HeLa & 115.23 µg/mL HCT116) than the reference drugs camptothecin ($IC_{50}$ = 38.07 µg/mL HeLa & 72.51 µg/mL HCT116). These results indicated stigmasterol as a potential therapeutic option which is compatible with the traditional use of plant and and these findings have been supported by molecular docking and ADME properties.

**Keywords: *Solanum trilobatum* leaf, Ethanol extract, GC-MS analysis, Molecular docking study, ADMET, Anticancer activity.**

## INTRODUCTION

Cancer is a type of aberrant tissue growth in which cells divide uncontrollably and autonomously, resulting in a gradual increase in the number of dividing cells [1]. Chemotherapy, radiation and chemically derived medications are all current treatments. Chemotherapy, for example, can put people under a lot of stress and harm their health even worse. As a result, there is a focus on alternative cancer treatments and therapies [2]. Despite numerous efforts, multidrug resistance remains a significant disadvantage in cancer chemotherapy, which has recently been the topic of extensive research [3]. Demand for anticancer therapy is increasing [4]. For thousands of years, nature has provided medicinal agents and an astounding number of modern medications have been obtained from natural sources, many of which are used in traditional medicine [5]. Medicinal plants are a vital source of life-saving pharmaceuticals for the world's ever-growing population. Traditional medicine plays a crucial role in providing healthcare to underdeveloped countries [6]. Plant secondary metabolites have been shown to be a great source of novel medicinal molecules [7]. The discovery and identification of new anticancer drugs with minimal immune system adverse effects has been an important target in many immunotherapy studies [8]. Traditional medicinal plants produce a number of chemicals with proven therapeutic qualities [9,10]. For decades, plants have been used to produce strong anticancer medications. The search for anticancer medications from plants began in the 1950s, with the discovery of vinca alkaloids (vinblastine and vincristine) and podophyllotoxin. This search lasted four decades, ending in the 1990s with the introduction of taxanes and camptothecins as anticancer medications.

Plants have a crucial part in the health of millions of people in many Indian communities through traditional use. *Solanum trilobatum*, a member of the Solanaceae family, has been found to treat a variety of ailments, including respiratory issues and bronchial asthma. *S. trilobatum* was shown to have hepatopro-

tective, antibacterial, larvicidal, antidiabetic, cytotoxic and anticancer properties. *S. trilobatum*'s leaves and stem have been shown to have antimitotic, anti-inflammatory and antiulcerogenic effects. The leaf extracts are used to promote male fertility and treat snake poison [11]. Biological screening of *S. trilobatum* indicated anticancer potential against specific types of cancer as well as usefulness as an adjuvant in cancer chemotherapy [12]. The dried powder of *S. trilobatum* leaves contained carbohydrates, saponins, phytosterols and tannins, whereas the stem portion contained carbohydrates, saponins, phytosterols, tannins, flavonoids, phenol and glycosides [13]. From the aforementioned fact, the phytochemical evaluation, molecular docking, ADMET features and anticancer activity of an ethanolic extract of *Solanum trilobatum* leaf are investigated.

## EXPERIMENTAL

**Plant material:** *Solanum trilobatum* leaves were collected from Vilavancode city, India, in June and July 2023. Prof. Dr. C. Babu, Department of Botany, Pioneer Kumaraswamy College, Nagercoil had recognized and validated this plant specimen. The leaves were thoroughly rinsed and washed before being dried at room temperature for 7-8 days. The plant leaves were carefully ground into a powder and kept in an airtight container for future use.

**Extract preparation:** The ethanolic extract was prepared by soaking 50 g of dried *S. trilobatum* leaves in 250 mL of ethanol using a Soxhlet extractor. The extraction process in a Soxhlet loop continues until the solvent becomes colourless [14]. The extracts were concentrated at room temperature, allowing the solvent to evaporate. The solvent was then kept in airtight containers and refrigerated at 4 ºC for subsequent use [15]. The extracts were combined and the solvent was evaporated at 40 ºC using a rotary evaporator working at reduced pressure.

**Phytochemical analysis:** The phytochemical assays were carried out on *Solanum trilobatum* leaves using the reported standard procedures [16]. To determine the extracts' component profiles, a number of qualitative and quantitative chemical tests were conducted.

**GC-MS analysis:** The phytochemical analysis of *S. trilobatum* was performed using GC-MS technique (Shimadzu GC MS QP2020). This system contained an autosampler, injector, gas chromatograph (GC-2010) and mass spectrometer. The GC-MS system utilized a capillary standard non-polar column SHRxi-5Sil-MS with dimensions of 30 m length, 0.25 mm diameter and 0.25 µm film thickness. The analysis employed an electron ionization equipment with a 70 eV electron ionization energy. The injection volume was 5 µL, with the carrier gas being 99.99% pure helium flowing at a rate of 1.20 mL/min with a split ratio of 10. The temperature program in the GC oven started at 50 ºC and was held isothermally for 2 min before ramping up to 280 ºC over 10 min. The mass spectra were recorded at 70 eV with a 0.3 s scan interval in the 50-500 *m/z* range. The entire GC analysis took 21 min to complete. In the quantitative analysis, the percentage of each detected component was calculated by dividing the component's average peak area by its overall peak area. Analysis and interpretation of mass spectra and chromatograms were performed using Shimadzu GC-MS real-time software.

**Identification of components:** The extensive data banks of the National Institute of Standards and Technology (NIST) [17] and WILEY [18] were used to study GC-MS mass spectra. These libraries give a diverse set of patterns for comparison. The mass spectra of the unidentified compounds were compared to those of recognized components from the NIST and WILEY database. Each constituent found in the test sample was thoroughly examined, including nomenclature, structural configuration, molecular weight and molecular formula.

