Identification of phytocompounds from *Houttuynia cordata* Thunb. as potential inhibitors for SARS-CoV-2 replication proteins through GC–MS/LC–MS characterization, molecular docking and molecular dynamics simulation

Sanjib Kumar Das¹ · Saurov Mahanta² · Bhaben Tanti³ · Hui Tag⁴ · Pallabi Kalita Hui¹

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Abstract
The COVID-19 pandemic caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a massive viral disease outbreak of international concerns. The present study is mainly intended to identify the bioactive phytocompounds from traditional antiviral herb *Houttuynia cordata* Thunb. as potential inhibitors for three main replication proteins of SARS-CoV-2, namely Main protease (Mpro), Papain-Like protease (PLpro) and ADP ribose phosphatase (ADRP) which control the replication process. A total of 177 phytocompounds were characterized from *H. cordata* using GC–MS/LC–MS and they were docked against three SARS-CoV-2 proteins (receptors), namely Mpro, PLpro and ADRP using Epic, LigPrep and Glide module of Schrödinger suite 2020-3. During docking studies, phytocompounds (ligand) 6-Hydroxyondansetron (A104) have demonstrated strong binding affinity toward receptors Mpro (PDB ID 6LU7) and PLpro (PDB ID 7JRN) with G-score of − 7.274 and − 5.672, respectively, while Quercitrin (A166) also showed strong binding affinity toward ADRP (PDB ID 6W02) with G-score -6.788. Molecular Dynamics Simulation (MDS) performed using Desmond module of Schrödinger suite 2020–3 has demonstrated better stability in the ligand–receptor complexes A104-6LU7 and A166-6W02 within 100 ns than the A104-7JRN complex. The ADME-Tox study performed using SwissADMEserver for pharmacokinetics of the selected phytocompounds 6-Hydroxyondansetron (A104) and Quercitrin (A166) demonstrated that 6-Hydroxyondansetron passes all the required drug discovery rules which can potentially inhibit Mpro and PLpro of SARS-CoV-2 without causing toxicity while Quercitrin demonstrated less drug-like properties but also demonstrated as potential inhibitor for ADRP. Present findings confer opportunities for 6-Hydroxyondansetron and Quercitrin to be developed as new therapeutic drug against COVID-19.

Sanjib Kumar Das and Saurov Mahanta have contributed equally to this work.

✉ Hui Tag
hui.tag@rgu.ac.in
✉ Pallabi Kalita Hui
pallabi2008rgu@gmail.com; pallabikalita@nitap.ac.in

¹ Department of Biotechnology, National Institute of Technology, Arunachal Pradesh, Yupia, Arunachal Pradesh 791112, India
² National Institute of Electronics and Information Technology, Guwahati, Assam 781008, India
³ Department of Botany, Gauhati University, Guwahati, Assam 781014, India
⁴ Pharmacognosy and Phytochemistry Research Laboratory, Department of Botany, Rajiv Gandhi University, Rono Hills, Doimukh, Arunachal Pradesh 791112, India
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Abbreviations
- ADME-Tox: Adsorption, digestion, metabolism, excretion and toxicity
- ADRP: ADP ribose phosphatase
- COVID-19: Coronavirus disease 2019
- MDS: Molecular dynamics simulation
- Mpro: Main protease
- PDB ID: Protein Data Bank Identity
- PLpro: Papain-like protease
- RMSD: Root-mean-square deviation
- RMSF: Root-mean-square fluctuation
- SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2

Introduction

Sudden outbreak of COVID-19 has become life threatening for millions of human population across the globe. World Health Organization (WHO) declared COVID-19 as the 6th public health emergency of international concern on 30th January 2020. WHO reported 760.2 million active cases of COVID-19 followed by 16.6 millions of confirmed deaths globally till the month of December, 2020 [1]. The COVID-19 is fundamentally caused by the infection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the human host and transmits to another human being through physical contact or droplets. Although antiviral drug and vaccine development are being undertaken for the prevention of the disease, presently, there are no effective therapeutic drug molecules available to date against COVID-19. SARS-CoV-2 is an enveloped virus that belongs to family of *Coronaviridae* containing single positive-stranded RNA and replicate inside the cytoplasm of human cell [2, 3]. SARS-CoV-2 genome encodes several proteins that are involved in the replication of viral genome. Inhibition of the expression of these receptor proteins may lead to the possibilities of anti-SARS-CoV-2 drug development for effective treatment of COVID-19. Three proteins of SARS-CoV-2, namely Main protease (Mpro), Papain-Like protease (PLpro) and ADP ribose phosphatase (ADRP), are reported to be mainly responsible for SARS-CoV-2 replication process.
The Main protease (Mpro) is present in SARS-CoV-2 and other coronaviruses (CoVs) which cleave two replicase polyprotein responsible for viral replication and maturation [5]. The papain-like protease (PLpro) is another proteolytic enzyme which is essential for processing of SARS-CoV-2 polyproteins to generate a functional replicase complex and initiate viral spread. PLpro is also involved in cleaving protein during post-translational modifications on host proteins as an eluding mechanism against host antiviral immune responses. PLpro cleaves ubiquitin and ISG15 which are known regulators of host innate immune pathways [6]. On the other hand, ADP ribose phosphatase (ADRP) is known enzymes of SARS-CoV-2 replication that convert ADP-ribose 1″-monophosphate (Appr-1″-p) to ADP-ribose (Appr), which regulate replication of the virus [7]. Therefore, inhibition of Mpro, PLpro and ADRP activities through bioactive phytochemical drug molecules would prevent replication mechanism of SARS-CoV-2.

In silico method employed for the screening of potential drug molecules is proven rapid and cost effective while compared with the trial and error methods using experimental studies. Molecular docking is an in silico approach with ability to screen and identify potential drug molecules from large and massive bioactive compound libraries. Currently, several molecular docking studies were carried out against SARS-CoV-2 receptors with some selected bioactive compounds (Table 1) and have been able to identify few potential compounds drug molecules effective against targeted receptors of COVID-19 [7, 9–14]. However, most of these docking studies relied on structure-based drug design (SBDD), as well as similarity searching and quantitative structure–activity relationship (QSAR) modeling [8]. Some recent cases of molecular docking studies of different molecular targets for SARS-CoV-2 are presented in Table 1.

The traditional healer of different ethnic communities used medicinal plants for treatment and prevention of various diseases including viral infections. Isolation of these bioactive phytocompounds from traditional medicinal plants may lead to the development of antiviral drug for effective treatment of COVID-19 [15]. *Houttuynia cordata* Thunb. is a herb belonging to the family *Saururaceae*, distributed in China, India, Bangladesh, Cambodia, East Himalaya, Hainan, Japan, Korea, Myanmar, Nepal, Taiwan, Thailand, Tibet (China), Vietnam, West Himalaya [16]. The whole plant is used in folk medicine for the treatment of cough, pneumonia, bronchitis, dysentery, dropsy, leukorrhea, urticaria, eczema, herpes simplex, acne, chronic sinusitis, stomach ulcer, infection, control wrinkle, chapped skin, septic, febrifuge, heatstroke, malaria, lung disorder, tonsillitis, skin ulcer, diarrhea, dysentery arthritis, appendicitis, and snake bite in Japan, Korea, Indonesia and Myanmar [17–19]. In Indian sub-continent, *H. cordata* is mainly used for the treatment and prevention of pneumonia, stomach disorder, sinusitis and heart disorders by the indigenous tribes of North East.

