Original Article

Molecular Docking Of Withaferin A From Withania Somnifera With The Proteins Associated With Cancer Therapy

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Abstract

Withaferin-A, a member of the withanolides class, is an essential phytoconstituent of Withania somnifera (Ashwagandha). These are naturally occurring C28-steroidal lactone triterpenoids groupings. For several decades, Withaferin-A was in use for several decade to treat various disorders. Design and development of an effective compound to combat cancer is clearly critical in the current circumstances. Therefore, it is of interest to document the molecular docking analysis data of the different cancer therapy with Withaferin-A from Withania somnifera in the context of cancer for further consideration. Here, we report the optimal interaction features of Withaferin A, different cancer receptors having low binding energies -9.93, -8.34, -9.93, -8.03, -9.69, -6.10 kcal/mol respectively) in this report. In order to gain additional insights, the interaction pattern of compounds with anti- cancer activities was studied.

Keywords: Drug-likeness properties, Molecular docking, Withaferin-A, Withania somnifera

1. Introduction

Withania somnifera is a native plant. One of the major phytoconstituents of this plant is Withaferin-A, which has been used for more than a few decades for its therapeutic uses in treating various disorders. Several reports are available, but the exact mechanism of their anti-cancer properties is still unknown.Withaferin-A (WA) is obtained from the Ashwagandha of the Solanaceae family. They are frequently referred as Indian ginseng, Ashwagandha or Indian winter cherry. The mechanism of cancer growth suppression by Withaferin-A is caused by the target-specific nature of Withaferin-A against tubulin (inherent of microtubules). A remarkable decrease in the amount of b-tubulin was observed during Withaferin-A usage in the treatments. Research was established to find the molecular functions that describe the anti-cancer properties of Withaferin-A.

Withaferin-A induces oxidative stress, which eventually determines the mitochondrial dysfunction and apoptosis in leukemia cells. Also, Withaferin-A has shown various pharmacological activities which have anti-inflammatory, anti-stress, anti-tumor, anticonvulsant and neuroprotective effects. They are naturally formed C_{28}-steroidal lactone terpenoids. Cancer, the fatal disease, has limited success in finding a suitable cure. Even though researchers are finding a way to know all the molecular pathways of Withaferin-A to fight against the cancer-causing agents, they also came across the fact that they have therapeutic action towards cancer, some of them even show cancer preventive activities.

The significant anti-cancer effects of this plant (Ashwagandha) are related to withanolides. Withaferin-A (4, 5, 6,22R) was the first anticancer compound isolated from the leaves of Withania somnifera. Withaferin-A is -4,27-dihydroxy-5,6-22,26-diepoxyergosta-2,24-diene-1,26-dione). The compound Withaferin-A is pictured in (**Error! Reference source not found.**). Here we provide a detailed and summarized review on Withaferin-A and its various decreasing types of cancer.

