IJPSR (2022), Volume 13, Issue 11 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 14 March 2022; received in revised form, 02 May 2022; accepted, 04 May 2022; published 01 November 2022

# PAPAIN LIKE PROTEASE: A POTENTIAL TARGET FOR MIDDLE EAST RESPIRATORY SYNDROME CORONAVIRUS (MERS-COV): THE BATTLE CONTINUES

Neeldeep Ganguly, Swagata Roy and Tamalika Chakraborty *

Guru Nanak Institute of Pharmaceutical Science and Technology, 157/F, Nilgunj Road, Sodepur Panihati - 700114, Kolkata, India.

**Keywords:**

Coronaviruses, Deacetylgedunin, Eucalyptol, Indinavir, MERS-CoV, Papain-like protease

**Correspondence to Author:**
**Ms. Tamalika Chakraborty**

Assistant Professor,
Guru Nanak Institute of Pharmaceutical Science and Technology, 157/F, Nilgunj Road, Sodepur Panihati - 700114, Kolkata, India.

**Email:** tamalika.chakraborty@gnipst.ac.in

**ABSTRACT:** Middle-East Respiratory Syndrome coronavirus (MERS-CoV) can trigger severe acute pneumonia, renal, digestive failure and even death. Coronaviruses express papain-like proteases (PLpro), multipurpose enzymes which had protease activity and can lacerate nonstructural proteins to manipulate the viral polyprotein responsible for replication. They also have deubiquitinating function, which can modify the innate immune response. The reduction of the infection of MERS-CoV is by Inhibition of PLpro with a ligand will wedge the cleavage progression of nonstructural protein. As a result, papain-like protease may be considered as a candidate for antiviral drug production. This current study focuses on screening of extracts from Neem and Eucalyptus for MERS-CoV that could be potentially used as an inhibitor against the disease. Blind molecular docking study was conducted by using Auto Dock followed by visualization using PyMol, which is examined in this existing study. Deacetylgedunin (Neem) and eucalyptol (Eucalyptus) showed successful binding to MERS-CoV papain-like protease based on measured parameters such as root Mean Square Deviation (RMSD), binding capacity and inhibiting constant. The compound Deacetylgedunin found in neem exhibited the lowest RMSD value of 16.388 Å and the highest binding energy of –8.28 kcal/mol. It also had the highest inhibition constant value of 851.36 nM and the lowest inhibition constant value of 851.36 nM. Since Deacetylgedunin gave a better result compared to Indinavir, hence it can be considered as a potential and safe alternative for the current medicine given for MERS-CoV disease.

**INTRODUCTION:** Coronaviruses are massively (2632 kb in size) wrapped single-stranded ribonucleic acid (RNA) viruses that can infect many mammals [5]. They belong to the *Coronaviridae* family, and sub-categorised as *Coronavirinae*. "

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(11).4476-84

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(11).4476-84

The infections are divided into four genera based on their genotypic and serological characteristics: Coronaviruses Alpha, Beta, Gamma, and Delta" [3, 4]. As of now, all distinguished CoVs that are equipped for contaminating humans fit into the initial two genera.

The alpha coronaviruses HCoV-NL63 (Human CoV-NL63) and HCoV-229E, as well as the beta coronaviruses HCoV-OC43 (Human CoV-OC43), HKU1 (Human CoV), SARS-CoV (Severe Acute Respiratory Syndrome CoV), and MERS-CoV (Middle Eastern Respiratory Syndrome CoV)" [4]. In the last two decades, betaCoVs have caused three

epidemics: SARS in 2002-03, MERS in 2012, and COVID-19, which was first identified in 2019 [9]. MERS-CoV first appeared in the Middle East in July 2019 and quickly spread to a few countries, infecting nearly 2300 people and causing widespread deaths [6, 10]. MERS-CoV has evolved into one of the world's most dangerous diseases since its discovery in 2012 in Saudi Arabia [1, 6]. According to the latest study, there were 1,806 confirmed MERS-CoV instances in September 2016, taking the lives of around 36% of people in 27 countries worldwide. The Middle East (Saudi Arabia, Jordan, Qatar, Kuwait, Yemen), Europe (the United Kingdom, Greece, France, Germany), Southeast Asia (Philippines, Malaysia, Thailand), and the United States of America (Saudi Arabia, Jordan, Qatar, Kuwait, Yemen). In 2015, 186 cases of MERS-CoV were recorded in South Korea" [9]. There is no affirmed at this point compelling antiviral medications or antibodies for treating MERS-CoV [1, 10]. As a result, genuine concerns and investigation into this illness are required to prevent a pandemic from coalescing, which is currently occurring.

"MERS-CoV is a single-stranded RNA virus that belongs to the *Coronavirinae* family's Beta Coronavirus group [11]. MERS-CoV has a large genome, varying in size from 26.2 to 37.1 kb, with a few auxiliary proteins (spike (S) glycoprotein, little envelope protein (E), essential film protein (M), and genome-related nucleocapsid protein (N)) and non-basic protein. PLpro, or papain-like protease, is a multifunctional proteolytic enzyme involved in the Coronavirus infection process and responsible for the cleavage of pp1a and pp1ab polyproteins into stable non-basic proteins [12-17]. The MERS-CoV replication cycle would be disrupted if the pathway for delivering non-auxiliary proteins, such as PL-pro, was repressed [1]. As a result of initial screening, this protease has been identified as a potential target for combating this illness [1].

