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Research Article

DIFERULOYLMETHANE IDENTIFIED AGAINST CYSTEINE PROTEASES AND TMPRSS2 USING *IN SILICO* APPROACH

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ABSTRACT

Objective: The present research aims to identify the bioactive compounds as therapeutic agents targeting cellular pathways in respiratory tract infection.

Methods: Insilico docking was performed using Autodock Vina and visualized by Discovery Studio. MD (Molecular Dynamic) simulation was performed to identify the structural dynamics of the protein in a complex with a ligand.

Results: The findings of the present study shows that curcumin (diferuloylmethane) exhibited the high binding affinity among ten natural ligands targeting TMPRSS2 (–7.2 kcal/mol), RdRp (–7.7 kcal/mol), 3CLpro (–7.6 kcal/mol), PLpro (–7.3 kcal/mol), and EndoU (–7.0 kcal/mol) and MD simulations showed structural stability with root mean square deviation (RMSD) values: TMPRSS2–curcumin (0.088–3.05 Å) confirmed by hydrogen bond analysis.

Conclusion: The study provides a rationale that curcumin can be used as a therapeutic agent against respiratory tract infection, which regulates the expression of both pro-and anti-inflammatory factors.

Keywords: Respiratory tract infection, Spices, MD simulation, Diferuloylmethane, Cysteine proteases, TMPRSS2

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INTRODUCTION

Respiratory tract infection has emerged as a serious concern globally, especially in winter, which is an influenza season and the severity of the disease increases, making the Severe acute respiratory syndrome combined with influenza a sinister [1]. Chen *et al.*, (2022) explained that the fidelity of viral enzymes is responsible for the high mutation rate caused by RNA-dependent RNA polymerase (RdRp) [2]. The spike (S) is a transmembrane protein that forms homotrimers and facilitates the binding of the virus to the host cells via the angiotensin-converting enzyme 2 (ACE-2) receptor. A furin-like protease Transmembrane serine protease 2(TMPRSS2) originating from the host cell cleaves the S (Spike) protein into two subunits S1 and S2, to support the viral growth and cell fusion, and helps to form replicase-transcriptase complex. The two important cysteine proteases, namely the 3-chymotrypsin-like protease (3CLpro) and the papain-like protease (PLpro), are essential for viral genome transcription and replication, along with promoting innate immune evasion [3]. Thus making 3CLpro and PLpro as an attractive drug target.

The growing interest in natural products as a source of new drugs can be attributed to many factors, including therapeutic needs, the wide range of both chemical structures and biological activities of natural secondary metabolites, and the adequacy of bioactive natural products as biochemical and molecular probes. Natural-derived compounds constantly become a worthy therapeutic alternative against several diseases, including respiratory viral infections like severe acute respiratory syndrome [4]. Identification of the antiviral properties of the natural products may help to understand the plausible mechanism of interaction and identification of the potential target of virus–host-specific interactions. In the present study, ten natural compounds (Ajoene, Allicin, Alliin, Bromhexine, Capsaicin, Curcumin, Colchicine, Diallyl disulfide, 6-Gingerol, Sakuranetin) were selected. These compounds are important compounds in garlic, onion, chillies, and are widely used as spices. They are known as immune boosters and are used in treating respiratory ailments [5].

In the present study, ten natural compounds (Ajoene, Allicin, Alliin, Bromhexine, Capsaicin, Diferuloylmethane (curcumin), Colchicine, Diallyl disulfide, 6-Gingerol, Sakuranetin were identified as an immune booster and are used in treating respiratory ailments [6]. *In silico* docking analysis was performed to study the efficacy of the selected compound against (RNA dependent RNA polymerase (RdRp), spike (S) glycoprotein, nonstructural protein-15 (NSP15) encodes for a uridylate-specific endoribonuclease (EndoU) enzyme, 3-chymotrypsin-like protease 3CLpro, PLpro, and TMPRSS2.

