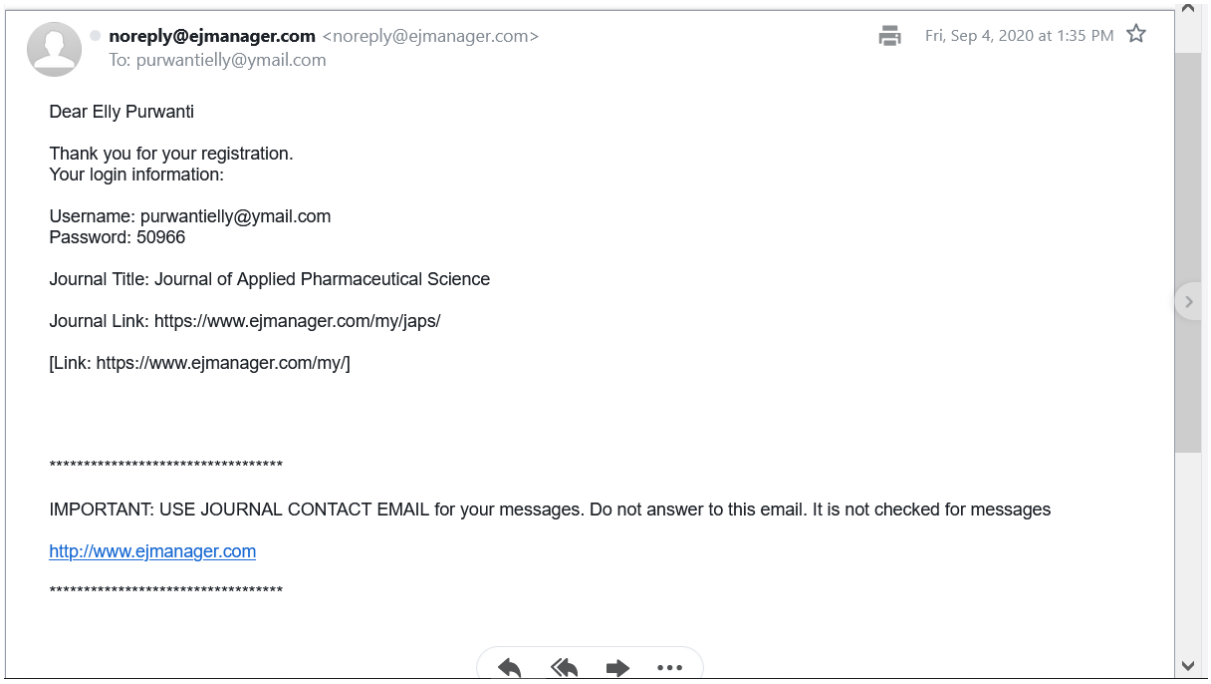
1. Author registered to Journal of Applied Pharmaceutical Science (4-9-2020)
    - Login information
2. Article submitted to Journal of Applied Pharmaceutical Science (4-9-2020)
    - Submission confirmation
3. First revision: Accepted with major revision (13-10-2020)
    - Article revision letter for authors
4. First revision submitted (22-10-2020)
    - Revised article submission confirmation
    - Revision letter to editor
    - Revised article with highlights
5. Second revision: Accepted with major revision (3-11-2020)
    - Article revision letter for authors
6. Second revision submitted (13-11-2020)
    - Revised article submission confirmation
    - Revision letter to editor
    - Revised article with highlights
7. Third revision: Minor revision (18-11-2020)
    - Article revision letter for authors
8. Third revision submitted (19-11-2020)
    - Revised article submission confirmation
    - Final draft
9. Article accepted for publication (23-11-2020)
    - Decision letter to authors
10. Galley proof sent to author (6-12-2020)
    - Notification letter
    - Author queries
11. Galley proof sent to author (10-12-2020)
    - Notification letter
12. Galley proof sent to author (15-12-2020)
    - Notification letter
13. Article published (2-6-2021)
    - Notification letter
    - Final paper for publication

1. Author registered to Journal of Applied Pharmaceutical Science (4-9-2020)
    - Login information



noreply@ejmanager.com <noreply@ejmanager.com>
To: purwantielly@ymail.com

Fri, Sep 4, 2020 at 1:35 PM

Dear Elly Purwanti

Thank you for your registration.
Your login information:

Username: purwantielly@ymail.com
Password: 50966

Journal Title: Journal of Applied Pharmaceutical Science

Journal Link: https://www.ejmanager.com/my/japs/

[Link: https://www.ejmanager.com/my/]

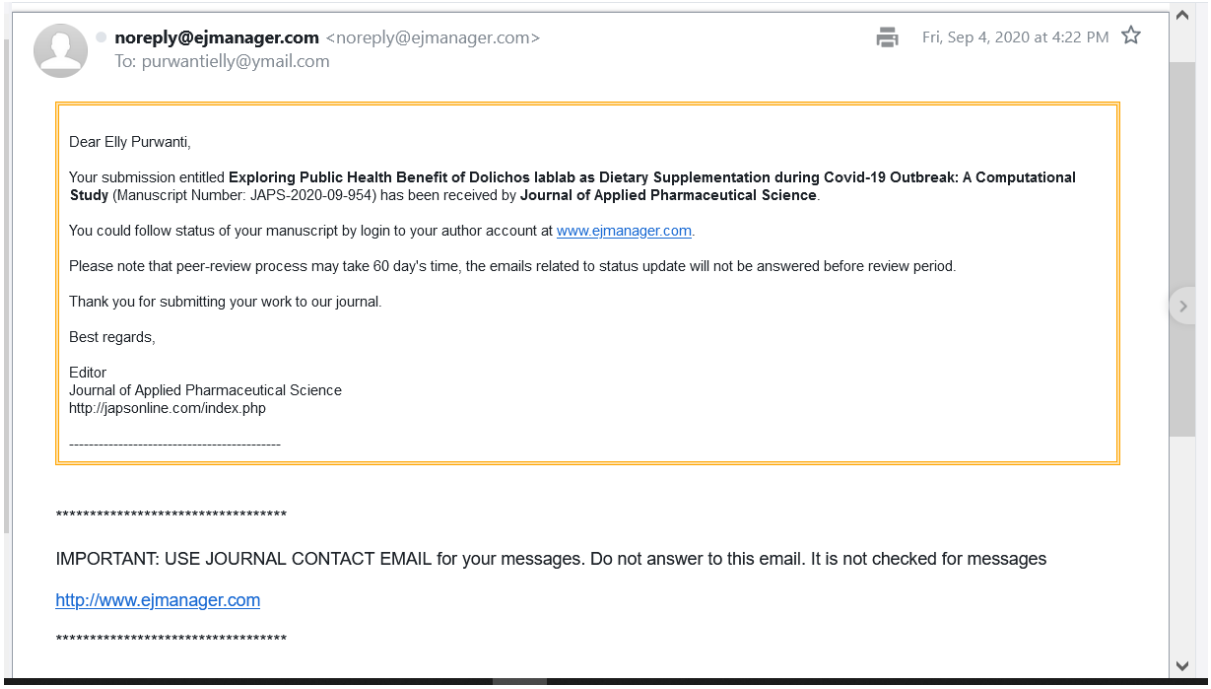
****

IMPORTANT: USE JOURNAL CONTACT EMAIL for your messages. Do not answer to this email. It is not checked for messages

http://www.ejmanager.com

****

2. Article submitted to Journal of Applied Pharmaceutical Science (4-9-2020)
    - Submission confirmation



noreply@ejmanager.com <noreply@ejmanager.com>
To: purwantielly@ymail.com

Fri, Sep 4, 2020 at 4:22 PM

Dear Elly Purwanti,

Your submission entitled **Exploring Public Health Benefit of Dolichos lablab as Dietary Supplementation during Covid-19 Outbreak: A Computational Study** (Manuscript Number: JAPS-2020-09-954) has been received by **Journal of Applied Pharmaceutical Science**.

You could follow status of your manuscript by login to your author account at www.ejmanager.com.

Please note that peer-review process may take 60 day's time, the emails related to status update will not be answered before review period.

Thank you for submitting your work to our journal.

Best regards,

Editor
Journal of Applied Pharmaceutical Science
http://japsonline.com/index.php

****

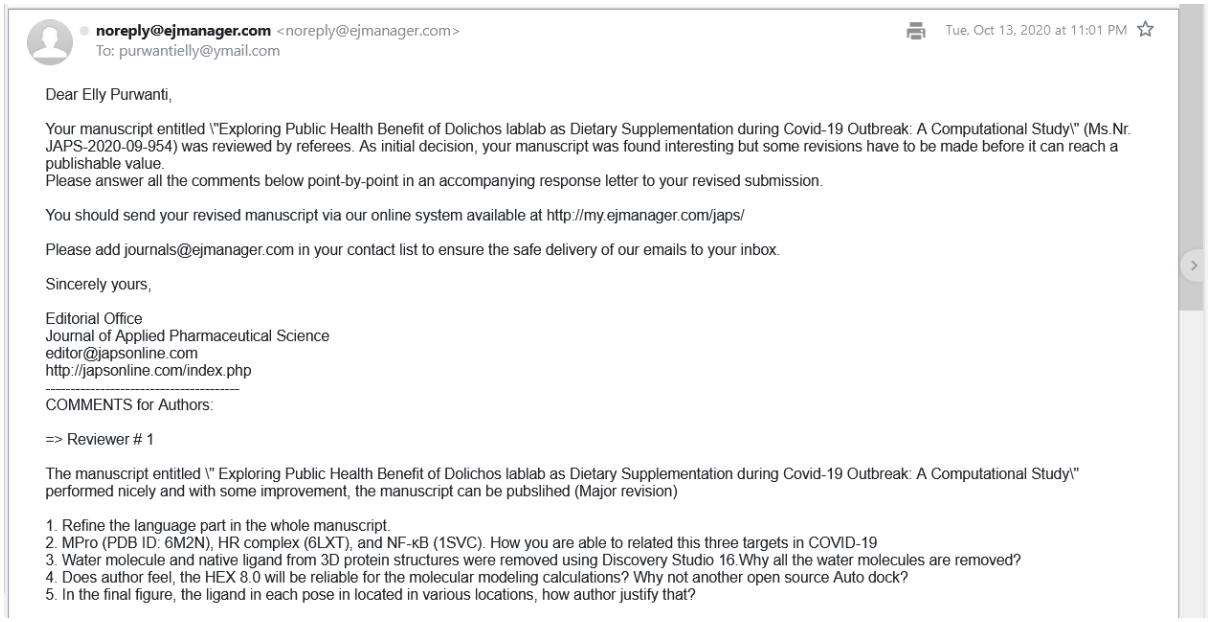
IMPORTANT: USE JOURNAL CONTACT EMAIL for your messages. Do not answer to this email. It is not checked for messages

http://www.ejmanager.com

****

3. First revision: Accepted with major revision (13-10-2020)

- Article revision letter for authors



noreply@ejmanager.com <noreply@ejmanager.com>
To: purwantielly@ymail.com

Tue, Oct 13, 2020 at 11:01 PM

Dear Elly Purwanti,

Your manuscript entitled \"Exploring Public Health Benefit of Dolichos lablab as Dietary Supplementation during Covid-19 Outbreak: A Computational Study\" (Ms.Nr. JAPS-2020-09-954) was reviewed by referees. As initial decision, your manuscript was found interesting but some revisions have to be made before it can reach a publishable value.
Please answer all the comments below point-by-point in an accompanying response letter to your revised submission.

You should send your revised manuscript via our online system available at http://my.ejmanager.com/japs/

Please add journals@ejmanager.com in your contact list to ensure the safe delivery of our emails to your inbox.

Sincerely yours,

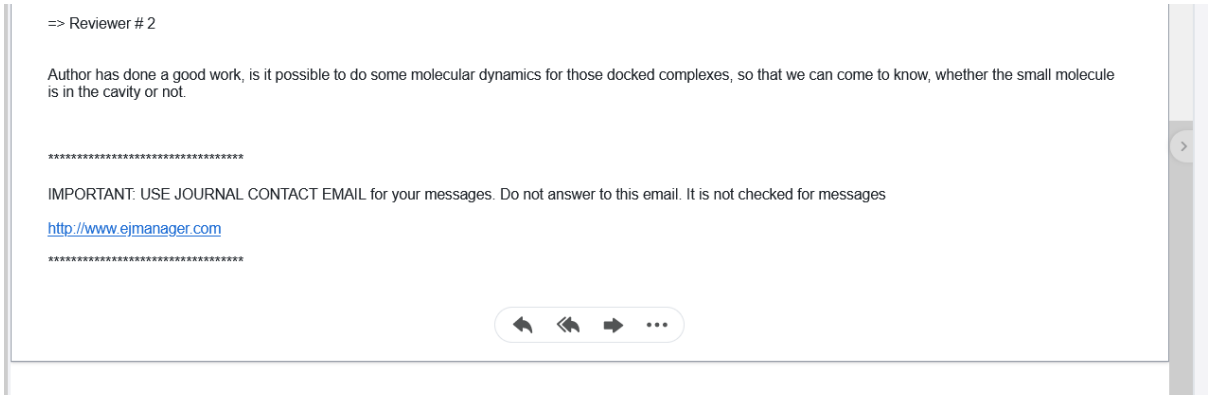
Editorial Office
Journal of Applied Pharmaceutical Science
editor@japsonline.com
http://japsonline.com/index.php

COMMENTS for Authors:

=> Reviewer # 1

The manuscript entitled \" Exploring Public Health Benefit of Dolichos lablab as Dietary Supplementation during Covid-19 Outbreak: A Computational Study\" performed nicely and with some improvement, the manuscript can be pubslihed (Major revision)

1. Refine the language part in the whole manuscript.
2. MPro (PDB ID: 6M2N), HR complex (6LXT), and NF-κB (1SVC). How you are able to related this three targets in COVID-19
3. Water molecule and native ligand from 3D protein structures were removed using Discovery Studio 16.Why all the water molecules are removed?
4. Does author feel, the HEX 8.0 will be reliable for the molecular modeling calculations? Why not another open source Auto dock?
5. In the final figure, the ligand in each pose in located in various locations, how author justify that?



=> Reviewer # 2

Author has done a good work, is it possible to do some molecular dynamics for those docked complexes, so that we can come to know, whether the small molecule is in the cavity or not.

****

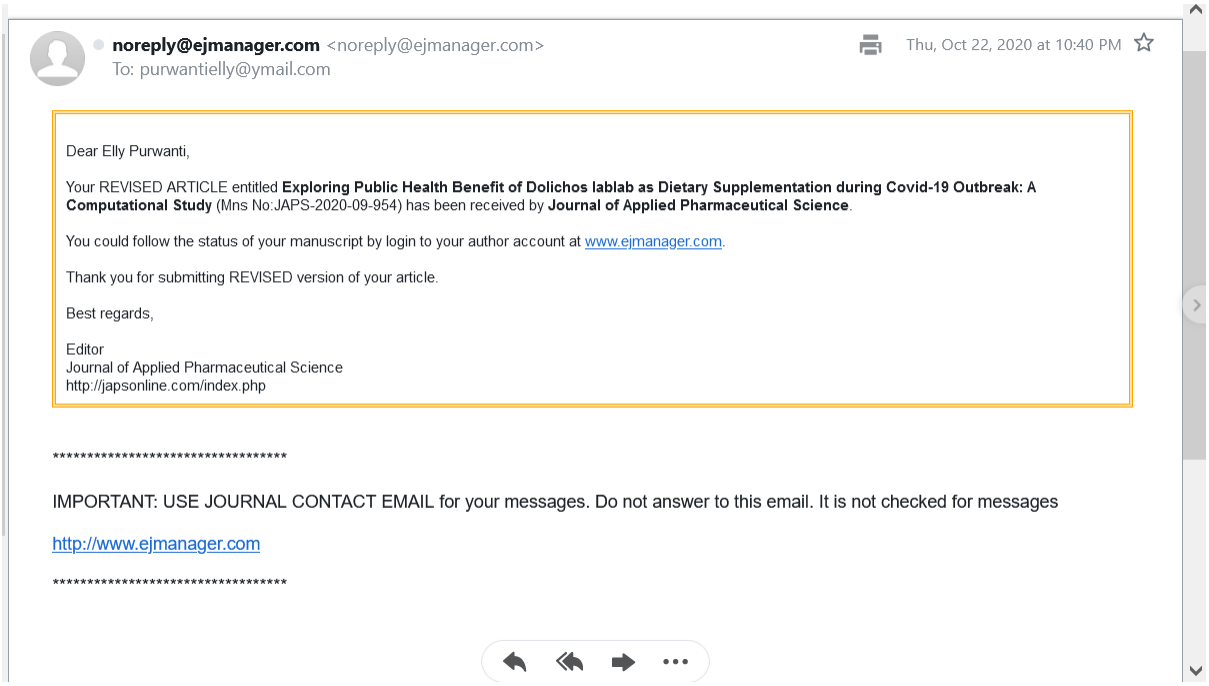
IMPORTANT: USE JOURNAL CONTACT EMAIL for your messages. Do not answer to this email. It is not checked for messages

http://www.ejmanager.com

****

4. First revision submitted (22-10-2020)

- Revised article submission confirmation



noreply@ejmanager.com <noreply@ejmanager.com>
To: purwantielly@ymail.com

Thu, Oct 22, 2020 at 10:40 PM

Dear Elly Purwanti,

Your REVISED ARTICLE entitled **Exploring Public Health Benefit of Dolichos lablab as Dietary Supplementation during Covid-19 Outbreak: A Computational Study** (Mns No:JAPS-2020-09-954) has been received by **Journal of Applied Pharmaceutical Science**.

You could follow the status of your manuscript by login to your author account at www.ejmanager.com.

Thank you for submitting REVISED version of your article.

Best regards,

Editor
Journal of Applied Pharmaceutical Science
http://japsonline.com/index.php

****

IMPORTANT: USE JOURNAL CONTACT EMAIL for your messages. Do not answer to this email. It is not checked for messages

http://www.ejmanager.com

****

- Revision letter to editor

October 22, 2020

To
The Editor
Journal of Applied Pharmaceutical Science

Subject: Submission of revised paper JAPS-2020-09-954

Thank you for your email notice of the status of our manuscript submission. We carefully reviewed and addressed the comments of the reviewer and updated the manuscript accordingly. Every revision from the manuscript was highlighted in yellow color. Also, our responses are given point-by-point below. We hope the revised version of our manuscript is now suitable for publication and look forward to hearing from you in due course.

Sincerely,

Elly Purwanti
Department of Educational Biology, Faculty of Teacher Training and Education,
University of Muhammadiyah Malang, East Java, Indonesia 65144
e-mail: purwantielly@ymail.com

Response to Reviewer 1:
Thank you for reviewing our articles. We have discussed by our team and answer your questions below:

1. We already revised and rechecked the grammar and language using grammar checker (premium version), and the score was outstanding. We hope word-by-word could be understand easily and our information could be delivered perfectly to the audiences.
2. As mentioned in our background section, MPro and HR are proteins responsible for viral infection and life cycle. Besides, NF-κB are transcription factor involved in gene regulation of inflammation process. Targeting MPro and HR aimed to look for the ability for preventing viral infection as well as disturbing its life cycle, while directing NF-κB was aimed to prevent hyper-inflammation during viral infection. With those two pathways, we hope that *Dolichos lablab* could have more benefits, i.e. by preventing viral infection/invasion and also regulating inflammation process to avoid severe damage of the tissue (controlling the virus as well as body response to viral infection).
3. To our knowledge, molecular docking was performed by setting the protein as rigid molecules while the ligand as flexible molecule. Keeping water molecules along with the protein structure will interfere the docking process and make the calculation bias. Therefore, removing water molecules was important during sample preparation for molecular docking purposes to get the closest representation of actual event.
4. We used HEX 8.0 to perform the protein-protein docking (e.g.: docking between the target proteins with its receptor after bounded with the studied ligands). As we know, AutoDock was designed for small molecule-protein docking. In addition, AutoDock also widely used for molecular docking studies rather than HEX with its good calculation algorithm, in particular for

small molecule-protein docking. Unfortunately, we have minim understanding regarding to the algorithm, so we cannot explain further regarding those calculations. However, because we also want to study the effect of the ligand to the interaction of target protein with its receptor after binding with target proteins, we used HEX to achieve those aims. As far as we know, HEX was designed to docking between small molecule-protein docking as well as protein-protein docking.

5. We have rotated the position of the complex in particular angle (as stated in the figure). With the comparison of the position of each complex in the same specific angle, we can visually describe the difference of the pose of each analyzed complex. Also, with the data of interacted residues in each analyzed complex, we could justify that the interaction was performed in different manner.

Response to Reviewer 2:

Thank you for reviewing our manuscript. We also acknowledge your suggestion regarding to molecular dynamics simulation. Unfortunately, with our current resources, we need more times to perform the molecular dynamics simulation. As stated in our data, we have more than five ligands which have the possibility to be an inhibitor for each studied protein. Our resources could process the molecular dynamics with 10 ns simulation time in 3-4 days for every complex. With those estimation, we could spend more than a month to get the data of molecular dynamics of each ligand as shown in table 1. We planned to do it separately to reinforce our current data.

- Revised article with highlights

Exploring Public Health Benefit of *Dolichos lablab* as Dietary Supplementation during Covid-19 Outbreak: A Computational Study

ABSTRACT

The emerging case of Coronavirus Disease-19 (Covid-19) caused by SARS-CoV-2 virus has become a global health issue. Since no available developed vaccine, health-promoting food has a vital role in maintaining the immune system against this disease. *Dolichos lablab* (DL), an unutilized high nutritional legume, has an excellent potential to cope with this pandemic with various health benefits phytochemical. This study appraised the possibility of phytochemical content from DL to prevent virus infection and hyper-inflammation in Covid-19 in silico. Several phytocemichals of DL from LC-HRMS analysis was docked with several SARS-CoV-2's proteins, including MPro and HR. Also, NF-κB docking was executed to pursue anti-inflammatory properties. The drug-likeness properties of screened phytochemicals were then evaluated using SwissADME. According to the results, there were sixteen phytochemicals with a high affinity to targeted proteins. Among those, five phytochemicals consistently gave low binding affinity to all targeted proteins. Those five phytochemicals' physicochemical properties, except for Rutin and (9cis)-Retinal, also coped with small-molecule bioavailability, permeability, and flexibility according to SwissADME calculations. In conclusion, DL has a high probability of complementing the medical effort as dietary supplementation to modulate the immune system and prevent viral infection.

Keywords: Covid-19, *Dolichos lablab*, Heptad Repeat, Main Protease, NF-κB

INTRODUCTION

The outbreak of COVID-19 has now become a significant global health issue. Until the August 2020 update, more than twenty million people have become infected, and nearly a million have died due to this pandemic (https://covid19.who.int/). This number is still

growing day by day, describing the war against Covid-19 continued. Although several companies already announced that they have proposed vaccine candidates entering the final phase of clinical trials, complementary medicine and dietary intervention still needed to prevent the severity of infected people and prevent the healthy people from getting infected (Di Matteo et al., 2020; Panyod et al., 2020). Therefore, exploring the proper diet for patients or healthy people to help against Covid-19 infection becomes essential.

Thwarting SARS-CoV-2 attachment and replication has become the main target for combating Covid-19 (Jha et al., 2020; McKee et al., 2020). Several proteins from SARS-CoV-2 have been modeled and appropriately studied as a target to decrease the number of positive cases (Dai et al., 2020; Xia et al., 2020; L. Zhang et al., 2020). Spike protein is the primary key for SARS-CoV-2 to enter the host's cell, consisting of unique parts called Heptad Repeat 1 (HR1) and HR 2 inside the Receptor Binding Domain (RBD) for performing membrane fusion after attachment (Bosch et al., 2004; Walls et al., 2020; Xia et al., 2020). With this critical role, HR1 and HR2 have been proposed as the main target to evade viral entry and infection (Xia et al., 2020). Another protein called Main Protease (MPro) also becomes the right candidate due to its vital role in viral replication and transcription (Hilgenfeld, 2014). Therefore, several studies also used MPro as a target to inhibit the severity of Covid-19 infection (Ahkam et al., 2020; Dai et al., 2020; Jin et al., 2020; Joshi et al., 2020).

