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IN-SILICO DOCKING OF BIOACTIVE COMPOUNDS DERIVED FROM *CASSIA AURICULATA* FLOWER EXTRACT AGAINST NS2B-NS3 PROTEASE OF DENGUE VIRUS

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Keywords:

NS2B-NS3 Protease, Dengue, Plant compounds, Docking, Receptor and Ligand

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ABSTRACT: Dengue is a fatal disease connected to the environment that spreads rapidly. As there is currently no effective vaccine against dengue and no exact treatment for the disease, controlling and preventing dengue fever outbreaks are essential steps for keeping people healthy. Dengue viruses are a member of the Flaviviridae family, mosquito-borne, and causative agents for dengue fever. Bioactive compounds obtained from natural products, including medicinal plants, are a potentially good target to control infections. Thus the current study was designed to find potential phytochemical inhibitors against the Dengue virus NS2B/NS3 protease, which can inhibit the viral replication and control the dengue vector *Aedes aegypti*. Larvicidal, functional group analysis (FTIR), and molecular docking studies results revealed that the bioactive compounds from the medicinal plant flower extract *Cassia auriculata* showed high inhibitory activity against the dengue protease enzyme NS2B-NS3 and *Aedes aegypti* vector. Out of fifteen compounds identified from GCMS, five compounds showed high inhibitory activity against the protease 2FOM in the docking analysis. The main antiviral approach of plant compounds comprises the inhibition of the dengue viral enzyme activity; therefore, controlling virus replication by these antiviral compounds were studied by computational tools. These findings conclude that the combination of selected compounds may serve as antiviral drugs for dengue infections.

INTRODUCTION: Dengue virus is considered an important mosquito-borne virus worldwide and possesses health care issue nowadays. Dengue infection become a serious health concern globally due to the high mortality rate and has no specific treatment, so the improvements of novel antiviral agents are now very essential. Globalization the increasing volume of trade and travel continuing growth, development, and climate change have contributed to the introduction of the Aedes genus mosquitoes and 2.5 billion people at risk worldwide. Dengue infections are caused by four closely related serotypes named DENV1, DENV2, DENV3, and DENV4 (DENgue Virus).

Similarly, the present investigation also deals with GC-MS and docking analysis of *Cassia auriculata* flower methanol extract derived compounds act as ligand molecule against the target DENV protease. Ligand binding is the primary step in the inhibition of enzymatic target, which is the basis for molecular docking. Since the natural drugs from plant sources have proven as significant with fewer side effects, based on this, the docking analysis was

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performed. Molecular docking approaches are generally used in the modern drug design process to understand the protein-ligand interactions [1].

Natural products from medicinal plants have been widely used in ancient times in India to control pests, vectors, and pathogens and to treat various disorders, including infectious diseases [2]. Due to vector-borne diseases and severe problems caused by mosquitoes, controlling them has become the main focus of various studies over the past few decades. Among the vectors, the *Aedes aegypti* carries the dengue virus that comes under the Flaviviridae family. Mosquito control is one of the most important protective methods to free from dengue.

Controlling mosquitoes in the larval stage is the ultimate target for insecticides because it is the stage of mosquito development, in which they mature, breed in water, and it is easy to deal with them in their habitat. Despite extensive research, there are currently no vaccines existing. DENV has five basic types of non-structural proteins (NS1, NS2, NS3, NS4, and NS5) along with subtypes are present which are associated with viral replication. These non-structural proteins from the replication complex which comprises the NS3 protease with its NS2B as a cofactor and serve as possible inhibitory target for antiviral agents. It is a primary target for discovering and developing dengue antiviral drugs; meanwhile, the NS2B/NS3B protease is required for virus replication and act as protease inhibitors. The population of dengue vectors that are responsible for the increase in viral deaths due to dengue is growing rapidly [3].

Many studies have reported that the medicinal plant extracts significantly inhibit the replication of various viruses and also effective against mosquito larvae. Phytoextracts are standard herbal preparations consisting of complex mixtures of one or more plants that have active components of plant parts; further they exhibit characteristic features that are included in the inhibition of mosquito growth and reproductive development. The phytochemical flavonoids played an important role in the cure of Dengue virus infections [4]. The leaf extract of ginger and turmeric could serve as a potential larvicidal agent against the *Ae. aegypti* larvae, the dengue vector [5].

