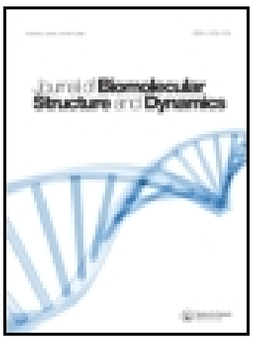

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Andrographolide as a potential inhibitor of SARS-CoV-2 main protease: an in silico approach

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ABSTRACT

SARS-CoV-2 virus which caused the global pandemic the Coronavirus Disease- 2019 (COVID-2019) has infected about 1,203,959 patients and brought forth death rate about 64,788 among 206 countries as mentioned by WHO in the month of April 2020. The clinical trials are underway for Remdesivir, an investigational anti-viral drug from Gilead Sciences. Antimalarial drugs such as Chloroquine and Hydroxychloroquine derivatives are being used in emergency cases; however, they are not suitable for patients with conditions like diabetes, hypertension and cardiac issues. The lack of availability of approved treatment for this disease calls forth the scientific community to find novel compounds with the ability to treat it. This paper evaluates the compound Andrographolide from *Andrographis paniculata* as a potential inhibitor of the main protease of SARS-COV-2 (Mpro) through *in silico* studies such as molecular docking, target analysis, toxicity prediction and ADME prediction. Andrographolide was docked successfully in the binding site of SARS-CoV-2 Mpro. Computational approaches also predicts this molecule to have good solubility, pharmacodynamics property and target accuracy. This molecule also obeys Lipinski's rule, which makes it a promising compound to pursue further biochemical and cell based assays to explore its potential for use against COVID-19.

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Andrographolide; in silico studies; SWISS-bioinformatics; SARS-CoV-2; plant compound

1. Introduction

The onset of symptoms such as fever, cough, fatigue, production of sputum, shortness of breath, sore throat, headache along with some with reports of diarrhoea and vomiting began to rise as the group of pneumonia cases from December 2019 and later they were identified as β-coronavirus in Wuhan, Hubei Province, China (Guan et al., 2020; Wang et al., 2020) The β- coronavirus was firstly named as 2019- novel coronavirus (2019-nCoV) on 12 January 2020 by WHO and formally named the disease as coronavirus 2019 (COVID- 19) and as a world emergency disease of cause and concern globally, International Committee of Coronavirus Study Group (CSG) recommended the use of the name as SARS- CoV- 2 which was published on 11 February 2020 (Guo et al., 2020). Through analyzing the viral sequence and evolutionary analysis, bat was suspected to be the natural host of the virus. And the virus might have been transferred to humans as their intermediate host by binding to ACE-2 Receptor (Angiotensin Converting Enzyme-2 Receptor) (Zhou et al., 2020). The first lethal case was reported on 11 January 2020. The infection from patients to healthcare workers was first verified on 20 January 2020. It was further reported that during Chinese New Year people migrated from Wuhan to various countries of the world. New cases evolved to other countries especially to various patients with no travel history to China which notified scientific and medical communities that local human to human transmission were seen in those countries (Rothe et al., 2020). Recently, the total number of cases around the world was recorded to be 1,203,959 confirmed cases with more than 64,788 deaths (https://www.worldometers.info/coronavirus/). Various types of treatments have been proposed which are mainly antiviral drugs, SARS- Cov and MERS- Cov antibodies are being used by clinicians and recent recommended combination therapy of hydroxychloroquine and azithromycin was studied and its results of open labelled non randomized clinical trial was published (Huang et al., 2020; Gautret et al., 2020).

