

Exploring Apoptotic Pathways in Cancer: Computational Investigation of *Plectronia parviflora* and *Agave cantala* extracts

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ABSTRACT

Cervical and breast cancer are significant global health concerns, demanding novel therapeutic strategies. This study evaluates the cytotoxic potential of hydro alcoholic extracts (1:1 water and alcohol) of *Plectronia parviflora* and *Agave cantala*, along with their isolated fractions (IF-PL and IF-AC), on HeLa cell lines. We assessed cellular viability using the MTT assay, apoptotic induction via flow cytometry, morphological changes using the Trypan Blue assay, and apoptosis-related marker modulation through Western blotting. Our findings indicate dose-dependent reductions in cell viability, particularly with the hydroalcoholic extracts, IF-PL, and IF-AC at concentrations of 45.75 µg/ml and 54.48 µg/ml. Notably, these agents significantly induce both early and late apoptosis, confirmed by flow cytometry analysis. Treated cells displayed typical morphological alterations, including condensed nuclei, cytoplasmic shrinkage, and denatured cell walls, emphasizing the cytotoxicity of the extracts. Western blot analysis revealed a significant increase in caspase-3 activity and a shift in the balance between pro-apoptotic BAX and anti-apoptotic BCL-XL proteins. Additionally, upregulation of IL-10 suggested an associated inflammatory response. These findings highlight the potential of *Plectronia parviflora* and *Agave cantala* extracts, along with their isolated fractions, as promising candidates for cancer therapy. Their cytotoxic effects, apoptosis induction, modulation of apoptosis-related markers, and morphological changes support their role as potential alternatives to conventional chemotherapy. Further exploration of their mechanisms and clinical applications is essential, offering potential for future advancements in cancer treatment. Additionally, in silico studies revealed promising interactions between the extracts and cancer targets, further supporting their therapeutic potential.

Keywords: Cervical & breast cancer, Tubulin – Colchicine complex (4O2B), Cholest-4-en-3-one, O-Terphenyl, and Colchicine.

1. INTRODUCTION

Over 10 million people will die from cancer in 2020, making it a leading cause of death. Cancer diagnoses among children are alarmingly high [1]. The prevalence of various cancer types varies from one country to another, with Cervical & breast cancer emerging as the most frequent in 23 countries [2]. Among the most prevalent cancers in women, Cervical & breast cancer are particularly noteworthy. HPV18 is likely to be at the root of virtually all Cervical & breast cancers, as well as other high-risk human papillomaviruses (HPV) [3]. The HeLa cell line, derived from aggressive glandular Cervical & breast cancer in 1951, was the first continuous culture of human cancer cells and has significantly contributed to our understanding of numerous cellular processes [4].

The unrestrained growth and rapid proliferation of cancer cells are fundamentally rooted in genetic alterations that manipulate cellular programs, thwarting apoptosis, the natural process of cell death [5]. Apoptosis is frequently delayed by genetic changes, inducing increased cell growth while delaying cell maturation and death. Current cancer treatment strategies primarily focus on inducing apoptosis in cancer cells, and chemotherapeutic agents aim to trigger this cellular mechanism, regulating the uncontrolled cell multiplication [6]. In the quest for alternative treatment options, herbal products have emerged as a potentially safe and viable alternative to conventional chemotherapy. Medicinal plants produce various bioactive

compounds, such as coumarins, xanthenes, alkaloids, and carotenoids, which have demonstrated significant anticancer potential in both in vitro and in vivo models. Secondary metabolites derived from these plants have shown promise as sources for developing novel cancer treatments [7].

The process of discovering and developing new drugs is both time-consuming and expensive. To reduce these challenges, advancements in technology like bioinformatics-based drug design simulations, or *in silico* approaches, can be utilized through the Computer-Aided Drug Design (CADD) method. This method leverages existing information about drugs and diseases, combined with computational simulations, to explore interactions between potential drug candidates and their target receptors. Molecular docking, a commonly used technique, predicts the formation of intermolecular complexes between drug molecules (acting as ligands) and target proteins or receptors, facilitating the screening of new compounds [8]. The stability of the ligand-receptor interaction over time and space can be further examined and confirmed using Molecular Dynamics (MD), which simulates the binding process in real-time [9].