MATERIALS AND METHODS

Retrieval of receptors

The crystal structures of receptors were retrieved from the protein data bank.(https://www.rcsb.org/). The IDs were as follows: TMPRRS2 (1Z8G) [7]; Spike glycoprotein (6VXX) [8]; RdRp (6NUR) [9]; 3CLpro (6M2N) [10];PLpro (6WX4) [11]; EndoU (6W01) [12]

Ligand retrieval and preparation for docking

The 3D structure of the 10 ligands was downloaded from Pub-Chem (https://pubchem.ncbi.nlm.nih.gov/) [13]. The downloaded compounds in PDB format were converted into PDBQT file format using Open Babel version 3.0. (O'Boyle *et al.*, 2011)[14] for docking and screening against the selected proteins.

Drug-like likeness study

Ten ligands were converted into SMILES files. Its Lipinski Rule properties were individually predicted by inputting its SMILES string and the prediction was done by the server (http://www.molinspiration.com/cgi-bin/properties) [13]. Molecular weight and LogP were the filters

representing the drug-like likeness; there are a few more parameters in this study, including the number of hydrogen bond donors (HBD), the number of hydrogen bond acceptors (HBA), the number of rotatable bonds and the surface area, were also predicted [15].

Protein preparation for docking

The 3D protein crystal structures were uploaded to Discovery Studio. The complexed ligand from the individual protein target was taken out from the complex and the protein was saved as pdb file. The pdb protein file was then uploaded into Autodock tools 1.5.6. The water molecules and heteroatom were deleted and polar hydrogens were added, followed by giving Kollman charge. The protein was then saved as pdbqt and ready for use [16].

In silico molecular docking and virtual screening

For the virtual screening of the ten natural compounds, the AutoDock Vina [17] protein-ligand docking platforms were used. The selected ten compounds were docked with RdRp, spike (S) glycoprotein, EndoU, 3CLpro, PLpro, and TMPRSS2. The compounds showing the highest interaction, specifically with all the catalytic residues of proteins, were only selected and used for further analysis. The docked result was analyzed using PyMOL [18] and BIOVIA Discovery Studio Visualizer [19]. All protein-ligand interaction images were prepared in BIOVIA Discovery Studio Visualizer. Table 1 shows the selected grid for the docking study.

Table 1: Grid values are as follows

Protein name	Grid box size			Centre of mass		
	X	Y	Z	X	Y	Z
RdRp	54	70	118	121.598	123.335	127.039
spike (S) glycoprotein	72	102	62	7.849	34.931	45.291
EndoU	52	68	40	-52.714	51.083	23.831
3CLpro	64	92	64	146.685	147.924	147.364
PLpro	50	44	126	-1.785	-15.533	-26.580
TMPRSS2	60	50	64	23.718	-2.669	11.354

Molecular dynamic (MD) simulation

The docking was run using Autodock Vina embedded in PyRx program with exhaustiveness = 64, covering 9 conformations for each ligand. The binding energy result was collected in csv file, whereas the best docking pose was selected and saved in pdbqt file ready for analysis. Docked protein-ligand complexes were subjected to molecular dynamic simulations using NAMD software [20]. MD simulations were performed using the CHARMM27 force field [21]. Visual molecular dynamics (VMD) was used to generate PSF files for both complexes. Both complexes were solvated in cubic water boxes containing transferable intermolecular potential with 3 points (TIP3P) water molecules. The box size was chosen so that there was 10 Å between the protein surface and the edges of the periodic box. A 12 Å cutoff distance was used to calculate short-range nonbonded interactions. The particle mesh Ewald (PME) method was used to calculate long-range electrostatic interactions. The SHAKE method was used to constrain all bonds involving hydrogen atoms. The system first performed 5000 steps of steepest descent with energy minimization. Then, the minimized system was used to perform simulations using an NVT ensemble. The Nosé–Hoover method was used to maintain a constant temperature. The simulated temperature was set in the range of 300 K. The simulation time for each simulated temperature was set to 10 ns. The time step of each simulation was set to 2 fs. Visualizations and data analysis were performed with VMD software.

