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Original Article

Studies on *in silico* and *in vitro* Anticancer Activity of Selected Plant Extract Phytocompounds against HeLa and HCT116 Cell Lines

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ABSTRACT

Background: This study explores the phytochemical composition, molecular interactions and cytotoxic potential of ethanolic extracts from four medicinal plants-*Solanum virginianum*, *Hibiscus rosa-sinensis, Piper betle* and *Eclipta alba*-against cervical (HeLa) and colon (HCT116) cancer cell lines. **Materials and Methods:** Qualitative phytochemical analysis was performed to identify bioactive compounds, including proteins, phenols, flavonoids and terpenoids. GC-MS analysis provided insights into key phytoconstituents such as fatty acids, steroids and diterpenoids. Molecular docking evaluated the binding affinities of major compounds like stigmasterol and skimmien against cancer targets, comparing their energies to the reference drug camptothecin. Cytotoxic activity was assessed using IC_{50} assays. **Conclusion:** *Eclipta alba* emerged as the most potent extract, showing the lowest IC_{50} values (30 µg/mL for HeLa and 24 µg/mL for HCT116). Molecular docking revealed strong binding affinities for certain phytoconstituents, suggesting potential mechanisms of action. The phytochemical profile highlighted diverse bioactive compounds contributing to the observed cytotoxic effects. This study identifies *Eclipta alba* as a promising candidate for cancer therapy, supported by its significant cytotoxicity and strong molecular interactions. The findings provide a foundation for further research into these plant extracts as complementary agents in cancer treatment.

Keywords: Anticancer activity, *Eclipta alba*, *Hibiscus rosa sinensis*, Molecular docking, Phytocompounds, *Piper betle*, *Solanum virginianum*.

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INTRODUCTION

Cancer is a multifaceted and life-threatening disease that remains one of the leading causes of death globally (Hanahan, 2022). According to the World Health Organization (WHO), cancer accounted for nearly 10 million deaths in 2020, with cervical cancer (caused by HeLa cells) and colorectal cancer (modeled by HCT116 cells) representing significant proportions of this burden. Current treatment options such as chemotherapy, radiation and surgery, while effective to varying degrees are often accompanied by severe side effects and limitations in selectivity towards malignant cells (Bray *et al*., 2022; Bray and Parkin, 2022). These challenges have prompted a global pursuit for safer, cost-effective and targeted anticancer therapies. One promising avenue involves exploring plant-derived bioactive compounds, which exhibit significant anticancer activity due to their ability to modulate multiple cancer pathways while maintaining biocompatibility (Sung *et al*., 2021).

Plant secondary metabolites, commonly referred to as phytocompounds, have gained substantial attention in recent years due to their diverse biological properties, including antioxidant, anti-inflammatory and anticancer activities (de Luna *et al*., 2023; Marques *et al*., 2021). Polyphenols, flavonoids, terpenoids and alkaloids, among others, have been shown to induce apoptosis, suppress angiogenesis and interfere with cancer-promoting pathways such as those mediated by Nuclear Factor-Kappa B (NF-κB), Mitogen-Activated Protein Kinase (MAPK) and Vascular Endothelial Growth Factor (VEGF) (Parekh *et al*., 2023; Situmorang *et al*., 2024). For example, extracts from *Asystasia gangetica*, enriched with polyphenols, demonstrated remarkable cytotoxicity in *in vitro* studies, alongside significant interactions with cancer-related proteins in molecular docking (*in silico*) analyses (Pyo *et al*., 2024; de Luna *et al*., 2023).

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Modern research methodologies combining *in silico* and *in vitro* approaches offer a robust platform to evaluate the anticancer potential of phytochemicals. *In silico* methods, including molecular docking and dynamics simulations, facilitate the prediction of compound-protein interactions, offering insights into the binding efficiency and mechanism of action of phytochemicals against critical cancer targets like Bcl-2 and VEGFR (Samadarsi *et al.*, 2022; Yousef *et al.*, 2022). These computational predictions, when coupled with *in vitro* studies such as cell viability assays, flow cytometry and ROS production analysis, provide a comprehensive understanding of the therapeutic potential of these compounds. For instance, a study on the bioactive compounds from *Annona macroprophyllata* demonstrated their ability to induce apoptosis in cancer cells through Bcl-2 inhibition, validated both computationally and experimentally (Ramírez-Santos *et al.*, 2024).

Cervical cancer, represented by HeLa cells, is predominantly caused by persistent infection with Human Papillomavirus (HPV), leading to genetic mutations and uncontrolled cell proliferation. On the other hand, colorectal cancer, modeled by HCT116 cells, is characterized by mutations in the Wnt/β-catenin signaling pathway, among others. Both cancer types are aggressive and require novel therapeutic strategies that can selectively target tumor cells. Plant-derived compounds, due to their structural diversity and multitarget potential, provide an ideal foundation for developing such therapies (Wang *et al.*, 2020; Sharma *et al.*, 2021).

This study focuses on evaluating the anticancer activity of selected phytocompounds from plant extracts using *in silico* and *in vitro* techniques. By analyzing their interactions with key cancer proteins and assessing their cytotoxicity against HeLa and HCT116 cell lines, the research aims to identify potent, natural anticancer agents with translational potential for clinical applications. The integration of computational and experimental approaches in this study underscores the importance of combining technological advancements with natural product research to address the global challenge of cancer treatment.

MATERIALS AND METHODS

Plant Material Collection

In this study, leaves of *Solanum virginianum, Hibiscus rosa-sinensis, Piper betle* and *Eclipta alba* were gathered from the Tirunelveli district of Tamil Nadu, India, during September and October 2023. Dr. C. Babu, Head and Associate Professor of Botany at Pioneer Kumaraswamy College, Nagercoil, identified and authenticated the plant specimens. The leaves were thoroughly rinsed to eliminate dust, laid out on plain paper and air-dried in the shade at room temperature for about 10 days. The dried leaves were then ground into a fine powder, which was stored in airtight containers for future use.

