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Molecular Docking Studies of Bioactive Constituents of Long Pepper, Ginger, Clove, and Black Pepper to Target the Human Cathepsin L Protease: As a Natural Therapeutic Strategy Against SARS-Cov-2

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Abstract: Human cathepsin L protease is involved in the cleavage of S protein of SARS-Cov-2 virus and activates the membrane fusion, which mediates the entry of the virus into the host cell. Thus, it suggests the cathepsin L protease is critical for the entry of SARS-Cov-2. Currently, chemically synthesized cathepsin L inhibitors are present, but the consumption of chemically synthesized drugs is also an alarming stage due to its side effects, illness, and age reduction. In this study, natural bioactive constituents of long pepper, ginger, clove, and black pepper that has been widely known for antiviral effect and other medicinal properties were used for molecular docking against the human cathepsin L receptor (PDB ID 2XU1). Molecular docking (using a software, AutoDock 4.2) was performed on bioactive constituents of long pepper, ginger, clove, and black pepper against the human cathepsin L protease and elucidates the binding energies, visualization, and analysis of interacting residue (using Discovery Studio) at the docking site of cathepsin L protease and compared the docking analysis of these bioactive constituents with preclinical cathepsin L inhibitor (Pub Chem CID: 16725315). The pharmacokinetic properties and toxicity evaluation were calculated by Datawarrior and Osiris Molecular Property explorer software, respectively. Many bioactive constituents from long pepper, ginger, clove, and black pepper have shown significant binding affinity, docking interactions and acceptable pharmacokinetic properties with the human cathepsin L protease. Piperolactam A constituent of long pepper and Kaempferol constituent of clove were found to be more acceptable natural therapeutic compounds among other selected bioactive constituents with the highest binding affinity (Kcal/mol) −9.4 and −9.3, respectively.

Keywords: molecular docking, SARS-Cov-2, human cathepsin L protease, natural bioactive components

1. Introduction

World Health Organization (WHO) has declared the COVID-19 outbreak a global epidemic that led to high morbidity and mortality across the world. Illness, common cold, pneumonia, and lower respiratory tract infection, cough, shortening of breath, and dyspnea belong to the symptoms of COVID-19-infected patients, Although, in more severe cases, this infection can also cause multiple organ failure and even death [1, 2]. According to WHO, in November 2002, there was an outbreak in the Guangdong province of china caused by the pathogenic virus known as SARS-Cov virus, affected 5 continents and 29 countries resulted in 800 cases and 774 death by 23 September 2003 and also affected the global economy around $180 billion. SARS-Cov-infected patients have also developed symptoms like severe acute respiratory illness, including fever, cough, and shortness of breath. Again in 2012, around 2494 diagnosed cases and 858 deaths were reported majority in Saudi Arabia due to the MERS-COV (The middle East Respiratory Syndrome Coronavirus) infection [3, 4]. Recently, COVID-19, a disease caused by SARS-Cov-2/2019-nCov, has been an outbreak from the city of Wuhan China to other Asian countries and other continents like Africa, America, and Europe are also affected [5]. Up until this point, the coronavirus disease outbreak (COVID-19) has spread to practically every nation on the planet, and the respiratory virus has claimed the lives of over 6.86 million individuals. The USA accounted for more than 1.16 million of these fatalities.

Based on the phylogenetic clustering, coronaviruses are envelope virus consisting of the positive-sense RNA genome and found to be the largest group of viruses belonging to the Coronaviridae family and placed under the Nidovirales order. The Coronaviridae family consists of one of the Coronavirinae subfamilies and further divided into 4 classes known as alpha, beta, gamma, and delta [6]. Pathogenic viruses (SARS-Cov,

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MERS-Cov, and SARS-Cov-2/2019) are belonging to the delta class of coronavirus [7]. Similar to SARS-Cov and MERS-Cov, SARS-Cov-2 attack the lower respiratory tract and cause viral pneumonia [8, 9]. Before human to human transmission, the Chinese horseshoe bat is believed to be a host of SARS-Cov-2 [10]. Cryo-EM structure analysis revealed the four structural components of coronaviruses: spike (S) glycoprotein, envelope (E) protein, membrane (M) protein, and nucleocapsid (N) protein [11]. Recent studies revealed the binding affinity of S protein of SARS-Cov-2 has 10–20 times higher affinity than the S protein of SARS-Cov [12]. Spike glycoprotein consists of extracellular S1 protein mediating the angiotensin-converting enzyme 2ACE-2 receptor binding present on the surface of the host cell and anchored S2 protein domain facilitates the fusion of SARS-Cov-2-ACE2 complex with the target cell membrane [13]. ACE-2 receptor binding to the S protein induces the changes in the conformation of the S protein and led to the formation of the ACE-2-SARS-Cov-2 complex [14]. Studies have also indicated that cathepsin L plays a critical role in priming the S protein of SARS-Cov-2 in the lysosome, protease activation of S protein, and play an important role in the entry of ACE-2SARS-Cov-2 complex through endocytosis. Furthermore, SARS-Cov-2 utilizes the endosomal acid protease (cathepsin L) for fusion active state and translocation of ACE2-SARS-Cov-2 complex to endosome [13, 14]. Therefore, we hypothesized that cathepsin L protease activity inhibitor may play a promising role in restricting the SARS-Cov-2 virus entry (Figure 1).

Antiviral drugs designing and synthesis are time-consuming and financial challenges that cannot be affordable by a large number of sections of society. Also, the consumption of drugs/antibiotics is also an alarming stage due to its side effects, illness, and age reduction. Recently, the highest mortality rate in SARS-Cov-2-infected patients underlying cardiovascular diseases, diabetes, blood pressure, and kidney diseases has been reported [2]. Patients having such comorbidities are more vulnerable to the side effect of chemically synthesized drugs resulted in a high mortality rate. Therefore, a natural therapeutic bioactive component needs to be studied to combat the SARS-CoV-2. In our study, natural bioactive components having an antiviral activity present in natural origin spices (long pepper, ginger, clove, and black pepper) were screened and in silico analysis was performed for the characterization of human cathepsin L inhibitor [15].

2. Material and Methods

2.1. Target protein

The human cathepsin L protease receptor (PBD ID 2XU1) is having a resolution of 1.45 Å and four chains A, B, C, and D used for molecular docking. The three-dimensional crystal structure of the receptor was retrieved from the Protein Data Bank (https://www.rcsb.org/structure/2XU1) as shown in Figure 2.

2.2. Receptor preparation

The crystal structure of the receptor file was saved as Protein.pdb. AutoDock 4.2 was used for removal of the water molecule and any non-protein atoms from the 3D structure of the receptor. Subsequently, the addition of polar hydrogen atoms and Kollman charges were added, followed by management of its conformer and the minimization process. Further, the Accelrys Discovery Studio 2.0 software package was used for the CHARMM27 force field.