Another perspective to support a patient's survival is by suppressing massive inflammation in lung tissue (Heck et al., 2020; W. Zhang et al., 2020). This inflammation, known as cytokine storms, occurs through deregulation of the immune response lead to disturbance of tissue homeostasis and severe organ damage (Ragab et al., 2020; Soy et al., 2020). Subsequently, injury in lung tissue give rise to breathe difficulties and increase the probability of the patient's death (Acosta and Singer, 2020; Lin et al., 2020). NF-κB, a kind of transcription

factor which controls several cytokines involved in cytokine storms like interleukin (IL)-1 and IL-6, has a good starting point to diminish the hyper-inflammation (Catanzaro et al., 2020; Conti et al., 2020; Soy et al., 2020). Previously, suppressing NF-κB could increase survival rate after coronavirus infection (DeDiego et al., 2014). Thus, targeting this transcription factor has a reasonable probability of improving patient survival.

Legumes have been a good source of nutrition for years. However, masses of legume species are still underutilized as a nutritious food. One of the rarely used legumes is *Dolichos lablab* (DL) (Minde et al., 2020). Not only for high nutritional content but DL also had an innumerable natural compound with numerous biological activities. Previously, DL has been explored for its antioxidant, antidiabetic, antimicrobial, even anti-inflammatory properties (Habib et al., 2017; Naeem et al., 2020; Rahman and Akhter, 2018). With those various health benefits, DL has promising candidates for dietary supplementation to avoid Covid-19 infection.

MATERIAL AND METHOD

Phytochemical Content Screening

Thermo Scientific Dionex Ultimate 3000 RSLCnano liquid chromatography (LC) coupled with Thermo Scientific Q Exactive Mass Spectrometry (MS) was run to identify phytochemical content inside the methanolic extract of DL. Hypersil GOLD aQ 50 x 1 mm x 1.9 µ particle size was installed in LC instrument as stationary phase, while the mobile phase consists of solvent A (0.1% Formic acid in water) and solvent B (0.1% Formic acid in Acetonitrile). The LC was operated under the following conditions: flow rate 40 µl/min, 30 min run time, 30°C column temperature. Obtained data were analyzed using Compound Discoverer with mzCloud MS/MS Library. Compound with mzCloud best match's score higher than 80 then directed for further analysis.

Ligand and Protein Structures Retrieval

The compounds from LC-HRMS analysis were used as the ligand. Three dimensional (3D) structure of the ligands was assessed through the PubChem database (supplementary file 1). Protein's 3D structures were retrieved from Protein Data Bank (https://www.rcsb.org/), i.e., MPro (PDB ID: 6M2N), HR complex (6LXT), and NF-κB (1SVC).

Molecular Docking

Water molecule and native ligand from 3D protein structures were removed using Discovery Studio 16. Energy minimization of the ligand structures was prepared using Open Babel integrated into PyRx 8.0 (O'Boyle et al., 2011). All compounds from LC-HRMS were screened using molecular docking to predict its interaction against protein targets. Protein-ligand docking was performed using AutoDock Vina integrated into PyRx 8.0 (Dallakyan and Olson, 2015; Trott and Olson, 2010) with a maximum grid size setting. HEX 8.0 was run for protein-protein docking using default setting and operated under Shape+DARS correlation type (Ritchie and Kemp, 2000). As a comparison, Hydroxychloroquine (HCQ, CID: 3652) (Procacci et al., 2020) and 4,6-dichloro-N-phenyl-1,3,5-triazine-2-amine (NI241, CID: 16766) (Kobayashi et al., 2016) were employed as a control inhibitor for MPro and NF-κB, respectively.

Data Analysis

Protein-ligand complex, which has binding energy lower than -7 kcal/mol, were directed into further analysis. Interacted residues in each protein-ligand complex and structure conformation were analyzed and visualized using Discovery Studio. Protein structure alignment was executed using PyMOL 2.3.2 with RMSD value determined as a structural difference among aligned proteins. The alignment was achieved by setting the HR1-HR2 complex as a reference structure.

Prediction of Drug Likeness Properties

Drug-likeness properties were analyzed according to Lipinski's Rule of 5 (LRO5) (Lipinski, 2004). Drug resemblance properties were determined using Swiss-ADME Physicochemical Properties (Daina et al., 2017), including molecular weight (MW), LogP value, and the number of H-bond donor, H-bond acceptor, rotatable bond, and Total Polar Surface Area (TPSA).

RESULT AND DISCUSSION

According to binding affinity, 16 compounds have been identified to have biological activities against SARS-CoV-2 infection and inflammation, which is 5 of them have binding energy lower than -7 kcal/mol consistently in every target protein. 19-Nortestosterone, Mesterolone, Oleanolic acid, Rutin, and Ursolic acid are the compounds that have low binding energy in MPro, HR-1, and NF- κB. MPro is the protein that can interact with more compounds tested with binding energy lower or equal with -7 kcal/mol. Based on binding affinity lower or similar to -7 kcal/mol, seven compounds could interact with HR-1, while six compounds have docked with NF-κB (table 1).

19-Norandrostenedione, 19-Nortestosterone, Galaxolidone, Mesterolone, Oleanolic Acid, Rutin, and Ursolic Acid are the compounds that have good affinity to HR-1. Attachment of those compounds could alter the HR1-HR2 binding motif represented by declining the HR complex's binding energy after bonded with those compounds compared to the HR complex without ligand (figure 1a). Among seven compounds that have an excellent affinity to HR-1, Rutin could modify HR1-HR2 interaction clearly. This was described by the RMSD value of the HR complex with Rutin inside compared to the HR complex alone, which has greater value than other complexes (figure 1b). Interaction of HR1-HR2 to form the helix bundle is the crucial step for SARS-CoV-2 membrane fusion (Liu et al., 2004; Ou et al., 2020). Altering helix bundle formation has been studied to prevent viral entry (Xia et al., 2020), suggesting that 19-Norandrostenedione, 19-Nortestosterone, Galaxolidone, Mesterolone,

Oleanolic Acid, Ursolic Acid, in particular Rutin, have an excellent potency to inhibit viral infection.

MPro is the target protein with plenty of interacted compounds. Compared to HCQ as control, all the compounds have lower binding energy. Each compound has its favorable binding region, presented by structural visualization (figure 2) or interacted residues between the ligand molecule and MPro (table S2). Among those compounds, only Daidzein has an interaction with catalytic residues of MPro at CYS:145. HCQ did not show any interaction with the catalytic residues (table S2). HIS:41 and CYS:145 have been known as essential residues during MPro enzymatic activity (Tahir ul Qamar et al., 2020). Inhibiting those residues has a promising effect on preventing virus replication and prevent viral spreading throughout the tissues (Ahkam et al., 2020; Gyebi et al., 2020; Hosseini-Zare et al., 2020; Tahir ul Qamar et al., 2020).

NF-κB is the transcription factor of several proteins related to infection response, including ~~several~~ cytokines related to defense mechanisms (Hayden et al., 2006; Schmitz et al., 2014). At critical condition, cytokine storms are the main factor that contributes to lung damage due to the overexpression of pro-inflammatory cytokines (Lin et al., 2020; Soy et al., 2020). Thus, targeting NF-κB as the main transcription factor for suppressing those cytokines' hyper-expression could play a vital role in augmenting patient survival (Catanzaro et al., 2020). From the docking result, six compounds could interact with NF- κB at 7 kcal/mol or lower. 19-Nortestosterone, Mesterolone, Oleanolic acid, and Ursolic acid could be bound with DNA binding sites (figure 3) based on residues involved in every compound-NF-κB complex (table S3). Those compounds could bind with several important residues involved in the DNA binding site, i.e., TYR:60 and HIS:144 (table S3) (Müller et al., 1995). Targeting DNA-binding sites of NF-κB has been employed to preclude chronic inflammation (Gilmore and Herscovitch, 2006; Gupta et al., 2010). Therefore, the presence of those compounds in the

NF-κB DNA-binding domain could reduce hyper inflammation by altering the pro-inflammatory cytokines' transcription process.

Several criteria have been developed by Lipinski for a small molecule to have good oral bioavailability, permeability, and flexibility (Lipinski, 2004). In order to have good oral bioavailability, the small molecule should have MW, LogP value, number of hydrogen bond donor, and acceptor less than 500 g/mol, 5, 5, 10, respectively (Lipinski, 2004). Also, a molecule with a TPSA value equal or less than 140 Å will perform good permeability, while the number of rotatable bonds less than 10 represents molecule flexibility (Chagas et al., 2018). Hence, SwissADME was employed to do the calculations related to LRO5. All of the analyzed compounds, except for Rutin and (9cis)-Retinal, have no violation of the Molecular Weight, LogP value, H-bond donor, H-bond acceptor, Rotatable bonds, and TPSA criteria. Rutin had low oral bioavailability and permeability with 610.52 g/mol of MW, 10 of H-bond donor, 16 of H-bond acceptor, and 269.43 $Å^2$ TPSA value. (9cis)-Retinal also not better than Rutin in terms of oral bioavailability and permeability with 8 of H-bond donor, 12 of H-bond acceptor, and 210.51 $Å^2$ TPSA value (table 2).

CONCLUSION

There were seven herbal compounds from DL, which have good potential as a preventive or complementary agent for Covid-19 treatment based on constant binding energy lower or equal to 7 kcal/mol with MPro or HR-1. However, Daidzein has better potency as an MPro inhibitor, while Rutin showed a worthy effect to prevent viral-host fusion by modifying HR complex structure orientation. As anti-inflammatory candidates, 19-Nortestosterone, Mesterolone, Oleanolic acid, and Ursolic acid have a satisfactory result as an NF-κB inhibitor. Lastly, all compounds with binding energy lower than or equal to -7 kcal/mol, but no for (9cis)-Retinal and Rutin, have good oral bioavailability, permeability, and flexibility.

ACKNOWLEDGEMENT

We would like to thank to Ministry of Research, Technology, and Higher Indonesia, Republic of Indonesia for funding this research under grant no. 229/SP2H/LT/DRPM/2019.

REFERENCES

Acosta, M.A.T., Singer, B.D., 2020. Pathogenesis of COVID-19-induced ARDS: implications for an aging population. Eur. Respir. J. https://doi.org/10.1183/13993003.02049-2020

Ahkam, A.H., Hermanto, F.E., Alamsyah, A., Aliyyah, I.H., Fatchiyah, F., 2020. Virtual prediction of antiviral potential of ginger (Zingiber officinale) bioactive compounds against spike and MPro of SARS-CoV2 protein: Berk. Penelit. HAYATI J. Biol. Res. 25, 52–57.

Bosch, B.J., Martina, B.E.E., Van Der Zee, R., Lepault, J., Haijema, B.J., Versluis, C., Heck, A.J.R., De Groot, R., Osterhaus, A.D.M.E., Rottier, P.J.M., 2004. Severe acute respiratory syndrome coronavirus (SARS-CoV) infection inhibition using spike protein heptad repeat-derived peptides. Proc. Natl. Acad. Sci. U. S. A. 101, 8455–8460. https://doi.org/10.1073/pnas.0400576101

Catanzaro, M., Fagiani, F., Racchi, M., Corsini, E., Govoni, S., Lanni, C., 2020. Immune response in COVID-19: addressing a pharmacological challenge by targeting pathways triggered by SARS-CoV-2. Signal Transduct. Target. Ther. 5. https://doi.org/10.1038/s41392-020-0191-1

Chagas, C.M., Moss, S., Alisaraie, L., 2018. Drug metabolites and their effects on the development of adverse reactions: Revisiting Lipinski's Rule of Five. Int. J. Pharm. 549, 133–149. https://doi.org/10.1016/j.ijpharm.2018.07.046

Conti, P., Ronconi, G., Caraffa, A., Gallenga, C., Ross, R., Frydas, I., Kritas, S., 2020. Induction of pro-inflammatory cytokines (IL-1 and IL-6) and lung inflammation by Coronavirus-19 (COVID-19 or SARS-CoV-2): anti-inflammatory strategies. J. Biol. Regul. Homeost. Agents 34, 327–331. https://doi.org/10.23812/CONTI-E

Dai, W., Zhang, B., Su, H., Li, J., Zhao, Y., Xie, X., Jin, Z., Liu, F., Li, C., Li, Y., Bai, F., Wang, H., Cheng, X., Cen, X., Hu, S., Yang, X., Wang, J., Liu, X., Xiao, G., Jiang, H., Rao, Z., Zhang, L.-K., Xu, Y., Yang, H., Liu, H., 2020. Structure-based design of antiviral drug candidates targeting the SARS-CoV-2 main protease. Science. https://doi.org/10.1126/science.abb4489

Daina, A., Michielin, O., Zoete, V., 2017. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci. Rep. 7, 42717. https://doi.org/10.1038/srep42717

Dallakyan, S., Olson, A.J., 2015. Small-molecule library screening by docking with PyRx. Methods Mol. Biol. Clifton NJ 1263, 243–250. https://doi.org/10.1007/978-1-4939-2269-7_19

DeDiego, M.L., Nieto-Torres, J.L., Regla-Nava, J.A., Jimenez-Guardeño, J.M., Fernandez-Delgado, R., Fett, C., Castaño-Rodriguez, C., Perlman, S., Enjuanes, L., 2014. Inhibition of NF-κB-Mediated Inflammation in Severe Acute Respiratory Syndrome Coronavirus-Infected Mice Increases Survival. J. Virol. 88, 913–924. https://doi.org/10.1128/JVI.02576-13

Di Matteo, G., Spano, M., Grosso, M., Salvo, A., Ingallina, C., Russo, M., Ritieni, A., Mannina, L., 2020. Food and COVID-19: Preventive/Co-therapeutic Strategies Explored by Current Clinical Trials and in Silico Studies. Foods 9, 1036. https://doi.org/10.3390/foods9081036

Gilmore, T.D., Herscovitch, M., 2006. Inhibitors of NF- κ B signaling: 785 and counting. Oncogene 25, 6887–6899. https://doi.org/10.1038/sj.onc.1209982

Gupta, S.C., Sundaram, C., Reuter, S., Aggarwal, B.B., 2010. Inhibiting NF-κB Activation by Small Molecules As a Therapeutic Strategy. Biochim. Biophys. Acta 1799, 775–787. https://doi.org/10.1016/j.bbagrm.2010.05.004

Gyebi, G.A., Ogunro, O.B., Adegunloye, A.P., Ogunyemi, O.M., Afolabi, S.O., 2020. 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. 1–13. https://doi.org/10.1080/07391102.2020.1764868

Habib, H.M., Theuri, S.W., Kheadr, E., Mohamed, F.E., 2017. DNA and BSA damage inhibitory activities, and anti-acetylcholinesterase, anti-porcine α-amylase and antioxidant properties of Dolichos lablab beans. Food Funct. 8, 881–887. https://doi.org/10.1039/c6fo01164k

Hayden, M.S., West, A.P., Ghosh, S., 2006. NF- κ B and the immune response. Oncogene 25, 6758–6780. https://doi.org/10.1038/sj.onc.1209943

Heck, T.G., Ludwig, M.S., Frizzo, M.N., Rasia-Filho, A.A., Homem de Bittencourt, P.I., 2020. Suppressed anti-inflammatory heat shock response in high-risk COVID-19 patients: lessons from basic research (inclusive bats), light on conceivable therapies. Clin. Sci. 134, 1991–2017. https://doi.org/10.1042/CS20200596

Hilgenfeld, R., 2014. From SARS to MERS: crystallographic studies on coronaviral proteases enable antiviral drug design. FEBS J. 281, 4085–4096. https://doi.org/10.1111/febs.12936

Hosseini-Zare, M.S., Thilagavathi, R., Selvam, C., 2020. Targeting severe acute respiratory syndrome-coronavirus (SARS-CoV-1) with structurally diverse inhibitors: a comprehensive review. RSC Adv. 10, 28287–28299. https://doi.org/10.1039/D0RA04395H

Jha, A.K., Kumar, R., Goenka, M.K., Dayal, V.M., 2020. Emerging Treatment and Prevention Strategies against COVID-19: A Brief Update. J. Dig. Endosc. 11, 69–72. https://doi.org/10.1055/s-0040-1712547

Jin, Z., Du, X., Xu, Y., Deng, Y., Liu, M., Zhao, Y., Zhang, B., Li, X., Zhang, L., Peng, C., Duan, Y., Yu, J., Wang, L., Yang, K., Liu, F., Jiang, R., Yang, Xinglou, You, T., Liu, Xiaoce,

Yang, Xiuna, Bai, F., Liu, H., Liu, Xiang, Guddat, L.W., Xu, W., Xiao, G., Qin, C., Shi, Z., Jiang, H., Rao, Z., Yang, H., 2020. Structure of M pro from SARS-CoV-2 and discovery of its inhibitors. Nature 582, 289–293. https://doi.org/10.1038/s41586-020-2223-y

Joshi, R.S., Jagdale, S.S., Bansode, S.B., Shankar, S.S., Tellis, M.B., Pandya, V.K., Chugh, A., Giri, A.P., Kulkarni, M.J., 2020. Discovery of potential multi-target-directed ligands by targeting host-specific SARS-CoV-2 structurally conserved main protease. J. Biomol. Struct. Dyn. 1–16. https://doi.org/10.1080/07391102.2020.1760137

Kobayashi, T., Tanuma, S., Kino, K., Miyazawa, H., 2016. New scaffolds of inhibitors targeting the DNA binding of NF-κB. Integr. Mol. Med. 3. https://doi.org/10.15761/IMM.1000242

Lin, S., Zhao, Y., Zhou, D., Zhou, F., Xu, F., 2020. Coronavirus disease 2019 (COVID-19): cytokine storms, hyper-inflammatory phenotypes, and acute respiratory distress syndrome. Genes Dis. https://doi.org/10.1016/j.gendis.2020.06.009

Lipinski, C.A., 2004. Lead- and drug-like compounds: the rule-of-five revolution. Drug Discov. Today Technol. 1, 337–341. https://doi.org/10.1016/j.ddtec.2004.11.007

Liu, S., Xiao, G., Chen, Y., He, Y., Niu, J., Escalante, C.R., Xiong, H., Farmar, J., Debnath, A.K., Tien, P., Jiang, S., 2004. Interaction between heptad repeat 1 and 2 regions in spike protein of SARS-associated coronavirus: implications for virus fusogenic mechanism and identification of fusion inhibitors. Lancet Lond. Engl. 363, 938–947. https://doi.org/10.1016/S0140-6736(04)15788-7

McKee, D.L., Sternberg, A., Stange, U., Laufer, S., Naujokat, C., 2020. Candidate drugs against SARS-CoV-2 and COVID-19. Pharmacol. Res. 157, 104859. https://doi.org/10.1016/j.phrs.2020.104859

Minde, J.J., Venkataramana, P.B., Matemu, A.O., 2020. Dolichos Lablab-an underutilized crop with future potentials for food and nutrition security: a review. Crit. Rev. Food Sci. Nutr. 1–13. https://doi.org/10.1080/10408398.2020.1775173

Müller, C.W., Rey, F.A., Sodeoka, M., Verdine, G.L., Harrison, S.C., 1995. Structure of the NF-kappa B p50 homodimer bound to DNA. Nature 373, 311–317. https://doi.org/10.1038/373311a0

Naeem, M., Shabbir, A., Ansari, A.A., Aftab, T., Khan, M.M.A., Uddin, M., 2020. Hyacinth bean (Lablab purpureus L.) – An underutilised crop with future potential. Sci. Hortic. 272, 109551. https://doi.org/10.1016/j.scienta.2020.109551

O'Boyle, N.M., Banck, M., James, C.A., Morley, C., Vandermeersch, T., Hutchison, G.R., 2011. Open Babel: An open chemical toolbox. J. Cheminformatics 3, 33. https://doi.org/10.1186/1758-2946-3-33

Ou, X., Liu, Y., Lei, X., Li, P., Mi, D., Ren, L., Guo, L., Guo, R., Chen, T., Hu, J., Xiang, Z., Mu, Z., Chen, X., Chen, J., Hu, K., Jin, Q., Wang, J., Qian, Z., 2020. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat. Commun. 11, 1620. https://doi.org/10.1038/s41467-020-15562-9

Panyod, S., Ho, C.-T., Sheen, L.-Y., 2020. Dietary therapy and herbal medicine for COVID-19 prevention: A review and perspective. J. Tradit. Complement. Med. 10, 420–427. https://doi.org/10.1016/j.jtcme.2020.05.004

Procacci, P., Macchiagodena, M., Pagliai, M., Guarnieri, G., Iannone, F., 2020. Interaction of hydroxychloroquine with SARS-CoV2 functional proteins using all-atoms non-equilibrium alchemical simulations. Chem. Commun. Camb. Engl. 56, 8854–8856. https://doi.org/10.1039/d0cc03558k

Ragab, D., Salah Eldin, H., Taeimah, M., Khattab, R., Salem, R., 2020. The COVID-19 Cytokine Storm; What We Know So Far. Front. Immunol. 11. https://doi.org/10.3389/fimmu.2020.01446

Rahman, S.A., Akhter, M.S., 2018. Antibacterial and cytotoxic activity of seeds of white hyacinth bean (Lablab purpureus L. sweet 'white') -. J. Adv. Biotechnol. Exp. Ther. 1, 49–54.

Ritchie, D.W., Kemp, G.J., 2000. Protein docking using spherical polar Fourier correlations. Proteins 39, 178–194.

Schmitz, M.L., Kracht, M., Saul, V.V., 2014. The intricate interplay between RNA viruses and NF-κB. Biochim. Biophys. Acta BBA - Mol. Cell Res. 1843, 2754–2764. https://doi.org/10.1016/j.bbamcr.2014.08.004

Soy, M., Keser, G., Atagündüz, P., Tabak, F., Atagündüz, I., Kayhan, S., 2020. Cytokine storm in COVID-19: pathogenesis and overview of anti-inflammatory agents used in treatment. Clin. Rheumatol. 39, 2085–2094. https://doi.org/10.1007/s10067-020-05190-5

Tahir ul Qamar, M., Alqahtani, S.M., Alamri, M.A., Chen, L.-L., 2020. Structural basis of SARS-CoV-2 3CLpro and anti-COVID-19 drug discovery from medicinal plants. J. Pharm. Anal. 10, 313–319. https://doi.org/10.1016/j.jpha.2020.03.009

Trott, O., Olson, A.J., 2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. J. Comput. Chem. 31, 455–461. https://doi.org/10.1002/jcc.21334

Walls, A.C., Park, Y.-J., Tortorici, M.A., Wall, A., McGuire, A.T., Veesler, D., 2020. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. Cell 181, 281-292.e6. https://doi.org/10.1016/j.cell.2020.02.058

Xia, S., Liu, M., Wang, C., Xu, W., Lan, Q., Feng, S., Qi, F., Bao, L., Du, L., Liu, S., Qin, C., Sun, F., Shi, Z., Zhu, Y., Jiang, S., Lu, L., 2020. Inhibition of SARS-CoV-2 (previously

2019-nCoV) infection by a highly potent pan-coronavirus fusion inhibitor targeting its spike protein that harbors a high capacity to mediate membrane fusion. Cell Res. 30, 343–355. https://doi.org/10.1038/s41422-020-0305-x

Zhang, L., Lin, D., Sun, X., Curth, U., Drosten, C., Sauerhering, L., Becker, S., Rox, K., Hilgenfeld, R., 2020. Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors. Science 368, 409–412. https://doi.org/10.1126/science.abb3405

Zhang, W., Zhao, Y., Zhang, F., Wang, Q., Li, T., Liu, Z., Wang, J., Qin, Y., Zhang, X., Yan, X., Zeng, X., Zhang, S., 2020. The use of anti-inflammatory drugs in the treatment of people with severe coronavirus disease 2019 (COVID-19): The Perspectives of clinical immunologists from China. Clin. Immunol. Orlando Fla 214, 108393. https://doi.org/10.1016/j.clim.2020.108393

Figure 1. Phytochemical effect on HR complex binding energy (A) and structural orientation (B). Figure A shows the blue ribbon represents the HR protein complex, while yellow spheres describe phytochemical ligand. At figure B, the blue ribbon represents the initial form of the HR complex without ligand, while the red ribbon represents the HR complex structure after bounded with the phytochemical ligands.

