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Prospecting for *Cressa cretica* to treat COVID-19 via *in silico* molecular docking models of the SARS-CoV-2

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ABSTRACT

The severe acute respiratory syndrome COVID-19 declared as a global pandemic by the World Health Organization has become the present wellbeing worry to the whole world. There is an emergent need to search for possible medications. *Cressa cretica* is reported to show antitubercular, antibacterial and expectorant property. In this research, we aim to prospect the COVID-19 main protease crystal structure (M^{pro}; PDB ID: 6LU7) and the active chemical constituents from *Cressa cretica* in order to understand the structural basis of their interactions. We examined the binding potential of active constituents of *Cressa cretica* plant to immensely conserved protein M^{pro} of SARS-CoV-2 followed by exploration of the vast conformational space of protein–ligand complexes by molecular dynamics (MD) simulations. The results suggest the effectiveness of 3,5-Dicaffeoylquinic acid and Quercetin against standard drug Remdesivir. The active chemical constituents exhibited good docking scores, and interacts with binding site residues of M^{pro} by forming hydrogen bond and hydrophobic interactions. 3,5-Dicaffeoylquinic acid showed the best affinity towards M^{pro} receptor which is one of the target enzymes required by SARS CoV-2 virus for replication suggesting it to be a novel research molecule. The potential of the active chemical constituents from *Cressa cretica* against the SARS-CoV-2 virus has best been highlighted through this study. Therefore, these chemical entities can be further scrutinized and provides direction for further consideration for *in-vivo* and *in-vitro* validations for the treatment of covid-19.

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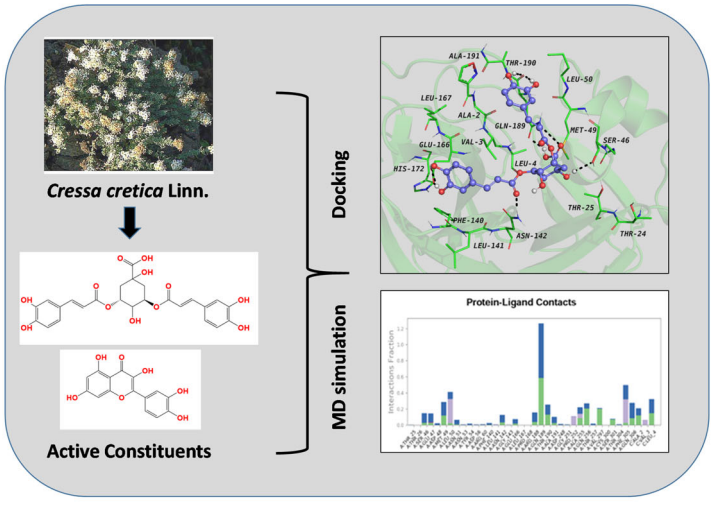
GRAPHICAL ABSTRACT



Abbreviations: MD: Molecular dynamics; ORF: Open reading frames; HIV: Human Immunodeficiency virus; RNA: Ribonucleic acid; OPLS: Optimized potentials for liquid simulations; NCDCV: Neonatal calf diarrhoea coronavirus; OC43: Orthocornavirinae family; RdRps: RNA-dependent RNA polymerase; MW: Molecular weight; PSA: Polar surface area; HBD: Hydrogen bond donor; RMSD: Root mean square deviation; RMSF: Root Mean Square Fluctuation; CAESAR: Computer assisted evaluation of industrial

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chemical substances according to regulation; CoMPARA: Collaborative modeling project for androgen receptor activity; IRFMN: Oestrogen receptor relative binding affinity model; ADI: Applicability domain index; WHO: World Health Organization; SDF: Spatial data file

1. Introduction

Novel coronavirus disease (COVID-19) has become a pandemic danger to the general wellbeing. It is a respiratory disease-causing fever, fatigue, dry cough; muscle aches, shortness of breath and some instances lead to pneumonia. Development of symptoms and their brutality of disease vary from patient to patient. The elderly people, children below 6 years and patients with the past medical history of asthma, diabetes, cardiac disorder are more susceptible to this disease due to weaker or conceded immune systems (Shah et al., 2020). The World Health Organization (WHO) has now declared a global emergency and pandemic for the coronavirus disease (COVID-19) that has been actively spreading around the globe (World Health Organization (WHO), 2020). SARS-CoV-2 virus consists of mRNA as genetic material after release into the host cell can be readily translated into protein. There are totally about 14 open reading frames (ORF) in the mRNA genome of virus. Every individual ORF is responsible for encoding a variety of structural and non-structural proteins required for viral existence plus its virulence influence. In the transformation phase of the viral genome, genes that encode non-structural polyprotein are first translates into ORF1a and ORF1b to generates two large overlapping polyprotein namely pp1a and pp1ab by contributing a ribosomal frame shifting event (Astuti & Ysrafil, 2020; Masters, 2006).

