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Computational studies of caffeoylquinic acids from Brazilian green propolis and its anti-viral potential against SARS-CoV-2 M^{pro}

Estudos computacionais dos ácidos cafeoylquínicos da própolis verde brasileira e seu potencial anti-viral contra a SARS-CoV-2 M^{pro}

Estudios computacionales de los ácidos cafeoilquínicos del propóleo verde brasileño y su potencial antiviral contra SARS-CoV-2 M^{pro}

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Abstract

Purpose: The present study evaluated the following caffeoylquinic acids, Neochlorogenic acid (**3-CQA**), Cryptochlorogenic acid (**4-CQA**), Chlorogenic Acid (**5-CQA**), Isochlorogenic acid A (**3,5-DCQA**) Isochlorogenic acid B (**3,4-DCQA**) and Isochlorogenic acid C (**4,5-DCQA**) found in Brazilian green propolis, regarding its physicochemical, pharmacokinetic and conformational characteristics. Additionally, the compounds were explored on the efficacy of inhibiting the pharmacological target SARS-CoV-2 M^{pro}, a protein involved in SARS-Cov-2 infection. Methods: The physicochemical and pharmacokinetic proprieties were obtained from the projected 2D structures of the respective compounds. The conformational characteristics were obtained from the three-dimensional models were subjected to geometric optimization by molecular mechanics under MMFF94 force field, subsequently, the calculation of partial atomic charges by employing AM1 semi-empirical methodology was performed. Molecular docking was performed, and the protein was loaded in PDB Data Bank (PDB ID 6LU7). Results: The physicochemical and pharmacokinetic results indicate that these phytochemicals have from medium to low potential for gastrointestinal tract absorption, principally in relation to the LogP values, and violated some druglikeness' criteria. However molecular docking showed that the compounds **3-CQA**, **4-CQA**, **5-CQA**, **3,5-DCQA**, **3,4-DCQA** and **4,5-DCQA** potentially binds with the active site of the SARS-CoV-2 M^{pro}, through stables complexes, with a docking score of -6.44, -6.11, -6.48, -6.26, -7.01 and -7.40 kcal/mol, respectively. Conclusion: The physicochemical and pharmacokinetic parameters, as well as Hydrogen-bond formation, energy landscape, indicate that the compound with the greatest therapeutic potential are **5-CQA**, **3,4-DCQA** and **4,5-DCQA** however this study necessitates further *in vitro* and *in vivo* experimental validation.

Keywords: Caffeoylquinic acids; Computer simulation; COVID-19; Propolis; SARS-CoV-2 infection.

Resumo

Objetivo: O presente estudo avaliou os seguintes ácidos cafeoilquínicos, ácido Neo-clorogênico (**3-CQA**), ácido Cripto-clorogênico (**4-CQA**), ácido Clorogênico (**5-CQA**), ácido Isoclorogênico A (**3,5-DCQA**) ácido isoclorogênico B (**3,4-DCQA**) e ácido isoclorogênico C (**4,5-DCQA**) encontrados na própolis verde brasileira, quanto às suas características físico-químicas, farmacocinéticas e conformacionais. Além disso, os compostos foram estudados quanto a ação farmacológica de tantativa de inibir o alvo SARS-CoV-2 M^{pro}, e uma proteína envolvida na infecção por SARS-Cov-2. Métodos: As propriedades físico-químicas e farmacocinéticas foram obtidas a partir das estruturas em projeto 2D dos respectivos compostos. As características conformacionais obtidas a partir dos modelos tridimensionais foram submetidas à otimização geométrica por mecânica molecular sob campo de força MMFF94, posteriormente foi realizado o cálculo das cargas atômicas parciais empregando a metodologia semi-empírica AM1. O encaixe molecular foi realizado e a proteína foi carregada no PDB Data Bank (PDB ID 6LU7). Resultados: Os resultados físico-químicos e farmacocinéticos indicam que esses fitoquímicos possuem potencial de médio a baixo para absorção no trato gastrointestinal, principalmente em relação aos valores de LogP, e violaram alguns critérios de druglikeness. No entanto, o docking molecular mostrou que os compostos **3-CQA**, **4-CQA**, **5-CQA**, **3,5-DCQA**, **3,4-DCQA** e **4,5-DCQA** potencialmente se ligam ao sítio ativo do SARS-CoV-2 M^{pro}, através de complexos de estábulos, com pontuação de docking de -6,44, -6,11, -6,48, -6,26, -7,01 e -7,40 kcal/mol, respectivamente. Conclusão: Os parâmetros físico-químicos e farmacocinéticos, assim como a formação de ligações de hidrogênio, carga energética, indicam que os compostos com maior potencial terapêutico são **5-CQA**, **3,4-DCQA** e **4,5-DCQA**, porém este estudo necessita de mais validação experimental *in vivo* e *in vitro*.

Palavras-chave: Ácidos cafeoilquínicos; Simulação computacional; COVID-19; Própolis; Infecção por SARS-CoV-2.

Resumen

Objective: El presente estudio evaluó los siguientes estudios cafeoilquínicos, Ácido Neoclorogénico (**3-CQA**), Ácido Criptoclorogénico (**4-CQA**), Ácido Clorogénico (**5-CQA**), Ácido Isoclorogénico A (**3,5-DCQA**) ácido isoclorogénico B (**3,4-DCQA**) e ácido isoclorogénico C (**4,5-DCQA**) encontrados en propóleo verde brasileño, en cuanto a sus características fisicoquímicas, farmacocinéticas y conformacionales. Además, se estudió la acción farmacológica de los compuestos similar a la del SARS-CoV-2 M^{pro} e una proteína relacionada con la infección por el SARS-CoV-2. Metodos: Se presentaron métodos químicos y farmacocinéticos basados en las estructuras de los proyectos: Las propiedades físicas e los métodos farmacocinéticos se basaron en las estructuras de los proyectos de los respectivos compuestos. Como los modelos de tensión molecular se realizaron a partir de modelos triómicos, se realizaron a partir de modelos triómicos, bajo modelos moleculares, se realizaron a partir de modelos triómicos, modelos moleculares, se realizaron a partir de modelos mecánicos semiparciales, se realizaron a partir de 4 modelos mecánicos semiparciales, se realizaron a partir de 4 modelos semiparciales modelos de mecanica Se realizó un análisis molecular e la proteína se cargó en el banco de datos PDB (PDB ID 6LU7). Resultados: Los resultados fisicoquímicos y cinéticos indican que estos fitoquímicos tienen un potencial farmacológico medio y bajo para la absorción en el tracto gastrointestinal, principalmente en relación con los valores de LogP, e violaron los criterios de similitud con los fármacos. Sin embargo, el molecular dio a conocer que los compuestos, **4-CQA**, **5-CQA**, **5-CQA**, **3,5-DCQA**, **3,4-DCQA** e se unen al SARS-CoV-2 M^{pro} activo, a través de complejos estables, con el ajuste de -6,44, -6,11, -6,48, -6,26, -7,01 e -7,40 kcal/mol, respectivamente. Conclusión: La farmacocinética física, química y química, así como la formación de puentes de hidrógeno, carga energética, indican que los compuestos con mayor potencial terapéutico son el **5-CQA**, **3,4-DCQA** e **4,5-DCQA**, pero este El estudio necesita una mayor validación experimental *in vivo* e *in vitro*.