Plectronia parviflora (Lam.) Bedd., also known as *Canthium coromandelicum* (Burm.f.) Alston and belonging to the Rubiaceae family (WFO, 2023), has gained recognition for its diverse medicinal properties. The leaves of *Canthium coromandelicum* are known for their antibacterial and antiretroviral properties, as well as their potential anti-obesity, anti-diabetic, wound healing, and antioxidant effects [10]. Key chemical constituents within this plant include kaempferol, β -sitosterol, Phytol, and hexadecanoic acid [11]. Various gastrointestinal disorders, such as gastric ulcers and constipation, have been treated with this plant in India, including gut worms in children, headaches, fevers, and snake bites [12].

Agave cantala, a member of the Agavaceae family, boasts a rich history of traditional medicinal use. Various parts of the plant have been employed in traditional remedies, claiming therapeutic properties in the treatment of conditions like purgative, scurvy, syphilis, edema, and urinary retention. Chemical analysis has revealed the presence of several constituents, including flavonoid-type glycosides, sterol-type glycosides, hecogenin, and tigogenin [13]. Despite the traditional uses and chemical knowledge of these plants, there has been limited research on their cytotoxic and apoptotic activities. As such, this study was designed to assess the cytotoxicity of hydroalcoholic extracts from *Plectronia* and *Agave* and the isolated compounds from these plants against HeLa cell lines. Additionally, this research delves into the mechanisms and effects of these plant constituents on the apoptotic stages of the cell lines.

2. MATERIALS AND METHODS

2.1 Molecular Docking

2.1.1 Protein and Ligand Preparation

This study utilized Auto-Dock Vina to perform molecular docking of ligands from *Plectronia parviflora* (PL1) and *Agave cantala* (PL2) against the crystal structure of the Tubulin-Colchicine complex (4O2B). Initially, the ligands were retrieved from PubChem, and their 3D structures were prepared for docking using the Open Babel software. The receptor, the crystal structure of the Tubulin-Colchicine complex (4O2B), was also prepared for docking. The AutoDock Vina protocol was set up by defining the grid box and search space with dimensions of 60 x 60 x 60. To ensure reliability, triplicate docking runs were performed, and the resulting binding affinities were evaluated. The optimal binding poses were selected based on the best binding affinities, which were then subjected to further analysis. Visualization of the binding modes and ligand-receptor interactions was conducted using PyMOL software [14].

2.1.2 Molecular dynamics

4O2B is a complex composed of colchicine and tubulin. The docking analysis identified a group of ligands that include Colchicine (STD), *Plectronia parviflora* (PL1), and *Agave cantala* (PL2). Ligand topologies were selected using an ATB server. Hydrogenation of heavy atoms was performed by GROMACS' pdb2gmx module. As a first step, 1500 steps of vacuum minimization were performed on prepared systems. An SPCE water model based on cubic periodic boxes was then used to solvate the molecules. The next step involved adjusting Na and Cl counterions to maintain 0.15 M salt concentrations. For the creation of the system, we referred to a previous study [PMID: 31514687]. NPT ensemble simulations were conducted for 100 ns on the structures generated by INPT equilibration. The trajectory of the simulation was analyzed with the GROMACS software package. In addition to RMSDs (Root Mean Square Deviations), RMSFs (Root Mean Square Fluctuations), radii of gyration (RGs), solvent accessible surfaces (SASAs), hydrogen bonds (H-Bonds), and PCAs (Principal Component Analysis), these tools also include Free Energy Landscapes (FELs). MM-PBSA was used to analyze the binding free energy of an inhibitor with proteins (Cholest-4-en-3-one, O-Terphenyl, and Colchicine). According to Kumari et al. (2014), they used the GROMACS utility `g_mmpbsa` to estimate binding free energy. The total number of frames over the past 50 ns was used to calculate Cholest-4-en-3-one, O-Terphenyl, and Colchicine. [15].

3. RESULTS

3.1 Molecular Docking

Our protein model was docked using Auto-dock Vina using molecular docking algorithms. A list of docking scores and 2D

interactions images of the selected ligands docked to the target proteins can be found in Table 1.

