Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Covid-19 treatment: Investigation on the phytochemical constituents of *Vernonia amygdalina* as potential Coronavirus-2 inhibitors

Johnson Olaleye Oladele a, *, Oyedotun Moses Oyeleke a, Oluwaseun Titilope Oladele b, Adenike Temidayo Oladiji c

a Biochemistry Unit, Department of Chemical Sciences, Kings University, Ode-Omu, Osun State, Nigeria
b Phytomedicine and Molecular Toxicology Research Laboratories, Department of Biochemistry, Osun State University, Osogbo, Nigeria
c Department of Biochemistry, University of Ilorin, Ilorin, Nigeria

ARTICLE INFO

**Keywords:** COVID-19, Antivirals, Phytochemicals, Medicinal plants, Molecular docking, *Vernonia amygdalina*

**ABSTRACT**

The upsurge in the current cases of COVID-19 poses a major threat on human health and population all over the globe. The emergence of new infectious diseases and increase in frequency of drug resistant viruses demand effective and novel therapeutic agents. In this study, we used bioinformatics approach to investigate the possible inhibitory potentials of phytochemical constituents of *Vernonia amygdalina* towards coronavirus-2 major protease. Pharmacodynamics, pharmacokinetics and toxicological profiles of the compounds were also examined using the pkCSM server. All the phytochemicals showed good binding affinity to the binding pocket of PDB ID 6LU7. It was observed that veronicoside A exhibited the highest binding affinity when compared to remdesivir, hydroxy-vernolide, vernodalin, vernodalol, and vernolide. The amino acids LEU272, LEU287, GLY275, TYR237, LYS236, THR198, THR199, ARG131, and LYS5 were showed as the key residues for veronicoside A binding to human SARS-COV2 major protease. The Pharmacodynamics and pharmacokinetics results suggested that all the tested phytochemicals have significant drug likeness properties and they could be absorbed through the human intestine. Furthermore, all the tested phytochemicals are not hepatotoxic and also exhibited non or relatively low toxic effects in human. Taken together, the results of this study indicated that all the tested phytochemicals are potential putative inhibitors of SARS-COV2 major protease with non or low toxicity effects. However, further experimental and clinical studies are needed to further explore their activities and validate their efficacies against COVID-19.

1. Introduction

Novel Coronavirus disease 2019 (COVID-19), ranked among the ninth deadliest world pandemic, is a highly infectious and severe acute respiratory disorder caused by a morbific virus called SARS-CoV-2 which is transmitted to humans via contact with infected persons and/or feeding on infected animals. The COVID-19 clinical manifestations are very similar to viral pneumonia such as fever, fatigue, cough, shortness of breath, and other complications. According to reports obtained on WHO and NCDC websites as of 27th June 2020, the coronavirus breakout in Wuhan, a city in Hubei Province of China in November 2019 and has spread to many countries in the world. This global pandemic has forced many nations to lock down their social and economic activities which in turn have adverse effects on the economy. Globally, more than ten million people have been confirmed infected with over 500,000 deaths. Nigeria is one of the countries seriously affected by the virus having over 25,000 cases and more than 500 mortalities [1,2]. Thus, there is an exigent need for effective and non-invasive treatment.

Coronaviruses (SARS-CoV) are non-segmented positive-sense single-stranded RNA viruses with a large viral RNA genome of diameter 80–120 nm [26]. They belong to the family of Coronaviridae, in the subfamily Orthocoronaviridae which consists of four genera namely: Alpha, Beta, Gamma, and Delta coronavirus [3]. Some of the modes of actions of SARS-CoV-2 include hyper-inflammation characterized by a sudden and fatal hyper-cytokinaemia with multi-organ failure [4]; immunosuppression; reduction of Angiotensin-Converting Enzyme 2 (ACE2) to enhance pulmonary vascular permeability and damage the alveoli [5]; and activated by ORF3a, ORF3b, and ORF7a via JNK pathway which induces lung damage [6].

* Corresponding author.
  E-mail addresses: oladelejohn2007@gmail.com, jo.oladele@kingsuniversity.edu.ng (J.O. Oladele).

https://doi.org/10.1016/j.comtox.2021.100161
Received 5 August 2020; Received in revised form 8 January 2021; Accepted 14 February 2021
Available online 18 February 2021
2468-1113/© 2021 Elsevier B.V. All rights reserved.
At present, there is no known effective treatment that can mitigate/inhibit the pathogenesis of coronavirus in infected patients, however, there is vaccine to prevent the wide spread of the virus. Available clinical interventions for COVID-19 patients are only palliative and limited to support. There is still urgent need for therapeutic agents as cases of infections is increasing on the daily basis. Therefore, many research groups around the world are currently focusing on developing novel antivirals using various in silico methods [28–30]. This research investigated the inhibitory potentials of some naturally occurring phytochemicals present in *Vernonia amygdalina* against COVID-19 major protease (6LU7). *Vernonia amygdalina*, commonly known as bitter leaf, is an indigenous African plant with a number of scientific proven medicinal importance [10,31–33]. These phytochemicals can be repurposed to mitigate the pathogenesis of the SARS-CoV-2 and thus put an end to the frightening associated mortality rate.

