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Research Article

# Molecular Docking Studies: Hepatitis C Viral-RNA-Dependent RNA Polymerase (RdRp) Inhibitory Activity of Siddha Herbal Formulation *Sittramutti Khirutham* (SK)

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## ABSTRACT

**Background:** Molecular docking has great potential in Siddha medicine, especially in herbal formulations where one can better understand the basic biochemical processes that the formulation addresses by understanding the molecular interactions between the formulation's lead molecules and receptors.**Aim and Objectives:** Molecular docking investigations of Siddha herbal formulation *Sittramutti khirutham* and screening the lead component interaction on the Hepatitis C virus-RNA-Dependent RNA Polymerase (RdRP).**Materials and Methods:** Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with Autodock tools (Morris, Goodsell, *et al*., 1998). Docking simulations were performed using the Lamarckian Genetic Algorithm (LGA) and the Solis and Wets local search method. The initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking.**Results and Conclusion:** The compounds present in SK like Curcumin, Maslinic acid, Arjungenin, Andrograpanin, Andrographolide, and Epiafzelechin reveal a maximum of 2-3 interactions with the core active amino acid residues present on the target protein enzyme Hepatitis C viral -polymerase (RdRp). Hence, these compounds of the test drug possess promising Hepatitis C viral-RNA-dependent RNA Polymerase (RdRp) inhibition activity. For prospective pharmacological validation of SK, the docking studies were an important step for its scientific justification.

**Keywords:** Hepatitis C virus, *Sittramutti khirutham*, Hepatitis C viral polymerase, Molecular docking, Siddha polyherbal medicine.

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## INTRODUCTION

Hepatitis C is an infectious liver disease caused by the Hepatitis C Virus (HCV).[1] HCV infection can be self-limited (viral elimination) or persistent.[2] An estimated 170 million people worldwide are thought to be infected with HCV, with more than 40% of instances occurring in Asian nations.[3] Approximately 15% of people experience viral clearance, characterized by undetectable HCV RNA in multiple blood tests from those with anti-HCV antibodies or acute infections.[4] About 85% of people get chronic HCV infection, which causes the virus to keep replicating and linger in their blood for the rest of their lives.[5] While some cases of acute HCV infection resolve on their own, 60-80% of these people have chronic illness as a result of the virus defeating the host's innate and adaptive immune systems.[6-9] The Siddha classical text *Sigicha Rathina Deepam* mentions the formulation named "*Sittramutti khirutham*" for the management of Hepatitis C Virus..[10] The primary components of this herbal combination are *Andrographis panniculatus, Sida cordifolia, Terminalia chebula, Terminalia bellrica, Phyllanthus embellica, Madhuca longifolia,* and *Curcuma longa*. The main compounds in this formulation were evaluated for their ability to inhibit "Hepatitis C viral-RNA-Dependent RNA Polymerase (RdRP)" to provide a more comprehensive research viewpoint. This might contribute to SK increased acceptance as a potent herbal anti-hepatitis or anti-viral composition.

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## MATERIALS AND METHODS

This study investigates Molecular Docking (MD) interactions between key phytochemicals from SK and the "Hepatitis C virus RNA-Dependent RNA Polymerase (RdRp) enzyme (PDB ID: 3MWV)". The docking focuses on evaluating hydrogen bonding with critical amino acid residues "(Asp220, Asp318, and Asp319)" in the active site, aiming to disrupt RdRp activity, inhibit viral RNA replication, and block NS5B protein synthesis. Phytochemicals studied include curcumin, gallic acid, maslinic acid, arjungenin, betulonic acid, andrograpanin, andrographolide, and epiafzelechin. Details of the target protein model are shown in Table 1 and Figure 1, with ligand properties in Table 2.

### Target Details and Receptor Structure

The 3D structure of the "Hepatitis C virus RNA-Dependent RNA Polymerase (RdRp)" is available in the Protein Data Bank (PDB) under the accession ID 3MWV, as illustrated in Figure 2.

The crystal structure of the Hepatitis C virus RNA-Dependent RNA Polymerase (RdRp) enzyme (PDB ID: 3MWV) was obtained from the Protein Data Bank. The protein was prepared by performing a clean-up process, which included the addition of missing hydrogen atoms as required. The AutoDock software was used to evaluate various orientations of the lead compounds relative to the target protein. The optimal docking pose was identified based on an analysis of the interaction studies.

