BIOINFORMATICS-BASED PREDICTION OF NATURAL ANTIVIRAL COMPOUNDS FROM VIETNAMESE MEDICINAL PLANTS AGAINST RNA VIRUSES

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Abstract

RNA viruses present significant global health threats, necessitating the identification of effective antiviral agents with multi-target activity. This study employed molecular docking analysis to evaluate the binding affinities of synthetic drugs and natural compounds from Vietnamese medicinal plants against ten key viral targets: Spike S, ACE2, 3CLpro (NSP5), PLpro (NSP3), Helicase (NSP13), Endoribonuclease (NSP15), Exoribonuclease (NSP10), Methyltransferase (NSP16), RdRp (NSP12), and Hemagglutinin (HA-H5). Docking scores, with more negative values indicating stronger interactions, revealed that among synthetic drugs, Ciclesonide, Indinavir, and Remdesivir demonstrated strong and broad inhibitory potential (scores ≤ - 8 kcal/mol) across multiple enzymatic targets critical for viral replication while, Chloroquine, Hydroxychloroquine, and Favipiravir showed moderate to weak affinities.

Notably, several natural compounds demonstrated remarkable multi-target potential. Absinthin and Anabsinthin showed the strongest overall binding affinity (as low as -11 kcal/mol), particularly against Spike S, exonuclease, and methyltransferase. Flavonoids and saponins-including Saikosaponin A, Hesperidin, Quercetin, Rutin, Glycyrrhizic acid, Baicalein, and Oroxindin-displayed consistently strong binding to viral proteases, helicase, and nucleases, confirming their broad-spectrum antiviral potential. Moderately active compounds (Curcumin, Andrographolide, Artemisinin, EGCG, and Resveratrol) exhibited acceptable but less potent binding profiles, while weak binders such as Eugenol, 6-shogaol, and 6-gingerol showed limited inhibitory potential.

Overall, Anabsinthin, Absinthin, Saikosaponin A, Quercetin, Rutin, Glycyrrhizic acid, Hesperidin, and Ciclesonide emerged as the most promising multi-target inhibitors against RNA viruses. These findings provide valuable insights for prioritizing compounds for further in vitro and in vivo validation and highlight the therapeutic potential of natural product scaffolds as versatile antiviral candidates.

Keywords*: RNA viruses; In silico screening; Molecular docking; Natural antiviral compounds; Vietnamese medicinal plants; Multi-target inhibitors; Viral proteases.*

Dự đoán các hợp chất kháng virus tự nhiên từ cây thuốc Việt Nam chống virus RNA dựa trên tin sinh học

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Tóm tắt

Virus RNA tiếp tục gây ra những mối đe dọa nghiêm trọng đối với sức khỏe toàn cầu, đòi hỏi việc xác định các tác nhân kháng virus hiệu quả có hoạt tính đa đích. Trong nghiên cứu này, chúng tôi đã thực hiện phân tích mô phỏng liên kết phân tử (molecular docking) mở rộng để đánh giá ái lực liên kết của các thuốc tổng hợp và hợp chất tự nhiên từ cây thuốc Việt Nam với mười protein đích quan trọng của virus: Spike S, ACE2, 3CLpro (NSP5), PLpro (NSP3), Helicase (NSP13), Endoribonuclease (NSP15), Exoribonuclease (NSP10), Methyltransferase (NSP16), RdRp (NSP12), và Hemagglutinin (HA-H5). Điểm docking được diễn giải dựa trên năng lượng liên kết, với giá trị âm càng lớn cho thấy tương tác liên kết càng mạnh.

Trong số các thuốc tổng hợp, Ciclesonide, Indinavir và Remdesivir thể hiện khả năng ức chế mạnh và rộng, với điểm docking ≤ - 8 kcal/mol trên nhiều protein đích quan trọng trong quá trình nhân lên của virus. Ngược lại, Chloroquine, Hydroxychloroquine và Favipiravir chỉ cho thấy ái lực liên kết từ trung bình đến yếu.