2. Materials and methods

2.1. Protein preparation

The crystal structure of SARS-COV2 major protease (PDB ID 6LU7) with resolution 2.16 Å was retrieved from the protein databank (www.rcsb.org). Prior to docking and analysis, the crystal structure was prepared by removing existing ligands and water molecules. Also, missing hydrogen atoms were added using Autodock v4.2 program, Scripps Research Institute [27]. Thereafter, non-polar hydrogens were merged while polar hydrogen was added and subsequently saved into pdbqt format in preparation for molecular docking.

2.2. Ligand preparation

The SDF structures of selected phytochemical constituents of the leaf extract of *Vernonia amygdalina*: hydroxyvernolide, vernodalin, vernodalol, vernolide, and veronicoside were retrieved from the PubChem database (www.pubchem.ncbi.nlm.nih.gov). The phytochemicals were converted to mol2 chemical format. Polar hydrogens were added while non-polar hydrogens were merged with the carbons and the internal degrees of freedom and torsions were set. The protein and ligand molecules were further converted to the dockable PDBQT format using Autodock tools.

2.3. Molecular docking

Docking of the phytochemicals to the targeted protein and determination of binding affinities was carried out using AutodockVina [7]. The PDBQT formats of the receptor and that of the phytochemicals were positioned at their respective columns and the software was run. The binding affinities of phytochemicals for the protein target were recorded. The phytochemicals were then ranked by their affinity scores. The molecular interactions between the receptor and phytochemicals with most remarkable binding affinities were viewed with Discovery Studio Visualizer, BIOVIA, 2016. The respective binding free energy was calculated by the Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) using HawkDock server (http://cadd.jzu.edu.cn/hawkdock/).

2.4. Molecular dynamics simulation

The conformational stability of the protein–ligand interactions was evaluated using molecular dynamics simulations analysis performed through iMODS server (http://imods.charonlab.org) by normal mode analysis (NMA) predicting properties such as deformability, mobility profiles, eigenvalues, variance, co-variance map and elastic network of the protein–ligand interactions [8].

2.5. ADMET analysis

The solubility, pharmacodynamics, pharmacokinetics and toxicological profiles of hydroxyvernolide, vernodalin, vernodalol, vernolide, veronicoside and remdesivir were computed based on their ADMET (absorption, distribution, metabolism, elimination, and toxicity) studies using pkCSM tool (http://biosig.unimelb.edu.au/pkcsms/prediction) as described by Pires et al. (2015). The canonical SMILES molecular structures of the compounds used in the studies were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov).

3. Results

3.1. Molecular docking analysis

The molecular docking analysis and visualization of 6LU7 binding with remdesivir, hydroxy-vernolide, vernodalin, vernodalol, vernolide, and veronicoside A is shown in Fig. 1, Fig. 2 and Table 1. Out of the all the tested compounds, veronicoside A exhibited the best docked score (−7.4 kcal/mol) with the SARS-COV2 major protease (6LU7). LEU272, LEU287, GLY275, TYR237, THR198, THR199, ARG131, LYS amino acid residues participating in the interaction at the binding pocket of the SARS-COV2 major protease (6LU7) (Fig. 1F). Vernolide exhibited (−7.2 kcal/mol) binding affinity with 6LU7. HIS41, MET49, GLN110 and PHE294 amino acid residues participating in the interaction at the binding pocket of 6LU7 (Fig. 1E). Vernodalin displayed (−6.7 kcal/mol) binding affinity with 6LU7. ASP153, GLN110, and PHE294 amino acid residues participating in the interaction at the binding pocket of 6LU7 (Fig. 1C). Remdesivir exhibited (−6.6 kcal/mol) binding affinity with 6LU7. ARG131, THR199, LYS137, ASP289, LEU272, LEU287, and MET276 amino acid residues participating in the interaction at the binding pocket of 6LU7 (Fig. 1A). Hydroxy-vernolide showed (−6.2 kcal/mol) binding affinity with 6LU7. HIS41, MET49, GLN189, GLU166, MET165, LEU167, and THR190 amino acid residues participating in the interaction at the binding pocket of 6LU7 (Fig. 1A). Hydroxy-vernolide showed (−7.4 kcal/mol) binding affinity with 6LU7. HIS41, MET49, GLN189, GLU166, MET165, LEU167, and THR190 amino acid residues participating in the interaction at the binding pocket of 6LU7 (Fig. 1B). Vernodalol exhibited (−6.1 kcal/mol) binding affinity with 6LU7. ASP197, THR199, TYR237, TYR239, LEU272, LEU286, and LEU287 amino acid residues participating in the interaction at the binding pocket of 6LU7 (Fig. 1D).