The plant *Cassia auriculata* is commonly known as avaram, a shrub that belongs to the *Caesalpiniaceae* family. It is dispersed throughout India's hot deciduous forests and widely used in traditional medicine [6], especially for rheumatism, conjunctivitis, and diabetes, and it also has many medicinal properties. Bioinformatics tools are majorly facilitating *in-silico* drug design and discovery. Molecular docking is one of the widely used computational techniques in biomedical research to find the binding interactions of small molecules against their target receptor proteins.

This technique provides a clear molecular vision of viral genes and the identification of novel inhibitory compounds against fatal viral infections. The high-throughput techniques such as crystallo-graphy and nuclear magnetic resonance methods have been greatly used to getting off the atomic structures of proteins at an increasing level. Once the 3D structure of the target, even from experiments or computing, occurs, a frequently used technique to design inhibitor molecules is Computer-Aided Drug Design (CADD) or Structure-Based Drug Design (SBDD). The most widely used method in SBDD is molecular docking.

The molecular docking of grayanotoxin compound with the encephalitis virus had been reported for their antiviral properties [7]. Hex is an interactive protein docking and molecular superimposition program [8]. Docking studies have been carried out to find the inhibitory activity of the compounds obtained from GC-MS analysis of methanol extract of the *Carica papaya* showed the best docking result for 5 compounds based on the binding energy [9]. In-silico approach of dengue vaccine candidate studies indicates that the vaccine is stable, antigenic, properly folded with proper binding to a broad cross-neutralizing murine monoclonal antibody against all DENV serotypes [10].

MATERIALS AND METHODS:

Plant Material Collection and Extract Preparation: The studied plant, *Cassia auriculata* flowers, were freshly collected from Viswanatham, Sivakasi, Viudhunagar District in the state of Tamil Nadu, India. The taxonomical description and botanical name of the plant were authentically identified by Dr. V. Ganesan, Taxonomist from PG

and Research Department of Botany, Ayya Nadar Janaki Ammal College, Sivakasi, and also identified with voucher specimen. The flowers were washed and rinsed thrice with double distilled water to remove the dirt, and other contaminants, then shade dried at room temperature to remove the moisture. The dried form of *Cassia auriculata* flower was ground to a fine powder and extracted twice with 95% methanol by the Soxhlet apparatus. The extracts were concentrated under reduced pressure in the rotary vacuum evaporator until the complete solvents evaporated at (>450 °C) to get a semisolid mass of crude extracts and then use freeze-dried (-800 °C) to obtain a solid residue.

Larvicidal Bioassay: The larvicidal activity was done as per the guidelines of the World Health Organization [11]. To 150 ml of water taken in beaker, appropriate volume of 1% stock solution of the *C. auriculata* flower methanol extract fraction was added and mixed to obtain different concentrations. To each concentration, 25 third instar larvae were released and provided with larval feed. Two controls (one with 150 ml water alone and the other with 150 ml of water containing the maximum volume of Me OH in the test sample) were maintained. Considering the percentage mortality of the larvae after 24 and 48 h in different concentrations (10, 20, 30, 40 & 50 ppm), Lc50 of the test fractions were calculated by using Probit analysis, IBM SPSS Statistics 23 software.

Fourier Transform Infrared Spectroscopy (FTIR): For FTIR Measurements *Cassia auriculata* flower was washed three times with 20 ml of deionized water to get rid of the free proteins/enzymes. FTIR spectra were obtained from KBr pellets prepared using 1.0 mg of powdered *Cassia auriculata* flower samples. The powdered sample was loaded in FTIR spectroscope [12] (Shimadzu, Japan), with a Scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

Gas Chromatography and Mass Spectroscopy (GC-MS) Analysis; The GC-MS analysis of the phytochemical present in the studied plant flower extract was carried out by using a GCMS-QP2010 Ultra chromatograph fitted with Rtx®-5MS (30m × 0.25mm I.D. df = 0.25 μm, Restek Corporation) and interfaced with a mass spectrometer 5975 °C (Agilent technologies). About 1 μl of the ethanol extract was injected into the GC-MS using a microsyringe. Helium gas was used as a carrier as well as eluent. Injector temperature was 250 °C; interface heating was 300 °C; ion source heating: 200 °C, EI mode; scan range was 40-600 amu. The identification of individual compounds was done using the Wiley/ NBS Registry of mass spectral database [13], the NIST 3.0 database.

Drug Scan Study: Based on Lipinski's rule of five, a drug scan was carried out to find whether the final selected ligand compounds have the pharmacological properties that act as drug candidates [14]. The structural details and the smiles notation of the selected five compounds were retrieved from Pub Chem / Drug bank database. The structures were determined by the online Smiles Translator tool. The Smiles notation of all the compounds was obtained from Drug bank, and Pub Chem was subjected to an online Smiles Translator tool to generate PDB and energy minimized 3D structure file. After building the structures of bioactive compounds as ligands successfully, geometry optimization and energy minimization were completed.

