VOLUME 9 (SPECIAL ISSUE 2) 2023

ISSN 2454 – 3055

Manuscripts under Special Issue are published under the theme "Yoga Practices and Alternative Therapies on Human Health"

Guest Editor: Dr. S. Mohanasundaram

Assistant Guest Editors: Mrs. K. Geetha

Mrs. M. Pennarasi

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 9 (Special Issue 2), 164-174 (2023)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Elucidation of Potent Inhibitors from Siddha Herbal Drug- Visha Jura Kudineer (VJK) Against RNA-Dependent RNA Polymerase, Dengue Viral Protein: An *In-Silico* Approach

Subathra T.[1*], Gomathi R.[2], Preetheekha E.[3], Mamallan A.[4], Shanmugapriya P.[1] and Srinivasan M.R.[2]

[1]Department of Nanju Maruthuvam, National Institute of Siddha, Tambaram Sanatorium, Chennai 47, India
[2]National Institute of Siddha, Tambaram Sanatorium, Chennai 47, India
[3]Medical Officer, Jain's Hospital, Tambaram, Chennai, India
[4]Kariyapatti Government Hospital, Aruppukottai, India

**Corresponding Author*

Received: 17th March, 2023; **Accepted:** 5th May, 2023; **Published online:** 5th August, 2023

https://doi.org/10.33745/ijzi.2023.v09ispl2.031

Abstract: The ancient traditional medicines are actively involved in therapeutics, especially in Siddha. In a recent past, dengue fever causes a significant impact to mankind all around the world. This study reports that the active biomolecules present in Siddha herbal can be used to ameliorate mosquito-borne viral infection. Visha Jura Kudineer (VJK) is a potent Siddha formulation prepared by 13 active herbs which has antiviral properties used to treat viral fevers. Molecular docking is an effective preliminary way to identify the activity of biomolecules against the protein of interest and studying its inhibitory function. This study mentioned the biomolecules present in VJK that was used to dock against Dengue Virus NS5 RNA- dependent RNA polymerase protein structure through Auto Dock software. The binding free energy obtained while docking for the compounds such as Cymol, Piperidine, Kaempferol, Ellagic acid, Andrographolide, Vasicoline, Glycyrrhetic acid, Withanolide are - 4.38 Kcal/mol, - 4.48 Kcal/mol, -6.17 Kcal/mol, -4.79 Kcal/mol, -5.67 Kcal/mol, -6.04 Kcal/mol, -7.25 Kcal/mol, -5.20 Kcal/mol, respectively. These findings confirm that VJK, Siddha drug may have possible beneficial effects in the treatment and management of Dengue. Further, wet lab assessments of VJK drug and its active mechanism against dengue viral fever yet to explore in future.

Keywords: Siddha medicine, Molecular docking, Herbal drug, Antiviral, Dengue, Visha Jura Kudineer

Citation: Subathra T., Gomathi R., Preetheekha E., Mamallan A., Shanmugapriya P. and Srinivasan M.R.: Elucidation of potent inhibitors from siddha herbal drug- Visha Jura Kudineer (VJK) against RNA-dependent RNA polymerase, dengue viral protein: An *in-silico* approach. Intern. J. Zool. Invest. 9(Special Issue 2): 164-174, 2023.

https://doi.org/10.33745/ijzi.2023.v09ispl2.031

Introduction

Dengue fever is caused by a vector-borne virus transmission of DENV through bites of two common mosquito species such as *Aedes aegypti* and *Aedes albopictus*. DENV belongs to Flaviviridae family and it is a single positive stranded RNA Virus. There are 4 serotypes identified towards DENV and those are antigenically considered as separate virus. The enzymatic activities of DENV protease and polymerase may be considered as the target to identify potential inhibitors while designing the antiviral drugs for dengue fever (Jenny *et al*., 2017).

Siddha system of medicine has enormous anti-viral potential drugs to treat different kinds of viral fevers. One recent research findings confirmed that the ethanolic extract of Siddha formulation Nilavembu kudineer has antiviral property against CHIKV and DENV infected cells. This Siddha polyherbal formulation Nilavaembu kudineer has found for its prophylactic activity which significantly prevents viral fever in humans. Similar such drug, Visha Jura Kudineer, a poly herbal Siddha formulation is being in use since ancient times to treat various fevers (Sathya *et al*., 2014).

Molecular docking analysis is one of the earlier steps in drug discovery process, to identify the pharmacological nature of bioactive molecules using Ligand-protein interaction. Binding of phytocomponents with the core amino acids of the targets by forming hydrogen bond will hinder the function of the enzyme RNA-dependent RNA polymerase since these are the prime mediators for dengue viral replication. Furthermore, the phytocomponents which inhibit this enzyme may act as a potential therapeutic agent for management of dengue fever (Jain *et al*., 2020).

