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# Facing COVID-19 Via Anti-Inflammatory Mechanism of Action: Molecular Docking and Pharmacokinetic Studies of Six Anti-Inflammatory Compounds Derived from *Passiflora edulis*

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Authors AM, PTM, DDT and KTNN wrote the first draft of the manuscript. Authors DSTT, VM, D. T. Mwanangombo, BZG and D. T. Mawete collected information on plants bioactivity. Authors DOO, BMN, JTK and GNB collected information on plant phytochemistry. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aim:** In the most severe case of the COVID-19, there is an excessive production of proinflammatory cytokines, being the main cause of mortality and morbidity. The present study aims at assessing the potential inhibitor effect of six phytochemicals with anti-inflammatory activity derived from *Passiflora edulis*, against the SARS-CoV-2 main protease.

**Materials and Methods:** Virtual screening by molecular docking (Autodock tool) was used to obtain the binding energies of ligand-protein complexes formed between each of the six ligands and the SARS-CoV-2 main protease. The six ligands were then submitted to ADME (absorption, distribution, metabolism and excretion) and toxicity analyses to understand their pharmacokinetic behavior, using SwissADME, preADMET and pkCSM webservers.

**Results:** Four high-docking score compounds were identified (both flavonoids) as hits, with the trend: ligand 4 (quercetin, -8.2 kcal/mol ) > ligand 1 (chrysin, -8.0 kcal/mol) > ligand 2 (kaempferol, -7.9 kcal/mol) > ligand 3 (luteolin, -7.7 kcal/mol)> ligand 5 (harmol, -6.7 kcal/mol) > ligand 6 (harmine, -6.4 kcal/mol). The pharmacokinetic behavior of the six ligands revealed that they can be easily absorbed and have good permeability and bioavailability. The toxicity predictions of the six compounds from *P. edulis* which is an editable fruit confirm that they are safe.

**Conclusion:** Several approaches are currently being used to tackle the COVID-19. Given the cytokine storm in the most severe case of the COVID-19, we adopted the strategy of combatting the disease by compounds that exhibit anti-inflammatory activity. The assessment of the efficiency of six phytochemicals from *P. edulis* against the SARS-CoV-2 Mpro and their pharmacokinetic profile revealed their potential inhibitor effect against the COVID-19 protein.


*Keywords: COVID-19; cytokine storm; passiflora edulis; anti-inflammatory activity; pharmacokinetic profile.*

## 1. INTRODUCTION

Two prior outbreaks have emerged in the world as epidemics over the past 20 years; Severe Acute Respiratory Syndrome (SARS-CoV-1) which was first reported in November 2002 in Guangdong, China, and Middle Eastern Respiratory Syndrome (MERS-CoV) which was first reported in Saudi Arabia in 2012 [1]. Recently, a case of unidentified pneumonia which was first reported in December 2019 in Wuhan, China, has become the third coronavirus outbreak, called COVID-19 [1]. This recent CoV outbreak is caused by the SARS-CoV-2. Data from www.worldometers.info/coronavirus states that, on January 19, 2021, COVID-19 had already infected 96,409,214 people, caused 2,059,503 mortalities and 68,932,463 recovered people around the world. Patients infected with SARS-CoV-2 develop fever, cough, fatigue, dyspnea, and sore throat [2]. In the most severe case, patients may rapidly develop respiratory failure with acute respiratory distress syndrome caused by the pneumonia, and even present extra-pulmonary clinical manifestations like kidney damage, liver dysfunction, heart failure, diarrhea, or headache [2-3]. While it is well-known that COVID-19 primarily affects the pulmonary system and that the immediate immune response is to produce proinflammatory cytokines to combat the pneumonia, Blanco-Mello *et al*. described a distinctive and unsuitable inflammatory response related to SARS-CoV-2 infection [4]. This wrong and weak immune response consists in the overproduction of proinflammatory cytokines (IL-1, IL-6, TNF-α, etc) that could favor virus replication and enhance complications related to severe cases of the disease [4].

In the active research for finding molecule that can treat COVID-19, two approaches are currently being used. One is to identify new uses for FDA-approved drugs (drug repurposing) [5-6], while the other one is to identify from plants biodiversity potential inhibitors or phytochemicals of SARS-CoV-2's main protease using molecular modeling approaches [6-7].

When using molecular modelling approaches in order to identify potentials inhibitors, particular emphasis is placed on the significance of binding affinity of ligand-protein complexes and on their drug-likeness properties [6,8]. However, it should be mentioned that the biological activities of molecules capable to treat COVID-19 are important [9-10]. Further, the most common trend is that an anti-COVID-19 molecule might exhibit

antiviral activity [11-14]. Nevertheless, what is abundantly clear in this moment is that the most important cause of COVID-19 related deaths is respiratory failure which is due to pneumonia, an acute inflammatory lung injury, which itself varies depending on the disease severity level, but also alveolar damage that can precipitate acute respiratory distress syndrome (ARDS) [15]. The innate immune response is then to produce pro-inflammatory cytokines and chemokines to contain and stop the infection. Pathophysiologically, previous studies have reported high levels of various cytokines, the so-called cytokine storm, and chemokines in the serum of SARS-CoV-2 patients [16-17]. In addition, Fidan and Aydoğdu recently reported that various pro-inflammatory cytokines such as IL-6, IL-1, the tumor necrosis factor (TNF-α) induce a migration of leukocytes into lungs, that then secrete the reactive oxygen species and proteases that damage capillary endothelium and alveolar epithelium [18].

Based on the relevant clinical characteristics, phytocompounds derived from *Passiflora edulis (P. edulis)* whose isolated molecules have several therapeutic properties such as anti-inflammatory, antioxidant, anti-microbial, anti-cancer, can represent treatment options for COVID-19 as supported in the next paragraphs.

