MALAYSIAN JOURNAL OF BIOCHEMISTRY & MOLECULAR BIOLOGY

The Official Publication of The Malaysian Society For Biochemistry & Molecular Biology (MSBMB)
http://mjbmb.org

NOVEL CORONAVIRUS (SARS-COV-2) MAIN PROTEASE: MOLECULAR DOCKING OF PUERARIN AS A POTENTIAL INHIBITOR

Oluwafemi Adeleke Ojo[1*], Adebola Busola Ojo[2], Odunayo Anthonia Taiwo[3], Olarewaju Oluba[1]

[1]*Department of Biochemistry, Landmark University, Omu-Aran, Nigeria*
[2]*Department of Biochemistry, Ekiti State University, Ado-Ekiti, Nigeria*
[3]*Department of Biochemistry, Chrisland University, Abeokuta, Nigeria*

*Corresponding author: ojo.adeleke@lmu.edu.ng

History
Received: 12th January 2021
Accepted: 4th April 2021

Keywords:

Puerarin, molecular docking studies, SARS-CoV-2 main protease, toxicity prediction

Abstract

SARS-CoV-2 is a single-stranded RNA virus that triggered the global pandemic Coronavirus Disease-2019 (COVID-2019). It has infected about 10,021,401 patients and brought forth mortality rate to about 499,913 among 216 countries as cited by WHO. Drugs including Chloroquine and Hydroxychloroquine derivatives are being administered in most urgent cases, although probable side effects on people with metabolic disorders. Thus, the unavailability of authorized drugs and treatment for this pandemic demands the research world to discover natural compounds with potency to cure it. This paper assesses the isoflavonoid puerarin from *Pueraria lobata* as a possible inhibitor of the main protease of SARS-COV-2 (Mpro) via *in silico* approach, for example, molecular docking, Lipinski's rule of five and toxicity prediction (ADME). Puerarin revealed a high binding affinity with the target site of SARS-CoV-2 main protease. This compound slightly meets the criteria of Lipinski's rule. It does not possess properties that could cause adverse effects in humans; thus, making puerarin a potential drug candidate to investigate its usage against COVID-19.

INTRODUCTION

Coronaviruses (CoVs), are single-stranded RNA viruses that can infect animals and humans, resulting in respirational, gastrointestinal, liver, and brain diseases [1]. Being the biggest recognized RNA viruses, CoVs are additionally grouped into four classes: α-coronavirus, betacoronavirus, γ-coronavirus and δ-coronavirus [2]. Thus far, six human coronaviruses (HCoVs) have been recognized, comprising the α-CoVs HCoVs-NL63 and HCoVs-229E and the β-CoVs HCoVs-OC43, HCoVs-HKU1, severe acute respiratory syndrome-CoV (SARS-CoV) [3], and Middle East respiratory syndrome-CoV (MERS-CoV) [4].

From late December 2019, the symptoms such as high fever, cough, tiredness, phlegm production, difficulty in breathing started to increase as a cluster of pneumonia were noticed and recognized as beta-coronavirus in Wuhan, Hubei Province, China [5]. The beta- coronavirus was primarily and officially termed as 2019- novel coronavirus (2019-nCoV) and coronavirus 2019 (COVID- 19) respectively by WHO while the International Committee of Coronavirus Study Group (CSG) suggested the usage of the term SARS-CoV-2 [6]. Through the sequential analysis study of the virus, bat was thought of as the biological host of the virus. This virus is transmitted between humans by binding to angiotensin converting enzyme-2 receptor [7].

Various medications have been suggested, which are largely antiviral drugs and are currently being used clinically [8, 9]. In the meantime, chloroquine phosphate and hydroxychloroquine sulphate are being administered for the urgent treatment of COVID-19 by the recommendations gotten from in vitro and some clinical studies data. However, the Food and Drug Administration (FDA) specified that these drugs are not yet accepted due to some recent experiments revealing that hydroxychloroquine can result in deleterious and reasonable austere effects in individuals

already being treated for metabolic disorders. Furthermore, treatment of hydroxychloroquine was perceived to impede inflammatory cytokines [10].

Due to hydroxychloroquine's side effects on the viral proteins associated with the virus's life cycle, a new effective drug candidate with less side effects needs to be established. *In silico* studies of chemically synthetic drugs, for example, Darunavir have been reported [11]. Also, several treatment target sites for handling COVID-19 have been established. SARS-CoV-2 main protease was preferred as a result of the potential in handling CoV-2 patients and stopping the multiplying process of the virus [12].