2.3. Ligand preparation

The NCBI-Pub Chem database has information on the bioactive components of black pepper, long pepper, ginger, and cloves. These

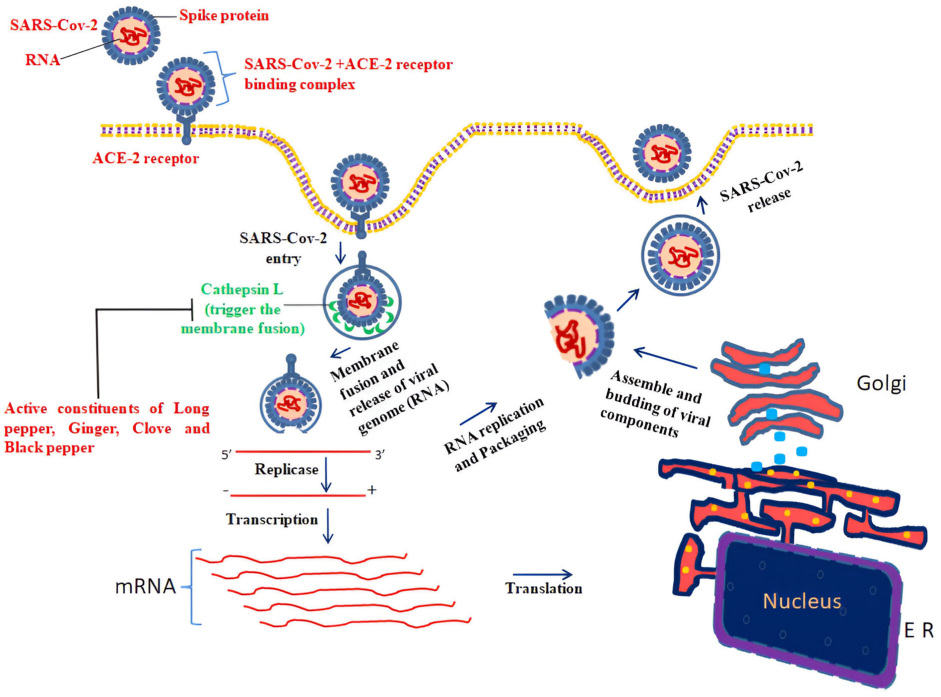

Figure 1. Graphical abstract

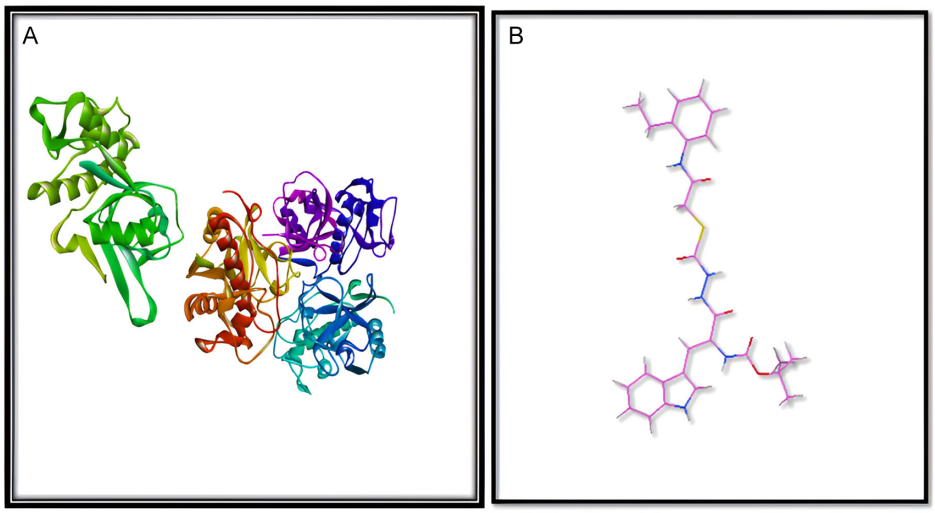

Figure 2. (A) Coronaviridae of human cathepsin L protease (PDB ID: 2XU1) having a resolution of 1.45 Å and four chains A, B, C and D. (B) cathepsin L inhibitor namely (Chemical name-*N*-[(1,1-Dimethylethoxy)carbonyl]-L-tryptophan-2-[[[2[(2ethylphenyl)amino]-2 oxoethyl]thio]carbonyl]hydrazide; Pub Chem CID: 16725315).

constituents are docked against the human cathepsin L receptor and are listed in Supplementary Table 1.

All naturally occurring bioactive constituents are downloaded from https://pubchem.ncbi.nlm.nih.gov/compound/16725315 in the SDF file. Further, Discovery Studio 2.0 software was used to convert the SDF file into the PDB file format. Subsequently, charges and torsion were added to the ligands, and pdbqt file was generated for docking studies.

2.4. Molecular docking

MGLTools 1.5.6 suite by using AutoDock tools version (4.2) was used for generating the input files in the pdbqt format by adding hydrogen bonds and gasteiger charges. Subsequently, to define the binding site, grid box was generated along the x (126), y (92), and z (84), respectively, with spacing 0.972 Å and 29.85, −10.33 and −19.18 as x-center, y-center, and z-center across the protein by using AutoGrid software. All these parameters were saved as a grid parameter file and further used by the AutoDock software to defining the active binding sites of ligands present in the receptor.

2.5. Analyzing and output visualization using Discovery Studio

The log and ligan_out.pdbqt file obtained from AutoDock 4.2 was used to predict the binding affinity and docking interactions of one ligand to the receptor. Binding energy and docking site (interacting and non-interacting molecules) of bioactive constituents of long pepper, ginger, clove, and Black pepper against human cathepsin L receptor were compared with the binding energy and docking site of cathepsin L inhibitor (Pub Chem CID: 16725315)+ cathepsin L receptor complex by using Discovery Studio software package.

2.6. Pharmacokinetic profiling and toxicity studies

Selected bioactive constituents of long pepper, ginger, clove, and black pepper were further evaluated for their pharmacokinetic properties and presence of any toxicity effects like mutagenicity, tumorigenicity, irritant effect, and reproductive effects by using Datawarrior software and Osiris Molecular Property explorer software, respectively [16] (Supplementary Table 3). The pharmacokinetic properties like calculated partition coefficient (cLogP), molecular weight, hydrogen bond donor, and acceptor sites were predicted based on Lipinski's rule of five and Veber.

