Evaluation of potency of the selected bioactive molecules from Indian medicinal plants with M\text{Pro} of SARS-CoV-2 through in silico analysis

Pinku Halder a, Upamanyu Pal a, Pranab Paladhi a, Saurav Dutta a, Pallab Paul a, Samudra Pal a, Debasmita Das a, Agnish Ganguly a, Ishita Dutta a, Sayarneel Mandal a, Anirban Ray b, Sujay Ghosh b, *

a Cyto genetics & Genomics Research Unit, Department of Zoology, University of Calcutta, Tarak nath Palti Siksha Prangan (Ballygunge Science College Campus), 35 Ballygunge Circular Road, Kolkata, West Bengal, 700019, India
b Department of Zoology, Bangabasi Morning College (affiliated to University of Calcutta), Kolkata, West Bengal, 700009, India

ABSTRACT

Background: The recent outbreak of the novel SARS-CoV-2 across the globe and the absence of specific drug against this virus lead the scientific community to look into some alternative indigenous treatments. India as a hub of Ayurvedic and medicinal plants can shed light on its treatment using specific active bio-molecules from these plants.

Objectives: Keeping our herbal resources in mind, we were interested to inquire whether some phytochemicals from Indian spices and medicinal plants can be used as alternative therapeutic agents in contrast to synthetic drugs.

Materials and methods: We used in silico molecular docking approach to test whether bioactive molecules of herbal origin such as hyperoside, nimbaflavone, ursolic acid, 6-gingerol, 6-shogaol and 6-paradol, curcumin, catechins and epigallocatechin, α-Hederin, pipeline could bind and potentially block the M\text{Pro} enzyme of the SARS-CoV-2 virus.

Results: Ursolic acid showed the highest docking score (–8.7 kcal/mol) followed by hyperoside (–8.5 kcal/mol), α-Hederin (–8.5 kcal/mol) and nimbaflavone (–8.0 kcal/mol), epigallocatechin, catechins, and curcumin also exhibited high binding affinity (Docking score –7.3, –7.1 and –7.1 kcal/mol) with the M\text{Pro}. The remaining tested phytochemicals exhibited moderate binding and inhibitory effects.

Conclusions: This finding provides a basis for biochemical assay of tested bioactive molecules on SARS-CoV-2 virus.

© 2021 The Authors. Published by Elsevier B.V. on behalf of Institute of Transdisciplinary Health Sciences and Technology and World Ayurveda Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a highly infectious virus for novel coronavirus disease -19 (COVID-19) disease that caused the recent outbreak in China in December 2019 and rapidly spread to the other parts of the globe owing to its extreme contagious nature. Its initial symptoms include fever, dry cough, tiredness, aches or pains, diarrhoea, difficulty in breathing, etc. In the human body, it probably settles through the angiotensin-converting enzyme 2 (ACE2) [1] receptor for entry into the host cell and the transmembrane protease, serine 2 (TMPRSS2) for viral spike protein priming [2,3]. The infection gradually took the shape of a pandemic with extremely high mortality [4,5] owing to lack of definite treatment regimen and medications against the virus as well as due to the presence of co-morbidities. Even the so-called developed nations like U.S. and several European countries failed to control the infection with their cutting-edge medical technologies at a very early phase. As a result, the World Health Organisation declared COVID-19 as a public health emergency of international concern [6].

The previous name of this betacoronavirus was 2019-nCoV. It was renamed as SARS-CoV-2 by the International Committee on Taxonomy of Viruses (ICTV) [7]. The genome of SARS-CoV-2 has been sequenced [8]. The whole genome sequence analysis of SARS-
CoV-2 shows 96.2% similarity with bat coronavirus (SARSr-CoV) [9,10], while it shows low sequence identity with that of SARS-CoV (about 79%) or MERS-CoV (about 50%) [11,12]. Since the onset of the COVID-19 pandemic, globally, researchers are involved in the rapid development of drugs and specific antiviral treatment strategies. Among all the SARS-CoV-2 targets the main protease (MPro, 3CLpro, nsp5) of the virus received major attention [13,14]. Some alternative targets like spike protein (S), RNA-dependent RNA-polymerase (RdRp, nsp12), NTPase/helicase (nsp13) and papain-like protease (PLpro, part of nsp3) have also been reported in some literature [15,16]. The SARS-CoV-2 MPro is a 33.8 kDa enzyme which plays a pivotal role in the cleavage of viral polyproteins (pp1a and pp1ab) in a site-specific (L-Q (S, A, G)) manner [17], resulting in the release of functional replicase enzyme which is crucial for transcription and replication of the virus [18–20]. Other essential enzymes which are involved in the replication process such as RdRp or nsp13 cannot fully function without this proteolytic action [13], making MPro a key enzyme in viral replication cycle. As a result, the inhibition of MPro could stop viral replication process and thus alleviate disease symptoms [21,22]. For drug discovery against SARS-CoV-2 virus, MPro is one of the most attractive viral targets. Some studies have already reported synthetic and natural inhibitors against SARS-CoV-2 MPro [17,23,24]; however, increase in substrate concentration often reduces the effectiveness of such inhibitors. Natural phytochemicals can provide safe and effective treatment by alleviating this limitation.