RESULTS AND DISCUSSION

Drug-like likeness study and biological profiling

The drug-like likeness is an important criterion for a compound to be a drug candidate. For the initial screening, the drug should satisfy the Lipinski Rule of Five. According to this, a drug should have a maximum molecular mass of less than 500 Daltons,<5 in the partition coefficient (log P), a maximum of 5 in the number of hydrogen bond donors (HBD), and lastly maximum of 10 in the number of hydrogen bond acceptor (HBA). The log P is the ratio of the concentration of the compound in noctanol over its concentration in water; thus, it states the balance of the compound's solubility in water during oral dissolution steps with its oral bioavailability of the compound in the blood system. Thus, the octanol-water partition coefficient log P<5. It is well known that a higher MW of the drug will exhibit a lower IC50 value and more show effective at lower concentrations, but if MW is>500 Dalton then permeability will decrease. The number of HBD or HBA indicates their polarity to interact with water during the dissolution process and molecular interaction during the pharmacodynamic step. The rotatable bonds influence the stability of the compound during pharmacokinetics and the receptor binding, as a less rotatable chain imparts more stable drug activity. The polar surface area (SA) determines the permeability of drugs across the cell membrane. The higher SA indicate less bioavailability [22]. In the present study, ten spices-based natural compounds were analyzed for drug likes study; the results are shown in table 2. All ten compounds satisfied the Lipinski Rule of Five and they have been used in traditional medicine treatment of inflammation, respiratory ailments, antioxidants, and antiviral agents.

Table 2: The drug-like likeness profile and biological properties of 10 compounds selected for docking

Ligands	Lipinski rule				Rotatable bonds	Surface area	Biological properties
	MW	log P	HBD	HBA			
Ajoene	234.4	1.7	0	4	8	207.85	Antiviral and antifungal
Allicin	162.3	1.3	0	3	5	145.51	Anti-infective, anti-metabolite, antilipemic and antioxidant
Alliin	177.22	-3.5	2	5	5	154.76	Antidiabetic, antilipemic, respiratory ailments, antioxidant,
Bromhexine	376.13	4.3	1	2	3	267.25	Used for respiratory ailments
Capsaicin	305.4	3.6	2	3	9	310.37	Used for neuropathic pain, analgesia
Diferuloylmethane	368.4	3.2	2	6	8	332.18	Anti-inflammatory, Chronic Obstructive Pulmonary Disease (COPD) and antioxidant
Colchicine	399.4	1	1	6	5	364.15	Antigout agent, anti-inflammatory
Diallyl disulfide	146.3	1.3	0	2	5	137.96	COPD, antioxidant and anti-inflammatory
6-Gingerol	294.4	2.5	2	4	10	295.61	Neuroprotective, respiratory protective, treatment of asthma
Sakuranetin	286.28	2.7	2	5	2	247.79	Anti-inflammatory treatment of asthma

The ten natural compounds Ajoene, Allicin, Alliin, Bromhexine, Capsaicin, Diferuloylmethane, Colchicine, Diallyl disulfide, 6-Gingerol, Sakuranetin (fig. 1) were docked with six proteins-TMPRSS2, spike (S) glycoprotein, RdRp, 3CLpro, PLpro and EndoU (fig. 2) using AutoDock Vina to find the interaction between the ligand and macromolecule.

