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Evaluation of Natural Compound as a Potential Drug Against DENV Non-structural Proteins: *In silico* Study

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ABSTRACT

Dengue fever is one of the most threatening epidemics of this era and there is no targeted vaccine and therapy. The present study was designed to elucidate the natural compounds as therapeutic targets for dengue virus. In this study, it is proposed that target approach for dengue drug discovery based on natural ligands obtained from plant extract of *C. papaya*. Nonstructural proteins were retrieved from the protein data bank and natural compounds were drawn using drawing tool, before docking. All of them were subjected to drug and absorption distribution metabolism toxicity analysis which shows satisfying results which leads to docking studies. Series of 8 compounds have been screened and docked for binding energy prediction and on the basis of lowest binding energy, the potential ligands like 2-Methoxy-4-vinylphenol, 9-Octadecyne and 9, 12, 15-Octadecatrienoic acid, (Z, Z, Z) are recommended for further studies.

Key words: Dengue, *C. papaya*, docking studies, proteins, non-structural proteins

INTRODUCTION

Dengue shock syndrome is the alarming threat worldwide. It is caused by airborn flavivirus transmmited through female mosquitoes. Increasing technology and urban development allows this epidemic condition to grow and transmit. Human to human transmision dramatically increase the risk. Very recent estimate in middle and South India approximately 500 cases met with drastic fate. Only symptomatic treatment is available to deal with the disease. Understanding the mechanism and mode of viral replication cycle is neccesary for target identification. Totally four serotypes existed among these, depending on severity, they are classified by WHO (2009) and Bhattacharjee and Bhattacharjee (2011).

The pathogenesis of severe Dengue Hemorrhagic Fever (DHF) is still at nascent stage, no vaccine is yet available for inhibition and the vector control measures are not promising. Dengue virus was isolated in India in 1944 but there are very few research centres involved in the core pathogenesis mechanism finding, drug discovery and development is also the tedious process until the mechanism is explained (Gupta *et al*., 2012). The natural herbal therapies have been tried against such an unknown infections since ancient times in India. Targets were selected from most prevalent strains of DENV2. Full genome of the dengue shows that the virus has structural and non-structural proteins; among this non-structural (NS) enzymes of the replication complex include

the NS3 protease and with its NS2B, cofactor, the NS3 helicase/nucleoside triphosphatase (NTPase)/RNA 5' triphosphatase (RTPase) and the NS5 methyltransferase/RNA-dependent RNA polymerase. Murthy *et al.* (1999), Erbel *et al.* (2006), Augustine Ocloo *et al.* (2012) hence these proteins were selected as target for the study.

The need of the hour is to develop natural antiviral drugs for dengue. Previously published data and evidences say local plant extract of *C. papaya* having the inhibitory effects on dengue, without scientific background. Papaya is also called "pawpaw" in English an herbaceous succulent plants that posses self supporting stems. Extensive research has been done on the medicinal properties of the leaves of Papaya and has found to be more effective as a antiseptic, blood purifier like biological activities (Gross, 2003; Ayoola and Adeyeye, 2010).

On the base of literature, this pilot study was aimed to evaluate the medicinal natural of compounds from *C. Papaya* and to study their *in silico* antiviral activity using docking studies.

MATERIALS AND METHODS

Preparation of protein structure: Selected 3 protein targets were downloaded from database Protein Data Bank (PDB). (PDB: http://www.rcsb.org/pdb/home/home.do). All water molecules were removed and on final stage hydrogen atoms were added to receptor molecule before docking.

Preparation of ligand structure: Total 8 important natural compounds available in the plant extract of *C. papaya* were selected on the basis of their biological activity reported. Structures of the phytochemicals were drawn using Chemsketch drawing tool and saved as structure data format.

Protein ligand interaction: Target proteins were then carried out for binding site studies for better understanding of interaction among the small and large molecules. This interaction is based on the geometry of target and flexibility of the ligand.

ADMET analysis: Small molecules obtained from the plant material are always screened for the potential drug like activity predictions, in this study all the compounds were screened for the drug likeness calculation using the pre-ADMET software (Lipinski *et al.*, 1997).

Absorption, Distribution, Metabolism and Excretion (ADME) were screened to analyse the drug like properties of the phytocompounds, calculation was performed by using the admet-SAR tool. SMILES of ligand were used as input and using the module different parameters were calculated such as blood brain barrier, aqueous solubility, metabolism and carcinogenicity (Cheng *et al.*, 2012).