Although there are no approved drugs for COVID-19, a number of clinical trials are in progress [25]. Lopinavir and ritonavir, combined with Chinese herbal medicines, were used in preliminary clinical studies [26]. Indian medicinal plants and spices are a rich hub of ingredients which can be utilized for drug designing because of their high therapeutical values [27]. Previous docking study already reported that phytochemicals such as hyperoside and nimbaflavone are good candidate drugs against influenza virus strains [28]. A recent study has suggested high binding efficacy of ursolic acid (Tulsi) against surface spike glycoprotein and RNA polymerase of SARS-CoV-2 virus [29]. Ajoene and allicin (Garlic) shows strong virucidal activity against selected viruses including, herpes simplex virus type 1, herpes simplex virus type 2, parainfluenza virus type 3, vaccinia virus, vesicular stomatitis virus, and human rhinovirus type 2 [30]. Curcumin has diverse antiviral activity against dengue virus, herpes simplex virus, Zika and chikungunya virus [31–33]. Previous study reported that catechins and epigallocatechin form green tea leaves have profound antiviral effects [34]. A very recent in silico molecular docking study revealed that piperine from black pepper can act as a potent inhibitor of the antiviral enzymes of dengue and Ebola viruses [35]. An in silico screening of herbal medicines for treatment of COVID-19 has also been reported [53]. Although several clinical trials are in progress to assess the potential effects of putative therapeutic agents, very limited data is available publicly regarding the in vitro and in vivo activities of the drugs that are currently involved in clinical trials for treatment of COVID-19 [25]. It has been reported that chloroquine phosphate shows anti-COVID-19 activity [54]. Several clinical trials are assessing the potential of protease inhibitors such as lopinavir and ritonavir that have been approved for treatment of other viral infections. Lopinavir and ritonavir were identified in earlier studies to target the MPro of the SARS virus.

In the present study, we utilized the recently available high resolution experimental structure of the main protease (MPro) of SARS-CoV-2 (Fig. 1) [55], as the target for molecular docking-based virtual screening. The predictions of this study will provide information that can be utilized for choice of candidate drugs for in vitro, in vivo and clinical trials.

2. Materials and methods

2.1. Protein and chemical compounds

The X-ray crystal structure of COVID-19 MPro in complex with an inhibitor N3 (PDB ID: 6LU7) [56], having 2.16 Å resolution was downloaded from RCSB Protein Data Bank (PDB) [57]. We collected Simplified Molecular Input Line Entry System (SMILES) code for 15 ligand molecules and then 3D structures were generated and downloaded in PDB format from CORINAClassic (https://www.mm.am.com/online_demos/corina_demo/).

For hyperoside and nimbaflavone, SMILES codes were downloaded from Neem Metabolites Structure Database [58]. For rest of the ligands, SMILES codes were collected from DrugBank (https://www.drugbank.ca/) and PubChem (https://pubchem.ncbi.nlm.nih.gov/). This in silico study was carried out by using AutoDock suite 4.2.6 for protein and ligand preparations, PyRx 0.8 for grid generation and protein-ligand docking, PyMol V1.7.4 (Educational suite 4.2.6 software. Protein preparation wizard was carried out by using AutoDock suite 4.2.6 software. Protein preparation wizard has the following steps: removal of water molecules, removal of inhibitor N3 from protein structure to obtained fresh protein for docking, addition of polar hydrogens, and addition of Kollman charges. After preparation protein molecule was exported in Protein Data Bank, Partial Charge (Q), & Atom Type (T) (PDBQT) format from AutoDock.

2.2. Protein preparation

SARS-CoV-2 MPro was prepared for molecular docking study by using AutoDock suite 4.2.6 software. Protein preparation wizard has the following steps: removal of water molecules, removal of inhibitor N3 from protein structure to obtained fresh protein for docking, addition of polar hydrogens, and addition of Kollman charges. After preparation protein molecule was exported in Protein Data Bank, Partial Charge (Q), & Atom Type (T) (PDBQT) format from AutoDock.

