Cameroonian medicinal plants as potential candidates of SARS-CoV-2 inhibitors

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ABSTRACT Coronavirus disease 2019 (COVID-19) is an ongoing pandemic instigated by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) which changed the daily train of the world’s population and cause several dead. Despite the significant efforts made in developing vaccines and therapeutic drugs, there is currently no available effective treatment against this new coronavirus infection, hence the need to continue research which is aimed at limiting the progression of this virus. The present study which has as objective to carry out in silico studies on the metabolites of some Cameroonian medicinal plants of the Asteraceae family with a view to propose potential molecules to fight against COVID-19. The selected plants are commonly used to treat respiratory infectious diseases, and for this reason they may contain some constituents which could exhibit an antiviral activity against SARS-CoV-2. In this work, a set of 74 naturally occurring compounds are computed with SARS-CoV-2 main protease protein (PDB ID: 6lu7) and spike protein (PDB ID: 6m0j) for their affinity and stability using binding energy analysis and molecular docking. Chrysoeriol-7-O-β-D-glucuronopyranoside (compound 16) has showed promising results including excellent Absorption, Distribution, Metabolism and Excretion (ADME) parameters as well as insignificant toxicity. Finally, the stability of this compound is complex with the two proteins validated through molecular dynamics (MD) simulation, they displayed stable trajectory and molecular properties with consistent interaction profile in molecular dynamics simulations. These findings call for further in vitro and in vivo challenges of phytoconstituents against the COVID-19 as a potential agent to fight the spread of this dramatic pandemic.

1. Introduction

Since December 2019, the pandemic of coronavirus has emerged in Wuhan, and was spreading throughout the rest of the world (World Health Organization, 2019). Also called nCoV-2 it is a virus transmitted between people through respiratory droplets and contact routes (Chan et al., 2020; Liu et al., 2020). It has been noted that, when the droplet particles are >5-10 μm in diameter they are referred to as respiratory droplets, and when they are <5 μm in diameter, they referred to a droplet nucleus (World Health Organization, 2014). The World Health Organization (WHO), on 11 February 2020 announced a new name for the epidemic disease caused by coronavirus, at the same time, International Committee on Taxonomy (ICTV) of Viruses has renamed the provisionally named 2019-nCoV as Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) (Gorbalenya et al., 2020).

COVID-19 is caused by novel coronavirus 2, which belong to a family of viruses that contain a relatively large single-stranded, positive-sense RNA genome of around 27-32 kilobases in length, coding four structural proteins including spike (S), Nucleocapsid (N), Membrane (M) and Envelope (E) (Fehr & Perlman, 2015; Pyrc et al., 2006). The ICTV has divided the family of coronavirus into four genera, alphacoronavirus, betacoronavirus, gammacoronavirus, and deltacoronavirus. The coronaviruses responsible of SARS-CoV-2, SARS-CoV and MERS-CoV belong to the betacoronavirus genus (Zhou et al., 2020). The viral binding to the human receptor cells occurs via the interaction of the spike protein of SARS-CoV-2, through its receptor-binding domain, with the human Angiotensin Converting Enzyme 2 (ACE-2) which is expressed in the respiratory tract, and favors entry of viral genome into the target cell (Zhou et al., 2020). After the penetration into the host cell, β-coronaviruses generate two large polyproteins (pp1a and...
pp1ab), also known as replicase polyproteins. RNA-dependent RNA polymerase (RdRp) and non-structural polyproteins (NSPs) are the 16 proteins released after proteolytically cleavage of replicase polyproteins, by cysteine proteases called 3-chymotrypsin-like protease (3CLpro) also called main protease (Mpro) set by the virus. RdRp and 15 other NSPs combined to form the transcription/replication complex, which is involved in the replication of the RNA + viral genome into RNA- and transcription. The 3CLpro enzyme controls coronavirus replication and it’s vital for its life cycle, so it would be a potential drug target for the case of SARS-CoV-2 inhibitor screening.

Since prehistoric, plants have been used by people for healing diseases. In fact, different parts of plants are used to treat several diseases such as, diarrhea, headache, inflammation, microbial infections and respiratory infections (Kumar et al., 2019). Much efforts has therefore been made in some African countries to find a solution to this pandemic; however, the effectiveness of certain formulations of medicinal plants has still not been approved by all against this new respiratory infection. In front of this upsurge, the search for new molecules, more active, less expensive and without too marked side effects is today an emergency for health workers, governments and the general public to prevent this spread.

Ethnobotanical surveys were carried out within healers, and the plants used to prepare recipes against respiratory tract diseases were collected from the Cameroonian flora (Mpondo et al., 2017). They are distributed to our knowledge into 15 families, the most represented of which is that of Asteraceae. These species are rich in various metabolites and their barks are the most demanded organs. The method of preparation and administration of the said recipes is based on decoction with water which will result to drinking and taken two to three times a day (Mpondo et al., 2017).

As part of the promotion of Cameroonian medicinal plants, various bioactive compounds have been isolated for their anticancer, antimicrobial, antiplasmodial activities. These results show that plants would be an important reservoir of bioactive molecules.

As nCoV-2 is a newly identified pattern, there is no effective drug neutralizing SARS-CoV-2. One expected way to find active molecules against this virus is to explore medicinal plants used in traditional medicine to fight respiratory diseases.