### Table 1 Molecular docking studies of different molecular targets for SARS-CoV-2

| Targeted receptors of SARS-CoV-2 | Experiment | Identified potential antiviral drug candidates for SARS-CoV-2 | References |
|---------------------------------|------------|------------------------------------------------------------|------------|
| Mpro                            | Molecular docking | Hesperidin, rutin, diosmin, apin, diacetylcumcumin, (E)-1-(2-Hydroxy-4-methoxyphenyl)-3-[3-[(E)-3-(2-hydroxy-4-methoxyphenyl)-3-oxoprop-1-enyl] phenyl] prop-2-en-1-one, and beta−(4-Methoxy-1,3- phenylene) bis(2’-hydroxy-4’-6’-dimethoxyacrylophenone | [9] |
| Mpro                            | Molecular docking and drug ability studies | Glycyrhrizin, bicyllogermecrene, trypanthrine, β-sitosterol, inindirubin, indican, indigo, hesperetin, crysophanic acid, rhein, berberine and β-caryophyllene | [10] |
| Spike (S) glycoprotein, Mpro and RNA-dependent RNA polymerase (RdRp) | Molecular docking | Silybin and withaferin A | [11] |
| Spike glycoprotein and ACE2 receptor | Molecular docking | Curcumin, nimbina, withaferin A, piperine, mangiferin, thebaene, berberine, rographolide resveratrol, quercetin, luteolin, naringenin, zingiberene, and gallic acid | [12] |
| Mpro, endoribonuclease (Nsp15/NendoU), ADP-ribose-1″-phosphatase (ADRP), RNA-dependent RNA polymerase (RdRp), 3CL-PRO, PL-PRO | Molecular docking | Sesquiterpene hydrocarbon (E)-β-farnesene, (E), (E)-α-farnesene, (E), (E)-β-farnesene, (E, E)-farnesol (E, E)-Farnesol | [7] |
| ACE2                            | Molecular Docking and molecular dynamics studies | saikosaponin D and amentoflavone | [13] |
|                                 |            | Hesperidin | [14] |
India [20–22]. The major bioactive phytocompounds including flavonoid, alkaloid, as well as essential oil, have been reported from various parts of *H. cordata* which possesses antimicrobial, hepatoprotective, anti-inflammatory, antioxidative, anticancer, anti-diabetic, anti-obesity and antiviral properties [17]. *Houttuynia cordata* was one of the most important ingredient of the herbal formulation used for the treatment of Severe Acute Respiratory Syndrome (SARS) outbreak of Southern China in 2003 [16]. Several scientific studies also reveal that the extract of *H. cordata* has the potential to inhibit replication of the various viral strains including SARS coronavirus (SARS-CoV), Chikungunya, Herpes simplex viruses, dengue virus serotype 2 (DEN-2), Influenza neuraminidase, pseudorabies herpes virus (PrV), Human noroviruses (HuNoVs), murine coronavirus and dengue virus infection and also possess innate immune modulation activities [16, 18, 23–29].

Therefore, considering the therapeutic importance of *H. cordata* with strong ethnopharmacological use background, the present study is mainly intended to characterize the bioactive phytocompounds by using gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–mass spectrometry (LC–MS) studies, and to perform molecular docking studies of bioactive phytocompounds against the three main proteins (target receptors) of SARS-CoV-2, namely Mpro, PLpro and ADRP, responsible for replication process of the virus, and to identify the bioactive phytocompounds (ligands) potential to inhibit SARS-CoV-2 replication proteins by determining the binding affinities of the ligands against selected receptors. We performed molecular docking study between the active phytochemicals of *H. cordata* using Epic, LigPrep and Glide module of Schrödinger suite 2020-3 followed by Molecular Dynamics simulation by Desmond module of Schrödinger suite 2020-3. We also performed Adsorption, Digestion, Metabolism, Excretion and Toxicity (ADME-Tox) study of the shortlisted phytocompounds using SwissADMEserver to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of the selected bioactive phytocompounds.

### Materials and methods

#### Collection of sample

The herb *H. cordata* was collected on 13 November 2019 from Ziro valley of Lower Subansiri District of Arunachal Pradesh, India (26°55′–28°21′ N and 92°40′–94°21′ E). The plant materials were identified and authenticated at ASSAM Herbarium, Botanical Survey of India, Eastern Regional Center, Shillong, Meghalaya, India. The accepted names and global distribution ranges were verified in [http://www.plantsoftheworldonline.org](http://www.plantsoftheworldonline.org) (POWO) hosted by Royal Botanic Garden, Kew, UK. The voucher specimen No. SD/HAU-0110 dated 13.11.2019 was deposited in the Herbarium of Arunachal University (HAU), Department of Botany, Rajiv Gandhi University, Rono Hills, Doimukh, Arunachal Pradesh, India for future references. The fresh herb harvested was washed in running tap water to remove microflora, dirt and soil particles and then shade dried at normal room temperature (20.0–27.0 °C) with proper ventilation. The minimum moisture content of the dried samples was maintained at 15% using Material Moisture Meters (Model Testo 606-1, Germany). The powdered samples were passed through 40-mesh sieve and stored for further use.

#### Characterization of bioactive phytocompounds

##### Preparation of crude extract

The crude extract was prepared by cold maceration of powder materials with methanol (solvent ratio 1:5). 50 g of powder sample was soaked in 250 ml of methanol and placed on magnetic stirrer for 48 h [30]. After 48 h, filtrate was collected through Whatman No. 1 filter paper and concentrated samples were then further subjected to dry in water bath (I THERM Model No. BTI 57) and then in hot air oven (I THERM Model No. BTI 29) at 40 °C, until the constant weight of extracts was observed. The crude extract obtained was further used for characterization of bioactive phytochemicals using GC–MS and LC–MS.

##### GC–MS analysis

The GC–MS characterization of crude extract of *H. cordata* was performed by injecting 2 μl of sample in a GC–MS–2010 Shimadzu instrument operating in EI mode at 70 eV with Restek-5MS column (30×0.25 mm film thickness 0.25 μm). The oven temperature was programmed as follows: kept at 60 °C for 2 min, then increased to 210 °C, at 3 °C min−1 and from 210–280 °C at the rate of 8 °C/min and held for 8 min at 280 °C. The injector temperature was 260 °C with normal injection mode. The flow rate of carrier helium gas was 1.21 ml/min. For the plant samples, the temperature program was set according to Jiang et al. [31]. Mass spectra were acquired using full scan monitoring mode with a mass scan range of 40–650 m/z. The chromatogram and mass spectra were evaluated using the XcaliburTM software embedded in the GC–MS/LC–MS system. Interpretation on mass spectrum GC–MS was conducted using the database of National Institute Standard and Technology (NIST), Wiley having more than 62,000 patterns and Dr. Duke’s phytochemical ethnobotanical databases (Duke 2018; phytochem. nal.usda.gov). The spectrum of the unknown component...
was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the compounds of the test materials were ascertained.