2.3. Ligand preparation

All 15 ligands (Fig.1) were prepared for docking using AutoDock suite 4.2.6 software. Ligand preparation has the following steps: addition of hydrogen atoms, removal of unwanted molecules, addition of all hydrogens, computation of Gasteiger charges, merging of non-polar hydrogens, generating ionization states at pH 7, tautomers, geometric characteristics, and low-energy ring conformations. After preparation, ligand molecules were exported in PDBQT format for docking in PyRx 0.8 software.
Fig. 1. 3D cartoon structure of SARS-CoV-2 main protease and all 15 ligands in stick form. A) Main protease  a) Hyperoside  b) Nimbaflavone  c) Eugenol  d) Ursolic acid ; e) 6-gingerol  f) 6-shogaol  g) 6-paradol  h) Curcumin  i) Catechins  j) Epigallocatechin  k) α-Hederin  l) Echinocystic acid diacetate  m) Ajoene  n) Allicin  o) Piperine.
2.4. Grid generation

The grid generation process was done in PyRx 0.8 software. It provided a square block at the active site of the protein for the accurate binding score with thermodynamic optimal energy. A grid box size of $x = 27.9382928446$, $y = 28.2467551684$, and $z = 30.0038760533 \text{ Å}$ points was generated to cover active amino acid residues that are important for docking [55]. The grid was centered at $x,y,z$ coordinates of $13.9467660792, 12.664485092, 68.4908850063$.

2.5. Molecular docking

Molecular docking was conducted with PyRx 0.8 software which uses AutodockVina wizard at the backend. 15 ligands were docked with generated grid of prepared protein. The exhaustiveness parameter that controls the extent of the search was chosen as 8, and 9 modes were generated for each ligand. The best ligand pose selection for the receptor was done based on the docking score and lowest Root Mean Square Deviation (RMSD) value.

2.6. Validation of docking score

For validation of docking results, we used another web server Webina 1.0.2 by Durrant Lab [59] with exactly same parameters. The best ligand pose selection for the receptor was done based on the docking score and lowest RMSD value.

2.7. Calculation of inhibition constant ($K_i$) from binding energy

After docking, best ligand poses for all 15 ligands were selected on the basis of binding energy $\Delta G_b$ (kcal/mol) and RMSD values. Then inhibition constant ($K_i$) (nM) were calculated from binding energy.

### Table 1

| Compounds               | Source                          | SMILE code collected from                  | AutoDock Binding Energy $\Delta G_b$ (kcal/mol) | No. of H-bonds | Interacting Residues | Inhibition constant ($K_i$) (nM) |
|-------------------------|---------------------------------|--------------------------------------------|-----------------------------------------------|----------------|----------------------|---------------------------------|
| Hyperoside              | Neem (Azadirachta indica)       | Neem Metabolite Structure Database         | $-8.6$                                        | 6              | Leu141, Ser144, His163, Arg188, Thr190, Gln192, His163 | 494.36                          |
| Nimbaflavone            | Neem (Azadirachta indica)       | Neem Metabolite Structure Database         | $-8$                                          | 1              | Leu141, Gly143, Ser144, Cys145, His163               | 1370.95                         |
| Eugenol                 | Clove (Syzygium aromaticum)     | DrugBank, DB09086                          | $-4.9$                                        | 6              | Leu141, Gly143, Ser144, Cys145, His163               | 256085.29                      |
| Ursolic acid            | Tulsi or holy basil (Ocimum sanctum) | Drugbank, DB15588                      | $-8.7$                                        | 3              | Thr24, Leu141, Ser144                                 | 421.27                          |
| 6-gingerol              | Ginger (Zingiber officinale)    | PubChem, 442793                           | $-5.8$                                        | 5              | Arg188, Gln189, Thr190, Gln192                        | 56008.89                       |
| 6-shogaol               | Ginger (Zingiber officinale)    | PubChem, 5281794                          | $-5.8$                                        | 4              | Arg188, Gln192, Thr190, Gln192                        | 56008.89                       |
| 6-paradol               | Ginger (Zingiber officinale)    | PubChem, 94178                            | $-5.7$                                        | 5              | Gln166, Arg188, Thr190, Gln192, Gln192               | 66387.61                       |
| Curcumin                | Turmeric (Curcuma longa)        | DrugBank, DB11672                          | $-7.1$                                        | 4              | Gly143, Ser144                                        | 6268.33                        |
| Catechins               | Tea plant (Camellia sinensis)   | PubChem, 1203                             | $-7.1$                                        | 2              | Thr26, Gln189                                         | 6268.33                        |
| Epigallocatechin        | Tea plant (Camellia sinensis)   | DrugBank, DB03823 [EXPT01331]             | $-7.3$                                        | 7              | Leu141, Ser144, Cys145, His163                       | 4461.61                        |
| α-Hederin               | Black cumin (Nigella sativa)    | PubChem, 73296                            | $-8.5$                                        | 4              | His163, Gln166, Gln189                               | 585.97                         |
| Echinocystic acid diacetate | Sponge gourd (Luffa cylindrica) | PubChem, 476534                           | $-6.7$                                        | 1              | Gln166                                               | 12249.81                       |
| Ajoene                  | Garlic (Allium sativum)         | PubChem, 5386591                          | $-4.1$                                        | 2              | Ser144                                               | 987829.94                      |
| Allicin                 | Garlic (Allium sativum)         | DrugBank, DB11780                         | $-3.6$                                        | 2              | Ser144                                               | 2288176.65                    |
| Piperine                | Black pepper (Piper nigrum)     | DrugBank, DB12582                         | $-6.8$                                        | 3              | Thr25, Ser144, Cys145                               | 10334.73                      |