Phytomolecules are rapidly produced, cheap and are the right candidate for compositions of drugs of interest. This model of repositioning phytocompounds in drug discovery will accelerate the process. Thus, the exploration for effective natural compounds with promising ability to inhibit the main protease [13]. *Pueraria lobata* also known as Kudza is a fast developing perennial vine arising from China. The plant is eatable with health-sustaining benefits of Kudzu arising from its flowers and roots, which comprise numerous phytomolecules such as isoflavones and saponins [14]. *Pueraria lobata* was reported to have antiviral property against HIV-1 replication by inhibiting the entry of HIV-1 into target cells [15]. The bioactive compound in *Pueraria lobata* is puerarin. Puerarin possesses many pharmacological activities, comprising anti-diabetic, anti-cancer, anti-inflammatory, and antiviral activities [16-19]. Puerarin has been reported to have antiviral properties against the Porcine epidemic diarrhea virus. As a result of these interests, this work engages the molecular docking analysis of puerarin against SARS- CoV-2 main protease.

MATERIALS AND METHODS

Molecular Interaction of Puerarin with SARS-CoV-2 Main Protease

Target Preparation and Docking

SARS-CoV-2 main protease 3D structure was downloaded from Protein Data Bank (PDB with ID number 6W63). To monitor the interaction modes of puerarin with SARS-CoV-2 main protease, the Maestro Molecular Modeling platform (version 11.5) by Schrödinger, LLC was used. The Protein Preparation Wizard module was used to prepare the structure. The Glide receptor grids constructed based on the co-crystalized ligand-binding sites in the Glide application (Glide, version) of Maestro. The center of each grid was arranged at the centroid of the crystalized ligand-binding site which is set with inner (acceptable space for the ligand center) and outer (search space surrounded all the ligand atoms) box sizes of 10 and 20 Å, respectively by virtual screening workflow [20]. The extra precision (XP) Workflow in Maestro was used to dock puerarin with SARS-CoV-2 main protease. During the molecular docking full flexibility of the protein and puerarin were considered. The maestro software was used to determine protein-ligand interaction.

ADMET Predictions

ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) analyses establish a drug molecule's pharmacokinetics. Lipinski's Rule of 5 (RO5) was aimed to set instructions for the druggability of novel molecular or chemical structures to differentiate between the druggable and non-druggable compounds [21]. Swissadme (http://www.swissadme.ch) and admetSAR (predated.bmdrc.kr) servers were used in this study to predict and describe significant druglikeness such as mutagenicity, toxicological dosage level and pharmacologically relevant properties of the compounds [22].

RESULTS AND DISCUSSION

The 3D structure of the puerarin and lopinavir was modelled and used as a target for docking simulation against SARS-CoV-2 (Figure 1). Puerarin and lopinavir structures (Figure 2) were downloaded from pubmedchem and prepared for the docking (3D) using Maestro Molecular Modeling platform.

With the aim of combating the high death rate caused by SARS-CoV-2 virus, numerous compounds are already in trial to give an antidote to this terrible disease plague. Numerous scholars have gone on several studies that can be employed to derive various therapeutic substitutes in the cure of the SARS-CoV-2 virus [23]. Furthermore, hydroxychloroquine is being given to emergency cases, though, it was reported to elevate the hydrogen ion concentration of protease and documented as a possible SARS-CoV-2 inhibitor [5, 23]. Although hydroxychloroquine is not without severe side effects but effective, there is a need to provide alternative measures with little or no side effects. Hence, natural plant products such as puerarin are safe, cheap, readily available with no known side effects.

