Search for therapeutics against COVID 19 targeting SARS-CoV-2 papain-like protease: an in silico study

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Research Article

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Abstract

**Background:** The global pandemic of novel coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an enveloped, positive-sense, single-stranded RNA betacoronavirus of the family Coronaviridae. Papain-like protease (PLpro) of SARS CoV-2 is an important target of COVID-19 because it is a multifunctional cysteine protease essential for coronaviral replication.

Large numbers of phytochemicals with varied chemical structures isolated from medicinal plants have been shown to possess antiviral activity. Some of these phytochemicals have been chosen on the basis of literature survey for this study. Reported inhibitors of the papain-like protease are taken as control and for QSAR study.

**Methods:** Three dimensional structure of target was downloaded from Protein Data Bank and docked with phytochemicals & inhibitors by using software FlexX. Inhibitors of the papain-like protease were taken from binding database and QSAR analysis was performed by using EasyQSAR software.

**Results:** Six phytochemicals: Baicalin, Rutin, Biopterin, Licoleafol, Luteolin and Quercetin shows stable bonding pattern with the target in compare to known inhibitors as it shows least score in docking, forms maximum number of hydrogen bonds with the active residues of the receptor. The predicted IC50 values of the phytochemicals are also better than the known inhibitors.

**Conclusion:** Based on present observation of docking score of both phytochemicals and known inhibitors, IC50 value of known inhibitors and predicted IC50 of phytochemicals, we suggests above mentioned six phytochemicals may be the Papain-like protease (PLpro) targeted potent drug leads against Covid-19.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes the global pandemic of novel coronavirus disease 2019 (COVID-19) began in Wuhan, China, in December 2019 and has since spread worldwide (1).

In human being COVID 19 experience as a mild to moderate respiratory problems and can be improved without any particular cure but senior peoples with diabetes, chronic respiratory diseases, cancer and cardiovascular disease are prone to high risk in this infection. The clinical practitioners report says patients with COVID-19 showed sign of sore throat, cough, fever, muscle pain, tiredness and viral pneumonia. This virus spread from diseased person to other through coughing and sneezing and can be avoided by keeping a proper distance with others and sanitizing hands with alcohol regularly. So practicing personal sanitation and public distancing is the only means to prevent from this deadly pandemic (2, 3). Several countries have enforced lockdown which is helping in confining the spread of the disease, however it has not been totally successful. In addition to loss of human lives, COVID-19 is causing rigorous economic losses to both developed and developing nations. According to WHO report as
of May 31, 2020, the virus has infected 5934936 people in more than 215 countries including a shocking 367166 deaths (2).

SARS-CoV-2 is a new member of betacorona virus in the Coronaviridae family (4). The virion of SARS-CoV-2 is consists of crown-shaped peplomers, 80-160 nm in diameter (5).

HCoVs generally are positive-sense single-stranded RNA (30kb) viruses. HCoVs are characterize by two groups of protein; structural such as Spike (S), Nucleocapsid (N) Matrix (M) and Envelope (E), and non-structural proteins such as RNA dependent RNA polymerase (RdRp) (nsp12) the Papain-like protease (PLpro) and 3C-like protease (3CLpro). PLpro is a crucial enzyme in the life cycle of RNA viruses, comprising coronaviruses. PLpro is a multifunctional cysteine protease that processes the viral polyprotein and host cell proteins by hydrolysing the peptide and isopeptide bonds in viral and cellular substrates leading to the virus replication. It is responsible for the cleavages of N-terminus of the replicase poly-protein to release Nsp1, Nsp2 and Nsp3, which is essential for correcting virus replication. PLpro also antagonize the host's innate immunity. As a vital enzyme in the process of coronavirus replication and infection of the host, PLpro is an accepted target for coronavirus inhibitors. It is very important for targeting PLpro to treat coronavirus infections (6, 7). Stripping ubiquitin and ISG15 from host-cell proteins to assist coronaviruses in their evasion of the host innate immune responses is an added function of PLpro. Inhibiting viral replication and inhibiting the dysregulation of signaling cascades in infected cells leading to cell death in surrounding and uninfected cells may be achieved by targeting PLpro (8). Therefore, the papain-like protease (PLpro) is an important target for antiviral drug design (9).

