Identification of Promising SARS-CoV-2 Main Protease (Mpro) and Spike Protein Inhibitors From Edible Mushroom: A Computational Approach

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Abstract

Coronaviruses infect lungs leading to death due to asphyxiation. SARS-CoV-2 is treated by targeting symptoms, repurposing drugs and plasma therapy. Several synthetic drugs are being prescribed that cause major side effects in liver, kidney and heart. Therefore new compounds with low toxicity must be investigated. We have identified antiviral compounds like Eritadenine, Gallic Acid, Ergosterol Peroxide and Pleuran from various edible mushrooms such as *Lentinula edodes*, *Agaricus bisporus*, *Pleutorus ostreatus* and *Hericium erinaceus* with evidence of literature review. The docking and simulation studies with the targets of SARS-CoV-2 such as Main Protease (M Pro) and Spike Protein were highly successful. *In silico* ADMET studies further proved that these compounds are druggable with low toxicity. These compounds have potential to prevent the cellular entry to prohibit assembly of new viruses inside the cell. But further studies are required to substantiate their bioactivity claim by *in vitro* and *in vivo* assay methods.

1. INTRODUCTION:

Nidovirales is the order from which the family of coronaviridae are classified they are further subdivided into four genera namely, α, β, γ and δ. The virus SARS-CoV-2 which has caused global pandemic and havoc is of the β genera of the coronaviridae family, which has four protein constructs where its frame is made of Nucleocapsid protein (N) which encompasses the single stranded viral RNA (ssRNA), Membrane protein (M), Envelope protein (E) and spike protein (S) [1]. These proteins are replicated and multiple copies of the virus are produced within the human cell as per the well-known mechanism as described: The spike protein of the SARS-CoV-2 virus attaches to the Angiotensin Converting Enzyme-2 (ACE-2) and with host factors as Cell Surface Serine Protease (TMPRSS-2) and enters the cell resulting in the release of the viral contents to the cell cytoplasm in which the viral ssRNA is spontaneously translated as individual non-structural proteins which act as regulatory proteins along with protective vesicles which contain genomic RNA and subgenomic mRNA for viral replication and transcription. And the resultant structural proteins move towards the endoplasmic reticulum which in-turn produces newly replicated viral genomic RNA and the viral structural construct when transporting through the Endoplasmic Reticulum to Golgi Intermediate Compartment (ERGIC) and the newly made viral constructs are released out of the cell via exocytosis[2].

SARS-CoV-2 infection causes COVID-19 which is an upper respiratory infection emerged from Wuhan, China also it spread as a pandemic leading to more than 208 million cases, 4.3 million deaths and 186 million recoveries worldwide [3].

The production of more number of viral particles then results in the increased infection and lysis of the alveolar epithelial cells, Type I and II pneumocytes following cytokine storm and inflammatory reaction in the lungs thereby leading to Acute Respiratory Distress Syndrome (ARDS). The recoveries from COVID-19 were made possible through treatment of symptoms, repurposing drugs, convalescent plasma therapy, and artificial oxygen supply to the patients. SARS-CoV-2 mainly causes damage in the lungs due to high concentration of ACE-2 receptors and presents drastic effects in the advanced stages of the disease progression as shock, ARDS, hypoxemia, coagulation defects, encephalopathy, acute kidney injury and heart failure resulting to the death of the affected person. The patients with symptoms are diagnosed with Reverse Transcriptase Polymerase Chain Reaction (RT-PCR), Basic blood work, Chest X ray, High Resolution Computer Tomography Chest, Serology and classified into five groups based on their severity of infection which are: Asymptomatic or Pre-symptomatic infection, Mild infection, Moderate infection, severe infection and Critical infection [4].

Treatments proposed according to the infection rate are: Mild infection the treatment for the symptoms is given and for Moderate infection treatment of symptoms is given individually or along with antibacterial medication only if a bacterial infection can be seen. For treatment with severe infection and critical infection the supplemental oxygen or non-invasive ventilation along with treatment of symptoms and interleukin 6 blockers (IL-6 Blockers), Ivermetcin, Hydroxychloroquine, Lopinavir or Ritonavir, Remdesivir and systemic corticosteroids are given according to the systemic evaluation or national health guidelines. However, these drugs are advised to be used with extreme caution as it may cause severe side effects and permanent damage the major organs such as kidney, liver and heart [5].

Due to these reasons natural product scientists have turned to nature for assistance to eradicate this dreadful disease. Natural products can have potential therapeutic use for the emerging diseases and also enable to eliminate them, it has been reported that 234 drugs for various diseases have been isolated and were approved from natural products for treating various diseases during the timeline of 1981 to 2014. About antiviral 64 small molecules and 138 antiviral drugs have been approved from the same time period [6]. Mushrooms belong to the separate Kingdom of Fungi and Phylum Basidiomycota. They are also an excellent source of natural products and antivirals which expresses antiviral activity in both enveloped and non-enveloped RNA viruses by mechanisms which can prohibit the attachment and entry, replication, assembly and release of the same. Such antiviral activity is possible through compounds which are in different forms of like analogues, derivatives, fraction or direct polysaccharides, triterpenoids, proteoglycan, proteins, lectins, polyphenols and enzymes[7]. A recent study involving *in silico* approach for Main Protease (MPro) inhibition through 36 compounds with edible and medicinal mushrooms with anti-HIV activity [8] but they fail to investigate and recognize compounds with wide antiviral activity especially in the light of compounds which prohibit or totally eradicate the upper respiratory viruses, they are only single targeted and they are not validated by molecular dynamics which predicts the stability of the docked molecule.