3.2. Molecular properties of the phytochemicals

Data in Table 2 revealed the results of the molecular properties of the phytochemicals used in this study. Veronicoside A was found to have the highest molecular weight of 814.699, followed by vernodalin with 392.404, hydroxy-vernolide with 378.377, vernolide with 362.378 and vernodalin with 360.362. Similarly, the surface area of the phytochemicals: veronicoside A, vernodalol, hydroxy-vernolide, vernolide, and vernodalin are 319.905, 162.317, 155.582, 150.788 and 150.152 respectively. Vernodalin has the highest lipophilicity of 0.8498, vernodalin has 0.4583, hydroxy-vernolide has 0.141 while veronicoside A has the least lipophilicity of 2.2824 (see Fig. 3).

3.3. Predicted absorption properties of the phytochemicals

The predicted absorption properties of each of the phytochemicals were reported in Table 3. The result showed that veronicoside A has the highest water solubility value of 2.886 while vernolide has the lowest value of −3.936. Vernolide has the highest permeability value of 0.804, but veronicoside A having the least permeability value of −1.469. Likewise, vernolide can be readily absorbed by the intestinal cells (100%) whereas veronicoside A may not be absorbed intestine. All the phytochemicals are substrate of P-glycoprotein and none of the phytochemicals are substrate of P-glycoprotein-I nor P-glycoprotein-II.
Fig. 1. Docking analysis and visualization of 6LU7 binding with (A) Remdesivir, (B) Hydroxy-vernalide, (C) Vernodalin, (D) Vernodalol, (E) Vernolide, (F) Veronicoside A.
Fig. 2. Binding-interaction analysis of 6LU7 binding with (A) Remdesivir, (B) Hydroxy-vernolide, (C) Vernodalin, (D) Vernodalol, (E) Vernolide, (F) Veronicoside A.
Table 1
Molecular docking analysis of the tested compounds against COVID-19 major protease (6LU7).

| Compound        | PubChem CID | Binding energies (kcal/mol) | ligand-amine acid interactions |
|-----------------|-------------|----------------------------|--------------------------------|
| Remdesivir      | 121,304,016 | -6.6                       | ARG131, THR199, LYS137, ASP289, LEU272, LEU287, MET276 |
| Hydroxy-vernonlde| 5,281,472   | -6.2                       | HIS41, MET49, GLN189, GLU166, MET165, LEU167, THR190 |
| Vernodalol      | 179,375     | -6.7                       | ASP153, GLN110, PHE294 |
| Vernolide       | 442,318     | -6.1                       | ASP197, THR199, TYR237, TVR239, LEU272, LEU286, LEU287 |
| Veronicoside A  | 44,258,142  | -7.2                       | HIS41, MET49, GLN189, GLU166, MET165, LEU167, THR190 |
|                 |             |                             | LEU272, LEU287, GLY275, TVR237, LYS236, THR198, THR199, ARG131, LYS5 |

3.4. Predicted in vivo distribution and cytochrome P450 promiscuity of the phytochemicals

Table 4 showed the predicted in vivo distribution of the phytochemicals. All the phytochemicals tested have relatively low steady-state volume of distribution. Also, the predicted result revealed that hydroxy-vernonlde has the highest unbound fraction in the human blood. All the phytochemicals have relatively low blood–brain barrier and CNS permeability values.

Table 5 displayed the predicted human cytochrome P450 promiscuity of the screened phytochemicals. All the compounds were neither substrate of CYP2D6 and CYP3A4 nor inhibitor of CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4. Expect vernoldol and vernolide that are substrate of CYP3A4.

3.5. Predicted in vivo clearance of the phytochemicals

The predicted clearance of each of the phytochemicals were reported in Table 6. Hydroxy-vernonlde has the highest total clearance rate of 1.267 while Veronicoside A has the least clearance rate of -0.356. None of the phytochemicals is substrate of renal organic cation transporter.

3.6. Predicted toxicological profile of the phytochemicals

Table 7 reported the predicted toxicological profile of all the tested phytochemicals. Hydroxy-vernonlde, vernoldol, and veronicoside A has no mutagenic potentials against bacteria (AMES toxicity) but vernoldol and vernolide could be toxic to bacteria. None of the phytochemicals has adverse effects on the hepatic or dermal cells. The phytochemicals are not inhibitors of human ether-a-go-go-related gene (hERG) hERG I and hERG II except veronicoside A which may inhibit hERG II. All the phytochemicals have relatively low maximum recommended tolerated dose values.

4. Discussion

Plants belonging to the Vernonia genus is principally known to house huge amounts of sesquiterpene lactones with several documented pharmacological and biological activities [9]. Vernonia amygdalina is a member of this group of plants and normally called bitter leaf. It is a medicinal plant used traditionally in the management and treatment of different diseases such as respiratory diseases, cough, reproductive diseases [10]. This present study examines the possible inhibitory activity of selected phytochemical constituents (hydroxyvernonlde, vernodalol, vernoldol, vernolide, and veronicoside) of the leaf extract of Vernonia amygdalina against SARS-COV2 major protease (6LU7).

