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DOCKING STUDY OF SELECTED RED *VITIS VINIFERA* PEEL CONSTITUENTS ON DENGUE VIRAL PROTEINS – AN *IN SILICO* APPROACH

[1]Smriti Chawla, [1]Pavithra K., [1]Rituparna Chatterjee, *[2]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

Abstract:

Dengue virus is the most prevalent arthropod-borne virus affecting humans today. The virus group consists of 4 serotypes that manifest with similar symptoms. It has seven major non structural proteins which are considered to be most effective for drug designing in the recent studies. The phytochemicals present in the Vitis vinifera peel extract are reported to have antifungal and anticancerous properties. Our work describes the study of the binding efficiency of the selected 9 compounds that are present in the Vitis vinifera peel with all the seven proteins through in silico methods. By our virtual screening and docking result, we found that the 2R, 3S-9-[1,3,4-Trihydroxy-2-butoxy methyl] guanine has highest binding affinity with the proteins and also we predicted the binding site amino acid residues and the type of hydrogen bonding.

Keywords: *Dengue virus, Vitis vinfera peel, binding energy, hydrogen bond*

Corresponding author:

Dr. Balasubramanian Sathyamurthy,

Professor, Department of Biochemistry,
Ramaiah College of Arts, Science and Commerce,
Bangalore – 560054
Email: balasramaiah@gmail.com

QR code

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INTRODUCTION:

Vitis vinifera (common grape vine) is a species of *Vitis* and belongs to the family *Vitaceae* [1]. There are between 5,000 to 10,000 different varietals of *Vitis Vinifera*. It is native to central Europe and southwestern Asia, although grapes are now cultivated throughout the world. Grapevine (*Vitis*) is considered to be one of the major fruit crops in the world based on economic value. Grapes (*Vitis vinifera)* are universally appreciated fruit for their delicacy, nutrition and accepted as functional food [2]. Grape peel constitutes on average 65% of total material present in the grape and it is a rich source of phenolic compounds [3]. The content of vitamin A in the peel of red grapes is found to be higher as compared with other parts of grapes. Red grapes have been used for thousands of years because of their nutritional and medicinal benefits [4]. Resveratrol is mainly contained in the skins of grapes. These grape seed and skin extract compounds act as antimutagenic and antiviral agents [5]. Resveratrol is also used to treat cancer. In addition to this, Resveratrol is also used to treat Alzheimer's disease [6]. Resveratrol present in Red grape peel is also said to have displayed antifungal activity against the human pathogenic fungi Candida albicans in concentrations of 10-20micro litres [7]. Grape skins are good sources of phytochemicals such as gallic acid, catechin, and epicatechin and are suitable raw materials for the production of antioxidative dietary supplements [8]. The skin of the red grapes are rich sources of saponins and pterostilbene which helps to accumulate cholesterol and prevents it from being absorbed in the blood so this helps to prevent obesity and cardiovascular diseases [9]. Catechin is usually the most important individual flavonol present in grape skin. The consumption of grape derived dietary flavonoids in the form of grape extracts and grape seeds powder has been shown to effectively suppress oxidative stress and prevent oxidative damage [10].

The GCMS results of methanolic red grape peel extract showed nearly 100 compounds. Out of the 100 compounds that were reported about 9 compounds showed higher retention time as compared to the others. Those compounds that had higher retention time includes 4H-Pyran-4-one,2,3-dihydro 3,5 dihydroxy-6-methyl (Antimicrobial, Melanin production inhibitor, Antioxidant activity), 2-Propyl-tetrahydropyran-3-ol (Anti infective agent in human microbial infections), 5-Hydroxy methylfurfual (Antioxidant, Antiproliferative agent), 4-Octen-3-one, 6-ethyl-7-hydroxy (Antifungal agent), Benzoic acid,4-hydrozy-3-methoxy-methyl ester (Useful in the treatment of Paramyxovirus viral infections, Modulators of ion channel), 2-Isopropoxyethylpropionate (Antibacterial activity, Treatment of inflammation and neoplastic disease), 2R, 3S – 9 - [1, 3, 4 – Trihydroxy –2-butoxy methyl]guanine (Antifungal activity) Ethanamine,N-ethyl-N-nitroso (Antitumoral activity and medicinal agent for treating patients suffering from disease caused by the monoaminooxidase excessive activity), 2 – t – Butyl – 4 – methyl – 5 – oxo - [1, 3] dioxolane - 4 – carboxylicacid (Antitubercular and Antibacterial activity) [11].

