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Predictive medicinal metabolites from *Momordica dioica* against comorbidity related proteins of SARS-CoV-2 infections

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ABSTRACT

Momordica dioica have proven medicinal potential of antidiabetic, antiviral and immune stimulating properties. Flavonoids and triterpenoids from *M. dioica* were more extensively investigated for antiviral, antidiabetic and immunomodulatory activities. In this present study, we have predicted the reported bioactive flavonoids and triterpenoids of the plant against the SARS-CoV-2 main protease, RNA-dependent RNA polymerase (RdRp), spike protein, angiotensin converting enzyme (ACE-2) receptor and dipeptidyl peptidase (DPP4) receptor through molecular docking and *in silico* ADME predictions methods. According to the binding affinities, the two triterpenoids, hederagenin and oleanolic acid exhibited the best docking scores with these proteins than the catechin and quercetin with compared to standard remdesivir, favipiravir and hydroxychloroquine. The *in vitro* protein-drug studies have also showed significant interaction of catechin and quercetin compounds than standard drugs. The *in silico* binding studies correlated with the *in silico* binding studies. Further, *M. dioica* being used as antidiabetic and its metabolite had significant interaction with DDP4, a comorbidity protein involved in aiding the viral entry. Out of all the natural ligands, quercetin was reported relatively good and safe for humans with high gastrointestinal tract permeability and poor blood brain barrier crossing abilities. Hence, *M. dioica* phytocompounds reflects promising therapeutic properties against SARS-CoV-2 infections under comorbid conditions such as diabetes, cardiovascular disease and kidney disorders.

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1. Introduction

Momordica dioica is a perennial dioecious climber creeper plant belonging to the Cucurbitaceae family. It has many nutritional and disease combating properties still it is an underutilized plant which needs to be explored to justify its therapeutical roles. All the parts of plant have been reported for secondary metabolites that includes alkaloids, steroids, triterpenoids, saponins, phenolic compounds and flavonoids (Figure 1). The roots have reported for anti-diabetic, anti-viral, immunostimulant, anti-inflammatory, anti-pyretic, antiseptic, anti-ulcerative, anti-toxic, anti-perspirant, anti-hemorrhoidal, bowel infection reducer and skin softening properties. Stearic acid, steroids like α-spinasterol octadecenoate, α-spinasterol-3-O-β-D-glucopyranoside, oleanolic acid, gypsogenin, hederagenin and triterpenoids like 3β-O-benzoyl-6-oxo-ursolic acid, 3β-O-benzoyl-11-oxo-ursolic acid, 3-O-β-D-glucopyranosyl hederagenin, 3-O-β-D-glucopyranosyl gypsogenin, 3-O-β-D-glucuronopyranosyl gypsogenin were identified in the methanolic extract of roots of this plant (Talukdar & Hossain, 2014). Based on literature survey, the flavonoids such as catechin & quercetin and phyto-steroids such as oleanolic acid and hederagenin have shown anti-viral and antidiabetic potentials that can serve as potential natural drug candidates against coronavirus (Hastantram et al., 2020; Thiruvengadam et al., 2016).

The COVID-19 pandemic is spreading across the world at an alarming rate and with lack of specific drugs and vaccines. The increasing mortality and morbidity rates necessitate the urge to discover and develop potential cure against the virus and the comorbid conditions. The best knowledge of medicinal plants has always been a savior that offer therapeutic effect with less side effects. Emerging data suggest that COVID-19 patients with other comorbid conditions such as diabetes, hypertension, cardiac and pulmonary diseases are more susceptible with increased mortality when compared to general populations (Singh et al., 2020). These subgroups of COVID-19 patients with associated comorbidities are due to higher expression of ACE-2 and DPP4 proteins that aid more viral entry and replication (Zou et al., 2020). Currently, no specific therapies for COVID-19 are available and investigations regarding the treatment of COVID-19 are lacking. There have been several attempts to check if existing solutions and compounds that could be repositioned for COVID-19 (Zou et al., 2020). The plant derived secondary

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Figure 1. *Momordica dioica* plant and its part illustrations.

metabolites and compounds have reported to possess antiviral properties. Therefore, this study focuses on the evaluation of outcome in diabetic patients with SARS-CoV-2 flavonoids and triterpenoid such as quercetin, catechin, oleanolic acid and hederagenin from *M. dioica* to predict inhibitory potential of main protease (M^{pro})/chymotrypsin-like protease ($3CL^{pro}$) (Figure 2(a)), RdRp (Figure 2(b)), spike protein with ACE-2 receptor (Figure 2(c)) and MERS-CoV with human DPP4 receptor (Figure 2(c)) using molecular docking analysis.

People with diabetes are at higher risk to develop complications with a corona virus infections. The recent data from the 2019 novel coronavirus (2019-nCoV) also confirms that diabetes, along with advanced age and/or presence of cardiovascular conditions, obesity, is a major risk factor for an adverse outcome. Similarly, Diabetes was also a major contributor to MERS-CoV disease severity and mortality (Iacobellis, 2020). Patients who have diabetes are often have associated with the weakened immune response, both concerning cytokine profile as well as changes in immune responses together with T-cell and macrophage activation. Weaker glycemic control harms several aspects of the immune response by a viral infection and in addition to the risk of potential bacterial secondary infection in the lungs. According to studies, the coronavirus predominantly infects human by allowing the cells to enter through the ACE-2 receptor. Spike proteins of coronavirus usually bind to the ACE receptors in the lungs. ACE-2 is a peptidase enzyme and a membrane glycoprotein, mainly expressed in epithelial cells of the lungs, intestine, kidney and blood vessels to regulate the renin-angiotensin-aldosterone system (RAAS). This enzyme is the main component that converts Ang-I to Ang-II (Das et al., 2020) and angiotensin-I (Ang-I) to peptides, angiotensin (1–7) and angiotensin (1–9), separately. Angiotensin (1–7) is responsible for the anti-inflammatory and antioxidant role (Das et al., 2020). Human dipeptidyl peptidase 4 (DPP4) was identified as a functional receptor for the spike protein of the SARS CoV-2 viral entry. DPP4 enzyme is a type II transmembrane glycoprotein, expressed ubiquitously in many tissues, including the immune cells. DPP4 degrades incretins such as glucagon like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide, ultimately leading to reduced insulin secretion and abnormal visceral adipose tissue metabolism (Iacobellis, 2020). The renin–angiotensin–aldosterone system (RAAS) component ACE2 and DPP4 are proteins dysregulated in diabetes (Cuschieri & Grech, 2020; Valencia et al., 2020). The entry of SARS-CoV-2 within the host cell triggers an inflammatory response, recruitment of T helper cells with the production of interferon gamma leading to a cytokine storm. Hence, considering the cellular mechanisms triggered by SARS-CoV-2 and the pathophysiology of diabetes, individuals with diabetes are more susceptible to a cytokine storm with potential organ damage if infected by COVID-19 (Iacobellis, 2020). ACE-2 is also expressed in the pancreas. So, the entry of the coronavirus into the pancreatic islets may cause an acute beta-cell dysfunction with a resultant acute hyperglycemic state. Hence, individuals with diabetes are vulnerable to COVID-19 infection leading to an uncontrolled hyperglycemic status (Cuschieri & Grech, 2020; Valencia et al., 2020). To summarize, potential mechanisms that may increase the susceptibility for COVID-19 in patients with DM includes higher affinity cellular binding and efficient virus entry, decreased viral clearance, diminished T cell function, increased susceptibility to hyperinflammation and cytokine storm syndrome, presence of cardiovascular diseases (Muniyappa & Gubbi, 2020). Liu et al., have successfully crystallized the main protease (M^{pro})/chymotrypsin-like protease ($3CL^{pro}$) from COVID-19 has been structured and repositioned in the Protein Data Bank (PDB) (Liu et al., 2020; Quimque et al., 2020). This protease represents a potential target for the inhibition of corona virus for entry and replication. In this study, the binding affinity of mentioned ligands to main protease (6LU7), spike protein with ACE2 receptor (6M0J), RdRp (6M71) and MERS-CoV with hDPP4 receptor (4L72) proteases was examined and compared with