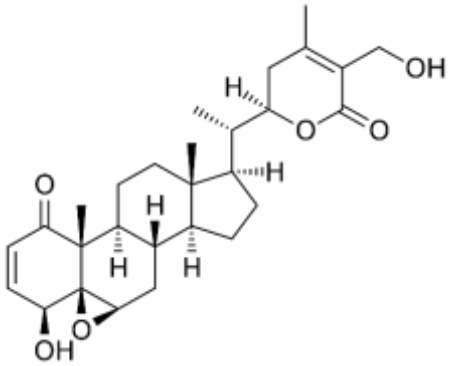


Fig. 1. Withaferin-A chemical structure

2. In vitro anti-cancer activity of Withaferin-A

The fluorescein diacetate propidium iodide dye exclusion test and glucose stimulation assay were used to assess the effect of a medication on pancreatic islet cell survival while controlling for aferin. Withaferin-A had no effect on islet cells, lowering their inflammatory response to cytokine exposure. 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-carboxymethoxyphenyl)-2-(4-carboxymethoxyethane) (4-sulfophenyl) The effects of Withaferin-A on the viability and proliferation of MCF-7 cells were investigated by using exclusion studies with -2H-tetrazolium (MTS) and trypan blue. Proliferation and viability have been demonstrated to be inhibited by Withaferin-A in MCF-7 cells [1]. Withaferin-A , an NF-B inhibitor, reduced neuromuscular junction denervation and clinical symptoms in TDP-43 (TAR DNA-binding protein 43) mice [2]. Another investigation discovered that Withaferin-A plays an active role in the HSF1-dependent stress response [3]. The effects of WA on corneal angiogenesis and retinal gliosis were investigated and treatment with WA reduced soluble and filamentous GFAP expression while inhibiting corneal neovascularization [4]. On isolated frog skin melanophores, the effects of pure Withaferin-A were investigated. In isolated skin melanophores, Withaferin-A evoked strong dose-dependent physiologically meaningful melanin dispersal effects that were fully inhibited by hyoscine and atropine [5]. It also inhibited LPS-induced cyclooxygenase (COX-2) protein and mRNA expression, as well as prostaglandin E2 (PGE (2)) production in BV2 murine microglial cells. Withaferin-A , according to these findings [93], suppresses LPS-induced PGE (2) synthesis and COX-2 expression.

The high binding energy required to link Withaferin-A to the active Hsp (Heat shock proteins) 90/Cdc37 complex boosts the thermodynamic stability of the association, according to computational study [6]. The effect of this compound on the module ability of the NF-B signalling pathway was investigated, and it was discovered that the native protein complexes with Withaferin-A had stable temporal trajectories [7]. Withaferin-A 's ability to inhibit mammalian proteasomes was explored, and it was discovered that it can inhibit mammalian proteasomes irreversibly and rapidly via acylation of the -5 subunit's N-terminal Thr1 [8]. Withaferin-A was tested for its ability to inhibit the Hsp90/Cdc37 chaperone/co-chaperone interaction complex. According to molecular modelling experiments [9]. The combination of Withaferin-A and 17-DMAG can be an efficient chaperone system inhibitor. Another study found that Withaferin-A , a dual vimentin and proteasome inhibitor, decreased E. coli-induced polymorph nuclear neutrophil (PMN) transmigration substantially [10]. During the recovery of a central nervous system injury, gliosis is a biological state characterised by the overexpression of the intermediate filaments glial fibrillary acidic protein and vimentin. A unique chemical probe is glial fibrillary acidic protein Withaferin-A [11]. Oral treatment of Withaferin-A to rats treated with 7,12-dimethylbenz(a) anthracene (DMBA) decreased tumour growth while also synchronising lipid peroxidation and antioxidant capacity [12].

The ability of Withaferin-A to suppress growth and differentiate in glioma (C6 and YKG1) cell lines was scrutinized and observed that Withaferin-A dramatically inhibits glioma cell proliferation in a dose dependent manner [13]. The effect of Withaferin-A on CF-related inflammation was also examined in an in vitro model [14]. When human kidney cancer cells and Caki cells are co-treated specifically with subtoxic doses of Withaferin-

A and a tumour necrosis factor-related apoptosis inducing ligand, apoptosis is accelerated. In human lung epithelial A549 cells, Withaferin-A decreases the expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), suggesting a function in airway inflammation. The effects of Withaferin-A on thermotolerance development and degradation in C57BL mice with B16F1 melanoma were studied.

Withaferin-A increases tumour responsiveness to recurrent heat by lowering thermotolerance and reducing recovery time [15]. The action of Withaferin In mice, the effects of monosodium urate crystals on inflammation were investigated. Withaferin outperformed the control group. A therapy reduced lysosomal enzyme levels, paw volume, inflammatory mediator tumour necrosis factor alpha, and lipid peroxidation [16]. Apoptosis is caused by Withaferin-A in conjunction with caspase-3 activation. The JNK and Akt pathways, as well as the inhibition of NF-kappaB activity, were discovered to be significant regulators of apoptosis in human leukaemia U937 cells in response to Withaferin-A [17]. Withaferin-A elevated Bax levels in response to MAPK signalling, resulting in the commencement of a mitochondrial death cascade. It has the potential to be used as a novel, low-cost chemotherapeutic medication to treat lymphoid and myeloid leukaemia [18]. When combined with the human filarial parasite Brugia malayi, Withaferin-A provides varied levels of protection in Mastomys coucha, with chemotype 101R protects strongly than other chemotypes.