*P. edulis* also known as passion fruit, exhibits potential effects for the treatment of inflammation. Several mechanisms, including the inhibition of proinflammatory cytokines: TNF-α and IL-1ß levels, enzyme: myeloperoxidase (MPO) and mediators: bradykinin, histamine, substance P, nitric oxide (NO) release and/or action, appear to account for *P. edulis'* actions. Interestingly, in a comparative study, Montanher *et al.* found that *P. edulis* was more effective than dexamethasone (0.5 in inhibiting both MPO and NO levels) [19]. This latter, which is considered as an important steroidal anti-inflammatory drug, might hold the promise for the treatment of COVID-19 as recently reported by Ledford [20]. Cazarin and co-authors reported in 2015 the anti-inflammatory activity of *P. edulis* leaves [21]. In a dextran sodium phosphate caused mice colitis model, *P. edulis* peel flour was found to reduce TNF-α, IL-1ß, IL-6, IL-12, and IL-17 [22]. Molecules responsible for this effect could be compounds like C-glycosyl flavonoids vicenin, orientin, chrysin, vitexin and kaempferol [23]. Finally, Harmol and harmine, two fluorescent harmala alkaloids showed anti-inflammatory activity by significantly inhibiting the NF-k signaling pathway [24-25].

With regards to the reactive oxygen species that are secreted by leucocytes, several studies highlighted the antioxidant activity of *P. edulis* fruit and leaf which can eliminate free radicals or inhibit their activity [26-27]. Finally, aqueous and ethanolic leaves extracts have shown *in vitro* effect on some viruses species including Herpes Simplex Virus Type 1 and 2, Varicella-Zoster Virus, etc. [28].

Since the morbidity and mortality of COVID-19 infections arise in part from the toxic hyperproduction of proinflammatory cytokines [3-4], the aim of this study is to assess the efficiency of six anti-inflammatory compounds derived from *P. edulis* against the SARS-CoV-2 main protease using molecular docking technique, in order to propose them as potential therapeutic for the COVID-19 disease.

## 2. MATERIALS AND METHODS

### 2.1 Literature Review

Based on information reported above, six phytochemicals [19] derived from *P. edulis* (passion fruit, see Fig. 1) with anti-inflammatory activity are chosen for this study. Chrysin or 5,7-dihydroxyflavone (5,7-dihydroxy-2-phenyl-4H-chromen-4-one), kaempferol or 3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one), luteolin or 2-(3,4-dihydroxyphenyl)-5,7-dihydroxychromen-4-one, quercetin or 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one, harmol or 1-Methyl-2,9-dihydropyrido[3,4-*b*]indol-7-one and harmine or 7-Methoxy-1-methyl-9H-pyrido[3,4-*b*] indole. The four first compounds are flavonoids while the two latter compounds are alkaloids. Chemical structures of compounds were retrieved from literature sources. Their 2D structures have been sketched using Marvin JS and their 3D structures retrieved from PubChem/NLM. Bibliographical references were made using a bibliographical software "Mendeley".

**Fig. 1. Leaves and flowers (left) and yellow passion fruits (right) of *P. edulis* (see Ref. [23])**

## 2.2 Molecular Docking

The most common tool to evaluate the strength of binding between ligand-protein interactions is molecular docking. The structure of the protein, 3-Chymotrypsin-Like protease (3CLpro) or the COVID-19 virus main protease (Mpro) which is among the most studied SARS-CoV-2 proteases was obtained from PDB (Protein Data Bank) database (PDB ID: 2GTB) and imported into chimera for visualizing the binding domain of the complex and identifying the amino acids in the binding pocket as well. The hydrogen atoms were added to the protein in order to correct the ionization and tautomeric states of the amino acid residues. Furthermore, water molecules and complexes bound to receptor molecule were removed before the docking. Incomplete side chains were replaced using Drunbrack rotamer library. In addition, the protein was subjected to energy minimization by applying the AMBER 14SB force field, and AM1-BCC was used for other residues with a maximum number of 200 steps at RMS gradient of 0.02. The optimized protein was saved in .pdbqt format and imported to PyRx for molecular docking which was carried out by means of Autodock Vina virtual screening tool [29]. The validation of the docking study was performed by re-docking the reference ligand into an appropriate protein cavity. Re-docking is accepted if the root mean square value (RMSD) is lower than 2.0 Å. Fig. 2 displays schematic structure of the SARS-CoV-2 Mpro/3CLpro (a) and the complex formed between the SARS-CoV-2 Mpro and 2GTB as a potential drug target for the new coronavirus-2 (b). According to Xu and co-workers, 2GTB is the main protease found in the coronavirus associated with the severe acute respiratory syndrome (SARS), and that the main protease in 2019-nCoV shares 96% similarity with that in SARS [30]. Further, some FDA-approved drugs such as lopinavir, ritonavir, and sofosbuvir, formerly used against SARS-CoV-1, MERS-CoV, HIV and Ebola virus are used for the management of patients with COVID-19 [31].

### 2.2.1 Preparation of ligands and pharmacokinetic profile

The selected compounds derived from various literature resources [23,32] were drawn using Marvin JS. Fig. 3 shows the 3D structures of the sketched compounds retrieved from PubChem/NLM and Fig. 4 displays their optimized structures with the Gaussian 09 set of codes [33] using HF/6-31+G(d,p) level of theory. The 3D ligands were then saved in .sdf format. Ligands optimization was performed by using universal force field (UFF) with conjugate gradients algorithm of 200 Steps, and then analyzed for pharmacokinetic properties. Bioinformatics resources have been employed in the prediction of ADME properties (Absorption, Distribution, Metabolism and Excretion) using the SwissADME database [34]. During the early stages of drug discovery, the ligand to be selected as a hit must be non-carcinogenic and non-hepatotoxic. The toxicity assessment (ADMET, T for Toxicity) that allows to predict the mutagenicity (Ames test) and carcinogenicity of the potential ligands was made using the preADMET server, Korea [35], while the hepatotoxicity and the oral rat acute toxicity were assessed using the pkCSM server [36].

