INNOVARE JOURNAL OF SCIENCES

Vol 10, 2022

ISSN - 2321-5496

Research Article

IN SILICO ANALYSIS AND DOCKING STUDY OF THE ACTIVE PHYTO COMPOUNDS OF *MORINGA OLEIFERA* AGAINST MARBURG VIRUS VP35 PROTEIN

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Received: 27 August 2022, Revised and Accepted: September 15, 2022

ABSTRACT

Objective: Marburg is a transmissible disease of the Filoviridae family. It infected a million people worldwide. Hence, an attempt was made to identify natural compounds from *Moringa oleifera*, having multiple medicinal values in Indian Ayurveda, to prevent the disease, using molecular docking, drug likeness prediction, absorption, distribution, metabolism, and excretion (ADME) analysis, and toxicity prediction.

Methods: Marburg main protein was retrieved from the protein data bank database. The ligands with poor binding and molecules that can affect docking were removed and docking is done with the PyRx tool. ADME and drug-likeness analysis were done using Swiss-ADME and absorption, distribution, metabolism, excretion, and toxicity (ADMET) lab web server.

Results: Ramachandran plot analysis shows the statistical distribution of the combinations of the backbone dihedral angles ϕ and ψ of the protein. Molecular docking studies show three compounds from *M. oleifera* have potential binding affinity to resist the main protein VP35 by preventing proteolytic cleavage, translation, and replication of the virus. ADMET profile and drug likeness and toxicity prediction showed that all three compounds Melanin, Diclazuril, and Tifentai were safe and possess drug-like properties.

Conclusion: The present study suggests that Melanin, Diclazuril, and Tifentai have significant binding affinity and they could inhibit the main protein VP35 and also helps to manage the therapeutic strategies against Marburg Virus.

Keywords: Marburg virus, VP35 protein, Docking, Nonstructural proteins, Viral replication, *Moringa oleifera*.

 DOI: http://dx.doi.org/10.22159/ijms.2022v10i1.46218. Journal homepage: https://innovareacademics.in/journals/index.php/ijs

INTRODUCTION

Marburg virus (MARV) disease is a rare but serious hemorrhagic fever that can affect both people and non-human primates. Marburg virus disease (MVD) is caused by the MARV, a virus of the filovirus family that is highly genetically unique. The other well-known members of the filovirus family are the six species of the Ebola virus. The MARV was first identified in 1967, and there have been outbreaks of hemorrhagic fever simultaneously in labs in Marburg and Frankfurt, Germany, and in Belgrade, Yugoslavia (now Serbia). Thirty-one people became ill after coming into contact with laboratory workers or those who had cared for them. This is a very small number, and it is not likely that anyone else became ill as a result. Seven people have died from the monkey-borne virus, which is a significant problem. The first people to become infected with the virus were researchers who were working with imported African green monkeys. One more case was diagnosed after it had already been diagnosed in an earlier round of testing. The reservoir host of the MARV is the African fruit bat, *Rousettus aegyptiacus*. Fruit bats that are infected with the MARV do not show any symptoms [1].

MVD is a disease that is highly fatal and is often associated with high CFRs (24–88%). During the early course of the disease, it is difficult to distinguish the clinical diagnosis of MVD from many other tropical fever diseases due to similar clinical manifestations. Other VHFs, especially Ebola virus disease, malaria, typhoid fever, leptospirosis, rickettsial infection, and plague should be excluded. Long-term exposure to mines or burrows used by *Rousettus* bat colonies can cause human MVD infection. Direct contact (through damaged skin or mucous membranes) with the blood, secretions, organs, or other bodily fluids of infected persons as well as with surfaces and items (e.g., bed linen and clothes), contaminated with these fluids, is how the MARV travels from one person to another. The first MVD epidemic in West Africa was identified in Guinea in 2021. Although there are presently no antiviral medications or vaccines that have been authorized to treat the virus, supportive care and the treatment of certain symptoms can help improve survival. A range of potential treatments, including blood products, immune therapies, and drug therapies, are being evaluated [2].

MVD is a viral hemorrhagic fever that can be caused by either of the two MARV es: MARV or Ravn virus [3]. Egyptian fruit bats are believed to be the normal carrier in nature and MARV Ribonucleic acid (RNA) has been isolated from them [4]. It causes Marburg hemorrhagic fever nervous system [5].

The ability of MARV to effectively undermine the host's innate immune response and subsequent adaptive immune responses has contributed in part to the high mortality rates [6]. Filoviral VP35 is an intriguing therapeutic target since protein performs many essential functions for virus reproduction, and its structure has been described with remarkable accuracy [7,8]. It is a multifunctional protein that participates in the viral polymerase complex as a cofactor. It has the potential to decrease the host's immune response, including the production of interferon (IFN), which is activated by Type I receptors in the retinoic acid-inducible gene (RLR) (RIG-I) [9]. The N-terminus of VP35 contains a spiral domain, while the C-terminus has an IFN inhibitor domain [10,11]. Potential protein inhibitors include the antivirals Favipiravir effective as medicine to defeat the MARV [12]. Other than these, some combinations of antiprotozoal drugs include Metronidazole, Atovaquone, and Benznidazole.

