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Original Research Article

Network Pharmacology and *In Silico* Investigation on *Saussurea lappa* for Viral Respiratory Diseases

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ABSTRACT

Respiratory viral diseases are prevalently affecting people of all ages, requiring extensive study into herbal medicine as a potential solution. Therefore, this study aimed to identify the *Saussurea lappa* (*S. lappa*) compounds and explain the molecular mechanisms against respiratory viral diseases. The molecular mechanisms of the compound against respiratory viral diseases was determined through network pharmacological methods using Cytoscape 3.10.0, GeneCards, OMIM, STRING 11.0, and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. The interaction of compounds with NFκB and TNFα were analyzed using molecular docking with dexamethasone as a control through PyRx Autodock Vina 9.0 and Biovia Discovery Studio. The results showed that *S. lappa* compounds activated defense mechanisms against viral infection, impacting genes associated with SARS-CoV-2 disease, and activating NF-κB and NRF2 signaling pathways. The molecular docking results, supporting the network pharmacology finding, indicated that the syrigaresinol compound, with several NF-κB binding residues, inhibited the inflammatory pathway by blocking the protein signal. Saussureamine A and C, with lower binding affinities for TNFα, showed higher effectiveness compared to dexamethasone, showing their potential to reduce inflammation. In addition, syrigaresinol and saussureamine A and C showed potential for reducing inflammation. These results showed the potential of *S. lappa* as an herb for defense against SARS-CoV-2.

Keywords: Molecular docking; Network pharmacology; *Saussurea lappa*; Viral respiratory diseases

Introduction

Natural bioactive compounds with pharmaceutical potential are derived from plants. However, these compounds are widely recognized as secondary plant metabolites, such as terpenoids, lignans, flavonoids, tannins, and alkaloids, which have various biological activities, including antibacterial, antiviral, and functional properties similar to the activity of drugs. [1,2] Several medicinal metabolites obtained from plants can hinder virus reproduction or impede cellular infection, hindering the transmission of the virus. In the current scenario, respiratory tract infections have led to significant morbidity and mortality rates worldwide. Several viral agents have the potential to trigger respiratory illnesses, leading to widespread outbreaks and, in specific cases, global pandemics. These include influenza viruses and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). [3]

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Saussurea lappa (*S. lappa*), also called *Saussurea costus*, is a perennial plant commonly used in herbal medicine and traditionally known as Qust (costus) or qusthul Hindi. The root extract of this plant possesses anti-ulcer, anti-spasmodic, anti-cancer, hepatoprotective, and anti-inflammatory effects, which are commonly used to treat rheumatic conditions and viral infections.[1,4,5] Abdallah (2017) and El-Far (2018) proved that *S. lappa* contains various compounds such as sesquiterpenes, flavonoids, coumarins, quinones, resins, phytosterols, lignans, and terpenes. Among the sesquiterpenes, dehydrocostulactone, dehydrocostunolide, and costunolide lappadilactone were predominantly isolated significant chemicals.[4,6] Based on metabolite screening with LCMS/MS, *S. lappa* ethanol extract contains numerous sesquiterpene lactone compounds, including saussureamine B, C, A, and dehydrocostus lactone. Vincent (2020) reported that the constituent syrigaresinol found in *S. lappa* showed antiviral efficacy against SARS-CoV-2 based an *in silico* study.[7] According to Polat (2023), the aqueous extract of *S. lappa* showed antiviral properties against SARS-CoV-2 both in vivo and in vitro.[8]

Given that SARS-CoV-2 triggers an inflammatory response, there is a growing interest in exploring herbal medications to attain antiviral, anti-inflammatory, antioxidant, and preventive outcomes. Among these herbal medicines, *S. lappa* is able to modulate the immune system by inhibiting the release of pro-inflammatory cytokines, which play an important role in the pathogenesis of infectious diseases.[9] Furthermore, herbal medicines, including those derived from *S. lappa*, play a crucial role in modulating functional immunity, controlling both adaptive and

default immunity. This modulation is accompanied by antivirus properties effective against various virus strains.[10]
A medicinal plant, known as herbal medicine, consists of various ingredients that work through various targets and pathways to provide health benefits. Network pharmacology is one of the most influential modern methods to show the molecular and pharmacology mechanisms of herbal medicines.[11] In network pharmacology, several active compounds are known that can interact with a variety of genes or proteins, explaining their targets, and diseases interactions. This indicates the potential of network pharmacology to comprehensively describe the effects of drugs on human biological systems by showing the interactions in tissue models.[12] Consequently, this study uses a pharmacological network method to determine how all of the compounds in *S. lappa* work at the molecular level in viral respiratory diseases. The results are expected to provide important insights for developing new herbal drugs to treat viral respiratory diseases.