## TOOL FOR STUDY

Docking calculations for the selected phytocomponents were performed against the Hepatitis C virus RNA-dependent RNA polymerase enzyme. Essential hydrogen atoms, Kollman charges, and solvation parameters were added using AutoDock tools. Affinity maps with dimensions of ×× Å grid points and 0.375 Å spacing were created using Auto grid. The van der Waals and electrostatic interactions were calculated using AutoDock parameters and distance-dependent dielectric functions.

Simulations employed the Lamarckian Genetic Algorithm (LGA) and Solis and Wets local search method. Ligand positions, orientations, and torsions were randomly initialized, with all bonds made flexible. Each docking run terminated after 250,000 energy evaluations with a population size of 150, applying 0.2 Å translational steps and quaternion/torsion steps of 5.[17-21]

## RESULTS

Eight bioactive lead compounds were identified from the herbs used in the Siddha formulation SK. Based on reported data, phytochemicals such as curcumin, maslinic acid, arjungenin, andrograpanin, andrographolide, and epiafzelechin demonstrated 2-3 interactions with the key active amino acid residues of the Hepatitis C virus RNA-Dependent RNA Polymerase (RdRp) enzyme. The results of the molecular docking studies, including the interactions between these compounds and the amino acid residues of the target protein (PDB ID: 3MWV), are summarized in Tables 3 and 4. Our results indicate that SK exhibits significant binding affinity towards the RdRp enzyme, suggesting its potential as a therapeutic agent against HCV. The binding energy of SK to RDRP was found to be comparable to that of known RdRp inhibitors. The inhibitory activity of SK against RdRp can be attributed to the presence of various phytochemicals, including flavonoids, alkaloids, and glycosides.[26] These compounds have been reported to exhibit antiviral activity against various viruses, including HCV.[25] The results of the molecular docking studies of the selected compounds against the Hepatitis C virus RNA-Dependent RNA Polymerase (RdRp), using the crystal structure with PDB ID 3MWV, are summarized in Table 3.

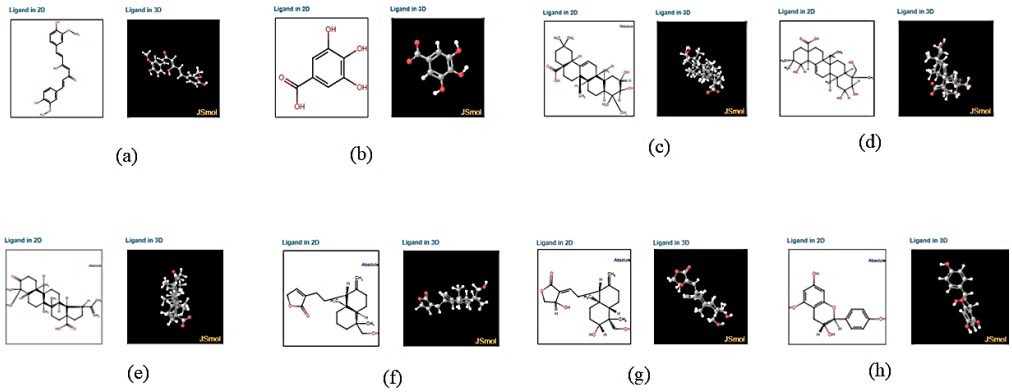



**Figure 1:** 2D And 3D Structure of Lead Compounds.

**3D- Structure of Hepatitis C viral -polymerase (RdRp) (PDB) - 3MWV**

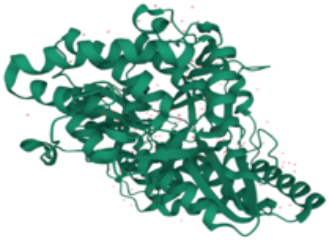


**Figure 2:** Q Receptor Structure.

(a) (b) (c)