Using the MDA1986, JMAR, UM-SCC-2, and JHU011 cell lines, researchers discovered that Withaferin-A had antiproliferative effect against head and neck squamous cell carcinoma (HNSCC) [19]. The oncogenic transcription factor STAT3 has been linked to a number of human malignancies, including breast cancer [108], and Withaferin-A suppresses both constitutive and IL-6-induced activation. The effect of Withaferin-A on the response of B16F1 melanoma to fractionated and acute radiation was studied with and without local hyperthermia. In fractionated regimens, Withaferin-A is a greater radiosensitizer than HT, and it improves the response of radioresistant tumours like melanoma [20]. In vimentin-expressing tumour cells, withaferin-A caused considerable apoptosis and vimentin cleavage, but not in normal mesenchymal cells. Furthermore, in a panel of soft tissue sarcoma xenograft tests, withaferin-A inhibited soft tissue sarcoma development, local recurrence, and metastasis [21]. In three colon cancer cell lines (HCT-116, SW-480, and SW-620), Withaferin-A is a bioactive molecule that inhibits Notch-1 signalling and downregulates prosurvival pathways such as Akt/NF-kappaB/Bcl-2.

The in vitro and in vivo efficacy and mechanism of Hsp90 inhibition of Withaferin-A in pancreatic cancer were investigated. Without requiring ATP, Withaferin-A binds to Hsp90 and suppresses its chaperone action. Dual inhibition of the transcription factors NF-kappaB and AP-1 Fra-1, as well as silencing of IL-6 promoter chromatin accessibility, were reported to reduce IL-6 gene transcription in metastatic breast cancer cells [22]. Withaferin- A anti-carcinogenic efficacy was investigated in Syrian golden hamsters exposed to 7, 12-dimethylbenz[a]anthracene (DMBA). When given orally for 14 weeks, Withaferin-A completely reduced tumour volume, tumour incidence, and tumour burden. To investigate the involvement of this compound in the molecular aetiology of oral cancer, researchers used immunological expression of the p53 and bcl-2 proteins. Withaferin-A protects the buccal mucosa of golden Syrian hamsters from 7, 12-dimethylbenz (a) anthracene (DMBA)-induced molecular alterations. Withaferin-A in human breast cancer cells, a therapy causes G2 and mitotic arrest. [24].

3. Drug -Likeness properties

ADMET Predictor is a computer tool developed to estimate pharmacokinetic properties of drug-like compounds based on their molecular structures for ADMET [25]. A drug/drug-like compound's strong bioactivity and low toxicity are not sufficient criteria to classify the chemical as a promising candidate. An improved pharmacokinetic profile is essential for a novel chemical that should be investigated during the drug/drug-like compound discovery process. As a result, it is critical to assess the ADMET profile of novel compounds early on in order to prevent wasting time and resources. As a result, we projected our W.A ADMET qualities. A chemical created with Swiss ADME online software [26].

To assess the pharmacokinetic features of Withaferin - A phytocompound, the compound's 2D structure was generated on Chemdraw Professional 16.0. Each structure was imported, and the structure smile was added

to the website's interface (http://swissadme.ch/). The SwissADME drug design study was carried out, and the ADMET properties were calculated [27].

ADMET stands for "absorption, distribution, metabolism, excretion, and toxicity" in pharmacokinetic/pharmacology, and it describes the disposition of a pharmacological compound in the body. The ADMET Predictor is a computer tool designed to estimate the pharmacokinetic parameters/properties of drug-like substances based on their molecular structures. The Swiss ADME web tool is free software that predicts the physicochemical qualities, absorption, distribution, metabolism, elimination, and pharmacokinetic properties of molecules, all of which are important factors in future clinical studies. It considers six critical physicochemical properties: lipophilicity, flexibility, saturation, polarity, solubility, and size [28].