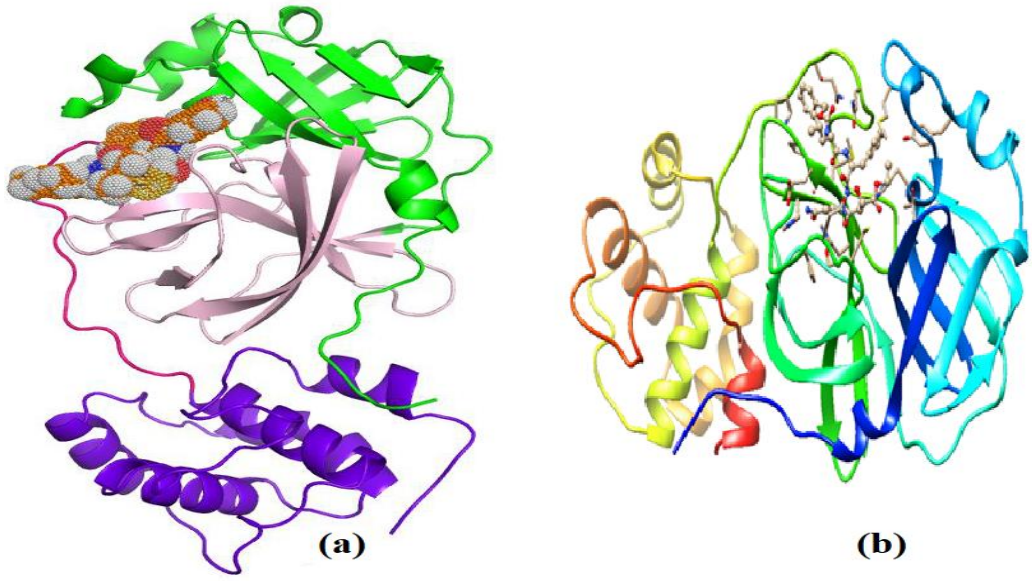


**Fig. 2. Schematic structure of the SARS-CoV-2 Mpro (a) and the complex formed between the SARS-CoV-2 Mpro and co-crystallized inhibitor 2GTB (b) (see Ref. 8)**