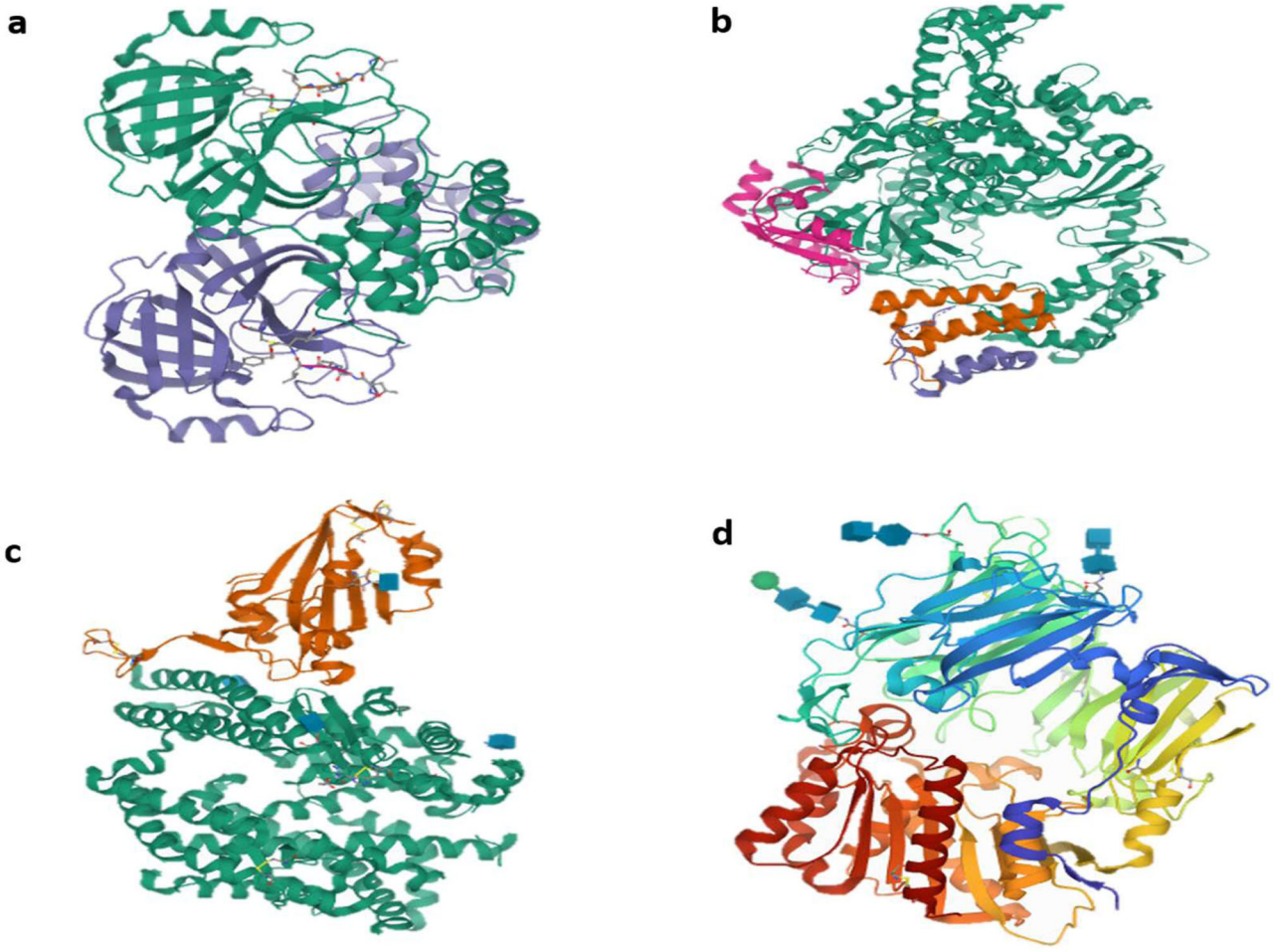

Figure 2. Structures of target proteins (a) Chymotrypsin-like protease, 3CLpro (PDB ID: 6LU7), (b) RNA dependent RNA polymerase, RdRp (PDB ID: 6M71), (c) Spike Protein with ACE2 receptor (PDB ID: 6M0J), (d) MERS-CoV with human DPP4 (PDB ID: 4L72).

existing anti-viral drugs like remdesivir, hydroxychloroquine and favipiravir.

Materials and methods

Structural properties of selected target proteins

The methodology for identification of active compounds with the proteins were followed by the protein selection, ligands and proteins, binding site prediction, molecular docking and prediction of drug likeliness and ADME assay (Figure 3).

Chymotrypsin-like protease ($3CL^{pro}$) or main protease (M^{pro})

The 3CLpro protease or main protease of SARS-CoV-2 does cleavage at the C-terminal of polyproteins at eleven sites to give rise to other non-structural proteins that are required in viral replication. The main protease has three domains I, II and III (Figure 2(a)). The active site having the catalytic dyad (Cys145 and His41) present between the domain I and II shows the protease activity. The third domain is connected to rest of the protein structure by a long loop and this gap contains a larger pocket relative to the active site. Thus, drugs that target this protease play a critical role in inhibiting the replication of the virus (Liu et al., 2020; Quimque et al., 2020).

RNA dependent RNA polymerase (RdRp)

The SARS-CoV2 RdRp model, consisting of 801 amino acids, was designed using the SARS-CoV RdRp as a homolog. The active site of RdRp is highly conserved and contains aspartate residues that protrude from the beta-turn joining the b15 and b16 (Figure 2(b)). With a vital role in viral genome replication, the active site residues of RdRp and most of the 5-Åregion surrounding it are surface accessible and can bind to free nucleotides, including ATP, GTP, CTP and UTP. Similar to the viral proteases, RdRp is regarded as a molecular target for the development of new drugs against SARS-CoV2 (Liu et al., 2020). The replication mechanism of SARS-CoV-2 is chiefly led by RdRp, a complex of nsp12, nsp8 and nsp7. The structure resembles a partially curled right-hand grip and can be assorted into three regions namely the palm, the thumb and the fingers (Quimque et al., 2020).

Spike protein (S protein) bounded to ACE2 receptor

The spike protein consists of three regions: Ectodomain (ED) region, Intracellular domain region and Transmembrane region. The S protein is type I-transmembrane (TM) protein which appear as clove shaped. The ED region consists of two receptor binding domains (RBD), S1 and trimeric stalk containing S2 subunit associated on C-terminal (Figure 2(c)). This

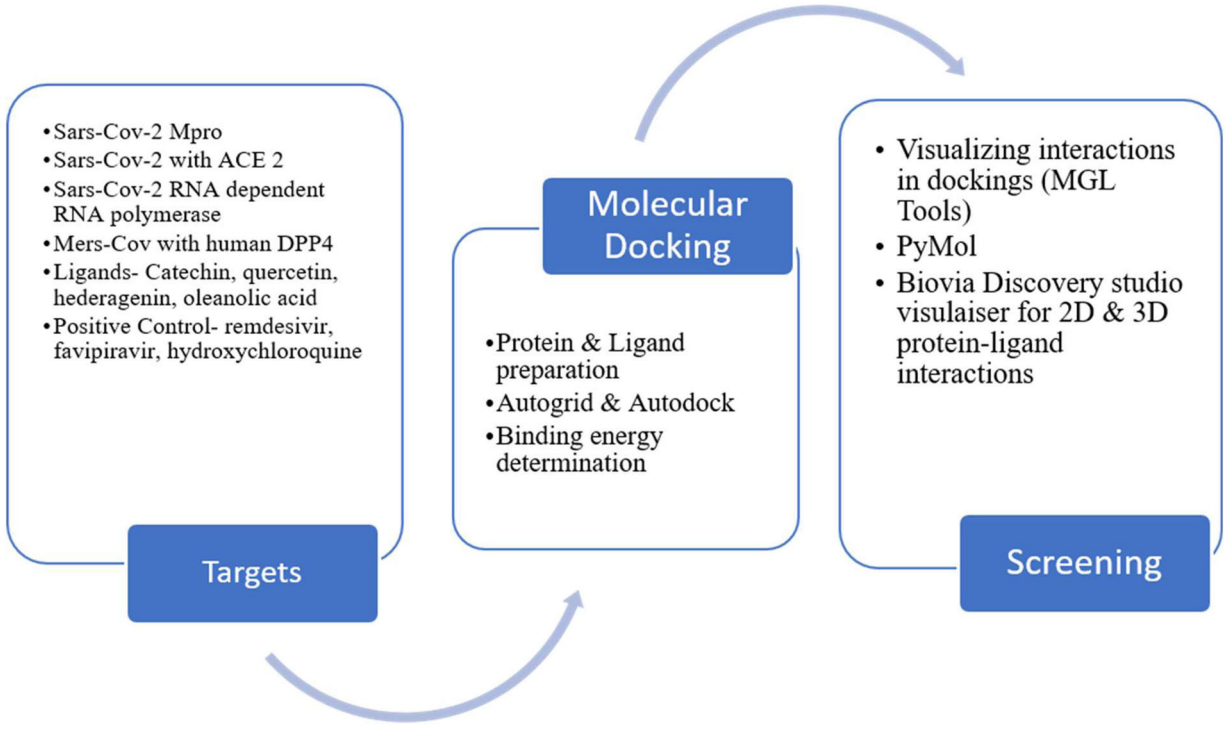

Figure 3. Pictorial illustration of approaches for identification of *in silico* interactions.

trimeric forms on the surface of the virus give the virus a crown-like appearance (Hoffmann et al., 2020; Matsumyama et al., 2020; Narkhede et al., 2020; Vijayakumar et al., 2020). The S protein is majorly responsible for viral entry into the host. It binds to host ACE-2 receptor and facilitates this binding. This protein is considered as a potential target for drug discovery because S1 domain and host ACE2 for SARS-CoV and Dipeptidyl peptidase-4 (DPP4) for MERS-CoV associated with host and viral membrane fusion mediated by S2 segment potentiate the coronavirus to release its RNA in host cell (Vijayakumar et al., 2020).

MERS-CoV associated with human DPP4

MERS-CoV engages the transmembrane dipeptidyl peptidase 4 (DPP4, also known as CD26) as the primary receptor. Middle East respiratory syndrome coronavirus (MERS-CoV) infects host cells through binding the receptor binding domain (RBD) on its spike glycoprotein to human receptor dipeptidyl peptidase 4 (hDPP4). hDPP4 extracellular domain consists of a variable N-terminal eight-blade β-propeller domain and a conserved C-terminal α/β-hydrolase domain (Figure 2(d)). The RBD–hDPP4 crystal structure showed that the viral RBD recognized blades IV and V of the DPP4 β-propeller domain (Song et al., 2014; Vankadari & Wilce, 2020).

Protein preparation

The high-resolution three-dimensional crystal structure of 3CLpro (PDB ID: 6LU7), RdRp (PDB ID: 6M71), S protein with ACE2 (PDB ID: 6M0J) and MERS-CoV with human DPP4 (PDB ID: 4L72) were retrieved from Protein Data Bank (PDB). It is an archived database for the three-dimensional crystal structures of biological macromolecules, worldwide. The macromolecule was prepared using AutoDock MGL Tools 1.5.6 by deleting the water molecules and adding polar hydrogen atoms and Kollman charges. The protein file was saved in the pdbqt format for further analysis.