In this study our focus is on edible mushrooms which accessible in super markets such as *Lentinula edodes* , *Agaricus bisporus* ,*Pleutorus ostreatus* and *Hericium erinaceus* . And we target their main compounds such as Eritadenine, Gallic acid, Pleuran and Ergosterol peroxide, these main compounds were chosen especially due to their ability to inhibit upper respiratory infections which are specifically caused by viruses from known existing literature. According to the literature, Eritadenine is a main compound of *L. edodes*and has been synthesized synthetically and was found to have antiviral activity against Poxviruses, Paramyxovirus, Rhabdovirus and Reovirus[9, 10]. *A. bisporus* has Gallic acid as its main component was reported to have activity against early viral entry of hepatitis C, Herpes Simplex Virus-1 (HSV-1), Human Immunodeficiency Virus-1 (HIV-1) and Influenza-A virus [11]. Pleuran a main compound of *P. ostreatus* was studied to have activity against Influenza –A virus, provided cellular immune response and respiratory tract infections in athletes [12, 13, 14]. Ergosterol Peroxide a main component from *H. erinaceus* was examined to have antiviral

activity against porcine delta coronavirus and other upper respiratory infections [15, 16, 17].

Due to these molecules has such antiviral activities, we target them against the Main Protease (Mpro) and the spike protein (S) of the SARS-CoV-2 virus in order to inhibit these targets and prohibit the entry and replication of the virus within the cell by using molecular docking tools and molecular dynamics simulation studies. We have also undertaken studies to assess the target accuracy and pharmacokinetic parameters by *in silico* analysis and compare them to the existing antivirals proposed against the SARS-CoV-2 with the targets mentioned in the literature.

2. MATERIALS AND METHODS:

2.1 DOCKING OF ERITADENINE, GALLIC ACID, ERGOSTEROL PEROXIDE AND PLEURAN ON SARS-COV-2 MAIN PROTEASE AND SPIKE PROTEIN:

2.1.1 LIGAND PREPARATION:

The ligands of interest Eritadenine (PubChem CID 159961), Gallic Acid (PubChem CID 370), Ergosterol (PubChem CID 5351516) were obtained from PubChem database [18]. Pleuran was designed using GlycamWeb webserver [19]. Molecules in 2D formats were converted to 3D mol2 format and protonated using OpenBabel[20].

2.1.2 RECEPTOR PREPARTION:

3D crystal structure of SARS-CoV-2 Main protease (PDB ID: 6LU7) and SARS-CoV-2 Spike protein (PDB ID: 6YOR) were obtained from RCSB PDB database [21]. Protein chains with appropriate chain identifiers for the protein were selected and retained other ligands present in the molecule were removed. Webservers 3DLigandSite[22] and RaptorX binding [23] was used to identify the active site in the protein given in the figure 1. The protein receptor was processed using the Dock Prep module of UCSF Chimera using AMBER parm99 partial charges.

2.1.3 DOCKING PARAMETERS:

All docking calculations were performed using Dock6 version (6.6). Active sites were identified and prepared by selecting spheres at a distance of 1–5 Å from the binding regions. Grid boxes with 5 Å and 15 Å margins were generated around the selected spheres for small and large ligands respectively. A standard flexible docking protocol with the maximum number of anchor orientations attempted was set to 1,000 and all 100,000 orientations was employed to sample internal degrees of freedom of the ligands. Grid scoring based on the intermolecular non-bonded terms (viz. van der Waals for steric and electrostatic interactions) was computed using AMBER force field ff99 to identify the best orientation of each ligand. Finally, the best-scored conformer was retained for scrutiny and further refinement and Amber-based scoring. All protein ligand interactions were detected and visualized using by the PLIP webserver [24] and LigPlot [25].

2.2 MOLECULAR DYNAMICS OF ERITADENINE, GALLIC ACID, ERGOSTEROL PEROXIDE AND PLEURAN ON SARS-COV-2 MAIN PROTEASE AND SPIKE PROTEIN:

The docked structures were used for molecular dynamics simulations using AMBER force field and TIP3P water model in GROMACS 2019. The parameters for docked ligands were prepared using Acypype. A vacuum energy minimization step was performed by employing the steepest descent algorithm for 50,000 steps and conjugate gradient minimization was performed for 5000 steps. Sodium (Na+) and chloride (Cl-) counter ions were added at a physiological concentration and the boundaries of the dodecahedron box were adjusted to 10Å in a unit cell. The complexes were again subjected to energy minimization for 50,000 steps for stabilization. Position restraint and unrestrained dynamics simulations and Parrinello- Rahman pressure- coupling bath was used for equilibration step of the system at temperature 300K using a Nose-Hoover thermostat under pressure of 1 atm for 100 ps. For simulations, all bonds were constrained using the LINCS algorithm. The Particle- Mesh Ewald method was used for electrostatic calculations by maintaining a cut-off distance of 1.4nm for coulomb and Van der Waals interactions, and finally, the production run for

10 ns was performed. GROMACS built-in functions were utilized to calculate RMSD, RMSF and Hydrogen bonds. Excel was used to produce graphs of the results.

2.3 ADME PREDICTION:

The prediction of Adsorption, Distribution, Metabolism and Excretion was done by obtaining the Simplified Molecular-Input Line-Entry System (SMILES) format of the ligands Eritadenine, Gallic acid, Ergosterol peroxide and Pleuran was taken from PubChem database and was provided as an input to the SWISS-ADMET website which analyses the lipophilicity, water solubility, druglikeness and medicinal chemistry of the compound submitted [26].

2.4 TARGET PREDICTION:

The target prediction of the compounds Eritadenine, Gallic acid, Ergosterol peroxide and Pleuran was analyzed by entering the SMILES and by choosing the *Homo sapiens* button to the Swiss target prediction website which in turn provides info about possible side effects or cross reactivity caused due to the effect of molecules in the physiological or genetic effect in the organs of the human body[27].

2.5 TOXICITY PREDICTION:

The toxicity of the ligands eritadenine, gallic acid, ergosterol peroxide and pleuran was analyzed by incorporating the SMILES data of these compounds from PubChem database to the ADMET Lab2.0 website and Toxicity option was selected and analyzed. ADMET Lab2.0 examines the ligand's toxicity among systemic organs in the human body, TOX21 Pathway and Toxicophore property rules ranging from acute toxicity, genotoxic carcinogenicity, non- genotoxic carcinogenicity, skin sensitization, aquatic toxicity, non-biodegradability and sureChEMBL Rule[28].