The disease Dengue Fever/Dengue Hemorrhagic Fever is caused by four closely related viruses (*Flaviviridae* family) DENV-1, DENV-2, DENV-3 and DENV-4. The four variants are indistinguishable clinically [12]. Dengue is a highly endemic infectious disease of the tropical countries and is rapidly becoming a global burden. Dengue disease varies from mild fever to severe conditions of dengue hemorrhagic fever and shock syndrome [13]. The viral genome consists of a positive-sense RNA of approximately 11kb and this genetic material encodes 3 structural proteins which include C, prM and E and 7 non-structural proteins that include the capsid protein, envelope protein, NS1 protein, trans – membrane domain of NS2A, NS2B/NS3 protease, NS3 helicase and NS5 protein. NS2B-NS3 is a heterodimeric protein of NS2B and NS3 protein. NS2B-NS3 protease is a crucial enzyme for the viral replication [14]. Intracellularly, the NS1 protein is reported to act as a cofactor in viral replication. It is presumed that through its interaction with NS4A/NS4B, and due to its location into the lumen of ER, NS1 serves as a scaffolding protein that is necessary for the stability of the viral replication complex [15]. NS2A functions in viral RNA synthesis. This NS2A protein is also important for viral assembly [16].

Bioinformatics is the application of computational tools to organize, analyze, understand, visualize and store information associated with biological macromolecule [17]. It develops methods for storing, retrieving, organizing and analyzing biological data. Protein Data Bank (PDB) is a bioinformatic tool which stores the structures of proteins, ligands and macromolecules [18]. Docking analysis can be conducted to analyse the fitness and interaction between the protein and the ligand in the form of energy. This interaction can be used as a pharmaceutical basis for drug production [19].

The main aim of our study is to find the best docking fit from the selected 9 compounds from *Vitis vinifera peel* extract with seven different non structural Dengue viral proteins

MATERIALS AND METHODOLOGIES:

Preparation of dengue viral proteins:

The protein data bank (PDB) was used to obtain the three-dimensional structure of the macromolecule. PDB is the single worldwide reservoir of primary structural data of a large number of biological macromolecules. Proteins of dengue virus were used for this study so the selected 7 proteins were downloaded from the Protein Data Bank and were saved in the PDB format. The downloaded proteins were viewed in Rasmol viewer [14].

Preparation of ligands:

Ligands selected were from the previous studies on GCMS analysis on *Vitis Vinifera peel* extract. 9 ligands were used for the study and the Ligands were constructed using ChemSketch [14]. Chemsketch is software that can be used to produce structures of organic molecules, names of organic molecules as well as Lewis structures, 3D structures, space filling models or ball and stick models, among other things. The constructed ligands were optimized to add the hydrogen bonds and the obtained structures were saved in mol format for docking analysis and named as A, B, C, D, E, F, G, H and I respectively.

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 [14]. iGEMDOCK provides the visualizations of the protein-compound interaction profiles and even the hierarchical clustering dendrogram of the compounds for post-screening analysis. **.** The proteins and the ligands were loaded and an output path was set. Standard docking parameters were set for docking (population size=200, generations =70 and Number of solutions =2) after which the docking process was initiated. After the docking process, the interaction profile and the interaction analysis was viewed to give us the information about the binding energies and the binding affinity of the compounds with the proteins. The best docking pose for the individual ligands were obtained for all the seven 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 further visualized in Ras Mol viewer. Ras Mol is computer software used to depict biological macromolecular structure.