Material and Methods

Data collection and structure determination
Secondary metabolites of *S. lappa* were obtained from previous study,[7,13] including Costunolide (5281437), Curcumen (92139), Dehydrocostus lactone (73174), Linsidomine (5219), Myristicin (4276), Pristimerin (159516), Saussureamine A (10427798), Saussureamine B (10360205), Saussureamine C (9998735), Saussureamine D (9975956), Spermine Hydrochloride (1103), Syrigaresinol (45027870), and Dexamethasone (5743) which serves as the positive control. The structures of all secondary metabolites were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/). The proteins NF-kB and TNFα, which are part of the anti-inflammatory pathway were used for molecular docking. Subsequently, the 3D conformations of NF-kB and TNFα were obtained from the protein data bank (https://www.rcsb.org/) using the PDB ID 4dn5 and 2e7a, respectively.

Target Gene Screening related to S. lappa
S. lappa-related target proteins were collected and screened using BindingDB (https://www.bindingdb.org/bind/index.jsp) in the "*Homo sapiens*" setting. The results obtained were limited to targets with a relevance score of > 0.700, which was considered to meet the standards.[14]

Collection and screening of Target Proteins related to viral respiratory disease.
Target proteins associated with viral respiratory disease were obtained by entering the keyword "viral respiratory disease" in the OMIM database (https://www.omim.org/) and GeneCards (https://www.genecards.org/).[15,16] The OMIM and GeneCards data results were limited to targets with a relevance value of ≥10.00. The target proteins collected from the database were aggregated and removed based on the existence of duplicates. Subsequently, the name of the target protein was standardized according to the Uniprot database.[17]

Network construction
Saussurea lappa-related target genes and proteins were imported into Cytoscape version 3.10.0 to construct the drug-compound-target-disease network. Additionally, the 'Network Analyzer' tool was used to analyze the topological properties.[18,19] The primary signaling pathways implicated in viral respiratory illness were investigated using the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis and Wikipathways. [20]

Molecular docking analysis
Molecular docking simulation was performed on AutoDock Vina on PyRx software. [21] The process was conducted using the parameters RMSD max of two and binding posture max of eight, which was repeated three times. Discovery Studio version 21.1.1 was used to determine the docking data and obtained two and three-dimensional picture of the ligand binding site on the target protein along with type of bond formed with the amino acid residues.

Results and Discussion

The 12 metabolites in the *S. lappa* root extract had 43 target proteins in the BindingDB database. These results indicated that the compounds strongly correlate with specific gene targets, which are predicted to play a role in various biological activities. This information provided important insights for research and development of the therapeutic potential of *S. lappa* and its constituent compounds.
Target proteins associated with viral infection disorders in 124 diseases were gathered from the two human genome databases, namely OMIM and GeneCards. A total of 101 target proteins were retrieved after the duplicates were eliminated.
Compound network topology describes the links between substances (nodes) and their implication on each other (edges) in pharmacological networks that control the activity of target genes. In the network shown in Figure 1, 12 chemicals have 12 edges and 84 target protein nodes. Each protein is a node in this network structure, and edges represent interactions between proteins. According to Zhou (2022), these interactions could entail the binding of chemicals to target genes, the control of gene expression, or biological regulation. [16] Figure 1 showed that 10 compounds in *S. lappa* (or 90% of the total compounds) have more than two target genes, indicating the impact of most compounds in the network on numerous target genes in respiratory viral infections. Protein-protein interaction (PPI) analysis of the target gene compounds in viral respiratory diseases aims to investigate the interactions of proteins in the biological processes of this disease, as shown in Figure 2. PPI refers to the functional interactions between two or more proteins that play essential roles in various cellular processes. This analysis is capable of providing information about cell signaling pathways in viral respiratory diseases.