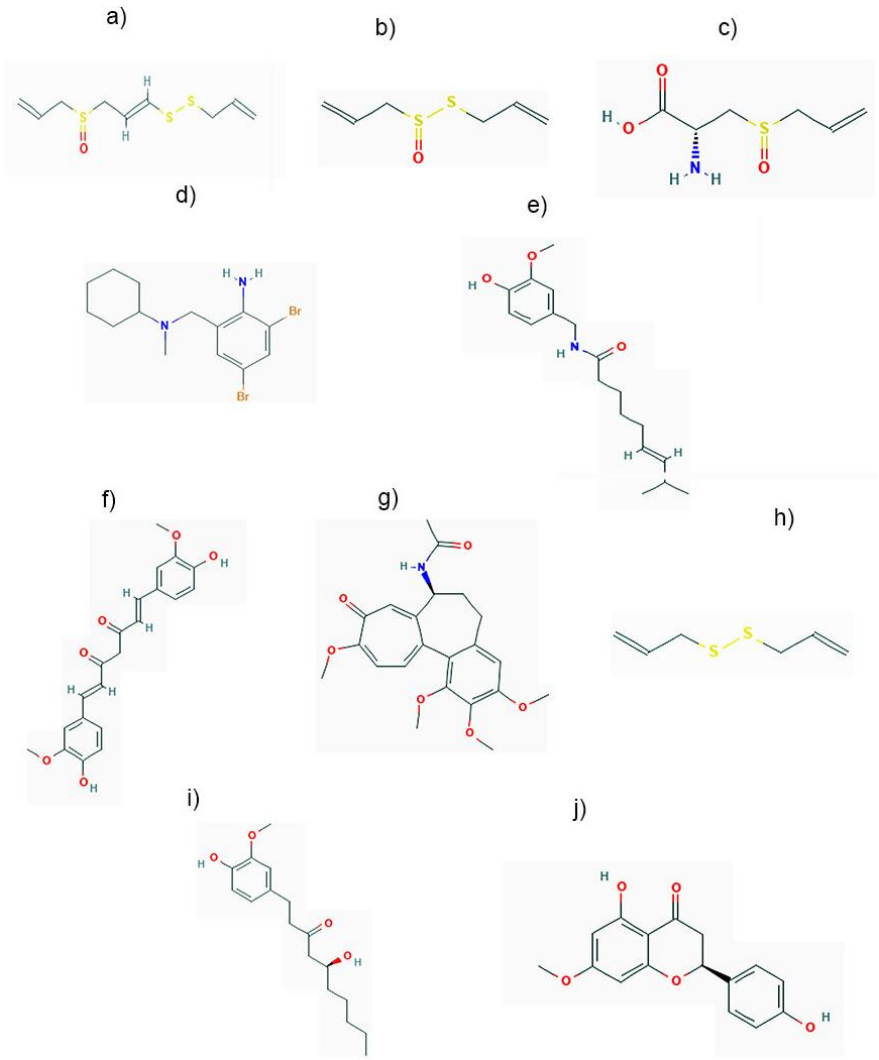

Fig. 1: Structure of ten compounds (ligands) selected for docking study: a) Ajoene b) Allicin c) Alliin d) Bromhexine e) Capsaicin f) Diferuloylmethane g) Colchicine h) Diallyldisulfidei) 6-Gingerol j) Sakuranetin

AutoDock vina docking results

In the present study, ten natural compounds Ajoene, Allicin, Alliin, Bromhexine, Capsaicin, Curcumin, Colchicine, Diallyl disulfide, 6-Gingerol, Sakuranetin were docked with six proteins-TMPRSS2, spike (S) glycoprotein, RdRp, 3CLpro, PLpro and EndoU using AutoDock Vina to find the interaction between the ligand and macromolecule. The present study will help to identify the binding affinity between the ligand and the receptor. The binding affinity of TMPRSS2 with ajoene, allicin, alliin, bromhexine, capsaicin, curcumin, colchicine, diallyl disulfide, 6-gingerol, sakuranetin was-3.9,-4.6,-4.3,-6.0,-6.5,-7.2,-6.3,-3.0,-6.2 and-4.0 kcal/mol. The highest affinity of TMPRSS2 was with curcumin as shown in table 3. Spike (S) glycoprotein exhibited docking scores of-3.0,-3.5,-3.5,-4.6,-4.5,-5.9,-6.3,-2.7,-4.8 and-3.3 kcal/mol with the ten ligands. Colchicine showed the highest binding affinity with spike (S) glycoprotein. RdRp showed-4.1,-4.9,-5.2,-6.4,-6.8,-7.7,-7.2,-3.4,-5.6 and-4.3kcal/mol. Curcumin showed a high binding affinity with RdRp. The two proteases 3CLpro and PLpro docking scores of (-4.3,-4.8,-4.6,-5.9,-5.8,-7.6,-6.7,-6.5,-6.2 and-5.2) and (-3.8,-4.7,-4.5,-5.2,-5.2,-7.3,-5.9,-3.0,-5.9 and-4.1kcal/mol), respectively. Both proteases exhibited high binding affinity with curcumin. EndoU exhibited binding affinity of-3.5,-4.3,-4.2,-5.5,-6.0,-7.0,-6.7,-3.5,-5.2 and-4.5kcal/mol with curcumin showing the highest affinity. The docking results are shown in table 3. The result shows that curcumin has a high binding affinity against important targets of Severe acute respiratory syndrome and can be used as an effective natural compound against virus replication. Colchicine showed a high binding affinity with spike protein which can prevent the binding of virus to the receptor. Fig. **3** depicts the region of protein where the ligand is binding and the amino acid associated with establishing the bonds. TMPRSS2 binds with diferuloylmethane and amino acid associated with the interaction (Gly-351, Val-Pro-187, Cys-349 and 381) hydrophobic interaction lue-187, Val-375, Tyr-377, and hydrogen bonds were formed with Ala-348, His-203, and ser-353. Colchicine bound with spike (S) glycoprotein at Asn-17, Cys-24, Asp-17 Glu-21, and lys-24. RdRp with diferuloylmethane showed hydrophobic interactions at Val-315, Arg-349, Ser-318, Phe-396 and Val-675, and hydrogen bonds were for with Asn-628 and Phe-396. 3CLpro interacted with diferuloylmethane at His-41,Asn-142, Cys-145 of chain A and Gly-143, and Glu-166 of chain B by hydrogen bonding. Diferuloylmethane hydrophobically interacted with PLpro at Thr-259, Tyr-305, Lys-306, and hydrogen bond with Gln-122, Lys-217, and Thr-259. Endonucleases interaction sites were Tyr-343 for hydrophobic interaction and Gln-245 and His-250 for hydrogen bonding.