3. RESULTS:

3.1 DOCKING:

The docking analysis of the compounds docked with Mpro and spike protein of SARS-CoV-2 yielded negative values for free energy respectively they were as follows. The grid box expressed negative values for free energies observed in Mpro they are -1.068 KJ/mol was observed for Eritadenine, -3.167 KJ/mol for Gallic acid, -0.420 KJ/mol for Ergosterol peroxide and -5.035 KJ/mol for Pleuran. Similarly, the values of free energies observed in Spike Protein were -0.783 KJ/mol, -2.140 KJ/mol, 1.027 KJ/mol and -5.241 KJ/mol respectively for the above four compounds. The binding confirmations of the docked protein of Mpro are hydrogen bonds and salt bridge formation for Eritadenine, hydrogen bonds were formed in Gallic acid, hydrogen bond and hydrophobic interactions were developed in Ergosterol peroxide and hydrogen bonds were formed with the molecule of Pleuran. Correspondingly, the binding confirmations of the docked protein of spike protein are hydrogen bonds were formed in the ligands of eritadenine, gallic acid and pleuran; the hydrogen bonds and hydrophobic interactions are developed in the compound Ergosterol peroxide.

3.1.1 ERITADENINE:

3.1.1.1 DOCKED POSE AND PROTEIN LIGAND INTERACTION:

The docked pose of the ligand eritadenine along with protein ligand interaction is given in the figures 2a, 2a1, 2b and 2b1. And protein ligand interactions are tabulated in Table-1.

3.1.2 GALLIC ACID:

3.1.2.1 DOCKED POSE AND PROTEIN LIGAND INTERACTION:

The docked pose of the ligand gallic acid along with protein ligand interaction is given in the figure 2c, 2c1, 2d and 2d1. And protein ligand interactions are tabulated in Table-2.

3.1.3 ERGOSTEROL PEROXIDE:

3.1.3.1 DOCKED POSE AND PROTEIN LIGAND INTERACTION:

The docked pose of the ligand ergosterol peroxide along with protein ligand interaction is given in the figure 2e, 2e1, 2f and 2f1. And protein ligand interactions are tabulated in Table-3.

3.1.4 PLEURAN

3.1.4.1 DOCKED POSE AND PROTEIN LIGAND INTERACTION:

The docked pose of the ligand pleuran along with protein ligand interaction is given in the figure 2g, 2g1, 2h and 2h1. And protein ligand interactions are tabulated in Table-4.

3.2 MOLECULAR DYNAMICS:

3.2.1 ROOT MEAN SQUARE DEVIATION (RMSD) AND ROOT MEAN SQUARE FLUCTUATION (RMSF):

The RMSD, RMSF produced from the docked compounds with targets Main Protease (MPro) and Spike Protein (S) are given as Figure 3a, 3b, 4a and 4b as follows:

3.2.2 HYDROGEN BONDING:

The hydrogen bonding which was formed during the molecular dynamics simulation was tabulated in Tables 5, 6, 7 and 8 for the ligand-protein bound complexes of Main Protease (MPro) and in Tables 9, 10, 11, and 12 for the docked complexes of Spike Protein (S).

3.3 ADME PREDICTION:

3.3.1 ERITADENINE:

The results which appeared after the submission of Eritadenine molecule in the SWISS-ADME database showed that the molecule, Eritadenine 18 heavy atoms, 7 hydrogen bond acceptors, 4 hydrogen bond donors and 147.38 $Å^2$ of Topological Polar Surface Area (TPSA). The lipophilicity parameters are optimal in nature. The estimated solubility of Eritadenine is -0.36 which points it to be in the very soluble class, *in silico* prediction of aqueous solubility with response to TPSA is about -0.75 which categorizes to very soluble class as well, water solubility by fragmental method puts the compound to very soluble class with assigning the value of -0.29. The pharmacokinetics properties determined that Eritadenine has low gastrointestinal absorption, non-blood brain barrier permeant, does not act as P-gp substrate and does not inhibit factors such as CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4. The molecule is skin permeable with log Kp rate about -9.14cm/s. And the molecule obeys Lipinski's rule of five and Muegge rules of druglikeness and also obeys Pan-Assay Interference Compounds (PAINS), Brenk, Leadlikeness rules of medicinal chemistry.

3.3.2 GALLIC ACID:

The results of Gallic acid contains 12 heavy atoms, 5 hydrogen bond acceptors, 4 hydrogen bond donors and TPSA is found to be 97.99 $Å^2$. The lipophilicity parameters were very optimal for the ligand. And water solubility parameters were soluble in nature as predicted values are -1.64, -2.34 and -0.04 with respect to estimated solubility (Log S-ESOL), *in silico* prediction of solubility with reference to TPSA (Log S- Ali) and water solubility by fragmental method (Log S- SILICOS-IT) which gives an inference of very soluble, soluble and soluble classes according to the values projected. Estimated pharmacokinetic data projects that the Gallic acid is highly absorbable gastrointestinally, non- blood brain barrier permeant, non-P-gp substrate, does not inhibit potential cytochrome P450 enzymes as mentioned above, also has skin permeability rate of -6.84 cm/s. And the compound obeys druglikeness rules such as Lipinski's, Veber and Egan. But the molecule doesn't obey any rules of medicinal chemistry.

3.3.3 ERGOSTEROL PEROXIDE:

The ligand Ergosterol peroxide has 31 heavy atoms, 3 hydrogen bond acceptors and 1 hydrogen bond donor and TPSA is about 38.69 $Å^2$. They are highly lipophilic in nature. The water solubility parameters are with predicted values of -6.46 for Log S- ESOL which is poorly soluble, -7.33 for Log S-Ali which is poorly soluble and -4.51 for Log S- SILICOS-IT which is moderately soluble. The pharmacokinetic estimated data exclaims

that the molecule is highly absorbable gastrointestinally, non- blood brain barrier permeant, non-P-gp substrate, does not inhibit potential cytochrome P450 enzymes such as CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4. It also has skin permeability rate of -4.15 cm/s. Ergosterol peroxide obeys druglikeness rules of Lipinski and Veber. There's no PAINS violation in medicinal chemistry rules.

