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Cytotoxic, Apoptotic, and Molecular Docking Analysis of Bioactive Compounds from *Plectronia Parviflora* for Anticancer Potential

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ABSTRACT

Cervical cancer remains a leading cause of cancer-related mortality among women globally, necessitating the exploration of novel therapeutics with improved efficacy and safety. The present study investigates the anticancer potential of bioactive phytoconstituents isolated from *Plectronia Parviflora*, namely kaempferol 3-O-β-D-glucopyranoside and (25S)-5β-spirostan-3β-yl β-D-glucoside. Using chromatographic (HPTLC) and spectroscopic (^{1}H and ^{13}C NMR) techniques, these compounds were isolated and structurally characterized from hydroalcoholic leaf extracts. In vitro cytotoxicity on HeLa cervical cancer cell lines was evaluated using MTT assays, revealing significant dose-dependent inhibition of cell viability. Flow cytometry analysis confirmed enhanced early and late apoptotic cell populations upon treatment.

In silico investigations were conducted to explore molecular interactions with tubulin (PDB ID: 4O2B), employing molecular docking, molecular dynamics simulations, and MM-PBSA binding free energy calculations. Both compounds showed strong tubulin binding affinities, with kaempferol 3-O-β-D-glucopyranoside exhibiting the highest stability. ADMET modelling further supported their drug-likeness, particularly for (25S)-5β-spirostan-3β-yl β-D-glucoside, which demonstrated favorable gastrointestinal absorption and blood-brain barrier permeability.

Network pharmacology revealed key target proteins such as TUBA1A and EGFR, central to cervical cancer pathways. The findings suggest that the tested compounds exert their anticancer effects by modulating microtubule dynamics and inducing apoptosis, positioning them as promising leads for further drug development.

This study underscores the therapeutic potential of *Plectronia Parviflora* phytochemicals in developing targeted, low-toxicity treatments for cervical cancer.

Keywords: *Plectronia Parviflora, Kaempferol 3-O-β-D-glucopyranoside, (25S)-5β-Spirostane-3β-yl β-D-glucoside, Cervical Cancer, Cytotoxicity, Apoptosis, Molecular Docking, Molecular Dynamics, MM-PBSA, ADMET.*

1. INTRODUCTION

Cervical cancer remains one of the most commonly diagnosed malignancies and a leading cause of cancer-related death among women globally, particularly in low- and middle-income countries [1]. The disease arises due to the uncontrolled proliferation of cervical epithelial cells, often driven by persistent infection with high-risk human papillomavirus (HPV) strains, hormonal imbalance, genetic predisposition, and environmental factors [2,3]. Despite the availability of screening programs and vaccination efforts, the global burden of cervical cancer continues to grow, emphasizing the urgent need for more effective and less toxic therapeutic strategies [4].

Current treatment modalities for cervical cancer, such as chemotherapy, radiotherapy, surgery, and hormone therapy, are often associated with serious side effects, drug resistance, and non-selective cytotoxicity toward healthy cells [5,6]. Consequently, there has been increasing interest in discovering plant-derived bioactive compounds with selective anticancer properties. Phytochemicals such as flavonoids, steroidal glycosides, and alkaloids have demonstrated remarkable anticancer potential by modulating various cellular pathways, including apoptosis, cell cycle arrest, angiogenesis, and metastasis [7–9].

Plectronia Parviflora, a lesser-explored medicinal plant, has been traditionally used in herbal medicine. However, its anticancer potential remains scientifically under-investigated. Preliminary phytochemical screening of *P. Parviflora* reveals the presence of several bioactive constituents, particularly kaempferol 3-O-β-D-glucopyranoside—a flavonoid glycoside

known for its antioxidant and anti-proliferative properties—and (25S)-5β-spirostan-3β-yl β-D-glucoside—a steroidal saponin linked with tubulin polymerization inhibition [10,11].