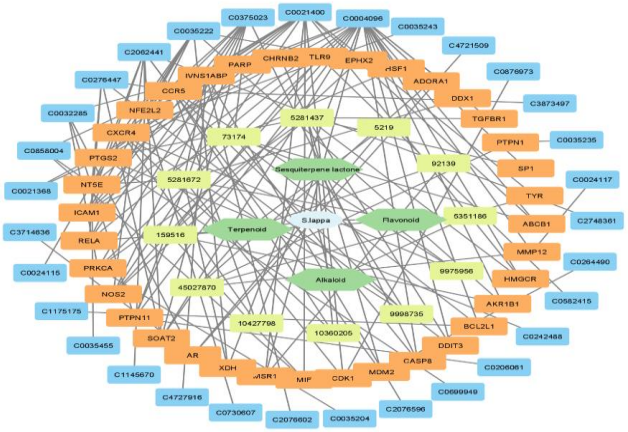

Figure 1. Compound network topology in *Saussurea lappa* with gene targets. Number of nodes: 84, number of edges: 158. Orange boxes represent target genes, yellow boxes represent compounds in *S. lappa*, and blue boxes represent diseases

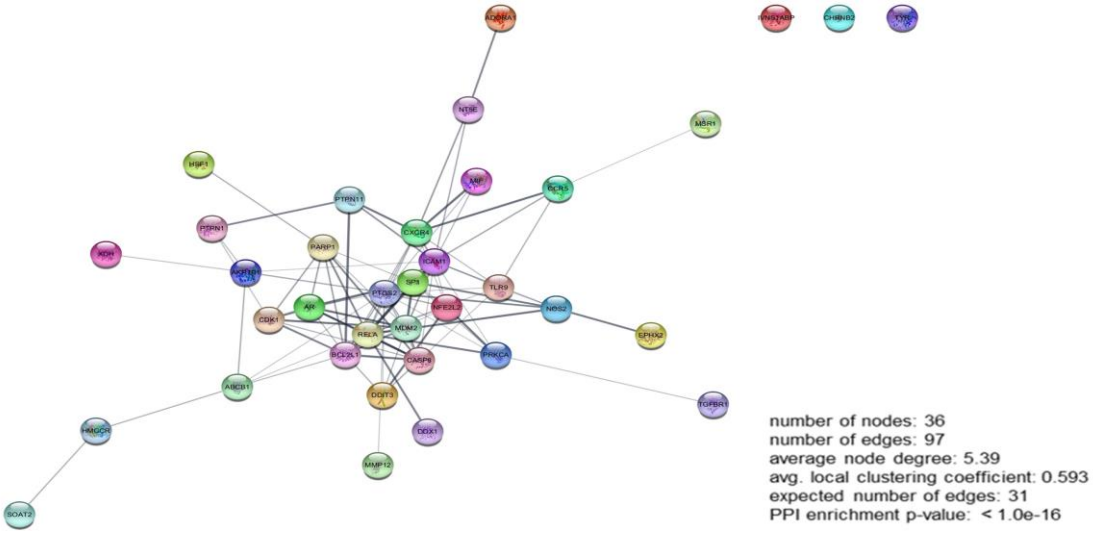

Figure 2. Protein-Protein Interaction (PPI) of compounds in *S. lappa* in the biological process of viral respiratory diseases. This interaction shows 36 nodes, which likely represent the proteins produced by the target gene, and 97 edges, representing the interactions between these proteins. The nodes and edges in this network represent physical or functional relationships between these proteins, suggesting a potential role in viral respiratory diseases.

Table 1: *Saussurea lappa* biological processes and signaling pathways associated with viral respiratory diseases based on KEGG analysis and Wikipathways.

ID	Number of genes involved	Category	Description	False discovery rate (FDR)
GO:0009615	7	GO Biological Process	Response to virus	8.25e-05
GO: 0016032	13	GO Biological Process	Viral Process	1.28e-07
GO:0019050	2	GO Biological Process	Suppression by virus of host apoptotic process	0.0016
GO:0019064	2	GO Biological Process	Fusion of virus membrane with host plasma membrane	0.0043
GO:0051607	4	GO Biological Process	Defense response to virus	0.0167
GO:0001618	4	GO Biological Process	Virus receptor activity	0.0050
hsa05164	4	KEGG	Influenza A	0.0032
WP5113	2	Wikipathways	Antiviral and anti-inflammatory effects of Nrf2 on SARS-CoV-2 Pathways	0.0189

In this study, the active compounds found in the root extract of *S. lappa* were put through a KEGG-based pathway and Wikipathway analysis. This analysis is presented in Table 1, providing information on the signaling pathways affected by the compounds in *S. lappa* and the associated biological processes.

The result of the genes ontology analysis showed that the compounds identified in *S. lappa* activated the target genes in various pathways related to the defense mechanism against viral infection. In this case, the activities included are the immune response to viruses, different viral processes, viruses stopping the host apoptotic process, viruses merging with the host plasma membrane, and the activation of virus receptors.

