Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
NAAS Rating: 3.53
www.plantsjournal.com
JMPS 2020; 8(4): 14-19

Received: 09-05-2020
Accepted: 10-06-2020

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Molecular docking of quassinoid compounds javanicolides A-F and H with C3-like protease (or 3CL^{pro}) of SARS and SARS-C0V-2 (COVID-19) and VP8* domain of the outer capsid protein VP4 of rotavirus A

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Abstract
Corona virus SARS-CoV-2, otherwise known as COVID-19 has created a pandemic from which as of June 13, 2020 the virus has infected 7,778,242 people throughout 213 countries of the world and caused deaths of 429,014 persons. COVID-19 is not the only virus troubling humans. Prior to SARS-CoV-2, there was SARS and MERS. Also according to the World Health Organization (WHO), rotaviruses are the most common causes of diarrheal episodes in children under 5 years of age and about 215,000 children die each year from rotavirus infections. Thus far there has been no discovery of therapeutics like drugs or vaccines effective against COVID-19. Four oral live attenuated rotavirus vaccines are available, but are less efficacious in low income countries. COVID treatments are costly and place a heavy financial burden on the society and the infected individual. Drugs like Ivermectin and Remdesivir are effective against only a fraction of the COVID patients; the drugs are yet to undergo full clinical trials against COVID-19. Against this backdrop, it is of utmost importance to explore the plant kingdom for phytochemical(s), which may be effective against multiple types of viruses. In this study we report the high binding affinities of several javanicolides (quassinoid group of compounds) to SARS and SARS-CoV-2 (COVID-19) C3-like protease (or 3CL^{pro}), and VP8* domain of the outer capsid protein VP4 of rotavirus A. The results suggest that the javanicins can turn out to be lead compounds for developing effective therapeutics against SARS, SARS-CoV-2 and rotavirus A.

Keywords: Javanicolide, simaroubaceae, SARS, SARS-CoV-2, rotavirus, molecular docking

Introduction
It has been hypothesized that interaction between human beings and viral agents was possibly a key factor in shaping human evolution and culture [1]. It is very much possible that although viral diseases have emerged much earlier, there were not enough human hosts in those periods to create a pandemic. However, the situation has changed; the humans are now densely populating the planet, which enables previously innocuous viruses to start a global pandemic. Many viral infections like human immunodeficiency virus (HIV), dengue, Nipah and Ebola now pose a direct threat to human existence. Other viral diseases, which are emerging as serious threats include rotaviruses and various types of corona viruses. The latter includes SARS (Severe Acute Respiratory Syndrome), MERS (Middle East Respiratory Syndrome), and SARS-CoV-2, the latter being the cause of the current pandemic. According to the World Health Organization (WHO), as of June 13, 2020 the virus (COVID-19) has infected 7,778,242 people throughout 213 countries of the world and caused deaths of 429,014 persons. Also according to WHO, rotaviruses are the most common causes of diarrheal episodes in children under 5 years of age and about 215,000 children die each year from rotavirus infections [2].
Rotaviruses are non-enveloped RNA viruses belonging to the Reoviridae family. The nucleocapsid is composed of three concentric shells; the outer layer contains two structural viral proteins (VP), namely VP4 and VP7 [3]. The VP8* domain of the VP4 protein (spike protein) mediates attachment of rotavirus to specific cell surface glycans [4].

Although VP8* is the least conserved among rotavirus structural proteins of the 37 P genotypes, VP8* is structurally conserved with a galectin-like fold [5]. The C3-like protease or 3CL^{pro} of both SARS and SARS-CoV-2 play a major role in the viral replication and have a highly similar 96% identity [6]. Thus this protease can be a prime target for designing inhibitors or testing potential inhibitor(s) in virtual screening approaches and the same principle applies for the VP8* domain of the VP4 protein of rotavirus A.

Existing rotavirus vaccine(s) efficacy ranges from 50 to less than 90% in severe diarrhea with moderate efficacy in lower socioeconomic countries [2]; the vaccines are prone to slightly elevated risk of a rare, serious condition called intussusception, which can result in potentially fatal bowel obstruction. Any vaccines or drugs to SARS and SARS-CoV-2 are yet to be discovered. As such, recent scientific attention has turned to the plant kingdom in an effort to discover plants and/or phytochemicals, which can be effective against SARS, SARS-CoV-2 and rotavirus. The *in vitro* anti-rotavirus activity of several medicinal plants has been reported from Brazil [7].

