BIOINFORMATION
Discovery at the interface of physical and biological sciences

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Volume 8(18)

Molecular docking analyses of *Avicennia marina*-derived phytochemicals against white spot syndrome virus (WSSV) envelope protein-VP28

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Received March 03, 2012; Accepted May 27, 2012; Published September 21, 2012

Abstract:
White spot syndrome (WSS) is one of the most common and most disastrous diseases of shrimp worldwide. It causes up to 100% mortality within 3 to 4 days in commercial shrimp farms, resulting in large economic losses to the shrimp farming industry. VP28 envelope protein of WSSV is reported to play a key role in the systemic infection in shrimps. Considering the most sombre issue of viral disease in cultivated shrimp, the present study was undertaken to substantiate the inhibition potential of *Avicennia marina*-derived phytochemicals against the WSSV envelope protein VP28**.** Seven *A. marina*-derived phytochemicals namely stigmasterol, triterpenoid, betulin, lupeol, avicenol-A, betulinic acid and quercetin were docked against the WSSV protein VP28 by using Argus lab molecular docking software. The chemical structures of the phytochemicals were retrieved from Pubchem database and generated from SMILES notation. Similarly the protein structure of the envelope protein was obtained from protein data bank (PDB-ID: 2ED6). Binding sites were predicted by using ligand explorer software. Among the phytochemicals screened, stigmasterol, lupeol and betulin showed the best binding exhibiting the potential to block VP28 envelope protein of WSSV, which could possibly inhibit the attachment of WSSV to the host species. Further experimental studies will provide a clear understanding on the mode of action of these phytochemicals individually or synergistically against WSSV envelope protein and can be used as an inhibitory drug to reduce white spot related severe complications in crustaceans.

Key words: White spot syndrome virus (wssv), VP28 envelope protein, Mangroves, *Avicennia marina*, Molecular docking, Phytochemicals

Background:
White spot syndrome virus (WSSV) causes the dreadful disease in crustaceans and cripples the fast growing aquaculture industry **[1].** WSSV belonging to the family nimaviridae is an enveloped, non-occluded, rod-shaped DNA virus infecting penaeid shrimps and other crustaceans. WSSV was first reported from farmed *Marsupenaeus japonicus* in Japan in 1993 and named the Penaeid rod-shaped DNA virus **[2]**. WSSV causes 100% mortality in cultured shrimp within 3 to 4 days. The principal clinical symptom of WSS is the presence of white spots in the exoskeleton of the infected shrimp. Other signs include a rapid reduction in food consumption, lethargy and reddening of appendages. WSSV also destroys the host cytoskeleton and shuts down the genes involved in host energy metabolism **[3]**. Envelope proteins play a critical role during early events of virus infection, especially in attachment in many host species. Of 39 structural proteins of WSSV, 22 are of enveloped proteins constructing the infection-related structure **[4].** VP28 envelope protein of WSSV plays a key role in the systemic infection in shrimp **[1].** It has been reported that VP28 binds to shrimp cells as an attachment protein and help the virus to enter the cytoplasm **[5].** Besides viral infection,

envelope proteins are important for viral assemblage **[6].** Mangrove plants are a rich source of medicinal compounds such as steroids, triterpenes, saponins, flavonoids, alkaloids and tannins **[7].** Over 349 metabolites have been isolated from mangrove species **[8].** Extracts from mangrove and mangrove associated plant species have proven their activity against human and animal pathogens. *Avicennia marina* (Forssk.) Vierh, is a predominant tree species of mangrove forests, and widely distributed along tropical and subtropical coastlines **[9].** This mangrove plant has been traditionally used for treatment of rheumatism, small pox, ulcers and other ailments **[10].** The serious impact of WSSV in shrimp culture industry worldwide and the broad host range call for an efficient control strategy against the virus. Therefore, the present study was undertaken to substantiate the inhibition potential of *A. marina*-derived phytochemicals against the WSSV envelope protein VP28**.**

Methodology:

Retrieval of protein Structure

The target envelope protein VP28 of White spot syndrome virus **[11]** (PDB ID: 2ED6), having the resolution of 2.0A° was retrieved from the protein data bank (PDB) (www.rcsb.org/pdb). Structural and active site studies of the protein were done by using CASTP (Computed Atlas of Surface Topography of Proteins) and pymol molecular visualization software.

