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RESEARCH ARTICLE

Investigation of Anti-cancer Potential of *Solanum virginianum* L. Fruit and Leaf Extracts by *In Vitro* and *In Silico* Studies

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Abstract

Background: *Solanum virginianum* L. is used as an ancient medicinal plant in *Ayurveda*. **Aim:** The present study aimed to elucidate the anticancer properties of *S. virginianum* through *in vitro* and *in silico* studies. **Methods:** The plant's fruits and leaves were subjected to hot extraction using methanol and water as a solvent. The cytotoxicity assay was carried out on MCF-7 (Adenocarcinoma breast cancer cell line) and HEK-293 (Human embryonic kidney cell line—a normal cell line). Cell migration inhibition assay and colony formation assay were performed conform on the MCF-7 cell line. The leaf aqueous extract was subjected to HR-LCMS/MS to evaluate different phytocompounds. The *in silico* study was performed by checking the binding affinity between NINE-selected phytocompounds and specific apoptotic target proteins. **Results:** Amongst all extracts, the leaf aqueous extracts showed promising results, as at the 72-h incubation period, the IC_{50} concentration was 10.4 ± 1.13 µg/mL. Leaf aqueous extract also showed significant results in cell migration inhibition assay and colony-forming assay as compared to fruits. Finding metabolites in aqueous leaf extract was done using HR-LCMS/MS, which revealed the presence of more than 30 phytocompounds. Nine selected phytocompounds were characterized for their ability to bind to specific apoptotic target proteins. Out of the selected compounds docked, ritterazine A showed very strong binding with a binding energy of above -9kcal/mol with all the target proteins. **Conclusion:** Based on this study, we can conclude that the leaf of *S. virginianum* has potent anti-cancer activities. Furthermore, there is potential for doing comprehensive research on the therapeutic applications of this plant in breast cancer treatment.

Keywords: Colony Formation, MTT, Migration Inhibition, Molecular Docking, Plant Extract, *S. virginianum*

1. Introduction

Over the last 30 years, breakthroughs in cancer therapeutics have occurred every year, but incidences and fatalities are also increasing. In therapeutic research, the heterogeneity of cancer creates new obstacles every time. Nevertheless, the recent scenario of cancer therapeutics and prevention calls for an in-depth understanding of molecular crosstalk leading to metastasis and tumorigenesis. The International Agency for Cancer Research Report Projects shows probable incidents of 24,500,000 and mortality of 12,500,000 by 2030[1]. The greatest recurrent types of cancer reported all over the world among men are lung, colorectal, liver, oesophagus and prostate. In contrast, breast, oral, colorectal, lung and cervical cancer are widespread among women[2].

Breast cancer is among the most lethal malignancies in women. It is well understood that genetic alterations promote cancer development. For example, tumour cells are formed when oncogenes are activated, and tumour suppressor gene pathways are altered. Tumor

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cells eventually lose control of cell growth signals, resulting in aberrant proliferation and the avoidance of apoptosis[3]. Researchers have been testing natural substances and their various constituents for breast cancer treatment to reduce toxicity and improve patients' survival. Breast cancer treatment has received exceedingly positive and encouraging results in such studies.

There has been a long history of medicinal plant use among Asian and African populations. Developed countries consume a variety of plants because of their health benefits. Either pure compounds or crude extracts provide unlimited opportunities for medicinal research. The main drawbacks of chemically derived drugs are resistance, toxicity, and adverse effects on microbiota; hence, recent medicinal research focuses on phytoconstituents as alternative synergistic medicine[4]. According to the World Health Organization (WHO), in a few countries, plant-derived compounds-based therapies remain the main ingredient of medicine, and there are developing countries that make use of naturally derived compounds in therapeutic treatments[5].

Solanum virginianum L. (Solanaceae family) is one of India's largest genera of flowering plants, with about 1500-2000 species. *S. virginianum* is a perennial herb with characteristics like bright green in colour and diffuse, prickly. It is commonly known as a yellow berried nightshade. In Gujarati, it is called Kantkari or Bhoyringani. This plant is available in overall regions of India, mostly in dry regions, roadside, and wastelands. Its root is used in Ayurveda, as in *Dashamoola*, while fruits are used in several infectious and inflammatory diseases[6]. The ancient Indian text *Materia medica* mentioned using *S. virginianum* leaves to cure many diseases like the decoction of the whole plant is used to cure gonorrhoea. Seeds and roots are useful to treat fever, asthma, cough and chest pain. The aqueous extract of the ripe fruits is used by tribal people in India as a conventional medicine to treat diabetic mellitus[7]. The stems, flowers, and fruits are advised to treat the vesicular lesions and sensations of burning in the foot. The young roots of *S. virginianum* are used to cure chronic skin diseases and, with great success, overcome psoriasis[8]. By local application, Konkan people also use it as a household treatment for anthrax pustules[9]. Earlier It has been reported that apigenin, solamargin and lupeol possess anticancer activities by apoptosis-induced cell death and cellular senescence in HepB3 cell line[10-12].

The current study aimed to explore the anticancer properties of phytochemical compounds found in *S. virginianum* using hydro-alcoholic and aqueous extracts. The research involved conducting *in vitro* and *in silico* studies to assess their anti-proliferative effects, highlighting the medicinal significance of these compounds.

