

**MOLECULAR DOCKING ANALYSES OF *CYNODON DACTYLON* DERIVED
PHYTOCHEMICALS AGAINST *WHITE SPOT SYNDROME VIRUS* (WSSV) STRUCTURAL
PROTEIN VP26**

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ABSTRACT: White spot disease is a major infectious disease of penaeid shrimps caused by the *white spot syndrome virus* (WSSV). The viral structural proteins are responsible for binding virus to the cellular membranes of the host that is being systematically infected. An In silico attempt was made to identify the potential drug to inhibit the WSSV spread of diseases. For that an effort, was made to deduce the antiviral potentiality of *Cynodon dactylon* derived phytochemicals with docking technique. To stimulate the structure based drug design the, 3D structure of the VP26 (PDB-ID: 2EDM), a tegument protein thought to be involved in the entry of WSSV nucleocapsid into the host nucleus, is retrieved from PDB database and docking studies are carried out with the sketched phytochemical structures using GOLD software. Among the phytochemicals screened, luteolin and apigenin shows the best binding affinity with binding energies of 42.51 and 38.92 K.cal/mol exhibiting the potential to block VP26 (2EDM) protein of WSSV. This study will be helpful in developing novel antiviral drugs from plant sources against aquatic important pathogens.

Key words: Antiviral drugs, *Cynodon dactylon*, docking, penaeid shrimps, White spot disease, *white spot syndrome virus*

INTRODUCTION

Global production of economically important penaeid shrimps in aquaculture has increased exponentially among the crustacean species. Indian white shrimp *Fenneropenaeus indicus*, is one among the shrimp species extensively farmed along the Indian Ocean especially in east coast of India. However, with rapid production and development in the industrial culture of *Fenneropenaeus indicus* faces serious problems by the prevalence of infectious diseases. Diseases which identified at all stages of shrimp culture and in capture fisheries in India are responsible for the declined production and vast economic losses. This is due to the outbreak of opportunistic microbial pathogens including viruses, bacteria, fungi and parasites (Bachere E et al 2004, Sivakamavalli J et al 2012, Rajan et al 2000, Austin B et al 1995). Viruses are considered to be the major pathogens that threaten the shrimp aquaculture industry worldwide as many viral infections may occur at different stages of shrimps which remain undetected till they result in massive disease outbreaks. Several viral diseases have been reported from cultured penaeid shrimps causing mortality, slow growth and deformations [Subramaniyan Manivannan et al 2004, Lightner et al 1998]. As nearly 20 viral pathogens that are known to infect penaeid shrimp, high mortalities and severe damages leading to decline in the global shrimp production over the last few decades is by the killer pathogen, *White Spot Syndrome Virus* (Primavera J.H. et al 1997, Rosenberry B et al 2000).

White spot syndrome virus (WSSV) is the causative organism of white spot disease (WSD) which causes rapid and high mortality up to 100% within a few days after onset of the viral infection causing massive economic losses to the penaeid shrimp aquaculture industry (Lightner D.V 1996). Moreover, it can also infect a large variety of other marine to freshwater crustacean species, including freshwater prawns, crabs, lobsters and crayfish that have been described not only to be hosts but also carriers of WSSV (Wang Y. C 1998). WSSV is a fast replicating and an extremely virulent infecting the vital organs of mesodermal and ectodermal origin, as evidenced by the presence of degenerated cells with hypertrophied nuclei in the infected tissues (Chou H.Y et al 1995, Chang 1996).

Clinical signs of WSSV include lethargy, a loose cuticle, sudden fall in food consumption, red discoloration of body and appendages, cuticle over abdomen segments and the appearance of white spots on the inner surface of the carapace and exoskeleton of the infected shrimp. WSSV also destroys the host cytoskeleton and shuts down the genes involved in host energy metabolism (Takahashi Y et al 1994, Wang B et al 2006).