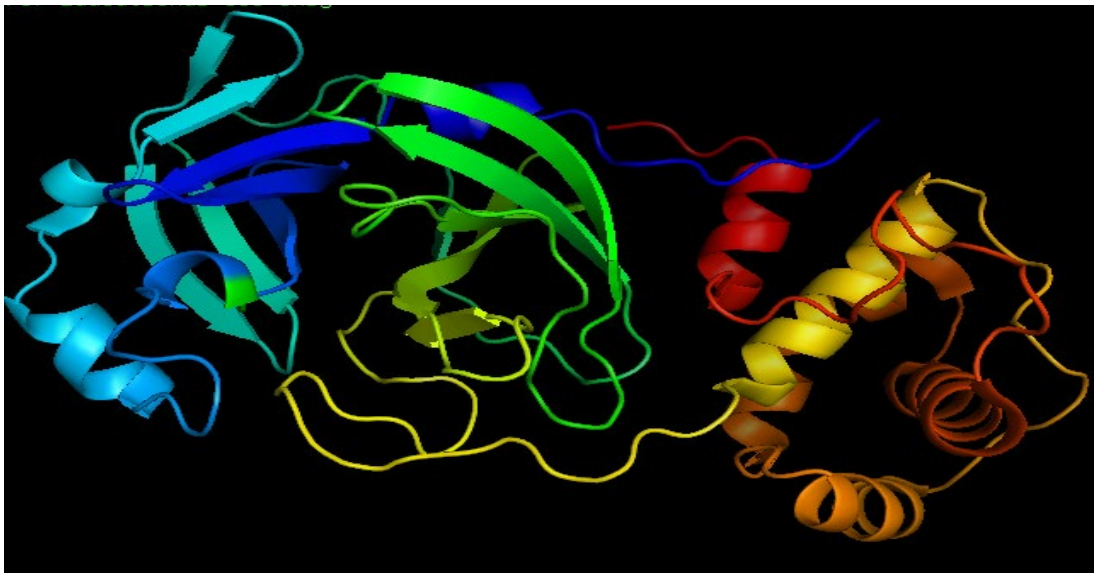

Figure 1. The three dimensional (3D) structure of SARS-CoV-2 main protease

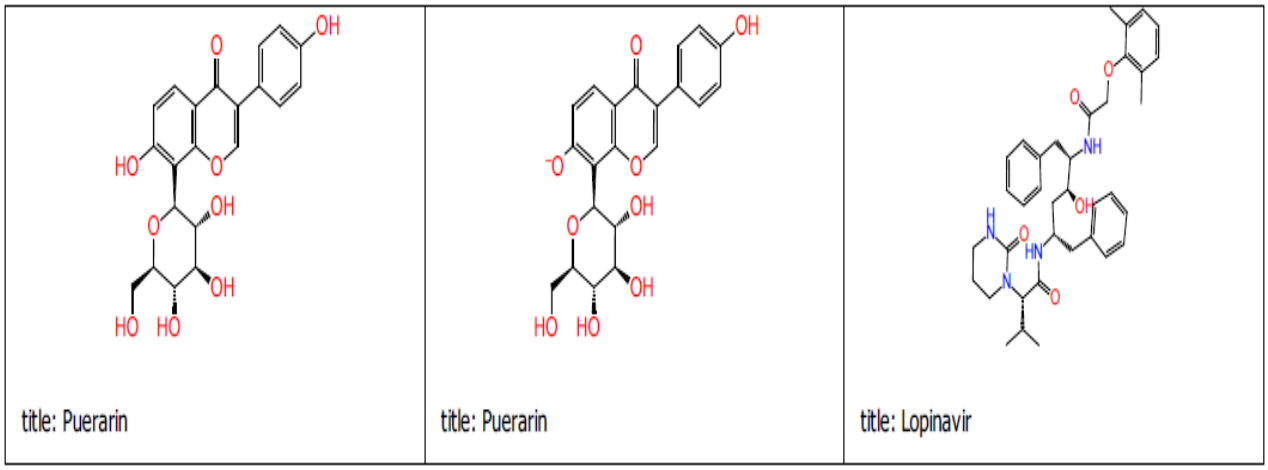

Figure 2. 3D structure of Puerarin (with –OH and –O) and Lopinavir

The molecular docking scores and binding affinity of puerarin with –OH, puerarin with –O and lopinavir (standard protease inhibitor) with SARS-CoV-2 protease produced negative values for free energy -8.070 and -4.658 Kcal/mol as well as lopinavir with -8.081 Kcal/mol in the grid box, indicating high interaction with the binding pocket (Table 1). Puerarin with –OH revealed a good docking score and higher binding interaction found rooted into the binding cavity of SARS-CoV-2, displaying all the major interaction (Figure 3) compared with lopinavir, a protease inhibitor (Figure 5). Also, lopinavir showed a good docking pose with SARS-CoV-2 than puerarin with –O which exhibited the lowest binding energy (Figure 4).