The SARS-CoV-2 virus polyprotein encodes two proteases, which share in its processing and release of the translated non-structural proteins. A) Main protease is called 3-CL-like or serine-type protease (M^{pro}) and B) Papain-like protease (PI^{pro}). The researchers are concentrating on both of these vital targets M^{pro} and PI^{pro} for drug discovery studies against the recent coronavirus epidemics. The mediation of nonstructural viral proteins and maturation by the main protease makes M^{pro} a very attractive target for the development of anti-coronavirus drugs. Thus, any inhibitors which inhibit the main protease ($3CL^{pro}$ or M^{pro}) and block the replication of SARS-CoV-2 would be effective and specific measures for the development of therapeutic agents or antiviral drugs against SARS-CoV-2 (Vlachakis et al., 2020). The first available crystal structure of COVID-19 proteins is M^{pro}, which was published in February 2020 (PDB ID:6LU7) (Kandeel & Al-Nazawi, 2020) provides structural insights for understanding of ligand binding to M^{pro}. As of now, no specific treatment available for copping this malady. Clinical trials undergoing at ClinicalTrials.gov (https://clinicaltrials.gov/) and WHO Solidarity concentrating on the repurposing of existing drugs (Altay et al., 2020; Viveiros Rosa & Santos, 2020). Scientist working in this area has suggested the use of some recognized broad-spectrum antiviral drugs such as Nucleoside analogs, HIV protease inhibitors and traditional Chinese medicines as hopeful treatment approach. Some antiviral drugs like Remdesivir, Ritonavir, Oseltamivir, Favinapir, Ganciclovir and Lopinavir are clinically tried against COVID-19 disease. Until any exact treatment procedure is accessible for COVID-19, the utilization of derivatives of recently realized antiviral drugs is a helpful technique (Hall & Ji, 2020). In resembling, different clinical trials are likewise now being experienced on nucleoside analogue medications, for example, Remdesivir, an antiviral medication demonstrated to be compelling against a wide scope of RNA infections *in vitro* (Elfiky, 2020a) and it is the only drug that is approved by FDA (National Institutes of Health, n.d.). However, the beneficial importance of Remdesivir remains uncertain (Siemieniuk et al., 2020). Favipiravir demonstrated a better effect in disease progression and viral clearance (Cai et al., 2020).

To encounter viral diseases, traditional plants are principally empowered in the greater part of the total populace (Mukhtar et al., 2008). Also, different assessment shows the valuable impact of traditional therapeutics in the usage of patients infected with a novel SARS-CoV-2 virus (Yang et al., 2020). Sanjeevani is among the most baffling and most sought-after herbs in Indian folklore, whose presence and personality are saturated with profound contention. *Cressa cretica* is a plant that is referred to by the name that mirrors the highlights of Sanjeevani (Sen, 2009). *Selaginella bryopteris, Dendrobium plicatile* and *Cressa cretica* have been anticipated as likely nominees for the Sanjeevani plant. Amongst them, *Cressa cretica* is a very common holophytic herb used in traditional medicine for cure of diabetes, ulcers, asthma, anthelmintic, stomachic, aphrodisiac, and beneficial in constipation, leprosy and urinary discharges. The leaf extract also shows antioxidant and antibacterial property for infections. It has a huge range of biologically active chemicals as Quercetin, Quercetin-3-*O*-glucoside, Kampferol-3-*O*-glucoside, Rutin, Syringaresinol-h-d-glucoside, Scopoletin, 3,5-dicaffeoylquinic acid, Creticane, Cressa tetracosanoate, Cressa tetratriacontanoic acid, Cressa triacontanone, Cressa naphthacenone, etc. that are chemically and structurally different (Afshari & Sayyed-Alangi, 2017; Priyashree et al., 2010; Rani et al., 2011; Suganthi et al., 2008).

With the conventional technique of drug discovery could take years, whereas *in silico* docking models from the most variable protein in the SARS-CoV-2 can search for the possible natural medications for the treatment of COVID 19. In this investigation, docking examines on the phytoconstituents of *Cressa cretica* were performed over restricting pocket of M^{pro} (protease) to locate the potential small natural molecule to encounter life-threatening coronavirus disease. The obtained results will help in the repurposing natural remedies to combat the recent dangerous COVID-19.

2. Material and methods

2.1. Protein preparations

In-silico analysis of phytoconstituents of *Cressa cretica* was performed on 2.16 Å crystal structure of COVID-19 M^{pro}, the main protease in complex with an inhibitor N3 (PDB ID:

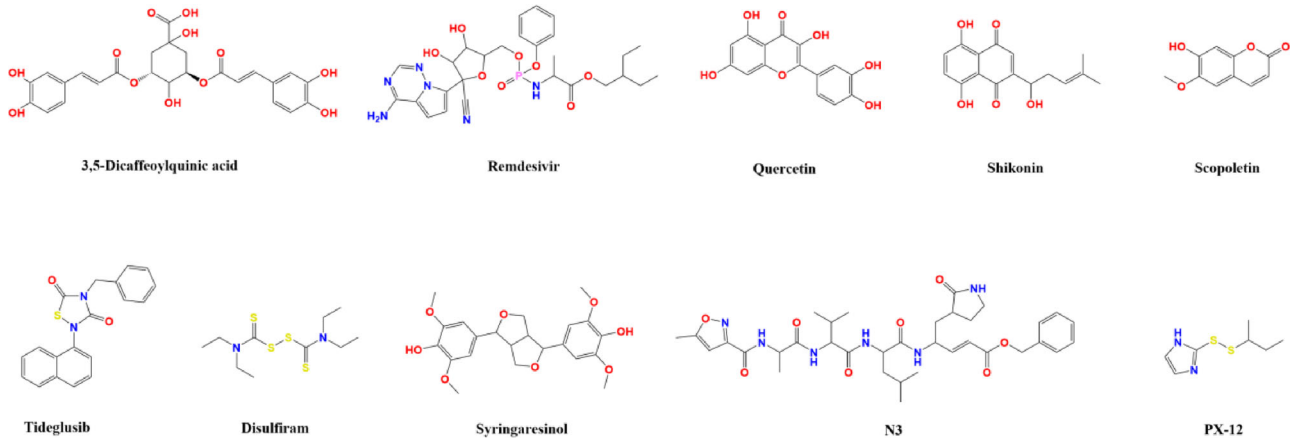

Figure 1. Chemical structure of all selected ligand molecules in docking studies.

6LU7, Resolution: 2.16 Å) which was retrieved from protein data bank (https://www.rcsb.org) Figure S1. Protein Preparation Wizard module of Maestro (Anang et al., 2018) was used to prepare and process protein structure which includes three main steps; import and process, review and modify and final refinement of the protein structure. Pre-process step includes assigning bond orders, hydrogen bond addition, creation of zero-order bonds to metals and disulphide bonds with the filling of missing side chains and missing loops using Prime. The waters beyond 5 Å was deleted and het states were generated using Epik pH 7.0 ± 0.0. The workspace was analysed and states were generated at pH 7.0 ± 0.0. In the refinement step, optimization of protein and removal of water molecules followed by minimization using OPLS3e as force field was performed (Jorgensen et al., 1996).

2.2. Ligand preparations

The structures of chemical constituents of *Cressa cretica* were retrieved in a MOL format from the PubChem database available on the NCBI website (https://pubchem.ncbi.nlm.nih.gov). The ligand N3 (N-[(5-methylisoxazol-3yl) carbonyl] alanyl-l-valyl-*n*∼1∼-((1R, 2Z)-4-(benzyloxy)-4-oxo-1-{[(3R)-2-oxopyrrolidin-3-yl] methyl} but-2-enyl)-l-leucinamide) was obtained from database of chemspider. CSID:4883311, http://www.chemspider.com/ChemicalStructure.4883311.html (accessed 04:56, May 12, 2020). Ten compounds were selected to target the main protease of SARS-CoV-2; five are known as potential inhibitors for M^{pro} enzyme, one of them is approved drug against different viral RdRps (Remdesivir) (C. Gordon et al., 2020) and 3,5-Dicaffeoylquinic acid, Quercetin, Scopoletin, Syringaresinol are active chemical constituent of the plant *Cressa cretica*. The chemical structures of all the ligands are depicted in Figure 1. All the structures were minimized using LigPrep module within Schrodinger using OPLS3e force field and pH 7.0 ± 0.0 was set as an ionization state (LigPrep, Schrödinger, LLC, New York, NY, 2020).

2.3. Receptor grid generation and molecular docking

The grid was generated by selecting co-crystallized inhibitor N3 within the minimised protein structure. Furthermore, the generated grid was used for docking of all prepared ligands using Glide employing extra precision (XP) docking module (Friesner et al., 2006; Release, 2017). Glide has been shown to calculate superior prediction in contrast to that of the other docking software; because it applies both empirical as well as force field terms to compute the finest binding pose and binding energy (Friesner et al., 2004).

2.4. Molecular dynamic (MD) simulation studies

Desmond with OPLS3e force field from Schrodinger was used to study the dynamic behaviour of all protein–ligand complexes in the presence of explicit water molecules (Harder et al., 2016). The obtained docking poses for selected compounds (3,5-Dicaffeoylquinic, Remdesivir and Quercetin) were used for MD simulation studies. The System Builder module was used for system preparation using the SPC module for solvation and volume occupancy in an orthorhombic box with periodic boundary conditions. The solvated system was neutralised by the addition of appropriate anion (Cl^-) and cation (Na^+) with a salt concentration of 0.15 mol/L. The generated solvated system was used for 100 ps minimization. The minimized system was then used for 100 ns MD simulation using NPT ensemble, 300 K temperature and pressure (1.013 bar). The MD trajectory analysis was performed using a simulation interaction diagram (Kotha et al., 2020).

Furthermore, the relative binding affinity of the ligands towards M^{pro} protein was determined using Prime Molecular Mechanics with Generalized Born Surface Area (MM-GBSA) Schrodinger, NY, 2019 (Release, 2017). The MM-GBSA (Genheden & Ryde, 2015) calculations were performed using VSGB (Li et al., 2011) and OPLS3 (Harder et al., 2016) as the solvent model and force field, respectively.