Computational docking studies: Docking calculations were carried out using Docking Server (Bikadi and Hazai, 2009). Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged and rotatable bonds were defined. Docking calculations were carried out on protein model. Essential hydrogen atoms, Kollman charges and salvation parameters were added affinity (grid) maps of 23 Å grid points and 0.375 Å spacing were generated using the auto grid program. Auto Dock parameter set-and distance-dependent dielectric functions were used in the calculation of the van der waals and the electrostatic terms, respectively.

Docking simulations were performed using the Lamarckian Genetic Algorithm (LGA) and the Solis and Wets local search method (Solis and Wets, 1981). Initial position, orientation and torsions

of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 10 different runs that were set to terminate after a maximum of 250,000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å and quaternion and torsion steps of 5 were applied.

RESULTS

Three-dimensional structures of all the three proteins of DENV2 were downloaded from PDB shown in Table 1.

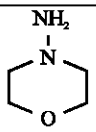
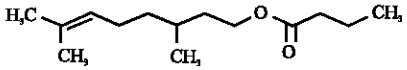
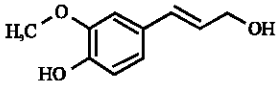
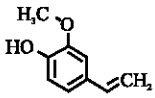
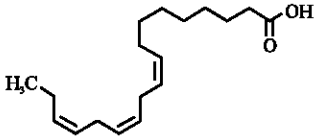
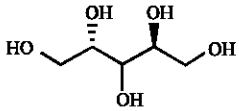
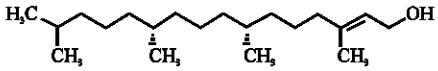
The GCMS analysis results of the plant gives some important phytochemicals (data not shown) which were selected for the docking purpose, structure and details given in Table 2.

ADMET analysis shows aqueous solubility of all compounds. Citronellyl butyrate and 9 octadecyne show level of good solubility. The Blood Brain Barrier (BBB) analysis generally used for penetration studies and in this study all the compounds except 2-Methoxy-4-vinylphenol and L-Arabinitol shows high penetration levels which mean they can cross the barrier and acts on

Table 1: Protein retrieved from PDB

Name of protein	PDB Id
NS3 protease helicase complex	2 VBC
Dengue NS2B-NS3 pro-complex	2 FOM
RNA dependent RNA polymerase NS5	1 L9K

Table 2: Isolated phytochemicals from *C. papya* used as a ligand

Name of compounds	Structure of compounds
N-Aminomorpholine	
Citronellyl butyrate	
4-Hydroxybenzyl alcohol, bis(pentafluoropropionate	
2-Methoxy-4-vinylphenol	
9, 12, 15-Octadecatrienoic acid, (Z, Z, Z)	
L-Arabinitol	
Phytol	
9-octadecyne	

Central nervous system. Cytochrome P (CYP450) is the important for drug metabolism. In this study, N-Aminomorpholine and 9-Octadecyne shows qualifies the test for inhibition. On the otherhand Citronellyl butyrate found to be more lethal due to its carcinogenic activity (Table 4 and 5).

In docking studies, NS3 and NS5 non-structural proteins were found to be more targeted by ligand, shows lowest binding energies, 2-Methoxy-4-vinylphenol, 9,12,15-Octadecatrienoic acid, (Z,Z,Z), 9-Octadecyne shows less binding energy less than -4.0 kcal mol^{-1} i.e., (-6.15) kcal mol^{-1} etc., scores shown in Table 3. While docking interaction of ligand with respective proteins shown in Fig. 1-3(a-h).

Table 3: Protein ligand interaction docking scores

Ligands/protein	NS3 (kcal mol^{-1})	NS5 (kcal mol^{-1})	Ns2+3 complex (kcal mol^{-1})
N-Aminomorpholine	-5.35	-4.27	-4.40
Citronellyl butyrate	-5.36	-5.38	-4.55
4-hydroxybenzyl alcohol, bis(pentafluoropropionate	-4.30	-4.34	-3.76
2-methoxy-4-vinylphenol	-5.48	-6.32	-3.46
9, 12, 15-octadecatrienoic acid (Z, Z, Z)	-5.76	-6.47	-3.90
L-Arabinitol	-4.07	3.63	-2.79
Phytol	-3.95	-5.08	3.28
9-octadecyne	-6.15	-5.42	-4.21