**LC–MS analysis**

The LC–MS characterization of the *H. cordata* was performed by injecting 10 µl of sample in a LC–MS Thermo Scientific Plus with Dionex Ultimate 3000 HPLC with column Hypersil Gold (C18) Diameter 150×2.1 mm, Particle Size 1.9 μ at room temperature. The mobile phases used were A (acetonitrile) and B (0.2% aqueous acetic acid, v/v). The run time was set for 20 min followed by flow rate of 0.6 mL/min. DAD detector was set at 280 nm for acquiring chromatograms. The mass spectrometer used was a Triple quadruple Mass Spectrometer (Thermo Scientific) equipped with ion sources ESI with Mass range for full scans m/z 50–6000. All raw data acquired were processed by METLIN database [32].

**Molecular docking**

**Preparation of receptor proteins**

The crystal structure of SARS-CoV-2 main protease in complex with an inhibitor N3 having PDB ID 6LU7 [33] (resolution 2.16 Å), wild type SARS-CoV-2 papain-like protease (PLPro) with inhibitor GRL0617 having PDB ID 7JRN (resolution 2.48 Å) and ADP ribose phosphatase of NSP3 from SARS-CoV-2 in the complex with ADP ribose having PDB ID 6W02 (resolution 1.5 Å) were downloaded from RCSB Protein Data Bank [34] and same were processed through protein preparation wizard of Epic module [35] of Schrödinger suite 2020-3. The proteins were prepared by removing similar binding sites, unnecessary water molecules and also refining bond orders. Missing chain atoms were added by using the prime module of Schrödinger suite 2020-3. Energy minimization of the proteins was performed using optimized potentials for liquid simulations-3 (OPLS3e) molecular force field with root-mean-square difference (RMSD) of crystallographic heavy atoms kept at 0.3 Å. The ligand binding information of N3, GRL0617 and ligand binding pocket of ADP ribose were used for prediction of the active site of the proteins.

**Preparation of ligand library**

The ligand library from the GC–MS and LC–MS analysis of *H. cordata*, having traditional medicinal linkage with antiviral treatment, was processed through the LigPrep module of Schrödinger suite 2020–3. During preparation of the ligands for docking, Lipinski Drug Discovery rule [36] was kept as filtering criteria to screen out unfavorable molecules from the dataset. 2D structures were converted to 3D structures, and optimized for their geometry, desalted and their chirality was corrected. The ionization and tautomeric states were generated between pH 6.8 and 7.2 by using Epik module of Schrödinger suite 2020-3. The ligands were minimized using OPLSe-3 force field in Schrödinger suite 2020-3 until a RMSD of 2.0 Å was achieved and the optimized ligands were used for docking analysis.

**Protein–Ligand docking using glide**

The filtered phytocompounds were prepared by LigPrep module of Schrödinger suite before proceeding for docking, and the docking was performed against each of the three selected receptor proteins, Mpro (PDB ID 6LU7), PLpro (PDB ID 7JRN) and ADRP (PDB ID 6W02) using Glide module of Schrödinger suite 2020-3. During the docking process, the receptor grid maps were prepared from the active site information available from the previously bound ligands with the receptor in crystal structure available from RCSB PDB. The binding modes with best docking score and best Glide gscores (G-Score) were selected. These scores perceive positive lipophilic, hydrogen bonding and metal–ligand associations and punish steric conflicts. The G-scoring capacity is mainly dependent on docking parameters like lipophilic perseverance in which the ligands are covered in the lipophilic pocket. The electrostatic forces and hydrogen bonding with ligands are other parameters to increase the binding affinity.

**Molecular dynamics simulation**

For Molecular Dynamics (MD) Simulation, the Desmond module through Maestro of Schrödinger suite 2020-3 was used in the Linux platform supported by 6 GB NVIDIA GeForce GTX graphics card. To study the stability of the docked complex with the ligand, an MD Simulation study of 100 nano second (ns) was performed [37]. The complex in the explicit solvent system with the OPLS3e force field was studied using the Desmond module of Schrödinger suite 2020-3. The molecular system was solvated with crystallographic water (TIP3P) molecules under orthorhombic periodic boundary conditions for a 10 Å buffer region. The overlapping water molecules are deleted, and the system was neutralized by adding Na+ as counter ions. An ensemble (NPT) of Nose–Hoover thermostat [38] and barostat was applied to maintain the constant temperature (300 K) and pressure (1 bar) of the systems, respectively. A hybrid energy minimization algorithm with 1000 steps of steepest descent followed by conjugate gradient algorithms was utilized.
Root-mean-square deviation (RMSD)

The structure and dynamic properties of the protein–ligand complexes were analyzed as the backbone RMSDs during the simulation period of 100 ns. The RMSD was measured as the average distance between the backbone atoms of the protein–ligand structures and it was derived from the following equation:

\[
\text{RMSD} = \sqrt{\frac{1}{N} \sum_{i=0}^{N} \delta^2_i}
\]

where \( N \) represents the total number of atoms considered in the calculation and \( \delta \) represents the distance between the \( N \) pairs of equivalent atoms.

The root-mean-square fluctuations (RMSF)

The root-mean-square fluctuations (RMSF) were assessed and plotted to equate the flexibility of each residue in the ligand–protein complexes. The RMSF of the protein–ligand complex denoted the minimized fluctuation for all the complexes.

ADME-Tox study

Adsorption, Digestion, Metabolism, Excretion and Toxicity (ADME-Tox) study of the shortlisted compounds was performed using SwissADMEserver (http://www.swissadme.ch) [39]. [SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci. Rep. (2017) 7:42717.]

Result

Characterization of bioactive phytocompounds

GC–MS analysis

GC–MS analysis of \( H. \) cordata has identified and quantified 80 phytocompounds and the result is summarized in Table 2. The chromatograms of GC–MS result are given as Fig. 1.

LC–MS analysis

The non-volatile compounds were identified by LC–MS. Ninety-seven phytocompounds were identified by LC–MS. The result of LC–MS is summarized in Table 3 and chromatogram is presented as Fig. 2.

Molecular docking

The molecular docking of ligands (bioactive phytocompounds from \( H. \) cordata) was carried out against three different receptors of SARS-CoV-2, namely Mpro (PDB ID 6LU7), PLpro (PDB ID 7JRN) and ADRP (PDB ID 6W02), respectively, by using an advanced molecular docking program, Glide of Schrödinger suite 2020-3 to determine the binding affinities of the ligands against selected receptors. The binding affinities of the ligands toward receptors were determined based on Docking Score and Glide gscore. Lower docking score and Glide gscore indicate better affinity. The results of the molecular docking study revealed that the bioactive phytocompound coded with A107 from \( H. \) cordata showed highest binding affinity toward Mpro (6LU7) with G-Score − 7.929 (Table 4). The bioactive phytocompounds A104, A120, A99 and A127 from \( H. \) cordata also showed significant binding affinities against Mpro (6LU7) with G-score − 7.274, − 7.024, − 6.883 and − 6.793, respectively (Table 4). Similarly, bioactive phytocompounds A105 showed highest binding affinity against PLpro (7JRN) with G-Score − 5.843, and bioactive phytocompounds A140, A104, A100 and A106 showed potential binding affinities against PLpro (7JRN) with G-score − 5.779, − 5.672, − 5.602 and − 5.227, respectively (Table 4). ADRP (6W02) is another important protein that is required for SARS-CoV-2 replication against which the bioactive phytocompounds A166 showed highest binding affinity with G–Score (− 6.788). The phytocompounds A165, A163, A167, A164 showed binding affinity against ADRP (6W02) with G – Score − 6.099, − 5.703, − 5.703 and − 5.206, respectively (Table 4). From the present study, it is suggested that the bioactive phytocompound A104 is capable of binding both Mpro (6LU7) and PLpro (7JRN) which has demonstrated multi-targets ability and have been identified as a viable drug molecule potential to inhibit replication proteins Mpro (6LU7) and PLpro (7JRN) of SARS-CoV-2.