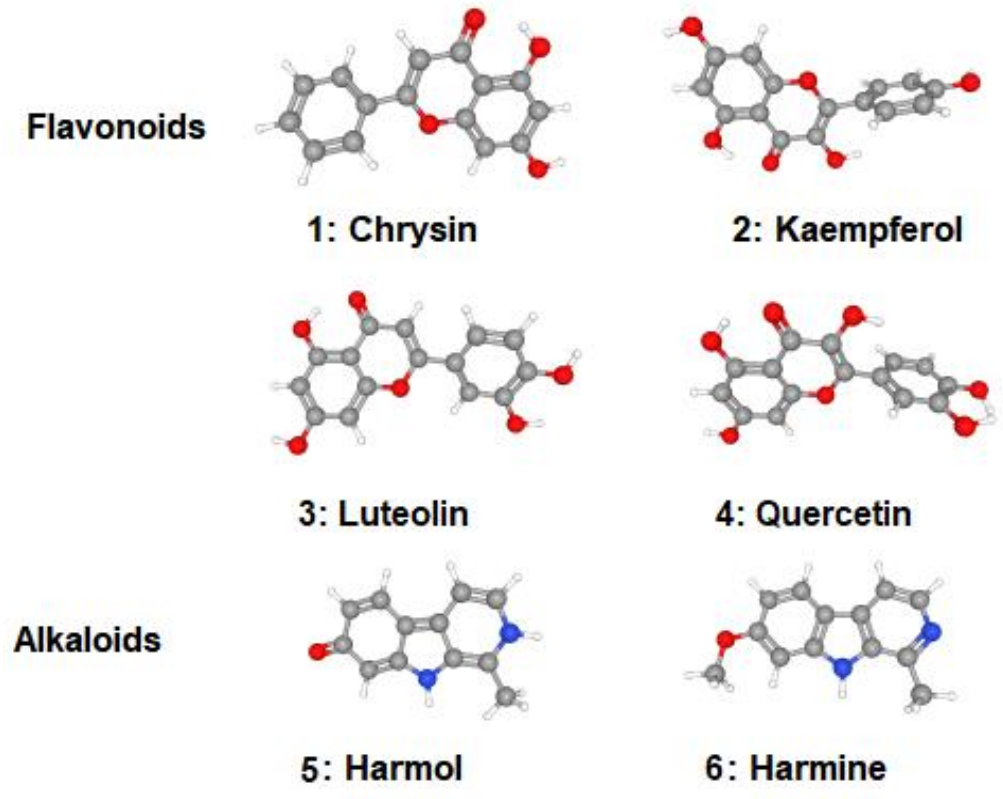


**Fig. 3. 3D Structures of selected flavonoids and alkaloids compounds 1–6 derived from *P. edulis***

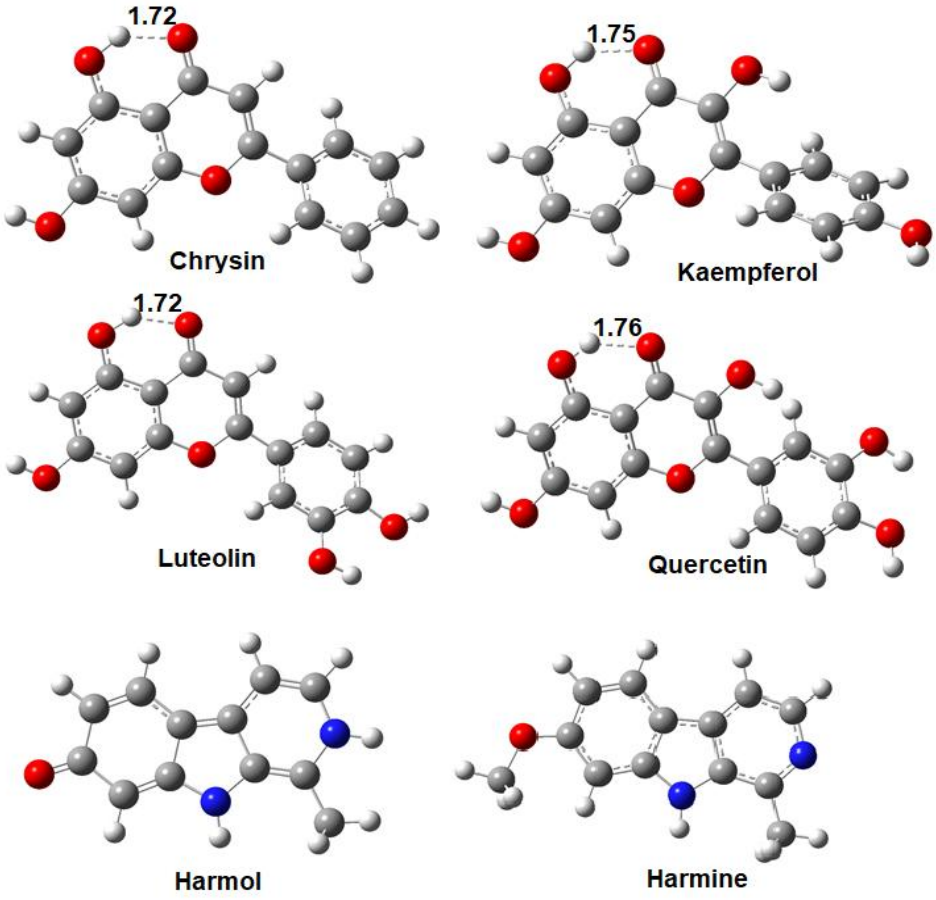


**Fig. 4. Optimized structures of compounds along with intramolecular H-bonding interactions (Å)**

## 3. RESULTS AND DISCUSSION

### 3.1 Energetics and Geometries

Noncovalent interactions, mainly H-bonds [37], van der Waals and π-π interactions (stacked/parallel and T-shaped/perpendicular conformations) [38] are forces that drive and determine the binding of ligand-protein interactions. The docking results obtained using AutoDock Vina virtual screening tool between ligands 1-6, the native or reference ligand with the SARS-CoV-2's main protease ($M^{pro}$ or 3CLpro) are gathered in Table 1.

Since Lopinavir and Nelfinavir, two FDA approved drugs for the treatment of human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome patients can represent potential treatment options for COVID-19 [39], they were used as drug standards for comparison.

The binding affinity values of the 6 ligands ranging from -6.40 to -8.22 kcal/mol place the four flavonoids compounds as the best docked ones to the SARS-CoV-2 main protease. The most strongly bound to the protease cavity is ligand 4 or quercetin (-8.22 kcal/mol), followed by ligand 1 or chrysin (-8.04 kcal/mol). The overall trend of the stability of complexes follows the pattern: ligand 4 > ligand 1> ligand 2 > ligand 3> ligand 5> ligand 6>. Based on data presented in this table, the binding energies of the four flavonoids compounds are somewhat close to those of Lopinavir/Nelfinavir and higher than that of the reference ligand. However, as part of principles that drive drug discovery, this does not mean that ligands 1-4 can automatically inhibit the virus action or ligands 5 and 6 cannot be

considered as hits, without establishing the pharmacokinetic properties of each ligand.

Turning next to the types of noncovalent interactions established between ligands and the SARS-CoV-2 Mpro, one can see in Fig. 5 below that the complexes are mainly stabilized by intermolecular hydrogen bonding interactions, supported by van der walls and π-π interactions. At this stage, the stability of ligands 1-4 which are flavonoids over ligands 5 and 6 can be explained by the presence of multiple OH groups that can be act simultaneously as hydrogen bonds acceptors (HBA) and donors (HBD) [40]. In addition, the establishment of intramolecular hydrogen bonding interaction in the ligands 1 (1.72 Å), 2 (1.75 Å), 3 (1.72 Å) and 4 (1.76 Å) strengthens the stability of the complexes formed between the SARS-CoV-2 Mpro and the four ligands.

The Molecular Electrostactic Potential (MEP) which is a powerful tool in understanding the interactions of two macromolecules such as a drug and its receptor [41-42], was calculated at the same level of theory used for the optimization. The MEP maps of the six ligands at ± 0.001 a.u. are displayed in Fig. 6. The electrostactic potential shows two regions, negative regions (in red) concentrated in the two first aromatic rings (ligands 1-4) and the O atom located in the second aromatic ring; and around the three rings of the ligands 5 and 6. The extreme positive regions (in yellow) are positive ones.

As shown in Fig. 6, the electrostactic potentials are consistent with the electrostatic interactions (i.e. H-bonds) and lipophilic interactions between hydrophobic amino acid residues of the SARS-CoV-2 Mpro and non-polar sites of ligands.

In addition, the presence of three aromatic rings in ligands 1-4 offers much possibilities to π-π interactions to take place. Such interactions that are important in the ligand-protein interactions are mainly stabilized by dispersion or van der Waals forces [38].

H-bonds parameters (distances and angles) between the protein target and ligands 1-6 along with the involved groups (ligands) and the amino acids residues of the Mpro engage in H-bonding interaction are summarized in Table 2.