Palabras clave: Ácidos cafeoilquínicos; Simulación por computadora; COVID-19; Propóleos; Infección por SARS-CoV-2.

1. Introduction

Currently statistical data of the SARS-CoV-2 pandemic show that more than 618 million people have been infected by this virus and almost 15 million people have died as a result of COVID-19 (Worldometers, 2022). Despite the fact that vaccination has already advanced in several countries around the world, and the possibility of using other drug treatments, the contaminations do not stop, competing daily with health systems, with the exposure of vulnerable populations and groups, the economic support of the financial system and the population, the mental health of people in times of confinement and fear for the risk of illness and death, access to essential such as food, medicines, transportation, among others. Therefore, new effective and adjuvant therapies should be investigated and discussed in order to contribute to the end of this pandemic.

Propolis is a resinous substance collected and processed by *Apis mellifera* bees from plant parts, buds and exudates

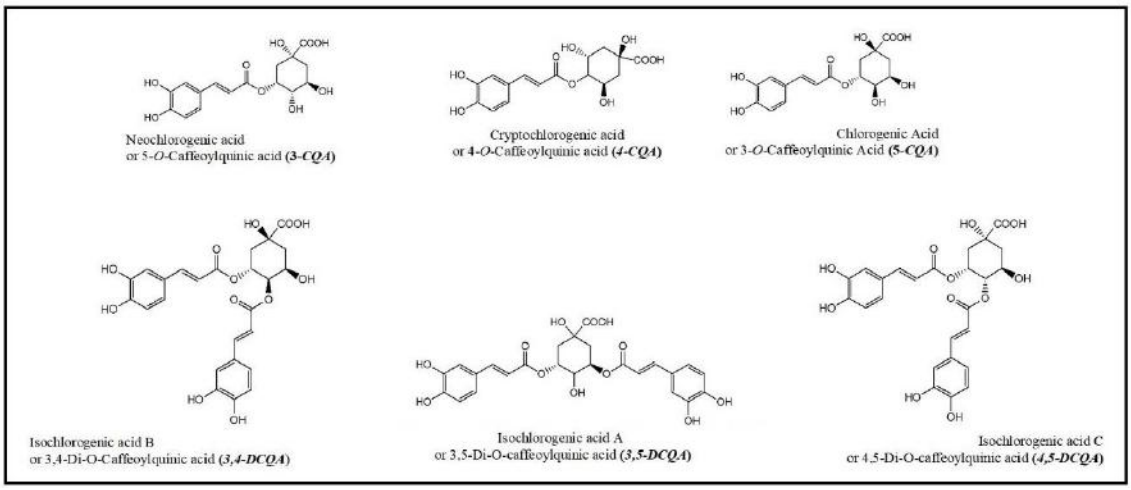
(Veiga et al, 2017). The chemical composition of propolis depends on several factors, such as the different types of plant sources used by bees during collection, the geographic origin of the plants, and the time of year when they are produced, but it is known that phenolic compounds represent the main bioactive constituents of propolis (Machado et al, 2012). The Brazilian green propolis in particular, presents as its main botanical source *Baccharis dracunculifolia* DC (Asteraceae) native to southeastern Brazil (Lupatini et al., 2016).

Besides exhibiting several important pharmacological activities, such as antioxidant (Veiga et al, 2017) anti-inflammatory, antibacterial (Lupatini et al., 2016), antiviral (Zulhendri et al., 2021) and antifungal (Bezerra et al., 2020), the employment of green propolis extract has been investigated previously in patients with COVID-19 (Ali; Kunugi, 2021; Refaat et al., 2021), showing that its addition to standard care procedures brought clinical benefits to hospitalized patients, especially evidenced by reduced hospital length of stay (Silveira et al., 2021).

The chemical marker of green propolis, Artepillin C, was detected by Kimoto (Kimoto et al., 1998) and it is present only in this type of propolis. Artepillin C showed significant results against SARS-CoV-2, as it selectively inhibits PAK1 (RAC/CDC42-activated kinase 1) kinase (Berretta et al., 2020), which when abnormally activated, can contribute with the development of various diseases, such as cancer, inflammation, parasitosis, and viral infections such as HIV and COVID-19 (De Oliveira; Bastos, 2011).

Many other active substances have been detected in Brazilian green propolis, the flavonoids kaempferide and kaempferol, aromatic acids and esters, terpenoids, amino acids, polysaccharides, hydrocarbons, fatty acids, steroids, caffeic acid and caffeoylquinic acids (Marcucci, 1995). The caffeoylquinic acids (Figure 1) are a wide class of secondary metabolites found especially in green propolis, medicinal plants and in the coffee.

Figure 1 – Chemical structure of caffeoylquinic acids from Brazilian Green Propolis.



Souce: Authors.

The first representative, **5-CQA**, was identified in 1920 by Karl Freudenberg (Freudenberg, 1920). In recent years, several naturally occurring caffeoylquinic acids have been isolated and structurally elucidated. These acids stand out for their large range of pharmacological activities, like antioxidant, antibacterial, antiparasitic, neuroprotective, anti-inflammatory, anticancer, antiviral, and antidiabetic effects (De Oliveira; Bastos, 2011).

Considering the relevant studies already published about the potent antiviral activity of caffeoylquinic acids (Ding et al., 2017; Li; et al., 2005; Mahmood et al., 1993; Urushisaki et al., 2011), this study aimed to design the structures of **3-CQA**, **4-CQA**, **5-CQA**, **3,5-DCQA**, **3,4-DCQA** and **4,5-DCQA** in order to investigate their physicochemical, pharmacokinetics and

conformational properties, and also their inhibitory potential against the major protease of SARS-CoV-2, the viral 3-chymotrypsin-like cysteine protease (M^{pro} or $3Cl^{pro}$), a cysteine protease present in β-coronaviruses and has been previously studied as a molecular target for the design and discovery of antivirals for the treatment of SARS (Malone et al., 2022), as a possible approach in combating COVID-19.

2. Methodology

2.1 Computer-aided studies

The three-dimensional models designed of each compound were subjected to geometric optimization by molecular mechanics under MMFF94 force field with the aid of Avogadro 1.2.0v software and, subsequently, the calculation of partial atomic charges by employing Austin Model 1 (AM1) semi-empirical methodology, implemented in MOAPC2016 software was performed. Geometry optimization and electrostatic partial atomic charges (CHELPG) were computed using the ab initio method HF/6-31G (ORCA 5.0.3). The files containing the three-dimensional information of each structure, as well as the electrostatic potential charge information, were input files for visualization using the JMol software (Rocha et al., 2006). The optimized geometry was then used as input file for calculating the energies of the frontier molecular orbitals highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO).