The ADMET result revealed physicochemical properties of the W.A phytocompound, including the rules of five (MW, iLOGP, HBAs, and HBDs) and several other properties such as molecular polar surface area (TPSA), number of rotatable bonds (ROTBs), number of aromatic heavy atoms, and number of aromatic heavy atoms, and among others as represented in the (**Table 1**) below. OSIRIS web tool software utilized to the toxicity prediction (**Fig. 1)** (www.Cheminfo.org).

Table1: Calculated ADME parameters and of the Withaferin-A Phytocompound

Comp	MW	iLogp	HBD	HBA	TPSA	RB	nAH	MR
W. A	470.61	3.39	2	6	96.36	3	34	127.49

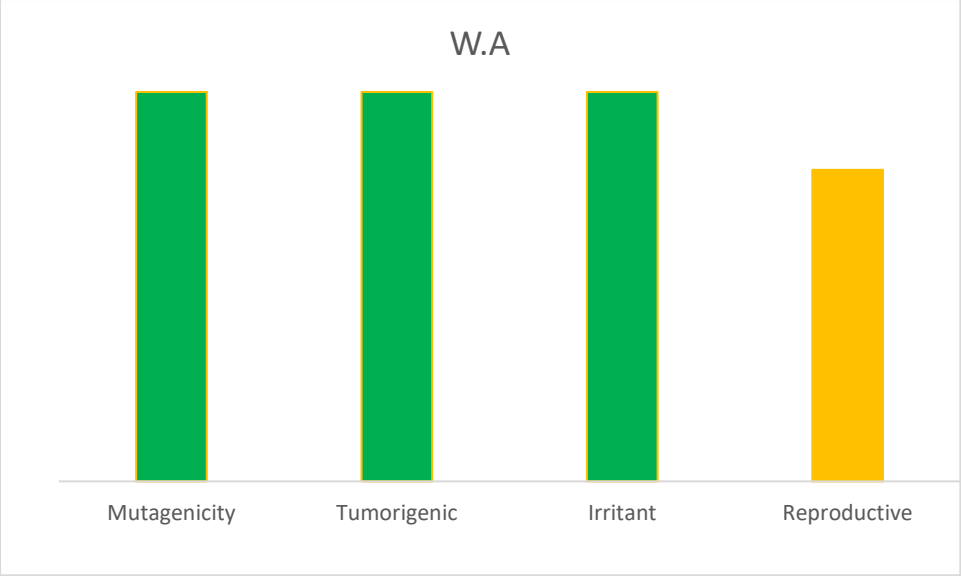


Fig.1. Toxicity prediction of Withaferin-A phytocompound

3.1. Preparation of Protein

The protein was utilised to forecast the behaviour of the chemicals indicated against the macromolecular targets of the Anti-tumour's activities Breast (PDB: 3ERT), Cervical (PDB: 4J96), Colon (PDB: 4UYA), Lung (PDB: 4ZXT), Prostate (PDB: 6XXP), Ovarian (PDB: 7SAP) (www.rcsb.org). We used the Swiss-PDB viewer software application to reduce crystal structure energy and then used Biovia Discovery Studio to eliminate all water

molecules and heteroatoms from the proteins. Following the protein synthesis procedure, H-bond optimization and the insertion of Kollmann charges, which were subsequently saved to pdbqt charges were performed.

3.2. Grid and Active Site Generation

The MMFF94 force field was employed in 2000 steps with a Van der Waals scaling factor of 1.50 and a charge cut off of 0.25. A 60 60 60 grid with a grid spacing of 0.503 was defined and used as the bounding box for the docking investigation. The docking and binding process has been described using the terms docking and critical analysis. The active sites were identified using the Drug Discovery Studio version and used as the ligand coordinates in the original target protein grids (BIOVIA Dassault Systems). The non-covalent interactions were discovered using Discovery Studios Software by the researcher [29].

3.3. Ligand–Receptor Interactions

The posture view of Lead was used to examine the interactions of Withaferin-A phytocompound with several anti-tumor protein targets in the docked complex [30]. We created a 2D and 3D pose view of the protein target phytocompound Withaferin-A and explored its lead.