Docking Analysis: The Energy minimized 3D structures of bioactive compounds identified from GCMS analysis of *Cassia auriculata* were docked with NS2B-NS3 Protease enzyme using HEX docking software. This software tool gives the active conformation of ligand and the binding sites of selected compounds with the active site of the target protein 2FOM. The structure of the target protein 2FOM (Protein Data Bank ID) NS2B-NS3 Protease enzyme was obtained from Protein Data Bank. This enzyme used as a receptor, and the five natural compounds derived from *C. auriculata* flower methanol extract acted as ligand molecule for docking. The interaction of these compounds with DENV protease can be determined via the docking studies by calculating their energy minimization value. Before docking, experiments were carried out once all the water molecules were removed.

Binding Site Prediction: The ligand-binding sites were analyzed using Q Site Finder, which locates energetically favorable binding sites of a receptor protein.

The binding site of amino acid residues for the dengue protease 2FOM receptor protein was predicted using Q Site Finder [15].

Pharmacological Properties Prediction Using ADMET Tools: The pharmacokinetic properties such as Absorption, Distribution, Metabolism, Excretion and the Toxicity of the best compounds identified from the docking score were predicted using the ADMET-SAR database.

The molecular structure of the best ligand was submitted to ADMET-SAR to examine their drug likeliness, different pharmacokinetic and pharma-codynamic parameters [16].

RESULTS AND DISCUSSION: The studied plant, *Cassia auriculata* flower aqueous extract, possesses some phytochemical properties such as terpenoids, tannins, flavonoids, saponins, and steroids, which were analyzed by preliminary tests and could be responsible for the larvicidal activities determined. The entire larvae bioassay test with *Cassia auriculata* flower methanol extract showed a significant increase in the mortality rate with the increase of concentration. In this study, the percent mortality of *Aedes aegypti* larvae after 24 h and 48 h treatment of flower extract showed 96% and 100 % respectively at a concentration of 50 ppm **Table 1**. The results also showed that the highest larvicidal activity against the third instar *Aedes aegypti* larvae with LC_{50} and LC_{90} values are being 8 ppm and 41 ppm at 24 h and 7 ppm and 28 ppm at 48 h, respectively. Similarly, the LC_{50} and Lc_{90} values for Amaltas (*Cassia fistula*) leave extract against 3rd instar larvae of Culex spp. after 24 h intervals were 203.492 and 542.804 ppm, respectively [17]. The previous studies reported that the larvicidal activity of *C. lanceolatus* fraction observed with the presence of insecticidal compounds such as C-methylated flavones [18]. The active compounds tested against *Cx. quinquefasciatus* and An. stephensi inhibited

TABLE 1: LARVICIDAL EFFICACY OF METHANOL *CASSIA AURICULATA* FLOWER EXTRACT AGAINST THE DENGUE VECTOR *AEDES AEGYPTI* THIRD INSTAR LARVAE

	Percent Mortality (ppm)					Lc50 (ppm)	95% (LCL-UCL)	Lc90 (ppm)	95% (LCL-UCL) Slope ± SE		χ^2 (df=3)	Reg. equation
	10	20	30	40	50							
24h	60	72	84	88	96	8.05	1.853 - 12.782	40.97	28.501-108.37	1.82±.534	0.902*	y = 0.88x + 53.6
48h	68	76	88	96	100	7.17	1.907 - 11.187	27.88	2.435-50.367	2.17±.606	2.57*	y = 0.84x + 60.4

Control-Nil mortality, UCL & LCL - Upper & Lower confidence Limit, X2 - Chi-square value, df - degrees of freedom, *Significant at $P < 0.05$ level.

A greater percentage of the treated larvae from emerging as adults extended their developmental duration and rendered them sterile to a greater extent [19]. The biological activity of this *Cassia auriculata* plant extract may be due to the presence of phytochemical compounds. Such compounds may together or independently contribute to producing toxic activity against the dengue vector species *Aedes aegypti*.

The FTIR spectrum of flower extract of *Cassia auriculata* is given in **Fig. 1**. The data on the peak values and the probable functional groups (obtained by FTIR analysis) present in the extract are presented in **Table 2**. The GC-MS chromatogram of methanol extract of *Cassia auriculata* flower showed prominent peaks indicating the presence of a total 15 phytochemical constituents **Fig. 2**.