2.2 Theoretical prediction of ADME/T, Physicochemical and Druglikeness parameters

To evaluate the pharmacokinetic properties of the designed compounds, the 2D structure of the compounds were drawn on Chemdraw Ultra 12.0. Each structure was imported and the structure smiley was entered at the interface of the website (http://swissadme.ch/). The SwissADME drug design study was run and the ADMET properties/parameters were generated (Daina; et al., 2017).

2.3 Molecular docking studies

Molecular docking simulations were preceded by redocking studies between the caffeoylquinic acids with employed Autodock 1.5.6 software. The active state geometry was prepared by removing the co-crystallized ligand N3. The atomic coordinates of SARS-CoV-2 M^{pro} were taken from the X-ray structure (PDB ID 6LU7, resolution 2.16Å). The docking procedure was validated by extracting and the inhibitor N3 from the crystal structure and docking them back into the receptor. A grid box with dimensions 60×60×60 centered at the coordinates X= − 10, Y= 13, and Z= 70 was used to represent active site (Keretsu; et al., 2020). The Lamarckian genetic algorithm (LGA) was used to perform the docking process, generating 10 conformations for each compound. Based on the binding energy and binding interactions with the receptor, a representative binding pose for the ligands was selected.

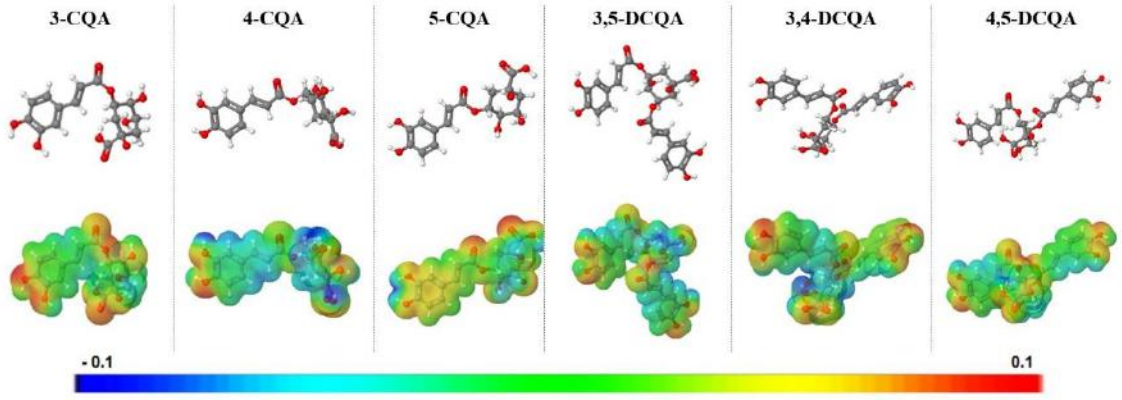
3. Results and Discussion

3.1 Molecular electrostatic potential (MEP)

Conformational analysis plays an important step in drugs development, then many compounds present flexibles molecules, which can adopt different conformations through rotation on single bonds. Despite conformational information can affect not only the physicochemical properties but also the biological activity of the substance (Uthuppan; Soni, 2013). Based on the principle of energy minimization, the structure can be optimized so that the most stable conformations are identified, from the lowest conformational energy. The three-dimensional structure of the caffeoylquinic acids **3-CQA**, **4-CQA**, **5-CQA**, **3,5-DCQA**, **3,4-DCQA** and **4,5-DCQA** are presented in Figure 2, as well as their electrostatic potential maps.

For all compounds, their molecular electrostatic potentials (MEPs) were also calculated on the total density surface of their molecular geometries. Negative electrostatic potential regions are represented in red color (high electronic density) while positive electrostatic potential areas are shown in dark blue color (low electronic density). The MEPs not only provide the positive and negative potential regions but also indicates the shape and size differences among the ligands for possible fitting/docking. The highest negative potentials are seen from the acidic H-atoms, which indicates its potential as favorite moiety for H-bond interactions for docking interactions.

Figure 2 – Electrostatic potential mapped onto the surfaces calculated to the five analogs.



Negative electrostatic potential regions are represented in red color (high electronic density) while positive electrostatic potential areas are shown in dark blue color (low electronic density). Souce: Authors.

In terms of volume, it can be observed that, compounds **4-CCQA**, **5-CQA** and **4,5-DCQA** show similarity. Although compound **4,5-DCQA** is disubstituted, its more stable conformation brings this condition. On the other hand, **3-CQA** presents a great difference in terms of volume, when compared to the other two monosubstituted acids. Regarding electronic density, a low electron density is observed in compound **4-CQA** in the region of the carboxylic acid grouping. The same is not observed in their isomers, **3-CQA** and **5-CQA**, where a high electron density is observed around the carboxylic acid groups. Minor differences in electronic density are observed in the **3,5-DCQA**, **3,4-DCQA**, and **4,5-DCQA** disubstituted acids, where a high electronic density is observed both under the carboxylic acid group and under the hydroxyls of the caffeic fragment, although the spatial volume of the three acids do not present considerable similarities.