The herbal ingredients present in this formulation VJK such *as Piper longum, Piper nigrum, Cyperusrotundus, Andrographis paniculata, Alpinia galangal* and *Terminalia chebula* had already reported for its antiviral effect individually. The active biomolecules such as Cymol, Piperidine, Kaempferol, Ellagic acid, Andrographolide, Vasicoline, Glycyrrhetic acid and Withanolide were selected from the present trial drug VJK for docking analysis against Dengue viral protein-RNA-dependent RNA polymerase structure to evaluate its binding nature (Stefano *et al*., 2019).

Though there are some antiviral drugs available to treat Dengue fever, still there is a vacant to identify a much more potent drug for the cure of dengue. At the same time, siddha formulations are traditionally being in clinical practice from olden days, scientific validation behind its therapeutic effect is the ultimate need of purpose. This study reports that the active biomolecules present in Siddha herbal can be used to ameliorate mosquito-borne viral infection.

Materials and Methods

Ingredients of VJK:

The herbs used as ingredients of VJK are *Piper nigrum, Piper longum, Alpinia galangal, Cyperus rotundus, Trichosanthus cucumerina, Mollungo cerviana, Withania somnifera, Andrographis paniculata, Terminalia chebula, Ocimum sanctum, Justicia adhatoda, Glycerrhiza glabra* and *Clerodendrum serratum.*

Ligand Extraction:

The structure of screened compounds such as Cymol, Piperidine, Kaempferol, Ellagic acid, Andrographolide, Vasicoline, Glycyrrhetic acid and Withanolide from plants used in Siddha drug VJK were retrieved from the Pubchem database (http://www.ncbi.nlm.nih.gov/pccompound) and used for the further studies.

Target Protein Extraction:

The receptor of Dengue Viral protein RNA-dependent RNA polymerase was downloaded from the Protein Data Bank (PDB) (http://www.rcsb.org/pdb/home.do) and the PDB ID is 2J7U as shown in Figure 1.

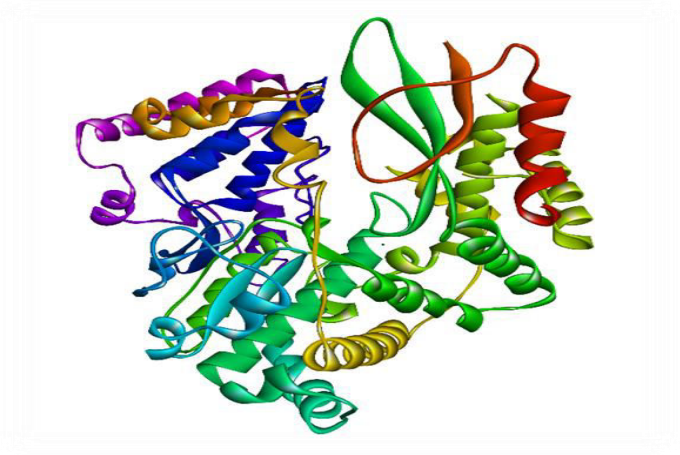


Fig. 1: Target Protein – Dengue virus NS5 RNA dependent RNA polymerase.

Protein preparation:

Crystalline structure of the target protein Human RNA-dependent RNA polymerase (2J7U) was retrieved from protein data bank and protein clean-up process was done and essential missing hydrogen atom were being added. Different orientation of the lead molecules with respect to the target protein was evaluated by Autodock 4 with the inclusion of polar hydrogens, rotatable bonds and merging of non-polar.

Active site prediction on the target protein:

Active amino acids involved in mediating the enzyme activity of Dengue viral protein structure (PDB ID 2J7U) was validated by using Ramachandran plot assessment and this plot was predicted by PROCHECK software. The Pepwheel analysis for the sequence of 2J7U protein was achieved through EMBOSS.

Protein-ligand docking:

Molecular docking study was carried out through Auto Dock version 4 for the prediction of binding affinity achieving through interactions between selected ligands from the Siddha formulation, VJK and target protein Dengue viral protein (PDB ID 2J7U).

Docking simulations:

Docking calculations were carried out using Auto Dock 4. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged and rotatable bonds were defined. Docking calculations were carried out for the screened molecules Cymol, Piperidine, Kaempferol, Ellagic acid, Andrographolide, Vasicoline, Glycyrrhetic acid and Withanolide against target protein Dengue Viral protein RNA-dependent RNA polymerase (2J7U). Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of Auto Dock tools. Affinity (grid) maps of ×× Å grid points and 0.375 Å spacing were generated using the Autogrid program. AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis and Wets local search method. Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 2 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied.

