





In Silico Approach of *Citrus sinensis* Peel Extract for Herpes Simplex Virus Type-1 Prevention and Phytopharmaceutical Preparation with Nano Lipid-Structured Carrier

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Abstract: Herpes Simplex Virus Type-1 (HSV-1) is a prevalent virus causing skin and genital infections. This study employs an in silico approach to predict *Citrus sinensis* peel extract's target mechanisms against HSV-1. The extract, known for its antiviral properties, aims to prevent HSV-1 infection through molecular interactions. Swiss Target and GeneCards databases identified active CSP ingredients for target prediction. Cytoscape and STRING databases constructed networks illustrating ingredient-target-disease and protein-protein interactions. The network revealed five compounds and 20 targets, with Genistein identified as the most promising CSP compound. Key targets for HSV-1 prevention include AKT1, BCL2, TNF, and STAT3. Molecular docking demonstrated a strong interaction between Genistein and AKT1, with a binding energy of -7.614 kcal/mol. Additionally, the various components of the CSP extract NLC formula were prepared using speed homogenization. NLC characterization met the criteria with a cream-pale color, pH of 6.23, droplet size of 560.3 nm, polydispersity index of 0.365, and zeta potential of -1.4 mV. These findings suggest the potential for further optimization in subsequent stages of research.

Keywords: *Citrus sinensis* peel; herpes simplex virus type-1; antiviral; network pharmacology; molecular docking; phytopharmaceutical.

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1. Introduction

Herpes is a type of skin and genital disease. Based on the location of the latent infection, herpes simplex virus (HSV) is the most common pathogen causing herpes disease [1]. HSV belongs to the Alpha Herpesvirinae subfamily and is divided based on differences in the infection area; HSV-1 is more common in oral-facial infections, while HSV-2 typically infects the genital area [2,3]. Research indicates that the majority of people infected with HSV are

more commonly infected with HSV-1 [4]. According to the World Health Organization (WHO) report, nearly 3.7 billion people under the age of 50 are living with HSV-1 infection [5].

Infections are typically caused by a weakened immune system in the body. Phytochemical compounds such as polysaccharides, terpenoids, alkaloids, flavonoids, and polyphenols have bioactivity as immunomodulatory agents [6]. Medicinal plant products have traditionally been used as alternative methods of treating various viral diseases [7]. In this context, there is a need to search for new compounds that can be used to treat viral infections due to the increasing resistance to existing antiviral drugs. Moreover, the widespread use of chemical drugs has shown resistance, particularly in individuals with compromised immune systems and bone marrow transplant recipients. To address the issue of viral resistance, it is crucial to develop new antiviral products with different mechanisms of action [8].

One natural substance that can be developed as a potential candidate for an effective antiviral against HSV-1 is *Citrus sinensis* peel (CSP). Previous research investigating the antiviral effect of *Citrus sinensis* against HSV-1 in vitro demonstrated that the extract exhibited a potent antiviral effect by disrupting host cell adsorption and various intracellular activities [9]. CSP is rich in diverse bioactive compounds known for their potent antioxidant properties and capacity to contribute to overall well-being, such as flavonoids and isoflavonoids [10,11]. The primary contents in CSP include hesperidin, naringin, rutin, and genistein [12]. The ethnomedicinal survey investigates the use of natural ingredients, which are highly promising for treating HSV infection, either by directly disrupting the virus's life cycle or indirectly enhancing the body's immunity [13]. The extract of sweet orange peel contains complex chemical components, namely multi-components and multi-targets, in its antiviral activity [14]. Typically, medicinal plants provide therapeutic effects through the synergistic action of several compounds contained within them.

Network pharmacology is a form of in silico study that can visually represent molecular pathways between drugs and targets. Network pharmacology can integrate biological system networks with drug target networks from a multi-target perspective [15,16]. Molecular docking is a computational method to predict the interaction between two molecules, resulting in a binding model [17]. The utilization of network pharmacology and molecular docking techniques to investigate the antiviral activity of sweet orange peel against HSV-1 is anticipated to identify potentially active compounds within the sweet orange peel. This approach allows for understanding the intricate interactions between these compounds and targets associated with the HSV-1 virus. Despite its potential, previous studies haven't analyzed the molecular proteins interacting with these compounds, which could provide important insights into pharmacological networks.

2. Materials and Methods

The materials used were CSP, 10% ethyl acetate, and distilled water. The test ligand used in this study is an active compound from sweet orange peel extract with antiviral properties against HSV-1, represented in '.PDB' format and SMILES (Simplified Molecular Input Line Entry Specification) code. The 3D receptor structure used for this research was downloaded from the Protein Data Bank (PDB) website. The in silico study steps for this study can be seen in Figure 1, which outlines the network pharmacology-based approach utilized in this investigation.

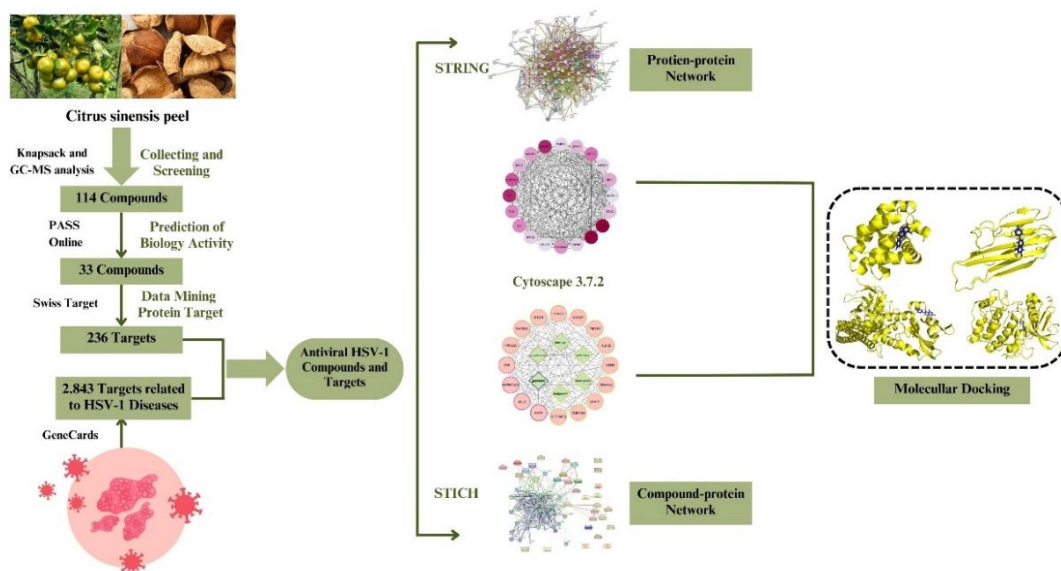


Figure 1. Flow chart of the network pharmacology-based study.