2. Materials and Methods

2.1 Cell Lines and Chemicals Used for Assay

Solanum virginianum L. dry plant powder was prepared in the laboratory through mechanical grinding. Methanol (cat# 65524, AR, 99.8%), Crystal violet (cat# 28376) and Dimethyl sulfoxide (DMSO) (cat# TC185) were of Sisco Research Laboratories (SRL) Pvt Ltd., India. Formaldehyde (Cas#50-00-0) was purchased from TCI Chemicals Pvt. Ltd., India. All cell lines HEK-293(Normal Cell line) and MCF-7 (ER+/PR+/Her2-) were obtained from the National Center for Cell Science (NCCS) in Pune, India. Trypan blue (cat# TC193), 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) (cat# TC191), Dulbecco's Modified Eagle Medium (DMEM) (cat# AL066A), Minimum Essential Medium (MEM) (cat# AL011S), 0.25% (w/v) Trypsin EDTA (cat# TCL047), 10,000 U Penicillin and 10mg Streptomycin per ml in 0.9% normal saline (Pen-strep)(cat# A001A) and Fetal Bovine Sera, Research Grade, Sterile Filtered, Heat Inactivated (FBS) (cat# RM9955) were purchased from HiMedia Laboratories Pvt. Ltd., India.

2.2 Plant Collection and Extract Preparation

2.2.1 Plant Material Collection and Authentication

In February, fresh plant parts of *S. virginianum* were collected from the Vadodara district (22°17' 30" N 73°07' 54"). The plant sample was identified and compared with Kew Herbarium K001153104 and K001152612. The voucher specimen (KU1) was deposited at Navrachana University.

2.2.2 Preparation of Extracts

The leaves (L), ripe fruits (RF) and unripe fruits (UNRF) were separated from the whole plant, washed

using distilled water, and subjected to shaded drying at room temperature. Separated plant parts were then powdered using a clean mixture grinder, stored in an airtight container, and stored in a dry place at room temperature for future processing.

2.2.3 Hydro-alcoholic and Aqueous Extraction

The shade-dried 30g of powdered plant material of unripe fruit, ripe fruit and leaf of *S. virginianum* were successively extracted with a Soxhlet apparatus (Durga Scientific Ltd., Vadodara, India) using 70% methanol as a solvent, and aqueous extraction was done by reflux method using de-ionized water as a solvent. The extraction was done for 40-48 hours until the plant material became colourless for each plant part. The crude extracts were filtered and evaporated. After the successful completion of the concentrated extraction process, weight determination of extracts was done, and their yield percentage was calculated and compared with the initial weight of plant materials. Dried extracts were stored at -20°C in a sterile, airtight container for further studies.

2.3 *In Vitro* Culture

The MCF-7 was harvested in high glucose DMEM while the HEK-293 cell line was cultured in MEM and through the study it was kept at 37°C and 5% CO_2 in a CO_2 incubator (Remi, India) with 95% humidity. All the culture media were added with 10% FBS and 1% antibiotics (Pen-strep). The complete growth medium was replaced every 2-3 days, and subculture was carried out with 90% confluency.

2.3.1 Cell Cytotoxicity Assay: IC_{50} Determination

Stock extracts were prepared (1 mg/mL) by dissolving the dried alcoholic extracts in 0.2% while aqueous extracts were reconstituted in autoclaved ddH_2O. The cytotoxic effect of all extracts was performed by using MTT with slight modifications[13]. 5×10^3 cells were counted and seeded in 96-well plates per well. 24h incubation in culture medium, different concentrations of extracts were given to cells (6.25µg/mL-100µg/mL) and similar concentrations of diluents (0.2% DMSO and only media) for further 24h, 48h, and 72h. After treatment, the medium was replaced with MTT solution (50 µL of 0.5 mg/mL per well) prepared in PBS and incubated for 3h at 37°C in a humidified incubator with 5% CO_2. After removing the MTT solution, 100 µL of DMSO was added to each well. After a half-hour incubation, optical density was measured at 492 nm by a micro-titre plate reader (Analytical Technologies Ltd., India). The % cell viability was calculated as {(A-B)/(A)} x 100, where A is the absorbance of the control (0.2% DMSO treated cells for hydro-alcoholic extracts or only media having cells for aqueous extracts) and B is the absorbance of the experimental group (extract treated cells). The concentration-effect curves predicted the IC_{50} of the extracts on cell lines using a graph pad prism. Doxorubicin was used as a positive control.

2.4 Migration Inhibition Determination Through Scratch Assay

Based on the MTT assay, the selected extracts were tested on an MCF-7 cell line with IC_{50} and sub-IC_{50} concentrations for wound healing assay[14]. For the MCF-7 cell line, aqueous leaf extract was stimulated. 1 x 10^5 cells/well were seeded in 12-well plates. After getting a proper confluent monolayer of cells, the scratch was made in the centre of the well. Further, the cells were treated with IC_{50} and sub-IC_{50} concentrations of extracts. The scratch area photographs were taken at 0 h 6h, and 12h at 10X using an inverted microscope (Olympus CKX53, Tokyo, Japan). For analyzing the cell migration, ImageJ software was used.