**Molecular docking analysis:** Using AutoDock Vina, the proteins from the Colon (5FGK) and Cervical (4J96) cancer cell lines were docked with the chemical components of a plant extract. Initially, ChemDraw 8.0 from the Chem Office software was used to create chemical structures of phytocompounds and assign appropriate 2D orientations. ChemBio3D was then employed to lower the energy of each chemical compound. The ligand structures were used as input for AutoDock Vina docking simulations [19-21]. Crystal structures of the colon and cervical receptor molecules were retrieved from the Protein Data Bank using the IDs 5FGK and 4J96, respectively. The target protein file was generated using AutoDock 4.0's auto preparation feature (MGL tools 1.5.7), which retained the associated protein residue. Protein preparation followed a standard procedure [22], which comprised the removal of co-crystallized ligands, specific water molecules and cofactors. The docking simulation settings were defined using a graphical user interface tool that generated a grid box. The grid box had dimensions of 30, 30 and 30 grid points in the x, y and z-directions, with a 0.375 grid point spacing. The grid dimensions for the colon protein (5FGK) were -6.278197, -19.093869 and 148.253541, while those for the cervical protein (4J96) were 37.442917, 11.807821 and 22.762488. The docking algorithm provided by AutoDock Vina was used to determine the optimal docked configuration between ligands and proteins. During the docking process, each ligand had up to nine conformers investigated. PyMOL and Discovery Studio Visualizer were then used to investigate the interactions between ligands and target receptors. The conformations with the lowest free binding energy were chosen for analysis, with interacting residues and hydrogen bonds depicted in stick models and ligands represented in different colours.

***In vitro* Anticancer activity:** The MTT assay was used to determine anticancer activity *in vitro*. Dispense 200l of cell suspension into each well of a 96-well plate, aiming for 20,000 cells per well. The test agent should be excluded from the initial seeding and the cells were incubated for around 24 h. After the incubation period, the recommended concentrations (20, 40, 60, 80, and 100 µg/mL) of the selected test agents into individual wells were introduced. Incubate the entire plate for an additional 24 h under controlled conditions of 37 ºC and 5% $CO_2$. After an additional incubation period, carefully remove the plates from the incubator. Discard the previously used culture media and introduce the MTT reagent into each well, with the objective of achieving a final concentration of 0.5 mg/mL, taking into account the entire volume. Aluminum foil should be wrapped around the plate to create a shield against light

and then incubated for another 3 h. After the third incubation phase, remove the MTT reagent and added 100 µL of solubilization solution (DMSO) to each well with gentle agitation using a gyratory shaker. Under exceptional conditions, especially in the densely populated cultures, it may be necessary to gently pipette the fluid up and down to achieve full disintegration of MTT formazan crystals. Determine absorbance at a wavelength of 570 nm using a spectrophotometer or an ELISA reader [23].

To calculate the percentage of cell viability, the following equation was used:

$$\text{Cell viability (\%)} = \frac{\text{Mean absorbance of treated cells}}{\text{Mean absorbance of untreated cells}} \times 100$$

For determining the $IC_{50}$ value, perform a linear regression analysis using the equation: Y = Mx + C, where the value of Y is 50% and the coefficients M and C are derived from the viability graph [24].

**ADMET studies:** Analyzing the pharmacokinetic parameters of potential drug candidates is critical in the early phases of drug research. The absorption, distribution, metabolism, excretion and toxicity of chemicals, known as ADMET, were determined utilizing online database servers pkCSM and SWISS ADME. The selected ligands' SMILES formats were generated from the PubChem database and submitted to the Swiss ADME software of the Swiss Institute of Bioinformatics (http://www.sib.swiss) and the pkCSM software (http://biosig.unimelb.edu.au/pkcsm/prediction). The web servers give trustworthy information to analyze physico-chemical parameters such as pharmacokinetics, water solubility, lipophilicity, toxicity and drug-likeness [25]. The drug-likeness of compounds was determined using the 'Lipinski's Rule of Five' [26], which indicates the oral bioavailability of selected ligands.

## RESULTS AND DISCUSSION

**Qualitative phytochemical analysis:** Table-1 displays the results of qualitative phytochemical tests on ethanolic extracts of *S. trilobatum* leaves. The ethanol extracts of *S. trilobatum* medicinal plants exhibited the substantial levels of terpenoids, alkaloids, steroids, phenolic, tannin, saponin, flavonoids, trace levels of glycosides, *etc*.

**GC-MS analysis of plant extract:** The GC-MS chromatogram of ethanolic extract of *S. trilobatum* contains 30 peaks (Fig. 1), indicating the presence of 30 compounds and chosen 10 of these compounds that appeared anticancer, such as lauric acid, loliolide, isochiapin B, stigmasterol, hexadecanoic acid, phytol, oleic acid, stearic acid, linoleic acid and α-tocopherol, as shown in Table-2.