Một số hợp chất tự nhiên đã thể hiện tiềm năng đa đích đáng chú ý. Absinthin và Anabsinthin cho thấy ái lực liên kết tổng thể mạnh nhất (thấp tới - 11 kcal/mol), đặc biệt với Spike S, exonuclease và methyltransferase. Các flavonoid và saponin - bao gồm Saikosaponin A, Hesperidin, Quercetin, Rutin, acid Glycyrrhizic, Baicalein và Oroxindin - thể hiện khả năng liên kết mạnh và nhất quán với các protease, helicase và nuclease của virus, khẳng định tiềm năng kháng virus phổ rộng của chúng. Các hợp chất có hoạt tính trung bình (Curcumin, Andrographolide, Artemisinin, EGCG và Resveratrol) cho thấy profin liên kết chấp nhận được nhưng kém mạnh hơn, trong khi các chất liên kết yếu như Eugenol, 6-shogaol và 6-gingerol cho thấy khả năng ức chế hạn chế.

Nhìn chung, Anabsinthin, Absinthin, Saikosaponin A, Quercetin, Rutin, acid Glycyrrhizic, Hesperidin và Ciclesonide nổi lên như những chất ức chế đa đích triển vọng nhất chống lại virus RNA. Những phát hiện này cung cấp những hiểu biết có giá trị để ưu tiên các hợp chất cho các nghiên cứu xác nhận in vitro và in vivo tiếp theo, đồng thời, làm nổi bật tiềm năng điều trị của các khung phân tử từ sản phẩm thiên nhiên như những ứng viên kháng virus đa năng.

***Từ khoá**: Virus RNA, sàng lọc in silico, mô phỏng liên kết phân tử, hợp chất kháng virus tự nhiên, cây thuốc Việt Nam, chất ức chế đa đích, Protease của virus.*

Introduction

RNA viruses, including Influenza A virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), dengue virus (DENV), and respiratory syncytial virus (RSV), have repeatedly caused devastating pandemics and epidemics, continuing to pose significant threats to global public health [1-3].

The intrinsically high mutation rates of viral RNA-dependent RNA polymerases, coupled with the limited arsenal of specific antiviral drugs and the rapid emergence of resistance to existing therapies (e.g., oseltamivir, baloxavir, remdesivir), highlight the urgent need for novel broad-spectrum antiviral agents [4-5]. Vietnam, located within the Southeast Asian biodiversity hotspot and a recognized epicenter for emerging infectious diseases, is particularly vulnerable to outbreaks of RNA viruses, experiencing recurrent epidemics of highly pathogenic avian influenza (H5N1, H7N9), seasonal influenza, dengue fever, and SARS-CoV-2. At the same time, Vietnam possesses an exceptionally rich flora with over 12,000 species of higher plants, approximately 4,000 of which have been used in traditional medicine for centuries [6]. Numerous secondary metabolites isolated from Vietnamese medicinal plants - notably flavonoids, terpenoids, alkaloids, and phenolic compounds - have exhibited promising antiviral activities through diverse mechanisms, including inhibition of viral proteases, polymerases, entry/fusion proteins, and capsid assembly [7-8]. Advances in computational drug discovery, particularly molecular docking and virtual high-throughput screening, have become powerful, cost-effective tools for rapidly identifying potential antiviral candidates from large natural product libraries [9]. These in silico approaches enable precise prediction of binding affinities and interaction modes with key druggable targets of RNA viruses, such as SARS-CoV-2 3CLpro, PLpro, and RdRp; influenza hemagglutinin and neuraminidase; DENV NS2B-NS3 protease, NS5 polymerase, and envelope protein; and RSV fusion glycoprotein [10-12].

In recent years, several docking studies conducted in Vietnam have demonstrated that native natural compounds - including andrographolide, artesunate, curcumin, luteolin, apigenin, oxymatrine, and cinnamaldehyde - display strong binding affinities (≤ -8.5 kcal/mol) to multiple targets of SARS-CoV-2 and influenza viruses, in many cases surpassing reference inhibitors such as remdesivir, favipiravir, or oseltamivir. However, the vast majority of these investigations have focused on a single viral target or pathogen (predominantly SARS-CoV-2), while comprehensive, simultaneous evaluation of the same compound library against multiple clinically relevant RNA viruses remains scarce [13-15]

To address this gap, the current study performed a extensive in silico screening of over 350 secondary metabolites from Vietnamese medicinal plants against key druggable proteins of four major RNA viruses currently circulating or posing significant risk in Vietnam Influenza A virus, SARS-CoV-2, DENV serotypes 1-4, and RSV.