Amongst the plants used by the Cameroonian traditional medicine used to prepare recipes against respiratory tract diseases (cough, dry cough and flu), those of the Asteraceae family are the most represented. This justifies our choice on the plants of this family. This choice was further supported by the strong implication of plants of the Artemisia genus in the treatment of Covid-19 pandemic, particularly in traditional African medicine. Intense reviewing of literature on Conyza sumatrensis (Chai et al., 2008), Artemisia incana (Çetin et al., 2009), Artemisia gorgonum Webb. (Ortet et al., 2010), Artemisia armeniaca (Mojarrab et al., 2011), Artemisia capillaris (Sheu & Tan, 1999), Artemisia ketone (Liu et al., 2002), Artemisia lavandulaeefolia (Liu et al., 2010), Artemisia argyi Levl. et Vant. (Yoshikawa et al., 1997), Artemisia annua (Liu et al., 2002; Shukla et al., 1997), Artemisia afra (Chagonda et al., 1999), Artemisia abyssinica (Tariku et al., 2010) and Artemisia asiatica (Kalemba, 1999) revealed the isolation and identification of several compounds, which, after eliminating of redundancies allowed to enumerate 74 compounds grouped in several classes such as anthocyanidins, tannins, polyphenols and flavonoids with various structural skeletons summarized here (Supplementary Material Table S1) in each species with their chemical structures.

2. Materials and methods

2.1. Construction of potential phytochemical database

Table S1 (Supplementary Material) lists 74 metabolites isolated from 12 plants of the Asteraceae family, identified from an extensive literature search conducted on Cameroonian medicinal plants used in the treatment of respiratory infections. The structures of these 74 plants metabolites, belonging to different classes, were converted into a single database format (SDF), using Discovery Studio 2020 software. Converted to most energy-stable structure, the ligands file was read in AutoDetect 2.1.4 under full protonation mode. After the optimization of the top candidates, we furthermore, conducted molecular docking analysis of the top candidates with SARS-CoV-2 main protease (coded PDB ID: 6lu7) and spike protein (coded PDB ID: 6m0j), which represents potential therapeutic targets for the inhibition of SARS-CoV-2 replication.

2.2. SARS-CoV-2 proteins/protein-domains preparation

According to the fact that proliferation and survival of SARS-CoV-2 relies on the process of replication, it is essential to find a way to impede the progression of virus replication.

The recently resolved 3 D structures of SARS-CoV-2 main protease (PDB code: 6lu7) in complex with a the covalent peptide N3 and the spike protein (PDB code: 6m0j)) in complex with 2-acetamido-2-deoxy-beta-D-glucopyranose were downloaded from the Protein Data Bank (www.rcsb.org). To prepare the selected proteins for molecular docking, the co-crystallized water and small molecules were removed, protein structures were protonated using reduce tool then non-polar hydrogens were removed using Discovery Studio. A grid box with a size of (x = 20, y = 20, z = 20) and center of (x = −10.641, y = 11.847, z = 68.346) was set to cover the N3 binding site in the 6lu7 (Chitta et al., 2021). While a grid box with a size of (x = 24, y = 24, z = 24) and a center of (x = −34.933, y = 8.672, z = 29.036) was set to cover the 2-acetamido-2-deoxy-beta-D-glucopyranose binding site in the 6m0j (Aouidate et al., 2020).

Based on binding free energy calculations using the molecular mechanics with generalized Born and surface area solvation (MM/GBSA) model and solvated interaction energy (SIE) methods, literature studies prompted us to consider antiviral drug Nelfinavir and Remdesivir as references ligands for our studied metabolites against SARS-CoV-2 main
protease and spike protein targets (Cao et al., 2020; Milani et al., 2021; Saqrane et al., 2021; Shannon et al., 2020; Xu et al., 2020).

2.3. Virtual screening of ligand with SARS-CoV-2 proteins/protein domains

Molecular docking is an up-to-date approach for screening suitable therapeutics with a precise drug target of deadly pathogens. This prevailing tool is used to explore the interaction pattern of ligand with their respective enzyme, thereby paving the way for drug discovery (Kitchen et al., 2004; Meng et al., 2011; Aouidate et al., 2018; Ouassaf et al., 2021). The docking analysis (binding affinity) of 74 plants metabolites with two SARS-CoV-2 main proteases (6lu7) and spike protein (6mol) was achieved by using AutoDock Tools 1.5.6 software (Trott & Olson, 2010) in order to evaluate the ligand binding with proteins of SARS-CoV-2 model. Molecules with lowest binding energy (best score) was then analyzed for interaction with the viral proteins in order to find the most probable docking conformation.

2.4. Drug profile analysis of top candidates

Each potential drug for oral administration must meet several basic criteria, such as low production cost, solubility, stability, kinetics of exposure to the tissues within an organism. But they must also comply with scales associated with pharmacological properties of absorption, distribution, metabolism, excretion and toxicity (Miteva et al., 2006) which is based on the rule of 5 formulated by Lipinski (Lipinski et al., 2001). This rule describes a set of criteria, making it possible to estimate the bioavailability of a compound by the oral from its two-dimensional structure. The SwissADME tools (http://www.swissadme.ch) were consequently used to assess physicochemical descriptors, pharmacokinetic properties, lipophilicity and water solubility of the top five metabolites (Minovski et al., 2012).