TABLE-1
PHYTOCHEMICAL ANALYSIS OF ETHANOL PLANT EXTRACT

| Phytochemical | Screening result for ethanolic extractants |
|---|---|
| | *Solanum trilobatum* |
| Protein (Millon's test) | +++ |
| Carbohydrate (Molisch's test) | ++ |
| Phenol (Ferric chloride test) | +++ |
| Tannins | +++ |
| Flavonoid (Alkaline reagent test) | ++ |
| Saponins (Foam test) | ++ |
| Glycosides (Salkowski's test) | + |
| Steroids (Chloroform test) | ++ |
| Terpenoids (Chloroform test) | +++ |
| Alkaloids (Wagner's test) | +++ |
| Reducing sugar (Fehling's test) | + |

Note: + = present in small concentration; ++ = present in moderately high concentration; +++ = present in very high concentration

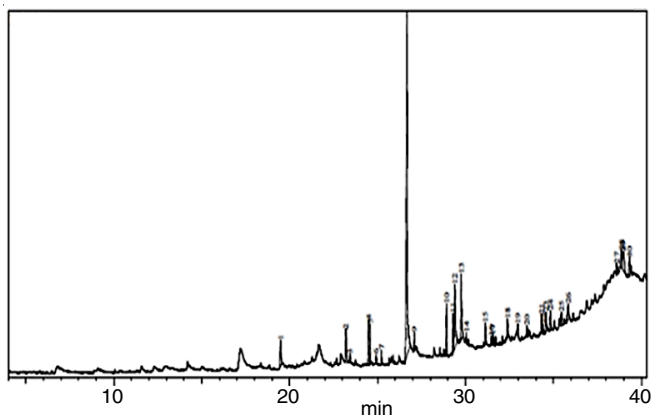


Fig. 1. GC-MS chromatogram for ethanolic extracts of *S. trilobatum*

**Molecular docking analysis:** The protein structures (Fig. 2) are depicted in the molecular docking analysis, along with their PDB IDs: 4J96 and 5FGK. Table-3 shows the docking scores and binding interactions for both standard compounds and ligands found in *S. trilobatum* (Fig. 3). The ligands targeting cervical and colon cell lines had binding affinities of -5.0 to -8.0 kcal/mol and -5.2 to -10.8 kcal/mol, respectively. Notably, standard camptothecin showed binding affinities of -9.5 kcal/mol and -10.2 kcal/mol for cervical and colon cancer cells,

TABLE-2
ISOLATION OF COMPOUNDS IN ETHANOL EXTRACT OF *Solanum trilobatum* BY GC-MS ANALYSIS

| Retention time | Peak area (%) | Name of the compound | m.f. | m.w. | Name of phytocompounds |
|---|---|---|---|---|---|
| 19.480 | 2.47 | Lauric acid | $C_{12}H_{24}O_2$ | 200 | Saturated fatty acids |
| 23.422 | 0.77 | Loliolide | $C_{11}H_{16}O_3$ | 196 | Monoterpenoid lactone |
| 32.974 | 1.17 | Isochiapin B | $C_{19}H_{26}O_6$ | 350 | Sesquiterpen lactone |
| 29.392 | 7.10 | Oleic acid | $C_{18}H_{34}O_2$ | 282 | Mono unsaturated fatty acids |
| 24.484 | 2.93 | Neophytadiene | $C_{20}H_{38}$ | 278 | Sesquiterpenoides |
| 27. 09 | 1.11 | Stearic acid | $C_{18}H_{36}O_2$ | 284 | Saturated fatty acids |
| 26.673 | 40.44 | *n*-Hexadecanoic acid | $C_{16}H_{32}O_2$ | 256 | Saturated fatty acids |
| 29.298 | 3.52 | Linoleic acid | $C_{18}H_{32}O_2$ | 280 | Polyunsaturated fatty acids |
| 35.850 | 2.27 | α-Tocopherol | $C_{29}H_{50}O_2$ | 430 | Antioxidant |
| 38.980 | 3.50 | Stigmasterol | $C_{29}H_{48}O$ | 412 | Tetracyclic triterpenes |

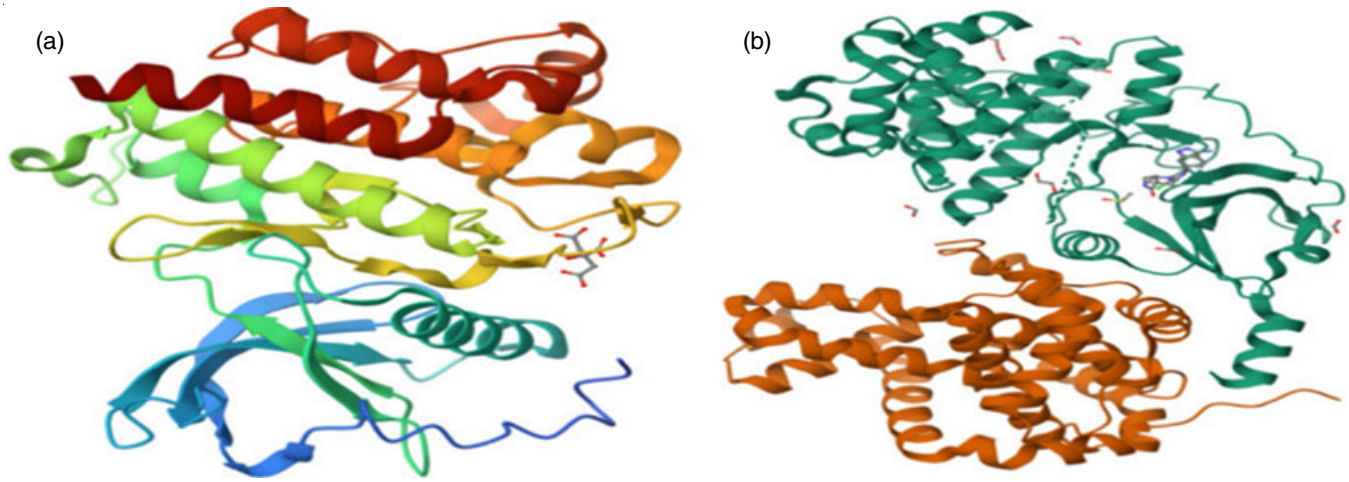


Fig. 2. Crystal structures of cervical and colon proteins (a) 4J96, (b) 5FGK

TABLE-3
BINDING ENERGY VALUES OF *Solanum trilobatum* ETHANOLIC LEAF EXTRACT PHYTOCOMPOUNDS TO TARGETED CERVICAL CANCER AND COLON CANCER CELL LINE PROTEINS