WSSV is a large enveloped, non-occluded, ovoid-to-bacilliform DNA virus belonging to the family *Nimaviridae* and the monotypic genus *Whispovirus* (Van Hulten et al 2001). Both genomic and proteomic approaches have revealed the WSSV virion structural protein components which contains a rod shaped nucleocapsid with a cross-hatched appearance, surrounded by an intermediate tegument layer and the outer lipid bilayer membrane envelope with a unique tail-like appendix at one end. The nucleocapsid is composed of 14 rings and has a circular double-strand DNA genome of approximately 300 kb (Wongteerasupaya C et al 1995, Durand S et al 1997, Tsai et al 2006, Nadala et al 1998). The structural proteins play vital roles in cell targeting, virus entry, assembly, and budding, as well as triggering host antiviral defenses (Campadelli-Fiume G et al 2007, Chazal N et al 2003, Mettenleiter TC et al 2004, Mettenleiter TC et al 2006, Rajca'ni J et al 2003). The virus particle consists of at least five major proteins VP28, VP26, VP24, VP19, and VP15. VP28 and VP19 are associated with the virion envelope and VP26, VP24, and VP15 with the nucleocapsid (Van Hulten et al 2000). The role of the envelope, nucleocapsid and their proteins in the establishment of the systemic infection process is when the virus enters host, the viral envelop proteins interact with endosomal proteins of shrimp, thereafter the naked viral nucleocapsid is transported into the shrimp nucleus where viral genome is released for its replication and dissemination (Sieczkarski SB et al 2002). The characterization of this identified structural proteins involved in shrimp infection has been suggested that they might be used as targets for vaccine design (Chang Y-S et al 2010). The currently available disease treatment applications against wssv are cost effective with undesirable side effects. Even though the positive effects of some antibiotics and synthetic drugs is in use, they are not recommended due to their residual effects, resistant strain development and other environmental hazards. To develop alternative practices against WSSV control attention should be given to find novel antimicrobial drugs, especially from plant sources. As part of the search for natural anti-viral agents from medicinal plants, *Cynodon dactylon* derived phytochemicals has been found which possess various potential medicinal properties against many microbial pathogens (Citarasu T et al 2006, Kanimozhi D et al 2012).

The present work investigates screening of potential phytochemicals from *Cynodon dactylon* to stimulate the structure-based design of new drugs that target protein VP26 from WSSV. VP26, a tegument or a matrix-like linker protein between the viral envelope and nucleocapsid is gained importance as it has been hypothesized that it may be instrumental in trafficking the WSSV nucleocapsid into the host nucleus via the cytoskeleton (Xie X et al 2005, Wan Q et al 2008). Therefore molecular docking studies are carried on retrieved WSSV protein VP26 (2EDM) with selected phytochemical molecules from *Cynodon dactylon*. Based on the energy values and hydrogen bond interactions of the docked complexes the compounds luteolin, apigenin shows strong binding affinities which could act as novel drug candidates for controlling WSSV infections.

MATERIALS AND METHODS

Retrieval of Protein Structure

The structure of the target protein VP26 of *White spot syndrome virus* is obtained from the protein databank. After evaluating number of entries, the best protein is selected based on good resolution and Ramachandran's plot analysis. The high resolution x-ray crystal structure of target protein VP26 (PDB ID: 2EDM), having the resolution of 2.20 Å is retrieved.

Phytochemicals screened

Phytochemicals namely Luteolin, Apigenin, beta-carotene, Orientin, Phytol, Tricosane, violaxanthin, vitexin identified from *Cynodon dactylon* are screened against the VP26 (2EDM) protein. Using ACD/ ChemSketch (12.0) software, the chemical structures of the phytochemical molecules are drawn and saved in mol2 format.

Molecular Simulation Studies

Ligand preparation

The sketched phytochemical molecules are later imported and minimized in Discovery Studio (DS) after adding hydrogen bonds. Using ligand preparation protocol of DS, these ligand molecules are prepared with constraint parameters such as ionization change; tautomer and isomer generation and all the duplicate structures are removed. By applying the forcefield CHARMM, minimization is carried out with the steepest descent algorithm which follows by the conjugant gradient algorithm till it satisfies the convergence gradient. 3D structure is generated by catalyst algorithm in DS.

Protein preparation

The crystal structure of the target protein VP26 (2EDM) of *White spot syndrome virus* is imported in to Discovery Studio. Water molecules are removed and the chemistry of the protein is corrected for missing hydrogens. The prepare protein protocol available in DS is employed to prepare the protein for further processing by standardizes atoms names, inserting missing atoms in incomplete residues, modeling missing loop regions, calculates pKa and protonates the protein. Default parameters were used. Following the above steps of preparation, the protein was then refined by energy minimization with appropriate parameters by applying CHARMM force field and using steepest descent algorithm followed by conjugant gradient algorithm until the convergence gradient is satisfied.

Active Site prediction

The possible binding sites of target protein VP26 (2EDM) are searched using Computed Atlas of Surface Topography of Proteins (CASTp). These include pockets located on protein surfaces and voids buried in the interior of proteins. CASTp includes a graphical user interface, flexible interactive visualization, as well as on-the-fly calculation for user uploaded structures. The number of active sites, surface area and volume of active site were analyzed.