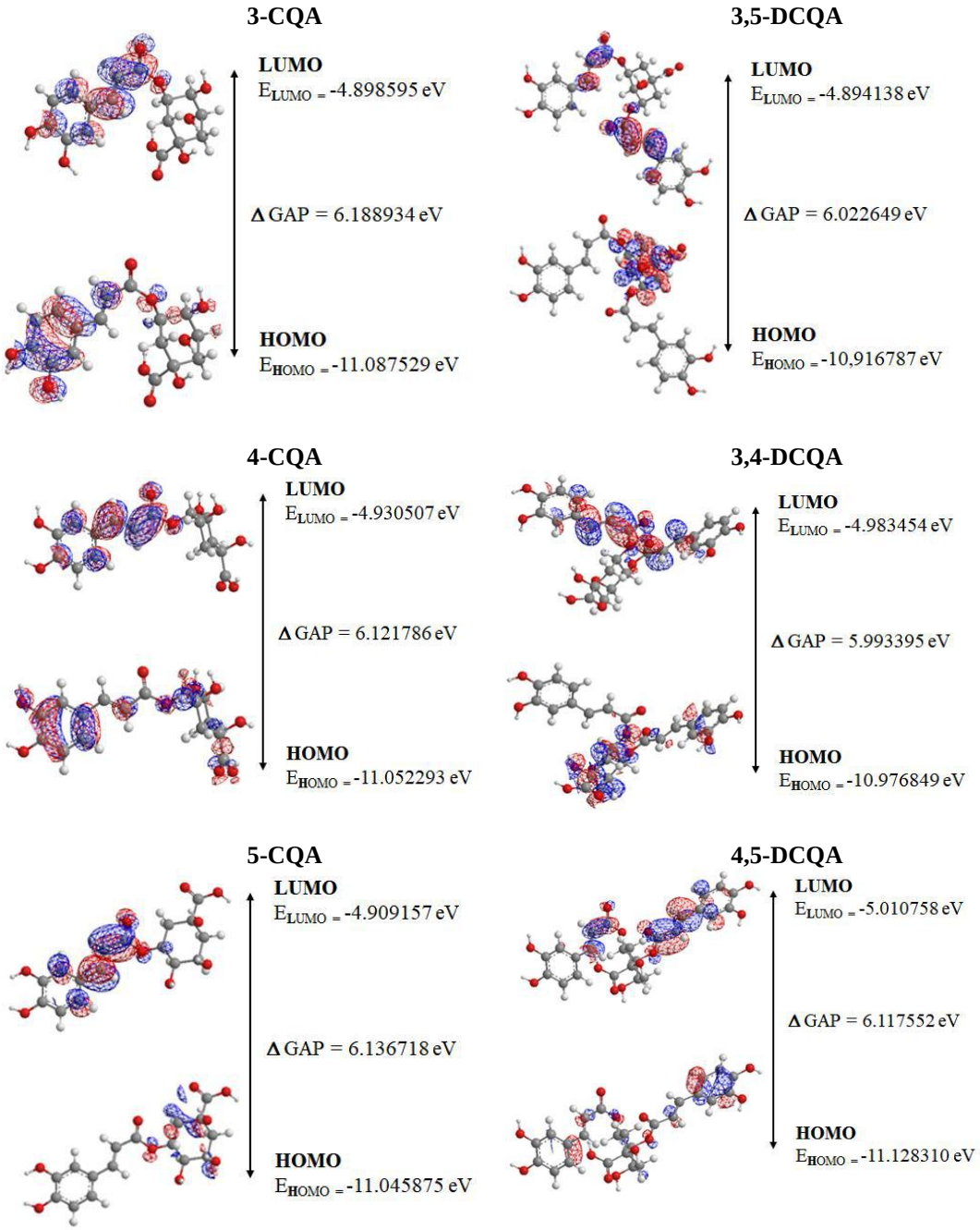
3.2 Molecular orbitals

The energies of the frontier orbitals, that is, the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO), were also calculated. These results are a quantitative descriptive that are widely used and play an important role in the chemistry reaction and in the formation of several complexes. The energy of HOMO is directly related to the potential ionization of the compound and the ability of the molecule to create nucleophiles. The energy of LUMO is directly related to electronic affinity, characterized by the susceptibility of the compound to nucleophiles in relation to them. The difference between the energies of the HOMO-LUMO orbitals, called GAP, is an important indicator of molecular stability.

Molecules with a low GAP value are generally reactive, while molecules with a high GAP value indicate high stability and low possibility to react with anthers compounds (Zhang; Musgrave, 2007). The Figure 3 presents the calculated values for the HOMO and LUMO orbitals of compounds **3-CQA**, **4-CQA**, **5-CQA**, **3,5-DCQA**, **3,4-DCQA** and **4,5-DCQA** as well as the GAP value. The electronic density of frontier orbitals is a useful way for the detailed characterization of donor-

acceptor interactions and the most of chemistry reactions occur at the location of the highest electronic density in the frontier orbitals.

Figure 3 – Frontier orbitals calculated for caffeoylquinic acids from Brazilian green propolis and their GAP values.



The GAP calculation was performed from the difference of the energy value of the LUMO orbital by the energy of the HOMO orbital. Souce: Authors.

Considering that the higher the energy of HOMO, the greater the electron-donor capacity and that the lower the energy of LUMO, the lower the resistance to accept electrons, we can define that the compound **5-CQA**, which has the highest GAP value may have high molecular stability. Compound **3,4-DCQA**, which has the lowest GAP value, can be considered the least stable compound. Thus, it is possible to predict that biological activity increases in the following sequence **5-CQA**<**3-CQA**<**4-CQA**<**4,5-DCQA**<**3,5-DCQA**<**3,4-DCQA**, respectively, corroborating the pharmacokinetic results obtained.

3.3 ADME/T, Physicochemical and Druglikeness parameters ADME/T parameters

The ADMET calculations were performed by comparison with similar substances, and the pharmacokinetic parameters were calculated from the SwissADME platform. In the pharmacokinetic studies we evaluated the intestinal absorption and the potential of the substance to cross or not the blood-brain barrier. The Table 1 presents the results obtained for the compounds **3-CQA**, **4-CQA**, **5-CQA**, **3,5-DCQA**, **3,4-DCQA** and **4,5-DCQA** from Brazilian green propolis.

Table 1 – ADME/T parameters of caffeoylquinic acids from Brazilian green propolis.

Compound	PAINs	BRENK	BBB	GI	Analyzed enzymes of the CYP450 complex				
					CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4
3-CQA	1	2	No	Low	No	No	No	No	No
4-CQA	1	2	No	Low	No	No	No	No	No
5-CQA	1	2	No	Low	No	No	No	No	No
3,5-DCQA	1	3	No	Low	No	No	No	No	No
3,4-DCQA	1	3	No	Low	No	No	No	No	No
4,5-DCQA	1	3	No	Low	No	No	No	No	No

PAINS: **P**an **A**ssay **IN**terference compound**S**; BRENK: Filter developed by Ruth Brenks; GI: intestinal absorption; BBB: blo barrier; CYP: Cytochrome P450. Souce: Authors.

All compounds evaluated have been demonstrated no potential ability to cross the BBB, indicating that these compounds don't have probability of any action in the central nervous system (CNS). As for GI, also the compounds **3-CQA**, **4-CQA**, **5-CQA**, **3,5-DCQA**, **3,4-DCQA** and **4,5-DCQA** showed low potential, with is in concordance with the others parameters here presented and are attributable to the low water/octanol partition coefficient (LogP) value obtained for these compounds. The LogP is applied in medicinal to understand the potential behavior of active compounds in the human system. Although the substances present low gastrointestinal absorption potential, the parameters analyzed in this study refer only to conditions related to a possible oral administration, and it is possible to improve through techniques such as physical and chemical modifications of drug, latentiation or nanotechnology. On the other hand, this may be a facilitator for the development of parenteral therapies, as water solubilization is favorable.

Regarding of the metabolism results, the following criteria were used: whether the substances undergo phase 1 metabolism, and whether they inhibit any enzyme of the CYP complex. Substances that inhibit two or more CYPs, in particular CYP3A4 and CYP2C9, may interfere in the metabolism of a large number of drugs and other substances, and may contribute to increasing their toxicity. For substances that inhibit only one CYP, there may be reduce the number of drugs that have a pharmacokinetic interaction with this complex. Non-inhibitory and non-inducing CYP substances are considered ideal substances, because they do not interfere with the metabolism of other drugs (Dolabela et al., 2018). Considering this information, any compound has been demonstrated potential for inhibition of any of the isoenzymes of the CYP450 complex studied, suggesting that these compounds have no potential to inhibit the metabolism of other active substances. one of the parameters indicatives of low toxicity.