| Name of the compound | Binding interaction | | | Cervical cancer |
|---|---|---|---|---|
| | Hydrogen | Electrostatic | Hydrophobic | |
| Lauric acid | LEU A:647, GLY A:646 & ASP A:626 | | PHE A:492 | -5.0 |
| Loliolide | ASP B:626, ALA B:491 & PHE B:492 | ARG B:630 | – | -6.5 |
| Isochiapin B | ASP B:644, ASN B:571 & LEU B:487 | MG B:805 | – | -6.8 |
| Oleic acid | ASP B:522 & ARG A:630 | – | LEU A:647 & PHE A:492 | -5.0 |
| Neophytadiene | – | – | ALA B:643, LEU B:633, VAL B:495, LYS B:517, LEU B:487 & VAL B:564 | -5.8 |
| Stearic acid | ASP B:644 & GLU B:534 | – | ALA B:515, ALA B:567, LEU B:487, TYR B:566, LEU B:633, VAL B:495 & ALA B:643 | -5.7 |
| *n*-Hexadecanoic acid | LEU A:647, GLY A:642 & ASP A:626 | – | ARG A:664 & PHE A:492 | -5.1 |
| Linoleic acid | ASN B:571 | – | TYR B:566, LEU B:487, ALA B:567, LEU B:633, VAL B:495, ALA B:515, ALA B:643, VAL B:564, ILE B:548, MET B:538 & LYS B:517 | -6.0 |
| α-Tocopherol | ASN A:571 | – | ALA B:643, ALA B:515, LEU B:633 & VAL B:495 | -7.8 |
| Stigmasterol | ALA A:491 | ASP A:527 | PHE B:492, ALA B:491 & VAL B:495 | -8.0 |
| Camptothecin std. drug | LYS517, ALA567, TYR566, ALA567 | - | LEU487, LEU633, VAL495, ALA515 | -9.5 |
| Name of the compound | Binding energy | | | Colon cancer |
| | Hydrogen | Electrostatic | Hydrophobic | |
| Lauric acid | ASN A:156 | – | VAL A:35, ALA A:50, LYS A:52, LEU A:158, PHE A:97, TYR A:32 & ILE | -5.2 |
| Loliolide | TYR A:32 | – | PHE A:97 | -7.1 |
| Isochiapin B | ARG A:356 | – | ALA A:100, ALA A:50, TYR A:32, PHE A:97, ALA A:172, LEU A:158 & TYR A:99 | -8.9 |
| Oleic acid | ALA A:100, ARG A:356 | – | PHE A:97, ILE A:79, VAL A:35, ALA A:172, LYS A:52 & ALA A:50 | -6.0 |
| Neophytadiene | – | – | PHE A:97, LEU A:70, PHE A:176, ALA A:172, ILE A:79, MET A:174, LYS A:52, TYR A:32, VAL A:35, ALA A:50, LEU A:158 & ALA A:100 | -6.9 |
| Stearic acid | ALA A:100 & ARG A & 356 | – | TYR A:32, LYS A:52, LEU A:158, VAL A:35, PHE A:97, ALA A:50, ILE A:79 & ALA A:172 | -5.5 |
| *n*-Hexadecanoic acid | ARG A:356 & ASP A:103 | – | TYR A:32, PHE A:97, ALA A:172, ILE A:79, ALA A:50, LEU A:158, LYS A:52 & VAL A35 | -5.7 |
| Linoleic acid | ALA A:100 | ARG A:356 | TYR A:32, VAL A:35, LYS A:52, ALA A:50, PHE A:97, ILE A:79, ALA A:172 & LEU | -6.7 |
| α-Tocopherol | ALA A:153 | – | TYR A:32, PHE A:97, LYS A:52, VAL A:35, ALA A:172 & ILE A:79 | -8.9 |
| Stigmasterol | LYS A:52 | – | HIS A:106, VAL A:27, VAL A:35, ALA A:50, ALA A:100, LEU A:158, ILE A:79, ALA A:172 & PHE A:97 | -10.8 |
| Camptothecin std. drug | LYS153, ASP173 | – | ALA172, ALA155, LEU158, ILE79 | -10.2 |