2.1. Preparation and extraction of CSP.

The CSP powder was sieved through a 60-mesh sieve. The extraction process was carried out using the Ultrasound-assisted Extraction (UAE) method with the Ultrasonic Processor Qsonica–Q500 instrument. A total of 5 g of sweet orange peel powder was extracted using distilled water and 10% ethyl acetate at a solvent-to-material ratio of 1:20 (w/v) for 15 minutes. The filtrate was evaporated using a rotary evaporator (Buchi, Germany) to obtain a concentrated extract [18].

2.2. Analysis of compound profiles using GC-MS.

The concentrated extract of CSP obtained from the extraction process was analyzed using Gas Chromatography Mass Spectrometry (GC-MS). GC-MS could only determine the volatile organic compounds [19]. The GC-MS analysis results, as ligands, were subsequently subjected to computational (in silico) network pharmacology and molecular docking methods to determine the compounds with the potential to target HSV-1.

2.3. Data mining of compounds and prediction biology activity in CSP.

Active compounds were identified from data mining the Knapsack herbal database (<http://www.knapsackfamily.com/>) and GC-MS analysis of 10% ethyl acetate CSP extract. The structure of each compound was further analyzed through the simplified molecular-input line-entry system (SMILES) in PubChem (<http://pubchem.ncbi.nlm.nih.gov>). The 2D structure was needed for druggability analysis in each component, besides the 3D structure for molecular docking analysis [20].

2.4. Prediction of biology activity.

The biological activities of compounds were assessed using the PASS server (<http://www.way2drug.com/passonline/g>), employing a structure-activity relationship (SAR) approach. Compounds with similar structures were expected to have similar activities, and a threshold of $P_a > 0.7$ in the PASS Server indicated a high likelihood of a compound being active in experiments [21].

2.5. Prediction of targeted protein.

The potentially most active compounds were further analyzed with Swiss Target Prediction (<http://www.swisstargetprediction.ch/>), using a targeted-focused compound library approach to make identifications. The principle was to predict new compounds' structural aspects and functional activities based on similarities to compounds in the database. The biochemical pathways of targeted proteins were then analyzed to study the processes that drugs could target [22].

2.6. Data mining protein target of HSV-1 diseases.

Disease targets were collected through the GeneCards database (<https://www.genecards.org/>) by searching for "Herpes Simplex Virus-1" in the search box. Venn diagrams were visualized using an online Venn diagram generator (<http://bioinformatics.psb.ugent.be/webtools/Venn/>) by entering a list of Herpes Simplex Virus-1 targets and CSP targets [21].

2.7. Pathway of protein-protein interaction.

Interactions between proteins or Protein-protein interactions (PPI) were analyzed through the website (<https://STRING-db.org/>), then select Multiple Proteins. Species were limited to the "Homo sapiens" group only, and interaction score >0.4. Visualization was carried out using the Cytoscape 3.8.2 application to analyze network topology using the network analysis function [23].

2.8. Pathway of compound-protein interaction.

Protein-compound interactions were analyzed through the website (<http://STITCH.embl.de/>), then select multiple proteins. Species were limited to the "Homo sapiens" group only, and interaction score >0.4. Visualization was carried out using the Cytoscape 3.8.2 application to analyze network topology using the network analysis function [24].

2.9. Molecular docking.

Targets exhibiting a high PPI score were selected for molecular docking to validate the connection between the active ingredients of the drug and their designated targets. The 2D structure of the compounds was sourced from the PubChem database (<http://pubchem.ncbi.nlm.nih.gov/>) and subsequently converted into 3D structures using Chem 3D, which were then obtained from PDB (<https://www.rcsb.Org/>). Water molecules and small molecule ligands were removed using PyMOL to prepare the target proteins and the targets were imported into AutoDock-tools 1.5.6 for hydrogenation, charge distribution, and atomic type assignment. Molecular docking was carried out using AutoDock Vina 1.1.2, and the results were refined using PyMOL software to generate molecular docking diagrams. The docking outcomes were further analyzed in LigPlot+ to assess the precise binding interactions between the ligands and proteins [25,26].

2.10. Nano lipid-structured carrier (NLC) preparation and characterization.

The formulation of CSP extract NLC is detailed in (Table 1), which list the raw materials and their respective concentrations used in the preparation process.

Table 1. Formulation of CSP extract NLC.

Raw materials	Concentration (%w/w)
CSP extract	0.55
Monostearin	9.9
Oleic acid	6.6
Tween 80	0.3
Span 80	5.2
Propilen glikol	1.5
Dapar fosfat pH 7,4	30.95

The CSP extract was formulated into NLC using the high-speed homogenization method. In the initial stage, the CSP extract was dissolved in oleic acid liquid lipid at a temperature of 80°C. Solid lipid monostearin was melted with Span 80 at 80°C. The mixture was stirred with an ultra turrax high shear homogenizer at 3400 rpm while adding the extract solution gradually. The next stage involved mixing the aqueous phase, including a pH 7.4 phosphate buffer, Tween 80, and propylene glycol, which were heated to 80°C in a separate container and dispersed gradually into the oil phase at a constant temperature and speed. The mixture was stirred with an Ultra-Turrax (5000 rpm) for 3 minutes and repeated for 5 cycles. The final stage consisted of cooling, accomplished by transferring the preparation from the high-shear homogenizer to a hot plate and stirring using a magnetic stirrer (500 rpm) until it reached room temperature. The characterization of NLC includes organoleptic tests, particle size, polydispersity index, TEM, and zeta potential [27].

