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IN SILICO STUDIES ON DENGUE AND POLIO VIRAL NON STRUCTURAL PROTEINS WITH SELECTED *MENTHA ARVENSIS* LEAVES CONSTITUENTS

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ABSTRACT

Mentha arvensis belongs to family Picornaviridae. The leaves of *Mentha arvensis* are used as herbs in Ayurvedic medicine. The plant has been shown to possess sedative–hypnotic, anti–inflammatory antioxidant, hepatoprotective antibacterial, antifertility and anti-Candida activities properties. The GCMS results showed the presence of 4 compounds in *Mentha arvensis* with a wide variety of biological activities. The comparative study of 5 non-structural proteins for dengue along with 6 non-structural proteins for Polio virus was carried out through In Silico methods. In this study we examined the binding affinities of 4 ligands with 11 proteins of both viruses. By our virtual screening and molecular docking result, we found that the 3, 7, 11, 15-Tetramethyl-2-hexadecen-1- ol had the highest binding affinities with all the 11 proteins and we also predicted the binding site amino acid residues and the type of hydrogen bonding.

KEYWORDS: Molecular docking, Dengue virus, Polio virus, *Mentha arvensis*, Hydrogen bond.

1. INTRODUCTION

Plants are important source of various bioactive compounds which have direct or indirect use in the treatment of various human ailments. From the time immemorial, human civilizations have been exploring and using various plants and plant products to cure the deadly diseases.[1] Thus, the medicinal plants are the "backbone" of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis. The medicinal plants are considered as a rich resources of ingredients which can be used in drug development and synthesis. Besides that these plants play a critical role in the development of human cultures around the whole world.[2] Therefore, due to the importance of oxidative stress in the pathophysiology of most of the hard curable diseases, the use of medicinal plants with antioxidant properties is important and should be considered more than before.[3] *Mentha arvensis* L. (Lamiaceae), commonly known as corn mint, menthol mint or Japanese mint came to India in 1952 from Japan.[4] The plant consist essential oils of monoterpenes like menthol, menthone, carvone and pulegone major constituents [5]. It is one of the most important spice extensively used as flavorings in food, cosmetic and pharmaceuticals throughout the world.[6] Therefore, the leaves of the plant are extensively used for various ailments like jaundice, digestive, diarrhea, cardio tonic, diuretic, inflammation of liver, peptic ulcer, bronchitis and skin diseases.[5]

GC-MS chromatogram of the methanolic extract of *Mentha arvensis* L. showed four highest peaks indicating the presence of 2,Cyclohexene-1-One-Methyl-5-(1-Methylethenyl), Benzaldehyde,2-Hydroxy-6-Methyl, 2-(2-Hydroxy-2-Phenylethyl)-3,5,6,-Trimethylpyrazine and 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol.[5] 2,Cyclohexene-1-0ne-2-Methyl-5-(1-Methylethenyl) is used as fragrance and flavor, potato sprouting inhibitor and antimicrobial agent.Benzaldehyde,2-Hydroxy-6-Methyl acts as pheromone of house dust mite.[7] 3,7,11,15-Tetramethyl-2-hexadecen-1-Ol is used as precursor for manufacture of synthetic form of Vitamin E[8] and Vitamin K.[9]

Dengue is a mosquito-borne systemic viral infection caused by any of the four antigenically related dengue viruses (DENV).[10] There are two well defined manifestations of dengue virus infection in humans, dengue fever and severe dengue (dengue hemorrhagic fever / dengue shock syndrome, DHF/DSS).[11] DENV is a positive-sense, single-stranded RNA virus with ~10.6kb genome.[12] There are seven non-structural proteins. NS1 protein attaches to plasma membrane of cells during infection.[13] NS2A 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.[14] NS2B-NS3 protease is a crucial enzyme for the viral replication .This protein is heterodimeric protein of NS2B and NS3 protein.[15] NS3 helicase is also called as NS3 ATPase,[16] a multi-domain dengue virus replication protein.[17] NS5 protein consists of Methyl Transferase [MTase] and RNA-dependent RNA polymerase [RdRp] domains, which catalyzes 5'– RNA capping/methylation and RNA synthesis, respectively, during viral genome replication.[18]

