World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 5.008

IN SILICO STUDIES ON DENGUE AND MEASLES VIRAL PROTEINS WITH SELECTED METHANOLIC EXTRACTS OF *CORIANDER SATIVUM* LEAVES CONSTITUENTS

[1]Aniruddha B. S., [1]Ashwini B. M., [1]Mahesh K., [1]Nagarjun N. and *[2]Dr. Balasubramanian Sathyamurthy

[1]Department of Biochemistry, Ramaiah College of Arts, Science and Commerce, Bangalore – 560054.
[2]Professor, Department of Biochemistry, Ramaiah College of Arts, Science and Commerce, Bangalore – 560054.

***Corresponding Author: Dr. Balasubramanian Sathyamurthy**
Professor, Department of Biochemistry, Ramaiah College of Arts, Science and Commerce, Bangalore – 560054.

Article Received on 18/10/2018 **Article Revised on 09/11/2018** **Article Accepted on 30/11/2018**

ABSTRACT

Coriander (*Coriandrum sativum L.*) has been reported to have many medicinal properties. Its plant, seeds, leaves and roots are edible, although they have very distinct flavours and uses. Coriander is also well known for its antioxidant, anti-diabetic, anti-mutagenic, antianxiety and antimicrobial activity along with analgesic and hormone balancing effect. Phytochemicals present in *Coriander Sativum* are also found to have and antifungal properties. In this study, the binding efficiency of 4 compounds that is present in the *Coriander Sativum* with two structural Dengue virus proteins and four structural measles virus proteins through Insilico methods. By molecular docking result, we found that the compound Squalene and 9, 12 octadecadienoic acid (Z,Z),2-hydroxy,1(hydroxymethy) propyl ester,2,3-dihydroxy propyl ester have highest binding affinity with the chosen viral proteins.

KEYWORDS: Dengue virus, measles virus, molecular docking, hydrogen bonding, binding affinities.

1. INTRODUCTION

Coriander (*Coriandrum sativum* L) is a spice obtained from the plant which belongs to the family Umbelliferae (Apiaceae). Coriander leaves are also known as cilantro. They are used as a herbal flavouring in preparation of sauces and salads.[1] They have been It contains a good amount of vitamins such as vitamin A,C and E.[2] Coriander seed oil is one of the 20 major essential oils in the world market and it is known to exert antimicrobial activity. *C. sativum* is an annual herb, which is native to Mediterranean and Middle Eastern regions. It grows 25–60 cm (9–24 inches) in height, and has thin, spindle-shaped roots, an erect stalk, alternate leaves, and small, pinkish-white flowers. The plant is cultivated for its aromatic leaves and seeds.[3]

GC-MS chromatogram of the methanolic extract of *Coriander sativum* showed four major peaks. 9, 12 octadecadienoic acid (Z, Z),2-hydroxy, 1hydroxymethylethylester,2,3-dihydroxy propyl ester , 9,12 octadecadienoic acid , n-hexadecanoic acid and squalene were the major components in the extract. Squalene is extensively utilized as a principal component of parenteral emulsions for drug and vaccine delivery.[4] It carries oxygen directly to the cell membranes. It is used as a supportive therapy in various types of cancer. It is a triterpene compound. Dietary squalene is absorbed well and transported by chylomicrons into circulation and then it is quickly taken up by the liver, where it is cyclized to sterols and bile acids. Squalene is an intermediate in the biosynthesis of phytosterol or cholesterol in plants or animals.[5] It acts as an anti-inflammatory agent. 9,12-octadecadienoic acid (Z, Z)- shows Hepatoprotective, Antihistaminic, hypocholesterolemic, Anti-eczemic activity, 9,12-octadecadienoic acid (Z, Z)- 2-hydroxy-1-(hydroxymethyl) ethyl ester 2,3-dihydroxy propyl ester shows Hepatoprotective, Antihistaminic, hypocholesterolemic, anti-eczemic, antibacterial, antimicrobial agent and used as antibiotic.[6]

