Original Article

In-silico screening and molecular docking studies of active constituents of Withania somnifera to investigate its kinase inhibitory activities

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Abstract

Background: Withania somnifera popularly known as Ashwagandha is a popular herbal medicine exhibits anti-cancer,anti-oxidant, anti-inflammatory, anti-stress, anti-tumour, haemopoietic andimmunomodulatory activity. Withania somnifera is used as a chemotherapeutic agent for cancertherapy. Kinases play important role in the different types of cancerprogressionmainly in carcinogenesis and metastasis, elucidated by understanding the basic molecular mechanisms of signalling of the cancer cell.

Materials and methods:The purpose of the study was to summarise the kinase inhibitory potential of active constituents of ashwagandha by in-silico analysis. The in-silico methods used in the study are Lipinski's rule of five, evaluation of in-silicotoxicity by admetSAR, analysis of electronic parameters and ADME properties and molecular docking using Schrodinger 2018-3 suit device Maestro 11.7.02 software.Thekinase targets considered in the study were CDK4, CDK6, EGFR, and VEGFR (PDB I.D: 5L2S,6P8E,7JXQ, and 4AGC).

Results: On the basis of the results of in-silico analysis, most of the constituents of ashwagandha exhibited good kinase inhibitory activity, out of which Withanolide A, Anaferin, Withaferin A and Withoxylactone were found to show strong inhibition for CDK6, EGFR, CDK4 and VEGFR respectively. Out of four target kinases, CDK6 and EGFR was found to be the better target for the constituents of Ashwagandha.

Keywords: Ashwagandha, In-silico analysis, Kinase enzymes, Molecular docking.

INTRODUCTION

The major components of today's early pharmaceutical research are the target and lead discovery [1,2].There are many benefits of in-silico methodology, including a decrease in the number of molecules produced and tested by the database searching for substrates or inhibitors, an increase in experiment speed through the determinationof the majority of pharmaceutical properties accurately from molecule structure alone, and eventually a decrease in the use of animals and reagents [3].

Withaniasomifera(Ws) is a popular Indian medicinal plant that belongs to the Solanaceae family and is also known as Ashwagandha, Indian ginseng, and Winter cherry. It is one of the most valuable plants in traditional Indian medicine systems.

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The roots of plants are the main parts that are widely used as therapeutic agents. The root contains active phytoconstituents likealkaloids, glycosides, steroids, amino acids, starch, volatile oil, starch and reducing sugar. The Ws roots contain 21.0 to 25.0% of fiber, 6.09 to 9.46 mg/g of starch, 0.39 to 0.82 mg/g of tannins, the minerals like K, Na, Ca, Cu, Zn, Al, Ca, Ni and, total sugars ranging from 2.25 to 9.25 mg/g, reducing sugars ranging from 0.15 to 2.10 mg/g, and 2.37 to 7.62 mg/g of non reducing sugars[4].Major constituents of Ws like withanolide D and withaferin Aexhibitsantitumour and cytotoxic properties. In addition to alkaloids, the plantalso consistsof saponins, phenols, flavonoids and phytophenols. Also, it is widely used in traditional medicine formulations as an antipyretic, analgesic[5], adaptogenic[6] and anti-inflammatory agent[7].

Cancer is characterised as a condition in which a collection of aberrant cells multiplies continuously without adhering to the laws of normal cell division.Signals that instruct normal cells to multiply, differentiate into new types of cells, or die are constantly being transmitted, butcancer cells become independent of these signals, resulting in uncontrolled growth and proliferation. In fact, nearly 90% of cancer-related deaths are caused by tumour spread, a process known as metastasis.[8].

Protein phosphate groups are transferred to proteins by kinases, whereas they are removed by phosphatases. Together, these two enzymatic systems control a variety of protein activities in a cell, frequently in responsiveness to outside stimuli.[9]. The human genome encodes about 538 recognised kinases, which maintain cellular function by activating protein function. Corresponding phosphatases counteract this action.[10,11].

Kinases play important role in the different types of cancer mainly in carcinogenesis and metastasis, elucidated by understanding the basic molecular mechanisms of signalling of cancer cells[12]. Because the vast majority of protein kinases encourage cell proliferation, migration, and survival. Human malignancies are increasingly being treated with synthetic and natural medicines that block both single- and multiple-kinase enzymes by using these kinases as therapeutic targets [13-16].