Docking studies

Molecular docking approaches helps to understand protein-ligand interactions that can strongly support and help the design of novel, more potent inhibitors by revealing the mechanism of their interactions. To increase the reliability and accuracy of VP26 inhibitors selection, the phytochemical ligand molecules are docked into the protein VP26 (2EDM) binding sites. The docking program GOLD has been employed to generate the bioactive binding poses of inhibitors in the active site of protein. GOLD 5.1 (Genetic Optimization for Ligand Docking; Cambridge Crystallographic Data Centre) uses a genetic algorithm to explore the conformational space of the ligands in addition to some flexibility of active site residues. Protein coordinates from the crystal structure were used to define the active site. Default GOLD parameters were used to produce the set of optimal conformations of both the ligand and the protein. The standard scoring function Goldscore is used to reach optimal accuracy for candidate compound selection. Each simulation is performed 10 times, yielding 10 docked conformations. The lowest energy conformations were regarded as the binding conformations between ligands and the protein. The interacting ability of a ligand molecule depends on the fitness score, greater the GOLD fitness score better the binding affinity.

ADME and Toxicity studies

ADME-Toxicity studies are performed through Accelrys Discovery Studio 2.0. The Absorption, Distribution, Metabolism, Excretion (ADME) and Toxicology studies are predicted which provides insights into the pharmacokinetic property of the screened phytochemical molecules from the plant *cynodon dactylon*. ADMET properties include absorption, aqueous solubility, blood brain barrier (BBB), hepatotoxicity, plasma protein binding (PPB) and cytochrome P450 CYP2D6_Probability enzyme inhibition study.

RESULTS AND DISCUSSION

Active Site prediction

The potential active site amino acids of VP26 from *white spot syndrome virus* are predicted using CASTp server. Among the 30 active sites predicted, pocket 1 found to be the best active site which contains methionine, glutamine, threonine, arginine, asparagine isoleucine and glycine amino acids.. The largest surface area of pocket 1 is found to be 59.6 Å with a volume of 72.6.

Docking studies

Molecular docking plays a significant role in structural based drug designing by predicting the binding orientation of small molecule drug candidates to their known 3D structures of the protein targets. To study the binding mode affinities and their energies of the chosen phytochemical molecules in the active sites of the protein VP26 (PDBID: 2EDM) molecular docking study is performed by GOLD. In GOLD, docking of the optimized protein and ligand molecules are carried out using the wizard with default parameters to explore the rotational flexibility of receptor hydrogens and ligand conformational flexibility. Protein coordinates from the crystal structure of VP26 (PDB ID: 2EDM), determined at a resolution of 2.20 Å were used to define the active site. The active site was defined with a 10 Å radius around the ligand and a set of 10 solutions were saved for each ligand. The docking run generated 10 different poses for each of the compound. Two important parameters have been considered for selecting potential compounds among the given input: (i) Hydrogen bond details of the top-ranked pose and (ii) prediction of binding energy of the best docked pose using scores calculated by GOLD scoring function. Apart from these, other input parameters for docking are also considered for evaluating the docking efficacy in our study.

Goldscore

GOLD uses a GoldScore fitness function for the calculation of optimized binding positions of ligand. It estimates the protein-ligand binding energies based on the interacting ability of a phytochemical molecules with the target protein VP26 (PDB ID: 2EDM). The GOLD fitness score is calculated from the contributions of hydrogen bond and vander Waals interactions between the protein and ligand, intramolecular hydrophobic interactions and strains of the ligand. Greater the GOLD fitness score better the binding affinity. Of the 10 generated conformations for each ligand, only top ranked docked complex score is taken and analyzed the binding modes. The Summary of docking information of the top ranked poses are tabulated in Table 1. From the overall docking scores we identified that luteolin is having a high fitness score of 42.51 K.cal/mol indicating high binding affinity with the target protein VP26 (2EDM). The order of binding affinities of all the compounds are luteolin> orientin> apigenin> phytol> vitexin> tricosane >violaxanthin> beta-carotene. The stability of the best docked pose of these compounds is evaluated by determining the hydrogen bonding between the protein and ligand.

Table 1: GoldScores of the ligand compounds docked with *white spot syndrome virus* VP26 (2EDM) protein active site.

