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EXPLORING WITHANOLIDE A AS A POTENTIAL THERAPEUTIC AGENT AGAINST ORAL CANCER: INSIGHTS FROM MOLECULAR DOCKING AND DFT CALCULATIONS

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ABSTRACT

Oral cancer continues to be a significant problem in the field of healthcare due to its intricate nature and wide range of causes. There has been recent attention on natural chemicals derived from medicinal plants as possible treatments for cancer. Withanolide A, extracted from Withania somnifera (ashwagandha), has demonstrated potential in treating oral cancer. Oral cancer, which impacts the lips, tongue, mouth, and throat, is a significant global health concern characterized by a bleak outlook in its late stages. This study investigates the capacity of Withanolide A to hinder crucial genes associated with oral cancer. The protein structures of the target genes (CALM3, ARRB1, HTT, FLNA) were obtained from the Protein Data Bank and prepared for docking. The structure of Withanolide A was obtained from the PubChem database. The molecular docking process was conducted using Autodock tools v1.5.4 and Autodock v4.2 to evaluate the interactions and binding energies. Analyzing electronic structures and characteristics, Density Functional Theory (DFT) computations were performed using Gaussian software. The molecular docking process revealed that CALM3 had the lowest binding energy, suggesting the highest binding affinity. ARRB1, HTT, and FLNA followed it. Withanolide A exhibited robust hydrogen bonding interactions with all the target proteins, with bond lengths of less than 3Å. DFT calculations validated the stability and reactivity of Withanolide A with four oral cancer targets. Withanolide A demonstrates substantial inhibition of CALM3 and HTT, with CALM3 displaying the most favourable binding energy. These findings emphasize the potential of Withanolide A as a specific treatment for oral cancer, requiring additional research to investigate its therapeutic uses.

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1. INTRODUCTION

The intricate and diverse causes of cancer pose a formidable challenge in the field of healthcare, hindering the development of productive therapeutic strategies [1]. In recent years, there has been an increasing interest in investigating the use of natural compounds obtained from medicinal plants as a potential method for treating cancer [2, 3]. Withanolide A, a bioactive compound found in the Withania somnifera plant, often known as ashwagandha, exhibits potential as a therapeutic agent, particularly in cancer therapy [4]. Withania somnifera, also known as Indian ginseng or winter cherry, has a long history of use in traditional medical systems like Ayurveda thanks to its adaptogenic and revitalizing properties [5]. Withanolide A, a biologically active molecule extracted from the roots and leaves of the Withania somnifera plant, has garnered significant attention for its potential therapeutic effects, particularly in cancer therapy [6]. Preclinical studies have demonstrated that Withanolide A has potent anticancer properties, including apoptosis induction, tumor growth inhibition, and

metastasis suppression in many types of cancer [7]. These effects are attributed to its ability to control important signaling pathways involved in cancer cell growth, survival, and dissemination [8].

Oral cancer, encompassing malignant neoplasms in the lips, tongue, mouth, and throat, poses a significant worldwide health burden, with escalating incidence rates worldwide [9]. Although there have been advancements in treatment procedures, the prognosis for patients with oral cancer remains bleak, especially in the advanced stages of the disease [10]. Therefore, there is an urgent need for innovative therapeutic approaches that can improve patient outcomes and boost quality of life [11]. Withanolide A has been discovered as a viable therapeutic medication for oral cancer based on strong preclinical evidence [12]. Studies have shown that Withanolide A inhibits the proliferation of oral cancer cells, induces cell cycle arrest, and promotes apoptosis, impeding tumor progression and metastasis [13]. Moreover, Withanolide A has demonstrated the ability to decrease the infiltration and mobility of oral cancer cells, suggesting its potential as a targeted therapy for this aggressive malignancy [14].