Structures and biological properties of selected natural ligands

Catechin is a polyphenolic antioxidant flavonoid with molecular formula $C_{15}H_{14}O_6$ has proven to possess antiviral and anti-inflammatory activities (Figure 4(a)). Quercetin is polyphenolic flavonoid belonging to class of organic compounds referred as flavonoids (Figure 4(b)). Quercetin with molecular formula $C_{15}H_{10}O_7$ has potential antiviral, anti-inflammatory, antiallergy and chemo-preventive activities (Figure 4(c)). Hederagenin, sapogenin with molecular formula $C_{30}H_{48}O_4$ (Figure 4(d)) and oleanolic acid, pentacyclic triterpenoid with molecular formula $C_{30}H_{48}O_3$ (Figure 4(d)).

Existing antiviral drugs were used as positive control (Wishart et al., 2006) (Drug Bank https://www.drugbank.ca/) (Figure 4(e–g)). Remdesivir was originally investigated as a treatment for Ebola virus, but has potential to treat a variety of RNA viruses. Its activity against the coronavirus (CoV) family of viruses, such as SARS-CoV and MERS-CoV, was described in 2017 and it is also being investigated as a potential treatment for SARS-CoV-2 infections. Remdesivir was granted an FDA Emergency Use Authorization on 1st May 2020. Remdesivir is a nucleoside analog used to inhibit the action of RNA polymerase that terminate the RNA transcription (Figure 4(e)) (Sheahan et al., 2020). Hydroxychloroquine is a racemic mixture consisting of an R and S enantiomer (Figure 4(f)). It is an

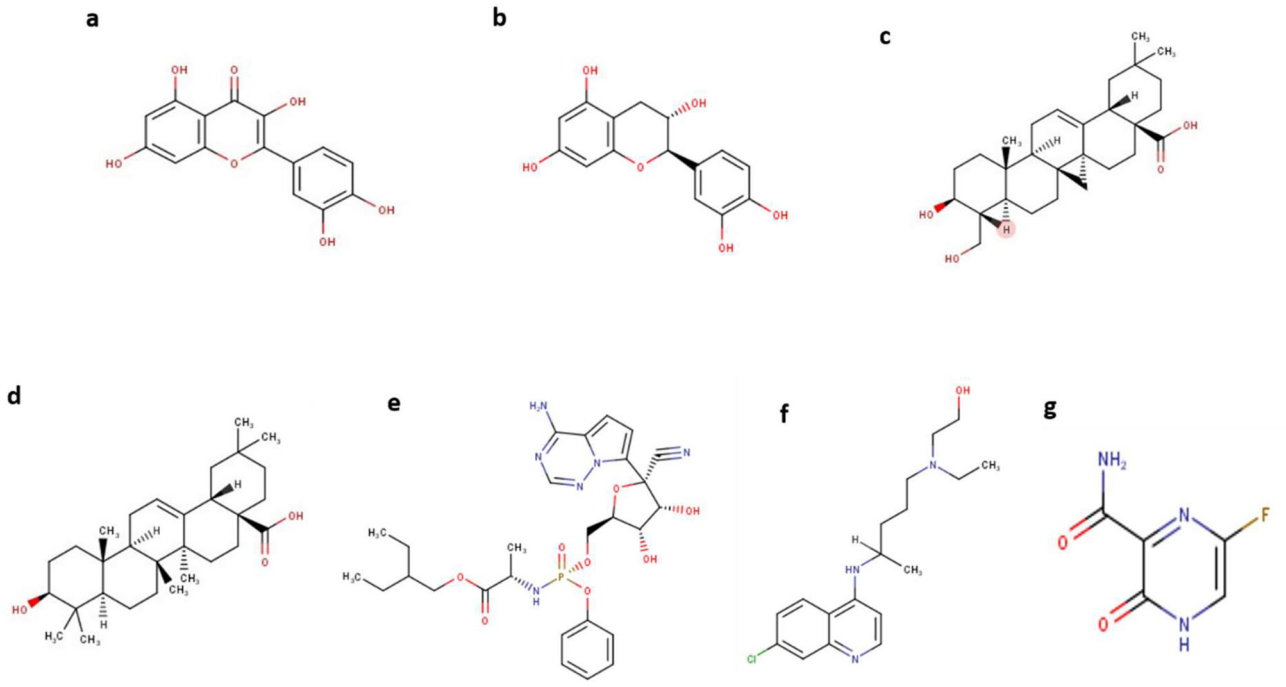

Figure 4. Structures of selected natural compounds: (a) Catechin, (b) Quercetin, (c) Hederagenin, (d) Oleanolic acid and standard control drugs (e) Remdesivir, (f) Hydroxychloroquine, (g) Favipiravir.

aminoquinoline which is commonly prescribed medication in the treatment of uncomplicated malaria, rheumatoid arthritis, chronic discoid lupus erythematosus and systemic lupus erythematosus (Liu et al., 2020). It was developed during World War II as a derivative of quinacrine and was granted FDA approval in 1955. Since after investigating, it was found that accumulation of hydroxychloroquine raises the pH which thereby prevents the virus particles (corona viruses) from utilizing their activity for fusion and entry into the cell, the FDA revoked an emergency use authorization on 15th June 2020. Hydroxychloroquine also inhibits terminal glycosylation of ACE2, the receptor that SARS-CoV and SARS-CoV-2 target for cell entry. Favipiravir is a modified pyrazine analog that was initially approved for therapeutic use in resistant cases of influenza (Figure 4(g)). Favipiravir has been investigated for the treatment of life-threatening pathogens such as Ebola virus, Lassa virus and now COVID-19. Favipiravir functions as prodrug and undergoes ribosylation and phosphorylation intracellularly to become the active favipiravir-RTP (Favipiravir-ribofuranosyl-5′-triphosphate). Favipiravir-RTP binds to and inhibits RNA dependent RNA polymerase (RdRp), which ultimately prevents viral transcription and replication. The RdRp catalytic domain is expected to be similar for other RNA viruses and hence it contributes to favipiravir's broad-spectrum coverage (Liu et al., 2020; Sheahan et al., 2020).

Ligand preparation

The three-dimensional structures of selected natural compounds and existing antiviral drugs, i.e. Catechin (PubChem CID: 9064), Quercetin (PubChem CID: 5280343), Hederagenin (PubChem CID: 73299), Oleanolic acid (PubChem CID: 10494), Remdesivir (PubChem CID: 121304016), Favipiravir (PubChem CID: 492405), Hydroxychloroquine (PubChem CID: 3652) were retrieved from PubChem database in sdf format. PubChem is a chemical substance and biological activities repository consisting of three databases, including substance, compound and bioassay databases [4]. The sdf format of these ligands were converted to pdb format using Open Babel software (Liu et al., 2020). The ligands were prepared for docking in AutoDock MGL Tools 1.5.6 in pdbqt format by removing water molecules, adding polar hydrogen atoms and computing Gasteiger charges.

Binding site prediction

In this study, the amino acid residues present at the active site of the proteases were predicted and determined from the related literature, using the metaPocket server (Huang, 2009; Morris et al., 2009) and through the Biovia Discovery Studio Visualizer 2020 (Design, 2014). The determination of the amino acids in the active site was used to analyze the Grid box and to carry out site-specific docking. Discovery Studio is an offline life sciences software that provides tools for protein, ligand and pharmacophore modeling (Hastantram et al., 2020).

Molecular docking studies

Molecular docking was conducted on the ligands using the AutoDock 4.2.6 (Morris et al., 2009) and AutoDock Vina (Trott & Olson, 2020) to get insight into their binding preferences within the active site of the receptor. The amino acids in the active site of the macromolecule was selected and the grid

box was used to obtain the X, Y and Z coordinates. Using the protease. pdbqt file, ligand pdbqt file and the X, Y and Z coordinates, binding affinity was determined using AutoDock Vina (Trott & Olson, 2020). On the basis of binding energies, the potency of selected natural compounds was compared to the control/reference drugs. The 3 D structure of the protease-ligand interaction and the 2 D structure of the molecular interaction were then visualized using AutoDock tools, PyMol 2.4 and Biovia Discovery Studio Visualizer (2020).

In silico *drug likeness, ADME prediction*

ADME of the selected natural ligands is studying the pharmacokinetic properties of the ligands when function in the bodily system. It is the study of what body does to the drug. The computational prediction of the absorption, distribution, metabolism and excretion (ADME) properties of ligands were done using SwissADME software (Daina et al., 2017). Lipinski's 'rule-of-five' which analyzes the biochemical features of a drug that may influence its absorption and permeation across cell membranes. Lipinski's criteria states that for a compound to exhibit drug likeness, at least three of the following criteria must be fulfilled such as no more than 5 hydrogen bond donors, no more than 10 hydrogen-bond acceptors, molecular mass less than 500 Daltons, an octanol-water partition coefficient or lipophilicity (log *P*) that does not exceed 5. Since all the numbers in these conditions of the rule are multiples of 5, hence the rule is called Lipinski's rule of five.

In vitro *validation of interaction studies by fluorescence quenching studies of viral proteins with compounds*

The phytocompounds in this studies such as Quercetin (117-39-5), Catechin (7295-85-4), Hederagenin (465-99-6), Oleanolic acid (508-02-1), Remdesivir (30354), Favipiravir (T-705), Hydroxychloroquine (747-36-4), Mpro-3CL Protease from coronavirus SARS-COV-2 (SAE0172), recombinant DPP4 (D4943) and recombinant spike protein S1 & S2 (S-ECD) protein (SAB5700592), was purchased from Sigma Aldrich, USA. The recombinant RNA dependent RNA Polymerase (MSB7135958) was purchased from MyBioSource Ltd. The protein-compounds interactions were studies by spectrofluorimetric assay at 280 nm excitation and emission at 290 nm at room temperature. Briefly, the protein at the concentration of 100 nm was incubated at different concentration of compounds ranging from 0.005 to 1 μM for 5 min at room temperature in 96-well plates. After 5 min, the plate was read at 290 nm emission that was excited at 280 nm. The IC_{50} concentration of compounds was calculated using the linear regression curve from dose-response studies.