Ligand name	Fitness value	S(hb_ext)	S(vdw_ext)	S(hb_int)	S(int)
Luteolin	42.51	6.99	32.82	0.00	-9.61
apigenin	38.92	1.92	31.89	0.00	-6.85
beta-carotene	28.02	0.00	44.15	0.00	-32.69
Orientin	39.49	11.68	29.50	0.00	12.75
phytol	36.33	0.00	35.95	0.00	-13.10
tricosane	35.29	0.00	42.04	0.00	-22.51
violaxanthin	29.08	0.59	36.46	0.00	-21.65
Vitexin	35.89	3.19	30.97	0.00	-9.88

Table 2: Hydrogen bond interactions between the *white spot syndrome virus* VP26 (2EDM) protein and phytochemical molecules from *cynodon dactylon*.

Protein	Ligand	Amino acids involved in H-Bonding with Ligand Atoms	H-Distance
VP26 (2EDM)	Luteolin	A: ARG51:NH1 - LUTEOLIN:O	2.689
		A: ASN186:OG1 - LUTEOLIN:C	2.520
		A: THR134:OG1 - LUTEOLIN:C	2.519
		A: GLY188:N - LUTEOLIN:O	2.449
		A: ASN131:OD1 - LUTEOLIN:C	2.125
		A: ASP132:CG - LUTEOLIN:C	1.843
	Apigenin	A: ARG51:NH1 - APIGENIN:O	2.878
		A: PRO137:O - APIGENIN:C	2.624
		A: THR134:OG1 - APIGENIN:C	2.695
		A: ASN186:ND2 - APIGENIN:O	2.155
		A: ASN132:CG - APIGENIN:C	1.721
	Orientin	A: ARG51:NH1 - ORIENTIN:O	2.797
		A: ARG136:NH2 - ORIENTIN:O	2.529
		A: THR134:OG1 - ORIENTIN:O	2.685
		A: ILE187:O - ORIENTIN:C	2.450
	Phytol	A: THR181:OG1 - ORIENTIN:O	3.627
		A: ASN131:ND2 - PHYTOL:O	2.831
	Violaxanthin	A: ARG51:NH1 - VIOLXANTHIN:O	3.332
Vitexin	A: ARG136:NE - VITEXIN:O	2.465	
	A: ASP132:OD1 - VITEXIN:C	2.286	
	A: THR134:OG1 - VITEXIN:C	2.531	
	A: ASN186:ND2 - VITEXIN:O	3.413	
	A: ASN186:O - VITEXIN:C	2.711	

Hydrogen Bond Interaction

By enlarging the interaction analysis the hydrogen bond interaction is contributed as major parameter. The Hydrogen bonding interactions of the protein with compounds are analyzed. Each of the compound having highest dockscore with VP26 (2EDM) are taken for H-Bond analysis which reveals the aminoacids involved in hydrogen bond formation. The amino acid residues involved in hydrogen bond interactions of VP26 (2EDM) with the ligands and their distances are depicted in Table 2.