Furthermore, the results indicate a warning for PAINS, an index that shows the tendency of these compounds to react non-specifically with numerous biological targets, suggesting a high possibility of selectivity. The results show a warning for all compounds analyzed regarding the presence of the catechol fragment, a structure considered biologically unstable and that can easily undergo redox reactions, resulting in benzoquinones (Baell; Holloway, 2010; Verheij, 2006). However, the catechol group has already shown promising experimental results in the treatment of viral diseases and cancer, besides being present in several naturally occurring flavonoids, such as quercetin. The quercetin was already studied against COVID-19 through a

computational study, demonstrating its ability to inhibit key enzymes of the SARS-Cov-2. The proven role of zinc in suppressing RdRp, leading to inhibition of viral replication, suggests its therapeutic value against SARS-CoV-2 infection. The mechanisms underlying the expression of candidate miRNA genes in the infection process and the roles of quercetin in their regulation need to be evaluated using *in vitro* and *in vivo* systems (Saakre; et al., 2021).

Brenk is another druglikeness filter that could be applied to study these molecules. It is able to compare the studied structure for its similarities with more than 105 fragments that are potentially toxic, chemically reactive, metabolically unstable or exhibit poor pharmacokinetic properties (Brenk et al., 2008; Daina; et al., 2017). Within this parameter, the monosubstituted caffeoylquinic acids, **3-CQA**, **4-CQA** and **5-CQA** analyzed showed two alerts, for the presence of catechol fragment and Michael acceptor. The disubstituted acids, **3,5-DCQA**, **3,4-DCQA** and **4,5-DCQA**, presented an additional warning for the presence of more than two ester groups. Regarding the Michael acceptor the indicated fragment is the α,β-insaturated carbonyl outside the ring, which is usually predominant in many natural compounds (Mulliner; WONDROUSCH; SCHÜÜRMANN, 2011). Although Michael acceptors exhibit a potent and broad spectrum of bioactivity, as electrophilic agents, Michael acceptors can form covalent bonds with nucleophilic sites of proteins and the DNA of biological organisms, resulting in diseases such as carcinogenicity, allergic contact dermatitis, and excessive toxicity (Jackson et al., 2017). Some studies show that Michael acceptor molecules, so far characterized by acting through a covalent mechanism, showed promising results in inhibitory interactions with amino acid residues of SARS-CoV-2 M^{pro}. Moliner et al (Arafet et al., 2021), demonstrate that α,β-insaturated ketones, act as an electrophilic "warhead" group responsible for covalent modification of M^{pro}.

The presence of more than two ester groups in compounds **3,5-DCQA**, **3,4-DCQA** and **4,5-DCQA** indicates a possible unfavorable pharmacokinetic activity (Brenk et al., 2008). In the case of caffeoylquinic acids, it has been demonstrated through *in vivo* studies by Rechner that the absorption of this class of compounds in the proximal gastrointestinal tract is lower than that of free hydroxycinnamic acids. The human gastrointestinal mucosa does not have esterases capable of hydrolyzing esterified acids, which significantly reduces the efficiency of absorption of caffeoylquinic acids in the gastric lumen and small intestine (Rechner et al., 2001).

3.4 Physicochemical parameters and Druglikeness parameters

The physicochemical properties of compounds with potential biological activity play an important role in the preliminary understanding of their pharmacokinetic properties, because before interacting with its target, the substance is involving in several biological processes that depend directly on these properties. The results obtained are shown in Table 2.

Table 2 – Physicochemical parameters of caffeoylquinic acids from Brazilian green propolis.

Compound	MF	MW (Da)	MR	*i*LOGP	TPSA (Å^2)	HBAs	HBDs	RB
3-CQA	$C_{16}H_{18}O_9$	354.31	83.50	-0.39	164.75	9	6	5
4-CQA	$C_{16}H_{18}O_9$	354.31	83.50	-0,53	164.75	9	6	5
5-CQA	$C_{16}H_{18}O_9$	354.31	83.50	-0,46	164.75	9	6	5
3,5-DCQA	$C_{25}H_{24}O_{12}$	516.45	126.90	0.79	211.28	12	7	9
3,4-DCQA	$C_{25}H_{24}O_{12}$	516.45	126.90	0.80	211.28	12	7	9
4,5-DCQA	$C_{25}H_{24}O_{12}$	516.45	126.90	0.63	211.28	12	7	9

Physicochemical properties calculated on SwissADME: MW: molecular weight; HBDs: hydrogen bonding donor; HBAs: hydrogen bonding acceptor; *i*LogP: octanol/water partition coefficient; TPSA: total polar surface area; RB: rotatable bonds. Souce: Authors.

The number of HBDs and HBAs is a key molecular descriptor for predicting the oral bioavailability of small drug candidates. It is generally assumed that hydrogen bond donors and acceptors impact passive diffusion across cell membranes, a

key event during drug absorption and distribution (Coimbra et al., 2021).

The TPSA, makes use of functional group contributions based on a large database of structures, is a convenient measure of polar surface area that avoids the need to calculate the 3D structure of the ligand or decide what the relevant biological conformation or conformations are (Prasanna; Doerksen, 2009), and is commonly used in medicinal chemistry for optimizing a drug's ability to permeate cells. Molecules with a TPSA greater than 140Å tend to be poor at permeating cell membranes (Pajouhesh; Lenz, 2005). For molecules to penetrate the blood-brain barrier, a TPSA less than 90Å is usually required (Clark, 1999).

RT is a measure of molecular flexibility and is important in determining the oral bioavailability of drugs. Only 4% of the molecules in the human metabolite data set have no rotatable bonds, while 32% have 1-10 rotatable bonds and 47% of the molecules have rotatable bonds in the range 36-50 (Khanna; Ranganathan, 2009).

Drug candidates are analyzed according to the LogP, considering analog optimization, because, lipophilicity is an important physicochemical parameter that contributes to the absorption, distribution, metabolism, excretion and toxicity of a drug. This in turn affects the solubility and permeability of a drug and contributes to its potency and selectivity (Leo; et al., 1971).

About MW the pharmaceutical and bioactive properties are currently unclear (Chae; et al., 2005). Overall, for oral drugs approved since 1983, there has been an increase in molecular mass, the median patented compound has a MW of 450 Da, while newer oral drugs, discovered since 1990, have a median MW of 432 Da (Leeson; SpringthorpE, 2007).

There are several types of filters used for the development of new drugs, which relate the physicochemical properties and possible pharmacokinetic characteristics of these substances. In this study we compare the results obtained for the filters of Lipinski, Ghose, Veber, Egan and Muegge (Table 3).