Preparation of Extracts

To prepare the extracts, 25 g of powdered material from each plant (*Solanum virginianum, Hibiscus rosa-sinensis, Piper betle* and *Eclipta alba*) were mixed with 250 mL of ethanol and extracted using a Soxhlet apparatus until the solvent turned colorless. The solvent was then evaporated at room temperature and the extract was preserved in airtight containers for further testing.

Phytochemical Analysis

Phytochemical analyses of the plants (*Solanum virginianum, Hibiscus rosa-sinensis, Piper betle* and *Eclipta alba*) were conducted following standard procedures established by RNS Yadav and Munin Agarwala (Yadav and Agarwala, 2011). The leaf samples were subjected to various qualitative tests to identify the presence of specific phytochemicals, including terpenoids, steroids, fatty acids, phenolic compounds, alkaloids, saponins and flavonoids.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC-MS analysis for the ethanolic extracts was conducted at Heber Analytical Instrumentation Facility (HAIF) at Bishop Heber College in Trichy using a SHIMADZU GC MS QP2020 system. This system included an autosampler, injector, gas chromatograph (GC-2010) and mass spectrometer, utilizing a capillary standard non-polar SHRxi-5Sil-MS column (30.0 m length, 0.25 mm diameter, 0.25 µm film thickness). The electron ionization apparatus operated at 70 eV, with a 5 µL injection volume and helium carrier gas (99.99% pure) flowing at 1.20 mL/min and a split ratio of 10. The GC oven was programmed to begin at 50°C, held for 2 min, then ramped to 280°C over 10 min. Data was collected in the m/z range of 50-500 with a 0.3-second scan interval, taking 21 min to complete. Percentage quantification for each identified component was calculated from the ratio of its peak area to the total peak area using Shimadzu GC-MS real-time software (Sehim *et al.*, 2023).

Component Identification

The GC-MS spectra were analyzed using the National Institute of Standards and Technology (NIST) (Stein, 2012) and WILEY (Hubschmann, 2015) databases, which contain extensive patterns for spectral comparison. Each unknown compound's spectra were compared with known profiles in these libraries to identify molecular structure, weight and formula.

Molecular Docking Studies

Molecular docking was performed to study interactions between proteins from Colon (5FGK) and Cervical (4J96) cancer cell lines and plant compounds, using AutoDock Vina (Trott and Olson, 2010). Chemical structures of the phytochemicals were built with ChemDraw 8.0 and ChemBio3D was used for energy minimization. Ligand structures were then used as input for AutoDock Vina for docking simulations (Elmezayen *et al.*, 2021).

Receptor structures were downloaded from the Protein Data Bank (5FGK for Colon, 4J96 for Cervical) and prepared with AutoDock 4.0's auto preparation feature. The docking grid box, defined to set simulation parameters, measured 30×30×30 grid points with 0.375-point spacing. Coordinates for Colon protein (5FGK) were -6.278197, -19.093869, and 148.253541; for Cervical protein (4J96), they were 37.442917, 11.807821, 22.762488. The AutoDock Vina algorithm identified optimal ligand-protein binding configurations, examining up to nine conformers for each ligand. PyMOL and Discovery Studio Visualizer tools analyzed the interactions, with the lowest free energy conformations selected for further examination, displaying hydrogen bonds and interacting residues in detail.

RESULTS

Qualitative Phytochemical Analysis

The Table 1 summarizes the qualitative phytochemical analysis of four different plant materials: *Solanum virginianum, Hibiscus rosa-sinensis, Piper betle* and *Eclipta alba*. Each plant was tested for the presence of various phytochemicals, including proteins, carbohydrates, phenols, flavonoids, saponins, steroids, terpenoids, alkaloids, glycosides and tannins. *Hibiscus rosa-sinensis* lacked detectable protein content. In Carbohydrates (Benedict Reagent Test): High carbohydrate presence was noted in *Solanum virginianum* and *Eclipta alba. Hibiscus rosa-sinensis* showed a low concentration, while *Piper betle* did not show carbohydrate presence. In Phenols (Ferric Chloride Test), *Hibiscus rosa-sinensis, Piper betle* and *Eclipta alba* showed a high phenol content, suggesting strong antioxidant potential in these plants. *Solanum virginianum* contained a moderate amount. In Flavonoids (Lead Acetate Test), *Hibiscus rosa-sinensis* and *Eclipta alba* showed moderate flavonoid content, whereas *Solanum virginianum* and *Piper betle* exhibited lower levels. In Saponins (Growth Test), Present at moderate levels in *Hibiscus rosa-sinensis* and in low concentrations in *Solanum virginianum* and *Eclipta alba*). *Piper betle* did not show any presence of saponins. In Steroids (Chloroform Test), Steroids were highly present in *Solanum virginianum*, while *Hibiscus rosa-sinensis, Piper betle* and *Eclipta alba* contained low levels. In Terpenoids (Chloroform Test), High terpenoid content was noted in *Solanum virginianum* and *Piper betle*, with moderate levels in *Hibiscus rosa-sinensis* and *Eclipta alba*. In Alkaloids (Wagner and Mayer Test), High concentrations of alkaloids were observed in *Solanum virginianum* and *Eclipta alba*, while *Hibiscus rosa-sinensis* and *Piper betle* showed moderate levels. In Glycosides (Brown Ring Test), High levels of glycosides were present in *Eclipta alba*, with moderate concentrations in *Solanum virginianum* and low levels in *Hibiscus rosa-sinensis* and *Piper betle*. In Tannins (NaOH Test), *Solanum virginianum, Eclipta alba* and *Hibiscus rosa-sinensis* all exhibited high tannin concentrations, while *Piper betle* showed no presence of tannins.