4. Molecular docking

We used molecular docking to investigate the deep molecular basis of interactions and to calculate the binding affinity of the current drugs with the anti-tumor active site. 3ERT, 4J96, 4UYA, 4ZXT, 6XXP, and 7SAP (crystallised compounds having anti-tumours) structures were prepared, and WA compounds were docked into the active site. (**Table 2**) shows the docking results of the ligands with WA using Docking calculations. With binding energies of -9.93, -8.34, -9.93, -8.03, -9.69, and -6.10 kcal/ mol.

Withaferin-A docking revealed excellent binding affinity with good interactions with key residues present in the catalytic site. 3ERT was docked into active sites with -9.93kcal/mol of binding energy, generating direct hydrogen bonds with 523-GLU,530-CYS, hydrophobic interactions, and pi-pi stacking interactions with 522-MET,525-LEU,526-TYR, according to docking interactions. 4J96 was docked into active sites with a binding energy of -8.34 kcal/mol and found to generate direct hydrogen bonds 494-GLN, 518-MET, 527-ASP, 531-LEU, 630-ARG hydrophobic interactions and pi-pi stacking interactions with 492-PHE, 495-VAL, 530-ASP, 644-ASP, 666-PRO.

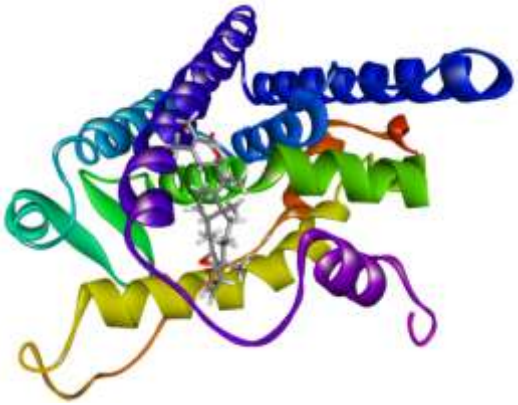
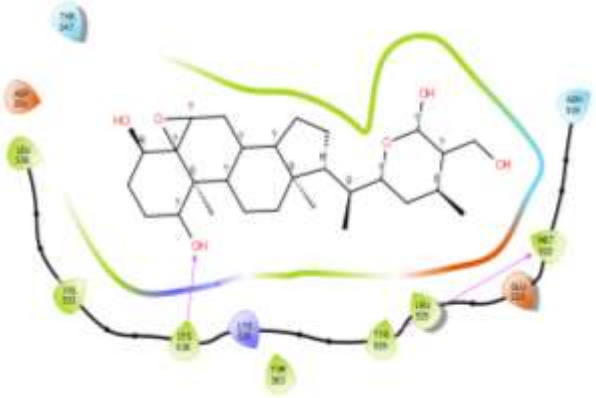
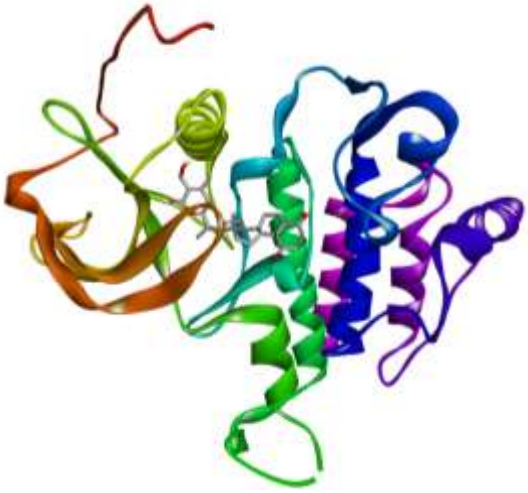
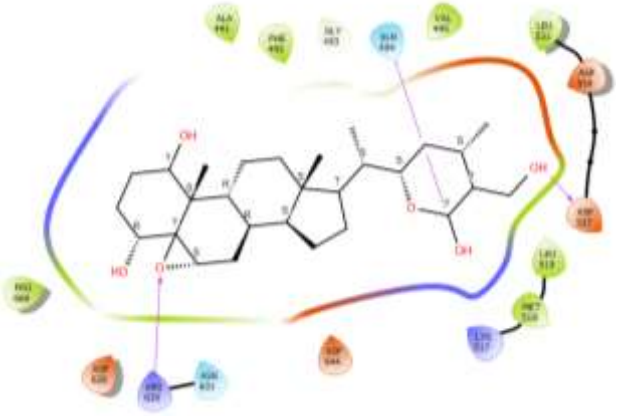
4UYA was docked into active sites with -9.93 kcal/mol of binding energy and shown to be forming direct hydrogen bonds 203-ALA,289-ASP,290-PHE hydrophobic interactions and pi-pi stacking interactions with 138-VAL,198-LEU,200-LEU,270-LEU,289-ASP.4ZXT was docked into active sites with -8.03 kcal/mol of binding energy and shown to be forming direct hydrogen bonds 54-LYS,71-GLU,153-SER,167-ASP hydrophobic interactions and pi-pi stacking interactions with 35-ALA,84-ILE,113-TYR,156-LEU.6XXP was docked into active sites with -9.69 kcal/mol of binding energy and shown to be forming direct hydrogen bonds 2-VAL,3-GLN,5-GLN,113-TYR hydrophobic interactions and pi-pi stacking interactions with 3-GLN,4-LEU,113-TYR.