Scientific attention has also turned to plants and associated phytochemicals for COVID-19 treatment. Baicalein, isorhamnetin, kaempferol, luteolin, and naringenin were some phytochemicals among the 10 plant-based compounds reported to be of possible therapeutic use against COVID-19 on the basis of molecular docking studies with COVID-19 proteins like the 3CL^{pro} [8]. A total of 253 compounds from Mongolian medicinal plants were screened for their effectiveness in binding to 3C-like protease of COVID-19 through the molecular docking method; of them, two compounds, phillyrin and chlorogenic acid were found to have potential therapeutic uses based on their binding to 3CL^{pro} [9].

Simaroubaceae family plants can be found in Indonesia, India and other tropical countries. Besides other ethnic uses, some of the plants like *Picrasma javanica* Blume have indigenous uses as an antiviral [10], making their phytochemicals an interesting object to study against viral diseases. For a comparative analysis we assessed through molecular docking studies whether the same phytochemicals can bind to specific targets of SARS, SARS-CoV-2 and rotavirus A. For purposes of this paper we selected 7 quassinoid compounds from plants belonging to this family, namely javanicolides A-F and H. Javanicolides A-F and H have been reported from seeds of *Brucea javanica* Merr. [11-14]. All phytochemicals were assessed for their binding energies and docking interactions with C3-like protease (or 3CL^{pro}) of SARS and SARS-C0V-2 (COVID-19) and VP8* domain of the outer capsid protein VP4 of rotavirus A.

Methods

A. Molecular docking studies with SARS and SARS-CoV-2 C3-like protease

Three-dimensional structure of COVID-19 and SARS major protease (3C-like protease)

The pdb file (6LU7) of the main protease of SARS-CoV-2 3C-like protease or SARS-CoV-2 3CL^{pro} as previously published by Professor Zihe Rao and his colleagues [15] was used in the present study. An inhibitor (called N3) was removed from the pdb file before using the protein's structure in our molecular docking studies. The active residues of SARS-CoV-2 3C-like protease are His41 and Cys145. Monomeric form of protein was used for molecular docking. The same protease from SARS (pdb: 3M3V) was used for docking studies with the same javanicolide phytochemicals. The two proteases (SARS-CoV 3CL^{pro} and SARS-CoV-2 3CL^{pro}) share a 96% sequence identity and have a highly similar three dimensional structure [16]. Binding of some selected javanicolides to SARS-CoV-2 3CL^{pro} have been shown to illustrate the binding site of the phytochemicals to the protease binding domain and the amino acids involved in the binding.

Compounds used in docking studies

We have studied javanicolides (quassinoid type of phytochemicals) known to occur in *Brucea javanica*. Ligand molecules were downloaded from Pubchem [17] in sdf format. They were optimized with the force field type MMFF94 using Openbable softwares and saved as pdbqt format.

Ligand molecular docking studies

We have conducted molecular docking (blind) using AutoDock Vina [18]. We report ΔG values as an average of five values from the docking program. In our figures, we show the pose of phytochemicals bound to SARS-CoV-2 main protease as obtained from PyMOL and displayed in Discovery Studio [19].

Phytochemicals

The structures of the javanicolides are shown in Figure 1.

B. Molecular docking studies with VP8* domain of the outer capsid protein VP4 of rotavirus A

Three-dimensional structure of receptor

We have used the pdb file (4YFW) of the VP8* domain of the outer capsid protein VP4 of human rotavirus A [20]. We have added polar hydrogen and removed water from the pdb structure. Monomeric form of the protein was used for molecular docking.

Compounds used in docking studies

We have studied the phytochemicals as described before in Section A of Methods.

Molecular docking

We have conducted molecular docking using AutoDock Vina in its blind mode where the GRID box used was large enough to cover the entire protein structure. An exhaustiveness of 16 was used. Poses were ranked on the basis of estimated free energy of interaction (ΔG, kcal/mol) and only the highest rank (that is with the lowest ΔG) pose for each ligand was considered. Also for each phytochemical, three independent docking runs were performed and the ΔG values were reported as average. Figures showing possible mode of interaction with the protein were made using PyMOL (https://pymol.org/2/) whilst the 2D ligand interaction diagrams were made using Discovery Studio as described above.