3.3.4 PLEURAN:

Pleuran has 41 hydrogen bond acceptors, 26 hydrogen bond donors and it has TPSA about 664.43 $Å^2$. They are highly lipophilic and less hydrophilic in nature. The pharmacokinetic data shows that the molecule is a non P-gp substrate, highly absorbable gastrointestinally, non- blood brain barrier permeant and does not inhibit potential cytochrome P450 enzymes such as CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4. The molecule obeys PAINS and Brenk medicinal chemistry rules.

3.4 TARGET PREDICTION:

After the analysis the target prediction of the ligands Eritadenine, Gallic acid, Ergosterol peroxide and Pleuran were given accordingly as the pie charts for top 15 hits for Eritadenine, all estimates for Gallic acid, top 15 hits for Ergosterol peroxide and all estimates for Pleuran (Figures 10-13) and the respective log output table with known protein complexes are given along with probability score with each binding to estimated protein complexes, the output table of results for the compounds are given in the supplementary file. As projected by the output file the compounds may reach the target with high accuracy to which it is targeted to. Whereas the target activities of Eritadenine, Gallic acid, Ergosterol peroxide and Pleuran are sought in literature and their validation is discussed in the discussion section to provide the fact of target accuracy.

3.5 TOXICITY PREDICTION:

3.5.1 SYSTEMIC TOXICITY:

The systemic toxicity properties of the ligands eritadenine, gallic acid, ergosterol peroxide and pleuran are tabulated in Table-13.

3.5.2 TOX21 PATHWAY:

The properties of the ligands which are studied for TOX21 pathway are given in the Table-14 as follows:

3.5.3 TOXICOPHORE RULES:

The toxicophore rules of the ligands of interest are tabulated in Table-15.

4. DISCUSSION:

Mushrooms are found to possess antiviral activity also they are reviewed to have therapeutic effect against inflammation and pneumonic superinfection against recent global pandemic of COVID-19[29]. We focused on the edible mushrooms which are commonly available in the markets rather than deep search in the forest and examined the main compounds which has broad activity against upper respiratory viruses via literature review and found out four compounds fall into the above categories mentioned. And targets Main Protease (Mpro) and Spike Protein were chosen to see the inhibitory effect of the ligands on the pre-entry stage and protein cleavage stage in the life cycle of the SARS-CoV-2 virus leading to disruption and decrease of the viral load *in vivo* . And also due to recent outbreak of different variants, whose mutation took place in the spike protein of the SARS-CoV-2 virus, thankfully they carry similar inhibition sites and the most mutation took place in the furin loops and so the spike protein was chosen as the target of interest [30].

The ligands were successfully docked against inhibition site of Main Protease with following docking scores -1.068 Kcal/ mol for Eritadenine, -3.617 Kcal/ mol for Gallic Acid, -0.420 Kcal/ mol for Ergosterol Peroxide and -5.035 Kcal/ mol for Pleuran respectively. Similarly, the ligands also docked successfully against Spike Protein with docking scores such as -0.783 Kcal/ mol for Eritadenine, -2.140 Kcal/ mol for Gallic Acid, 1.027 Kcal/ mol for Ergosterol Peroxide and -5.241 Kcal/ mol for Pleuran respectively. And the docked complexes

exhibited convergence within 10 ns of molecular dynamics whose RMSD values ranged from 1Å to 2.5Å for all ligands which has been bound to Main protease and RMSF values ranged from 5Å to 6.5Å. Similarly, RMSD values ranged from 1Å to 2Å and also the RMSF values ranged from 0.5Å to 3.5Å respectively in the Spike Protein target, therefore all protein ligand complexes are stable in both targets according to results in RMSD analysis as well as in RMSF analysis. All complexes expressed strong hydrogen bonding throughout the time of molecular dynamics simulation through which it gives an inference that the protein ligand complexes are stable [31]. All other compounds except pleuran did express good druggability properties during *in silico* ADME analysis. Similarly, during target analysis the compounds except pleuran showed good targeting against protease enzymes. But in literature pleuran has been found to express good activity against protease enzymes and also effective in progressivity towards being a drug and also has immunomodulatory effect [32]. Based on toxicity all ligands expressed minimum toxicity levels except eritadenine but are mostly non-toxic to the important organs such as liver, heart and kidneys based on the simulation projected in the results.

When comparing these compounds with already repurposed drugs such as Lopinavir , Famciclovir, Drunavir, Amprenavir and N3 whose docking scores are -9.918 Kcal/ mol, -7.546 Kcal/ mol, -7.505 Kcal/ mol, -8.655 Kcal/ mol, -7.5 Kcal/ mol respectively. And the repurposed drugs which are mentioned above exhibit higher docking score than the compounds of interest [33, 34]. The ligand of interest has lesser docking score and has more significance. Therefore the compounds which were studied can be used for therapeutic purposes against COVID-19. We the authors also propose as the inhibition site of all spike proteins in variants of concern are similar, one particular drug or a compound which has been studied to have inhibitory effect can be used to treat SARS-CoV-2.

5. CONCLUSION:

The small compound eritadenine has exhibited excellent properties against all aforementioned categories of target, toxicity and ADME so it could be propagated as a drug to combat the SARS-CoV-2. Whereas the other compounds also possessed such immunomodulatory properties as mentioned in the existing literature mentioned above. However, this work is foundational, but the results are promising and further studies of these ligands *in vitro* and *in vivo* should be studied to decipher the therapeutic role of these compounds.

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7. TABLES:

Compound	Protein	No of H Bonds	Residue Receptor	Ligand	Bond Length (Å)	Docking Score (Kcal/ mol)
Eritadenine	Mpro	3	Gly 143 (H) His 164 (H) His 41 (H)	N2 O2 O2	3.05 2.85 4.39	-1.068 -1.068 -1.068
	Spike	5	Asn 343 (H) Asn 343 (H) Val 367 (H) Ser 371 (H) Trp 436 (H)	O.co2 O2 O2 O3 N2	3.03 2.43 3.23 3.32 3.45	-0.783 -0.783 -0.783 -0.783 -0.783

Table-1. Interaction of SARS-CoV-2 Mpro and Spike Protein with Eritadenine Ligand.