2.5. Prediction of toxicological properties

Toxicological purpose is the most main considerations in case of the development of new drugs. The toxicity evaluation was carried out on the top molecule’s candidates using an online server (ProTox-II16) which gives predicted values of toxicity, cytotoxicity, mutagenicity, carcinogenicity, and immunotoxicity. Oral toxicity was also expected using the same online server. Some parameters were counted in this study such as Ames toxicity, Human Maximum Tolerated Dose (MTDD), hERG I/II: hERG I and II Inhibitors, Hepatotoxicity (HP), Skin Sensitisation (SS), Minnow toxicity (MT). Chronic Oral Rat Toxicity (LOAEL) and Oral Rat Acute Toxicity (LD50) were also predicted based on the analysis of two-dimensional similarity to compounds with known lethal doses.

2.6. Molecular dynamics simulations

The maximum binding affinity towards SARS-CoV-2 spike protein and SARS-CoV-2 main protease protein was shown by chrysoeriol-7-O-β-D-glucuronopyranoside. These two complexes were further studied by mimicking the real physiological conditions conditions to analyse their stability and dynamics. The molecular dynamics (MD) simulation was carried out using Gromacs-2018.1 packages with amber99sb-ILDN force field (Berendsen et al., 1995). The proteins and their complexes (with minimum binding energy) were solvated in triclinic box with TIP3P water model. The proteins/complexes were neutralized by adding equal number of sodium or chlorine counter ions. The topology of ligands was generated using antechamber in AmberTools19. The energy of systems was minimized using the steepest descent minimization of 50000 steps to remove the weak van der Waals contacts. The systems were then equilibrated for NVT and NPT for 1 ns each. The 100 ns standard MD simulation was carried out for both proteins and their complexes and coordinates were saved for the entire simulation period. The PBC (periodic boundary conditions) corrections were made prior to analysis. Using Gromacs utility, root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), radius of gyration (Rg), solvent accessible surface area (SASA), secondary structures of proteins, and hydrogen bonds were analyzed. MM-PBSA calculation was performed to calculate the binding energy of chrysoeriol-7-O-β-D-glucuronopyranoside with proteins (Kumari et al., 2014).

3. Results and discussion

3.1. Virtual screening of ligand with SARS-CoV-2 proteins domains

To estimate the binding affinity of the 74 ligands, with SARS-CoV-2 main protease (PDB ID: 6lu7) and spike protein (PDB ID: 6mol), molecular docking using AutoDock Tools 1.5.6 software was performed. The results of the docking analysis are described in Table 1. The negative and low value of binding energy obtained demonstrates a favorable conformation between ligands and the SARS-CoV-2 proteins-domain selected.

3.2. Molecular docking simulation

Structural conformation analyzes of the complex constituted of the five-top ligand with main protease protein were carried out to unpick the drug surface hotspot of the target. The ligand binding pattern and interacting residues with their respective positions were also investigated, moreover, the type of interactions and the number of amino acid residues of SARS-CoV-2 interacting with each ligand are highlighted. In this analysis, five most active compounds (12, 15, 16, 46 and 51) from the phytochemical database interacted with the active site amino acid residues through interacting forces like hydrogen bonding (conventional and carbon hydrogen bonds) and hydrophobic interaction (Pi-alkyl, Pi-
donor Hydrogen Bond, Pi-Pi T-Shaped, Pi-Sigma and -Pi-stacked interactions).

Table 2 summarizes the result of five studied ligands chosen based on the most negative docking score. This corresponds to rutin (51), kaempferol-3-O-beta-D-glucopyranoside (46), quercetin-3-O-beta-D-glucopyranoside (15), pyrrolemic acid-3-O-(6’-O-[4-hydroxybenzyol]-beta-D-glucopyranoside) (12) and chrysoeriol-7-O-beta-D-glucuronopyranoside (16).

The binding energy values of the five compounds range from -8.0 to -9.1 kcal/mol and from -5.8 to -7.4 kcal/mol for SARS-CoV-2 main protease and spike protein, respectively. While that of binding affinity of Nelfinavir with 6lu7 is -7.8 kcal/mol and the binding affinity of Remdesivir with 6m0j is -6.8 kcal/mol. This indicates that these compounds could be of an affinity comparable to that of the references. Rutin exhibited the highest binding affinity with the dual-target scaffold for the two proteins SARS-CoV-2 main protease 6lu7 (-9.1 kcal/mol) and spike protein 6m0j (-7.4 kcal/mol).

Nelfinavir attached in the main protease enzyme is showing its inhibitory activity by forming hydrogen bonds with Glu166 and Gln189, and hydrophobic interaction with His41, Cys145 and Met165 (Figure 1F). On the other hand, Remdesivir attached in the spike protein is showing its inhibitory activity by forming hydrogen bonds with Phe342, Asn343 and Ser373, and hydrophobic interaction with Leu335, Phe342, Val367, Leu368 and Leu441 (Figure 2F).

3.2.1. SARS-CoV-2 main protease (PDB ID: 6lu7)
Rutin (51) isolated from Artemisia annua, binds at the target site of main protease protein with five hydrogen bonds; Tyr54, Asn142, Phe140, Cys145 and His164 at the respective distances 2.51 Å, 3.17 Å, 3.04 Å, 2.33 Å and 2.73 Å. This ligand also exhibited one pi-sulfur bond interaction with the amino acid residues Cys145 at the distance 5.12 Å and two-pi-alkyl interactions with the amino acid residue Met49 at distances 4.44 Å and 5.12 Å, which are hydrophobic Pi-sigma interactions largely involve charge transfer that helps in intercalating the drug in the binding site of the enzyme (6lu7). This observation reveals that amino acids from 49 to 54 and 140 to 164 positions were crucial for the binding of SARS-CoV-2 main protease 6lu7 with Rutin (Figure 1A).

