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Network ethnopharmacological and molecular docking based evaluation of the anti-breast cancer activity for ayurvedic botanicals acting on hypoxia-inducible factor-1

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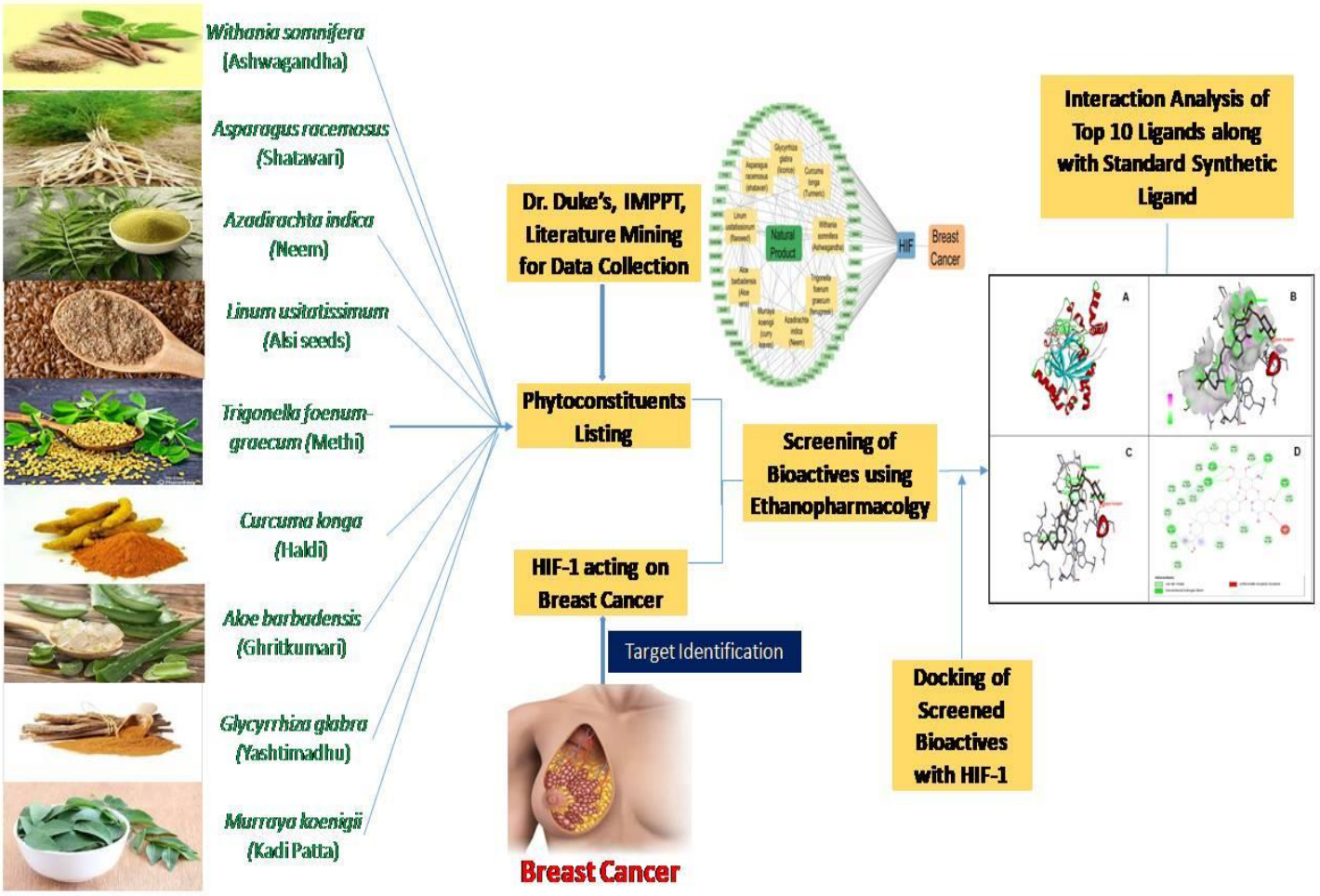
ABSTRACT

Graphical Abstract: Assessment of the Anti-Breast Cancer Potential of Ayurvedic Botanicals Acting on HIF-1 via Network Ethnopharmacology and Molecular Docking Methods



The recognized root causes of breast cancer aggressiveness, resistance to therapy, and poor prognosis encompass hypoxia-inducible factors (HIFs), the HIF-dependent cancer hallmarks angiogenesis and metabolic reconfiguration.There is enough evidence to suggest that HIF is involved in the progression of breast cancer.Information for the network was gathered from the following databases: Dr. Duke's, IMPPAT, PubChem, Binding DB, UniProt, and DisGeNET. The network was created using the Cytoscapeprogramme. Screened bioactives having similarity index more than 0.6 from polypharmacology approach were subjected to docking studies with HIF-1 (PDB ID: 1H2K) with PyRx software and the ligands with good docking score was further explored for molecular docking interaction analysis. The number of bioactives having interaction with HIF-1, equal to or greater than 0.6 from Ashwagandh, Shatavari, Neem, Alsi seeds, Methi, Haldi, Ghritkumari, Yashtimadhu, and KadiPatta was 12, 9, 2, 6, 12, 11, 5, 10, and 3, respectively. On performing the docking against the target HIF-1for top molecules with Araboglycyrrhizin (-10.1kcal/mol), Asparanin-A (-9.2 kcal/mol), Shatavarin-I (-9.2 kcal/mol), Shatavarin-X (-9.1 kcal/mol), Somniferanolide (-9.1 kcal/mol), Somniferawithanolide (-9.7 kcal/mol), Trigofoenoside-A (-9.2 kcal/mol), Trigofoenoside-F (-9.4 kcal/mol), Trillin (-9.9 kcal/mol), and Withanolide (-9.2 kcal/mol), the binding energies and molecular interactions of the ligands were fairly good as compared with standard synthetic ligand Acriflavine (-7.6 kcal/mol). The study elucidated an in silico molecular mechanism of HIF-1 inhibition by various bioactive phytoconstituents from selected plants. Comprehending the logic behind anti-breast cancer action was made easier with the help of experimental evidence of the network findings.

Keywords: Breast Cancer, Hypoxia-Inducible Factor,Network Pharmacology,Molecular docking, Ayurvedic Botanicals.

INTRODUCTION

Integration of Ayurveda into our current health care research programs is critical to making progress in global wellness and in disease prevention and control, especially for cancer. Ayurveda promotes restoration of the innate healing mechanisms existing in the body for optimal immunity, resilience, and health. Ayurveda also has an abundant resource of botanical products containing diverse pharmacoactive ingredients and millennia of experience of clinical applications for health benefits. But there is a lack of evidence-based research to demonstrate its efficacy and potential [1]. The potential of Ayurvedic medicine needs to be explored further with modern scientific validation approaches for better therapeutic leads [2].

More than 410,000 women die each year as a result of breast cancer, which affects an estimated one million women each year [3].Most nations have breast cancer as the most prevalent cancer diagnosed in women, accounting for a quarter of all malignancies diagnosed in women. There are more than half (52 percent) of new breast cancer diagnoses in economically developing nations and more than half (62 percent) fatalities from the illness [4].Up to the year 2030, nearly 10 million new cases of breast cancer would have been discovered, making it the most prevalent disease in the world. When a woman is diagnosed with breast cancer and undergoes treatment, she and her family are subjected to a wide range of negative effects on their physical and emotional health as well as their quality of life, as well as their financial well-being [5].

Breast cancer may be fought using natural chemicals, which can block malignant cell growth, and regulate cancer-related processes. When it comes to cancer prevention and therapy, natural products have played a significant role. Anticancer capabilities have been found in more than 3000 plant species [6]. As contrast to conventional chemotherapy, the side effects of plant-derived natural compounds are far less severe. In order to delay, diminish, or reverse the occurrence of breast cancer in women at high risk, it is required to develop effective therapies. Natural products may be an effective means of chemoprevention for breast cancer since they have less side effects and a lower toxicity than manufactured drugs [7].