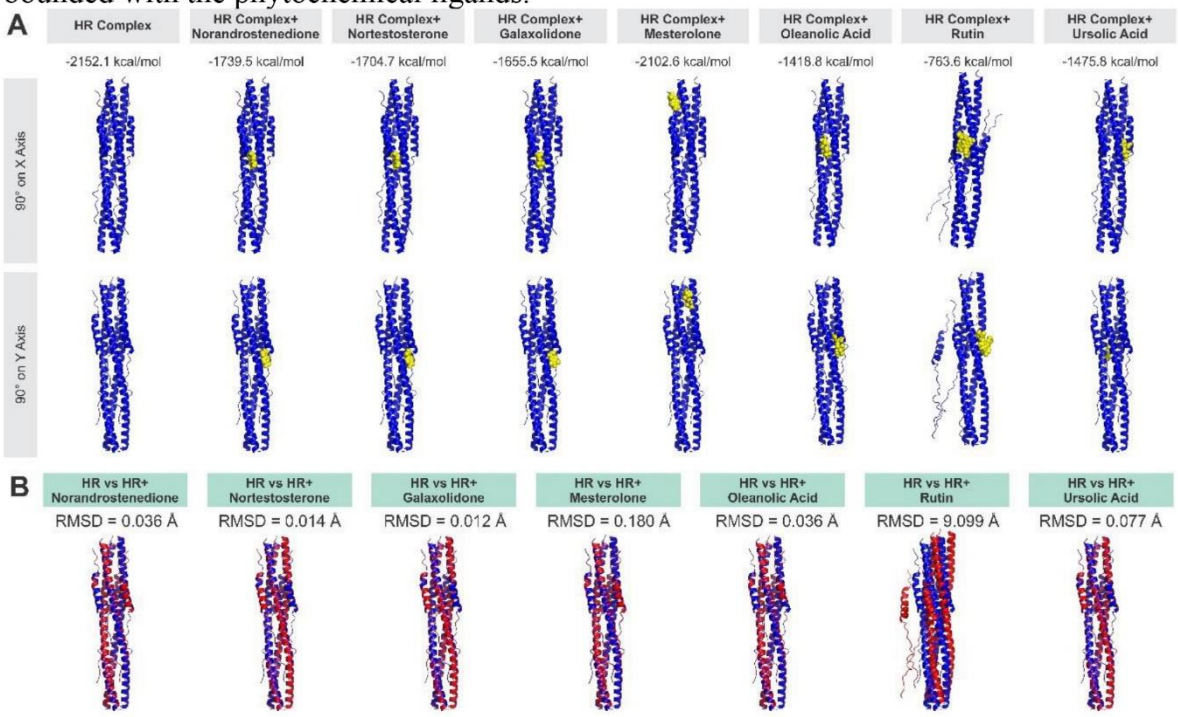


Figure 2. Visualization of MPro docked with its inhibitor and phytochemical ligand: HCQ (A), (9cis)-Retinal (B), 19-Norandrostenedione (C), 19-Nortestosterone (D), 3-Hydroxy-3,5,5-trimethyl-4-(3-oxo-1-buten-1-ylidene)cyclohexyl β-D-glucopyranoside (E), Benzoic Acid (F), Daidzein (G), Dimethomorph (H), Galaxolidone (I), Ilicic Acid (J), Isoquercetin (K), Mesterolone (L), Oleanolic Acid (M), Psilotachyin B (N), Rutin (O), and Ursolic Acid (P).

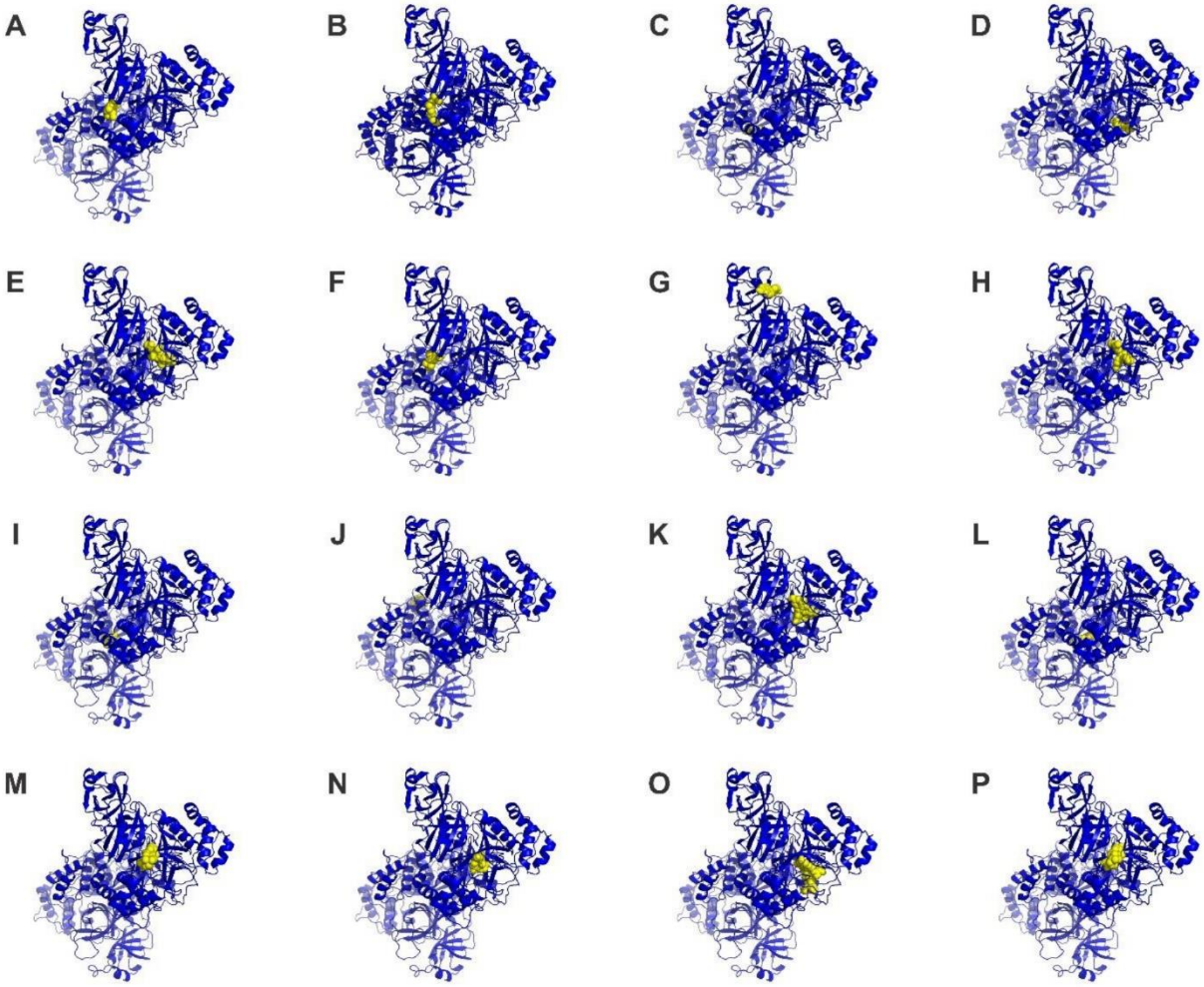
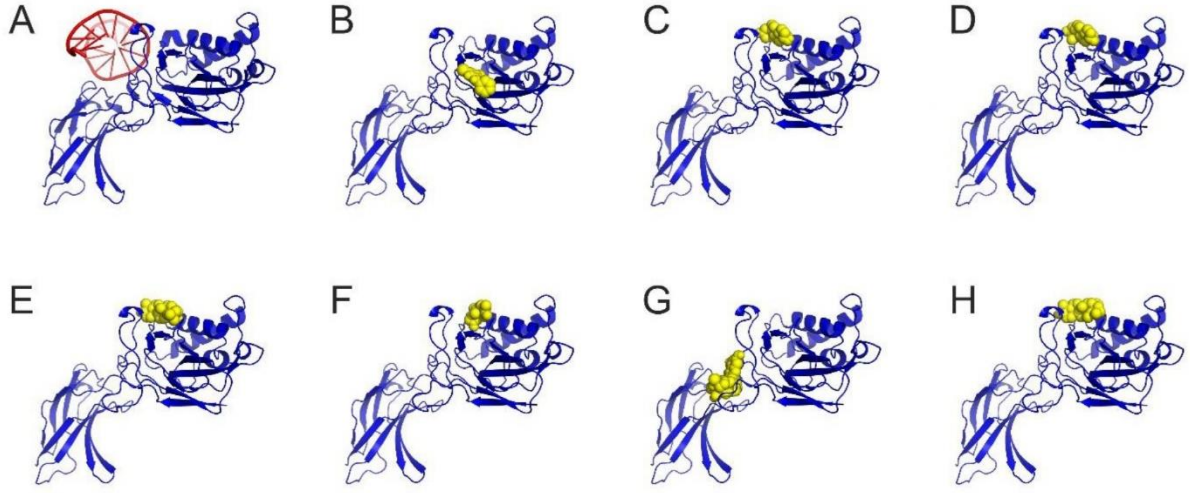
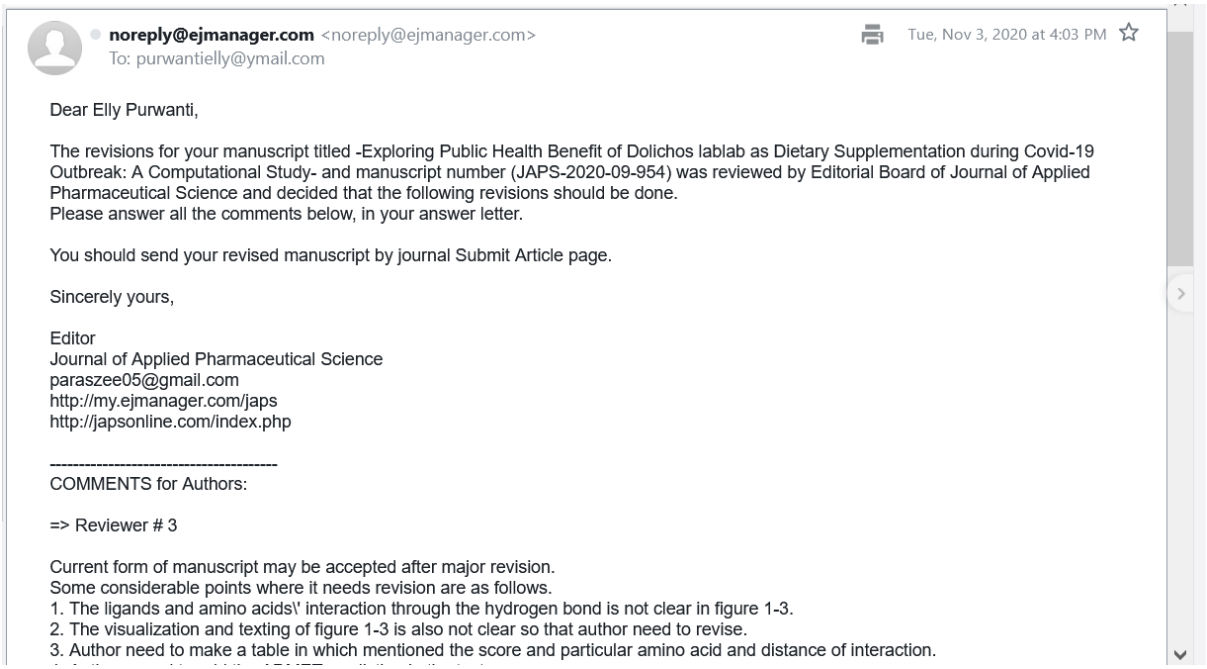


Figure 3. Structural visualization of NF- κB binds with DNA (A), NI241 (B), 19-Nortestosterone (C), Mesterolone (D), Oleanolic Acid (E), Psilotachyin B (F), Rutin (G), and Ursolic Acid (H).



5. Second revision: Accepted with major revision (3-11-2020)

   - Article revision letter for authors



noreply@ejmanager.com <noreply@ejmanager.com>
To: purwantielly@ymail.com

Tue, Nov 3, 2020 at 4:03 PM

Dear Elly Purwanti,

The revisions for your manuscript titled -Exploring Public Health Benefit of Dolichos lablab as Dietary Supplementation during Covid-19 Outbreak: A Computational Study- and manuscript number (JAPS-2020-09-954) was reviewed by Editorial Board of Journal of Applied Pharmaceutical Science and decided that the following revisions should be done.
Please answer all the comments below, in your answer letter.

You should send your revised manuscript by journal Submit Article page.

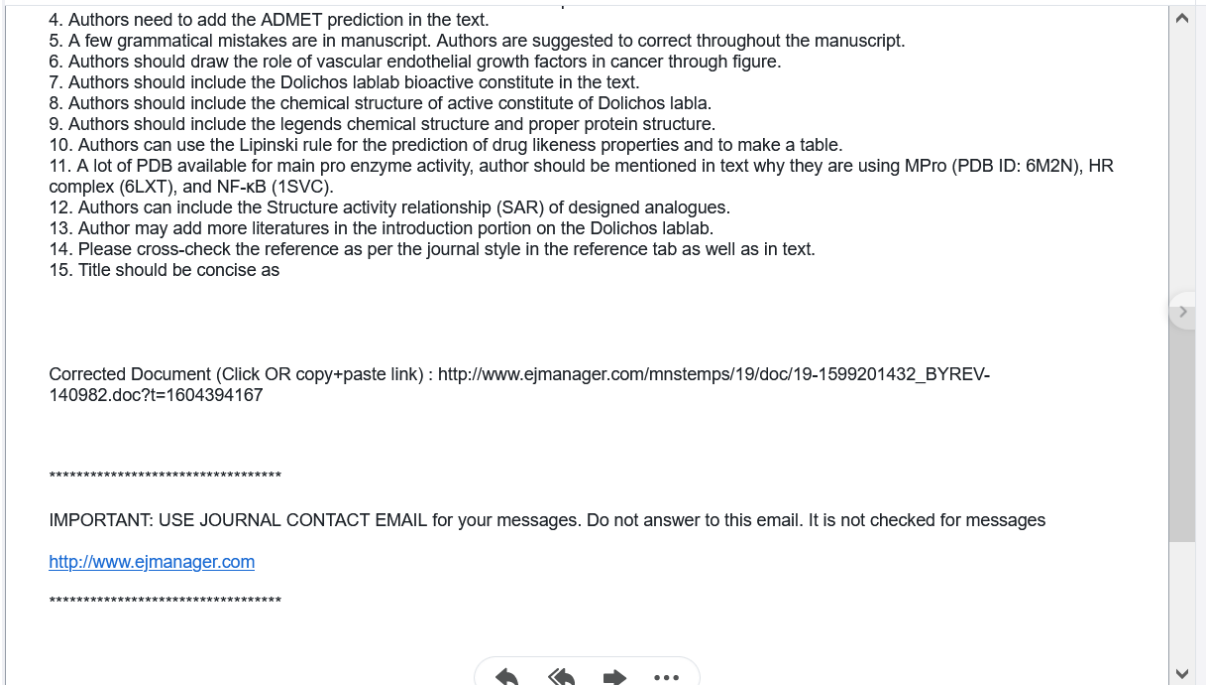
Sincerely yours,

Editor
Journal of Applied Pharmaceutical Science
paraszee05@gmail.com
http://my.ejmanager.com/japs
http://japsonline.com/index.php

COMMENTS for Authors:

=> Reviewer # 3

Current form of manuscript may be accepted after major revision.
Some considerable points where it needs revision are as follows.
1. The ligands and amino acids\' interaction through the hydrogen bond is not clear in figure 1-3.
2. The visualization and texting of figure 1-3 is also not clear so that author need to revise.
3. Author need to make a table in which mentioned the score and particular amino acid and distance of interaction.



4. Authors need to add the ADMET prediction in the text.
5. A few grammatical mistakes are in manuscript. Authors are suggested to correct throughout the manuscript.
6. Authors should draw the role of vascular endothelial growth factors in cancer through figure.
7. Authors should include the Dolichos lablab bioactive constitute in the text.
8. Authors should include the chemical structure of active constitute of Dolichos labla.
9. Authors should include the legends chemical structure and proper protein structure.
10. Authors can use the Lipinski rule for the prediction of drug likeness properties and to make a table.
11. A lot of PDB available for main pro enzyme activity, author should be mentioned in text why they are using MPro (PDB ID: 6M2N), HR complex (6LXT), and NF-κB (1SVC).
12. Authors can include the Structure activity relationship (SAR) of designed analogues.
13. Author may add more literatures in the introduction portion on the Dolichos lablab.
14. Please cross-check the reference as per the journal style in the reference tab as well as in text.
15. Title should be concise as

Corrected Document (Click OR copy+paste link) : http://www.ejmanager.com/mnstemps/19/doc/19-1599201432_BYREV-140982.doc?t=1604394167

****

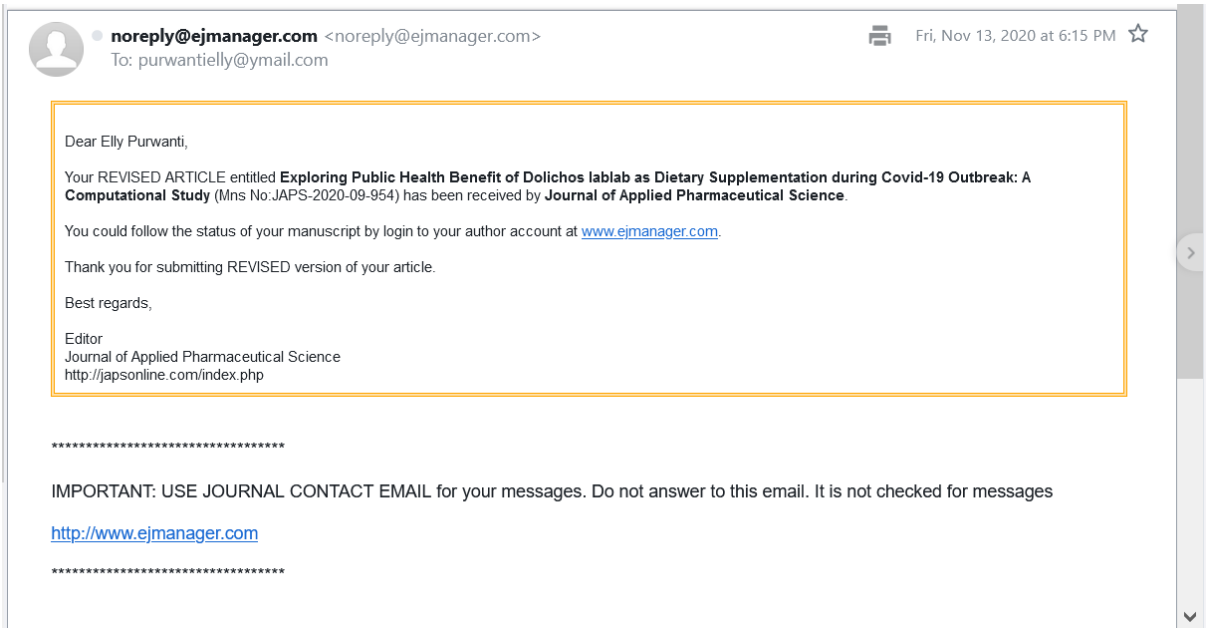
IMPORTANT: USE JOURNAL CONTACT EMAIL for your messages. Do not answer to this email. It is not checked for messages

http://www.ejmanager.com

****

6. Second revision submitted (13-11-2020)

- Revised article submission confirmation



noreply@ejmanager.com <noreply@ejmanager.com>
To: purwantielly@ymail.com

Fri, Nov 13, 2020 at 6:15 PM

Dear Elly Purwanti,

Your REVISED ARTICLE entitled **Exploring Public Health Benefit of Dolichos lablab as Dietary Supplementation during Covid-19 Outbreak: A Computational Study** (Mns No:JAPS-2020-09-954) has been received by **Journal of Applied Pharmaceutical Science**.

You could follow the status of your manuscript by login to your author account at www.ejmanager.com.

Thank you for submitting REVISED version of your article.

Best regards,

Editor
Journal of Applied Pharmaceutical Science
http://japsonline.com/index.php

****

IMPORTANT: USE JOURNAL CONTACT EMAIL for your messages. Do not answer to this email. It is not checked for messages

http://www.ejmanager.com

****

- Revision letter to editor

October 22, 2020

To
The Editor
Journal of Applied Pharmaceutical Science

Subject: Submission of revised paper JAPS-2020-09-954

Thank you for your e-mail notice of the status of our manuscript
submission. We carefully reviewed and addressed the comments of the reviewer and updated the manu script accordingly. Also, our responses are given point-by-point below.
We hope the revised version of our manuscript is now suitable for publication and look forward to hearing from you in due course.

Sincerely,

Elly Purwanti
Department of Educational Biology, Faculty of Teacher Training and Education,
University of Muhammadiyah Malang, East Java, Indonesia 65144
e-mail: purwantielly@ymail.com

Response to Reviewer 3:
Thank you for reviewing our articles. We have discussed by our team and answer your questions below:

1. We have included the hydrogen bond and other interaction in supplementary files 2. In our opinion, to save space in the article, we only show the structural visualization of the complexes and put the detailed interaction in the table of supplementary files 2. Please let us know if it is better to show the ligand interaction in separate figures in the main manuscript.
2. We have revised the texting of our figure's caption. We hope it fulfills the discussion we addressed.
3. We have added and replaced the supplementary table into supplementary files 2, including the specific amino acid and distance of interaction.
4. In our kind opinion, we have discussed the ADMET prediction in the result and discussion session in line with our result regarding the physicochemical properties of potential ligands.
5. We have revised some sentences and recheck them using a grammar checker. The results are excellent and ready to be published. Please let us know if there is a mistake in some sections.
6. We were sorry, but we have no discussion about cancer and angiogenesis, so we have no action regarding this comment.
7. We have added some bioactive constitute of *Dolichos lablab* according to previous research, as shown in the introduction section.
8. We used many compounds, and the result of the possible compound also has more than ten compounds. We decided not to include the chemical structure of *Dolichos lablab* bioactive in the text due to page efficiency. The audiences could address the chemical structure based on PubChem ID in our supplementary files 1.

9. We apologize for not understanding your advice. Please tell us more about this point. To our knowledge, there is no specific legend for protein and chemical structure. Commonly, it only explains which one the protein as well as the ligand.
10. We have added this section in our initial submitted manuscript and explained the result in table 2.
11. There was no specific reason to choose the 3D PDB structure of the proteins used in this study. However, according to a previous study, we choose those 3D protein structures that used the same protein structure to obtain their aims of the research.
12. Thank you for your suggestion. Unfortunately, we did not design the analogs. We used the natural compound as obtained from LC-HRMS analysis. Therefore, we did not have an idea to add SAR study in this manuscript.
13. We have added additional literature in the introduction section, mainly related to the portion of *Dolichos lablab.*
14. We have crosschecked the references and citations in our text. Because we used automatic citation tools (Zotero), we sure that the citations in the text are already included in the references list.
15. We have discussed with our team and decided not to change our title. Please let us know if you have a better suggestion regarding our manuscript title.