Table 1: Docking scores and interactions of the top 3 phytoconstituents, each from *Plectronia parviflora* and *Agave cantala*, with 4O2B

Protein-Ligand Complex	Plant Name	Docking score	Interactions
4O2B-TOP1 [91477]	<i>Plectronia parviflora</i> .	-8.0	VAL236, CYS239, LEU240, GLN245, LEU246, ALA248, ASP249, LEU250, LYS252, LEU253, ASN256, ALA314, ILE316, LYS350, THR351
4O2B-TOP2 [252224966]	<i>Plectronia parviflora</i>	-7.9	VAL236, CYS239, LEU240, LEU246, ALA248, ASP249, LYS252, LEU253, ASN256, ALA314, ALA315, ILE316, LYS350, THR351, ALA352
4O2B-TOP3 [552099]	<i>Plectronia parviflora</i>	-7.8	VAL236, CYS239, LEU240, LEU246, ALA248, ASP249, LEU253, ASN256, MET257, THR312, VAL313, ALA314, ALA315, ILE316, ASN347, ASN348, LYS350, THR351, ALA352
4O2B-TOP1 [6766]	<i>Agave cantala</i>	-8.9	VAL236, LEU240, LEU246, ALA248, ASP249, LYS252, LEU253, ASN256, ALA314, ALA315, ILE316, LYS350, ALA352,
4O2B-TOP2 [457801]	<i>Agave cantala</i>	-8.8	VAL236, LEU240, LEU246, ALA248, ASP249, LEU250, LYS252, LEU253, ASN256, ILE316, LYS350, ILE368,
4O2B-TOP3 [614842]	<i>Agave cantala</i>	-8.7	VAL236, CYS239, LEU240, LEU246, ALA248, ASP249, LEU250, LEU253, ASN256, MET257, ALA314, LYS350,
4O2B-STD [Colchicine]	<i>Plectronia parviflora</i> and <i>Agave cantala</i>	-8.2	CYS239, GLN245, LEU246, ALA248, ASP249, LEU250, LYS252, LEU253, ALA254, ASN256, MET257, THR312, VAL313, ALA314, ALA315, ILE316, ASN348, LYS350, THR351, ALA352, VAL353, ILE368,

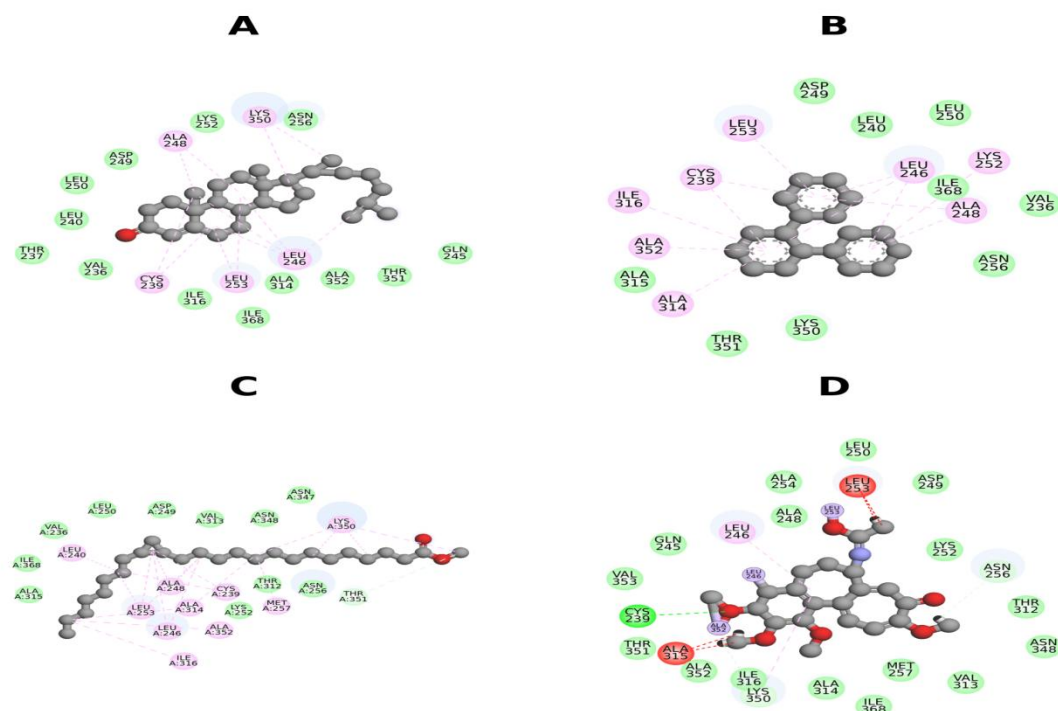


Figure 1: The 2D images of the protein-ligand interaction of complexes (Plectronia parviflora)