Cancers are known for their hypoxia. In hypoxic cancerous tissues, HIF-1 (hypoxia-inducible factor-1), a key subunit of HIF (hypoxia-inducible factor), is overexpressed and stimulates the transcription of numerous oncogenes. HIF-1 enhances tumor angiogenesis, metastasis, metabolism, and immune evasion, according to mounting data [8]. The ability of the solid malignancy to produce hypoxia-inducible factors (HIFs), which control hundreds of target genes, allows it to overcome the hypoxic state. It has been demonstrated that many cancers, including breast cancer, overexpress HIF-1. The hypoxic area is found in nearly all solid tumors. Excessive cancer cell proliferation and aberrant blood vessel creation and anatomy are the causes of the hypoxia inside the cancerous tumor. According to reports, 25–40% of the breast cancer microenvironment exhibits a hypoxic area. HIFs and their targets are important for the enrichment of cancer stem cells; nevertheless, there is evidence that focusing on HIF may lower the number of cancer stem cells and enhance the effectiveness of chemotherapy [9].

A plethora of medicinal plants have gained the attention of researchers towards their therapeutic effects against various diseases, including their anti-carcinogenic properties. Phytochemicals play an important role in the initiation, development, and advancement of carcinogenesis, as well as in suppressing or reversing the early stages of cancer or the invading potential of premalignant cells and also regulates cell proliferation and apoptosis signaling pathways in transformed cells [10].

The goal of the study is to investigate, using in-silico techniques, the anti-breast cancer activity of multiple Ayurvedic botanicals that are

traditionally recognized for their anti-cancerous action toward known breast cancer targets. In order to uncover bioactives targeting HIF-1, the design applies a network ethnopharmacological approach to the initial screening of phytochemicals from confident Ayurvedic botanicals. A molecular docking tool is then used to further explore the screened bioactives for binding affinity and interaction analysis. Major investigations will identify leads as naturally occurring, bioactive substances that efficiently inhibit HIF-I as compared to standard synthetic molecule in the treatment and prevention of breast cancer.

MATERIAL AND METHODS

Network pharmacological studies

Data mining for Phytochemicals

In this investigation, the phytoconstituents from selected Ayurvedic botanicals that have been traditionallydocumented to possess anti-breast cancer were employed. Dr. Duke's Phytochemical and Ethnobotanical Databases online platform, Indian Medicinal Plants, Phytochemistry and Therapeutics 2.0, and literature mining were used to gather information about the phytoconstituents of selectedAyurvedic botanicals [11-12].

For the investigation, the structure data file (sdf) formats, which are freely available in the 3D structures of phytoconstituents, were utilized. In PubChem, look up the common names and precise structures of the phytoconstituents from selected Ayurvedic botanicals [13].

Establishment of Target

The RCSB Protein Data Bank (RCSB PDB) was used to gather data on hypoxia-inducible factor-1 (HIF-1) and its role in cancer progression.The species are confined to human sources, and target is identified through its UniProt ID at RCSB PDB [14]. The HIF-1as therapeutic target related to breast cancer was searched by using DisGeNET database [15]. Through UniProt database, standard name for protein target was found [16].

Screening of bioactives by polypharmacology

The sdfscontaining the structures of phytoconstituents from selected Ayurvedic botanicals were uploaded to the Binding DB (https://www.bindingdb.org) for the purpose of predicting the binding of bioactives to HIF-1 for the treatment of breast cancer. Binding DB recommends similarity index more than 0.4 to study compound target-association. Those bioactiveshaving a similarity index between 0.6 and 1 has been chosen for study. The multiple databases that Binding DB is connected to were leveraged to extract additional data on the target. The UniProt IDs provided in Binding DB were used to retrieve the protein symbols from UniProt. DisGeNET was searched for associations between the bioactive target and breast cancer.

Network Construction

Cytoscape3.10.0.was used to visually represent the network, analyze, and update the data. The data pairs of selected Ayurvedic botanicalswith bioactive PCIDs, bioactive PCIDs of with HIF-1, and HIF-1with breast cancer were built in excel programmed files. The data pairs were imported and created a network map of the therapeutic components and disease target.The nodes in the network diagram stand in for selectedAyurvedic botanicals, bioactives, HIF-1, and breast cancer, while the edges show how the nodes are connected. The network was examined using the 'Network Analyzer' function.

Docking studies

Selection of ligand

Screened bioactives from network pharmacology approach having similarity index more than 0.6 and found to be associated with HIF-1 for breast cancer activity were subjected to dockingstudies against HIF-1(1H2K). Factor Inhibiting HIF-1 alpha in complex with HIF-1 alpha fragment peptide (1H2K) was chosen for study based on classification as transcription activator/inhibitor, organism as Homo sapiens, and no mutation type.The HIF-1 (1H2K) protein can imitate its structure to facilitate docking.Docking study was performed usingPyRx software and the ligands with good docking score were further explored for detail in-silico studies.

Preparation of ligand

The 3-D structure of inhibitors with their respective PubChem CID were redeemed and saved in .sdf format. Furthermore, ligand preparations were continued by taking the 3-D structure of all the ligands and were introduced in BIOVIA Discovery Studio 2021 software (a tool for viewing, sharing, and analyzing protein and modeling data in 3D interactions) for conversion of 3D structure from .sdf to.pdb format. The prepared ligands were saved in PDB format for further docking studies [17]. Hydrogen bond interactions are also calculated and mentioned, presence of H-bonds depicts stable interaction between ligand and protein. BIOVIA Discovery Studio 2021 Client is used to depict Hydrogen bonds, 2-D images and protein-ligand interactions images for a good visualization of the docking [18].

Preparation of protein

The crystal structure of target proteins was retrieved from PDB with PDB ID: 1H2K and was carried further for more studies of docking process.The validation and establishment of the 1H2K protein target was achieved through comprehensive molecular simulation characterization and comparison with other proteins that possess proangiogenic features in the RCSB PDB database.

Molecular docking

Molecular Docking is an important component of computer-assisted drug discovery. It helps in predicting the intermolecular framework formed between a protein and ligand and outputs the appropriate binding between the molecules. Docking was performed by PyRx softwareprogram. PyRx is virtual screening software for computational drug discovery that can be used to screen libraries of compounds against potential drug targets [19].

The best conformation with the lowest docked energy was chosen from the docking search. The interactions of complex protein-ligand

conformations including hydrogen bonds and bond lengths were analyzed using BIOVIA Discovery Studio 2021 software.

RESULTS AND DISCUSSION

Network pharmacological studies

Phytochemical data mining

The botanical of Ashwagandha (*Withaniasomnifera*) is reported to contain 52 phytoconstituents; Shatavari (*Asparagus racemosus*) is reported to contain 28 phytoconstituents; Neem (*Azadirachtaindica*) is reported to contain 23 phytoconstituents; Alsi seeds (*Linumusitatissimum*) is reported to contain 60 phytoconstituents; Methi (*Trigonellafoenumgraecum*) is reported to contain 65 phytoconstituents; Haldi (*Curcuma longa*) is reported to contain 98 phytoconstituents; Ghritkumari (*Aloe barbadensis*) is reported to contain 21 phytoconstituents; Yashtimadhu (*Glycyrrhizaglabra*) is reported to contain 100 phytoconstituents and KadiPatta (*Murrayakoenigii*) is reported to contain 86 phytoconstituents.