Compound	Protein	No of H Bonds	Residue Receptor	Ligand	Bond Length (Å)	Docking Score (Kcal/ mol)
Gallic Acid	Mpro	7	Leu 141 (H) Leu 141 (H) Gly 143 (H) Ser 144 (H) Cys 145 (H) Glu 166 (H) Gln 189 (H)	O2 O2 O2 O2 O2 O.co2 O2	2.12 1.92 3.11 2.4 2.81 2.23 2.69	-3.617 -3.617 -3.617 -3.617 -3.617 -3.617 -3.617
	Spike	4	Asn 343 (H) Asn 343 (H) Ser 371 (H) Phe 343 (H)	O2 O2 O.co2 O2	2.33 2.06 2.44 3.36	-2.140 -2.140 -2.140 -2.140

Table-2. Interaction of SARS-CoV-2 Mpro and Spike Protein with Gallic Acid Ligand.

Compound	Protein	No of H Bonds	Residue Receptor	Ligand	Bond Length (Å)	Docking Score (Kcal/ mol)
Ergosterol Peroxide	Mpro	2	Asn 142 (H) Glu 166 (H)	O3 O2	3.22 3.68	-0.420 -0.420
	Spike	4	Ser 373 (H) Val 367 (H) Asn 370 (H) Phe 374 (H)	O3 O2 O2 O2	2.71 3.83 3.96 3.96	1.027 1.027 1.027 1.027

Table-3. Interaction of SARS-CoV-2 Mpro and Spike Protein with Ergosterol Peroxide Ligand.

Compound	Protein	No of H Bonds	Residue Receptor	Ligand	Bond Length (Å)	Docking Score (Kcal/ mol)
Pleuran	Mpro	12	Pro 168 (H) Thr 169 (H) Thr 26 (H) Thr 26 (H) Gly 138 (H) Gly 170 (H) Arg 188 (H) Gln 189 (H) Glu 166 (H) Glu 166 (H) His 41 (H) His 142 (H)	O2 O2 O3 O2 O2 O2 O2 O3 O2 O3 O2 O2	3.47 2.98 3.1 2.47 3.51 2.95 2.72 3.15 2.37 2.26 4.85 5.30	-5.035 -5.035 -5.035 -5.035 -5.035 -5.035 -5.035 -5.035 -5.035 -5.035 -5.035 -5.035

Compound	Protein	No of H Bonds	Residue Receptor	Ligand	Bond Length (Å)	Docking Score (Kcal/ mol)
	Spike	19	Thr 376 (H)	O3 O3 O3 O2	2.13 2 2.14	-5.241 -5.241
			Thr 376 (H)	O3 O3 O3 O3	2.88 2.74 2.73	-5.241 -5.241
			Thr 376 (H)	O2 O3 O3 O3	2.83 2.87 3.15	-5.241 -5.241
			Gly 404 (H)	N2 O3 O2 O3	3.17 1.92 1.91	-5.241 -5.241
			Arg 408 (H)	O2 O2 O2	2.9 2.83 2.65	-5.241 -5.241
			Arg 408 (H)		2.54 3.13 2.58	-5.241 -5.241
			Arg 408 (H)		1.92	-5.241 -5.241
			Asn 343 (H)			-5.241 -5.241
			Ser 373 (H)			-5.241 -5.241
			Ser 375 (H)			-5.241
			Tyr 508 (H)			
			Tyr 508 (H)			
			Trp 436 (H)			
			Asn 440 (H)			
			Ala 372 (H)			
			Asn 349 (H)			
			Ser 375 (H)			
			Ser 373 (H)			
			Asn 440 (H)			

Table-4. Interaction of SARS-CoV-2 Mpro and Spike Protein with Pleuran Ligand.

Donor	**Hydrogen**	**Acceptor**	**% Existence**
UNK307O11	UNK307H24	ASP187O	98.402
UNK307N7	UNK307H18	HIS164O	52.647
GLN189NE2	GLN189HE21	UNK307O15	6.693
HIS41NE2	HIS41HE2	UNK307N7	3.297

Table-5. Dominant Hydrogen Bond Donors and Acceptors in MD Simulation Between Mpro and Eritadenine of SARS-CoV-2.

Donor	**Hydrogen**	**Acceptor**	**% Existence**
LIG307O1	LIG307H3	HIS164O	50.05
HIS163NE2	HIS163HE2	LIG307O2	44.356
LIG307O	LIG307H2	SER144OG	36.863
HIS163NE2	HIS163HE2	LIG307O	24.975
GLU166N	GLU166H	LIG307O3	10.49
GLY143N	GLY143H	LIG307O1	9.59
LIG307O	LIG307H2	HIS164O	8.691
LIG307O2	LIG307H4	PHE140O	3.596
SER144N	SER144H	LIG307O	3.397
ASN142ND2	ASN142HD21	LIG307O4	3.297
ASN142ND2	ASN142HD21	LIG307O3	2.098
LIG307O2	LIG307H4	GLU166OE2	1.798
GLU166N	GLU166H	LIG307O4	1.099

Donor	Hydrogen	Acceptor	% Existence
LIG307O	LIG307H2	LEU141O	1.099

Table-6. Dominant Hydrogen Bond Donors and Acceptors In MD Simulation Between Mpro and Gallic Acid of SARS-CoV-2.

Donor	Hydrogen	Acceptor	% Existence
SER46N	SER46H	LIG307O	27.373
SER46N	SER46H	LIG307O1	14.985
THR45OG1	THR45HG1	LIG307O2	7.293
THR45OG1	THR45HG1	LIG307O1	5.095
SER46OG	SER46HG	LIG307O1	3.996
SER46OG	SER46HG	LIG307O	3.197
THR45OG1	THR45HG1	LIG307O	1.099

Table-7. Dominant Hydrogen Bond Donors and Acceptors In MD Simulation Between Mpro and Ergosterol Peroxide of SARS-CoV-2.