The scratch area % was calculated using the following formula[14]:

in % = (A_{th} /A_{t0}) x 100, where A_{t0} is the area gap at 0 h while the area at A_{th} is the area gap at 6 h or 12 h.

2.5 Tumor Formation Inhibition Determination Through Colony Formation Assay

The viable cells of both cell lines were plated at a 1x 10^3 cells/well density in 6-well plates. After 24 h, the cells were treated with selected extracts at IC_{50} and sub-IC_{50} concentrations and incubated further for 24 h. After 6 days, cells were fixed in 500 µL fixative (37% Formaldehyde). After removing the fixative, the cells were stained with 0.5% crystal violet for 30 min at room temperature. After removing the dye by washing the plate under running tap water, the pictures of colonies were captured and counted, calculated as the colony formation %[15].

2.6 Identification of Metabolites of *S. virginianum* Leaf Sample Through High-Resolution Liquid Chromatography and Mass Spectrometry (HR-LCMS/MS) Analysis

For HR-LC-MS/MS analysis, the instrument used for analysis is Agilent Technologies 6550-funnel, Q-TOF, LC/MS, USA. The column type is a Hypersil gold column (C18 X 2.1 mm-3Micron). The solvent composition was a binary combination of 0.1% formic acid in water (A) and 90% acetonitrile, 10% water, and 0.1% formic acid (B), and the flow rate was 300 µL/ min. The LC-QTOF MS analysis was performed in dual (positive and negative) ion modes. iFunnel Q-TOF Mass Spectrometer segment of the instrument was set at a capillary tension of 3500 V, a gas flow rate 13 L/min at a temperature of 250°C, a sheath gas flow rate 11 L/min at a temperature of 300°C, and a 35-psi nebulizer gas flow pressure[16].

2.7 *In Silico* Analysis

2.7.1 Ligand Preparation

The structure of selected phyto-compounds was derived from the PubChem database in SDF format, and it was converted into a PDB file format by the Online Smile Translator.

2.7.2 Drug Likeliness Prediction of Ligand

2.7.2.1 Lipinski Rule of '5' Analysis

According to Lipinski's "rule of five", a reasonable candidate for use as an orally active drug should have no more than one violation of the following criteria: <5 hydrogen bond donors, <10 hydrogen bond acceptors, molecular weight < 500, logP< 5, and no more than one violation of the above criteria. After this, the "prepare ligands" module was applied to the remaining molecules to generate multiple conformations.

2.7.2.2 ADME/T Properties Analysis

In ADME/T properties, the parameters selected for the analysis are intestinal absorption (in human % absorption), blood-brain barrier permeability, CYP2D6 substrate, CYP2D6 inhibitor, total clearance, AMES toxicity, Oral Rat acute toxicity, Oral rat chronic toxicity and Hepatotoxicity.

2.7.2.3 Preparation of Protein

The 3D crystal structure of cell cycle proteins [Cylcin D1(PDB id: 2w99_A); Cyclin D3(PDB id: 2w99_B); CDK4(PDB id: 3g33_A); CDK6(PDB id: 1g3n_A); CDK6(PDB id: 1g3n_A); P18(PDB id: 1g3n_B); p21(PDB id: 1axc_B); p27(PDB id: 1jsu_C)] and apoptotic proteins [BAX(PDB id: 2k7w_B); BAK(PDB id: 2yv6_A); Bcl-2(PDB id: 1g5m_A); Bcl-XL(PDB id: 1g5j_A); caspase-3(PDB id: 1gfw_A); caspase-9(1nw9_B)] were downloaded from protein databank. AutoDock Tools in PyRx conducted the structure preparation of protein and saved it as a PDBQT file format.

2.7.3 Molecular Docking

Docking investigations were done using the PyRx program. The interaction between ligands and proteins was created, displayed, and evaluated using Discovery Studio. Throughout this *in silico* experiment, an exhaustiveness of 10 was chosen for docking, and the mode number was set at 10 to produce more accurate and dependable results.

2.8 Statistical Analysis

All experimentally analysed data are shown as the mean ± SEM of three different measurements per extract. To analyze the different variances between the experimental groups, we carried out a one-way analysis of variance (ANOVA) by Dunnett's post hoc method. The statistical significance is shown as * $p \leq 0.05$, ** as $p \leq 0.01$, *** as $p \leq 0.001$, **** as $p \leq 0.000$, and was performed using Graph-pad Prism software 8.