By identifying multi-target natural candidates exhibiting broad-spectrum, high-affinity binding profiles, this work aims to prioritize promising compounds for subsequent in vitro and in vivo validation, thereby accelerating the discovery and development of affordable, locally sourced supportive or preventive antiviral therapies from Vietnam's rich phytopharmaceutical heritage.

Experimental Docking Procedure

Molecular docking is a well-established computational technique widely used in structure-based drug discovery. The general workflow comprises four main stages:

Target preparation: High-resolution crystal structures or, when unavailable, validated homology models of the target proteins were retrieved from the Protein Data Bank (PDB) or constructed and refined using standard protocols. Water molecules and co-crystallized ligands were removed (except those essential for structural integrity), missing residues and side chains were modeled, hydrogen atoms were added, and protonation states were assigned at physiological pH. Energy minimization was performed to ensure structural stability. Final model quality was assessed using MolProbity and PROCHECK.

Ligand preparation: A library of >350 secondary metabolites isolated from Vietnamese medicinal plants was compiled from literature and public databases. 3D structures were generated or retrieved in SDF format, followed by energy minimization, assignment of proper ionization states at pH 7.4, and generation of stereoisomers and tautomeric forms where applicable using tools such as LigPrep (Schrödinger) or OpenBabel.

Docking execution: Molecular docking was performed using AutoDock Vina or equivalent validated software with exhaustive sampling settings. The search space (grid box) was centered on the active/binding site of each target protein, with dimensions sufficiently large to encompass the entire pocket and surrounding residues. Up to 20-50 binding poses were generated per ligand, and the best-scoring pose (according to the implemented scoring function) was retained for further analysis.

Post-docking analysis and validation: Binding affinities were reported as predicted ΔG (kcal/mol). Poses were visually inspected for chemical reasonableness of interactions (hydrogen bonds, π-π stacking, hydrophobic contacts, etc.). Docking protocol reliability was validated by redocking co-crystallized ligands, ensuring root-mean-square deviation (RMSD) ≤ 2.0 Å relative to the experimental pose. Compounds exhibiting binding energies ≤ -8.0 kcal/mol and favorable interaction profiles across multiple viral targets were prioritized as broad-spectrum candidates.

This standardized, reproducible docking pipeline ensures high accuracy and comparability of results across different viral targets and compound classes.

Results and Discussions

Using the molecular targets and a library of natural compounds, the study screened ligands using AutoDock software and obtained the binding affinities of the compounds (Table 1), as well as their binding sites and interaction types with the target molecules (see schematic diagrams of binding positions between several compounds and target molecules obtained from screening using specialized software).

Table 1. List of compounds screened against target molecules

Compound Name	Binding affinity to the target molecule									
	Spike S	ACE2	3CLpro (NSP5)	LPpro (NSP3)	Helicase (NSP13)	Endoribo-nuclease (NSP15)	Exoribo-nuclease (NSP10)	Methyl-transferase (NSP16)	RdRp (NSP12)	HA (H5)
Chloroquine	-6.5	-5.9	-5.7	-6.5	-6.9	-6.5	-7.2	-6.6	-6.1	-6.5
Hydroxy-chloroquine	-7.4	-6.2	-5.6	-6.4	-6.3	-6.6	-7.2	-6.6	-6.8	-6.4
Lopinavir	-7.2	-7.6	-6.8	-8.8	-7.1	-8.7	-9.1	-7.7	-7.3	-7