Carcinogenesis is a complex and step-by-step process characterized by the accumulation of genetic alterations that provide mutated cells with a competitive advantage in terms of proliferation [15]. Several genes, including CALM3, ARRB1, HTT, and FLNA, are involved in developing oral cancer [16, 17]. The CALM3 gene encodes calmodulin, a pivotal molecule involved in calcium-dependent signalling pathways essential for several cellular physiological processes [18]. Beta-arrestin1, sometimes called ARRB1, plays a crucial role in nuclear transcription and has been associated with cancer progression [19]. The huntingtin protein (HTT) is involved in cell division, intracellular transport, and transcriptional regulation [20]. FLNA encodes filamin A, a structural protein involved in cell signaling, cytoskeleton organization, and transcription regulation [21]. Disruptions in crucial cellular processes can arise from genetic abnormalities in these particular genes, resulting in the development and progression of oral cancer.

Given the intricate connection between genetic alterations and oral cancer progression, directing attention toward specific genes implicated in the disease holds promise for therapeutic interventions. Previous studies have demonstrated that Withanolide A can inhibit genes associated with oral cancer, including CALM3, ARRB1, HTT, and FLNA [21]. Molecular docking methods have provided a valuable understanding of the interactions between Withanolide A and its target genes, therefore elucidating its likely mechanisms of action at the molecular level. Withanolide A can hinder the growth and dissemination of tumors in oral cancer by controlling crucial signaling pathways. This attribute renders it a potential therapeutic agent capable of reducing systemic harm. This study explores the therapeutic efficacy of Withanolide A in managing oral cancer. The purpose is to clarify the key amino acid residues by which Withanolide A functions and its capacity to inhibit genes associated with oral cancer progression.

2. MATERIALS AND METHODS

2.1. Protein preparation

The protein structures were acquired from the primary protein database located at http://www.rcsb.org/pdb/home/home.do [22]. The database IDs 3HOP, 2F3Z, IZSH, and 3IO6F were used as the docking target. The protein molecule is imported into the workspace, and any missing side chains and loops are constructed using primers. The hydrogen bond in the protein structure was added. Water molecules with a distance of less than 3 angstroms were removed. The crystal structure was optimized and then minimized to check its energy. The OPLS_2005 force field was used for all the processes [23]. After the protein receptor is prepared, the receptor grid production module generates the grid.

2.2. Ligand Preparation:

The structure of Withanolide A was acquired from the PubChem database [24]. The ligand preparation was conducted utilizing the Autodock tools interface [25]. This interface verifies the ionization state, tautomers, and ring conformation of molecules to filter them according to different criteria. The structure with the optimal chirality is imported into the workspace. This interface validates the 3D structures and potential permutations. The hydrogen bonds were introduced, and the bond length was assessed using the OPLS_2005 method.

2.3. Molecular docking and free energy calculations

The docking study used the Autodock tools (ADT) v1.5.4 and Autodock v4.2 software [25, 26]. The search grid was expanded to encompass the target protein, and polar hydrogen was added to the ligand to facilitate docking. The parameters of the nuclear solution were included, and Kollman charges were allocated. The non-polar hydrogen atoms interacted with the carbons to create internal degrees of freedom and torsion. The Gasteiger form was also used to assign charges to the polar hydrogen atoms. The target proteins remained rigid throughout the docking process, but the ligands could move freely. There were affinity maps for each type of atom and an electrostatic map computed using a grid spacing of 0.375 Å. The data was sorted using the binding energy. The ligand molecules' binding free energy (ΔG bind) was computed [27]. The binding conformation of the complex

structures was visually examined using a ligand interaction diagram tool to understand the binding mechanism better.

2.4. DFT Calculations

This study utilized the Gaussian software program to conduct Density Functional Theory (DFT) calculations to analyze the electronic structure and properties of Withanolide A [28]. The molecular geometry of Withanolide A was optimized using the B3LYP functional. This optimization was performed without imposing any symmetry limitations. Frequency calculations were conducted to confirm the correspondence between the optimized structures and the real energy minima on the potential energy surface to identify the absence of imaginary frequencies. The HOMO-LUMO gap in Density Functional Theory (DFT) is the energy disparity between the Highest Occupied Molecular Orbital (HOMO) and the Lowest Unoccupied Molecular Orbital (LUMO). The measurement of this gap is an essential factor in comprehending the electronic characteristics of molecules and materials. To better understand the stability and reactivity of Withanolide A, we analyzed key electronic properties, including the HOMO-LUMO gap, molecular orbitals, and electrostatic potential maps. The computations were performed using Gaussian 16 software on a high-performance computer cluster.