**Figure 3:** Curcumin.

**Table 1: Compound retrieved from herbal sources.**

| Herbs | Phytochemicals |
|---|---|
| *Curcuma longa* L. | Curcumin[11] |
| *Terminalia chebula* Retz. | Gallic acid<br>Maslinic acid[12] |
| *Terminalia bellirica* (Gaertn.)Roxb. | Arjungenin[13] |
| *Phyllanthus Emblica* L. | Betulonic acid[14] |
| *Andrographis paniculata* (Burm.f.) Nees | Andrograpanin<br>Andrographolide[15] |
| *Madhuca longifolia* (J.Konig) J.F.Macbr. | Epiafzelechin[16] |
| *Pavonia zeylanica* L. | Unable to retrieve the phytocompounds |

The amino acid residue interactions of the lead compounds with Hepatitis C virus RNA-Dependent RNA Polymerase (RdRp) (PDB ID: 3MWV) are presented in Table 4.

Docking Poses of Various Herbs Depicted in Figures 3-10 (a) interaction with Hepatitis C viral -Polymerase (RdRp) (PDB) -3MWV, (b and c) 2D Interaction Plot Analysis.

## DISCUSSION

Molecular docking is a widely used technique to predict the pharmacological effectiveness of drugs or formulations. This approach holds significant promise in Siddha medicine, especially for herbal formulations. By examining the atomic-level interactions between the active molecules in these formulations and their target receptors, it is possible to gain insights into the fundamental biochemical processes the formulation aims to influence.[22,23]

Hepatitis C Virus (HCV) is a major global health issue, with approximately 50 million people affected worldwide and around 1 million new infections annually, according to the World Health Organization (WHO). The current standard treatment for HCV involves a combination of Direct-Acting Antivirals (DAAs), which have greatly improved therapeutic outcomes.[24,25] However, the high cost of these drugs and the development of Resistance-Associated Variants (RAVs) have highlighted the need for alternative treatment options.

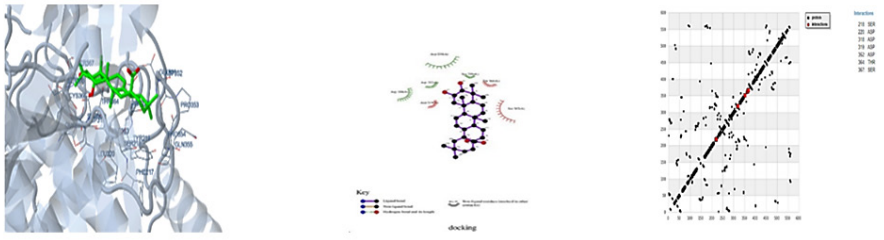
Siddha medicine, a traditional system of healing practiced in India and Sri Lanka, offers a wealth of herbal remedies with potential antiviral properties.[26-28] SK is one such herbal

**Table 2: Ligand Properties of the Compounds Selected for Docking Analysis.**

| Compound | Molar weight g/mol | Molecular Formula | H Bond Donor | H Bond Acceptor | Rotatable bonds |
|---|---|---|---|---|---|
| Curcumin | 368.4 g/mol | $C_{21}$ $H_2O$ $O_6$ | 2 | 6 | 8 |
| Gallic acid | 170.12g/mol | $C_7H_6O_5$ | 4 | 5 | 1 |
| Maslinic acid | 472.7 g/mol | $C_{30}$ $H_{48}$ $O_4$ | 3 | 4 | 1 |
| Arjungenin | 504.708 g/mol | $C_{30}H_{48}O_6$ | 5 | 6 | 2 |
| Betulonic acid | 454.7 g/mol | $C_{30}$ $H_{46}$ $O_3$ | 1 | 3 | 2 |
| Andrograpanin | 318.4 g/mol | $C_{20}H_{30}O_3$ | 1 | 3 | 4 |
| Andrographolide | 350.4 g/mol | $C_{20}H_{30}O_5$ | 3 | 5 | 3 |
| Epiafzelechin | 274.27 g/mol | $C_{15}H_{14}O_5$ | 4 | 5 | 1 |

(a) (b) (c)

**Figure 4:** Gallic acid.



(a) (b) (c)

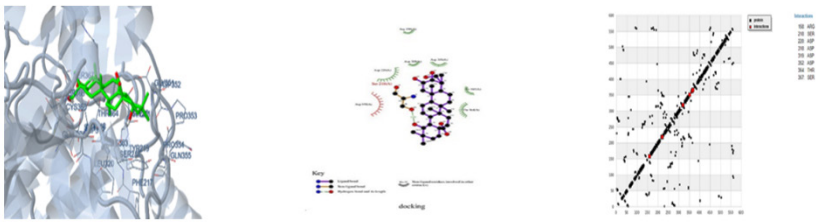
**Figure 5:** Maslinic acid.