The dengue virus, a member of the genus *Flavivirus* of the family Flaviviridae, is an arthropode-borne virus that includes four different serotypes (DEN-1, DEN-2, DEN-3, and DEN-4).[7,8] These viruses contain ten proteins out of which three are structural proteins and seven are non structural proteins.[9] The seven non structural proteins are capsid protein, envelope protein, NS1 protein, transmembrane domain of NS2A, NS2B/NS3 protease, NS3 helicase and NS5 protein. NS2B-NS3 protease is a crucial enzyme for the viral replication.[10] This is hetero dimeric protein of NS2B and NS3protein. The N-terminal of the NS3 protein forms some associates with the NS2B cofactor. NS2B/ NS3 protease has an important role in the viral life cycle.[11] Envelope protein

is a structural protein which participates in the viral assembly. The protein utilized for the study is the envelope protein domain III of dengue type 4 viruses. It is classified under structural protein immune system. Capsid protein is one of the structural proteins, which takes part in the encapsidation of the viral genome. The capsid protein used for this study was from dengue virus type 2 (strain Puerto Rico/PR159-S1/1969).[12] The protein used for this study was the trans-membrane domain of theNS2A of dengue virus type 2. NS2A is a non structural protein and it is a component of viral replication complex which is functionally active in the assembly of the virion and also it acts as an antagonist to the host immune response.[13] NS3 helicase belongs to the non-structural and a multi-domain dengue virus replication protein.[14] The protein used for this study is the non-structural 5 (NS5) protein from the dengue virus type 3 (strain Sri Lanka / 1266 / 2000). This protein is classified under the transferases. The RNA – dependent – RNA – polymerase domain of the NS5 protein is involved in the replication of the viral genome. RNA is synthesized via "de novo" by NS5 protein.

Measles is a highly contagious disease caused by the measles virus. The disease spreads through air by the aerosols produced from coughing or sneezing. . Measles virus belongs to the morbilivirus genus and *Paramoxyviridae* family. It is a single stranded RNA virus with a negative sense. Measles virus, a negative strand RNA virus, packages its genome into large, helical superstructures formed by the nucleoprotein (N) that assembles on the RNA genome.[18] Measles virus contains five structural proteins which include HA (Hemaglutinin), P(Phospoprotein), N(Nucleocapsid), F (Fusion) and M (Matrix). It has three non-structuralptoteins which include C, V and R protein.[19]

Fusion-enhancing substitutions in the extracellular domain of the MV fusion (F) protein (T461I and S103I/N462S/N465S), which are found in multiple SSPE (Subacute sclerosing panencephalitis) virus isolates, promote MV spread in human neuroblastoma cell lines and brains of suckling hamsters.[15] Hemagglutinin (H) protein is antigenically stable. The H protein is responsible for receptor binding, and is the main target of neutralizing antibodies.[16] The measles virus (MV) nucleoprotein NP associates with the viral RNA genome to form the N-RNA complex, providing a template for viral RNA synthesis.[17] Bioinformatics is an interdisciplinary branch of science which utilizes statistics, computer and mathematics to analyse biological data.[20] Bioinformatics is utilized for many researches to identify many aspects such as evolution. Protein Data Bank (PDB) is a protein storage bioinformatics tool. It contains the structures of large numbers of proteins, ligands and other macromolecules. Docking analysis can be conducted for the protein and the ligand to analyse the fitness and the interaction with each other. This interaction could be used as the pharmaceutical approach for drug production.[21]

3. MATERIALS AND METHODOLOGIES

3.1. Preparation of Dengue and Measles viral proteins

The protein data bank (PDB) was used to obtain the three-dimensional structure of the macromolecule. PDB contains large number of proteins which are experimentally determined and stored in this site. For this study only those proteins of Dengue and Measles virus were taken that are numbered and identified in PDB. The structures are downloaded and saved either in mm CIF or PDB format. Proteins of dengue and measles virus were used for this study. The 3D structure of all the six proteins were downloaded from PDB and saved in PDB format. The downloaded proteins were viewed in Py-Mol viewer.[22,23]

3.2. Preparation of ligands

Ligands selected were from the previous studies on GCMS analysis on *Coriander sativum* leaves extract. 4 ligands were used for the study. Ligands were constructed using ChemSketch. The constructed ligands were optimized to add the hydrogen bonds and the obtained structures were saved in mol for docking analysis and named as A, B ,C and D respectively.