Poliovirus has a diameter of 25 to 30 nm. Its outer coat or capsid is composed of 60 protomers each made of 4 virion proteins VP1, VP2, VP3, and VP4 arranged in icosahedral symmetry. All the 4 virions are made of 8 strands of protein arranged in β sheet array forming a β barrel. Due to the intermingling of various proteins, loops are created, which serve as antigenic sites for combination with corresponding antibodies. Three serotypes of poliovirus have been recognized as types 1, 2, and 3. The prototype strains are Brunhilde and Mahoney strains for type 1, Lansing and MEFI for type 2, and Leon and Saukett for type 3.[19] Polio virus (PV) is the causal agent of paralytic poliomyelitis, an acute disease of the central nervous system (CNS) resulting in flaccid paralysis.[20] Polio virus belongs to the enterovirus subgroup, family *Picornaviridae*. Thus, the PV genome is composed of a single-stranded RNA copy of positive polarity of about 7.4Kb.[21] There are 7 non-structural proteins. 2A protein (Cysteine protease) that cleaves viral polyprotein and specific host proteins, 2B protein plays an essential role in the virus replication cycle by acting as a viroporin and rearranges intracellular membrane , 2C protein as a role in the nucleotide binding and forms replication complexes, 3A inhibit secretion and interacts with replication membrane, 3B protein needed for viral replication as a primer, 3C protein that cleaves viral polypeptide and 3D protein an RNA dependent RNA polymerase. In addition, non-structural proteins of polio virus having large effects on host intra-cellular membrane structure and function. Thus, the symptoms of polio are fever, fatigue, headache, stiffness in the neck, pain in the limbs and weakness in the limbs.[20]

Bioinformatics has been rapidly growing, keeping pace with the expansion of genome sequence data. Recent technological development of large-scale gene expression analysis using DNA microarrays and proteomics experiments has further boosted the importance of bioinformatics methods. The integration of wet experiments and the use of bioinformatics analyses have become an indispensable part of the biological and clinical research of this century.[22]

Thus, this is due to its role in the development of computers able to determine the peptide sequence, programs to recognize and display structures for use in X-ray crystallography and computational methods for protein sequence comparison, allowing us to infer the evolutionary connections among kingdom.[23]

The aim of our study is to compare the best docking fit for the selected *Mentha arvensis* leaves constituents with the Dengue and Polio virus non-structural proteins.

2. MATERIALS AND METHODOLOGIES

2.1. Preparation of dengue 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. The structures are downloaded and saved either in mm CIF or PDB format. Proteins of dengue virus were used for this study. The 3D structure of all the five proteins were downloaded from PDB and saved in PDB format. The downloaded proteins were viewed in Py-Mol viewer.[24]

2.2. Preparation of ligands

Ligands selected were from the previous studies on GCMS analysis on *Mentha arvensis* leaves extract.[5] 4 ligands were used for the study. Ligands were constructed using ChemSketch.[24] 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.

2.3. 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.[16] 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 five dengue 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.[25]

3. RESULTS

3.1. Total Binding Energy (kcal/mol) profile for Dengue and Polio virus non- structural proteins with 4 ligands.

Table 1: The Total Binding Energy (kcal/mol) profile for Dengue and Polio virus non- structural proteins with 4 ligands.