- Revised article with highlights

Exploring Public Health Benefit of *Dolichos lablab* as Dietary Supplementation during Covid-19 Outbreak: A Computational Study

ABSTRACT

The emerging case of Coronavirus Disease-19 (Covid-19) caused by the SARS-CoV-2 virus has become a global health issue. Since no available developed vaccine, health-promoting food has a vital role in maintaining the immune system against this disease. *Dolichos lablab* (DL), an unutilized high nutritional legume, has an excellent potential to cope with this pandemic with various health benefits phytochemical. This study appraised the possibility of phytochemical content from DL to prevent virus infection and hyper-inflammation in Covid-19 in silico. DL's phytochemicals from LC-HRMS analysis were docked with several SARS-CoV-2's proteins, including MPro and HR. Also, NF-κB docking was executed to pursue anti-inflammatory properties. The drug-likeness properties of screened phytochemicals were then evaluated using SwissADME. According to the results, there were sixteen phytochemicals with a high affinity to targeted proteins. Among those, five phytochemicals consistently gave low binding affinity to all targeted proteins. Those five phytochemicals' physicochemical properties, except for Rutin and (9cis)-Retinal, also coped with small-molecule bioavailability, permeability, and flexibility according to SwissADME calculations. In conclusion, DL has a high probability of complementing the medical effort as dietary supplementation to modulate the immune system and prevent viral infection.

Keywords: Covid-19, *Dolichos lablab*, Heptad Repeat, Main Protease, NF-κB

INTRODUCTION

The outbreak of COVID-19 has now become a significant global health issue. Until the November 2020 update, more than fifty million people have become infected, and over a million have died due to this pandemic (https://covid19.who.int/). This number is still

growing day by day, describing the war against Covid-19 continued. Although several companies already announced that they have proposed vaccine candidates entering the final phase of clinical trials, complementary medicine and dietary intervention still needed to prevent the severity of infected people and prevent the healthy people from getting infected (Di Matteo et al., 2020; Panyod et al., 2020). Therefore, exploring the proper diet for patients or healthy people to help against Covid-19 infection becomes essential.

Thwarting SARS-CoV-2 attachment and replication has become the main target for combating Covid-19 (Jha et al., 2020; McKee et al., 2020). Several proteins from SARS-CoV-2 have been modeled and appropriately studied as a target to decrease the number of positive cases (Dai et al., 2020; Xia et al., 2020; L. Zhang et al., 2020). Spike protein is the primary key for SARS-CoV-2 to enter the host's cell, consisting of unique parts called Heptad Repeat 1 (HR1) and HR 2 inside the Receptor Binding Domain (RBD) for performing membrane fusion after attachment (Bosch et al., 2004; Walls et al., 2020; Xia et al., 2020). With this critical role, HR1 and HR2 have been proposed as the main target to evade viral entry and infection (Xia et al., 2020). Another protein called Main Protease (MPro) also becomes the right candidate due to its vital role in viral replication and transcription (Hilgenfeld, 2014). Therefore, several studies also used MPro as a target to inhibit the severity of Covid-19 infection (Ahkam et al., 2020; Dai et al., 2020; Jin et al., 2020; Joshi et al., 2020).

Another perspective to support a patient's survival is suppressing massive inflammation in lung tissue (Heck et al., 2020; W. Zhang et al., 2020). This inflammation, known as cytokine storms, occurs through deregulation of the immune response lead to disturbance of tissue homeostasis and severe organ damage (Ragab et al., 2020; Soy et al., 2020). Subsequently, injury in lung tissue gives rise to breathing difficulties and increases the patient's death (Acosta and Singer, 2020; Lin et al., 2020). NF-κB, a kind of transcription factor which

controls several cytokines involved in cytokine storms like interleukin (IL)-1 and IL-6, has a good starting point to diminish the hyper-inflammation (Catanzaro et al., 2020; Conti et al., 2020; Soy et al., 2020). Previously, suppressing NF-κB could increase the survival rate after coronavirus infection (DeDiego et al., 2014). Thus, targeting this transcription factor has a reasonable probability of improving patient survival.

Legumes have been a good source of nutrition for years. However, masses of legume species are still underutilized as nutritious food. One of the rarely used legumes is *Dolichos lablab* (DL) (Minde et al., 2020). Not only for high nutritional content, but DL also had an innumerable natural compound with numerous biological activities. Several bioactive compounds were reported to be contained in DL, including gallic acid, 4-hydroxy-3-methoxybenoic acid, p-coumaric acid, ferulic acid, cinnamic acid, catechin, and Rutin. Also, saturated and unsaturated fatty acids, terpenoids, and steroids were found as a constituent inside DL beans (Baba et al., 1983; Bahtiar et al., 2017; Habib et al., 2017b; Yoshikawa et al., 1998). Previously, DL has been explored for its antioxidant, antidiabetic, antimicrobial, even anti-inflammatory properties (Habib et al., 2017a; Naeem et al., 2020; Rahman and Akhter, 2018; Yin et al., 2018). With those various health benefits, DL has promising candidates for dietary supplementation to avoid Covid-19 infection.

MATERIAL AND METHOD

Phytochemical Content Screening

Thermo Scientific Dionex Ultimate 3000 RSLCnano liquid chromatography (LC) coupled with Thermo Scientific Q Exactive Mass Spectrometry (MS) was run to identify phytochemical content inside the methanolic extract of DL. Hypersil GOLD aQ 50 x 1 mm x 1.9 µ particle size was installed in LC instrument as stationary phase, while the mobile phase consists of solvent A (0.1% Formic acid in water) and solvent B (0.1% Formic acid in Acetonitrile). The LC was operated under the following conditions: flow rate 40 µl/min, 30

min run time, 30°C column temperature. Obtained data were analyzed using Compound Discoverer with mzCloud MS/MS Library. Compound with mzCloud best match's score higher than 80 then directed for further analysis.

Ligand and Protein Structures Retrieval

The compounds from LC-HRMS analysis were used as the ligand. Three dimensional (3D) structure of the ligands was assessed through the PubChem database (supplementary file 1). Protein's 3D structures were retrieved from Protein Data Bank (https://www.rcsb.org/), i.e., MPro (PDB ID: 6M2N), HR complex (6LXT), and NF-κB (1SVC) according to the previous study (Muzaffer et al., 2017; Su et al., 2020; Xia et al., 2020).

Molecular Docking

Water molecule and native ligand from 3D protein structures were removed using Discovery Studio 16. Energy minimization of the ligand structures was prepared using Open Babel integrated into PyRx 8.0 (O'Boyle et al., 2011). All compounds from LC-HRMS were screened using molecular docking to predict its interaction against protein targets. Protein-ligand docking was performed using AutoDock Vina integrated into PyRx 8.0 (Dallakyan and Olson, 2015; Trott and Olson, 2010) with a maximum grid size setting. HEX 8.0 was run for protein-protein docking using the default setting and operated under Shape+DARS correlation type (Ritchie and Kemp, 2000). As a comparison, Hydroxychloroquine (HCQ, CID: 3652) (Procacci et al., 2020) and 4,6-dichloro-N-phenyl-1,3,5-triazine-2-amine (NI241, CID: 16766) (Kobayashi et al., 2016) were employed as a control inhibitor for MPro and NF-κB, respectively.

Data Analysis

Protein-ligand complex, which has binding energy lower than -7 kcal/mol, were directed into further analysis. Interacted residues in each protein-ligand complex and structure conformation were analyzed and visualized using Discovery Studio. Protein structure

alignment was executed using PyMOL 2.3.2 with RMSD value determined as a structural difference among aligned proteins. The alignment was achieved by setting the HR1-HR2 complex as a reference structure.

Prediction of Drug Likeness Properties

Drug-likeness properties were analyzed according to Lipinski's Rule of 5 (LRO5) (Lipinski, 2004). Drug resemblance properties were determined using Swiss-ADME Physicochemical Properties (Daina et al., 2017), including molecular weight (MW), LogP value, and the number of H-bond donor, H-bond acceptor, rotatable bond, and Total Polar Surface Area (TPSA).

RESULT AND DISCUSSION

According to binding affinity, 16 compounds have been identified to have biological activities against SARS-CoV-2 infection and inflammation, which is 5 of them have binding energy lower than -7 kcal/mol consistently in every target protein. 19-Nortestosterone, Mesterolone, Oleanolic acid, Rutin, and Ursolic acid are the compounds that have low binding energy in MPro, HR-1, and NF- κB. MPro is the protein that can interact with more compounds tested with binding energy lower or equal to -7 kcal/mol. Based on binding affinity lower or similar to -7 kcal/mol, seven compounds could interact with HR-1, while six compounds have docked with NF-κB (table 1).

19-Norandrostenedione, 19-Nortestosterone, Galaxolidone, Mesterolone, Oleanolic Acid, Rutin, and Ursolic Acid are the compounds that have good affinity to HR-1. Attachment of those compounds could alter the HR1-HR2 binding motif represented by declining the HR complex's binding energy after bonded with those compounds compared to the HR complex without ligand (figure 1a). Among seven compounds that have an excellent affinity to HR-1, Rutin could modify HR1-HR2 interaction. This was described by the RMSD value of the HR complex with Rutin inside compared to the HR complex alone, which has greater value than

other complexes (figure 1b). Interaction of HR1-HR2 to form the helix bundle is the crucial step for SARS-CoV-2 membrane fusion (Liu et al., 2004; Ou et al., 2020). Altering helix bundle formation has been studied to prevent viral entry (Xia et al., 2020), suggesting that 19-Norandrostenedione, 19-Nortestosterone, Galaxolidone, Mesterolone, Oleanolic Acid, Ursolic Acid, in particular Rutin, have an excellent potency to inhibit viral infection.

MPro is the target protein with plenty of interacted compounds. Compared to HCQ as control, all the compounds have lower binding energy. Each compound has its favorable binding region, presented by structural visualization (figure 2) or interacted residues between the ligand molecule and MPro (supplementary file 2). Among those compounds, only Daidzein and (9cis)-Retinal have an interaction with catalytic residues of MPro at HIS:41. HCQ did not show any interaction with the catalytic residues (supplementary file 2). HIS:41 and CYS:145 have been known as essential residues during MPro enzymatic activity (Tahir ul Qamar et al., 2020). Inhibiting those residues has a promising effect on preventing virus replication and prevent viral spreading throughout the tissues (Ahkam et al., 2020; Gyebi et al., 2020; Hosseini-Zare et al., 2020; Tahir ul Qamar et al., 2020).

NF-κB is the transcription factor of several proteins related to infection response, including cytokines related to defense mechanisms (Hayden et al., 2006; Schmitz et al., 2014). At critical condition, cytokine storms are the main factor that contributes to lung damage due to the overexpression of pro-inflammatory cytokines (Lin et al., 2020; Soy et al., 2020). Thus, targeting NF-κB as the main transcription factor for suppressing those cytokines' hyper-expression could play a vital role in augmenting patient survival (Catanzaro et al., 2020). From the docking result, six compounds could interact with NF- κB at 7 kcal/mol or lower. Although the ligands did not bind with the vital residues involved in NF-κB-DNA binding site, several interacted amino acids taken place adjacent to those binding sites (supplementary file 2) suggesting their potentials as NF-κB inhibitor (Müller et al., 1995). Targeting DNA-

binding sites of NF-κB has been employed to preclude chronic inflammation (Gilmore and Herscovitch, 2006; Gupta et al., 2010). Therefore, the presence of those compounds in the NF-κB DNA-binding domain could reduce hyper inflammation by altering the pro-inflammatory cytokines' transcription process.

Several criteria have been developed by Lipinski for a small molecule to have good oral bioavailability, permeability, and flexibility (Lipinski, 2004). The oral bioavailability of small molecules is determined by several criteria, including MW, LogP value, the number of hydrogen bond donors, and acceptor less than 500 g/mol, 5, 5, and 10, respectively (Lipinski, 2004). Also, a molecule with a TPSA value equal or less than 140 Å will perform good permeability, while the number of rotatable bonds less than 10 represents molecule flexibility (Chagas et al., 2018). Hence, SwissADME was employed to do the calculations related to LRO5. All of the analyzed compounds, except for Rutin and (9cis)-Retinal, have no violation of the Molecular Weight, LogP value, H-bond donor, H-bond acceptor, Rotatable bonds, and TPSA criteria. Rutin had low oral bioavailability and permeability with 610.52 g/mol of MW, 10 of H-bond donor, 16 of H-bond acceptor, and 269.43 $Å^2$ TPSA value. (9cis)-Retinal also not better than Rutin in terms of oral bioavailability and permeability with 8 of H-bond donor, 12 of H-bond acceptor, and 210.51 $Å^2$ TPSA value (table 2).

CONCLUSION

There were seven herbal compounds from DL, which have good potential as a preventive or complementary agent for Covid-19 treatment based on constant binding energy lower or equal to 7 kcal/mol with MPro or HR-1. However, Daidzein has better potency as an MPro inhibitor, while Rutin showed a worthy effect to prevent viral-host fusion by modifying HR complex structure orientation. As anti-inflammatory candidates, 19-Nortestosterone, Mesterolone, Oleanolic acid, and Ursolic acid have a satisfactory result as an NF-κB

inhibitor. Lastly, all compounds with binding energy lower than or equal to -7 kcal/mol, but no for (9cis)-Retinal and Rutin, have good oral bioavailability, permeability, and flexibility.

ACKNOWLEDGEMENT

We want to thank the Ministry of Research, Technology, and Higher Indonesia, the Republic of Indonesia, for funding this research under grant no. 229/SP2H/LT/DRPM/2019.

REFERENCES

Acosta, M.A.T., Singer, BD, 2020. Pathogenesis of COVID-19-induced ARDS: implications for an aging population. European Respiratory Journal. https://doi.org/10.1183/13993003.02049-2020

Ahkam, A.H., Hermanto, F.E., Alamsyah, A., Aliyyah, I.H., Fatchiyah, F., 2020. Virtual prediction of antiviral potential of ginger (Zingiber officinale) bioactive compounds against spike and MPro of SARS-CoV2 protein: BERKALA PENELITIAN HAYATI JOURNAL OF BIOLOGICAL RESEARCHES 25, 52–57.

Baba, J., Yokota, T., Takahashi, N., 1983. Brassinolide-related New Bioactive Steroids from Dolichos lablab Seed. Agricultural and Biological Chemistry 47, 659–661. https://doi.org/10.1080/00021369.1983.10865704

Bahtiar, A., Vichitphan, K., Han, J., 2017. Leguminous Plants in the Indonesian Archipelago: Traditional Uses and Secondary Metabolites. Nat Prod Commun 12, 461–472.

Bosch, B.J., Martina, B.E.E., Van Der Zee, R., Lepault, J., Haijema, B.J., Versluis, C., Heck, A.J.R., De Groot, R., Osterhaus, ADME, Rottier, P.J.M., 2004. Severe acute respiratory syndrome coronavirus (SARS-CoV) infection inhibition using spike protein heptad repeat-derived peptides. Proc. Natl. Acad. Sci. USA 101, 8455–8460. https://doi.org/10.1073/pnas.0400576101

Catanzaro, M., Fagiani, F., Racchi, M., Corsini, E., Govoni, S., Lanni, C., 2020. Immune response in COVID-19: addressing a pharmacological challenge by targeting pathways triggered by SARS-CoV-2. Signal Transduct Target Ther 5. https://doi.org/10.1038/s41392-020-0191-1

Chagas, C.M., Moss, S., Alisaraie, L., 2018. Drug metabolites and their effects on the development of adverse reactions: Revisiting Lipinski's Rule of Five. Int J Pharm 549, 133–149. https://doi.org/10.1016/j.ijpharm.2018.07.046

Conti, P., Ronconi, G., Caraffa, A., Gallenga, C., Ross, R., Frydas, I., Kritas, S., 2020. Induction of pro-inflammatory cytokines (IL-1 and IL-6) and lung inflammation by Coronavirus-19 (COVI-19 or SARS-CoV-2): anti-inflammatory strategies. J. Biol. Regul. Homeost. Agents 34, 327–331. https://doi.org/10.23812/CONTI-E

Dai, W., Zhang, B., Su, H., Li, J., Zhao, Y., Xie, X., Jin, Z., Liu, F., Li, C., Li, Y., Bai, F., Wang, H., Cheng, X., Cen, X., Hu, S., Yang, X., Wang, J., Liu, X., Xiao, G., Jiang, H., Rao, Z., Zhang, L.-K., Xu, Y., Yang, H., Liu, H., 2020. Structure-based design of antiviral drug candidates targeting the SARS-CoV-2 main protease. Science. https://doi.org/10.1126/science.abb4489

Daina, A., Michielin, O., Zoete, V., 2017. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep 7, 42717. https://doi.org/10.1038/srep42717

Dallakyan, S., Olson, A.J., 2015. Small-molecule library screening by docking with PyRx. Methods Mol. Biol. 1263, 243–250. https://doi.org/10.1007/978-1-4939-2269-7_19

DeDiego, M.L., Nieto-Torres, J.L., Regla-Nava, J.A., Jimenez-Guardeño, J.M., Fernandez-Delgado, R., Fett, C., Castaño-Rodriguez, C., Perlman, S., Enjuanes, L., 2014. Inhibition of NF-κB-Mediated Inflammation in Severe Acute Respiratory Syndrome Coronavirus-Infected Mice Increases Survival. J Virol 88, 913–924. https://doi.org/10.1128/JVI.02576-13

Di Matteo, G., Spano, M., Grosso, M., Salvo, A., Ingallina, C., Russo, M., Ritieni, A., Mannina, L., 2020. Food and COVID-19: Preventive/Co-therapeutic Strategies Explored by Current Clinical Trials and in Silico Studies. Foods 9, 1036. https://doi.org/10.3390/foods9081036

Gilmore, T.D., Herscovitch, M., 2006. Inhibitors of NF- κ B signaling: 785 and counting. Oncogene 25, 6887–6899. https://doi.org/10.1038/sj.onc.1209982

Gupta, S.C., Sundaram, C., Reuter, S., Aggarwal, B.B., 2010. Inhibiting NF-κB Activation by Small Molecules As a Therapeutic Strategy. Biochim Biophys Acta 1799, 775–787. https://doi.org/10.1016/j.bbagrm.2010.05.004

Gyebi, G.A., Ogunro, O.B., Adegunloye, A.P., Ogunyemi, OM, Afolabi, SO, 2020. 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 1–13. https://doi.org/10.1080/07391102.2020.1764868

Habib, H.M., Theuri, S.W., Kheadr, E., Mohamed, F.E., 2017a. DNA and BSA damage inhibitory activities, and anti-acetylcholinesterase, anti-porcine α-amylase and antioxidant properties of Dolichos lablab beans. Food Funct 8, 881–887. https://doi.org/10.1039/c6fo01164k

Habib, H.M., Theuri, S.W., Kheadr, E.E., Mohamed, F.E., 2017b. Functional, bioactive, biochemical, and physicochemical properties of the Dolichos lablab bean. Food Funct. 8, 872–880. https://doi.org/10.1039/C6FO01162D

Hayden, M.S., West, A.P., Ghosh, S., 2006. NF- κ B and the immune response. Oncogene 25, 6758–6780. https://doi.org/10.1038/sj.onc.1209943

Heck, T.G., Ludwig, M.S., Frizzo, M.N., Rasia-Filho, A.A., Homem de Bittencourt, PI, 2020. Suppressed anti-inflammatory heat shock response in high-risk COVID-19 patients: lessons from basic research (inclusive bats), light on conceivable therapies. Clin Sci (Lond) 134, 1991–2017. https://doi.org/10.1042/CS20200596

Hilgenfeld, R., 2014. From SARS to MERS: crystallographic studies on coronaviral proteases enable antiviral drug design. FEBS J. 281, 4085–4096. https://doi.org/10.1111/febs.12936

Hosseini-Zare, M.S., Thilagavathi, R., Selvam, C., 2020. Targeting severe acute respiratory syndrome-coronavirus (SARS-CoV-1) with structurally diverse inhibitors: a comprehensive review. RSC Adv. 10, 28287–28299. https://doi.org/10.1039/D0RA04395H

Jha, A.K., Kumar, R., Goenka, M.K., Dayal, V.M., 2020. Emerging Treatment and Prevention Strategies against COVID-19: A Brief Update. Journal of Digestive Endoscopy 11, 69–72. https://doi.org/10.1055/s-0040-1712547

Jin, Z., Du, X., Xu, Y., Deng, Y., Liu, M., Zhao, Y., Zhang, B., Li, X., Zhang, L., Peng, C., Duan, Y., Yu, J., Wang, L., Yang, K., Liu, F., Jiang, R., Yang, Xinglou, You, T., Liu, Xiaoce, Yang, Xiuna, Bai, F., Liu, H., Liu, Xiang, Guddat, L.W., Xu, W., Xiao, G., Qin, C., Shi, Z., Jiang, H., Rao, Z., Yang, H., 2020. Structure of M pro from SARS-CoV-2 and discovery of its inhibitors. Nature 582, 289–293. https://doi.org/10.1038/s41586-020-2223-y

Joshi, R.S., Jagdale, S.S., Bansode, S.B., Shankar, S.S., Tellis, M.B., Pandya, V.K., Chugh, A., Giri, A.P., Kulkarni, M.J., 2020. Discovery of potential multi-target-directed

ligands by targeting host-specific SARS-CoV-2 structurally conserved main protease. J. Biomol. Struct. Dyn. 1–16. https://doi.org/10.1080/07391102.2020.1760137
Kobayashi, T., Tanuma, S., Kino, K., Miyazawa, H., 2016. New scaffolds of inhibitors targeting the DNA binding of NF-κB. Integr Mol Med 3. https://doi.org/10.15761/IMM.1000242
Lin, S., Zhao, Y., Zhou, D., Zhou, F., Xu, F., 2020. Coronavirus disease 2019 (COVID-19): cytokine storms, hyper-inflammatory phenotypes, and acute respiratory distress syndrome. Genes & Diseases. https://doi.org/10.1016/j.gendis.2020.06.009
Lipinski, C.A., 2004. Lead- and drug-like compounds: the rule-of-five revolution. Drug Discov Today Technol 1, 337–341. https://doi.org/10.1016/j.ddtec.2004.11.007
Liu, S., Xiao, G., Chen, Y., He, Y., Niu, J., Escalante, C.R., Xiong, H., Farmar, J., Debnath, A.K., Tien, P., Jiang, S., 2004. Interaction between heptad repeat 1 and 2 regions in spike protein of SARS-associated coronavirus: implications for virus fusogenic mechanism and identification of fusion inhibitors. Lancet 363, 938–947. https://doi.org/10.1016/S0140-6736(04)15788-7
McKee, D.L., Sternberg, A., Stange, U., Laufer, S., Naujokat, C., 2020. Candidate drugs against SARS-CoV-2 and COVID-19. Pharmacol Res 157, 104859. https://doi.org/10.1016/j.phrs.2020.104859
Minde, J.J., Venkataramana, P.B., Matemu, A.O., 2020. Dolichos Lablab-an underutilized crop with future potentials for food and nutrition security: a review. Crit Rev Food Sci Nutr 1–13. https://doi.org/10.1080/10408398.2020.1775173
Müller, C.W., Rey, F.A., Sodeoka, M., Verdine, G.L., Harrison, S.C., 1995. Structure of the NF-kappa B p50 homodimer bound to DNA. Nature 373, 311–317. https://doi.org/10.1038/373311a0
Muzaffer, U., Paul, V.I., Rajendra Prasad, N., 2017. Molecular docking of selected phytoconstituents with signaling molecules of Ultraviolet-B induced oxidative damage. In Silico Pharmacol 5. https://doi.org/10.1007/s40203-017-0035-z
Naeem, M., Shabbir, A., Ansari, A.A., Aftab, T., Khan, M.M.A., Uddin, M., 2020. Hyacinth bean (Lablab purpureus L.) – An underutilised crop with future potential. Scientia Horticulturae 272, 109551. https://doi.org/10.1016/j.scienta.2020.109551
O'Boyle, N.M., Banck, M., James, C.A., Morley, C., Vandermeersch, T., Hutchison, G.R., 2011. Open Babel: An open chemical toolbox. Journal of Cheminformatics 3, 33. https://doi.org/10.1186/1758-2946-3-33
Ou, X., Liu, Y., Lei, X., Li, P., Mi, D., Ren, L., Guo, L., Guo, R., Chen, T., Hu, J., Xiang, Z., Mu, Z., Chen, X., Chen, J., Hu, K., Jin, Q., Wang, J., Qian, Z., 2020. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nature Communications 11, 1620. https://doi.org/10.1038/s41467-020-15562-9
Panyod, S., Ho, C.-T., Sheen, L.-Y., 2020. Dietary therapy and herbal medicine for COVID-19 prevention: A review and perspective. Journal of Traditional and Complementary Medicine 10, 420–427. https://doi.org/10.1016/j.jtcme.2020.05.004
Procacci, P., Macchiagodena, M., Pagliai, M., Guarnieri, G., Iannone, F., 2020. Interaction of hydroxychloroquine with SARS-CoV2 functional proteins using all-atoms non-equilibrium alchemical simulations. Chem. Commun. (Camb.) 56, 8854–8856. https://doi.org/10.1039/d0cc03558k
Ragab, D., Salah Eldin, H., Taeimah, M., Khattab, R., Salem, R., 2020. The COVID-19 Cytokine Storm; What We Know So Far. Front. Immunol. 11. https://doi.org/10.3389/fimmu.2020.01446

Rahman, S.A., Akhter, M.S., 2018. Antibacterial and cytotoxic activity of seeds of white hyacinth bean (Lablab purpureus L. sweet 'white') -. Journal of Advanced Biotechnology and Experimental Therapeutics 1, 49–54.