3.3. Docking study

Docking studies were conducting using iGEMDOCK software. IGEMDOCK (Generic Evolutionary Method for molecular Docking) is a graphical-automatic drug design system for docking, screening and post-analysis. The proteins and the ligands were loaded and the out path was set. Standard docking parameters were used for docking (population size=200, generations =70 and Number of solutions =2). The docking process was initiated. After the docking process, the best docking pose for the individual ligands can be obtained for all the two dengue and four measles viral proteins. The best binding pose, the binding affinity and the total binding energy values were saved in the output folder. The saved files were visualized in Py-Mol viewer.

4. RESULTS

4.1. Total Binding Energy (kcal/mol) profile for Dengue and Measles viruses proteins with 4 ligands

Table 1: The Total Binding Energy (kcal/mol) profile for Dengue and Measles viruses structural proteins with 4 ligands.

Ligand	Compound name	Dengue virus		Measles virus			
		Capsid protein	Envelope protein	Nucleoprotein	HA protein	Phospoprotein	Fusion protein
A	9,12 octadecadienoic acid (Z,Z),2-hydroxy, 1hydroxymethylethylester2,3-dihydroxy propyl ester	-92.70	-112.30	-95.60	-94.1	-91.00	-98.75
B	9,12 octadecadienoic acid	-90.90	-78.70	-90.00	-91.20	-79.50	-71.60
C	n-hexadecanoic acid	-82.60	-85.00	-89.40	-89.00	-72.50	-79.06
D	Squalene	-95.20	-84.10	-103.60	-99.60	-82.10	-84.71

4.2. H – bond profile for Dengue and Measles viruses structural proteins with 4 ligands

Table 2: H – Bond profile for Dengue and Measles viruses structural proteins with 4 ligands.

Ligand	Compound name	Dengue virus		Measles virus			
		Capsid protein	Envelope protein	Nucleoprotein	HA protein	Phospoprotein	Fusion protein
A	9,12 octadecadienoic acid (Z,Z),2-hydroxy, 1hydroxymethylethylester2,3-dihydroxy propyl ester	H-M	H-M H-S	H-S	H-S H-M	H-M H-S	H-M H-S
B	9,12 octadecadienoic acid	H-S	-	H-S H-M	H-S	H-S	H-S
C	n-hexadecanoic acid	H-S	H-M	H-S	H-S	H - S H-M	H-M
D	Squalene	-	-	-	-	-	-

4.3. Amino acid position profile for Dengue and Measles viruses protein with 4 ligands

Table 3: Amino acid position profile for Dengue and Measles viruses structural proteins with 4 ligands.

Ligand	Compound name	Dengue virus		Measles virus			
		Capsid protein	Envelope protein	Nucleoprotein	HA protein	Phospoprotein	Fusion protein
A	9,12 octadecadienoic acid (Z,Z),2-hydroxy, 1hydroxymethylethylester2,3-dihydroxy propyl ester	Arg(100)	Arg(629) Lys(625)	Arg(150)	Asp(343) Asp(343)	Gly(39) His(297)	Asp(476) Arg(477)
B	9,12 octadecadienoic acid	Thr(62)	-	Arg(184) Ala(181)	Ser(218) -	Try(497)	Arg (490)
C	n-hexadecanoic acid	Arg(68)	Arg(629)	His(89)	Arg(580)	His(26) Gly(28)	Ala(491)
D	Squalene	-	-	-	-	-	-