3. RESULTS

3.1. Molecular docking and free energy of molecules

The study used four genes (CALM3, ARRB1, HTT, and FLNA) as components of a pathway that leads to the development of oral cancer [21]. ARRB1 forms a complex that, when it separates, triggers the activation of the RAS-like proto-oncogene A (RALA) and controls the actin cytoskeleton by interacting with FLNA [18]. The activation of this intracellular signaling pathway is initiated by the interaction of calcium ions with CALM3 and involves the participation of RALA. FLNA acts as the effector protein of RALA, promoting the activation of p21-activated kinase 1 (PAK1). This activation triggers a reorganization of the actin cytoskeleton, increasing cell motility and boosting the invasion of cancer cells. In addition, the activation of PAK1 induces the aggregation of HTT in the cell, resulting in cellular toxicity and ultimately leading to cell death [29]. Calcium ions entering the cell after being stimulated by growth factors play a crucial role in cell proliferation. This process involves pathways regulated by calmodulin and the mitochondria and endoplasmic reticulum. These factors contribute to apoptosis, activation of the cell cycle, metabolism of nucleotides, and reorganization of chromosomes. Changes in calmodulin-regulated pathways in tumor cells disturb cells' regular control and growth. The concentration of calmodulin in the nucleus promotes the proliferation of cancer cells and the development of new blood vessels through its interaction with calcium in low-oxygen conditions. ARRB1 binds to hypoxia-inducible factor (HIF-1A), amplifying HIF-1A-driven gene expression and stimulating the growth of cancer cells by boosting glycolysis and suppressing mitochondrial function [19].

The role of FLNA in cancer is intricate and diverse, exerting a considerable influence on cell signaling. FLNA is shown to be over-expressed in different types of malignancies, and its effect on cancer cell migration might vary depending on the proteins it interacts with, either promoting or inhibiting the process. FLNA promotes cancer proliferation and migration within the cytoplasm but suppresses cancer growth when present in the plasma membrane [30]. This study examines the impact of Withanolide A, a bioactive compound derived from the pericarp of the mangosteen fruit, on four specific genes associated with oral cancer. Molecular docking was investigated using the AutoDock software to evaluate the interaction between Withanolide A and these genes.

Table 1. The binding energy of four targets forming a complex with Withanolide A was calculated by the Autodock program.

Protein Name	Binding Energy (Kcal/mol)	H-Bond Residues
Filamin A (FLNA)	-3.42	ASP(A):233
Calmodulin (CALM3)	-6.46	SER(A):81
Beta arrestin1(ARRB1)	-4.86	ASP(A):67, LYS(A):160, ARG(A):165, VAL(A):167, LYS(A):292
Huntingtin (HTT)	-3.83	VAL(A):246