Although A107 and A105/A140 have the highest predicted affinity toward Mpro (6LU7) and PLPro (7JRN), the phytocompound A104 was selected for further analysis because of its ability to bind both the receptors. Binding affinities with formation of H-bonds and other non-bonding interactions of the complexes are presented in Fig. 3a, b. On the other hand, the best binding ligand A166 was selected for further analysis against the target protein ADRP and the interactions of A166 with ADRP (6W02) are presented in Fig. 3c.

Molecular dynamics (MD) simulation

The protein–ligand complexes with the shortlisted ligands A104 and A166 were performed by MD simulation study to examine the stability of the compounds to the binding
Table 2 List of phytocompounds identified and quantified from *H. cordata* by GC–MS

| SL No | Code name | Compound name | Peak area% | Molecular mass (g/mol) |
|-------|-----------|---------------|------------|------------------------|
| 1     | A1        | Propane, 1,1-dimethoxy- | 0.12       | 104.15                 |
| 2     | A2        | Dimethyl fumarate | 0.20       | 144.12                 |
| 3     | A3        | Butanedioic acid, dimethyl ester | 0.53 | 146.14               |
| 4     | A4        | 1,3,7-Octatriene, 3,7-dimethyl-, (E) | 0.25 | 136.23               |
| 5     | A5        | Dimethyl malate | 0.64       | 162.14                 |
| 6     | A6        | 1-Nonanol | 0.12       | 144.25                 |
| 7     | A7        | Methyl phenylacetate | 0.14 | 150.17               |
| 8     | A8        | cis-4-methoxy thujane | 0.20 | 129.20               |
| 9     | A9        | Nonanoic acid, methyl ester | 0.27 | 172.26               |
| 10    | A10       | 1,1-Dimethoxynonane | 0.16 | 188.31               |
| 11    | A11       | 2-Undecanone | 1.06       | 170.29                 |
| 12    | A12       | Methyl Decanoate | 6.88       | 186.29                 |
| 13    | A13       | n-Decanoic acid | 1.15       | 172.26                 |
| 14    | A14       | DL-Proline, 5-oxo-, methyl ester | 0.38 | 143.14               |
| 15    | A15       | Undecanoic acid, methyl ester | 0.15 | 200.32               |
| 16    | A16       | Decanoic acid, TMS derivative | 0.23 | 244.44               |
| 17    | A17       | 14-Bromo-2-methyl-tetradec-1-en-3-ol | 1.23 | 305.29               |
| 18    | A18       | Trimethylsilyl p-(trimethylsilyloxy)benzoate | 0.48 | 282.48               |
| 19    | A19       | Phenol, 2,4-bis(1,1-dimethylethyl)- | 0.32 | 278.50               |
| 20    | A20       | Dodecanoic acid, methyl ester | 0.62 | 214.34               |
| 21    | A21       | 1-Hexadecene | 0.21       | 224.42                 |
| 22    | A22       | Quinic acid | 1.28       | 192.17                 |
| 23    | A23       | gamma-Nonalactone | 0.23 | 156.22               |
| 24    | A24       | Dodecanaldimethylacetal | 1.19 | 230.39               |
| 25    | A25       | Methyl tetradecanoate | 0.39 | 242.40               |
| 26    | A26       | 1-Nonadecene | 0.22       | 266.50                 |
| 27    | A27       | 6,10,14-Trimethylpentadecan-2-one | 0.28 | 268.50               |
| 28    | A28       | Neophytadiene | 0.91       | 278.50                 |
| 29    | A29       | 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate, (2E,7R,11R)- | 0.44 | 338.60               |
| 30    | A30       | 9-Hexadecenoic acid, methyl ester, (Z)- | 0.45 | 268.40               |
| 31    | A31       | Hexadecanoic acid, methyl ester | 7.69 | 270.50                |
| 32    | A32       | n-Hexadecanoic acid | 4.86 | 256.42                |
| 33    | A33       | Eicosyltrifluoroacetate | 0.28 | 394.60               |
| 34    | A34       | Heptadecanoic acid, methyl ester | 0.24 | 284.50               |
| 35    | A35       | Palmitic Acid, TMS derivative | 0.41 | 328.60               |
| 36    | A36       | 2(3H)-Furanone, 5-heptyldihydro- | 0.26 | 184.27               |
| 37    | A37       | n-Nonadecanol-1 | 3.62 | 284.50               |
| 38    | A38       | 9,12-Octadecadienoic acid (Z, Z)-, methyl ester | 5.37 | 294.47               |
| 39    | A39       | 6-Octadecenoic acid, methyl ester, (Z)- | 7.05 | 296.50               |
| 40    | A40       | cis-11-Octadecenoic acid methyl ester | 0.44 | 296.50               |
| 41    | A41       | 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate, (2E,7R,11R)- | 4.57 | 338.60               |
| 42    | A42       | Methyl stearate | 3.81 | 298.50               |
| 43    | A43       | 9,12-Octadecadienoic acid (Z, Z)-, methyl ester | 5.37 | 294.47               |
| 44    | A44       | cis-9-Hexadecenal | 1.89 | 238.41               |
| 45    | A45       | Octadec-9-enoic acid | 0.21 | 282.50               |
| 46    | A46       | 1-Octadecanol, TMS derivative | 0.11 | 342.67               |
| 47    | A47       | Octadecanoic acid | 1.02 | 284.50               |
| 48    | A48       | Phytol, TMS derivative | 0.20 | 368.71               |
| 49    | A49       | Ethyl 9,12-hexadecadienoate | 0.28 | 280.40               |
site of 6LU7, 7JRN and 6W02 according to their predicted affinity as mentioned in Table 4. MD simulation deals with studying the behavior of protein and ligand for a particular time. The whole simulation was subjected for 100 ns in the production phase for the ligand complexes. The structure and dynamic properties of the protein–ligand complexes were analyzed as the backbone RMSDs during the simulation period of 100 ns. The RMSD plot for 6LU7 and 6LU7 bounded with A104 presented in Fig. 4a reveals that during the initial 60 ns of MD simulation, the ligand-bound protein showed significant stability, however, it was slightly unstable after 60 ns of simulation. Similarly, RMSD plot of 7JRN and 7JRN bonded with A104 is presented in Fig. 4b, where the ligand-bound complex established a stabilization between 50 and 70 ns of simulation. However, the RMSD plot 6W02 bonded with A166 presented in Fig. 4c showed instability from 0 to 19 ns of simulation, and after 20 ns, it became stabilized up to 30 ns. Although a slight deviation was observed between 30 and 60 ns, the complexes stabilized after 60 ns.

Ligand RMSD, the radius of Gyration (rGyr), Molecular Surface Area (MolSA), Solvent Accessible Surface Area (SASA), Polar Surface Area (PSA) of ligands with respect to the reference conformation were also studied during the MD simulation which is presented in Fig. 5a–c.