5. DISCUSSION

From the above tables, the 3D structure coordinates of two dengue proteins and four measles proteins were optimized and 4 compounds from methanolic extracts of *Coriander Sativum* was identified. Evaluations of binding conformation of these 4 compounds with two dengue as well as four Measles viral proteins are

performed using iGEMDOCK. From docking study, we listed binding affinities of 4 compounds based on ligand binding energy (Table- 1). The binding pose for each ligand molecule into the dengue and Measles viral proteins are analyzed and the one having lowest ligand binding energy with these proteins among the different poses are generated. The lower energy scores represent better protein-ligand target binding affinity compared to higher energy score. In this we took only the structural proteins of dengue and measles virus. Considering their values, we get to know that compound 'D' has lowest binding energy (-92.7kcal/mol) than other analogues towards Dengue Capsid protein. For dengue Envelope protein, compound 'A' has lowest binding energy (-112.3 kcal/mol). For measles virus nucleoprotein, compound D has the lowest binding energy (-103.6 kcal/mol), compound D for Hemagglutinin protein (-99.6 kcal/mol), Compound A for phospoprotein (-91 kcal/mol) and fusion protein (-98.75 kcal/mol).

5.1. Structural proteins of Dengue virus

5.1.1. The Total Binding Energy for Dengue virus Capsid protein with 4 ligands

From Table – 1, 2 and 3 the docking simulation of 4 ligands were performed for Dengue virus Capsid protein. From the docking study, we observed that compound – D has best binding affinity with the target Capsid protein with the binding energy value of -95.2 kcal/mol. Interaction analysis of binding mode of compound –D in dengue virus Capsid protein reveals that it does not show any hydrogen bond interaction . A close-up view of the Total Binding Energy (kcal/mol) profile for Dengue virus Capsid protein with 4 ligands is shown in Fig.1

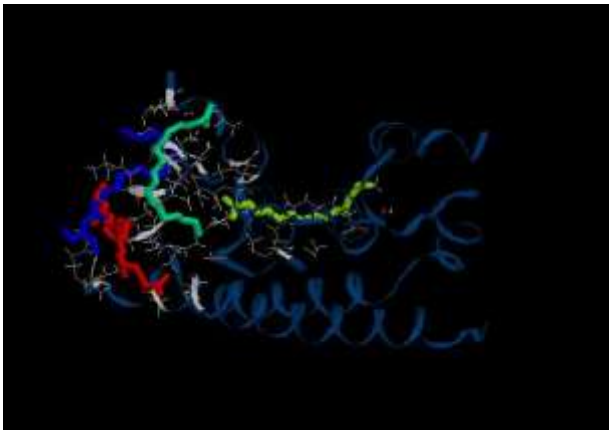

Figure 1: A view of the capsid protein binding energy with 4 ligands.

5.1.2. Total Binding Energy for Dengue virus envelope protein with 4 ligands

From table 1, 2and 3, the docking simulation of 4 ligands were performed for Dengue virus envelope protein. From the docking study, we observed that compound – A has best binding affinity with the target envelope protein with the binding energy value of -112.3 kcal/mol. Interaction analysis of binding mode of compound –A in dengue virus envelope protein reveals that it forms two hydrogen bond with low energy, with Arg(629) and Lys(625) residues. A close-up view of the Total Binding Energy (kcal/mol) profile for Dengue virus envelope protein with 4 ligands: is shown in Figure 2.

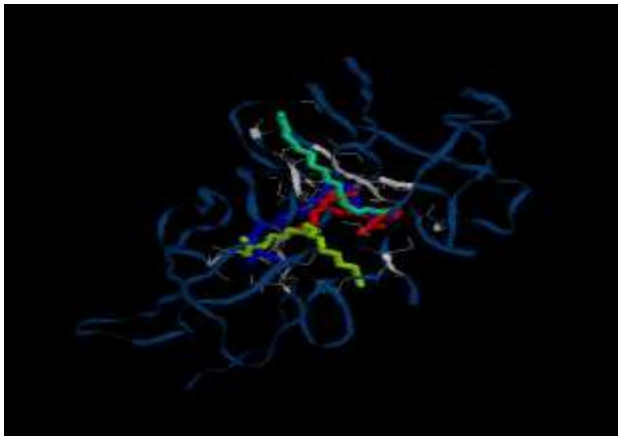

Fig. 2: The Total Binding Energy for Dengue virus envelope protein with 4 ligands.