The Docking results suggest that, the kaempferol-3-O-beta-D-glucopyranoside obtained (46) from Artemisia annua has good orientation shape with active site by hydrogen bonds with Thr26, Tyr54, Phe140, Asp187 and Gly143 at distances 1.91 Å, 2.56 Å, 2.19 Å, 1.96 Å and 4.16 Å respectively. The hydrophobic interaction with Cys145 (5.07 Å) and Met49 (4.53 Å and 5.10 Å) and the presence of numerous hydrogen bond interaction may explain the good interaction of the molecule with the receptor. Figure 1B also shows that the amino acids from 26 to 54 and 140 to 187 positions are crucial for the binding interactions of kaempferol-3-O-beta-D-glucopyranoside (46) with main protease protein. In comparison with Rutin, docking study of kaempferol-3-O-beta-D-glucopyranoside in SARS-CoV-2 main protease protein shows equal conventional hydrogen bond, carbon hydrogen bond, two pi-alkyl interactions with Met49 at distances 4.53 Å and 5.10 Å, but they are distinguished by a pi-sulfur bond with Rutin and one another pi-alkyl bond with kaempferol-3-O-beta-D-glucopyranoside.

It can be seen in Figure 1C that quercetin-3-O-beta-D-glucopyranoside (15) (Conyza sumatrensis and Artemisia annua) is surrounded by four-hydrogen bond interactions with Tyr54, Leu141, His163 and Gly143 at distances 2.25 Å, 1.87 Å, 2.58 Å and 3.50 Å, respectively, two pi-alkyl interactions with the amino acid residue Met49 and one pi-sulfur bond interaction with the amino acid residues Cys145 at respective distances of 4.52 Å, 5.07 Å and 5.20 Å. In comparison with Rutin, the docking study of quercetin-3-O-beta-D-glucopyranoside in SARS-CoV-2 main protease protein also shows two pi-alkyl interaction one pi-sulfur bond and one carbon hydrogen interactions...
bond. But the difference is observed with four conventional hydrogen bonds with rutin and three conventional hydrogen bonds with quercetin-3-O-β-D-glucopyranoside. The number of conventional hydrogen bond could play a major role in the pharmacological effect of the ligands.

The binding ability of chrysoeriol-7-O-β-D-glucuronopyranoside (16) (Conyza sumatrensis) is good, there are three hydrogen bonds formed with amino acids Asn142, Cys145 and Gln192 at distances 2.65 Å, 3.56 Å and 3.39 Å. The stability of compound 12 in the binding site is also attributed to the pi-Alkyl bond is with Met165 (5.09, 5.44 Å), Met49 (3.59 Å) and Pro168 (4.93 Å) and one Pi-Pi T-Shaped with His41 (4.10 and 5.55) (Figure 1D). The amino acids at the positions 41-49 and 142-192 were decisive for the binding

Table 2. Structures of the best docking score compounds with their binding energy (kcal/mol).

| Plants            | Phytochemical structure + Phytochemical name | Binding energy |
|-------------------|---------------------------------------------|----------------|
| Artemisia armeniaca | Rutin                                        | –9.1           |
|                   |                                              | –7.4           |
| Artemisia armeniaca | Kaempferol-3-O-β-D-glucopyranoside          | –8.9           |
|                   |                                              | –6.2           |
| Conyza sumatrensis | Quercetin-3-O-β-D-glucopyranoside           | –8.8           |
|                   |                                              | –5.8           |
| Conyza sumatrensis | Pyromeconic acid-3-O-(6''-O-[4-hydroxybenzoyl]-β-D-glucopyranoside) | –8.0           |
|                   |                                              | –6.7           |
| Conyza sumatrensis | Chrysoeriol-7-O-β-D-glucuronopyranoside     | –8.0           |
|                   |                                              | –7.4           |
interactions of SARS-CoV-2 main protease protein with chrys­
oeriol-7-O-β-D-glucuronopyranoside. The connections of pyromeconic acid-3-O-(6"-O-[4-hydroxybenzoyl]-β-D-glucopyranoside) (Conyza sumatrensis) the main protease protein listed in Figure 1E above, indicates the presence of hydrogen bond interaction with Leu141, Ser144, His163, Arg188 and Met165 at distances 1.74 Å, 2.86 Å, 2.45 Å, 2.07 Å and 3.35 Å, respectively. The conformational energy of compound 16 is also minimized by the presence of one pi-alkyl interaction with Cys145, one Pi-sulfur interaction with Met49. Pi-Pi T-Shaped with His41 is besides responsible for the stability of pyromeconic acid-3-O-(6"-O-[4-hydroxybenzoyl]-β-D-glucopyranoside).

This study showed five compounds with the highest binding affinities, the active site of SARS-CoV-2 main protease protein. The following amino acids, Arg188, Asn142, Asp187, Cys145, Gln192, Gly143, His163, His164, His41, Leu141, Met165, Met49, Phe140, Pro168, Ser144, Thr26 and Tyr54 were found to play an important role in the most favorable conformations, also they were found to be the key residues interacting in the active site of the target. Moreover, Tyr54, Met165 (H-Bonding interactions), Cys145 and Met49 (Hydrophobic interactions) were the most dominant, so they were involved in many cases as possible to form the docked complexes. The results also revealed that amino acids at positions 41-54 and 140-192 were crucial for the binding interactions of SARS-CoV-2 main protease protein. It also appears that the H-bond involving Met49 (8 interactions) and Cys145 (6 interactions) are very important for the stability of the docked conformation. Compounds with the best docking score are found to have interacted with about eight to nine amino acid residues. These results also illustrate that an increased binding affinity with the target protein does not result in a greater number of H bonds or pi-alkyl bonds.