TABLE 2: FUNCTIONAL GROUPS OF *CASSIA AURICULATA* FLOWER EXTRACT REVEALED BY FTIR

S. no.	Peak area	Compound	Band
1	837.05	Alkyl halides	C-Cl stretch
2	1070.42, 1116.71, 1157.21& 1230.5	Aliphatic amines	C-N stretch
3	1362.61	Aromatic amines	C-N stretch
4	1453.26	Aromatics	C-C stretch
5	1508.23	Nitro compound	N-O symmetric stretch
6	1613.34	Primary amines	N-H bond
7	2926.78	Alkanes	C-H stretch

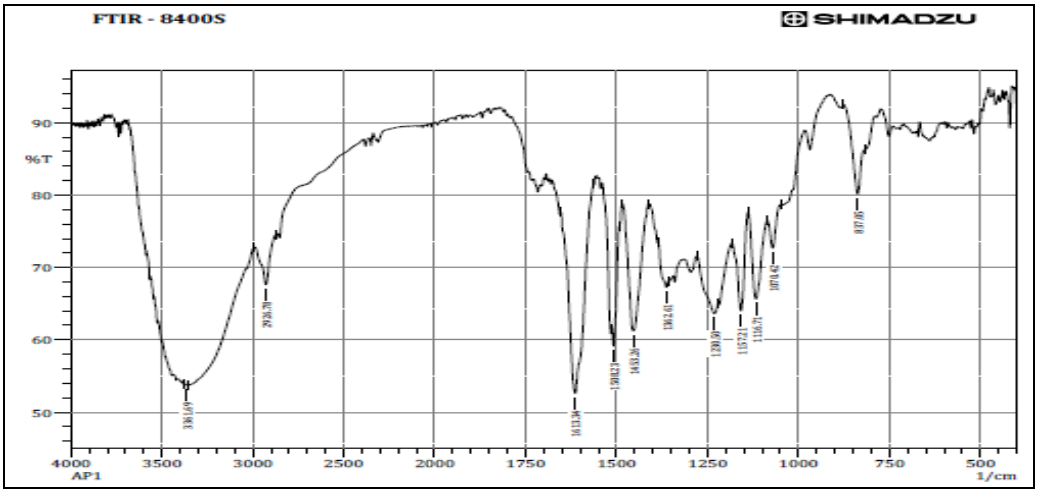

FIG. 1: FTIR SPECTRUM OF *CASSIA AURICULATA* FLOWER EXTRACT

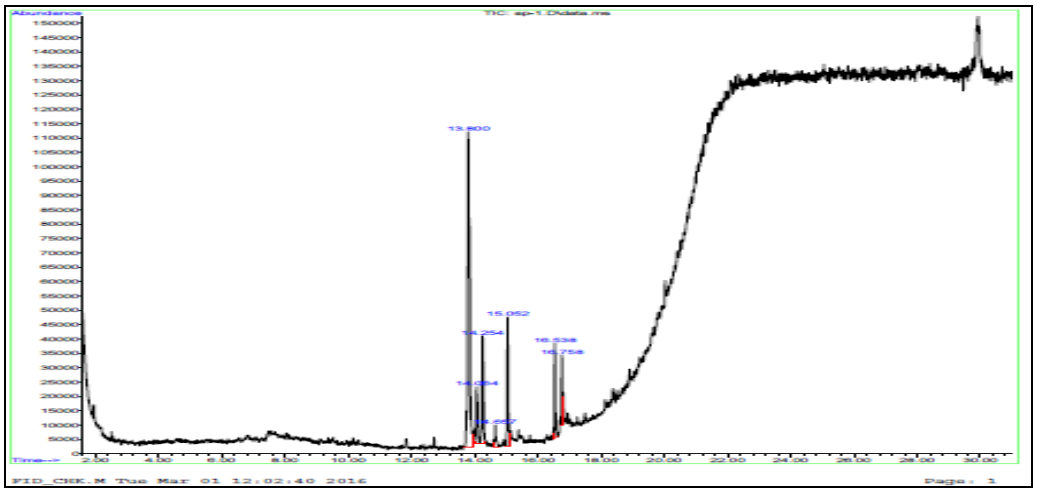

FIG. 2: GCMS CHROMATOGRAM OF THE *CASSIA AURICULATA* FLOWER EXTRACT

Out of the fifteen compounds found in GC-MS analysis **Table 3**, only five compounds were docked with 2FOM, which satisfies the Lipinski's rule of five. Molecular docking study was carried out with the following phytochemical compounds, namely 3, 7, 11, 15- Tetramethyl -2- Hexadecen -1-ol, 2R-Acetoxymethyl -1, 3, 5 -trimethyl, Palmitic acid, Methyl ester 8, 11, 14 –hepta deca trienoate and Bicyclo 3, 1, 1 heptane. The Lipinski's parameters for these compounds were collected from PubChem or Drug bank, and computed properties were outlined in **Table 4**.