Meanwhile, Food and Drug Administration (FDA) has stated that both Chloroquine phosphate and Hydroxychloroquine sulphate are not approved of treating COVID-19. And upon certain *in vitro* and some clinical data chloroquine phosphate and hydroxychloroquine sulphate was advised to be the treatment for COVID-19 and enough randomized trials on these compounds to be provided and allowed the administration of the above drugs to be used for emergency (https://www.fda.gov/emergency-use-authorization#covidtherapeutics). Hydroxychloroquine may have inhibitory mechanism over the viral processes and

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metabolisms. They may be involved in other mechanisms as inhibition of ACE2 cellular receptor, acidification of the cell membrane preventing the entry of virus and modulation of immune response through respective cytokine release (COVID-19 Drug Therapy-Elsevier, 09 March 2020). But recent studies have shown that the hydroxychloroquine can also cause drug poisoning and severe or moderate adverse effects in individuals who are already taking treatments for diabetic and hypersensitive patients, the same patient group who are found to be affected severely by COVID-19. Administration of hydroxychloroquine has found to inhibit pro-inflammatory cytokines which finally leads to Acute Respiratory Distress Syndrome (ARDS) (Guastalegname & Vallone, 2020). It has been found out that adverse neuropsychiatric condition was seen in post treatment of hydroxychloroquine which is hypothesized that it specifies the lysosomal dysfunction leading to psychiatric symptoms, which initiated the normal state of the patient who has been administered with the drug (Ali & Jones et al., 2018). Lethal adverse effect of retinal toxicity was seen in patient with acute renal impairment when administered with hydroxychloroquine (Tailor et al., 2012). A study of high doses of hydroxychloroquine along with atorvastatin in diabetic patients showed highest decline of blood glucose in patients (Wondafrash et al., 2020). When Antimalarial drug, hydroxychloroquine when administered to patients with dermatomyosis, non-life threatening cutaneous reactions are seen most in dermatomyosis patients than cutaneous lupus erythrematosus (Pelle & Callen, 2002) and many side effects has been reported. And according to the website, (https://www.guidetopharmacology.org/coronavirus.jsp) many ligands which are synthetic in nature have been proposed for the treatment of COVID-19 and are in clinical trials and are in process of peer review.

Due to these high adverse effects and the site of target through which Hydroxychloroquine acts on the viral proteasome, spike proteins and proteins involved in the life cycle of the virus are unknown (COVID-19 Drug Therapy-Elsevier, 09 March 2020). A potential natural, non- synthetic drug compound has to be found with minimal side effects. The drugs which are necessary to act on the targets such as ACE-2 receptors, TMPRSS2, SARS-CoV-2 and CD147 (https://www.guidetopharmacology.org/coronavirus.jsp) are in the process of being found in order to decrease the prognosis of the disease and life cycle of the virus. *In silico* studies of chemically synthetic drugs such as Paritaprevir and Raltegravir, Doultegravir and Bictegravir for the targets 3CLpro and 2′-OMTase (Khan, Jha, et al., 2020), theophylline and pyrimidone derivatives as possible inhibitors of RNA bound N terminal domain (Sarma et al., 2020) and Remdesivir, Saquinavir and Darunavir also with two natural compounds, flavone and coumarine derivatives for the inhibition of 3CL pro (Khan, Zia, et al., 2020) have been published. Though there are many targets are found for the treatment of COVID-19, the main protease (M^{pro}) of SARS- CoV-2 was chosen due to interest of treating infected patients, to stop the multiplication of virus within the cells, through which M^{pro} was involved in the release of polypeptides which are functional extensive proteolysis and cleavage of the enzyme itself from the sites of genome, pp1a and ppa1ab (Jin et al., 2020).

Plant compounds are an ideal of finding drug components of interest and most economical one to produce quickly as possible. This is known as the concept of repurposing the natural phytomolecules which will hasten the drug discovery process. During a search for such potent plant compounds we found a recent study on potential plant compounds which are able to inhibit the M^{pro} is in a process of publication (Khaerunnisa et al., 2020), while discussing the findings on the paper with group of Siddha and Ayurvedic doctors, they have advised us to examine the properties of the traditional available plant, several plant molecules obtained from medicinal plants were explored. The outcome was that *Andrographis paniculata* which was found to have anti-viral property and already reported in ancient texts could be investigated as a good bet. We examined the main compound in *Andrographis paniculata* and found that the main compound in the plant was Andrographolide. The plant compound has been evidenced to have anti-inflammatory, anti-cancer, anti-obesity and anti-diabetes (Dai et al., 2019). Andrographolide was found to have antiviral properties over many types of viral infections (Gupta et al., 2017) and was found to have activity against Chikungunya (Wintachai et al., 2015) potential inhibitor of herpes simplex virus type-1 (Seubsasana et al., 2011). Also, during the outbreak of dengue in India at 2006, aqueous extracts of *Andgrographis paniculata* were given through the advice of Ministry of AYUSH (Ayurveda, Unani, Siddha and Homeopathy) department of India which led to decrease in cases and infection of the disease as a preventive measure even to normal people acting as a immune booster. The study of anti-dengue activity was found and published through quantification of dengue viral inhibition and showed most antiviral inhibitory effects when DENV 1-4 infected Vero cells with maximum non-toxic dose (Krishnasamy et al., 2018). Due to these antiviral activities the compound Andrographolide was chosen.