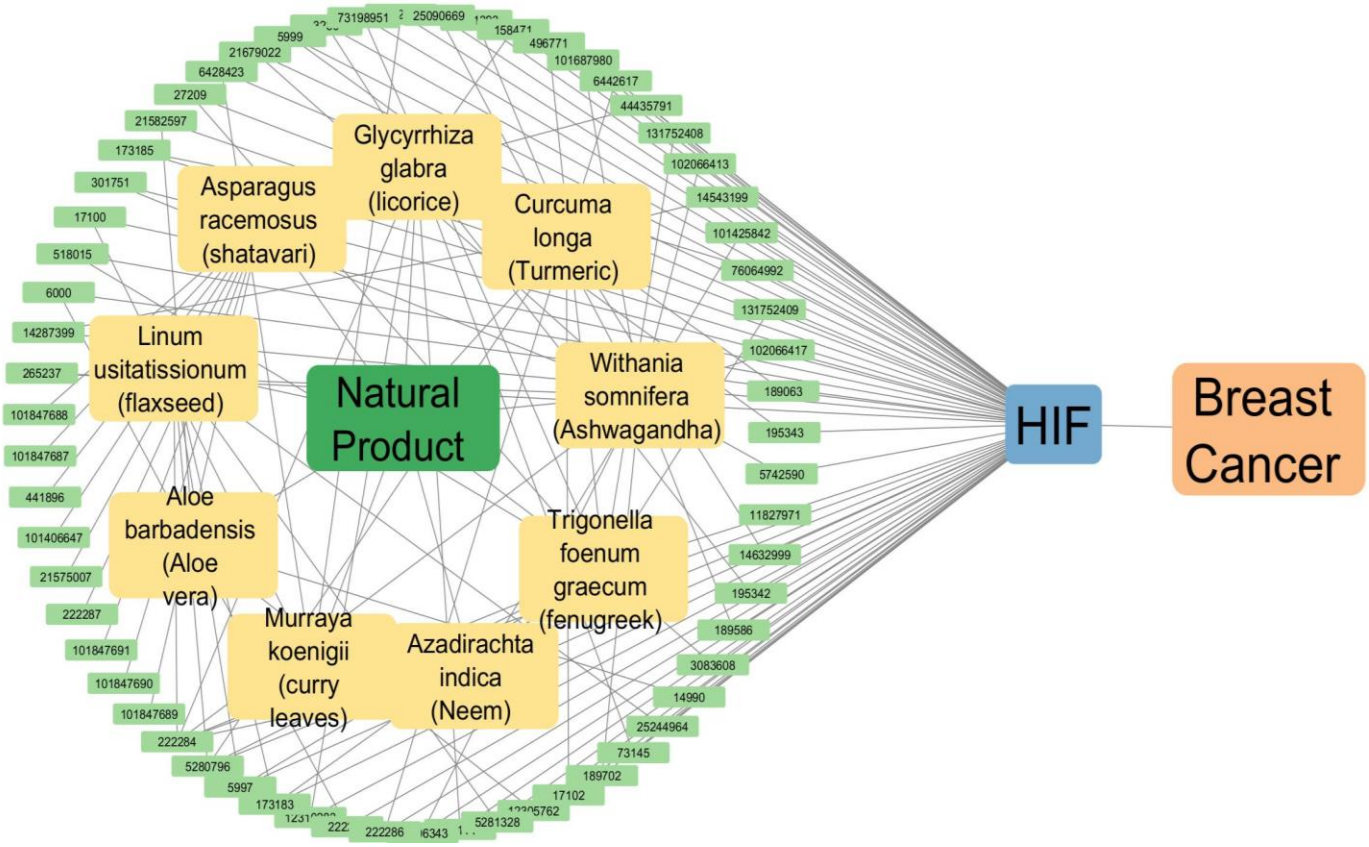
Establishment of Target

For HIF-1 data located as PDB ID: 1H2K, Protein name: Hypoxia-Inducible Factor, Organisms(s): Homo sapiens, Sequence Length= 349, Uniprot ID -Q9NWT6, Gene Names: HIF1AN, FIH1.

Screening of bioactives by polypharmacologyfor target

The number of bioactives having interaction with HIF-1, equal to or greater than 0.6 (high scoring bioactives) from Ashwagandha (*Withaniasomnifera*) was 12; from Shatavari (*Asparagus racemosus*) was 9; from Neem (*Azadirachtaindica*) was 2; from Alsi seeds (*Linumusitatissimum*) was 6; from Methi (*Trigonellafoenumgraecum*) was 12; from Haldi (*Curcuma longa*) was 11; from Ghritkumari (*Aloe barbadensis*) was 5; from Yashtimadhu (*Glycyrrhizaglabra*) was 10 and from KadiPatta (*Murrayakoenigii*) was 3 **(Figure 1)**. The screened bioactives from selected Ayurvedic botanicals using polypharmacology approach was subjected for network construction and analysis using Cytoscape v.3.10.0 software.

Figure 1: Interaction network of bioactives from selected Ayurvedic botanicals with HIF-1 in breast cancer.



Molecular docking Studies

The best conformation with the lowest docked energy was chosen from the docking search. It was observed that the highest binding affinitywas demonstrated by Araboglycyrrhizin (-10.1 kcal/mol). On performing the docking against the targetHIF-1 (1H2K) with Araboglycyrrhizin(-10.1 kcal/mol) **(Figure 2)**, Asparanin-A (-9.2 kcal/mol) **(Figure 3)**, Shatavarin-I (-9.2 kcal/mol)**(Figure 4)**, Shatavarin-X (-9.1 kcal/mol) **(Figure 5)**, Somniferanolide (-9.1 kcal/mol)**(Figure 6)**, Somniferawithanolide (-9.7 kcal/mol)**(Figure 7)**, Trigofoenoside-A (-9.2 kcal/mol)**(Figure 8)**, Trigofoenoside-F (-9.4 kcal/mol)**(Figure 9)**, Trillin (-9.9 kcal/mol)**(Figure 10)**, and Withanolide (-9.2 kcal/mol) **(Figure 11)**, it was observed that the binding energiesof theligandswerefairly good as compared with standard synthetic ligand Acriflavine (-7.6 kcal/mol) **(Figure 12)**. None of the compounds violated the Lipinski's rule of five. Docking analysis for HIF-1 inhibition by screened top 10 bioactivesand standard synthetic Acriflavine ligand is given in (**Table 1)**