TABLE 3: BIOACTIVE COMPOUNDS OF *CASSIA AURICULATA* FLOWER EXTRACT IDENTIFIED BY GCMS

S. no.	Compound name	Molecular formula	Molecular weight (g/mol)
1	11-Tetradecyn-1-ol acetate	$C_{16}H30O_2$	254.4082
2	Oxacycloheptadec-8-en-2-one	$C_{16}H_{28}O_2$	252.3923
3	7-Heptadecyne	$C_{17}H_{31}Cl$	270.88104
4	6-Methyloctahydro coumarin	$C_{10}H_{16}O_2$	168.233
5	3, 7, 11, 15-Tetramethyl-2-hexadecene	$C_{20}H_{40}O$	296.531
6	Bicyclo[3.1.1]heptane-2-thiol- 2, 6, 6-trimethyl	$C_{10}H_{18}$	138.2499
7	1, 2-Dimethyl-4-trifluoroacetoxy…	$C_{10}H_{14}$	134.2182
8	Cyclohexene, 1,2-dimethyl-	C_8H_{14}	110.19676
9	2R-Acetoxymethyl-1, 3, 5-trimethyl	$C_{17}H_{30}O_3$	282.4183
10	n-Hexadecanoic acid	$C_{16}H_{31}O_2$	258.41
11	Levomenthol	$C_{10}H_{20}O$	156.2652
12	Palmitic acid	$C_{16}H_{32}O_2$	256.42
13	7, 10, 13-Hexadecatrienoic acid	$C_{16}H_{26}O_2$	250.376
14	9, 12, 15-Octadecatrien-1-ol…	$C_{18}H_{32}O$	264.446
15	Methyl ester 8,11,14- heptadecatrienoate	$C_{18}H_{30}O_2$	278

TABLE 4: PHARMACOLOGICAL PROPERTIES OF LIGANDS – LIPINSKI RULE OF FIVE

Phytochemical Name	Pubchem/ Drugbank ID	Molecular Formula	Molecular Weight g/mol	XlogP	Hydrogen bond donor	Hydrogen bond acceptor
Bicyclo[3,1,1]heptane 2-thiol- 2,6,6-trimethyl	101742879	$C_{10}H18$	418.64	6.2	1	4
Methyl ester 8, 11, 14 heptadecatrienoate	6433658	$C_{19}H_{32}O_7$	292.46	6.7	1	2
3,7,11,15-Tetramethyl -2-hexadecene	9971827	$C_{20}H_{40}O$	296.53	8.2	1	1
2R-Acetoxymethyl -1,3,5-trimethyl	550401	$C_{17}H_{30}O_3$	282.42	3.8	1	5
Palmitic Acid	985	$C_{16}H_{32}O_2$	256.42	6.4	1	2

Hex is a docking tool for predicting receptor-ligand binding modes and for virtual screening. The 3D X-Ray diffraction structure of Dengue virus NS2B – NS3 protease (2FOM) was retrieved from the Protein Data Bank, which acts as a receptor. Their binding sites were predicted using Q Site Finder **Fig. 3**. The predicted amino acid binding sites of the receptor are given in **Table 5**.

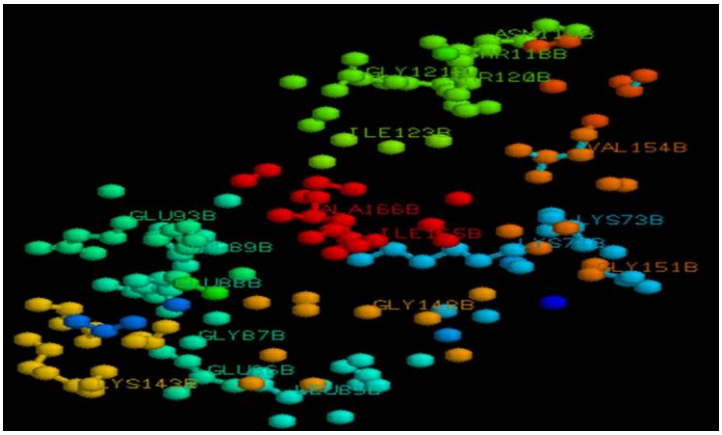

FIG. 3: POTENTIAL LIGAND BINDING SITES OF NS2B&NS3 PROTEASE

TABLE 5: LIGAND BINDING SITES OF 2FOM

Residue	Molecular Atom ID
ILE	123,165
ASN	119,152, 167
VAL	72,95,146,147,154,155
ALA	164, 166
THR	118, 120,122, 156
LYS	73, 74, 84, 117, 142, 143
GLY	87, 92,121, 124, 148, 151, 153
LEU	76, 85, 149
ASP	71, 75, 141
TRP	49, 69, 83, 89
GLU	66,86, 88, 93, 94
MET	59
GLY	110