5.2. Structural proteins of measles virus

5.2.1. The Total Binding Energy for Measles virus Nucleoprotein protein with 4 ligands

From Table 1, 2 and 3, the docking simulation of 4 ligands were performed for Measles virus Nucleoprotein. From the docking study, we observed that compound – C has best binding affinity with the target Nucleoprotein with the binding energy value of -103.4kcal/mol. Interaction analysis of binding mode of compound –C in dengue virus Nucleoprotein reveals that it does not form any hydrogen bond. A close-up view of the Total Binding Energy (kcal/mol) profile for Ebola virus Nucleoprotein with 4 ligands: is shown in Figure 3.

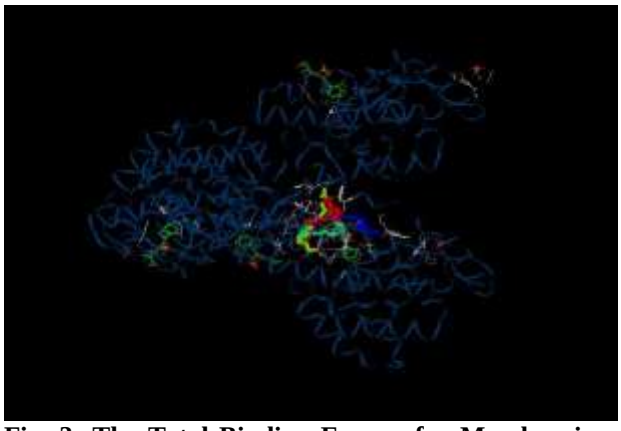

Fig. 3: The Total Binding Energy for Measles virus nucleoprotein protein with 4 ligands.

5.2.2. Total binding energy of Hemagglutinin protein of measles virus

From Table 1, 2 and 3, the docking simulation of 4 ligands were performed for Measles virus Hemaglutinin. From the docking study, we observed that compound – D has best binding affinity with the target Hemaglutinin with the binding energy value of -103.4kcal/mol. Interaction analysis of binding mode of compound –D in

dengue virus Hemaglutinin reveals that it does not form any hydrogen bond . A close-up view of the Total Binding Energy (kcal/mol) profile for Measles virus Hemagglutinin with 4 ligands: is shown in Figure 4.

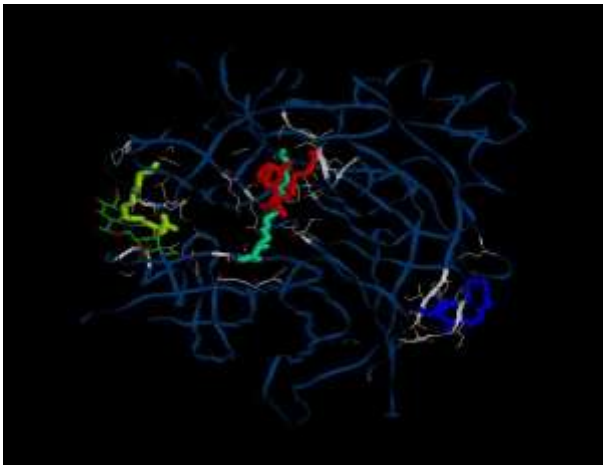

Fig. 4: The Total Binding Energy for Measles virus Hemagglutinin protein with 4 ligands.

5.2.3. Total binding energy for Measles virus of Fusion protein

From Table 1, 2 and 3, the docking simulation of 4 ligands were performed for Measles virus fusion protein. From the docking study, we observed that compound – A has best binding affinity with the target Fusion protein with the binding energy value of -98.75 kcal/mol. Interaction analysis of binding mode of compound –A in dengue virus Fusion protein reveals that it forms two hydrogen bonds with the Gly (39) and His(297) residues. A close-up view of the Total Binding Energy (kcal/mol) profile for Measles virus Fusion protein with 4 ligands is shown in Figure 5.