MERS-CoV and SARS-CoV require host proteases to activate their envelope glycoproteins to gain access to cells via endosomal or non-endosomal trails [7]. Antiviral agents that inhibit host cell proteases are being studied [18]. For both MERS-CoV and SARS-CoV, the serine protease TMPRRSS2 intervenes *via* the non-endosomal pathway [4, 19, 20–22]. Camostat mesylate, which has been used to treat chronic pancreatitis, inhibits MERS-CoV, SARS-CoV, and influenza virus TMPSSR2-mediated glycoprotein actuation [4, 18, 23–25]. K11777, a cysteine protease inhibitor, is currently being studied in clinical trials for the treatment of parasitic contaminations. K11777 has broad antiviral activity against coronaviruses (MERS-CoV, SARS-CoV, HCoV-229E), filoviruses (EBOV, Marburg infection), and paramyxoviruses (MERS-CoV, SARS-CoV, HCoV-229E) (Nipah infection) [4, 26]. "Zhou et al. discovered that Camostat and K11777 had inhibitory action against SARS-CoV whereas K11777 just repressed EBOV, implying that these viruses had separate host protease requirements. Both MERS-CoV and SARS-CoV are inhibited in vitro by E-64-D, an inhibitor of an endosomal cysteine protease currently in Phase III preliminaries to treat muscular dystrophy" [27]. Filovirus cell entry is also hampered by E-64-D [28]. When choosing protease inhibitors for antiviral therapeutic claims, keep in mind that viruses depend on explicit serine or cysteine host proteases. Subsequently, an expanded comprehension of the relationship between viral pathogenesis and host proteases will resolve the unsurpassed treatment alternatives for viral diseases.

Neem (*Azadirachta indica*), a traditional Indian medicinal herb, has been utilizedas a therapy to treat a variety of acute and chronic ailments in Asia and Africa since antiquity [18]. The insecticidal, antimicrobial, antimalarial, antibacterial, antiviral, and spermicidal properties of different plant components have been found to be useful in the treatment of some bacterial pathogens [29]. Antiviral activity of Neem crude extracts was discovered against the extremely pathogenic avian influenza virus (H5N1) [25, 30]. Gedunin is abundant in *Azadirachta indica A. Juss.* fruit epicarps, with a higher proportion in young green fruits than ripe fruits [6, 31]. Gedunin concentration in A. indica leaves is less than 0.1 percent, with a residual presence in the rest of the plant [6, 31]. Antibacterial, antifungal, antiallergic, anti-inflammatory, antimalarial, anticancer, insecticidal, and neuroprotective properties have all been attributed to gedunin [6, 32].

Essential oils (EOs) are complex mixtures of volatile phytochemicals from a variety of families, including monoterpenes, sesquiterpenes, and phenylpropanoids [25]. A number of researchers have observed the effects of Eos [14]. Antiviral activity of these EOs has been demonstrated against a variety of viruses, including Human Immunodeficiency Virus (HIV), human herpesviruses (HSV), influenza virus (IFV), and yellow fever virus, and avian influenza [7, 33, 34]. "The essential ingredient used in eucalyptus oil from all eucalyptus plants is eucalyptol (1, 8 cineole); they discovered that Eucalyptus is virucidal [35]. Antivirals such as protease inhibitors (indinavir, saquinavir, and lopinavir/ritonavir) and RNA polymerase blockers (remdesivir) are being tested against SARS-CoV-2 [2, 13, 36]. The use of bioinformatics for small molecule discovery has become increasingly important in drug development in recent years [3]. Molecular docking, among other research methods, has been extensively used to investigate the binding affinity of small molecules with the functional groups of the target protein [3]. Virtual screening methods have recently identified promising antiviral compounds against a wide range of viruses, including influenza, Dengue fever, and, more specifically, CoVs, while others have demonstrated the superiority of molecular dynamics models in the search for feasible antiviral and drug rejection pathways [2, 3]. PLpro is a highly conserved protease found across CoVs proposed as a target for MERS-CoV blockers [3]. In this study, a molecular docking approach was employed to explore probable PLpro inhibitors for MERS-CoV from plant extracts of Neem and Eucalyptus and compared with Indinavir which could be conceivably utilized as an alternate inhibitor against the disease.

## MATERIALS AND METHODS

**Retrieval of Receptor Protein:** The Protein Data Bank (PDB) can be accessed via the RCSB-PDB site: http://www.rcsb.org/pdb for probing three-dimensional protein structure"[2]. Protein Data Bank (PDB) is the overall database of underlying and observational data of organic macromolecules, set up in Brookhaven National Laboratories. It gathered structural and observational data of the macromolecules, distinctively protein data recovered by the X-ray crystallographic and Nuclear magnetic resonance (NMR) techniques. Papain Like protease (PLpro) (PDB ID: 4PT5) structure was acquired from PDB. We composed papain-like protease and tapped on retrieved released data coordinating the queryicon then the molecule name was featured, then we tapped on the view/analyze/save macromolecule icon. Under the dataretrieval segment, we tapped on the save Protein data bankicon. A spring-up menu appeared, at that point, we tapped on the express save for experts' icon and saved it on a hard drive.

**Scan for Ligands and Drugs:** "PubChem" was used to obtain the 3-dimensional structures of deacetylgedunin (CID 3034112) and eucalyptol (CID 2758) [2]. PubChem is a directory of biomolecules and biological processes, with three databases in its library: molecules, compounds, and bioassays. Since we were keen on downloading the structure of the compound, by tapping on the download button over the structures, we could, without much of a stretch, download the structures in various formats like SDF, JSON, XML, ASNT. We had saved it in SDF format then the structures were transformed into pdbqt format by the assistance of Open Babel GUI.