According to Figure 3, the KEGG analysis showed that the two compounds found in *S. lappa*, dehydrocostus lactone and costunolide, targeted four genes associated with respiratory tract infections, particularly Influenza A. These four genes were identified as CASP8, ICAM1, ReIA/NF-κB, and PRKCA. Caspase-8 (CASP8) directly activates CASP3, initializing the apoptotic signaling pathway. Additionally, CASP8 activated Bid and initiated the mitochondrial pathway during apoptosis. Wang (2021) reported the anti-inflammatory role of caspase-8 during Influenza A virus infection. [22]

Figure 4 showed that the chemicals in *S. lappa* were thought to fight viruses and lower inflammation by activating Nrf2 on the SARS-CoV-2 pathway. The proteins engaged in this pathway were ReIA/NF-κB and NFE2L2. Figure 4 further illustrates the programmed cell death (PCD) signaling pathway activation by costunolide. This route is vital in triggering the innate immune response, serving as the primary defense against infections. Caspases have a role in PCD, immune response, and regulation of homeostasis. Caspase-8 has a specific role in the PCD pathway, including pyroptosis, apoptosis, and necroptosis.

ICAM1 and NF-κB stimulate the Toll-like receptor and RIG-I-like receptor signaling pathways. Infection with the influenza virus enhances the production of ICAM-1, resulting in the activation of NFκB and the induction of steady-state messenger RNA (mRNA) levels. Consequently, the activation of NF-κB hindered the replication of the influenza virus.[23] PKCα also plays a role in the MAPK signaling pathway, which activates Raf and controls MAPK/ERK kinase (MEK) ½ phosphorylation, regulating ERK ½ activation, thereby increasing viral protein expression. Mondal (2017) stated that during viral infection, nucleoprotein (NP) oligomerization and ribonucleoprotein complex (RNP) assembly are controlled by activated PKCα through interaction with the viral PB2 polymerase component. Consequently, viruses exploit PKCα as a host to control RNP assembly, a crucial stage in transitioning from primary transcription to genome replication. [24]