Results and Discussion

The binding energies of javanicolides A-F and H are shown in Table 1. All the javanicolides tested demonstrated good binding affinities to not only the C3-like protease of SARS and SARS-CoV-2 but also (with the exception of javanicolide A) to the VP8* domain of the outer capsid protein VP4 of human rotavirus A. The results suggest that these quassinoid compounds can possibly act as inhibitors to various viral proteases, assuming that their binding would inhibit the respective viruses. To our knowledge, this is the first study of

javanicolide binding studies (through molecular docking) to key proteins of three virus species, which play a major role in replication of the respective viruses. Thus this group of compounds can potentially play simultaneous therapeutic roles in multi-viral diseases.

Table 1.

Phytochemical	Binding energy (ΔG, kcal/mol)		
	COVID-19	SARS	Rotavirus
Javanicolide A	-7.7	-7.7	-6.7
Javanicolide B	-7.3	-7.9	-7.3
Javanicolide C	-7.1	-7.4	-7.4
Javanicolide D	-7.5	-7.5	-7.6
Javanicolide E	-7.2	-7.7	-7.6
Javanicolide F	-7.0	-7.3	-7.7
Javanicolide H	-7.4	-7.7	-7.7

The highest binding energy (-7.7 kcal/mol) with the C3-like protease of SARS-CoV-2 was obtained with javanicolide A, and the lowest binding energy of -7.0 kcal/mol with the same protease was obtained with javanicolide F. With C3-like protease of SARS, the highest binding energy (-7.9 kcal/mol) was obtained with javanicolide B, and the lowest (-7.3 kcal/mol) with javanicolide F. Against the VP8* domain of the outer capsid protein VP4 of human rotavirus A, the highest binding energies of -7.7 kcal/mol each were obtained with javanicolides F and H, and the lowest (-6.7 kcal/mol) was obtained with javanicolide A. The interacting residues of the inhibitor N3 with the C3-like protease of SARS-CoV-2 include amino acids His41, Met49, Phe140, Leu141, Asn142, Gly143, His163, His164, Glu166, Leu167, Pro168, Gln189, Thr190, and Ala191. However, possibly binding of both the active amino acid residues His41 and Cys145 of the C3-like protease to the same ligand position enhances binding affinity of the protease to the ligand, as can be seen in comparing javanicolides A, F and D interactions with the protease (Figures 2-4) and their binding energies (Table 1).

In rotaviruses, initial cell attachment occurs following recognition of specific cellular glycans; this is mediated by the distally located VP8* domain of the spike protein VP4 [20-22]. VP8* of human rotavirus recognizes histo-blood group antigens (HBGAs) in a type-specific manner [23]. Rotavirus strains may or may not (depending on the strain) recognize the terminal N-acetyl neuraminic (sialic) acid residues of carbohydrates on the host cells for attachment. As a result, neuraminidase treatment leads to decrease in interaction between host cell and virus. Even virus resistant to neuraminidase treatment might react with sialic acids located in a different context [24]. For inhibition of viral attachment, an emerging strategy is to develop multivalent sialic acid-based inhibitors [25]. Thus javanicolides may be acting through substitution of sialic acid as an attachment to VP8* domain of the spike protein VP4.