**Retrieving Structure of Indinavir:** The structure of Indinavir (ACCESSION NO: DB00224) was obtained from Drug Bank (https://go.drugbank.com/) [2]. After composing the name of Indinavir we tapped on download then right tapped on PDB and saved it on the hard drive.

**Energy Minimization Utilizing ModRefiner:** The Mod Refinement server plays out a refinement utilizing the ModRefiner [2]. We entered the email address, then transferred the papain protease model into the PDB arrangement, and afterward, we tapped on the 'submit' button. In terms of backbone topology, hydrogen bonds, and side-chain placement, one aim of ModRefiner was to get the underlying beginning models nearer to their original conformation. It also results in a significant change in the actual design of local systems. The autonomous software also supports ab initio full-atomic control, in which the underlying or comparative models do not influence the refined model.

**Molecular Docking and Simulation:** The Blind Molecular docking investigation of the compound

over papain-like protease (PLpro) was contemplated utilizing Autodock (http://autodock.scripps.edu/) [2]. The above compounds' SDF files were downloaded from PubChem, translated into PyRx and then processed into Ligands. Since this is a blind molecular docking, no ligand parameters are optimized, and the program automatically selected the grid coordinates. The main ligand to inhibit MERS-CoV PLpro was the one with the lowest Gibbs free binding energy, the smallest RMSD value, the highest inhibition constant (pKi), and the most significant binding interaction [1, 2]. The ligand with the lowest Gibbs free binding energy, the smallest RMSD value, the highest inhibition constant (pKi) and the most significant binding interaction was chosen as the best ligand to inhibit MERS-CoV PLpro [1, 2].

**Receptor-ligand Binding Analysis:** Auto dock created docking pair of protein and ligand were saved in pdb format and were visualized in PyMol 2.4 visualization [2]. It leads to the three-dimensional molecular representation of small molecules, organic macromolecules, such as proteins, density, surfaces, and trajectories. It additionally incorporates molecular editing, raytracing, and films. The ligand-binding sites as well as the ligand's surrounding amino acids, were also visualized. Molecular interactions within certain hydrogen bonds between proteins and ligands were analyzed, and the distance between hydrogen atoms was calculated as a result. The residue side chains that structure a brimming with life cavity or cleft where the ligands or atoms or various proteins are competent to bind are called active sites in Site Prediction proteins.

## RESULTS AND DISCUSSION

**3D Structure Validation of MERS-CoV PLpro:** The protein 3d structure of PDB ID: 4PT5 was determined in this experiment (Fig. I). This protein structure was discovered employing X-beam diffraction with a target of 1.79, implying that its 3D structure is more similar to the first protein structure than the 3D structure with a higher crystal resolution value [1, 2]. The MERS-CoV PLpro structure was portrayed using the 4PT5 structure in order to find a potential inhibitor of this protein in this study.

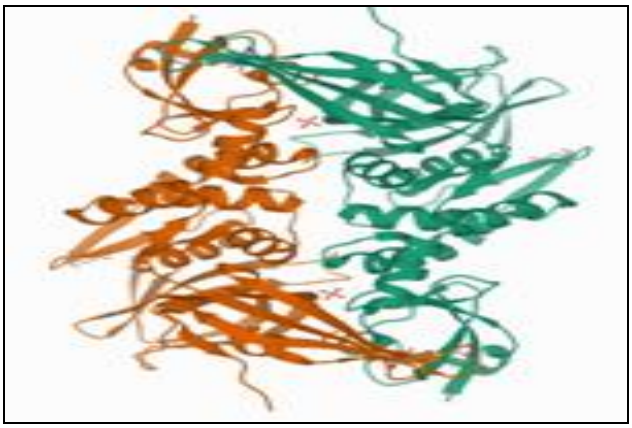

**FIG. 1: PAPAIN PROTEASE STRUCTURE OBTAINED FROM PDB**

**Structure Validation of Deacetylgedunin (Neem) and Eucalyptol (Eucalyptus) from PubChem:** The structure of Deacetylgedunin from Neem (CID 3034112) and Eucalyptol from Eucalyptus (CID 2758) were acquired from PubChem **Fig. 2.**

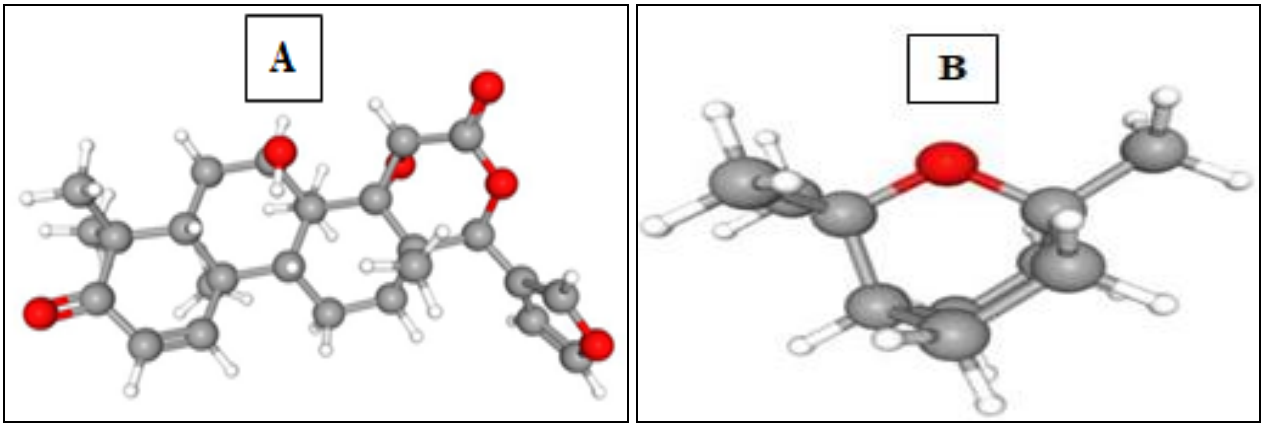


**FIG. 2: STRUCTURE OF DEACETYLGENUDIN (NEEM) DEPICTED AS "A" AND EUCALYPTOL (EUCALYPTUS) DEPICTED AS "B" OBTAINED FROM PUBCHE**