3. Results and Discussion

3.1. Database of active components within the CSP extract.

The exploration of active components within the CSP extract, utilizing the Knapsack database, revealed the identification of 87 compounds. Additionally, an analysis of CSP extract using GC-MS identified 27 compounds. The GC-MS chromatogram of the CSP extract is illustrated in (Figure 2). The biological activity of metabolite compounds in CSP was analyzed with the PASS server probability that a compound is active (Pa) set at a threshold of 0.7. Of the total 114 compounds found in the CSP extract, 33 exhibited collective properties encompassing antiviral, antioxidant, and anti-inflammatory activities. This discovery holds potential as a prospective candidate for treating HSV-1.

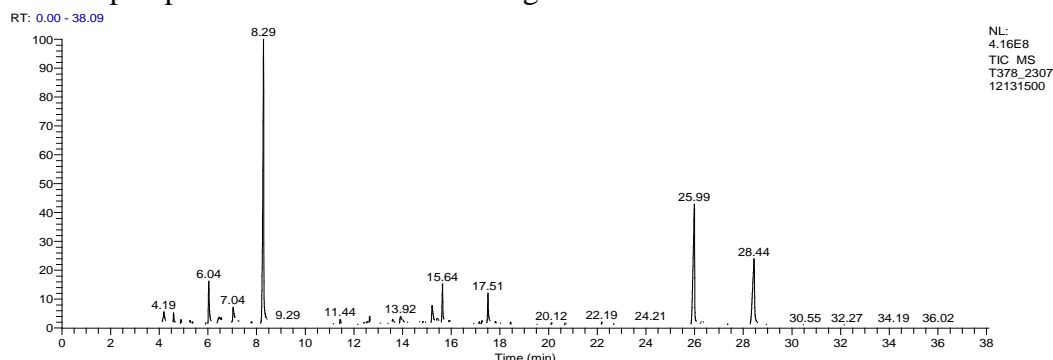


Figure 2. GC/MS chromatogram of CSP extract.

3.2. Data mining protein target involved in HSV-1.

SWISS target prediction identified that 33 compounds of CSP were documented to have 236 protein-specific targets, and 2,843 genes from GeneCard were identified as being related to HSV-1 treatment. Matching HSV-1 treatment-related targets with CSP compound targets yielded 115 potential action targets (Figure 3). Intersection targets were obtained to preliminarily identify the candidate targets of action of CSP in the treatment of HSV-1.

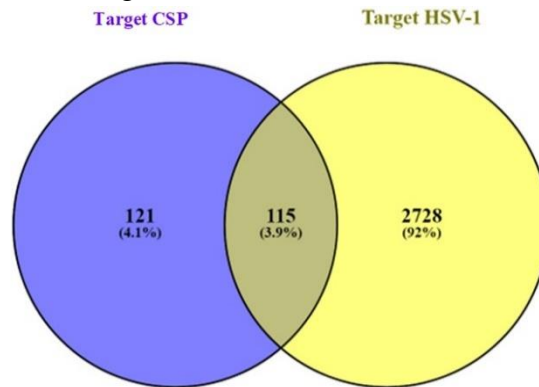


Figure 3. The Venn diagram of drug and disease Targets. The purple circle signifies the CSP compound's target, while the yellow circle denotes the target associated with the HSV-1 infection.

3.3. Protein-protein network.

The above data were imported into the STRING database and selected organism as Homo sapiens to get the protein interaction network relationship. Then, removed the free nodes and imported the results into the Cytoscape 3.7.2 version for visualization (Figure 4).

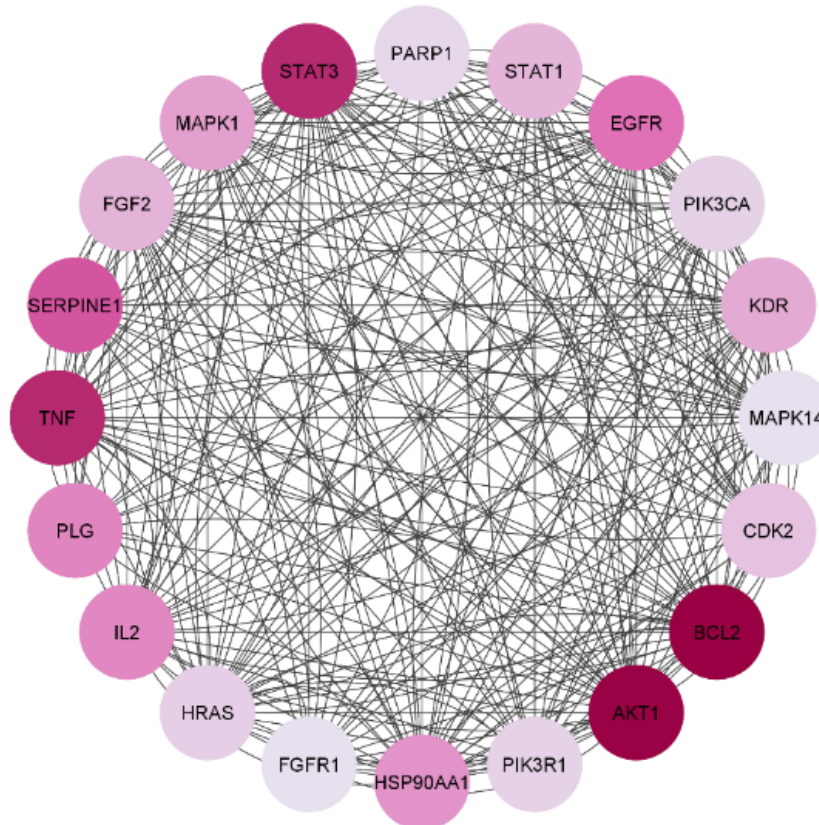


Figure 4. Protein-protein interaction network of CSP target genes.