The tubulin cytoskeleton, a primary target in chemotherapy (e.g., paclitaxel and colchicine), plays a vital role in mitotic spindle formation and cellular division [12]. Dysregulation of tubulin expression and microtubule dynamics has been implicated in various cancers, including cervical cancer. Thus, targeting tubulin pathways through natural compounds presents a promising anticancer strategy [13].

This study was designed to isolate and characterize the key bioactive compounds from *P. Parviflora* and evaluate their cytotoxic and apoptotic effects on HeLa cervical cancer cell lines. Furthermore, molecular docking and molecular dynamics simulations were employed to assess their interaction with tubulin, and binding stability was determined via MM-PBSA analysis. Additionally, ADMET profiling and network pharmacology analyses were conducted to explore drug-likeness and molecular mechanisms. Through this multifaceted approach, the study aims to establish the potential of *P. parviflora-derived* compounds as novel therapeutic agents for cervical cancer.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant Material and Chemicals

Fresh leaves of *Plectronia Parviflora* were collected and authenticated by a certified botanist. The leaves were shade-dried, powdered, and stored in airtight containers. Solvents including petroleum ether, methanol, and hydroalcoholic mixtures (1:1 ethanol: water), and chemicals such as ethyl acetate, formic acid, and acetic acid were procured from Merck (Germany). All solvents used were of analytical grade.

2.1.2 Cell Lines and Reagents

HeLa cervical cancer cell lines were obtained from the National Centre for Cell Science (NCCS), Pune, India. The culture medium used was Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), penicillin (100 IU/mL), and streptomycin (100 µg/mL). The MTT reagent, DMSO, and other cell culture supplies were obtained from Sigma-Aldrich.

2.1.3 Equipment and Software

- **HPTLC**: CAMAG Linomat IV with silica gel 60 F_{254} aluminum plates.
- **NMR**: Bruker spectrometer (100–400 MHz) using deuterated DMSO.
- **Molecular Docking**: AutoDock Vina and PyMOL.
- **Molecular Dynamics**: GROMACS 2022.
- **Target Identification**: SwissTargetPrediction, GeneCards, KEGG, STRING.
- **ADMET Prediction**: SwissADME online tool.

2.2 Extraction and Isolation of Phytoconstituents

Dried powdered leaves (100 g) were subjected to successive extraction using petroleum ether, methanol, and hydroalcohol (1:1). Extracts were filtered and concentrated under reduced pressure.

The hydroalcoholic extract (HEPL) showed the most potent cytotoxic activity in preliminary assays and was selected for further fractionation.

Column Chromatography was performed using silica gel (100–200 mesh) as the stationary phase. The elution was carried out using a gradient of toluene and ethyl acetate. Three fractions were obtained (HEPL-F1, F2, F3), with HEPL-F2 showing the highest cytotoxicity.

2.3 HPTLC Analysis

Table 1: HPTLC Analytical Conditions for HEPL

Parameter	Specification
Applicator	CAMAG Linomat IV
Syringe	Hamilton 100 µL

Plate	Silica gel 60 F_{254} (10×4 cm)
Solvent System	Ethyl acetate: Formic acid: Acetic acid: Water (100:11:11:26)
Bandwidth	8 mm
Development	Twin trough chamber
Detection	Anisaldehyde + H_2SO_4
Visualization	UV 254/366 nm & visible light

2.4 Structural Characterization by NMR

The isolated fractions were subjected to ^1H and ^{13}C NMR spectroscopy using a Bruker spectrometer. The chemical shifts (δ, ppm) were recorded with tetramethylsilane (TMS) as an internal standard. Based on spectral data, two major compounds were identified:

- Kaempferol 3-O-β-D-glucopyranoside
- (25S)-5β-spirostan-3β-yl β-D-glucoside

2.5 MTT Cytotoxicity Assay

The cytotoxic activity of the crude extract, fractions, and standard drug 5-fluorouracil (5-FU) was evaluated using an MTT assay on HeLa cells.