Table 1: List of Phytocomponents selected for docking based on herbs present in *VJK*

S. No.	Herbs	Tamil name	Family name	Bioactive molecules
1	*Piper longum*	Thipilli	Piperaceae	Cymol
2	*Piper nigrum*	Milagu	Piperaceae	Piperidine
3	*Alpinia galangal*	Peraraththai	Zingiberaceae	Kaempferol
4	*Terminalia chebula*	Kadukkai	Combretaceae	Ellagic acid
5	*Andrographis paniculata*	Nilavaembu	Acanthaceae	Andrographolide
6	*Justicia adhatoda*	Adathodai	Acanthaceae	Vasicoline
7	*Glycerrhiza glabra*	Athimathuram	Fabaceae	Glycyrrhetic acid
8	*Withania somnifera*	Amukkura	Solanaceae	Withanolide

Results and Discussion

The compound siddha herbal formulation Visha Jura Kudineer includes *Piper longum, Piper nigrum, Alpinia galangal, Terminalia chebula, Andrographis paniculata, Justicia adhatoda, Glycerhiza glabra* and *Witahnia somnifera*. The selected phytochemicals are taken for docking (Table 1).

Ramachandran plot assessment:

The predicted RNA-dependent RNA polymerase Dengue Viral protein Structure (2J7U) was validated by using Ramachandran plot using PROCHECK software which shows the protein molecule contains amino acids of 573 residues in that 463 in most favored region, 36 in additionally allowed region, 0 in generally allowed region and 4 were disallowed region (Fig. 2).

Docking Analysis:

Molecular docking is a very good beneficial tool in drug discovery to identify the potency of lead biomolecules against target proteins (Choudhary and Singh, 2018). Docking process indicates the binding affinity level between the lead molecules and target proteins. The potency of the drug is assessed by the higher the negativity in that value (Karthiga *et al*., 2010). 2D and 3D Ligand structure of bioactive compounds and molecular docking 3D pose and HD Plot results of selected biomolecules of plants in Siddha drug VJK, the target Dengue Viral protein RNA- dependent RNA polymerase (2J7U) is shown in the Tables 2 and 3 (Chao and Lin, 2010).

The phyto-compounds are ranked based on amino acid residue interactions of Human RNA-dependent polymerase-Dengue virus for molecular docking (Table 4).

Total of 8 bioactive lead molecules from reported data of the herbs belongs to the Siddha drug VJK which includes Glycyrrhetic acid, Kaempferol, Vasicoline, Andrographolide, Withanolide, Ellagic acid, Piperidine and Cymol got the binding energy value of -7.25 Kcal/mol, -6.17 Kcal/mol, -6.04 Kcal/mol, -5.67 Kcal/mol, -5.20 Kcal/mol, -4.79 Kcal/mol, -4.48 Kcal/mol, -4.38 Kcal/mol, respectively (Table 5).

The amino acid residue which observed during the interaction sequential analysis indicates its potential enzymatic action of RNA-dependent RNA polymerase enzyme present in Dengue Virus protein molecule (Table 6) (Evanthia *et al*., 2014).

These amino acids binding indicates the higher chances of enzyme inhibition. Out of eight compounds the phytocomponents such as Vasicoline, Glycyrrhetic acid, Withanolide and