The root-mean-square fluctuations (RMSF) were assessed and plotted to equate the flexibility of each residue in the ligand–protein complexes. The RMSF of the protein–ligand complex denoted the minimized fluctuation for all the complexes. The RMSF did not deviate much during the simulation period of 100 ns, and the average RMSF values were kept constant for all the complexes and the same is presented in Fig. 6a–c.

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Table 2 (continued)

| SL No | Code name | Compound name                                                                 | Peak area% | Molecular mass (g/mol) |
|-------|-----------|--------------------------------------------------------------------------------|------------|------------------------|
| 50    | A50       | 3.alpha., 5.alpha.-cyclo-ergosta-7,9(11), 22t-triene-6.beta.-ol                 | 0.30       | 394.63                 |
| 51    | A51       | n-Nonadecanol-1                                                                | 1.36       | 284.50                 |
| 52    | A52       | 3.alpha., 5.alpha.-cyclo-ergosta-7,9(11), 22t-triene-6.beta.-ol                 | 0.68       | 394.63                 |
| 53    | A53       | 9-Octadecenal, (Z)-                                                            | 0.30       | 266.50                 |
| 54    | A54       | Eicosanoic acid, methyl ester                                                   | 1.12       | 326.60                 |
| 55    | A55       | Docosanoic acid, methyl ester                                                   | 0.93       | 354.60                 |
| 56    | A56       | Tricosanoic acid, methyl ester                                                   | 0.44       | 368.60                 |
| 57    | A57       | Hexadecanoic acid, 2-hydroxy-, methyl ester                                     | 0.43       | 316.50                 |
| 58    | A58       | 3-Isobutyl-9,10-dimethoxy-2,3,4,6,7,11b-hexahydro-1H-pyrido[2,1-a]-isoquinolin-2-amine | 0.30       | 318.50                 |
| 59    | A59       | Tetracosanoic acid, methyl ester                                                | 0.80       | 382.70                 |
| 60    | A60       | 1-Methyladenine                                                                | 0.13       | 281.27                 |
| 61    | A61       | Methyl 2-hydroxy-heptadecanoate                                                | 0.24       | 314.50                 |
| 62    | A62       | 2H-1,3-Benzoxazine, octahydro-2-(4-methoxyphenyl)-, trans-                      | 4.00       | 217.31                 |
| 63    | A63       | Pentacosanoic acid, methyl ester                                                | 0.14       | 396.70                 |
| 64    | A64       | Methyl 2-hydroxy-tetracosanoate                                                | 1.04       | 398.70                 |
| 65    | A65       | 1-Hentetracontanol                                                             | 0.36       | 593.10                 |
| 66    | A66       | 2-Hydroxy-5-methoxybenzaldehyde, 3-methylbutyl ether                           | 0.43       | 222.28                 |
| 67    | A67       | Hexacosanoic acid, methyl ester                                                 | 0.32       | 410.70                 |
| 68    | A68       | 6,7-Dihydroindoxazene, 3-undecyl                                              | 0.27       | 275.40                 |
| 69    | A69       | Spirost-5-en-3-ol, acetate, (3.beta., 25R)-                                    | 0.24       | 456.70                 |
| 70    | A70       | Octacosanoic acid, methyl ester                                                | 1.09       | 438.80                 |
| 71    | A71       | Ergost-5-en-3-ol, (3.beta., 24R)-                                              | 0.72       | 400.70                 |
| 72    | A72       | Stigmasterol                                                                  | 0.63       | 412.70                 |
| 73    | A73       | Diosgenin                                                                     | 2.26       | 414.60                 |
| 74    | A74       | gamma.-Sitosterol                                                              | 5.82       | 414.70                 |
| 75    | A75       | 9,19-Cyclolanost-24-en-3-ol, (3.beta.-)                                        | 0.98       | 426.70                 |
| 76    | A76       | gamma.-Sitostenone                                                            | 0.97       | 412.70                 |
| 77    | A77       | Silane, (dotriacontyloxy)trimethyl-                                            | 0.67       | 539.00                 |
| 78    | A78       | Olean-12-En-28-Oic Acid, 2.Beta., 3.Beta.,                                   | 2.93       | 455.70                 |
| 79    | A79       | Stigmastane-3,6-dione, (5.alpha.-)                                            | 0.74       | 428.70                 |
| 80    | A80       | C(14A)-Homo-27-norgammacer-14-ene, 3.beta.-methoxy                            | 0.79       | 456.70                 |
The hydrogen bond interaction between the bioactive compounds and the active site residues of the proteins was also analyzed during the entire duration of simulation. For 6LU7 and A104, H-bond was observed in His-163; 7JRN and A104 in ASN-167 and GLN-269 and 6W02 and A166 in ASP-22, LEU-126, ALA-129, ILE-131 and ALA-154 and are presented in Fig. 7a–c along with other interactions formed during the 100 ns of simulation.

Result of the interaction study between the shortlisted ligands and the receptor proteins at various steps of the Molecular Dynamics simulation is reported in Figs. 7a–c and 8a–c. Fig. 7a–c demonstrates the Protein interactions with the ligand observed throughout the simulation. Receptor–ligand interactions are generally categorized into four types viz. Hydrogen Bonds, Hydrophobic, Ionic and Water Bridges. Each interaction type contains more specific subtypes, which were also explored through the ‘Simulation Interactions Diagram’ presented in Fig. 8a–c. The stacked bar charts are normalized over the course of the trajectory, for example, a value of 0.5 suggests that 50% of the simulation time, the specific interaction is maintained. Values over 1.0 are possible as some protein residue makes multiple contacts of same subtype with the ligand. Timeline representation of the interactions and contacts (H-bonds, Hydrophobic, Ionic, Water bridges) is also summarized in each of the lower panel of Fig. 7a–c. The interaction analysis between 6LU7 and A104 during the entire duration of 100 ns of MDs shows that the amino acid His 163, His 164, Glu 166, Asp 187, Arg-188, Thr 190 and Gln 192 have formed hydrogen bonds with different atoms of A104. Similarly, Asn 267, Gly 266 and Gln 269 of 7JRN have shown hydrogen bond contacts with the ligand A104. Lastly, the interaction analysis between 6W02 and A166 reveals that hydrogen bonds were formed between the ligand with amino acids namely Ala 22, Ile 23, Leu 126, Ser 128, Ala 129, Ile 131, Ala 154 and Phe 156.
Table 3  List of phytocompounds identified from *H. cordata* by LC–MS