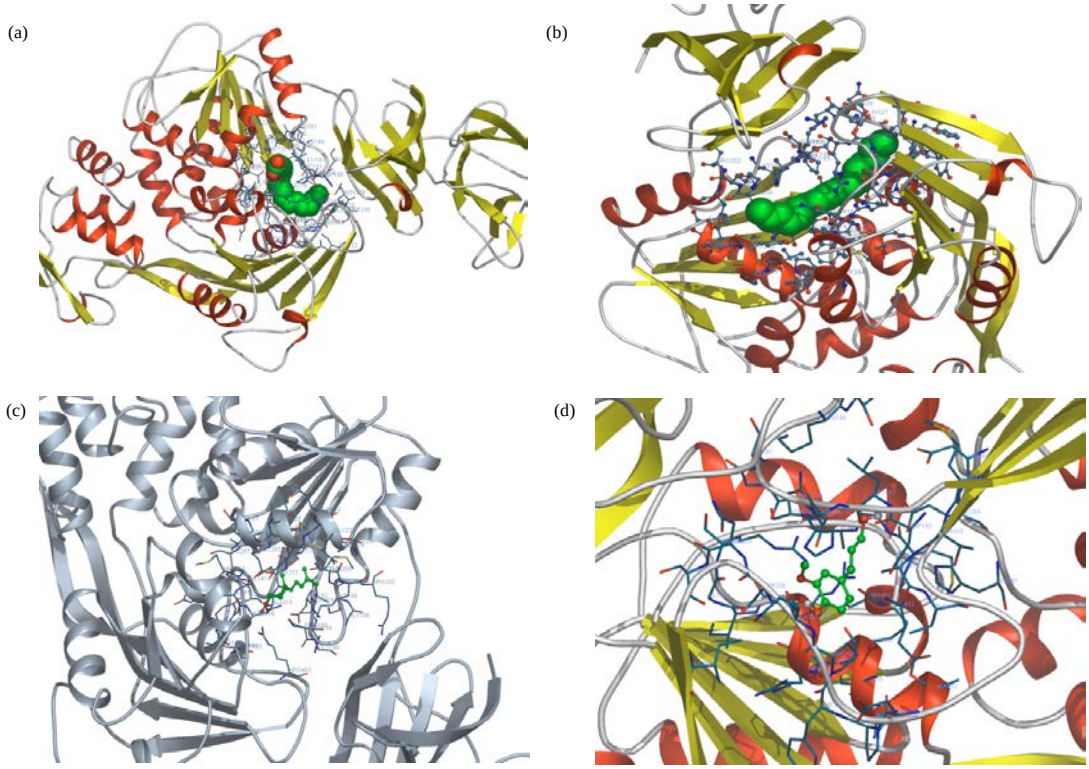


Fig. 1(a-f): Continue

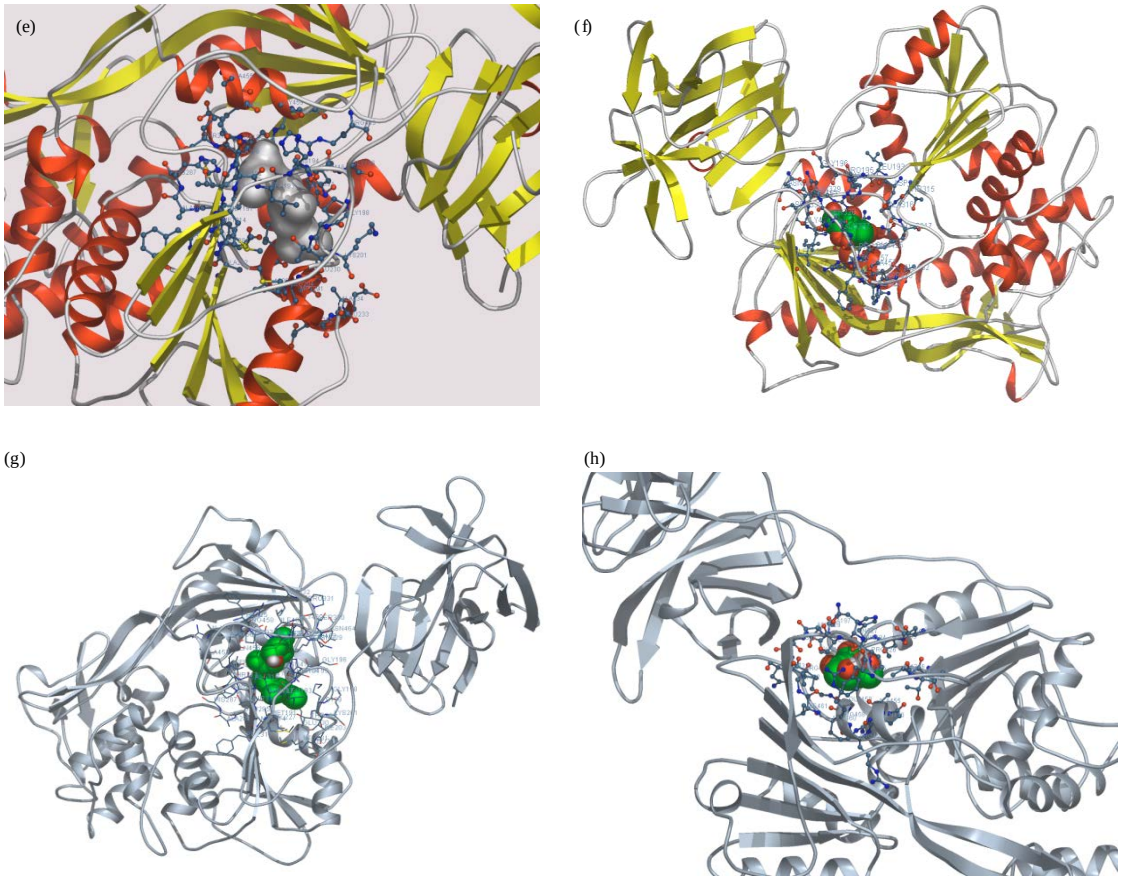


Fig. 1(a-h): NS3 protein docked with following ligand (a) 9, 12, 15-Octadecatrienoic acid, (Z, Z, Z) (b) 9 Octa decayne, (c) Citronyll butyrate, (d) 4-Hydroxybenzyl alcohol, bis(pentafluoropropionate), (e) 2-Methoxy-4-vinylphenol (f) L-Arabinitol and (g) Phytol and (h) N-Aminomorpholine