At present, there is no evidence from randomized clinical trials (RCTs) that any possible therapy improves outcomes in patients with either suspected or confirmed COVID-19, therefore, there is an urgent need for effective drugs (10).

Plants have naturally developed over the years in diverse weather conditions on earth and have been bestowed with rich composite of secondary metabolites/phytochemicals with wide pharmacokinetic spectrum. Around 2500 medicinal plant species have been recognized worldwide to treat a myriad of infictions and ailments (11, 12). A large number of compounds of varied chemical structures isolated from medicinal plants possess antiviral activity (13-19) (Table 1).

Experimental approaches for the study of interactions between drug compounds and target proteins are expensive and time consuming. In silico approaches propose techniques to examine hypotheses of new putative drugs by reducing the cost and shortening the time.

Therefore, the present study was conducted to identify potential inhibitors of SARS-CoV-2 papain-like protease from natural compounds using in silico approaches. Reported inhibitors of the Papain-like protease are taken as control and for QSAR study.

Table 1: Some antiviral phytochemicals
| Sl. No. | Phytochemicals       | Plant (part)                      |
|----------|----------------------|-----------------------------------|
| 1        | Calanolide A         | *Calophyllum lanigerum*           |
| 2        | Curcumin             | Turmeric etc.                     |
| 3        | Eugenol              | *Syzygium aromaticum*             |
| 4        | Collinin             | *Zanthoxylum schinifolium*        |
| 5        | Ellagic acid         | *Phyllanthus urinaria*            |
| 6        | Resveratrol          | grapes, blueberries, raspberries, mulberries and peanuts |
| 7        | Galangin             | *Helichrysum aureonitens* (shoots) |
| 8        | Leachianone G        | *Morus alba* L.                   |
| 9        | Kaempferol           | apples, grapes, tomatoes, green tea, potatoes, onions, broccoli, squash, cucumbers, lettuce, green beans, peaches, blackberries, raspberries, and spinach etc. |
| 10       | epigallocatechin gallate | *Camellia sinensis*               |
| 11       | epigallocatechin     | *Camellia sinensis*               |
| 12       | epicatechin gallate  | *Camellia sinensis*               |
| 13       | epicatechin          | *Camellia sinensis*               |
| 14       | catechin             | *Camellia sinensis*               |
| 15       | Camptothecin         | *Ophiopogon mungos* (leaves)      |
| 16       | Caffeine             | *Theobroma cacao* L. and *Coffea* sp. |
| 17       | Emetine              | *Cephaelis ipecacuanha*           |
| 18       | Oliverine            | *Polythia oliveri*               |
| 19       | Schumannificine      | *Schumannophyton magnificum* (bark) |
| 20       | Aframisin            | *Wisteria brachybotrys*          |
| 21       | Formononetin         | *Wisteria brachybotrys*          |
| 22       | Tematin              | *Evodia madagascariensis*        |
| 23       | Wogonin              | *Scutellaria baicalensis*         |
| 24       | Podophyllotoxin      | *Podophyllum peltatum*            |
| 25       | Cochinolide          | *Homalium*                        |
|   | Compound                        | Plant/Species                          |
|---|---------------------------------|---------------------------------------|
| 26| Dolabellane                     | *Dolabella californica*               |
| 27| Sageone                         | *Salvia officinalis*                  |
| 28| Silymarin                       | *Silybum marianum*                    |
| 29| Cyanidol                        | *Silybum marianum*                    |
| 30| Salaspermic acid                | *Trityrygium wilfordii*               |
| 31| Platanic acid                   | *Syzigium claviflorum* (leaves)       |
| 32| Baicalin                         | *Scutellaria baicalensis* (roots)     |
| 33| Chalcones                       | *Glycyrrhiza inflate* (roots)         |
| 34| Dammarenolic acid               | *Aglaia sp.* (bark)                   |
| 35| Decanoylphorbol-13 acetate      | *Croton mauritianus* (leaves)         |
| 36| Excoecarianin                   | *Phyllanthus urinaria* (whole plant)  |
| 37| Loliolide                       | *Phyllanthus urinaria* (whole plant)  |
| 38| Honokiol                        | Magnolia tree (roots, bark)           |
| 39| Jubanines                       | *Ziziphus jujuba* (roots)             |
| 40| Limonoids                       | *Swietenia macrophylla* (stem)        |
| 41| Oleanane                        | *Camellia japonica* (flowers)         |
| 42| Quercetin                       | *Embelia ribes* (seeds)               |
| 43| Saikosaponins                   | *Bupleurum kaoi* (roots)              |
| 44| Sennoside A                     | *Rheum palmatum* (roots)              |
| 45| Silvestrol                      | *Aglaia foieolata* (leaves, bark)    |
| 46| SJP-L-5                         | *Schisandra micrantha* (roots)        |
| 47| Spiroketalenol                  | *Tanacetum vulgare* (rhizome)         |
| 48| Swerilactones                   | *Swertia mileensis* (whole plant)     |
| 49| Xanthohumol                     | *Humulus lupulus* (whole)             |
|    | 50 | Oxyresveratrol | Artocarpus lakoocha (Heartwood) |
|----|----|----------------|---------------------------------|
| 51 |  Saikosaponin B2 | Bupleurum kaoi (Root) |
| 52 |  Tangeretin | Citrus reticulate (Pericarps) |
| 53 |  Nobiletin | Citrus reticulate (Pericarps) |
| 54 |  Jatrophane ester | Euphorbia amygdaloides spp. (Whole plant) |
| 55 |  Glycyrrhizic acid | Glycyrrhiza radix (Roots) |
| 56 |  Quercetin 3-rhamnoside | Houttuynia cordata (Aerial parts) |
| 57 |  Samarangenin B | Limonium sinense (Root) |
| 58 |  LPRP-Et-97543 | Liriope platyphylla (Root) |
| 59 |  Pterocarnin A | Pterocarya stenoptera (Bark) |
| 60 |  Chalepin | Ruta angustifolia (Leaves) |
| 61 |  Pseudane IX | Ruta angustifolia (Leaves) |
| 62 |  Manassantin B | Saururus chinensis (Root) |
| 63 |  Dicaffeoylquinic acids | Schefflera heptaphylla (Leaf stalks) |
| 64 |  Scopadulcic acid B | Scoparia dulcis L. (Whole plant) |
| 65 |  5,7,4'-trihydroxy-8-methoxyflavone (F36) | Scutellaria baicalensis (Root) |
| 66 |  Naringin | grape and orange (skin) |
| 67 |  Myricetin | Myrica cerifera |
| 68 |  Inophyllum_B | Calophyllum inophyllum |
| 69 |  Inophyllum_P | Calophyllum inophyllum |
| 70 |  Pericalline | Catharanthus roseus / C. lanceus |
| 71 |  Chrysophanic acid | Dianella longifolia |
| 72 |  Nordihydroguaiaretic acid | Larrea divaricata |
|   | Ligand                          | Plant Name                        |
|---|--------------------------------|-----------------------------------|
| 73| Retrojusticidin B              | *Phyllanthus myrtifolius*         |
| 74| Emodin                         | *Rheum sp.* and *Polygonum sp.*  |
| 75| Gingerol                       | *Zingiberis rhizome*             |
| 76| Anthraquinone                  | *Dianella longifolia*            |
| 77| Methyl rosmarinate             | *Hyptis atrorubens Poit*         |
| 78| Licoleafol                     | *Glycyrrhiza uralensis*          |
| 79| Amaranthin                     | *Amaranthus tricolor*             |
| 80| Calceolarioside B              | *Fraxinus sieboldiana*           |
| 81| Actinophnine                   | *Actinodaphne hookeri*           |
| 82| Bioterin                       | *Crithidia fasciculata*          |
| 83| Buchapine                      | *Euodia roxburghiana*            |
| 84| Caribine                       | *Hymenocallis arencola*          |
| 85| Lycorine                       | *Clivia miniata*                 |
| 86| Fisetin                        | *Rhus spp.*                      |
| 87| Morin                          | *Chlorophora tinctoria L.* Gaud  |
| 88| Luteolin                       | *Matricaria inodora L.*          |
| 89| Rutin                          | *Fagopyrum esculentum*           |
| 90| Taxifolin                      | *Acacia catechu*                 |
| 91| Oleanolic acid                 | *Prosopis glandulosida*          |
| 92| Betulinic acid                 | *Syzygium claviflorum*           |

**Materials And Methods**

**The Ligands**

Antiviral phytochemicals were taken based on literature survey and known inhibitors of the papain-like protease of SARS CoV-2 were taken from the Binding Database (20). The structure of these phytochemicals and known inhibitors of the Papain-like protease were retrieved from PubChem Compound and by drawing using ChemOffice tools. The three dimensional structure of these compounds in sdf format were generated using OpenBabel software (21).