GC-MS Analysis

Table 2 summarizes the key phytocompounds found in the ethanolic extracts of *Solanum virginianum, Eclipta alba, Hibiscus rosa-sinensis* and *Piper betle*, as identified by GC-MS analysis.

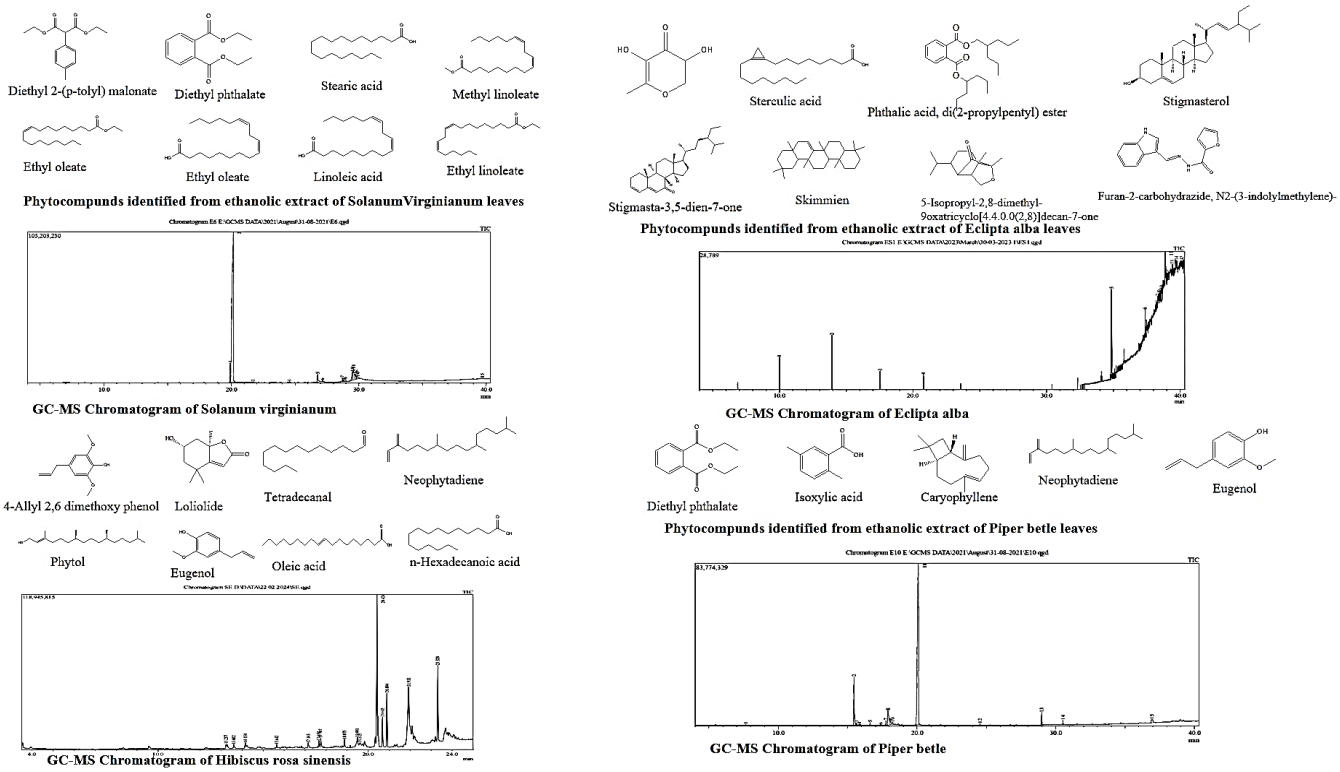

Figure 1: GC-MS chromatogram of *Solanum virginianum, Eclipta alba, Hibiscus rosa sinensis* and *Piper betle*.

Table 1: Qualitative phytochemical analysis of plant materials.

Phytochemical analysis	Plant material			
	Solanum virginianum	*Hibiscus rosa sinensis*	*Piper betle*	*Eclipta alba*
Protein (Xanthoprotein test).	++	_	++	+++
Carbohydrate (Benedict reagent test).	+++	+	-	+++
Phenol(Ferric chloride test).	++	+++	+++	++
Flavonoid(Lead acetate test).	++	+ ++	+	++
Saponins (Growth test).	+	++	-	+
Steroid(Chloroform test).	+++	+	+	+
Terpenoids (Chloroform test).	+++	++	+++	++
Alkaloid(Wagner and Mayer test).	+++	++	++	+++
Glycoside (Brown ring test).	++	+	+	+++
Tannin (NaOH test).	+++	++	-	+++

Note: + → present in small concentration; ++ → present in moderately high concentration; +++ → present in very high concentration; - → absent.