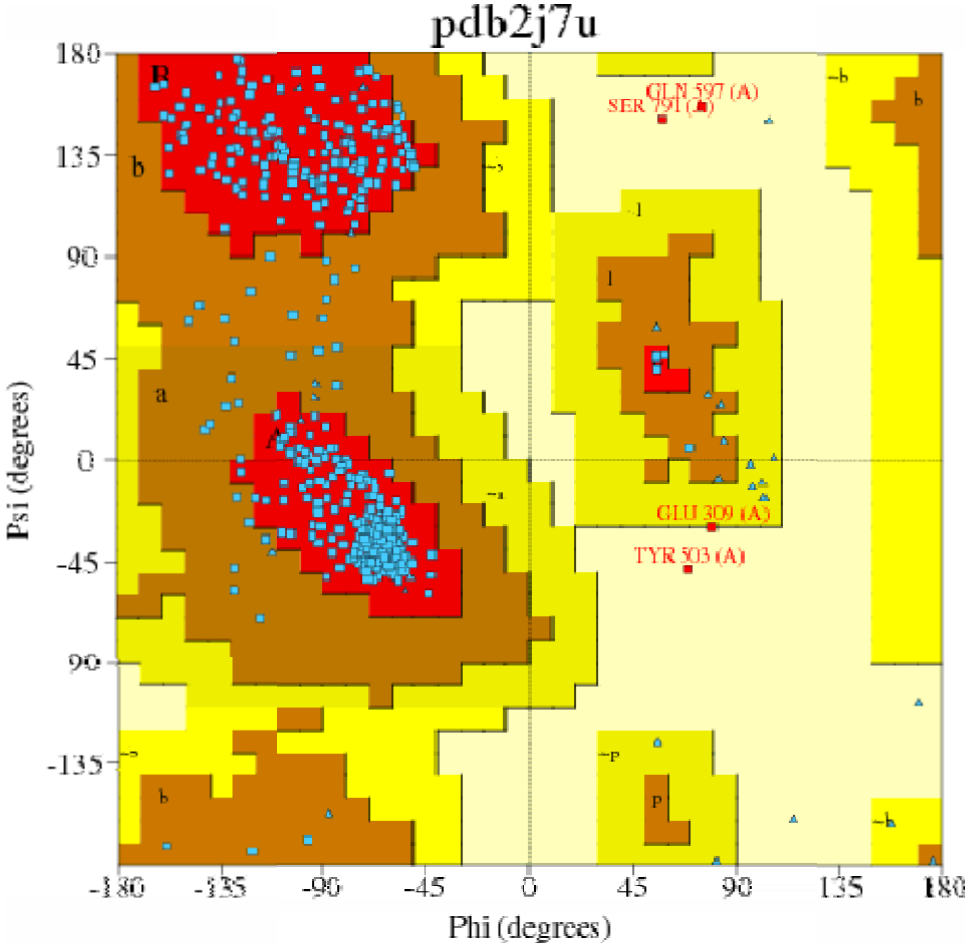
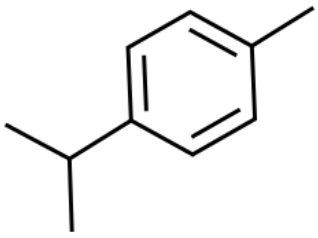
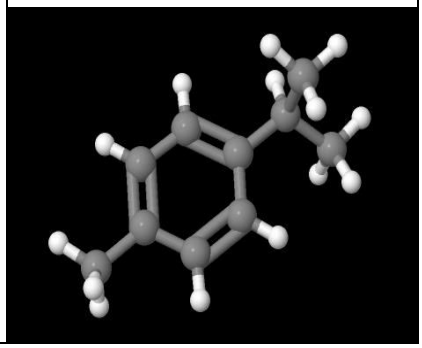
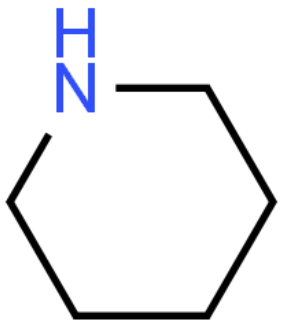
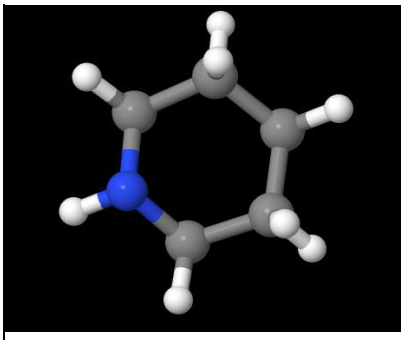
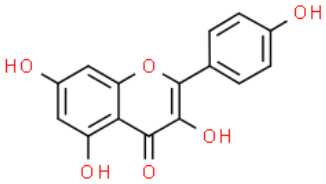
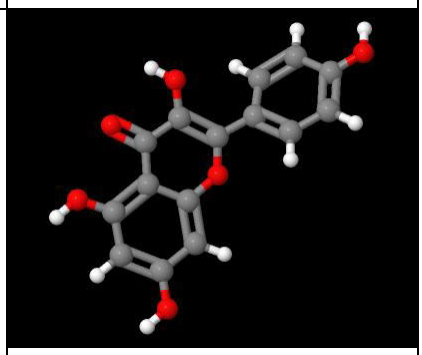
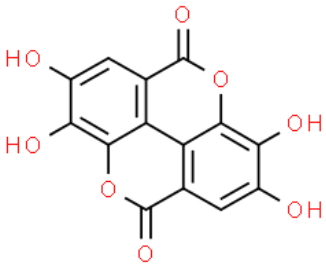
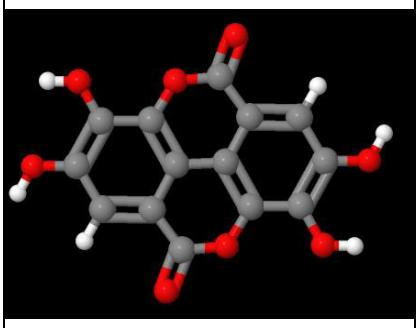
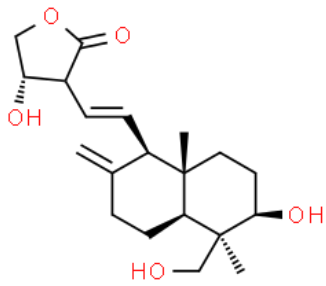
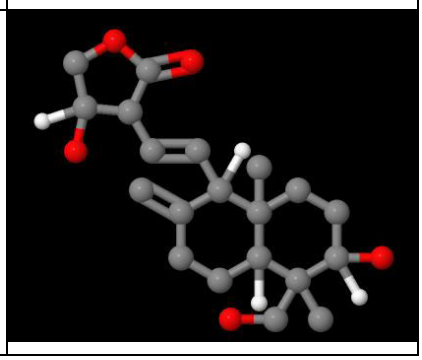


Fig. 2: Ramachandran Plot.