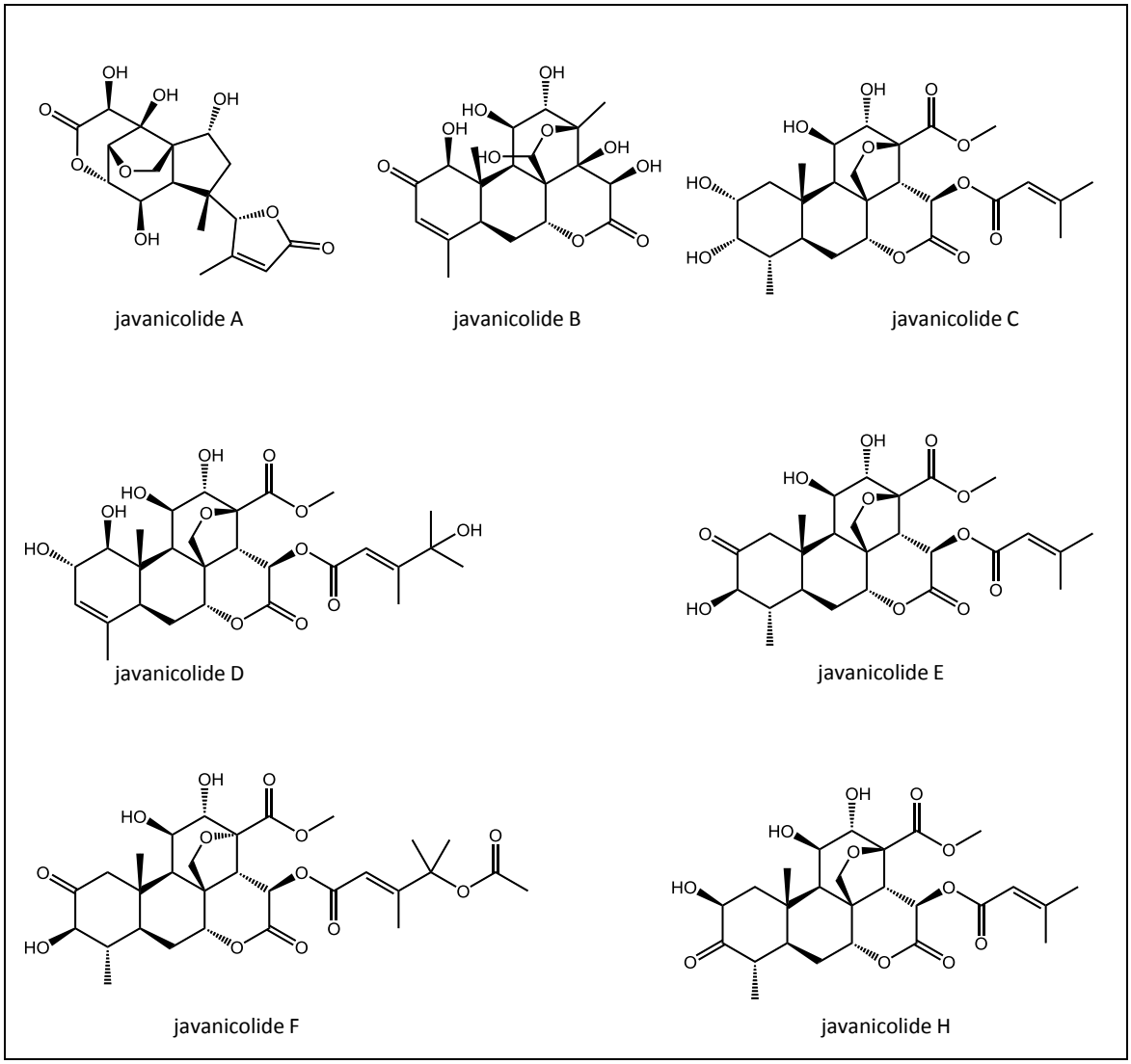

Fig 1: Structure of the javanicolides.