Due to these interests, this paper involves the *in silico* analysis of Andrographolide against crystal structure of SARS-CoV-2 main protease which is provided with an inhibition site (PDB ID: 6LU7) (Zhang et al., 2020), prediction of ADME (https://www.swissadme.ch), Target Prediction (https://www.swisstargetprediction.ch) were done by using Swiss-Bioinformatics online Tools. The prediction of toxicity of Andrographolide was seen using pkCSM online web tool (https://biosig.unimelb.edu.au/pkcsm/).

2. Materials and methods

2.1. Docking of andrographolide on sars-cov-2 main protease

2.1.1. Receptor preparation

The SARS-CoV-2 main protease (Figure 1a and 1b) (PDB ID: 6LU7) (Jin et al., 2020) was used as the receptor. The inhibitor was selected and removed. The receptor preparation was done using the Dock Prep tool of UCSF-Chimera. Hydrogens were added and optimized by a hydrogen bonding network and allowed the method to determine the Histidine protonation state. The receptor was saved in mol2 format

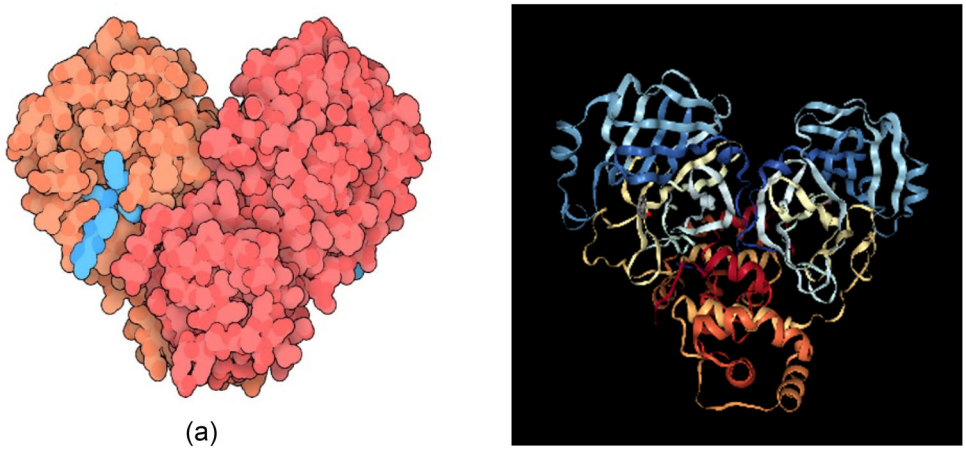

Figure 1. (a) SARS- CoV-2 main protease with inhibitor in turquoise; (b) Crystal structure of SARS- CoV-2 main protease with inhibitor.

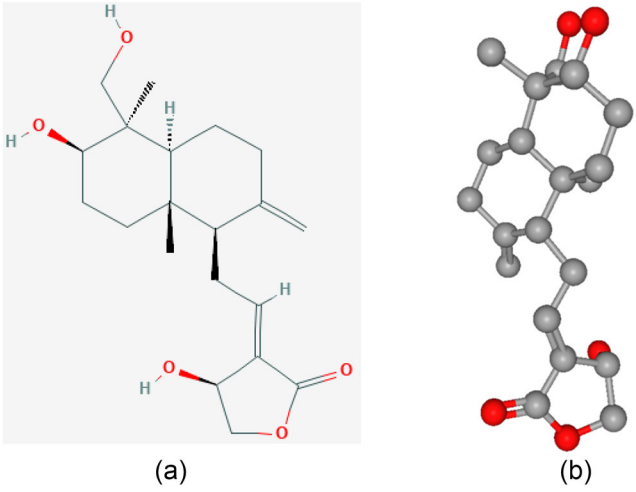

Figure 2. (a) 2D structure of andrographolide; (b) 3D structure of andrographolide.