The color of the circle varied between the essentiality values by mapping type. There are 20 nodes and 291 edges. Calculated by the Network Analyzer plug-in in Cytoscape, the

average degree value of the node is 33,818; the average Betweenness Centrality is 0,01; and the average Closeness Centrality is 0.036. Among them, there are 4 nodes whose degree values, Betweenness Centrality and Closeness Centrality, are all greater than the average (Figure 4). AKT1 (AKT Serine/Threonine Kinase1), BCL (BCL2 Apoptosis Regulator), TNF (Tumor Necrosis Factor), and STAT3 (Signal Transducer And Activator Of Transcription 3). They may be the primary targets for CSP to play a role (Table 2).

Table 2. Most activated pathways by CSP extract

Protein target	Betweenness centrality	Degree	Closeness centrality
BCL2	0.06454262850042086	58	0.9142857142857143
AKT1	0.06399379337497282	58	0.9142857142857143
TNF	0.052525451009453064	52	0.8421052631578947
STAT3	0.052133834337594456	58	0.9142857142857143

HSV-1 infection triggers a long-lasting immune response characterized by chronic inflammation, which leads to the formation of scars, thinning, and new blood vessel growth in the cornea [28]. The interaction between the host and the virus results in significant alterations in host protein tissue, disrupting signaling pathways and creating a diseased environment. The virus-induced increase in heparanase (HPSE), a key player in HSV-1 replication, initiates a signaling cascade that promotes the activation of AKT protein through phosphorylation [29]. Nonetheless, it is worth noting that AKT1 is a versatile protein involved in various cellular functions, including cell growth, survival, metabolism, and immune system regulation [30,31].

HSV-1 employs various mechanisms to evade host cell apoptosis, and part of this evasion involves interacting with the BCL2 family of proteins. By interfering with apoptosis, the virus ensures the survival of infected cells and facilitates its replication and spread within the host organism. This complex interplay between HSV-1 and the host cell's apoptotic machinery is crucial to the virus's pathogenicity [32,33].

TNF plays a significant role in the host's immune response to HSV-1 infection. It helps orchestrate the immune defense against the virus by promoting inflammation, activating immune cells, and stimulating the production of antiviral factors. This interplay between TNF and the immune response is essential for controlling HSV-1 and other viral infections while highlighting the importance of maintaining a balanced immune response to prevent excessive inflammation and tissue damage [34].

STAT3 plays an essential role in viral infection. STAT3 plays a crucial role in the host's immune defense against HSV-1, and its activation is a fundamental part of the body's protective response [35]. Nevertheless, the virus has developed strategies to disrupt the STAT3 pathway to evade the immune system. This intricate interaction between STAT3 and HSV-1 exemplifies the continual battle between the host's immune defenses and viral invaders, with the host working to safeguard itself. At the same time, the virus seeks to establish infection [35].

3.4. Compound-protein network.

The potential mechanism of compounds was identified based on prediction analysis. The data were imported into Cytoscape 3.7.1 to construct a visual network diagram of the CSP compounds and intersection targets. Following the 33 compounds of CSP, 20 targets from STRING were documented, and the network contained 22 nodes (including six compounds and 16 genes) and 125 edges (Figure 5). Stronger associations are represented by thicker borders in the figure represented in purple. Protein interactions with chemical compounds are shown with thick lines, while potential chemical compound interactions with potential proteins are

shown through thin lines. Results showed that Genistein is the most potential compound for potential targets AKT1, BCL2, TNF, and STAT3.

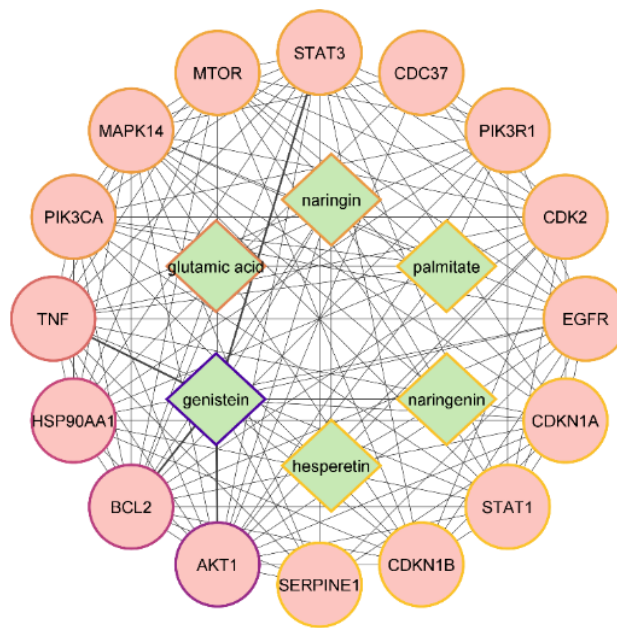


Figure 5. Compound-protein interaction network of CSP. The orange nodes represent the intersection target gene, and the green nodes represent the active components.

Genistein, derived from isoflavonoids, has been a research subject due to its anti-inflammatory characteristics. Genistein could serve as a promising compound in drug development for its anti-inflammatory properties. Regarding its impact on HSV-1, Genistein has exhibited the ability to impede the replication of HSV-1 [36]. Genistein is involved in inhibiting the penetration stage of HSV-1 into host cells. Virus penetration into host cells is the initial step in the virus replication cycle [37].

Genistein has demonstrated the ability to disrupt the interaction between HSV-1 virions (virus particles) and host cell membranes [38]. In this way, this compound can inhibit the virus's ability to enter host cells. Apart from that, Genistein also affects the stages of virus replication in host cells. The compound has been found to inhibit the activity of the DNA polymerase enzyme required by HSV-1 for replication and the formation of new virus particles. This means that Genistein can hinder the production of new viruses within infected host cells. Furthermore, this compound has the potential to interact with viral structural components, such as glycoproteins, that play a role in virus attachment to host cells. Therefore, Genistein can interfere with the virus's ability to attach to host cells and initiate infection [39,40].