RESULTS AND DISCUSSION:

Total Binding Energy (kcal/mol) profile for Dengue virus protein with 9 ligands:

Table – 1: The Total Binding Energy (kcal/mol) profile for Dengue virus' protein with 9 ligands

Ligand	Compound name	Envelope protein	NS1 protein	Trans membrane domain of NS2A	NS2B / NS3 protease	NS3 helicase	NS5 protein	Capsid protein
A	4H Pyran-4-one,2,3-dihydro 3,5 dihydroxy-6- methyl	-66.4	-85.14	-54.74	-60.95	-68.39	-75.17	-67.35
B	2-Propyl-tetrahydropyran-3-ol	-61.34	-80.39	-64.05	-75.19	-69.37	-67.57	-72.06
C	5-Hydroxy methylfurfural	-66.82	-70.57	-53.06	-64.51	-66.29	-68.24	-66.14
D	4-Octen-3-one 6 ethyl-7-hydroxy	-64.06	-79.25	-61.79	-65.12	-82.26	-73.34	-77.94
E	Benzoic acid 4-hydroxy-3-methoxy-methyl ether	-73.69	-78.56	-69.59	-77.26	-80.14	-76.43	-77.97
F	2-Isopropoxyethylpropionate	-62.24	-75.48	-59.8	-59.49	-65.22	-68.13	-71.5
G	2R, 3S-9-[1,3,4-Trihydroxy-2-butoxy methyl] guanine	-101.11	-119.05	-94.92	-103.41	-114.86	-117.17	-97.79
H	Ethanamine, N-ethyl-N-nitroso	-50.23	-57.94	-44.25	-50.07	-59.32	-52.28	-63.81
I	2-t-Butyl-4-mehtyl-5-oxo-[1,3]dixoxlane-4-carboxylic acid	-76.02	-74.21	-61.64	-73.14	-83.93	-81.37	-80.66

H – Bond profile for Dengue virus' protein with 9 ligands:

Table – 2: H – Bond profile for Dengue virus' protein with 9 ligands

Ligand	Compound name	Envelope protein	NS1 protein	Trans membrane domain of NS2A	NS2B/NS3 protease	NS3 helicase	NS5 protein	Capsid protein
A	4H Pyran-4-one,2,3-dihydrro 3,5 dihydroxy-6- methyl	-18.64	-23.82	-12.81	-18.54	-22.9	-18.84	-25.38
B	2-Propyl-tetrahydropyran-3-ol	-13.04	-13.14	-9.48	-9.5	-14.69	-10.5	-12.66
C	5-Hydroxy methylfurfural	-21.47	-14.01	-13	-19.21	-12.95	-20.33	-21.94
D	4-Octen-3-one 6 ethyl-7-hydroxy	-17.41	-3.99	-13	-9.91	-12.78	-10.43	-7
E	Benzoic acid 4-hydroxy-3-methoxy-methyl ether	-18.89	-7.38	-16.06	-16.22	-19.87	-11.74	-13.53
F	2-Isopropoxyethylpropionate	-12.27	-3.5	-9.45	-3.5	-10.58	-6.76	-10.24
G	2R, 3S-9-[1,3,4-Trihydroxy-2-butoxy methyl] guanine	-35.36	-26.14	-22.21	-31.3	-35.47	-49.93	-25.48
H	Ethanamine, N-ethyl-N-nitroso	-17.15	-12	-12.94	-21.96	-22.62	-18.29	-27.94
I	2-t-Butyl-4-mehtyl-5-oxo-[1,3]dixoxlane-4-carboxylic acid	-18.92	-19.38	-11.3	-23.57	-37.13	-28.31	-22.94

Amino acid position profile for Dengue virus' protein with 9 ligands:

Table – 3: Amino acid position profile for Dengue virus' protein with 9 ligands

Ligand	Compound name	Envelope protein	NS1 protein	Trans membrane	NS2B/NS3 protease	NS3 helicase	NS5 protein	Capsid protein
A	4H Pyran-4-one-2,3-dihydrro 3,5 dihydroxy-6-methyl	Ile(618)	Asn(255)	Gly(3)	Ser(135)	Gln(467)	Asn(253)	Arg(41) Leu(46)
B	2-Propyl-tetrahydropyran-3-ol	Arg(619)	Ile(243)	Ile(2) Gly(3)	Trp(83) Asn(152)	Gln(456)	Ala(41) His(53) Lys(253)	Arg(41) Arg(68)
C	5-Hydroxy methylfurfural	Arg(619)	Ser(185)	Ile(2) Gly(3)	Gly(87)	Gln(384)	Lys(105)	Arg(32) Phe(47)
D	4-Octen-3-one 6 ethyl-7-hydroxy	Ile(618) Lys(625) Arg(629) Ile(630)	Asp(197)	Ile(2) Gly(3) Thr(7)	Ala(166)	Gln(384) Ser(386)	Asn(69)	Arg(41)
E	Benzoic acid 4-hydroxy-3-methoxy-methyl ether	Ile(618)	His(181) Asp(197)	Asp(1)	Arg(55)	His(194)	Arg(352)	Arg(41)
F	2-Isopropoxyethylpropionate	Gly(628)	Lys(245)	Ile(2) Gly(3)	Leu(149)	Gln(467)	Arg(448)	Arg(41)
G	2R, 3S-9-[1,3,4-Trihydroxy-2-butoxy methyl] guanine	Lys(613)	Ser(252) Lys(245) Ile(242)	Gly(3)	Trp(83) Ala(164)	Leu(216)	Val(255)	Arg(32)
H	Ethanamine, N-ethyl-N-nitroso	Thr(579)	Ile(243)	Asp(1)	Thr(120)	Thr(200)	Ser(56)	Thr(25) Thr(62)
I	2-t-Butyl-4-mehtyl-5-oxo-[1,3]dioxlane-4-carboxylic acid	Lys(625)	A(197)	Gly(3)	Arg(55) Asn(58)	Arg(209)	Asp(808)	Arg(41)

DISCUSSION:

From the Table – 1, Table – 2 and Table – 3, the 3D structure coordinates of seven non structural proteins of dengue virus is optimized and five compounds from *Vitis Vinifera* peel extract are identified. The total binding energy of these compounds with the dengue virus proteins was calculated using the software iGEMDOCK. Evaluations of binding conformation of the selected 9 compounds with seven non-structural dengue viral proteins are performed using iGEMDOCK. From the mentioned docking study, we have listed the binding affinity of 9 compounds based on ligand binding energy (Table.1). The depicted binding pose for each ligand molecule into the non structural dengue viral protein is analyzed and the one having lowest ligand binding energy with these proteins among the different poses are generated. The lower energy values represent better protein-ligand target binding affinity compared to higher energy value. Among the 9 analogs, compound G is found to have lower ligand binding energy (binding energy value = -101.11 kcal/mol), than other analogs for Envelope protein and even for the other proteins like with that of NS1 protein (binding energy value= -119.05 kcal/mol), Trans membrane domain of NS2A (binding energy value= -94.92 kcal/mol), NS2B / NS3 protease (binding energy value= -103.41 kcal/mol), NS3 helicase (binding energy value= -114.86 kcal/mol), NS5 protein (binding energy value= -117.17 kcal/mol) and Capsid protein (binding energy value = -97.79 kcal/mol). We further analyzed the docked pose for finding the binding mode of compound "G" in to seven non structural dengue proteins to validate the reasonable binding conformations.