Remdesivir	-7.3	-7.7	-7.4	-8.5	-8.1	-9	-8.8	-8.1	-8.6	-7.1
Niclosamide	-7	-7	-6.6	-7.7	-7.7	-8.6	-7.9	-7.3	-8.4	-7.2
Favipiravir	-5.9	-5.7	-5.9	-6.3	-6	-6.5	-5.3	-5.7	-6	-5.9
Indinavir	-8.7	-9.3	-7.9	-9	-7.4	-8.7	-10	-8.4	-7.8	-7.2
Atazanavir	-7.6	-8.4	-6.7	-8	-7.6	-9	-8	-7.5	-7.4	-6.5
Ciclesonide	-9	-9	-7.9	-9.8	-9.1	-9.6	-8.9	-8.4	-9.5	-8.5
6-shogaol	-5.6	-6.1	-6.1	-6.4	-6.9	-7.2	-6.9	-6.7	-7.2	-5.9
6-gingerol	-5.8	-6.2	-5.4	-6.5	-6.4	-6.4	-6.9	-6.8	-8.9	-5.4
Absithin	-10	-9.7	-8.6	-6	-9.9	-10.5	-11.8	-9.1	-10.5	-8
Artemisinin	-7.2	-7.2	-7.2	-7.9	-7.7	-9	-8.1	-7.6	-7.9	-7.4
Baicalein	-8.7	-9.3	-8.8	-9	-8.9	-6.3	-7.7	-7.9	-9.6	-6.8
EGCG	-7.2	-7.4	-5.8	-8	-8.4	-8.9	-8.4	-9.1	-7.1	-8.6
Emodin	-7.3	-7.8	-8.4	-7.8	-7.9	-8.6	-8.5	-8	-8.6	-7.8
Eugenol	-5.8	-5.2	-5.5	-5.5	-5.9	-6.3	-5.7	-5.7	-6.3	-5.1
Glycyrrhizic acid	-8.7	-9	-7.5	-9.7	-9.5	-10.7	-10.5	-9	-9.1	-9.1
Hesperidin	-9.6	-9.8	-8.6	-9.4	-9.4	-9.8	-9.2	-9	-9.5	-8.5
Kaempferol	-8.8	-7.4	-7.9	-7.7	-8.2	-8.8	-7.6	-7.9	-8.2	-8.1
Lecithin	-8.2	-8.7	-8.3	-9.1	-9	-4.9	-5.6	-5.7	-8.8	-7.8
Myricerin	-7.7	-7.9	-8	-8.1	-8.6	-8.8	-7.6	-8.5	-8.8	-7.6
Licorine	-7.3	-7.5	-6.9	-7.7	-9.2	-7.6	-7	-8.1	-8.5	-7.4
Oroxindin	-8.9	-9.1	-8.1	-8.5	-9.1	-8.8	-8.7	-8.9	-9.4	-7.8
Quercetin	-8.9	-9.8	-9.2	-9.3	-10.3	-8.2	-8.9	-8.7	-9	-8.1
Rutin	-9.2	-8.9	-7.8	-9.7	-10	-9.6	-10	-8.6	-9	-8.6
Resveratrol	-7.3	-7	-7	-7.1	-10	-7.5	-7.7	-7.2	-7.5	-6.8
Saikosaponin A	-9.5	-9.6	-8.9	-10.8	-10.1	-10.2	-9	-10.2	-10.5	-9.2
Curcumin	-7.3	-7.4	-7.1	-7.4	-7.5	-8.6	-7.7	-7.4	-8.4	-6.5
Andrographolide	-8.8	-7.4	-7.5	-7.6	-8	-7.3	-7.8	-7.2	-7.3	-6.9
14-deoxy-andrographolide	-7.8	-7.3	-7.4	-7.5	-7.3	-7.3	-7.5	-7	-7.3	-7.2
Pyrazofurin	-7.1	-7.3	-7.1	-6.9	-7.4	-7.6	-7.6	-7.1	-7.3	-6.8
Rabivirin	-7.2	-7	-7	-6.7	-6.9	-7.1	-7.7	-6.8	-7.1	-6.5
Anabsinthin	-11	-9.9	-8.7	-9.3	-9.2	-9.9	-11.7	-9.6	-9.8	-8.9

Chemical drugs: Chloroquine, Hydroxychloroquine, Lopinavir, Remdesivir, Favipiravir, Indinavir, Atazanavir, Ciclesonide.

Library of natural compounds used in the screening study: Anabsitin, 14-Deoxyandrographolide, Chrysoeriol-8-C-glucoside, 3,5-Dicaffeoylquinic acid, 3-O-Caffeoylquinic acid, Isosakuranetin, Quercetin-3-glucuronide, Anabsinthin, 6-Shogaol, 6-Gingerol, Absitin, Artemisinin, Baicalin, EGCG, Emodin, Eugenol, Glycyrrhizin, Quercetin, Hesperidin, Kaempferol, Lectin, Myricetin, Licorice, Andrographolide, Oroxindin, Rutin, Resveratrol, Saikosaponin, Curcumin, Pyrazofurin, Ribavirin.