The structure of the dengue receptor protease (2FOM) has in total 2 chains and the Fasta format of the sequence is shown in **Fig. 4**:

>2FOM:A|PDBID|CHAIN|SEQUENCEGSHMLE
ADLELERAADVRWEEQAEISGSSPILSITISED
GSMSIKNEEEEQTLGGGGSGGGG>2FOM:B|P
DBID|CHAIN|SEQUENCEAGVLWDVPSPPPVG
KAELEDGAYRIKQKGILGYSQIGAGVYKEGT
FHTMWHVTRGAVLMHKGKRIEPSWADVKK
DLISYGGGWKLEGEWKEGEEVQVLALEPGK
NPRAVQTKPGLFKTNTGTIGAVSLDFSPGTSG
SPIVDKKGKVVGLYGNGVVTRSGAYVSAIAN
TEKSIEDNPEIEDDIFRK

The 3D structure of receptor protein (PDB ID: 2FOM) in PDB format has been uploaded in the Hex docking tool. The GCMS identified compounds 3D structure in PDB format was downloaded from Pub Chem / Drug bank and also uploaded in the Hex tool as ligand files. In this study, protein-ligand interactions and their docking scores were determined for the five phytochemicals present in the *Cassia auriculata* with 2FOM receptor using an automated docking model. The final docked structure is completely energy minimized with the lowest energy conformation. The molecules with the lowest energy minimum will have the stable conformation and result in drug stability. From these results, the molecule showing their respective binding energies were evaluated

based on the binding affinity [docked energy (kcal/mol)] with the receptor. Among the five selected phytochemicals, the bioactive compound methyl ester scored the highest drug-likeness character. The conformations of the various ligands with the receptor and their binding energy values were calculated. The docked poses of the molecules were represented in **Fig. 5**.