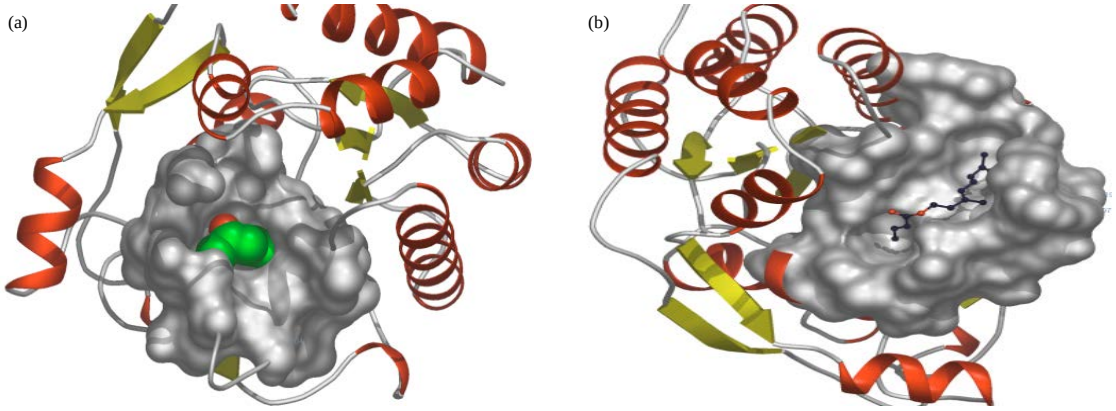


Fig. 2(a-h): Continue

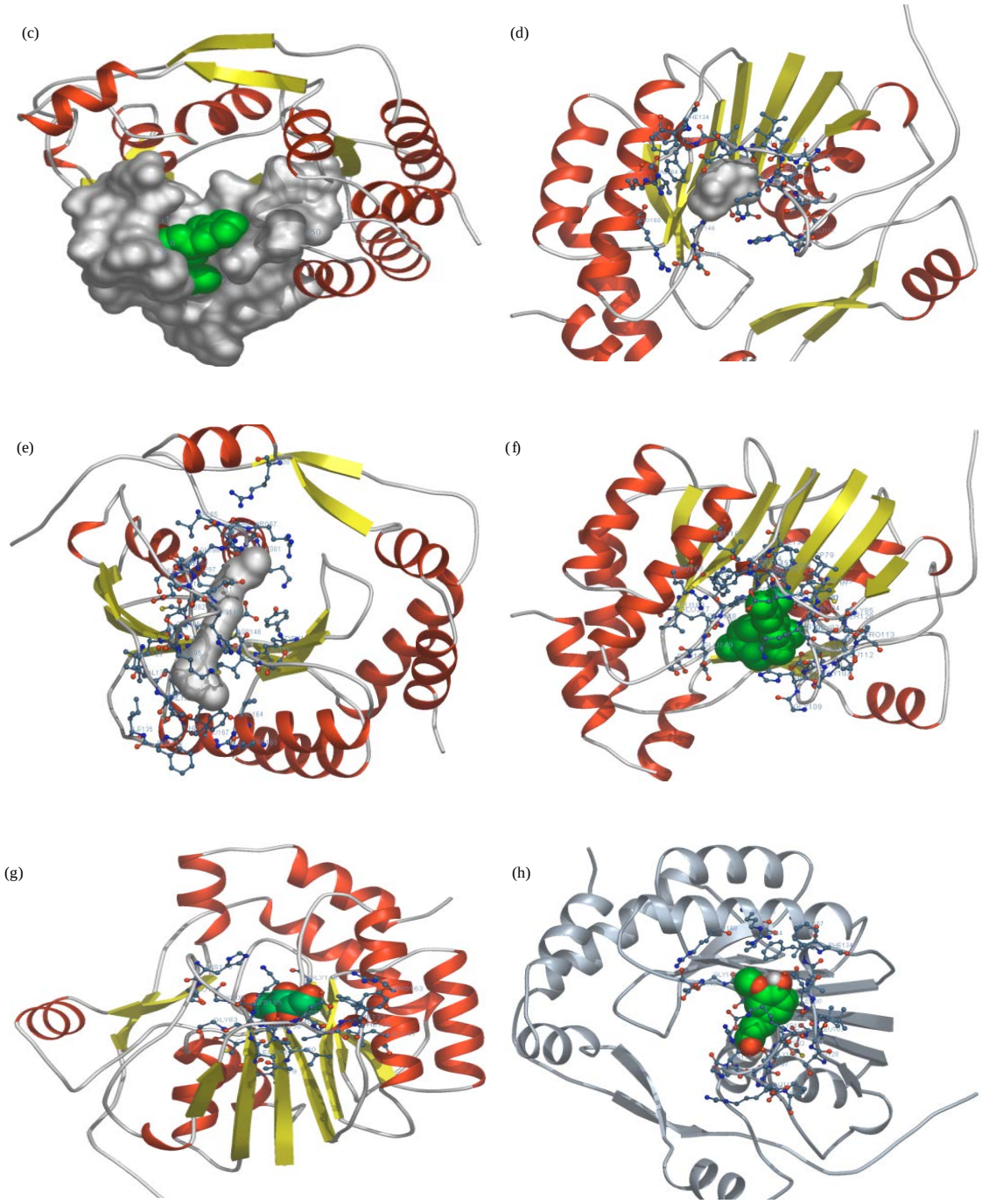


Fig. 2(a-h): NS5 protein docked with following ligand (a) Citronyll butyrate, (b) 2-Methoxy-4-vinylphenol, (c) 9, 12, 15-Octadecatrienoic acid, (Z, Z, Z), (d) N-Aminomorpholine, (e) 9-Octa decayne, (f) Phytol, (g) L-Arabinitol and (h) 4-Hydroxybenzyl alcohol bis(Pentafluoropropionate)