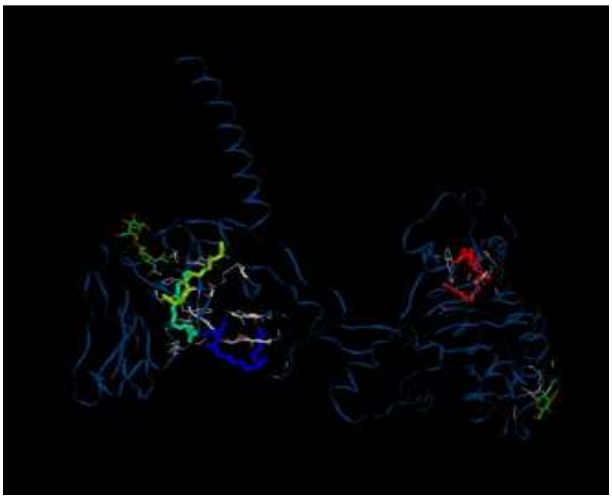

Figure 5: The Total Binding Energy for Measles virus Fusion protein with 4 ligands.

5.2.4. Total binding energy of Phospoprotein for measles virus

From Table 1, 2 and 3, the docking simulation of 4 ligands were performed for Measles virus Phospoprotein. From the docking study, we observed that compound – A has best binding affinity with the targetphospoprotein with the binding energy value of -91 kcal/mol. Interaction analysis of binding mode of compound –A in dengue virus Phospoprotein reveals that it forms two hydrogen bonds with the Asp(476) and Arg(477) residues. A close-up view of the Total Binding Energy (kcal/mol) profile for Measles virus phospoprotein with 4 ligands is shown in Figure 6.

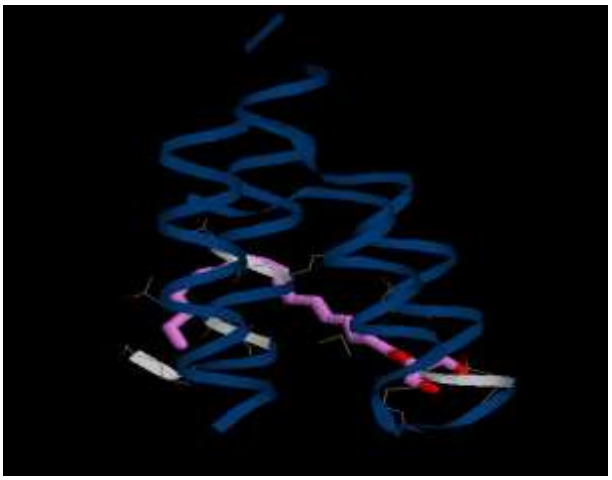

Figure 6: The Total Binding Energy for Measles virus Phospoprotein with 4 ligands.

6. CONCLUSION

Our molecular docking studies explored the binding affinity of 4 ligands of methanolic extract of *coriander sativum* leaf with two dengue viral proteins and four measles viral proteins. These constitute only the structural proteins of both the viruses. Measles virus has nucleoprotein, phospoprotein, hemagglutinin protein and fusion protein. Dengue virus has capsid and envelope protein as the structural proteins. Compounds A (9, 12 octadecadienoic acid (Z, Z), 2-hydroxy, 1hydroxymethylethylester),2,3-dihydroxypropyl ester and D (Squalene) have the best results when compared to other compounds. After comparison of binding energy and amino acid residues, we get to know that all four compounds have different hydrogen bonding and different binding energies. This conclusion is drawn based on screening analysis of the compounds with the viral proteins. Compound A has highest binding energy for dengue viral proteins whereas Compound D has highest binding energy for Measles viral proteins. Hence we conclude that both compound A and D can be considered as the suitable drug targets for dengue and measles viruses.

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