Ayurveda is a venerable medical system with a long history dating back to the Vedas. It is a valuable resource for keeping people healthy. Its unique approach to life, health, and cures makes it one of the most reliable sources of information on the topic. Ayurveda is a well-known

health system that pays close attention to geriatrics, rejuvenation, nutrition, immunology, genetics, and higher consciousness [13].

Moringa oleifera (Miracle tree) is one of the most commonly cultivated species of the monogeneric family *Moringaceae*, which is native to south Asia. This treasured and cultivated plant grows rapidly and is highly nutritious. Every part of this product is suitable for both nutritional and commercial purposes. The leaves of this tree are worth paying attention to, as they are a significant part of its overall structure. The leaves are an excellent source of minerals, Vitamins, and other essential plant chemicals. Traditional medicine is an effective tool for curing a variety of diseases in several countries. Clinical studies now suggest that this approach may be on the right track. *M. oleifera* leaves are packed with essential Vitamins and minerals, including Vitamins A and C. These leaves could help prevent malnutrition and related diseases [14].

M. oleifera has antiviral properties [15,16] as well as it is widely used as a nutritional herb and contains valuable pharmacological action such as anti-asthmatic, anti-diabetic, hepatoprotective, anti-inflammatory, anti-fertility, anti-cancer, anti-microbial, anti-oxidant, cardiovascular, anti-ulcer, CNS activity, anti-allergic, wound healing, analgesic, and antipyretic activity. The leaves of *M. oleifera* have Antiepileptic, anti-convulsant, Anti-diabetic, Cardiovascular activity/anti-hypertensive, Anthelmintic, and CNS activity. The roots and leaves have anti-oxidant and radical scavenging activity also roots have anti-fertility activity. The bark of *M. oleifera* has anti-urolithiatic and anti-inflammatory activity [17]. In this study, an attempt was made to identify new active and stable inhibitors of MARV core protein 4GH9 from a total of 461 different active phyto components of the *M. oleifera* plant.

MATERIALS AND METHODS

Protein preparation

The three-dimensional crystal structure of the VP35 protein RNA binding domain (Protein data bank (PDB) ID: 4GH9) of the MARV was retrieved from the (Research Collaboratory for Structural Bioinformatics) PDB (https://www.rcsb.org/) [9,18]. It has a single chain A consisting total of 146 amino acids. It has a crystal resolution of 1.65 Å. Protein crystal structures are prepared prior to docking in order to optimization of hydrogen bonds and remove atomic clashes. Protein preparation was done using a standard protocol of Discovery studio visualizer 21.1. Water molecules and heteroatoms from the proteins were removed followed by the addition of the polar hydrogen. Further, active site prediction of prepared protein was done.

Ramachandran plot

Ramachandran plot is a plot of torsional angles phi and psi of the amino acids contained in a peptide. Ramachandran plot analysis was done using the web-based PDB sum server of EMBL-EBI (http://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/GetPage.pl) [19]. The PDB ID of Protein (4GH9) was submitted and an analysis of the Ramachandran plot was run with outliers labeled by residue type, residue number, and chain and displaying all the labels.

Ligand selection

For the documentation of potential inhibitors of VP35, a total of 461 active phytocompounds from *M. oleifera* were retrieved from the different literature. Structures of phytocompounds were retrieved from the PubChem compound database (https://pubchem.ncbi.nlm.nih.gov/) in the 3D SDF (Three-Dimensional Structure Data File) format [20]. Ligand preparation was carried out by optimization of ligand, energy minimization, and conversion of ligands to 3D PDB format using the PyRx tool.

Molecular docking

The molecular docking method allows us to characterize how small molecules behave in the binding site of target proteins and to better understand basic biochemical processes by simulating the interaction between a small molecule and a protein at the atomic level [21]. PyRx is a virtual screening tool software was used for the molecular docking study [22]. Using the PyRx tool, all 461 active phytocompounds of *M. oleifera* were docked with VP35 (PDB ID: 4GH9). For docking study, prepared receptors and ligand files were selected to set the target. Protein was loaded and converted into macromolecule for docking, then ligands were imported using the open-babel tab in the tool and prepared [23]. After defining protein and ligand molecules, the grid box was defined by maximizing to check all the possibilities of ligand to bind with protein. After adjusting all the things, docking was started by clicking on the forward button. After completion of docking, we got a table consisting binding affinity of each ligand. Top 3 ligands were selected for further study based on the highest binding affinity of the ligand. Selected top 3 compounds were saved in PDB file format. Two-dimensional (2D)-3D interactive visualization study was performed using Discovery studio visualizer 21.1 [24].