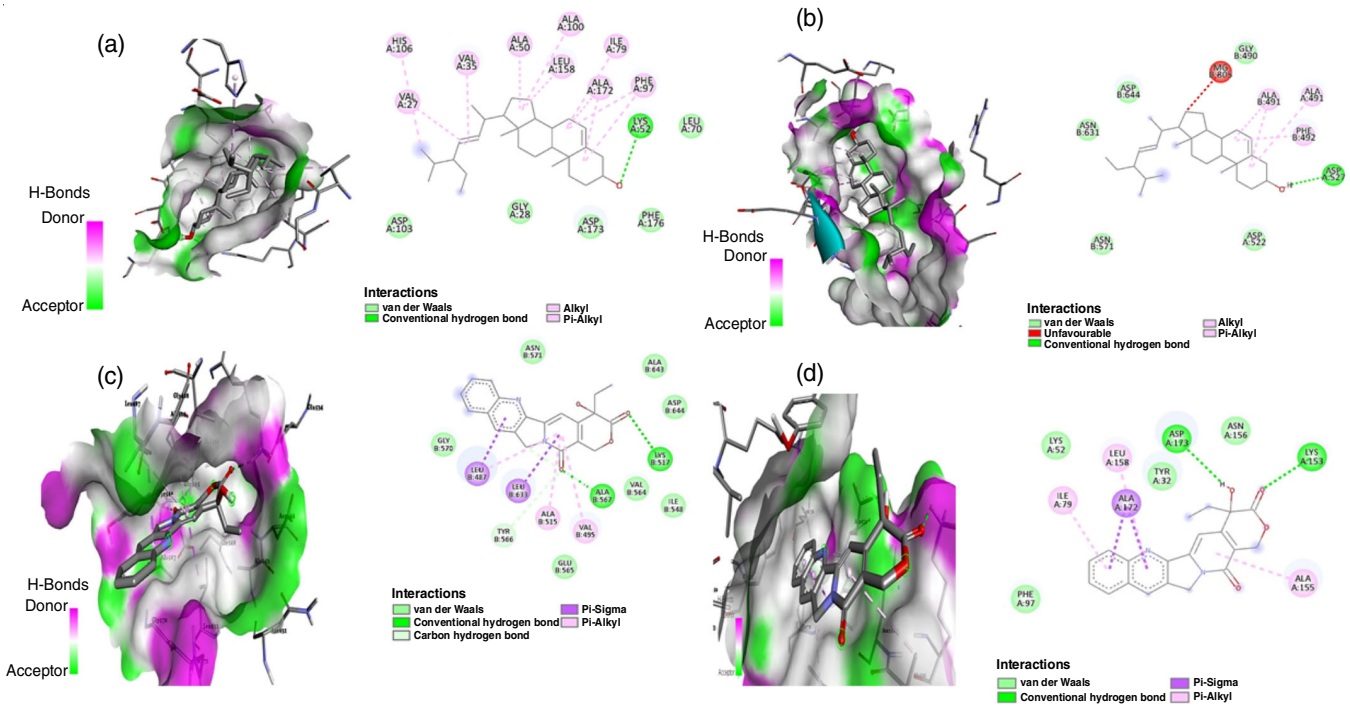


Fig. 3. 2D and 3D best interaction of molecular docking of stigmasterol with (a) cervical cell line (b) colon cell line and standard drug doxorubicin with (c) cervical cell line (d) colon cell line

respectively. Stigmasterol has proven to be the most potent inhibitor of these compounds and showed a strong inhibitory effects across the board, with binding affinities of -8.0 kcal/mol (4J96) and -10.8 kcal/mol (5FGK). Furthermore, the results of the molecular docking investigation revealed that stigmasterol has a high potential as an inhibitor of both cervical (4J96) and colon (5FGK) cancer cell line proteins.

***In vitro* anticancer evaluation:** The MTT method was used to determine the cytotoxicity of *S. trilobatum*. The anticancer activity was evaluated against a variety of cancer cell lines, *e.g.* HeLa and HCT116 cell lines and the results revealed that the viability (%) decreased as the quantity of isolated molecule increased. The results showed that stigmasterol inhibited many cancer cells in a dose-dependent manner. The percentage cell viability of the compound in cervical cancer varied dramatically depending on the concentration. On the HeLa cell line, the compound had a 45.04% viability at 100 µg/mL. However, at 20 µg/mL, the extract exhibited 74.34% viability. The percentage cell viability of stigmasterol in colon cancer changed significantly with concentration. On the HCT116 cell line, stigmasterol had a 49.84% viability at 100 µg/mL, however at 20 µg/mL, the extract showed 84.33% viability. Thus, both cytotoxic and anticancer in human colourectal adenocarcinoma (HCT116) cells, with an $IC_{50}$ value of 115.23 µg/mL after 24 h of incubation, as shown in Table-4. Camptothecin (20 µM/mL) served as a standard control for the study, with an $IC_{50}$ of 72.51 µg/mL. Figs. 4 and 5 illustrate the cytotoxicity of stigmasterol against the HeLa cell lines.

**Drug-likeness and oral bioavailability analysis:** The pharmacokinetic parameters of potential therapeutic candidates should be studied early in the drug discovery process. Lipinski and his team set the following criteria for drug-like compounds:

TABLE-4
% CELL VIABILITY VALUES AND OBSERVED $IC_{50}$ VALUE OF METHYLPREDNISOLONE ACETATE AGAINST HeLa AND HCT116 CELLS AFTER THE TREATMENT PERIOD OF 24 h

| Culture condition | % Cell viability (HeLa) | $IC_{50}$ conc. (µg/mL) (HeLa) | % Cell viability (HCT116) | $IC_{50}$ conc. (µg/mL) (HCT116) |
|---|---|---|---|---|
| Untreated | 100 | 83.14 | 100 | 115.23 |
| Std control (CPT-20 µM) | 38.07 | | 72.51 | |
| 20 µg/mL | 74.34 | | 84.33 | |
| 40 µg/mL | 63.78 | | 80.26 | |
| 60 µg/mL | 56.55 | | 75.59 | |
| 80 µg/mL | 50.90 | | 69.75 | |
| 100 µg/mL | 45.04 | | 49.84 | |

molecular weight (MW) < 500 Da, number of hydrogen bond donors (HBDs) < 5, number of hydrogen bond acceptors (HBAs) < 10 and octanol-water partition coefficient (Log P) < 5. There should only be one violation [27]. The isolated compound's HA, MW, HBD, HBA and Log P values are all within the range and no compound violated multiple rules. The Swiss ADME web application was used to determine the oral bioavailability and other physico-chemical parameters of the isolated medication and standards (Table-5). According to Daina & Zoete [28], the bioavailability radar quickly determines the significant physico-chemical features and drug-likeness of the selected compounds and standards.