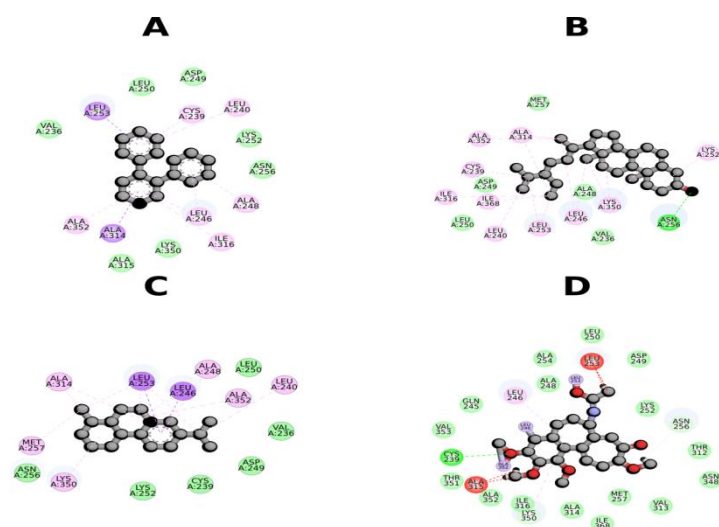


Figure 2: The 2D images of the protein-ligand interaction of complexes (Agave cantala)

3.2 Molecular Dynamics

To understand how proteins interact with ligands, all-atom MD simulations are an excellent tool. Computer-aided drug development has changed considerably as a result of molecular system analysis, which gives scientists access to detailed atomic-level information about molecules. By using MD simulations, we were able to determine which peptides bind to which peptides. In addition to RMSD, RMSF, Rg, SASA, and intermolecular hydrogen bonding, proteins and protein-ligand complexes were also able to be calculated. Furthermore, the Free Energy Landscape and Principal Component Analysis were performed for the 100-ns trajectory of the simulation.

3.2.1 Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), Radius of gyration (Rg), and Solvent accessible surface area (SASA)

Figure 3 shows the RMSD values over time used to determine the stability of the protein-tyrosine phosphatase complex. According to the simulation, both systems reach equilibrium during the simulation after 10 nanoseconds. The RMSD values revealed that even though Cholest-4-en-3-one, O-Terphenyl, and Colchicine alone were present in the docked complex, it was stable for up to 100 ns. A RMSD calculation showed that Protein alone, Cholest-4-en-3-one, O-Terphenyl, and Colchicine had RMSD values of 0.22 ± 0.02 nm, 0.19 ± 0.02 nm, 0.22 ± 0.02 nm, and 0.21 ± 0.02 nm, respectively. O-Terphenyl, Colchicine, and Protein alone are all stable systems based on simulation results.