Absorption, distribution, metabolism, and excretion (ADME) analysis

The term ADME in pharmacokinetics and pharmacology refers to how a drug is disposed of within an organism. The performance and pharmacological activity of the compound as a drug are affected by the four criteria because they all have an impact on drug levels and the kinetics of drug exposure to tissues. Sometimes toxicity is also taken in a consideration, called an absorption, distribution, metabolism, excretion and toxicity (ADMET) [25]. In this research study, Top 3 compounds having highest binding affinity were taken for the drug likeness test and ADMET analysis. Drug-likeness and ADMET analysis was done using SWISS-ADME (http://www.swissadme.ch/) [26] and ADMETLAB (https://admetmesh.scbdd.com/service/evaluation/index) [27]. Boiled-Egg analysis was also carried out with the SWISS-ADME tool [28]. Lipinski's rule of five was considered for ADME analysis. Lipinski's rule of five forecasts whether a drug-likeness would be successful or not when a molecule complies with two or more of the conditions,

a. Molecular mass<500 Dalton,
b. $\log_p$<4.15,
c. H-bond donor<5
d. H-bond acceptor<10 and
e. 40<molar refractivity<130.

RESULTS

Ramachandran plot

In the Ramachandran plot (Fig. 1), considering protein geometry as below,

Residues in most favored regions [A, B, L]	101	97.1%
Residues in additional allowed regions [a,b,l,p]	3	2.9%
Residues in generously allowed regions [~a,~b,~l,~p	0	0.0%
Residues in disallowed regions	0	0.0%
Number of non-glycine and non-proline residues	104	100.0%
Number of end-residues (excl. Gly and Pro)		2
Number of glycine residues (shown as triangles)		7
Number of proline residues		9
Total number of residues		122

Molecular docking

Molecular docking study revealed that various active phytocompounds from *M. oleifera* show significant binding affinity with VP35. The list of top 3 phytocompounds from *M. oleifera* has the highest binding affinity with VP35 (Table 1).

Results of molecular docking using PyRx, we found that out of 461 compounds from *M. oleifera* 10 compounds show significant binding affinity (>7 Kcal/mol) with VP35. From docking results, we selected the top 3 compounds (Table 2) for drug-likeness Prediction and ADME analysis.

Molecular visualization

Interactions between receptor-ligand of the top 3 phytocompounds having the highest binding affinity were visualized using Discovery Studio Visualizer 21.1. Docked ligands were saved in PDB file format

using PyRx and then opened with purified protein VP35. Different 2D and 3D interactions of phytocompounds with VP35 were observed. In a diagram of 2D interactions, we observed the different interactions such as Van der Waals forces, conventional and carbon-hydrogen bonds, Pi-sulfur interactions, alkyl, and Pi-alkyl interactions, Pi-Pi T-shaped interactions, and unfavorable interactions.

Melanin

Melanin forms different 2D-3D interactions with 4GH9. It includes conventional hydrogen bonding with residues THR 272, ASP 274, and LYS 244; carbon-hydrogen bonding with PHE 273 residue only; Pi-Sigma bonding with ALA 251 formed as shown in Fig. 2.

Diclazuril

Diclazuril forms different 2D-3D interactions with 4GH9. It includes conventional hydrogen bonding with residue SER 242 only, Pi-Sigma bonding with VAL 279 only; also forms Alkyl and Pi-Alkyl bonding with ILE 238, PRO 282, LYS 237, and VAL 234 as shown in Fig 3.

Tifentai

Tifentai forms different 2D-3D interactions with 4GH9. It includes conventional hydrogen bonding with residue LYS 211. It also forms one Pi-Donor hydrogen bond with GLN 233. It also forms Pi-Alkyl with LYS 237, Lys 241, ALA 210, LEU 215, ALA 214, and VAL 234 as shown in Fig. 4.

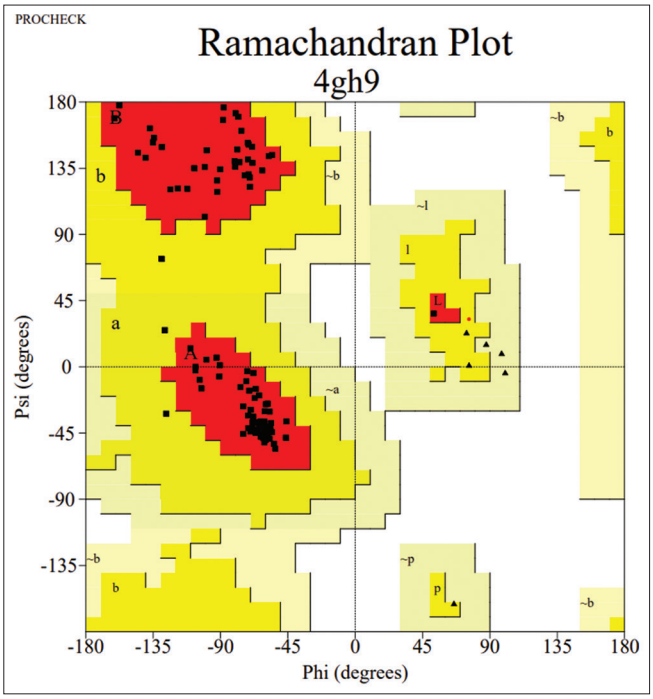

Fig. 1 Ramachandran Plot analysis of 4GH9.