As shown in the result, veronicoside A exhibited the highest affinity with 6LU7. This indicates that veronicoside can be a putative inhibitor of coronavirus-2. Veronicoside has been reported to have radical scavenging and antioxidant activities. It has also been documented to have cytoxicity activities against Hep-2 (human larynx epidermoid carcinoma), RD (human rhabdomyosarcoma), and L-20B (transgenic murine cells) cell lines [11]. Several species of plants containing veronicoside are being used in traditional medicine to treat influenza, respiratory diseases, hernia, cough, laryngopharyngitis, cancer, hemoptysis, and are also used as an antiscorbutic and expectorant [12].

Vernoldol and vernolide also showed a good binding affinity with the coronavirus-2 major protease, suggesting them as potential suitable inhibitors of the virus. Vernoldol and vernolide have been reported to exhibit antiproliferative activities [13] against lung A549 (adenocarcinomnic human alveolar basal epithelial cells), HeLa, and MDA-MB-23 (human breast cancer) cell lines and induced apoptosis on HepG2 cells with G2/M phase cell cycle arrest [14]. They have potential to be used as lead compounds in the development of a therapeutic natural product for treatment of cancers in the lungs, breast or liver. These phytochemicals may also offer help in inhibiting the proliferative activities of SARS-COV2 in the host thereby mitigate the pathogenesis of COVID-19.

Sini et al. [15] has reported vernoldol has a good activator of Nrf2. NF-E2-related factor-2 (NRF2) is a transcriptional factor that binds to and facilitates the activation of the ARE-dependent gene. Under basal conditions, NRF2 is sequestered in the cytoplasm and its expression is maintained to be low due to constant polyubiquitination. In response to different kinds of stress, NRF2 is significantly induced and translocates into the nucleus, where it activates the antioxidant response element (ARE)-dependent gene expression in association with small Maf proteins and other coactivators. Thus, causing the release of phase II cytoprotective enzymes such as γ-glutamylcysteine ligase (γ-GCS), NAD[P]H:quinone oxidoreductase-1 (NQO1), heme oxygenase-1 (HO-1), and glutathione S-transferase (GST) which protect the cells against the attack of the stress. Since oxidative stress has been reported as one of the features of COVID-19, vernoldol can help to extenuate it by activation of NRF2.

Nuclear Factor kappa-light-chain-enhancer of activated B-cells (NF-κB) pathway has been implicated in the mode of actions of SARS-COV2 [16,17] leading to a cytokine profile resembling secondary haemophagocytic lymphohistiocytosis (sHLH) with a hyperinflammatory syndrome characterized by a fulminant and severe hypercytokinaemia with multiorgan failure. This is characterized by increased tumor necrosis factor-α, interleukin (IL)-2, IL-7, interferon-γ inducible protein 10.
granulocyte-colony stimulating factor, macrophage inflammatory protein 1-α, and monocyte chemoattractant protein 1 [18]. However, vernolide and vernodalol have been documented to show marked inhibitory activity on STAT3/NF-κB [15]. Therefore, vernolide and vernodalol could protect against COVID-19-induced multiorgan failure by suppressing the hyperinflammatory syndrome via inhibition of NF-κB.

The results of the solubility, pharmacodynamics, pharmacokinetics and toxicological profiles of remdesivir, hydroxy-vernolide, vernodalin, vernodalol, vernolide, and veronicoside A are presented in Tables 2–7.

Fig. 3. 2D structures of (A) Remdesivir, (B) Hydroxy-vernolide, (C) Vernodalin, (D) Vernodalol, (E) Vernolide, (F) Veronicoside A.
The profiles were investigated as a systemic virtual screening of drugs and potential drugs. This is done as alternative to in vivo examinations which are essential complements in drug discovery. The Lipinski’s rule is a major criterion to evaluate drug likeliness and to determine if a compound with a particular pharmacological and biological actions has physical and chemical properties that could favour its activities in human. The molecular properties of the compounds based on the computed partition coefficient (log P) showed that the phytochemicals have relatively good lipophilicity as the logP values were less than 5 [19,20]. All the tested phytochemicals could be maintained in the system at appropriate concentrations.

Table 3
Predicted absorption properties of the phytochemicals.

| Model name                  | Remdesivir | Hydroxy vernolide | Vernodalin | vernodalol | Vernolide | Veronicoside A |
|-----------------------------|------------|-------------------|------------|------------|-----------|----------------|
| Water solubility (log mol/L)| -3.07      | -3.894            | -3.382     | -3.192     | -3.936    | -2.886         |
| Caco2 permeability (log Papp in 10⁻⁶ cm/s) | 0.635 | 0.8 | 0.469 | 0.279 | 0.804 | -1.469 |
| Intestinal absorption (% Absorbed) | 71.109 | 96.455 | 96.144 | 75.395 | 100 | 0 |
| Skin permeability (log Kp)   | -2.735     | -2.908             | -3.222     | -3.447     | -3.086    | -2.735         |
| P-glycoprotein substrate     | Yes        | Yes                | Yes        | Yes        | Yes       | Yes            |
| P-glycoprotein I inhibitor   | Yes        | No                 | No         | No         | No        | No             |
| P-glycoprotein II inhibitor  | No         | No                 | No         | No         | No        | No             |

Caco2: Human colon adenocarcinoma-2.