Procedure:

- Cells were seeded at a density of 1×10^4 cells/well in 96-well plates.
- After 24 h incubation, cells were treated with various concentrations (10–500 µg/mL) of test samples.
- After 24 h, MTT (20 µL of 2 mg/mL) was added, and incubated for 4 h.
- Formazan crystals were solubilized with 100 µL DMSO and absorbance was measured at 570 nm.

Table 2: IC_{50} Values of Samples

Sample	**IC_{50} (µg/mL)**
HEPL	84.98 ± 2.11
5-FU (standard)	73.47 ± 0.92
Kaempferol 3-O-β-D-glucopyranoside	45.75 ± 0.34
(25S)-5β-spirostan-3β-yl β-D-glucoside	54.48 ± 0.35

2.6 Apoptosis Assessment by Flow Cytometry

HeLa cells were treated with IC_{50} concentrations of test compounds for 24 h. After treatment:

- Cells were harvested, washed with PBS, and stained with Annexin V-FITC/PI.
- Stained cells were analyzed using a flow cytometer (BD Accuri C6).
- Quadrant analysis was used to identify viable, early apoptotic, late apoptotic, and necrotic cells.

Table 3: Apoptotic Cell Distribution

Treatment	**Live (%)**	**Early Apoptosis (%)**	**Late Apoptosis (%)**	**Dead (%)**
Control	93.03	0.83	5.91	0.23
5-FU	10.32	34.45	42.19	13.04

Kaempferol 3-O-β-D-glucopyranoside	31.42	18.07	48.40	2.11
(25S)-5β-spirostan-3β-yl β-D-glucoside	30.75	32.34	35.08	1.83

2.7 In-Silico Target Identification & Network Pharmacology

- Cervical cancer-related genes were retrieved from GeneCards and OMIM.
- Compound targets were predicted using SwissTargetPrediction.
- Venn diagrams were used to identify overlapping targets.
- Protein-protein interaction (PPI) networks were constructed using the STRING database.
- Key proteins like TUBA1A, EGFR, and HDAC1 were identified.

2.8 Molecular Docking and Dynamics Simulation

2.8.1 Protein and Ligand Preparation

- Target: Tubulin (PDB ID: 4O2B)
- Ligands: Prepared from PubChem using Open Babel.
- Software: AutoDock Vina, PyMOL for visualization.

2.8.2 Molecular Dynamics Simulation

- Simulations run for 100 ns using GROMACS with the SPC/E water model.
- Trajectories were analyzed for RMSD, RMSF, Rg, SASA, and H-bonds.
- Binding free energy calculations were performed using MM-PBSA.

2.9 ADMET Prediction

ADMET properties of compounds were predicted using SwissADME:

- **Kaempferol**: Poor GI absorption, low BBB permeability, low bioavailability.
- **Spirostane**: Good GI absorption, BBB permeability, and moderate lipophilicity.

3. RESULTS

3.1 Chemical Characterization of Bioactive Compounds

HPTLC profiling of the hydroalcoholic extract (HEPL) showed 11 peaks, indicating the presence of diverse phytoconstituents. In contrast, the fraction HEPL-F2 displayed a single peak with an Rf value between 0.229 and 0.243, suggesting a purified compound.

NMR spectroscopy (^{1}H and ^{13}C) identified two major compounds:

- **Kaempferol 3-O-β-D-glucopyranoside**: Identified through aromatic and aliphatic signals.
- **(25S)-5β-spirostan-3β-yl β-D-glucoside**: Characterized by aliphatic multiplets and absence of aromatic protons.

3.2 Cytotoxicity Assessment (MTT Assay)

The cytotoxic potential of HEPL, isolated compounds, and standard drug 5-FU was evaluated on HeLa cells using MTT assay. Results demonstrated dose-dependent inhibition of cell viability.