The application of guidelines linked to the concept of "drug similarity", such as Lipinks' rule (Lipinski et al., 2001), has achieved wide acceptance as an approach to reduce attrition in the discovery and development of active substances. In this regard Lipinks proposed the "Rule of 5", which could predict the probability of a small molecule being active when administered orally. This rule states that, new compounds should have molecular masses less than 500 Da (MM < 500), LogP less than 5 (LogP < 5), up to 10 hydrogen acceptors (HBA ≤ 10), and 5 or fewer hydrogen donors (HBD ≤ 5).

Another quantitative characterization based on profiles of physicochemical properties, such as LogP, molar refractivity, molecular weight, and number of atoms, as well as a qualitative characterization based on the occurrence of important functional groups and substructures, was developed by Ghose and collaborators (Ghose; et al., 1999). Based on this study, one should consider LogP between -0.4 and 5.6, with an average value of 2.52. For molecular weight, the qualification range is between 160 and 480, with a mean value of 357. For molar refractivity, the qualification range is between 40 and 130, with an average value of 97. For total number of atoms, the qualification range is between 20 and 70, with an average value of 48.

Veber et al (Veber et al., 2002) also analyzed the physicochemical parameters of approximately 1100 drug candidates. The study conducted at GlaxoSmithKline showed that reduced molecular flexibility, as measured by the number of rotatory bonds and low total polar surface area (TPSA) area or total hydrogen bond count (sum of donors and acceptors) are considered important predictors of good oral bioavailability, independently of molecular weight. As such, Veber demonstrated that the presence of ≤10 rotatable bonds and TPSA ≤140 are the parameters that relate to good bioavailability of drugs in the oral phase.

The descriptors employed by Egan and collaborators (Egan; et al., 2000) included in their studies, TPSA and LogP, to successful oral absorption of new drugs, based on the physical processes involved in membrane permeability. Model validation showed that a good rate of successful predictions (up to 92%) have LogP ≤5.88 and TPSA ≤131.6, and molecular weight of the

compounds showed no relevance.

The results of Mueggen's filter were developed through a statistically significant overlap between drugs with recognized biological activity, such as compounds similar to drugs already in active use (Muegge; et al., 2001). Mueggen defined that the appropriate parameters for good oral bioavailability, are molecular weight between 200 and ≤ 600 Da, LogP between -2 and ≤5, TPSA ≤150, number of rings ≤7, number of carbons >4, heteroatoms >1, rotatable bonds ≤15, HBAs≤10 and HBDs ≤5.

Table 3 – Druglikeness parameters of caffeoylquinic acids Brazilian green propolis.

Compound	Lipinski	Ghose	Veber	Egan	Muegge	ABS (%)
3-CQA	1	1	1	1	2	52.16
4-CQA	1	1	1	1	2	52.16
5-CQA	1	1	1	1	2	52.16
3,5-DCQA	3	1	1	1	3	36.10
3,4-DCQA	3	1	1	1	3	36.10
4,5-DCQA	3	1	1	1	3	36.10

%ABS was expressed by the equation %ABS = 109 – (0.345 × TPSA) = high ABS 100–67%, medium ABS 66–33%, and low ABS 32–0%. Souce: Authors.

As diasteroisomers of each other, the caffeoylquinic acids **3-CQA**, **4-CQA** and **5-CQA** and the **3,5-DCQA**, **3,4-DCQA** and **4,5-DCQA** have different physicochemical properties closely related to their chemical structure (Stefaniak et al., 2005). For the studied compounds, only differences in their calculated LogP values were observed. This occurs because the differences in the relative hydrophilicities-lipophilicities of the positioned groups promotes the interactions with the solvents (Parker, 1978).

Regarding Lipinski parameters, the caffeoylquinic acids, **3-CQA**, **4-CQA** and 5-**CQA**, showed only one violation with respect to HBSs>5. The violation presented by compounds **3-CQA**, **4-CQA** and **5-CQA** considering the Ghose filter is related to the values of LogP<-0.4. For both the Veber and Ergan settings the violation was demonstrated in relation to the value of TPSA>140Å and TPSA>131.6Å, respectively. The Muggen filter was violated for the HBDs > 5, and the TPSA value >150Å. For the caffeoylquinic acids, **3,5-DCQA**, **3,4-DCQA** and **4,5-DCQA**, values of molecular mass >500 Da, HBAs>10 and HBDs>5, violate three parameters of the Lipinski Rule. The Ghose filter detected only one violation, with respect to the molecular mass value, which is >480 Da. For both the Ergan and Veber filters, the violation was demonstrated with respect to the TPSA>140Å and TPSA>131.6Å values, respectively. In the Muggen definitions, the three isomers do not agree with respect to the values of TPSA>150, HBAs>10 and HBDs>5. Finally, through the equation developed by Zhao et al (Zhao et al., 2002), which relates the values of LogP, molecular polar surface area, number of hydrogen bond acceptors and donors, and Abraham descriptors, the overall potential on absorption of these substances was calculated, and classified in the medium absorption ranking, with values between 36.10 and 52.15% absorption. Konishi (Konishi; et al., 2006) and Lafay (Lafay et al., 2006) has demonstrated through animal experiments with in situ gastric infusion of **5-CQA** that absorption initiates in the stomach, where a small portion is absorbed intact, and in its hydrolyzed form as caffeic, isoferulic acid and ferulic acid in the small intestine. In a study with ileostomized humans, Hollman (Olthof; et al., 2001) was able to show that the absorption of monocaffeoylquinic acids can be as high as 50%, which corroborates with the results calculated in this study.

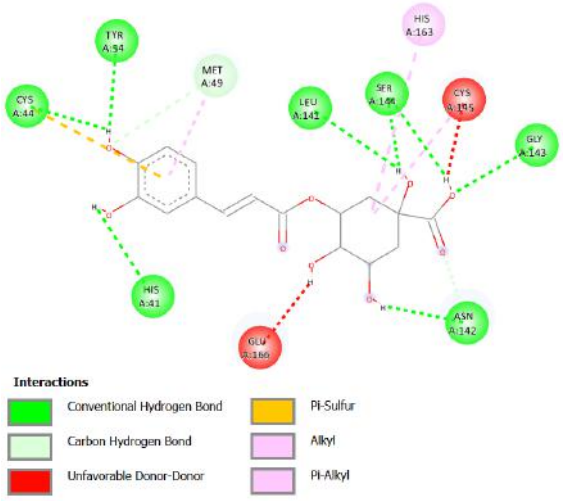
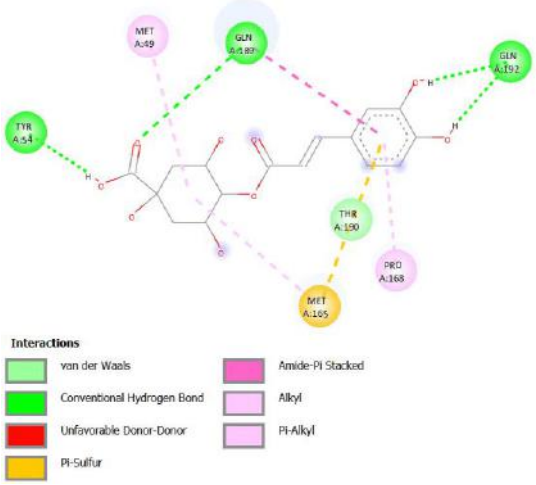
Consistent results by Mateos et al demonstrated that, after absorption, free monoesterified caffeoylquinic acids can undergo phase II enzymatic action in the intestinal mucosa and subsequently in the liver or other tissues, forming sulfated, glycuronated, and/or methylated conjugates by the action of sulfo-transferases, UDP-glycotransferases, and catechol-*O*-