Table 4
Predicted in vivo distribution of the phytochemicals.

| Model name                  | Remdesivir | Hydroxy vernolide | Vernodalin | vernodalol | Vernolide | Veronicoside A |
|-----------------------------|------------|-------------------|------------|------------|-----------|----------------|
| VDss (human) (log L/kg)     | 0.307      | 0.198             | -0.236     | -0.197     | 0.156     | 0.246         |
| Fraction unbound (human) (Fa) | 0.005 | 0.551 | 0.419 | 0.509 | 0.452 | 0.225 |
| BBB permeability (log BB)   | -2.056     | -0.423             | -0.684     | -0.48      | -0.566    | -2.686         |
| CNS permeability (log PS)   | -4.675     | -3.179             | -3.061     | -3.049     | -3.092    | -6.228         |

Vdss: Steady-state volume of distribution, BBB: Blood-brain barrier, CNS: Central nervous system.

Table 5
Predicted human cytochrome P450 promiscuity of the phytochemicals.

| Model name                  | Remdesivir | Hydroxy vernolide | Vernodalin | vernodalol | Vernolide | Veronicoside A |
|-----------------------------|------------|-------------------|------------|------------|-----------|----------------|
| CYP2D6 substrate            | No         | No                | No         | No         | No        | No             |
| CYP3A4 substrate            | Yes        | No                | No         | No         | No        | Yes            |
| CYP1A2 inhibitor            | No         | No                | No         | No         | No        | No             |
| CYP2C19 inhibitor           | No         | No                | No         | No         | No        | No             |
| CYP2C9 inhibitor            | No         | No                | No         | No         | No        | No             |
| CYP2D6 inhibitor            | No         | No                | No         | No         | No        | No             |
| CYP3A4 inhibitor            | No         | No                | No         | No         | No        | No             |

Table 6
Predicted in vivo clearance of the phytochemicals.

| Model name                  | Remdesivir | Hydroxy vernolide | Vernodalin | vernodalol | Vernolide | Veronicoside A |
|-----------------------------|------------|-------------------|------------|------------|-----------|----------------|
| Total clearance (log ml/min/kg) | 0.198 | 1.267 | 0.725 | 0.747 | 1.184 | -0.356 |
| Renal OCT2 substrate        | No         | No                | No         | No         | No        | No             |

OCT2: Organic cation transporter 2.

Table 7
Predicted toxicological profile of the phytochemicals.

| Model name                  | Remdesivir | Hydroxy vernolide | Vernodalin | vernodalol | Vernolide | Veronicoside A |
|-----------------------------|------------|-------------------|------------|------------|-----------|----------------|
| AMES toxicity               | No         | No                | Yes        | Yes        | No        | No             |
| Max. Tolerated dose (human) (log mg/kg/day) | 0.15 | 0.11 | 0.236 | 0.501 | -0.324 | 0.361 |
| hERG I inhibitor            | No         | No                | No         | No         | No        | No             |
| hERG II inhibitor           | Yes        | No                | No         | No         | No        | Yes            |
| Oral Rat Acute Toxicity (LD50) (mol/kg) | 2.043 | 3.949 | 2.285 | 2.388 | 3.467 | 2.475 |
| Oral Rat Chronic Toxicity (LOAEL) (log mg/kg_bw/day) | 1.639 | 2.087 | 1.768 | 1.971 | 1.107 | 5.441 |
| Hepatotoxicity              | Yes        | No                | No         | No         | No        | No             |
| Skin sensitization          | No         | No                | No         | No         | No        | No             |
| T. pyriformis toxicity (log ug/L) | 0.285 | 0.287 | 0.314 | 0.29 | 0.295 | 0.285 |
| Minnow toxicity (log mM)    | 0.291      | 3.852             | 2.36       | 3.646      | 3.007     | 10.719         |

AMES: Salmonella typhimurium reverse mutation assay, Max.: Maximum hERG: Human ether-a-go-go-related gene.

The profiles were investigated as a systemic virtual screening of drugs and potential drugs. This is done as alternative to in vivo examinations which are essential complements in drug discovery. The Lipinski’s rule is a major criterion to evaluate drug likeliness and to determine if a compound with a particular pharmacological and biological actions has physical and chemical properties that could favour its activities in human. The molecular properties of the compounds based on the computed partition coefficient (log P) showed that the phytochemicals have relatively good lipophilicity as the logP values were less than 5 [19,20]. All the tested phytochemicals could be maintained in the system at appropriate concentrations.