Donor	Hydrogen	Acceptor	% Existence
GLU166N	GLU166H	LIG307O7	46.254
LIG307O27	LIG307H74	THR26O	34.965
LIG307O37	LIG307H6O	GLU166OE1	30.969
GLU166N	GLU166H	LIG307O14	29.271
GLY143N	GLY143H	LIG307O41	28.472
GLN192N	GLN192H	LIG307O2	26.873
LIG307O26	LIG307H73	HIS41NE2	23.576
LIG307O41	LIG307H78	ASN142OD1	20.579
CYS145N	CYS145H	LIG307O41	19.081
LIG307O37	LIG307H6O	PHE140O	17.283
GLN189NE2	GLN189HE21	LIG307O26	17.083
LIG307O2	LIG307H2O	GLN192O	15.984
LIG307O1	LIG307HO1	GLN192O	15.185
GLN192N	GLN192H	LIG307O1	14.585
LIG307O11	LIG307H46	PRO168O	14.585
LIG307O38	LIG307H75	GLY170O	13.686
LIG307O9	LIG307H44	GLU166OE1	13.287
PHE140N	PHE140H	LIG307O38	11.688
LIG307O40	LIG307H77	HIS164O	10.789
HIS41NE2	HIS41HE2	LIG307O19	10.49
HIS172NE2	HIS172HE2	LIG307O37	10.19
LIG307O38	LIG307H75	PHE140O	10.09
LIG307O21	LIG307H68	ASN142OD1	7.093
LIG307O7	LIG307H42	GLU166O	6.593
LIG307O10	LIG307H45	PRO168O	6.494
HIS41NE2	HIS41HE2	LIG307O13	6.094
LIG307O37	LIG307H6O	GLU166OE2	4.995
LIG307O26	LIG307H73	HIS41ND1	4.695
LIG307O40	LIG307H77	ARG188O	4.296

Donor	Hydrogen	Acceptor	% Existence
LIG307O25	LIG307H72	PRO168O	4.096
LIG307O4	LIG307H4O	GLN189O	3.796
LIG307O21	LIG307H68	GLU166OE2	3.696
LIG307O11	LIG307H46	THR169O	3.596
LIG307O39	LIG307H76	GLY170O	3.197
GLN189NE2	GLN189HE21	LIG307O6	3.097
ASN142ND2	ASN142HD21	LIG307O41	2.797
GLN189NE2	GLN189HE21	LIG307O4	2.597
LIG307O3	LIG307H3O	GLN189O	2.398
LIG307O38	LIG307H75	THR169O	2.398
LIG307O1	LIG307HO1	GLU166O	2.298
LIG307O23	LIG307H70	PHE140O	2.298
ASN142ND2	ASN142HD21	LIG307O22	2.198
LIG307O15	LIG307H49	ASN142OD1	2.198
LIG307O13	LIG307H48	THR26O	1.998
THR26N	THR26H	LIG307O20	1.898
GLN189NE2	GLN189HE21	LIG307O40	1.898
LIG307O38	LIG307H75	GLU166OE1	1.898
GLY143N	GLY143H	LIG307O27	1.798
LIG307O22	LIG307H69	ASN142OD1	1.798
LYS137NZ	LYS137HZ1	LIG307O25	1.698
ASN142ND2	ASN142HD21	LIG307O37	1.698
LIG307O1	LIG307HO1	GLN189O	1.598
LIG307O39	LIG307H76	PRO168O	1.598
ASN142ND2	ASN142HD21	LIG307O15	1.499
ASN142ND2	ASN142HD21	LIG307O27	1.399
LIG307O20	LIG307H51	THR26O	1.399
ASN142ND2	ASN142HD21	LIG307O8	1.299
LIG307O4	LIG307H4O	GLN189OE1	1.199
LIG307O18	LIG307H50	THR169O	1.099
LIG307O41	LIG307H78	ASN119OD1	1.099

Table-8. Dominant Hydrogen Bond Donors and Acceptors In MD Simulation Between Mpro and Pleuran of SARS-CoV-2.

Donor	Hydrogen	Acceptor	% Existence
UNK529N7	UNK529H18	VAL367O	14.885
ASN343ND2	ASN343HD21	UNK529O15	8.591
UNK529N7	UNK529H18	PHE342O	8.192
PHE338N	PHE338H	UNK529N1	7.892
UNK529N7	UNK529H18	ASN343OD1	7.892
ASN343ND2	ASN343HD21	UNK529N3	7.193
SER373OG	SER373HG	UNK529N7	7.093
UNK529O15	UNK529H26	ASN343OD1	6.793
ASN343ND2	ASN343HD21	UNK529O11	5.894
ASN343ND2	ASN343HD21	UNK529N1	3.596
SER373OG	SER373HG	UNK529N1	3.596
UNK529O11	UNK529H24	ASN343OD1	3.397
TRP436NE1	TRP436HE1	UNK529N7	2.597

Donor	Hydrogen	Acceptor	% Existence
PHE338N	PHE338H	UNK529N7	2.298
SER371OG	SER371HG	UNK529O13	2.098
ASN343ND2	ASN343HD21	UNK529N7	1.998
SER371OG	SER371HG	UNK529O14	1.798
SER373OG	SER373HG	UNK529O13	1.499
SER373OG	SER373HG	UNK529O14	1.499
UNK529O11	UNK529H24	SER373O	1.299

Table-9. Dominant Hydrogen Bond Donors and Acceptors In MD Simulation Between Spike Protein and Eritadenine of SARS-CoV-2.

Donor	Hydrogen	Acceptor	% Existence
UNK529N7	UNK529H18	VAL367O	14.885
ASN343ND2	ASN343HD21	UNK529O15	8.591
UNK529N7	UNK529H18	PHE342O	8.192
PHE338N	PHE338H	UNK529N1	7.892
UNK529N7	UNK529H18	ASN343OD1	7.892
ASN343ND2	ASN343HD21	UNK529N3	7.193
SER373OG	SER373HG	UNK529N7	7.093
UNK529O15	UNK529H26	ASN343OD1	6.793
ASN343ND2	ASN343HD21	UNK529O11	5.894
ASN343ND2	ASN343HD21	UNK529N1	3.596
SER373OG	SER373HG	UNK529N1	3.596
UNK529O11	UNK529H24	ASN343OD1	3.397
TRP436NE1	TRP436HE1	UNK529N7	2.597
PHE338N	PHE338H	UNK529N7	2.298
SER371OG	SER371HG	UNK529O13	2.098
ASN343ND2	ASN343HD21	UNK529N7	1.998
SER371OG	SER371HG	UNK529O14	1.798
SER373OG	SER373HG	UNK529O13	1.499
SER373OG	SER373HG	UNK529O14	1.499
UNK529O11	UNK529H24	SER373O	1.299

Table-10. Dominant Hydrogen Bond Donors and Acceptors In MD Simulation Between Spike Protein and Gallic Acid of SARS-CoV-2.