Figure 2: 2D model for docking interactions of Araboglycyrrhizin against HIF-1

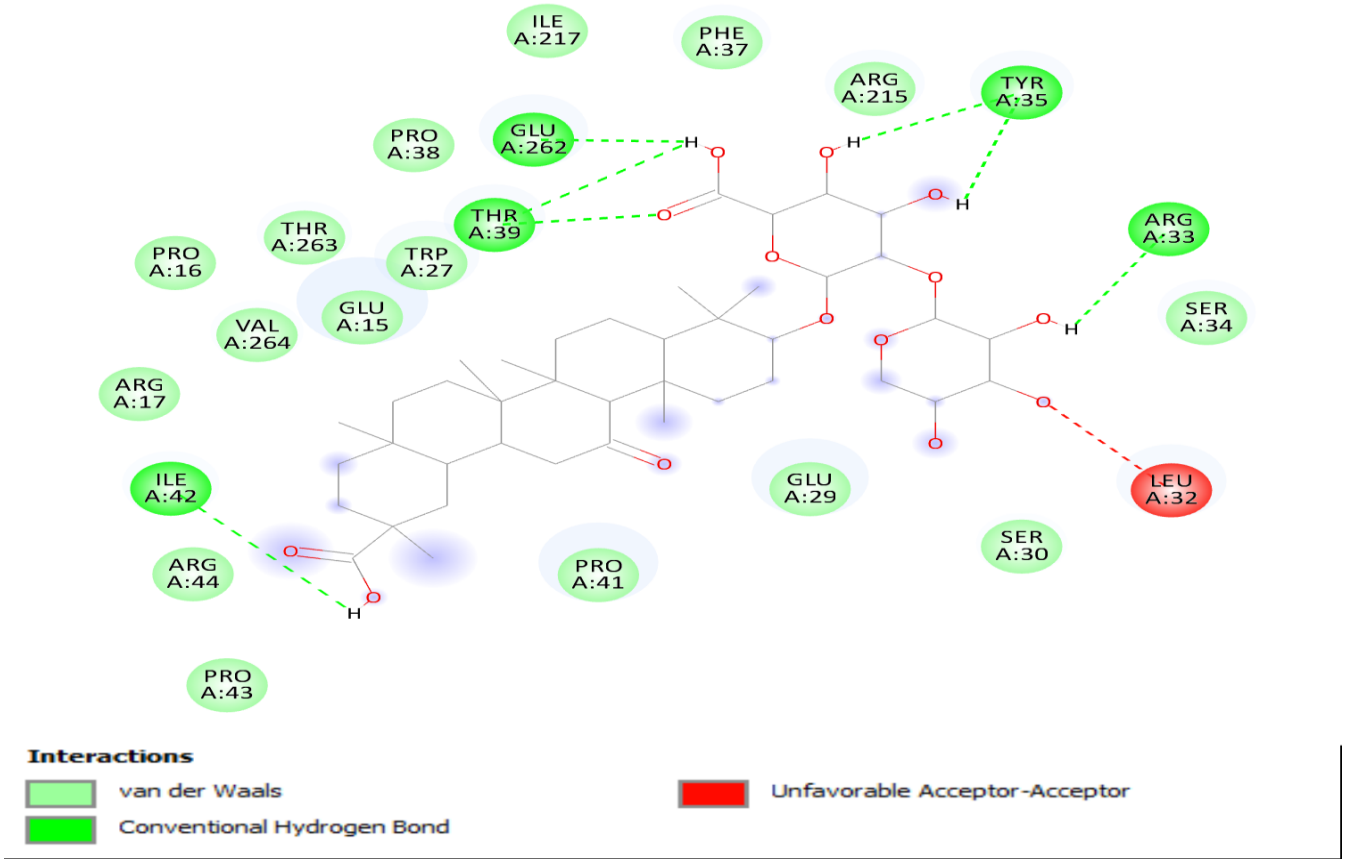


Figure 3: 2D model for docking interactions of Asparanin against HIF-1

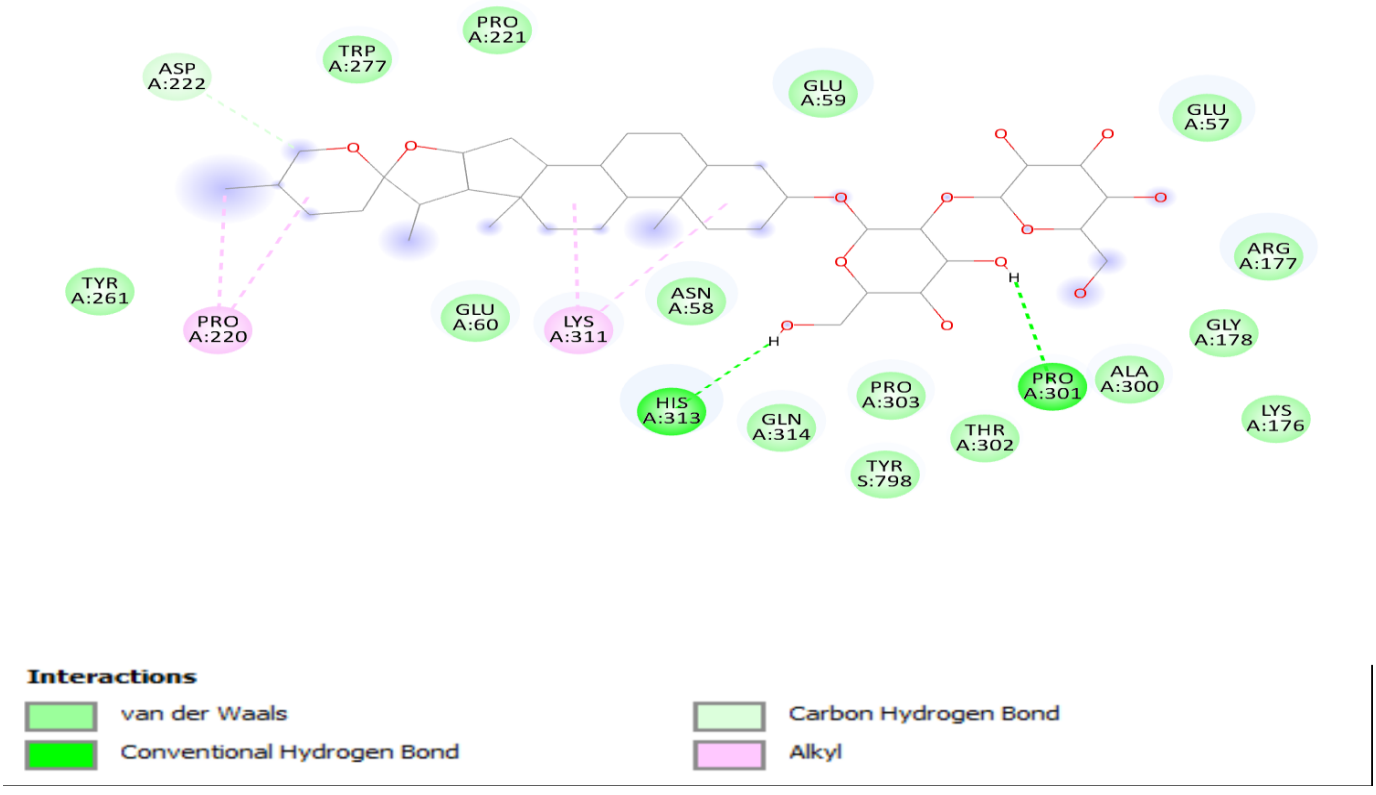


Figure 4: 2D model for docking interactions of Shatavarin-I against HIF-1

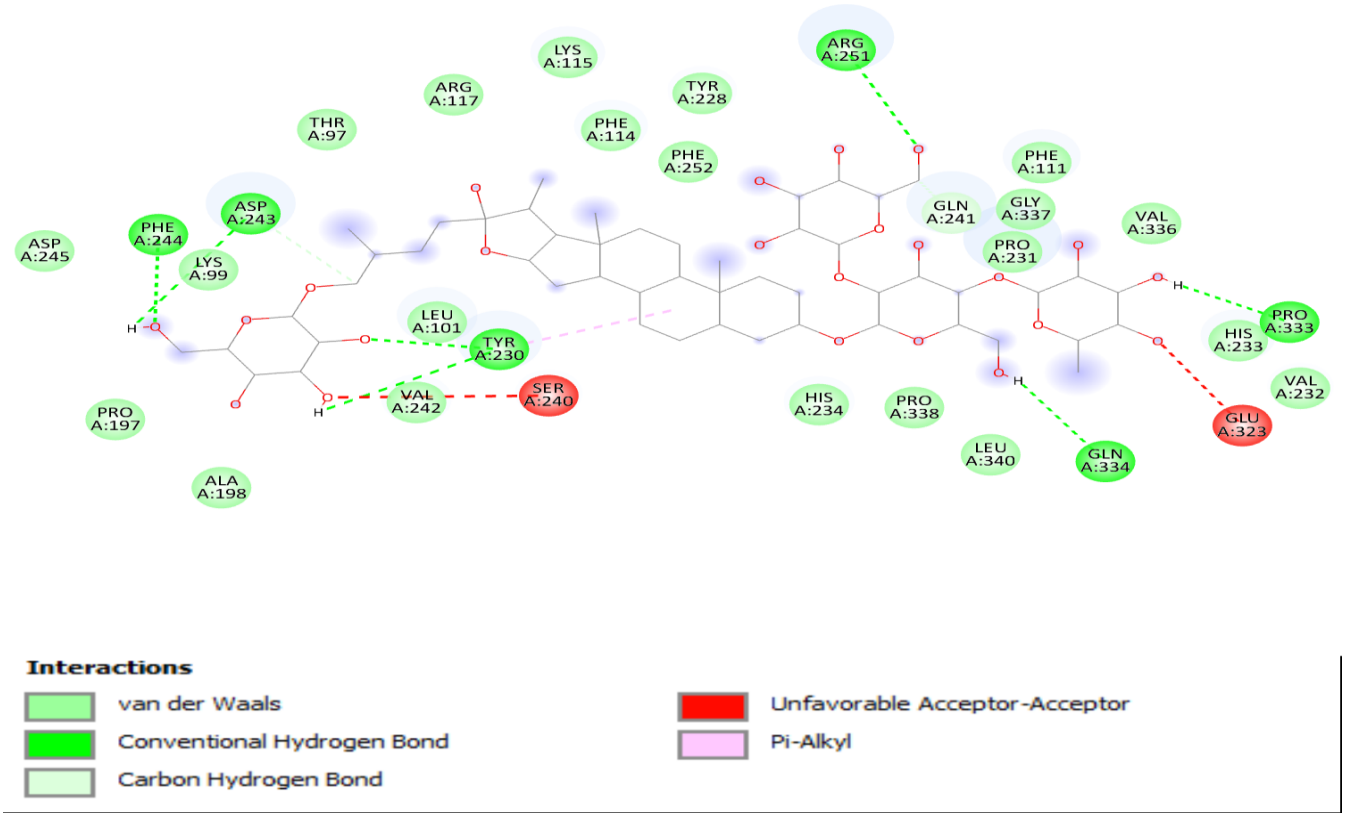


Figure 5: 2D model for docking interactions of Shatavarin-X against HIF-1.