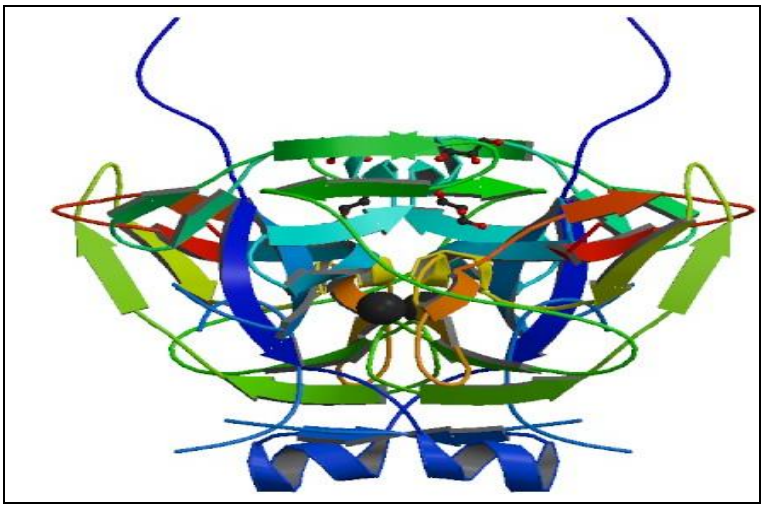

FIG. 4: THE STRUCTURE OF RECEPTOR PROTEIN NS2B-NS3 PROTEASE (2FOM) OF DENGUE

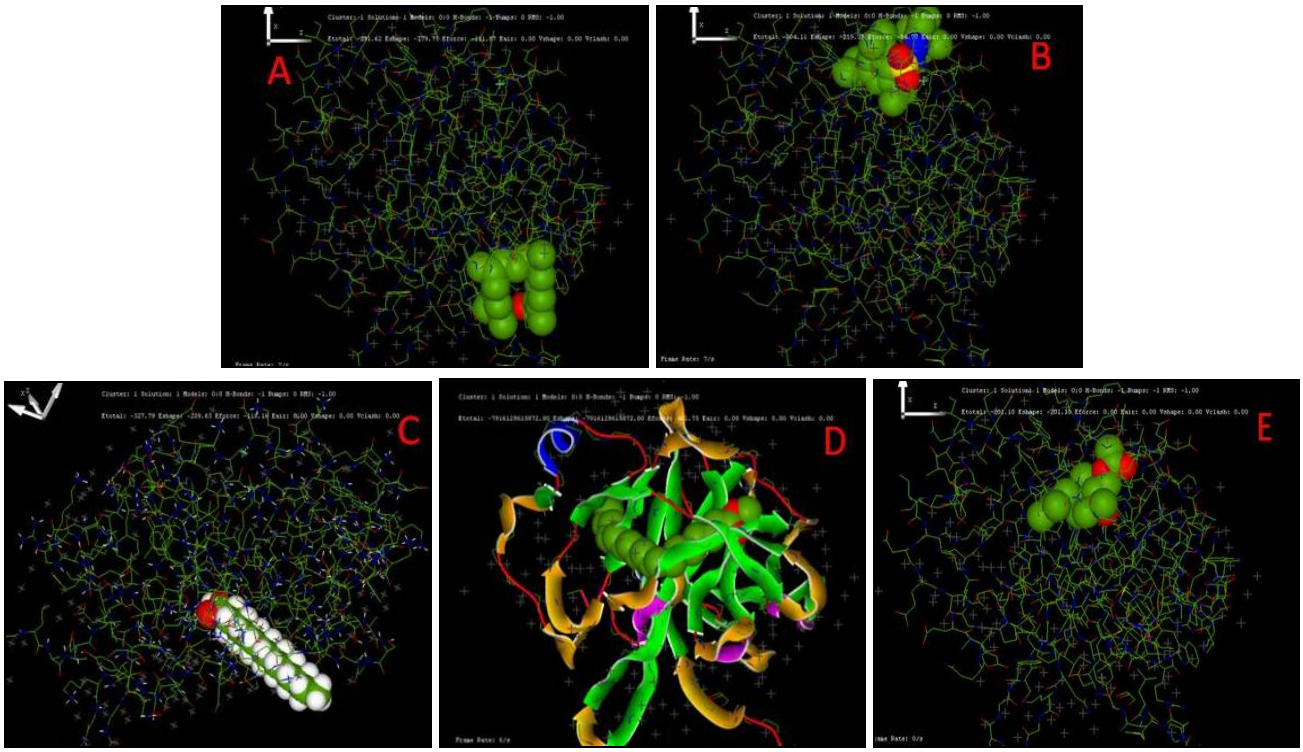

FIG. 5: MOLECULAR DOCKING OF NS2B-NS3 PROTEASE RECEPTOR WITH NATURAL ACTIVE LIGANDS (SPACE FILL MODEL)

A. 2R-Acetoxymethyl-1, 3, 5-trimethyl; B. 3, 7, 11, 15-Tetramethyl-2-hexadece; C. Bicyclo [3.1.1]heptane; D. Palmitic Acid; E. Methyl ester 8, 11, 14-heptadecatrienoate: From the analysis of docking energy, it was found that the compound methyl ester has low energy minimization (E-min -792.00) and maximization values (E-max -677.00) reported in **Table 6**. Similar studies have been carried out with the ethyl acetate extract of *T. purpurea* root GC-MS analyze compound, deguelin showed its predicted E-min value is -305.9 by docking [8]. Likewise, the docking study of NS3-NS2B chimera Canthin-6-one 9-O-beta-gluco- pyranoside, Kushenol W and Kushenol K have been observed with a binding score of −12.26, −11.68 and −11.30 kcal/mol, respectively

[20]. In the current study, the best conformation was selected upon the least binding energy; explicitly the methyl ester showed the best interaction with the target NS2B- NS3 protease. ADMET profile for the methyl ester ligand was evaluated using the ADMET-SAR database, which was predicted as the best ligand based on their lowest energy conformation **Table 7**. The results indicated that it's non-carcinogenic and biodegradable, and hence it is predicted to be fit for drug development and for human consumption also.

ADMET profiling of this potential compound shows that it has no side effects on absorption. Docking to different ligands confirmations is due to a change of a receptor-specific conformation. Therefore, the prediction of these selected compounds from the docking study would help to screen the compound against dengue virus and other flavivirus family and also this drug may use to inhibit dengue virus replication. As a result of virtual screening, methyl ester was found to be a good analog.