3. Result

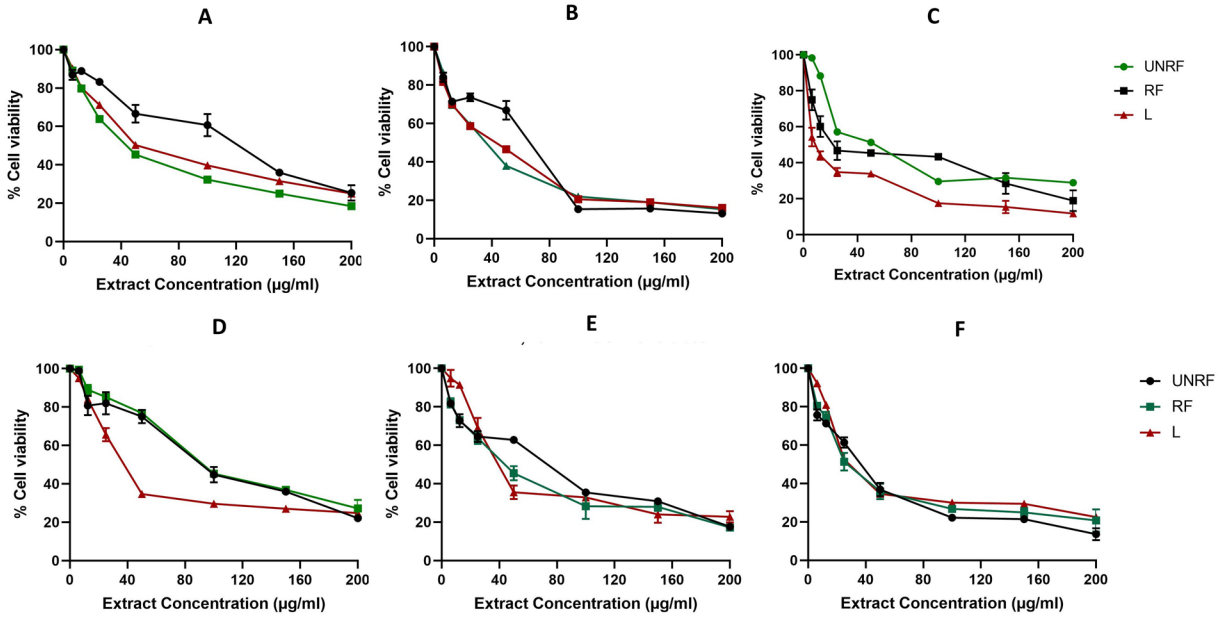
3.1 *S. virginiaum* Extracts Show Cytotoxicity in MCF-7

The anti-proliferative effect of *S. virginianum* extracts was determined through the dose-based assay for three periods at 24 h, 48 h and 72 h, respectively. The aqueous and hydro-alcoholic extracts treatment was subjected to cells (Figure 1). The highest cytotoxicity at the lower dose was observed for *S. virginianum* aqueous leaf extract on MCF-7 cell line at 24 h incubation. At 48 h and 72 h, all extracts show good control over the proliferation of cells. There is also a clear inhibition of cells with decreasing cell survival on dose increase. The results of the MTT assay have been indicated in terms of their Inhibitory

concentration, 50% (IC_{50}) values generated from their respective graphs. The overall MTT result shows that unripe fruit and ripe fruit extracts showed cytotoxicity on HEK-293 cells (Figure 2) but interestingly it was not affected by aqueous leaf extract, so only aqueous leaf extract was considered for further analysis.

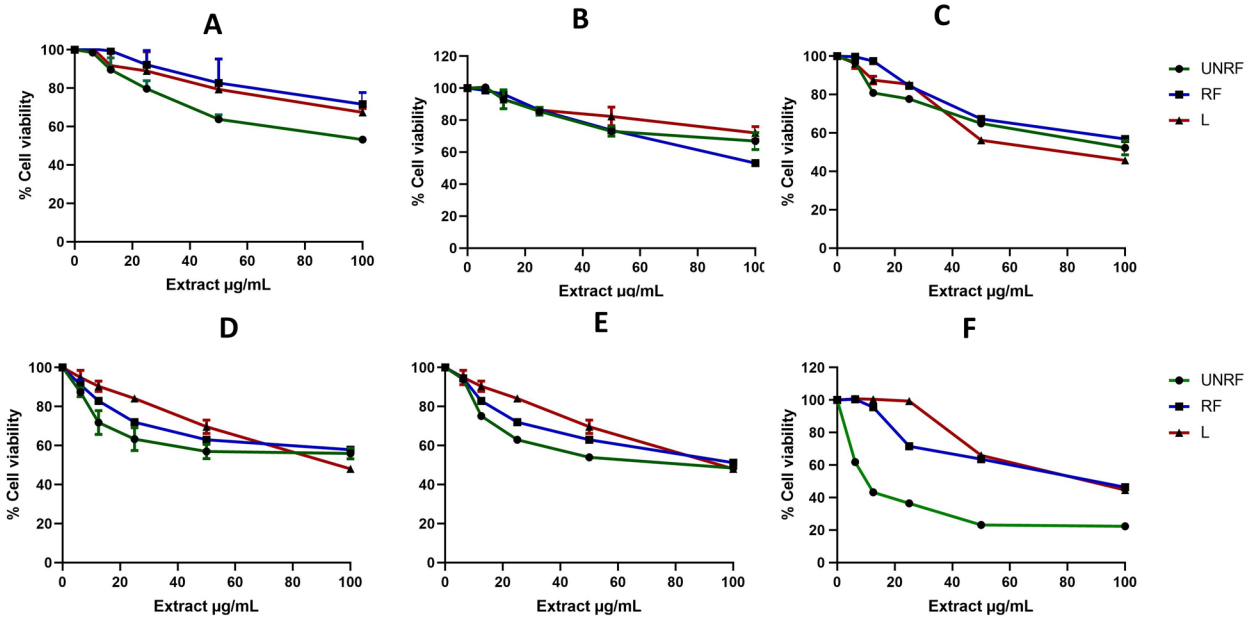
3.2 *S. virginianum* Leaf Extract Inhibits Cell Migration

Figure 3 depicts the findings of the Cell Migration Assay. The scratch area at 0 h for all groups was considered 100%. In the control group, the cells were migrating, and due to that, the scratch area %



The dose response curve of plant extracts (6.25-200 µg/ml) on MCF-7 cell line were plotted to evaluate IC50 concentration. Present graph shows the cell viability at 24 hr,48 hr and 72 hr respectively for aqueous extract treatment (1A,1B,1C) ad hydro-alcoholic extract treatment(1D,1E,1F).