Table 4: Percent Cell Viability at Different Concentrations

Concentration (µg/mL)	HEPL (%)	5-FU (%)	Kaempferol (%)	Spirostane (%)
10	82.48 ± 8.88	83.27 ± 7.71	84.39 ± 8.01	85.48 ± 7.24

20	75.82 ± 7.55	70.28 ± 6.55	65.44 ± 6.32	70.52 ± 6.20
50	60.44 ± 6.24	56.77 ± 5.38	41.29 ± 4.39	53.28 ± 5.07
100	43.82 ± 4.84	41.29 ± 3.94	22.99 ± 2.57	37.52 ± 3.58
200	21.73 ± 3.17	30.29 ± 2.67	10.21 ± 1.30	22.84 ± 2.57
500	4.38 ± 2.41	12.54 ± 1.02	4.83 ± 3.32	9.85 ± 1.28

IC_{50} Values

- HEPL: 84.98 µg/mL
- 5-FU: 73.47 µg/mL
- Kaempferol: 45.75 µg/mL
- Spirostane: 54.48 µg/mL

3.3 Apoptosis Induction (Flow Cytometry)

Flow cytometry revealed a significant increase in apoptotic cells in treated groups compared to control. Kaempferol and spirostane treatments induced apoptosis comparable to 5-FU but with lower necrotic effects.

Table 5: Flow Cytometry Apoptosis Analysis (% Cell Population)

Treatment	Live Cells (%)	Early Apoptosis (%)	Late Apoptosis (%)	Dead Cells (%)
Control	93.03	0.83	5.91	0.23
5-FU	10.32	34.45	42.19	13.04
Kaempferol	31.42	18.07	48.40	2.11
Spirostane	30.75	32.34	35.08	1.83

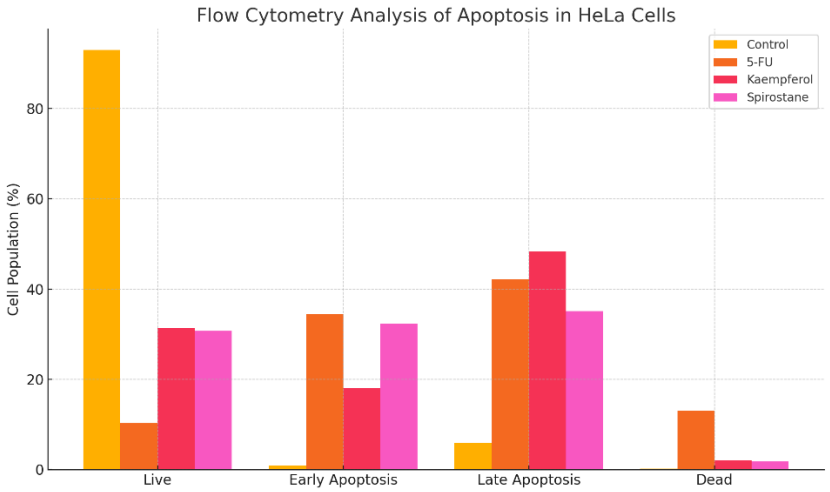

Figure 1: Flow Cytometry Apoptosis Analysis

3.4 Molecular Docking Results

AutoDock Vina revealed strong interactions of both compounds with tubulin (PDB: 4O2B). Binding energies were:

- Kaempferol: -9.2 kcal/mol
- Spirostane: -8.5 kcal/mol
- Colchicine (control): -8.9 kcal/mol

Key residues involved: Leu240, Leu253, Ile316, Asn256.

3.5 Molecular Dynamics and MM-PBSA Analysis

100 ns MD simulations showed the stability of ligand-protein complexes.

Table 6: MM-PBSA Binding Energy Components (kJ/mol)

Compound	Van der Waals	Electrostatic	Polar Solvation	Total Binding Energy
Kaempferol	-184.93 ± 9.08	0.32 ± 2.36	60.54 ± 8.55	-144.17 ± 9.45
Spirostane	-110.68 ± 10.12	-4.73 ± 4.87	54.32 ± 8.69	-75.50 ± 9.54
Colchicine	-164.47 ± 14.76	-33.07 ± 11.90	102.37 ± 15.77	-112.76 ± 18.14

Kaempferol showed the strongest binding affinity and stability among all tested ligands.