Ritchie, D.W., Kemp, G.J., 2000. Protein docking using spherical polar Fourier correlations. Proteins 39, 178–194.

Schmitz, M.L., Kracht, M., Saul, V.V., 2014. The intricate interplay between RNA viruses and NF-κB. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research 1843, 2754–2764. https://doi.org/10.1016/j.bbamcr.2014.08.004

Soy, M., Keser, G., Atagündüz, P., Tabak, F., Atagündüz, I., Kayhan, S., 2020. Cytokine storm in COVID-19: pathogenesis and overview of anti-inflammatory agents used in treatment. Clin Rheumatol 39, 2085–2094. https://doi.org/10.1007/s10067-020-05190-5

Su, H., Yao, S., Zhao, W., Li, M., Liu, J., Shang, W., Xie, H., Ke, C., Hu, H., Gao, M., Yu, K., Liu, H., Shen, J., Tang, W., Zhang, L., Xiao, G., Ni, L., Wang, D., Zuo, J., Jiang, H., Bai, F., Wu, Y., Ye, Y., Xu, Y., 2020. Anti-SARS-CoV-2 activities in vitro of Shuanghuanglian preparations and bioactive ingredients. Acta Pharmacologica Sinica 41, 1167–1177. https://doi.org/10.1038/s41401-020-0483-6

Tahir ul Qamar, M., Alqahtani, S.M., Alamri, M.A., Chen, L.-L., 2020. Structural basis of SARS-CoV-2 3CLpro and anti-COVID-19 drug discovery from medicinal plants. J Pharm Anal 10, 313–319. https://doi.org/10.1016/j.jpha.2020.03.009

Trott, O., Olson, A.J., 2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. J Comput Chem 31, 455–461. https://doi.org/10.1002/jcc.21334

Walls, A.C., Park, Y.-J., Tortorici, M.A., Wall, A., McGuire, A.T., Veesler, D., 2020. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. Cell 181, 281-292.e6. https://doi.org/10.1016/j.cell.2020.02.058

Xia, S., Liu, M., Wang, C., Xu, W., Lan, Q., Feng, S., Qi, F., Bao, L., Du, L., Liu, S., Qin, C., Sun, F., Shi, Z., Zhu, Y., Jiang, S., Lu, L., 2020. Inhibition of SARS-CoV-2 (previously 2019-nCoV) infection by a highly potent pan-coronavirus fusion inhibitor targeting its spike protein that harbors a high capacity to mediate membrane fusion. Cell Res. 30, 343–355. https://doi.org/10.1038/s41422-020-0305-x

Yin, J., Seo, C.-S., Hwang, I.H., Lee, M.W., Song, KH, 2018. Anti-Obesity Activities of Chikusetsusaponin IVa and Dolichos lablab L. Seeds. Nutrients 10, 1221. https://doi.org/10.3390/nu10091221

Yoshikawa, M., Murakami, T., Komatsu, H., Matsuda, H., 1998. Medicinal foodstuffs. XII. Saponin constituents with adjuvant activity from hyacinth bean, the seeds of Dolichos lablab L. (1): Structures of lablabosides A, B, and C. Chem Pharm Bull (Tokyo) 46, 812–816. https://doi.org/10.1248/cpb.46.812

Zhang, L., Lin, D., Sun, X., Curth, U., Drosten, C., Sauerhering, L., Becker, S., Rox, K., Hilgenfeld, R., 2020. Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors. Science 368, 409–412. https://doi.org/10.1126/science.abb3405

Zhang, W., Zhao, Y., Zhang, F., Wang, Q., Li, T., Liu, Z., Wang, J., Qin, Y., Zhang, X., Yan, X., Zeng, X., Zhang, S., 2020. The use of anti-inflammatory drugs in the treatment of people with severe coronavirus disease 2019 (COVID-19): The Perspectives of clinical immunologists from China. Clin Immunol 214, 108393. https://doi.org/10.1016/j.clim.2020.108393

Figure 1. Structural orientation and binding energy of HR complex after bound with phytochemical ligands (A) and structural alignment of HR complex before and after bound with phytochemical ligands (B). The blue ribbon in figure A represents the HR protein complex, while yellow spheres describe phytochemical ligand. In figure B, the blue ribbon represents the initial form of the HR complex without ligand, while the red ribbon represents the HR complex structure after bound with the phytochemical ligands.

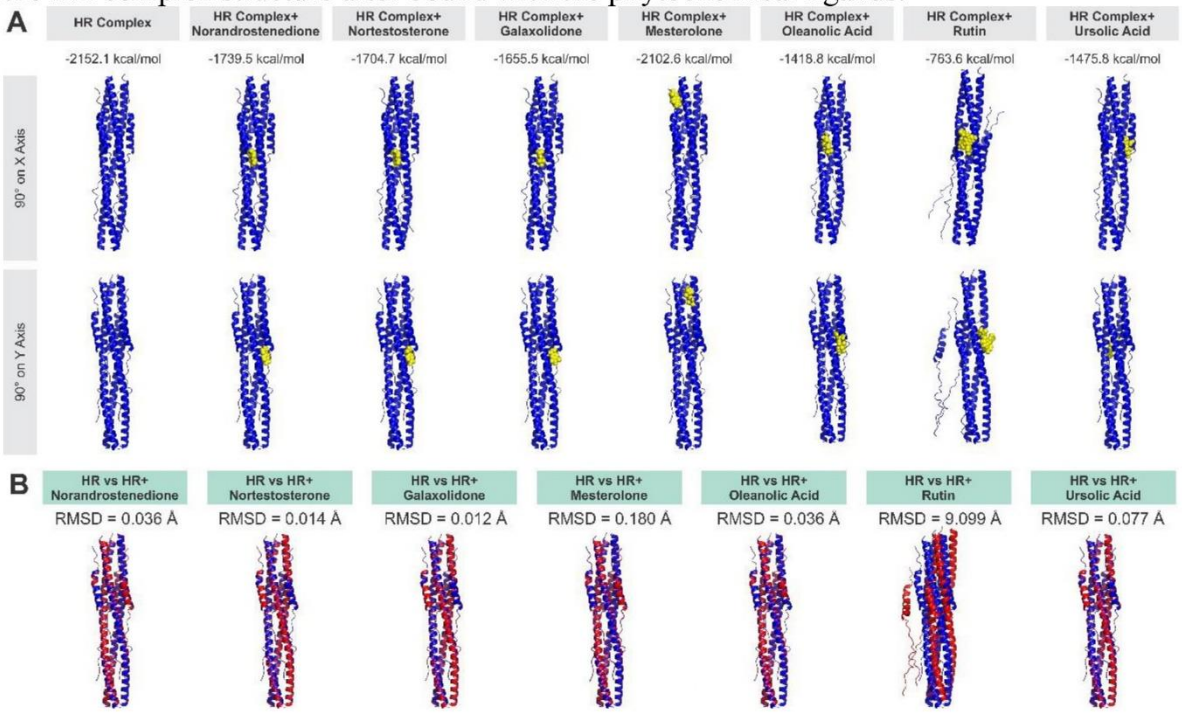


Figure 2. Structural visualization of MPro after bound with its inhibitor and phytochemical ligands: HCQ (A), (9cis)-Retinal (B), 19-Norandrostenedione (C), 19-Nortestosterone (D), 3-Hydroxy-3,5,5-trimethyl-4-(3-oxo-1-buten-1-ylidene)cyclohexyl β-D-glucopyranoside (E), Benzoic Acid (F), Daidzein (G), Dimethomorph (H), Galaxolidone (I), Ilicic Acid (J), Isoquercetin (K), Mesterolone (L), Oleanolic Acid (M), Psilotachyin B (N), Rutin (O), and Ursolic Acid (P).

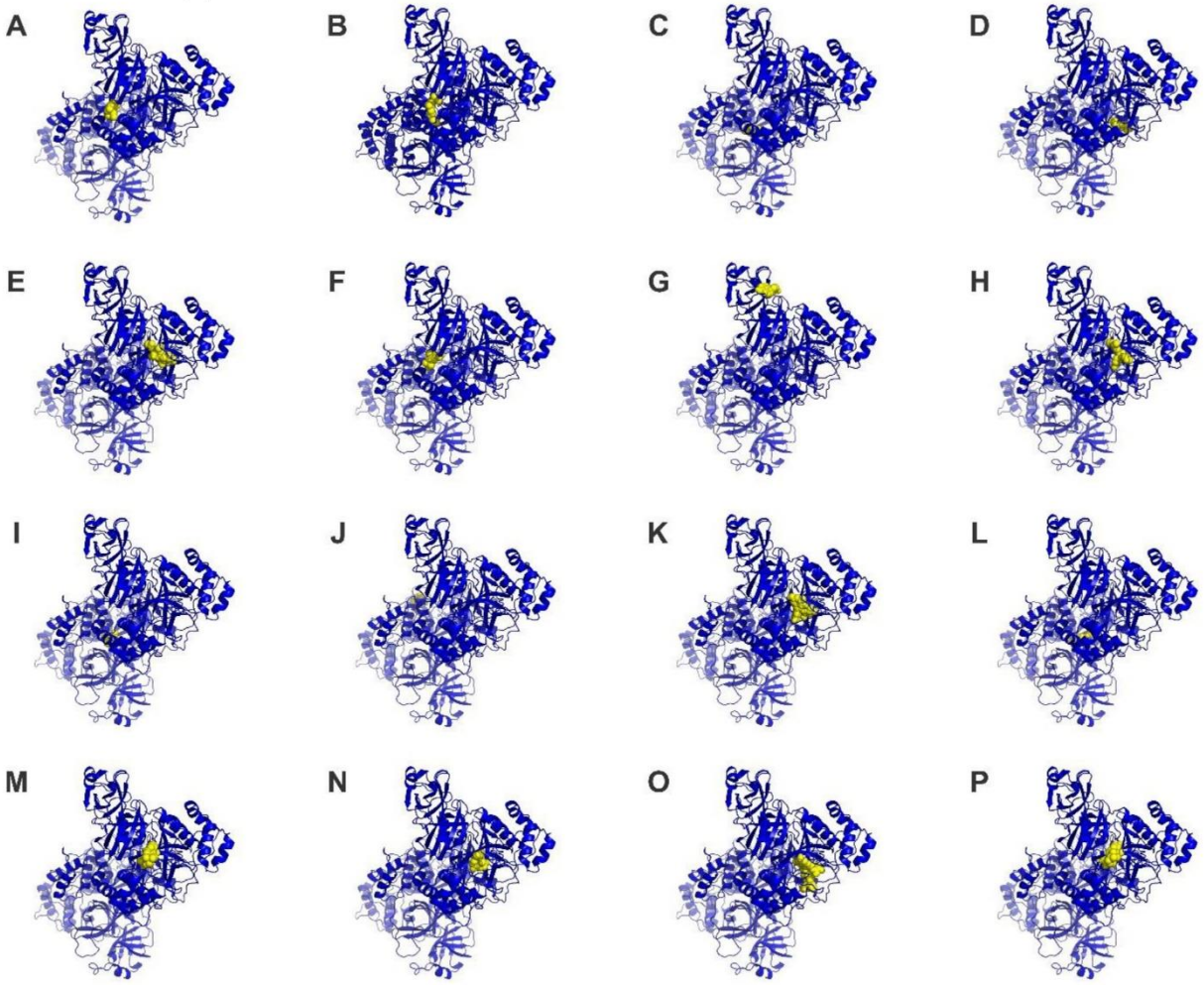
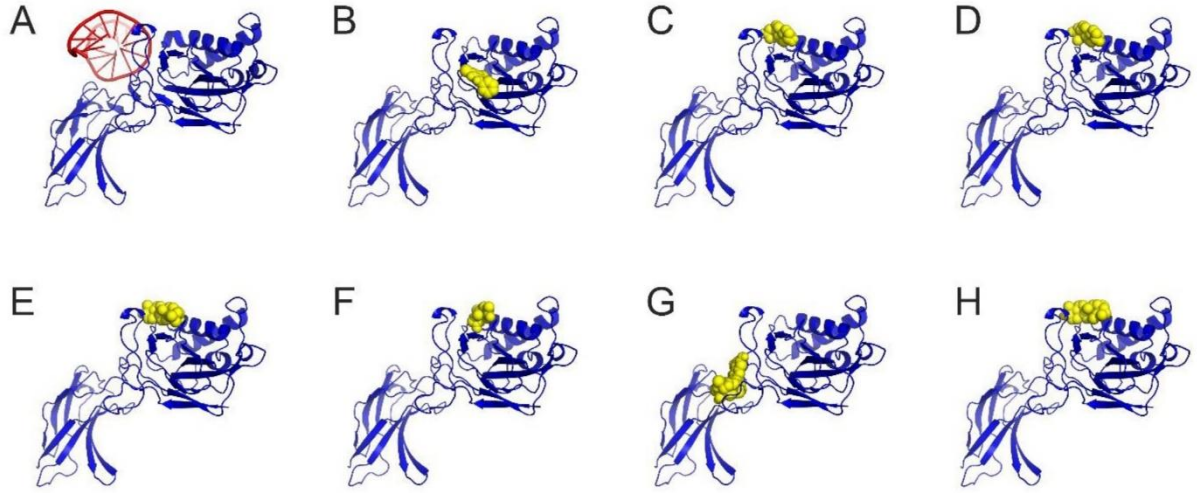
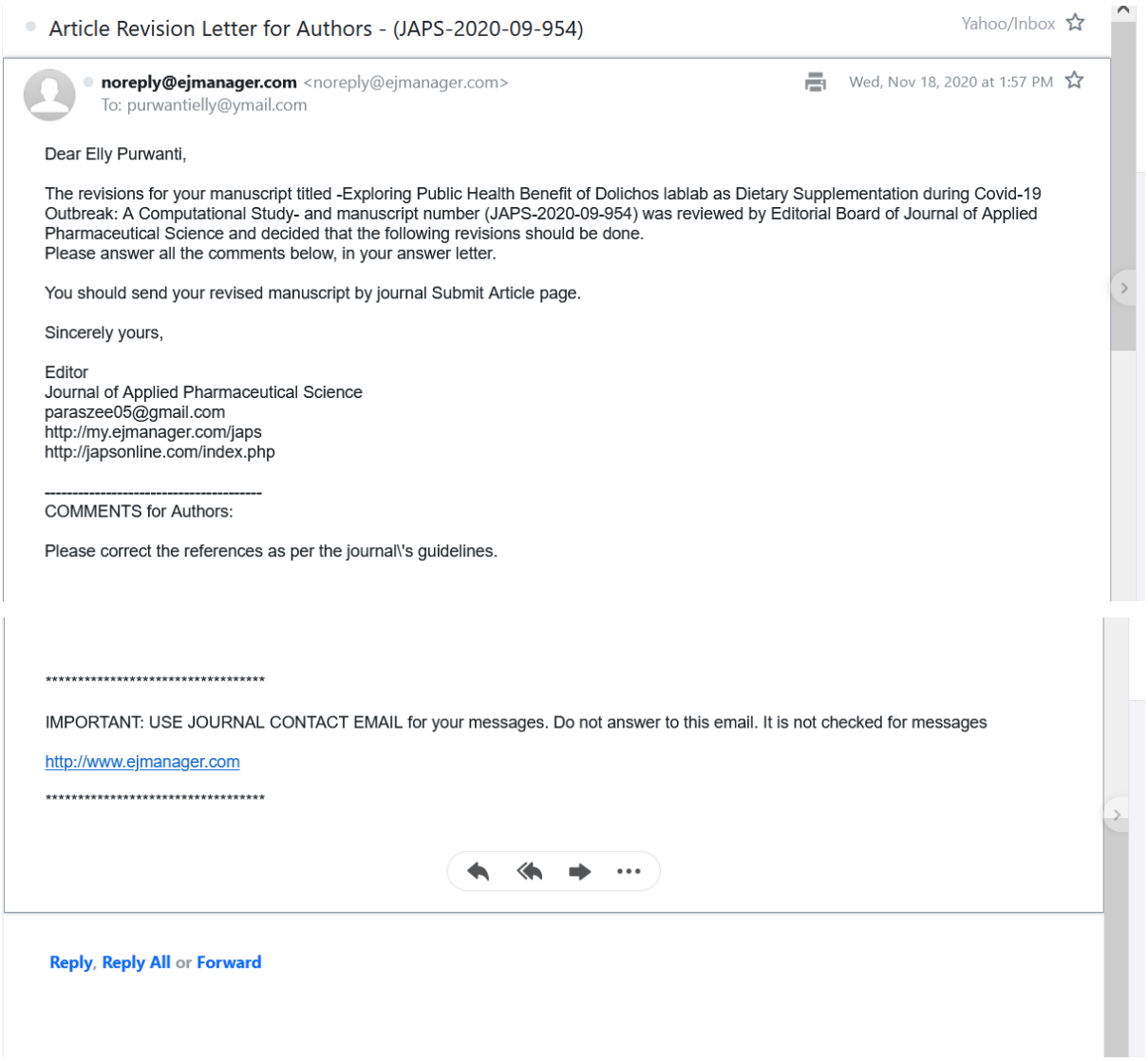


Figure 3. Structural visualization of NF-κB after bound with DNA (A), NI241 (B), 19-Nortestosterone (C), Mesterolone (D), Oleanolic Acid (E), Psilotachyin B (F), Rutin (G), and Ursolic Acid (H).



7. Third revision: Minor revision (18-11-2020)

- Article revision letter for authors



Article Revision Letter for Authors - (JAPS-2020-09-954)

Yahoo/Inbox

noreply@ejmanager.com <noreply@ejmanager.com>
To: purwantielly@ymail.com

Wed, Nov 18, 2020 at 1:57 PM

Dear Elly Purwanti,

The revisions for your manuscript titled -Exploring Public Health Benefit of Dolichos lablab as Dietary Supplementation during Covid-19 Outbreak: A Computational Study- and manuscript number (JAPS-2020-09-954) was reviewed by Editorial Board of Journal of Applied Pharmaceutical Science and decided that the following revisions should be done.
Please answer all the comments below, in your answer letter.

You should send your revised manuscript by journal Submit Article page.

Sincerely yours,

Editor
Journal of Applied Pharmaceutical Science
paraszee05@gmail.com
http://my.ejmanager.com/japs
http://japsonline.com/index.php

COMMENTS for Authors:

Please correct the references as per the journal\'s guidelines.

****

IMPORTANT: USE JOURNAL CONTACT EMAIL for your messages. Do not answer to this email. It is not checked for messages

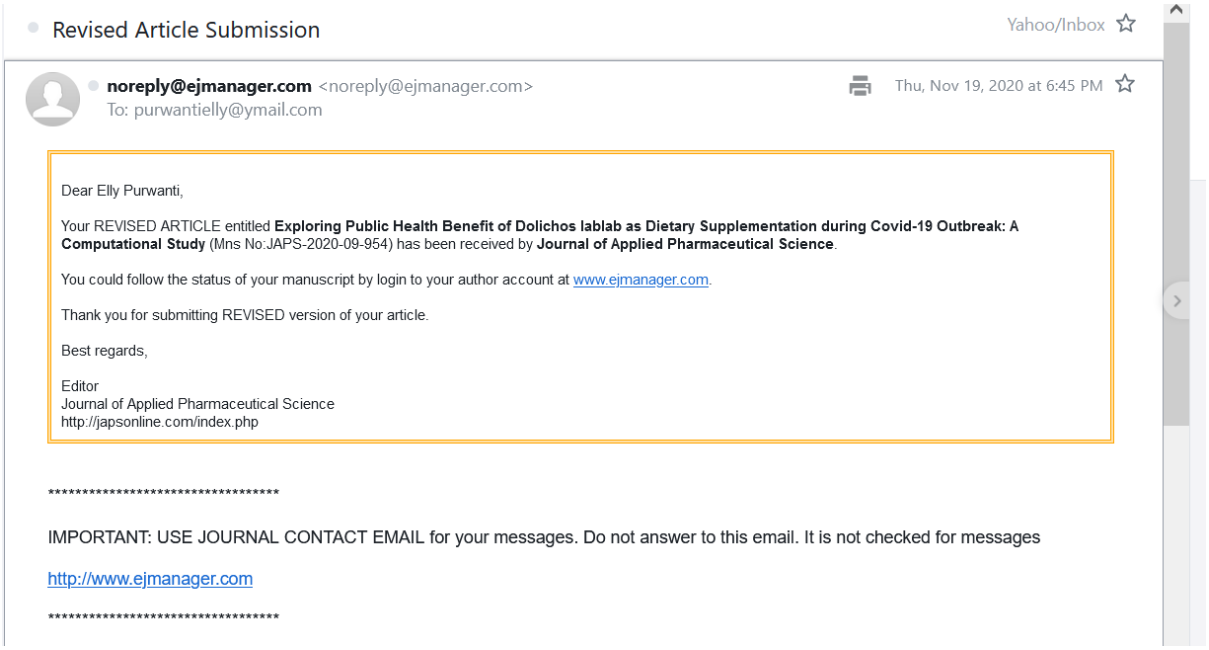
http://www.ejmanager.com

****

Reply, **Reply All** or **Forward**

8. Third revision submitted (19-11-2020)

- Revised article submission confirmation



Revised Article Submission

Yahoo/Inbox

noreply@ejmanager.com <noreply@ejmanager.com>
To: purwantielly@ymail.com

Thu, Nov 19, 2020 at 6:45 PM

Dear Elly Purwanti,

Your REVISED ARTICLE entitled **Exploring Public Health Benefit of Dolichos lablab as Dietary Supplementation during Covid-19 Outbreak: A Computational Study** (Mns No:JAPS-2020-09-954) has been received by **Journal of Applied Pharmaceutical Science**.

You could follow the status of your manuscript by login to your author account at www.ejmanager.com.

Thank you for submitting REVISED version of your article.