Table 4: ADMET Properties of the compounds

Rules for ADMET	N-Aminomorpholine	Citronellyl butyrate	4-Hydroxybenzyl alcohol,	2-Methoxy-4-vinylphenol
CMC like rule	Not qualified	Qualified	Qualified	Not qualified
CMC like rule violation fields	Mol. Weight	--	--	Mol. Weight
CMC like rule violations	4	0	0	1
Lead like rule	Violated	Violated	Violated	Suitable if its binding affinity is greater than 0.1 microM
Lead like rule violations	1	1	2	0
Lead-like rule violation fields	SKlog_D value	SKlog_D value	Mol wt.,SKlogD	-----
MDDR like rule	Nondruglike	Mid structure	Mid structure	Mid-structure
MDDR like rule violation fields	No_Rings, No_Rotatable_bonds	No_Rings, No_Rotatable_bonds	No_Rings, No_Rotatable_bonds	No_Rings, No_Rotatable_bonds
MDDR like rule violations	3	2	2	2
Rule of five	Suitable	Suitable	Suitable	Suitable
Rule of five violation fields	----------	------	---	--
Rule of five violations	0	0	0	0
WDI like rule	In 90% cutoff	Out of 90% cutoff	In 90% cutoff	Out of 90% cutoff
WDI like rule violation fields	---	Balaban_index_JX	---------	Balaban_index_JX
WDI like rule violations	0	3	0	1
Rules for ADMET	9,12,15-Octadecatrienoic acid (Z, ZZ)	L-Arabinitol	Phytol	9-Octadecyne
CMC like rule	Not qualified	Not qualified	Not qualified	Not qualified
CMC like rule violation fields	Alop98 value	Alop98 value	Alop98 value	Alop98 value
CMC like rule violations	1	3	1	1
Lead like rule	Violated	Violated	Violated	Violated
Lead like rule violations	1	1	1	1
Lead-like rule violation fields	SkLog D	SkLog D	SkLog D	SkLog D
MDDR like rule	Mid-structure	Mid-structure	Mid-structure	Mid-structure
MDDR like rule violation fields	No_Rings, No_Rotatable_bonds	No_Rings, No_Rotatable_bonds	No_Rings, No_Rotatable_bonds	No_Rings, No_Rotatable_bonds
MDDR like rule violations	2	2	2	2
Rule of five	Suitable	Suitable	Suitable	Suitable
Rule of five violation fields	LogD	---	SklogD	SklogD
Rule of five violations	1	0	1	1
WDI like rule	Out of 90% cutoff	Out of 90% cutoff	Out of 90% cutoff	Out of 90% cutoff
WDI like rule violation fields	AlopP98_value, Balaban_index_JX, Kier_flexibility	Balaban_index_JX	AlopP98_value, Balaban_index _JX, Kier_flexibility	AlopP98_value,Balaban_index_ JX, Kier_flexibility
WDI like rule violations	5	1	5	5