Phytochemicals screened

Seven phytochemicals namely stigmasterol, triterpenoid, betulin, lupeol, avicenol-A, betulinic acid and quercetin identified from *A. marina* of the coastal mangrove ecosystems **[7, 12-14]** were screened against the VP28 envelope protein. The phytochemical molecules were retrieved from the pubchem database and the chemical structures were generated from SMILES notation (Simplified Molecular Input Line Entry Specification) by using the Chemsketch Software (www.acdlabs.com).

Active site prediction

Active site of the target protein were predicted by using "Active site prediction tool" from SCFBio Server (http://www.scfbio-iitd.res.in/dock/ActiveSite.jsp) which requires a "pdb" file as an input and this tool explains the total number of active sites along with information on their amino acid sequence, cavity points and the average volume of the cavity.

Docking methods

Argus Lab 4.0.1, most common and freely available software was used for docking analysis (to calculate the binding energy requirements of different ligands with VP28 envelope protein of WSSV). The inhibitor and target protein were geometrically optimized and "Argus dock" docking engine was used. Calculation type was set to "Dock" mode whereas "flexible mode" was selected for the ligand. Grid resolution was set to 0.40A°. Least energy represented the easy binding character of ligand and receptor.

Ligand binding sites prediction

After docking the docked structure was saved as ".pdb" file and further explored to predict the binding sites using "ligand explorer" software. The predicted binding sites, based on the binding energy, and amino acids make up the binding cavity. Here ligand binding site represents the site where the ligands most efficiently bind with the protein, among all the active site.

Drug likeliness prediction

Ligand property was predicted by using "Lipinski drug Filters" (http://www.scfbio-iitd.res.in/utility/LipinskiFilters.jsp). Lipinski rule of five helps in distinguishing drug-like and non-drug-like properties and predicts high probability of success or failure due to drug likeliness for molecules. The Lipsinki filter helps in early preclinical assessment and thereby avoiding costly late stage preclinical and clinical failures.

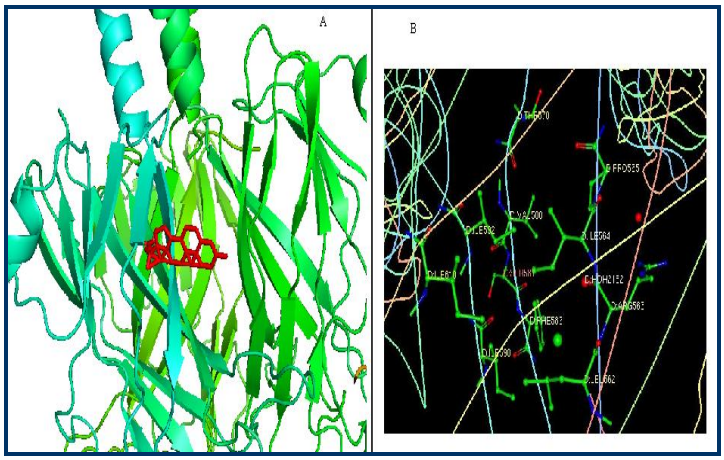

Figure 1: Molecular visualization of stigmasterol **(A)** Protein-ligand interaction (Pymol software) **(B)** Amino acids in the binding pocket, ARG563, ILE564, THR578, PRO565 (Beta strand), LEU562 (Coil) (RCSB Ligand explorer)

Discussion:

White spot syndrome virus is the most virulent and the largest animal virus known to affect all crustaceans in particular cultured shrimp **[1, 15].** Hence, developing an efficient treatment method is of the greatest urgency for aquaculture to survive. Argus lab molecular docking software 4.0.1 was used to dock seven *A. marina* derived phytochemicals namely stigmasterol, triterpenoid, betulin, lupeol, avicenol-A, betulinic acid and quercetin against the WSSV envelope protein VP28. Totally 135 active sites were predicted in the target protein by the "Active site prediction tool". This high number of active sites may be due to the high structure weight (220675.19) **[11].** The docking interaction of the protein and ligand, and the predicted ligand binding site residues are shown in **(Figure 1a)** and **(Figure 1b)** respectively. The docked ligand molecules were selected based on docking energy and good interaction with the active site residues and the results are shown in **Table 1 (see supplementary material).** Of seven phytochemicals, three were found potent namely stigmasterol, lupeol and betulin which exhibited minimum docking score of -15.0363, -11.9573 and -11.5012 Kcal/mol respectively. Lesser the docking score more is the binding capacity of the ligand. Antiviral activity of *A. marina* against herpes simplex virus type 1 and vaccine strain of poliovirus has already been ascertained **[16].** Apart from antiviral activities few studies on the anti-parasitic, antifungal and antibacterial, antimalarial and anticandidal activities as well as cytotoxicity of *A. marina* has also been reported **[17-19, 7].** In majority of the previous studies on anti WSSV property of plant extracts, there have been very few attempts to purify the components responsible for anti WSSV activity. All these