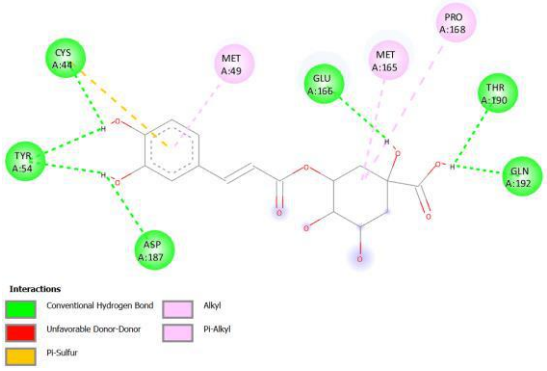
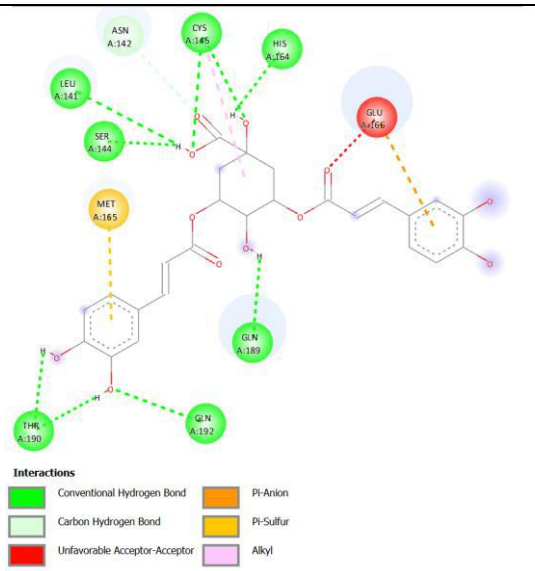
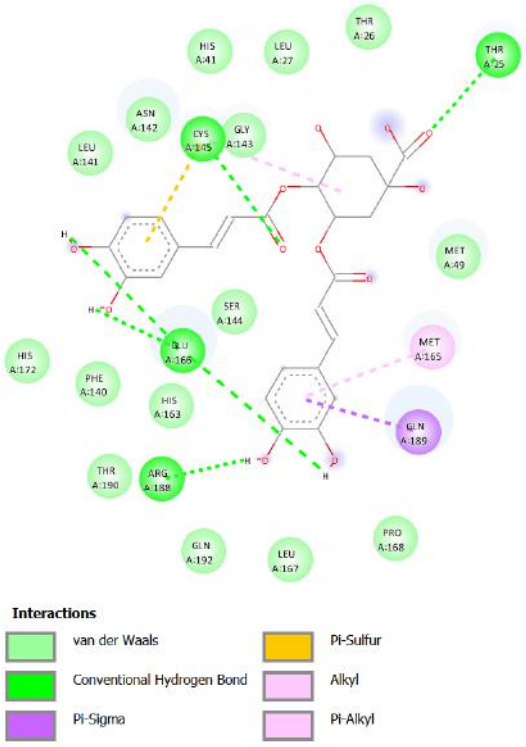
methyltransferases, respectively. These conjugates represent a metabolic detoxification process common to many xenobiotics, which facilitates biliary and urinary elimination by increasing the hydrophilicity of the compounds (Mateos; et al., 2006).

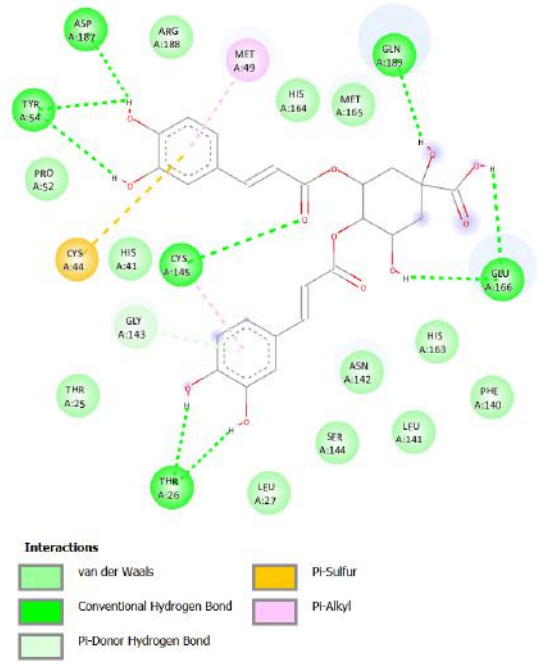
3.5 Molecular docking studies

In the present study, we found the potential interaction of caffeoylquinic acids, present in green Brazilian propolis, with an important pharmacological target for SARS-CoV-2 mediated infection in humans. All the compounds analyzed showed potential for various types of interaction with several amino acids present in the active site of the macromolecule, however, it is considered that the greater the number of hydrogen interactions between the ligand and the target protein, the greater the stability of the ligand/receptor complex. The best conformers were filtered using binding energy, which is related considering the number of hydrogen bonds and presented in Table 4.

Table 4 – Interactions of COVID-19 Main Protease (6LU7) amino acid residues with ligands at receptor sites.

Complex	Binding affinity, ΔG (Kcal/mol)	Amino acids involved and distance (Å) in hydrogen binding interactions	Interaction with 6LU7 active site
3CQA /6LU7	-6.44	MET49 (3.45), HIS41 (2.45), CYS44 (2.53), TYR54 (2.25), LEU141 (2.85), ASN142 (3.37), **GLY143 (2.98)**, SER144 (2.01), SER144 (3.03)	
4CQA /6LU7	-6.11	TYR54 (1.78), **GLN189 (3.24),** GLN192 (2.10), GLN192 (2.66)	

5CQA /6LU7	-6.48	CYS44 (2.29), TYR54 (2.44), TYR54 (2.21), **GLU166 (2.06)**, ASP187 (2.06), **THR190 (2.57)**, GLN192 (2.94)	
3,5-DCQA /6LU7	-6.26	LEU141 (2.73), SER144 (2.17), ASN142 (3.12), CYS145 (3.21), CYS145 (2.95), HIS164 (2.20), **GLN189 (2.28)**, **THR190 (1.83)**, **THR190 (1.99)**, GLN192	
3,4-DCQA /6LU7	-7.01	THR25 (3.34), CYS145 (2.66), **GLU166 (2.04)**, **GLU166 (1.85)**, **GLU166 (1.89)**, ARG188 (2.08)	

4,5-DCQA /6LU7	-7.40	THR26 (2.15), THR26 (1.96), TYR54 (2.90), TYR54 (2.43), GLY143 (3.25), CYS145 (3.47), **GLU166 (2.31), GLU166 (2.15),** ASP187 (2.02), **GLN189 (2.19)**	

Souce: Authors.