Figure 1. Anti-proliferative activity of *S. virginianum* extracts on human breast cancer cell line MCF-7.



The dose response curve of plant extracts (6.25-200 µg/ml) on MCF-7 cell line were plotted to evaluate IC_{50} concentration. Present graph shows the cell viability at 24 hr, 48 hr and 72 hr respectively for aqueous extract treatment (2A, 2B, 2C) ad hydro-alcoholic extract treatment (2D, 2E, 2F).

Figure 2. Safety testing of *S. virginianum* extract on non-cancerous cell line HEK-293.

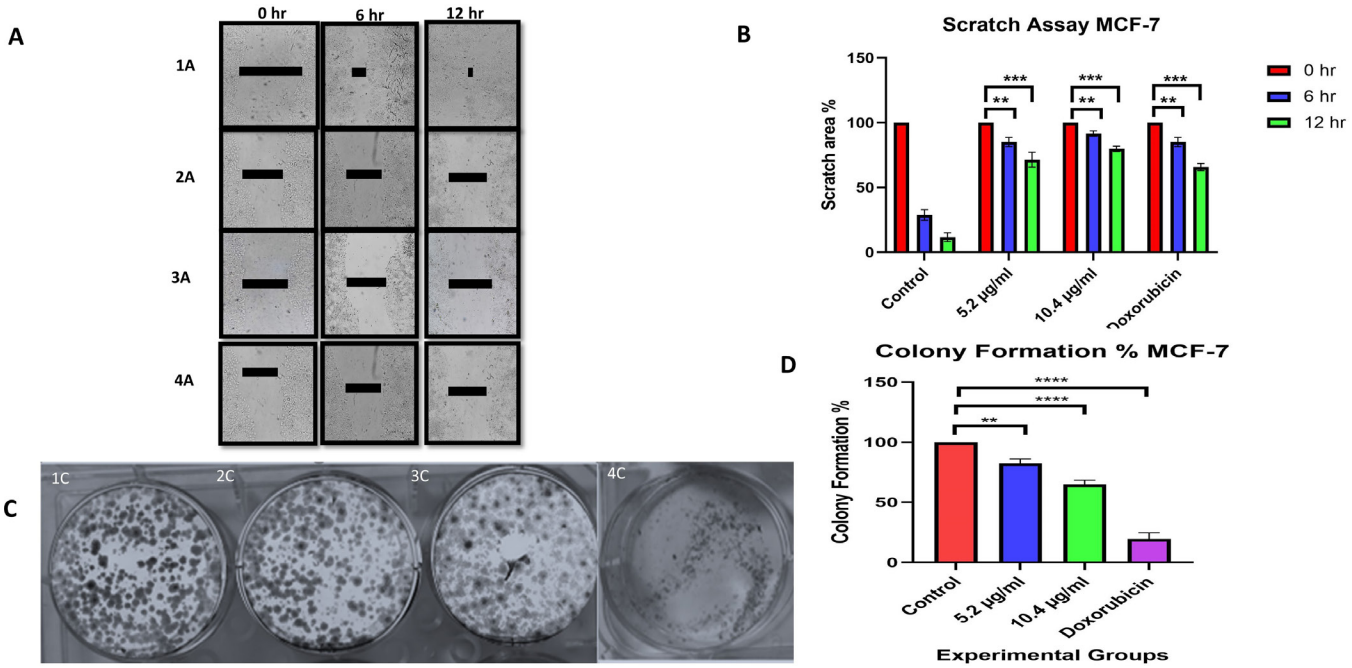


Figure 3. Effect of leaf aqueous extract on scratch area % determination and colony formation.

decreased to 25%. As the wound closed, the scratch area value was 10% at 12 h. Inhibition of cell migration rate at IC_{50} and Sub IC_{50} concentrations revealed a time-dependent trend. When the cells were exposed to 5.2 µg/mL aqueous leaf extract, the migration rate was slow as the scratch area was 61% at 6 h and 75% at 12 h. At the IC_{50} concentration of 10.4 µg/mL, the scratch area was 89% at 6 h and 79% at 12 h, indicating that the cell migration rate decreased significantly with higher concentrations of aqueous leaf extract compared to control.

3.3 *S. virginianum* Leaf Extract Reduces Tumorigenicity Efficacy

The effect of aqueous leaf extract on colony-formation ability in MCF-7 was investigated to assess the difference between the colony-forming ability of treated cells with untreated cells. The colonies are represented in Figure 3. The number of colonies observed was significantly lesser with extract exposure in comparison with the untreated cells. Colony-forming % was remarkably decreased in the case of cells treated with extracts compared to control cells (Figure 3). These results put forward the direction that aqueous leaf extract showed high anti-tumour activity in MCF-7.