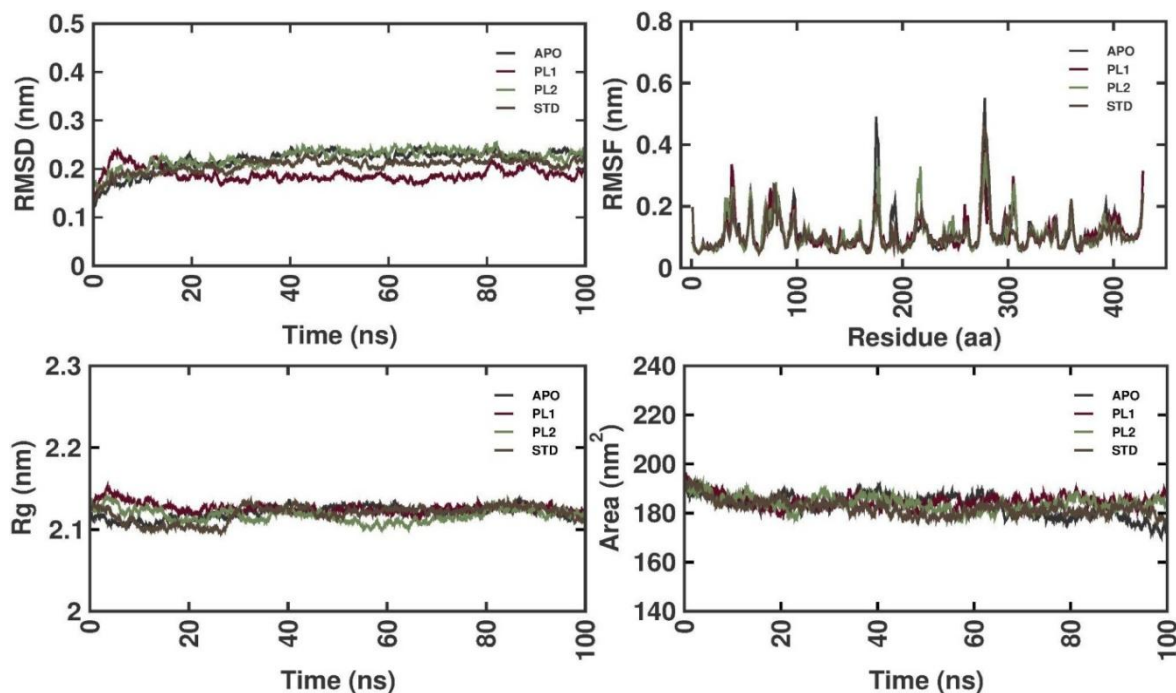


Figure 3: RMSD, RMSF, Rg, and SASA conformational dynamics analysis of Protein alone, Cholest-4-en-3-one, O-Terphenyl, and Colchicine

RMSF is used to measure protein residue fluctuations and flexible regions during MD simulations. To determine how ligands affect proteins' RMSF, we simulate their binding to proteins. Protein structures such as sheets and helices have low RMSF values, while loop regions have high RMSF values. In this study, RMSF values were calculated and plotted for Protein alone, Cholest-4-en-3-one, O-Terphenyl, and Colchicine complexes. These calculations determined that Protein alone, Cholest-4-en-3-one, O-Terphenyl, and Colchicine had RMSFs of 0.11 ± 0.06 nm, 0.11 ± 0.05 nm, 0.11 ± 0.05 nm, and 0.11 ± 0.05 nm, respectively. In terms of RMSF distributions, Protein alone, Cholest-4-en-3-one, O-Terphenyl, or Colchicine complexes do not differ significantly.

This figure shows the evolution of Rg values for proteins, cholesterol, terphenyl, and colchicine. According to averaging the Rg values for Protein alone, Cholest-4-en-3-one, O-Terphenyl, and Colchicine, these values are 2.12 ± 0.01 nm, 2.13 ± 0.01 nm, 2.12 ± 0.01 nm, and 2.12 ± 0.01 nm, respectively. It determines how accessible a protein is to solvents. As part of the investigation, SASA values were calculated for protein alone, Cholest-4-en-3-one, O-Terphenyl, and Colchicine complexes. 183.30 nm, 184.81 nm, 184.00 nm, and 182.09 nm are the average values for protein alone, Cholest-4-en-3-one, O-Terphenyl, and Colchicine, respectively. It appears that SASA values remain equilibrated throughout the simulation, without experiencing any significant fluctuations.

3.2.2 Intra and Inter Hydrogen Bond

In interactions between Protein alone, Cholest-4-en-3-one, O-Terphenyl, and Colchicine, hydrogen bonds play an essential role in ensuring stability. Using time-dependent analyses, Protein alone, Cholest-4-en-3-one, O-Terphenyl, and Colchicine complexes were compared. Based on the theoretical wavelengths of 347.98 nm for Protein, 354.73 nm for Cholest-4-en-3-one, 354.11 nm for O-Terphenyl, and 359.48 nm for Colchicine Complex, we calculated the theoretical wavelengths based on P, CH, P-1, and P-2. Despite the less frequent formation of hydrogen bonds, the APO form of the protein exhibits a higher level of stability than Protein alone, Cholest-4-en-3-one, O-Terphenyl, or Colchicine.