Table 1. Showing binding energy in Kcal/mol of puerarin with SARS-CoV-2

Compounds	Docking Score (Kcal/mol)
Puerarin with -OH	-8.070
Puerarin with -O	-4.658
Lopinavir	-8.081

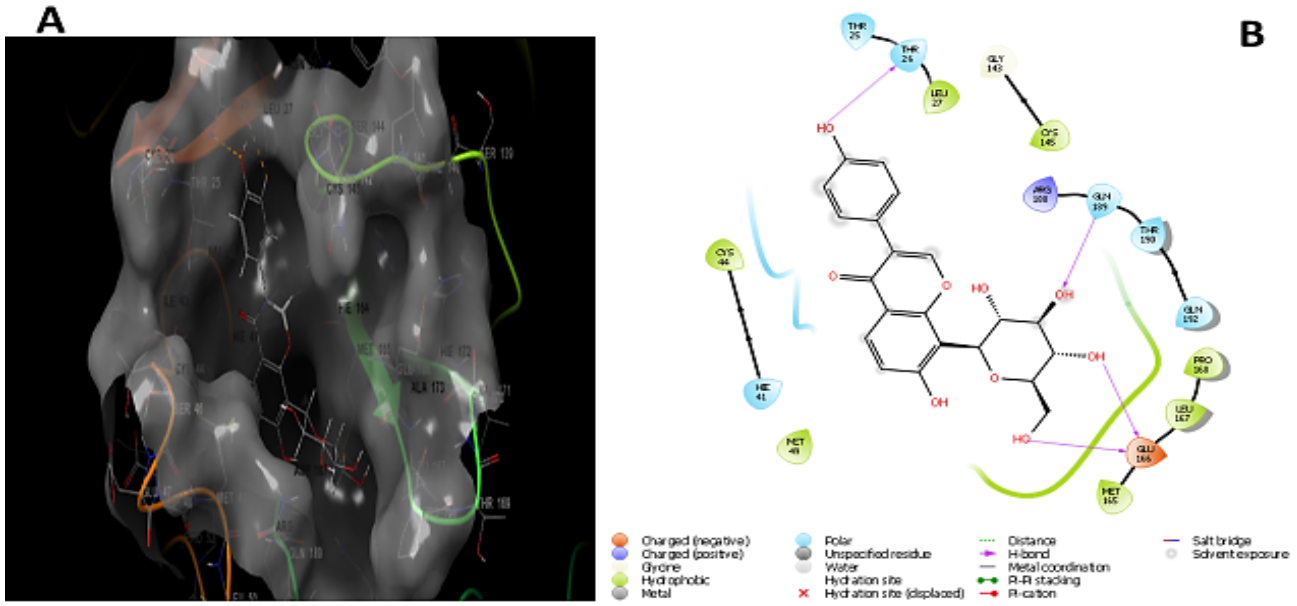

Figure 3. Binding pose and binding site of puerarin (OH) with SARS-CoV-2 main protease (Panel A), molecular interaction of puerarin with amino acid residues within the binding pocket of SARS-CoV-2 main protease (Panel B)