Statistical analysis

Results were expressed as mean ± SD of triplicate experiments. The statistical analysis was performed using the SPSS software. The comparison between the compounds was performed by one-way ANOVA test. Effects of doses in the further experiments were analyzed using the Mann-Whitney U tests. Results were considered statistically significant with $p < 0.05$ at 95% confidence levels by two tailed tests.

Results

SARS-CoV-2 main protease, RdRp, spike protein with ACE2 receptor and MERS-CoV with hDPP4 receptor, the four natural ligands Catechin, Quercetin, Hederagenin and Oleanolic acid showed best docking scores as compared to reference drugs such as Remdesivir, Favipiravir and Hydroxychloroquine upon molecular modeling. The binding affinities with surrounding amino acid residues of ligand interactions (Table 1).

Sars-CoV-2 M^{pro} with ligands and control drugs

Through *in silico* docking studies, the four natural ligands and three control drugs were analyzed for their binding affinity to the main protease (6LU7). The obtained binding affinities are Catechin (−6.7 kcal/mol), Quercetin (−6.6 kcal/mol), Hederagenin (−8.0 kcal/mol), Oleanolic acid (−8.5 kcal/mol), Favipiravir (−5.7 kcal/mol), Hydroxychloroquine (−5.3 kcal/mol) and Remdesivir (−6.4 kcal/mol) (Table 1). The triterpenoids oleanolic acid and hederagenin showed the highest affinity to the protease. The hederagenin was properly positioned between Arg131, Thr199, Leu286 and Asp289 (Figures 5 and 6). The Arg131 and Thr199 shared hydrogen bonding with Hederagenin. While the Oleanolic acid was positioned between Arg131, Thr199, Tyr237, Leu272 and Leu286. The residues Leu272, Tyr237 and Leu286 share alkyl and pi-alkyl interaction with oleanolic acid while Thr199 and Arg131 shared hydrogen bonding. The amino acid residues are Arg131, Thr199 and Leu286 are common in both the cases, since the compounds show structural similarity and both of them belong to same class of plant metabolites. These ligands showed potentially high biological activity as compared to the control drugs in terms of binding affinities. The amino acid residue interactions at the binding site and the docked pose of binding of natural ligand or control drug to the protease.

Studies of SARS-CoV-2 RdRp with ligands and control drugs

With the help of *in silico* docking studies, the four natural ligands and three control drugs were analyzed for their binding affinity to the SARS-CoV-2 RdRp (6M71). The obtained binding affinities are (Table 1) Catechin (−6.8 kcal/mol), Quercetin (−7.6 kcal/mol), Hederagenin (−8.2 kcal/mol), Oleanolic acid (−8.3 kcal/mol), Favipiravir (−5.1 kcal/mol), Hydroxychloroquine (−5.5 kcal/mol) and Remdesivir (−6.9 kcal/mol). The triterpenoids Oleanolic acid and Hederagenin showed the highest affinity to the protease. The hederagenin was properly positioned between Lys621 and Trp800 (Figures 7 and 8). The Trp800 shared Pi-Alkyl interaction with Hederagenin while Lys621 showed unfavorable donor-donor bond to hederagenin moiety. While the Oleanolic acid was positioned between Cys622 and Trp800. The Trp800 shared Pi-Alkyl interaction with Oleanolic acid as similar to Hederagenin while Cys622 showed Alkyl bond to hederagenin

Table 1. Binding energy values and site residues of ligands against the coronavirus proteases.

Ligands	Coronavirus proteases and host receptors							
	Main protease (6LU7)		RNA dependent RNA polymerase (6M71)		Spike protein with ACE2 receptor (6M0J)		MERS-CoV with human DPP4 receptor (4L72)	
	Binding affinity (kcal/mol)	Binding site residues	Binding affinity (kcal/mol)	Binding site residues	Binding affinity (kcal/mol)	Binding site residues	Binding affinity (kcal/mol)	Binding site residues
Catechin	−6.7	Thr199, Leu286, Leu287	−6.8	Tyr619, Asp760, Asp761, Lys798, Trp800	−7.5	Phe40, Gly352, Asp382, Phe390, Arg393	−7.2	Ser275, Ala282, Thr283, Gly335
Quercetin	−6.6	Arg131, Lys137, Thr199, Tyr239, Leu286, Asp289	−7.6	Val315, Arg349, Glu350, Pro461, Asn628, Pro677	−7.8	Ala348, Gly352, Asp382, Phe390, Arg393, Asn394, His401	−7.8	Phe269, Asn281, Ala282, Asp537(B), Val561(B), Ala562(B)
Hederagenin	−8.0	Arg131, Thr199, Leu286, Asp289	−8.2	Lys621, Trp800	−8.3	Asn33, His34, Pro389	−7.9	Leu366, Ala409, Leu410, Phe461
Oleanolic acid	−8.5	Arg131, Thr199, Tyr237, Leu272, Leu286	−8.3	Cys622, Trp800	−8.5	Asn33, His34, Pro389, Lys417(E)	−8.2	Phe364, Leu366, Ala409, Leu410, Phe461
Favipiravir	−5.7	Gln110, Thr111, Asn151, Thr292, Asp295	−5.1	Ser15(C), Gln19(C), Lys411, Pro412, Tyr546, Asp846	−5.3	Arg454(E), Arg457(E), Lys458(E), Asp467(E), Ser469(E), Glu471(E)	−5.6	Ile185, Trp187, Tyr225, Gln227, Phe269, Ala282, Ser284
Hydroxychloroquine	−5.3	Arg131, Thr199, Tyr239, Leu272, Met276, Leu286, Leu287, Asp289	−5.5	Phe441, Ile548, Arg836, Ala840, Phe843, Val844, Asp845, Val848, Leu854, Arg858,	−6.2	Phe40, Trp349, Asp350, Asp382, Tyr385, Phe390, Arg393,	−5.4	His363, Phe364, Leu366, Ala409, Leu410
Remdesivir	−6.4	Arg131, Lys137, Thr199, Tyr237, Tyr239, Leu272, Leu287, Asp289	−6.9	Arg249, Thr394, Cys395, Phe396, Arg457, Pro461, Pro677	−7.9	Phe40, Ala348, Trp349, Asp350, His378, Tyr385, Phe390, Asn394	−7.4	Ser212, Trp215, Asp302, Trp305, Arg358

moiety. The amino acid residue Trp800 is common in both the cases, since the compounds show structural similarity and both of them belong to same class of plant metabolites. These ligands showed potentially high biological activity as compared to the control drugs in terms of binding affinities.

Studies of SARS-CoV-2 spike protein with ACE2 receptor with ligands and control drugs

In silico docking studies of the four natural ligands and three control drugs to the spike protein with ACE2 receptor (6M0J) for their binding affinity were noted. The obtained binding affinities are (Table 1) Catechin (−7.5 kcal/mol), Quercetin (−7.8 kcal/mol), Hederagenin (−8.3 kcal/mol), Oleanolic acid (−8.5 kcal/mol), Favipiravir (−5.3 kcal/mol), Hydroxychloroquine (−6.2 kcal/mol) and Remdesivir (−7.9 kcal/mol). The triterpenoids, Oleanolic acid and Hederagenin showed the highest affinity to the protease. The hederagenin was properly positioned between Asn33, His34, Pro389 (Figures 9 and 10). The His34 and Pro389 shared alkyl and pi-alkyl interaction with hederagenin. While the Oleanolic acid was positioned between Asn33, His34, Pro389, Lys417 (E). The residues His34, Pro389, Lys417 share alkyl and pi-alkyl interaction with oleanolic acid. The amino acid residues are Asn33, His34 and Pro389 are common in both the cases, except Lys417 of E chain of the protease which is included in interaction of oleanolic acid to the protease, since the compounds show structural similarity and both of them belong to same class of plant metabolites. These ligands showed high potentially high biological activity as compared to the control drugs in terms of binding affinities.

Studies of MERS-CoV with human DPP4 receptor with ligands and control drugs

The *in silico* docking studies of the four natural ligands and three control drugs to the MERS-CoV with DPP4 receptor for their binding affinity were noted. The obtained binding affinities are (Table 1) Catechin (−7.2 kcal/mol), Quercetin (−7.8 kcal/mol), Hederagenin (−7.9 kcal/mol), Oleanolic acid (−8.2 kcal/mol), Favipiravir (−5.6 kcal/mol), Hydroxychloroquine (−5.4 kcal/mol) and Remdesivir (−7.4 kcal/mol). The triterpenoids Oleanolic acid and Hederagenin showed the highest affinity to the protease. The hederagenin was properly positioned between Leu366, Ala409, Leu410, Phe461 (Figures 11 and 12). The Leu366, Ala409, Leu410 shared alkyl interaction with Hederagenin whereas Phe461 showed unfavorable donor-donor interaction with hederagenin. While the Oleanolic acid was positioned between Phe364, Leu366, Ala409, Leu410, Phe461. The residues Leu366, Ala409, Leu410 shared alkyl interaction with oleanolic acid while Phe364 and Phe461 shared hydrogen bonding with the Oleanolic acid moiety. The amino acid residues are Leu366, Ala409, Leu410, Phe461 are common in both the cases, since the compounds show structural similarity and both of them belong to same class of plant metabolites. These ligands showed high potentially high biological activity as compared to the control drugs in terms of binding affinities.