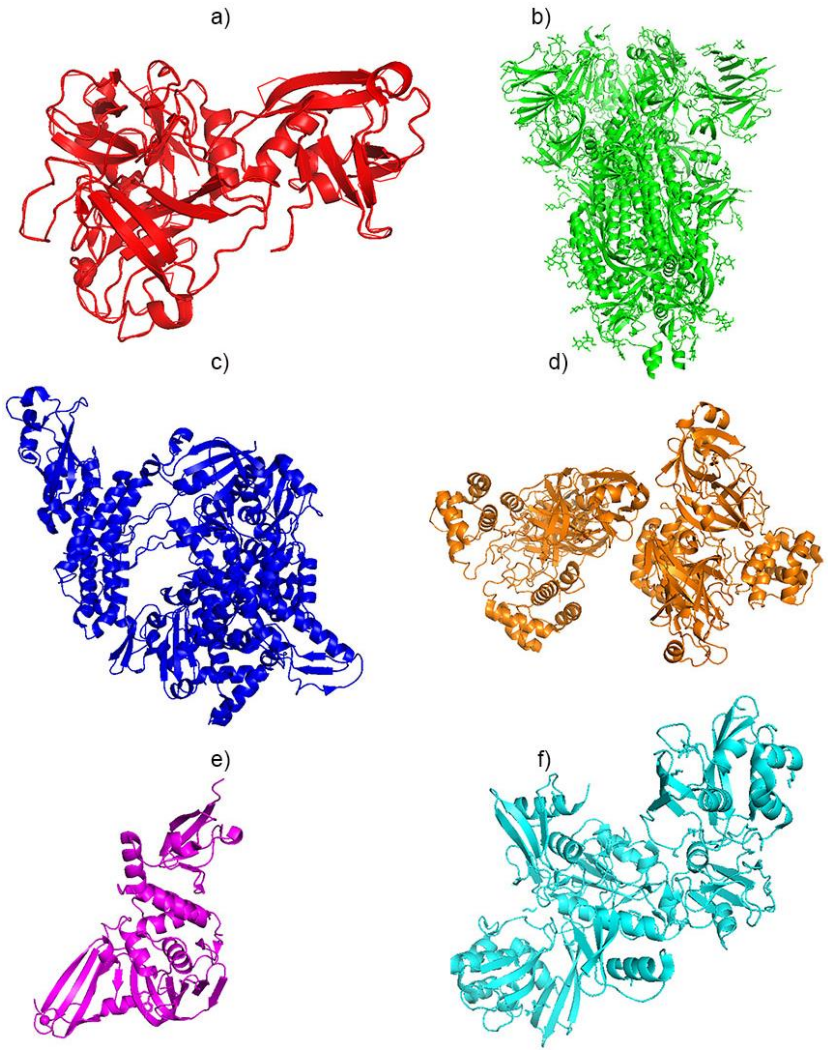

Fig. 2: Structure of proteins (receptor) for docking study: a) TMPRRS2 (1Z8G) b)Spike glycoprotein (6VXX) c) RdRp (6NUR) d) 3CLpro (6M2N) e)PLpro (6WX4) and f)EndoU (6W01)