2.5. ADME and toxicity studies

The selected phytoconstituents were further checked for drug-likeness properties according to the Lipinski rule. During drug development, safety is usually the foremost important issue, therefor Toxicology prediction of small molecules is vital to predict the amount of tolerability before being ingested into the animal models. VEGA-QSAR (http://

Table 1. Ligands binding interaction parameter with the main protease of SARS-CoV-2 (PDB ID: 6LU7).

Sr. No.	PubChem CID/ ChemSpider ID	Name	MW (g/mol)	log P	HBDH	Topo-logical PSA (Å^2)	XP docking score (kcal/mol)	Lipinski rule violation
1	6474310	3,5-Dicaffeoylquinic acid	516.4	1.5	19	211	−6.375	3
2	121304016	Remdesivir	602.6	1.9	17	204	−6.278	2
3	5280343	Quercetin	302.23	1.5	12	127	−5.314	0
4	479503	Shikonin	288.29	3.0	8	94.8	−4.091	0
5	5280460	Scopoletin	192.17	1.5	5	55.8	−3.545	0
6	11313622	Tideglusib	334.4	4.3	3	65.9	−2.100	0
7	3117	Disulfiram	296.5	3.9	4	121	−1.977	0
8	100067	Syringaresinol	418.4	2.2	10	95.8	−1.740	0
9	4883311	N3	680.791	1.74	19	198	−1.705	2
10	219104	PX-12	188.3	2.3	4	79.3	−1.506	0

Table 2. Binding interactions of ligands with the binding site of main protease of SARS-CoV-2 (PDB ID: 6LU7).

		Interactions (PDB-6LU7)	
Sr. No.	**Ligands**	H-Bonding	Hydrophobic
1	3,5-Dicaffeoylquinic acid	LEU4, MET49, GLN189, THR190, GLN256, ALA255, VAL297, SER301	ALA2, LEU50, ARG188, PHE305
2	Remdesivir	LEU4, THR24, THR25, GLU166, GLN189	VAL3, MET49, LEU50 and PRO168
3	Quercetin	THR24, THR26, ASN28, HIS41, ASN119, ASN142 and GLN189	

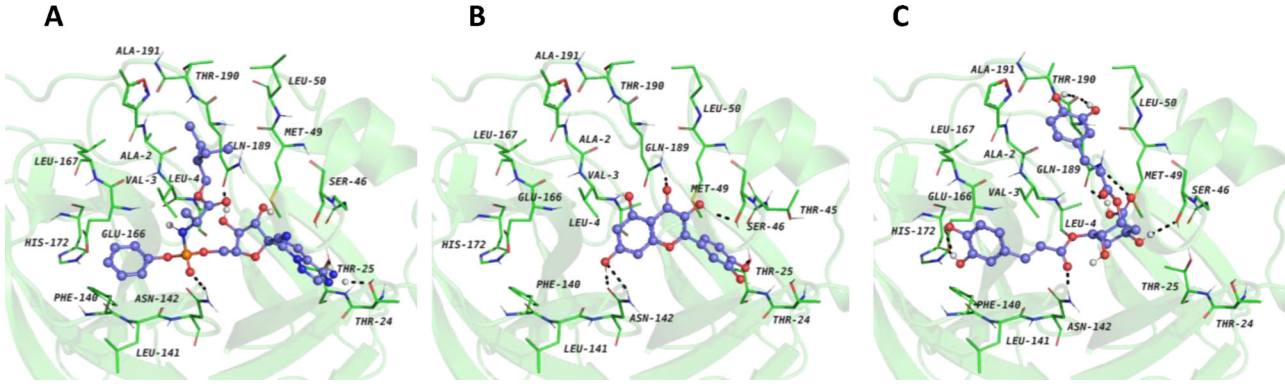

Figure 2. Docked pose of A) Remdesivir B) Quercetin and C) 3,5-Dihydrocaffeolyquinic acid against M^{pro} protease (PDB ID: 6LU7). The ligand is shown in ball and stick representation whereas residues forming binding pocket of M^{pro} are shown as green sticks. Hydrogen bond interactions are shown with black dotted lines.

www.vega-qsar.eu/) is integrating *In silico* QSAR models and read-across method for a number of toxicological data outcomes (Rogiers et al., 2020). To analysed ligands for toxicological properties, SMILES notations or SDF files uploaded followed by selecting required models for generating numerous information about structure related effects. The results also show structural alerts in chemical structure based on known mutagenic and carcinogenic structural analog (Benfenati et al., 2019).

3. Result and discussion

The main aim of the study was to prospect active chemical constituents of *Cressa cretica* to a highly conserved protein, M^{pro} of SARS-CoV-2, therefore, we performed molecular docking studies of all chemical constituents of *Cressa cretica* followed by identification of top hits which is discussed in the first section. Furthermore, the docking poses of ligands showing highest docking score were evaluated through MD simulations, calculated free energy of binding for the drugs using MM-GBSA. The results are presented in the second section.

3.1. Molecular docking studies

All the prepared ligands shown in Figure 1 were docked (XP module) on the prepared protein (PDB ID: 6LU7) successfully and XP docking score was analysed.

The XP docking score for all the ligands is listed in Table 1 along with their molecular properties.