(rec_charged.mol2) using untransformed coordinates and Sybyl-style hydrogen naming. Hydrogen present in the structure were removed from the protein and saved in pdb format (rec_noH.pdb).

2.1.2. Ligand preparation

The 3 D structure of Andrographolide (Figure 2a and 2b) (PubChem CID:5318517) was downloaded from Pubchem (https://pubchem.ncbi.nlm.nih.gov/compound/Andrographolide). Hydrogen was added to the ligands using the AddH function in structure editing tools in UCSF-Chimera. Charges were added to the ligands using AM1-BCC method. The ligand file was saved in mol2 format (lig_charged.mol2) using untransformed coordinates.

2.1.3. Docking

The molecular surface of the receptor (rec_noH.pdb) was prepared using the Write DMS Tool in UCSF-Chimera. Spheres outside the surface were generated using sphgen function having 4.0 in Angstroms as the maximum sphere radius and 1.4 in Angstroms as the minimum sphere radius. The cluster present in the binding site of the inhibitor was chosen using showsphere function. A box was created around the chosen cluster, having extra margins enclosed of 5.0 in Angstroms in all the 6 directions. A grid was generated using the program grid of DOCK6. Flexible docking parameters were employed for Andrographolide.

2.2. Adme prediction

ADME (Adsorption, Distribution, Metabolism and Excreation) is important to analyze the pharmacodynamics of the proposed molecule which could be used as a drug. SWISS-ADME is a website (https://www.swissadme.ch) which allows the user to draw their respective ligand or drug molecule or include SMILES data from PubChem and provides the parameters such as lipophilicity (iLOGP, XLOGP3, WLOGP, MLOGP, SILICOS-IT, Log P_0/w), water solubility- Log S (ESOL, Ali, SILICOS-IT), drug likeness rules (Lipinski, Ghose, Veber, Egan and Muegge) and Medicinal Chemistry (PAINS, Brenk, Lead-likeness, Synthetic accessibility) methods are analyzed (Daina et al., 2017). The data from PubChem which consists of SMILES of Andrographolide (https://pubchem.ncbi.nlm.nih.gov/compound/Andrographolide) was entered into the search bar and was analyzed.

2.3. Target prediction

Molecular Target studies are important to find the phenotypical side effects or potential cross reactivity caused by the action of small biomolecules (Keiser et al., 2007; Gfeller et al., 2014). Swiss Target Prediction website (https://www.swisstargetprediction.ch) was logged on and the ZINC number for Andrographolide (ZINC3881797) was entered on to the search bar and was analyzed.

2.4. Toxicity prediction

Toxicology prediction of small molecules is important to predict amount of tolerability of the small molecule before