Intestinal absorption and Caco2 permeability are parameters that determine the ultimate bioavailability of drug candidates. The tested phytochemicals (hydroxy-vernolide, vernodalin, vernodalol, vernolide, and veronicoside A) have relatively low Caco2 permeability potential (<8 × 10⁻⁶ cm/s) and could be absorbed through the human intestine [21]. However, ADMETSAR1 as predicted that remdesivir is subcellular localization in the lysosome [22]. Furthermore, the observed
lipophilicities have an association with Caco2 permeability but corre-
lated negatively with water solubility potentials of the tested phyto-
chemicals. This result is in tandem with the findings of Yazdanian et al [23] who used the human colon adenocarcinoma (Caco-2) cell line assay to document no correlation between the drug permeability and measured lipophilicity. All the tested phytochemicals were predicted to be substrates of P-glycoprotein, a member of the ATP-binding cassette transporter and an efflux membrane transporter found chiefly in epithelial cells. On the other hand, none of the phytochemicals is pre-
dicted as P-glycoprotein inhibitors except remdesivir. This indicate that they don’t alter the normal physiological activities of P-glycoprotein including restricting the active uptake and the distribution of drugs [24].

The volume of distribution calculated using a steady-state volume of
distribution (VDDs) as predicted showed that vernodalin has the lowest
theoretical dose required for uniform distribution in the plasma when compared with other tested phytochemicals (hydroxy-vernoldin, ver-
nodalin, vernoldin, and veronicoside A). VDDS showed the distribution
of drug in the tissue and plasma. The degree of diffusing across plasma
membrane increases in this order hydroxyvernodalin < vernodalin < vernoldin < veronicoside A < remdesivir measured as the fraction that is in the unbound state. The predicted evaluation on the
nervous system distribution of the compounds revealed that lip-
ophilicity of the phytochemicals correlates to the degree of permeability
across the central nervous system and the blood–brain barrier.

Cytochrome P450 is a group of enzymes that perform crucial func-
tions in drug metabolism. They play a major role in the activation of
drugs and also in the toxicity effects of the drugs. Only vernoldin, ver-
nodalin and remdesivir is substrate of CYP3A4, all other tested phyto-
chemicals were neither substrate of CYP2D6 nor CYP3A4. None of the phytochemicals is inhibitors of CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4. The lipophilicity of the drug appears to correlate nega-
tively to metabolism-related toxicity. Furthermore, none of the tested phytochemicals were a substrate of renal organic cation transporter, this
implies that they are possibly cleared through other available routes
such as sweat, bile, etc. Also, veronicoside A was observed to have the
least total clearance while hydroxyvernoldin has the highest. Drug
clearance is related to bioavailability and is crucial for determining
dosing rates to achieve steady-state concentrations.

The toxicological assessment of the tested phytochemicals revealed
that all the tested compounds expect remdesivir are not skin sensitive
dermal toxic) or hepatotoxic. Similarly, only vernoldin and vernodalin
are bacterial mutagenic potential drugs using the Ames toxicity ex-
amination. However, all the compound showed high level of toxicity to
Tetratymena pyriformis toxicity test. None of the phytochemicals is an
inhibitor to hERG I but veronicoside A and remdesivir may be inhibitors
to hERG II. Inhibition of the hERG potassium channel could result in
delayed ventricular repolarisation leading to a severe disturbance in the
normal cardiac rhythm and disrupt hepatic functions [25]. Acute and
chronic toxicity were also carried out on the tested phytochemicals to
determine the safety of the compounds when administered. Exposure to
low-moderate doses/concentrations of xenobiotics over long period of
time is of significant concern in many treatment strategies or in-
terventions. Chronic studies are designed to identify the lowest dose of a
compound that can result in adverse effects (LOAEL), and the highest
dose at which no adverse effects are observed (NOAEL).

5. Conclusion

Taken together, the results from this study showed that all the tested phytochemicals exhibited significant binding affinity to the binding
pocket of SARS-COV2 major pro tease suggesting them as potential
molecules that could mitigate/inhibit SARS-COV2. Binding of these
phytochemicals to SARS-COV2 could inhibit or interfere the pathogen-
esis of COVID-19 thereby preventing its cellular entry and proliferation.
Three of the tested phytochemicals (veronicoside A, vernoldin and
vernoldin) showed higher binding affinity with 6LU7 than remdesivir,
an established antiviral drug, thus, suggested them as better antiviral
agents. The pharmacodynamics and pharmacokinetics properties of the
phytochemicals showed that they would be good drug candidates and
the toxicological evaluations showed that the phytochemical has rela-
tively low or no toxic effect in human. However, further experimental
and clinical studies are needed to further explore their activities and
validate their efficacies against COVID-19.

Declaration of Competing Interest

The authors declare that they have no known competing financial
interests or personal relationships that could have appeared to influence
the work reported in this paper.