3.5. Molecular docking.

The top-ranked potential targets in the PPI network and their corresponding compounds were validated via molecular docking. A molecular docking strategy was employed to confirm the interactions between the active compounds predicted by CSP and the target protein associated with HSV-1 diseases. Molecular docking was carried out on four target proteins that met the criteria of high connectivity value, were targeted by Genistein, and were involved in HSV-1 diseases. This study will dock the most promising Genistein compound with four potential protein targets involved in HSV-1 diseases: BCL2, AKT1, TNF, and STAT3.

The stability of the conformational energy of the ligand binding to the receptor results in a lower energy level, which enhances the likelihood of interaction. Binding energy is a

parameter for evaluating the potential binding between the ligand and receptor. A negative binding energy indicates the potential for ligand-receptor binding. Generally, ligand-receptor binding energy of ≤ -5.0 kcal/mol signifies a solid binding affinity, with lower binding energies indicating increased stability in the interaction. The most favorable docking interaction was observed between AKT1 and Genistein as the ligand, yielding a binding energy of -7.614 kcal/mol, as detailed in Table 3 and depicted in Figure 6.

Table 3. Molecular docking result of the potential protein target and Genistein.

Potential protein target	Binding energies (kcal/mol)	Hydrogen bond	Hydrophobic bond
AKT1	-7.614	Lys158, Ala230	Tyr229, Ala177, Thr291, Met281, Val64, Leu156, Phe438, Val64, Gly1157, Gly1159
BCL2	-6.370	-	Phe112, Phe153, Ala149, Phe104, Asp111, Leu137
TNF	-5.792	Leu120	His15, Tyr151, Tyr59, Tyr119
STAT3	-5.072	Gln644	Tyr657, Tyr640, Glu638, Pro639

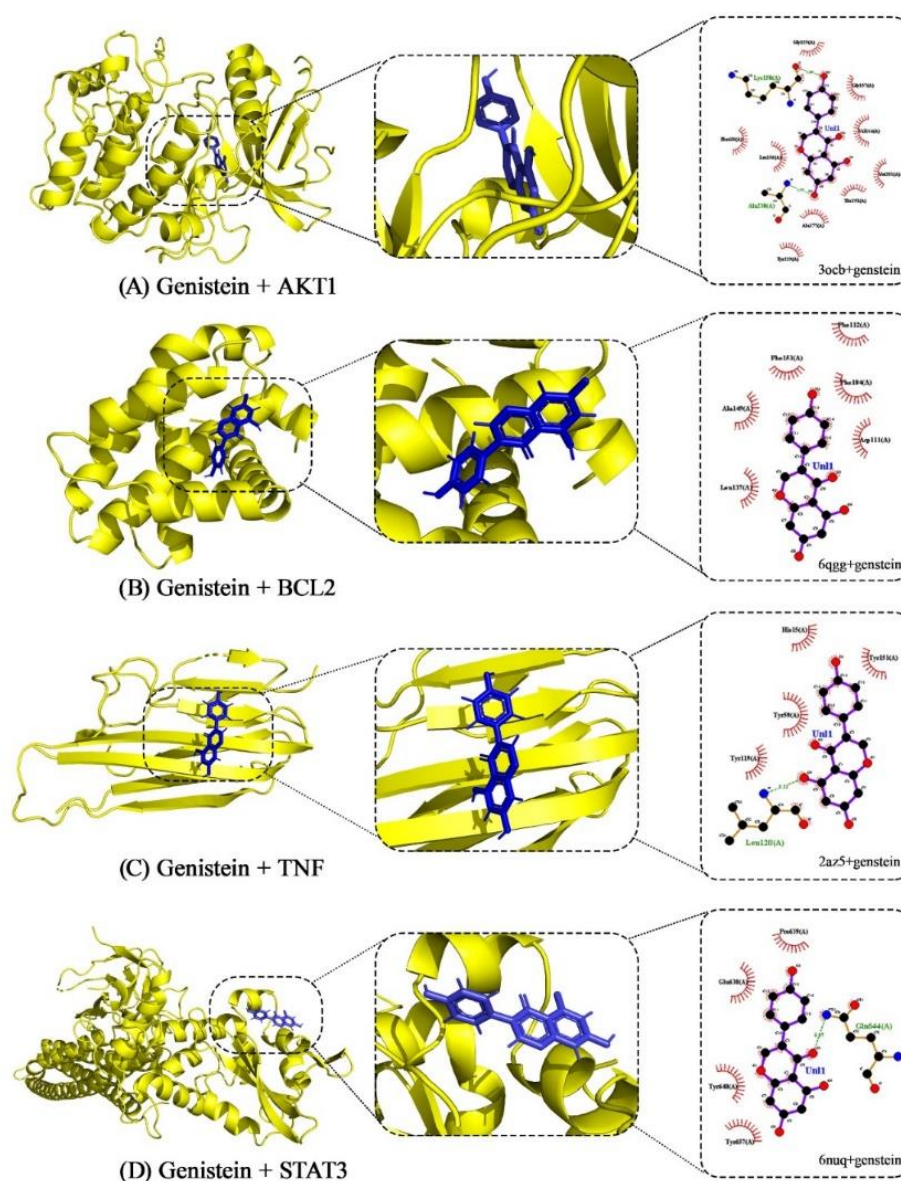


Figure 6. Molecular docking diagram of chemical composition to target: (A) Genistein+BCL2; (B) Genistein+AKT1; (C) Genistein+TNF; (D) Genistein+STAT3.

Hydrogen and hydrophobic bonds are two interactions that play important roles in molecular docking. Hydrogen bonds are attractive forces between a hydrogen atom covalently

<https://nanobioletters.com/>

bonded to an electronegative atom (such as oxygen or nitrogen) and another electronegative atom with a lone pair of electrons. Hydrophobic bonds are also called hydrophobic interactions or van der Waals forces. They are caused by the transient fluctuations of the electron clouds around the atoms, which create temporary dipoles that attract each other. Hydrophobic bonds are also weak but can be significant when involving large surface areas. Hydrophobic bonds can stabilize the binding of a ligand to a receptor by reducing the entropy of the system and increasing the effective concentration of the ligand [41].