The Total Binding Energy (kcal/mol) profile for Dengue virus envelope protein with 9 ligands:

From Table – 1, Table – 2, Table – 3 and Figure – 1, the docking simulation of the 9 ligands were performed for Dengue virus envelope protein. From the docking study, we observed that compound –G has best binding affinity with the target envelope protein with the binding energy value of -101.11kcal/mol. Interaction analysis of binding mode of compound –G in dengue virus envelope protein reveals that it forms one hydrogen bond of low energy with Lys(613) residues. A close-up view of the Total Binding Energy (kcal/mol) profile for Dengue virus envelope protein with 9 ligands is shown in Fig.1.

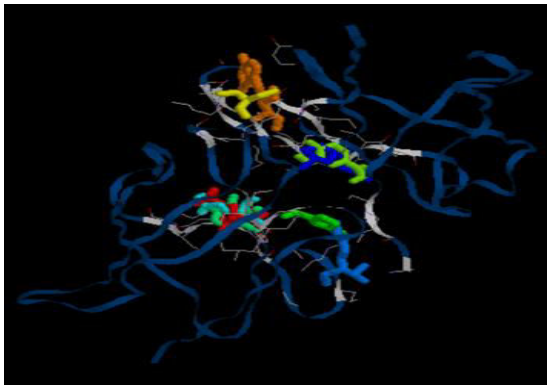

Fig.1: The Total Binding profile for Dengue virus envelope protein with 9 ligands:

2.1. The Total Binding Energy (kcal/mol) profile for Dengue virus NS1 protein with 9 ligands:

From Table – 1, Table – 2, Table – 3 and Figure – 2, the docking simulation of 9 ligands were performed for Dengue virus NS1 protein. From the docking study, we observed that compound G has best binding affinity with the target NS1 protein with the binding energy value of -119.05kcal/mol. Interaction analysis of binding mode of compound G in dengue virus NS1 protein reveals that it forms two hydrogen bonds of low energy with Ser(252), Lys(245) and Ile(242) residue. A close-up view of the Total Binding Energy (kcal/mol) profile for Dengue virus NS1 protein with 9 ligands is shown in Fig.2.

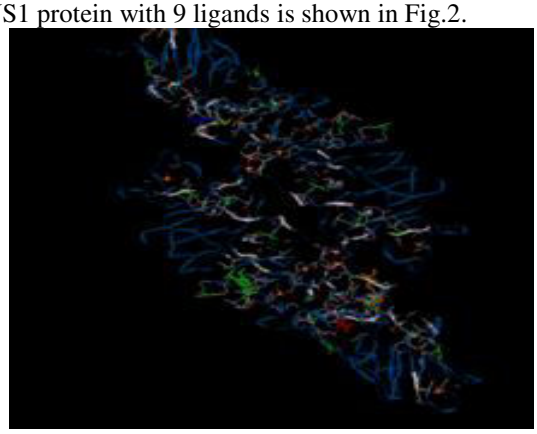

Fig.2: The Total Binding profile for Dengue virus NS1 protein with 9 ligands

2.2. The Total Binding Energy (kcal/mol) profile for Dengue virus Trans membrane domain of NS2A with 9 ligands:

From Table – 1, Table – 2, Table – 3 and Figure – 3, the docking simulation of 9 ligands were performed

for Dengue virus Trans membrane domain of NS2A. From the docking study, we observed that compound G has best binding affinity with the target Trans membrane domain of NS2A with the binding energy value of -94.92kcal/mol. Interaction analysis of binding mode of compound G in dengue virus Trans membrane domain of NS2A reveals that it forms one hydrogen bond with low energy, with Gly(3) residue. A close-up view of the Total Binding Energy (kcal/mol) profile for Dengue virus transmembrane domain of NS2A with 5 ligands is shown in Fig.3.