The pink area in Fig. 6 represents the optimal positioning for each of the bioavailable qualities (LIPO, SIZE, INSOLU, POLAR, INSATU and FLEX). The isolated drug and standards LIPO (lipophilicity) were measured using the octanol-water partition coefficient (XLOGP3). The isolated compounds and conventional medicines must follow the Lipinski order of five

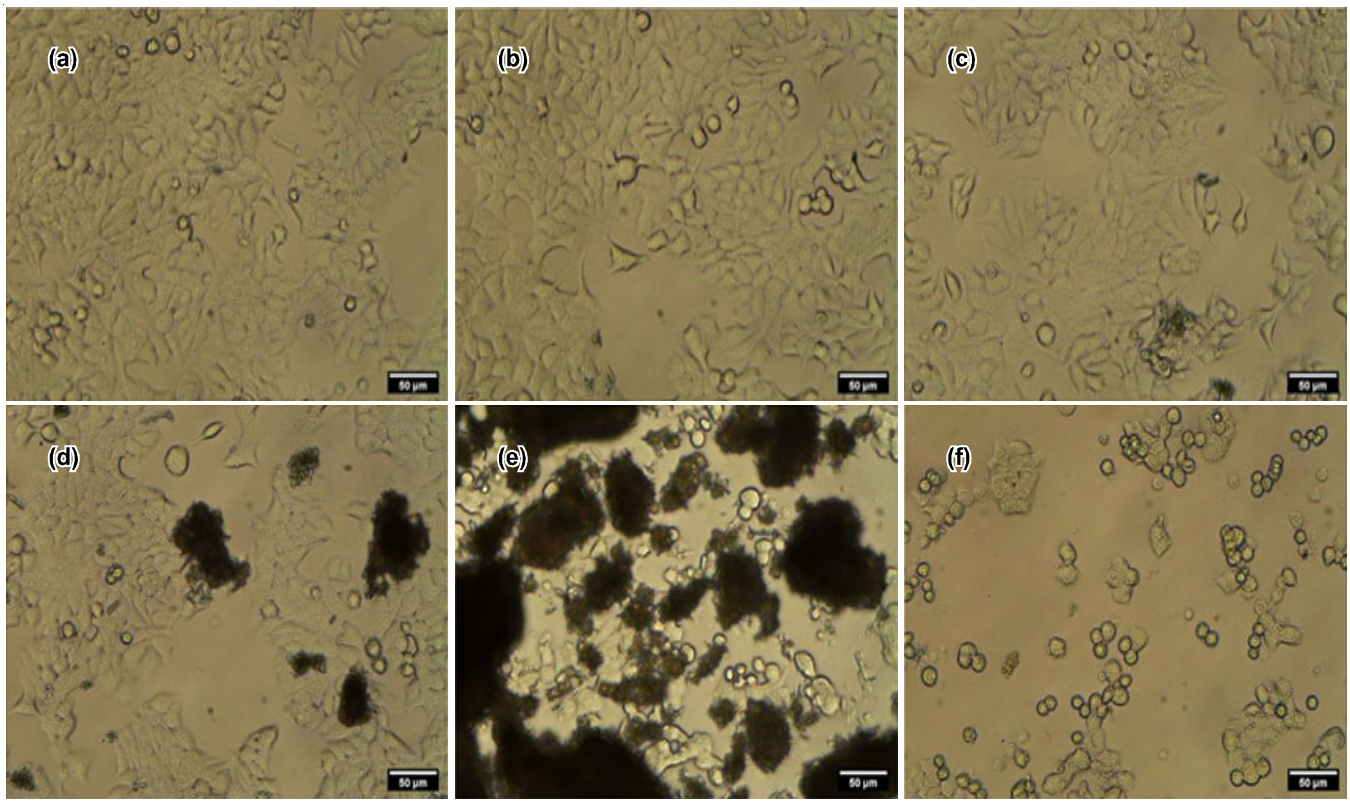


Fig. 4. Viability of HeLa cell line treated with stigmasterol in MTT assay (a) 20 µg/mL shows 74.34% viability, (b) 40 µg/mL extract shows 63.78% viability, (c) 60 µg/mL shows 56.55% viability, (d) 80 µg/mL shows 50.90% viability, (e) 100 µg/mL shows 45.04% (f) vehicle control shows 38.07% viability