Best regards,

Editor
Journal of Applied Pharmaceutical Science
http://japsonline.com/index.php

****

IMPORTANT: USE JOURNAL CONTACT EMAIL for your messages. Do not answer to this email. It is not checked for messages

http://www.ejmanager.com

****

- Final draft

Exploring Public Health Benefit of *Dolichos lablab* as Dietary Supplementation during Covid-19 Outbreak: A Computational Study

Elly Purwanti[1], Feri Eko Hermanto[2], Janjte Wiliem Souhaly[2], Wahyu Prihanta[1], Tutut Indria Permana[1]

[1]*Department of Educational Biology, Faculty of Teacher Training and Education, University of Muhammadiyah Malang, East Java, Indonesia 65144*

[2]*Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, Malang, East Java, Indonesia 65145*

ABSTRACT

The emerging case of Coronavirus Disease-19 (Covid-19) caused by SARS-CoV-2 virus has become a global health issue. Since no available developed vaccine, health-promoting food has a vital role in maintaining the immune system against this disease. *Dolichos lablab* (DL), an unutilized high nutritional legume, has an excellent potential to cope with this pandemic with various health benefits phytochemical. This study appraised the possibility of phytochemical content from DL to prevent virus infection and hyper-inflammation in Covid-19 in silico. Hundreds of phytochemical from LC-HRMS analysis was docked with several SARS-CoV-2's proteins, including MPro and HR. Also, NF-κB docking was executed to pursue anti-inflammatory properties. The drug-likeness properties of screened phytochemicals were then evaluated using SwissADME. According to the results, there were sixteen phytochemicals with a high affinity to targeted proteins. Among those, five phytochemicals consistently gave low binding affinity to all targeted proteins. Those five phytochemicals' physicochemical properties, except for Rutin and (9cis)-Retinal, also coped with small-molecule bioavailability, permeability, and flexibility according to SwissADME calculations. In conclusion, DL has a high probability of complementing the medical effort as dietary supplementation to modulate the immune system and prevent viral infection.

Keywords: Covid-19, *Dolichos lablab*, Heptad Repeat, Main Protease, NF-κB

INTRODUCTION

The outbreak of COVID-19 has now become a significant global health issue. Until the August 2020 update, more than twenty million people have become infected, and nearly a million have died due to this pandemic (https://covid19.who.int/). This number is still increasing day by day, describing the war against Covid-19 continued. Although several companies already announced that they have proposed vaccine candidates entering the final phase of clinical trials, complementary medicine and dietary intervention still needed to prevent the severity of infected people and prevent the healthy people from getting infected (Di Matteo et al., 2020; Panyod et al., 2020). Therefore, exploring the proper diet for patients or healthy people to help against Covid-19 infection becomes essential.

Thwarting SARS-CoV-2 attachment and replication has become the main target for combating Covid-19 (Jha et al., 2020; McKee et al., 2020). Several proteins from SARS-CoV-2 have been modeled and appropriately studied as a target to decrease the number of positive cases (Dai et al., 2020; Xia et al., 2020; L. Zhang et al., 2020). Spike protein is the primary key for SARS-CoV-2 to enter the host's cell, consisting of unique parts called Heptad Repeat 1 (HR1) and HR 2 inside the Receptor Binding Domain (RBD) for performing membrane fusion after attachment (Bosch et al., 2004; Walls et al., 2020; Xia et al., 2020). With this critical role, HR1 and HR2 have been proposed as the main target to evade viral entry and infection (Xia et al., 2020). Another protein called Main Protease (MPro) also becomes the right candidate due to its vital role in viral replication and transcription (Hilgenfeld, 2014). Therefore, several studies also used MPro as a target to inhibit the severity of Covid-19 infection (Ahkam et al., 2020; Dai et al., 2020; Jin et al., 2020; Joshi et al., 2020).

Another perspective to support a patient's survival is by suppressing massive inflammation in lung tissue (Heck et al., 2020; W. Zhang et al., 2020). This inflammation, known as cytokine storms, occurs by dysregulation of the immune response, disturbing tissue homeostasis, leading to tissue damage (Ragab et al., 2020; Soy et al., 2020). With an injury in lung tissue, the patients will suffer from breathing and died (Acosta and Singer, 2020; Lin et al., 2020). NF-ϰB, a kind of transcription factor which controls several cytokines involved in cytokine storms, like interleukin (IL)-1 and IL-6, has a good starting point to diminish the hyper-inflammation (Catanzaro et al., 2020; Conti et al., 2020; Soy et al., 2020). Previously, suppressing NF-ϰB could increase survival after coronavirus infection (DeDiego et al., 2014). Thus, targeting this transcription factor has a reasonable probability of improving patient survival.

Legumes have been a good source of nutrition for years. However, masses of legume species are still underutilized as a nutritious food. One of the rarely used legumes is *Dolichos lablab* (DL) (Minde et al., 2020). Not only for high nutritional content but DL also had an innumerable natural compound with numerous biological activities. Previously, DL has been explored for its antioxidant, antidiabetic, antimicrobial, even anti-inflammatory properties (Habib et al., 2017; Naeem et al., 2020; Rahman and Akhter, 2018). With those various health benefits, DL has promising candidates for dietary supplementation to avoid Covid-19 infection.

MATERIAL AND METHOD

Phytochemical Content Screening

Thermo Scientific Dionex Ultimate 3000 RSLCnano coupled with Thermo Scientific Q Exactive MS, was used to identify phytochemical content inside the methanolic extract of DL. Hypersil GOLD aQ 50 x 1 mm x 1.9 µ particle size was installed in LC instrument as stationary phase, while the mobile phase consists of solvent A (0.1% Formic acid in water) and solvent B (0.1% Formic acid in Acetonitrile). The LC was operated under the following conditions: flow rate 40 µl/min, 30 min run time, 30°C column temperature. Obtained data were analyzed using Compound Discoverer with mzCloud MS/MS Library. Compound with mzCloud best match's score higher than 80 then directed for further analysis.

Ligand and Protein Structures Retrieval

The compounds from LC-HRMS analysis were used as the ligand. Three dimensional (3D) structure of the ligands was assessed through the PubChem database (supplementary file 1). Protein's 3D structures were retrieved from Protein Data Bank (PDB), i.e., MPro (PDB ID: 6M2N), HR complex (6LXT), and NF-ϰB (1SVC).

Molecular Docking

Water molecule and native ligand from 3D protein structures were removed using Discovery Studio 16. Energy minimization of the ligand structures was prepared using Open Babel integrated into PyRx 8.0 (O'Boyle et al., 2011). All compounds from LC-HRMS were screened using molecular docking to predict its interaction against protein targets. Protein-ligand docking was performed using AutoDock Vina integrated into PyRx 8.0 (Dallakyan and Olson, 2015; Trott and Olson, 2010) with a maximum grid size setting. HEX 8.0 was run for protein-protein docking using default setting and operated under Shape+DARS correlation type (Ritchie and Kemp, 2000). As a comparison, Hydroxychloroquine (HCQ, CID: 3652) (Procacci et al., 2020) and 4,6-dichloro-N-phenyl-1,3,5-triazine-2-amine (NI241, CID: 16766) (Kobayashi et al., 2016) were employed as a control inhibitor for MPro and NF- ϰB, respectively.

Data Analysis

Protein-ligand complex, which has binding energy lower than -7 kcal/mol, were directed into further analysis. Interacted residues in each protein-ligand complex and structure conformation were analyzed and visualized using Discovery Studio. Protein structure alignment was executed using PyMOL 2.3.2 with RMSD value determined as a structural difference among aligned proteins. The alignment was achieved by setting the HR1-HR2 complex as a reference structure.

Prediction of Drug Likeness Properties

Drug-likeness properties were analyzed according to Lipinski's Rule of 5 (LRO5) (Lipinski, 2004). Drug resemblance properties were determined using Swiss-ADME Physicochemical Properties (Daina et al., 2017), including molecular weight (MW), LogP value, and the number of H-bond donor, H-bond acceptor, rotatable bond, and Total Polar Surface Area (TPSA).

RESULT AND DISCUSSION

According to binding affinity, 16 compounds have been identified to have biological activities against Covid-19 treatment, which is 5 of them have binding energy lower than -7 kcal/mol consistently in every target protein. 19-Nortestosterone, Mesterolone, Oleanolic acid, Rutin, and Ursolic acid are the compounds that have low binding energy in MPro, HR-1, and NF-ϰB. MPro is the protein that can interact with more compounds tested with binding energy lower or equal with -7 kcal/mol. Based on binding affinity lower or similar to 7 kcal/mol, seven compounds could interact with HR-1, while six compounds have docked with NF-ϰB (table 1).

19-Norandrostenedione, 19-Nortestosterone, Galaxolidone, Mesterolone, Oleanolic Acid, Rutin, and Ursolic Acid are the compounds that have good affinity to HR-1. Attachment of those compounds could alter the HR1-HR2 binding motif represented by declining the HR complex's binding energy after bonded with those compounds compared to the HR complex without ligand (figure 1a). Among seven compounds that have an excellent affinity to HR-1, Rutin could modify HR1-HR2 interaction clearly. This was described by the RMSD value of the HR complex with Rutin inside compared to the HR complex alone, which has greater value than other complexes (figure 1b). Interaction of HR1-HR2 to form the helix bundle is the crucial step for SARS-CoV-2 membrane fusion (Liu et al., 2004; Ou et al., 2020). Altering helix bundle formation has been studied to prevent viral entry (Xia et al., 2020), suggesting that 19-Norandrostenedione, 19-Nortestosterone, Galaxolidone, Mesterolone, Oleanolic Acid, Ursolic Acid, in particular Rutin, have an excellent potency to inhibit viral infection.

MPro is the target protein with plenty of interacted compounds. Compared to HCQ as control, all the compounds have lesser binding energy compared to HCQ. Each compound has its favorable binding region, presented by structural visualization (figure 2) or interacted residues between the ligand molecule and MPro (table S2). Among those compounds, only Daidzein has an interaction with catalytic residues of MPro at CYS:145. HCQ did not show any interaction with the catalytic residues (table S2). HIS:41 and CYS:145 have been known as essential residues during MPro enzymatic activity (Tahir ul Qamar et al., 2020). Inhibiting those residues has a promising effect on preventing virus replication and prevent viral spreading throughout the tissues (Ahkam et al., 2020; Gyebi et al., 2020; Hosseini-Zare et al., 2020; Tahir ul Qamar et al., 2020).

NF-ϰB is the transcription factor of several proteins related to infection response, including several cytokines related to defense mechanisms (Hayden et al., 2006; Schmitz et al., 2014). At critical condition, cytokine storms are the main factor that contributes to lung damage due to the overexpression of pro-inflammatory cytokines (Lin et al., 2020; Soy et al., 2020). Thus, targeting NF-ϰB as the main transcription factor for suppressing those cytokines' hyper-expression could play a vital role in increasing patient survival (Catanzaro et al., 2020). From the docking result, six compounds could interact with NF- ϰB at 7 kcal/mol or lower. 19-Nortestosterone, Mesterolone, Oleanolic acid, and Ursolic acid could be bound with DNA binding sites (figure 3) based on residues involved in every compound-NF-ϰB complex (table S3). Those compounds could bind with several important residues involved in the DNA binding site, i.e., TYR:60 and HIS:144 (table S3) (Müller et al., 1995). Targeting DNA-binding sites of NF-ϰB has been employed to preclude chronic inflammation (Gilmore and Herscovitch, 2006; Gupta et al., 2010). Therefore, the presence of those compounds in the NF-ϰB DNA-binding domain could reduce hyper inflammation by altering the pro-inflammatory cytokines' transcription process.

Several criteria have been developed by Lipinski for a small molecule to have good oral bioavailability, permeability, and flexibility (Lipinski, 2004). In order to have good oral bioavailability, the small molecule should have MW, LogP value, number of hydrogen bond donor, and acceptor less than 500 g/mol, 5, 5, 10, respectively (Lipinski, 2004). Also, a molecule with a

TPSA value equal or less than 140 Å will perform good permeability, while the number of rotatable bonds less than 10 represents molecule flexibility (Chagas et al., 2018). Hence, SwissADME was employed to do the calculations related to LRO5. All of the analyzed compounds, except for Rutin and (9cis)-Retinal, have no violation of the Molecular Weight, LogP value, H-bond donor, H-bond acceptor, Rotatable bonds, and TPSA criteria. Rutin had low oral bioavailability and permeability with 610.52 g/mol of MW, 10 of H-bond donor, 16 of H-bond acceptor, and 269.43 $Å^2$ TPSA value. (9cis)-Retinal also not better than Rutin in terms of oral bioavailability and permeability with 8 of H-bond donor, 12 of H-bond acceptor, and 210.51 $Å^2$ TPSA value (table 2).

CONCLUSION

There were seven herbal compounds from DL, which have good potential as a preventive or complementary agent for Covid-19 treatment based on constant binding energy lower or equal to 7 kcal/mol with MPro or HR-1. However, Daidzein has better potency as an MPro inhibitor, while Rutin showed a worthy effect to prevent viral-host fusion by modifying HR complex structure orientation. As anti-inflammatory candidates, 19-Nortestosterone, Mesterolone, Oleanolic acid, and Ursolic acid have a satisfactory result as an NF-$\varkappa$B inhibitor. Lastly, all compounds with binding energy lower than or equal to 7 kcal/mol, but no for (9cis)-Retinal and Rutin, have good oral bioavailability and permeability flexibility.

REFERENCES

Acosta, M.A.T., Singer, B.D., 2020. Pathogenesis of COVID-19-induced ARDS: implications for an aging population. Eur. Respir. J. https://doi.org/10.1183/13993003.02049-2020

Ahkam, A.H., Hermanto, F.E., Alamsyah, A., Aliyyah, I.H., Fatchiyah, F., 2020. Virtual prediction of antiviral potential of ginger (Zingiber officinale) bioactive compounds against spike and MPro of SARS-CoV2 protein: Berk. Penelit. HAYATI J. Biol. Res. 25, 52–57.

Bosch, B.J., Martina, B.E.E., Van Der Zee, R., Lepault, J., Haijema, B.J., Versluis, C., Heck, A.J.R., De Groot, R., Osterhaus, A.D.M.E., Rottier, P.J.M., 2004. Severe acute respiratory syndrome coronavirus (SARS-CoV) infection inhibition using spike protein heptad repeat-derived peptides. Proc. Natl. Acad. Sci. U. S. A. 101, 8455–8460. https://doi.org/10.1073/pnas.0400576101

Catanzaro, M., Fagiani, F., Racchi, M., Corsini, E., Govoni, S., Lanni, C., 2020. Immune response in COVID-19: addressing a pharmacological challenge by targeting pathways triggered by SARS-CoV-2. Signal Transduct. Target. Ther. 5. https://doi.org/10.1038/s41392-020-0191-1

Chagas, C.M., Moss, S., Alisaraie, L., 2018. Drug metabolites and their effects on the development of adverse reactions: Revisiting Lipinski's Rule of Five. Int. J. Pharm. 549, 133–149. https://doi.org/10.1016/j.ijpharm.2018.07.046

Conti, P., Ronconi, G., Caraffa, A., Gallenga, C., Ross, R., Frydas, I., Kritas, S., 2020. Induction of pro-inflammatory cytokines (IL-1 and IL-6) and lung inflammation by Coronavirus-19 (COVI-19 or SARS-CoV-2): anti-inflammatory strategies. J. Biol. Regul. Homeost. Agents 34, 327–331. https://doi.org/10.23812/CONTI-E

Dai, W., Zhang, B., Su, H., Li, J., Zhao, Y., Xie, X., Jin, Z., Liu, F., Li, C., Li, Y., Bai, F., Wang, H., Cheng, X., Cen, X., Hu, S., Yang, X., Wang, J., Liu, X., Xiao, G., Jiang, H., Rao, Z., Zhang, L.-K., Xu, Y., Yang, H., Liu, H., 2020. Structure-based design of antiviral drug candidates targeting the SARS-CoV-2 main protease. Science. https://doi.org/10.1126/science.abb4489

Daina, A., Michielin, O., Zoete, V., 2017. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci. Rep. 7, 42717. https://doi.org/10.1038/srep42717

Dallakyan, S., Olson, A.J., 2015. Small-molecule library screening by docking with PyRx. Methods Mol. Biol. Clifton NJ 1263, 243–250. https://doi.org/10.1007/978-1-4939-2269-7_19

DeDiego, M.L., Nieto-Torres, J.L., Regla-Nava, J.A., Jimenez-Guardeño, J.M., Fernandez-Delgado, R., Fett, C., Castaño-Rodriguez, C., Perlman, S., Enjuanes, L., 2014. Inhibition of NF-κB-Mediated Inflammation in Severe Acute Respiratory Syndrome Coronavirus-Infected Mice Increases Survival. J. Virol. 88, 913–924. https://doi.org/10.1128/JVI.02576-13

Di Matteo, G., Spano, M., Grosso, M., Salvo, A., Ingallina, C., Russo, M., Ritieni, A., Mannina, L., 2020. Food and COVID-19: Preventive/Co-therapeutic Strategies Explored by Current Clinical Trials and in Silico Studies. Foods 9, 1036. https://doi.org/10.3390/foods9081036

Gilmore, T.D., Herscovitch, M., 2006. Inhibitors of NF- κ B signaling: 785 and counting. Oncogene 25, 6887–6899. https://doi.org/10.1038/sj.onc.1209982

Gupta, S.C., Sundaram, C., Reuter, S., Aggarwal, B.B., 2010. Inhibiting NF-κB Activation by Small Molecules As a Therapeutic Strategy. Biochim. Biophys. Acta 1799, 775–787. https://doi.org/10.1016/j.bbagrm.2010.05.004

Gyebi, G.A., Ogunro, O.B., Adegunloye, A.P., Ogunyemi, O.M., Afolabi, S.O., 2020. 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. 1–13. https://doi.org/10.1080/07391102.2020.1764868

Habib, H.M., Theuri, S.W., Kheadr, E., Mohamed, F.E., 2017. DNA and BSA damage inhibitory activities, and anti-acetylcholinesterase, anti-porcine α-amylase and antioxidant properties of Dolichos lablab beans. Food Funct. 8, 881–887. https://doi.org/10.1039/c6fo01164k

Hayden, M.S., West, A.P., Ghosh, S., 2006. NF- κ B and the immune response. Oncogene 25, 6758–6780. https://doi.org/10.1038/sj.onc.1209943

Heck, T.G., Ludwig, M.S., Frizzo, M.N., Rasia-Filho, A.A., Homem de Bittencourt, P.I., 2020. Suppressed anti-inflammatory heat shock response in high-risk COVID-19 patients: lessons from basic research (inclusive bats), light on conceivable therapies. Clin. Sci. 134, 1991–2017. https://doi.org/10.1042/CS20200596

Hilgenfeld, R., 2014. From SARS to MERS: crystallographic studies on coronaviral proteases enable antiviral drug design. FEBS J. 281, 4085–4096. https://doi.org/10.1111/febs.12936

Hosseini-Zare, M.S., Thilagavathi, R., Selvam, C., 2020. Targeting severe acute respiratory syndrome-coronavirus (SARS-CoV-1) with structurally diverse inhibitors: a comprehensive review. RSC Adv. 10, 28287–28299. https://doi.org/10.1039/D0RA04395H

Jha, A.K., Kumar, R., Goenka, M.K., Dayal, V.M., 2020. Emerging Treatment and Prevention Strategies against COVID-19: A Brief Update. J. Dig. Endosc. 11, 69–72. https://doi.org/10.1055/s-0040-1712547

Jin, Z., Du, X., Xu, Y., Deng, Y., Liu, M., Zhao, Y., Zhang, B., Li, X., Zhang, L., Peng, C., Duan, Y., Yu, J., Wang, L., Yang, K., Liu, F., Jiang, R., Yang, Xinglou, You, T., Liu, Xiaoce, Yang, Xiuna, Bai, F., Liu, H., Liu, Xiang, Guddat, L.W., Xu, W., Xiao, G., Qin, C., Shi, Z., Jiang, H., Rao, Z., Yang, H., 2020. Structure of M pro from SARS-CoV-2 and discovery of its inhibitors. Nature 582, 289–293. https://doi.org/10.1038/s41586-020-2223-y

Joshi, R.S., Jagdale, S.S., Bansode, S.B., Shankar, S.S., Tellis, M.B., Pandya, V.K., Chugh, A., Giri, A.P., Kulkarni, M.J., 2020. Discovery of potential multi-target-directed ligands by targeting host-specific SARS-CoV-2 structurally conserved main protease. J. Biomol. Struct. Dyn. 1–16. https://doi.org/10.1080/07391102.2020.1760137

Kobayashi, T., Tanuma, S., Kino, K., Miyazawa, H., 2016. New scaffolds of inhibitors targeting the DNA binding of NF-κB. Integr. Mol. Med. 3. https://doi.org/10.15761/IMM.1000242

Lin, S., Zhao, Y., Zhou, D., Zhou, F., Xu, F., 2020. Coronavirus disease 2019 (COVID-19): cytokine storms, hyper-inflammatory phenotypes, and acute respiratory distress syndrome. Genes Dis. https://doi.org/10.1016/j.gendis.2020.06.009

Lipinski, C.A., 2004. Lead- and drug-like compounds: the rule-of-five revolution. Drug Discov. Today Technol. 1, 337–341. https://doi.org/10.1016/j.ddtec.2004.11.007

Liu, S., Xiao, G., Chen, Y., He, Y., Niu, J., Escalante, C.R., Xiong, H., Farmar, J., Debnath, A.K., Tien, P., Jiang, S., 2004. Interaction between heptad repeat 1 and 2 regions in spike protein of SARS-associated coronavirus: implications for virus fusogenic mechanism and identification of fusion inhibitors. Lancet Lond. Engl. 363, 938–947. https://doi.org/10.1016/S0140-6736(04)15788-7

McKee, D.L., Sternberg, A., Stange, U., Laufer, S., Naujokat, C., 2020. Candidate drugs against SARS-CoV-2 and COVID-19. Pharmacol. Res. 157, 104859. https://doi.org/10.1016/j.phrs.2020.104859

Minde, J.J., Venkataramana, P.B., Matemu, A.O., 2020. Dolichos Lablab-an underutilized crop with future potentials for food and nutrition security: a review. Crit. Rev. Food Sci. Nutr. 1–13. https://doi.org/10.1080/10408398.2020.1775173

Müller, C.W., Rey, F.A., Sodeoka, M., Verdine, G.L., Harrison, S.C., 1995. Structure of the NF-kappa B p50 homodimer bound to DNA. Nature 373, 311–317. https://doi.org/10.1038/373311a0

Naeem, M., Shabbir, A., Ansari, A.A., Aftab, T., Khan, M.M.A., Uddin, M., 2020. Hyacinth bean (Lablab purpureus L.) – An underutilised crop with future potential. Sci. Hortic. 272, 109551. https://doi.org/10.1016/j.scienta.2020.109551

O'Boyle, N.M., Banck, M., James, C.A., Morley, C., Vandermeersch, T., Hutchison, G.R., 2011. Open Babel: An open chemical toolbox. J. Cheminformatics 3, 33. https://doi.org/10.1186/1758-2946-3-33

Ou, X., Liu, Y., Lei, X., Li, P., Mi, D., Ren, L., Guo, L., Guo, R., Chen, T., Hu, J., Xiang, Z., Mu, Z., Chen, X., Chen, J., Hu, K., Jin, Q., Wang, J., Qian, Z., 2020. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat. Commun. 11, 1620. https://doi.org/10.1038/s41467-020-15562-9

Panyod, S., Ho, C.-T., Sheen, L.-Y., 2020. Dietary therapy and herbal medicine for COVID-19 prevention: A review and perspective. J. Tradit. Complement. Med. 10, 420–427. https://doi.org/10.1016/j.jtcme.2020.05.004

Procacci, P., Macchiagodena, M., Pagliai, M., Guarnieri, G., Iannone, F., 2020. Interaction of hydroxychloroquine with SARS-CoV2 functional proteins using all-atoms non-equilibrium alchemical simulations. Chem. Commun. Camb. Engl. 56, 8854–8856. https://doi.org/10.1039/d0cc03558k

Ragab, D., Salah Eldin, H., Taeimah, M., Khattab, R., Salem, R., 2020. The COVID-19 Cytokine Storm; What We Know So Far. Front. Immunol. 11. https://doi.org/10.3389/fimmu.2020.01446

Rahman, S.A., Akhter, M.S., 2018. Antibacterial and cytotoxic activity of seeds of white hyacinth bean (Lablab purpureus L. sweet 'white') -. J. Adv. Biotechnol. Exp. Ther. 1, 49–54.