3.2.2. SARS-CoV-2 spike protein (PDB ID: 6m0j)
Rutin (51), interacts with the active site amino acid residues of SARS-CoV-2 Spike protein through hydrogen bonding with amino acids; Asn437 (2.66 Å), Arg509 (2.66 Å) and Ser373
(2.27 Å), also through hydrophobic interactions Trp436 (3.54 Å), Phe374 (4.96 Å) and Ser373 (2.38 Å). This observation reveals that amino acids 373 and 374 then 436 to 509 positions were crucial for the binding of SARS-CoV-2 spike protein (6m0j) with Rutin (Figure 2A).

Kaempferol 3-O-β-D-glucopyranoside (46) (Figure 2B) interacts with conventional Hydrogen bond with Trp436 (2.39 Å) and through Hydrophobic interactions Phe342 (4.79 Å) of the type Pi-Pi Stacked, 373 (3.27 Å) of the Pi-Donor hydrogen bonds and Leu368 (5.39 Å) of the type Pi-Alkyl bond. The amino acids at the positions range from 342 to 373 and 436 were decisive for the binding interactions of SARS-CoV-2 spike protein with Kaempferol 3-O-β-D-glucopyranoside.

Figure 2C shows that Quercetin-3-O-β-D-glucopyranoside (15), interacts with the active amino acid residues of the spike protein such as Val362 (2.88 Å) and Asp364 (1.90 Å) via Conventional hydrogen bonds, Val367 (3.87 Å), Val367 (5.42 Å) via Pi-Alkyl and Leu335 (5.41 Å) through hydrophobic Pi-Sigma bonds. Figure 2C also shows that the amino acids from 335 to 367 positions are crucial for the binding interactions of Quercetin-3-O-β-D-glucopyranoside with spike protein. According to his best docking score, it may be said that Val362, Asp364, Val367 and Leu335 the main amino acids of SARS-CoV-2 spike protein with potential candidates.

Chrysoeriol-7-O-β-D-glucuronopyranoside (16) was found to bind to the SARS-CoV-2 spike protein active site, with two conventional hydrogen bonds Cys336, and Val362 at the respective distances of 1.99 Å and 2.80 Å. The compound also displayed four hydrophobic contacts mediated by the amino acids Leu368 (3.98 Å), Phe374 (5.25 Å), Val367 (4.30 Å) and Ser373 (2.67 Å) (Figure 2D).

The docking results suggest that Pyromeconic acid-3-O-(6-O-[4-hydroxybenzoyl]-β-D-glucopyranoside) (12) has good orientation shape with active site by hydrogen bonds with Asn343 (2.58 Å, 2.55 Å and 3.61 Å), Ser373 (2.26 Å and 2.46 Å) and Ala344 (2.15 Å). We can also observe hydrophobic interaction with Leu441 (3.93 Å), Phe342 (4.56 Å) and Ley368 (4.86 Å). The presence of many Hydrogen bond interactions in this complex explains the good interaction of the molecule with the receptor (Figure 2E).

From the results of molecular docking of SARS-CoV-2 spike protein receptor and the 5 top ligands, we can...
conclude that Asn, Leu, Phe, Ser and Val are the most represented amino acid to form the docked complexes, particularly Ser, Asn, and Val at 373, 343 and 367 positions respectively. Ser373, Asn343 and Val362 are the most prominent H-binding residues and Leu368, Val367, His41, Phe374, and Phe342 are the most prominent hydrophobic binding residues. Results also revealed that, the amino acids from 335 to 374 and 436 to 441 positions were crucial for the binding interactions of SARS-CoV-2 spike protein (6m0j), from 335 to 374 and indicating their low flexibility.

### 3.3. In silico ADME analysis

To evaluate the drug profiles of the five potential metabolites for SARS-CoV-2 proteins inhibition, some parameters that influence the performance and pharmacological activity of a drug exposure to the tissues within an organism were investigated (Balani et al., 2005). The results of drug likeness and ADME prediction using SwissADME are summarized in Tables 3 and 4.

#### 3.3.1. Prediction of molecular properties

Lipinski’s rule consists of four different restrictions, which defined parameters that can identify a potential drug candidate having probable absorption and permeability problems. From the results shown in Table 3, it can be said that all the molecules studied have good solubility in water, which is an important parameter, since it is related to dissolution, which is necessary for drug absorption. This property is also related to the molecular weight, since compound 16 has a molecular weight greater than 500 g/mol, it may little absorbed from the intestine. Structures 51, 46, 15 and 12 violate Lipinski’s rule regarding the value of the number of hydrogen bond donors (more than 5 HBD) and hydrogen bond acceptors (more than 10 HBA). Each candidate presents a polar surface area (PSA) greater than 140 Å, demonstrate that they could decrease permeability and oral bioavailability. Nevertheless, all compounds have less than 10 rotatable bonds which are in favor of binding to their target to avoid entropic penalty and indicating their low flexibility.