**Validation of Structure of Indinavir:** The structure of Indinavir was obtained from the Drug Bank (ACCESSION NO.: DB00224) **Fig. 3.**

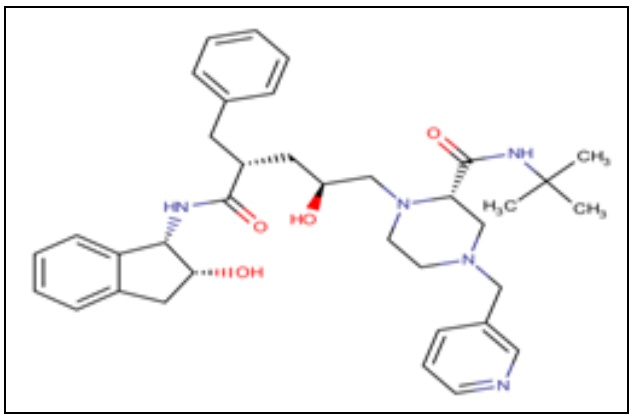


**FIG. 3: INDINAVIR STRUCTURE FROM DRUG BANK**

**Energy Minimisation:** Prior to molecular docking, the 3D protein structure of MERS-CoV PLpro was condensed and configured. These loops were completed using ModRefiner (JOB ID: M26648) [1]. Protonation of the MERS-CoV PLpro structure added a hydrogen atom to the protein structure [1]. Even though hydrogen atoms are required for molecular docking and molecular dynamic simulation, this step was necessary. In general, hydrogen atoms are not accessible in 3D protein structures formed by X-ray crystallography due to instrument constraints. The addition to the treatment of each particle in the protein structure was then determined using the partial or full charge of the MERS-CoV PLpro [1]. The MERS-CoV PLpro forcefield was also used to determine a molecular structure's preferred conformity and the potential energy for each conformation [1, 2]. Despite its suitability for macromolecule structures like enzymes, ModRefiner was chosen as the MERS-CoV PLpro forcefield in this study [1]. In addition, the 'Gas Phase' boundary was chosen in the solvation mode [1]. After this progression was directed, the 3D protein structure of MERS-CoV PLpro was prepared to move on to the next level. Energy conservation was the responsibility of ModRefiner **Fig. 4** and **5** [1].

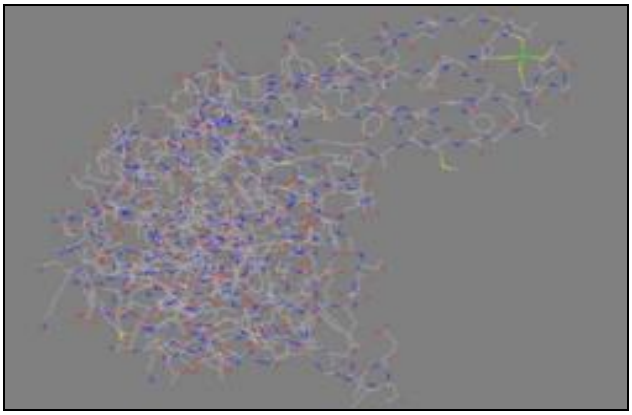

**FIG. 4: UNREFINED PROTEIN IN PYRX**

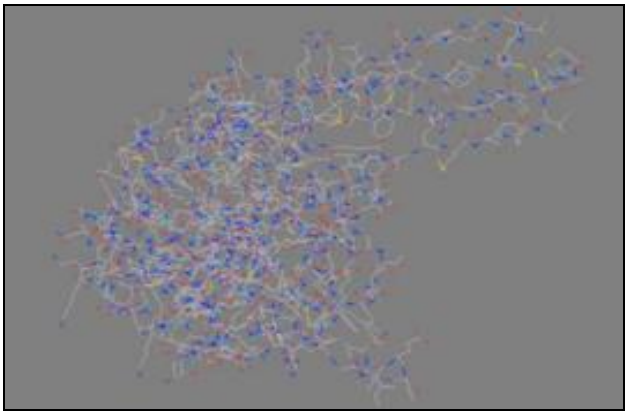

**FIG. 5: REFINED ENERGY MINIMIZED PROTEIN IN PYRX**

**Molecular docking using Auto Dock**

The ligands Deacetylgedunin and Eucalyptol were docked to Papain-like protease (PLpro) using Auto Dock. **Table 1** shows the molecular docking results of different parameters like RMSD values, Binding energy and Inhibiting constant.