References

[1] WHO Covid-19 briefing 6th May 2020. https://who.int/dg/speeches/detail/who-
director-general-s-openingremarks-at-the-media-briefing-on-covid-19-6–may-
2020.
[2] NCDC. Coronavirus COVID-19. https://covid19.ncdc.gov.ng (Accessed May 19,
2020).
[3] J.-W. Chan, K.-W. To, H. Tse, D.Y. Jin, K.-Y. Yuen, Interspecies transmission and
emergence of novel viruses: lessons from bats and birds, Trends Microbiol. 21 (2013)
544–555.
[4] C. Huang, Y. Wang, X. Li, Clinical features of patients infected with 2019 novel
coronavirus in Wuhan, China, Lancet 395 (2020) 497–506.
[5] G. Li, E. De Clercq, Therapeutic options for the 2019 novel coronavirus (2019-
CoV), Nat. Rev. Drug Discov. 19 (2020) 149–150, https://doi.org/10.1038/s41573-
020-00016-6.
[6] D.X. Liu, T.S. Fung, K.K.L. Chong, A. Shukla, R. Hilgenfeld, Accessory proteins of
SARS-CoV and other coronaviruses, Antivir. Res. 109 (2014) 97–104, https://
doi.org/10.1016/j.antiviral.2014.06.012.
[7] O. Trott, A.J. Olson, AutoDock Vina: improving the speed and accuracy of docking
with a new scoring function, efficient optimization, and multithreading, J. Comput.
Chem. 31 (2) (2010) 455–461, https://doi.org/10.1002/jcc.21334.
[8] J.R. Lopez-Blanco, J.J. Aлина, E.S. Quintanar-Ort, P. Chacon, IM006: internal
coordinates normal mode analysis server, Nucleic Acids Res. 42 (Web Server issue)
(2014) W271–W276, https://doi.org/10.1093/nar/gku339.
[9] Y.H. Kuo, T.J. Kuo, A.S. Yu, M.D. Wu, C.W. Ong, Two novel sesquiterpene lactones,
cytotoxic vernoldin A and B, from Vernonia cinerae, Chem. Pharm. Bull. (Tokyo) 51
(2003) 425–426, https://doi.org/10.1248/cpb.51.425.
[10] J.O. Oladele, O.M. Oyeleke, O.T. Oladele, O.D. Babatope, O.O. Awosanya,
Nitrobenzeno-induced hormonal disruption, alteration of steroidogenic pathway,
and oxidative damage in rat: protective effects of Vernonia amynalia, Clin.
Phytochem. 6 (2020) 1–3.
[11] I. Saracoğlu, F.H. Oztunca, A. Nagatou, U.S. Harpud, Iridoid content and biological
activities of Veronica canadensis subsp. canadensis and V. Cymbalaria, Pharm.
Biol. 49 (11) (2011) 1150–1157.
[12] J.G. Graham, M.L. Quinn, D.S. Fabricant, N.R. Farnsworth, Plants used against
cancer - an extension of the work of Jonathan Hartwell, J. Ethnopharmacol. 73 (3)
(2000) 347–377.
[13] T. Ito, S. Amaitai, N.N. Win, T. Kodama, H. Morita, New sesquiterpene lactones,
vernoladin and A, from the seeds of Vernonia amynalia in Uygur and their
antiproliferative, Bioorg. Med. Chem. Lett. 26 (2016) 3608–3611, https://doi.
org/10.1016/j.bmcl.2016.06.099.
[14] Sanit Thongnenta, Pomsula Chawengkram, Siripong Lirdprapamongkol, Chatchakorn
Evirutong, Jutapit Boonsombat, Prasat Kittiakop, Jimsunon Svasti, Somkas Rakwatrava, Vernoaldinarlimer, A, sesquiterpene lactone
dimer from Vernonia extensa and anti-tumor effects of vernoldin, vernolepin, and
vernoldin on HepG2 liver cancer cells, Bioorg. Chem. 92 (2019) 103197.
[15] Janmaria Simini, Estrella Millán, Solomon M. Ahay, Annette Habuetzelli, Giovanni
Appendino, Eduardo Muñoz, Oscar Taglialatela-Scafati, Poly-Electrophilic
Sesquiterpene Lactones from Vernonia cinerea, Pharm. Biol. 49 (9) (2011) 377.
[16] K.-L. Xin, K.-S. Yuen, C. Castano-Rodriguez, Z.-W. Ye, M.-L. Yeung, S.-Y. Fung,
S. Yuan, C.-P. Chan, K.-Y. Yuen, L. Enjuanes, D.-Y. Jin, Severe acute respiratory
syndrome coronavirus ORF3a protein activates the NLRP3 inflammasome by
promoting TRAF3-dependent ubiquitination of ASC, FASEB J. 31 (8) (2019)
8865–8877.
[17] J.O. Oladele, E.I.O. Ajayi, O.M. Oyeleke, O.T. Oladele, B.D. Olowookere, B.M.
Adeyinji, O.I. Oyewole, Curative potentials of Nigerian medicinal plants in COVID-
19 treatment: a mechanistic approach, Jordan J. Biol. Sci. (2020), in press.
[18] C. Huang, Y. Wang, X. Li, et al., Clinical features of patients infected with 2019
novel coronavirus in Wuhan, China, Lancet 395 (2020) 497–506.
[19] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Experimental and
computational approaches to estimate solubility and permeability in drug
discovery and development settings, Adv. Drug Deliv. Rev. 23 (1–3) (1997) 3–25,
https://doi.org/10.1016/0169-409X(96)00423-1.
[20] J.D. Hughes, J. Blagg, D.A. Price, S. Bailey, G.A. Decrescenzo, R.V. Devraj, E. Ellsworth, Y.M. Fobian, M.E. Gibbs, R.W. Gilles, N. Greene, E. Huang, T. Krieger-Burke, J. Loesel, T. Wager, L. Whiteley, Y. Zhang, Physiochemical drug properties associated with in vivo toxicological outcomes, Bioorg. Med. Chem. Lett. 18 (17) (2008) 4872–4875, https://doi.org/10.1016/j.bmcl.2008.07.071.