$: Nil.
$^a$ Hotspot residue.
2.8. Molecular dynamics simulation study

The protein and docked protein-ligand complexes were subjected to Molecular Dynamics (MD) Simulation using MDWeb web portal [60]. GROMACS FULL MD setup was performed using AMBER-99SB* force field. Simple Box Solvent Molecular Dynamics (NPT) operations were performed using the following settings: Total time 2 fs, Temperature 300 K, Total time 10 ns, Output frequency 500 steps and Total 10,000 snapshots, to obtained MD trajectory file. Water molecules and ions were removed from trajectories to obtained dry trajectory. The RMSD and B-factor fluctuations along the residues were calculated for the protein and all docked protein-ligand complexes and plotted to compare the protein backbone stability. Due to limitation in computing power, no further analyses were performed.

3. Results

This study was done to identify possible compounds that can bind to the M\textsuperscript{pro}, which may be used as a potential drug target for SARS-CoV-2. We tested 15 bioactive compounds from Indian spices and medicinal plants that have been previously reported for their antiviral activity [28,31,34,61–65]. These compounds can bind with the M\textsuperscript{pro} with a docking score of –8.7 to –3.6 kcal/mol (Table 1). Ursolic acid (Drugbank ID DB15588), a compound of Tulsi, reported to have antiviral activity [29], had highest docking score –8.7 (kcal/mol) than others (Table 1), and formed three hydrogen bonds (H-bonds) with Thr24, Leu141*, Ser144* residues of M\textsuperscript{pro} (Fig. 2).

Hyperoside and nimbaflavone were predicted to have a docking score of –8.6 and –8.0 kcal/mol (Table 1). Hyperoside forms six H-bonds with Thr24, Leu141*, Ser144*, His163*, Arg188*, Thr190*. Gln192

Fig. 2. The binding site of SARS CoV-2 M\textsuperscript{pro}, represented as a mesh, shows ligand interactions. Amino acid residues that formed polar H-bonds with ligands are highlighted with red circle in Ligplot. (a) Hyperoside (b) Nimbaflavone (c) Eugenol (d) Ursolic acid (e) 6-gingerol (f) 6-shogaol (g) 6-paradol (h) Curcumin (i) Catechins.

P. Halder, U. Pal, P. Paladhi et al. Journal of Ayurveda and Integrative Medicine 13 (2022) 100449
residues whereas nimbaflavone exhibited single H-bond with His163* residue of SARS-CoV-2 M^pro (Fig. 2). Eugenol (DrugBank ID DB09086), the principal bioactive compound of clove, exhibited a docking score of $-4.9$ kcal/mol (Table 1), and formed six H-bonds with Leu141*, Gly143, Ser144*, Cys145, His163* residues of viral M^pro (Fig. 2). Natural compounds of ginger i.e., 6-gingerol (PubChem ID 442793), 6-shogaol (PubChem ID 5281794) and 6-paradol (PubChem ID 94378) were predicted to have a docking score of $-5.8$, $-5.8$ and $-5.7$ kcal/mol (Table 1). 6-Gingerol forms five interacting H-bonds with Arg188*, Gln189, Thr190*, Gln192 residues, 6-shogaol forms four interacting H-bonds with three residues of SARS-CoV-2 M^pro (Arg188*, Thr190*, Gln192), whereas 6-paradol exhibited five interacting H-bonds with Glu166, Arg188*, Thr190*, Gln192 residues of the viral M^pro (Fig. 2). Curcumin (DrugBank ID