**TABLE 1: MOLECULAR DOCKING RESULTS WITH RMSD VALUE**

| S. no. | Protein | Ligand | RMSD value | Binding energy | Inhibiting constant |
|---|---|---|---|---|---|
| **1** | Papain like protease (PLpro) | Indinavir | 37.287 Armstrong | -6.98 kcal/mol | 7.63 µM |
| **2** | Papain like protease (PLpro) | Deacetylgedunin (Neem) | 16.388 Armstrong | -8.28 kcal/mol | 851.36 µM |
| **3** | Papain like protease (PLpro) | Eucalyptol (Eucalyptus) | 23.319 Armstrong | -6.99 kcal/mol | 7.53 µM |

**Molecular Visualisation using PyMOL:** The interaction between MERS-CoV papain, like deacetylgedunin, eucalyptol and indinavir, is shown in Figures 6, 7 and 8. The compound deacetylgedunin used in neem seeds had the maximum binding energy of –8.28 kcal/mol and inhibition constant of 851.36 nM, based on the current blind docking findings [12]. In contrast to the blind docking findings for Eucalyptol present in Eucalyptus, the drug Indinavir had the lowest

binding energy of – 6.98 kcal/mol, and its inhibition constants Ki at 25C (298K) was 7.63 M [12]. Other than the above compounds, the ligand selected from Neem extracts exhibited the highest inhibition constant against Papain Like Protease (PLpro) (PDB ID: 4PT5).