The results of the docking screening reveal the binding affinities of various compounds with RNA virus-related target proteins.

Evaluation Principles

More negative docking scores indicate stronger binding affinities, which correspond to a higher potential to inhibit the target. Typically, a docking score ≤ -8 kcal/mol is considered a "strong" interaction, scores between -6 and -8 kcal/mol are regarded as moderate, and scores above -6 kcal/mol are relatively weak.

Synthetic Drugs

ChloroquineandHydroxychloroquine: These compounds show moderate binding affinities across all targets (ranging from -5.3 to -7.4 kcal/mol) and do not exhibit particularly strong interactions with any specific protein.

Lopinavir, Remdesivir, Niclosamide, Indinavir, Atazanavir, and Ciclesonide: Most of these compounds show docking scores ≤ -8 kcal/mol with proteases (3CLpro, LPpro), helicase, and exonuclease/endoribonuclease, suggesting a strong potential to inhibit key enzymes involved in viral replication.

Favipiravir: Exhibits relatively low binding affinities (-5.3 to -6.5 kcal/mol), indicating limited potential.

Observation: Ciclesonide, Indinavir, and Remdesivir demonstrate strong docking scores against multiple enzymes, supporting their potential for multi-target mechanisms.

Natural Compounds

Strong affinity (≤ -9 kcal/mol): Absithin and Anabsinthin: Docking scores range from -10 to -11 kcal/mol with Spike S, Exoribonuclease, and Methyltransferase, highlighting strong multi-target potential. Saikosaponin A, Hesperidin, Quercetin, Rutin, Glycyrrhizic acid, Baicalein, Oroxindin: These compounds show docking scores ≤ -9 kcal/mol with proteases, helicase, and exonuclease/endoribonuclease, indicating strong multi-target inhibitory potential. Ciclesonide (synthetic drug) also appears in this high-affinity group.

Moderate affinity (-6 to -8 kcal/mol): Curcumin, Andrographolide, Artemisinin, EGCG, Resveratrol, Myricerin: These compounds exhibit moderate inhibition potential.

Weak affinity (-5 to -6 kcal/mol): Eugenol, 6-shogaol, 6-gingerol: These compounds show low binding affinity and limited inhibitory potential.

Key Targets

Spike S: Involved in viral entry via ACE2 interaction. Strongest binding observed with Anabsinthin (-11), Absithin (-10), Hesperidin (-9.6), and Saikosaponin A (-9.5).

3CLpro and LPpro (NSP5 & NSP3): Key proteases for viral replication. Strong binding observed with Ciclesonide, Lopinavir, Indinavir, Saikosaponin A, Quercetin, and Baicalein.

Helicase (NSP13), Endoribonuclease (NSP15), Exoribonuclease (NSP10), Methyltransferase (NSP16), RdRp (NSP12): Natural compounds such as Absithin, Anabsinthin, Glycyrrhizic acid, Saikosaponin A, Quercetin, and Rutin show strong docking, indicating broad multi-target potential.

Summary and Strategic Recommendations

High-potential multi-target compounds: Anabsinthin, Absithin, Saikosaponin A, Quercetin, Rutin, Glycyrrhizic acid, Hesperidin, and Ciclesonide. These compounds demonstrate strong binding to Spike S and multiple essential viral enzymes (protease, polymerase, exonuclease).

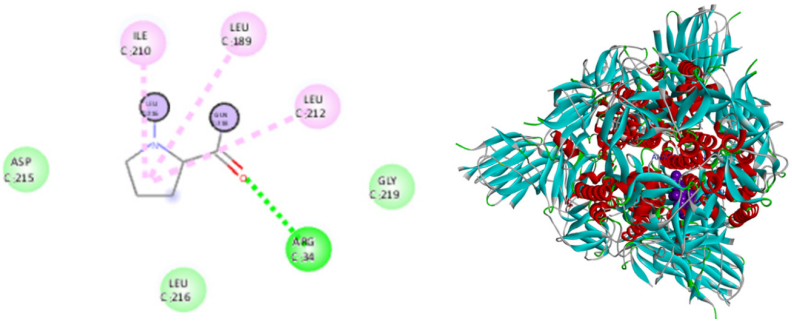
Moderate-potential compounds: Curcumin, Andrographolide, Artemisinin, EGCG, Baicalein, Myricerin. These compounds exhibit moderate binding and could be combined with other compounds to enhance multi-target efficacy.