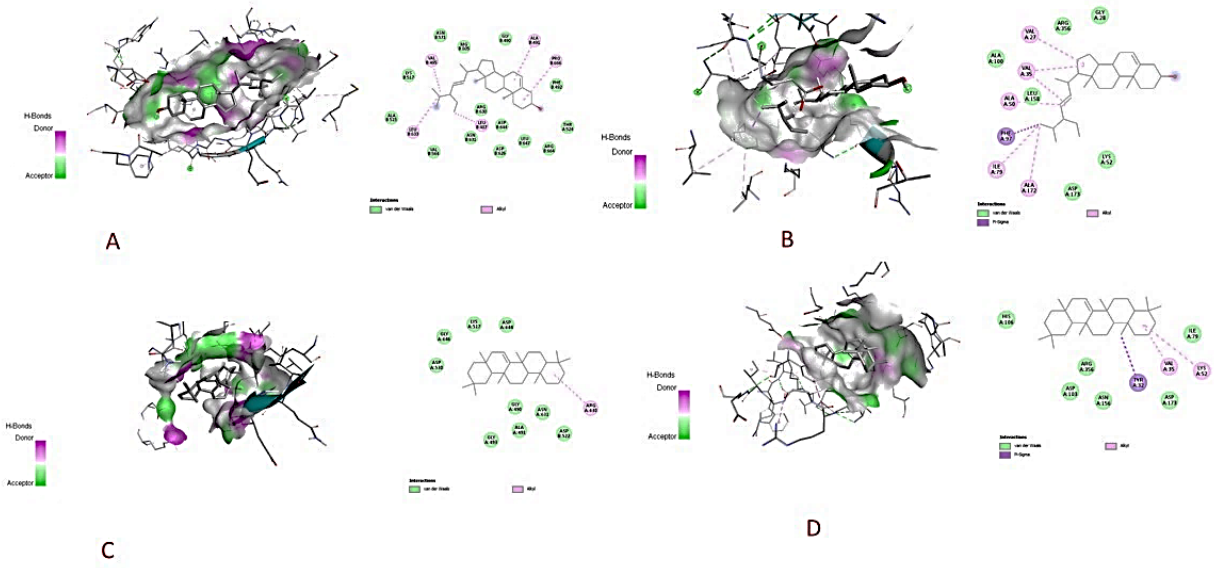

Figure 2: 2D and 3D structure of stigmasterol (A and B) and Skimmien (C and D) interaction with HeLa and HCT116 cell line.

Each plant extract contained various compounds, categorized by retention time, percentage peak area, compound name, molecular formula, molecular weight and phytochemical classification. For *Solanum virginianum*, prominent compounds include Stearic acid (6.12%), Hexadecanoic acid (4.12%) and Phytol (5.78%), indicating a high concentration of fatty acids and diterpenoids. The plant also contains ester derivatives like Diethyl 2-(p-tolyl) malonate and Ethyl oleate, suggesting potential anti-inflammatory properties. In *Eclipta alba*, the main components were n-Hexadecanoic acid (26.98%) and 9-octadecynoic acid (26.95%), both fatty acids recognized for their anti-inflammatory and antimicrobial effects. Other noteworthy compounds include Stigmasterol and Stigmasta-3,5-dien-7-one, which are steroids with cholesterol-lowering and possible anticancer benefits. Additionally, the presence of Phytol (0.703%) as a diterpenoid and Skimmien (1.702%) as a triterpenoid supports the plant's therapeutic potential. The primary compounds in *Hibiscus rosa-sinensis* include Hexadecanoic acid (22.65%) and Neophytadiene (5.28%), both known for anti-inflammatory and antimicrobial properties. Phytol (13.72%) and Loliolide, a benzofuran derivative, contribute antioxidant effects, making this plant suitable for skin-related applications. For *Piper betle*, notable compounds were Diethyl phthalate (6.78%) and Neophytadiene (7.42%), indicating antimicrobial and antioxidant activity. Additional compounds like Eugenol and Caryophyllene, both known for strong anti-inflammatory and analgesic effects,

Table 2: Major phytocompounds in ethanolic extracts of *Solanum Virginianum, Eclipta Alba, Hibiscus rosa sinensis* and *Piper betle* by GC-MS chromatogram.

Name of the Plant	Retention Time	Peak Area%	Name of the Compound	Molecular formula	Molecular weight	Name of the phytocompounds
Solanum virginianum	20.160	4.31	Diethyl 2-(p-tolyl) malonate.	$C_{14}H_{18}O_4$	250	Ester derivative
	19.910	1.46	diethyl phthalate.	$C_{12}H_{14}O_4$	222	Ester derivative
	29.930	6.12	Stearic acid.	$C_{18}H_{36}O_2$	284	Acid
	26.775	4.12	Hexadecanoic acid.	$C_{16}H_{32}O_2$	256	Acid
	25.468	1.05	Methyl linoleate.	$C_{19}H_{34}O_2$	294	Ester derivative
	26.710	2.04	Linoleic acid.	$C_{20}H_{36}O_2$	308	Acid
	27.835	3.46	Ethyl oleate.	$C_{20}H_{38}O_2$	310	Ester derivative
	28.964	2.01	Ethyl linoleate.	$C_{20}H_{36}O_2$	308	Ester
	29.465	5.78	phytol	$C_{20}H_{40}O$	296	Diterpenoid
Eclipta alba	15.423	26.978	n-Hexadecanoic acid.	$C_{16}H_{32}O_2$	256	Acid
	16.521	0.139	Sterculic acid.	$C_{19}H_{34}O_2$	294	Mono saturated fatty acid
	18.612	0.723	2-(3,4-Methylenedioxyphenyl) cyclohexenone.	$C_{13}H_{14}O_3$	218	Ketone Derivative
	21.534	26.948	9-octadecynoic acid.	$C_{18}H_{32}O_2$	280	Acid
	22.651	3.455	Oleic Acid.	$C_{18}H_{34}O_2$	282	Acid
	23.461	4.968	Octadecanoic acid.	$C_{18}H_{36}O_2$	284	Acid
	24.541	0.348	Phthalic acid, di(2-propylpentyl) ester.	$C_{24}H_{38}O_4$	390	Benzoate Ester
	25.631	0.703	Phytol	$C_{20}H_{40}O$	296	Diterpenoid
	26.428	3.082	Stigmasterol.	$C_{29}H_{48}O$	412	Steroid
	27.564	15.456	Stigmasta-3,5-dien-7-one.	$C_{29}H_{46}O$	410	Steroid
	28.214	1.702	Skimmien.	$C_{30}H_{50}$	410	Triterpenoid
Hibiscus rosa sinensis	18.875	1.73	4-Allyl 2,6- dimethoxy phenol.	$C_{11}H_{16}O_3$	196	Phenol
	19.491	1.58	Loliolide	$C_{14}H_{28}O_2$	299	Benzofuran
	19.615	0.78	Tetradecanoic acid	$C_{20}H_{38}$	278	Acid
	20.685	5.28	Neophytadine	$C_{16}H_{32}O_2$	333	Diterpenoid
	21.933	22.65	Hexadecanoic acid	$C_{20}H_{40}O$	296	Acid
	23.320	13.72	Phytol	$C_{11}H_{16}O_3$	196	Diterpenoid
Piper betle	20.090	6.78	Diethyl phthalate	$C_{12}H_{14}O_4$	222	Ester Derivative
	15.455	2.53	Eugenol	$C_{10}H_{12}O_2$	164	Phenyl propanoid
	17.900	1.79	Isoxylic acid	$C_9H_{10}O_2$	150	Benzoic acid
	16.610	2.03	Caryophyllene	$C_{15}H_{24}$	204	Sesquiterpenoid
	24.535	7.42	Neophytadiene	$C_{20}H_{38}$	278	Diterpenoid