An essential pathway in signallingtumor angiogenesis is formed by the growth vascular endothelial growth factor (VEGF) family and their receptors. Numerous malignancies, including cervical, gastric, and lung cancers, have been examined to determine the prognostic importance of VEGF expression. By encouraging the growth of aberrant blood vessels, VEGF also has a significant impact on tumourspread[17].Another target for cancer immunotherapy is the tyrosine kinase receptor family known as the epithelial growth factor receptor (EGFR) family. It has been shown to play both physiologic and carcinogenic roles in a variety of cancers. Various EGFR pathways function as proto-oncogenes in a number of malignancies, including gastrointestinal, oral, and breast cancers. Up to 60% of ovarian epithelial malignancies over-express the EGFR gene, and activation of this gene is linked to a worse prognosis for patients and an increase in the malignant tumour phenotype. EGFR is a desirable target for therapeutic interventions because it plays a role in several aspects of cancer progression, including tumour initiation, angiogenesis, and metastasis [18].The dysregulation of the cyclin-dependent kinase pathway in luminal breast cancer opens up a potential treatment avenue for estrogen receptor positive breast cancer. For women with advanced HR positive breast cancer, the CDK4/6 inhibitors palbociclib, ribociclib, and abemaciclib have been approved by the US Food and Drug Administration for use in combination with endocrine therapy[19]. The transition of the cell cycle from the G1 to the S phase is specifically regulated by CDKs 4 and 6, and CDK4/6 inhibitors effectively stop cancer cell growth by causing G1 cell cycle arrest[20, 21].

Our study was designed to assess the kinase inhibitory potential of ashwagandha's active components using in-silico analysis, with the intention of finding a potent anti-cancer lead molecule.

Materials and methods:

Determination of Lipinski's rule of five:

Physico-synthetic characteristics and the presence of several pharmacophoric highlights are one of the major measures that affected the molecules' behaviour in the residing framework. This multitude of variables issignificant mediator of good oral BA. Lipinski's standard of 5 wasprimarily employed to check fundamental atomic features connected with the PK of the compounds[22]. In agreement with Lipinski's rule of five, if a candidate molecule is orally active, the molecular weight must be < 500, log P (octanol/water partition coefficient) is lower than 5, hydrogen bond donors (OH and NH groups) and acceptors (N and O) should not be more than 5 and 10 respectively, If a compound follows these rules with not more than 1 violation then compound is inclined to be orally active. Number of rotatable bonds (nRotb) $\leq$ 10 andtopological polar surface area (tPSA) must be < 140 Å.

All the structures were drawn using Chem bio draw software. Smiles notations were generated for each structure. By using molinspiration software MW, Log P, the number of Hydrogen bond donors/acceptors, tPSA and nrotb were determined.

Analysis of electronic parameters and ADME properties:

Electronic parameters and ADME properties are mainly determined usingQikprop of Schrodinger 2018-3 suite device Maestro 11.7.012 [23].

Evaluation of in-Silicotoxicity of active constituents of ashwagandha:

One of the significant issues in the many phases of drug

discovery is toxicity, Hence it is vital to extract the toxicity profile of the compounds [24,25,26].It was mostly done utilizingthe admetSAR information base which is freely accessible.

Molecular docking studies:

Glide module of Schrodinger 2018-3 suits device Maestro 11.7.012 was used to carry out molecular docking studies. Crystal structures of all the kinase targets with good were taken from Protein Data Bank (PDB). Different kinases selected are CDK4, CDK6, EGFR and VEGFR with PDB ID: 5L2S,6P8E,7JXQ and 4AGC respectively.

Results and discussion

All the tested compounds were found to obey Lipinski's rule of 5, They have good log P values <5 except withasomidienone (log P =5.96). Molecular weight <500 Daltons except for viscosalactone B and withaoxylactone which is having a slightly higher molecular weight of 502.6 Daltons. Hydrogen bond donors and acceptors are in the acceptable range. Hence all the tested compounds were expected to exhibit good oral bioavailability. The number of rotatable bonds and tPSA of all the tested compounds were found to be less than 10 and 140 A0 respectively, which indicates the molecules are favourably flexible and expected to have better absorption and bioavailability (Tables 1 and 2). Most of the compounds evaluated were exhibited good physicochemical and electronic properties. All of the compounds have reactive functional groups within the range 0-2 except withaoxylactone. Except tropane and pseudotropine which as no reactive functional group. All of the tested compoundshave a zero dipole suggests that the molecules are non-polar. The steric effects of the compounds were determined by measuring the total polarizability. All of the tested compounds are within the range of 14.539-50.507 values are comparable with that of standards (Table 2).