![Fig. 3. The binding site of SARS CoV-2 M^pro, represented as a mesh, shows ligand interactions. Amino acid residues that formed polar H-bonds with Ligands are highlighted with red circle in Ligplot. (a) Epigallocatechin (b) α-Hederin (c) Echinocystic acid diacetate (d) Ajoene (e) Allicin (f) Piperine.]

| Compound             | AutoDock Binding Energy ΔGib (kcal/mol) | No. of H-bonds | Interacting Residues                                                                 | Inhibition constant [K(J) [nM]] |
|----------------------|-----------------------------------------|----------------|------------------------------------------------------------------------------------|---------------------------------|
| Ursolic acid         | $-8.7$                                   |                | Thr24, Leu141*, Ser144*                                                            | 421.27                          |
| Hyperoside           | $-8.6$                                   | 6              | Leu141*, Ser144*, His163*, Arg188*, Thr190*, Gln192                                | 494.36                          |
| α-Hederin            | $-8.5$                                   |                | His163*, Glu166, Gln189                                                            | 585.97                          |
| PF-00835231          | $-8.4$                                   | 8              | His141, Leu141*, Gly143*, Ser144*, Cys145, Gln189                                  | 3764.107                        |
| Nimbaflavone         | $-8$                                     | 1              | His163*                                                                            | 1370.95                         |
| Remdesivir           | $-7.7$                                   | 5              | Phe140, Leu141*, Gly143*, Ser144*, His163*                                        | 2260.33                         |
| Quercetin            | $-7.4$                                   | 8              | Leu141*, Gly143*, Ser144*, Cys145, His163*                                       | 694.55                          |
| Epigallocatechin     | $-7.3$                                   | 7              | Leu141*, Ser144*, Cys145, His163*                                                 | 4461.61                         |
| Curcumin             | $-7.1$                                   | 4              | Gly143*, Ser144*                                                                  | 6268.33                         |
| Catechins            | $-7.1$                                   | 2              | Thr26, Gln189                                                                     | 6268.33                         |
| Piperine             | $-6.8$                                   | 3              | Thr25, Ser144*, Cys145                                                           | 10334.73                        |
| Echinocystic acid diacetate | $-6.7$                                   | 1              | Glu166                                                                           | 12249.81                        |
| 6-gingerol           | $-5.8$                                   | 5              | Arg188*, Gln189, Thr190*, Gln192                                             | 56008.89                        |
| 6-shogaol            | $-5.8$                                   |                | Arg188*, Thr190*, Gln192                                                        | 56008.89                        |
| 6-paradol            | $-5.7$                                   |                | Glu166, Arg188*, Thr190*, Gln192                                               | 66387.61                        |
| Eugenol              | $-4.9$                                   | 6              | Leu141*, Gly143, Ser144*, Cys145, His163*                                    | 256083.29                       |
| Ajoene               | $-4.1$                                   |                | Nil                                                                              | 987829.94                       |
| Allicin              | $-3.6$                                   | 2              | Ser144*, Cys145                                                                   | 2288176.65                      |

* Hotspot residue.
Table 3 Predicted binding energies (Kcal/mol) of all 15 phytochemicals and 3 compounds in PyRx 0.8 and Webina 1.0.2

| Compounds     | Binding Energy (Kcal/mol) | Docking with PyRx 0.8 | Docking with Webina 1.0.2 |
|---------------|---------------------------|-----------------------|---------------------------|
| Hyperoside    | -8.6                      | -8.6                  |                           |
| Nimbalflavone | -8                        | -8                    |                           |
| Eugenol       | -4.9                      | -4.9                  |                           |
| Ursolic acid  | -8.7                      | -8.7                  |                           |
| 6-gingerol    | -5.8                      | -5.6                  |                           |
| 6-shogaol     | -5.8                      | -5.8                  |                           |
| 6-paradol     | -5.7                      | -5.3                  |                           |
| Curcumin      | -7.1                      | -6.9                  |                           |
| Catechins     | -7.1                      | -7.1                  |                           |
| Epigallocatechin | -7.3                  | -7.3                  |                           |
| α-Hederin     | -8.5                      | -8.6                  |                           |
| Echinocysic acid diacate | -6.7                  | -6.9                  |                           |
| 6-gingerol    | -4.1                      | -4.2                  |                           |
| 6-shogaol     | -3.6                      | -3.5                  |                           |
| 6-paradol     | -6.8                      | -7                    |                           |
| Ajone         | -4.9                      | -4.9                  |                           |
| Allicin       | -4.1                      | -4.1                  |                           |
| Piperine      | -3.6                      | -3.7                  |                           |
| Quercetin     | -7.4                      | -7.4                  |                           |
| Remdesivir    | -7.7                      | -7.6                  |                           |
| PF-00835231   | -8.4                      | -8.4                  |                           |