3.4 Bioactive Compound Screening by High Resolution-Liquid Chromatography-Mass Spectroscopy

More than 30 phyto-compounds were found in both the extracts in HRLC MS/MS analysis. The list (list is provided in Supplementary Tables S1-S2, with chromatograms in Supplementary Figures S1-S2) of phyto compounds for each extract contains some steroidal alkaloids like Solasonin, Solamargin, and Solamarin already reported in this species[15]. However, many compounds like gambiriin A3, jubanine A, vicinin 2 and allivicin were not reported in this species to date. In *S. virginianum* aqueous leaf extract, one flavonoid, one terpenoid, two amino acid derivatives, two coumarins, two glycosides and eight alkaloids were identified, whereas, in *S. virginianum* hydro alcoholic extract, six flavonoids, two terpenoids, two amino acid derivatives, two carbohydrate derivatives, three polyphenols, three glycosides and three alkaloids were identified through LC-MS/MS analysis. Out of these, some compounds were selected for an *in silico* analysis to prove the anticancer role of the given extract.

3.5 Molecular Docking Study

The selected targets and their respective PDB IDs are given in Table 1. Selected phyto-compounds were

Table 1. PDB id of selected target proteins

S. No.	Cell cycle proteins	PDB ID with Chain
1	Cylcin D1	2w99_A
2	Cyclin D3	2w99_B
3	CDK4	3g33_A
4	CDK6	1g3n_A
5	P18	1g3n_B
6	p21	1axc_B
7	p27	1jsu_C

docked with the selected cancer targets using PyRx software, and the interacted complex is illustrated in supplementary figures S3-S15 Supplementary Figures. The ADME/T property analysis and Lipinski rule of '5' Analysis are shown in Tables 2 and 3 respectively. For each compound's docking, many poses were generated. Based on the binding energy, the pose was ranked. The pose with high binding energy was selected for each compound, and their scoring parameters are shown in Table 4.

4. Discussion

Solanum virginianum L. has important medicinal properties as its roots are used in *Dashamoola*. It also has ethnobotanical importance as tribal people use this plant to reduce inflammation and control respiratory diseases[6,7]. Previous studies have shown the importance of *S. virginianum* fruits. The fruit extract was tested on the MCF-7 breast cancer cell line[16,17]. However, nobody has studied the anticancer activity of *S. virginianum* leaf extract. The present study reveals that in comparison with unripe and ripened fruits, the leaf extract has acceptable anticancer activity besides showing no toxicity at lower doses on normal cells. In comparing hydro-alcoholic and aqueous extracts of *S. virginianum* plant parts, aqueous leaf extract shows definite anti-proliferative activity on the MCF-7 cell line. Saraswathi *et al.*, working on *S. virginianum* dried fruit ethanolic and aqueous extracts, have shown anticancer properties in the HepG2 cell line. In their study, the aqueous and ethanolic extracts of dried *S. virginianum* fruits showed anti-proliferative activity against the HepG2 cell line[18]. Fruits show high anti-proliferative activity on MCF-7 compared to leaf; however, it also shows the same effect on normal cell lines (HEK-293). Hence, *S. virginianum* leaves can be considered for further clinical analysis.

The role of natural products in establishing antioxidant- and anticancer-based lead molecules for cancer treatment is becoming increasingly important. In synthetic medical chemistry, computational algorithm methods are widely documented, yet their use in the field of natural phytocompounds is not evaluated in depth. The identified phytocompounds the cell cycle and apoptotic proteins were investigated for molecular docking. Molecular docking studies revealed interactions between active site residues and predicted compounds, leading to the development of new inhibitors[19,20]. The previous Quantitative Structure-Activity Relationship (QSAR) model suggests atomic volume, charges, and electronegativity as key factors in inhibitor design, with hypoxia-related human carbonic anhydrase IX enzyme playing a key role in metastasis[21]. Molecular docking is an alternative method used to analyse potential physiologically active drug candidates. Intermolecular flexible docking simulations were conducted to determine energy values from the docked conformations of the complexes. In the present study, Table 1 displays the PDB id for each of the specified target proteins. The phytochemicals have been subjected to docking to the target proteins, thereby enhancing their action. Nevertheless, the selected phytocompounds over here adhere to the ADMET properties; hence our study indicates that the phytocompounds identified from extracts are not going to be harmful and toxic to humans (Table 2). Most of the ligands exhibited higher binding affinity towards the target proteins. Inhibition was assessed by the binding energy of the most favourable ligand posture, calculated in kcal/mol. Table 4 lists the binding poses and corresponding energy values.