| Sl. No | Code name | Compound name                                                                 | Molecular mass (g/mol) |
|--------|-----------|--------------------------------------------------------------------------------|------------------------|
| 1      | A81       | 3-methyl sulfolene                                                             | 132.02                 |
| 2      | A82       | Niacinamide                                                                    | 122.05                 |
| 3      | A83       | 3-Oxo-3-ureidopropanoate                                                       | 146.03                 |
| 4      | A84       | S-(4,5-Dihydro-2-methyl-3-furanyl) ethanethioate                               | 138.04                 |
| 5      | A85       | Urocanic acid                                                                  | 138.04                 |
| 6      | A86       | 3-hydroxy-3-methyl-Glutaric acid                                               | 162.05                 |
| 7      | A87       | Magnesium dipropionate                                                          | 170.04                 |
| 8      | A88       | 1-Naphthoic acid                                                               | 172.05                 |
| 9      | A89       | 2-Ketogulonolactone                                                            | 176.03                 |
| 10     | A90       | Dibenzo-p-dioxin                                                               | 184.05                 |
| 11     | A91       | p-Hydroxybenzophenone                                                           | 198.06                 |
| 12     | A92       | ethyl-2-amino-4-methyl-Thiazole-5-Carboxylate                                  | 186.04                 |
| 13     | A93       | Dinitrosopentamethylenetetramine                                               | 186.08                 |
| 14     | A94       | Nor-Acetyl-L-glutamine                                                          | 188.08                 |
| 15     | A95       | Porphobilinogen                                                                | 226.09                 |
| 16     | A96       | (22,4′Z)-2-(5-Methylthio-4-penten-2-ynylidene)-1,6-dioxaspiro[4.4]non-3-ene     | 234.07                 |
| 17     | A97       | Clofibrate Acid                                                                | 214.03                 |
| 18     | A98       | N-Glycolyl-D-glucosamine                                                        | 237.08                 |
| 19     | A99       | 2-(7′-Methylthio)heptylmalic acid                                               | 278.11                 |
| 20     | A100      | 8-Hydroxydesmethylondansetron                                                   | 295.13                 |
| 21     | A101      | L-N-(1H-Indol-3-ylacetyl)aspartic acid                                          | 290.09                 |
| 22     | A102      | Purpuritenin B                                                                  | 292.11                 |
| 23     | A103      | Tuliposide B                                                                    | 294.09                 |
| 24     | A104      | 6-Hydroxyondansetron                                                           | **309.15**            |
| 25     | A105      | Demethylcitalopram                                                             | 310.15                 |
| 26     | A106      | Ethopropazine                                                                   | 312.17                 |
| 27     | A107      | Fluvoxamine acid                                                                | 318.12                 |
| 28     | A108      | podocarpic acid                                                                 | 274.16                 |
| 29     | A109      | Cinnavalininate                                                                  | 300.03                 |
| 30     | A110      | Diazinon                                                                        | 304.10                 |
| 31     | A111      | Laurencenone A                                                                  | 332.05                 |
| 32     | A112      | Maximaflavone J                                                                 | 336.14                 |
| 33     | A113      | 1,3-Diaza-spiro[4.6]undecane-2,4-dione                                          | 336.19                 |
| 34     | A114      | 2′-Oxquinidine                                                                  | 340.18                 |
| 35     | A115      | Isocycloneosamandaridine                                                        | 345.23                 |
| 36     | A116      | 9-hydroperoxy-12,13-dihydroxy-10-octadecenoic acid                             | 346.23                 |
| 37     | A117      | 5alpha-Androstane-2beta-fluoro-17beta-ol-3-one acetate                          | 350.22                 |
| 38     | A118      | Lochnericine                                                                    | 352.18                 |
| 39     | A119      | Tephrowatsin A                                                                  | 354.18                 |
| 40     | A120      | Kanzonol V                                                                      | 376.16                 |
| 41     | A121      | 5(S),6(R)-Lipoxin A4-d5                                                          | 357.25                 |
| 42     | A122      | 10-Deoxygeniposidic acid                                                        | 358.12                 |
| 43     | A123      | Malachite green                                                                  | 364.17                 |
| 44     | A124      | 6alpha,9-Difluoro-11beta-hydroxypregn-4-ene-3,20-dione                         | 366.20                 |
| 45     | A125      | 9,10-dihydroxy-Octadecanedioic acid                                             | 346.23                 |
| 46     | A126      | Gentian Violet                                                                   | 371.24                 |
| 47     | A127      | 15beta-Hydroxy-7alpha-mercapto-pregn-4-ene-3,20-dione 7-acetate                 | 404.20                 |
| 48     | A128      | Albafuran A                                                                     | 378.18                 |
| 49     | A129      | Fludrocortisone                                                                 | 380.20                 |
| Sl. No | Code name | Compound name | Molecular mass (g/mol) |
|-------|-----------|---------------|-----------------------|
| 50    | A130      | Ajaconine     | 359.24                |
| 51    | A131      | 3,12-Dioxochola-1,4,9(11)-tri-en-24-oic Acid | 382.21                |
| 52    | A132      | Quercetol B   | 368.19                |
| 53    | A133      | 5-Megastigmen-7-yne-3,9-diol 9-glucoside | 370.19                |
| 54    | A134      | Pirenperone   | 393.18                |
| 55    | A135      | 2,2-Dimethyl-3-(4-methoxyphenyl)-4-ethyl-6-(1-pyrrolidinylmethyl)-2H-1-benzopyran-7-ol | 393.23                |
| 56    | A136      | 20-Dihydrodexamethasone | 394.22                |
| 57    | A137      | 12α-Hydroxy-3-oxochola-1,4,6-trien-24-oic Acid | 384.23                |
| 58    | A138      | Actinonin     | 385.25                |
| 59    | A139      | trans-Methylbixin | 408.23                |
| 60    | A140      | Sesamin       | 354.35                |
| 61    | A141      | Pregn-4-en-20-one,3β,17-dihydroxy-6a-methyl-,17-acetate | 388.26                |
| 62    | A142      | N-Carboxytocainideglucuronide | 412.15                |
| 63    | A143      | 3β-Hydroxy-6-oxo-5α-cholan-24-oic Acid | 390.28                |
| 64    | A144      | Linopirdine   | 391.17                |
| 65    | A145      | Naltrindole   | 414.19                |
| 66    | A146      | Fluorometholone 17-acetate | 418.21                |
| 67    | A147      | Tris(butoxyethyl)phosphate | 398.24                |
| 68    | A148      | 4-Quinolone-3-Carboxamide CB2 Ligand | 422.25                |
| 69    | A149      | Myriocin      | 401.27                |
| 70    | A150      | 1α,25-dihydroxy-21-nor-20-oxavitamin D3/1α,25-dihydroxy-21-nor-20-oxacholecalciferol | 404.29                |
| 71    | A151      | Usambarensine | 432.23                |
| 72    | A152      | 5,5’-(4-Tetradecene-1,4-diyl)bis[1,3-benzenediol]; 5,5’-[(4Z)-4-Tetradecene-1,14-diyl]di(1,3-benzenediol) | 412.26                |
| 73    | A153      | 6-keto Testosterone Enanthate | 414.28                |
| 74    | A154      | Clindamycin sulfoxide | 440.17                |
| 75    | A155      | Suillin       | 440.29                |
| 76    | A156      | 1-heptadecanoyl-sn-glycerol 3-phosphate | 424.26                |
| 77    | A157      | Condelphine   | 449.28                |
| 78    | A158      | cholest-4,14-dien-15,20-diol-3,16-dione | 428.29                |
| 79    | A159      | Dihydrocetrol | 452.29                |
| 80    | A160      | Canthothenic acid | 454.31                |
| 81    | A161      | 17-phenyl trinor Prostaglandin F2α serinol amide | 461.28                |
| 82    | A162      | 26,26,26-trifluoro-25-hydroxy-27-norvitamin D3/26,26,26-trifluoro-25-hydroxy-27-norcholecalciferol | 442.31                |
| 83    | A163      | Isoquercitrin  | 464.40                |
| 84    | A164      | 3,5-Didecanoylpyridine | 387.60                |
| 85    | A165      | Quercetin     | 302.25                |
| 86    | A166      | Quercitrin    | 484.40                |
| 87    | A167      | Hyperoside    | 464.41                |
| 88    | A168      | Amastatin     | 474.27                |
| 89    | A169      | Progeldanamycin | 475.29                |
| 90    | A170      | 26,26,26-trifluoro-25-hydroxyvitamin D3/26,26,26-trifluoro-25-hydroxycholecalciferol | 454.31                |
| 91    | A171      | Calystatin A  | 456.32                |
| 92    | A172      | Borrelidin    | 489.31                |
| 93    | A173      | Murrayenol    | 470.34                |
| 94    | A174      | Dipyridamole  | 504.32                |
| 95    | A175      | Rhodoxanthin  | 562.38                |
| 96    | A176      | Canthaxanthin | 564.39                |
| 97    | A178      | 3-Benzoyloxy-6-oxo-12-ursen-28-oic acid | 574.36                |