In silico *drug likeness and ADME prediction*

For the *in silico,* absorption, distribution, metabolism, excretion (ADME) analysis, the four natural *M. dioica* metabolites Catechin, Quercetin, Hederagenin and Oleanolic acid were

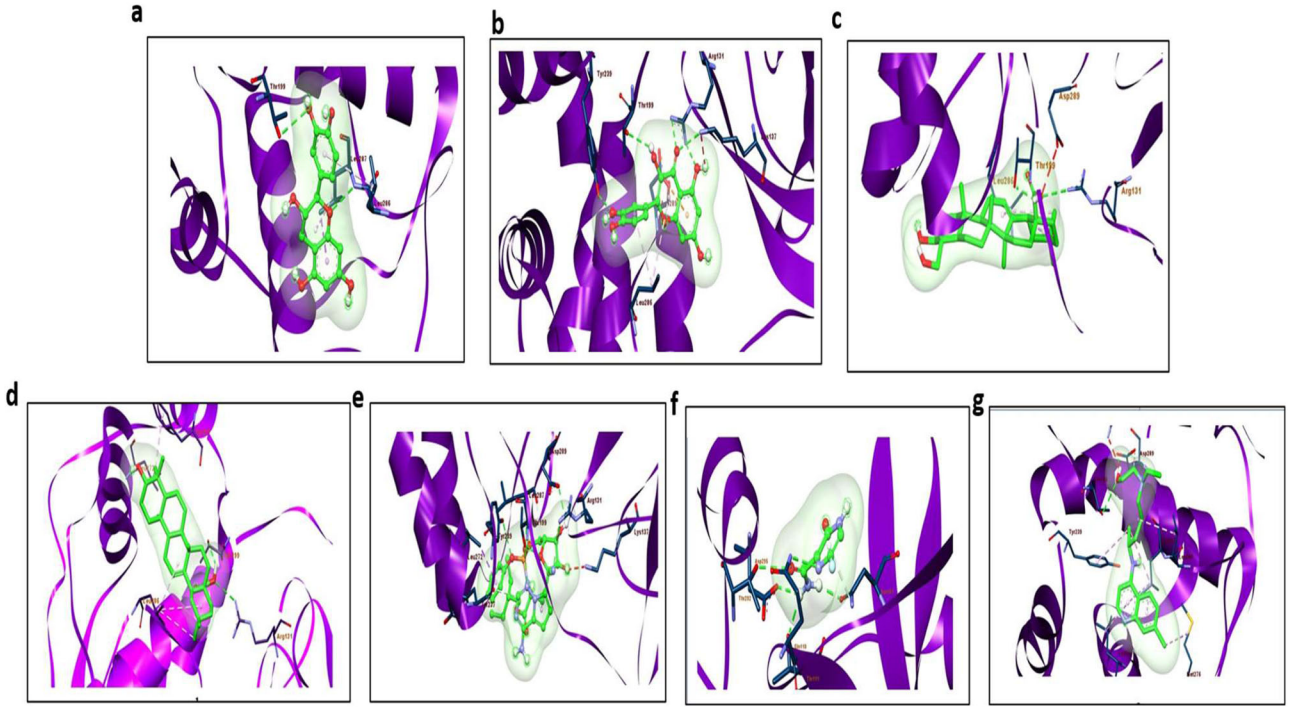

Figure 5. Docked poses of (a) Catechin, (b) Quercetin, (c) Hederagenin, (d) Oleanolic acid, (e) Remdesivir, (f) Favipiravir and (g) Hydroxychloroquine against SARS-CoV-2 main protease (PDB ID: 6LU7).

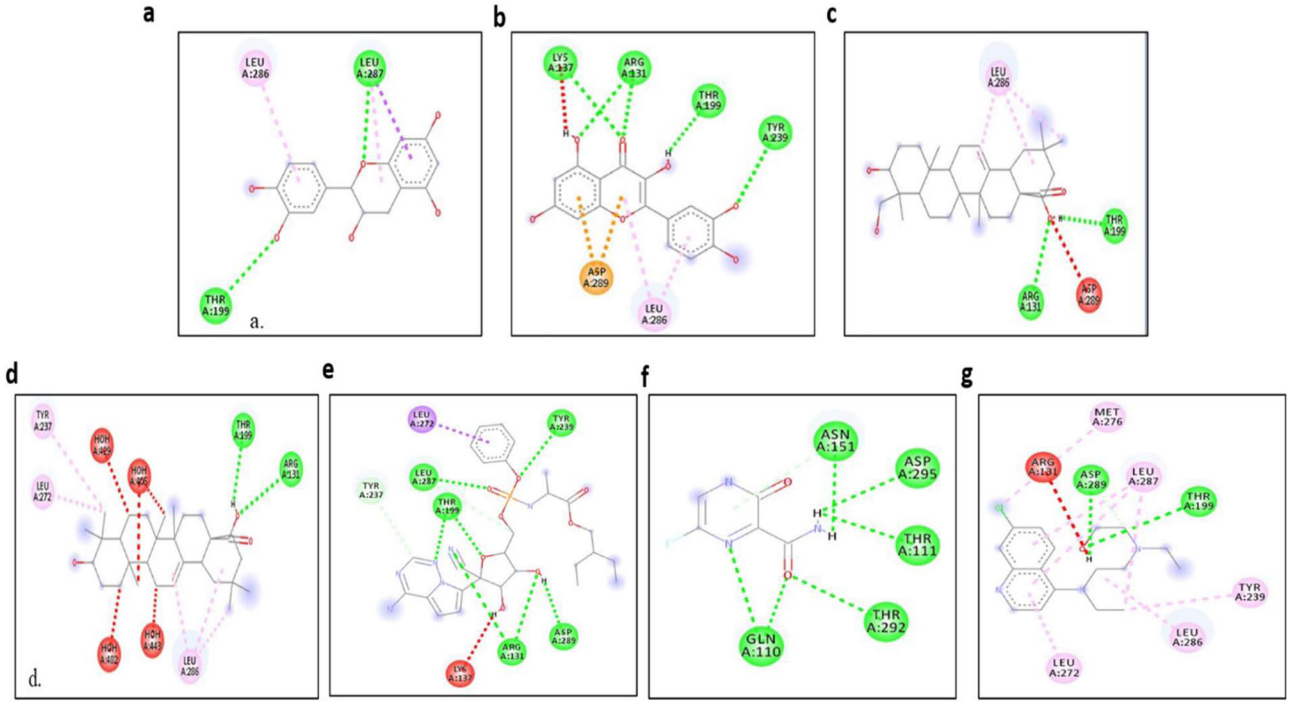

Figure 6. Binding interaction analysis of ligand (a) Catechin, (b) Quercetin, (c) Hederagenin, (d) Oleanolic acid, (e) Remdesivir, (f) Favipiravir, (g) Hydroxychloroquine with SARS-CoV-2 main protease (PDB ID: 6LU7).

submitted to and screened using the Swiss ADME tool (Daina et al., 2017) to predict their overall pharmacokinetic profile and drug-likeness ability based on the Lipinski's Rule of Five. This rule majorly focuses the molecular weight, lipophilicity and the presence of number of hydrogen bond donors and acceptors in the compound (Table 2) (Quimque et al., 2020).

The BOILED-Egg (brain or intestinal estimated permeation predictive model), which is an intuitive graphical plot of the functions of lipophilicity and apparent polarity as described by the parameters WLOGP (atomistic octanol-water partition coefficient) and TPSA (topological polar surface area), respectively, was used to predict passive intestinal absorption and brain permeation of the compounds (Figure 13). Compounds located in the yellow region (yolk) have a high probability of BBB penetration while those in the white region have the propensity for passive absorption through

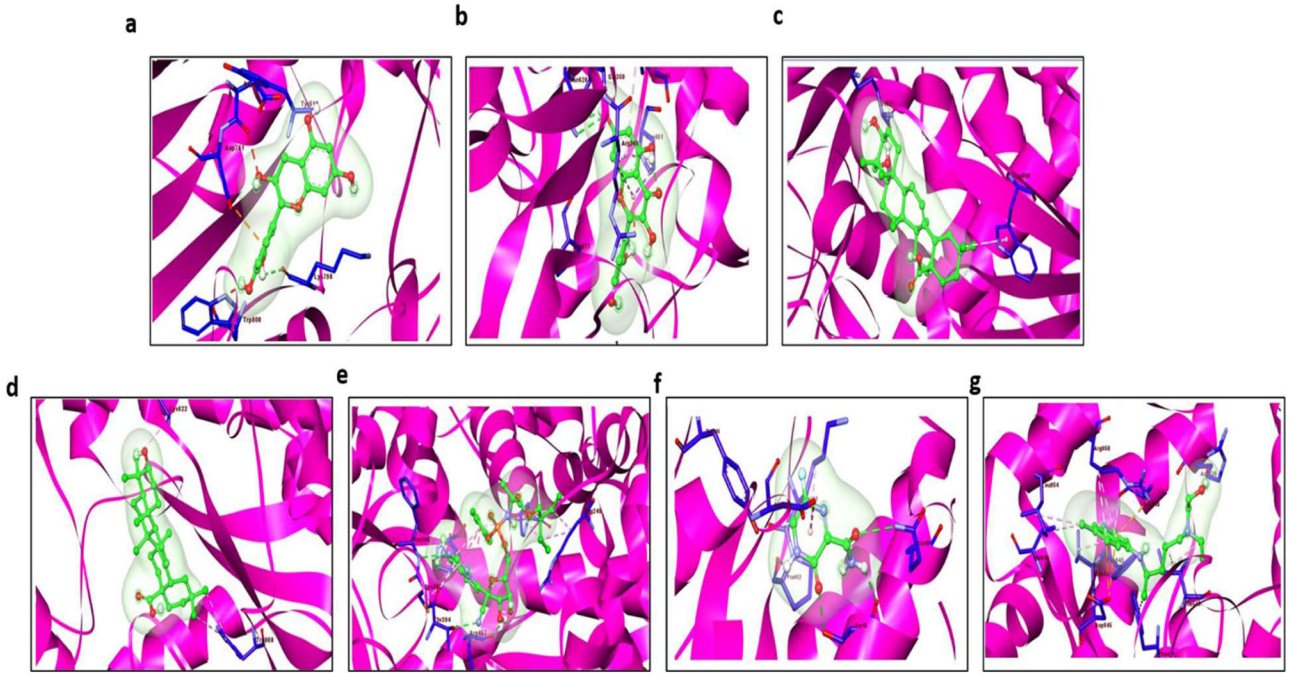

Figure 7. Docked poses of (a) Catechin, (b) Quercetin, (c) Hederagenin, (d) Oleanolic acid, (e) Remdesivir, (f) Favipiravir, (g) Hydroxychloroquine against SARS-CoV-2 RNA dependent RNA Polymerase (PDB ID: 6M71).