**The receptor**

The crystal structure of the papain-like protease of SARS CoV-2 was downloaded from RCSB Protein Data Bank ([http://www.rcsb.org](http://www.rcsb.org)). It was deposited by Osipiuk, J et al. on 22nd March 2020 and released on 1st April 2020. The protein has three chains (Chain A, B and C) of 317 residues determined by X-ray diffraction method at a resolution of 2.70 Å. The PDB id of the protein is 6W9C.
**Active site identification**

The active sites of the receptor were identified by the FlexX software during receptor preparation process.

**Protein – Ligand interaction using FlexX**

Docking is a term used for computational schemes that attempt to find the best matching between two molecules: a receptor and ligand (22). The receptor was docked with known inhibitors of the Papain-like protease and phytochemicals using software FlexX (23). The active site amino acids were defined in the target molecule during the target preparation. The SDF file of all the compounds was loaded in FlexX as docking library. The output file gave the energy values in Kcal/mol. For each docked molecule, this value was noted down.

**Quantitative Structure Activity Relationship (QSAR) studies**

The QSAR analysis (24) was performed by taking the known inhibitors of the papain-like protease. The QSAR descriptors viz. Molar Refractivity, Molar volume, Parachor, Polarizability and Monoisotopic mass were generated for each of the molecule using ACD ChemSketch softwares. The activities have been calculated by taking the inverse logarithm of IC50 values. The descriptors were tabulated in a MS Excel Sheet against their bioactivities (log IC50⁻¹). The descriptors and activities were loaded in Easy QSAR software for multiple linear regression analysis. From the regression, the QSAR equation was generated and the IC50 values of best docked phytochemicals were was predicted.

**Results**

Interaction energies between ligand and receptor play the most crucial role in drug designing. In this work, the papain-like protease of SARS CoV-2 (PDB ID: 6W9C) was selected as drug target and the interactions of the compounds were studied using FlexX software. The docking results of phytochemicals with target are described in table 2 and the docking results of papain-like protease inhibitors with target are described in table 3. The docking poses of best docked phytochemicals and inhibitors are shown in Figures (Figure 1 – Figure 9). Phytochemicals: Baicalin, Quercetin, Licoleafol, Biopterin, Luteolin and Rutin show much more binding affinity with the target in comparison to the reported inhibitors of the papain-like protease.