Ligands	Compound Name	Dengue Virus					Polio Virus					
		NS1	Trans membrane domain of NS2A	NS2B/NS3 protease	NS3 helicase	NS5 protein	2A	2C	3A	3B	3C	3D
A	2,Cyclohexene,1,One-2-Methyl-5-(1-Methylethenyl)	-66.8	-56.1	-48.6	-55.9	-58.2	-72.8	-63.2	-557.12	-281.1	-56.3	-64.0
B	Benzaldehyde,2-Hydroxy-6-Methyl-	-66.6	-55.7	-59.4	-62.6	-65.6	-74.2	-64.1	-580.65	-311.1	-69.2	-67.6
C	2-(2-Hydroxy-2-Phenylethyl)-3,5,6-Trimethylpyrazine	-74.8	-71.4	-74.3	-72.5	-76.8	-91.8	-80.2	-604.38	-442.0	-83.9	-82.1
D	3,7,11,15-Tetramethyl-2-Hexadecen-1- ol	-75.7	-81.9	-86.2	-91.7	-96.6	-102.4	-85.9	-728.57	-453.7	-76.2	-89.7

3.2. H – Bond profile for Dengue and Polio virus non- structural proteins with 4 ligands.

Table – 2: The Total Binding Energy (kcal/mol) profile for Dengue and Polio virus non-structural proteins with 4 ligands.

Ligands	Compound Name	Dengue Virus					Polio Virus					
		NS1	Trans membrane domain of NS2A	NS2B/NS3 protease	NS3 helicase	NS5 protein	2A	2C	3A	3B	3C	3D
A	2,Cyclohexene,1,One-2-Methyl-5-(1-Methylethenyl)	H-M	H-S H-M	H-S	H-S H-M	H-S	H-M	H-M	H-M	H-S	H-S H-M	H-S H-M
B	Benzaldehyde,2-Hydroxy-6-Methyl-	H-M	H-S	H-S H-M	H-S	H-S H-M	H-S H-M	H-S H-M	H-S H-M	H-M H-M	H-S H-M	H-M H-M
C	2-(2-Hydroxy-2-Phenylethyl)-3,5,6-Trimethylpyrazine	H-M	H-M	H-S H-M	H-M	H-S H-M	H-S H-M	H-S H-M	H-S H-M	H-S H-M	H-S H-M	H-S H-M
D	3,7,11,15-Tetramethyl-2-Hexadecen-1-ol	-	-	H-M	-	H-M	H-M	H-M	H-S H-M	H-M	H-M	H-M

3.3. Amino acid position profile for Dengue and Polio virus non- structural proteins with 4 ligands.

Table – 3: Amino acid position profile for Dengue and Polio virus non-structural proteins with 4 ligand.

Ligands	Compound Name	Dengue Virus					Polio Virus					
		NS1	Trans membrane domain of NS2A	NS2B/NS3 protease	NS3 helicase	NS5 protein	2A	2C	3A	3B	3C	3D
A	2,Cyclohexene,1,One-2-Methyl-5-(1-Methylethenyl)	Ser(185)	Ile(2) Gly(3)	Trp(83)	Ala(452)	Asp(131)	Leu(139)	Val(338)	Ser(31) Gln(32)	Lys(10)	Ala(61)	Ala(340) Ser(341)
B	Benzaldehyde,2-Hydroxy-6-Methyl-	Ser(185)	Ile(2) Gly(3)	Gly(87)	Ser(321)	Lys(105)	Leu(139)	Ala(340)	Asn(23)	Lys(9)	Thr(73) Ile(56)	Ala(231)
C	2-(2-Hydroxy-2-Phenylethyl)-3,5,6-Trimethylpyrazine	Asp(180)	Gly(3)	Leu(85) Gly(87)	Ser(321)	Glu(111)	Arg(114)	Leu(229) Ala(340) Ser(341) Glu(364)	Gln(27) Gln(27)	Pro(11) Asn(12)	Gly(44)	His(413)
D	3,7,11,15-Tetramethyl-2-Hexadecen-1- ol	-	-	Gly(87)	-	Gly(85) Gly(86)	Phe(40)	Val(338)	Tyr(37)	Asn(8)	Thr(142)	Ala(231)