**Table 3:** Summary of the molecular docking studies of compounds against Hepatitis C viral -polymerase (RdRp) (PDB) - 3MWV.

| Compound | Est. Free Energy of Binding | Est. Inhibition Constant, Ki | Electrostatic Energy | Total Intermole. Energy | Interact. Surface |
|---|---|---|---|---|---|
| Curcumin | -6.04 kcal/mol | 37.22 uM | -0.34 kcal/mol | -6.80 kcal/mol | 792.224 |
| Gallic acid | -5.89 kcal/mol | 48.52 uM | -1.83 kcal/mol | -5.40 kcal/mol | 440.727 |
| Maslinic acid | -8.18 kcal/mol | 1.02 uM | -0.21 kcal/mol | -7.96 kcal/mol | 823.14 |
| Arjungenin | -8.76 kcal/mol | 379.52 nM | -0.82 kcal/mol | -8.96 kcal/mol | 828.679 |
| Betulonic acid | -6.73 kcal/mol | 11.74 uM | -1.14 kcal/mol | -7.31 kcal/mol | 867.857 |
| Andrograpanin | -6.86 kcal/mol | 9.35 uM | -0.06 kcal/mol | -7.92 kcal/mol | 652.501 |
| Andrographolide | -6.76 kcal/mol | 11.13 uM | -0.20 kcal/mol | -7.53 kcal/mol | 617.651 |
| Epiafzelechin | -4.53 kcal/mol | 478.23 uM | -0.58 kcal/mol | -5.49 kcal/mol | 563.784 |

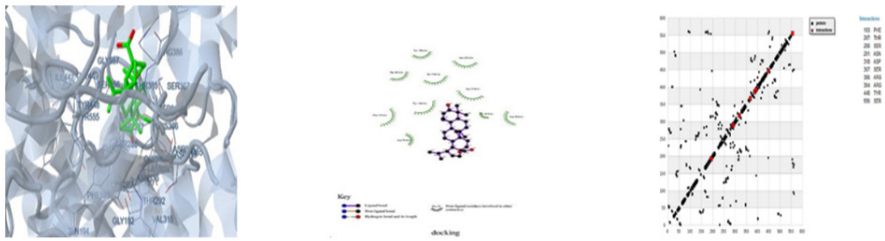
**Table 4:** Amino acid interactions of lead compounds with HCV RdRp (PDB ID: 3MWV).

| Compounds | Interaction | Amino Acid Residues | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Curcumin | 3 | 158 | 218 | 220 | 318 | 319 | 352 | | | |
| | | ARG | SER | ASP | ASP | ASP | ASP | | | |
| Gallic acid | 1 | 48 | 52 | 158 | 223 | 224 | 225 | 226 | 318 | |
| | | ARG | VAL | ARG | CYS | PHE | ASP | SER | ASP | |
| Maslinic acid | 3 | 218 | 220 | 318 | 319 | 352 | 364 | 367 | | |
| | | SER | ASP | ASP | ASP | ASP | THR | SER | | |
| Arjungenin | 3 | 158 | 218 | 220 | 318 | 319 | 352 | 364 | 367 | |
| | | ARG | SER | ASP | ASP | ASP | ASP | THR | SER | |
| Betulonic acid | 1 | 193 | 287 | 288 | 291 | 318 | 367 | 386 | 394 | 448 |
| | | PHE | THR | SER | ASN | ASP | SER | ARG | ARG | TYR |
| Andrograpanin | 2 | 218 | 220 | 319 | 352 | 355 | 364 | | | |
| | | SER | ASP | ASP | ASP | GLN | THR | | | |
| Andrographolide | 2 | 218 | 220 | 319 | 352 | 364 | 367 | | | |
| | | SER | ASP | ASP | ASP | THR | SER | | | |
| Epiafzelechin | 3 | 218 | 220 | 318 | 319 | 352 | | | | |
| | | SER | ASP | ASP | ASP | ASP | | | | |