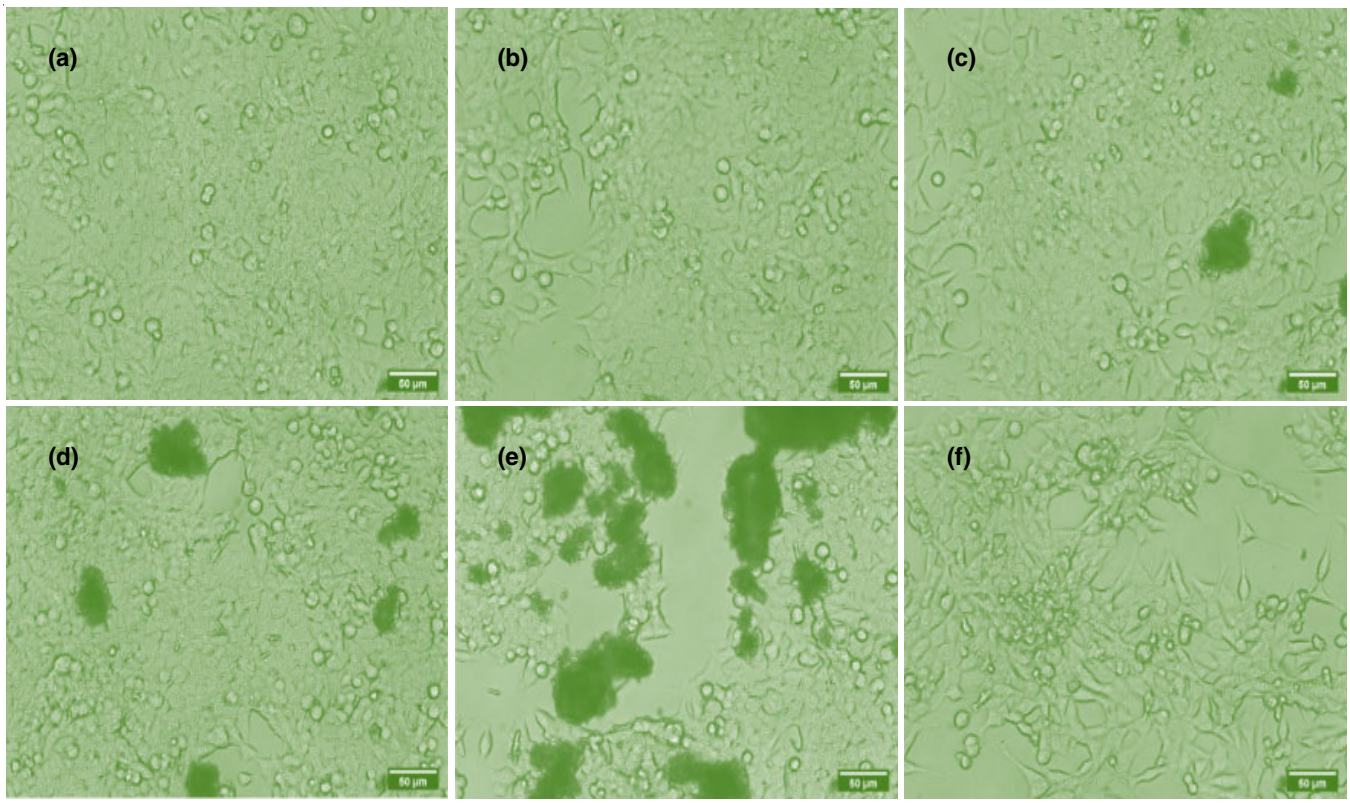


Fig. 5. Viability of HCT116 cell line treated with methylprednisolone acetate in MTT assay (a) 20 µg/mL shows 84.33% viability, (b) 40 µg/mL extract shows 80.26% viability, (c) 60 µg/mL shows75.59% viability, (d) 80 µg/mL shows 69.75% viability, (e) 100 µg/mL shows 49.84%, (f) vehicle control shows 72.51% viability

TABLE-5
*In silico* ADMET PROPERTIES

| Phyto compounds | Absorption | | | | Distribution | | |
|---|---|---|---|---|---|---|---|
| | Water solubility (Log mol/L) (Swiss Adme) | Intestinal human absorption (%) (Pkcsm) | Skin permeability (Log Kp) (Pkcsm) | TPSA (Å$^2$) (Swiss Adme) | VDSS (PKCSM) | BBB Permeability (Logbb) (Pkcsm) | CNS permeability (Logps) (Pkcsm) |
| Stigmasterol | -7.46 Poorly soluble | 94.97 | -2.783 | 20.23 | -0.178 | 0.771 | -1.652 |
| Camptothecin std. drug | -3.49 Soluble | 99.562 | -2.744 | 81.42 | 0.074 | -0.54 | -2.472 |

| Phyto compounds | Metabolism | | | | | Excertion | |
|---|---|---|---|---|---|---|---|
| | CYP1A2 (Swiss Adme) | CYP2C19 (Swiss Adme) | CYP2C9 (Swiss Adme) | CYP2D6 (Swiss Adme) | CYP3A4 (Swiss Adme) | Total clearance (Log ML/(Pkcsm) Min/Kg) (Pkcsm) | Renal Oct2 substrate (Pkcsm) |
| Stigmasterol | No | No | No | No | No | 0.618 | No |
| Camptothecin std. drug | No | No | No | No | No | 0.726 | No |

| Phyto compounds | Toxicity | | | | Bioavailability score (Swiss Adme) | Lipinski rule (Swiss Adme) |
|---|---|---|---|---|---|---|
| | H ERG I inhibitor (Pkcsm) | H ERG II inhibitor (Pkcsm) | Skin sensitization (Pkcsm) | Acute oral toxicity (Ld50 Mg/Kg) (Pkcsm) | | |
| Stigmasterol | No | Yes | No | 2.54 | 0.55 | 1 Violation |
| Camptothecin std. drug | No | Yes | | 50mg | 0.55 | No violation |