4. DISCUSSION

Considering all the tables from Table – 1, Table – 2 and Table - 3, the 3D structure coordinates of five proteins of dengue and six proteins of polio viruses are optimized and 4 compounds from *Mentha arvensis* leaves extract are identified. The total binding energy of the compounds with all the eleven proteins was calculated using iGEMDOCK. Evaluations of binding conformation of these 4 compounds with five dengue and six polio viral non -structural 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 polio 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. Considering the non- structural proteins of Dengue virus, among the 4 analogs,NS1 protein('D', binding energy value= -75.7kcal/mol) , Trans membrane domain of NS2A ('D', binding energy value= -81.9kcal/mol), NS2B / NS3 protease ('D', binding energy value= -86.2kcal/mol), NS3 helicase ('D', binding energy value= -91.7kcal/mol) and NS5 protein ('D', binding energy value= -96.6 kcal/mol) . And the non- structural proteins of polio virus have, 2A ('D', binding energy value= -102.4kcal/mol), 2C ('D', binding energy value= -85.9kcal/mol),3A("D", binding energy value= -728.57kcal/mol),3B("D", binding energy value= -453.7kcal/mol),3C("C", binding energy value= -83.9kcal/mol) and 3D("D", binding energy value= -89.7kcal/mol). We found that the compound "D" was found to have the best binding affinity with five dengue and six polio non-structural viral proteins.

4.1. Non-Structural proteins of Dengue Virus

4.1.1. The Total Binding Energy for Dengue virus NS1 protein with 4 ligands

From Table – 1, Table -2 and Table -3, the docking simulation of 4 ligands were performed for Dengue virus NS1 protein. From the docking study, we observed that compound – D has best binding affinity with the target NS1 protein with the binding energy value of -75.7 kcal/mol. Interaction analysis of binding mode of compound –D in dengue virus NS1 protein reveals that there are no hydrogen bond with low energy residue. A close-up view of the Total Binding Energy (kcal/mol) profile for Dengue virus NS1 protein with 4 ligands: is shown in Fig.1.

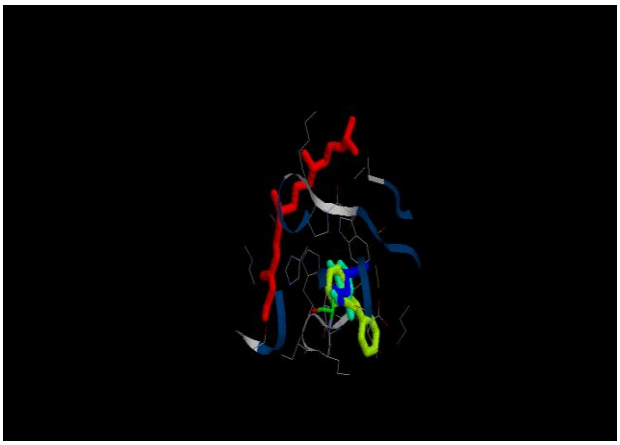

Fig. 1: The Total Binding profile for Dengue virus NS1 protein with 4 ligands.

4.1.2. The Total Binding Energy for Dengue virus Trans membrane domain of NS2A with 4 ligands

From Table – 1, Table – 2 and Table -3, the docking simulation of 4 ligands were performed for Dengue virus Trans membrane domain of NS2A. From the docking study, we observed that compound – D has best binding affinity with the target Trans membrane domain of NS2A with the binding energy value of -81.9 kcal/mol.

Interaction analysis of binding mode of compound –D in dengue virus NS2A protein reveals that there is no hydrogen bond with low energy residue. A close-up view of the Total Binding Energy (kcal/mol) profile for Dengue virus Trans membrane domain of NS2A with 4 ligands: is shown in Fig.2.