Table 1: Top 3 phytocompounds from *M. oleifera* having the highest binding affinity with VP35

S. No	PubChem Compound ID	Name of Phytocompound	Binding Energy(Kcal/mol)
1	CID_6325610	Melanin	−7.7
2	CID_456389	Diclazuril	−7.6
3	CID_9832120	Tifentai	−7.5

Drug-likeness prediction, ADMET analysis, and toxicity prediction

Based on five aspects, Lipinski's rule of five helps to distinguish between compounds that are drug-like and non-drug like molecules. Drug-likeness prediction for the best-docked compounds was done with Lipinski's rule of five and ADME analysis was performed using the Swiss-ADME web server and ADMETLAB 2.0. Boiled-Egg analysis was also carried out using the Swiss-ADME tool (Table 2) and ProTox 2 prediction server used to calculate toxicity (Table 3). Furthermore, with the help of the Swiss-ADME tool, BOILED EGG analysis was carried out for the prediction of passive brain access blood brain barrier (BBB) and gastrointestinal absorption human intestinal absorption (HIA) of selected phytocompounds (Fig. 5).

In addition, best-docked compounds were analyzed within the standard scale for their water solubility ($\log_s$), HIA, and permeability glycoprotein substrate, BBB, carcinogenic effects, and Lipinski's rule validation (Table 4).

Comparison of Swiss ADMET lab and ProTox-2 with pass server

In the ADMET lab, all 3 compounds follow the Lipinski rule and with compared to the ProTox-2 server all 3 compounds have class 4 but Diclazuril has the highest LD50 (1100 mg/kg) than other compounds. However, in PASS Server Melanin shows the antiviral properties against 6 viruses, diclazuril shows antiviral properties against 1 virus, and Tifentai shows the antiviral properties against 11 viruses.

Secondary structure prediction

In secondary structure prediction Fig. 6, the VP35 has a sequence length of 146 amino acids, a number of aligned proteins 17, and a number of matched PDB structures 10. In predicted features, the protein helix is in blue, strands in red, and others in yellow. The 3D structure of a protein is shown in Fig. 7.

DISCUSSION

In modern medicine, MARV disease is treated with anti-viral and other therapies individually and in combination too. Natural phytocompounds from medicinal plants such as *M. oleifera* can be used, as they are less toxic than synthetic compounds. *In silico* methods such as molecular docking, ADME analysis, molecular dynamic simulation, and Toxicity prediction have shown to be beneficial for further research to analyze the binding affinity, interactions, and stability of the ligands with a target. In addition to affecting the host's immunity, VP35 is an attractive therapeutic target because the protein performs many functions necessary for viral replication and its structure has been described with high precision. It is a multifunctional protein that acts as a cofactor in the viral polymerase complex. It can suppress the host's immune response, including the production of IFN, which is activated by type I receptors on the retinoic acid-inducible gene (RLR).

Hence, MARV VP35 can be considered a significant target. Ramachandran plot shows the geometry of protein with 122 residues that show good protein.

A current study revealed the role of phytocompounds from *M. oleifera* which have multiple medicinal properties according to Ayurveda. The research study states that some of the phytocompounds from *M. oleifera* have been found effective against MARV Disease. For the research

Table 2: ADME analysis of best-docked compounds based on Lipinski's rule

S. No	Ligand Name	Molecular Weight(g/mol)	H-Bond Donor	H-Bond Acceptor	Log^P	Molar Refractivity
1.	Melanin	318.28	2	4	1.20	93.74
2.	Diclazuril	407.64	1	4	3.65	99.49
3.	Tifentai	444.52	2	4	3.89	126.35