Note that logP measures how hydrophilic or hydrophobic a chemical substance is, and therefore it is closely related to bioavailability. Although the value of log P of each compound is negative, they are highly likely to exhibit absorption or permeability problems. Moreover, with regards to drug likeness, all the compounds show 1 to 4 violations of Lipinski’s parameters Number of violations, which depends of drug dissolution in gastrointestinal tract. Structures 51, 46, 15 and 12 violate Lipinski’s rule for oral drugs, chrysoeriol-7-O-D-glucurono-pyranoside (16) have one violation and therefore still fall under the category of orally bioavailable compounds. The other compounds with a count of 3 to 4 violations may have bioavailability issues and cannot be considered as order proceeding with drug discovery.

#### 3.3.2. Prediction of ADMET properties

The absorption consists in the passage of a molecule through the lipid bilayer of membranes from cells in the gut, which depends of drug dissolution in gastrointestinal tract. The predictive tests of absorption use here are; Caco-2, Human Intestinal Absorption (HIA), Skin permeability (logKp), P-glycoprotein substrate and P-glycoprotein I or II inhibitor. Given that intestinal absorbance value below 30% indicates poor absorbance; Table 4 shows a poor absorbance for 51...
(13.185%) and a good absorbance for 46, 16, 15 and 12 with 50.024, 42.785, 59.82 and 56.398 values respectively. Although each candidate was water soluble, they showed low skin absorption, we have also found that compounds 46, 15, 12 and 16 present a combination of high intestinal absorption, low Caco-2 permeability and low blood-brain barrier values, which indicate that they show a very little chance to cross the blood-brain barrier.

Drug distribution refers to the delivery of the bioactive compound to different compartments of the body. Some parameters have been investigated in silico with notably the volume of distribution (Vdss), the fraction unbound (fu), the blood-brain barrier (BBB) and the penetration of the central nervous system (CNS).

BBB permeation prediction was revealed no BBB permeability among the studied top drug candidates. It can also be observed that the compound 51 which has a molecular weight of 610g/mol and BBB permeability weaker than the others compounds with a MW from 394 to 464g/mol. MW is an important parameter, as reduced absorption and lower penetration of BBB is associated with an increase molecular weight. The ability of the five compounds to be capable of penetrating the BBB and affecting the desired receptor and to give a biological response could be weak. Lipinski’s rules can be used to identify compounds that show good CNS penetration. A low value indicates strong hydrophilicity, Table 4 indicates that all compounds screened have a tolerable range.

### 3.3.3. Prediction of metabolism properties

As the cytochrome P450 monooxygenase (CYP) enzymes play a pivotal role in drug metabolism, they have been extensively investigated, especially 2D6 and 3A4 which are the most important forms in human. Analysis of inhibition effects with CYP2D6 and CYP3A4 substrate revealed that none of the candidates had such interaction. Hence, potential adverse effects resulting from drug interactions upon oral administration of these compounds are unlikely. Results also show that compounds 51, 46, 16, 15 and 12 could not undergoes metabolism via CYP2D6 and CYP3A4 enzyme (Table 4).

### 3.3.4. Prediction of excretion properties

Concerning their renal organic cation transporter 2 (OCT2), all compounds are shown to be unsuitable. Hence, potential adverse effects resulting from drug interactions upon oral administration of these compounds are unlikely.

### 3.4. Toxicity pattern analysis of top drug candidates

Passing the rule five is no guarantee that a compound is drug-like. Knowledge of the degree of toxicity of the candidate compounds to become a drug is crucial. For this purpose, toxicity assessment was performed (Table 4). The results show that very few analogs have deviated toxicity prediction. None of the compounds showed any undesired effects such as skin sensitization and a negative result in AMES test that suggests a non-mutagenicity of these compounds. Compound 12 was found to be relatively toxic among the five candidates with relative hERG I/II effect. However, compound 16 show considerable Hepatotoxicity.

Estimated LD50 for 12, 15, 16, 46 and 51 were 2551, 1230, 1022, 1186 and 1555 (×10^3 mg/kg) respectively, which correspond to the values greater than 15000 mg/kg. In oral rat acute toxicity, all the candidates are in category V in which the compounds were practically non-toxic (LD50 values > 5000 mg/kg).

The five compounds show reasonable absorption and distribution properties, which could be considered as permeable compounds with poor distribution into the brain, also they present moderate clearance property and show no hERG inhibition or AMES toxicity. We can also conclude that these compounds could not be substrates or inhibitors for the subtypes of cytochrome CYP2D6 and CYP3A4, therefore probably could not be metabolized, thus a low chance of drug-drug interactions. This effect suggests them as promising inhibitors for main protease protein and spike receptor of SARS-CoV-2.

ADMET studies showed that chrysoeriol-7-O-β-D-glucuronopyranoside (compound 16) was in accordance with the Lipinski’s rules by causing no more than one violation, and was shown to possess excellent predicted absorption, distribution, metabolism and toxicity parameters, suggestive of their development as orally-active lead molecules. So, this compound has been chosen as model for MD to investigate their stability in main protease protein (PDB ID: 6lu7) and spike protein (PDB ID: 6m0j) binding sites.