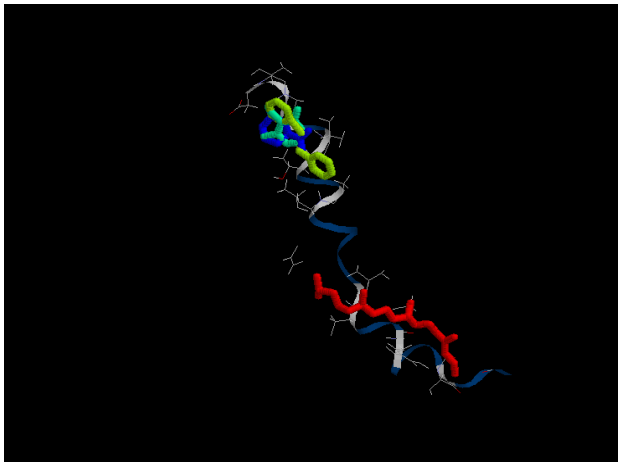

Fig. 2: The Total Binding profile for Dengue virus Trans membrane domain of NS2A with 4 ligands.

4.1.3. The Total Binding Energy for Dengue virus NS2B / NS3 protease with 4 ligands

From Table – 1, Table – 2 and Table – 3, the docking simulation of 4 ligands were performed for Dengue virus NS2B / NS3 protease. From the docking study, we observed that compound – D has best binding affinity with the target NS2B / NS3 protease with the binding energy value of -86.2 kcal/mol. Interaction analysis of binding mode of compound –D in dengue virus NS2B / NS3 protease reveals that it forms one hydrogen bond with low energy, with Gly(87) residue. A close-up view of the Total Binding Energy (kcal/mol) profile for Dengue virus NS2B / NS3 protease with 4 ligands: is shown in Fig.3.

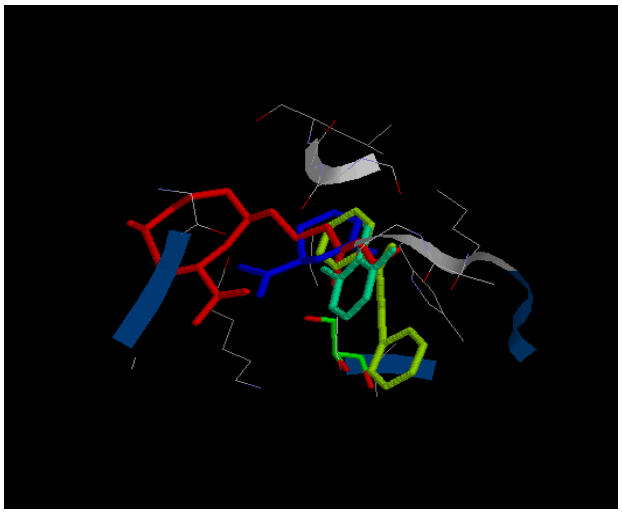

Fig.3: The Total Binding profile for Dengue virus NS2B / NS3 protease with 4 ligands.

4.1.4. The Total Binding Energy for Dengue virus NS3 helicase with 4 ligands

From Table – 1, Table – 2 and Table – 3, the docking simulation of 4 ligands were performed for Dengue virus NS3 helicase. From the docking study, we observed that compound – D has best binding affinity with the target NS3 helicase with the binding energy value of -91.7 kcal/mol. Interaction analysis of binding mode of compound –D in dengue virus NS3 helicase reveals that there is no hydrogen bonds with low energy residues. A close-up view of the Total Binding Energy (kcal/mol) profile for Dengue virus NS3 helicase with 4 ligands: is shown in Fig.4.

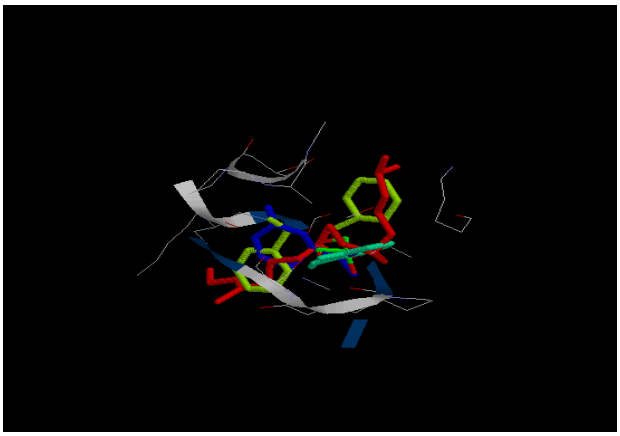

Fig. 4: The Total Binding profile for Dengue virus NS3 helicase with 4 ligands.