It can be seen that ligands 1, 2, 3 and 4 form five conventional hydrogen bonds with the active site of the SARS-CoV-2 main protease, whereas the two weakest complexes have three and one hydrogen bonds for the ligand 5 and the ligand 6, respectively. The strongest (shortest) hydrogen bonding interaction is established between the residue of the amino acid ASP187 of the Mpro with the O-H group of the quercetin ligand (1.78 Å). The two adducts form the strongest complex.

## 3.2 Physicochemical Properties and ADME-T Profiles

Computational chemistry methods and bioinformatics tools are widely used in the field of drug design and for understanding the electronic properties of various drug-molecules [43-48]. Physicochemical property is an important parameter of a molecule that influences efficacy, safety or metabolism which could be predicted by using Lipinski's rule of five (RO5) that is: molecular mass < 500; Hydrogen-bond donors (HBD) ≤ 5; Hydrogen-bond acceptors (HBA) < 10; and Log P < 5 [49]. Prediction of *in silico* physicochemical parameters of the six ligands are grouped in Table 3.

**Table 1. Binding affinity (kcal/mol) of 2GTB and Ligands 1-6 with SARS-CoV-2 $M^{pro}$**

| Receptor PDB id | Ligands | Binding affinity (ΔG in Kcal/mol) |
|---|---|---|
| 2GTB | Lopinavir | -8.4 |
| | Nelfinavir | -8.1 |
| | 1 | -8.0 |
| | 2 | -7.9 |
| | 3 | -7.7 |
| | 4 | -8.2 |
| | Ref. Ligand | -7.4 |
| | 5 | -6.7 |
| | 6 | -6.4 |