According to the analysis of docking results, the interactions (listed in Table 2) between 3,5-Dicaffeoylquinic acid and Quercetin are highly consistent with that of Remdesivir and even they represent the most promising inhibitors of the SARS-CoV-2 M^{pro}. The results of the molecular docking showed that the tested compound 3,5-Dicaffeoylquinic acid gives the lowest binding energy (−6.375 kcal/mol) in complex with 6LU7, which is the best score when compared to other docked compounds. Quercetin (−5.314 kcal/mol) gives score agreeable to the one given by Remdesivir (−6.278 kcal/mol).

Docking of selected ligands shows various kinds of interactions with active site of M^{pro} protein indicating the possible binding of these ligands to M^{pro} protein. Ligand 3,5-Dihydrocaffeolyquinic acid shows eight hydrogen bond interactions with LEU4, MET49, GLN189, THR190, GLN256, ALA255, VAL297 and SER301. Additionally, it also forms hydrophobic

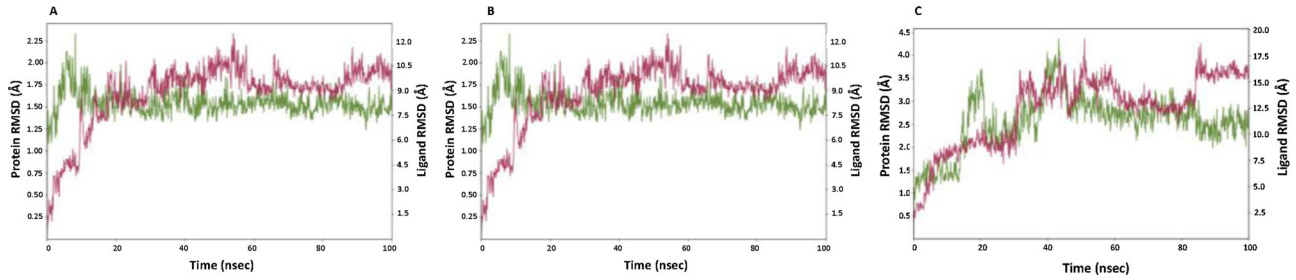

Figure 3. 1) RMSD of the protein backbone along the simulation trajectory for the protein and all the docked complexes. The overall structure of M^{pro} did not change much after the binding of (A) Remdesivir B) Quercetin and C) 3, 5-Dicaffeoylquinic acid.

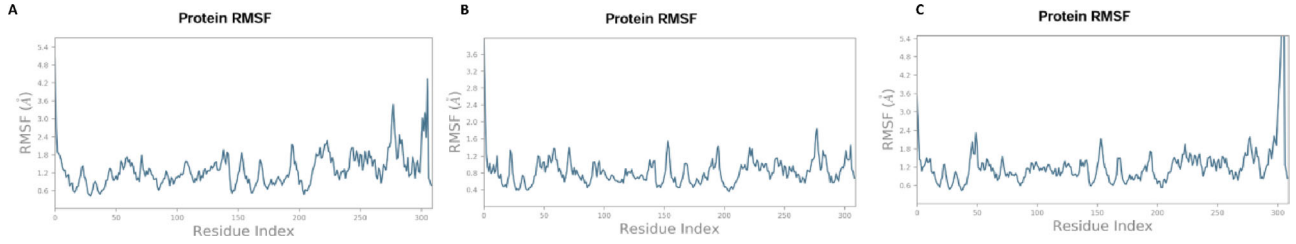

Figure 4. RMSF of the amino acids comprising the M^{pro}. No abrupt fluctuations were observed in any region of the protein with the three ligands A) Remdesivir B) Quercetin and C) 3, 5-Dicaffeoylquinic acid.

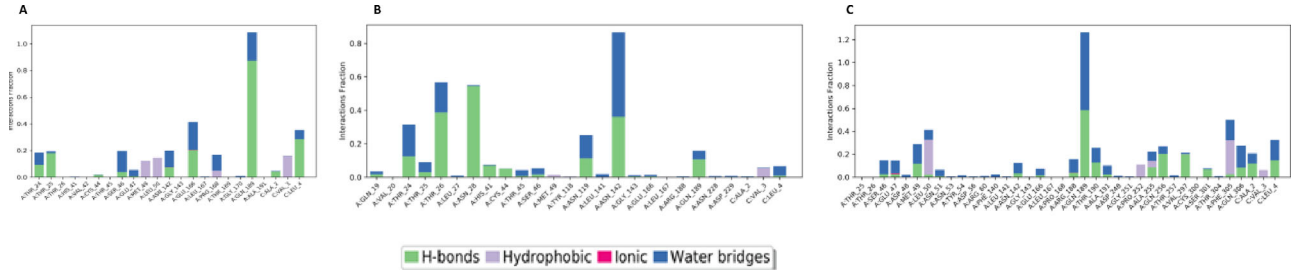

Figure 5. Hydrogen bond occupancy of various important residues of the main protease during the simulation run in case of binding with A) Remdesivir B) Quercetin and C) 3,5-Dicaffeoylquinic acid.

interactions with ALA2, LEU50, ARG188 and PHE305. On the other hand, Remdesivir formed five hydrogen bonds with LEU4, THR24, THR25, GLU166 and GLN189, and four hydrophobic interactions with VAL3, MET49, LEU50 and PRO168. This is well aligned with the reported docking studies of Remdesivir (Elfiky, 2020a, 2020b; Shannon et al., 2020). Similarly, Quercetin also interacted with different amino acids such as THR24, THR26, ASN28, HIS41, ASN119, ASN142 and GLN189 residues by forming H-bond interactions. The docking poses for 3,5-Dihydrocaffeolyquinic acid, Remdesivir and Quercetin is depicted in Figure 2 and Figure S2 in supporting information (SI).