4.1.5. The Total Binding Energy for Dengue virus NS5 protein with 4 ligands:

From Table – 2, Table – 2 and Table – 3, the docking simulation of 4 ligands were performed for Dengue virus NS5 protein. From the docking study, we observed that compound – D has best binding affinity with the target NS5 protein with the binding energy value of -96.6kcal/mol. Interaction analysis of binding mode of compound –D in dengue virus NS5 protein reveals that it forms one hydrogen bonds with low energy, with one Gly (85) and with Gly (86) residues. A close-up view of the Total Binding Energy (kcal/mol) profile for Dengue virus NS5 protein with 4 ligands: is shown in Fig.5.

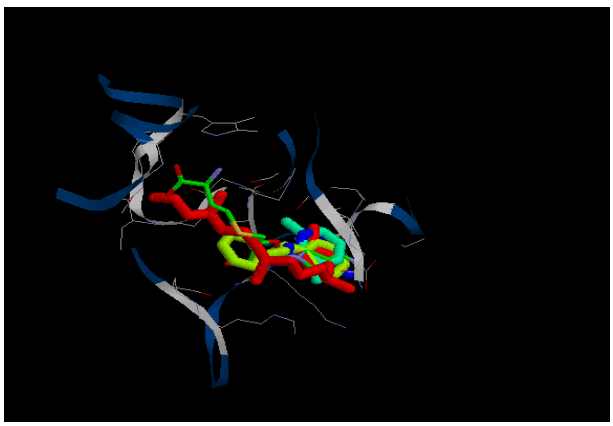

Fig. 5: The Total Binding profile for Dengue virus NS5 protein with 4 ligands.

4.2. Non-Structural proteins of Polio Virus

4.2.1. The Total Binding Energy for Polio virus 2A protein with 4 ligands:

From Table – 1, Table – 2 and Table – 3, the docking simulation of 4 ligands were performed for Polio virus 2A protein. From the docking study, we observed that compounds – D has best binding affinity with the target 2A protein with the binding energy values of -102.4 kcal/mol. Interaction analysis of binding mode of compounds –D in Polio virus 2A protein reveals that it forms one hydrogen bond with low energy, with Phe(40) residues. A close-up view of the Total Binding Energy (kcal/mol) profile for Polio virus 2A protein with 4 ligands: is shown in Fig.6.

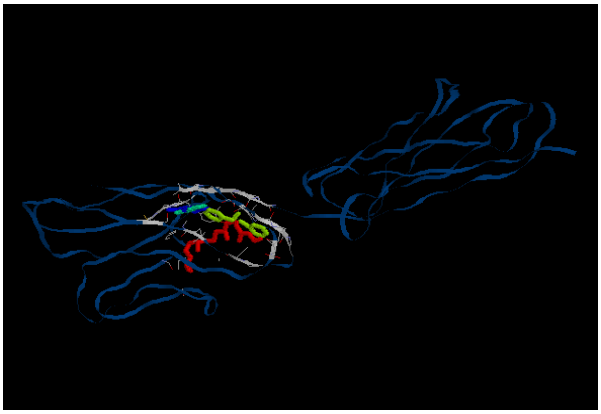

Fig. 6: The Total Binding profile for Polio virus 2A protein with 4 ligands.

4.2.2. The Total Binding Energy for Polio virus 2C protein with 4 ligands

From Table – 1, Table – 2 and Table – 3, the docking simulation of 4 ligands were performed for Polio virus 2C protein From the docking study, we observed that compound – D has best binding affinity with the target 2C protein with the binding energy value of -85.9kcal/mol. Interaction analysis of binding mode of compounds-D in Polio virus 2C protein reveals that it forms one hydrogen bond with low energy, with Val (338) residues. A close-up view of the Total Binding Energy (kcal/mol) profile for Polio virus 2C protein with 4 ligands: is shown in Fig.7.