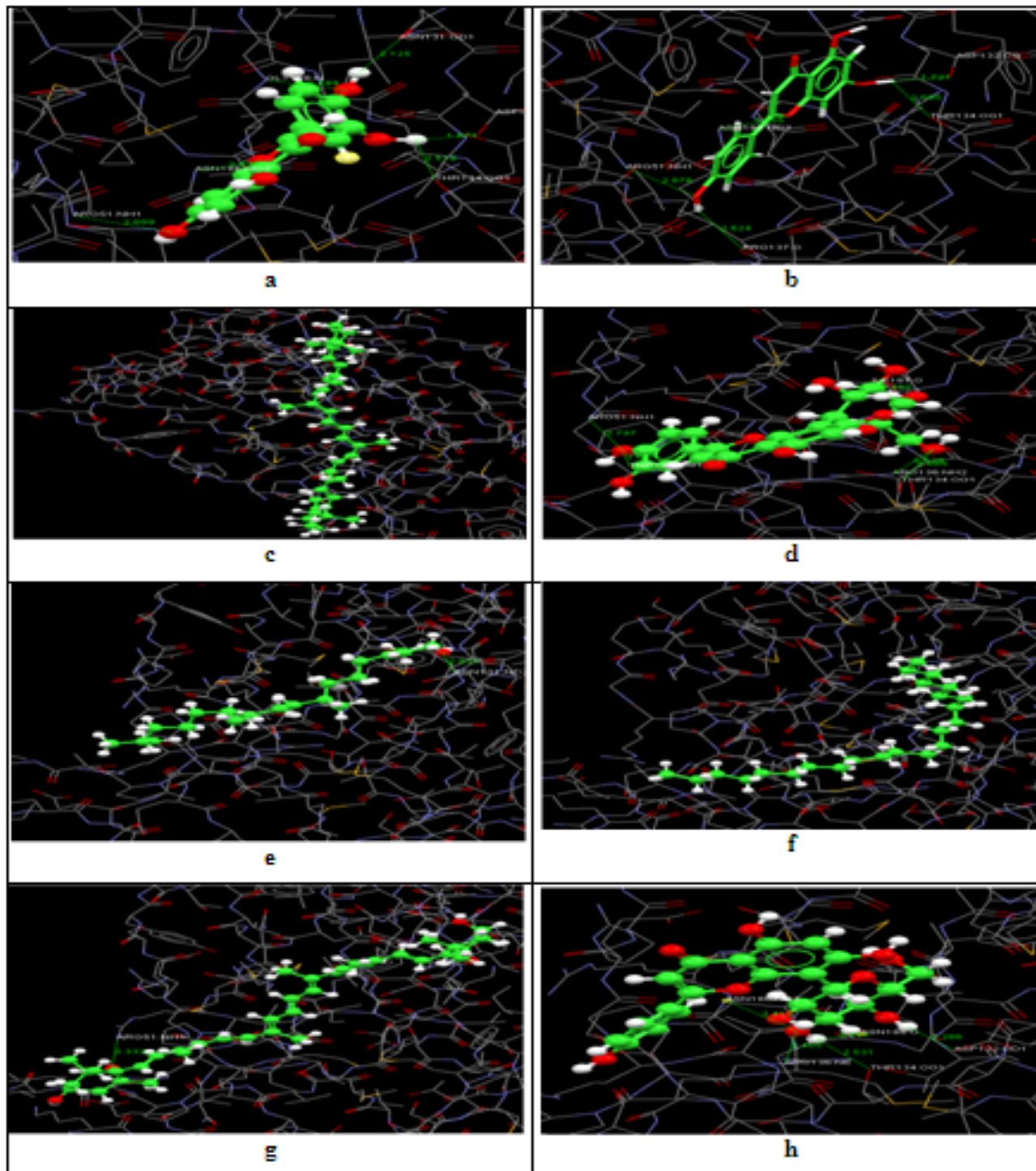


Figure 1: Bonded and non-bonded interactions of *white spot syndrome virus* protein VP26 (2EDM) with (a) Luteolin (b) apigenin (c) beta-carotene (d) Orientin (e) phytol (f) tricosane (g) violaxanthin (h) Vitexin. The protein is shown in line representation and the ligand is shown in ball and stick model. Green dashed lines indicate hydrogen bonds and white letters showing the amino acids involved in the bonding

ADME and Toxicity studies

The energy minimized phytochemical molecules from the plant *Cynodon dactylon* are further examined by their pharmacokinetic and toxicity studies using ADMET descriptors analysis protocol in DS. Interpretation of the values was done using standards provided by DS. All the parameters calculated are tabulated in the Table 3.

Table 3: Absorption, distribution, metabolism, excretion and toxicity (ADMET) of phytochemical molecules from the plant *Cynodon dactylon*

Ligand name	ADMET_BBB	ADMET_Absorption_Level	ADMET_Solubility	ADMET_Hepatotoxicity	ADMET_CYP2D6_Probability	ADMET_PPB_Level	ADMET_A logP98
Luteolin	4	0	-2.856	1	1	2	2.168
Apigenin	3	0	-2.977	1	1	2	2.41
Beta-carotene	4	3	-9.866	0	0	2	11.998
Orientin	4	3	-3.318	1	1	2	-0.224
Phytol	4	3	-5.73	0	0	2	7.337
Tricosane	4	3	-8.426	0	0	2	10.864
Violaxanthin	4	3	-4.655	0	0	2	7.001
Vitexin	4	3	-2.746	1	1	2	0.018

CONCLUSION

The present study initiates to find the antiviral activity of the Indian medicinal plant *Cynodon dactylon* against white spot disease caused by *white spot syndrome virus*. Docking studies are carried out in order to know to which extent the derived phytochemicals from *Cynodon dactylon* could inhibit the activity of the viral protein VP26 (2EDM) from WSSV. The results reveal that of the eight phytochemical molecules, luteolin and apigenin shows strong binding affinity that can act as the most potential drugs for treating the viral infections of WSSV.

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