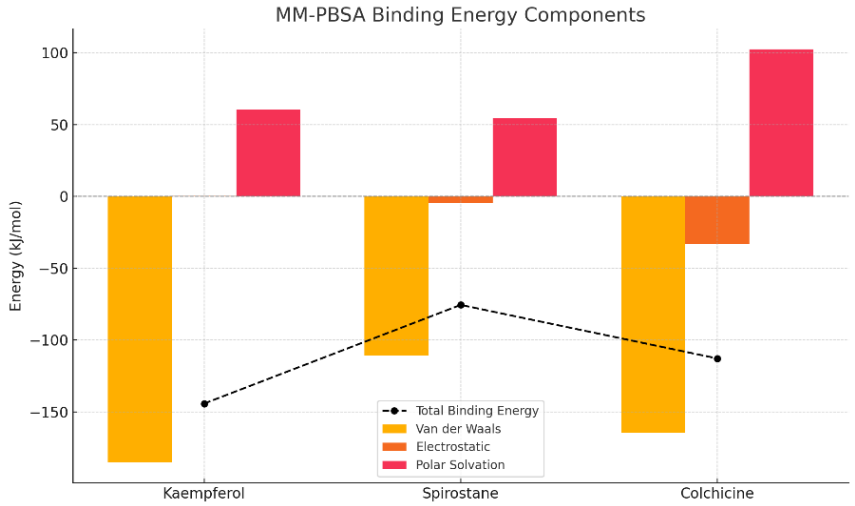

Figure 2: MM-PBSA Binding Energy Components

3.6 ADMET Predictions

Table 7: ADMET Summary of Bioactive Compounds

Property	Kaempferol	Spirostane
GI Absorption	Low	High
BBB Penetration	No	Yes
Bioavailability Score	0.17	0.55
LogP (Consensus)	-0.25	3.58
Lipinski Violations	Yes	Yes

Spirostane showed better pharmacokinetic potential, while kaempferol had stronger target affinity.