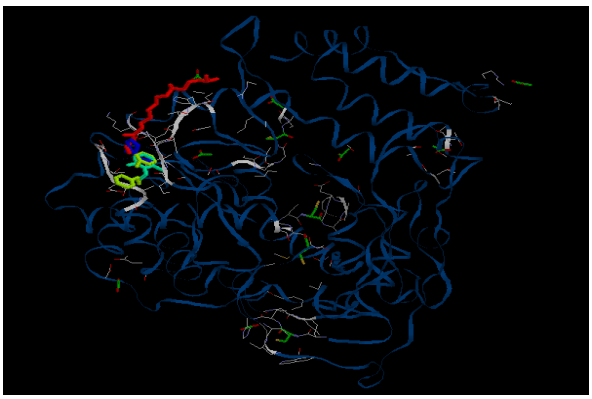

Fig. 7: The Total Binding profile for Polio virus 2C protein with 4 ligands.

4.2.3. The Total Binding Energy for Polio virus 3A protein with 4 ligands

From Table – 1, Table – 2 and Table – 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 3A protein with the binding energy value of -728.57kcal/mol. Interaction analysis of binding mode of compound –D in Polio virus 3A protein reveals that it forms two hydrogen bond with low energy, with Tyr(37), residue. A close-up view of the Total Binding Energy (kcal/mol) profile for Polio virus 3A protein with 4 ligands: is shown in Fig.8.

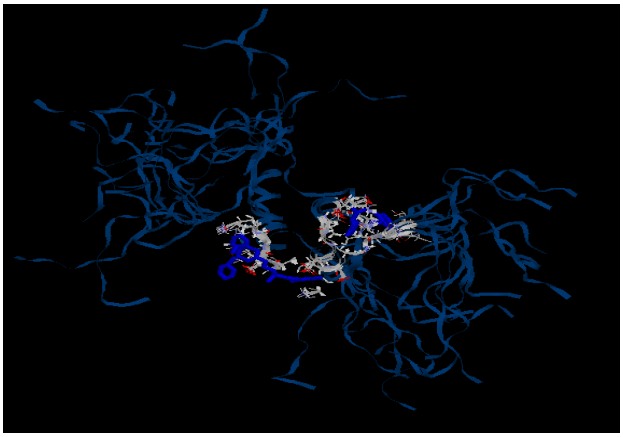

Fig. 8: The Total Binding profile for Polio virus 3A protein with 4 ligands.

4.2.4. The Total Binding Energy for Polio virus 3B protein with 4 ligands

From Table – 1, Table – 2 and Table – 3, the docking simulation of 4 ligands were performed for Polio virus 3B protein. From the docking study, we observed that compound – D has best binding affinity with the target 3B protein with the binding energy value of -453.7 kcal/mol. Interaction analysis of binding mode of compound –D in Polio virus 3B protein reveals that it forms one hydrogen bond with low energy, with Asn(8) residues. A close-up view of the Total Binding Energy (kcal/mol) profile for Polio virus 3B protein with 4 ligands: is shown in Fig.9.

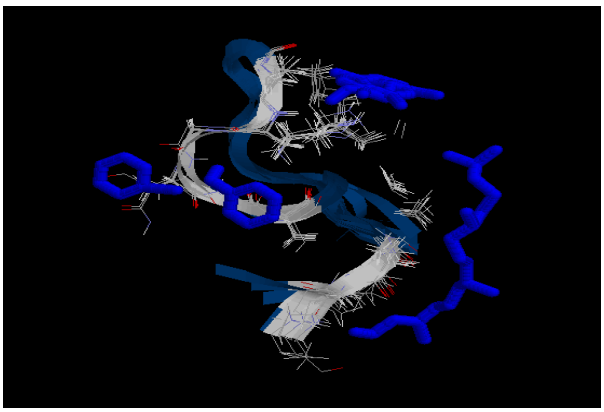

Fig. 9: The Total Binding Energy (kcal/mol) profile for Polio virus 3B protein with 4 ligands.