Donor	Hydrogen	Acceptor	% Existence
SER373OG	SER373HG	LIG529O1	51.948
SER373OG	SER373HG	LIG529O	51.349
SER371OG	SER371HG	LIG529O	8.092
SER373OG	SER373HG	LIG529O2	4.396
SER371OG	SER371HG	LIG529O1	2.597

Table-11. Dominant Hydrogen Bond Donors and Acceptors In MD Simulation Between Spike Protein and Ergosterol Peroxide of SARS-CoV-2.

Donor	Hydrogen	Acceptor	% Existence
SER375OG	SER375HG	LIG529O5	91.309
THR376OG1	THR376HG1	LIG529O11	81.419
LIG529O4	LIG529H4O	ALA372O	76.723
LIG529O8	LIG529H43	GLN506OE1	71.628
LIG529O12	LIG529H47	ASN437O	71.429
TRP436NE1	TRP436HE1	LIG529O13	66.234
LIG529O20	LIG529H51	PHE342O	64.635
ARG509NH1	ARG509HH11	LIG529O20	62.637
LIG529O27	LIG529H74	ALA344O	48.152
LIG529O39	LIG529H76	GLY404O	45.654
LYS378NZ	LYS378HZ1	LIG529O18	34.466
GLN506NE2	GLN506HE21	LIG529O15	17.582
LIG529O15	LIG529H49	GLN506OE1	16.184
LIG529O7	LIG529H42	SER373O	12.388
TRP436NE1	TRP436HE1	LIG529O12	9.79
SER375OG	SER375HG	LIG529O1	9.391
ASN440ND2	ASN440HD21	LIG529O12	8.492
GLN506NE2	GLN506HE21	LIG529O22	8.192
LIG529O20	LIG529H51	ASN343O	7.592
LIG529O10	LIG529H45	SER375O	7.393
ASN439ND2	ASN439HD21	LIG529O15	6.893
LIG529O40	LIG529H77	ASN440OD1	6.893
ASN440ND2	ASN440HD21	LIG529O14	6.294
LIG529O3	LIG529H3O	ALA372O	6.094
LIG529O39	LIG529H76	ASP405OD1	5.894
LIG529O27	LIG529H74	ASN343OD1	5.894
ASN440ND2	ASN440HD21	LIG529O33	5.594
ARG408NH1	ARG408HH11	LIG529O39	4.695
TYR508OH	TYR508HH	LIG529O10	4.695
LIG529O12	LIG529H47	ASN440OD1	4.695
LIG529O27	LIG529H74	ASN343O	4.196
ALA344N	ALA344H	LIG529O27	3.896
LIG529O39	LIG529H76	ASP405O	3.696
ARG408N	ARG408H	LIG529O39	3.497
ASN437N	ASN437H	LIG529O6	3.197
LIG529O21	LIG529H68	ASN440OD1	3.097
ASN343ND2	ASN343HD21	LIG529O27	2.398
LIG529O8	LIG529H43	ASN437OD1	2.398
ASN439ND2	ASN439HD21	LIG529O8	2.298
LIG529O20	LIG529H51	ALA344O	1.998
LIG529O21	LIG529H68	ASN440ND2	1.898
LIG529O27	LIG529H74	PHE342O	1.798
LIG529O13	LIG529H48	PHE342O	1.698
ALA344N	ALA344H	LIG529O20	1.499
SER375OG	SER375HG	LIG529O6	1.399
ARG408NE	ARG408HE	LIG529O39	1.199
LIG529O27	LIG529H74	THR345OG1	1.199
LYS378NZ	LYS378HZ1	LIG529O25	1.099

Table-12. Dominant Hydrogen Bond Donors and Acceptors In MD Simulation Between Spike Protein and Pleuran of SARS-CoV-2.

Ligands	hERG Blockers ++	H-HT++	DILI++	AMES ++	ROAT ++	FDAMDD ++	SS ++	CARC++	EC++	EI ++
ERA +	- §	- §	- §	- §	- §	- §	- §	- §	- §	- §
GA +	- §	- §	+ §	-§	- §	- §	+ §	- §	+ §	+ §
ERPO +	- §	- §	- §	-§	+ §	+ §	- §	- §	- §	- §
PLEU +	- §	- §	- §	-§	- §	- §	+§	- §	- §	- §

Table-13. Systemic toxicity parameters of the ligands

+ Eritadenine (ERA), Ergosterol Peroxide (ERPO), Gallic Acid (GA), Pleuran (PLEU)

++ AMES Test, Carcinogenicity (CARC), Drug Induced Liver Injury (DILI), Eye corrosion (EC), Eye Irritation (EI), human Ether-a-go-go related potassium channel blockers (hERG), Human Hepatotoxicity (H-HT), Rat Oral Acute Toxicity (ROAT), Respiratory Toxicity (RT) and Skin Sensitization (SS).

SS Negative or no toxicity (-), Positive or in probability of being actively toxic (+).

Ligands	NR-AR ++	NR-AR- LBD ++	NR-AhR ++	NR- Aromatase++	NR- ER ++	NR-ER-LBD ++	NR-PP
ERA +	- §	- §	-§	- §	- §	- §	- §
GA +	- §	- §	+ §	- §	+ §	- §	- §
ERPO +	- §	- §	- §	+ §	- §	+ §	- §
PLEU +	+ §	+§	- §	+ §	+ §	+ §	- §

Table-14. Tox21 Pathway Parameters for the ligands

+ Eritadenine (ERA), Ergosterol Peroxide (ERPO), Gallic Acid (GA), Pleuran (PLEU).