Table 1. In-silico analysis of Lipinski's rule of five for constituents of Ashwagandha

Active Constituents	LogP	Molecular weight	H bond donors	H bond acceptors	nvoilation
Withanolide A	4.15	470.61	6	2	0
Withanolide F	2.85	488.62	7	4	0
Withaferin A	3.86	470.61	6	2	0
Withanone	4.15	470.61	6	2	0
Sominone	4.63	458.64	5	3	0
Withaoxylactone	2.21	502.60	8	3	1
Withasomidienone	5.96	452.63	4	0	1
Tropane	1.63	125.22	1	0	0
Pseudotropine	0.48	141.21	2	1	0
Cuscohygrine	0.86	224.35	3	0	0
Dl-isopelletierene	0.30	127.19	2	1	0
Anaferine	1.38	224.35	3	2	0
Anahygrine	1.12	224.35	3	1	0
Viscosolactone B	4.43	502.69	6	3	1
Gefitinib	4.19	446.91	7	1	0
Palbociclib	2.96	447.4	9	2	0
Pazopanib	3.27	437.53	9	3	0

Table 2. Evaluation of physicochemical and electronic parameters

Compounds	tPSA	Nrotb	#rtvfg	Dipole	Polarizability
Range	<140a^2	<10	0-2	1-12.5	13-70
Withanolide A	105.605	5	2	0	47.957
Withanolide F	130.176	7	1	0	47.387
Withaferin A	111.567	5	2	0	47.342
Withanone	100.436	4	2	0	47.966
Sominone	97.99	6	1	0	47.741
Withaoxylactone	141.347	6	3	0	46.749
Withasomidienone	74.173	4	1	0	50.507

Tropane	3.607	0	0	0	18.055
Pseudotropine	24.427	1	0	0	15.368
Cuscohygrine	33.527	4	1	0	26.665
Dl-isopelletierene	43.263	4	1	0	14.539
Anaferin	52.653	2	1	0	26.099
Anahygrine	44.234	4	1	0	25.955
Viscosolactone B	131.628	6	2	0	47.304
Gefitinib	61.213	8	0	0	44.493
Palbociclib	107.343	3	0	0	50.244
Pazopanib	113.027	6	0	0	46.248

The tested compounds show satisfactory ADME properties. The apparent Caco2 cell permeability of compounds showed excellent results, tropane and pseudotopine were expected to have excellent intestinal permeability which is greater than standard drugs. Estimated values for blood/brain partition co-efficient for the tested constituents were withinthe recommended range.Most of the compounds are comparable with standard drugs. Pseudotropine, cuscohygrine, anaferin, anahygrine show greater results than standard drugs, hence expected to have excellent BBB permeability. With regard to the prediction of human oral absorption most of the compounds have high oral absorption as that of standard drugs except withasomidienone. Based on the evaluation, good skin permeability is expected for all the constituents.Binding to human serum albumin (protein binding) is nearly equivalent to standard reference drugs(Table 3).

Table 3. Determination of in-silico ADME properties

Compounds	Qppcaco (mm/sec)	Qplogbb	Qppmdck	Qplogk_p	Qplogkhsa	Human oral absorption
Range	<25 poor, >500 great	-3 to 1.2	<25 poor, >500 great	-8 to -1	-1.5 to 1.5	1,2 or 3 for low , medium or high
Withanolide A	478.744	-1.047	223.141	-3.219	0.395	3
Withanolide F	167.12	-1.646	71.538	-4.074	0.266	2
Withaferin A	300.309	-1.243	134.792	-3.745	0.337	3
Withanone	583.264	-0.858	276.235	-3.251	0.537	3
sominone	223.69	-1.494	98.038	-4.029	0.846	3
withaoxylactone	210.015	-1.405	91.576	-4.183	-0.296	3
withasomidienone	916.16	-0.75	450.034	-2.859	0.896	1
tropane	2470.586	0.101	1454.789	-1.098	-0.161	3
pseudotropine	1063.829	0.611	585.161	-4.197	-0.528	3
cuscohygrine	324.394	0.854	179.323	-5.802	-0.59	2
dl-isopelletierene	525.628	0.281	273.096	-4.696	-0.541	3
anaferin	152.272	0.533	79.18	-6.441	0.172	3
anahygrine	169.752	0.598	89.049	-6.398	-0.263	2
Viscosolactone B	156.075	-1.56	66.441	-4.431	0.119	3
Gefitinib	1044.673	0.309	2291.935	-2.683	0.351	3
Palbociclib	179.894	-0.554	85.702	-4.922	0.15	3
Pazopanib	185.135	-1.847	81.718	-3.295	0.266	3