**Table 2: Docking results of Phytochemicals with Papain-like protease of SARS CoV-2**
| Phytochemicals       | Docking Score (Kcal/mol) | Residues involved in the hydrogen bonding                  |
|----------------------|--------------------------|-----------------------------------------------------------|
| Calanolide A         | -5.9827                  | ARG138, ASN146                                            |
| Curcumin             | -11.9951                 | ASN12, ASN13                                              |
| Eugenol              | -10.6228                 | ARG138, ASN146, TYR83                                     |
| Collinin             | -3.9488                  | ARG138, ASN146, ASN13                                     |
| Ellagic acid         | -14.0599                 | LYS105, TRP106, ASP286, ALA288                            |
| Resveratrol          | -11.7275                 | TYR56, TYR72, ALA131                                     |
| Galangin             | -16.4712                 | ASN109, CYS111, TYR112, GLY163, GLN269, GLY271           |
| Leachianone G        | -11.2836                 | THR74, THR75, AN156                                      |
| Kaempferol           | -15.1889                 | ASN109, CYS111, TYR112, GLY163, GLN269, GLY271           |
| Epigallocatechin gallate | -13.9114              | HIS89, LYS92, TRP93, TRP106, ASP108, CYS155, ASN156, LYS157 |
| Eepigallocatechin    | -18.7043                 | TYR72, TYR83, ALA131, ARG138, ASN146                     |
| Epicatechin gallate  | -13.6344                 | ASP12, ASN13, TYR83, ARG138, ASN146                      |
| Epicatechin          | -16.2121                 | TYR83, ALA131, ARG138, ASN146                            |
| Catechin             | -16.2121                 | TYR83, ALA131, ARG138, ASN146                            |
| Camptothecin         | -10.1170                 | TRP106, ASP286, ALA288                                   |
| Caffeine             | -7.9904                  | THR74, THR75                                             |
| Emetine              | -12.2723                 | THR74, ASP76, LYS92, ASN156                              |
| Oliverine            | -14.6060                 | LYS105, ASP108, GLU167                                   |
| Schumannificine      | -17.5028                 | LYS92, ASP108, LYS157, GLU161                            |
| Aframosin            | -10.6251                 | TYR83, ARG138, ASN146                                   |
| Formononetin         | -11.5464                 | TYR72, ARG138                                            |
| Ternatin             | -8.2402                  | LYS105, ASP108, TYR264                                   |
| Wogonin              | -17.5758                 | ASP12, TYR83, ARG138, ASN146                             |
| Podophyllotoxin      | -8.0189                  | THR74, THR75, ASN156                                     |
| Cochinolide          | -14.2751                 | ASN13, TYR83, ARG138, ASN146                             |
| Molecule                          | Score  | Docking Information                  |
|----------------------------------|--------|--------------------------------------|
| Dolabellane                      | -7.4013| ASN146, THR74, THR75, ASP76          |
| Sageone                          | -11.6264| ASN13, TYR56, ARG138, ASN146         |
| Silymarin                        | -13.3862| ASN13, TYR72, ALA131, ALA135, ARG138|
| Cyanidol                         | -12.8967| HIS89, TRP106, LYS157, LEU162        |
| Salaspermic acid                 | NOT DOCKED|                                            |
| Platanic acid                    | -7.3902| THR74, THR75, ASP76                  |
| Baicalin                         | -34.3309| ASN13, TYR71, TYR83, ASP134, ARG138  |
| Dammarenolic acid                | -0.1305| ASN13, ARG138                        |
| Excoecarianin                    | NOT DOCKED|                                            |
| Loliolide                        | -10.4500| ASN13, ARG138, ASN146                |
| Honokiol                         | -7.9547| TYR56, ALA131, ASN146                |
| Oleanane                         | NOT DOCKED|                                            |
| Quercetin                        | -24.9869| ASP108, ASN109, CYS111, TYR112, GLY163, GLN269, GLY271|
| Sennoside A                      | -8.1751| ASN109, VAL159, LEU162, GLN269       |
| Silvestrol                       | -1.8664| ASN13, TYR71, TYR72, ARG138          |
| SJP-L-5                          | -13.2672| ASN13, ARG138, ASN146                |
| Xanthohumol                      | -9.1494| LYS92, LYS105, TRP106, LYS157, GLU161|
| Spiroketalenol                   | -12.9512| THR74, THR75, ASP76                  |
| Licochalcone                     | -10.8747| ALA131, ASN146                       |
| Chalcone                         | -14.1573| ARG138                               |
| Decanoylphorbol-13 acetate       | 6.3441 | LYS92, LYS105, TRP106, LYS157, GLU161|
| Jubanine A                       | -1.8017| LYS157, GLU167                       |
| Jubanine B                       | NOT DOCKED|                                            |