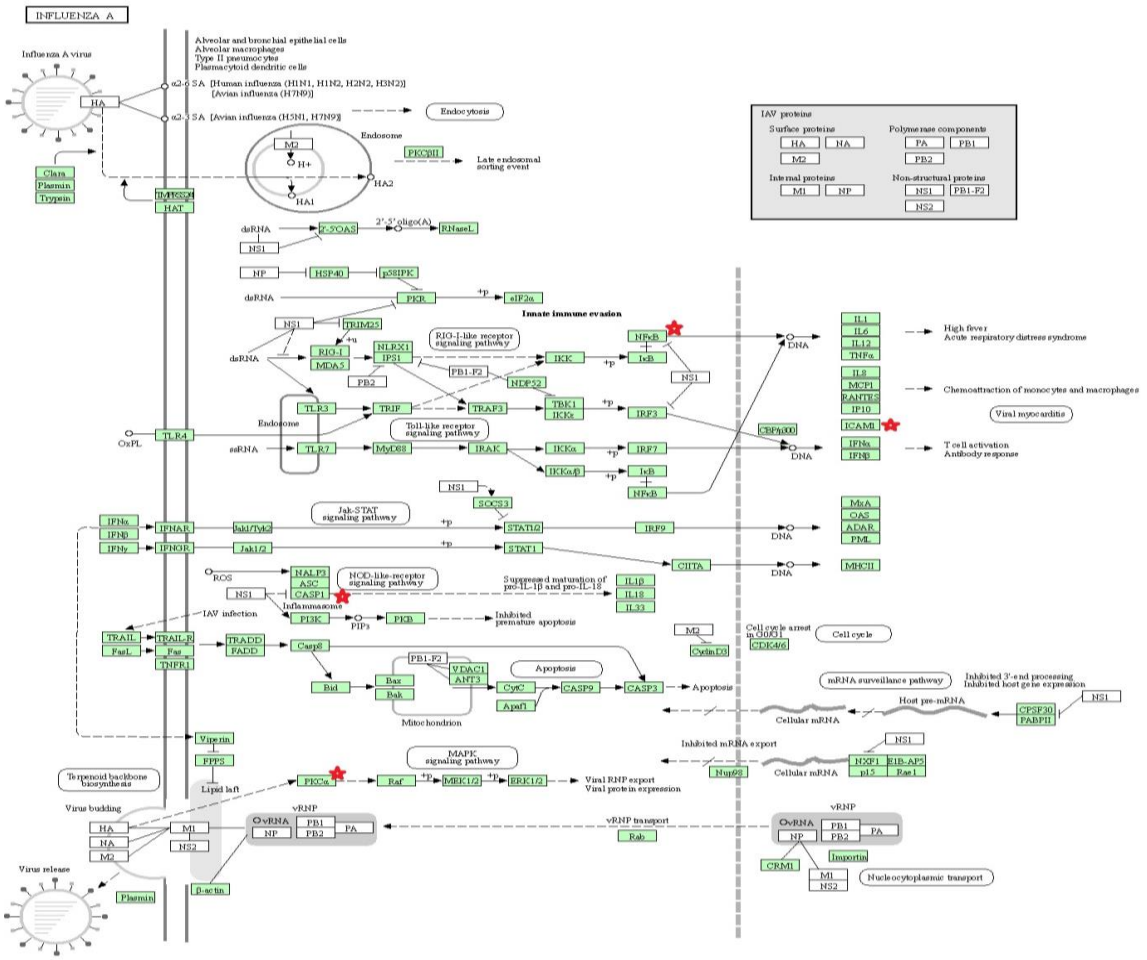

Figure 3: The role of compounds in *S. lappa* in influenza A pathway

The star marks indicate the target gene for respiratory tract infections due to Influenza A (CASP8, ICAM1, RELA/NF-κB, and PRKCA). CASP8: Caspase 8; ICAM: Intercellular Adhesion Molecule 1: ReIA/NF-κB: RelA/Nuclear Factor-κB; PRKCA: Protein Kinase C Alpha.

Based on Figure 4, it can be inferred that under typical circumstances, erythroid-related factor 2 (NFE2L2), also known as NRF2, interacts with Kelch-like ECH-associated protein 1 (KEAP1), situated in the cytoplasm. Simultaneously, infection with SARS-CoV-2 leads to the occurrence of oxidative stress. Consequently, NRF2 is transported to the nucleus and bind to anti-oxidant response elements, triggering the transcription of genes that protect cells from damage. This is followed by the stimulation of the NF-κB signaling pathway by ReIA/NF-κB, contributing to the inflammatory process triggered by SARS-CoV-2. NF-κB regulates the essential transcriptional genes responsible for activating the expression of target genes implicated in inflammation. [25]

Of the 12 active compounds of *S. lappa* tested with NF-κB, syrigaresinol showed the lowest binding affinity (-9.5 kcal/mol) compared to the other compounds, as shown in Table 2. The syrigaresinol molecule shows a binding affinity value comparatively lower than that of dexamethasone, precisely measuring at -8.2 kcal/mol. These results indicate that the syrigaresinol compound shows higher efficacy in inhibiting the inflammatory pathway by effectively blocking the protein signal than the control group, which relies solely on binding mechanisms. Furthermore, syrigaresinol has the highest residues that bond to the active site of NF-κB as shown in Table 2 and Figure 5.

Saussureamine A and C showed bonding affinity values of -9.2 kcal/mol to TNFα. Both compounds showed binding affinities relatively lower than dexamethasone, precisely measured at -8.2 kcal/mol. This suggested that saussureamine A and C were more effective than dexamethasone in inhibiting inflammatory pathways by blocking protein signals, as shown in Table 2 and Figure 6.

Dexamethasone was selected as a control in this model due to its ability to reduce inflammation and suppress the immune system quickly. These properties are achieved by blocking the production of pro-inflammatory cytokines, such as IL-1, IL-2, IL-6, IL-8, and TNFα, which are linked to the severity of SARS-CoV-2. [26] The bond affinity value represents the quantitative measure of the bond strength formed between a ligand, referring to a specific chemical composition, and a receptor, which indicated a protein. According to a previous study,[27] a lower bond affinity value showed a more significant and stable interaction between the ligand and the receptor. The bonding affinity quantifies the energy liberated when a bonding molecule engages in an interaction and establishes a bond with the recipient. Consequently, ligands require more incredible energy to establish stronger interactions with receptors when the bond affinity values are lower.[28]

The NF-κB signaling system plays a crucial role in inducing the expression of pro-inflammatory cytokines, overseeing several biological functions, including inflammatory reactions, cellular proliferation, and programmed cell death. Severe COVID-19 patients experience an increase in pro-inflammatory cytokines due to the activation of NF-κB by SARS-CoV-2, which produces pro-inflammatory cytokines. These cytokines are closely related to the development and progression of COVID-19.[29,30]