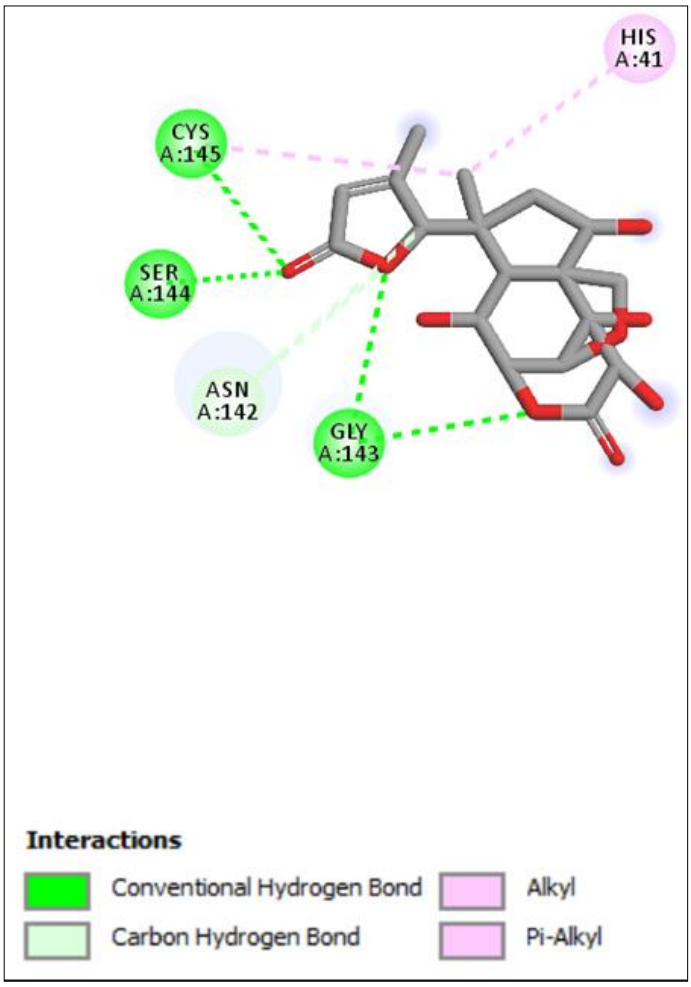

Fig 2: Depiction of javanicolide A interactions with C3-like protease of SARS-CoV-2. To be noted is that the compound binds to both active amino acid residues of the binding pocket of the protease, namely His41 and Cys145. Also His41 and Cys145 bind to the same site, which would strengthen the binding.

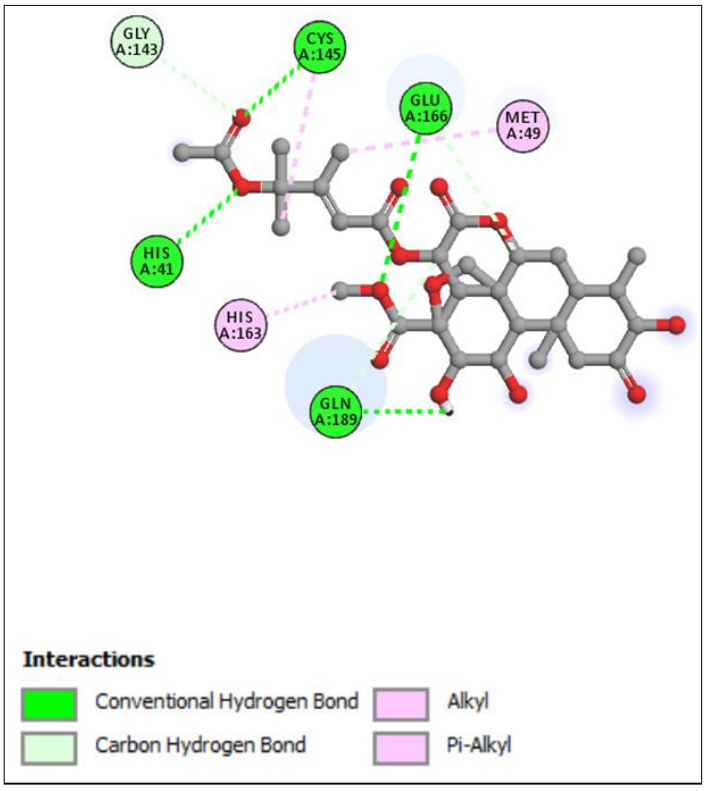

Fig 3: Depiction of javanicolide F interactions with C3-like protease of SARS-CoV-2. To be noted is that the compound binds to both active amino acid residues of the binding pocket of the protease, namely His41 and Cys145. However, unlike javanicolide A, javanicolide F does not interact with Ser144, thus making hydrogen bonding weaker.