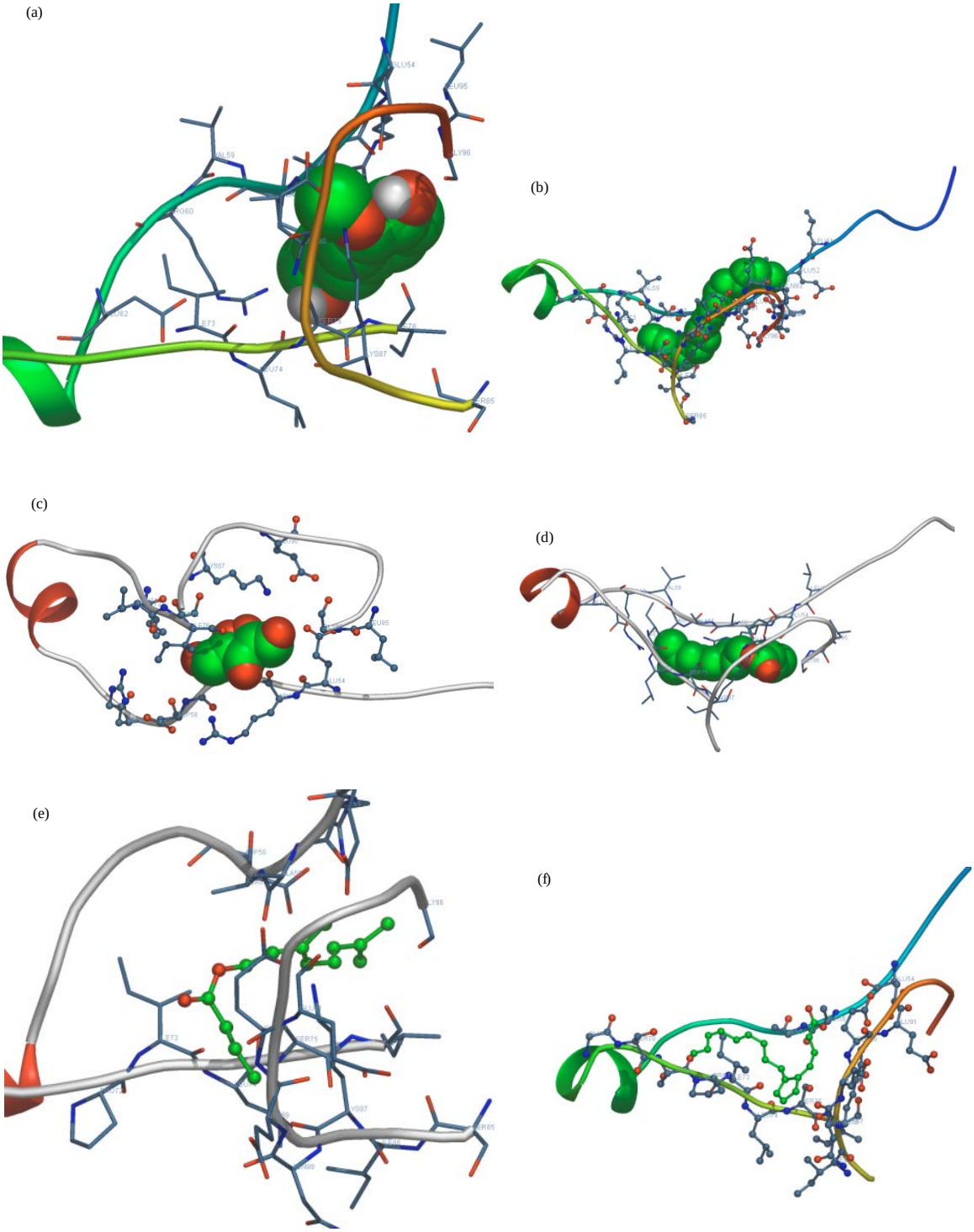


Fig. 3(a-h): Continue

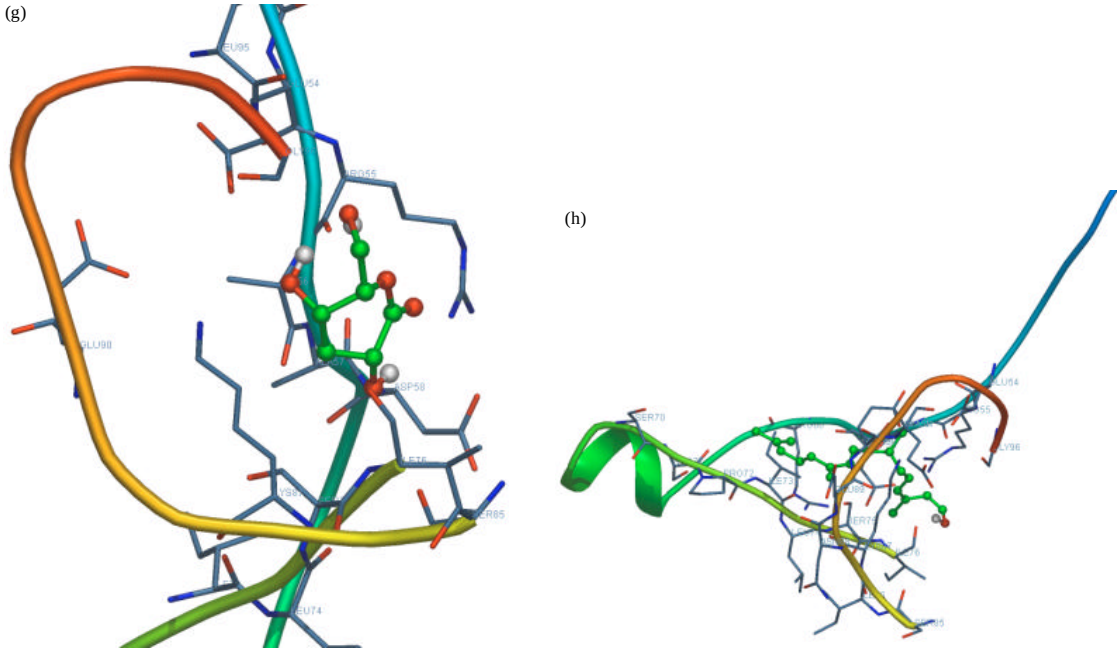


Fig. 3(a-h): NS2+NS3 complex protein docked with following ligand (a) 4-Hydroxybenzyl alcohol, (b) 9-Octa decayne bis(Pentafluoropropionate), (c) L-Arabinitol, (d) Citronyll butyrate, (e) 2-Methoxy-4-vinylphenol, (f) 9, 12, 15-Octadecatrienoic acid, (Z, Z, Z), (g) N-Aminomorpholine and (h) Phytol