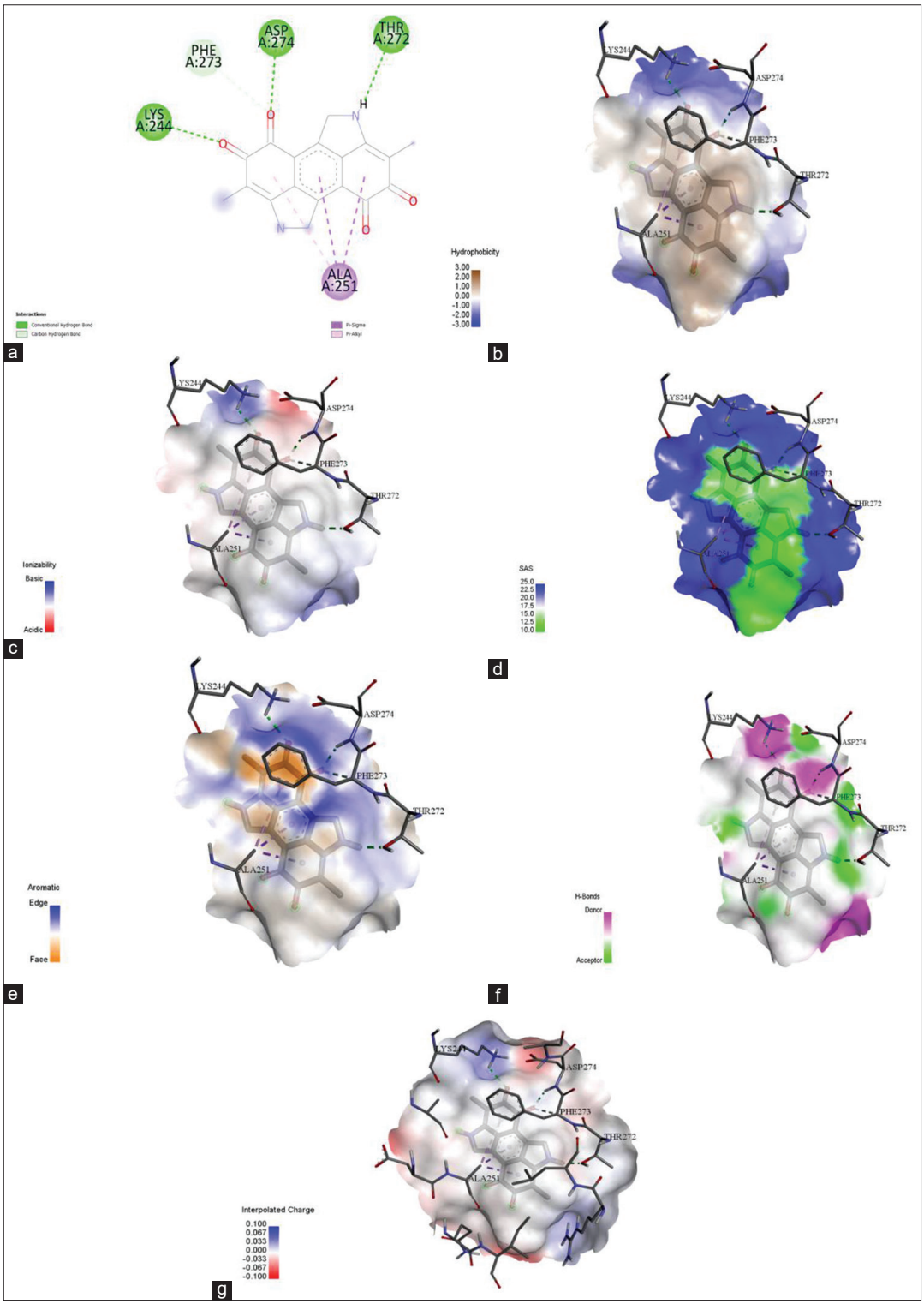

Fig. 2: 2D and 3D diagram of interactions of Melanin with Marburg VP35 (a) 2D Structure (b) Hydrophobicity (c) Ionizability (d) SAS (e) Aromatic (f) H-bonds (g) Interpolated Charges

Table 3: Toxicity Prediction using ProTox-2 Server

S. No.	Ligand Name	Predicted LD50(mg/kg)	Predicted Toxicity Class	Avg Similarity	Prediction accuracy
1.	Melanin	1000	4	53.68%	67.38%
2.	Diclazuril	1100	4	42.49%	54.26%
3.	Tifentai	550	4	73.55%	69.26%