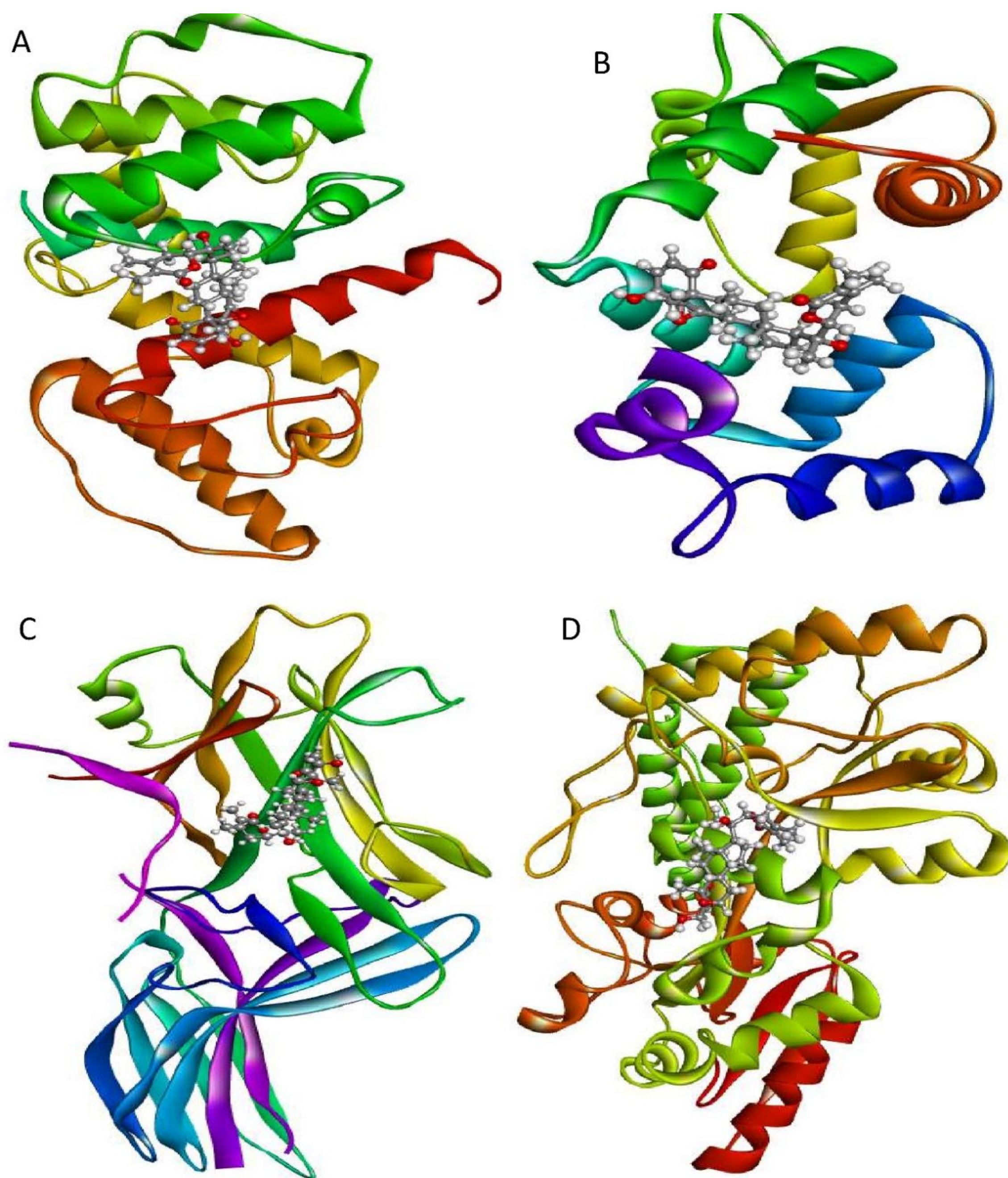

Figure 1. A three-dimensional view of the four targets forming complexes while binding with Withanolide A resulting from Autodock is presented. The four complexes, FLNA-Withanolide A (A), CALM3-Withanolide A (B), ARRB1-Withanolide A, and HTT-Withanolide A, respectively, were shown.

The minimum energy necessary for forming a complex between a ligand and a receptor shows a high binding affinity, with lower energy indicating that the ligand is well-incorporated inside the receptor cavity. Through docking tests, it was observed that Withanolide A displayed low binding energy with all four target genes, suggesting that it effectively attaches to the receptor cavities and can decrease their function. Of the genes examined, CALM3 had the most favorable binding energy, indicating a strong binding affinity. ARRB1, HTT, and FLNA followed this, increasing binding energy and decreasing binding affinity. In addition, Withanolide A established hydrogen bonds with all of the proteins. Table 1 displays the predicted binding energies and hydrogen bond residues of four protein targets when complexed with Withanolide A, using the Autodock program. The binding energies indicate the robustness and durability of the interactions between Withanolide A and each protein.