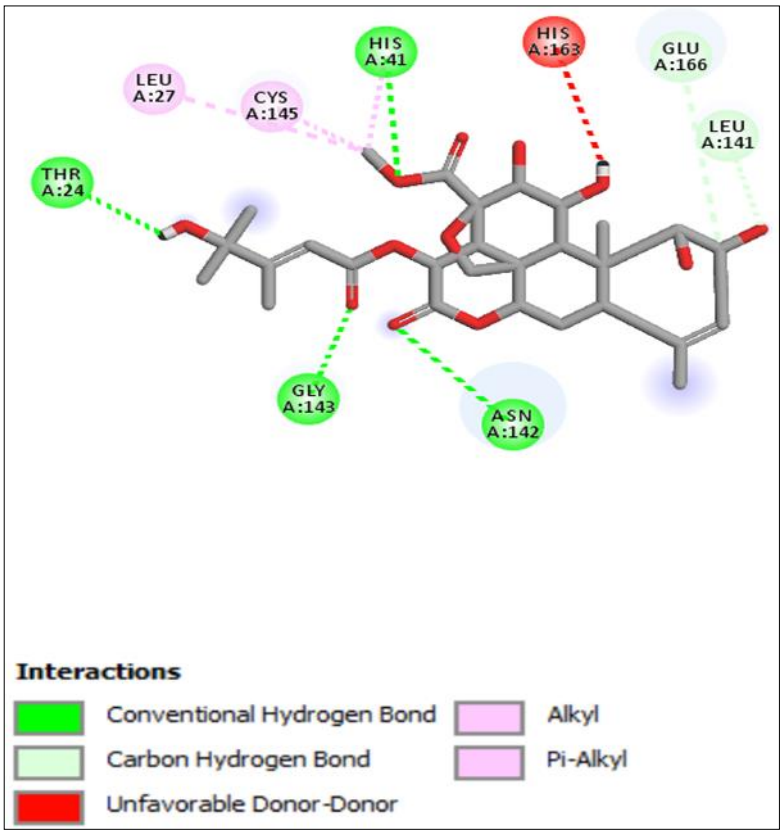

Fig 4: Depiction of javanicolide D interactions with C3-like protease of SARS-CoV-2. To be noted is that the compound binds to both active amino acid residues of the binding pocket of the protease, namely His41 and Cys145. Also His41 and Cys145 bind to the same site, which would strengthen the binding more than say javanicolide F.

Conclusions

Molecular docking analysis of binding of various javanicolides with C3-like protease (or $3CL^{pro}$) of SARS and SARS-C0V-2 (COVID-19) and VP8* domain of the outer capsid protein VP4 of rotavirus A suggest that these compounds merit potential for further anti-viral laboratory experiments towards potential use as anti-viral drugs.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Acknowledgements

This work was funded solely by the authors.

References

1. de Souza Leal É, de Andrade Zanotto PM. Viral diseases and human evolution. Mem Inst Oswaldo Cruz. 2000; 95(1):193-200.
2. World Health Organization (WHO). Immunization, Vaccines and Biologicals. Rotavirus, 2018. [https://www.who.int/immunization/diseases/rotavirus/en/]
3. Payne DC, Stockman LJ, Gentsch JR, Parashar UD. VPD Surveillance Manual, 4th Edition. Rotavirus, Chapter 13, 2008.
4. Xu S, Liu Y, Tan M, Zhong W, Zhao D, Jiang X *et al*. Molecular basis of P[6] and P[8] major human rotavirus VP8* domain interactions with histo-blood group antigens. bioRxiv preprint, 2019. Doi: https://doi.org/10.1101/512301, January 4.
5. Trojnar E, Sachsenröder J, Twardziok S, Reetz J, Otto PH, Johne R. Identification of an avian Group A rotavirus containing a novel VP4 gene with a close relationship to those of mammalian rotaviruses. J Gen Virol. 2013; 94(1):136-142.
6. Chen YW, Yiu C.-PB, Wong K.-Y. Prediction of the SARS-CoV-2 (2019 nCoV) 3C-like protease ($3CL^{pro}$) structure: virtual screening reveals velpatasvir, ledipasvir, and other drug repurposing candidates [version 2; peer review: 3 approved). F1000Research. 2020; 9:129. [https://doi.org/10.12688/f1000research,22457.2]
7. Gonçalves JLS, Lopes RC, Oliveira DB, Costa SS, Miranda MMFS, Romanos MTV *et al*. *In vitro* anti-rotavirus activity of some medicinal plants used in Brazil against diarrhea. J Ethnopharmacol. 2005; 99(3):403-407.
8. Huang Y.-F, Bai C, He F, Xie Y, Zhou H. Review on the potential action mechanisms of Chinese medicines in treating Coronavirus disease 2019 (COVID-19). Pharmacol Res. 2020; 158:104939. [doi: https://doi.org/10.1016/j.phrs.2020.104939]
9. Yu J.-W, Wang L, Bao L.-D. Exploring the active compounds of traditional Mongolian medicine in intervention of novel coronavirus (COVID-19) based on molecular docking method. J Functional Foods. 2020. [https://doi.org/10.1016/j.jff.2020.104016]
10. Alves IABS, Miranda HM, Soares LAL, Randau KP. Simaroubaceae family: botany, chemical composition and biological activities. Rev Bras Farmacogn. 2014; 24:481-501.
11. Kim IH, Suzuki R, Hitotsuyanagi Y, Takeya K. Three novel quassinoids, javanicolides A and B, and javanicoside A, from seeds of *Brucea javanica*. Tetrahedron. 2003; 59(50):9985-9989.
12. Kim IH, Takashima S, Hitotsuyanagi Y, Hasuda T, Takeya K. New quassinoides, javanicolides C and D and javanicosides B-F from seeds of *Brucea javanica*. J Nat Prod. 2004; 67(5):863-868.
13. Liu JH, Zhao N, Zhang GJ, Yu SS, Wu LJ, Qu J *et al*.