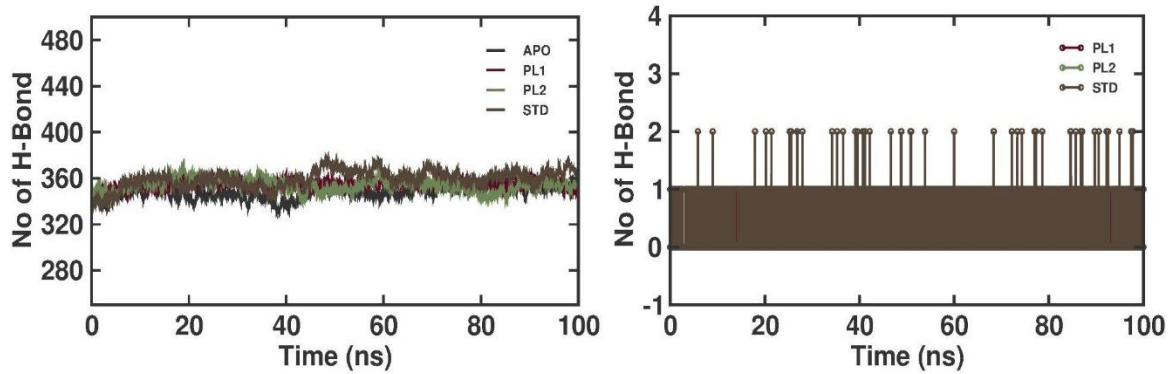


Figure 4: Intramolecular hydrogen bonds of Protein alone, Cholest-4-en-3-one, O-Terphenyl, and Colchicine

Hydrogen bonds are crucial for protein-ligand interactions. Our study examines hydrogen bond behavior over time, Cholest-4-en-3-one, O-Terphenyl, and Colchicine and plotted the results (Figure 4).

3.3 Principal Component Analysis (PCA)

This study examined the collective movements of Protein alone, Cholest-4-en-3-one, O-Terphenyl, and Colchicine using PCA. Protein molecules must be tracked globally by an EV. Consequently, we performed PCA to study O-Terphenyl, Cholest-4-en-3-one, and Colchicine's conformational dynamics (Figure 5). As shown in Figure 5, stable complexes of O-Terphenyl, Colchicine, and Cholest-4-en-3-one are found on both EVs based on time evolution. All three compounds, as well as Protein by itself, have significant conformational similarities.

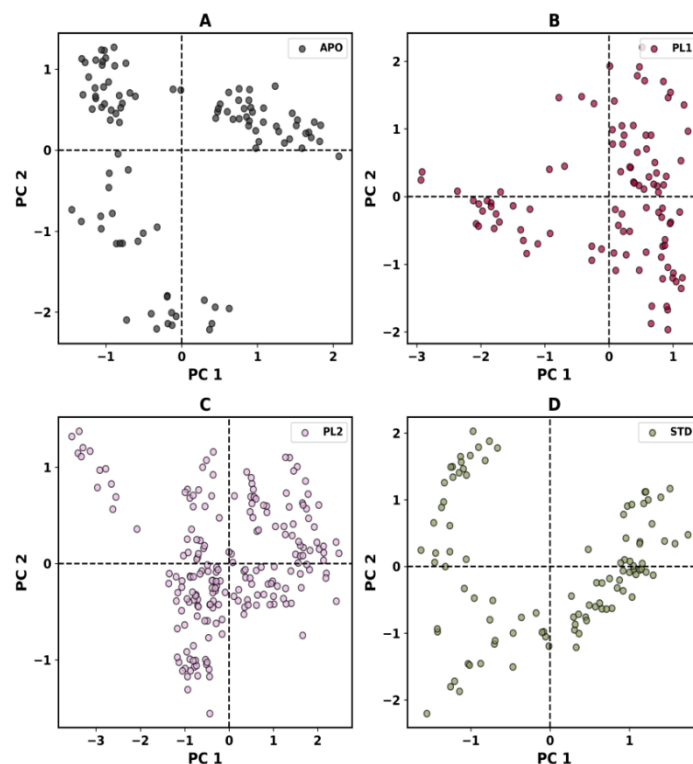


Figure 5: Principal component analysis 2D projection plot shows the conformation sampling of Protein alone, Cholest-4-en-3-one, O-Terphenyl, and Colchicine