DB11672), the active compound of turmeric, had a docking score of -7.1 kcal/mol (Table 1) and formed four H-bonds with two interacting residues of M<sup>pro</sup> (Gly143<sup>α</sup>, Ser144<sup>ε</sup>) (Fig. 2). Two main naturally occurring compounds of tea plant, catechins (PubChem ID 1203) and epigallocatechin (DrugBank ID DB03823), predicted to have a docking score of -7.1 kcal/mol and -7.3 kcal/mol respectively (Table 1). Catechins formed two H-bonds with Thr26, Gln189 residues of viral M<sup>pro</sup> (Fig. 3) whereas epigallocatechin exhibited seven interacting H-bonds with Leu141<sup>ε</sup>. Docking results were validated by Webina 1.0.2 web server (scores < 8.5 kcal/mol). PF-00835231 (PubChem CID 11561899) that can target SARS-CoV-2 M<sup>pro</sup> (Table 2). We compared docking results of all 15 phytochemicals with three well-known drugs viz., quercetin (DrugBank ID DB04216), remdesivir (DrugBank ID DB14761), and PF-00835231 (PubChem ID 11561899) that can target SARS-CoV-2 M<sup>pro</sup> (Table 2). Quercetin had a docking score prediction -7.4 kcal/mol. Remdesivir was predicted to have a docking score of -7.7 kcal/mol. PF-00835231 had a docking score prediction -7.4 kcal/mol. Comparison of the docking results of all 15 phytochemicals showed that ursolic acid (-8.7 kcal/mol), hyperoside (-8.6 kcal/mol) and α-Hederin (-8.5 kcal/mol) have greater binding affinity with M<sup>pro</sup> of SARS-CoV-2 virus than quercetin, remdesivir and PF-00835231, which are currently being used in COVID-19 treatment (Table 2).

4. Discussion

In this study, we evaluated the efficacy of 15 bioactive compounds from Indian spices and medicinal plants as potential inhibitors of SARS-CoV-2 M<sup>pro</sup>. All these compounds have some antiviral properties as reported in published literature. Hyperoside, a neem secondary metabolite, has potential effects against influenza virus nucleoprotein [28]. Ursolic acid exhibits strong antiviral activity against rotavirus [66]. 6-Gingerol and 6-paradol show high efficacy against hepatitis C virus [67]. Green tea catechins and epigallocatechin have been reported to have antiviral effects against numerous viruses such as herpes simplex virus, hepatitis B virus, hepatitis C virus, etc. [34]. Previous study reported that eugenol can inhibit human herpes virus in vitro and in vivo [62]. Curcumin can inhibit Zika and chikungunya viruses [31]. Garlic compound allicin is highly effective against human cytomegalovirus, influenza B, herpes simplex virus type 1, herpes simplex virus type 2 [68].

The molecular docking approach provides an opportunity to test different drugs against SARS-CoV-2 M<sup>pro</sup> in combination with N3 inhibitor. Recent docking study showed that the inhibitor N3 can bind to the substrate binding pockets of new COVID-19 M<sup>pro</sup> [56]. This substrate binding pockets are located within a cleft between domain I and II and are highly conserved among all SARS-CoV-2 M<sup>pro</sup>’s which make it a good target for designing drugs for anti-COVID-19 activity [56]. Similarly, we analysed the above mentioned bioactive compounds from Indian spices and medicinal plants which may act as potential drug targets for SARS-CoV-2 M<sup>pro</sup>. Out of the 15 bioactive compounds, 9 exhibited very high docking scores (scores > -6.5 kcal/mol) (Table 1). Highest docking score was exhibited by ursolic acid (-8.7 kcal/mol) which is the principal bioactive compound of Tulsi leaf extract. The α-Hederin exhibited second highest docking score of -8.5 kcal/mol (Table 1). Neem secondary metabolites, hyperoside and nimbaflavone exhibited...
docking score as −8.6 and −8.0 kcal/mol (Table 1). Curcumin exhibited docking score of −7.1 kcal/mol (Table 1). Compounds of tea plant i.e., catechins and epigallocatechin exhibited a docking score of −7.1 kcal/mol and −7.3 kcal/mol respectively (Table 1). Piperine, the bioactive compound from black pepper yielded docking score −6.8 kcal/mol (Table 1). Echinocystic acid diacetate yielded docking score −6.7 kcal/mol (Table 1). We identified five hotspot residues namely Leu141, Ser144, His163, Arg188, Thr190 (Table 1) on the sequence of SARS-CoV-2 Mpro which exhibited effective interaction with all the tested bioactive compounds. These residues can be targeted for potential drug designing to block SARS-CoV-2 Mpro. In addition, we compared (Table 2) the binding capacity of these tested bioactive compounds with widely used popular drugs against SARS-CoV-2 infection across the world. These widely used drugs are quercetin, remdesivir, PF-00835231. Interestingly, three of our tested compounds namely ursolic acid, hyperoside, α-hederin exhibited stronger binding and inhibitory potential against SARS-CoV-2 Mpro compared to quercetin, remdesivir, PF-00835231.