Table 3: Docking score of ligand and receptor (AutoDock Vina)

S. No.	Ligands	Receptor					
		TMPRRS2	Spike glycoprotein	RdRp	3CLpro	PLpro	EndoU
		(Docking score kcal/mol)					
1	Ajoene	-3.9	-3.0	-4.1	-4.3	-3.8	-3.5
2	Allicin	-4.6	-3.5	-4.9	-4.8	-4.7	-4.3
3	Alliin	-4.3	-3.5	-5.2	-4.6	-4.5	-4.2
4	Bromhexine	-6.0	-4.6	-6.4	-5.9	-5.2	-5.5
5	Capsaicin	-6.5	-4.5	-6.8	-5.8	-5.2	-6.0
6	Diferuloylmethane	-7.2	-5.9	-7.7	-7.6	-7.3	-7.0
7	Colchicine	-6.3	-6.3	-7.2	-6.7	-5.9	-6.7
8	Diallyldisulfide	-3.0	-2.7	-3.4	-6.5	-3.0	-3.5
9	6-Gingerol	-6.2	-4.8	-5.6	-6.2	-5.9	-5.2
10	Sakuranetin	-4.0	-3.3	-4.3	-5.2	-4.1	-4.5

Molecular dynamics simulation

MD simulation was carried out for six proteins, with the ligand having the highest binding affinity based on results obtained from the Autodock vina score. The MD simulations a stable as evidenced by the time-dependent evaluation of backbone root mean square deviation (RMSD). The RMSD was calculated during the production phase using the respective initial minimized structure as the reference structure. RMSD value for TMPRRS2-curcumin (cur) (0.088 to 3.05); spike glycoprotein-colchicine (0.10 to 4.45);RdRp-cur (0.05 to 2.85); 3CLpro-cur (0.04 to 3.30); PLpro (0.06 to 2.80); EndoU (0.52 to 2.18) as shown in fig. 4a. Hydrogen bonds are another important factor that influences protein stability. Here, a distance cutoff of 3.5 Å and an angle cutoff of 30° were applied in the hydrogen bond calculation. The study showed that the curcumin was entirely buried in the interior of receptors forming a strong hydrogen bond interaction (fig. 4b).