| 3-Hydroxy Caruilignan C          | -8.3390| ASN13, TYR56, ARG138                 |
| Limonin                          | NOT DOCKED|                                            |
| Oxyresveratrol                   | -14.8726| ASN13, TYR56, TYR72, ALA131         |
| Saikosaponin B2                  | NOT DOCKED|                                            |
| Molecule                        | Score    | Interacting Residues                                      |
|--------------------------------|----------|----------------------------------------------------------|
| Tangeretin                     | -6.8313  | ASN13, ARG138, ASN146                                     |
| Nobiletin                      | -6.0527  | ASN13, ARG138, ASN146                                     |
| Jatrophan ester                | NOT DOCKED|                                                          |
| Glycyrrhizic acid              | NOT DOCKED|                                                          |
| Quercetin 3-rhamnoside         | -20.3139 | ASN13, TYR56, TYR72, TYR83, ARG138, ASN146               |
| LPRP-Et-97543                  | -13.2511 | ASP108, GLU167, GLY271                                    |
| Chalepin                       | -7.9836  | ASN13, TYR56                                             |
| Manassantin B                  | -8.8858  | THR74, THR75, TRP93, ASN156, LYS157, GLU161             |
| Dicaffeoylquinic acid          | -12.6497 | ASN13, TYR56, TYR72, ARG138, ASN146                      |
| Scopadulcic acid B            | NOT DOCKED|                                                          |
| Naringin                       | -9.1107  | ASP12, ASN13, TYR71, ARG138                              |
| Myricetin                      | -14.3847 | ASP108, ASN109, CYS111, TYR112, GLY163, GLN269, GLY271   |
| Inophyllum_B                   | -11.4238 | ARG138, ASN146                                           |
| Inophyllum_P                   | -11.4238 | ARG138, ASN146                                           |
| Pericalline                    | -12.1713 | TRP106, GLU167                                           |
| Chrysophanic acid              | -22.4865 | ASP12, ASN13, TYR56, ARG138                              |
| Nordihydroguaiaretic acid      | -9.8957  | TRP106, GLY163, TYR268, ASP286, ALA288                   |
| Retrojusticidin B             | -12.5426 | LYS105, TRP106, ALA288                                    |
| Emodin                         | -23.1325 | ASN13, TYR56, ALA131                                     |
| Gingerol                       | -5.3377  | TYR71, TYR83, ARG134, ASN146                             |
| Anthraquinone                  | -11.6131 | ASN13, ARG138                                           |
| Methyl rosmarinate             | -13.9423 | LYS105, ASP108, ASP286, ALA288                           |
| Licoleafol                     | -26.5293 | LYS92, LYS105, TRP106, LYS157, LEU162, GLU167           |
| Amaranthin                     | -20.2696 | ASP12, ASN13, TYR56, ARG138, GLU143, ASN146             |
| Calceolarioside B              | -15.0832 | ASN13, TYR56, GLU167, ASN146                             |
| Inhibitor                      | Score     | Binding Site                                                                 |
|-------------------------------|-----------|------------------------------------------------------------------------------|
| Papaverine                    | -8.1881   | TYR72, ARG138, LYS45, THR74, THR75, ASP76                                  |
| Biopterin                     | -26.9995  | ASN109, GLY160, GLN269                                                      |
| Buchapine                     | -7.3976   | THR74, THR75                                                                |
| Caribine                      | -14.7955  | LYS105, LYS157, GLU167                                                      |
| Lycorine                      | -15.1457  | LYS105, ASP108, GLU161, GLU167                                              |
| Fisetin                       | -16.3059  | TYR83, ALA131, ARG138                                                      |
| Morin                         | -17.0390  | ASN109, GLY160, LEU162, GLN269                                              |
| Luteolin                      | -25.9438  | ASP108, ASN109, CYS111, TYR112, LY163, GLN269, GLY271                      |
| Rutin                         | -27.0507  | ASP12, ASN13, TYR83, ASP134, ARG138, ASN146                                 |
| Taxifolin                     | -15.0153  | ASN13, TYR72, ASP134, ARG138                                                |
| Oleanolic acid                | NOT DOCKED|                                                                              |
| Betulinic acid                | NOT DOCKED|                                                                              |
| 5,7,4' trihydroxy-8-methoxyflavone | -15.7340 | TYR71, TYR83, ARG138, ASN146                                               |
| Swerilactone A                | NOT DOCKED|                                                                              |
| Swerilactone B                | NOT DOCKED|                                                                              |