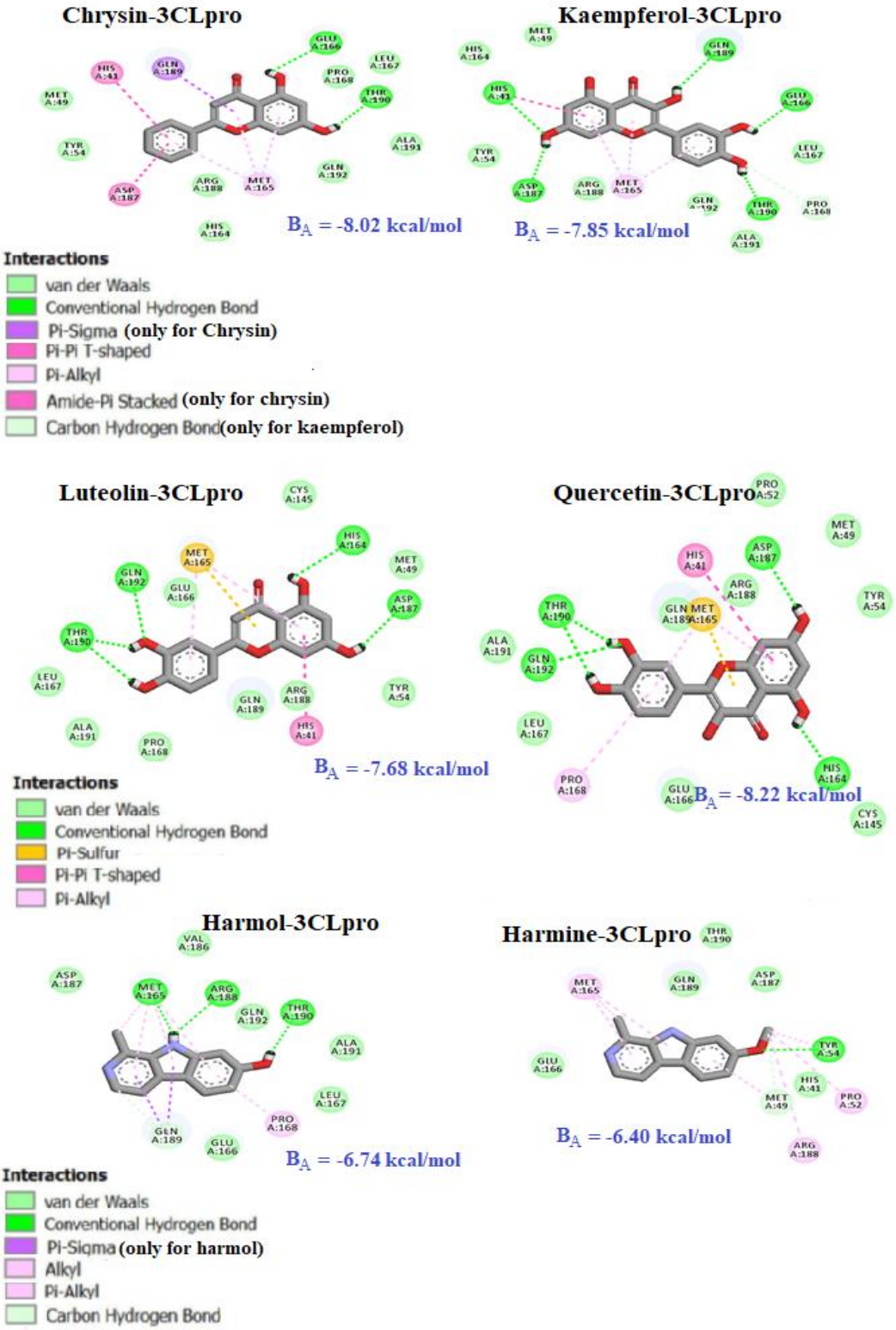


**Fig. 5. Interaction map of ligands 1-6 with the main protease of coronavirus 3CLpro**

**Table 2. Hydrogen-bonds parameters derived from docking of ligands 1-6 with SARS-CoV-2 Mpro**

| Ligand | AA residues | Ligand group | δ (Å) | θ(°) |
|---|---|---|---|---|
| 1 | GLU166 | O-H | 1.99 | 155 |
| | HIS189 | O-H | 2.17 | 163 |
| | THR180 | O-H | 2.01 | 142 |
| | ASP187 | O-H | 2.18 | 140 |
| | HIS41 | $O=C_2$ | 2.17 | 153 |
| 2 | HIS41 | O-H | 2.30 | 163 |
| | ASP187 | O-H | 2.29 | 135 |
| | GLU166 | O-H | 1.86 | 153 |
| | THR190 | $O=C_2$ | 2.24 | 158 |
| | GLN189 | O-H | 1.97 | 160 |
| 3 | THR190 | O-H | 2.15 | 145 |
| | THR190 | O-H | 2.15 | 150 |
| | GLN192 | O-H | 2.36 | 144 |
| | HIS164 | O-H | 2.32 | 130 |
| | ASP187 | O-H | 2.00 | 170 |
| 4 | GLN192 | O-H | 2.10 | 144 |
| | THR190 | O-H | 2.20 | 155 |
| | THR190 | O-H | 2.22 | 150 |
| | HISP164 | O-H | 1.99 | 140 |
| | ASP187 | O-H | 1.78 | 165 |
| 5 | ARG188 | H-N | 2.06 | 145 |
| | THR190 | H-O | 1.83 | 165 |
| | MET165 | H-N | 2.04 | 151 |
| 6 | TYR54 | H-N | 2.18 | 160 |

Inspection of Table 3 shows that all ligands meet every single criterion of Lipinski's rule of five and thus fully obey the rule. Consequently, all the investigated ligands are predicted to be easily absorbed and have good permeability and bioavailability. According to Ghose and co-workers, the molecular refractivity is an ubiquitous parameter for a drug molecule that cannot exceed 130 $m^3.mol^{-1}$ and not to be under 40 $m^3.mol^{-1}$ [50].

None violation is observed here for all the investigated ligands as can be seen in Table 3. Finally, as pointed out by Cerqueira and co-authors, for optimal drug absorption and distribution, the polar surface area (PSA) values cannot be higher than 140 Å [51]. Once again, none violation is observed here. Three potential candidates for the inhibition of the SARS-CoV-2 3CLpro have PSA values almost two to three times less than the recommended value (ligands 1, 5 and 6), while ligands 2, 3 and 4 have PSA values higher than 100 Å.

The next step to deal with is to establish the ADME/T profiles of each ligand. In fact, a major issue after identifying stables complexes, that is, hit compounds, is to evaluate their ADME parameters and cardiotoxicity.

These pharmacokinetic properties are very important parameters in the computer-aided drug discovery since they allow one to retract some hits from early-stage trials. The ADME properties are evaluated by using SwissADME and pkCSM servers, but other parameters such as the Blood-Brain Barrier (BBB), the Human Intestine Absorption (HIA) and the skin permeability come from the preADMET server. The selected endpoints for toxicity are Ames test and Rodent Carcinogenicity (rat) in preADMET server, hepatotoxicity and oral rat acute toxicity ($LD_{50}$) in pkCSM server. These parameters are gathered in Table 4.

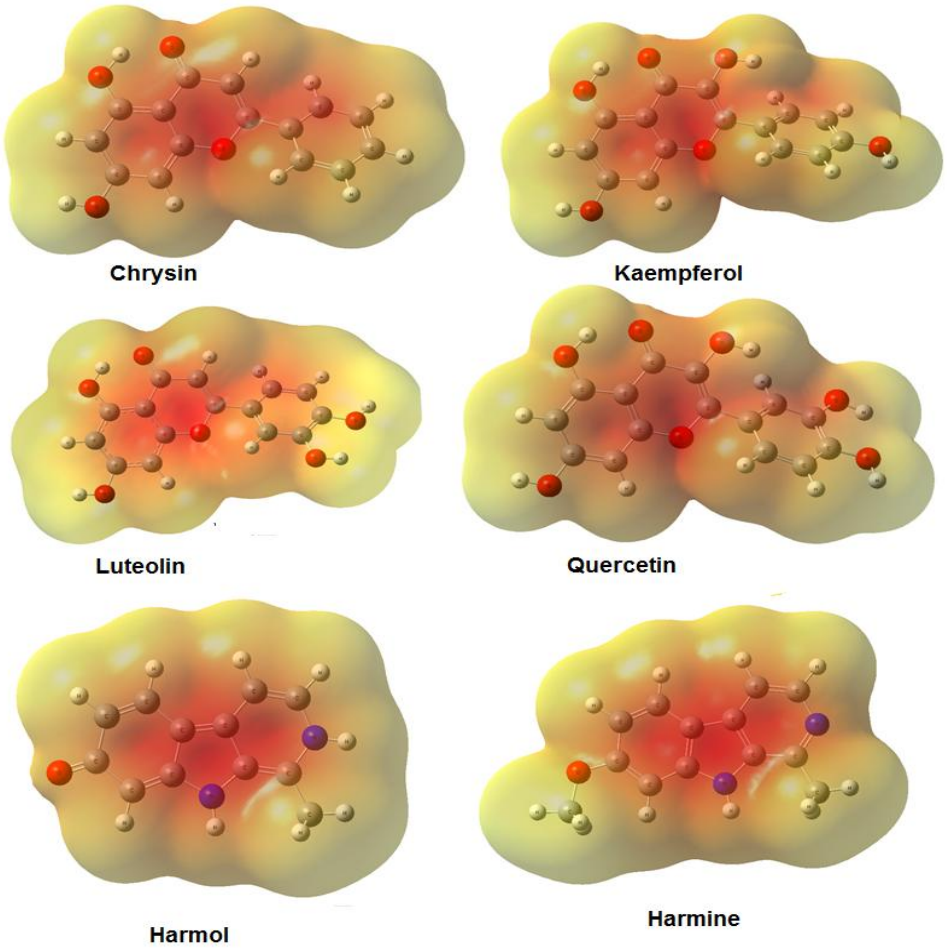


**Fig. 6. MEPs of the six selected compounds at ± 0.001 a.u**

According to the binding affinity values, it was derived the following decreasing order in the complexes formed between ligands and the SARS-CoV-2 3CLpro or Mpro: Ligand 4 > Ligand 1 > Ligand 2 > Ligand 3> Ligand 5 > Ligand 6. This order only reflects the thermodynamic stability of complexes. However, the stability over time of the ligand in a protein interaction site depends on further factors. For a ligand to be used for therapeutic purposes, its absorption, distribution, metabolism, excretion and toxicity are aspects to take into account. To pursue further, it is worthy to point out that first of all, such a ligand must be non-hepatotoxic and non-carcinogenic [52].