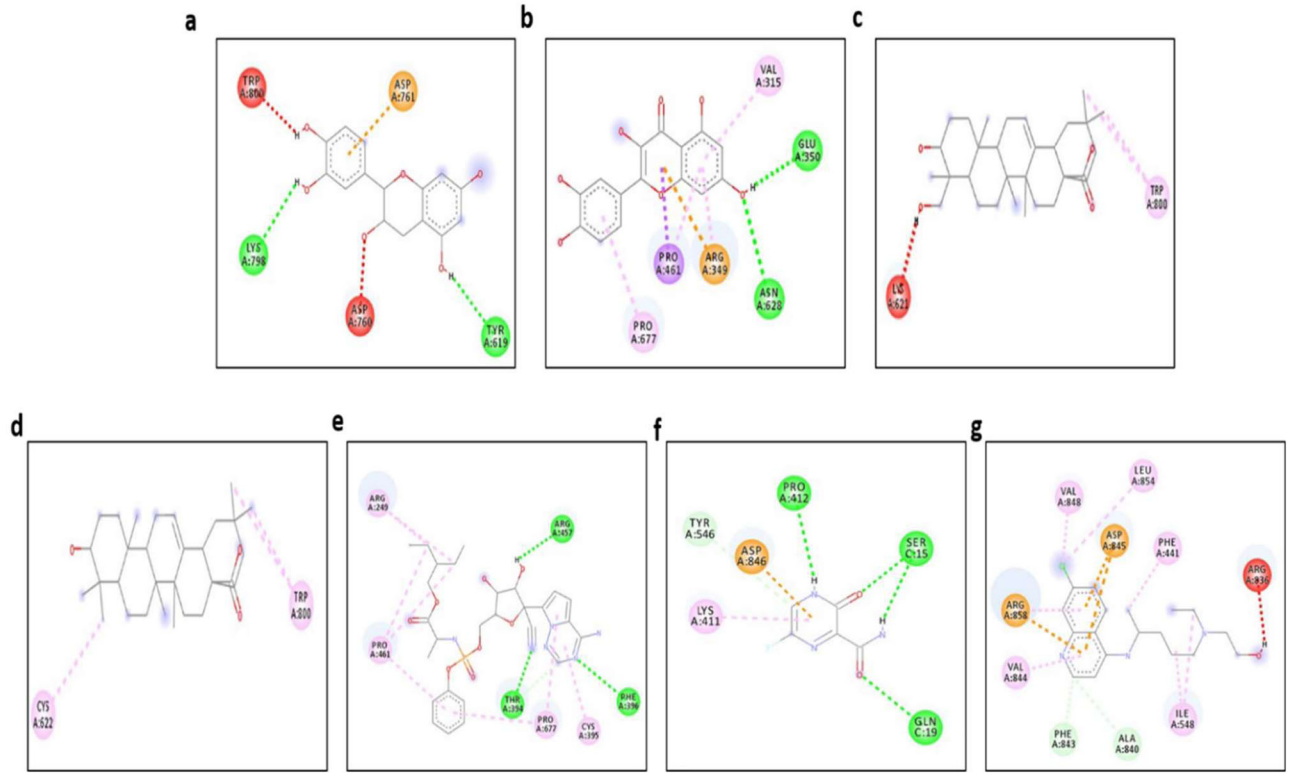

Figure 8. Binding interaction analysis of ligand (a) Catechin, (b) Quercetin, (c) Hederagenin, (d) Oleanolic acid, (e) Remdesivir, (f) Favipiravir, (g) Hydroxychloroquine with SARS-CoV-2 RNA dependent RNA Polymerase (PDB ID: 6M71).

the GI tract. Out of the four metabolites, Catechin, Quercetin and Hederagenin have predicted to have good Gastrointestinal (GI) tract absorption. In spite of having good binding affinity to all the viral proteins as well as proteins with comorbidities, Oleanolic acid does not even lie in the white portion of the BOILED-egg which means Oleanolic acid predicted to get poorly absorbed in the GI tract (Quimque et al., 2020).

Since the compounds were also screened for Lipinski's rule of five that predicts drug likeness properties of the natural compounds/metabolites from *M. dioica*, Catechin, Quercetin and Hederagenin showed good bioavailability and

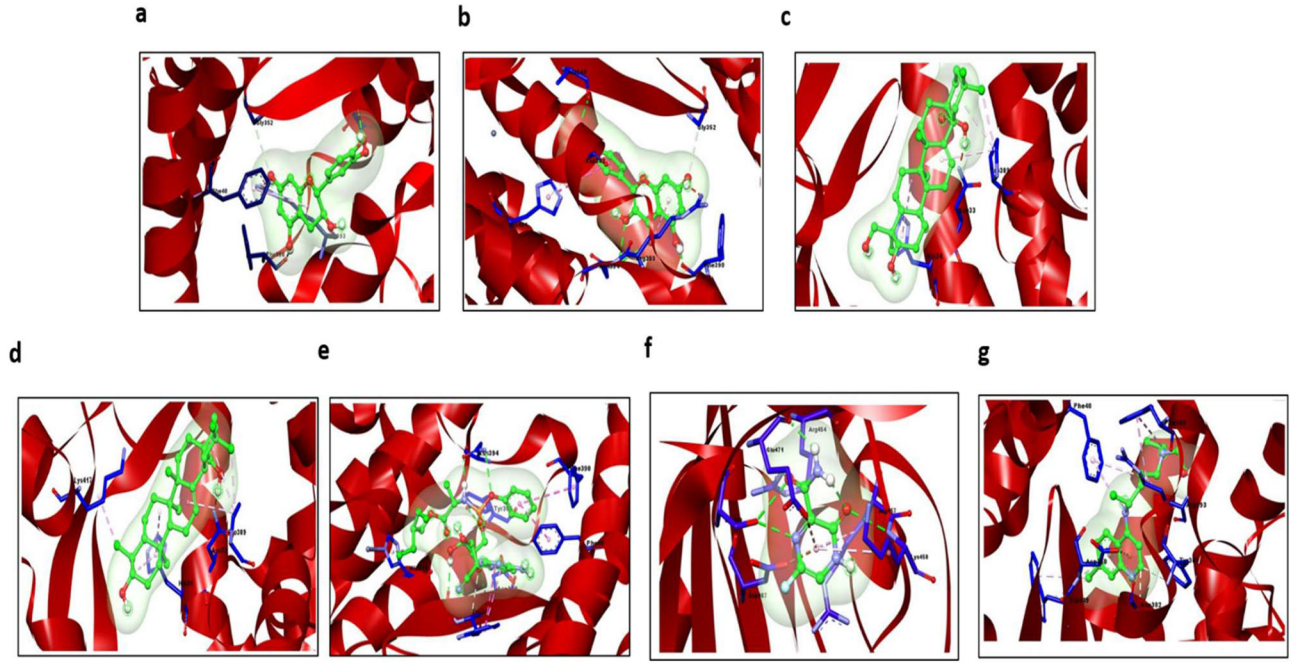

Figure 9. Docked poses of (a) Catechin, (b) Quercetin, (c) Hederagenin, (d) Oleanolic acid, (e) Remdesivir, (f) Favipiravir, (g) Hydroxychloroquine against SARS-CoV-2 spike protein with ACE2 receptor (PDB ID: 6M0J).