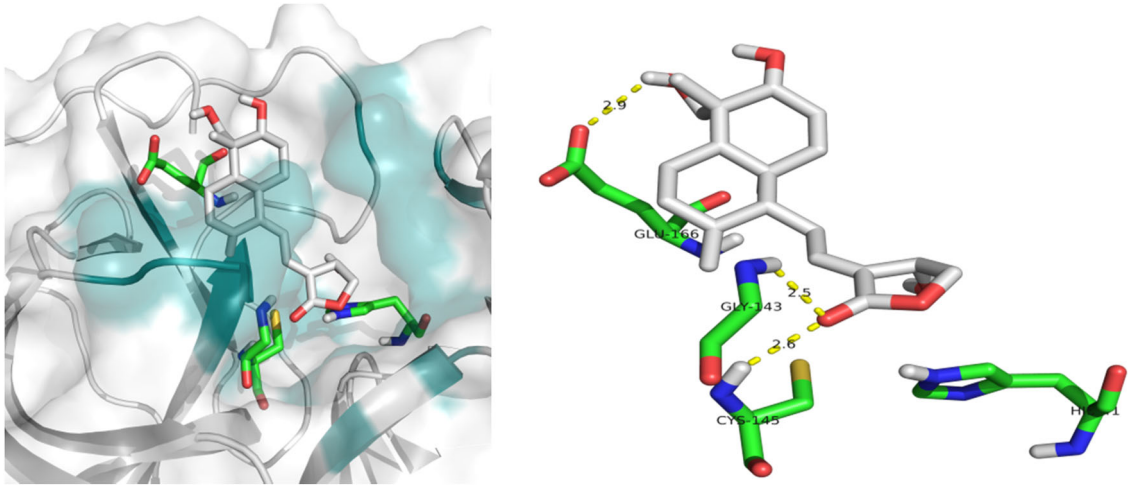

Figure 3. (a) Docked Ligand in the binding pocket of the protein. The binding pocket of the protein is in light blue and the surface of the protein in white; (b) Residues interacting with the ligand and their hydrogen bonds distance. The Ligand is given in white, interacting residues in green and hydrogen bonds in yellow.

TABLE 1. Interaction of the SARS-CoV2 Main Protease with Andrographolide Ligand.*

Compound	No of H- Bonds	Residue Receptor	Ligand	Bond Length (Å)	Docking Score (Kcal/ mol)
Andrographolide	**4**	**Cys145(H)**	**O2**	**2.46**	**-3.094357**
		Gly143(H)	**O2**	**2.62**	**-3.094357**
		Glu166(H)	**O3**	**2.93**	**-3.094357**
		Glu166(H)	**O3**	**2.92**	**-3.094357**

*Prediction of noncovalent interactions for PDB structure using PLIP v1.4.4 (Salentin,S. et al. PLIP: fully automated protein-ligand interaction profiler. Nucl. Acids Res. (1 July 2015) 43 (W1): W443-W447. doi: 10.1093/nar/gkv315).

being ingested into the human and animal models. pkCSM is an online database in which the small molecule can be drawn virtually or can be analyzed by submitting the SMILES of the same. The website can provide details of toxicology effects in the fields of AMES Toxicity, human maximum tolerance dose, hERG-I inhibitor, hERG-II inhibitor, LD50, LOAEL, Hepatotoxicity, Skin Toxicity, *T. pyriformis* toxicity and Minnow toxicity. The website was logged on and the SMILES of the Andrographolide data from PubChem was searched and submitted into the website and toxicity mode was selected (Pires et al., 2015).

3. Results

3.1. Docking

The docking analysis of the compound with SARS-CoV-2 protease generated negative values for free energy -3.094357 KJ/mol in the grid box, suggesting high affinity for the binding pocket. All the binding conformations of the compound in the active binding pocket involved both H-bond and salt bridge interaction. The compound did bind to the protease with 4 hydrogen bonds with 3 residues namely Gly143, Cys145 and Glu166 as shown (Figure 3a and 3b). All details of the atoms involved in bonding with ligands, bond lengths, docking energies and salt bridges are given in Table 1.

3.2. Adme prediction

The ADME prediction which was done using SWISSADME database came with the results following after submission of the small biomolecule, Andrographolide. The physiochemical properties of the compound are, number of 25 heavy atoms, 5 hydrogen bond acceptors, 3 hydrogen bond donors, molar refractivity of 95.21and topological polar surface area (TPSA) of the molecule is found to be 86.99Å^2. The lipophilicity of the molecule, iLOGP is 2.45, XLOGP3 is 2.16, WLOGP is 1.96, MLOGP is 1.98, SILICOS-IT is 2.94 and Consensus P_0/W is 2.30.

Water Solubility properties calculated are ESOL -3.18, solubility of 2.36e^{-02}mg/ml and of soluble class; Ali -3.62, solubility of 8.42e^{-02}mg/ml and of soluble class, SILICOS-IT -2.69, solubility of 7.22e^{-01}mg/ml and of soluble class. Pharmacokinetic data predicted was found to be of high Gastrointestinal absorption (GI), not blood brain barrier permeant, acts as a P-gp substrate, does not inhibit CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4 cytochromes. Skin permeation kinetics (Log K_p) was found to be -6.90 cm/s.