align with the traditional use of *Piper betle* in oral care and as an anti-infective treatment (Figure 1).

Molecular docking Analysis

Table 3 provides an overview of the binding energy values for various phytocompounds from *Solanum virginianum, Eclipta alba, Hibiscus rosa-sinensis* and *Piper betle* when docked with proteins associated with cervical and colon cancer cell lines. The Table includes each compound's binding interactions (hydrogen, electrostatic and hydrophobic) and binding energy values in kcal/mol, where more negative values indicate stronger binding affinities. Compounds from all four plants displayed a range of binding affinities with the cancer proteins, with energies between -5.0 and -11.4 kcal/mol. Notably, *Eclipta alba*'s Stigmasterol and Skimmien had the strongest affinities, showing values up to -10.1 kcal/mol against cervical cancer proteins and -11.4 kcal/

Table 3: Binding energy values of *Solanum virginianum, Hibiscus rosa sinensis, Piper betle* and *Eclipta alba* phytocompounds to targeted cervical cancer and colon cancer cell line proteins.

Solvent	Name of the Compound	Binding Interaction			Cervical cancer	Binding Interaction			Colon cancer
		Hydrogen	Electrostatic	Hydrophobic		Hydrogen	Electrostatic	Hydrophobic	
Solanum virginianum	Diethyl 2- (p-tolyl) malonate	LYS517:HZ3		LEU487, ALA643, TYR566	-6.9	LYS52, ASP1 73	-	ALA50, ALA100, PH E97,TYR99, PHE17 6	-6.9
	diethyl phthalate	PHE492, GLY493, LYS517	ASP644	PHE492	-6.1	LYS52, ASP1 73	-	PHE97, TYR32	-6.3
	Stearic acid	ASP644,	-	VAL495,ALA515 ,ALA567,LEU633 ALA643,TYR566	-5.7	ALA100	-	VAL27, VAL35, AL A50, LYS52, LEU15 8, ALA172	-5.9
	Hexadecanoic acid	GLY646, LEU647,	-	LEU647, ARG664, PHE492	-5.0	ARG356,	-	VAL35, ALA50, LY S52, ALA172, TYR3 2, PHE97	-5.7
	Methyl linoleate	LYS517, ASP644,	-	VAL495,ALA515 ALA567,LEU633, ALA643,TYR566	-5.7	ASP103, ALA 155	-	VAL35, ALA50, LY S52, ILE79, ALA172 , TYR32, PHE97PHE 176	-6.6
	Linoleic acid	ASN571	-	VAL495,ALA515, LYS517,VAL564, ALA567,ALA64 3,TYR566	-6.0	-	-	VAL35, ALA50, LY S52, ALA172, PHE9 7	-6.7
	Ethyl oleate	ASN571	-	VAL495,ALA515 ,LYS517,VAL564 ALA567,ALA643 , TYR566	-5.7	ALA100	-	VAL35, LYS52, AL A172, TYR32, PHE9 7	-6.4
	phytol	-	-	VAL35,ALA50, LYS52,ILE79, LEU158,ALA172, TYR32,PHE97	-6.6	-	-	VAL35, ALA50, LY S52, ILE79, LEU158, ALA172, TYR32, PH E97	-6.6
Eclipta alba	Furan-2-carbohydrazide, N2-(3-indolylmethylene)-	LYS517	MG805	LEU487, LEU633	-7.7	ASP173, LYS 52	LYS52, GLU66	: PHE97	-8.2
	Sterculic acid	GLY646, LEU647	-	LEU647,ARG6	-5.7	ALA100		VAL35, ALA50, LY	-6.4