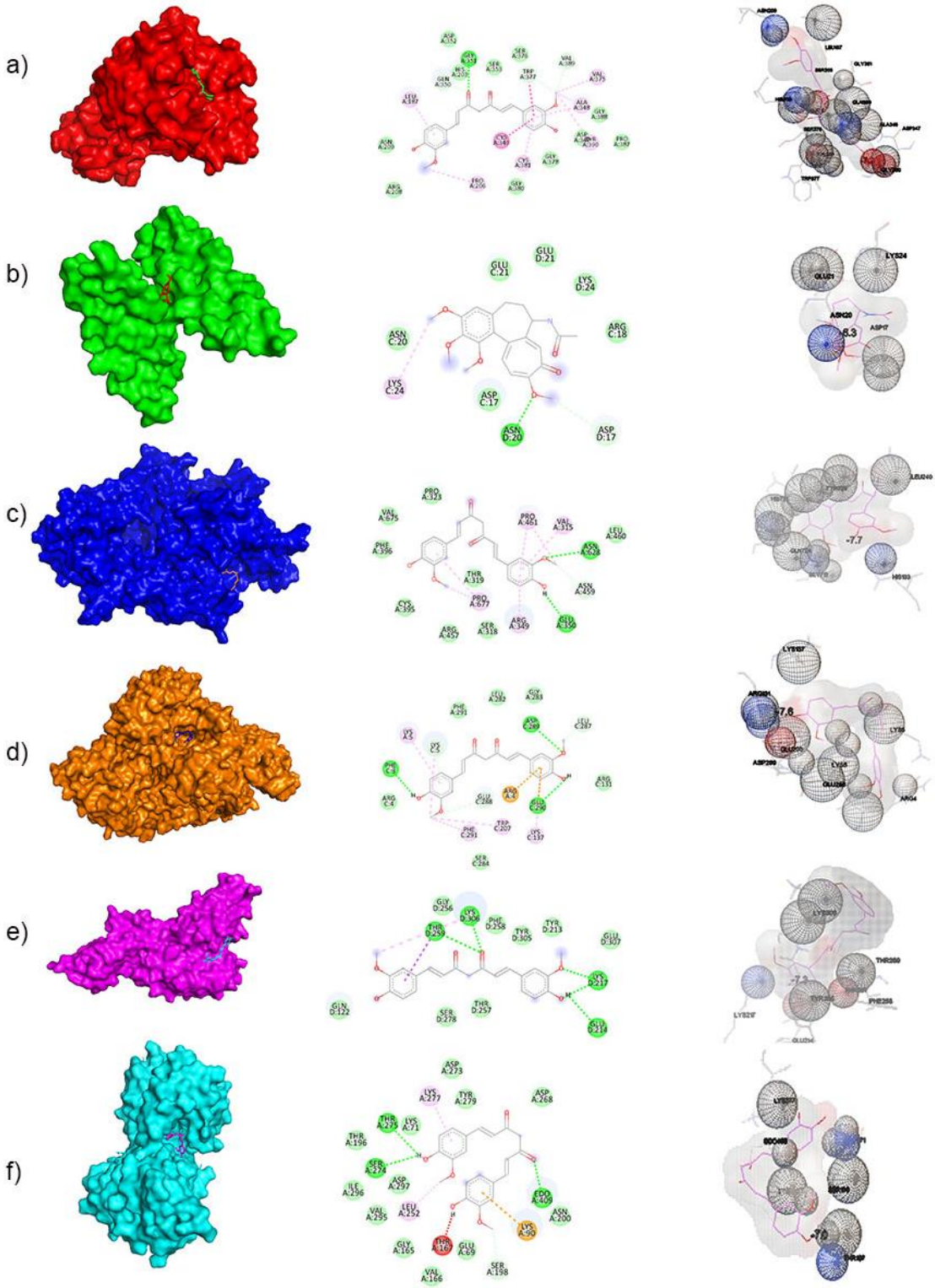

Fig. 3: Interaction between the proteins and ligand. a) TMPRRS2 interaction with diferuloylmethane b)Spike glycoprotein interaction with colchicine c) RdRp interaction with diferuloylmethane d) 3CLpro interaction with diferuloylmethane e)PLpro interaction with f) EndoU interaction with diferuloylmethane

The result obtained from Autodock vina showed that diferuloylmethane exhibited the highest binding energy with TMPRSS2, proteases (3CLpro and PLpro), endonucleases and RdRp, whereas spike protein exhibited high binding affinity with colchicine. Moreover, diferuloylmethane is low toxic, antioxidant, and anti-inflammatory, and it is plausible to be considered to be used as a therapeutic drug for respiratory ailments. Diferuloylmethane exerts protective effects by regulating the expression of both pro-and anti-inflammatory factors such as IL-6, IL-8, IL-10, and COX-2, promoting the apoptosis of PMN cells scavenging the reactive oxygen species (ROS), which exacerbates the inflammatory response [4]. The study provides a rationale that diferuloylmethane can be used as a therapeutic agent against influenza and severe acute respiratory syndrome infection.