4J96 was docked into active sites with -6.10 kcal/mol of binding energy and shown to be forming direct hydrogen bonds 52-TYR,57-HIS,98-SER,100-LYS,101-ASP hydrophobic interactions and pi-pi stacking interactions with 97-ARG,99-GLU,100-LYS **(Fig.2).**

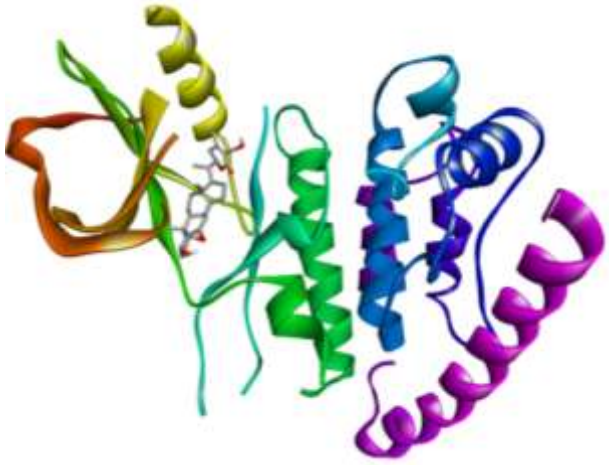
Table 2 Docking scores of Withaferin-A in silico binding with the anti-tumour's proteases

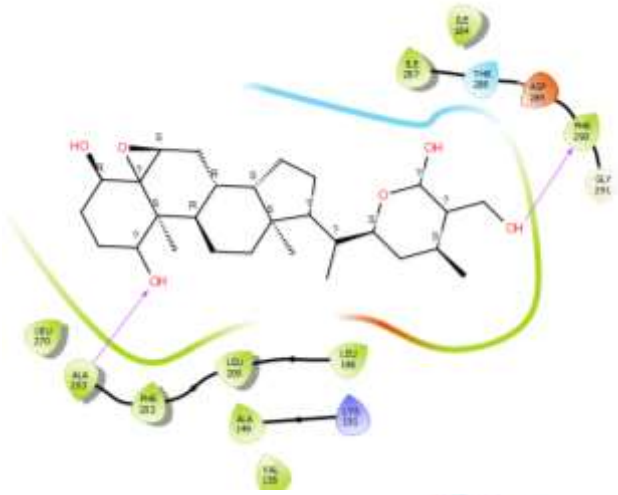
Comp	PDB IDS	Binding energy	H-BONDS & Distance	Hydrophobic and Pi-Pi Stackings & Distance
	3ERT	-9.93	523-GLU-2.59	522-MET-3.95
			530-CYS-2.21	525-LEU-3.82
				526-TYR-3.67
	4J96	-8.34	494-GLN-2.19	492-PHE-3.51
			518-MET-2.50	495-VAL-3.29
			527-ASP-2.19	530-ASP-3.76