3.6. Nanostructured lipid carriers (NLC) preparation and characterization.

The NLC system was prepared using the high-shear homogenization method. The characterization of CSP extract NLC visually shows that milk has a cream-pale color. This is due to the mixing of the oil phase and water phase at the same temperature. CSP extract NLC has the aroma of oil and the odor of CSP. The pH measurement results of the preparation were 6.23. During storage at room temperature for 14 days, it did not change, indicating the work of surfactants as physical stabilizers (Table 4).

Table 4. Characterization result of CSP extract NLC.

Characterization	Result
Color	cream pale
Aroma	Oily and tea aroma
pH	6,23
Viskositas	21,83 cP
Particle Size	560,3 nm
Polydispersity index	0,365
Zeta potential	-1,4 mV

The smaller the particle size, the more surface area the particles will have so that drug absorption will be faster and bioavailability will increase. The results of the measurement of CSP extract NLC using a particle size analyzer (PSA) showed a value of 560,3 nm. The polydispersity index describes the particle size distribution. The polydispersity index measures the homogeneity of the particles with a value between 0.0 and 1.0. The polydispersity index provides information about the stability of the system. The smaller the polydispersity index value, the more homogeneous the globule size and the narrower the particle size distribution. A PDI value close to 0 indicates the emulsion is homogeneously distributed, while a PDI value exceeding 0.5 indicates the emulsion has high non-uniformity. The results of the PDI of CSP extract NLC measurement are 0.365.

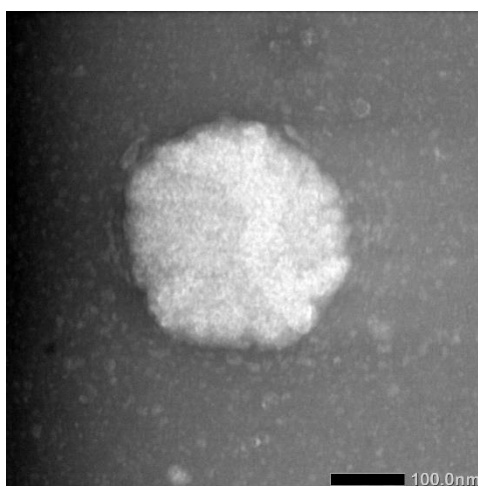


Figure 7. Transmission electron micrographs of CSP extract-loaded NLC dispersion.

Zeta potential is an indicator of the colloidal stability of a system [42]. High zeta potential values (positive or negative) indicate a more stable dispersion of particles because particles with high charges tend to repel each other. TEM was conducted to investigate the morphology of CSP extract NLC. It was evident from TEM images that nanoparticles were almost spherical with smooth morphology appearing as white dots, well dispersed and separated on the surface (Figure 7).

4. Conclusions

Genistein is the main compound contained in CSP extract, which, in comparative studies, shows antiviral, antioxidant, and anti-inflammatory activities that play a role as a prospective candidate for treating HSV-1. The most potent compound in CSP is Genistein, and critical targets for preventing HSV-1 infection are AKT1, BCL2, TNF, and STAT3. Genistein, a type of isoflavonoid derivative, is involved in inhibiting the penetration stage of HSV-1 into host cells. It was known that the limitations of the network pharmacology method and the complexity of drug components in decoction and human metabolic reactions still require further animal experiments for verification. CSP extract NLC formula consists of monostearin, oleic acid, pH 7.4 phosphate buffer, Tween 80, and propylene glycol, which meet the criteria. The results of this screening can be continued at a more comprehensive optimization stage.