Table 3: Docking results of Known inhibitors with Papain-like protease of SARS CoV-2
| Inhibitors (ID) | Docking Score (Kcal/mol) | Residues involved in the hydrogen bonding |
|----------------|--------------------------|------------------------------------------|
| BDBM31524      | -16.0618                 | TYR56, ARG138                            |
| BDBM31531      | -17.4198                 | TYR56, TYR71, ALA131, ARG138            |
| **BDBM31523**  | **-20.2673**             | ASN13, TYR56, ARG138                     |
| BDBM50007789   | -12.5947                 | ASP108, LYS157, LEU162, GLU167           |
| BDBM31530      | -19.0533                 | TYR56, ASP134, ARG138                    |
| BDBM31528      | -16.2484                 | TYR56, ARG138                            |
| BDBM154574     | -11.6219                 | ASP108, LYS157, LEU162, GLU167           |
| BDBM31520      | -16.3075                 | TYR56, ARG138                            |
| BDBM31524      | -16.0614                 | TYR56, ARG138                            |
| BDBM31527      | -16.5289                 | TYR56, ARG138                            |
| BDBM31529      | -16.4990                 | ASN13, TYR56, ARG138                     |
| BDBM31526      | -16.6231                 | TYR56, ARG138                            |
| BDBM31508      | -15.3900                 | TYR56, ARG138                            |
| BDBM154573     | -12.0729                 | ASN13, TYR83, ASN146                     |
| BDBM31525      | -12.0982                 | TYR56, ARG138                            |
| BDBM31514      | -12.1381                 | TYR56, ARG138                            |
| BDBM31512      | -14.7425                 | TYR56, ARG138                            |
| BDBM31509      | -15.6676                 | TYR56, ARG138                            |
| BDBM31521      | -10.6302                 | THR74, THR75                             |
| **BDBM31522**  | **-24.0149**             | TYR56, TYR71, ALA131, ARG138            |
| BDBM31510      | -11.8777                 | TYR56, ARG138                            |
| **BDBM31516**  | **-21.3324**             | TYR56, TYR71, ALA131, ARG138            |
| BDBM31511      | -18.2742                 | ASN13, TYR56, ARG138                     |
| BDBM31513      | -9.5794                  | TYR56, ARG138                            |