Thein-silico AMES toxicity studies can detect a wide range of toxic compounds that can cause genetic damage and gene mutations. It is one of the most widely used methods for determining whether a chemical can cause mutations in bacteria's DNA. Among the 14tested active constituents all the constituents were non-Ames toxic. The probability value ranges from 72.70% - 91.92%. All the constituents were found to be non-carcinogenic with a high probability so they are all anticipated to be non-carcinogenic. The value ranges from 90.16% -97.40%. The LD50 value is presently the basis for the toxicological classification of chemicals. Among the 14 constituents, 7 of them fall into category III of acute oral toxicity where LD50values ranges within 500mg to 5000mg. Other constituents were found to be moderately toxicby the

in-silico toxicity screening (Table 4).

Table 4. Evaluation of toxicity of the active constituents

Active constituents	AMES Toxicity		Carcinogenecity		Acute oral toxicity		Rat acute toxicity LD50 mol/kg	Fish toxicity pLC50 mg/L
	Result	Probability	Result	Probability	Result	Probability		
Withanolide A	-	0.7270	-	0.9650	I	0.8987	3.2351	0.8987
Withanolide F	-	0.9144	-	0.9740	I	0.4263	3.1892	1.0143
Withaferin A	-	0.9192	-	0.9549	I	0.5780	3.5404	0.7353
Withanone	-	0.7270	-	0.9650	I	0.4368	3.2351	0.8987
Sominone	-	0.8725	-	0.9604	III	0.6884	2.9436	0.8468
Withoxylactone	-	0.8905	-	0.9655	I	0.5720	3.5326	0.9391
Withasomidienone	-	0.8299	-	0.9458	III	0.3733	3.1864	-0.1782
Tropane	-	0.7456	-	0.9653	II	0.5207	2.4830	1.4854
Pseudotropine	-	0.7551	-	0.9700	III	0.6562	2.2645	2.0126
Cuscohygrine	-	0.8030	-	0.9016	III	0.6033	2.4980	1.9941
dl-isopelletierene	-	0.8524	-	0.9082	III	0.7333	2.0700	1.8263
Anaferine	-	0.8331	-	0.9356	III	0.7233	2.0177	2.7477
Anahygrine	-	0.7940	-	0.9478	III	0.6838	2.2939	2.0853
Viscosalactone B	-	0.8905	-	0.9655	I	0.5720	3.5326	0.9391
Geftinab	-	0.5000	-	0.9218	III	0.7006	2.5141	1.3361
Palbociclib	+	0.6017	-	0.8110	III	0.5394	2.3711	1.6017
Pazopanib	-	0.6543	-	0.8567	III	0.6642	2.3961	1.7277

Docking study was carried out by taking different targets of kinases likeCDK4,CDK6, EGFR,VEGFR (PDB ID: 6P8E,5L2S,7JXQ,4AGC). 6P8E is X-ray crystal structure of CDK4 in complex with cyclinD1 and P27 (Resolution 2.30 A0). 5L2S is a It is X-ray co-crystal structure of CDK6 with boundAbemaciclib(Resolution 2.27 A0).7JXQ is a EGFR kinase (T790M/V948R) in complex with allosteric inhibitor JBJ-09-063 (Resolution 1.83 A0). 4AGC is X-ray crystal structure of VEGFR2 (Juxtramembrane and kinase domains) in complex with Axtinib (Resolution 2.0 A0).From the molecular docking the affinityof binding was expressedbythe docking score. In the CDK4, the active constituents withaferin A(-4.989), withaoxylactone(-4.983), anaferin(-4.042) showed good docking scores with comparison to reference drugs, by exhibiting better interaction with the protein. In CDK6, the active constituents displayeda docking score within the range of -7.154 to -0.602.Withanolide A was found to be the best constituent to interact with 5L2S (CDK6) a with docking score of -7.154, which was found to be greater than the standard CDK inhibitors. In the EGFR target the active constituents displayed docking score within the range of -7.012 to -2. 943. Among all the active constituents anaferin (-7.012) and anahygrine (-6.946) got good docking scores than the reference standards. In case of VEGFR, the active constituents displayed docking score within the range of -6.843 to -0.792. Among all the active constituents withaoxylactone(-6.843) got good docking score which is higher compared to reference drugs(Table 5).