Druglikeness factors was found to be of drug like compound which obeys Lipinski's Rules with no violation, also obeys druglikeness score rules such as Ghose, Veber, Egan, Muegge and with 0.55 Bioavailability score. Medicinal chemistry parameters were found to be of no PAINS alert, violates Brenk's laws with two alerts of being an isolated alkene, one michael acceptor, no lead likeness with molecular weight of greater than 350 and synthetic accessibility of 5.06 rate.

3.3. Target prediction

The target prediction analysis was displayed in the Web page with the following observations the top 25 of the

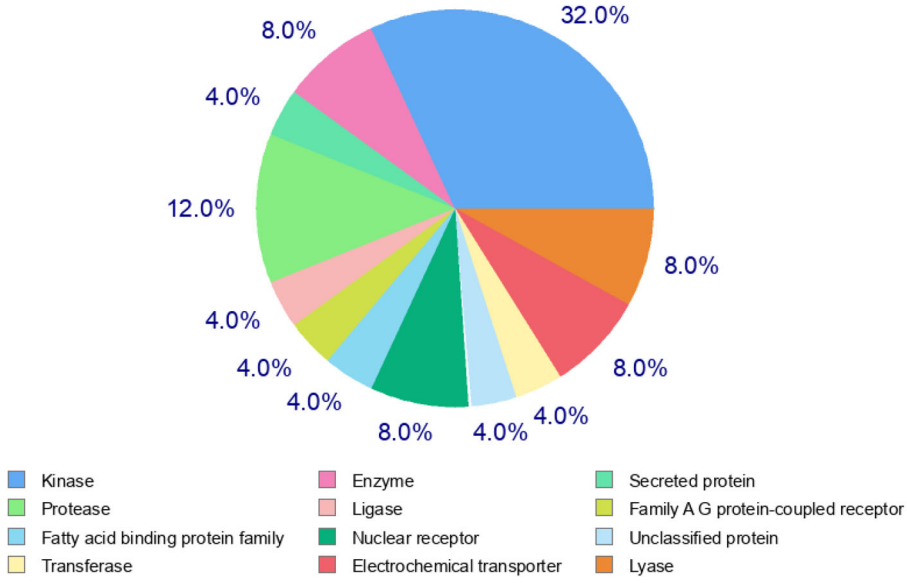

Figure 4. Top-25 of Target Predicted for Andrographolide.

results were given as a pie-chart (Figure 4). The pie chart predicts 32% of Kinase, 12% of protease, 4% of Fatty acid binding protein family, 4% of Transferases, 8% of Enzymes, 4% of Ligases, 8% of Nuclear Receptors, 8% of electrochemical transporters, 4% of Secreated protein, 4% of Family AG protein coupled receptor, 4% of unclassified protein and 8% of Lyase. The output table consistting of Target, Common Name, Uniprot ID, ChEMBL-ID, Target Class, Probability and Known actives in 2 D/3D are given in the Supplementary-1. The possible sites of target which the compound may bind to are mostly the targets which are predicted by the software and the probability score are very less that is from 0.10560 to 0.0972. This makes an inference that the small compound may have high target attraction towards the specific binding site it is directed to.

3.4. Toxicity prediction

The toxicity predicted was displayed in the website and the results is as follows, The Andrographolide does not have AMES toxicity, Maximum tolerated dose for human is about 0.128 log mg/kg/day, it does not inhibit hERG-I and hERG-II, Acute oral rat toxicity (LD_{50}) was found to be 2.162 mol/kg, Chronic oral rat toxicity (LOAEL) was found to be 1 log mg/kg_bw/day, does not produce hepatotoxicity, it does not cause skin sensitivity, 0.491 log μg/L causes *T. pyriformis* toxicity and 1.37 log mM causes Minnow toxicity.