There was significant correlation with R square value of 82% (The Rsq value should be definitely high for a good QSAR equation, Higher Rsq means higher fitting of the equation to the given data, hence better predictions it will provide for new test data). The Adjusted Rsq is 73% therefore the difference between Rsq and adjusted Rsq is less (High difference in Rsq and Adjusted Rsq indicates weaker overall...
prediction). The F statistics value of the test is 5.01 and the critical F value is 2.20 (The F statistics of the test should be greater than Critical F otherwise the generated equation is inefficient).

The equation generated out of QSAR analysis is as follows:

\[
\text{Activity} = -6.36683 + 40.54242 \text{ (Molar refractivity)} + -0.02928 \text{ (Molar volume)} + 0.012697 \text{ (Parachor)} + -1.02268E+26 \text{ (Polarizability)} + 0.003573 \text{ (Monoisotopic mass)}
\]

From the above QSAR equation the IC 50 value of Baicalin, Quercetin, Licoleafol, Biopterin, Luteolin and Rutin were predicted and shown in table 4. The multiple regression plot (linear) of QSAR analysis is shown in figure 10.

| Inhibitors (ID) | IC 50 (nM) | Phytochemicals | Predicted IC 50 (nM) |
|-----------------|------------|----------------|---------------------|
| BDBM31524       | 230        | Baicalin       | 69.1831             |
| BDBM31531       | 460        | Quercetin      | 933.2543            |
| BDBM31523       | 560        | Licoleafol     | 1548.817            |
| BDBM50007789    | 980        | Biopterin      | 19498.45            |
| BDBM31530       | 1180       | Luteolin       | 977.237             |
| BDBM50007789    | 1200       | Rutin          | 0.18197             |
| BDBM31530       | 1300       |                |                     |
| BDBM31528       | 1400       |                |                     |
| BDBM154574      | 2260       |                |                     |
| BDBM31520       | 2300       |                |                     |
| BDBM31524       | 2640       |                |                     |
| BDBM31527       | 2700       |                |                     |
| BDBM31529       | 4800       |                |                     |
| BDBM31526       | 5200       |                |                     |
| BDBM31508       | 8700       |                |                     |
| BDBM154573      | 10900      |                |                     |
| BDBM31525       | 11100      |                |                     |
| BDBM31514       | 12100      |                |                     |
| BDBM31512       | 13500      |                |                     |
| BDBM31509       | 14800      |                |                     |
| BDBM31521       | 22600      |                |                     |
| **BDBM31522**   | **24800**  |                |                     |
| BDBM31510       | 29100      |                |                     |
| BDBM31516       | 46100      |                |                     |
| BDBM31511       | 90000      |                |                     |
| BDBM31513       | 149000     |                |                     |
Discussion

The least score in docking was preferred for considering better ligand as it indicates more stability in binding (22). The interactions of phytochemicals and the Papain-like protease inhibitors with target were screened based on hydrogen bonding based prediction (25). Among the inhibitors, three inhibitors: BDBM31523, BDBM31522 and BDBM31516 show more binding affinity with target. The docking score of BDBM31523 is -20.2673 Kcal/mol and forms four hydrogen bonds with active site residues. BDBM31522 forms five hydrogen bonds with the residues of binding pocket with a docking score of -24.0149 Kcal/mol. BDBM31516 binds with the target with a docking score of -21.3324 Kcal/mol and forms five hydrogen bonds.