A literature review revealed that extracts of the selected plants were reported to possess antiviral activity at various concentrations (Shahat et al., 2004; Sunita et al., 2011). 3,5-Di-*O*-caffeoylquinic acid possessed potent anti-respiratory syncytial virus activity (IC_{50} of 2.33 mM), antibacterial activity against *Vibrio cholera, Vibrio parahaemolyticus, Bacillus cereus* (Li et al., 2005; Ooi et al., 2006) and strongest DPPH radical scavenging activity (Devrnja et al., 2017). The role of Quercetin as potential antiviral agents is well known since 1951 and it is also found to diminish infectivity of bovine and human coronaviruses, NCDCV and OC43, respectively, by half at a concentration of 60 μg/mL. Quercetin, have ability to block the entry of SARS-CoV into host cells. Quercetin antagonized HIV-luc/SARS pseudo typed virus entry (EC_{50} of 83.4 μM) (Meyer-Almes, 2020; Russo et al., 2020).

Table 3. MM-GBSA values for the selected ligands.

Ligands	MM-GBSA DG bind (kcal/mol)
3.5-Dicaffeoylquinic acid	−45.62
Remdesivir	−50.14
Quercetin	−40.64
Shikonin	−25.29
Scopoletin	−21.34
Tideglusib	−30.59
Disulfiram	−10.27
Syringaresionol	−28.11
N3	−34.54
PX12	−11.38

The screened chemical constituents displayed higher docking scores, stronger binding energies, and better interactions with the conserved catalytic residue than Remdesivir. To further prove the effectiveness of phytoconstituents for COVID-19 therapy, the best three compounds having the highest docking scores based on XP docking method, namely 3,5-Dicaffeoylquinic acid, Remdesivir and Quercetin were selected for MD simulation studies.

3.2. Molecular dynamic simulation studies

MD simulation studies were carried out to understand the stability of protein ligand interaction. As discussed earlier,

Table 4. Toxicological data of selected active phytoconstituents (QSAR Models).

Toxicity study	Toxicity test	3,5-Dicaffeoyl quinic acid	Quercetin	Remdesivir
Mutagenicity	Mutagenicity (Ames test) CONSENSUS model – assessment	NON-Mutagenic (Consensus score: 0.5)	Mutagenic (Consensus score: 1)	Mutagenic (Consensus score: 0.1)
	Mutagenicity (Ames test) CONSENSUS model – prediction	NON-Mutagenic	Mutagenic	Mutagenic
	Mutagenicity (Ames test) model (CAESAR) – assessment	0.823**	1.0***	0.505*
	Mutagenicity (Ames test) model (CAESAR) – prediction	NON-Mutagenic	Mutagenic	NON-Mutagenic
	Mutagenicity (Ames test) model (SarPy/IRFMN) – assessment	0.745**	0.5*	0.559*
	Mutagenicity (Ames test) model (SarPy/IRFMN) – prediction	NON-Mutagenic	NON-Mutagenic	NON-Mutagenic
	Mutagenicity (Ames test) model (ISS) – assessment	0.737**	0.85**	0.506*
	Mutagenicity (Ames test) model (ISS) – prediction	NON-Mutagenic	Mutagenic	Mutagenic
	Mutagenicity (Ames test) model (KNN/Read-Across) – assessment	0.645*	1.0***	0.619*
	Mutagenicity (Ames test) model (KNN/Read-Across) – prediction	NON-Mutagenic	Mutagenic	Mutagenic
Carcinogenicity	Carcinogenicity model (CAESAR) – assessment	outside applicability domain of the model	0.793**	0.156*
	Carcinogenicity model (CAESAR) – prediction	Carcinogen	NON-Carcinogen	NON-Carcinogen
	Carcinogenicity model (ISS) – assessment	0.5*	0.85**	0.506*
	Carcinogenicity model (ISS) – prediction	NON-Carcinogen	Carcinogen	Carcinogen
	Carcinogenicity model (IRFMN/ ISSCAN-CGX) – assessment	0.666**	1.0***	0.492*
	Carcinogenicity model (IRFMN/ ISSCAN-CGX) – prediction	Possible NON-Carcinogen	Carcinogen	Carcinogen
	Carcinogenicity oral classification model (IRFMN) – assessment	0.5*	0.525*	0.354*
	Carcinogenicity oral classification model (IRFMN) – prediction	NON-Carcinogen	Carcinogen	Carcinogen
	Carcinogenicity oral Slope Factor model (IRFMN) – assessment	0.85**	0.541*	0.199*
	Carcinogenicity oral Slope Factor model (IRFMN) – prediction [log(1/(mg/kg-day))]	1.26	0.8	3.59
	Carcinogenicity inhalation classification model (IRFMN) – assessment	0.5*	0.75**	0.287*
	Carcinogenicity inhalation classification model (IRFMN) – prediction	NON-Carcinogen	Carcinogen	Carcinogen
	Carcinogenicity inhalation Slope Factor model (IRFMN) – assessment	0.85**	0.394**	0.199*
	Carcinogenicity inhalation Slope Factor model (IRFMN) – prediction [log(1/(mg/kg-day))]	0.03	1.22	1.21
Developmental Toxicity	Developmental Toxicity model (CAESAR) – assessment	0.74**	0.886**	0.5*
	Developmental Toxicity model (CAESAR) – prediction	NON-Toxicant	Toxicant	NON-Toxicant
	Developmental/Reproductive Toxicity library (PG) – assessment	0.5*	0.881**	Toxicant (low reliability)
	Developmental/Reproductive Toxicity library (PG) – prediction	NON-Toxicant	Toxicant	Toxicant
Zebrafish	Zebrafish embryo AC50 (IRFMN/ CORAL) – assessment	520.15 ug/L (low reliability)	179309.14 ug/L (low reliability)	192108.37 ug/L (low reliability)
	Zebrafish embryo AC50 (IRFMN/ CORAL) – prediction [log(umol/l)]	0	2.77	2.5
Oestrogen	Estrogen Receptor Relative Binding Affinity model (IRFMN) – assessment	0.866***	0.99***	0.354*
	Estrogen Receptor Relative Binding Affinity model (IRFMN) – prediction	Inactive	Active	Inactive
	Estrogen Receptor-mediated effect (IRFMN/CERAPP) – assessment	0.868***	0.797**	0.724**