Bioactive quassinoids from the seeds of *Brucea javanica*. J Nat Prod. 2012; 75(4):683-688.
14. Yan XH, Chen J, Di YT, Fang X, Dong JH, Sang P *et al*. Anti-tobacco mosaic virus (TMV) quassinoids from *Brucea javanica* (L.) Merr. J Agric Food Chem. 2010; 58(3):1572-1577.
15. Liu X, Zhang B, Jin Z, Yang H, Rao Z. The crystal structure of COVID-19 main protease in complex with an inhibitor N3. 2020; https://www.rcsb.org/structure/6LU7, accessed June 15, 2020. [https://doi.org/10.2210/PDB6LU7/PDB]
16. Zhang L, Lin D, Sun X, Curth U, Drosten C, Sauerhering L *et al*. Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors. Science. 2020; 368(6489):409-412. [doi: 10.1126/science.abb3405]
17. Ihlenfeldt WD. PubChem. In: *Applied Chemoinformatics*, 2018, 245-258. [https://doi.org/10.1002/9783527806539.ch6e]
18. Trott O, Olson A. Autodock Vina: Improving the Speed and Accuracy of Docking. J Comput Chem. 2010; 31(2):455-461. [https://doi.org/10.1002/jcc.21334.AutoDock]
19. Studio D. Dassault Systemes BIOVIA, Discovery Studio Modelling Environment, Release 4.5. Accelrys Software Inc. 2015, 98-104.
20. Hu L, Ramani S, Czako R, Sankaran B, Yu Y, Smith DF *et al*. Structural Basis of Glycan Specificity in Neonate-Specific Bovine-Human Reassortant Rotavirus. Nature Commun. 2015; 6(1):8346. [doi: 10.1038/ncomms9346]
21. Tate JE, Burton AH, Boschi-Pinto C, Steele AD, Duque J, Parashar UD. WHO-coordinated Global Rotavirus Surveillance Network. 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. Lancet Infect Dis. 2012; 12(2):136-141.
22. Estes MK, Greenberg HB. In: Fields Virology (DM Knipe and P Howley, Eds.), Lippincott, Williams & Wilkins, 2013, 1347-1401.
23. Huang P, Xia M, Tan M, Zhong Y, Wei C, Wang L *et al*. Spike protein VP8* of human rotavirus recognizes histo-blood group antigens in a type-specific manner. J Virol. 2012, 4833-4843.
24. Isa P, Arias CF, López S. Role of sialic acids in rotavirus infection. Glycoconj J. 2006; 23:27-37.
25. Rustmeier NH, Strebl M, Stehle T. The symmetry of viral sialic acid binding sites-implications for antiviral strategies. Viruses. 2019; 11:947. [doi: 10.3390/v11100947]