4. Discussion

The need of the hour is a therapy for SARS-CoV-2 virus, many small molecules like Remdesivir are in trial to provide cure for this dreadful viral outbreak. Hydroxychloroquine and azithromycin complex is being advised to be given for the affected in case of emergency, though it has been studied to increase the pH of the protease and is published as a potent inhibitor of SARS-CoV-2 infection and spread (Wang et al., 2020; Heald-Sargent & Gallagher, 2012; Vincent et al., 2005). The Hydroxychloroquine and azithromycin complex mentioned above may be potent, but the adverse side effects they bring to the patients are very alarming as shared already, with these drastic side effects, the need to bring equally or more potent alternatives in the form of plant derived drug is very essential as they are much safe and have no known side effects. As we are interested only in finding pure potent plant compounds without adding any analogs or derivatives we found that the plant compound, Andrographolide intriguing due to its awesome properties. The drug compound, Andrographolide can be isolated and produced easily by extracting from the plant *Andrographis paniculata*. When the compound was analyzed by *in silico* computational docking tools it successfully docked against the inhibitor region of the main protease of SARS-CoV-2 virus with docking score of -3.094357 Kcal/mol, the docking score showed great binding when compared to synthetic compounds when they are docked against M^{pro} such as disulfiram, tideglusib and shikonin which are -46.16 Kcal/mol, −61.79 Kcal/mol and -17.35 Kcal/mol (Jin et al., 2020). And it also shows great binding score when compared against recently proposed combination of three drugs namely, lopinavir, ostelmivir and ritonavir whose binding scores are -4.1Kcal/mol,-4.65 Kcal/mol and -5.11 Kcal/mol (Muralidharan et al., 2020). Even some plant molecules which are studied to inhibit the main protease of SARS-CoV-2 failed to prove their binding score when compared with the Andrographolide. They are compounds such as kaempferol -9.41 Kcal/mol, quercetin -8.58 Kcal/mol, demethoxycurcumin -8.17 Kcal/mol, curcumin -7.31 Kcal/mol, catechin –7.05 Kcal/mol, epichatechin gallate- 7.24 Kcal/mol, zingerol -6.67 Kcal/mol and gingerol -5.40 Kcal/mol respectively (Khaerunnisa et al., 2020). Even proposed inhibitor of M^{pro} such as PRD_002214 has a docking score of -10.466 Kcal/mol (Bouchentof & Missoum, 2020) which proclaims that Andrographolide has better properties than other proposed inhibitors.

The compound possesses excellent properties of druggability as well small biomolecule. The molar refractivity of the compound confirms that the drug compound is permeable through particular membranes and can remain constant even in the midst of strong or weak solute-solvent, solvent-solvent interactions. And exemplary TPSA says that it has great transport properties. Through lipophilicity of the drug compound we can know that the compound has ideal property for oral and intestinal absorption and is able to be absorbed sub-lingual as well. Through water solubility properties predicted the drug is free soluble.

The pharmacokinetic data predicted about absorption and permeability echoes the predicted values of lipophilicity and solubility, they also predict that is able to release phosphate from ATP and simultaneous binding of ADP to the glycoprotein, thus the compound acts as a p-gp substrate. The compound, Andrographolide does not inhibit liver metabolism by inhibiting CYP1A2, it does not stop the metabolism of several therapeutic drugs especially, anti-ulcer, anti- malarial, anti-convulsant, anesthetic and sedative drugs via inhibiting CYP2C19, it does not stop metabolism of anti-hypersensitive drugs, β blockers, anti-arrhythmic drugs and anti-depressants via inhibition of CYP2D6, it does not stop the metabolism of anti-clotting agents, anti-seizure, management of type-II diabetes, anti-hypertensive, Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) via CYP2D9 and it does not stop oxidation of steroids, fatty acids and xenobiotics as well as for hormone synthesis and breakdown through CYP3A4.

Druglikeness factor rules were obeyed accordingly without any violation to this compound which describes the compound can act as a drug in the biological systems. Medical Chemistry parameters exclaims that, zero Pan-Assay Interference Compounds (PAINS) alert for the compound meaning that it is a progressive compound worthy of testing for biochemical assays. The toxicity prediction says that the compound, Andrographolide is safe and can be given as a drug with the value of tolerance prescribed for human consumption as predicted by the website. Though these properties are appreciable *in silico*, due to extensive lockdown and work from home command through the government the findings was not continued in a wet lab. So, further studies of *in vitro* and clinical studies dealing with SARS-CoV-2 should be considered for further studies.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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