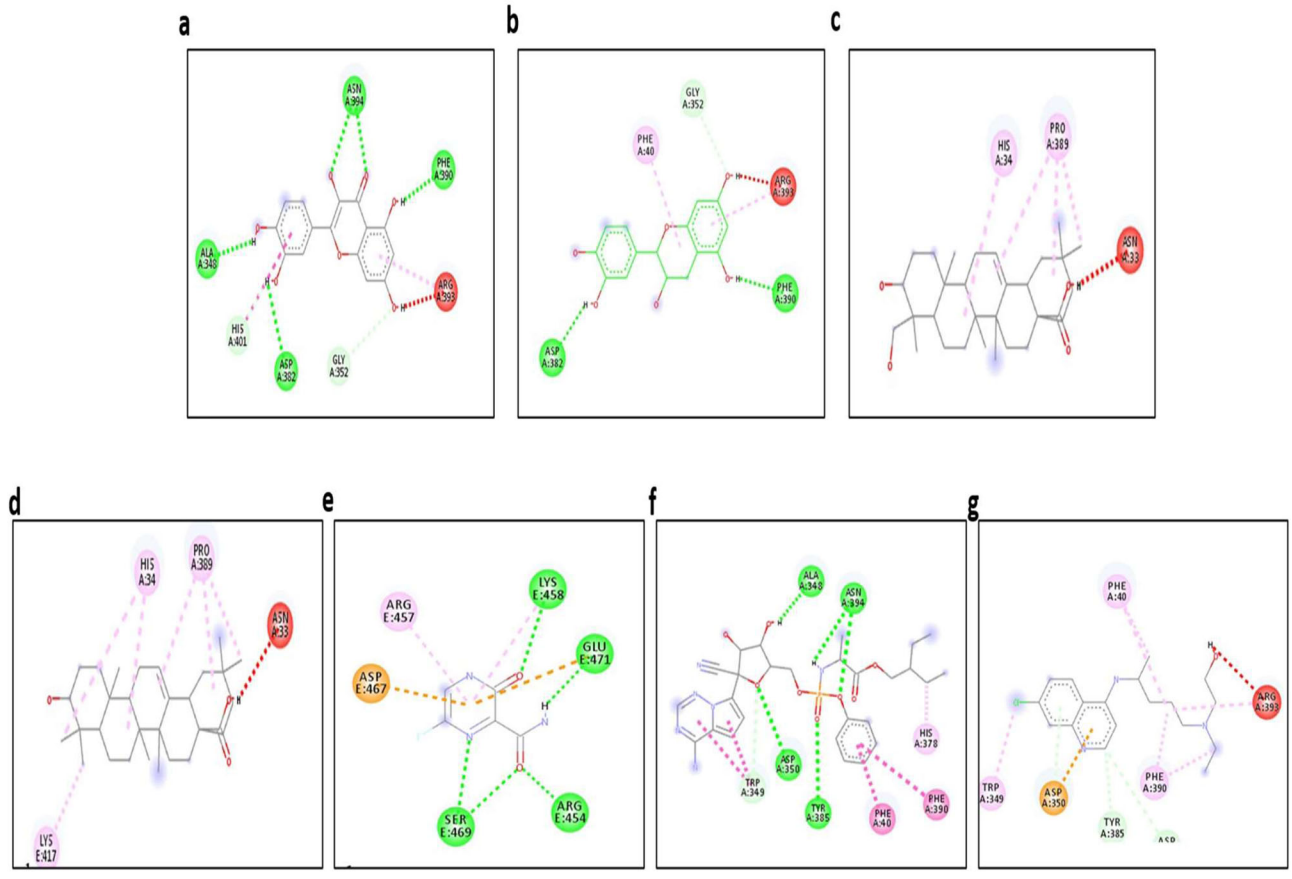

Figure 10. Binding interaction analysis of ligand (a) Catechin, (b) Quercetin, (c) Hederagenin, (d) Oleanolic acid, (e) Remdesivir, (f) Favipiravir, (g) Hydroxychloroquine with SARS-CoV-2 spike protein with ACE2 receptor (PDB ID: 6M0J).

drug-likeness by fulfilling Lipinski's criteria. Other than these criteria, Catechin and Quercetin have good solubility which means good ADME properties execution. Hederagenin showed moderate solubility while Oleanolic acid showed poor solubility. Quercetin has the best solubility among all the compounds involved in this study. Molar refractivity of compounds should lie between 40 to 13. This rule is maintained in case of catechin and quercetin while not for

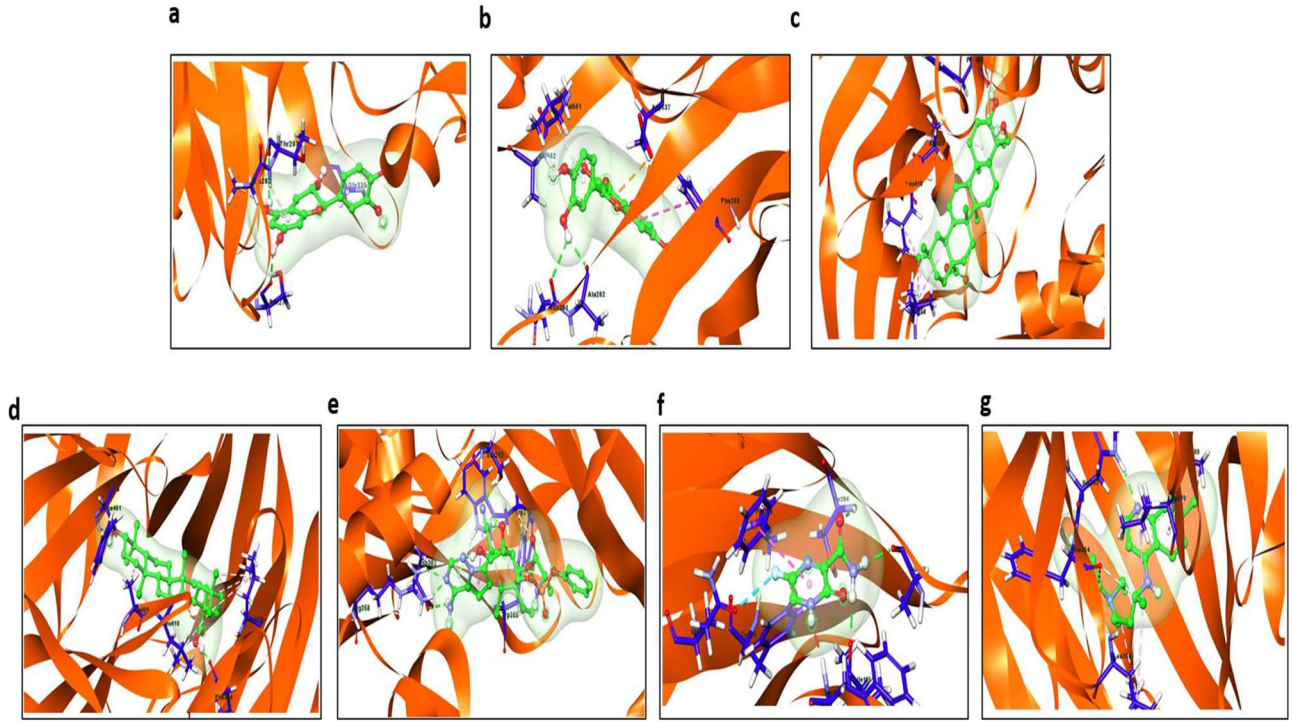

Figure 11. Docked poses of (a) Catechin, (b) Quercetin, (c) Hederagenin, (d) Oleanolic acid, (e) Remdesivir, (f) Favipiravir, (g) Hydroxychloroquine against MERS-CoV with human DPP4 receptor (PDB ID: 4L72).

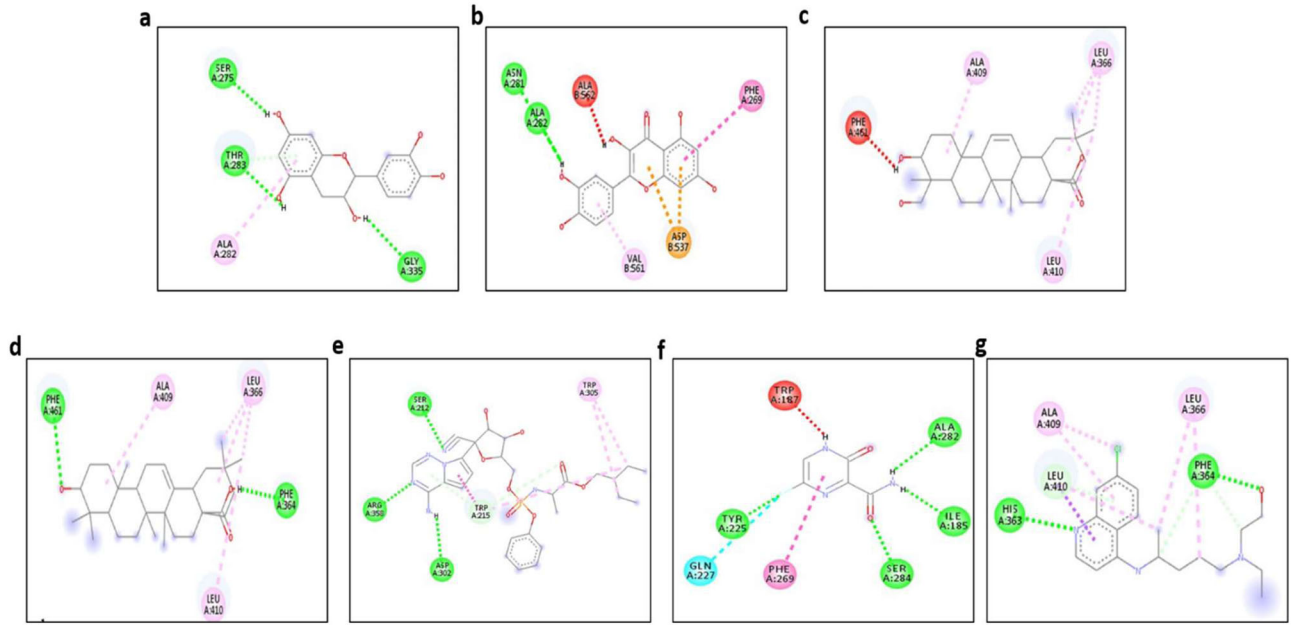

Figure 12. Binding interaction analysis of ligand (a) Catechin, (b) Quercetin, (c) Hederagenin, (d) Oleanolic acid, (e) Remdesivir, (f) Favipiravir, (g) Hydroxychloroquine with MERS-CoV with Human DPP4 receptor (PDB ID: 4L72).

Hederagenin and Oleanolic acid. The molar refractivity values for catechin and quercetin are 74.33 and 78.03, respectively.

In vitro *validation of interaction studies by fluorescence quenching studies of viral proteins with compounds*

The *in vitro* interaction studies using fluorescent quenching results have suggested the compounds had varied range of interactions with target viral proteins. The best interactions were observed with the quercetin with the spike (IC_{50} of $0.052 \pm 0.03\,\mu M$) and DPP4 proteins (IC_{50} of $0.057 \pm 0.02\,\mu M$) comparatively less interaction with main proteases (IC_{50} of $0.062 \pm 0.02\,\mu M$) and RNA-dependent RNA Polymerase proteins (IC_{50} of $0.082 \pm 0.03\,\mu M$) when compared to positive controls remdesivir, favipiravir and hydroxychloroquine. Compared to remdesivir (IC_{50} of $0.092 \pm 0.03\,\mu M$), quercetin had excellent interaction with RNA-dependent RNA Polymerase proteins and might be efficient inhibitor than standard remdesivir drug (Table 3). Catechin also exhibited

Table 2. Lipinski's Rule of five for *in silico* ADME analysis of compounds.