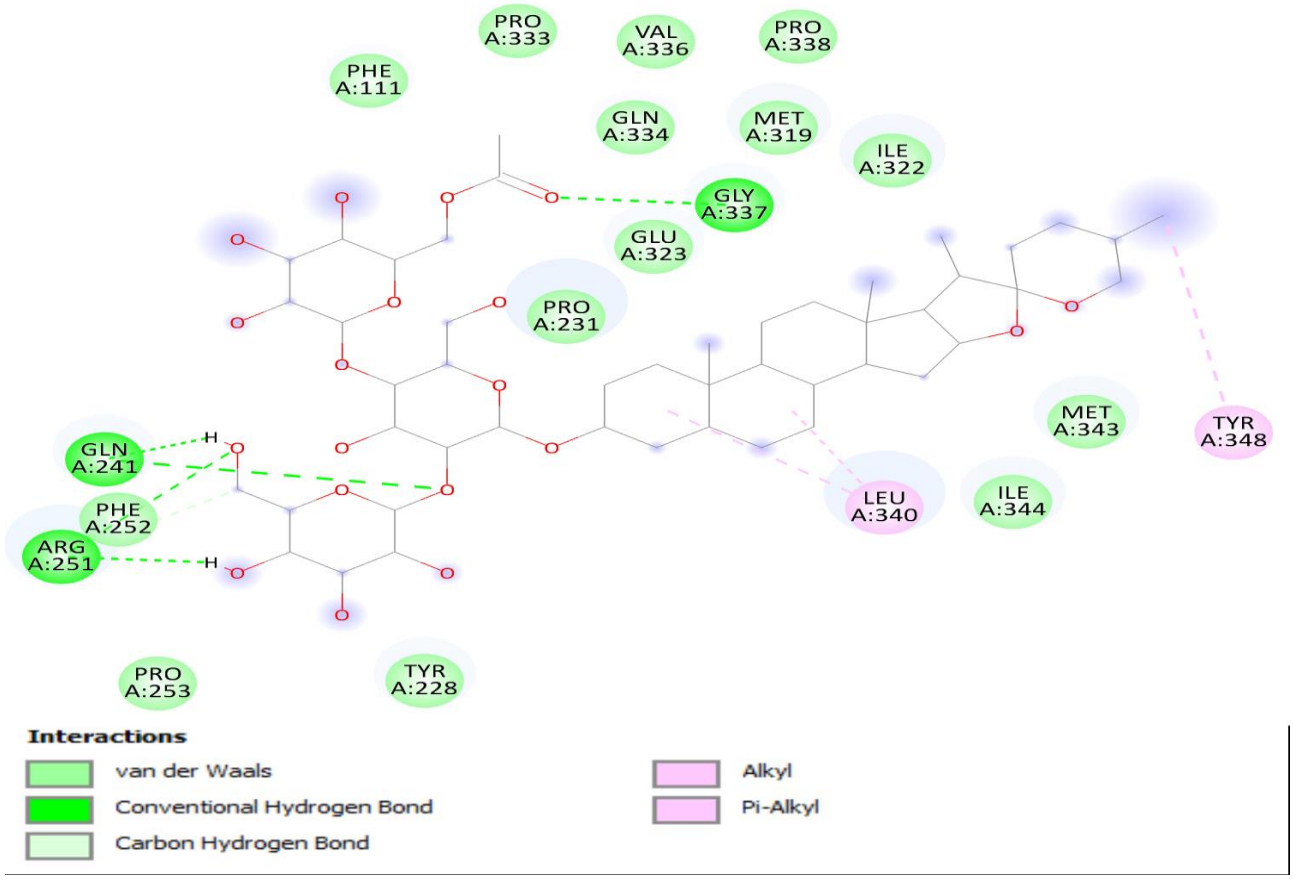


Figure 6: 2D model for docking interactions of Somniferanolide against HIF-1

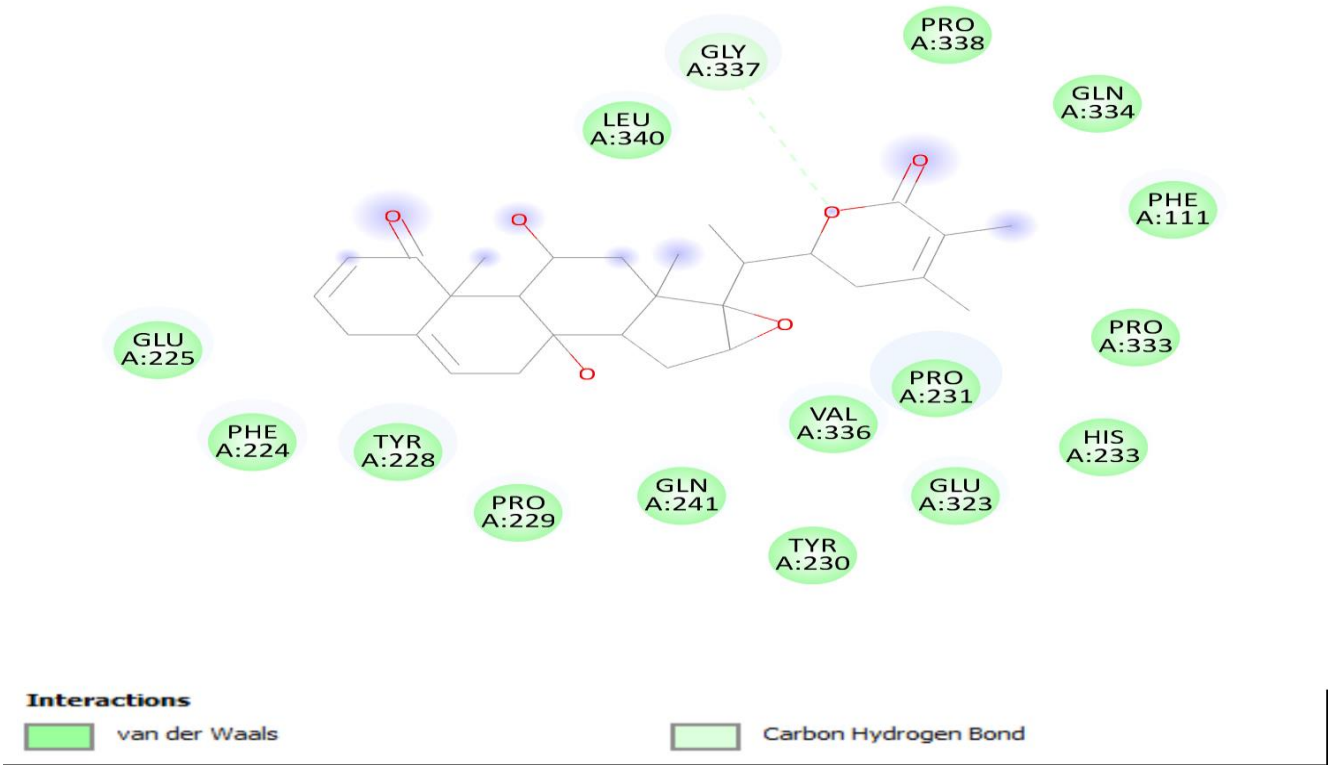


Figure 7: 2D model for docking interactions of Somniferawithanolide against HIF-1