Scrutiny of toxicity outcomes in Table 4 reveals that all potential ligands are non-hepatotoxic. With regards to the carcinogenicity, the results predict the carcinogenic activity only for the ligand 2. This encouraging result of toxicity assessment allows us to go back to ADME properties. The ability of a drug molecule to cross into the brain is an important propriety to improve the efficacy of drugs (reduce side effects and toxicities). The BBB values for the potential candidates are all positive and the lowest value is found in ligand 4 (0.173) which forms the strongest complex with the SARS-CoV-2 main protease. The probability of intestinal absorption by human is very high, and on the one hand almost the same for ligands 1, 5 and 6; and on the other hand almost the same for ligands 2, 3 and 4. Ligand 4 has the smallest probability (79.44 %) of being absorbed by human intestine, in contrast with its binding affinity with the COVID-19 protease. The recommended value of the skin permeability or log $K_p$ for a drug molecule is set at more than -2.5 cm/h. Interestingly, the computed log $K_p$ values range

from -3.3 to -4.7 cm/h. Finally, the bioavailability score which is evaluated to 0.55 confirms that ligands 1-6 have good absorption and distribution since all potential candidates may have more than 10% of bioavailability in rat [53].

The Cytochrome P450 inhibition as metabolic indicators including CYPs: 1A2, 2C19, 2C9, 2D6 and 3A4 are also predicted. Nevertheless, only CYP2D6 and CYP3A4 are responsible for drug metabolism [51]. Interestingly, the three best candidates according to their binding affinity are found to be non-inhibitors of CYP2D6 and CYP3A4 except hits 1 and 6 that affect the CYP3A4, and hit 6 which in addition affects the CYP2D6. This result rules the ligand 6 out from the list of potential candidates for the inhibition of the SARS-CoV-2 main protease, a result which is moreover in good agreement with its lower free enthalpy (-6.40 kcal/mol).

Turning next to excretion also called elimination, the total clearance is directly linked to the renal OCT2 (organic cation transporter 2) substrate that offers helpful information on potential contraindications. The six selected compounds are predicted to be not renal OCT2 substrates. This means that all the six phytocompounds can be eliminated through the OCT2 substrate. Surprisingly, the total clearance values of the investigated compounds vary almost inversely with their binding affinities values. In addition, ligands 2 and 3 which are tautomers, have the same molecular weight and have exactly the same total clearance.

Closing finally the ADME-T profiles of the six compounds, their oral acute toxicity ($LD_{50}$) are classified in category or class 4, meaning that they are slightly toxic (Globally Harmonized System: 300 < Category 4 ≤ 2000) and can thus be considered as safe.

Bacteria, fungi, viruses can cause serious infections and diseases in the world. Some of these infections and diseases are curable while some of them are not (i.e. HIV, COVID-19). Even for those that are curable, there is sometimes multiple drug resistance. Since humanity exists, plants have been one of man's best friends. While it is obvious that plants are a source of vitamins, proteins, minerals, they are as well source of phytocompounds containing excellent therapeutic properties: antioxidant, anti-inflammatory, antifungal, antimicrobial, and antitumor activities. *Passiflora edulis*, also known as passion fruit, passion flower, purple granadilla, or "maracuja" (DR Congo or Brazil), is widely cultivated for its edible fruit. With numerous biological activities, the passion fruit also contains vitamins A, C, E, K, minerals such as Zn (0.10 g), Mg (29 mg), K (348 mg), Ca (12 mg), etc [54]. On the one hand, vitamins A and K could help to fight the COVID-19 [55]. On the other hand, these chemical elements mainly Zn, although indispensable as enzymatic co-factors, a slight increase in their intracellular concentration inhibits the replication of retroviruses including SARS-CoV-1 [56] important in the management of COVID-19. Owing to its numerous therapeutic activities like antioxidant activity, antifungal activity, antitumor activity, antianxiety activity, and antihypertensive activity, *P. edulis* has traditional or ethno medicinal uses in many countries. Of complex phytochemistry, its secondary metabolites have numerous health benefits and very recently, as stated above, Jabareen and co-workers reported in an experimental study the antiviral activity of *P. edulis* leaves on some viruses' species including Herpes Simplex Virus Type 1 and 2, Varicella-Zoster Virus, etc [28]. Since *P. edulis* exhibits anti-inflammatory capabilities essential to stem the cytokine storm, this study is conducted in order to identify potential inhibitors from a set of six phytochemicals endowed with anti-inflammatory activity. The six selected compounds (four flavonoids and two alkaloids) reacted with the SARS-CoV-2 3CLpro or Mpro, and an order of thermodynamic stability was obtained.