The ORF3a, M, ORF7a, and N proteins of SARS-CoV-2 further contribute to the activation of the NF-κB pathway. The ORF7 protein stimulates the production of NF-κB-regulated cytokines, including IL-1α, IL-1β, IL-6, IL-8, IL-10, TNFα, and IFNβ. Cytokines and chemokines frequently show high levels in severely impacted COVID-19 individuals.[31] The molecular docking results show that the chemicals found in *S. lappa* can hinder the production of the pro-inflammatory cytokines NFκB and TNFα.

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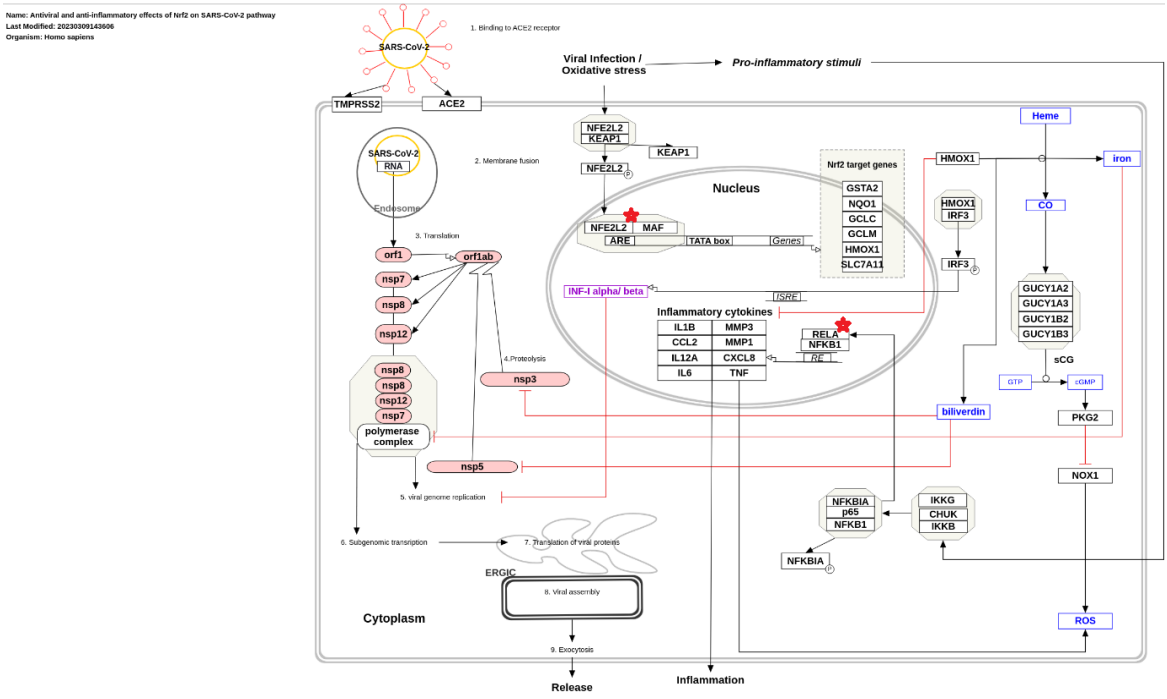

Figure 4: The role of compounds in *S. lappa* in the SARS-CoV-2 pathway
The star marks indicate the target gene for respiratory tract infections due to SARS-CoV-2 (NFE2L2 and RELA/NF-κB). NFE2L2: Nuclear factor erythroid 2 related factor 2; ReIA/NF-κB: ReIA/Nuclear Factor-κB.