Ritchie, D.W., Kemp, G.J., 2000. Protein docking using spherical polar Fourier correlations. Proteins 39, 178–194.

Schmitz, M.L., Kracht, M., Saul, V.V., 2014. The intricate interplay between RNA viruses and NF-κB. Biochim. Biophys. Acta BBA - Mol. Cell Res. 1843, 2754–2764. https://doi.org/10.1016/j.bbamcr.2014.08.004

Soy, M., Keser, G., Atagündüz, P., Tabak, F., Atagündüz, I., Kayhan, S., 2020. Cytokine storm in COVID-19: pathogenesis and overview of anti-inflammatory agents used in treatment. Clin. Rheumatol. 39, 2085–2094. https://doi.org/10.1007/s10067-020-05190-5

Tahir ul Qamar, M., Alqahtani, S.M., Alamri, M.A., Chen, L.-L., 2020. Structural basis of SARS-CoV-2 3CLpro and anti-COVID-19 drug discovery from medicinal plants. J. Pharm. Anal. 10, 313–319. https://doi.org/10.1016/j.jpha.2020.03.009

Trott, O., Olson, A.J., 2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. J. Comput. Chem. 31, 455–461. https://doi.org/10.1002/jcc.21334

Walls, A.C., Park, Y.-J., Tortorici, M.A., Wall, A., McGuire, A.T., Veesler, D., 2020. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. Cell 181, 281-292.e6. https://doi.org/10.1016/j.cell.2020.02.058

Xia, S., Liu, M., Wang, C., Xu, W., Lan, Q., Feng, S., Qi, F., Bao, L., Du, L., Liu, S., Qin, C., Sun, F., Shi, Z., Zhu, Y., Jiang, S., Lu, L., 2020. Inhibition of SARS-CoV-2 (previously 2019-nCoV) infection by a highly potent pan-coronavirus fusion inhibitor targeting its spike protein that harbors a high capacity to mediate membrane fusion. Cell Res. 30, 343–355. https://doi.org/10.1038/s41422-020-0305-x

Zhang, L., Lin, D., Sun, X., Curth, U., Drosten, C., Sauerhering, L., Becker, S., Rox, K., Hilgenfeld, R., 2020. Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors. Science 368, 409–412. https://doi.org/10.1126/science.abb3405

Zhang, W., Zhao, Y., Zhang, F., Wang, Q., Li, T., Liu, Z., Wang, J., Qin, Y., Zhang, X., Yan, X., Zeng, X., Zhang, S., 2020. The use of anti-inflammatory drugs in the treatment of people with severe coronavirus disease 2019 (COVID-19): The Perspectives of clinical immunologists from China. Clin. Immunol. Orlando Fla 214, 108393. https://doi.org/10.1016/j.clim.2020.108393

Table and Illustration

Table 1. Phytochemical with binding energy less than or equal to -7 kcal/mol during screening process using molecular docking

Compound	Binding Energy (kcal/mol)		
	HR-1	MPro	NF-κB
(9cis)-Retinal	-6.2	**-7.6**	-5.9
19-Norandrostenedione	**-7.3**	**-7.6**	-6.6
19-Nortestosterone	**-7.6**	**-7.2**	**-7.3**
3-Hydroxy-3,5,5-trimethyl-4-(3-oxo-1-buten-1-ylidene)cyclohexyl β-D-glucopyranoside	-6.8	**-7.5**	-6.3
Benzoic Acid	-6.4	**-7.1**	-5.4
Daidzein	-6.3	**-7.4**	-6.1
Dimethomorph	-6.1	**-8.6**	-6.3
Galaxolidone	**-7.4**	**-7.8**	-6.3
Ilicic Acid	-6.5	**-7.3**	-5.8
Isoquercetin	-6.7	**-8.8**	-6.8
Mesterolone	**-7.1**	**-7.5**	**-7.3**
Oleanolic acid	**-7.8**	**-9.1**	**-8.2**
Psilostachyin B	-6.2	**-7.7**	**-7.1**
Rutin	**-7.5**	**-9.1**	**-7.3**
Ursolic acid	**-8.1**	**-9.5**	**-7.9**
Hydroxychloroquine	-	**-6.5**	-
NI241	-	-	-5.4

Table 2. Physicochemical properties of screened phytochemicals according to SwissADME measurement

Compound	**Mol. Weight (g/mol)**	**LogP value**	**H-bond donor**	**H-bond acceptor**	**Rotatable bonds**	**TPSA**
(9cis)-Retinal	464.38	2.11	8	12	4	210.51 Å²
19-Norandrostenedione	272.38	2.6	0	2	0	34.14 Å²
19-Nortestosterone	274.4	2.79	1	2	0	37.30 Å²
3-Hydroxy-3,5,5-trimethyl-4-(3-oxo-1-buten-1-ylidene)cyclohexyl β-D-glucopyranoside	386.44	2.21	5	8	4	136.68 Å²
Benzoic Acid	250.33	2.74	2	3	3	57.53 Å²
Daidzein	254.24	1.77	2	4	1	70.67 Å²
Dimethomorph	387.86	3.67	0	4	6	48.00 Å²
Galaxolidone	272.38	3.17	0	2	0	26.30 Å²
Ilicic Acid	252.35	2.26	2	3	2	57.53 Å²
Mesterolone	304.47	3.09	1	2	0	37.30 Å²
Oleanolic acid	456.7	3.92	2	3	1	57.53 Å²
Psilostachyin B	262.3	2.17	0	4	0	52.60 Å²
Quercetin	284.44	3.76	0	1	5	17.07 Å²
Rutin	610.52	2.43	10	16	6	269.43 Å²
Ursolic acid	456.7	4.01	2	3	1	57.53 Å²

Figure 1. Phytochemical effect on HR complex binding energy (A) and structural orientation (B). Figure A shows the blue ribbon represents the HR protein complex, while yellow spheres describe phytochemical ligand. At figure B, the blue ribbon represents the initial form of the HR complex without ligand, while the red ribbon represents the HR complex structure after bounded with the phytochemical ligands.

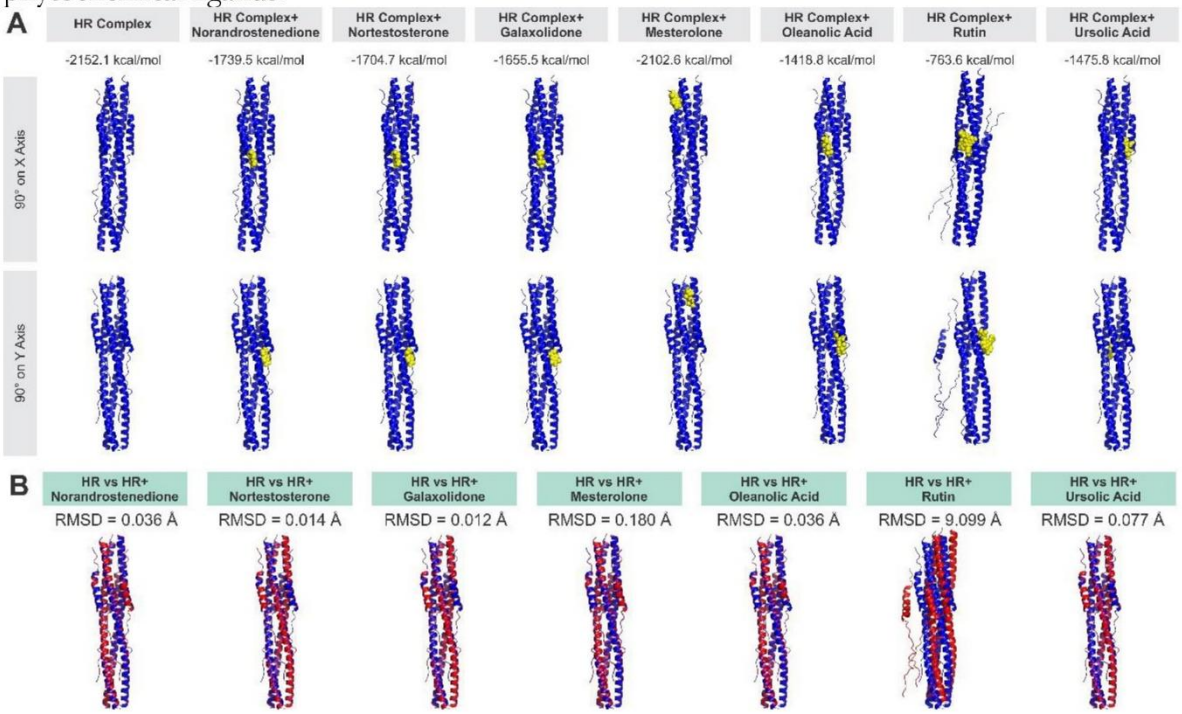


Figure 2. Visualization of MPro docked with its inhibitor and phytochemical ligand: HCQ (A), (9cis)-Retinal (B), 19-Norandrostenedione (C), 19-Nortestosterone (D), 3-Hydroxy-3,5,5-trimethyl-4-(3-oxo-1-buten-1-ylidene)cyclohexyl β-D-glucopyranoside (E), Benzoic Acid (F), Daidzein (G), Dimethomorph (H), Galaxolidone (I), Ilicic Acid (J), Isoquercetin (K), Mesterolone (L), Oleanolic Acid (M), Psilotachyin B (N), Rutin (O), and Ursolic Acid (P).

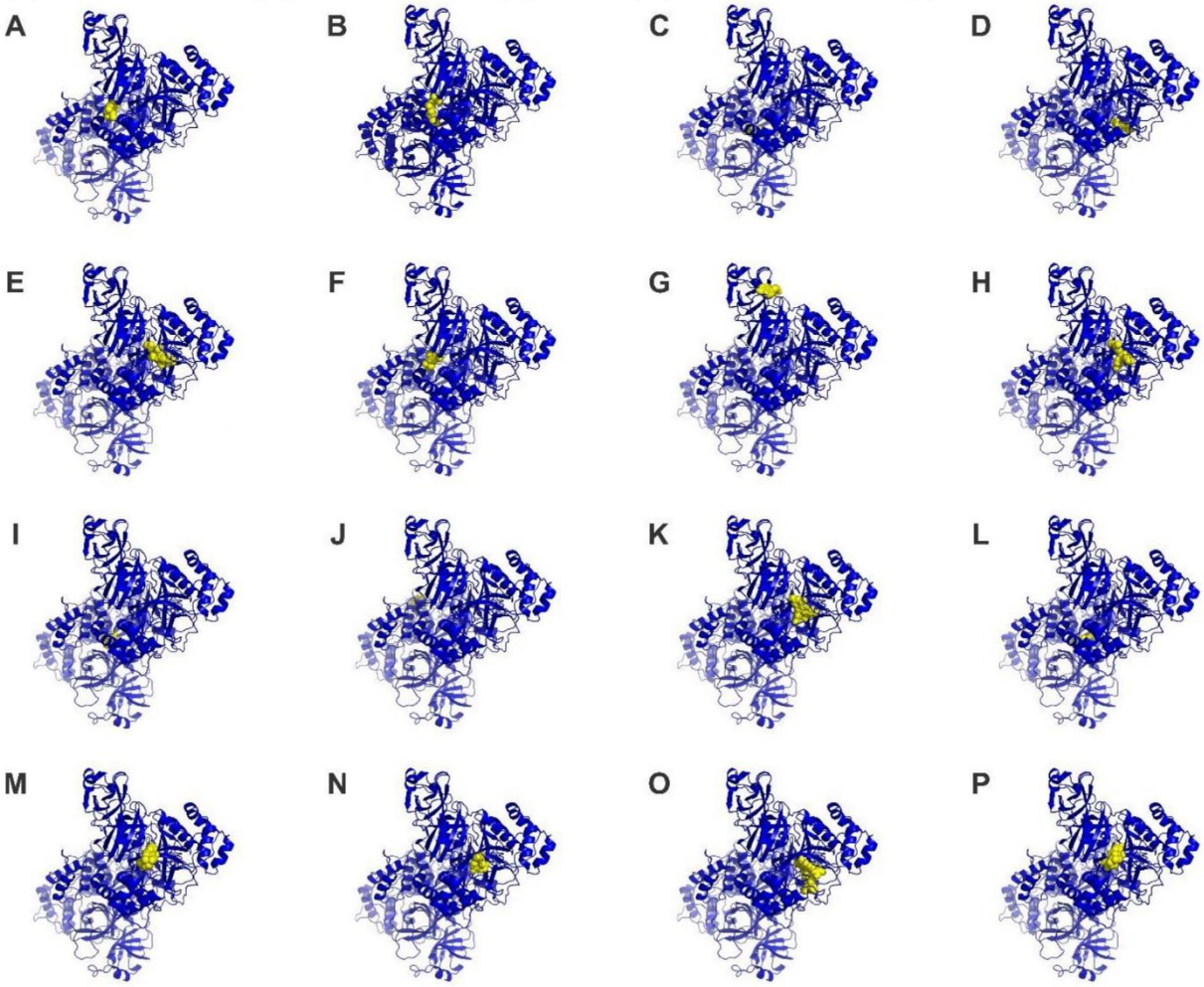
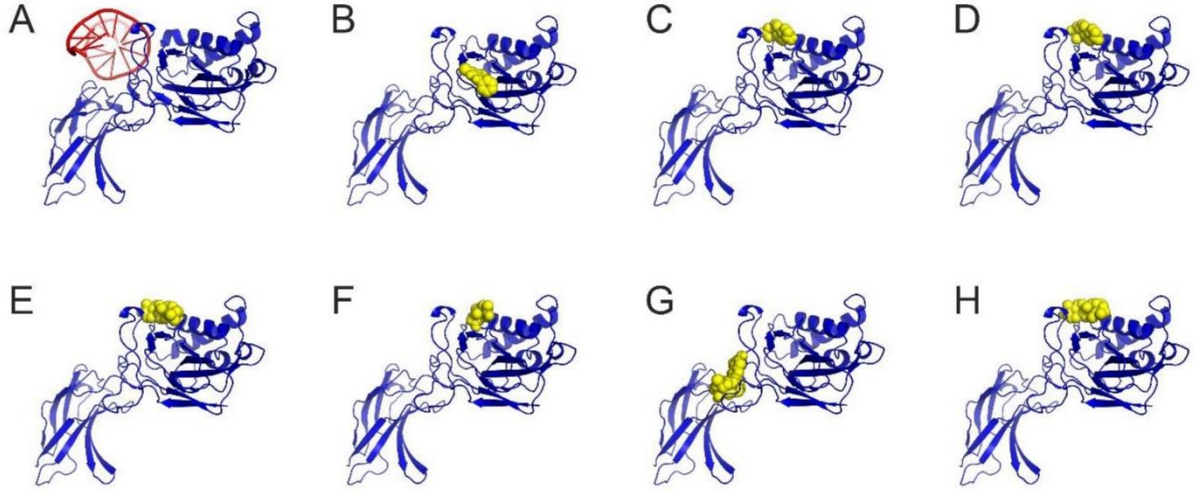
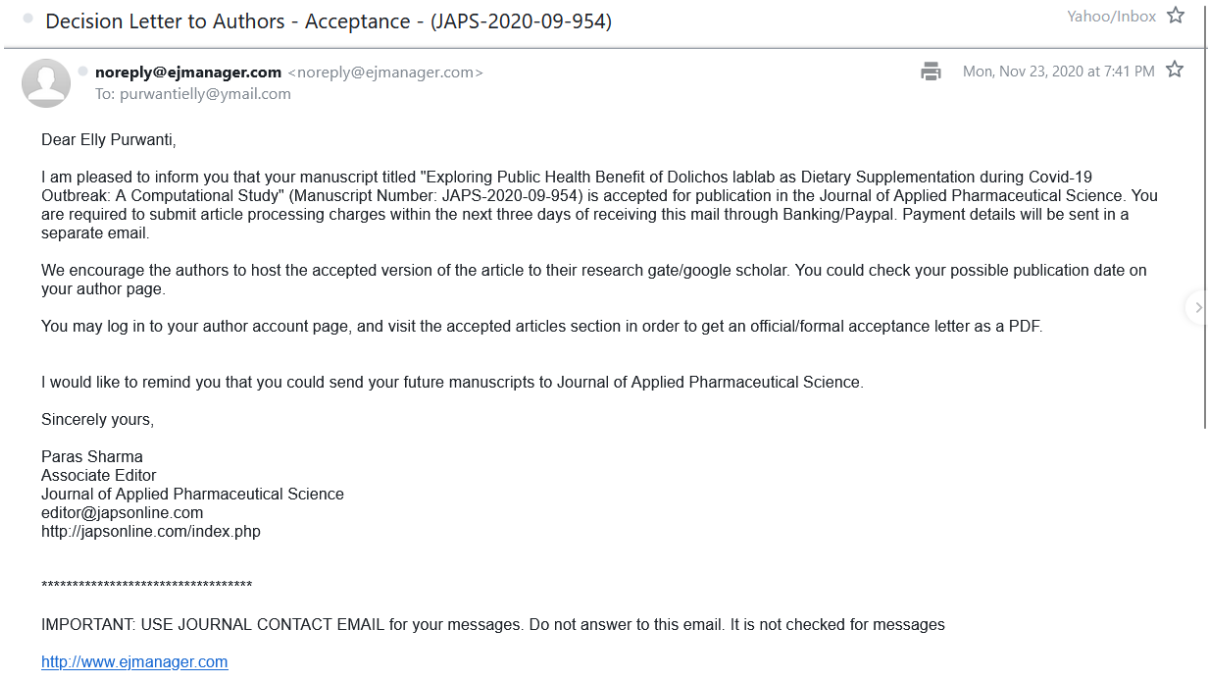


Figure 3. Structural visualization of NF- ϰB binds with DNA (A), NI241 (B), 19-Nortestosterone (C), Mesterolone (D), Oleanolic Acid (E), Psilotachyin B (F), Rutin (G), and Ursolic Acid (H).



9. Article accepted for publication (23-11-2020)

- Decision letter to authors



Decision Letter to Authors - Acceptance - (JAPS-2020-09-954)

Yahoo/Inbox

noreply@ejmanager.com <noreply@ejmanager.com>
To: purwantielly@ymail.com

Mon, Nov 23, 2020 at 7:41 PM

Dear Elly Purwanti,

I am pleased to inform you that your manuscript titled "Exploring Public Health Benefit of Dolichos lablab as Dietary Supplementation during Covid-19 Outbreak: A Computational Study" (Manuscript Number: JAPS-2020-09-954) is accepted for publication in the Journal of Applied Pharmaceutical Science. You are required to submit article processing charges within the next three days of receiving this mail through Banking/Paypal. Payment details will be sent in a separate email.

We encourage the authors to host the accepted version of the article to their research gate/google scholar. You could check your possible publication date on your author page.

You may log in to your author account page, and visit the accepted articles section in order to get an official/formal acceptance letter as a PDF.

I would like to remind you that you could send your future manuscripts to Journal of Applied Pharmaceutical Science.

Sincerely yours,

Paras Sharma
Associate Editor
Journal of Applied Pharmaceutical Science
editor@japsonline.com
http://japsonline.com/index.php

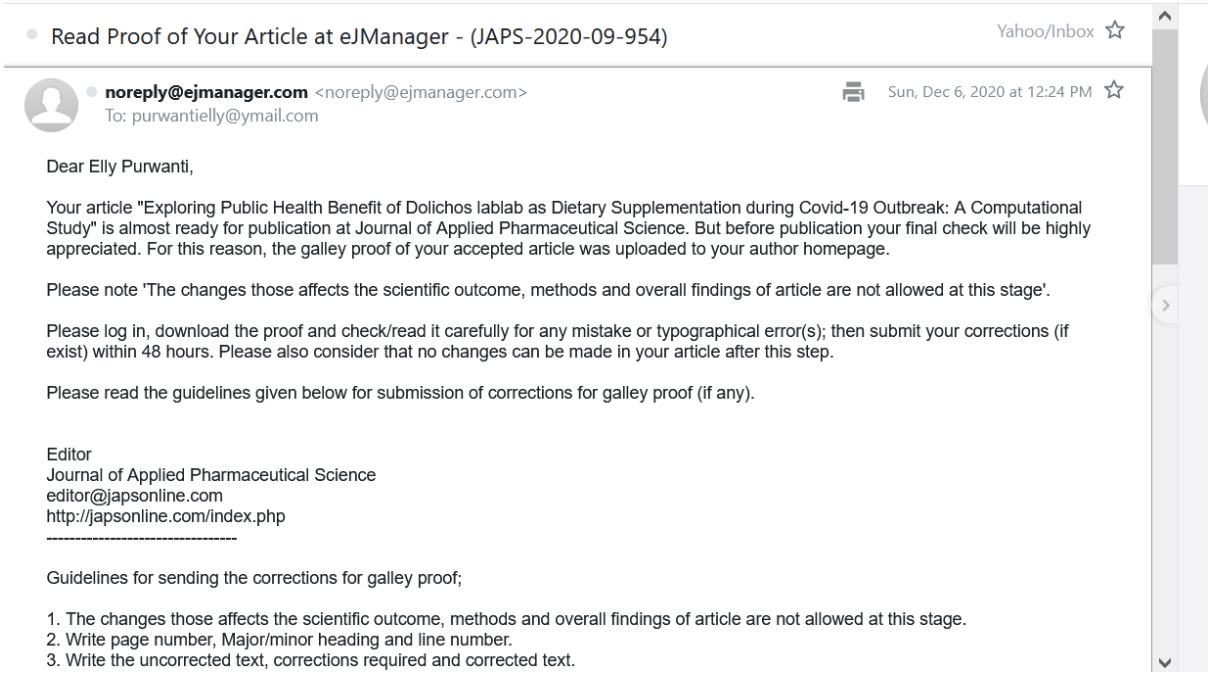
****

IMPORTANT: USE JOURNAL CONTACT EMAIL for your messages. Do not answer to this email. It is not checked for messages

http://www.ejmanager.com

10. Galley proof sent to author (6-12-2020)

- Notification letter



Read Proof of Your Article at eJManager - (JAPS-2020-09-954)

Yahoo/Inbox

noreply@ejmanager.com <noreply@ejmanager.com>
To: purwantielly@ymail.com

Sun, Dec 6, 2020 at 12:24 PM

Dear Elly Purwanti,

Your article "Exploring Public Health Benefit of Dolichos lablab as Dietary Supplementation during Covid-19 Outbreak: A Computational Study" is almost ready for publication at Journal of Applied Pharmaceutical Science. But before publication your final check will be highly appreciated. For this reason, the galley proof of your accepted article was uploaded to your author homepage.

Please note 'The changes those affects the scientific outcome, methods and overall findings of article are not allowed at this stage'.

Please log in, download the proof and check/read it carefully for any mistake or typographical error(s); then submit your corrections (if exist) within 48 hours. Please also consider that no changes can be made in your article after this step.

Please read the guidelines given below for submission of corrections for galley proof (if any).