3.4 Free Energy Landscapes (FELs)

An important method for understanding protein folding mechanics and stability is to use free energy landscapes (FELs). An FEL plot allows us to identify the most stable conformational ensembles in a protein structure. Using FEL plots (Figure 6), we indicated more stable, low-energy conformations for PC1 and PC2. Protein alone can be characterized by values ranging

from 0 to 16 kJ/mol, and Cholest-4-en-3-one, O-Terphenyl, and Colchicine can be characterized by values ranging from 0 to 20 kJ/mol. As can be seen from the FEL plots, Protein alone, Cholest-4-en-3-one, O-Terphenyl, and Colchicine show a single global minimum. Colchicine, Cholest-4-en-3-one, and O-Terphenyl do not affect the target structure in a significant way and therefore stabilize it.

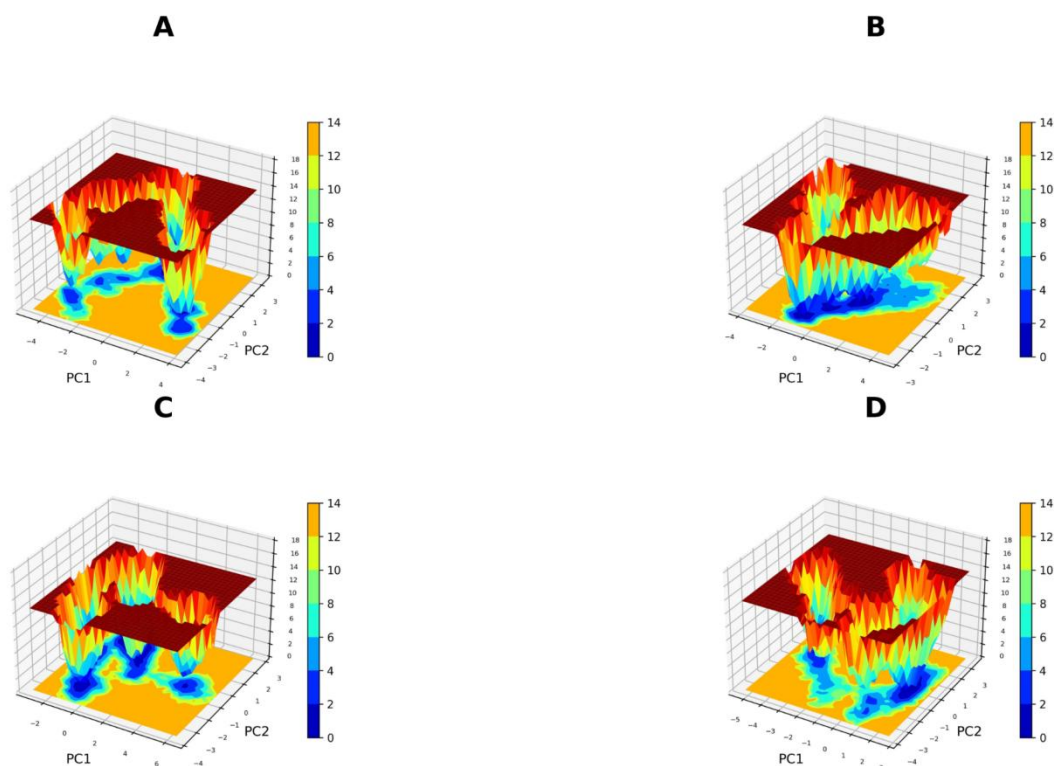


Figure 6: The free energy landscape plots for (A) Protein alone (B) Cholest-4-en-3-one (C) O-Terphenyl (D) Colchicine Complex

3.5 MM - PBSA

Cholest-4-en-3-one, O-Terphenyl, and Colchicine were tested for their relative binding strengths to summery energy proteins in this study. MM-PBSA calculations are used to compare Cholest-4-en-3-one, O-Terphenyl, and Colchicine. Calculating residue interaction energy requires simulation of a trajectory.