3. Results

3.1. Binding energy and docking pose of human cathepsin L inhibitor (Pub Chem CID: 16725315)

The inhibitor of human cathepsin L, as determined by molecular docking simulation against human cathepsin L, has a binding energy of 9.2 Kcal/mol (Supplementary Table 1). The docking ligand interacts with amino acid residues, such as PRO D:15, LEU D:45, PRO C:15, ARG D:44, SER C:47, LEU C:45, and GLY D:43, that are present at the receptor's active site (cathepsin L) through π, π-alkyl, unfavorable donordonor, and conventional hydrogen (H) bonds (Figure 4). Conversely, non-interacting amino acid residues were also discovered to be present at the surface of the docking ligand. These residues included THR D:14, VAL D:13, GLY C:11, PHE C:39, ILE D:46, GLY C:43, PHE D: 39, ARG C:44, LYS D:10, GLU D:9, LYS C:17, and VAL C:13.

3.2. Binding energy and docking pose of active constituents of long pepper (*Piper longum*)

Among the constituents of long pepper, Piperolactam A was found to be the highest binding energy (−9.4 Kcal/mol) as shown in Supplementary Table 1, which is greater than the binding energy of human cathepsin L inhibitor. Moreover, amino acid residue, i.e., ARG C:44; LEU C:45; PHE D:39; PHE C:39; LEU D:45; LYS D:10; GLY C:11; GLY C:43, GLY D:43, and ARG D:44 present at the surface of docking Piperolactam A (Figure 5) is similar to the amino acid residue present at docking pose of cathepsin L inhibitor-receptor complex. Also, LEU C:45 and LEU D:45 are the common interacting amino acid residues present at the docking pose of both Piperolactam

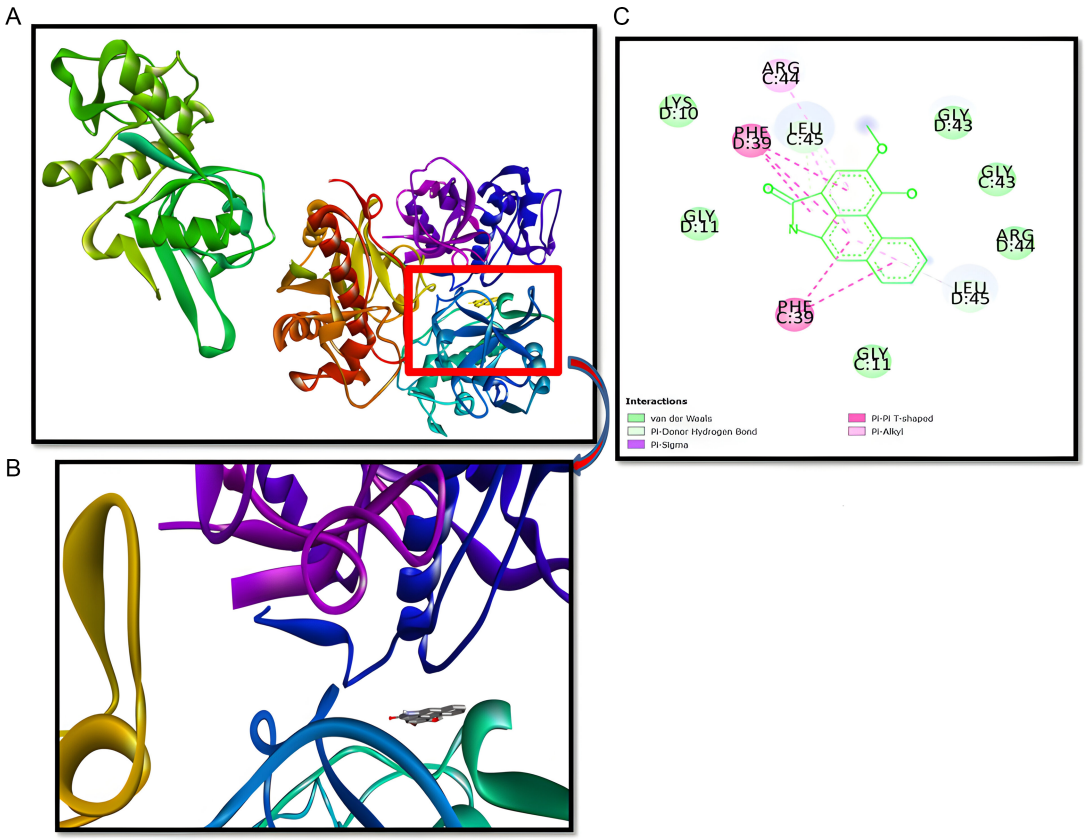

Figure 3. (A) The structure of human cathepsin L protease-Cathepsin L inhibitor complex. The red box indicating the docking pocket of cathepsin L inhibitor (Gray color). (B) Zoom-in view of the docking site of cathepsin L inhibitor. (C) The two-dimensional binding interactions of cathepsin L inhibitor show the main interactions with amino acid residue, i.e., PRO D:15; LEU D:45; PRO C:15; ARG D:44; SER C:47; LEU C:45 and GLY D:43 present at the active site of receptor (human cathepsin L protease) by alkyl, pi-alkyl, unfavorable donor-donor, and conventional hydrogen bond.

A+ cathepsin L and inhibitor + cathepsin L receptor complex. The similarity in the interacting and non-interacting amino acid residue indicates that Piperolactam A, a constituent of long pepper, can also act as an inhibitor of human cathepsin L.

Bisdemethoxycurcumin was found to be docked with the binding energy (−8.5 Kcal/mol) at the active site of cathepsin L. The human cathepsin L receptor's active site is contacted by amino acid residues such as ARG D:8, PRO D:15, and PRO C:15 with π-alkyl and alkyl bonds, PHE D:39 with π-πT shaped bond, ARG C:44 with an unfavorable bond, and GLY C:11 and GLY D:43 with typical hydrogen bonds.

Caumaperine exhibits the binding energy (−7.8 Kcal/mol) with the amino acid residue, i.e., PRO C:15; PRO D:15; PHE D:39, and ARG C:44 with the alkyl or π-alkyl bond interaction present at the active site of human cathepsin L receptor. Amino acid residue, i.e., PRO D:15; THR C:14; LEU C:45; GLY C:43; LEU D:45; THR D:14; VAL C:13; PHE C:39; GLY C:11, GLY D:43, and VAL D:13 found at the surface at the docking pose of Caumaperine is similar to the docking pose of inhibitor + cathepsin L receptor complex, whereas (PRO D:15) is the common interacting amino acid present at both the receptor complexes.