Table 2: 2D and 3D structures of ligands from the herbs used in the formulation VJK

S. No.	Compounds	2D Structures	3D Structures
1	Cymol		

2	Piperidine		
3	Kaempferol		
4	Ellagic acid		
5	Andrographolide		

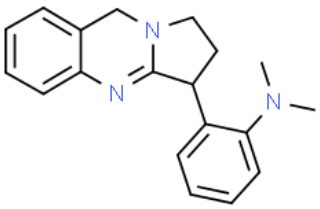
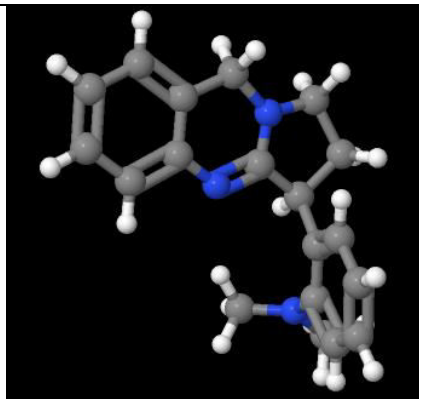
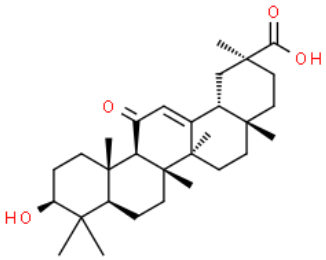
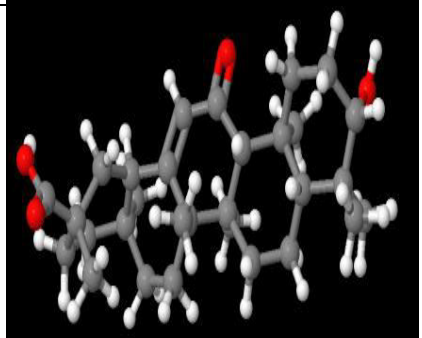
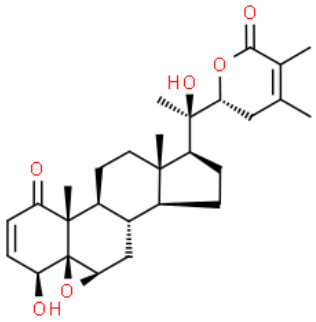
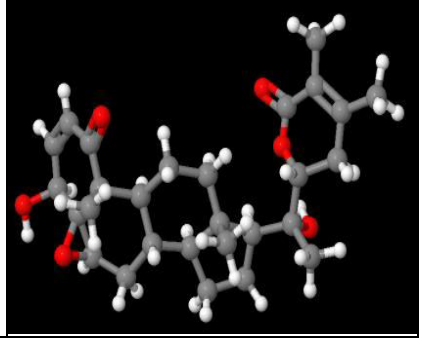
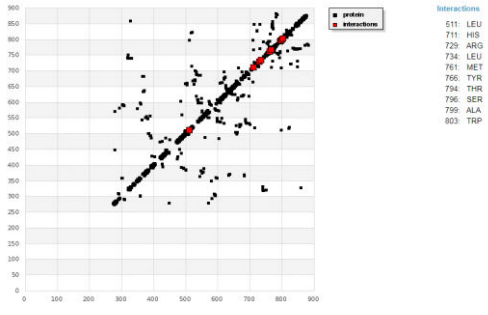