Bold words signify main compound’s potential to inhibit SARS-CoV-2 replication proteins
Fig. 2 LC–MS chromatogram of phytocompounds identified from *H. cordata*

Table 4 Result of the docking experiment performed between the receptors and the ligand library

| Compounds code | Compounds name                                                        | Docking score | Glide ligand efficiency | Glide gscore | Glide lipo | Glide hbond |
|----------------|-----------------------------------------------------------------------|---------------|-------------------------|--------------|------------|-------------|
| **Result of Docking between 6LU7 and the ligand library** |                                                                       |               |                         |              |            |             |
| A107           | Fluvoxamine acid                                                      | −7.929        | −1.01                   | −7.929       | −2.108     | −0.36       |
| A104           | 6-Hydroxyondansetron                                                  | −7.147        | −0.884                  | −7.274       | −2.472     | −0.122      |
| A120           | Kanzonol V                                                            | −7.024        | −0.762                  | −7.024       | −3.279     | −0.588      |
| A127           | 15beta-Hydroxy-7alpha-mercapto-pregn-4-ene-3,20-dione 7-acetate      | −6.793        | −0.737                  | −6.793       | −2.162     | −0.304      |
| A99            | 2-(7′-Methylthio) heptylmalic acid                                     | −6.746        | −0.834                  | −6.883       | −2.181     | −0.048      |
| **Result of Docking between 7JRN and the ligand library** |                                                                       |               |                         |              |            |             |
| A105           | Demethylcitalopram                                                    | −5.842        | −1.413                  | −5.843       | −1.943     | −0.2        |
| A140           | Sesamin                                                               | −5.779        | −1.537                  | −5.779       | −2.961     | 0           |
| A104           | 6-Hydroxyondansetron                                                  | −5.544        | −1.341                  | −5.672       | −2.087     | 0           |
| A100           | 8-Hydroxydesmethylandansetron                                         | −5.464        | −1.321                  | −5.602       | −1.83      | −0.164      |
| A106           | Ethopropazine                                                         | −5.226        | −1.277                  | −5.227       | −2.377     | 0           |
| **Result of Docking between 6W02 and the ligand library** |                                                                       |               |                         |              |            |             |
| A166           | (Quercitrin)                                                          | −6.759        | −0.211                  | −6.788       | −1.841     | −0.162      |
| A165           | (Quercetin)                                                           | −6.067        | −0.276                  | −6.099       | −2.301     | 0           |
| A163           | (Isoquercitrin)                                                       | −5.674        | −0.172                  | −5.703       | −1.815     | 0           |
| A167           | (Hyperoside)                                                          | −5.674        | −0.172                  | −5.703       | −1.815     | 0           |
| A164           | (3,5-Didecanoylpyridine)                                              | −5.206        | −0.186                  | −5.206       | −3.108     | −0.158      |

Bold words signify main compound’s potential to inhibit SARS-CoV-2 replication proteins
ADME-Tox study

SwissADME server was used to study the ADME-Tox properties of A104 (6-Hydroxyondansetron) and A166 (Quercitrin), and the results are presented in Table 5:

Discussion

Bioinformatics tools and techniques are widely used in drug discovery sector. In silico-based drug design is an approach which plays an important role in modern drug discovery.
Fig. 4  a RMSD plot of 6LU7 and 6LU7 bounded with A104.  
b RMSD plot of 7JRN and 7JRN bonded with A104.  
c RMSD plot 6W02 and 6W02 bonded with A166

(A) RMSD plot of 6LU7 and 6LU7 bounded with A104

(B) RMSD plot of 7JRN and 7JRN bonded with A104

(C) RMSD plot 6W02 and 6W02 bonded with A166
Fig. 5  Ligand RMSD: Root-mean-square deviation of ligands with respect to the reference conformation. The radius of Gyration (rGyr): Representation of the ‘extendedness’ of the ligands. Molecular Surface Area (MolSA): Molecular surface calculation with 1.4 Å probe radius. This value is reciprocal to a van der Waals surface area. Solvent Accessible Surface Area (SASA): Surface area of the respective ligands accessible by a water molecule is presented. Polar Surface Area (PSA): Solvent accessible surface area in the ligands contributed only by oxygen and nitrogen atoms. a A104 complexed with 6LU7 b A104 complexed with 7JRN and c A166 with 6W02
Fig. 6  RMSF plots for Protein a 
RMSF plot for 6LU7  b RMSF 
plot for 7JRN  c RMSF 6W02
It reduces the cost and takes less time to search out lead molecules. This structural-based drug designing study provides the probable drug compound for a particular disease which can be further confirmed by in vivo, as well as in vitro experimental studies [40, 41]. The outbreak of COVID-19 caused by SARS-CoV-2 has thrown millions of human lives out of gear within one year timescale. The virus has not only disrupted the global healthcare networks but it also severely affected the global economy [42]. Therefore, there is an urgent need of antiviral drugs from bioactive phytocompounds for the inhibition of SARS-CoV-2 replication proteins. The SARS-CoV-2 is a single-stranded RNA virus which requires mainly three proteins, namely Main protease (Mpro), Papain-like protease (PLpro) and ADP ribose phosphatase (ADRP), for their replication. The inhibition of the expression of these proteins may lead to the development of potent antiviral effective against treatment of COVID-19. Bioactive phytocompounds from traditional medicinal plants are reported to possess several therapeutic properties. In the present study, we explored the possibilities and demonstrated that at least two bioactive phytocompounds, namely 6-Hydroxyondansetron (Fig. 9a) and Quercitrin (Fig. 9b), quantified from *Houttuynia cordata* Thunb. possess potential anti-SARS-CoV-2 activities and demonstrated potential to inhibit Main protease (Mpro), Papain-like protease (PLpro) and ADP ribose phosphatase (ADRP). Previous study reported that the extract of *H. cordata* inactivates SARS-CoV 3CL pro and inhibits the viral replication [9]. It also demonstrated antiviral activity against Chikungunya viral strains [18], Herpes simplex viruses-1 (HSV-1) and Herpes simplex viruses-2 (HSV-2) [21], and dengue virus serotype 2 (DEN-2) [24]. The bioactive phytocompounds isolated from the herb, rutin (8.8%), hyperin (26.7%), isoquercitrin (9.9%), and quercitrin (31.7%) reduced influenza A virus (IAV)-induced acute lung injury (ALI) in mice by inhibiting Influenza neuraminidase and Toll-like receptor signaling [25]. Our present studies have characterized and identified a total of 177 phytocompounds from the herb *H. cordata* by GC–MS and LC–MS analysis. The molecular docking of ligands (bioactive phytocompounds from *H. cordata*) against three different receptors of SARS-CoV-2, namely Mpro (PDB IDs 6LU7), PLpro (7JRN) and ADRP