3.3. Binding energy and docking pose of active constituents of ginger

Zingiberene was discovered to be docked at the surface of the cathepsin L receptor with a binding energy of -7.2 Kcal/mol and amino acid residues, namely PRO D:15, PHE D:39, LEU C:45, ARG C:44, LEU D:45, and PHE C:39, which interact with the docking ligand via alkyl and π-alkyl bonds, respectively. The amino acid residues found at the surface of the docking pose of the zingiberene-cathepsin L receptor complex are similar to the amino acid residues found at the docked pose of the inhibitor + cathepsin L receptor complex, indicating the similar docking site of both the zingiberene and inhibitor ligand. The interacting amino acid residues are PRO D:15, LEU C:45, LEU D:45, and the non-interacting amino acid residues are PHE D:39, GLY D:43 (Figure 3).

6-Gingerdiol forms a π-alkyl, π-πT shaped, and conventional hydrogen bond with the amino acid residues, namely GLY D:196; GLU D:191; TYR D:198; VAL D:16; VAL D:13; TYR C:198; GLU C:191, with a binding energy of -7.1 Kcal/mol. Amino acid residue was discovered near the surface of, namely VAL D:13, THR D:14, THR C:14, and PRO D:15. 6. The inhibitor-cathepsin L receptor complex and gingerdiol-cathepsin L are comparable. The amino acid residues identified at the surface of 6-gingerdiol-cathepsin L (VAL D:13, THR D:14, THR C:14, and PRO D:15) are comparable to those observed in the inhibitor-cathepsin L receptor complex.

6-Gingerol was found to be docked at the surface of cathepsin L receptor with the binding energy (−7.0 Kcal/mol) and the amino acid residues, i.e., PRO C:15; VAL D:13; PRO D:15; GLU C:191; ARG D:8, are interacting via alkyl, π-alkyl, carbon-hydrogen bond, unfavorable donor–donor, and conventional hydrogen bond with

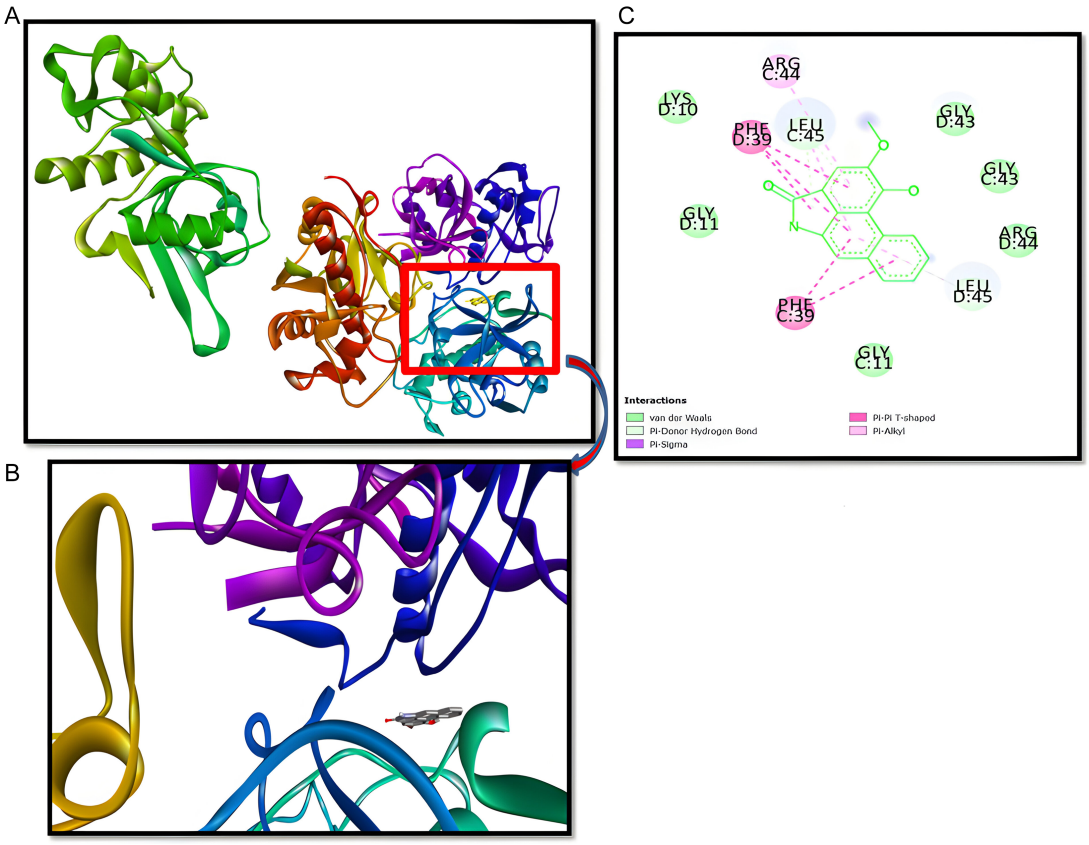

Figure 4. (A) The structure of human cathepsin L protease-Piperolactam A complex. The red box indicating the docking pocket of Piperolactam A (Gray color). (B) Zoom-in view of the docking site of Piperolactam A. (C) The two-dimensional binding interactions of Piperolactam A show the main interactions with amino acid residue, i.e., ARG C:44; LEU C:45; PHE D:39; PHE C:39 and LEU D:45 present at the active site of receptor (human cathepsin L protease) by Van der Waals, p-Donor hydrogen bond, pi-alkyl, pi-sigma, and pi-pi T-shaped bond.

the docking ligand. The interacting amino acid residues PRO C:15 and PRO D:15 interact, while the non-interacting amino acid residues found at the surface of the docking pose of 6-Gingerol include GLY C:11, THR C:14, THR D:14, LEU D:45, and LEU C:45. These residues are similar to those found at the docked pose of the inhibitor + cathepsin L receptor complex, indicating that the 6-Gingerol molecule also poses a similar docking site at the surface of the human cathepsin L receptor in comparison with the docking site of the inhibitor ligand.

8-gingerodione forms π-alkyl, π-π T-shaped, and conventional hydrogen bonds with amino acid residues, such as TYR D:12, LEU D:45, PHE C:39, GLY D:43, and ARG C:44, having a binding energy of −6.3 Kcal/mol. The surface of the 8-gingerdione-cathepsin L receptor complex had the interacting amino acid residue (LEU D:45) and the non-interacting amino acid residues (LYS D:10, PHE D:39, ILE C:46, LEU C:45 and GLY C:11, ARG D:44 and GLY C:43). These residues are similar to those found in the inhibitor-cathepsin L receptor complex.