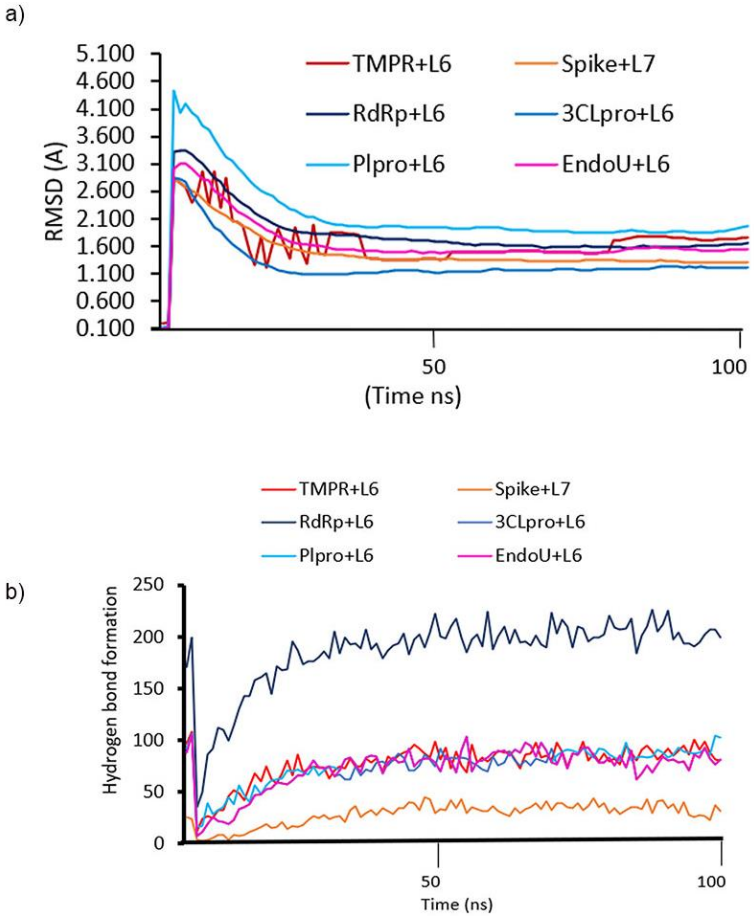

Fig. 4: MD simulation of curcumin with receptors, a) RMSD plots for five MD simulations. The Root means square deviation (RMSD) of backbone atoms was shown for the initial minimized structure for all the six simulations, such as TMPRRS2 interaction with curcumin; Spike glycoprotein interaction with colchicine; RdRp interaction with curcumin; 3CLpro interaction with curcumin; PLpro interaction with curcumin; EndoU interaction with curcumin. b) Changes in the hydrogen bond number for simulation with ligand and receptor

Spices used in food are rich in natural compounds with medicinal properties, including anti-inflammatory, antibacterial, antiviral and antioxidants. Turmeric, onion and garlic are commonly used spices and are rich in biologically active molecules like curcumin, allicin, and many other organosulfur compounds. These compounds are traditionally used in the treatment of many ailments including used as antiviral and respiratory ailments [23]. The present study focuses on finding the binding affinity of the spices-based compounds with influenza and severe acute respiratory syndrome proteases, endonucleases, RdRp and TMPRSS2. The result obtained from Autodock vina showed that curcumin exhibited the highest binding energy with TMPRSS2, proteases (3CLpro and PLpro), endonucleases and RdRp and spike protein exhibited high binding affinity with colchicine. Antiviral activity of curcumin was observed against various viruses, including hepatitis viruses, SARS coronavirus, influenza viruses, human immunodeficiency virus (HIV), herpes simplex virus, dengue virus, chikungunya virus etc [24]. Previously, it was reported that curcumin binds to inhibit severe acute respiratory syndrome by targeting replication and proteases [25]. The present *In silico* study showed that curcumin can efficiently bind to target different proteins of SARS-CoV-2. Moreover, as curcumin is known for its low toxicity, antioxidant, anti-inflammatory, and antiviral activity, it is plausible to consider that curcumin could be used as a therapeutic drug for respiratory ailments as curcumin exerts protective effects by regulating the expression of both pro-and anti-inflammatory factors such as IL-6, IL-8, IL-10, and COX-2, promoting the apoptosis of PMN cells, and scavenging the reactive oxygen species (ROS), which exacerbates the inflammatory response [26].

CONCLUSION

The pandemic outbreak of respiratory infection by severe acute respiratory syndrome combined with influenzahas affected the healthcare system and economy globally. *In silico* study was performed to study the binding efficacy of spice-based natural compounds against proteases, spike glycoprotein, endonucleases, RdRp and TMPRSS2. The results demonstrated that curcumin showed the highest binding efficacy with targets associated with different cellular pathways, further inhibiting the growth and replication of viruses and regulating cytokines storm makingit an ideal candidate as an anti-viral drug. The study provides a rationale that curcumin can be used as a therapeutic agent against respiratory tractinfections.

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AUTHORS CONTRIBUTIONS

Seema Kumari is the sole author. The author has conceptualized,conducted the experimental studies, written and approved the final manuscript.

CONFLICT OF INTERESTS

The author declares no conflicts of interest

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