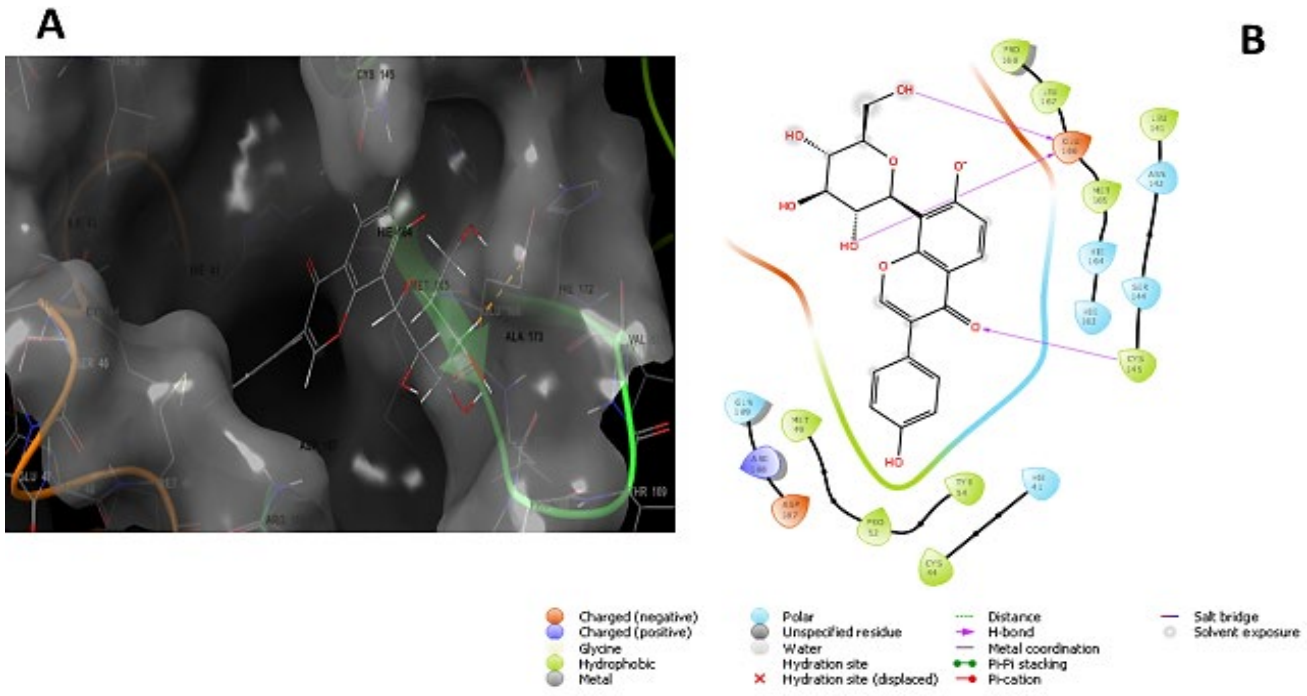

Figure 4. Binding pose and binding site of puerarin isomer (with –O) with SARS-CoV-2 main protease (Panel A), molecular interaction of puerarin isomer with amino acid residues within the binding pocket of SARS-CoV-2 main protease (Panel B)