Table 5. Molecular docking studies

Compound	**Docking score**			
	6P8E	5L2S	7JXQ	4AGC
Withanolide A	-3.909	-7.154	-5.602	-
Withanolide F	-3.289	-6.937	-4.425	-
Withaferin A	-4.989	-6.035	-3.3	-
Withanone	-3.565	-5.231	-3.657	-
Sominone	-2.95	-5.147	-3.241	-
Withaoxylactone	-4.983	-4.737	-4.764	-6.843

Withasomidienone	-1.575	-3.976	-3.183	-
Tropane	-2.026	-3.793	-3.372	-0.893
Pseudotropine	-3.569	-3.45	-2.943	-0.792
Cuscohygrine	-0.653	-3.309	-5.437	-3.860
dl-isopelletierene	-3.147	-2.814	-4.781	-4.359
Anaferin	-4.042	-2.527	-7.012	-4.508
Anahygrine	-0.987	-1.809	-6.496	-3.601
Viscosalactone B	0.108	-0.602	-3.859	-
Geftinab	-4.012	-7.278	-6.312	-6.162
Palbociclib	-2.593	-7.126	-3.685	-2.057
Pazopanib	-0.88	-6.154	-6.127	-6.134

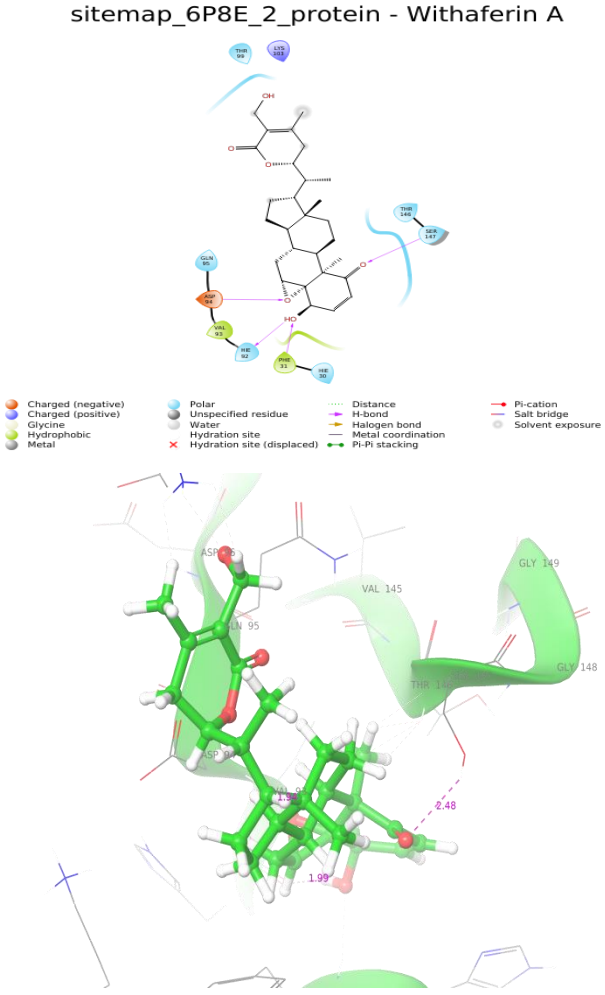

Figure 1: Interaction of Withaferin A with 6P8E

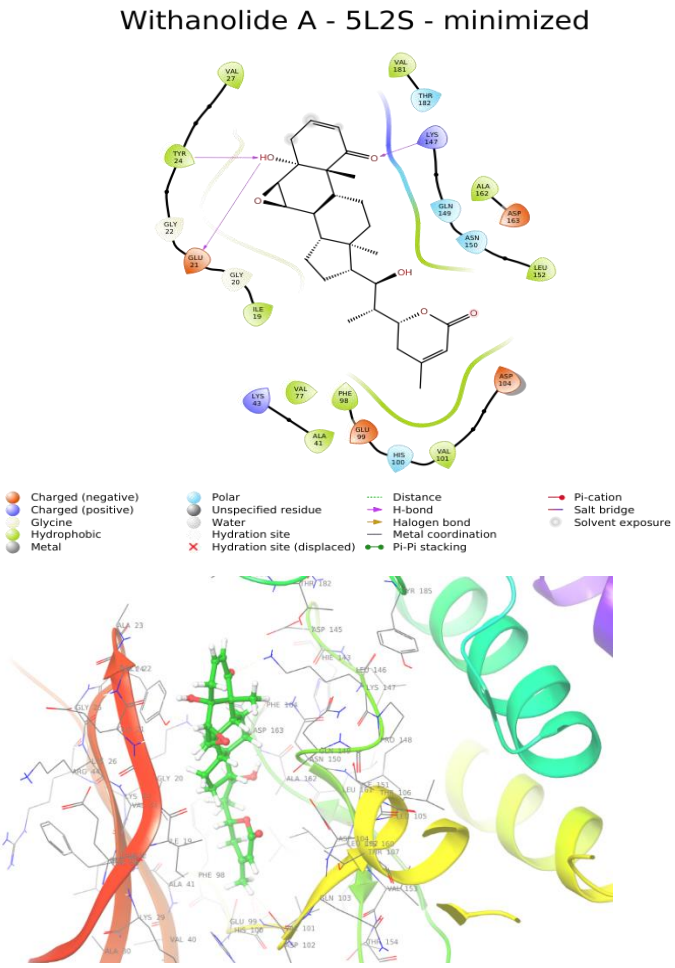

Figure 2: Interaction of Withanolide A with 5L2S

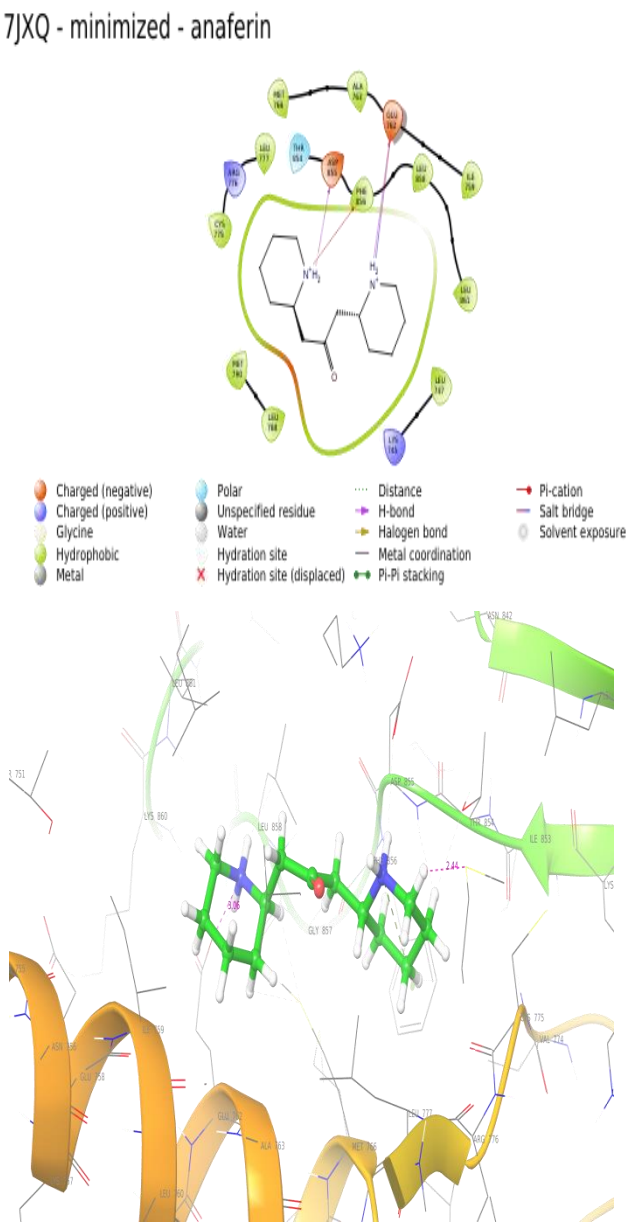

Figure 3: Interaction of Anaferin with 7JXQ

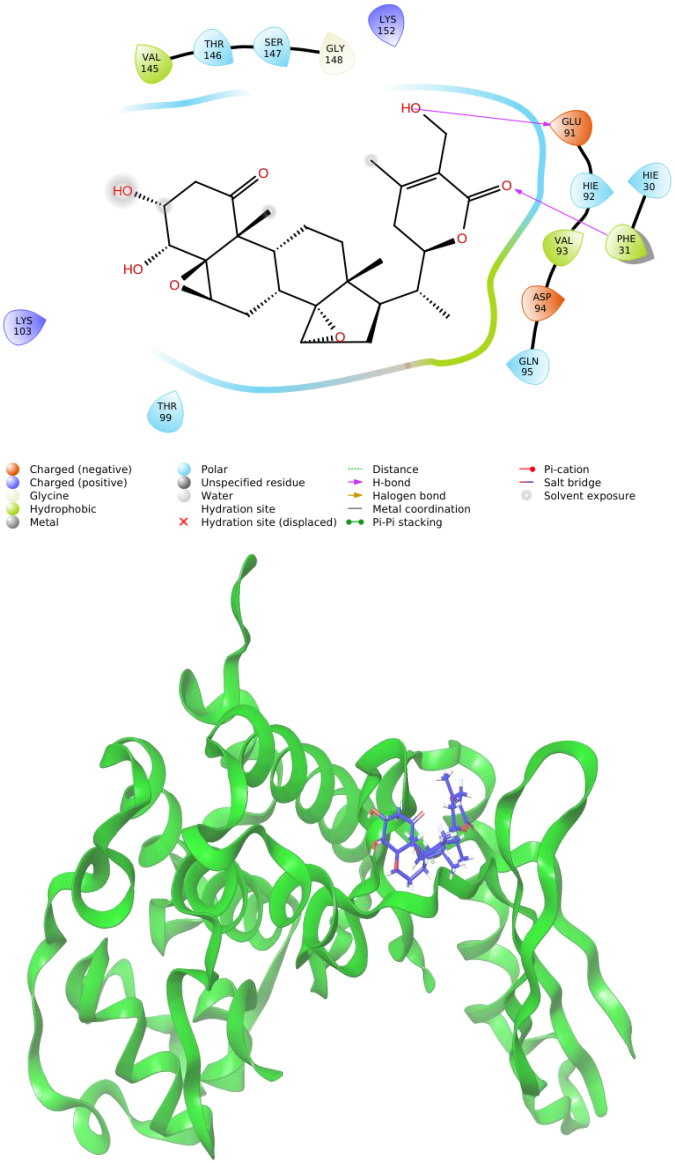

Figure 4: Interaction of Withaoxylactone with 4AGC

Conclusion