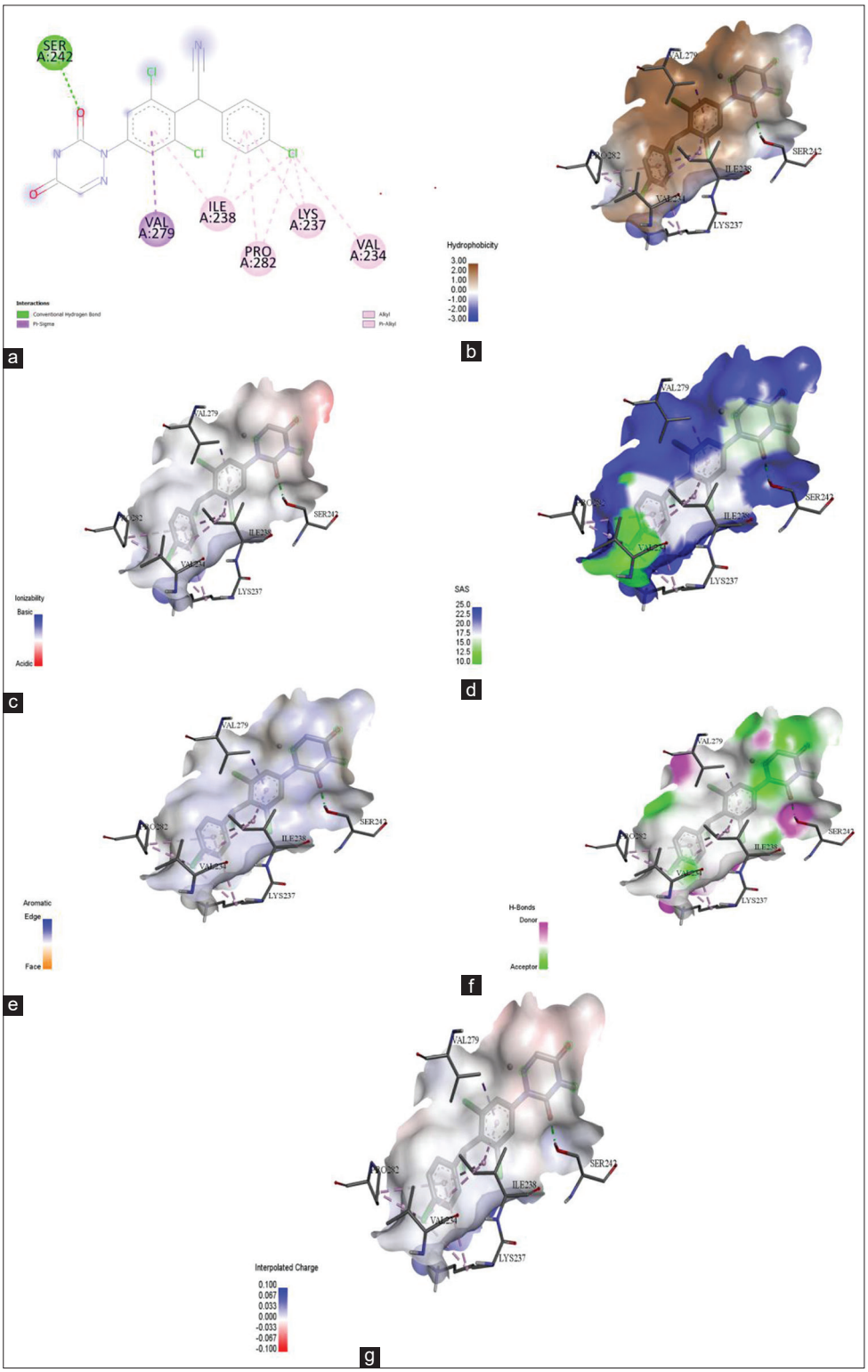

Fig. 3: 2D and 3D diagram of interactions of Diclazuril with Marburg VP35. a) 2D Structure b) Hydrophobicity c) Ionizability d) SAS e) Aromatic f) H-bonds f. Interpolated Charges

Table 4: ADME Analysis using ADMET lab 2.0

S. No.	Ligand Name	LogS	HIA	Pgp-sub	BBB	Carcinogenicity	Lipinski's Rule
1.	Melanin	−6.095	0.892	0.308	0.085	0.101	0 violations
2.	Diclazuril	−4.37	0.004	0.001	0.593	0.173	0 violation
3.	Tifentai	−4.418	0.546	0.003	0.114	0.484	0 violation

HIA: human intestinal absorption, BBB: Blood Brain Barrier, ADME: Absorption, distribution, metabolism, and excretion, Pgp-sub: Permeability glycoprotein substrate