In the present study, the residual interaction revealed the amino acid of the protein to which the ligand binds. In terms of binding energy, all the identified compounds demonstrated robust affinity with the target proteins. Out of 9 compounds docked, Ritterazine A showed very strong binding with all the selected target proteins. It shows an above 9kcal/mol binding energy with all the target proteins and the amino acid Glu-255 formed the strong binding with Cyclin-D1. Cyclin-D3 forms the interaction with Ritterazine A through the amino acids GLN-182, and PHE-287. The amino acid residues like ASP-144, GLU-228, and ALA-167 of CDK4 form

Table 2. ADMET properties analysis of selected phyto-compounds

Compound name	Intestinal absorption (human) (% Absorbed)	BBB permeability (log BB)	CYP2D6 substrate (Yes/No)	CYP2D6 inhibitor	Total Clearance (log ml/min/kg)	AMES toxicity (Yes/No)	Oral Rat Acute Toxicity (LD50) (mol/kg)	Oral Rat Chronic Toxicity (LOAEL) (log mg/kg-bw/day)	Hepato toxicity (Yes/No)
Ritterazine A	100	-0.987	No	No	-1.57	No	3.523	2.104	Yes
Koryogin senoside R1	31.722	-1.888	No	No	0.468	No	3.313	2.876	No
CaffeoylQuinic acid	36.377	-1.407	No	No	0.307	No	1.973	2.982	No
Aconine	64.628	-1.273	No	No	0.05	Yes	2.607	2.991	No
Fabianine	94.094	0.32	No	No	1.013	No	2.239	1.708	No
Myricitrin	43.334	-1.811	No	No	0.303	No	2.537	3.386	No
Pedaliin	38.429	-2.017	No	No	0.42	No	2.565	4.339	No
Quinic acid	32.274	-2.737	No	No	0.639	No	1.128	3.529	No
Sulfametho pyrazine	79.093	-0.077	No	No	0.643	No	2	1.935	Yes

Table 3. Lipinski rule of 5 analysis for selected compounds

Compound name	Mol. wt	Log P	Rotatable bonds	acceptors	donors	Surface area
Ritterazine A	913.206	5.7269	0	12	5	389.945
Koryoginsenoside R1	869.099	2.2468	12	15	9	360.625
CaffeoylQuinic acid	354.311	-0.6459	4	8	6	141.587
Aconine	499.601	-1.7874	6	10	5	205.648
Fabianine	219.328	3.141182	1	2	1	97.724
Myricitrin	464.379	0.1943	3	12	8	183.901
Pedaliin	478.406	-0.2359	5	12	7	190.586
Quinic acid	192.167	-2.3214	1	5	5	74.056
Sulfamethopyrazine	280.309	0.8682	4	6	2	110.057

Table 4. Target-ligand interaction score

Target: Cylcin D1				
S. No.	Ligands	Binding affinity	rmsd/ub	rmsd/lb
1	Ritterazine A	-9.6	0	0
2	Koryoginesenoside R1	-7.6	0	0
3	Caffeoylquinic acid	-6.8	0	0
4	Aconine	-6.5	0	0
5	Fabianine	-6.2	0	0
6	Myricitrin	-7.5	0	0
7	Pedalin	-7.2	0	0
8	Quinic acid	-5.3	0	0
9	Sulfamethopyrazine	-6.1	0	0
Target: CDK6				
1	Ritterazine A	-10.8	0	0
2	Koryoginesenoside R1	-8	0	0
3	Caffeoylquinic acid	-7.5	0	0
4	Aconine	-6.1	0	0
5	Fabianine	-7	0	0
6	Myricitrin	-7.6	0	0
7	Pedalin	-8.5	0	0
8	Quinic acid	-5.6	0	0
9	Sulfamethopyrazine	-6.9	0	0

Table 4. Continued...

Target: Cylcin D3				
1	Ritterazine A	-9.5	0	0
2	Koryoginesenoside R1	-8.3	0	0
3	Caffeoylquinic acid	-8.6	0	0
4	Aconine	-6.1	0	0
5	Fabianine	-7.1	0	0
6	Myricitrin	-9.8	0	0
7	Pedalin	-8.7	0	0
8	Quinic acid	-6.5	0	0
9	Sulfamethopyrazine	-7.3	0	0
Target: CDK4				
1	Ritterazine A	-9.9	0	0
2	Koryoginesenoside R1	-7.7	0	0
3	Caffeoylquinic acid	-7.5	0	0
4	Aconine	-6.3	0	0
5	Fabianine	-5.6	0	0
6	Myricitrin	-8.3	0	0
7	Pedalin	-8.2	0	0
8	Quinic acid	-6	0	0
9	Sulfamethopyrazine	-6.9	0	0
Target: p18				
1	Ritterazine A	-9.5	0	0
2	Koryoginesenoside R1	-7.3	0	0
3	Caffeoylquinic acid	-6.5	0	0
4	Aconine	-5.5	0	0
5	Fabianine	-5.7	0	0
6	Myricitrin	-7.4	0	0
7	Pedalin	-7	0	0
8	Quinic acid	-5	0	0
9	Sulfamethopyrazine	-5.5	0	0
Target: p21				
1	Ritterazine A	-7.1	0	0
2	Koryoginesenoside R1	-5.7	0	0
3	Caffeoylquinic acid	-4.8	0	0
4	Aconine	-4.3	0	0
5	Fabianine	-4.2	0	0
6	Myricitrin	-5.8	0	0
7	Pedalin	-5.4	0	0
8	Quinic acid	-3.9	0	0
9	Sulfamethopyrazine	-4.7	0	0
Target: p27				
1	Ritterazine A	-10.3	0	0

Table 4. Continued...