Our study suffers from some potential limitations. It would have been better to use GROMACS full package for entire molecular dynamics simulation analyses. We did not use MM/PBSA and MM/GBSA program for calculating free binding energy calculation of each bioactive compound. We could not determine the inhibitory effects in term of IC50 value for each of these tested drug. Moreover, our finding needs wet lab experimental validation.
5. Conclusion
In the present study, we selected and tested 15 bioactive compounds against SARS-CoV-2 Mpro. These compounds are found among Indian spices and medicinal plants and exhibit antiviral properties. Among them, nine compounds namely ursoic acid, z-Hederin, hyperoside, nimbaflavone, curcumin, catechins, epigallocatechin, piperrine, and echinocystic acid diacetate exhibited very high docking score against SARS-CoV-2 Mpro. We also identified a set of hotspot residues on the peptide chain of the viral protease which are important for protein–ligand interactions and can be targeted for designing novel drugs against SARS-CoV-2 Mpro. Moreover, through comparative analyses we demonstrated that three bioactive agents have higher potential to inhibit the SARS-CoV-2 Mpro, than the drugs that are being used widely across the globe in treatment of COVID-19. This comparative analyses makes us optimistic to develop herbal drugs without any side-effects in near future. This is particularly important as many cases have been reported from across the globe regarding secondary drug complications among the patients following recovery from SARC-CoV-2 infection. Our study will help researchers to carry similar analyses for other drugs and Ayurvedic bioactive agents and all these would contribute effectively to win the battle against SARS-CoV-2 infections.

Source(s) of funding
The work is financially supported by Department of Science and Technology, Government of West Bengal, India, Grant no. SG/WB/DST/S&T 1000114/2016.

Conflict of interest
None.

Acknowledgement
The infrastructural support and instrumental facilities were provided by UGC-UGE II, DST-FIST, UGC-PURSE program at University of Calcutta. Pinku Halder is thankful to University Grants Commission, Government of India for providing him NET-JRF fellowship for his research.

Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaime.2021.05.003.