Compound name	MW (<500) (g/mol)	#H acceptors (<10)	#H donors (<5)	Lipophilicity MLogp (<5)	Lipinski #violations	Drug Likeness
Catechin	290.27	6	5	0.24	0	Yes
Quercetin	302.24	7	5	−0.56	0	Yes
Hederagenin	472.70	4	3	4.97	1	Yes
Oleanolic acid	456.70	3	2	5.82	1	Yes

MW-Molecular Weight, # H-Hydrogen bond.

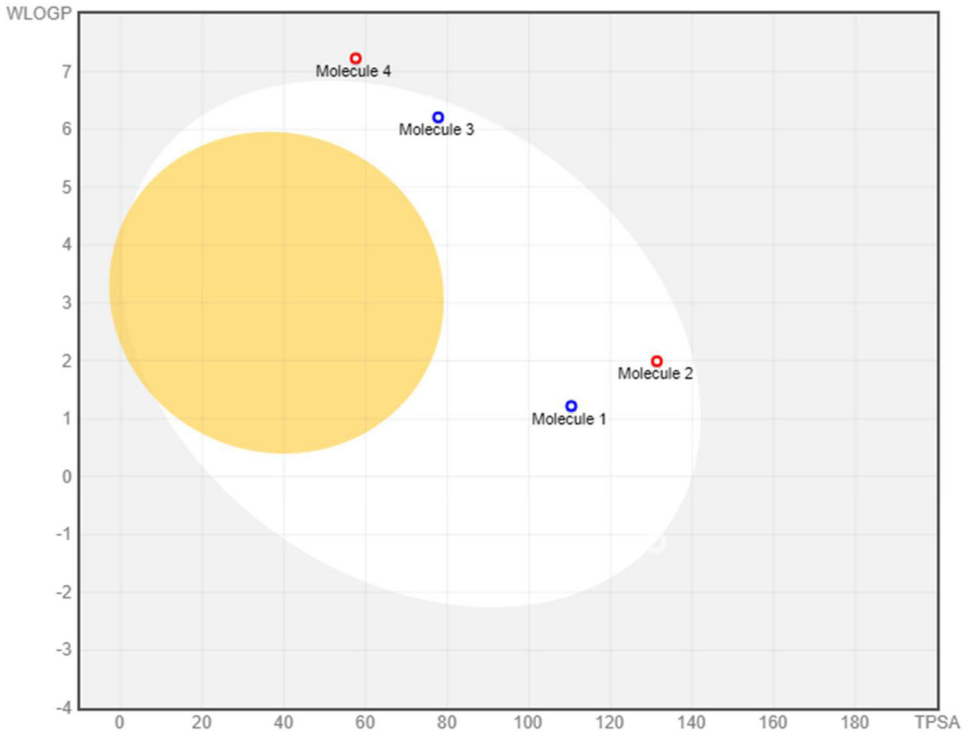

Figure 13. Prediction of gastrointestinal (GI) tract and brain permeation of selected natural compounds: Catechin (Molecule 1), Quercetin (Molecule 2), Hederagenin (Molecule 3) and Oleanolic acid (Molecule 4) by brain or intestinal estimated permeation predictive model (BOILED-Egg) method.

Table 3. *In vitro* validation of interaction studies by fluorescence quenching studies of viral proteins with compounds.

	Protein quenching (IC_{50} in μM)			
Compound name	Main protease	RNA dependent RNA polymerase	Spike protein	DPP4 protein
Catechin	$0.093 \pm 0.02^{**}$	$0.071 \pm 0.05^{**}$	$0.065 \pm 0.02^{**}$	$0.032 \pm 0.01^{**}$
Quercetin	$0.062 \pm 0.02^{**}$	$0.082 \pm 0.03^{**}$	$0.052 \pm 0.03^{**}$	$0.057 \pm 0.02^{**}$
Hederagenin	$0.129 \pm 0.04^{**}$	$0.096 \pm 0.05^{**}$	$0.115 \pm 0.05^{**}$	$0.093 \pm 0.02^{**}$
Oleanolic acid	$0.167 \pm 0.11^{**}$	$0.173 \pm 0.07^{**}$	$0.091 \pm 0.06^{**}$	$0.117 \pm 0.04^{**}$
Favipiravir	0.247 ± 0.27	$0.187 \pm 0.08^{**}$	0.262 ± 0.05	0.527 ± 0.27
Hydroxychloroquine	$0.182 \pm 0.17^{**}$	0.551 ± 0.04	0.671 ± 0.04	0.726 ± 0.18
Remdesivir	0.217 ± 0.12	$0.127 \pm 0.03^{**}$	0.092 ± 0.03	0.783 ± 0.17

**Statistically significance at $p < 0.05$ by the Mann-Whitney U tests.

nearly to effects of similar to quercetin with DPP4 (IC_{50} of 0.032 ± 0.01 μM), spike protein (IC_{50} of 0.065 ± 0.02 μM), RNA dependent RNA polymerase (IC_{50} of 0.071 ± 0.05 μM) and main protease (IC_{50} of 0.093 ± 0.02 μM). Other compounds hederagenin and oleanolic acid had moderate level of interaction with the SARS-CoV-2 viral proteins (Table 3).

Discussion

Coronaviruses have evolved since history from MERS-CoV, SARS-CoV-1 and SARS-CoV-2 causing threat to human population. There is absence of specific drugs for the cure, many studies have carried out underlying the importance of existing viral drugs that were used for HIV or Ebola viruses or for the development of medicinal herbs as drugs against this virus till as the world is waiting for the development of vaccine. The presence of existing life-threatening disorders to an individual like diabetes, cardiovascular diseases and obesity are prone to worsen the COVID-19 complications. Research and development on SARS and MERS may offer insights that would be beneficial to the development of therapeutic and preventive agents for COVID-19. Identification of targets is important for identifying drugs with high target specificity and/or uncovering existing drugs that could be repurposed to treat SARS-CoV-2 infections (Dhama et al., 2020; Harrison, 2020; Kilanski & Baker, 2015;

Li & Clercq, 2020; Lin et al., 2020; Mandadapu et al., 2013; Prajapat et al., 2020; Prasanth et al., 2020; Solaimanzadeh, 2020; Totura & Bavari, 2019; Trott & Olson, 2020; Wishart et al., 2006; Yang et al., 2020; Zhang et al., 2020; Zhou et al., 2020).

Use of plant metabolites is very promising as our Indian System of Medicine and Ayush Health Ministry focuses the importance of natural drugs. Medicinal drugs from cucurmin, shatavari (Chikhale et al., 2020), cinnamon (Prasanth et al., 2020) or specific classes of plant metabolites are researched upon using *in silico* approaches to predict the effectiveness, efficacy and safety of the natural products (Kilanski & Baker, 2015; O'Boyle et al., 2011).

The natural products from plants like *M. dioica* are known to be antidiabetic in nature as it belongs to the *Cucurbitaceae* family. So, selection of this plant was based on its diverse pharmacological activities such as antiviral, antidiabetic, antioxidant, immune-stimulant, easy cultivation approaches and resemblance to structure of coronavirus. The *in silico* studies, ADME analyses and *in vitro* interaction studies have suggested the important natural product-based hits for the comorbid and SARS-CoV-2 viral proteins. The molecular modeling and *in silico* pharmacokinetic analysis had put a great contribution and light to further wet lab analysis for drug discovery and development from *M. dioica* molecules and their kinetics as well as cell lines model studies are in progress. However, these natural molecules have significant in the drug discovery for multitargets of SARS-CoV-2 related infections.

Conclusion

The present study reports the predicted efficacy of flavonoids and triterpenoids from *M. dioica* plant against the viral proteases and proteases having comorbidities of the coronavirus. These metabolites in *M. dioica* have been previously reported to have antiviral, antidiabetic and immunomodulatory properties that are primarily necessary to be effective against the SARS-CoV-2 pandemic. Currently, as in the COVID-19 pandemic, there is rapid replicability of viral particles and lack of specific drugs and vaccines. So, it is need to apply concepts of Phyto pharmaceuticals to fight against the coronavirus and keep oneself away from the physical and mental stress. The phytoconstituents catechin, quercetin, hederagenin and oleanolic acid could serve as potential medicinal agents in COVID-19. In this present study, we screened the potential biological activities of phytoconstituents from spine gourd against the viral and comorbid proteases of SARS-CoV-2 using molecular modeling and to compared its efficacy in terms of binding energy with the drugs currently used in treatment of COVID-19.

The metabolites considering as oral drugs were also screened for its virtual ADME properties. Among the metabolites, the triterpenoids hederagenin and oleanolic acid showed the best results of binding interaction with the viral macromolecules but according to *in silico* ADME predictions they both are relatively less efficient. This efficiency can be assured and experimented with strategies using wet lab analysis. The quercetin metabolite is best in terms of both analysis parameters such as binding affinity and drug likeness behavior. The reference drugs showed comparatively very less binding affinity toward viral proteases. Therefore, these compounds can be employed as therapeutic alternatives and can be utilized for further development of multitargeting drugs against SARS-CoV-2 infections. The present work may be used as a template for future discovery and development of novel multitargeting compounds against the viral disease. To the best of our knowledge, this study of potentially inhibiting properties of *M. dioica* toward coronavirus viral and comorbid proteins is novel.

Disclosure statement

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

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