4.2.5. The Total Binding Energy for Polio virus 3C protein with 4 ligands

From Table – 1, Table – 2 and Table – 3, the docking simulation of 4 ligands were performed for Polio virus 3c. From the docking study, we observed that compound – C has best binding affinity with the target 3C with the binding energy value of – 83.9kcal/mol. Interaction analysis of binding mode of compound –C in Polio virus 3C reveals that it forms one hydrogen bond with low energy, with Gly(44) residue. A close-up view of the Total Binding Energy (kcal/mol) profile for Polio virus 3C with 4 ligands: is shown in Fig.10.

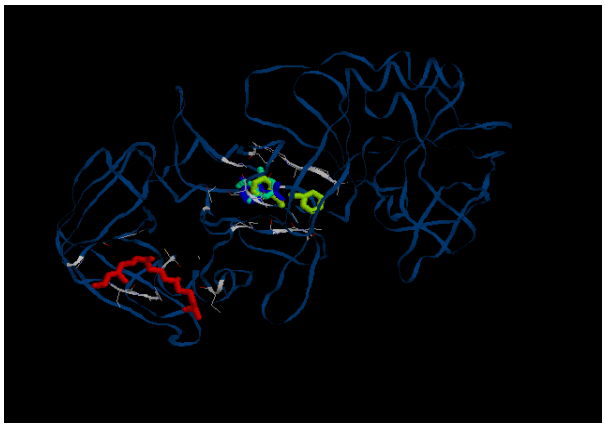

Fig. 10: The Total Binding profile for Polio virus 3C protein with 4 ligands.

4.2.6. The Total Binding Energy for Polio virus 3D protein with 4 ligands

From Table – 1, Table – 2 and Table – 3, the docking simulation of 4 ligands were performed for Polio virus 3D protein. From the docking study, we observed that compound – D has best binding affinity with the target 3D protein with the binding energy value of -89.7 kcal/mol. Interaction analysis of binding mode of compound –D in Polio virus 3D protein reveals that it forms one hydrogen bonds with low energy, with Ala(231) residue. A close-up view of the Total Binding Energy (kcal/mol) profile for Polio virus 3D protein with 4 ligands: is shown in Fig.11.

Fig. 11: The Total Binding profile for Polio virus 3D protein with 4 ligands.

5. CONCLUSION

Our molecular docking studies explored the possible binding modes of 4 compounds that are present in *Mentha arvensis* leaves extract with five proteins of Dengue virus and six proteins of Polio virus. Dengue virus consists of, NS1 protein, Trans membrane domain of NS2A, NS2B/NS3 protease, NS3 helicase, and NS5 protein; Polio virus consists of 2A, 2C, 3A, 3B, 3C and 3D protein complex. It revealed that all the 4 compounds show minimum affinity with all the proteins. The compound 'D' (3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol) showed the best results compared to other compounds. On comparing the binding energy and the binding site residues, we found that all the compounds will differ in either of them for hydrogen bond formation. The conclusion which is drawn from our virtual screening and docking result are that the Compound 'D' has highest binding affinity with most of the non-structural proteins of Dengue virus and Polio virus and therefore it can be used as an effective drug target for Dengue virus as well as Polio virus. Hence, the Compound 'D' may be considered as the effective drug target for both dengue and Polio virus because it can effectively bind to most of the proteins of both the viruses. Though, there are many reports on the *in vitro* analysis of these compounds and its medicinal and toxic properties, there are no in silico studies that predict the binding and active regions especially with these proteins. Our study is probably the first such attempt to predict the binding site and the binding residues. However, validation of our results through *in vivo* and *in vitro* experiments and also with animal models will enlighten hope for the future development of more potent drugs for the treating Dengue and Polio virus.

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