### 3.5. Molecular dynamics simulations

The complexed conformation with lowest binding energy was used as initial conformations to study the dynamics using MD simulation. The initial analysis of the proteins and their complexes were performed by calculating RMSD of the backbone with respect to their respective initial structures as shown in Figure 3. The RMSD of both the complexes of compounds 16 were similar to their respective proteins. The average RMSD of main protease and compound 16-main protease complex was 0.188 ± 0.027 and 0.180 ± 0.018 nm, respectively. Similarly, average RMSD of spike protein and compound 16-spike protein complex was obtained as 0.131 ± 0.016 and 0.148 ± 0.016 nm, respectively. These values show that compound 16 was quite stable with both the proteins in aqueous environment. It is interesting to note that RMSD curves for both complexes were similar to their respective proteins with a negligible deviation within an acceptable range.

The RMSF of the Cα atoms of both proteins were calculated by averaging all conformations during 100 ns simulation for the determination of dynamic behavior of amino acids present in the proteins. Moreover, RMSF also provide useful insights regarding the structural fluctuations and flexibility of different regions of protein. The RMS fluctuations are inversely related to the stability of residues. The RMSFs of Cα atoms of spike protein and main protease alone and in
complex with compound 16 are shown in Figure 4. The RMSF of compound 16-main protease complex was similar to that of main protease alone. The RMSF of nearly all residues were below 0.2 nm indicating the stability of main protease even in presence of compound 16. A similar result was found for spike protein in which ligand-protein complex exhibited a similar fluctuation in residues. However, residues 362–372 of spike protein alone showed more fluctuation compared to compound 16-spike protein complex. This shows that the binding of compound 16 in this region decreased the structural flexibility and residues became more stable after interaction. The average RMSF of atoms of compound 16 was also calculated as shown in Figure 5. The RMSF of compound 16 showed some variations indicating the dynamical shift of the ligand from their initial position in both proteins.

The mass-weighted root mean square distance of a collection of atoms from their common center of mass is defined as radius of gyration ($R_g$). The $R_g$ is considered as a stability indicator of protein-ligand complex during molecular dynamics. The globular or compact proteins show less variations in their $R_g$ compared to the expanded form proteins. The changes in $R_g$ of spike protein, main protease, and their complexes with compound 16 is shown in Figure 6. The average $R_g$ of SARS-CoV-2 main protease alone was found to be...
2.188 ± 0.013 nm that was insignificantly changed in presence of compound 16. A similar result was obtained for spike protein where negligible changes in Rg was recorded over the entire simulation period. The SASA of spike protein, main protease, and their complexes with compound 16 was also calculated as shown in Figure 7. SASA of both complexes were similar to their respective proteins. This further validates the stability of compound 16 with SARS-CoV-2 spike protein and SARS-CoV-2 main protease protein.

The interactions of compound 16 with main protease and spike protein was studied by calculating the hydrogen bond profiles (Figure 8). The average number of hydrogen bonds between compound 16 and main protease was found to be 1.469. Similarly, the average number of hydrogen bond for compound 16 and spike protein interaction was found to be 1.329. Compound 16 formed slightly greater number of hydrogen bonds with main protease than spike protein. Moreover, it was observed that hydrogen bond existence between the ligand and both proteins was found over the entire simulation period.

The effect of binding of compound 16 with SARS-CoV-2 spike protein and SARS-CoV-2 main protease on their secondary structure was assessed. The % of secondary structural components of main protease, spike protein and their complexes with compound 16 are shown in Figure 9. The α-helix and β-sheet main protease was found to be 17.72 and 27.24% respectively, which remained approximately unchanged even after interaction with compound 16. Similarly, insignificant changes on the secondary structure of spike protein were observed after the binding of compound 16. This validates the structural stability of SARS-CoV-2 spike protein and SARS-CoV-2 main protease complexed with compound 16. To further verify the stability of the both proteins with compound 16, the energies (total energy and potential energy) of the system were also calculated (Figure 10). The total energy and potential energy of both proteins alone and in complex with compound 16 remained stable during over the entire simulation period.

The detailed insight of the various forces involved in the interaction of compound 16 with main protease and spike protein was studied by analyzing the MM-PBSA calculations. The MM-PBSA binding energies were calculated for 100 snapshots taken at uniform intervals of the entire MD simulation (Table 5). In typical drug-protein interactions, major non-covalent interactions are hydrophobic, hydrogen, electrostatic, Van Der Waals interactions. These interactions either contribute positively or negatively to the overall binding energy (BE) (Siddiqui et al., 2019). The interaction of compound 16 with main protease was most favored by electrostatic (Elec) interactions followed by Van Der Waals (VDW) forces. There was also a small contribution of solvent accessible surface area (SASA) energy in the overall binding energy.
Figure 8. (A) Number of hydrogen bonds between compound 16 and main protease. (B) Number of hydrogen bonds between compound 16 and spike protein.

Figure 9. (A) Percentage of secondary structure in main protease and compound 16-main protease complex during the simulation period. (B) Percentage of secondary structure in spike protein and compound 16-spike protein complex during the simulation period.

Figure 10. (A) The potential energy and the total energy of the main protease and compound 16-main protease complex. (B) The potential energy and the total energy of the spike protein and compound 16-spike protein complex.