Table 5: ADME analysis values

Ligands/test	Aqueous solubility	BBB+	CYP450	AMES toxicity	Carcinogens
N-Aminomorpholine	-0.6090	0.9873	0.9148	0.5348^{-}	0.8709^{-}
Citronellyl butyrate	-3.8244	0.9371	0.8819	0.9281^{+}	0.5692^{+}
4-Hydroxybenzyl alcohol, bis(pentafluoropropionate)	-3.3436	0.9821	0.8627	0.8107^{-}	0.7691^{-}
2-Methoxy-4-vinylphenol	-1.9439	0.8480	0.7598	0.9132^{-}	0.8519^{-}
9, 12, 15-octadecatrienoic acid (Z, Z, Z)	-3.0676	0.9314	0.7735	0.9132^{-}	0.6502^{-}
L-Arabinitol	-0.5593	0.6671	0.8802	0.8869^{-}	0.7894^{-}
Phytol	-2.4720	0.9375	0.7910	0.9132^{-}	0.5055^{-}
9-octadecyne	-4.5010	0.9761	0.8415	0.9929^{-}	0.6452^{+}

DISCUSSION

Eight naturally available compounds were critically evaluated against target and for drug likeliness activity using different parameters. Analysis shows that all compounds are suitable for the drug Lipinski Rule Five, but out of 8 and 3 compound such as 2-Methoxy-4-vinylphenol, 9,12,15-Octadecatrienoic acid, (Z,Z,Z) and 9 octadecyne were strictly qualifies the parameters which can refer the compound as a drug molecule.

There are number of recent high quality research reporting drug designing in dengue. Most are relevant and sharing the knowledge about the *Flavivirus* and the cell infection mechanism but yet except some of the leading pharma industries no plant based antiviral drug development is initiated. In the present day, there are no drugs against dengue as far as stated (Halstead, 2008; Gubler *et al*., 2007; Noble *et al*., 2010).

DENV having the Non-structural protein 3 (NS3) is responsible for proteolysis of the dengue viral RNA polyprotein as well as carrying out various enzymatic reactions that are mandatory for replication of the dengue virus. In addition, NS5 can stimulate the NTPase activity of NS3 which is necessary for unwinding of dsRNA substrates by helicase activity during viral replication. Arakaki *et al.* (2002) and Salonen *et al.* (2005) hence, the different non-structural dengue proteins were selected as a target which always plays a role in viral replication. Therefore, *C. papaya* was selected for evaluation of its natural antiviral active compounds. Previous studies stated the medicinal properties of the plant against dengue at pathological level but without scientific evidence. Since, the ancient times, *C. papaya* recommended for viral infection, wound healing etc. Hence, this *in silico* attempt was made to elucidate the mechanism and to develop a hypothesis explaining inhibitory activity of compounds available in *C. papaya* against virus at cellular level (Saklani and Kutty, 2008, Ayoola and Adeyeye, 2010; Dawkins *et al.*, 2003; Cowan, 1999).

Current docking results confirms that isolated compounds having medicinal properties and can bind more effectively with the respective targets in dengue virus type 2. Among the 8 compounds 3 show promising result against the NS3 and NS5 proteins. 2-Methoxy-4-vinylphenol, 9, 12, 15-Octadecatrienoic acid, (Z,Z,Z) interact with pockets available in target proteins. Both the compounds interact mainly with RNA dependent polymerase which directly inhibits the viral replication. This would open the doors for new drug discovery and drug development in the field of dengue. The proteomics based studies are important in targeted drug delivery system hence this study reveals the docking part after the complete proteomics of targeted proteins of dengue virus.

Dengue has emerged as a most important viral infection in the 21st century. The complexity of dengue infection and the difficulties in vaccine design has prompted us to study the role of antiviral compounds in *C. papaya*. The crude extract of the plant has been used in treatment of dengue however, without enough scientific evidence. This study will throw some light on the antiviral role of the compounds present in the extract which will play an important role in drug development.

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