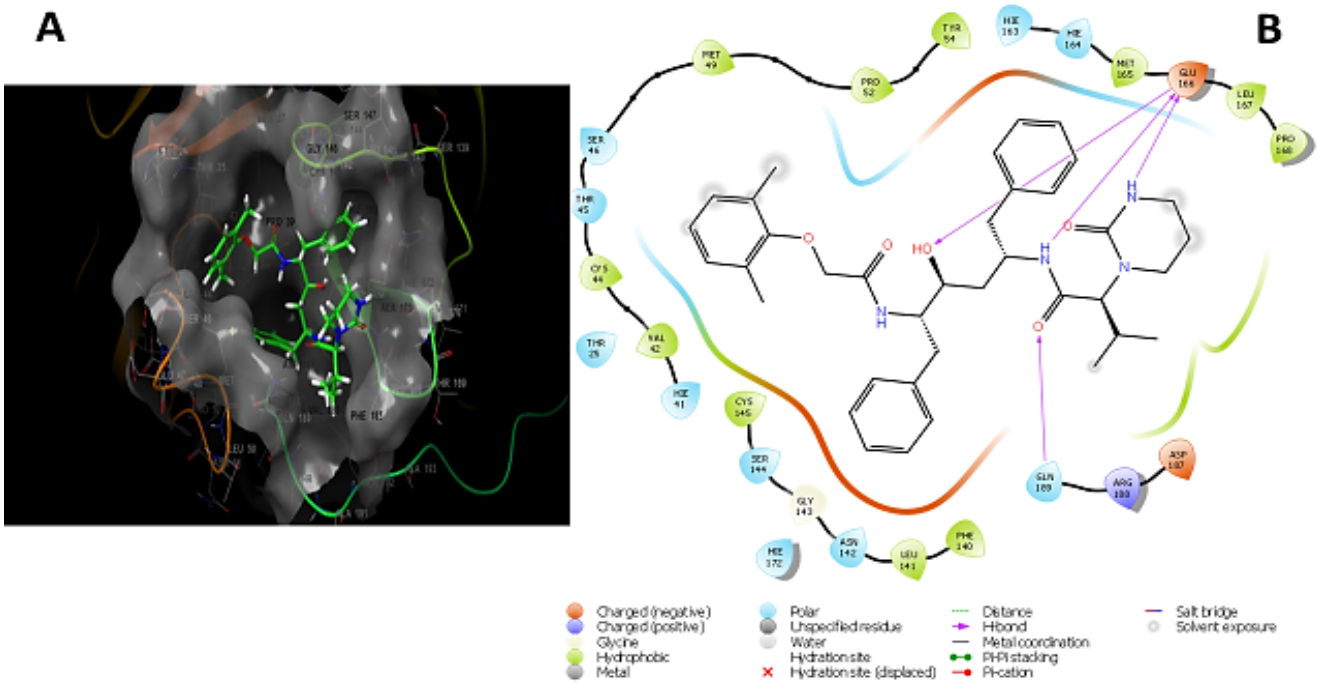

Figure 5. Binding pose and binding site of lopinavir with SARS-CoV-2 main protease (Panel A), molecular interaction of lopinavir with amino acid residues within the binding pocket of SARS-CoV-2 main protease (Panel B)

Puerarin, a potent bioactive compound extracted from *Pueraria lobata* was used in this study due to its overwhelming properties. This compound was analyzed using molecular simulation tools against the SARS-CoV-2 main protease virus showing a high binding affinity (-8.070 Kcal/mol) with the active site residues compared to lopinavir, a protease inhibitor with a binding energy value of -8.081 Kcal/mol. Although, some bioactive compounds reported to inhibit SARS-CoV-2 protease was not capable of proving their binding affinity when likened to puerarin. Examples of such compounds include quercetin -8.58 Kcal/mol, demethoxycurcumin -8.17 Kcal/mol and kaempferol -9.41 Kcal/mol, respectively [13]. Thus indicating that puerarin has a better inhibitory property against SARS-CoV-2 protease.

Data presented in Table 2 shows that puerarin slightly meets all the requirements of the rule of five. Puerarin has H-bond donor value of 6. Lipinski's rule of five is a technique used to assess the drug-likeness of a chemical compound to ascertain whether a chemical compound has certain biological activities or pharmacological properties that would make it a possible effective oral drug in humans [25]. It also aids in illustrating the high possibility of failure or success due to drug-likeness of molecules fulfilling the underlying rules such as; molecular mass ≤ 500 Dalton, high lipophilicity, which is represented as LogP, should be less than 5, hydrogen bond donors not greater than 5, hydrogen bond acceptor not more than 10 and molar refractivity should be between 40-130 [24, 25].

Table 2: The evaluation of the oral drug-likeness of the puerarin using Lipinski's rule of five filters

Compound	Molecular weight (Dalton)	Log P	Number of HBD	Number of HBA	Molar refractivity	XLogP
Puerarin	414.381	1.77	6	9	104.59	0.01
Lopinavir	628.80	6.39	4	5	187.92	5.92

HBD; Hydrogen bond donor; HBA: Hydrogen bond acceptor

Data as predicted using admetSAR server revealed the prospective ADME profiles of the puerarin. ADMET properties, as derived from admetSAR server, reveal that puerarin had no significant ADME properties that could cause antagonistic effects in humans (Table 3). It was predicted to have high human intestinal absorption (HIA), not blood brain barrier permeant, inhibits CYP2C19, CYP2C9 and CYP3A4 cytochromes, and plasma protein binding value was found to be 54.383253. Skin and MDCK (Madin Darby canine kidney) permeability values was -4. 6011 and 3.99199 respectively. The result in this study (AMES Test) shows puerarin is not a potential mutagen and non-carcinogen.

Table 3: ADMET properties of puerarin predicted from admetSAR

Properties	Puerarin	Lopinavir
BBB	0.0372427	0.9916
HIA	54.397793	0.6593
Caco-2	6.03338	0.8856
Ames Toxicity	Non-toxic	Non-toxic
Carcinogenicity	Non- carcinogen	Non- carcinogen
Pgp inhibition	Non	Inhibitor
CYP 2C19 inhibition	Inhibitor	Inhibitor
CYP 2C9 inhibition	Inhibitor	Non
CYP 2D6 inhibition	Non	Non
CYP 2D6 substrate	Non	Non
CYP 3A4 inhibition	Inhibitor	Inhibitor
CYP 3A4 substrate	Weakly	Substrate
Plasma Protein Binding	54.383253	1.157
Pure water solubility mg/L	377.993	-3.4144
Skin Permeability	-4.6011	-5.93

Abbreviations: ADMET**:** absorption, distribution, metabolism, and excretion-toxicity; BBB: Blood brain barrier; Caco-2: Caco-2 permeability; CYP, cytochrome; HIA: human intestinal absorption; P-gp: permeability glycoprotein.