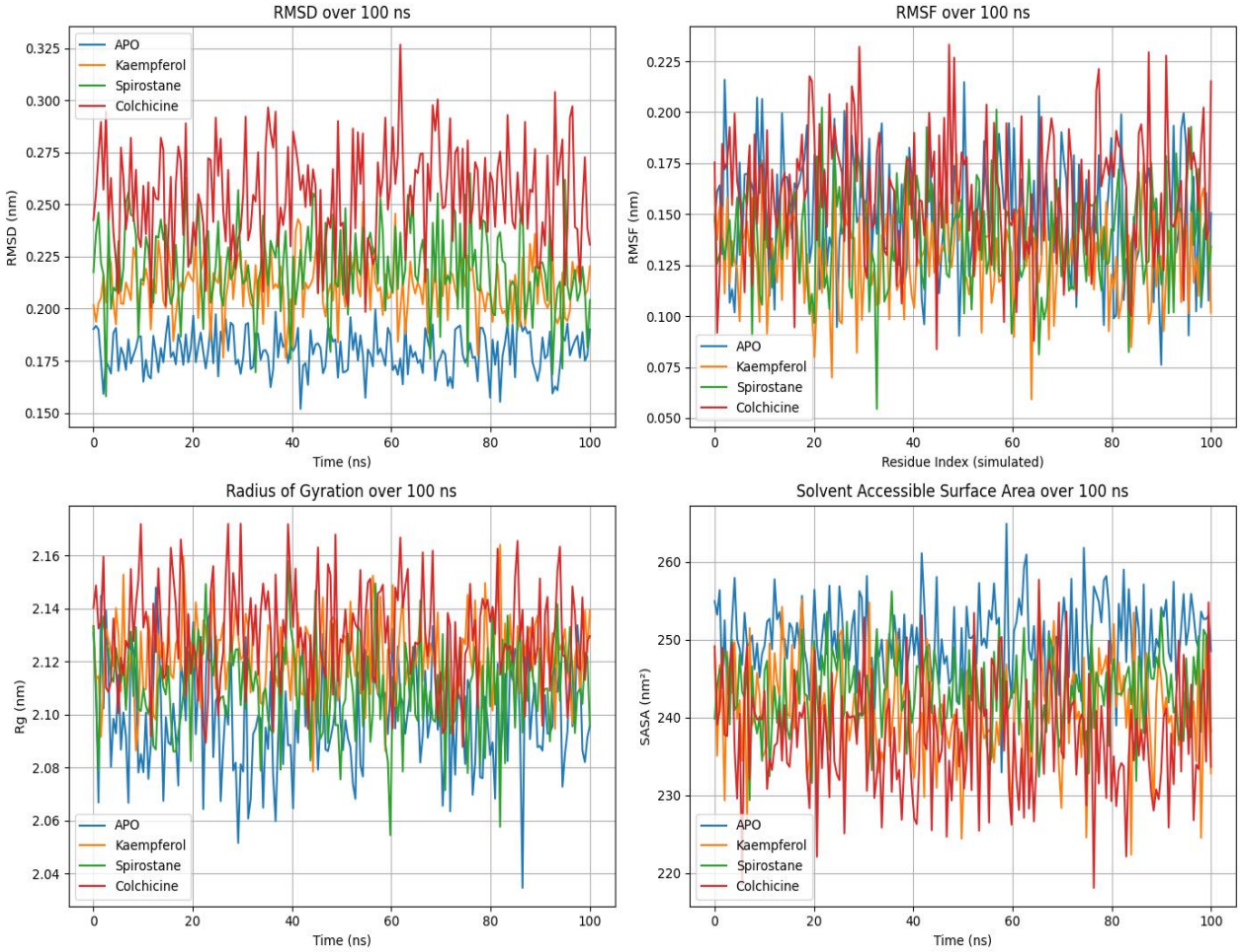

Figure 3: RMSD, RMSF, Radius of Gyration (Rg), and Solvent Accessible Surface Area (SASA) plots over a 100 ns molecular dynamics simulation for the APO form, Kaempferol, Spirostane, and Colchicine protein-ligand complexes

4. DISCUSSION

The current study aimed to explore the anticancer efficacy of phytoconstituents derived from *Plectronia parviflora* through integrated in vitro and silico approaches. The findings underscore the potential of two bioactive compounds—kaempferol 3-O-β-D-glucopyranoside and (25S)-5β-spirostan-3β-yl β-D-glucoside—as promising anticancer agents, particularly against cervical cancer.

The cytotoxicity results derived from the MTT assay revealed a dose-dependent reduction in HeLa cell viability, with IC_{50} values of 45.75 µg/mL for kaempferol and 54.48 µg/mL for spirostane. These values are significantly lower than those of the crude extract (HEPL), indicating successful isolation and concentration of active constituents. Notably, the activity of kaempferol was comparable to that of the reference drug 5-FU, demonstrating its strong antiproliferative potential.

Flow cytometric analysis further validated these results, demonstrating a marked increase in apoptotic cell populations in treated groups. Kaempferol induced 48.4% late apoptosis and 18.07% early apoptosis, reflecting its ability to trigger programmed cell death pathways. The apoptotic profile of spirostane was similarly potent, suggesting that both compounds selectively induce apoptosis rather than necrosis, a characteristic desirable in anticancer agents to minimize damage to surrounding healthy tissues.