10-Gingerdione was found to be docked at the surface of cathepsin L receptor with the binding energy (−6.4 Kcal/mol) and the amino acid residue, i.e., PHE D:39; LEU D:45; PHE C:39, ILE C:46, and GLY D:43 are interacting via alkyl, π-alkyl, π-π T shaped bond, and conventional hydrogen bond with the docking ligand. The surface of the 8-gingerdione-cathepsin L receptor complex had the interacting amino acid residue (LEU D:45) and the non-interacting amino acid residues (LYS D:10, PHE D:39, ILE C:46, LEU C:45 and GLY C:11, ARG D:44 and GLY C:43). These residues are similar to those found in the inhibitor-cathepsin L receptor complex.

10-Paradosal forms an unfavorable acceptor-acceptor interaction with the human cathepsin L receptor upon docking, with a binding energy of −7.1 Kcal/mol. It then interacts with the GLU D:9 amino acid residue. Non-interacting amino acid residues were discovered on the surface of 10-Paradol-cathepsin L is comparable to inhibitor-cathepsin L receptor complex. These residues include LYS C:17, GLU C:86, PRO C:15, SER C:47, THR C:14, LYS D:10, and GLU C:87

10-Shagaol exhibits the binding energy (−5.4 Kcal/mol) with the amino acid residue, i.e., LEU C:45; PHE D:39 and PHE C:39 with conventional hydrogen bond interaction present at the active site of human cathepsin L receptor. Interacting amino acid residue, i.e., LEU C:45 and non-interacting amino acid residue, i.e., ARG C:44; GLY C:11; ARG D:44; ILED:46; LEU D:45, GLY D:43 and GLY C:43 found at the surface at the docking pose of 10-Shagaol–cathepsin L receptor complex is similar to the docking pose of inhibitor + cathepsin L receptor complex.

6-Paradol interacts with the amino acid residue, i.e., TYR C:91 and GLU D:9 with pi-alkyl and conventional hydrogen bond present at the active site of human cathepsin L receptor and exhibits the binding energy (−5.6 Kcal/mol). Amino acid residue, i.e., GLU D:9; LYS C:17; GLU C:86; GLU C:87; PRO C:15; THR C:14 and SER C:47 found at the surface at the docking pose of 6-Paradol–cathepsin L receptor complex is similar to the docking pose of inhibitor + cathepsin L receptor complex.

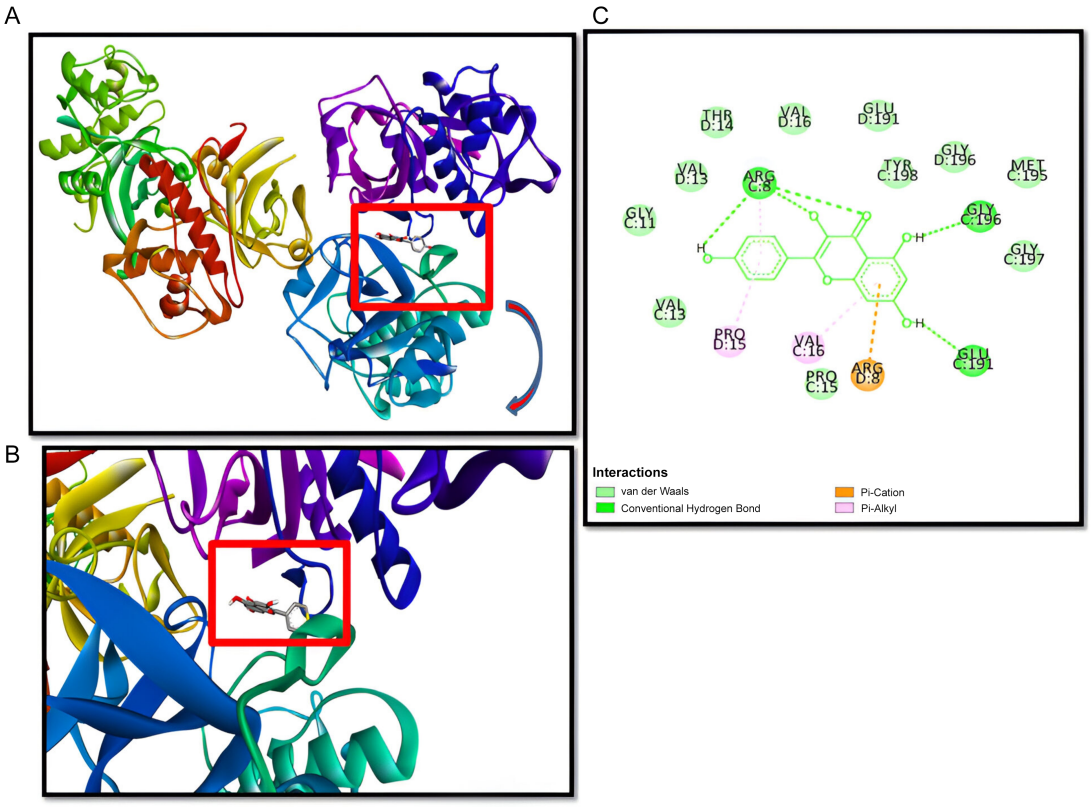

Figure 5. (A) The structure of human cathepsin L protease Kaempferol complex. The red box indicating the docking pocket of Kaempferol (Gray color). (B) Zoom-in view of the docking site of Kaempferol. (C) The two-dimensional binding interactions of Kaempferol show the main interactions with amino acid residue, i.e., ARG C:8; PRO D:15; VAL C:16; ARG D:8; GLU C:191, and GLU C:195 present at the active site of receptor (human cathepsin L protease) by Van der Waals, conventional hydrogen bond, pi-cation, and pi alkyl bond.

3.4. Binding energy and docking pose of active constituents of clove

Kaempferol, a type of natural flavonoid, was found to be docked at the surface of cathepsin L receptor with the binding energy (−9.3 Kcal/mol) as shown in Supplementary Table 1 and the amino acid residue, i.e., ARG C:8; PRO D:15; VAL C:16; ARG D:8; GLU C:191; and GLU C:195 present at the docking site of cathepsin L receptor interact via Van der Waals, conventional hydrogen bond, π-cation, and π-alkyl bond, respectively, with the docking ligand (Figure 6). The amino acid residues found at the surface of the Kaempferol docking pose are similar to the amino acid residues found at the docked pose of the inhibitor + cathepsin L receptor complex, indicating the similar docking site of both the Kaempferol and inhibitor ligand. PRO D:15 is the interacting amino residue, and THR D:14, VAL D:13, GLY C:11, and VAL C:13 are the non-interacting amino acid residues.