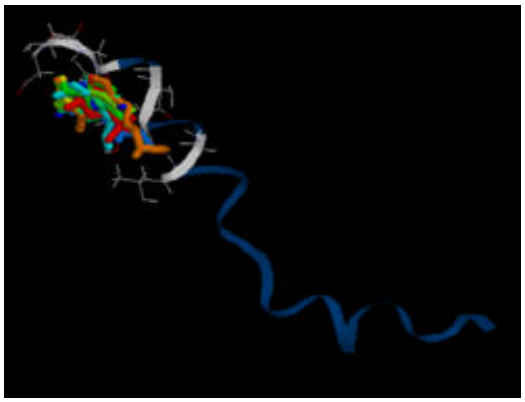

Fig.3: The Total Binding profile for Dengue virus Trans membrane domain of NS2A with 9 ligands

The Total Binding Energy (kcal/mol) profile for Dengue virus NS2B / NS3 protease with 9 ligands

From Table – 1, Table – 2, Table – 3 and Figure – 3 the docking simulation of five ligands were performed for Dengue virus NS2B / NS3 protease. From the docking study, we observed that compound –G has best binding affinity with the target NS2B / NS3 protease with the binding energy value of -103.41kcal/mol. Interaction analysis of binding mode of compound – D in dengue virus NS2B / NS3 protease reveals that it forms two hydrogen bond with low energy, with Trp(83) and Ala(164) residue. A close-up view of the Total Binding Energy (kcal/mol) profile for Dengue virus NS2B / NS3 protease with 9 ligands is shown in Fig.4.

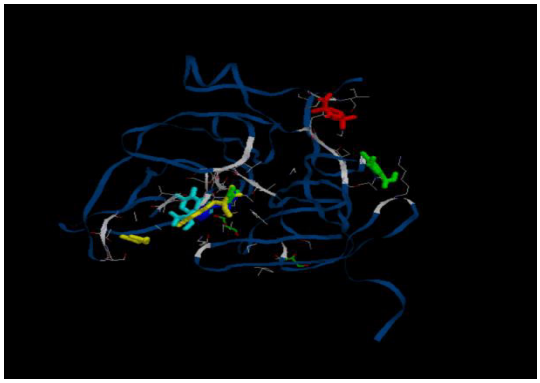

Fig.4: The Total Binding profile for Dengue virus NS2B / NS3 protease with 9 ligands

2.3. The Total Binding Energy (kcal/mol) profile for Dengue virus NS3 helicase with 9 ligands:

From Table – 1, Table – 2, Table – 3 and Figure – 5, the docking simulation of 9 ligands were performed for Dengue virus NS3 helicase**.** From the docking study, we observed that compound G has best binding affinity with the target NS3 helicase with the binding energy value of -114.86 kcal/mol. Interaction analysis of binding mode of compound G in dengue virus NS3 Helicase reveals that it forms one hydrogen bond with low energy with Leu(216) residue.A close-up view of the Total Binding Energy (kcal/mol) profile for Dengue virus NS3 helicase with 9 ligands is shown in Fig 5.

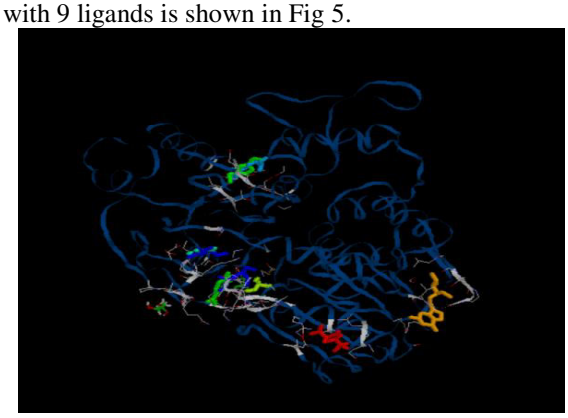

Fig.5: The Total Binding profile for Dengue virus NS3 helicase with 9 ligands

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2.6.
2.7.
2.8.

The Total Binding Energy (kcal/mol) profile for Dengue virus NS5 protein with 9 ligands:

From Table – 1, Table – 2, Table – 3 and Figure – 6, the docking simulation of 9 ligands were performed for Dengue virus NS5 protein. From the docking study, we observed that compound G has best binding affinity with the target NS5 protein with the binding energy value of -117.17kcal/mol. Interaction analysis of binding mode of compound G in Dengue virus NS5 protein reveals that it forms one hydrogen bond with low energy with Val(255) residues. A close-up view of the Total Binding Energy (kcal/mol) profile for Dengue virus NS5 protein with 9 ligands is shown in Fig.6.