Low-potential compounds: Eugenol, 6-shogaol, 6-gingerol, and Favipiravir. In the context of these targets, these compounds are less promising.

Some molecular interaction images between target proteins and the active compounds

Binding of Spike S with Anabsanthin

Figure 1. Model of Spike S Binding with Anabsanthin



The binding model shows that the ligand is positioned within a hydrophobic pocket formed mainly by ILE C:210, LEU C:189, LEU C:212, and LEU C:216, which stabilize the ligand through multiple hydrophobic and pi-alkyl interactions. A key polar interaction is formed with ARG C:34, acting as an anchoring residue that contributes significantly to binding affinity. Nearby residues such as ASP C:215 and GLY C:219 are close to the ligand but do not form direct bonds. In the 3D structure, the ligand is embedded deeply within a cavity surrounded by α-helices and β-sheets, indicating good pocket complementarity and stable accommodation within the active site. Overall, the ligand interacts through a combination of hydrophobic contacts and a critical hydrogen bond or electrostatic interaction, suggesting a stable and favorable binding pose.

Binding of Spike S with 6-Gingerol

Figure 2. Model of Spike S Binding with 6-Gingerol

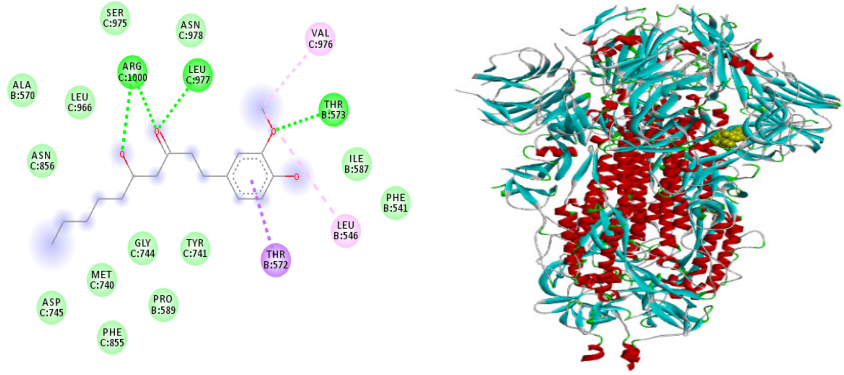
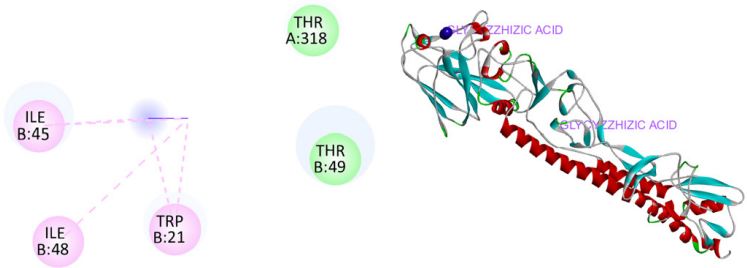


Figure 2 shows the binding model of 6-Gingerol with the SARS-CoV-2 Spike S protein, in which the compound fits into a hydrophobic pocket within the receptor-binding domain (RBD). Its hydroxyl and carbonyl groups form stabilizing hydrogen bonds with nearby polar residues, while the aliphatic chain engages in hydrophobic interactions with non-polar amino acids. The aromatic ring of 6-Gingerol further contributes to stability through π-alkyl or aromatic contacts. These combined interactions suggest that 6-Gingerol may interfere with the Spike-ACE2 recognition process, potentially reducing viral attachment and entry into host cells.