				64,PHE492				S52, LEU158, ALA1 72, TYR32,PHE97	
	2-(3,4-Methylenedioxy phenyl) cyclohexanone	-	LYS517,GLU534	VAL564, VAL495, ALA515	-7.8	LYS52	-	TYR32, VAL35, AL A50, ALA100,TYR9 9	-7.9
	5-Isopropyl-2,8-dimethyl-9oxatricycl[4.4 .0.0(2,8)]decan- 7-one	ASN571	-	ALA567, TYR566	-7.3	-	-	PHE97	-8.3
	Oleic Acid	ARG630		ARG664, PHE492	-5.4	-	-	VAL35, ALA50, LY S52, LEU158, ALA1 72, TYR32, PHE97	-6.5
	Phthalic acid, di(2-propylpentyl) ester	LYS517, GLY646	LYS517	VAL495, LEU6 47, PHE492	-6.3	LYS52	-	TYR32,ALA50,ILE 79,ALA100,LEU15 8,ALA172,PHE97,T YR99,PHE176	-7.4
	Stigmasterol	-	-	LEU487, ALA491, VAL4 95	-8.4	-	-	VAL27,VAL35,VA L35,ALA50,ALA17 2	-10.1
	Stigmasta-3,5- dien-7-one	-	-	LEU647, PHE492	-8.3	-	-	VAL35,ALA155, LEU158,ALA172, PHE 97	-9.6
	Skimmien			PHE492	-10.0			VAL35,LYS52	-11.4
Hibiscus rosa sinensis	4-Allyl 2,6 dimethoxy phenol	ASN571	-	LEU633, ALA567, ALA643, TYR566	-7.4	LYS52		PHE97, ALA50, TY R32, PHE97,PHE17 6	-6.2
	Loliolide	ALA567, TYR566	-	-	-6.5			VAL27,VAL35,VA L35,ALA50, ALA172	-7.1
	Tetradecanoic acid	-	-	VAL35,ALA50, LYS52,ALA172, TYR32, PHE97	-5.8	-	-	VAL35, ALA50, LY S52, ALA172, TYR3 2,PHE97	-5.8
	Neophytadine	-	-	LEU487, VAL495, LA515, LYS517, VAL564, LEU633,	-5.7			VAL35,ALA50,LY S52,ILE79,ALA100, LEU158,ALA172,T	-6.9
				ALA643				YR32, PHE97	
	Hexadecanoic acid	ASP644	-	LEU487,VAL495, ALA515, ALA567, LEU633, ALA643, TYR566	-5.3	ALA100	-	VAL35, LYS52, AL A172, TYR32, PHE9 7	-5.7
	Phytol	-	-	VAL35, ALA50, LYS52,ILE79, LEU158, ALA172, TYR32, PHE97	-6.6	-	-	VAL35, ALA50, LY S52, ILE79, LEU158, ALA172, TYR32, PH E97	-6.6

Piper betle	Diethylphthalate	PHE492, GLY493, LYS517	ASP644	PHE492	-6.1	LYS52, ASP1 73	-	PHE97, TYR32	-6.3
	Eugenol	LYS517, ASP644	-	ALA517, ALS567 ALA643, TYR566	-5.9	ASP173	LYS52	PHE97, ALA50TYR 32	-6.2
	glycidyl phenylether	ALA491, PHE492	LYS517, GLU534	-	-5.2	-	-	VAL35, TYR32	-5.5
	Isoxylic acid	ALA491, LYS517	B: LYS517, GLU5 34, ASP644	PHE492	-6.3	ARG356	-	PHE97,	-6.4
	Caryophyllene	-	-	LEU487, VAL495, ALA515,ALA567, LEU633, TYR566	-7.4	-	-	ALA50.ILE79, ALA 172, PHE97	-8.3
	Neophytadiene	-	-	LEU487,VAL495, ALA515,LYS517, VAL564,LEU633, ALA643	-5.7			VAL35,ALA50,LY S52,ILE79,ALA100, LEU158,ALA172,T YR32,PHE97	-6.9
Reference drug - Camptothecin		LYS517, ALA567 TYR566, ALA56	-	LEU487, LEU633, VAL495, ALA51	-9.5	LYS153, ASP 173	-	ALA172,ALA155,L EU158,ILE79	-10.2

mol against colon cancer proteins, suggesting their potential efficacy in targeting cancer-related proteins. Frequent hydrogen bonding with amino acids such as LYS517, ASP173 and PHE97 was observed, which are crucial for stabilizing protein-ligand complexes (Figure 2). Hydrophobic interactions, particularly with residues like VAL35, ALA50 and TYR566, also played a significant role in enhancing the compounds' affinities for cancer proteins. In *Solanum virginianum*, Diethyl 2-(p-tolyl) malonate and Stearic acid showed moderate affinities (-6.9 and -5.7 kcal/mol, respectively) with cervical cancer proteins, indicating potential for anticancer activity via protein inhibition. In *Eclipta alba*, Furan-2-carbohydrazide demonstrated strong binding (-8.2 kcal/mol) with colon cancer proteins, likely due to its hydrogen bonding and electrostatic interactions.

Hibiscus rosa-sinensis exhibited moderate binding in compounds such as 4-Allyl 2,6-dimethoxy phenol (-7.4 kcal/mol) with cervical cancer proteins, aided by hydrogen bonding and hydrophobic effects. In *Piper betle*, Eugenol and Caryophyllene showed affinities of -5.9 and -7.4 kcal/mol, respectively, with cervical cancer proteins, supporting their potential as complementary agents in cancer therapy. The reference drug Camptothecin demonstrated binding energies of -9.5 kcal/mol (cervical cancer) and -10.2 kcal/mol (colon cancer). Several plant compounds, including Stigmasterol, Skimmien and Stigmasta-3,5-dien-7-one, achieved or exceeded these values, suggesting they may serve as natural alternatives or complements to conventional cancer treatments.