Rhammetin was found to be docked at the surface of cathepsin L receptor with the binding energy (−8.9 Kcal/mol) and the amino acid residue, i.e., LEU D:45; GLY D:43 and ARG C:44 present at the docking site of cathepsin L receptor interacts via Van der Waals, alkyl, and π-alkyl bond, respectively, with the docking ligand. The amino acid residues found at the surface of the docking pose of hammetin are similar to amino acid residues found at the docked pose of the inhibitor + cathepsin L receptor complex. The interacting amino acid residues are LEU D:45 and GLY D:43, while the non-interacting amino acid residues are ARG C:44, PHE C:39, GLY C:11, ARG D:44, GLY C:43, LEU C:45, GLU D:9, and LYS D:10.

βCadinene exhibits the binding energy (8.0 Kcal/mol) docked at the surface of cathepsin L receptor with the amino acid residue, i.e., VAL C:16; VAL D:16; ARG D:8; VAL D:13 and PRO C:15 with Van der Waal bond and alkyl bond. The surface-located interacting amino acid residue (PRO C:15) and non-interacting amino acid residues (VAL D:13, PRO D:14, VAL C:13, THR, and C:14) of the βCadinenecathepsin L receptor complex are similar to the inhibitor + cathepsin L receptor complex docking position.

Alpha-cedrene was found to be docked at the surface of cathepsin L receptor with the binding energy (−8.0 Kcal/mol) and the amino acid residue, i.e., VAL C:16; TYR C:198; PRO D:15; ARG C:8; VAL D:13; ARG D:8, and PRO C:15 present at the docking site of cathepsin L receptor interact via Van der Waals, alkyl and π-alkyl bond, respectively, with the docking ligand. Amino acid residues found at the surface of the docking pose of alpha-cedrene are similar to those found at the docked pose of the inhibitor + cathepsin L receptor complex. Specifically, PRO D:15 and PRO C:15 are the interacting amino residues, and VAL D:13 and VAL C:13 are the non-interacting amino acid residues.

Alpha-muurolene was found to be docked at the surface of cathepsin L receptor with the binding energy (−7.6 Kcal/mol) and the amino acid residue, i.e., TYR D:198; VAL C:16; ARG C:8; VAL C:13; PRO C:15; ARG D:8 and VAL D:13 present at the docking site of cathepsin L receptor interact via Van der Waals, alkyl, and π-alkyl bond, respectively, with the docking ligand. The amino acid residue found at the surface of the docking pose of alpha-muurolene is similar to the amino acid residue found at the docked pose of inhibitor + cathepsin L receptor complex. Specifically,

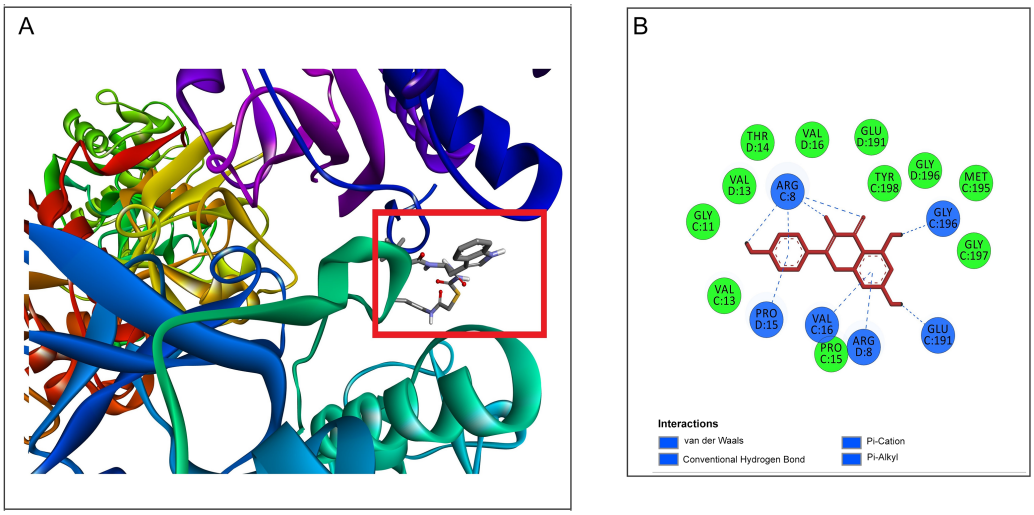

Figure 6. (A) The structure of human cathepsin L protease Kaempferol complex. The red box indicates the docking pocket of Kaempferol (gray color). (B) The two-dimensional binding interactions of Kaempferol show the main interactions with amino acid residue. i.e., amino acid residue, i.e., PRO D:15 is the interacting amino residue and THR D:14; VAL D:13; GLY C:11 and VAL C:13 are the non-interacting amino acid residue was found at the surface of the docking pose of Kaempferol is similar to amino acid residue found at the docked pose of inhibitor + cathepsin L receptor complex indicating the similar docking site of both the Kaempferol and inhibitor ligand.

PRO C:15 is the interacting amino acid residue, and VAL C:13; THR D:14; PRO D:15; THR C:14 is the non-interacting amino acid residue.

Eugenitin exhibits the binding energy (−7.5 Kcal/mol) docked at the surface of cathepsin L receptor with the amino acid residue, i.e., PHE D:39; LEU D:45; ARG C:44 and LEU C:45 with π- π T-shaped, alkyl, and π-alkyl bond. LEU D:45 and LEU C:45 are the interacting amino acid residue and VAL D:13; PRO D:14; VAL C:13; THR, GLY D:43 and C:14 are non-interacting amino acid residue found at the surface of βCadinene –cathepsin L receptor complex is similar to the docking pose of inhibitor + cathepsin L receptor complex.

Jasmone was found to be docked at the surface of cathepsin L receptor with the binding energy (−6.0 Kcal/mol) and the amino acid residue, i.e., PRO C:15; TYR C:91; PHE C:28 and LYS C:17 present at the docking site of cathepsin L receptor interact via Van der Waals, alkyl, and π-alkyl bond, respectively, with the docking ligand. The interacting amino acid residue, PRO C:15, and the non-interacting amino acid residues, LYS C:17, THR C:14, GLU D:9, GLU C:86, and SER C:47, were discovered at the surface of the docking pose of Jasmone. These amino acid residues are similar to those found at the docking pose of the inhibitor + cathepsin L receptor complex.

Eugenol exhibits the binding energy (−5.3 Kcal/mol) docked at the surface of cathepsin L receptor with the amino acid residue, i.e., PHE D:39; LEU D:45 and ARG C:44 with π-πT shaped, alkyl, and π-alkyl bond. The interacting amino acid residue on the surface of the eugenol-cathepsin L receptor complex is LEU D:45, while the non-interacting amino acid residues are PHE D:39, ARG C:44, PHE C:39, GLY C:43, GLY D:43, and LEU C:45. This surface arrangement is comparable to the docking position of the inhibitor + cathepsin L receptor complex.