Some phytochemicals exhibited better binding efficacy with the target. Among them Baicalin, Rutin, Biopterin, Licoleafol, Luteolin and Quercetin binds more strongly with the target in comparison to the inhibitors and other phytochemicals.

Baicalin a flavonoid obtained from roots of the plant *Scutellaria baicalensis* interferes and inhibits dengue virus (DENV-2) at various stages of the virus replication cycle (26) has the highest docking score (-34.3309 Kcal/mol) with the receptor among all the phytochemicals and inhibitors also forms seven hydrogen bonds with the receptor.

Rutin have antiviral effect against avian influenza strain H5N1 (27), a naturally occurring flavonoid found in many foods, especially buckwheat (*Fagopyrum esculentum*) strongly docked with the target forming ten hydrogen bonds with a docking score of -27.0507 Kcal/mol.

Biopterin isolated from *Crithidia fasciculata* possessing antiviral activity (14) shows strong binding affinity with the receptor, forms eight hydrogen bonds with a docking score of -26.9995 Kcal/mol.

Licoleafol a prenylated antiviral flavanone isolated from *Glycyrrhiza uralensis* (28) which forms eight hydrogen bonds with target and binds with a docking score of -26.5293 Kcal/mol.

Luteolin an antiviral flavone against herpes and poliomyelitis viruses isolated from *Matricaria inodora* L. plant (14) has binding efficacy with the target with a docking score of -25.9438 Kcal/mol and forms eight hydrogen bonds with the receptor.

Quercetin exhibit remarkable activities against picornaviruses and vesicular stomatitis virus (14) a potent antioxidant flavonoid found mostly in onions, grapes, berries, cherries, broccoli, and citrus fruits shows good binding affinity with the target, forms eight hydrogen bonds with a docking score of -24.9869 Kcal/mol.

The predicted IC50 values of above mentioned phytochemicals were much less than the most of the inhibitors (Table 4).
The Papain-like protease (PLpro) is a multifunctional cysteine protease that processes the viral polyprotein and host cell proteins by hydrolysing the peptide and isopeptide bonds in viral and cellular substrates leading to the virus replication. Targeting PLpro with antiviral drugs may have an advantage in not only inhibiting viral replication but also inhibiting the dysregulation of signaling cascades in infected cells that may lead to cell death in surrounding, uninfected cells (6, 7 and 8).

Six phytochemicals: Baicalin, Rutin, Biopterin, Licoleafol, Luteolin and Quercetin shows stable bonding pattern with the target in compare to known inhibitors as it shows least score in docking, forms maximum number of hydrogen bonds with the active residues of the receptor. The predicted IC50 values of the phytochemicals are also better than the known inhibitors. Therefore, these six phytochemicals have more potentiality to inhibit the Papain-like protease.

**Conclusion**

Based on present observation of docking score of both phytochemicals and known inhibitors, IC50 value of known inhibitors and predicted IC50 of phytochemicals, we suggest six phytochemicals: Baicalin, Rutin, Biopterin, Licoleafol, Luteolin and Quercetin may be the Papain-like protease (PLpro) targeted potent drug leads against Covid-19. However, further studies are required to validate the same in vivo or in vitro.

**Declarations**

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**Figures**
Figure 1

Docking pose of inhibitor BDBM31523 with target
Figure 2

Docking pose of inhibitor BDBM31522 with target
Figure 3

Docking pose of inhibitor BDBM31516 with target
Figure 4

Docking pose of phytochemical Baicalin with target
Figure 5
Docking pose of phytochemical Rutin with target
Figure 6

Docking pose of phytochemical Biopterin with target
Figure 7

Docking pose of phytochemical Licoleafol with target
Figure 8

Docking pose of phytochemical Luteolin with target
Figure 9

Docking pose of phytochemical Quercetin with target
Figure 10

The multiple regression plot (linear) for inhibitors