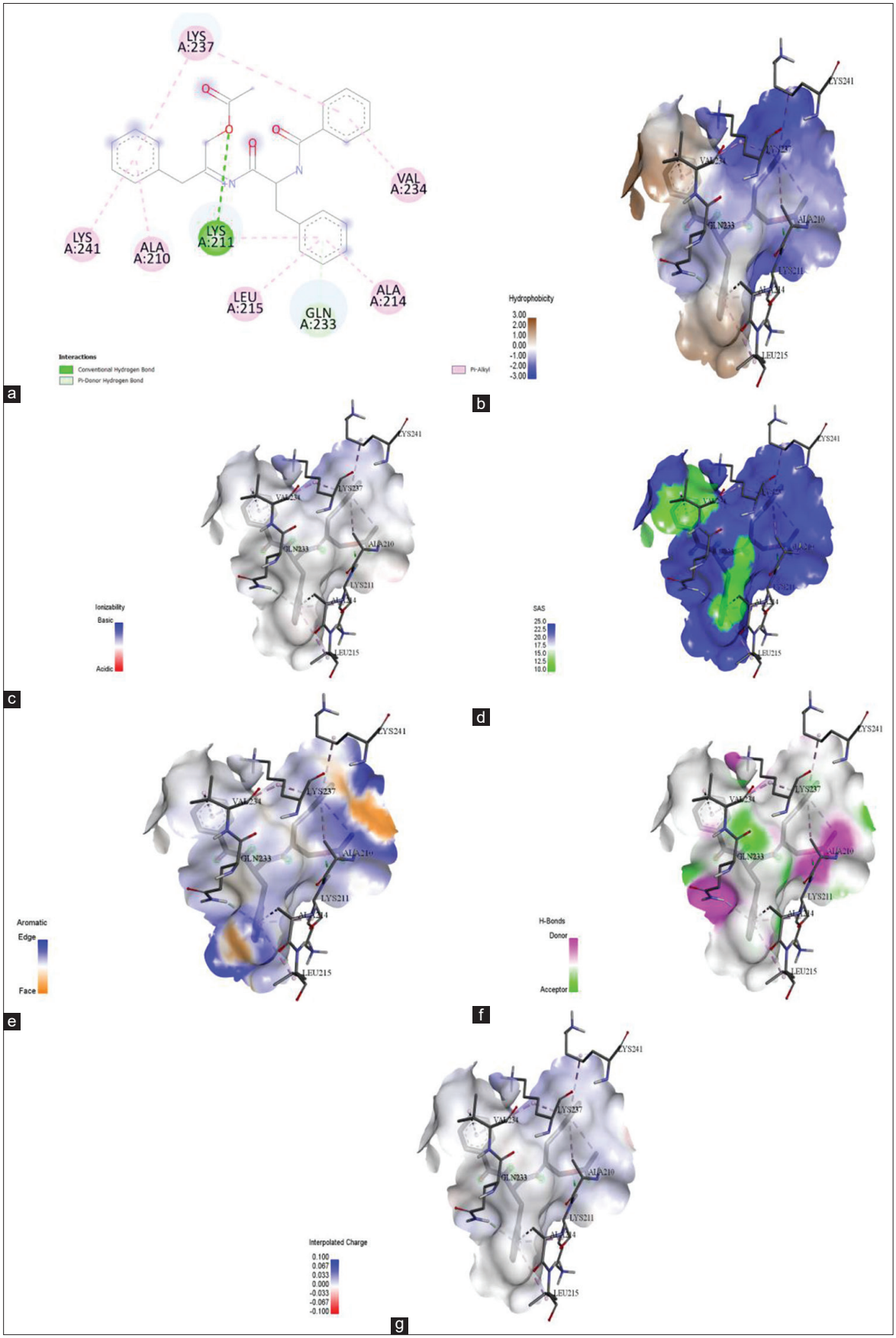

Fig. 4: 2D and 3D diagram of interactions of Tifentai with Marburg VP35 (a) 2D Structure (b) Hydrophobicity (c) Ionizability (d) SAS (e) Aromatic (f) H-bonds (g) Interpolated Charges

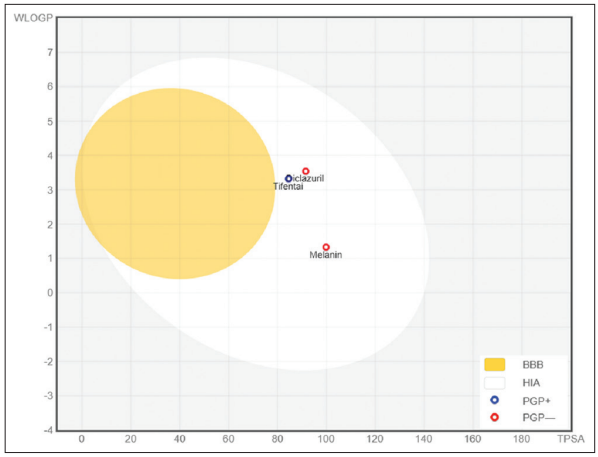

Fig. 5: Boiled-Egg analysis: Melanin, Diclazuril and Tifentai

study, VP35 protein (4GH9) was analyzed for the Ramachandran plot to validate the purity of the protein, and there were no outliers and poor rotamers observed. The 122 residues of protein were observed. For *in silico* study, the binding affinity of all the 461 phytocompounds was checked against MARV VP35 (PDB ID: 4GH9) using a molecular docking approach. Among them, three phytocompounds from *M. oleifera*, namely, Melanin, Diclazuril, and Tifentai have shown significant binding affinity. After the molecular docking study, all three compounds were further studied for ADME analysis to validate the drug likeness and ProTox-2 to validate the toxicity. The binding of these phytochemicals with VP35 helps in inhibits the RNA replication of the virus. Among these identified phytocompounds, Melanin and Diclazuril can be predicted as potential inhibitors based on their significant binding affinity, drug-likeness properties, ADMET prediction, toxicity prediction (based on Predicted LD50), and properties of biologically active substances (PASS Server). These phytocompounds are helps to inhibit RNA synthesis of viral protein into a host cell and also found safe and effective against MARV or MARV disease without toxicity.