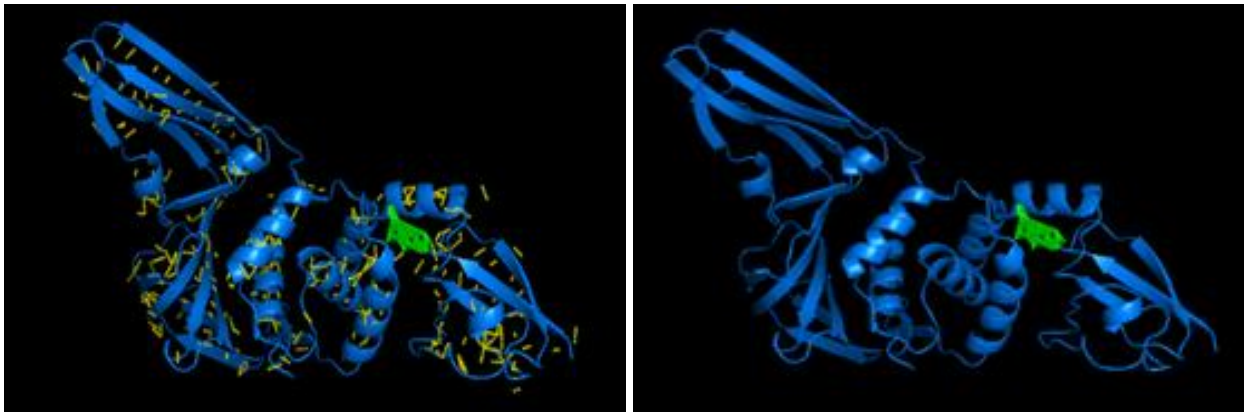

**FIG. 6: INTERACTION OF MERS-COV PLPRO AND DEACETYLGEDUNIN FROM NEEM VISUALISED ON PYMOL**

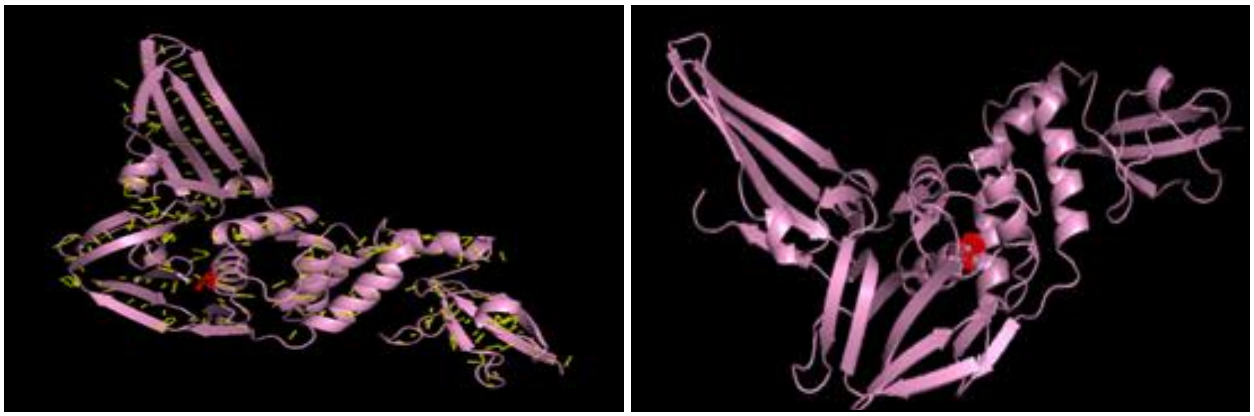

**FIG. 7: INTERACTION OF MERS-COV PLPRO AND EUCALYPTOL FROM EUCALYPTUS VISUALISED ON PYMOL**

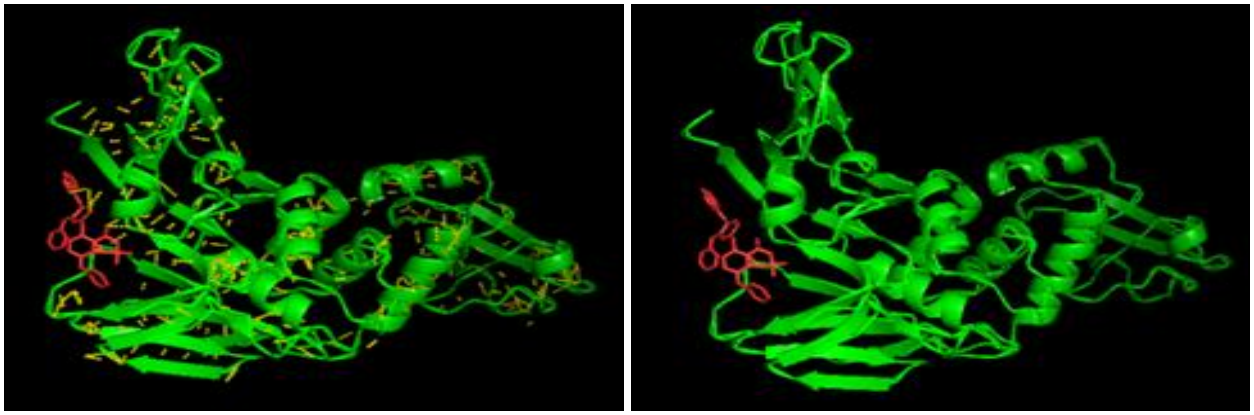

**FIG. 8: INTERACTION OF MERS-COV PLPRO AND INDINAVIR VISUALISED ON PYMOL**

Computational and structural biology strategies have quickened the revelation of novel drugs used to treat viral illnesses [2]. Herbal remedies and naturally occurring products afford a rich reserve for novel antiviral drug development. Documentation of the antiviral mechanisms from these natural mediators has elucidated where they interact with the viral lifecycle, such as viral entry, reproduction, assemblage, release, and the directing of virus–host-specific interactions. CoV infections influence humans in different manners, such as respiratory, digestive, cold, fever, and liver frameworks. Proteases play an essential part in viral replication, so they're frequently used as

protein targets in implementing antiviral medications [2]. In MERS-CoV, the PLpro protein plays an important role in the viral proteolytic expansion. It has already been investigated as a promising target protein for preventing the spread of infection by hindering the cleavage of a viral polyprotein [2, 22].

Proteases are prime targets for viral replication restraint, according to Lu and Wang [4, 14]. Since the protein sequences of the SARS-CoV Main protease and the 2019-nCoV Main protease are 96 percent indistinguishable, recipient proteases can be utilized as potential biomarkers [2, 11]. We surveyed the structural biological facets of accessing and recovering a Papain-like Protease (PLpro) receptor structure from the PDB database [8]. Utilizing Auto dock, the ligands Deacetylgedunin, Eucalyptol and Indinavir were docked to Papain-Like Protease (PLpro). **Fig. 1** and **2** show the structure of receptors and ligands, respectively. The consequences of Auto Dock with RMSD values are shown in **Table 1.**

RMSD data revealed a high affinity of the ligand for the protein structure, with nearby and global RMSD values [2]. **Table 1** Further investigation may aid in determining the function of these residues, mostly in drug binding mechanisms. One of the significant physical characteristics that influences protein configuration and function is structural flexibility [2, 3]. Though elevated expansion in kinetic energy and protein flexibility could even dislocate non-covalent associations as in denaturation, a sharp decrease in flexibility can cause protein denaturation as found in cold denaturation [2, 3]. To perform their native function under physiological conditions, proteins require a basic level of flexibility [3]. In this frame of reference, an inhibitor can modify a protein's flexibility and reduce its enzymatic activity by binding to it [3]. Compared with Indinavir, a protease inhibitor recommended for the treatment of MERS, the binding energy score of Deacetylgedunin was greater and showed the lowest RMSD value [16]. To this end, Deacetylgedunin can be considered a high-potential anti-MERS-CoV plant drug candidate for managing this disease. Finally, the inadequacy of wet-lab approval is a drawback in our studies. Still, we anticipate that computational biology research and its integration with wet-lab data will be fruitful in identifying potential anti-PLpro components [8].

**CONCLUSION:** Natural phytochemicals provide a valuable and influential reservoir of chemical compounds with antiviral properties [5]. Chemical modifications to these structures, guided by computer-based docking simulations, may improve efficacy and selectivity [5]. This study aimed to examine deacetylgedunin from neem leaves/seeds, and eucalyptol from eucalyptus essential oil could be utilized to hinder the MERS-CoV disease lane. Deacetylgedunin exhibited greater binding affinity and lowered binding energy than Eucalyptol and Indinavir. As a result, we assume that deacetylgedunin, which is found in medicinal plants, may act as a potential inhibitor of MERS-CoV PLpro and might become a viable treatment option. Since these compounds may be toxic at certain concentrations, in vitro and *in-vivo* screening is required to determine safe and therapeutic concentrations before clinical trials in humans can be conducted. More research should be directed towards their approval using in-vitro and in-vivo models to pave the way for these compounds in drug discovery.

**ACKNOWLEDGEMENT:** The authors express ardent gratitude towards the Department of Biotechnology, Guru Nanak Institute of Pharmaceutical Science and Technology, 157/F, Nilgunj Road, Panihati, Kolkata - 700114, West Bengal, for rendering constant assistance while writing this review.

**CONFLICTS OF INTEREST:** The authors declare no conflicts of interest.

**REFERENCES:**

1. Wu A, Peng Y, Huang B, Ding X, Wang X, Niu P, Meng J, Zhu Z, Zhang Z, Wang J, Sheng J, Quan L, Xia Z, Tan W, Cheng G & Jiang T: Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China. Cell Host & Microbe 2020; 27(3): 325-328. https://doi.org/10.1016/j.chom.2020.02.001
2. Mani J S, Johnson JB, Steel JC, Broszczak DA, Neilsen PM, Walsh KB & Naiker M: Natural product-derived phytochemicals as potential agents against coronaviruses: A review. Virus Research 2020; 284: 197989. https://doi.org/10.1016/j.virusres.2020.197989
3. Chu DK, Pan Y, Cheng S, Krishnan P, Liu Y, Ng DY, Wan CK, Yang P, Wang Q, Peiris M & Poon LL: Molecular diagnosis of a novel coronavirus (2019-nCoV) causing an outbreak of pneumonia. Clinical Chemistry