[21] A.O. Adeoye, B.J. Oso, I.F. Olaoye, H. Tijanic, A.I. Adebayo, Repurposing of chloroquine and some clinically approved antiviral drugs as effective therapeutics to prevent cellular entry and replication of coronavirus, J. Biomol. Struct. Dyn. (2020), https://doi.org/10.1080/07391102.2020.1765876.

[22] F. Cheng, W. Li, Y. Zhou, J. Shen, Z. Wu, G. Liu, P.W. Lee, Y. Tang, admetSAR: A comprehensive source and free tool for assessment of chemical ADMET properties, J. Chem. Inf. Model. 52 (11) (2012) 3099–3105, https://doi.org/10.1021/acs.jcim.9b00969.

[23] M. Yazdanian, S.L. Glynn, J.L. Wright, A. Havì, Correlating partitioning and caco-2 cell permeability of structurally diverse small molecular weight compounds, Pharm. Res. 15 (9) (1998) 1490–1494, https://doi.org/10.1023/A:1011930411574.

[24] K.M. Srivalli, P.K. Lakshmi, Overview of P-glycoprotein inhibitors: a rational outlook, Braz. J. Pharmacut. Sci. 48 (3) (2012) 353–367, https://doi.org/10.1590/S1984-82502012000300002.

[25] B.J. Oso, E.B. Oyewo, A.T. Oladiji, Influence of ethanolic extracts of dried fruit of Xylopiaaethiopica (Dunal) A. Rich on haematological and biochemical parameters in healthy Wistar rats, Clin. Phytosci. 5 (1) (2019) 9, https://doi.org/10.1186/s40816-019-0104-4.

[26] J.O. Oladele, E.I.O. Ajayi, O.M. Oyelke, O.T. Oladele, B.D. Olowokere, B. M. Adeniyi, O.I. Oyewole, A.T. Oladiji, A systematic review on COVID-19 pandemic with special emphasis on Curative potentials of medicinal plants, Heliyon (2020), https://doi.org/10.1016/j.heliyon.2020.e04897.

[27] G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell, A. J. Olson, AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility, J. Comput. Chem. 16 (2009) 2785–2791.

[28] J.O. Oladele, O.M. Oyelke, O.T. Oladele, B.D. Olowokere, B.J. Oso, A.T. Oladiji, Kolaviron (kolaflavanone), apigenin, fisetin as potential coronavirus inhibitors: in silico investigation, Res. Square Preprints (2020), https://doi.org/10.21203/rs.3.rs-51350/v1.

[29] V.K. Bhardwaj, R. Singh, J. Sharma, V. Rajendran, R. Purushit, S. Kumar, Identification of bioactive molecules from tea plant as SARS-CoV-2 main protease inhibitors, J. Biomol. Struct. Dyn. (2020), https://doi.org/10.1080/07391102.2020.1766572.

[30] G.A. Gyebi, O.B. Ogunro, A.P. Adegunloye, O.M. Ogunyemi, S.O. Afolabi, Potential inhibitors of coronavirus 3-chymotrypsin-like protease (3CLpro): an in silico screening of alkaloids and terpenoids from African medicinal plants, J. Biomol. Struct. Dyn. (2020), https://doi.org/10.1080/07391102.2020.1764868.

[31] J.O. Oladele, O.M. Oyelke, B.O. Akindolie, B.D. Olowokere, O.T. Oladele, Vernonia amygdalina abates oxidative hepatic damage and inflammation associated with nitrobenzene in rat, Jordan J. Biol. Sci. (2021). in press.

[32] J.O. Oladele, O.M. Oyelke, B.D. Olowokere, O.T. Oladele, Bitter leaf (Vernonia amygdalina) modulates nitrobenzene-induced renal damage in rats via suppression of oxidative-inflammatory activities, Serbian J. Exp. Clin. Res. (2021). in press.

[33] J.O. Oladele, O.M. Oyelke, O.T. Oladele, M. Olaniyi, Neuroprotective mechanism of Vernonia amygdalina in a rat model of neurodegenerative diseases, Toxicol. Rep. 7 (2020) 1223–1232, https://doi.org/10.1016/j.toxrep.2020.09.005.