Author Contributions

All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Wijayanti, H.; Aritasya. Potensi Imunomodulator Herbal Ekstrak Etanol Daun Pepaya Varietas Calina terhadap Struktur Jaringan Limpa Tikus Putih Galur Wistar. *Jurnal Veteriner* **2021**, *22*, 531-539, <https://doi.org/10.19087/jveteriner.2021.22.4.531>.
2. Irianti, M.; Fitriana, W.; Arifianti, A.; Rahmasari, R. Herpes Simplex Virus Tipe 1: Prevalensi, Infeksi dan Penemuan Obat Baru. *Sainstech Farma* **2020**, *13*, 21-26.
3. Kumar, A.; De, S.; Moharana, A.K. Inhibition of herpes simplex virus-1 infection by MBZM-N-IBT: in silico and in vitro studies. *Virol J.* **2021**, *18*, 103, <https://doi.org/10.1186/s12985-021-01581-5>.
4. Haryono, W.; Wibianto, A. Herpes Zoster Oftalmikus Sinistra pada Geriatri dengan Komplikasi. *Jurnal Medical Professional* **2021**, *48*, 236-235.
5. Rani, A.; Kalaimathi, K.; Jayasree, S.; Srinivasan, P.; Vijayakumar, S.; Ramasubbu, R.; Priya, N. Exploring the Drug Potential of Phytochemicals as a Novel Therapeutic Drug Candidate for Herpesvirus: An In-silico Evaluation. *Chemistry Africa* **2022**, *6*, <https://doi.org/10.1007/s42250-022-00529-8>.
6. Mauludya, N.; Tallei, T.; Ginting, B.; Idroes, R.; Illian, D.; Faradilla, M. Analysis of flavonoid compounds of Orange (*Citrus sp.*) peel as anti-main protease of SARS-CoV-2: A molecular docking study. *IOP Conference Series: Earth and Environmental Science* **2022**, *951*, 012078, <https://doi.org/10.1088/1755-1315/951/1/012078>.
7. Indriyani, N.N.; Anshori, J.A.; Permadi, N.; Nurjanah, S.; Julaeha, E. Bioactive Components and Their Activities from Different Parts of *Citrus aurantifolia* (Christm.) Swingle for Food Development. *Foods* **2023**, *12*, 2036, <https://doi.org/10.3390/foods12102036>.
8. Alberto, M.; Manzur, M.; Luciardi, M.; Amparo, M.; Cartagena, E.; Arena, M. Citrus sinensis Essential Oils an Innovative Antioxidant and Antipathogenic Dual Strategy in Food Preservation Against Spoilage Bacteria. *Antioxidants* **2023**, *12*, 1-18, <https://doi.org/10.3390/antiox12020246>.
9. El-Serehy, H.; Al-Rasheid, K.A.; Al-Misned, F.; Mortuza, M.G. Citrus sinensis peel extract induced in vitro effects on herpes simplex virus. *Journal of Pure and Applied Microbiology* **2014**, *8*, 3807-3812.
10. Long, X.Y.; Zeng, X.G.; Yan, H.T.; Xu, M.J.; Zeng, Q.T.; Xu, C.; Xu, Q.M.; Liang, Y.; Zhang, J. Flavonoids composition and antioxidant potential assessment of extracts from Gannanzao Navel Orange (*Citrus sinensis* Osbeck Cv. Gannanzao) peel. *Natural Product Research* **2021**, *35*, 702-706, <https://doi.org/10.1080/14786419.2019.1593162>.
11. El-Kersh, D.M.; Ezzat, S.M.; Salama, M.M.; Mahrous, E.A.; Attia, Y.M.; Ahmed, M.S.; Elmazar, M.M. Anti-estrogenic and anti-aromatase activities of citrus peels major compounds in breast cancer. *Scientific* **2021**, *11*, 7121, <https://doi.org/10.1038/s41598-021-86599-z>.
12. Abdelghffar, E.A.; El-Nashar, H.A.S.; Al-Mohammadi, A.G.A.; Eldahshan, O.A. Orange fruit (*Citrus sinensis*) peel extract attenuates chemotherapy-induced toxicity in male rats. *Food & Function* **2021**, *12*, 9443-9455, <https://doi.org/10.1039/d1fo01905h>.
13. Kandar, C.C. Medicinal Plants Against Herpes Simplex Virus (HSV) Type 2 Infections: Ethnopharmacology, Chemistry, Clinical, and Preclinical Studies. In *Anti-Viral Metabolites from Medicinal Plants*, Pal, D., Ed.; Springer International Publishing: Cham, **2022**; pp. 1-40, https://doi.org/10.1007/978-3-030-83350-3_5-1.
14. Tutunchi, H.; Naeni, F.; Ostadrahimi, A.; Hosseinzadeh-Attar, M.J. Naringenin, a flavanone with antiviral and anti-inflammatory effects: a promising treatment strategy against COVID-19. *Phytotherapy Research* **2020**, *34*, 3137-3147, <http://dx.doi.org/10.1002/ptr.6781>.
15. Zhuang, Y.; Qin, K.; Yang, B.; Liu, X.; Cai, B.; Cai, H. Prediction of the targets of the main components in blood after oral administration of: *Xanthii Fructus*: A network pharmacology study. *RSC Advances* **2018**, *8*, 8870-8877, <https://doi.org/10.1039/c8ra00186c>.
16. Westerhoff, H.V. Network-based pharmacology through systems biology. *Drug Discovery Today: Technologies* **2015**, *15*, 15-16, <https://doi.org/10.1016/j.ddtec.2015.05.001>.
17. Huang, S.Y. Exploring Binding Pathways And Mechanisms Of Protein-Ligand Interactions. *Wiley Interdisciplinary Reviews: Computational Molecular Science* **2020**, *10*, 1448, <https://doi.org/10.1038/s41467-021-23157-1>.
18. Safdar, M.N.; Kausar, T.; Jabbar, S.; Mumtaz, A.; Ahad, K.; Saddozai, A.A. Extraction and Quantification of Polyphenols from Kinnow (*Citrus reticulata* L.) Peel Using Ultrasound and Maceration Techniques. *Journal of Food and Drug Analysis* **2017**, *25*, 488-500, <https://doi.org/10.1016/j.jfda.2016.07.010>.