			531-LEU-3.52	644-ASP-3.88
			630-ARG-2.66	666-PRO-3.68
	4UYA	-9.93	203-ALA-2.54	138-VAL-3.30
			289-ASP-2.38	198-LEU-3.62
			290-PHE-2.26	200-LEU-3.79
Withaferin-A				270-LEU-3.31
				289-ASP-3.93
	4ZXT	-8.03	54-LYS-1.96	35-ALA-3.64
			71-GLU-2.05	84-ILE-3.94
			153-SER-2.92	113-TYR-3.73
			167-ASP-2.28	156-LEU-3.35
	6XXP	-9.69	2-VAL-2.56	3-GLN-3.42
			3-GLN-1.93	4-LEU-3.70
			5-GLN-2.73	113-TYR-3.83
			113-TYR-2.04	
	7SAP	-6.10	52-TYR-2.57	97-ARG-3.20
			57-HIS-2.66	99-GLU-3.49
			98-SER-2.12	100-LYS-3.78
			100-LYS-2.66	
			101-ASP-3.13	

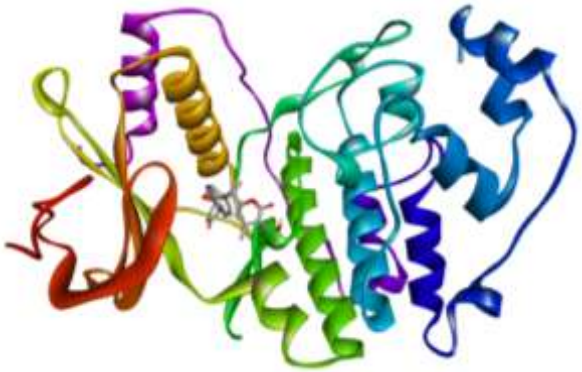
PDB ID	3D POSE STRUCTURE	2DLIGAND INTERACTION
3ERT		
4J96		

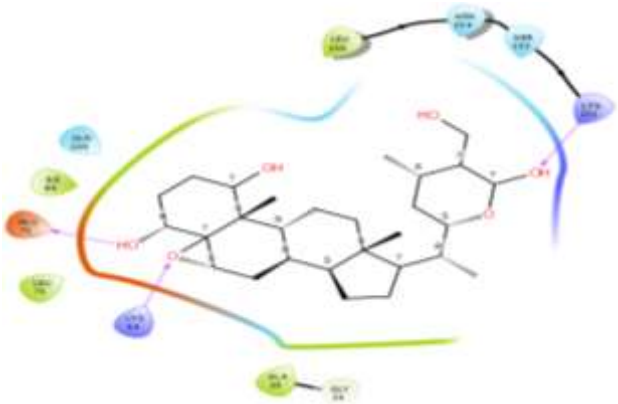
4UYA



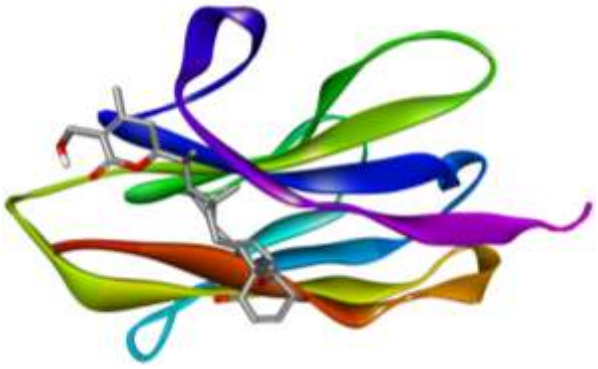


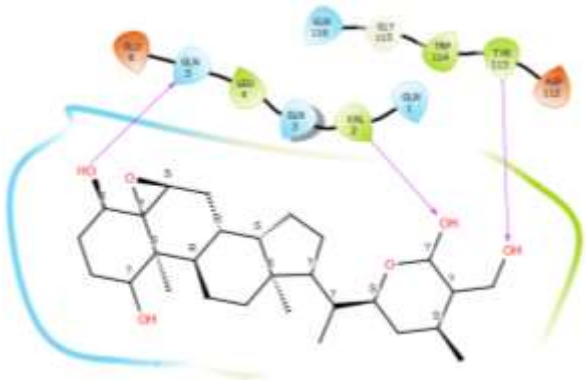
4ZXT



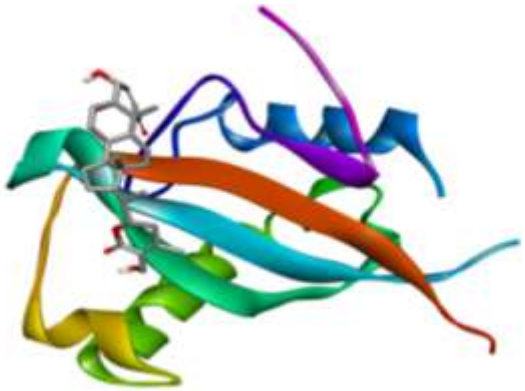


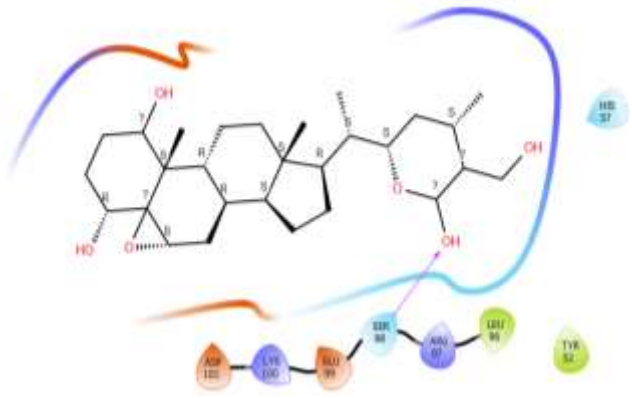
6XXP





7SAP





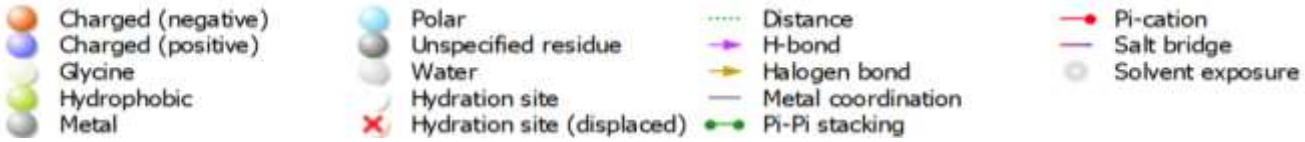


Fig.2. H-bond and contacts created by WA are shown in this docking simulation snapshot in complex with WA. Various interactions generated by WA at the catalytically active site are shown in a 2D depiction snapshot.

5. Conclusion

The current data could be quite beneficial for drug computational screening. Understanding the chemical interactions between ligand and receptor may aid in the identification of anti-tumor target specific medicinal compounds derived from this research. The recent study also found that Withaferin-A, a potential natural anticancer drug, is a good target. The Lipinski criterion for drug-like qualities was met, and the natural chemicals caused little or no harm. The molecular docking results of the protein complexed with the ligand Withaferin-A are stable, with the complex's energies lower than those of the un-docked protein.

Finally, the compound's inhibitory potential toward reasonable anticancer activities was shown by the present docking, predicted IC_{50} value, and molecular docking analysis. New interesting research will also be carried out on Withaferin-A in the future for its anti-cancer properties, thereby opening a great future aspect for it to become an important phytochemical in various cancer treatment therapies and general population-related diseases. Withaferin-A is said to have antimetastatic, anti-inflammatory, immunosuppressive properties, and many more. Whether it be the treatment of Cancer or COVID-19 or any other form of the disease, Withaferin-A has proved to have a great potential in being a very profitable and essential phytochemical that can be used as a therapeutic agent.

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Declaration of conflicts of interest

The authors declare they have no relevant conflicts of interest.

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