Puerarin revealed exceptional druggability properties, which confirms its molar refractivity, which indicates its ability to pass through specific biomembranes amid weak or strong interactions. Furthermore, the lipophilicity of puerarin reveals its characteristics for oral absorption. Though puerarin slightly obeys the Lipinski rule of five, it can act as a drug candidate which is laudable of testing in biological systems.

The ADME data, predicted that puerarin had no significant ADME properties that can lead to serious side effects in humans. It is also a non-inhibitor of p-gp inhibition indicating the ability to liberate phosphate group from adenosine triphosphate and concurrent bonding of adenosine diphosphate to glycoprotein. The capability of puerarin to infiltrate blood brain barrier is of remarkable benefit, as it suggests a therapeutic effect against glucose – neurotoxicity. The prediction of human absorption via Caco-2 based penetration assay is routinely performed during drug development. However, puerarin could satisfy as a good drug as it has an easy passage through the blood-brain barrier, human intestinal absorption and Caco-2 penetration. Puerarin is also not mutagen neither carcinogenic, as indicated in the data present.

The safety and toxicity of medicinal plants remain a big concern to health practitioners, particularly modulation of cytochrome P450 enzyme family (CYP) and P-glycoprotein (P-gp) due to the therapeutic consequence they have, as CYPs are the most recognized enzymes involved in the biotransformation of drug [24]. Hence, the predicted inhibition of CYP2C9, CYP2C19, and CYP3A4 by puerarin proposes its significant role in drug metabolism. Puerarin does not halt the metabolism of numerous beneficial drugs through the inhibition of CYP2C19, oxidation of steroids, fatty acids and biotransformation of drugs, in addition to the synthesis and degradation of hormones via the inhibition of CYP3A4. Taken together, puerarin has a good binding affinity with SARS-CoV-2 slightly meeting the Lipinski's rule of five and has good ADME toxicity profile. The ADMET prediction revealed puerarin as a safe compound that can be given as a drug. Although the properties are considerable *in silico* data, additional findings from *in vitro*, clinical, and preclinical investigations addressing SARS-CoV-2 should be deliberated.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

REFERENCES

1. Weiss SR, Leibowitz JL. Coronavirus pathogenesis. Adv Virus Res 2011; 81:85-164.
2. Yang D, Leibowitz JL. The structure and functions of coronavirus genomic 3' and 5' ends. Virus Res 2015; 206:120-33.
3. Drosten C, Günther S, Preiser W. Identification of a Novel Coronavirus in Patients with Severe Acute Respiratory Syndrome. N Engl J Med 2020; 348:1967-76.
4. Zaki AM, Boheemen Sv, Bestebroer TM, Osterhaus ADME, Fouchier RAM. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med 2012; 367:1814-20.
5. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, Wang B, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. *JAMA*, 2020; 323(11), 1061.
6. Guo Y, Cao Q, Hong Z, Tan Y, Chen S, Jin H, Tan K, Wang D, Yan Y. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak – an update on the status. *Military Medical Research*, 2020; 7(1).

7. Zhou P, Yang X, Wang X, Hu B, Zhang L, Zhang W, Si H, Zhu Y, Li B, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*, 2020; 579(7798), 270-273

8. Gautret P, Lagier J, Parola P, Hoang VT, Meddeb L, Mailhe M, Doudier B, Courjon J, Giordanengo V, Vieira VE, Dupont HT, Honoré S, Colson P, Chabrière E, La Scola B, Rolain J, Brouqui P, Raoult D. Hydroxychloroquine and azithromycin as a treatment of COVID-19: Results of an open-label non-randomized clinical trial. *International Journal of Antimicrobial Agents,* 2020; 105949.

9. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The Lancet*, 2020; 395(10223), 497-506.

10. Guastalegname M, Vallone A. Could chloroquine/Hydroxychloroquine be harmful in coronavirus disease 2019 (COVID-19) treatment? *Clinical Infectious Diseases* 2020; https://doi.org/10.1093/cid/ciaa321.

11. Khan SA, Zia K, Ashraf S, Uddin R, Ul-Haq Z. Identification of chymotrypsin-like protease inhibitors of SARS-Cov-2 via integrated computational approach. *Journal of Biomolecular Structure and Dynamics*, 2020; 1-10.