TABLE 6: ENERGY MINIMIZATION & ENERGY MAXIMIZATION SCORES OF DOCKING

GC-MS derived ligands	Energy minimization	Energy maximization
2R-Acetoxymethyl-1,3,5-trimethyl	-199.90	-142.30
3,7,11,15-Tetramethyl-2-hexadece	-198.28	-146.44
Bicyclo[3.1.1] Heptanes	-233.69	-152.23
Palmitic Acid	-209.63	-182.59
Methyl ester 8, 11, 14 heptadecatrienoate	-792.00	-677.00

TABLE 7: ADMET PREDICTED PROFILE OF METHYL ESTER LIGAND

Model	**Result**	**Probability**
Absorption		
Blood-Brain Barrier	BBB+	0.978
Human Intestinal Absorption	HIA+	0.9904
P-glycoprotein Substrate	Non-substrate	0.6904
P-glycoprotein Inhibitor	Non-inhibitor	0.7569
Renal Organic Cation Transporter	Non-inhibitor	0.8832
Distribution		
Subcellular localization	Plasma membrane	0.5877
Metabolism		
CYP450 2C9 Substrate	Non-substrate	0.8437
CYP450 2D6 Substrate	Non-substrate	0.8946
CYP450 3A4 Substrate	Non-substrate	0.6235
CYP450 1A2 Inhibitor	Non-inhibitor	0.5574
CYP450 2C9 Inhibitor	Non-inhibitor	0.9432
CYP450 2D6 Inhibitor	Non-inhibitor	0.9519
Excretion & Toxicity		
Human Ethr-a-go-go-Related GeneInhibition	Weak inhibitor	0.8742
	Non-inhibitor	0.9112
AMES Toxicity	Non AMES toxic	0.8828
Biodegradation	**Ready biodegradable**	0.7277
Acute Oral Toxicity	III	0.7281
Carcinogenicity (Three-class)	Non-required	0.7586
ADMET Predicted Profile --- Regression		
Model	**Value**	**Unit**
Absorption		
Aqueous solubility	-3.4604	LogS
Caco-2 Permeability	1.1601	LogPapp,
Distribution, Metabolism, Excretion & Toxicity		
Rat Acute Toxicity	1.6333	LD50, mol/kg
Fish Toxicity	0.7919	pLc50, mg/L
Tetrahymena Pyriformis Toxicity	0.895	pIGC50, ug/L

CONCLUSION: In Asia, *Aedes aegypti* is the main vector of dengue. It is geographically spread in both tropical and subtropical areas. This mosquito survives 26-30 °C in optimum and humidity of 70-80%. Dengue transmission occurs through the bites of mosquito that contains the

virus. The infected mosquito will contain a virus for its whole life, and it can transmit virus [21]. Biological active compounds have been used as eco-friendly vector management, and botanical pesticides are cheap and easy to control against dengue vector. Flavonoids present in medicinal plants hold anti-viral activity and can be used as a vaccine against viruses [22]. For the past few years, the binding study of phytocompounds through computational techniques before their lab manufacturing and investigation has become a common and valuable practice among researchers. The molecular docking approach can be used to model the interaction between a small molecule and a receptor at the atomic level, which allows us to characterize the behavior of small molecules in the binding site of target proteins.

The computer-aided development of a series of drug-like low molecular weight compound is capable of inhibiting the helicase enzyme of GGE virus. The results derived from a repertoire of multi-disciplinary bioinformatics and statistical methods would enhance the understanding of the mechanism of action of the GGE viral helicase enzyme [23]. Recently, phytochemical-based inhibitors and vaccine candidates against specific nonstructural Dengue viral proteins have been reported, but none of them has been proved effective against heterotypic dengue infections [24]. From the current study, the authors concluded that methyl ester will be a potential inhibitor and have effective interactions in the binding site of NS2B-NS3 protease (2FOM). ADMET based drug scan tool predicted the drug-likeness of the proposed ligand of the dengue vector inhibitor. It analyses the compounds based on four parameters as Absorption, Distribution, Metabolism and Excretion [25].

The results of the ADMET properties study found that methyl ester could act as a drug molecule. This study will help to understand how the bioactive compounds could be used as lead molecules for the treatment of dengue by inhibiting NS2B/NS3 protease. Therefore, the prediction of these selected drugs from the Docking study will serve the public for dengue treatment. The identified ligands have good pharmacokinetic properties and biodegradable which fulfill the parameter of Lipinski's rule. Extensive researches have been performed to develop vaccines against this dengue infection by various researchers but these efforts have not proven successful till today. There is a need to design new approaches to fight this viral infection. Using plants to develop a drug in this way offers many advantages like higher expression, lower production cost, easier distribution, and better safety as there are no animal or human pathogens, which increases the biosafety aspect.

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