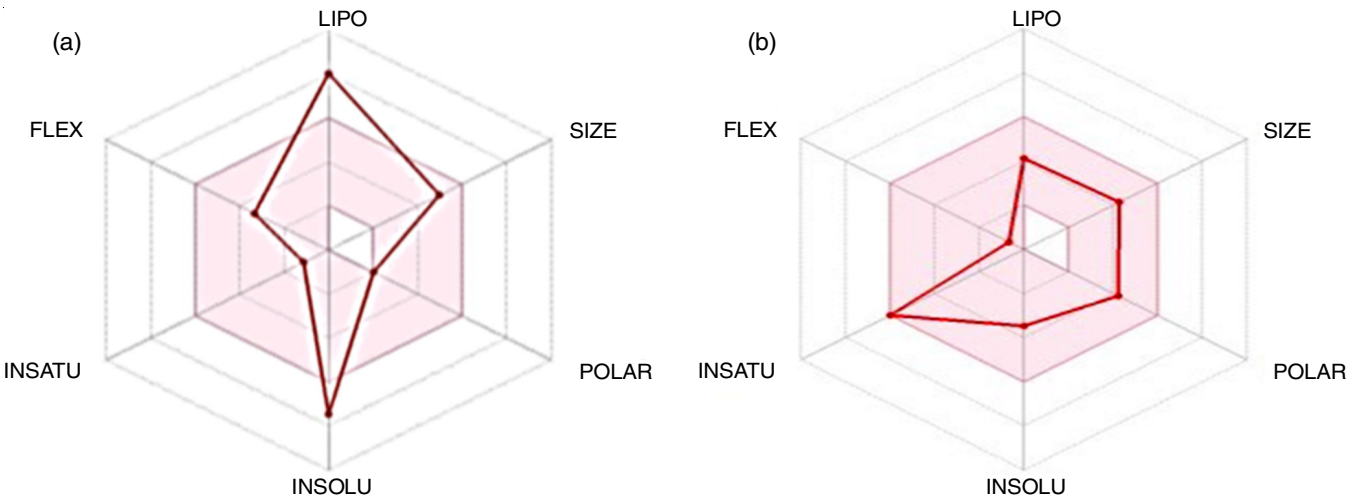


Fig. 6. Bioavailability radar picture of (a) stigmasterol and (b) camptothecin

(Ro5), which states that a potential drug candidate's SIZE (m.w.) should not exceed 500 g mol$^{-1}$. Stigmasterol was found to have good solubility, whereas standards camptothecin also had good solubility according to the isolated compound and standard INSOLU (insolubility) criteria, as indicated by their ESOL (Log S) and ESOL class. Total polarity surface area (TPSA), which has a range of values between 20 and 130 Å, was utilized to determine the POLAR (polarity) of the relevant chemicals and standards. Standards are used to calculate the proportion of carbon $sp^3$ (CSP3), which should be between 0.25 and 1, as well as the number of rotatable bonds, which should not exceed 9. These measures are utilized to quantify the isolated compound's unsaturation and flexibility, as well as to determine INSATU (unsaturation) and FLEX (flexibility). When compared to conventional camptothecin, the isolated compound was found to meet INSATU standards. In present work, stigmasterol has the highest oral bioavailability when considering its physico-chemical properties.

The ADMET properties revealed that isolated compound and antibiotics have a strong probability of being absorbed in the human stomach, with HIA + values of 94.97% and 99.6%, respectively. Stigmasterol exhibits superior 0 HIA values compared to the traditional drugs. Moreover, stigmasterol exhibits significant ability to cross the blood-brain barrier, making it a crucial pharmacokinetic characteristic for the development of medications. While standard drugs have near-negative BBB potential, this is unlikely to be a problem because the study's purpose isn't to find possible medications that target brain receptors in the same way as antipsychotics, antiepileptics and antidepressants work. Furthermore, the log S values of an isolated compound are within the expected range of a therapeutic molecule's water solubility, indicating that the chemical has a strong

absorption and distribution potential (-1 to -5). Additionally, the metabolic activity of the selected drugs choices was predicted using microsomal enzymes (cytochrome P450 inhibitors). Stigmasterol and standards have little impact on cytochrome P450 and hence show potential for the medical applications. In addition, the isolated compounds and standards have superior clearance and thus lack renal OCT2 substrate, resulting in a loss of potency within the body. On the other hand, isolated molecule VI (non-toxic) is used in the lead optimization stage of drug discovery, whereas normal drug class III is extremely toxic. It is important to observe that the standard prohibits access to the hERG II channel, as a result, stigmasterol represents a potentially safe and effective treatment candidate for the target receptor.

## Conclusion

The ethanolic extract of *Solanum trilobatum* yielded a significant array of highly active 10 compounds, with stigmasterol emerging as among the most significant separation. Stigmasterol has the largest overall inhibitory action of the medicines examined, as evidenced by its binding affinities of -10.8 kcal/mol for 5FGK and -8.0 kcal/mol for 4J96. Furthermore, the anticancer efficacy against two human cancer cell lines, colon and cervical, was comparable to the standard camptothecin. Stigmasterol had $IC_{50}$ values of 83.14 µg/mL for HeLa and 115.23 µg/mL for HCT116, indicating a possible role in the cancer treatment. According to the ADMET parameters, stigmasterol surpassed its competitors in terms of desirable drug-like properties. This chemical not only performed well in molecular docking experiments, but it also displayed outstanding biological activity, indicating that it could be a leading contender for therapeutic development. The convergence of molecular docking data, drug-likeness characteristics and ADMET analysis validates the experimental results. Overall, these results strongly suggest that the identified compounds from ethanolic extract of *S. trilobatum* leaf hold significant promise as possible therapeutic leads.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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