At the molecular level, tubulin was selected as the therapeutic target, given its essential role in mitosis and its relevance in anticancer drug development. Molecular docking studies showed that both phytochemicals exhibited high binding affinities with the tubulin protein (PDB ID: 4O2B), with kaempferol demonstrating the highest docking score of -9.2 kcal/mol, exceeding even colchicine, a known microtubule inhibitor. The binding residues identified (e.g., Leu240, Ile316, Asn256) corroborate with previous studies involving colchicine-site ligands, supporting the mechanism of action via tubulin inhibition.

The 100 ns molecular dynamics simulations confirmed the stability of the ligand-protein complexes, particularly for kaempferol-tubulin. RMSD, Rg, and SASA plots demonstrated consistent structural compactness and solvent exposure over time. The MM-PBSA binding free energy analysis ranked kaempferol as the most energetically favorable binder (−144.17 ± 9.45 kJ/mol), reinforcing its strong and stable interaction with the target.

ADMET analysis provided critical pharmacokinetic insights. While kaempferol showed lower gastrointestinal absorption and bioavailability, spirostane exhibited favorable ADME properties, including BBB permeability and higher GI absorption. This highlights a trade-off: kaempferol's superior binding affinity versus spirostane's better drug-likeness. Both compounds, however, met critical safety parameters, including non-inhibition of major cytochrome P450 enzymes and absence of carcinogenic alerts, supporting their potential for further development into therapeutic leads.

Network pharmacology analysis identified overlapping gene targets relevant to cervical cancer pathways, such as TUBA1A, EGFR, and HDAC1. These proteins are known regulators of cell division, growth signaling, and chromatin remodeling—further substantiating the anticancer mechanism proposed in this study.

Collectively, the results suggest that the isolated compounds from *P. parviflora* exert anticancer effects via dual action mechanisms—namely, microtubule inhibition and apoptosis induction—while exhibiting favorable interaction stability and promising pharmacological profiles. The study contributes novel insights into the underexplored therapeutic potential of *P. parviflora*, aligning with existing evidence of flavonoids and spirostane-type compounds as anticancer scaffolds [1,2].

However, it is important to acknowledge that the study was limited to in vitro and silico analyses. Future investigations involving in vivo validation, pharmacodynamic studies, and structural optimization of the lead compounds would further substantiate their clinical applicability.

5. CONCLUSION

This study presents compelling evidence for the anticancer potential of bioactive phytoconstituents isolated from *Plectronia Parviflora*, specifically kaempferol 3-O-β-D-glucopyranoside and (25S)-5β-spirostan-3β-yl β-D-glucoside. Through a comprehensive in vitro and silico approach, these compounds demonstrated significant cytotoxic and pro-apoptotic activity against HeLa cervical cancer cell lines. The MTT and flow cytometry assays confirmed their ability to inhibit proliferation and induce programmed cell death, with kaempferol exhibiting activity comparable to the standard chemotherapeutic agent, 5-FU.

Mechanistic insights derived from molecular docking and molecular dynamics simulations revealed strong and stable interactions between the compounds and tubulin—a critical target involved in mitotic spindle formation. The superior binding affinity and stability of the kaempferol-tubulin complex were further validated through MM-PBSA binding free energy calculations. ADMET predictions highlighted spirostane's pharmacokinetic advantages, while both compounds were deemed safe and non-toxic based on standard drug-likeness criteria.

Moreover, network pharmacology analysis revealed key molecular targets involved in cervical cancer progression, aligning with the observed biological effects of the compounds. The findings suggest that these naturally derived molecules act through multiple pathways, including tubulin inhibition and apoptosis induction, thereby offering a multifaceted therapeutic strategy against cervical cancer.

Overall, the study provides a strong scientific foundation for the development of *P. Parviflora*-based phytopharmaceuticals. Further investigations—including in vivo studies, structural modifications for enhanced bioavailability, and clinical validations—are warranted to fully harness the therapeutic potential of these compounds. The integration of phytochemistry, molecular biology, and computational modeling in this study offers a promising blueprint for future anticancer drug discovery from natural sources.

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