(*continued*)

Table 4. Continued.

Toxicity study	Toxicity test	3,5-Dicaffeoyl quinic acid	Quercetin	Remdesivir
	Estrogen Receptor-mediated effect (IRFMN/CERAPP) – prediction	NON-active	Active	NON-active
Androgen	Androgen Receptor-mediated effect (IRFMN/COMPARA) – assessment	0.872***	0.5*	0.612*
	Androgen Receptor-mediated effect (IRFMN/COMPARA) – prediction	NON-active	Active	NON-active
Thyroid	Thyroid Receptor Alpha effect (NRMEA) – assessment	0.958***	0.94***	0.866***
	Thyroid Receptor Alpha effect (NRMEA) – prediction	Inactive	Inactive	Inactive
	Thyroid Receptor Beta effect (NRMEA) – assessment	0.958***	0.94***	0.866***
	Thyroid Receptor Beta effect (NRMEA) – prediction	Inactive	Inactive	Inactive
Skin sensitivity	Skin Sensitization model (CAESAR) – assessment	0.706**	0.368*	0.5*
	Skin Sensitization model (CAESAR) – prediction	NON-Sensitizer	Sensitizer	NON-Sensitizer
	Skin Sensitization model (IRFMN/JRC) – assessment	outside applicability domain of the model	0.5*	0.5*
	Skin Sensitization model (IRFMN/JRC) – prediction	Sensitizer	NON-Sensitizer	Sensitizer
Hepatotoxicity	Hepatotoxicity model (IRFMN) – assessment	0.801***	0.781**	0.5*
	Hepatotoxicity model (IRFMN) – prediction	NON-Toxic	Toxic	Toxic

[*low reliability prediction; **medium reliability prediction; and ***high reliability prediction].

three best ligands having highest docking score were selected for MD simulations studies.

Remdesivir was considered as the standard drug molecule in the treatment of covid-19 (Costanzo et al., 2020; C. J. Gordon et al., 2020; Jean & Hsueh, 2020; Wu et al., 2020). Backbone root mean square deviation (RMSD) analysis was carried out to evaluate the stability of Remdesivir into the binding pocket of M^{pro} protein. 100 ns MD simulations of Remdesivir showed that simulations converged after ~60 ns yielding final RMSD within 3 Å. Thus, the last 40 ns simulations were considered for further calculations. It was observed that Remdesivir formed H-bond with various amino acid residues such as LEU4, THR24, THR25, GLU166 and GLN189 whereas hydrophobic bond interaction with VAL3, MET49, LEU50 and PRO168. The RMSD and RMSF plot of protein–ligand and the ligand protein contacts for Remdesivir are shown in Figures 3 and 4, respectively.

Similarly, the interactions of 3,5-Dicaffeoylquinic acid at different time intervals were analysed and check for the stability which showed that, at 40 ns, the proteins got stabilized and ligand was forming interaction with the protein (RMSD difference = 2.5 Å). 3,5-Dicaffeoylquinic acid was forming H-bond interactions with LEU4, MET49, GLN189, THR190, GLN256, ALA255, VAL297 and SER301 while hydrophobic interactions with ALA2, LEU50, ARG188 and PHE305. The RMSD of 3,5-Dicaffeoylquinic acid is shown in Figure 3.