19. Deliiski, N.; Dzurenda, L.; Tumbarkova, N.; Angelski, D. Computation of Temperature Conductivity of Frozen Wood during its Defrosting. *Drvna industrija* **2015**, *66*, 87-96, <https://doi.org/10.5552/drind.2015.1351>.
20. Bina, L.; Nyanyu, S.; Kasman, M.; Puput, S.; Abdurrahman, H. Aktivitas Antioksidan Dan Studi In Silico Ekstrak Buah Pala (*Myristica Fragrans* Houtt). *Pharmamedica Journal* **2022**, *7*, 28-40, <https://doi.org/10.47219/ath.v7i1.142>.
21. Firzannida, F.; Bagaskara, S.; Savira, S.; Fadnurrahim, A.; Rofida, S. Network Pharmacology Of Black Cumin (*Nigella Sativa* L.) As A Candidate Of OMAI In Colorectal Cancer: In Silico Study. *Indonesian Journal of Biotechnology* **2022**, *27*, 87, <https://doi.org/10.22146/ijbiotech.70699>.
22. Shiyan, S.; Zubaidah, P.G. Chemometric Approach to Assess Response Correlation and its Classification in simplex centroid design for Pre-Optimization stage of Catechin-SNEDDS. *Res. J. Pharm. Technol.* **2021**, *14*, 5863–5870, <https://doi.org/10.52711/0974-360X.2021.01020>.
23. Akbar, N.D.; Nugroho, A.K.; Martono, S. REVIEW ARTICLE: OPTIMIZATION OF SNEDDS FORMULATION BY SIMPLEX LATTICE DESIGN AND BOX BEHNKEN DESIGN. *J. Ilm Farm. Bahari* **2021**, *13*, 90–100.
24. Khan, A.; Liaqat, A.; Masood, A.; Ali, S.S.; Ali, L.; Alshammari, A.; Alasmari, A.F.; Mohammad, A.; Waheed, Y.; Wei, D.Q. Exploring the medicinal potential of Dark Chemical Matters (DCM) to design promising inhibitors for PLpro of SARS-CoV-2 using molecular screening and simulation approaches. *Saudi pharmaceutical journal* **2023**, *31*, 10, <https://doi.org/10.1016/j.jsps.2023.101775>.
25. Nguyen, N. T.; Nguyen, T. H.; Pham, T.N.H.; Huy, N.T.; Bay, M.V.; Pham, M.Q.; Nam, P.C.; Vu, V.V.; Ngo, S.T. Autodock Vina Adopts More Accurate Binding Poses but Autodock4 Forms Better Binding Affinity. *Journal of Chemical Information and Modeling* **2020**, *60*, 204-211, <https://doi.org/10.1021/acs.jcim.9b00778>.
26. Ravindranath, P.A.; Forli, S.; Goodsell, D.S.; Olson, A.J.; Sanner, M.F. AutoDockFR: Advances in Protein-Ligand Docking with Explicitly Specified Binding Site Flexibility. *PLoS Computational Biology* **2015**, *11*, 12, <https://doi.org/10.1371/journal.pcbi.1004586>.
27. Ortiz, A.C.; Yanez, O.; Huenuleo, E.S.; Morales, J.O. Development of a Nanostructured Lipid Carrier (by a Low-Energy Method Comparison of Release Kinetics and Molecular Dynamics Simulation. *Pharmaceutics* **2021**, *13*, 531-550, <https://doi.org/10.3390/pharmaceutics13040531>.
28. Carpenter, D.; Hsiang, C.; Jiang, X.; Osorio, N.; BenMohamed, L.; Jones, C.; Wechsler, S.L. The herpes simplex virus type 1 (HSV-1) latency-associated transcript (LAT) protects cells against cold-shock-induced apoptosis by maintaining phosphorylation of protein kinase B (AKT). *Journal of neurovirology* **2015**, *21*, 568–575, <https://doi.org/10.1007/s13365-015-0361-z>.
29. Koujah, L.; Suryawanshi, R.K.; Shukla, D. Pathological processes activated by herpes simplex virus-1 (HSV-1) infection in the cornea. *Cellular and molecular life sciences: CMLS* **2019**, *76*, 405–419, <https://doi.org/10.1007/s00018-018-2938-1>.
30. Zhao, J.; Qin, C.; Liu, Y.; Rao, Y.; Feng, P. Herpes Simplex Virus and Pattern Recognition Receptors: An Arms Race. *Frontiers in immunology* **2021**, *11*, 613799, <https://doi.org/10.3389/fimmu.2020.613799>.
31. Huang, Q.; Hou, J.; Yang, P. Antiviral activity of mitoxantrone dihydrochloride against human herpes simplex virus mediated by suppression of the viral immediate early genes. *BMC Microbiol* **2019**, *19*, 274, <https://doi.org/10.1186/s12866-019-1639-8>.
32. Roberts, A.W. Therapeutic development and current uses of BCL-2 inhibition. Hematology. American Society of Hematology. *Education Program* **2020**, *1*, 1–9, <https://doi.org/10.1182/hematology.2020000154>.
33. Qian, S.; Wei, Z.; Yang, W.; Huang, J.; Yang, Y.; Wang, J. The role of BCL-2 family proteins in regulating apoptosis and cancer therapy. *Frontiers in oncology* **2022**, *12*, 985363, <https://doi.org/10.3389/fonc.2022.985363>.
34. Jang, D.i.; Lee, A.H.; Shin, H.Y.; Song, H.R.; Park, J.H.; Kang, T.B.; Lee, S.R.; Yang, S.H. The Role of Tumor Necrosis Factor Alpha (TNF- α) in Autoimmune Disease and Current TNF- α Inhibitors in Therapeutics. *Int. J. Mol. Sci* **2021**, *22*, 2719, <https://doi.org/10.3390/ijms22052719>.
35. Ike, A.C.; Onu, C.J.; Ononugbo, C.M.; Reward, E.E.; Muo, S.O. Immune Response to Herpes Simplex Virus Infection and Vaccine Development. *Vaccines* **2020**, *8*, 302, <https://doi.org/10.3390/vaccines8020302>.
36. Goh, Y. X.; Jalil, J.; Lam, K.W.; Husain, K.; Premakumar, C.M. Genistein: A Review on its Anti-Inflammatory Properties. *Frontiers in pharmacology* **2022**, *13*, 820969, <https://doi.org/10.3389/fphar.2022.820969>.

37. Zakaryan, H.; Arabyan, E.; Oo, A.; Zandi, K. Flavonoids: promising natural compounds against viral infections. *Archives of virology* **2017**, *162*, 2539–2551, <https://doi.org/10.1007/s00705-017-3417-y>.
38. Sohn, S.I.; Pandian, S.; Oh, Y.J.; Kang, H.J.; Cho, W.S.; Cho, Y.S. Metabolic Engineering of Isoflavones: An Updated Overview. *Frontiers in plant science* **2021**, *12*, 670103, <https://doi.org/10.3389/fpls.2021.670103>.
39. Alshehri, M.M.; Sharifi-Rad, J.; Herrera-Bravo, J.; Jara, E.L.; Salazar, L.A.; Kregiel, D.; Uprety, Y.; Akram, M.; Iqbal, M.; Martorell, M.; Torrens-Mas, M.; Pons, D.G.; Daştan, S.D.; Cruz-Martins, N.; Ozdemir, F.A.; Kumar, M.; Cho, W.C. Therapeutic Potential of Isoflavones with an Emphasis on Daidzein. *Oxid Med Cell Longevity* **2021**, 6331630, <https://doi.org/10.1155/2021/6331630>.
40. Xu, L.; Zhong, X.L.; Xi, Z.C.; Li, Y.; Xu, H.X. Medicinal plants and natural compounds against acyclovir-resistant HSV infections. *Front. Microbiol.* **2022**, *13*, 1025605, <https://doi.org/10.3389/fmicb.2022.1025605>.
41. Owoloye, A.J.; Ligali, F.C.; Enejoh, O.A.; Musa, A.Z.; Aina, O.; Idowu, E.T.; Oyebola, K.M. Molecular docking, simulation and binding free energy analysis of small molecules as PfHT1 inhibitors. *PLoS one* **2022**, *17*, e0268269, <https://doi.org/10.1371/journal.pone.0268269>.
42. Pratiwi, G.; Ramadhiani, A.R.; Shiyan, S. Understanding the combination of fractional factorial design and chemometrics analysis for screening super-saturable quercetin-self nano emulsifying components. *Pharmacia* **2022**, *69*, 273-284, <https://doi.org/10.3897/pharmacia.69.e80594>.

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