2020; 66(4): 549-555. https://doi.org/10.1093/clinchem/hvaa029
4. Cui J, Li F & Shi ZL: Origin and evolution of pathogenic coronaviruses. Nature Reviews Microbiology 2018; 17(3): 181–192. https://doi.org/10.1038/s41579-018-0118-9
5. Yang Y, Peng F, Wang R, Yang M, Guan K, Jiang T, Xu G, Sun J & Chan C: Corrigendum to "The deadly coronaviruses: The 2003 SARS pandemic and the 2020 novel coronavirus epidemic in China" [J. Autoimmun. (2020). 109C (2020) 102434]. Journal of Autoimmunity, 111, 102487. https://doi.org/10.1016/j.jaut.2020.102487
6. Zhou H, Yang J, Zhou C, Chen B, Fang H, Chen S, Zhang, X, Wang L & Zhang L: (2021). A Review of SARS-CoV2: Compared With SARS-CoV and MERS-CoV. Frontiers in Medicine, 8. https://doi.org/10.3389/fmed.2021.628370
7. The WHO MERS-CoV Research Group. (2013). State of knowledge and data gaps of Middle East respiratory syndrome coronavirus (MERS-Cov) in humans. PLoS Currents. https://doi.org/10.1371/currents.outbreaks.0bf719e352e7478f8ad85fa30127ddb8
8. Pillaiyar T, Manickam M & Jung S: (2015). Inhibitors of melanogenesis: A patent review (2009 – 2014). Expert Opinion on Therapeutic Patents, 25(7), 775-788. https://doi.org/10.1517/13543776.2015.1039985
9. Corrigendum to "Coronavirus disease-19: The first 7,755 cases in the Republic of Korea" [Osong public health res Perspect 2020; 11(2): 85–90]. (2020). Osong Public Health and Research Perspectives, 11(3), 146-146. https://doi.org/10.24171/j.phrp.2020.11.3.09
10. Zhang YY, Li BR & Ning BT: (2020). The Comparative Immunological Characteristics of SARS-CoV, MERS-CoV, and SARS-CoV-2 Coronavirus Infections. Frontiers in Immunology, 11. https://doi.org/10.3389/fimmu.2020.02033
11. De Groot RJ, Baker SC, Baric RS, Brown CS, Drosten C, Enjuanes L, Fouchier RA, Galiano M, Gorbalenya AE, Memish ZA Perlman S, Poon LL, Snijder EJ, Stephens GM, Woo PC, Zaki AM, Zambon M & Ziebuhr J: Middle East respiratory syndrome coronavirus (MERS-Cov): Announcement of the coronavirus study group. Journal of Virology 2013; 87(14), 7790-7792.
12. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, Hu Y, Tao ZW, Tian JH, Pei YY, Yuan ML, Zhang YL, Dai, FH, Liu Y, Wang QM, Zheng JJ, Xu L, Holmes EC & Zhang YZ: A new coronavirus associated with human respiratory disease in China. Nature, 579(7798), 2020; 265–269. https://doi.org/10.1038/s41586-020-2008-3
13. Dai L & Gao GF: Viral targets for vaccines against COVID-19. Nature Reviews Immunology 2020; 21(2): 73–82. https://doi.org/10.1038/s41577-020-00480-0
14. Moustaqil M, Ollivier E, Chiu HP, Van Tol S, Rudolffi-Soto P, Stevens C, Bhumkar A, Hunter DJB, Freiberg AN, Jacques D, Lee B. Sierecki E & Gambin Y: SARS-CoV-2 proteases PLpro and 3CLpro cleave IRF3 and critical modulators of inflammatory pathways (NLRP12 and TAB1): implications for disease presentation across species. Emerging Microbes & Infections 2021; 10(1)" 178–195. https://doi.org/10.1080/22221751.2020.1870414
15. Kilianski A, Mielech AM, Deng X & Baker SC: (Assessing activity and inhibition of Middle East respiratory syndrome coronavirus papain-like and 3C-Like proteases using luciferase-based biosensors. Journal of Virology 2013; 87(21): 11955-11962. https://doi.org/10.1128/jvi.02105-13
16. Baez-Santos YM, Mielech AM, Deng X, Baker S & Mesecar AD: Catalytic function and substrate specificity of the papain-like protease domain of nsp3 from the Middle East respiratory syndrome coronavirus. Journal of Virology 2014; 88(21): 12511-12527. https://doi.org/10.1128/jvi.01294-14
17. Cheng K, Cheng S, Chen W, Lin M, Chuang S, Cheng I, Sun C & Chou C: Thiopurine analogs and mycophenolic acid synergistically inhibit the papain-like protease of Middle East respiratory syndrome coronavirus. Antiviral Research 2015; 115: 9-16. https://doi.org/10.1016/j.antiviral.2014.12.011
18. Rahbar Saadat Y, Hosseiniyan Khatibi SM, Zununi Vahed S & Ardalan M: (2021). Host Serine Proteases: A Potential Targeted Therapy for COVID-19 and Influenza. Frontiers in Molecular Biosciences, 8. https://doi.org/10.3389/fmolb.2021.725528
19. Gierer S, Bertram S, Kaup F, Wrensch F, Heurich A, Kramer-Kuhl A, Welsch K, Winkler M, Meyer B, Drosten C, Dittmer U, Von Hahn T, Simmons G, Hofmann H & Pohlmann S: The spike protein of the emerging Betacoronavirus EMC uses a novel coronavirus receptor for entry, can be activated by TMPRSS2, and is targeted by neutralizing antibodies. Journal of Virology 2013; 87(10): 5502-5511. https://doi.org/10.1128/jvi.00128-13
20. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler, T., Erichsen, S., Schiergens, T. S., Herrler, G., Wu NH, Nitsche A, Müller MA, Drosten C & Pöhlmann S: SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell 2020; 181(2): 271–280.e8. https://doi.org/10.1016/j.cell.2020.02.052
21. Matsuyama S, Nagata N, Shirato K, Kawase M, Takeda M & Taguchi F: Efficient activation of the severe acute respiratory syndrome coronavirus spike protein by the Transmembrane protease TMPRSS2. Journal of Virology 2010; 84(24): 12658-12664. https://doi.org/10.1128/jvi.01542-10
22. Schönfelder K, Breuckmann K, Elsner C, Dittmer U, Fistera D, Herbstreit F, Risse J, Schmidt K, Sutharsan S, Taube C, Jöckel KH, Siffert W, Kribben A & Möhlendick, B: 2021 Transmembrane serine protease 2 Polymorphisms and Susceptibility to Severe Acute Respiratory Syndrome Coronavirus Type 2 Infection: A German Case-Control Study. Frontiers in Genetics, 12. https://doi.org/10.3389/fgene.2021.667231
23. Hosoya M, Matsuyama S, Bab M, Suzuki H & Shigeta S: Effects of protease inhibitors on replication of various myxoviruses. Antimicrobial Agents and Chemotherapy 1992; 36(7): 1432-1436. https://doi.org/10.1128/aac.36.7.1432
24. Kawase M, Shirato K, Van der Hoek L, Taguchi F & Matsuyama S: Simultaneous treatment of human bronchial epithelial cells with serine and cysteine protease inhibitors prevents severe acute respiratory syndrome coronavirus entry. Journal of Virology 2012; 86(12): 6537-6545. https://doi.org/10.1128/jvi.00094-12
25. Shirato K, Kawase M & Matsuyama S: Middle East respiratory syndrome coronavirus infection mediated by the Transmembrane serine protease TMPRSS2. Journal of Virology 2013; 87(23): 12552-12561. https://doi.org/10.1128/jvi.01890-13
26. Zhou Y, Vedantham P, Lu K, Agudelo J, Carrion R, Nunneley JW, Barnard D, Pöhlmann S, McKerrow JH, Renslo AR & Simmons G: Protease inhibitors targeting coronavirus and filovirus entry. Antiviral Research 2015; 116: 76-84. https://doi.org/10.1016/j.antiviral.2015.01.011
27. Xiang R, Yu Z, Wang Y, Wang L, Huo S, Li Y, Liang R, Hao Q, Ying T, Gao Y, Yu F & Jiang S: Recent advances