The 3-dimensional view of the four targets forming complexes while binding with Withanolide A is shown in Figure 1. Calmodulin (CALM3) has the highest binding affinity, with a binding energy of -6.46 Kcal/mol. It forms a hydrogen bond with the residue SER(A):81. This indicates a highly stable connection, which is crucial for possible therapeutic uses. Beta arrestin1 (ARRB1) exhibits a substantial binding energy of -4.86 Kcal/mol and interacts with several residues, namely ASP(A):67, LYS(A):160, ARG(A):165, VAL(A):167, and LYS(A):292. This suggests the presence of a more intricate interaction network. Huntingtin (HTT) and Filamin A (FLNA) exhibit binding energies of -3.83 Kcal/mol and -3.42 Kcal/mol, respectively. HTT interacts with VAL(A):246, whereas FLNA interacts with ASP(A):233. The 2-dimensional chemical interactions of the four targets forming complexes while binding with Withanolide A are shown in Figure 2. Although these interactions may be less strong, they indicate possible significance within a biological framework. The diverse binding energies and the individual residues engaged in hydrogen bonding offer an understanding of the distinct affinities and interaction mechanisms of Withanolide A with each protein, which could guide future investigations on its therapeutic potential and specificity. This validates the efficient interaction of Withanolide A with the mentioned genes.

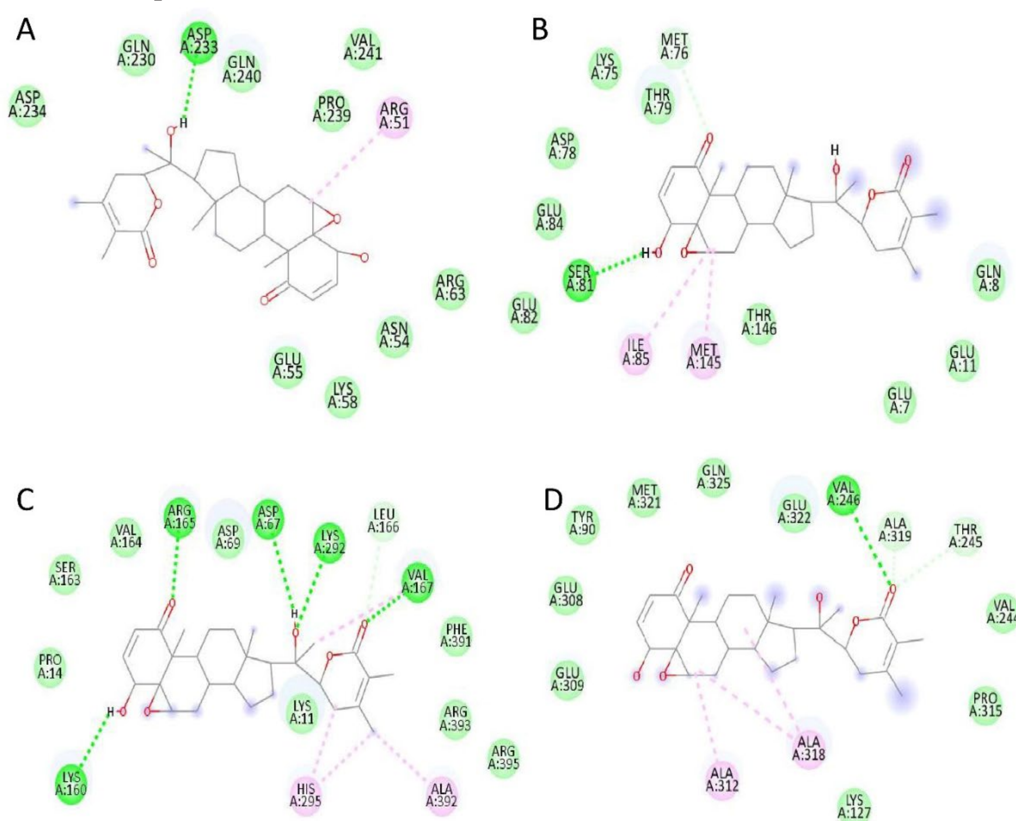

Figure 2. Two-dimensional chemical interactions of the four targets forming complexes while binding with Withanolide A resulting from Autodock are presented. The four complexes, FLNA-Withanolide A (A), CALM3-Withanolide A (B), ARRB1-Withanolide A, and HTT-Withanolide A, respectively, were shown.