The six caffeoylquinic acids and their binding energy, corresponding 2D structure, the interacting residues, and the hydrogen bonding were calculated, in order to compare their potential interactions with the Michael acceptor, N3, proposed by by Yang et al 59 which shows promising inhibitory activity against the target SARS-CoV-2 M^{pro}. The interaction significant binding residues of M^{pro}, which shows promising inhibitory activity against the target SARS-CoV-2 M^{pro}. The interaction significant binding residues of M^{pro} with the compound, native inhibitor, N3 (-6.30 kcal/mol) are, PHE140, GLY143, HIS163, GLU166, GLU 166, GLN189 and THR190.

The main interactions found for the complex **3-CQA/6LU7** (-6.44 Kcal/mol) were with MET49 and GLY143, for the complex **4-CQA/6LU7** (-6.11 Kcal/mol) was GLN189, and finally to the complex **5-CQA/6LU7** (-6.48 Kcal/mol) were GLU166 and THR190. The docking main interactions results for the disubstituted caffeoylquinic acids were for the **3,5-DCQA/6LU7** (-6.26 Kcal/mol) ASN142, GLN189, THR190 and THR190, for the **3,4-DCQA/6LU7** (-7.01 Kcal/mol) the residues showing main hydrogen interactions were GLU166, GLU166, GLU166 and finally the **4,5-DCQA/6LU7** (-7.40 Kcal/mol Kcal/mol) complex interacted with the main residues, GLY143, GLU166, GLU166 and GLN189.

Furthermore, the compounds ***3-CQA*** and **3,5-DCQA** showed unfavorable interactions with the CYS145, GLU166 and GLU166 respectively. Unfavorable bonds affect the activity stability of the drug. The formation of any kind of unfavorable bond between/in protein-ligand complex reduces the stability of the complex as these types of bonds indicate a force of repulsion occurring between two molecules and an atom.

Hydrophobic interactions were also achieved by the studied complexes. The Table 6 presents the type of bond found for each amino acid of the M^{pro} protein.

Table 5 – Hydrofobic interations with 6LU7 active site.

Complex	Van der Waals	Pi-Sulfur	Alkyl	Pi-Alkyl	Amide Pi Stacked	Pi-Sigma
3CQA/ 6LU7	MET49, PRO52, **PHE140,** HIS164, ASP187	CYS44	CYS145	MET49, **HIS163**	---	---
4CQA/ 6LU7	HIS41, PRO52, HIS164 **GLU166,** LEU167, ASP187, ARG188, **THR190,** ALA191	MET165	MET49, MET165	PRO168	**GLN189**	---
5CQA/ 6LU7	HIS41, PRO52, LEU167, ARG188, **GLN189**	CYS44	MET165, PRO168	MET49	---	---
3,5-DCQA / 6LU7	HIS41, **PHE140, GLY143,** PRO168, ARG188, ALA191	MET165, **GLU166**	CYS145	---	---	---
3,4-DCQA / 6LU7	LEU25, THR27, HIS41, **PHE140,** LEU141, ASN142, MET49, HIS172, **HIS163,** SER144, **THR190,** GLN192, LEU167, PRO168	CYS145	CYS145	MET165	---	**GLN189**
4,5-DCQA / 6LU7	THR25, HIS41, PRO52, **PHE140,** LEU141, ASN142, SER144, **HIS163,** HIS164, MET165	CYS44	---	MET49, CYS145	---	---

Souce: Authors.

The significant residues that interacted through forces classified as hydrophobic were the PHE14O and HIS163 to the complex 3-CQA/6LU7, GLU166, GLN189 e THR190 to the complex 4-CQA/6LU7, GLN 189 to the complex 5-CQA/6LU7, PHE140, GLY143 and GLU166 to the complex 3,5-DCQA/6LU7, PHE140, HIS163, GLN189 and GLN189 to the complex 3,4-DCQA/6LU7, and PHE140 and GLY143 to the complex 4,5-DCQA/6LU7.

Considering just the main amino acids involved in the inhibition of the M^{pro} protein, and all the types of interactions the compounds **3-CQA**, **4-CQA**, **5-CQA**, **3,5-DCQA**, **3,4-DCQA** and **4,5-DCQA** has the potential to bind 3, 4, 3, 6, 7 and 5 to the receptor, respectively.

The mechanism of M^{pro} inhibition by Michael acceptor compounds has also been reported by Klein (Klein et al, 2020), firstly the formation of a reversible complex with the protease occurs, which then undergoes a chemical step (nucleophilic attack by Cys) leading to the formation of a stable covalent bond.

Analyzing all the parameters obtained in this study, we consider that compound **5-CQA**, **3,4-DCQA** and **4,5-DCQA** are particularly promising for future studies to be conducted, since these compounds showed potential for interaction with important amino acids of the active site of M^{pro}. Despite the low LogP value, this parameter may not be relevant for the development of parenteral therapies. However, further studies conducted *in vitro* and *in vivo* are essential, because the flexibility of the protein is not considered in commonly applied docking protocols, which play a crucial role in the activity, and furthermore, the compound may have an unexpected mode of action or a different binding target.

4. Conclusion

Plant-derived compounds have therapeutic potential against various diseases, including viral diseases. Thus, computer-based identification of potential phytochemicals inhibiting drug targets of SARS-CoV-2 are currently highly

important. Natural compounds have lower toxicity and side effects, making them an appropriate candidate for the development of new therapies. In the present molecular docking study, six major caffeoylquinic acids mainly present in Brazilian green propolis were analyzed and showed high significant binding potential at the active sites of M^{pro} protein. Especially the compounds compound **5-CQA**, **3,4-DCQA** and **4,5-DCQA** which showed a large number of interactions, and showed no potential for unfavorable interactions. The physicochemical, pharmacokinetic, conformational and druglikess properties indicate some disadvantage for oral use of these substances, especially considering the LogP values achieved. Overall, we conclude that caffeoylquinic acids may represent an interesting class for the development of therapies targeting SARS-CoV-2, besides showing to be promising raw materials for the proposition of molecular modifications to obtain even more potent compounds.

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