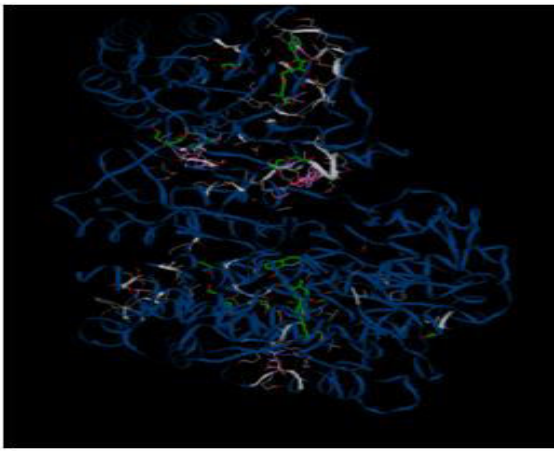

Fig.6: The Total Binding profile for Dengue virus NS5 protein with 9 ligands:

The Total Binding Energy (kcal/mol) profile for Dengue virus Capsid protein with 9 ligands:

From Table – 1, Table – 2, Table – 3 and Figure – 7, the docking simulation of five ligands were performed for Dengue virus Capsid protein. From the docking study, we observed that compound G has best binding affinity with the target Capsid protein with the binding energy value of -97.79kcal/mol. Interaction analysis of binding mode of compound G in dengue virus Capsid protein reveals that it forms one hydrogen bond of low energy with residue Arg(32). A close-up view of the Total Binding Energy (kcal/mol) profile for Dengue virus Capsid protein with 9 ligands is shown in Fig.7.

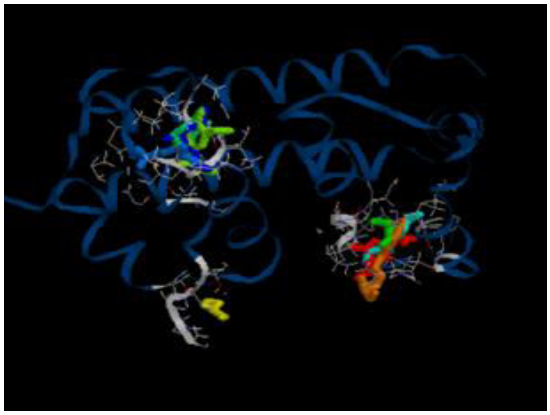

Fig.7: The Total Binding profile for Dengue virus Capsid protein with 9 ligands:

CONCLUSION:

Our molecular docking studies explored the possible binding modes of 9 compounds that were chosen from the GC-MS results of the *Vitis Vinifera* peel extract with seven non structural proteins of the dengue virus which include envelope protein, NS1 protein, Transmembrane domain of NS2A, NS2B/NS3 protease, NS3 helicase, NS5 protein and capsid protein. Among all the 9 compounds that were docked with the proteins the compound G (2R, 3S-9-[1, 3, 4-Trihydroxy-2-butoxy methyl] guanine) shows best result compared to other compounds. On comparing the results of the binding energy and the binding site residues, we found that all compounds differ either in their binding modes or with the binding site residues for hydrogen bond formation. The conclusion drawn from our docking studies was that since Compound G has highest binding affinity with all of the proteins hence it can be used as an effective and potent drug target for Dengue virus. Though, there are many reports on the *in vitro* analysis of these compounds and its antioxidant properties, but there are no *In silico* studies that predict the binding and active regions especially with these proteins. Our study is probably the first approach to this methodology. However, validation of our results through *invivo* and *invitro* experiments along with animal models will enlighten hope for the future development of more potent and effective drugs for treating Dengue.

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