Compared with Lopinavir and Nelfinavir that are proteases inhibitors recommended for the treatment of SARS and MERS [31], the binding affinities of the four top compounds, both flavonoids, are very close to those of two anti-HIV drugs, and even a bit higher to that of the ligand reference. Indeed, the ligand 1 or chrysin has anti-inflammatory, antibacterial and antioxidant activities [57-58], while ligand 2 or kaempferol exhibits antitumor, antioxidant and anti-inflammatory capabilities [59]. In fact, our previous study showed that *Aloe vera* represents a potential treatment for COVID-19 [60], and three of its phytochemicals were identified as potential inhibitors of SARS-CoV-2 main protease, in which two compounds exhibit anti-inflammatory effect [8]. The anti-inflammatory activity of luteolin in experimental animal models was reported by Ziyan and co-workers [61], and recently, a study by Lesjak and co-authors showed antioxidant and anti-inflammatory activities of quercetin and its derivatives [62].

**Table 3. Predicted *in silico* physicochemical parameters using SwissADME online tool**

| Ligand | Formula | MW (Da) | Log P | HBD | HBA | PSA (Å) | Refractivity ($m^3.mol^{-1}$) | Violations | Log S |
|---|---|---|---|---|---|---|---|---|---|
| 1 | $C_{15}H_{10}O_4$ | 254.24 | 2.27 | 2 | 4 | 70.67 | 71.97 | 0 | -4.19 |
| 2 | $C_{15}H_{10}O_6$ | 286.24 | 1.70 | 4 | 6 | 111.13 | 76.01 | 0 | -3.31 |
| 3 | $C_{15}H_{10}O_6$ | 286.24 | 1.86 | 4 | 6 | 117.31 | 76.01 | 0 | -3.71 |
| 4 | $C_{15}H_{10}O_7$ | 302.24 | 1.63 | 5 | 7 | 131.36 | 78.03 | 0 | -3.16 |
| 5 | $C_{12}H_{10}N_2O$ | 198.22 | 1.68 | 2 | 1 | 48.65 | 61.39 | 0 | -2.18 |
| 6 | $C_{13}H_{12}N_2O$ | 212.25 | 2.07 | 1 | 2 | 37.91 | 65.06 | 0 | -4.05 |

*With MW= Molecular weight, Log P = Lipophilicity, PSA = Polar Surface Area, Log S = water solubility*

**Table 4. ADME-T profile of ligands 1-6**

| Parameter | Ligand 1 | Ligand 2 | Ligand 3 | Ligand 4 | Ligand 5 | Ligand 6 |
|---|---|---|---|---|---|---|
| **Absorption & Distribution** | | | | | | |
| BBB | 0.933 | 0.286 | 0.368 | 0.173 | 0.320 | 3.798 |
| HIA (%) | 92.644 | 79.439 | 81.132 | 77.207 | 94.263 | 92.827 |
| Skin permeability (log $K_p$) | -3.346 | -4.323 | -4.280 | -4.433 | -4.662 | -4.386 |
| Bioavailability score | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 |
| **Metabolism** | | | | | | |
| CYP2D6 | No | No | No | No | No | Yes |
| CYP3A4 | Yes | No | No | No | No | Yes |
| **Excretion** | | | | | | |
| Total clearance | 0.48 | 0.50 | 0.50 | 0.41 | 0.59 | 0.62 |
| Renal OCT2 substrate | No | No | No | No | No | No |
| **Toxicity** | | | | | | |
| Ames test | Yes | No | No | No | Yes | Yes |
| Hepatotoxicity | No | No | No | No | No | No |
| Carcinogenicity (rat) | Negative | Positive | Negative | Negative | Negative | Negative |
| Oral rat acute toxicity ($LD_{50}$, in mol/kg) | 2.486 | 2.197 | 2.455 | 2.471 | 2.781 | 2.999 |
| | 1243 | 1099 | 1228 | 1236 | 1391 | 1450 |

*Values in bold are expressed in mg/kg*

One can remember that the hyperproduction of proinflammatory cytokines is the main reason that causes morbidity and mortality in SARS-CoV-2 patients [3-4,63]. However, further considerations have to be taken into account. The current epidemiological data suggests that men are more affected than women by the COVID-19 virus. Kopel *et al* reported that women are less susceptible to viral infections than men due to their mounting of more robust immune responses [64]. Oertelt-Prigione hypothesized that this effect could result from an increase in the production of cytokines, chemokines, and interferons in females than males [65]. Another interesting facet is to take into account the component of race in the manifestations of the COVID-19. In fact, previous studies pointed out that minority groups may be more susceptible to COVID-19 infections given their low socioeconomic status that may be a contributing factor to the higher presence of comorbidities and thus to the prevalence of COVID-19 infections [66-67]. Pathophysiologically, even if the biological mechanism of the six anti-inflammatory compounds is unknown, the application of anti-inflammatory molecules is a mechanistically-sound strategy for treatment development [63]. Indeed, previous papers dealing with drug repurposing used for COVID-19 suggest that most of drugs which have been used for the treatment of COVID-19 act on host proinflammatory cytokines [68-69]. For instance, tocilizumab, an anti-interleukin-6 (IL-6) blocks IL-6 receptor and revert the cytokine storm production [68]. Certolizumab is an anti-TNF-α, azithromycin and chloroquine act on IL-6 and TNF-α cytokine targets [3].

## 4. CONCLUSION

The strategy adopted in this work consisted in exploring the inhibitory power of six phytochemicals derived from *P. edulis*. Given the cytokine storm that could favor virus replication and enhance complications related to severe cases of the disease, six anti-inflammatory compounds were each paired with the SARS-CoV-2 main protease in order to evaluate first their thermodynamic stability. The docking affinity scores showed that ligands 1-4 (flavonoids) are more stables than ligands 5 and 6 (alkaloids). Then, the Lipinski's rule of five and the pharmacokinetic studies showed that these phytochemicals have good ADME-T profiles, mainly the flavonoids compounds. Consequently, chrysin, kaempferol, luteolin and quercetin as the four top compounds can represent potential therapeutic options for COVID-19 via a mechanism that involves the inhibition of toxic pro-inflammatory cytokines. As a future perspective, we recommend further *in vivo* trials for the experimental validation of our findings.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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