Table 5. Binding free energy (kJ/mol) for the interaction of compound 16 with SARS-CoV-2 main protease and SARS-CoV-2 spike protein for 100 snapshots of MD simulation.

| Type of energy       | Main protease (6lu7) | Spike protein (6m0j) | 2-acetamido-2-deoxy-beta-D-glucopyranose |
|----------------------|----------------------|----------------------|------------------------------------------|
| $\Delta E_{VdW}$     | $-143.614 \pm 1.450$ | $-155.455 \pm 2.267$ | $-30.997 \pm 2.735$                     |
| $\Delta E_{ele}$     | $-317.059 \pm 4.389$ | $-40.162 \pm 1.424$  | $-20.621 \pm 2.168$                     |
| $\Delta E_{PSE}$     | $216.103 \pm 5.503$  | $170.300 \pm 3.244$  | $37.954 \pm 4.589$                      |
| $\Delta E_{ASA}$     | $-17.670 \pm 0.130$  | $-17.444 \pm 0.190$  | $-4.783 \pm 0.374$                      |
| $\Delta E_{BE}$      | $-262.256 \pm 2.439$ | $-66.745 \pm 2.640$  | $-64.024 \pm 2.569$                     |

$\Delta E_{VdW}$: Van Der Waal energy, $\Delta E_{ele}$: Electrostatic energy, $\Delta E_{PSE}$: Polar solvation energy, $\Delta E_{ASA}$: Solvent accessible surface area energy, $\Delta E_{BE}$: Binding energy.
In compound 16 and spike protein interaction, Van Der Waals (VDW) forces were more prominent compared to electrostatic interactions. However, polar solvation energy (PSE) impaired the interaction of compound 16 with both the proteins. The average, total average binding energy for the interaction of compound 16 with main protease and spike protein was found to be $-262.256 \pm 2.439$ and $-64.024 \pm 2.569 \text{kJ/mol}$, respectively. It is interesting to note that the MM-PBSA binding energy of compound 16 with both the proteins ($6lu7$ and $6m0j$) was more compared to the respective controls. This shows the stronger binding affinity of compound 16 compared to the controls ($N3$ for $6lu7$ and $2$-acetamido-$2$-deoxy-$\beta$-D-glucopyranose for $6m0j$).

Various in silico research of phytoconstituents from naturel compounds were investigated to effectively combat the COVID-19. In a study published in April 2020, three compounds of Moroccan medicinal plants origin (Crocin, Digitoxigenin and b-Eudesmol) were showing a high binding affinity and interact to the conserved residues of the substrate-binding pocket of SARS-CoV-2 Mpro (Aanouz et al., 2020). A study by Saravanan et al. (2020) suggests five Indian medicinal plants derived antiviral compounds (Amentoflavone, Lectin, glycyrrhizicacid, Hypericin and Torvoside) as inhibitors against the coronavirus based on molecular docking study and a 40 ns molecular dynamics simulation. In the same way, the main chemical constituents of another Indian medicinal herb Tinospora cordifolia (berberine, $\beta$-sitosterol, coline, tetrahydropalmatine and octacosanol) were proposed as an antiviral drug against SARS-CoV-2 (Chowdhury, 2020). In a recent study, two compounds of an Indonesian natural medicinal product, Sulawesi propolis (isorhamnetin and glyasperin A), are proposed as potential inhibitor of of SARS-CoV-2 (Khayrani et al., 2021). Yu et al. proposed two active compounds of traditional Mongolian medicine (Phylliryn and chlorogenic acid) that can be used as potential inhibitors of COVID-19 for further research and development (Yu et al., 2020). Thus, current study has evaluated the Asteraceae family of Cameroonian plants and has recommended chrysoeriol-7-O-$\beta$-D-glucuronopyranoside as a suitable lead candidate with good efficacy against the SARS-CoV-2 based on molecular docking study and a 100 ns molecular dynamics simulation.

4. Conclusion

SARS-CoV-2 virus has spread exponentially across the globe since the advent of COVID-19, resulting in a pandemic. The only way to break the chain of virus transmission seems to be vaccination or the growth of herd immunity; however, the existence of similar mutated strains and the failure of initially recommended drugs have posed fears for the near future. As a result, successful treatments are urgently needed to ease the pandemic’s burden and lower mortality rates. To treat this viral respiratory infection, few oral formulations based on medicinal plants have been proposed in Africa but without scientific concepts. The present in silico study, highlight the binding affinity and interactions analysis of SARS-COV-2 main protease ($6lu7$) and spike receptor binding domain ($6m0j$) with 74 natural molecules obtained from Cameroonian medicinal plants intervening in the treatment of respiratory infection and belongs to the Asteraceae family. Five compounds give the best preliminary results with lowest docking scores (between $-8.0$ and $-9.1 \text{ kcal/mol}$ for $6lu7$ and between $-5.8$ and $-7.4 \text{ kcal/mol}$ for $6m0j$) and were found to have significant affinity with the selected target. The binding energy of chrysoeriol-7-O-$\beta$-D-glucuronopyranoside (compound 16) with SARS-CoV-2 spike protein and SARS-CoV-2 main protease were found to be $-7.4$ and $-8.0 \text{ kcal/mol}$ which indicates that this compound could be of an affinity comparable to that of the references (Remdesivir and Nelfinavir). Moreover, MM-PBSA binding energies of compound 16 with both the proteins were found to be more than their respective controls. Molecular docking was performed, and the results also illustrate that H-bonding and hydrophobic interactions are crucial for the stability of docked complexes. Since the success of a drug through the body is based on the pharmacokinetics parameters, the best-identified hits were submitted for further analysis. Investigation of adsorption, distribution, metabolism, excretion and toxicity parameters helps to filter among the top hits and identify one compound, the compound 16, as a suitable lead candidate. Due to the complexity of human being, an in vitro research is an imperative before clinical trials in the aim to confirm that this molecule is an efficient antiviral compound against COVID-19.

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