3.5. Binding energy and docking pose of active constituents of black pepper

A bioactive constituent of black pepper (Pub Chem CID 44292388) was found to be docked at the surface of cathepsin L receptor with the binding energy (−6.7 Kcal/mol) and the amino acid residue, i.e., PRO C:15; PRO C:90; and ASN C:18 interact via Van der Waals, conventional hydrogen bond, carbon hydrogen bond, and alkyl bond, respectively, with the docking ligand. The interacting amino acid residue, PRO C:15, and the non-interacting amino acid residues, THR C:14, GLU D:9, SER C:47, and GLU C:86, were discovered to be identical to the amino acid residues observed at the docked posture of the inhibitor-cathepsin L receptor complex.

Piperolein B exhibits the binding energy (−6.8 Kcal/mol), docked at the surface of cathepsin L receptor with the amino acid residue, i.e., LEU C:45; PHE D:39; LEU D:45; ILE C:46; VAL C:106 and SER C:85 with alkyl, π-alkyl bond, and π-π donor hydrogen bond. The interacting amino acid residue, PRO C:15, and the non-interacting amino acid residues, LYS C:17, THR C:14, GLU D:9, GLU C:86, and SER C:47, were discovered at the surface of the docking pose of Jasmone. These amino acid residues are similar to those found at the docking pose of the inhibitor + cathepsin L receptor complex.

Sabinene exhibits the binding energy (−6.1 Kcal/mol), docked at the surface of cathepsin L receptor with the amino acid residue, i.e., LEU C:45; PHE C:39; LEU D:45; ARG C:44 and PHE D:39 with alkyl and pi-alkyl bond. The interacting amino acid residues on the surface of the sabinene-cathepsin L receptor complex are LEU C:45 and LEU D:45, while the non-interacting amino acid residues are PHE D:39, PHE C:39, ARG C:44, GLY D:43, and ARG D:44. These positions are comparable to the docking pose of the inhibitor + cathepsin L receptor complex.

3.6. Pharmacokinetic and toxicity evaluation

Active constituents of long pepper, ginger, clove, and black pepper having similar docking site interactions and acceptable range of binding energy were further evaluated their drug-likeness, pharmacokinetic properties, and toxicity effects based on Lipinski's-Rule-of-five. Supplementary Table 2 displays the range of acceptable pharmacokinetic properties for the active constituents of long pepper, ginger, clove, and black pepper. These properties include acceptable molecular weight (less than 500 g/mol),

acceptable hydrogen bond acceptor (less than 10), acceptable hydrogen bond donor (less than 5), acceptable TPSA (two-dimensional polar surface area; less than 140 Å), acceptable lipophilicity property (less than 0.4 to 5.6), and good and moderate water solubility for all active constituents, with the exception of 10-gingerdione, 10-paradol, and 10-shagool, which have poor water solubility. Also, no mutagenic, tumorogenic, irritant, and reproductive effect revealed by toxicity profiling of active constituents of long pepper, ginger, clove, and Black as shown in Supplementary Table 3.

4. Discussion

Similar to SARS-Cov virus, the entry stage of SARS-Cov-2 virus is also having the three-step process, i.e., 1- S protein-ACE2 receptor binding, 2- changes in the conformation of S protein, 3- involvement of endosomal cathepsin L protease enzyme activates the S protein fusion activity with the endosome [17, 18]. Previously, it was believed that the fusion activity with the endosomal membrane requires an absolute acidic environment or low pH. It has been demonstrated by another investigation that the viral S protein can induce membrane fusion at neutral pH levels with the aid of the protease enzyme cathepsin L [19]. According to in vitro research [20, 21], cathepsin L inhibitors like E63c, E64d, and MDL28170 limit the entry of the SARS-Cov virus or SARS-Pseudovirus. Therefore, the current in-silico study has targeted the human cathepsin L protease enzyme to block the initial entry stage of SARS-Cov-2 with the bioactive constituents of long pepper, ginger, clove, and black pepper. Also, in our current study, we hypothesized these active constituents as herbal medicine in the treatment of viral infection and also having multiple benefits with a lower risk of adverse side effects. Recently, WHO and few clinical studies have reported most of the SARS-Cov-2-infected patients died due to comorbidities conditions. Therefore, there should be an effective therapeutic in such a way that it may not aggravate the patient's condition.

Long pepper is recognized for its medicinal properties and its active constituents are commonly used for the treatment of various diseases such as viral hepatitis, respiratory infections, chronic bronchitis, asthma, cough, chronic malaria, constipation, diarrhea, cholera, gonorrhea, and tumors [22, 23]. Also, long pepper is known for its remarkable improvement in inflammatory diseases, depression, and diabetes, and cancer patients reported the inhibitory activity of active constituents of long pepper on the hepatitis B virus infection [24]. In vitro antiviral activity and anticancer activity of methanol and chloroform extraction of long pepper against the vesicular stomatitis Indian virus and human parainfluenza virus have also been demonstrated by Priya and Kumari [25]. Therefore, in the present study, we have performed the molecular docking of bioactive constituents of long pepper and analyzed its binding energy and docking ligand interactions with the human cathepsin L protease enzyme and compared with the binding energy and docking pose interactions of human cathepsin L inhibitor (Pub Chem CID: 16725315). The finding from this in silico study suggests that all the active constituents as shown in Supplementary Table 1 have acceptable binding energy and docking interactions with the active site of human cathepsin L protease. We have determined that Piperolactam A (CID: 30810116), Bisdemethoxycurcumin (CID: 5315472), and Caumaperine (CID 10131321) are the most acceptable active constituents based on a comparison of their binding energy (−9.3 Kcal/mol) and docking interactions of the inhibitor + cathepsin L receptor complex. These parameters include binding energy, interacting and non-interacting amino residue present at the docking site, pharmacokinetic and toxicity evaluation profiles. Furthermore, the binding energy of Piperolactam A (−9.4 Kcal/mol) is larger than the binding energy of human cathepsin L inhibitor (−9.3 Kcal/mol). Also, amino acid residue ARG C:44; LEU C:45; PHE D:39; PHE C:39; LEU D:45; LYS D:10; GLY C:11; GLY C:43 and ARG D:44 out of them LEU C:45 and LEU D:45 forming a stable alkyl and π-alkyl bond at the docking site of Piperolactam A is similar to the inhibitor + cathepsin L receptor complex revealing the potency of Piperolactam A to act as inhibitory action on the human cathepsin L protease. Similarly, the docking pose of Bisdemethoxycurcumin and Caumaperine was also found in a similar docking pose with the inhibitor + cathepsin L receptor complex, Although the binding energy of Bisdemethoxycurcumin (−8.5 Kcal/mol) and Caumaperine (−7.8 Kcal/mol) was lower than the human cathepsin L inhibitor (−9.3 Kcal/mol), in an acceptable range that can also be considered a promising inhibitor of cathepsin L to restrict the early entry of SARS-Cov-2 virus into the cell.