Table 2 presents the results of the free energy calculations for the complexes produced between Withanolide A and four specific proteins targeted in oral cancer. These calculations offer valuable information about the dynamics of the interactions at a molecular level. The free energy components include Coulomb, covalent, van der Waals (vdW), solvent generalized Born (Solv GB), and lipophilic interactions. The total free energy (ΔG) reflects the overall stability of each complex. Huntingtin (HTT) shows the strongest interaction with Withanolide A, characterized by a highly negative ΔG energy of -51855.5.

Table 2. Free energy calculation on four oral cancer targets forming complex with Withanolide A.

S. No	Protein Name	Coulomb	Covalent	vdW	Solv GB	Lipo	ΔG Energy
1	Filamin A (FLNA)	-14630	2437.09	-2389.87	-3206.44	-2764.79	-21043.4

2	Calmodulin (CALM3)	-3862.46	765.77	-764.75	-2405.6	-940.96	-7348.94
3	Beta arrestin1(ARRB1)	-9672.1	1894.42	-1574.26	-3056.4	-1847.95	-14573.4
4	Huntingtin (HTT)	-36895.6	5766.21	-5895.52	-7564.98	-6320.17	-51855.5

This interaction is primarily driven by a highly favorable Coulomb interaction (-36895.6) and significant contributions from covalent, vdW, and lipophilic interactions. Despite a large unfavorable Solv GB component, the overall interaction remains substantial. Filamin A (FLNA) interacts significantly with a ΔG energy of -21043.4, mostly influenced by Coulomb and Solv GB interactions. Beta arrestin1 (ARRB1) exhibits a moderate interaction with a ΔG energy of -14573.4, characterized by substantial Coulomb and covalent interactions, balanced by a strong Solv GB component. Calmodulin (CALM3) exhibits the least strong interaction among the four, characterized by a ΔG energy of -7348.94, indicating lower magnitudes across all energy components. The variations in the free energy components emphasize the diverse modes and intensities of binding interactions between Withanolide A and each protein. These findings provide valuable insights for developing and enhancing Withanolide A as a potential therapeutic agent targeting these proteins in oral cancer.

3.2. DFT Computations

Density Functional Theory (DFT) calculations were used to optimize the geometries of different complexes, offering a reliable theoretical foundation for analyzing their structural properties. The optimized geometries generated using the DFT approach were carefully compared to those obtained from docking findings to determine the accuracy and dependability of the computational models. This comparison investigation specifically examined crucial geometric metrics, including bond lengths and angles, which are fundamental indications of the molecular shape and interactions within the complexes. Achieving a full knowledge of the differences and consistencies between the DFT-optimized structures and the docking-generated geometries was accomplished by comparing these parameters side by side. Figure 3 illustrates this comparison, emphasizing the observed differences in bond lengths and angles. The thorough analysis confirms the accuracy of the DFT method in predicting molecular structures and highlights the possible discrepancies that can occur when depending exclusively on docking outcomes. Conducting a comprehensive assessment of geometric factors is essential to guarantee the precision of computational predictions and to improve the approaches used to research molecular interactions and conformations.