(A) Various interactions between 6LU7 and A104

Fig. 7  a Various interactions between 6LU7 and A104. b Various interactions between 7JRN and A104. c Various interactions between 6W02 and A166
respective, revealed that the bioactive phytocompounds (ligand) coded with A104 (6-Hydroxyondansetron) have shown more binding affinities toward Mpro (6LU7) and PLpro (7JRN) with G-score $-7.274$ and $-5.672$, respectively, while bioactive phytocompound A166 (Quercitrin) has demonstrated the best binding affinity toward ADRP (6W02) with G-score $-6.788$. Therefore, the molecular dynamics simulation study of both the bioactive phytocompounds A104 and A166 was performed. From the MD simulation analysis, it is evident that the complexes A104-6LU7 and A166-6W02 have shown better stability than the A104-7JRN complex within 100 ns duration. The interaction analysis during the entire duration of MDs, as presented in Fig. 7a–c and Fig. 8a–c, and its subsequent analysis also support good binding efficiency of the selected ligands to the receptor proteins. MDs study ultimately identified and confirmed A104 and A166 as potential inhibitors for Mpro, PLpro and ADRP which can prevent replication machinery of SARS-CoV-2. ADME-Tox study also reveals that the bioactive phytocompound A104 (6-Hydroxyondansetron) passes all the required drug discovery rules and possesses multi-target capabilities which can become a potential inhibitor for the Mpro (6LU7) and PLpro (7JRN) protein of SARS-CoV-2 without causing toxicity with minimum
chance of resistance development in near future. On the other hand, the bioactive phytocompound A166 (Quercitrin) has demonstrated relatively less drug-like properties and has single target capability but can also be used as potential inhibitor against ADRP (6W02) of SARS-CoV-2. Some evidence of clinical trials of different quercitrin derivatives such as quercetin and isoquercitrin are currently emerging for the treatment of COVID-19 in few countries [43]. In Pakistan, clinical trial for adjuvant benefits of Quercetin Phytosome with COVID-19 is reported to undergoing at Liaquat University Hospital Jāmshoro, Sindh in community-based subjects with confirmed SARS-CoV-2 infection. Researchers from Kanuni Sultan Suleyman Training and Research Hospital, Istanbul Turkey are also reported to perform the clinical trial on effect of Quercetin for Prophylaxis and treatment of COVID-19 [43]. Clinical trials are also reported to undergoing in France to evaluate efficacy of the masitinib and isoquercetin combination in moderate and severe COVID-19 patients [43]. However, clinical trial evidence on 6-Hydroxyondansetron (A104) against SARS-CoV-2 replication proteins are yet to be reported. Thus, 6-Hydroxyondansetron with multi-target capabilities as potential inhibitor for Mpro (6LU7) and PLpro (7JRN) replication proteins of SARS-CoV-2 can be effectively used as potential drug candidate for treatment of COVID-19 after in vitro and in vivo clinical trials.

**Conclusion**

The present studies have characterized 177 phytocompounds from *H. cordata* through GC–MS/LC–MS and they were docked against three receptor proteins, namely Main protease (Mpro), Papain-like protease (PLpro) and ADP ribose

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**Fig. 7** (continued)
Fig. 8  

(A) Interaction diagram; (I) 6LU and A104 interaction at 0 ns (II) 6LU and A104 interaction at 100 ns

(B) Interaction diagram; (I) 7JRN and A104 interaction at 0 ns (II) 7JRN and A104 interaction at 100 ns

Fig. 8  

a Interaction diagram; (I) 6LU and A104 interaction at 0 ns (II) 6LU and A104 interaction at 100 ns. b Interaction diagram; (I) 7JRN and A104 interaction at 0 ns (II) 7JRN and A104 interaction at 100 ns. c Interaction diagram; (I) 6W02 and A166 at 0 ns (II) 6W02 and A166 at 100 ns
phosphatase (ADRP), of SARS-CoV-2 responsible for controlling the replication process. The result of molecular docking studies clearly revealed that the compound code A104 (6-Hydroxyondansetron) has shown more binding affinity toward two SARS-CoV-2 receptor proteins Mpro (PDB ID 6LU7) and PLpro (PDB ID 7JRN) with G-score −7.274 and −5.672, respectively, thus could be used as potential inhibitor for Mpro and PLpro to prevent the replication process of SARS-CoV-2. On the other hand, phytocompounds code A166 (Quercitrin) is also identified

Fig. 8 (continued)

Fig. 9 Chemical structure and properties of two bioactive phytocompounds with highest binding affinity with SARS-CoV-2 receptors [Mpro (PDB IDs 6LU7), PLpro (7JRN) and ADRP (6W02)]. a 6-Hydroxyondansetron (A104). [Properties: PSA: 60.05; ALogP: 2.3925; Stereo Center Count: 1; Hydrogen Acceptor Count: 3; Hydrogen Donor Count: 1; Composition: C: 69.9%, H: 6.2%, N: 13.6%, O: 10.3%; Formula Weight: 309.36236; Exact Mass: 309.147726878; Molecular Formula: C_{18}H_{19}N_{3}O_{2}]. b Quercitrin (A166). [Properties: PSA: 186.36; ALogP: 0.5892; Stereo Center Count: 5; Hydrogen Acceptor Count: 11; Hydrogen Donor Count: 7; Composition: C: 56.3%, H: 4.5%, O: 39.3%; Formula Weight: 448.3769; Exact Mass: 448.10056146; Molecular Formula: C_{21}H_{20}O_{11}].
as another promising inhibitor as it has shown best binding affinity toward protein ADRP (PDB ID 6W02) of SARS-CoV-2. Among the two-identified bioactive phytocompounds (ligands), 6-Hydroxyondansetron (A104) has demonstrated more promising properties as drug candidate since it has shown more binding affinity toward multiple receptors. This is very significant as chances of development of drug resistance are much lower in case of multi-target capabilities as inhibitor. The Molecular Dynamic Simulation analysis confirmed that the complex A104-6LU7 and A166-6W02 have shown better stability than the A104-7JRN complex within 100 ns duration. ADME-Tox study further demonstrated that out of two bioactive phytocompounds evaluated, 6-Hydroxyondansetron (A104) passes all the required drug discovery rules, and thus confirmed as viable drug candidate which can be used as potential inhibitor for the two SARS-CoV-2 replication proteins (Mpro and PLpro). However, Quercitrin (A166) has demonstrated relatively less drug-like properties but can also be used as potential inhibitor for ADRP.

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Author’s contribution Authors PKH, HT, BT and SM are senior research guides and mentors who designed the experiment and drafted the manuscript in consultation with all the co-authors. Author SKD, SM, PKH and BT significantly contributed to docking and molecular dynamics simulation and identification of the phytocompounds while HT, PKH and SKD significantly contributed to phytochemistry and bioactive phytocompounds analysis.

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Declarations

Conflict of interest Authors declare no conflict of interest.

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