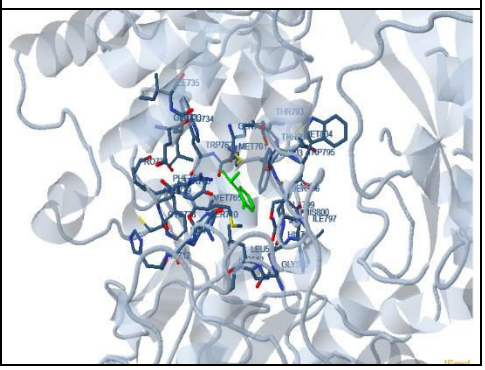
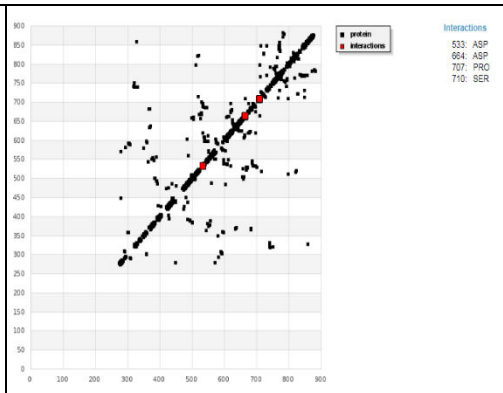
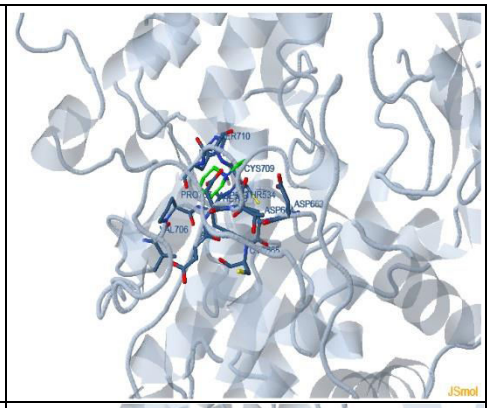
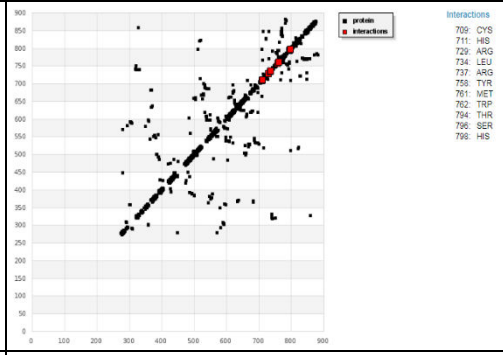
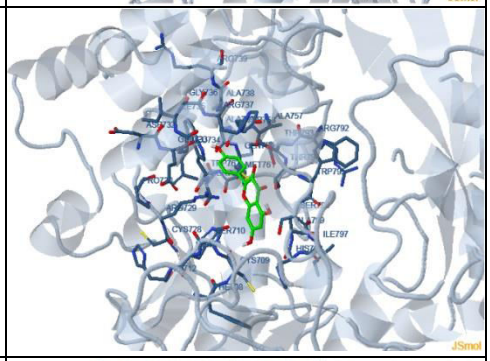
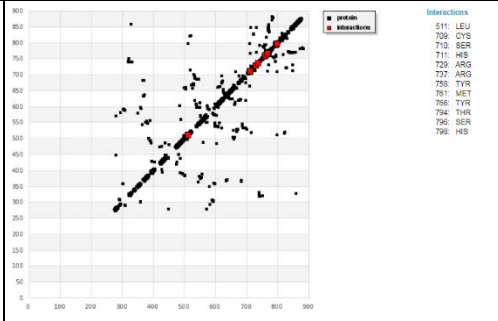
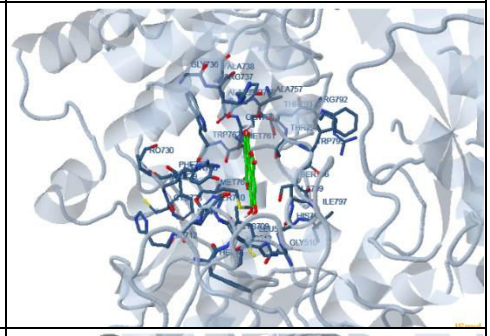
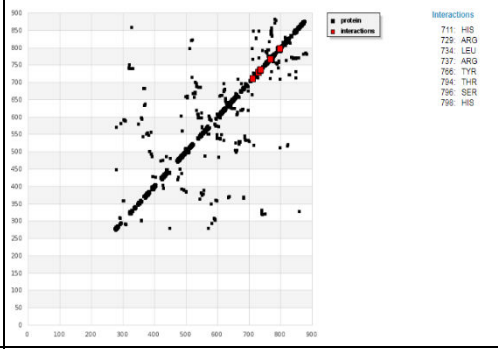
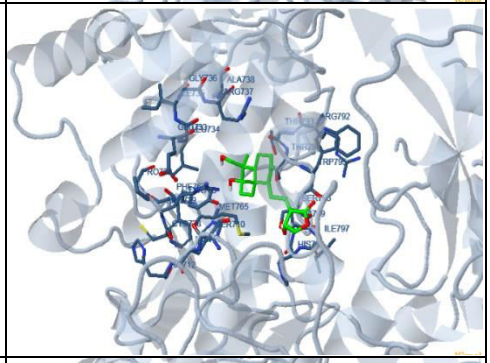
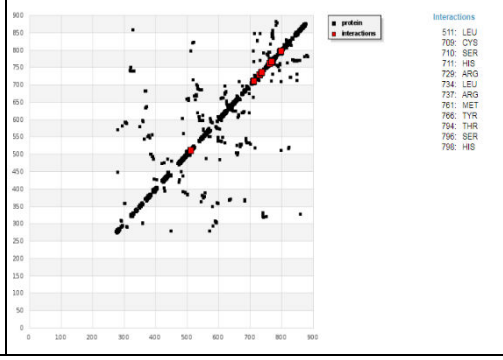
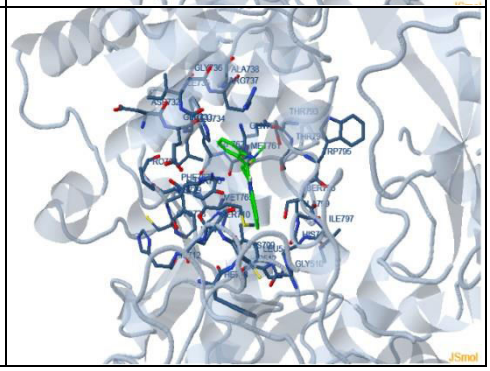
6	Vasicoline		
7	Glycyrrhetic acid		
8	Withanolide		