Ginger also known as Zingiber officinale Roscoe, belong to the Zingiberaceae, has been commonly used as a spice in food. Several studies have demonstrated its herbal medicinal properties in various biological activities, i.e., antimicrobial, antioxidant, anti-inflammatory, anticancerous and also having potential in the treatment of respiratory diseases, cardiovascular diseases, obesity, and neurodegenerative diseases [26, 27]. Moreover, preclinical evaluation has revealed the antiviral activity of the ginger extract against the Hepatitis C virus (HCV) [28]. Chang et al. [29] has reported the antiviral activity of fresh ginger by blocking the viral attachment and internalization process resulted in a reduction in plaque formation in the respiratory tract induced by human respiratory syncytial virus. Therefore, in our present study active constituents of ginger were screened through a molecular docking process and analyzed the binding affinity against the human cathepsin L protease and measured the potentiality of active constituents of ginger to block the internalization process of the SARS-Cov-2 virus. Based on our result, we screened out the selected active constituents of ginger, i.e., Zingiberene (B.E.−7.2 Kcal/mol), 6-Gingerdiol (B.E.−7.1 Kcal/mol), 6-Gingerol (B.E.−7.0 Kcal/mol), 8-Gingerdione (B.E.−6.3 Kcal/mol), 10-Gingerdione (B.E.−6.4 Kcal/mol), 10-Paradol (B.E.−5.7 Kcal/mol), 10-Shagaol (−5.4 Kcal/mol), and 6-Paradol (B.E.−5.6 Kcal/mol) showed acceptable binding energy and similar docking pose with the comparison with the docking pose of inhibitor + cathepsin L receptor complex. Thus, the finding suggests the significant interactions of active constituents of ginger against the cathepsin L protease indicating the potential of selected active constituents of ginger to act as an inhibitor.

Clove (*Syzygium aromaticum*) is one of the traditional spice use in food, preservative, and medicinal herbs. In the last few decades, several studies have confirmed the antiviral, antibacterial, antioxidant, and anti-cancerous properties of clove [30]. A phenolic compound extracted from cloves such as Eugenol, eugenol acetate, and gallic acid is commonly used in pharmaceutical industries for their biological applications [31]. In vitro study has also demonstrated the antiviral activity of aqueous extract of both clove and ginger against the feline calicivirus [32]. Eugenol, β-caryophyllene, and β-Medinformatics Vol. 00 Iss. 00 2024 08caryophyllene, an active ingredient of clove, have demonstrated the inhibitory action against the Herpes simplex virus type-1 (HSV-1) [33]. Consequently, in our present study, essential active constituents of the clove are screened and targeted against the active site of human cathepsin L protease. We screened out the active constituents of clove, i.e., Kaempferol (B.E. −9.3 Kcal/mol), βCadinene (B.E. −8.0 Kcal/mol), Alpha-cedrene (B.E. −8.0 Kcal/mol), Alpha-muurolene

(B.E. −7.6 Kcal/mol), Eugenitin (B.E. −7.5 Kcal/mol), Jasmone (B.E. −6.0 Kcal/mol), and Eugenol (B.E. −5.3 Kcal/mol) based on the acceptable range of binding energy and similar docking site with the docking site of inhibitor+ cathepsin L receptor complex. Several studies have also demonstrated the antiviral activity of Kaempferol and its derivatives such as Kaempferol 3-O-α-L-rhamno pyranoside, and Kaempferol-7-o-glucoside against the viruses, i.e., human cytomegalovirus (HCV), Japanese encephalitis virus, and influenza virus-like H1N1 and H9N2 [34, 35].

Black pepper (*Piper nigrum* L.) is one of the spices used in food but also included under the medicinal herbs due to its antimicrobial, antioxidant, antiproliferative, and gastroprotective module properties [15]. Mair et al. [36] have also observed the antiviral and anti-proliferative properties in an in vitro study of active constituents of black pepper. Consequently, in our in silico study, we found the acceptable range of binding energy of following active constituents of black pepper against the human cathepsin L receptor, i.e., Pub Chem CID 44292388 (B.E. −6.7 Kcal/mol), Piperolein B (B.E. −6.8 Kcal/mol), and Sabinene B (B.E. −6.8 Kcal/mol). Also, these active constituents show a similarity in the docking pose with the inhibitor + cathepsin L receptor complex indicating the potentiality of these active constituents to restrict the activity of cathepsin L protease.

The Lipinski rule of five predictors states that when there are hydrogen bond donors that are at least five and hydrogen bond acceptors that are at least ten to avoid, proper absorption and penetration are more likely. Bioactive molecules should have a molecular weight of no more than 500 g/mol, a lipophilicity (Clog P) of no more than 5, and a topological polar surface area (TSPA) of no more than 140 Å for the measurement of polar surface area [37, 38]. Consequently, pharmacokinetic properties of selected active constituents of long pepper, ginger, clove, and Black pepper are found suitable in various parameters of Lipinski rule of five (Supplementary Table 2) such as molecular weight, hydrogen bond donor, hydrogen bond acceptor, topological polar surface area (TPSA), water solubility, and lipophilicity for better bioavailability to the cell.

5. Conclusion

Piperolactam A and Kaempferol were found to be a more acceptable natural therapeutic compounds among other selected bioactive constituents, which possess to act as an inhibitor to target the human cathepsin L protease as they are having equal or higher binding energy as shown in Supplementary Table 1 and similar docking pose comparatively with the inhibitor + cathepsin L receptor complex. So, it can be concluded that these bioactive constituents not only act a promising inhibitor of the human cathepsin L protease in the lysosome and restrict the entry of the SARS-Cov-2 virus but also having possibilities to overcome the comorbidities conditions and multiple organ injuries in the treatment of SARS-Cov-2-infected patients. However, still, a need for the in vivo antiviral activity should be further tested in animal models to put forward therapeutic repositioning of bioactive constituents of long pepper, ginger, clove, and black pepper.

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Ethical Statement

This study does not contain any studies with human or animal subjects performed by any of the authors.

Conflicts of Interest

P. S. Bisen is an Editorial Board Member for *Medinformatics*, and was not involved in the editorial review or the decision to publish this article. The authors declare that they have no conflicts of interest to this work.

Data Availability Statement

The data that support the findings of this study are publicly available: Protein Data Bank: https://www.rcsb.org/structure/2XU1; Pub Chem: https://pubchem.ncbi.nlm.nih.gov/compound/16725315.

Supplementary Information

The supplementary tables are available at: https://doi.org/10.47852/bonviewMEDIN32021518

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