In vitro anticancer Activity

Table 4 compares the IC_{50} values of phytocompounds from *Solanum virginianum*, *Hibiscus rosa-sinensis*, *Piper betle* and *Eclipta alba* with the reference drug camptothecin against two cancer cell lines: HeLa (cervical cancer) and HCT116 (colon cancer). Among the plant extracts, *Eclipta alba* showed the lowest IC_{50} values, with 30 µg/mL for HeLa and 24 µg/mL for HCT116, indicating the highest potency. *Solanum virginianum* displayed IC_{50} values of 69 µg/mL (HeLa) and 59 µg/mL (HCT116), demonstrating moderate activity. *Hibiscus rosa-sinensis* had IC_{50} values of 86 µg/mL (HeLa) and 72 µg/mL (HCT116), the highest values among the extracts, suggesting lower potency. *Piper betle* exhibited IC_{50} values of 75 µg/mL (HeLa) and 66 µg/mL (HCT116), reflecting moderate efficacy (Figures 2 and 3). The reference drug camptothecin showed IC_{50} values of 32 µg/mL (HeLa) and 28 µg/mL (HCT116), indicating greater effectiveness than all plant extracts except *Eclipta alba* (Figure 4). *Eclipta alba* demonstrated notable promise as an anti-cancer agent, showing IC_{50} values comparable to the reference drug camptothecin, positioning it as a strong candidate for further research in cervical and colon cancer treatment. In contrast, other extracts such as *Hibiscus rosa-sinensis* and *Piper betle* exhibited lower potency, suggesting more limited effectiveness.

DISCUSSION

The qualitative analysis reveals that each plant material possesses unique phytochemical profiles, which can be related to potential therapeutic benefits. For example: *Eclipta alba* exhibited high levels of protein, carbohydrate, alkaloid, glycoside and tannins, making it a good candidate for antioxidant and anti-inflammatory studies. *Solanum virginianum* showed diverse phytochemical presence, particularly in steroids, terpenoids and alkaloids, suggesting potential antimicrobial and anti-inflammatory properties. *Hibiscus rosa-sinensis* contained notable levels of

Table 4: IC_{50} **values of *solanum virginianum, Hibiscus rosa sinensis, Piper betle* and *Eclipta alba* phytocompounds to targeted cervical cancer and colon cancer cell line proteins.**

Sl. No.	Plant Extract	IC_{50} (µg/mL)	
		HeLa Cell	HCT116 Cell
1	*solanum virginianum*	69	59
2	*Hibiscus Rosa sinensis*	86	72
3	*Piper betle*	75	66
4	*Eclipta alba*	30	24
5	Reference drug - Camptothecin	32	28

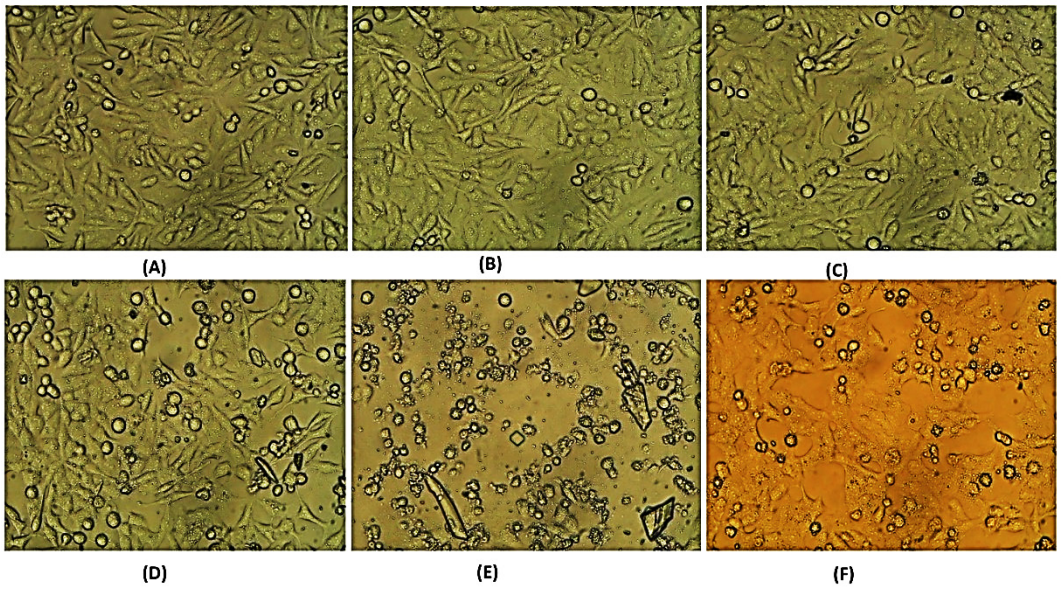

Figure 3: Viability of HeLa cell line treated with *Eclipta alba* extract in MTT assay A. 10 µg/mL shows 69.48% viability, B. 20 µg/mL extract shows 61.16% viability, C. 40 µg/mL shows 53.01% viability, D. 80 µg/mL shows 35.98% viability, E. 160 µg/mL shows 15.93% F. vehicle control shows 53.64% viability.