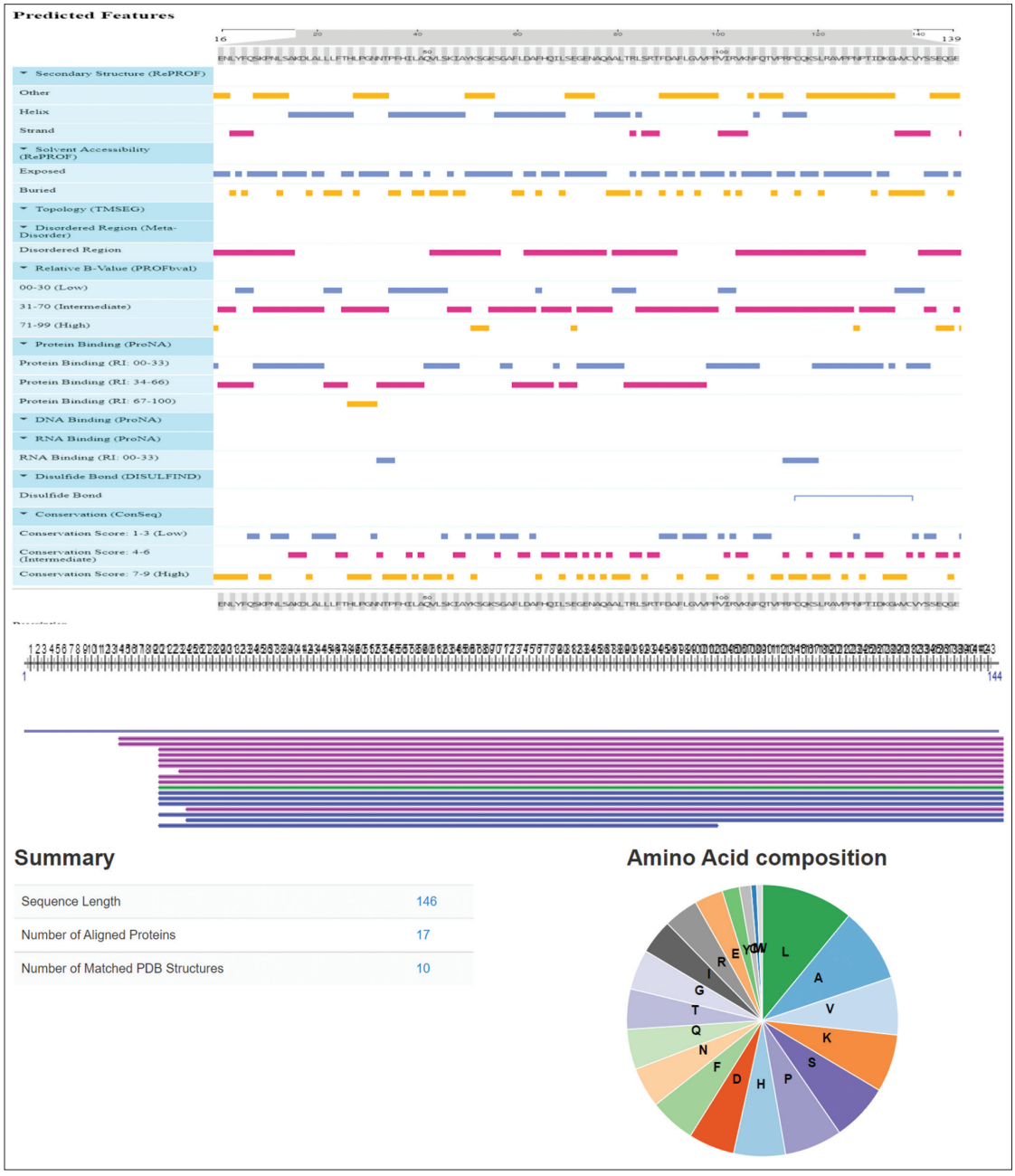

Fig. 6: Secondary structure prediction of VP35 Protein

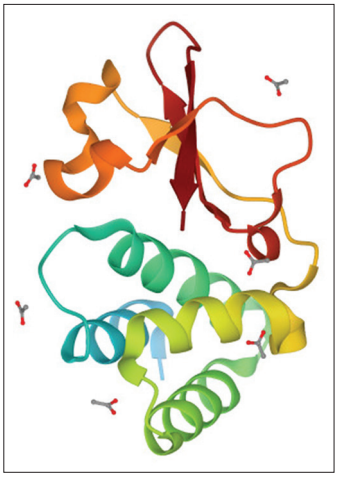

Fig. 7: 3D structure of VP35 RNA binding domain (4GH9) VP35 protein

CONCLUSION

This study aims to identify natural plant compounds from *M. oleifera* as therapeutic agents for Marburg disease. It is caused by the MARV, which was a major epidemic in Africa in the 19th century. The MARV VP35 protein has great potential and is a necessary target for the prevention of MARV disease. VP35 is a key protein essential for viral RNA synthesis to replicate within the host. Targeting the VP35 protein with natural phytocompounds inhibits RNA synthesis and thus resists further replication and proliferation. From this study, it can be concluded that two Phyto compounds of *M. oleifera* were predicted to suppress the action of VP35 by preventing RNA synthesis that contributes to host cell damage. These Phyto compounds have high inhibitory potency and the highest binding affinity with VP35. Best docked botanical compounds with drug-like properties, safe ADMET profile, toxicity prediction, and potency will aid in the development of optimized MARV inhibitors and treatment of MARV disease with Ayurvedic therapy.

ACKNOWLEDGMENT

We thank BioNome to provide support in training as well as in research projects.

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