investigations were focused mainly on crude extracts from a single plant or combination of plants **[20].** All the docked phytochemicals passed the Lipinski drug filter as evident in the **Table 1 (see supplementary material).** Hence, stigmasterol which showed minimum docking score could be considered for further *in vitro* and *in vivo* studies. The potential of mangrove-derived compounds has been already corroborated by molecular docking studies in our laboratory against, sterol containing protein (AeSCP-2), breast cancer protein (BRCA1) and dihydrofolate reductase **[21-23].** Recently, a few studies have made efforts to develop polyclonal antibodies (pAbs) against specific viral peptides such as VP28 and VP19 and have obtained promising results with indoor experiments **[24].** Envelope protein, VP28 is involved in systemic infection of shrimp **[1].** Hence blocking this protein can possibly impede the entry of WSSV in the host. However, further *in vitro* and *in vivo* experiments are needed to demonstrate the effectiveness of stigmasterol for inhibition of WSSV.

Conclusion:

The results obtained from this study would be useful in both understanding the inhibitory mode of *A. marina*-derived phytochemicals as well as in rapidly and accurately predicting the activities of newly designed inhibitors on the basis of docking scores. The present study showed that of seven phytochemicals, stigmasterol could be the most potential inhibitory source against VP28 envelope protein of WSSV. However, ratification of the mechanism of envelope protein for a viral infection can provide important molecular targets. We anticipate that further exploration of the functions of envelope proteins, including VP24, will facilitate a better understanding of the molecular mechanism underlying WSSV infection for control of viral infection.

Acknowledgement:

Authors are thankful to the authorities of Annamalai University for providing necessary facilities to carry out this work, and Department of Science & Technology, Govt. of India, New Delhi for providing INSPIRE fellowship (SK Sahu).

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Edited by P Kangueane

Citation: Sahu *et al*. Bioinformation 8(18): 897-900 (2012)

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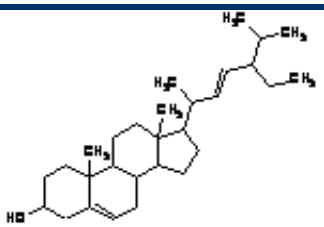
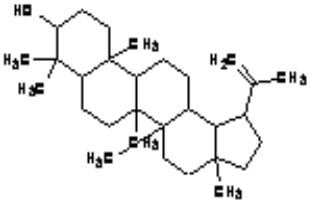
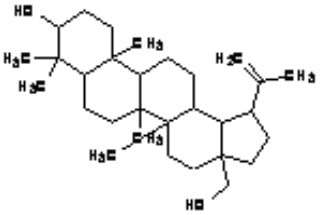
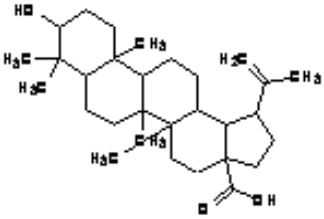
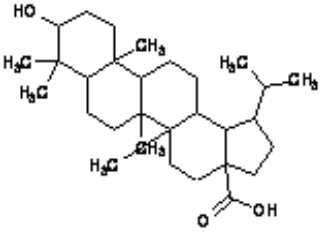
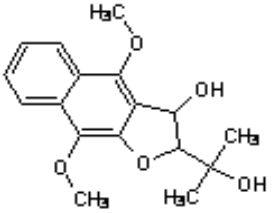
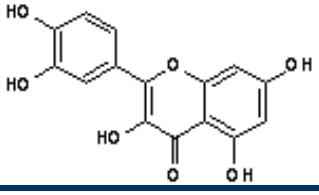
Supplementary material:

Table 1: Docking results of *A. marina*-derived phytochemicals against VP28 envelope protein of WSSV

Compound Name	Pubchem ID	2D structure of Compound	Molecular Weight (g/mol)	Hydrogen donor/ acceptor	Docking Energy Level (Kcal/mol)
Stigmasterol	5280794		269.082	(1,1)	-15.0363
Lupeol	259846		426.717	(1,1)	-11.9573
Betulin	221023		442.716	(2,2)	-11.5012
Betulinic acid	64971		456.700	(2,3)	-10.8796
Triterpenoid	9804218		458.604	(2,3)	-10.8786
Avicenol A	11208912		304.337	(2,5)	-8.06299
Quercetin	5280343		302.235	(5,7)	-6.967