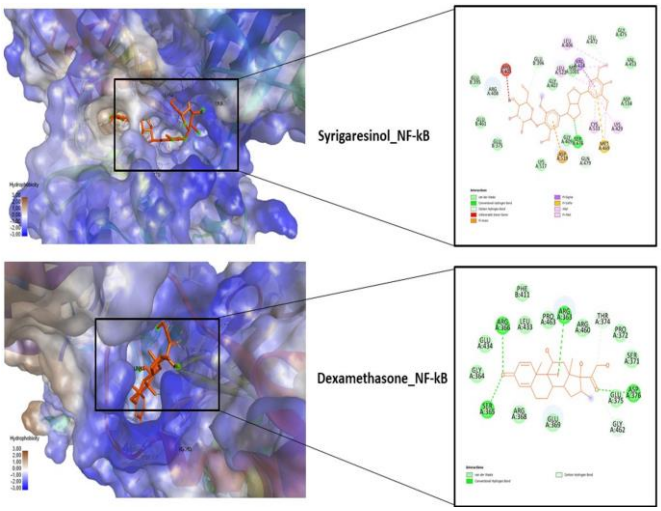

Figure 5: Interaction between syrigaresinol and dexamethasone against NF-κB

Conclusion

In conclusion, this study showed that *S. lappa* compounds activated genes in defense mechanisms against viral infection, including response, viral processes, and fusion of virus and host plasma membranes. These compounds also acted as anti-influenza agents by upregulating or downregulating genes like CASP8, ICAM1, ReIA, and PRKCA. Furthermore, the chemicals found in *S. lappa* affected two specific genes associated with SARS-CoV-2 sickness, activating NF-κB and NRF2 signaling pathways, crucial in the inflammation mechanisms. The syrigaresinol compound, with its high NF-kB binding residues, effectively inhibited the inflammatory pathway by blocking protein signals. Saussureamine A and C, with lower binding affinity to TNFα, showed higher efficacy than dexamethasone, showing their potential in reducing inflammation. This study suggested that *S. lappa* served as a defense against SARS-CoV-2 through the inflammatory pathway. Further in vivo studies will be required to confirm the present study.

Conflict of Interest

The authors declare no conflict of interest.

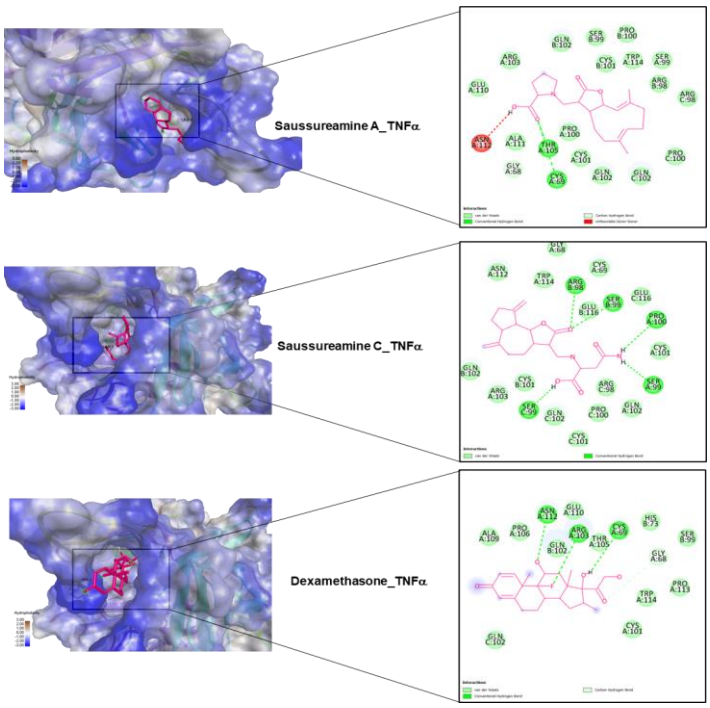

Figure 6: Interaction between saussureamine A, saussureamine C and dexamethasone against TNFα

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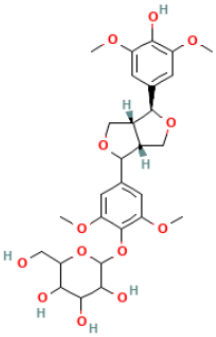
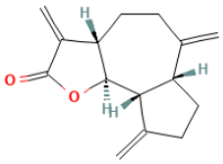
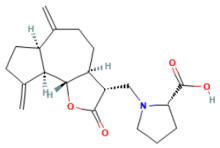
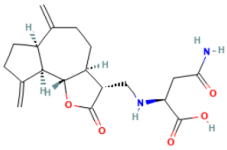
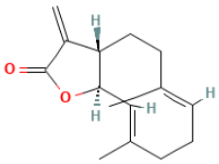
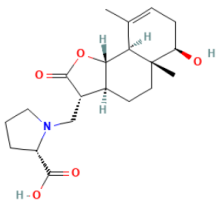
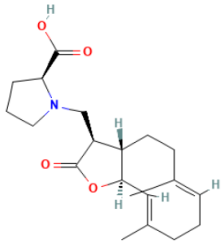
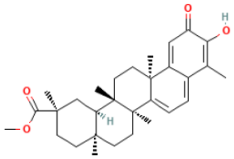
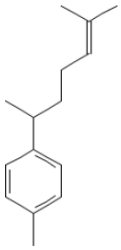
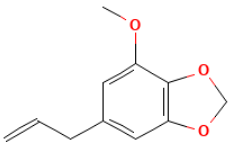
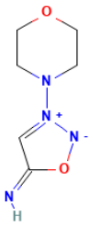
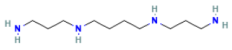
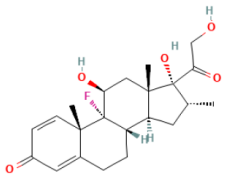
Table 2: Molecular interaction of *S. lappa* compounds and dexamethason with NFκB and TNFα-targeted proteins