Table 3: Molecular docking analysis 3D pose and HD plot results of selected biomolecules of plants in siddha drugpolymerase (2J7U) VJK target Dengue Viral protein RNA-dependent RNA

S. No.	Bioactive Compounds	HB plotting analysis	3D Docking pose
1	Cymol		

2	Piperidine		
3	Kaempferol		
4	Ellagic acid		
5	Andrographolide		
6	Vasicoline		

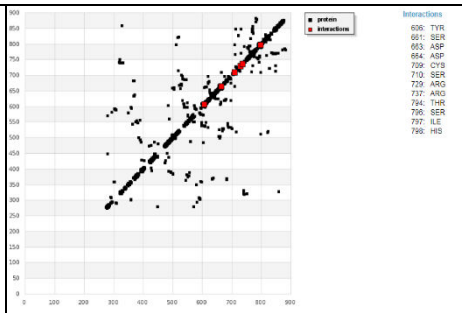
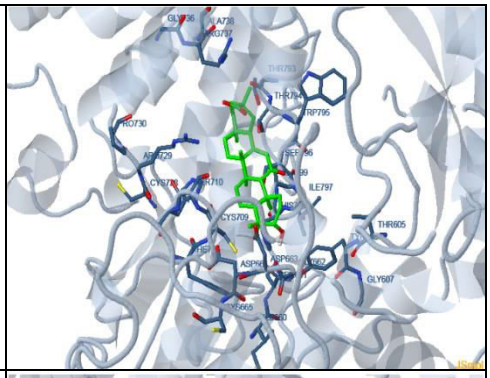
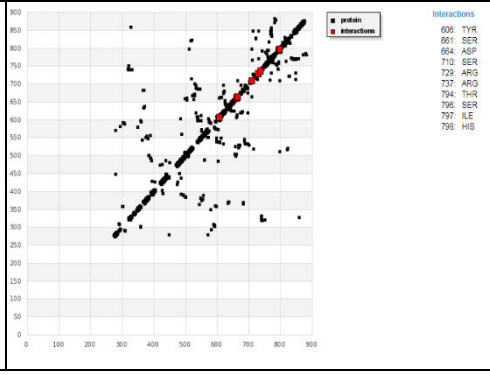
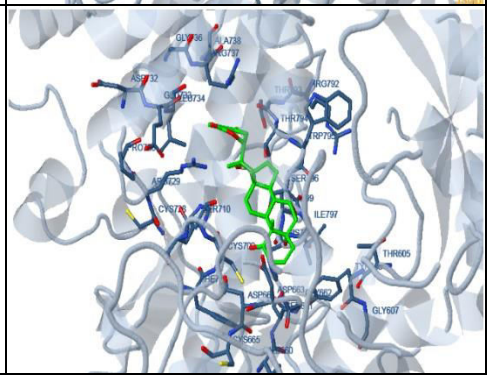
7	Glycyrrhetic acid		
8	Withanolide		

Table 4: Ranking of compounds based on Amino acid residue interactions of Human RNA- dependent RNA polymerase – Dengue Virus

Name of Phytocomponents	**Number of Interactions**	**Rank**
Cymol	1	III
Piperidine	1	III
Kaempferol	2	II
Ellagic acid	3	I
Andrographolide	2	II
Vasicoline	3	I
Glycyrrhetic acid	3	I
Withanolide	3	I

Table 5: Summary of the molecular docking studies of compounds against Human RNA-dependent RNA polymerase – Dengue Virus- PDB 2J7U Receptor