in developing small-molecule inhibitors against SARS-CoV-2. Acta Pharmaceutica Sinica B 2022; 12(4): 1591–1623. https://doi.org/10.1016/j.apsb.2021.06.016

28. Chandran K: Endosomal proteolysis of the Ebola virus glycoprotein is necessary for infection. Science 2005; 308(5728): 1643-1645. https://doi.org/10.1126/science.1110656
29. Gupta SC, Prasad S, Tyagi AK, Kunnumakkara AB & Aggarwal BB: Neem (Azadirachta indica): An Indian traditional Panacea with modern molecular basis. Phytomedicine 2017; 34: 14-20. https://doi.org/10.1016/j.phymed.2017.07.001
30. Schoonheim-Klein M, Ong TS & Loos BG: Implementation process of all periodontal competences and assessments as proposed in the 2010 European consensus meeting into the existing local undergraduate curriculum. European Journal of Dental Education 2015; 20(4): 197-205. https://doi.org/10.1111/eje.12161
31. Koumaglo K, Akogo Y, Dotse K, Gbeassor M & Arnason JT: Evaluation de la teneur en gédunine dans differents organes d'Azadirachta indica A. Juss. Comptes Rendus Chimie 2004; 7(10-11): 1047-1050. https://doi.org/10.1016/j.crci.2003.12.032
32. Hitotsuyanagi Y, Mitsui K, Fukaya H & Takeya K: Fluorinated and rearranged gedunin derivatives. Phytochemistry Letters 2012; 5(3): 486-489.
33. Asif M, Saleem M, Saadullah M, Yaseen HS & Al Zarzour R: COVID-19 and therapy with essential oils having antiviral, anti-inflammatory, and immunomodulatory properties. Inflammopharmacology 2020; 28(5): 1153-1161. https://doi.org/10.1007/s10787-020-00744-0
34. Ma L & Yao L: Antiviral effects of plant-derived essential oils and their components: An updated review. Molecules 2020; 25(11): 2627.
35. Goodger JQ, Senaratne SL, Nicolle D & Woodrow IE: Correction: Foliar essential oil glands of eucalyptus subgenus eucalyptus (Myrtaceae) are a rich source of flavonoids and related non-volatile constituents. PLOS ONE 2016; 11(5): e0155568. https://doi.org/10.1371/journal. pone.0155568
36. Dyall J, Gross R, Kindrachuk J, Johnson RF, Olinger GG, Hensley LE, Frieman MB & Jahrling PB: Middle East Respiratory Syndrome and Severe Acute Respiratory Syndrome: Current Therapeutic Options and Potential Targets for Novel Therapies. Drugs 2017; 77(18): 1935–1966. https://doi.org/10.1007/s40265-017-0830-1

**How to cite this article:**
Ganguly N, Roy S and Chakraborty T: Papain like protease: a potential target for middle-east respiratory syndrome coronavirus (mers-cov): the battle continues. Int J Pharm Sci & Res 2022; 13(11): 4476-84. doi: 10.13040/IJPSR.0975-8232.13(11).4476-84.