12. Jin Z, Du X, Xu Y, Deng Y, Liu M, Zhao Y, Zhang B, et al. Structure of Mpro from COVID-virus and discovery of its inhibitors. *Nature*. 2020; https://doi.org/10.1038/s41586-020-2223-y

13. Khaerunnisa S, Kurniawan H, Awaluddin R, Suhartati S, Soetjipto S. Potential inhibitor of COVID-19 main protease (Mpro) from several medicinal plant compounds by molecular docking study. 2020; https://doi.org/10.20944/preprints202003.0226.v1

14. Li G, Zhang Q, Wang Y. Chemical constituents from roots of *Pueraria lobata*. Zhongguo Zhong Yao Za Zhi. 2010;35:3156–60.

15. Mediouni S, Jablonski JA, Tsuda S, Richard A, Kessing C, Andrade MV, Biswas A, Even Y, Tellinghuisen T, Choe H, Cameron M, Stevenson M, Valente ST. Potent suppression of HIV‑1 cell attachment by Kudzu root extract. *Retrovirology.* 2018 15:64.

16. Wu, K., T. Liang, X. Duan, L. Xu, K. Zhang and R. Li. Anti-diabetic effects of puerarin, isolated from Pueraria lobata (Willd.), on streptozotocin-diabetogenic mice through promoting insulin expression and ameliorating metabolic function. Food Chem. Toxicol. 60: 341–347, 2013.

17. Gan M, Yin X. Puerarin induced in mantle cell lymphoma apoptosis and its possible mechanisms involving multi-signaling pathway. Cell Biochem Biophys. 2015;71(1):367–373.

18. Zhu C, Wu Y, Jiang Z, Zheng C, Wang L, Yang X, et al. Dietary soy isoflavone attenuated growth performance and intestinal barrier functions in weaned piglets challenged with lipopolysaccharide. Int Immunopharmacol. (2015) 28:288–94.

19. Ullah MZ, Khan AU, Afridi R, Rasheed H, Khalid S, Naveed M, et al. Attenuation of inflammatory pain by puerarin in animal model of inflammation through inhibition of pro-inflammatory mediators. Int Immunopharmacol. 2018; 61:306-16.

20. Bagherzadeh K, Daneshvarnejad K, Abbasinazari M, Azizan H. In silicon repositioning for dual inhibitor discovery of SARS-CoV-2 (COVID-19) 3C-Like protease and papain-like peptidase. 2020; Doi:10.20944/preprints202004.0084.v1

21. Ojo OA, Aruleba RT, Adekiya TA, Sibuyi NR, Ojo AB, Ajiboye BO, Oyinloye BE, Fadaka AO. Deciphering the interaction of Puerarin with cancer macromolecules: An *in silico* investigation. *Journal of Biomolecular structure and dynamics* 2020; doi: 10.1080/07391102.2020.1819425.

22. Elekofehinti OO, Opeyemi I, Ayodeji F, Damilare A, Bulu Y, Rocha JBT, Ejelonu OC, Kamdem JP, Akinlosotu O. Discovery of potential vistatin activators using in silico docking and ADME predictions as therapy for type-2- diabetes. Beni –suef University Journal of Basic and applied Sciences 2018; Doi.org/10.1016/j.bjbas.2018.02.007

23. Lipinski CA. Lead- and drug-like compounds: the rule-of-fiverevolution. Drug Discov Today Technol 2004; 1: 337-41.

24. Oyinloye BE, Adekiya TA, Aruleba T, Ojo OA, Ajiboye BO. Structure based docking 4studies of GLUT4 towards exploring selected phytochemicals from *Somalia xanthocarpum* as a therapeutic target for the treatment of cancer. *Current Drug Discovery Technologies*, 2019**;** 16(4): 406-416 doi: 10.2174/1570163815666180801152110**.**

25. Ojo OA, Afon AA, Ojo AB, Ajiboye BO, Okesola MA, Aruleba RT, Adekiya TA, Oyinloye BE. *Spondias mombim* L. (Anacardiaceae): chemical fingerprints, inhibitory activities and molecular docking on key enzymes relevant to erectile dysfunction and Alzheimer's diseases. J Food Biochem, 2019; 43(3):e12772; doi:10.1111/jfbc.12772