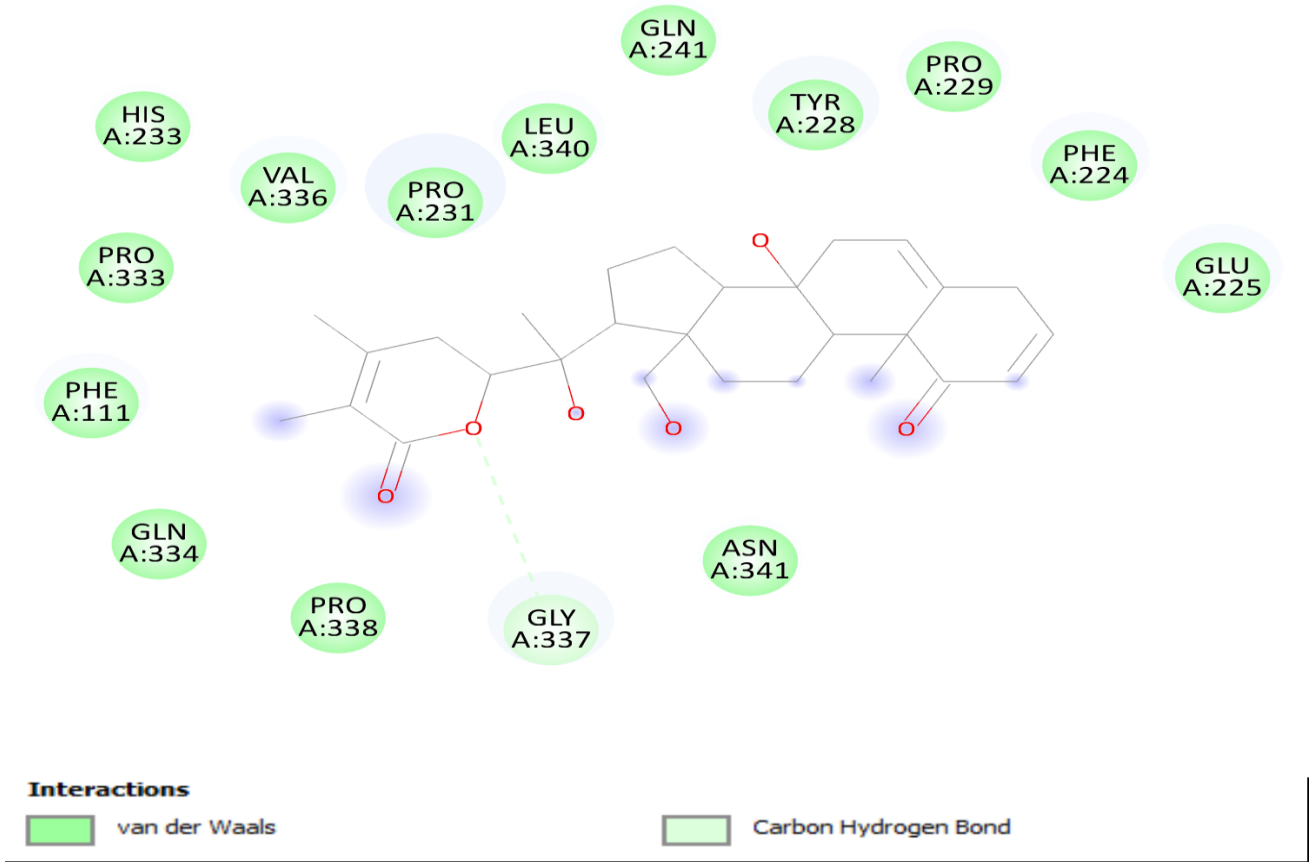


Figure 8: 2D model for docking interactions of Trigofoenoside-A against HIF-1

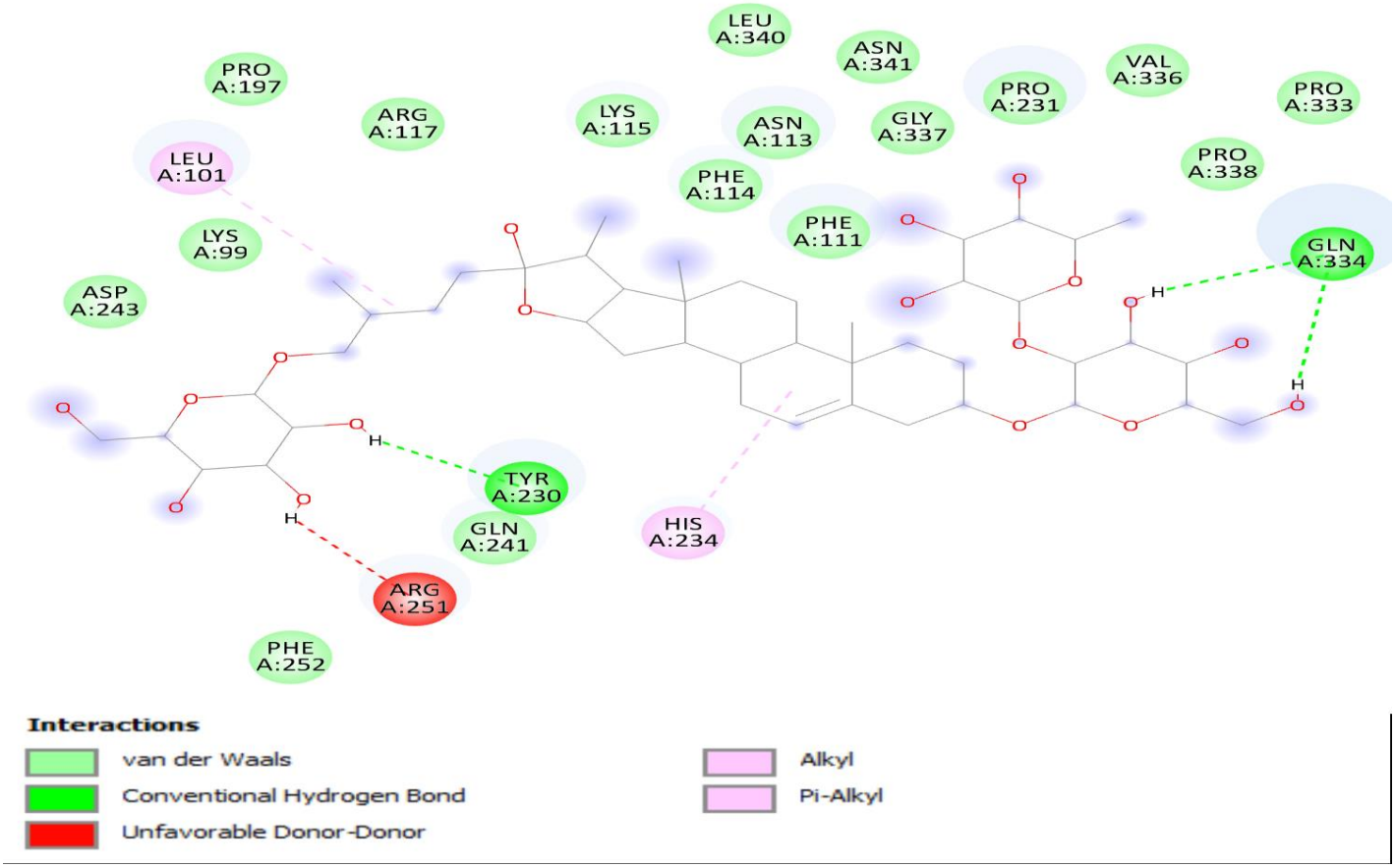


Figure 9: 2D model for docking interactions of Trigofoenoside-F against HIF-1

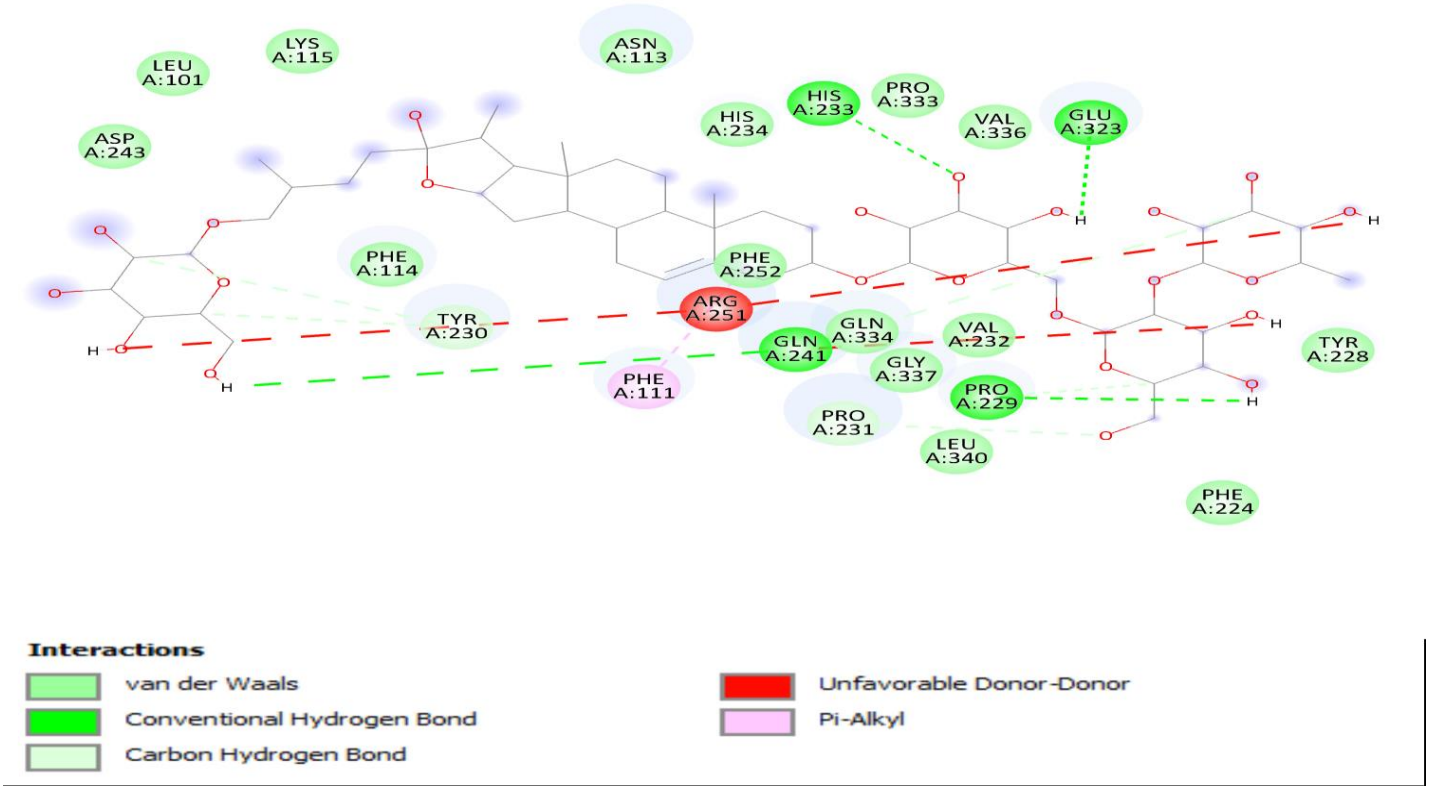


Figure 10: 2D model for docking interactions of Trillin against HIF-1

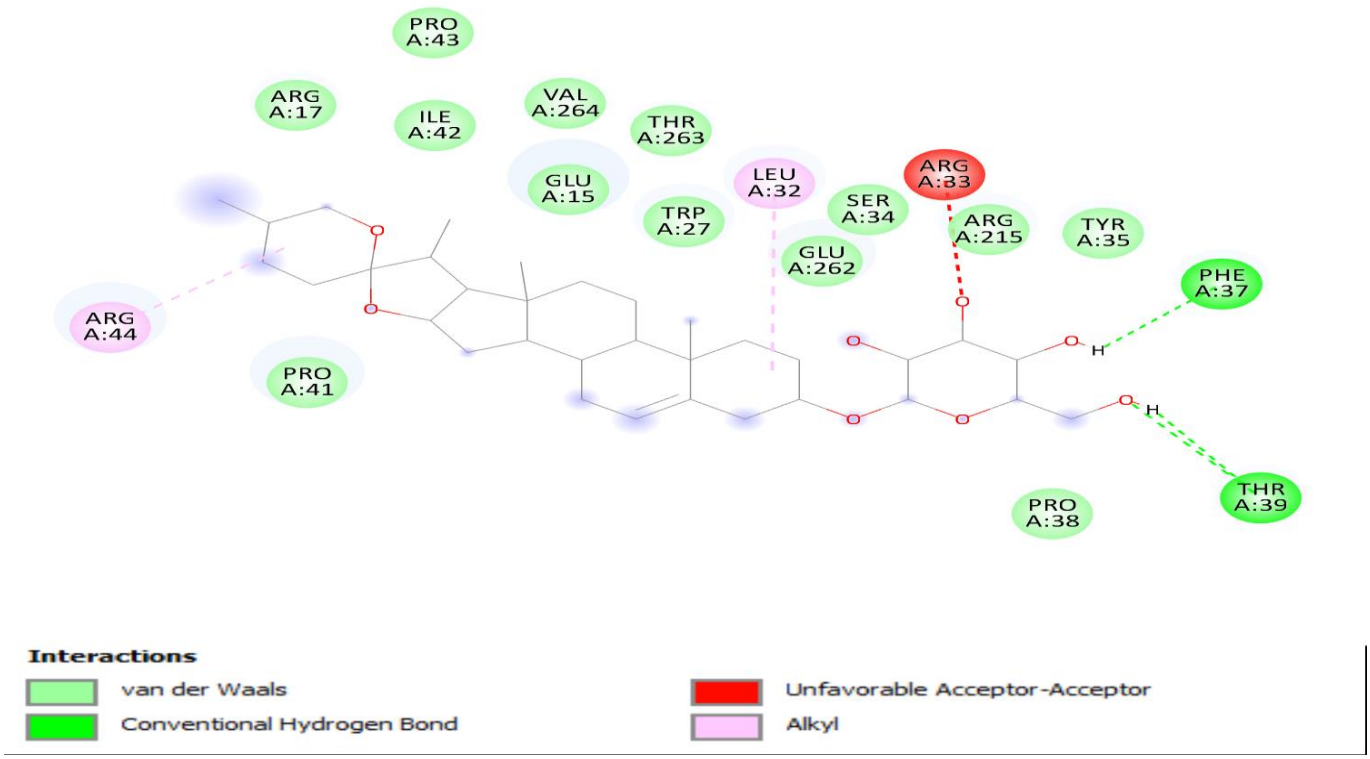


Figure 11: 2D model for docking interactions of Withanolide A against HIF-1

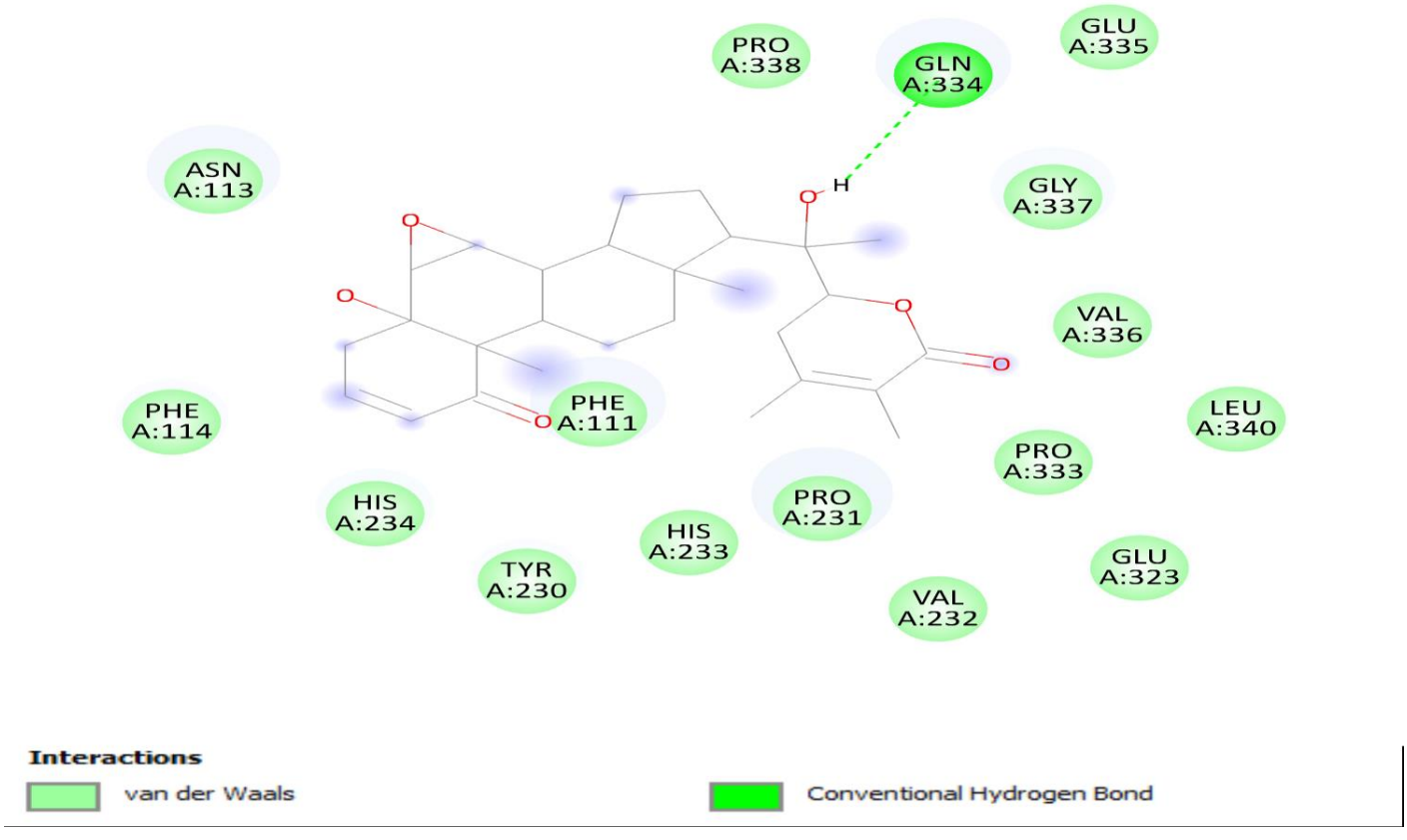


Figure 12: 2D model for docking interactions of Standard Synthetic Acriflavine against HIF-1

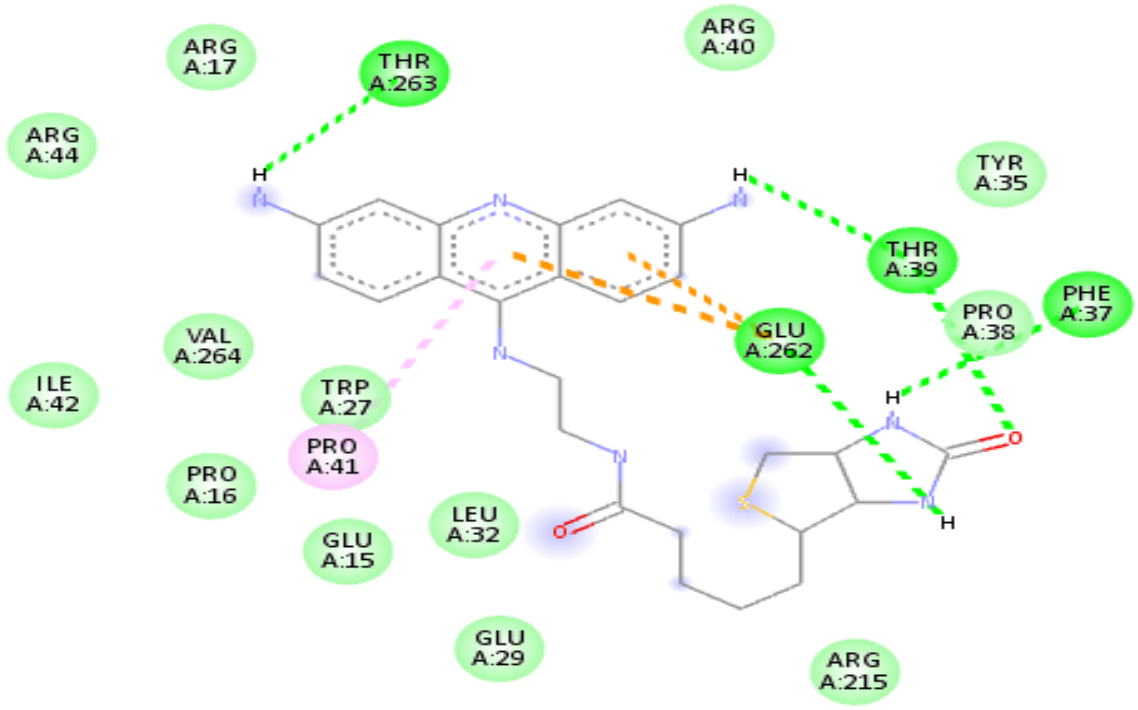


Table 1: Docking interaction analysis for HIF-1 inhibition by screened top bioactives ligands

Ligand Name	Binding Energy (kcal/mol)	Interaction			
		Hydrogenbonding	Electrostatic (Pi-Anion)	Hydrophobic (Alkyl)	Hydrophobic (Pi-Alkyl)
Araboglycyrrhizin	-10.1	A:THR39, A:THR39, A:THR39, A:GLU262, A:TYR35, A:TYR35, A:ARG33, A:ILE42	-	-	-