References
[1] Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature 2003;426(6965):450—50.
[2] Matsuyama S, Nagata N, Shirato K, Kawase M, Takeda M, Taguchi F. Efficient activation of the severe acute respiratory syndrome coronavirus spike protein by the transmembrane protease TMPRSS2. J Virol 2010;84(24):12658—64.
[3] Glowacka I, Bertram S, Müller MA, Allen P, Souleix E, Pfefferle S, et al. Evidence that TMPRSS2 activates the severe acute respiratory syndrome coronavirus spike protein for membrane fusion and reduces viral control by the humoral immune response. J Virol 2011;85(9):4122—34.
[4] Wu F, Zhao S, Yu B, Chen Y-M, Wang W, Song Z-G, et al. A new coronavirus associated with human respiratory disease in China. Nature 2020;579(7798):765—9.
[5] Zhao S, Lin Q, Ran J, Musa SS, Yang G, Wang W, et al. Preliminary estimation of the basic reproduction number of novel coronavirus (2019-nCoV) in China, from 2019 to 2020: a data-driven analysis in the early phase of the outbreak. Int J Infect Dis 2020;92:214—7.
[6] Liu C-C, Shih T-P, Ko W-C, Tong H-J, Houeh P-R. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): the epidemic and the challenges. Int J Antimicrob Agents 2020;55(3):105928.
[7] Coronavirus Study Group of the International Committee on Taxonomy of Viruses. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol 2020;5(4):536—44. https://doi.org/10.1038/s41564-020-0695-z.
[8] Vasudevan PD, Potdar VA, Chaudhary DA, Agrawal M, JadHAV SM, et al. Full-genome sequences of the first two SARS-CoV-2 viruses from India. Indian J Med Res 2020;151(2 & 3):200—9.
[9] Zhou F, Yang X-L, Wang X-G, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020;579(7798):270—3.
[10] Parskevis D, Kostaki EC, Magorkinis G, Panayotakopoulos G, Sourvigos N, Tsiodra S. Full-genome evolutionary analysis of the novel corona virus (2019-nCoV) rejects the hypothesis of emergence as a result of a recent recombinant event. Infect Genet Evol 2020;79:104212.
[11] Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet 2020;395(10224):503—7.
[12] Tang X, Wu C, Li X, Song Y, Yao O, Xue W, et al. On the origin and continuing evolution of SARS-CoV-2. Nat Sci Rev 2020;7(6):1012—23. https://doi.org/10.1093/nsr/nwaa036.
[13] Thiel V, Ivanov KA, Pfitz CR, Hertzog T, Schelle B, Bayer S, et al. Mechanisms and enzymes involved in SARS coronavirus genome expression. J Gen Virol 2003;84(Pt 9):2305—15.
[14] Yang H, Yang M, Ding X, Liu Y, Lou Z, Zhou Z, et al. The crystal structures of severe acute respiratory syndrome virus main protease and its complex with an inhibitor. Proc Natl Acad Sci USA 2003;100(23):13190—5.
[15] Hilgenfeld R, Peisir M. From SARS to MERS: 10 years of research on highly pathogenic human coronaviruses. Antivir Res 2013;100(1):286—95.
[16] Wu C, Liu Y, Yang Y, Zhang P, Zhong W, Wang Y, et al. Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods. Acta Pharm Sin B 2020;10(5):766—88. https://doi.org/10.1016/j.apsb.2020.02.008.
[17] Ma C, Sacco MD, Hurst B, Townsend JA, Hu Y, Szeto T, et al. Boceprevir, GC-376, and calpain inhibitors II, XII inhibit SARS-CoV-2 viral replication by targeting the viral main protease. Cell Res 2020;30(8):678—92.
[18] Ziebuhr J, Snijder EJ. Virus-encoded proteinases and proteolytic processing in the Nidovirales. J Gen Virol 2000;81(4 Pt 4):853—79.
[19] Hegyi A, Ziebuhr J. Conservation of substrate specificities among coronavirus main proteases. J Gen Virol 2002;83(3 Pt 3):595—9.
[20] Du Q-S, Wang S-Q, Zhu Y, Wei D-Q, Guo H, Sirois S, et al. Polyprotein cleavage of SARS CoVMpro and chemical modification of the octapeptide. Peptides 2004;25(11):1857—64.
[21] Liang P-H. Characterization and inhibition of SARS-coronavirus main protease. Curr Top Med Chem 2006;6(4):361—76.
[22] Yang H, Bartlam M, Rao Z. Drug design targeting the main protease, the Achilles' heel of coronaviruses. Curr Pharmaceut Des 2006;12(35):4573—90.
[23] Zhu L, George S, Schmidt MF, Al-Gharabli SI, Rademann J, Hilgenfeld R. Peptide aldehyde inhibitors challenge the substrate specificity of Mpro, a key viral enzyme. J Med Chem 2011;54(6):1616—26.
[24] Kumar AH. Molecular docking of natural compounds from Tulsi (ocimum tenuiflorum) against SARS-CoV-2 main proteases. J Biol Sci 2020;10(2):122—27.
[25] Lu H. Drug treatment options for the 2019-new coronavirus (2019-nCoV). BEMS Reports 2020;6(1):11.
[26] Lai C-C, Shih T-P, Ko W-C, Tang H-J, Hsueh P-R. Severe acute respiratory syndrome: the species Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19). J Med Virol 2020;92:105924.
[27] Introduction and importance of medicinal plants and herbs National Health Portal of India. Available from: https://www.nhp.gov.in/introduction-and-importance-of-medicinal-plants-and-herbs-ntl.
[28] Ahmad A, Javed MR, Rao AQ, Husain T. Designing and screening of universal drug from neem (Azadirachta indica) and standard drug chemicals against influenza virus nucleoprotein. BMC Comp Alternative Med 2016;16(1):519.
[29] Kumar AH. Molecular docking of natural compounds from Tulsi (ocimum sanctum) and neem (azadirachta indica) against SARS-CoV-2 protein targets. BEMS Reports 2020;6(1):11—3.
[30] Weber ND, Andersen DO, North JA, Murray BK, Lawson LD, Hughes BG. In vitro virucidal effects of Allium sativum (garlic) extract and compounds. Planta Med 1992;58(5):417—23.
[31] Yadav PD, Mordica C, Cesaro T, Grasso L, Vittorelli T, Vignuzzi M. Curcumin inhibits Zika and chikungunya virus infection by inhibiting cell binding. Acta Virol 2017;61(2):148—58.
[32] Padilla-S L, Rodriguez A, Gonzales MM, Gallego OJ, Castano OJ. Inhibitory effects of curcumin on dengue virus type 2-infected cells in vitro. Arch Virol 2014;159(3):573—9.