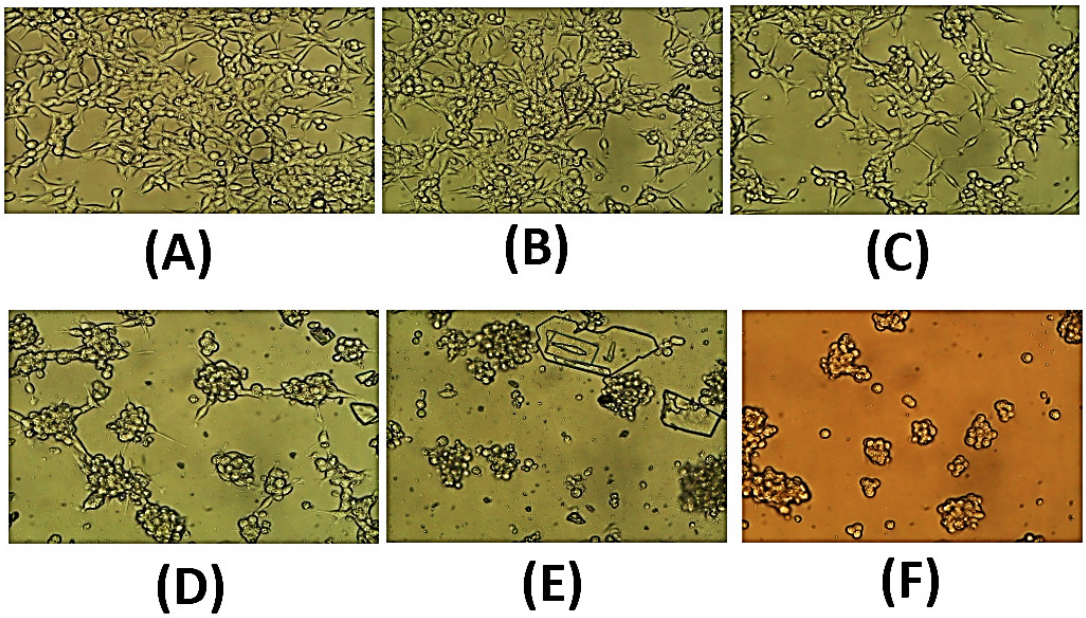

Figure 4: Viability of HCT116 cell line treated with *Eclipta alba* extract in MTT assay A. 10 µg/mL shows 66.22% viability, B. 20 µg/mL extract shows 43.57% viability, C. 40 µg/mL shows 31.81% viability, D. 80 µg/mL shows 20.55% viability, E. 160 µg/mL shows 15.03% F. vehicle control shows 28.32% viability.

phenols, flavonoids and tannins, supporting its known antioxidant and skin-soothing effects. *Piper betle* showed a strong presence of terpenoids and phenols, which may contribute to its known anti-microbial properties. This analysis suggests the potential for using these plants in therapeutic formulations, highlighting the importance of each plant's unique phytochemical profile for different health applications.

The GC-MS analysis demonstrates that each plant has a distinct phytochemical profile with potential therapeutic applications. *Solanum virginianum* and *Eclipta alba* are notably rich in fatty acids and sterols, which may account for their anti-inflammatory and cholesterol-lowering properties. *Hibiscus rosa-sinensis* contains a wealth of antioxidants and anti-inflammatory compounds, suggesting its suitability for skincare applications. *Piper betle* features a variety of essential oils and anti-inflammatory agents, aligning with its traditional uses in oral and respiratory health. These findings underscore the medicinal potential of these plants and emphasize the need for further research to explore and confirm the therapeutic effects of these bioactive compounds.

This docking analysis highlights the ability of plant-based compounds, particularly from *Eclipta alba* and *Solanum virginianum*, to interact with cancer-related proteins through hydrogen bonding and hydrophobic interactions, with binding affinities close to those of Camptothecin. These findings point to compounds like Stigmasterol and Skimmien as promising candidates for further investigation in developing plant-derived therapies for cervical and colon cancer, laying a foundation for future *in vitro* and *in vivo* studies to confirm their efficacy.

Overall, HCT116 cells (colon cancer) exhibited slightly lower IC_{50} values across all extracts than HeLa cells, indicating a higher sensitivity to these phytocompounds. These findings highlight *Eclipta alba*'s potential for future cancer studies, while *Solanum virginianum, Hibiscus rosa-sinensis* and *Piper betle* may serve as valuable options in combination therapies or as complementary treatments.

CONCLUSION

The phytochemical analysis, docking studies and IC_{50} assays highlight *Eclipta alba* as having the most promising anti-cancer potential, showing the highest cytotoxicity and binding affinity among the extracts tested. *Solanum virginianum, Hibiscus rosa-sinensis* and *Piper betle* also exhibited moderate activity, with compounds like stigmasterol and skimmien achieving binding affinities close to those of camptothecin. These findings indicate that *Eclipta alba* is a strong candidate as a natural anti-cancer agent, meriting further *in vivo* research to assess its effectiveness. The distinct phytochemical profiles of each extract suggest potential for developing multi-targeted therapeutic approaches, using these plants individually or in combination with established anti-cancer drugs.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

HeLa: Human Cervical Cancer Cell Line; **HCT116:** Human Colorectal Carcinoma Cell Line; **in silico:** Computational Biological Modeling; **in vitro:** Experimental Studies in Controlled Laboratory Environment; **DMSO:** Dimethyl Sulfoxide; **MTT:** 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide; **IC_{50}:** Half Maximal Inhibitory Concentration; **ATCC:** American Type Culture Collection; **RPMI:** Roswell Park Memorial Institute Medium; **FBS:** Fetal Bovine Serum; **PBS:** Phosphate Buffered Saline; **DNA:** Deoxyribonucleic Acid; **RNA:** Ribonucleic Acid; **mM:** Millimolar; **µM:** Micromolar; **µg/mL:** Microgram per Milliliter; **nm:** Nanometer; **DAPI:** 4′,6-Diamidino-2-phenylindole; **FITC:** Fluorescein Isothiocyanate; **PI:** Propidium Iodide; **ELISA:** Enzyme-Linked Immunosorbent Assay; **PCR:** Polymerase Chain Reaction; **RT-PCR:** Reverse Transcription Polymerase Chain Reaction; **GAPDH:** Glyceraldehyde 3-Phosphate Dehydrogenase; **SD:** Standard Deviation; **SEM:** Standard Error of Mean; **ANOVA:** Analysis of Variance; **DMEM:** Dulbecco's Modified Eagle Medium; **CO_2:** Carbon Dioxide; **°C:** Degree Celsius.

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