2	Koryoginesenoside R1	-7.5	0	0
3	Caffeoylquinic acid	-5.8	0	0
4	Aconine	-5.3	0	0
5	Fabianine	-5.3	0	0
6	Myricitrin	-6.1	0	0
7	Pedalin	-6.1	0	0
8	Quinic acid	-4.7	0	0
9	Sulfamethopyrazine	-5	0	0
Target: BAX				
1	Ritterazine A	-6.3	0	0
2	Koryoginesenoside R1	-5.3	0	0
3	Caffeoylquinic acid	-5.1	0	0
4	Aconine	-4.2	0	0
5	Fabianine	-4.5	0	0
6	Myricitrin	-5.3	0	0
7	Pedalin	-5.6	0	0
8	Quinic acid	-3.8	0	0
9	Sulfamethopyrazine	-4.7	0	0
Target: BAK				
1	Ritterazine A	-10.3	0	0
2	Koryoginesenoside R1	-8.8	0	0
3	Caffeoylquinic acid	-7.2	0	0
4	Aconine	-7.2	0	0
5	Fabianine	-6.3	0	0
6	Myricitrin	-7.7	0	0
7	Pedalin	-7.7	0	0
8	Quinic acid	-5.7	0	0
9	Sulfamethopyrazine	-6.5	0	0
Target: BCL-2				
1	Ritterazine A	-10.7	0	0
2	Koryoginesenoside R1	-7.2	0	0
3	Caffeoylquinic acid	-7.6	0	0
4	Aconine	-6.4	0	0
5	Fabianine	-6	0	0
6	Myricitrin	-8.3	0	0
7	Pedalin	-7.9	0	0
8	Quinic acid	-5.5	0	0
9	Sulfamethopyrazine	-6.2	0	0
Target: BCL-XL				
1	Ritterazine A	-10.4	0	0
2	Koryoginesenoside R1	-7.4	0	0
3	Caffeoylquinic acid	-6.8	0	0

Table 4. Continued...

4	Aconine	-6	0	0
5	Fabianine	-6.8	0	0
6	Myricitrin	-7	0	0
7	Pedalin	-8	0	0
8	Quinic acid	-5.8	0	0
9	Sulfamethopyrazine	-6	0	0
Target: Caspase 3				
1	Ritterazine A	-9	0	0
2	Koryoginesenoside R1	-6.1	0	0
3	Caffeoylquinic acid	-6.2	0	0
4	Aconine	-5.4	0	0
5	Fabianine	-5.7	0	0
6	Myricitrin	-6.3	0	0

Table 4. Continued...

7	Pedalin	-6.5	0	0
8	Quinic acid	-4.6	0	0
9	Sulfamethopyrazine	-5.3	0	0
Target: Caspase 9				
1	Ritterazine A	-11.4	0	0
2	Koryoginesenoside R1	-6.8	0	0
3	Caffeoylquinic acid	-6.6	0	0
4	Aconine	-5.4	0	0
5	Fabianine	-5.9	0	0
6	Myricitrin	-7.2	0	0
7	Pedalin	-7.4	0	0
8	Quinic acid	-5.9	0	0
9	Sulfamethopyrazine	-6.3	0	0

a strong binding with Ritterazine A. Likewise the CDK6 form a strong hydrogen bond interaction with Ritterazine A through the amino acid residues PHE-39, and HIS -100 and it's also formed the Pi-anion interaction with the GLU-99. p18 and Ritterazine A form the interaction through the ARG-79. p21 forms the unfavourable interaction with the residue of LEU-157. p27 forms the strong hydrogen bond interaction with Ritterazine A through the LYS-81, and TYR-88 also forms Pi-Alkyl interaction with residue TRP-76. BAX and Ritterazine A formed only one interaction with TYR-162. Likewise, BAK and Ritterazine A formed only one hydrogen bond interaction through GLN-94. The target Bcl-2 formed one hydrogen bond and one Pi-Anion interaction through the residues of SER-49 and ASP-10 respectively. Bcl-xl and Ritterazine A form the pi-alkyl interaction through the ARG-104. Caspase-3 and Ritterazine A do not form a significant interaction, but it had very good binding energy. Caspase-3 and Ritterazine A had strong binding through the hydrogen bond and pi-alkyl interaction with the residues of LYS-410 and LEU-145 respectively. All these types of interaction confirmed that Ritterazine A had very good activity against all selected target proteins. Hence saying a slight modification in its structure retaining the pharmacophore feature would be a better way to retain this molecule and a drug candidate.

The observations also showed a good number of hydrogen bonds and non-bonded interactions with all the target proteins. The hydrogen bond implies that the ligand has a high affinity for binding with the protein, and a high negative score suggests that the target protein has a high affinity for binding. The docking score and interaction details of the target proteins with all plant-derived compounds are shown in Table 4 and supplementary figures S3-S15 Supplementary Figures.

5. Conclusion

The major findings of this study determine the *in vitro* and *in silico*-based anticancer activity of *S. virginianum* in which leaf extracts show promising activity compared to fruit extracts. The molecular interaction between target proteins and nine selected phytocompounds, Ritterazine A, showed effective binding. Our lab is still working on the isolation and characterization of individual effective phytocompounds and their anticancer activities against other types of cancer cell lines and target proteins. The present work will become preliminary supportive data to explore their molecular targets in cancer cells, which may be examined in further research. Further advanced studies are required to confirm the pharmacological properties of the molecules for promising cancer therapeutic purposes.

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