++ Androgen Receptor (NR-AR), Androgen Receptor Ligand Binding Domain (NR- AR- ABD), Antioxidant Response Element (SR-ARE), Aryl Hydrogen Receptor (NR- AhR), Aromatase enzyme (NR-Aromatase), ATPase family AAA domain-containing protein 5 (SR-ATAD5), Estrogen Receptor (NR-ER), Estrogen Receptor Ligand Binding Domain (NR-ER-LBD), Heat Shock Factor Response Element (SR-HSE), Mitochondrial Membrane Potential (SR-MMP), Peroxisome Proliferator- Activated Receptor Gamma (NR-PPAR-γ) and p53 binding site (SR-p53).

§ Negative or no toxicity (-), Positive or in probability of being actively toxic (+).

Ligands	ATR++	GCR ++	NON-GCR ++	SSR++	AQTR ++	NONBIOR++	SChEMBLR++
ERA +	No Alerts	No Alerts	No Alerts	No Alerts	No Alerts	1 Alert	No Alerts
GA+	No Alerts	No Alerts	No Alerts	7 Alerts	No Alerts	1 Alert	1 Alert
ERPO +	No Alerts	No Alerts	No Alerts	No Alerts	1 Alert	No Alerts	1 Alert
PLEU+	No Alerts	No Alerts	No Alerts	No Alerts	2 Alerts	1 Alert	No Alerts

Table-15. Toxicophore Rules

[+] Eritadenine (ERA), Ergosterol Peroxide (ERPO), Gallic Acid (GA), Pleuran (PLEU)

[++] Acute Toxicity Rule (ATR), Aquatic Toxicity Rule (AQTR), Genotoxic Carcinogenicity Rule, Non Biodegradable Rule (NONBIOR), Non Genotoxic Carcinogenicity Rule (NON-GCR), SureChEMBL Rule (SChEMBLR), Skin Sensitization Rule.

8. FIGURE LEGENDS:

Figure_1	a- Main Protease (MPro) of SARS-CoV-2 with active residues in green. b- Spike Protein of SARS-CoV-2 with active residues in green.
Figure_2	a- Docked eritadenine in the pocket of Mpro of SARS-CoV-2 where the pocket is in greyish white and the ligand in Licorice. a1- Eritadenine interaction with Mpro in 2D structure is given in crimson, bond formation in green and amino acids to which they were formed in black and other amino acidic interaction in yellow. b- Docked eritadenine in the pocket of Spike Protein of SARS-CoV-2 where the pocket is in greyish white and the ligand in Licorice. b1- Eritadenine interaction with Spike Protein in 2D structure is given in crimson, bond formation in green and amino acids to which they were formed in black and other amino acidic interaction in yellow. c- Docked Gallic Acid in the pocket of Mpro of SARS-CoV-2 where the pocket is in greyish white and the ligand in Licorice. c1- Gallic Acid interaction with Mpro in 2D structure is given in crimson, bond formation in green and amino acids to which they were formed in black and other amino acidic interaction in yellow. d- Docked Gallic Acid in the pocket of Spike Protein of SARS-CoV-2 where the pocket is in greyish white and the ligand in Licorice. d1- Gallic Acid interaction with Spike Protein in 2D structure is given in crimson, bond formation in green and amino acids to which they were formed in black and other amino acidic interaction in yellow. e- Docked Ergosterol Peroxide in the pocket of Spike Protein of SARS-CoV-2 where the pocket is in greyish white and the ligand in Licorice. e1- Ergosterol Peroxide interaction with Mpro in 2D structure is given in crimson, bond formation in green and amino acids to which they were formed in black and other amino acidic interaction in yellow. f- Docked Ergosterol Peroxide in the pocket of Spike Protein of SARS-CoV-2 where the pocket is in greyish white and the ligand in Licorice. f1- Ergosterol Peroxide interaction with Spike Protein of SARS-CoV-2 in 2D structure is given in crimson, bond formation in green and amino acids to which they were formed in black and other amino acidic interaction in yellow. g- Docked Pleuran in the pocket of Mpro of SARS-CoV-2 where the pocket is in greyish white and the ligand in Licorice. g1- Pleuran interaction with Mpro in 2D structure is given in crimson, bond formation in green and amino acids to which they were formed in black and other amino acidic interaction in yellow. h- Docked Pleuran in the pocket of Spike Protein of SARS-CoV-2 where the pocket is in greyish white and the ligand in Licorice h1- Pleuran interaction with Spike Protein of SARS-CoV-2 in 2D structure is given in crimson, bond formation in green and amino acids to which they were formed in black and other amino acidic interaction in yellow.

Figure_1	a- Main Protease (MPro) of SARS-CoV-2 with active residues in green. b- Spike Protein of SARS-CoV-2 with active residues in green.
Figure_3	a- Root Mean Square Deviation (RMSD) of Ligands bound to Main Protease of SARS-CoV-2 (PDB ID: 6LU7). b- Root Mean Square Fluctuation (RMSF) of Ligands bound to Main Protease of SARS-CoV-2 (PDB ID: 6LU7).
Figure_4	a- Root Mean Square Deviation (RMSD) of Ligands bound to Spike Protein of SARS-CoV-2 (PDB ID: 6YOR). b- Root Mean Square Fluctuation (RMSF) of Ligands bound to Spike Protein of SARS-CoV-2 (PDB ID: 6YOR).
Figure_5	Top 15 Targets for Eritadenine
Figure_6	All Targets for Gallic Acid
Figure_7	Top 15 Targets for Ergosterol Peroxide
Figure_8	All Targets for Pleuran

9. SUPPORTING INFORMATION:

The supporting information containing the *in silico* target prediction from Swiss Target Prediction is given as supporting information along with this article.

The supporting information file has output files from SwissTargetPrediction website for entered ligands of interest such as eritadenine, gallic acid, ergosterol peroxide and pleuran. Each of the output file has possible targets for the same ligand, the target's common name, Uniprot ID of the targets, ChEMBL ID of individual targets, Target Class and Probability in a tabular format.

10. DATA AVAILABILITY STATEMENT:

The data that support the findings of this study are available from the corresponding author upon reasonable request.

11. CONFLICTS OF INTEREST:

The authors declare that there is no conflict of interest.