Compounds	Binding Free energy Kcal/mol	Inhibition constant Ki µM (*mM)(**nM)	Electrostatic energy Kcal/mol	Intermolecular energy Kcal/mol	Total Interaction Surface
Cymol	-4.38	611.96	0.01	-4.68	451.91
Piperidine	-4.48	518.58	-1.50	-5.05	359.90
Kaempferol	-6.17	29.84	-0.91	-6.61	631.54
Ellagic acid	-4.79	307.80	0.04	-5.00	578.96
Andrographolide	-5.67	70.01	0.01	-7.04	733.95
Vasicoline	-6.04	37.67	0.76	-5.95	688.31
Glycyrrhetic acid	-7.25	4.84	-0.71	-7.91	871.52
Withanolide	-5.20	154.19	-7.96	-0.07	888.11

Table 6: Amino acid Residue Interaction of Lead and Standard against Human RNA-dependent RNA polymerase – Dengue Virus

Name of Phytocomponents	Number of Interactions	Amino Acid Residue- Binding											
Cymol	1	511 LEU	711 HIS	729 ARG	734 LEU	761 MET	766 TYR	794 THR	796 SER	799 ALA	803 TRP		
Piperidine	1	533 ASP	664 ASP	707 PRO	710 SER								
Kaempferol	2	709 CYS	711 HIS	729 ARG	734 LEU	737 ARG	758 TYR	761 MET	762 TRP	794 THR	796 SER	798 HIS	
Ellagic acid	3	511 LEU	709 CYS	710 SER	711 HIS	729 ARG	737 ARG	758 TYR	761 MET	766 TYR	794 THR	796 SER	798 HIS
Andrographolide	2	711 HIS	729 ARG	734 LEU	737 ARG	766 TYR	794 THR	796 SER	798 HIS				
Vasicoline	3	511 LEU	709 CYS	710 SER	711 HIS	729 ARG	734 LEU	737 ARG	761 MET	766 TYR	794 THR	796 SER	798 HIS
Glycyrrhetic acid	3	606 TYR	661 SER	663 ASP	664 ASP	709 CYS	710 SER	729 ARG	737 ARG	794 THR	796 SER	797 ILE	798 HIS
Withanolide	3	606 TYR	661 SER	664 ASP	710 SER	729 ARG	737 ARG	794 THR	796 SER	797 ILE	798 HIS		

Ellagic acid has all 3 interactions (100%), similarly Kaempferol and Andrographolide possess 2 significant interactions (75%), hence it was observed that these compounds have significant RNA-dependent RNA polymerase enzyme present in Dengue Virus inhibition activity.

Conclusion

Based on the results of the computational analysis, it was concluded that the compounds such as Vasicoline, Glycyrrhetic acid, Withanolide,Ellagic acid, Kaempferol and Andrographolide present in the formulation VJK possess significant inhibition of RNA- dependent RNA polymerase enzyme (PDB ID:2J7U) present in Dengue Virus. Thereby, it is concluded that this formulation may have promising Dengue viral inhibition activity. Further preclinical and clinical investigations are needed for the safe and effective use of siddha drug VJK.

Acknowledgements

The authors gratefully acknowledge the financial support provided by the Kerala State Council for Science Technology and Environment (KSCSTE), Government of Kerala, India.

References

Chao WW and Lin B. (2010) Isolation and identification of bioactive compounds in *Andrographis paniculata* (Chuanxinlian). Chin Med. 5: 17.

Choudhary N and Singh V. (2018) A census of *P. longum*'s phytochemicals and their network pharmacological evaluation for identifying novel drug-like molecules against various diseases, with a special focus on neurological disorders. PLoS One 13(1): e0191006.

Evanthia L, George S, Demetrios KV and Zoe C. Structure-based virtual screening for drug discovery: principles, applications and recent advances. Curr Top Med Chem. 14: 1923-1938.

Jain J, Kumar A, Narayanan V, Ramaswamy RS, Sathiyarajeswaran P, Shree Devi MS, Kannan M and Sunil S, (2020) Antiviral activity of ethanolic extract of Nilavembu Kudineer against dengue and chikungunya virus through *in vitro* evaluation. J Ayurveda Integr Med. 11(3): 329-335.

Jenny GH, Low Eng Eong Oo and Subhash GVasudevan (2017) Current Status of Dengue Therapeutics Research and Development, J Infect Dis. 215(Suppl 2): S96-S102.

Karthiga Reshmi, Sathya E Devi PS. (2010) Isolation of piperdine from *Piper nigrum* and its antiproliferative activity. J Med Plants Res. 4: 1535-1546.

Sathya B, Velpandian V, Ramani M and Kumar MP. (2014) A primitive approach on review of siddha herbs, herbo-mineral formulation exhibiting antiviral activity. Int J Pharm Bio Sci. 5(4): 138-147.

Stefano F, Ruth H, Michael EP, Michel S and David SG, Arthur JO. (2016) Computational protein- ligand docking and virtual drug screening with the AutoDock suite. Nat Protoc. 11: 905-919.