Binding of Endoribo-nuclease with Glycyrrhizic acid

Figure 3. Model of Endoribo-nuclease Binding with Glycyrrhizic acid



The binding model illustrates how Glycyrrhizic acid interacts within the protein's ligand-binding pocket through a network of hydrophobic and polar contacts. Key residues surrounding the ligand include Ile45, Ile48, and Trp21 of chain B, which form a predominantly hydrophobic environment that stabilizes the ligand through van der Waals interactions. Thr49 in chain B and Thr318 in chain A contribute additional stabilization via potential hydrogen-bonding interactions stemming from their polar -OH groups. In the three-dimensional structure, Zhizhic Acid is positioned along a groove between helical and β-sheet regions, fitting into a pocket defined largely by hydrophobic surfaces with supportive polar contacts. Altogether, the arrangement of residues suggests that Zhizhic Acid is accommodated in a well-defined hydrophobic cavity, with threonine-mediated hydrogen bonds enhancing the overall binding affinity.

The selected bioactive compounds after screening were based on the

following criteria: (1) having high binding affinity to the target molecules; (2) being abundant in Vietnamese medicinal plants; and (3) possessing synergistic mechanisms of action without mutual antagonism. Based on recently published literature, pharmacopoeias, and the book *"Medicinal Plants and Remedies of Vietnam"* (2006) by Prof. Do Tat Loi, as well as classical medical texts and traditional prescriptions, we selected key Vietnamese medicinal plants as raw materials to obtain the bioactive compounds used to develop a product capable of inhibiting RNA viruses that cause influenza.

Discussion

The present study employed a comprehensive in silico approach combining bioinformatics and molecular docking to evaluate the antiviral potential of natural compounds from Vietnamese medicinal plants against multiple RNA virus targets [9]. The results demonstrate that several phytochemicals exhibit strong multi-target inhibitory profiles comparable to, or surpassing, those of established synthetic antiviral drugs. These findings highlight the therapeutic potential of traditional medicinal resources [7,14] and underscore the utility of computational screening in expediting antiviral drug discovery [9-10].

Validation Through Synthetic Drug Benchmarking

Synthetic antiviral drugs served as critical positive controls to validate our computational pipeline. Ciclesonide, Indinavir, and Remdesivir consistently exhibited high binding affinities (≤ -8 kcal/mol) across essential viral proteins, including proteases (3CLpro, PLpro), polymerases (RdRp), and nucleases (NSP10, NSP15). These results align with their documented broad-spectrum antiviral mechanisms and clinical efficacy [5]. Conversely, Chloroquine, Hydroxychloroquine, and Favipiravir displayed weaker and less consistent interactions, concordant with clinical observations of limited therapeutic benefit. This strong correlation between in silico predictions and known pharmacological outcomes validates the reliability of our docking methodology and parameter settings [10].

Multi-Target Inhibitory Potential of Natural Compounds

Among natural compounds, a structurally diverse array - encompassing terpenoids, flavonoids, and saponins- demonstrated remarkable inhibitory potential [7-8]. Absinthin and Anabsinthin emerged as the most potent candidates, achieving binding energies as low as -11 kcal/mol. Their strong interactions with Spike S (viral entry), exonuclease (NSP10), and methyltransferase (NSP16) suggest a multifaceted mechanism targeting both viral attachment and post-entry RNA processing. This multi-target profile is strategically advantageous for combating RNA viruses, which exhibit high mutation rates and frequently develop resistance to single-target therapeutics [4-5].

Flavonoids-including Quercetin, Rutin, Hesperidin, Baicalein, and Oroxindin-demonstrated robust binding to 3CLpro, PLpro, helicase (NSP13), and viral nucleases [8]. These compounds are abundant in Vietnamese medicinal plants [14], possess well-characterized pharmacological safety profiles, and offer structural versatility for chemical optimization. Their consistent activity across proteolytic and replicative machinery suggests they can disrupt multiple stages of the viral life cycle, from polyprotein processing to genome replication. This broad mechanistic coverage may significantly reduce the probability of viral escape mutations [4].

Saponins, particularly Saikosaponin A and Glycyrrhizic acid, also exhibited notable multi-target inhibition. These findings corroborate existing experimental evidence of their antiviral and immunomodulatory properties [7], reinforcing their potential as dual-action therapeutic agents. The prevalence of these compounds in traditional Vietnamese herbal formulations further supports their accessibility and cultural relevance for drug development initiatives in the region [6,14].