On the other hand, Quercetin was showing stable interactions throughout the simulation period (100 ns) which indicates the stability of the ligand in the binding site pocket of the protein (RMSD Difference = 2.8 Å). Quercetin was interacted with different amino acid such as THR24, THR26, ASN28, HIS41, ASN119, ASN142 and GLN189 residues by forming H-bond interactions. The RMSF plot for Quercetin is shown in Figure 4.

Even though hydrogen bonds are weaker compared to ionic and covalent bonds, they are exploited the most for design of new drug candidate (Bhardwaj et al., 2020; Yunta, 2017). H-bonds are important contributor for the specificity of molecular recognition. The free energy for H-bonds usually ranges from of −12 to −20 kJ/mol, and the binding potential of a ligand rises by almost one order of magnitude per H-bond. Therefore, we observed into the H-bonding pattern of Remdesivir, 3,5-Dicaffeoylquinic acid and Quercetin over the entire 100 ns simulation trajectory.

MD simulations trajectories revealed that 3,5-Dicaffeoylquinic acid (Figure 5(C)) was making more H-bonds in comparison to Remdesivir (Figure 5(A)) and Quercetin (Figure 5(B)) over the entire simulation trajectory. All the selected molecules (3,5-dicaffeoylquinic, Remdesivir and Quercetin) maintained the molecular interactions with the protein. Overall, the interactions analysis showed that at any fraction of time, 3,5-Dicaffeoylquinic acid was making better contacts and better consistency compared to Remdesivir. This suggests that 3,5-Dicaffeoylquinic acid has good affinity towards the substrate-binding pocket of M^{pro} and could probably be natural and readily available drugs for the inhibition of SARS-CoV-2 functional activity. Refer Table S1 for ligand interactions with amino acid residues of protein at different time intervals.

Furthermore, we also carried out MM-GBSA calculations to estimate binding energies or affinity (dG Bind) of ligands. The results of MM-GBSA calculations are shown in Table 3.

Based on the MM-GBSA calculation, the dG bind values for top three molecules such as Remdesivir, 3.5-Dicaffeoylquinic acid and Quercetin was found to −50.14, −45.62 and −40.64, respectively. The obtained binding energies of 3.5-Dicaffeoylquinic acid is closed to Remdesivir

indicating stronger binding to M^{pro} protein. Based on both the results, the order of binding affinity was found to be Remdesivir, 3.5-Dicaffeoylquinic acid and Quercetin. Therefore, 3.5-Dicaffeoylquinic acid and Quercetin might be a novel therapeutic for M^{pro} inhibition and could be helpful in the treatment of coronavirus infection.

3.3. Toxicity results

To access toxicological data, QSAR modelling method performed using VEGA-QSAR (Table 4). The software incorporated algorithm provides evaluation of reliability prediction as Applicability domain index (ADI) value. We used positive results with ADI >0.5, as indicators of reliability effect; low ($0.5 < ADI < 0.6$), medium ($0.6 < ADI < 0.8$) and high ($0.8 < ADI < 1$). The dicaffeolquinic acid does not show mutagenicity (CONSENSUS model, CAESAR, SarPy/IRFMN, ISS and KNN/Read-Across, assessment and prediction) (Votano et al., 2004), does not have carcinogenicity (ISS model, IRFMN/ISSCAN-CGX, IRFMN (oral, inhalation and slope factor model) assessment and prediction) (Fjodorova et al., 2010), do not show developmental toxicity (CAESAR model, PG model assessment and prediction) (Simms et al., 2020), no adverse health effects to humans and ecological species (IRFMN/COMPARA, assessment and prediction) (Mansouri et al., 2020), Inactive for oestrogen and androgen mediated effect (IRFMN/CERAPP, assessment and prediction) (Cotterill et al., 2019; Mansouri et al., 2020) and found to be inactive for Thyroid hormone receptor α/β (NRMEA, assessment and prediction). compounds does not have skin sensitivity (CAESAR model, assessment and prediction) (Chaudhry et al., 2010). No hepatotoxic potential (IRFMN, assessment and prediction). Thus, overall 3,5-Dicaffeoylquinic acid can be suitable candidate for further *in vitro* and *in vivo* assessment for its inhibitory potential against SARS-CoV-2.

4. Conclusion

In summary, results obtained by molecular docking revealed that 3, 5-Dicaffeoylquinic acid from *Cressa cretica* shows highest binding energy as compared to Remdesivir and may inhibit M^{pro} protein required to cut mRNA and for viral assembly. Likewise, the interaction with various amino acid residues of M^{pro} were maintained throughout the 100 ns of molecular dynamic simulations. 3,5-Dicaffeoylquinic acid showed best affinity towards COVID-19 main protease (M^{pro}) of SARS-CoV-2 suggesting it to be novel research molecule. Thus, chemical constituents of *Cressa cretica* become effective to fight against the new corona virus and provide an imminent research attention as they mark the desire interaction with main protease (M^{pro}), which implies a possible antiviral activity. These results encourage further *in vitro* and *in vivo* investigations and also encourage traditional use of *Cressa cretica* preventively and will provide vital information on novel scaffolds for further lead optimization.

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