No	Compounds		Molecular structure	NF-κB		TNFα	
				Binding Affinity (kcal/mol)	**Residues***	**Binding Affinity (kcal/mol)**	**Residues***
1.	Syrigaresinol (45027870)			-9,5	**A:SER476**, **A:GLN479**, **A:LEU472**, A:ARG408, B:GLU396, **A:VAL414**, **A:MET469**, A:LEU406, **A:LEU522**, **A:LYS429**, **A:CYS533**, **A:LEU522**, A:ARG405	-9,0	A:GLU110, **C:ARG98**, **B:SER99**, **C:PRO100**, **A:ALA109**, **A:PRO106**
2.	Dehydrocostus lactone (73174)			-8,8	**VAL414, B:CYS533,**	-7,7	**B:ARG98**, **B:SER99**
3.	Saussureamine (10360205)	B		-8,5	A:THR401, B:LEU404, A:HIS402, A:ARG405	-8,7	B:**ARG98**, **B:SER99**, **C:ARG98**, **A:CYS69**
4.	Saussureamine (9998735)	C		-8,5	**A:ASP519**, **A:SER476**, A:ARG408, A:LEU406, **A:VAL414**, **A:LEU522**	-9,2	**B:ARG98**, **B:SER99**, **A:PRO100**
5.	Costunolide (5281437)			-8,3	A:ARG363, A:ARG460, A:PRO463	-7,4	**B:ARG98**
6.	Saussureamine (9975956)	D		-8,3	B:GLU413, B:GLN403, A:LEU404, B:ARG405, B:HIS415	-7,9	C:THR86, C:SER84, C:HIS87, C:SER84, C:GLN89,
7.	Saussureamine (10427798)	A		-8,1	A:ARG363, A:ARG460, A:GLY462, A:PRO463	-9,2	**A:CYS69**, **A:THR105**, A:GLY68, **A:ASN112**
8.	Pristimerin (159516)			-7,8	B:ARG408, B:GLN479, B:LYS517, A:PRO372, A:LYS373, B:GLY407,	-7,6	A:ASN19, A:LEU29

				B:SER476, **B:ASP519** A:LYS373		
9.	Curcumen (92139)		-7,1	**VAL414**, **A:LEU522,** **A:MET469**, A:ALA427, **A:CYS533**, **A:LYS429**, A:LEU406, A:LEU471, **A:LEU522**, A:LEU406, **A:ALA427**	-5,5	**A:ASN112, A:CYS69**, **A:CYS101**, **A:PRO106**, **A:CYS69**
10.	Myristicin (4276)		-6,4	**A:VAL414**, **A:LEU522**, **A:MET469**, A:VAL414, **A:ALA427**, **A:LYS429**, **A:CYS533**, A:LEU406, A:LEU471	-6,0	**B:ARG98**, **C:SER99, B:CYS101**, **C:CYS69**
11.	Linsidomine (5219)		-5,5	B:ARG460, B:GLY462, PRO463	-6,3	**A:GLN102**, **C:ARG98**, **C:SER99**, **C:PRO100**, **C:ARG98**, **B:CYS101**
12.	Spermine Hydrochloride (1103)		-4,5	A:ASP519, A:ASN520, A:LEU406, A:GLN479,	-4,7	**B:SER99**, **C:PRO100**, **A:CYS101**, **A:SER99**, **B:PRO100**, **A:ASN112**
13.	**Dexamethasone (5743)**		**-8,2**	A:ARG363, A:SER365, **A:ARG366**, A:ASP376, :GLY462, A:THR374	**-8,2**	**A:ARG103**, **A:ASN112**, **A:CYS69**, A:GLY68

*Note. Residues in bold are active sites of NF-κB and TNFα proteins.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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