Asparanin	-9.2	A:PRO301, A:HIS313, A:ASP222	-	A:PRO220 A:LYS311 A:LYS311 A:PRO220	-
Shatavarin I	-9.2	A:TYR230, A:PHE244, A:ARG251, A:GLN334, A:PRO333, A:TYR230, A:ASP243, A:GLN241, A:ASP243	-	-	A:TYR230
Shatavarin X	-9.1	A:GLN241, A:ARG251, A:GLY337, A:ARG251, A:GLN241, A:ARG251	-	A:LEU340 A:LEU340	A:TYR348
Somniferanolide	-9.1	A:GLY337	-	-	-
Somniferawithanolide	-9.7	A:GLY337	-	-	-
Trigofoenoside-A	-9.2	A:GLN334, A:GLN334, A:TYR230	-	A:LEU101	A:HIS234
Trigofoenoside-F	-9.4	A:HIS233, A:GLU323, A:PRO229, A:GLN241, A:PRO231, A:PRO229, A:GLN241, A:TYR230, A:TYR230,	-		A:PHE111
Trillin	-9.9	A:THR39, A:THR39, A:PHE37	-	A:LEU32 A:ARG44	-
Withanolide A	-9.2	A:GLN334			
Std Synthetic Acriflavine	-7.6	A:THR39, A:THR39, A:THR263, A:THR39, A:PHE37, A:GLU262	A:GLU262 A:GLU262	- -	A:PRO41

CONCLUSION

The study demonstrated the in silico investigations of the cellular processes by which various bioactive phytoconstituents from various Ayurvedic botanicals affect the suppression of HIF-1. This study further reveals the possibility of traditionally used Ayurvedic botanicals with established anti-cancer action to treat breast cancer by targeting the HIF-1 protein. Through a networking method,

ehanopharmacological screening revealed bioactives from defined phytoconstituents from convinced Ayurvedic botanicals. Experimental validation of the network findings would aid in the understanding of the rationale behind the anti-breast cancer action and benefit bioactive formulation-based drug discovery.

Docking score indicated that the tested bioactives inhibited the target with good binding energies and interactions when compared to a standard synthetic ligand. Docking research demonstrated that screened natural bioactive compounds have stronger molecular interactions than standard, making them superior ligands. The ligands that have been screened can be thoroughly characterized using additional insilico tools, in vitro and in vivo research, and other methods fortreatment and prevention of breast cancer.

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