Moderate and Weak Binders: Implications for Therapeutic Strategy

Moderately active compounds - Curcumin, Andrographolide, Artemisinin, EGCG, and Resveratrol - exhibited acceptable but suboptimal binding profiles (typically -7 to -8 kcal/mol) [13,15]. While these may not qualify as lead compounds for monotherapy, their moderate activity positions them as viable candidates for combination therapy or as adjuvants to enhance the efficacy of more potent antivirals. Synergistic formulations leveraging these compounds

could improve therapeutic outcomes while minimizing toxicity.

In contrast, weak binders such as Eugenol, 6-shogaol, and 6-gingerol showed limited direct inhibitory potential (> -7 kcal/mol). This suggests that their reported antiviral effects, if any, are likely mediated through indirect mechanisms such as immunomodulation [7] or require structural modifications to enhance target specificity and binding affinity [9].

Mechanistic Advantages and Resistance Mitigation

The multi-target inhibitory capacity observed in top-ranking compounds is particularly significant in the context of RNA virus biology. RNA viruses, including coronaviruses and influenza, evolve rapidly through high replication error rates and recombination events [4]. Single-target drugs are prone to resistance development, whereas compounds that simultaneously inhibit multiple essential viral proteins create a higher genetic barrier to resistance [5]. Natural products, with their inherent chemical diversity and polypharmacological properties, represent an underutilized reservoir for developing such multi-target antivirals [7, 9].

Limitations and Future Perspectives

This study is inherently limited by its computational nature [10]. While molecular docking provides valuable insights into binding potential, it does not account for factors such as bioavailability, metabolic stability, cellular permeability, or cytotoxicity. Experimental validation through enzymatic inhibition assays (e.g., fluorescence resonance energy transfer or enzyme kinetics) [11-12], cell-based antiviral assays (e.g., plaque reduction or cytopathic effect inhibition), and pharmacokinetic profiling is essential to confirm biological activity and therapeutic viability.

Additionally, the study did not evaluate potential off-target effects or drug-drug interactions, which are critical for safety assessment. Future research should prioritize:

In vitro validation using viral enzyme assays and cell culture models [11-12]

Structure-activity relationship (SAR) studies to optimize lead compounds [9]

In vivo efficacy studies in animal models of viral infection

Toxicological profiling and ADMET (absorption, distribution, metabolism, excretion, toxicity) characterization

Investigation of synergistic combinations among top candidates [5]

Broader Implications

This study bridges traditional medicinal knowledge with modern computational drug discovery, demonstrating that Vietnamese medicinal plants represent a valuable, yet underexplored, source of antiviral scaffolds [6-7, 14]. The identification of multi-target inhibitors not only advances the rational design of natural product-based therapeutics but also provides a foundation for phytopharmaceutical development tailored to regional needs and resources.

Conclusion

This bioinformatics-driven investigation identified several natural compounds from Vietnamese medicinal plants with potent and broad-spectrum antiviral activity against RNA viruses. Anabsinthin, Absinthin, Saikosaponin A, Quercetin, Rutin, Hesperidin, and Glycyrrhizic acid emerged as the most promising multi-target inhibitors, exhibiting consistently favorable docking energies (≤ -8 to -11 kcal/mol) across critical viral proteins involved in entry, proteolysis, RNA replication, and genome maintenance. Among synthetic drugs, Ciclesonide also demonstrated comparable multi-target potential.

These findings validate the therapeutic relevance of traditional medicinal resources in modern antiviral research and highlight the efficiency of computational screening as a rapid, cost-effective strategy for early-stage drug discovery. The multi-target nature of these compounds addresses the challenge of viral resistance and positions them as promising candidates for further development.

Experimental validation through in vitro enzymatic assays, cell-based antiviral studies, and in vivo efficacy testing is warranted to confirm biological activity, optimize pharmacological profiles, and advance these candidates toward preclinical and clinical evaluation. This work establishes a strong foundation for developing natural product-based antivirals to combat current and emerging RNA virus threats, with particular emphasis on leveraging Vietnam's rich biodiversity and traditional medicine heritage.

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