Table 2: MM – PBSA of Cholest-4-en-3-one, O-Terphenyl, and Colchicine

System	van der Waal energy(kJ/mol)	Electrostatic energy(kJ/mol)	Polar solvation energy(kJ/mol)	Binding energy(kJ/mol)
Cholest-4-en-3-one	-184.929 +/- 9.087	0.318 +/- 2.363	60.542 +/- 8.552	-144.166 +/- 9.450
O-Terphenyl	-110.680 +/- 10.121	-4.735 +/- 4.871	54.324 +/- 8.686	-75.503 +/- 9.540
Colchicine	-164.474 +/- 14.762	-33.067 +/- 11.904	102.372 +/- 15.768	-112.762 +/- 18.137

4. DISCUSSION

Cervical & breast cancer affects approximately 660,000 women a year, and it causes 350,000 deaths. Due to the ineffectiveness of conventional Cervical & breast cancer treatments, natural phytochemicals are gaining popularity. In this

study, potential tubulin-interacting drugs were discovered in *Plectronia parviflora* and *Agave cantala* using a computational analysis. As compared to Standard Colchicine, *Agave cantala* O-Terphenyl exhibited greater molecular interactions.[16]As with conventional colchicine, it works similarly to *Plectronia parviflora*-derived Standard Colchicine. Additionally, molecular dynamics simulations were used to study how protein-ligand complexes behave under physiological conditions. Physiological and physicochemical data were integrated to create a MD simulation.[17]A higher RMSF value indicates that the protein-ligand complex is less compact, whereas a lower RMSD value indicates that the compounds are more stable. Our analysis of the RMSD values over time showed that O-Terphenyl, Colchicine, and the protein-tyrosine phosphatase complex were stable throughout the simulation. It was found that the concentration of protein alone remained stable without significant fluctuations throughout the experiment, whereas the concentration of cholest-4-en-3-one remained stable without significant fluctuations during the experiment. [18]The RMSF values revealed that the binding of Cholest-4-en-3-one, O-Terphenyl, and Colchicine to the protein did not significantly affect the overall flexibility of the protein. The average RMSF values for Protein alone and the complexes were consistent, all around 0.11 ± 0.05 to 0.06 nm, indicating minimal changes in the protein's structural dynamics. This suggests that the ligand binding did not disrupt the stability or flexibility of the protein, further supporting the stability of the docked complexes.

The Rg values indicated that the dynamic stability and compactness of the Protein alone and its complexes with Cholest-4-en-3-one, O-Terphenyl, and Colchicine remained consistent, with average Rg values around 2.12 to 2.13 ± 0.01 nm. Additionally, the SASA analysis provided insights into the solvent accessibility of the protein. The average SASA values for the Protein alone and the complexes ranged from 182.09 ± 3.81 nm to 184.81 ± 3.45 nm, demonstrating that all systems exhibited stable equilibration throughout the simulation period without significant fluctuations. A MD simulation showed hydrogen bonds, hydrophobic interactions, and ionic bonds between the ligand and the target protein.[19]It was possible to establish a possible binding between the ligand and protein throughout the simulation because the ligand-protein interaction was stable. A compound's MM-GBSA values also indicate how well it interacts with the target protein over time. Based on MD simulations, earlier research findings about optimal properties for all compounds were confirmed. These in-silico results indicate that the screened compounds may interact positively with target proteins, increasing their consistent and reliable properties. These compounds may be useful in the future for treating lethal cancers such as Cervical & breast cancer, which may lead to their use as therapeutic agents. Based solely on in-silico computational analysis, further testing in the lab is needed to confirm the clinical and molecular implications of these findings. For determining whether the screened phytochemicals have clinical potential as Cervical & breast cancer treatments, the following steps must be taken in support of the bioinformatic analysis presented in this article.

5. CONCLUSION

The use of *Plectronia parviflora* and *Agave cantala* in addition to conventional treatments may prove beneficial in the treatment of Cervical & breast cancer. Even though the compounds had lower affinity energies and higher binding strengths, they were still able to demonstrate enhanced biological activity against the target tubulin receptors. Because they are capable of forming complexes in various combinations, they may prove to be an attractive starting point for designing and developing Cervical & breast cancer drugs. Researchers identified agents that inhibit tumor growth in *Plectronia parviflora* and *Agave cantala* from this study, which could lead to potential new treatments for Cervical & breast cancer.

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