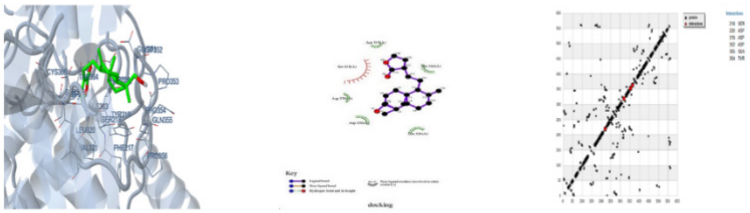
(a) (b) (c)

**Figure 6:** Arjungenin.



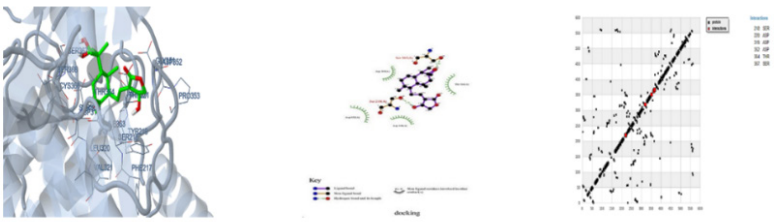
(a) (b) (c)

**Figure 7:** Betulonic acid.



(a) (b) (c)

**Figure 8:** Andrograpanin.



(a) (b) (c)

**Figure 9:** Andrographolide.

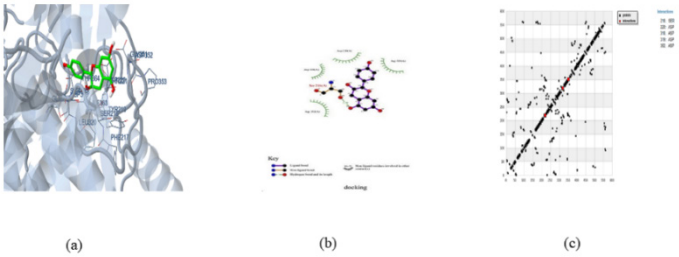



**Figure 10:** Epiafzelechin.

formulation traditionally used to treat various conditions, including liver-related disorders.

In this study, we applied molecular docking techniques to explore the potential inhibitory effects of SK on HCV RNA-Dependent RNA Polymerase (RdRp), a crucial enzyme involved in the replication of the virus.

## CONCLUSION

The computational analysis results suggest that bioactive compounds such as curcumin, maslinic acid, arjungenin, andrograpanin, andrographolide, and epiafzelechin in SK exhibit strong binding to the Hepatitis C virus RNA-Dependent RNA Polymerase (RdRp) by interacting with key amino acids at the enzyme's active site. Our study supports the potential inhibitory effect of SK on HCV RdRp, indicating that these compounds may block RNA replication and the synthesis of Non-Structural (NS5B) proteins, crucial for HCV survival. Therefore, these phytochemicals could serve as potential therapeutic agents for managing HCV.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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## ABBREVIATIONS

**SK:** *Sittramutti Khirutham*; **HCV:** Hepatitis C Virus; **RdRp:** RNA-dependent RNA Polymerase; **MD:** Molecular Docking; **LGA:** Lamarckian Genetic Algorithm; **PDB:** Protein Data Bank; **NS5B:** Non-Structural Protein 5B; **DAAs:** Direct-Acting Antivirals; **RAVs:** Resistance-Associated Variants; **2D:** Two-Dimensional; **3D:** Three-Dimensional; **AutoDock:** Automated Docking Software; **ADMET:** Absorption, Distribution, Metabolism, Excretion, and Toxicity.

## ETHICAL APPROVAL

As the present study is an *in vitro* investigation, it does not involve the use of human or animal subjects and therefore, ethical clearance is not applicable.

## AUTHOR CONTRIBUTION

Conceptualization: ME; Data collection and compilation: ME and SR; Manuscript Writing: ME, SR, AB and LR; Proofreading and editing: ME, SR, AB and LR.

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