For in-silico screening, the 14 reported constituents of ashwagandha were selected. All of the assessed constituents provided excellent results for the preliminary in-silico screening, which included the evaluation of physicochemical and electronic parameters, ADME properties, and in-silico toxicity screening. Molecular docking studies were performed to determine the binding efficiency of these constituents for different kinases involved in different types of cancer cell growth and proliferation like EGFR, VEGFR, CDK4 and CDK6. Most of the compounds exhibited good kinase inhibitory activity, out of which Withanolide A, Anaferin, Withaferin A and Withoxylactone were found to show strong inhibition for CDK6, EGFR, CDK4 and VEGFR respectively. Out of 4 target kinases, CDK6 and EGFR was found to be the better target for the constituents of

Ashwagandha.These active constituents can be tested by in-vitro and in-vivoscreening for the confirmation of their anti-cancer and kinase inhibitory action.

Summary

Popular Indian ethnomedicinal Withaniasomifera, often known as Ashwagandha, is a member of the Solanaceae family. The primary plant portions that are frequently employed as therapeutic agents are the roots. Inhibitors of kinases will be a potent target for the cancer therapy because of the critical function that kinases play in the proliferation and metastasis of cancer cells in various forms of cancer. In the current investigation, kinases like EGFR, VEGFR, CDK4 and CDK6 that are implicated in different types of cancer cell proliferation were targeted in order to assess the kinase inhibitory ability of recognised Withaniasomifera components. Most of thecompounds exhibited good kinase inhibitory activity, out of which Withanolide A, Anaferin,Withaferin A and Withoxylactone were found to show strong inhibition for CDK6, EGFR,CDK4 and VEGFR respectively. CDK6 and EGFR were identified to be the best targets out of 4 target kinases. These active constituents can be tested by in-vitro and in-vivoscreening for the confirmation of their anti-cancer and kinase inhibitory action. Hence Withania somifera's components, including Withanolide A, Anaferin, Withaferin A, and Withoxylactone, can serve as the lead molecule for additional investigation.

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