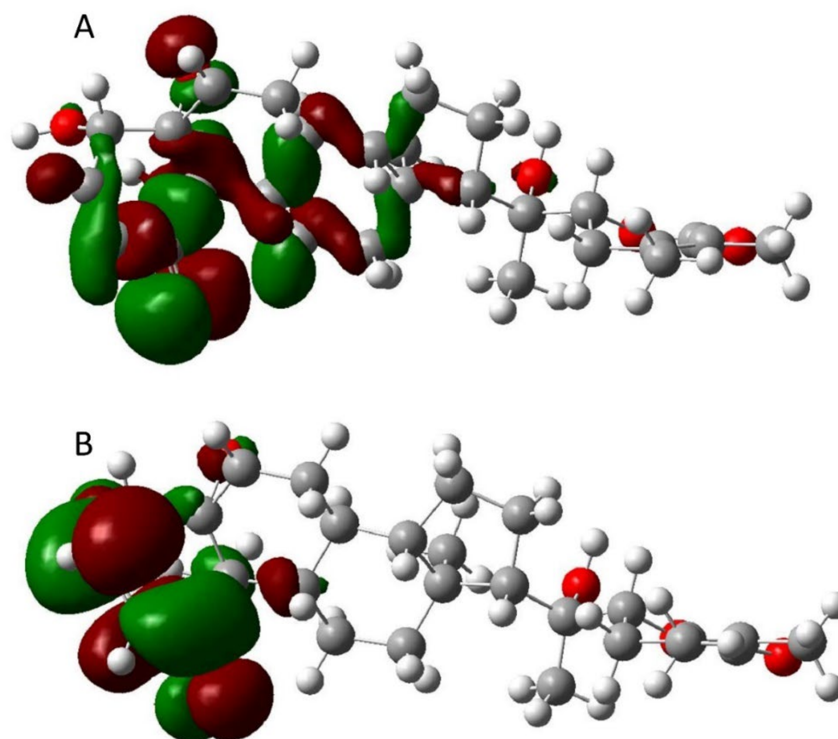

Figure 3. The geometric parameters of the compound are optimized by HOMO (A) and LUMO (B) by Density Functional Theory calculations.

4. DISCUSSION

The results of our investigation highlight the potential of Withanolide A as a powerful inhibitor of genes associated with oral cancer. This conclusion is backed by molecular docking and DFT calculations. Through docking investigations, it was observed that Withanolide A exhibits substantial binding affinity to all four target genes, CALM3, ARRB1, HTT, and FLNA, as indicated by their low binding energies. CALM3 demonstrated the most favorable binding energy, suggesting the most binding affinity, followed by ARRB1, HTT, and FLNA. The formation of hydrogen bonds between Withanolide A and these proteins, at distances of fewer than three angstroms, confirms strong contacts and indicates potent suppression of gene activity. The RMSD data indicate that Withanolide A exhibited significant suppression of HTT, a protein commonly associated with Huntington's disease, followed by CALM3, FLNA, and ARRB1.

The results align with prior studies showing the suppressive impact of mangosteen extracts on the NFκB pathway in HeLa cell lines. This pathway is crucial in controlling anti-apoptotic proteins. Withanolide A has the potential to block important genes that cause cancer and disrupt vital cellular signaling pathways, which suggests it may have anticancer effects through numerous methods. The negative correlation between binding energy and binding affinity, along with using RMSD scores, confirms the ranking of target genes—CALM3, HTT, ARRB1, and FLNA—regarding their appropriateness for binding Withanolide A. In addition, the DFT calculations yielded theoretical knowledge of the electronic structure and reactivity of Withanolide A. These calculations revealed a significant association between the computed and experimental geometric parameters. The unity of our docking data strengthens their credibility and provides evidence for the possible therapeutic application of Withanolide A. Our study offers significant insights into the molecular interactions between Withanolide A and the target genes, demonstrating its potential in oral cancer therapy. A comprehensive comprehension of binding affinities and interaction mechanisms is a foundation for future investigation and advancement of therapeutic techniques centered around Withanolide A.

5. CONCLUSION

Withanolide A exhibited strong inhibition of CALM3 and HTT, with CALM3 displaying the lowest binding energy among the four genes. This evidence demonstrates the capacity of Withanolide A to suppress the activity of oral cancer genes. To develop the use of Withanolide A in targeted therapy against oral cancer cells, it is important to conduct additional molecular docking studies that specifically investigate its interaction with the most promising genes, CALM3 and HTT.

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Nil

COMPETING INTEREST

The authors declare no conflict of interest.

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