Editor
Journal of Applied Pharmaceutical Science
editor@japsonline.com
http://japsonline.com/index.php

Guidelines for sending the corrections for galley proof;

1. The changes those affects the scientific outcome, methods and overall findings of article are not allowed at this stage.
2. Write page number, Major/minor heading and line number.
3. Write the uncorrected text, corrections required and corrected text.

4. Do not send unnecessary corrections.

For example;

Correction: 1
Page no 1
Major heading: Material and Methods
Minor Heading: Plant material
Line no: 4
Uncorrected text: ..leaves of the plant was collected
Corrected text: ..leaves of the plant were collected

Correction: 2
Page no 1
Line no: 4
Major heading: Material and Methods
Minor Heading: Animal
Uncorrected text: ..Animals were keep at
Corrected text: ..Animals were kept at

[Link: http://my.ejmanager.com/japs/]

****

IMPORTANT: USE JOURNAL CONTACT EMAIL for your messages. Do not answer to this email. It is not checked for messages

http://www.ejmanager.com

****

- Author queries

AQ1 : Ok
AQ2 : LC-HRMS= Liquid Chromatography – High-Resolution Mass Spectrometry
CID = Compound Identity Number/ID
DARS = Decoys As the Reference State [Note: it's common to mention as "Shape+DARS" – page 002, line no. 16 because it's one of algorithm options in HEX software]
AQ3 : Ok
AQ4 :
Page no.: 001
Major Heading: Introduction
Column: Right
Line no.: 8
Uncorrected text: **Zhang et al., 2020**
Corrected text: **Zhang et al., 2020a.**

Page no.: 002
Major Heading: Introduction
Column: Left
Line no.: 5
Uncorrected text: **Zhang et al., 2020**
Corrected text: **Zhang et al., 2020b**.

AQ5 : **Dai et al 2020** (Vol. 368, Issue 6497): p. 1331-1335,
Gyebi et al 2020 (Article in press, online available but not assigned in specific volume and issue number yet),
Joshi et al 2020 (Article in press, online available but not assigned in specific volume and issue number yet)
Minde et al (Article in press, online available but not assigned in specific volume and issue number yet)
AQ6 : **Lin et al 2020 (**Article in press, online available but not assigned in specific volume and issue number yet)
AQ7 **:** Ok
AQ8 :
Page no.: 003
Major Heading: Results and Discussion
Column: Right
Line no.: 5
Uncorrected text: From the docking result, six compounds could interact with NF-κB at 7 kcal/mol or lower.
Corrected text: From the docking result, six compounds could interact with NF-κB at 7 kcal/mol or lower (table 1, figure 3).

Page no.: 003
Major Heading: Results and Discussion
Column: Right
Line no.: 7
Uncorrected text: Although the ligands did not bind with the vital residues involved in NF-κB DNA-binding site, several interacted amino acids take place adjacent to these binding sites (Supplementary File 2),

Corrected text: Although the ligands did not bind with the vital residues involved in NF-κB DNA-binding site, several interacted amino acids take place adjacent to these binding sites (figure 3 and Supplementary File 2),

Journal of Applied Pharmaceutical Science Vol. 0(00), pp 001-006, December, 2020
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2018.8801
ISSN 2231-3354

Exploring public health benefits of *Dolichos lablab* as a dietary <AQ1> supplement during the COVID-19 outbreak: A computational study

Elly Purwanti[1*], Feri Eko Hermanto[2], Jantje Wiliem Souhaly, Wahyu Prihanta, Tutut Indria Permana

[1]Department of Educational Biology, Faculty of Teacher Training and Education, University of Muhammadiyah Malang, East Java, Indonesia.
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, Malang, East Java, Indonesia.

ARTICLE INFO

Received on: 04/09/2020
Accepted on: 23/11/2020
Available online: XX

Key words:
COVID-19, *Dolichos lablab*, heptad repeat, main Protease, NF-κB.

ABSTRACT

The emerging case of coronavirus disease-19 (COVID-19) caused by the severe acute respiratory syndrome-coronavirus (SARS-CoV-2) virus has become a global health issue. Since there is no available developed vaccine, health-promoting foods play a vital role in maintaining the immune system against the disease. *Dolichos lablab* (DL), an unutilized highly nutritional legume, has an excellent potential to cope with this pandemic with various health benefit phytochemicals. This study appraised the possibility of phytochemical content from DL to prevent virus infection and hyperinflammation in COVID-19 *in silico*. DL's phytochemicals from LC-HRMS <AQ2> analysis were docked with several SARS-CoV-2 proteins, including main protease and HR. Also, NF-κB docking was executed to pursue anti-inflammatory properties. The drug-likeness properties of screened phytochemicals were then evaluated using SwissADME. According to the results, there were 16 phytochemicals with a high affinity to targeted proteins. Among those, five phytochemicals consistently gave a low binding affinity to all targeted proteins. Those five phytochemicals' physicochemical properties, except for rutin and (9cis)-retinal, also coped with small-molecule bioavailability, permeability, and flexibility according to the SwissADME calculations. In conclusion, DL has a high probability of complementing the medical effort as dietary supplementation to modulate the immune system and prevent viral infection.

INTRODUCTION <AQ3>

The outbreak of coronavirus disease-19 (COVID-19) has now become a significant global health issue. Until the November 2020 update, more than 50 million people have become infected, and over a million have died due to this pandemic (https://covid19.who.int/). This number is still growing day by day, describing the war against COVID-19 as continued. Although several companies already announced that they have proposed vaccine candidates entering the final phase of clinical trials, complementary medicine and dietary intervention still needed to prevent the severity of infected people and prevent healthy people from getting infected (Di Matteo *et al*., 2020; Panyod *et al*., 2020). Therefore, exploring the proper diet for patients or healthy people to help against COVID-19 infection becomes essential.

Thwarting severe acute respiratory syndrome-coronavirus (SARS-CoV-2) attachment and replication have become the main target for combating COVID-19 (Jha *et al*., 2020; McKee *et al*., 2020). Several proteins from SARS-CoV-2 have been modeled and appropriately studied as a target to decrease the number of positive cases (Dai *et al*., 2020; Xia *et al*., 2020; <AQ4> Zhang *et al*., 2020). Spike protein is the primary key for SARS-CoV-2 to enter the host's cell, consisting of unique parts called heptad repeat 1 (HR1) and HR2 inside the receptor binding domain (RBD) for performing membrane fusion after attachment (Bosch *et al*., 2004; Walls *et al*., 2020; Xia *et al*., 2020). With this critical role, HR1 and HR2 have been proposed as the main targets to evade viral entry and infection (Xia *et al*., 2020). Another protein called main protease (MPro) has also become the right candidate due to its vital role in the viral replication and transcription (Hilgenfeld, 2014). Therefore, several studies also used MPro as a target to inhibit

*Corresponding Author
Elly Purwanti, Department of Educational Biology, Faculty of Teacher Training and Education, University of Muhammadiyah Malang, East Java, Indonesia. E-mail: purwantielly @ ymail.com

the severity of the COVID-19 infection (Ahkam *et al*., 2020; Dai *et al*., 2020; Jin *et al*., 2020; Joshi *et al*., 2020).

Another perspective to support a patient's survival is suppressing massive inflammation in lung tissue (Heck *et al*., 2020; Zhang *et al*., 20[illegible]This inflammation, known as cytokine storms, occurs through the deregulation of the immune response, leading to the disturbance of tissue homeostasis and severe organ damage (Ragab *et al*., 2020; Soy *et al*., 2020). Subsequently, injury in the lung tissue gives rise to breathing difficulties and speeds up the patient's death (Acosta and Singer, 2020; Lin *et al*., 2020). NF-κB, a kind of transcription factor which controls several cytokines involved in cytokine storms like interleukin- (IL-) 1 and IL-6, has a good starting point to diminish the hyperinflammation (Catanzaro *et al*., 2020; Conti *et al*., 2020; Soy *et al*., 2020). Previously, suppressing NF-κB could increase the survival rate after coronavirus infection (DeDiego *et al*., 2014). Thus, targeting this transcription factor has a reasonable probability of improving patient survival.

Legumes have been a good source of nutrition for years. However, masses of legume species are still underutilized as nutritious food. One of the rarely used legumes is *Dolichos lablab* (DL) (Minde *et al*., 2020). DL not only has a high nutritional content but also has innumerable natural compounds with numerous biological activities. Several bioactive compounds were reported to be contained in DL, including gallic acid, 4-hydroxy-3-methoxybenoic acid, p-coumaric acid, ferulic acid, cinnamic acid, catechin, and rutin. Also, saturated and unsaturated fatty acids, terpenoids, and steroids were found as a constituent inside DL beans (Baba *et al*., 1983; Bahtiar *et al*., 2017; Habib *et al*., 2017b; Yoshikawa *et al*., 1998). Previously, DL was explored for its antioxidant, antidiabetic, antimicrobial, and even anti-inflammatory properties (Habib *et al*., 2017a; Naeem *et al*., 2020; Rahman and Akhter, 2018; Yin *et al*., 2018). With those various health benefits, DL is a promising candidate for dietary supplementation to avoid COVID-19 infection.

MATERIAL AND METHOD

Phytochemical content screening

Thermo Scientific Dionex Ultimate 3,000 RSLCnano liquid chromatography (LC) coupled with Thermo Scientific Q Exactive Mass Spectrometry (MS) was run to identify the phytochemical content inside the methanolic extract of DL. Hypersil GOLD aQ 50 × 1 mm × 1.9 µ particle size was installed in the LC instrument as stationary phase, while the mobile phase consists of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile). The LC was operated under the following conditions: flow rate of 40 µl/minutes, 30 minutes run time, and 30°C column temperature. The obtained data were analyzed using Compound Discoverer with mzCloud in the MS/MS Library. Compound with mzCloud best matched a score higher than 80, and then directed for further analysis.

Ligand and protein structures retrieval

The compounds from LC-HRMS analysis were used as the ligand. The three-dimensional (3D) structure of the ligands was assessed through the PubChem database (Supplementary File 1). The protein's 3D structures were retrieved from Protein Data Bank (https://www.rcsb.org/), i.e., MPro (PDB ID: 6M2N), HR complex (6LXT), and NF-κB (1SVC) according to previous studies (Muzaffer *et al*., 2017; Su *et al*., 2020; Xia *et al*., 2020).

Molecular docking

Water molecules and native ligand from the 3D protein structures were removed using Discovery Studio 16. Energy minimization of the ligand structures was prepared using Open Babel integrated into PyRx 8.0 (O'Boyle *et al*., 2011). All compounds from LC-HRMS were screened using molecular docking to predict its interaction against protein targets. Protein–ligand docking was carried out using AutoDock Vina integrated into PyRx 8.0 (Dallakyan and Olson, 2015; Trott and Olson, 2010) with a maximum grid-size setting. HEX 8.0 was run for protein–protein docking using the default setting and operated under Shape + DARS correlation type (Ritchie and Kemp, 2000). As a comparison, hydroxychloroquine (HCQ, CID: 3,652) (Procacci *et al*., 2020) and 4,6-dichloro-N-phenyl-1,3,5-triazine-2-amine (NI241, CID: 167,66) (Kobayashi *et al*., 2016) were employed as a control inhibitor for MPro and NF- κB, respectively.

Data analysis

The protein–ligand complex, which has a binding energy lower than −7 kcal/mol, was directed for further analysis. Interacted residues in each protein–ligand complex and structure conformation were analyzed and visualized using Discovery Studio. The protein structure alignment was executed using PyMOL 2.3.2 with the RMSD value determined as a structural difference among aligned proteins. The alignment was achieved by setting the HR1–HR2 complex as a reference structure.

Prediction of drug-likeness properties

Drug-likeness properties were analyzed according to Lipinski's Rule of 5 (LRO5) (Lipinski, 2004). Drug resemblance properties were determined using the SwissADME physicochemical properties (Daina *et al*., 2017), including molecular weight (MW), LogP value, the number of H-bond donors, H-bond acceptor, rotatable bond, and total polar surface area (TPSA).

RESULT AND DISCUSSION

According to the binding affinity, 16 compounds were identified to have biological activities against SARS-CoV-2 infection and inflammation; five of them have a binding energy lower than −7 kcal/mol consistently in every target protein. 19-Nortestosterone, mesterolone, oleanolic acid, rutin, and ursolic acid are the compounds that have a low binding energy in MPro, HR1, and NF-κB. MPro is the protein that can interact with more compounds tested with a binding energy lower than or equal to −7 kcal/mol. Based on the binding affinity lower than or equal to −7 kcal/mol, seven compounds could interact with HR1, while six compounds were docked with NF-κB (Table 1).

19-Norandrostenedione, 19-nortestosterone, galaxolidone, mesterolone, oleanolic acid, rutin, and ursolic acid are the compounds that have good affinity to HR1. Attachment of these compounds could alter the HR1–HR2 binding motif represented by declining the HR complex's binding energy after being bonded with those compounds compared to the HR complex without ligand

Table 1. Phytochemical with binding energy less than or equal to −7 kcal/mol during screening process using molecular docking.

Compound	Binding energy (kcal/mol)		
	HR-1	MPro	NF-κB
(9cis)-Retinal	−6.2	−7.6	−5.9
19-Norandrostenedione	−7.3	−7.6	−6.6
19-Nortestosterone	−7.6	−7.2	−7.3
3-Hydroxy-3,5,5-trimethyl-4-(3-oxo-1-buten-1-ylidene)cyclohexyl β-D-glucopyranoside	−6.8	−7.5	−6.3
Benzoic acid	−6.4	−7.1	−5.4
Daidzein	−6.3	−7.4	−6.1
Dimethomorph	−6.1	−8.6	−6.3
Galaxolidone	−7.4	−7.8	−6.3
Ilicic acid	−6.5	−7.3	−5.8
Isoquercetin	−6.7	−8.8	−6.8
Mesterolone	−7.1	−7.5	−7.3
Oleanolic acid	−7.8	−9.1	−8.2
Psilostachyin B	−6.2	−7.7	−7.1
Rutin	−7.5	−9.1	−7.3
Ursolic acid	−8.1	−9.5	−7.9
Hydroxychloroquine (MPro Inhibitor)	–	−6.5	–
NI241 (NF-κB inhibitor)	–	–	−5.4

Bold typed font indicates the value equal or lower than −7.0 kcal/mol.

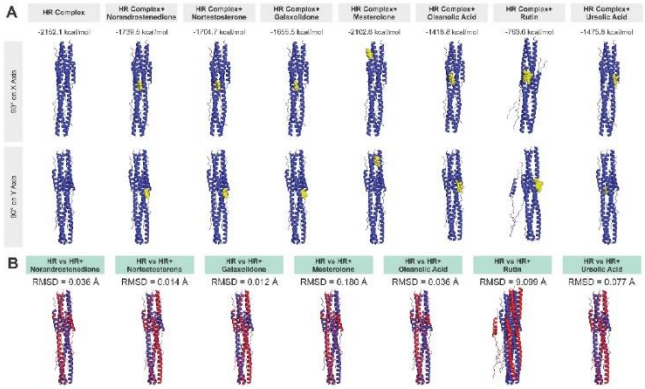


Figure 1. Structural orientation and binding energy of the HR complex after being bound with phytochemical ligands (A) and structural alignment of HR complex before and after being bound with phytochemical ligands (B). The blue ribbons in Figure A represent the HR protein complex, while the yellow spheres describe the phytochemical ligand. In Figure B, the blue ribbons represent the initial form of the HR complex without the ligand, while the red ribbons represent the HR complex structure after being bound with the phytochemical ligands.

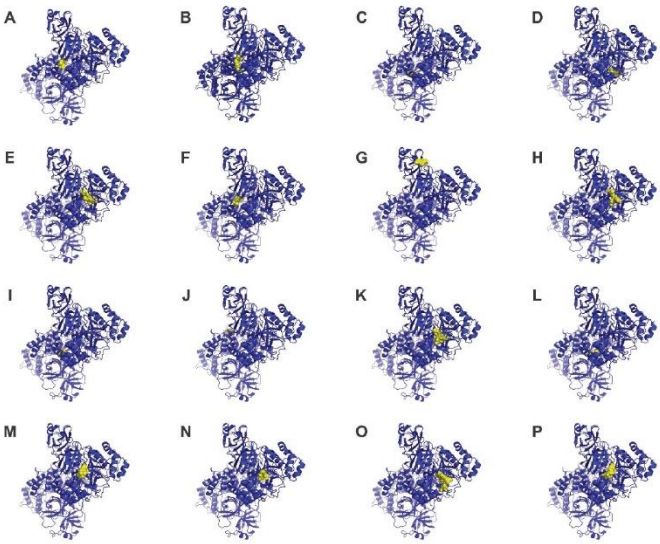


Figure 2. Structural visualization of MPro after being bound with its inhibitor and phytochemical ligands: HCQ (A), (9cis)-retinal (B), 19-norandrostenedione (C), 19-nortestosterone (D), 3-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-buten-1-ylidene)cyclohexyl β-D-glucopyranoside (E), benzoic acid (F), daidzein (G), dimethomorph (H), galaxolidone (I), ilicic acid (J), isoquercetin (K), mesterolone (L), oleanolic acid (M), psilostachyin B (N), rutin (O), and ursolic acid (P).

(Fig. 1a). Among the seven compounds that have an excellent affinity to HR1, rutin could modify the HR1–HR2 interaction. This was described by the RMSD value of the HR complex with rutin inside compared to the HR complex alone, which has a greater value than other complexes (Fig. 1b). Interaction of HR1–HR2 to form the helix bundle is the crucial step for SARS-CoV-2 membrane fusion (Liu *et al.*, 2004; Ou *et al.*, 2020). Altering the helix bundle formation has been studied to prevent viral entry s (Xia *et al.*, 2020), suggesting that 19-norandrostenedione, 19-nortestosterone, galaxolidone, mesterolone, oleanolic acid, ursolic acid, and in particular rutin have an excellent potency to inhibit viral infection.

MPro is the target protein with plenty of interacted compounds. Compared to HCQ as control, all the compounds have a lower binding energy. Each compound has its favorable binding region, presented by structural visualization (Figure. 2) or interacted residues between the ligand molecule and MPro (Supplementary File 2). Among those compounds, only daidzein and (9cis)-retinal have an interaction with catalytic residues of MPro at HIS:41. HCQ did not show any interaction with the catalytic residues (Supplementary File 2). HIS:41 and CYS:145 have been known as essential residues during MPro enzymatic activity (Tahir ul Qamar *et al.*, 2020). Inhibiting these residues has a promising effect on preventing virus replication and prevent viral spreading throughout the tissues (Ahkam *et al.*, 2020; Gyebi *et al.*, 2020; Hosseini-Zare *et al.*, 2020; Tahir ul Qamar *et al.*, 2020).

NF-κB is the transcription factor of several proteins related to infection response, including cytokines related to defense mechanisms (Hayden *et al.*, 2006; Schmitz *et al.*, 2014). aIn this critical condition, cytokine storms are the main factors that contribute to lung damage due to the overexpression of proinflammatory cytokines (Lin *et al.*, 2020; Soy *et al.*, 2020). Thus, targeting NF-κB as the main transcription factor for suppressing those cytokines' hyperexpression could play a vital role in augmenting patient survival (Catanzaro *et al.*, 2020). From the docking result, six compounds could interact with NF-κB at 7 kcal/mol or lowe[illegible]though the ligands did not bind with the vital residues involved in NF-κB DNA-binding site, several interacted amino acids take place adjacent to these binding sites (Supplementary File [illegible]suggesting their potentials as NF-κB

Table 2. Physicochemical properties of screened phytochemicals according to SwissADME measurement.

Compound	Mol. weight (g/mol)	LogP value	H-bond donor	H-bond acceptor	Rotatable bonds	TPSA
(9cis)-Retinal	464.38	2.11	8	12	4	210.51 Å²
19-Norandrostenedione	272.38	2.6	0	2	0	34.14 Å²
19-Nortestosterone	274.4	2.79	1	2	0	37.30 Å²
3-Hydroxy-3,5,5-trimethyl-4-(3-oxo-1-buten-1-ylidene)cyclohexyl β-D-glucopyranoside	386.44	2.21	5	8	4	136.68 Å²
Benzoic acid	250.33	2.74	2	3	3	57.53 Å²
Daidzein	254.24	1.77	2	4	1	70.67 Å²
Dimethomorph	387.86	3.67	0	4	6	48.00 Å²
Galaxolidone	272.38	3.17	0	2	0	26.30 Å²
Ilicic acid	252.35	2.26	2	3	2	57.53 Å²
Mesterolone	304.47	3.09	1	2	0	37.30 Å²
Oleanolic acid	456.7	3.92	2	3	1	57.53 Å²
Psilostachyin B	262.3	2.17	0	4	0	52.60 Å²
Quercetin	284.44	3.76	0	1	5	17.07 Å²
Rutin	610.52	2.43	10	16	6	269.43 Å²
Ursolic acid	456.7	4.01	2	3	1	57.53 Å²

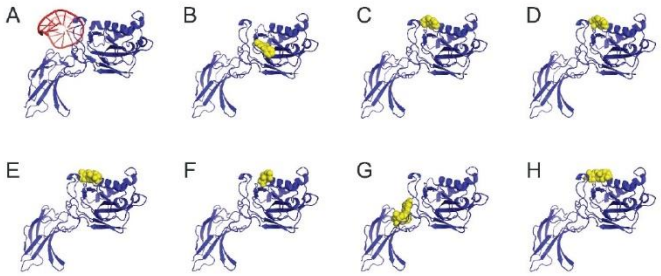


Figure 3. Structural visualization of NF-κB after being bound with DNA (A), NI241 (B), 19-nortestosterone (C), mesterolone (D), oleanolic acid (E), psilostachyin B (F), rutin (G), and ursolic acid (H). <AQ8>

inhibitors (Müller *et al.*, 1995). Targeting DNA-binding sites of NF-κB has been employed to preclude chronic inflammation (Gilmore and Herscovitch, 2006; Gupta *et al.*, 2010). Therefore, the presence of those compounds in the NF-κB DNA-binding domain could reduce hyperinflammation by altering the proinflammatory cytokines' transcription process.

Several criteria have been developed by Lipinski for a small molecule to have good oral bioavailability, permeability, and flexibility (Lipinski, 2004). The oral bioavailability of small molecules is determined by several criteria, including MW, LogP value, the number of hydrogen bond donors, and acceptor less than 500 g/mol, 5, 5, and 10, respectively (Lipinski, 2004). Also, a molecule with a TPSA value equal or less than 140 Å will carry out good permeability, while the number of rotatable bonds less than 10 represents molecule flexibility (Chagas *et al.*, 2018). Hence, SwissADME was employed to do the calculations related to LRO5. All of the analyzed compounds, except for rutin and (9cis)-retinal, have no violations of the MWmolecular weight, LogP value, H-bond donor, H-bond acceptor, rotatable bonds, and TPSA criteria. Rutin had a low oral bioavailability and permeability with 610.52 g/mol of MW, 10 of H-bond donor, 16 of H-bond acceptor, and 269.43 Å² TPSA value. (9cis)-Retinal is also not better than rutin in terms of oral bioavailability and permeability with 8 of H-bond donor, 12 of H-bond acceptor, and 210.51 Å² TPSA value (Table 2).

CONCLUSION

There are seven herbal compounds from DL, which have good potential as a preventive or complementary agent for COVID-19 treatment based on the constant binding energy lower or equal to 7 kcal/mol with MPro or HR1. However, daidzein has better potency as an MPro inhibitor, while rutin showed a worthy effect to prevent viral-host fusion by modifying the HR complex structure orientation. As anti-inflammatory candidates, 19-nortestosterone, mesterolone, oleanolic acid, and ursolic acid have a satisfactory result as NF-κB inhibitors. Lastly, all compounds with a binding energy lower than or equal to −7 kcal/mol, except for (9cis)-retinal and rutin, have good oral bioavailability, permeability, and flexibility.

ACKNOWLEDGMENTS

The authors acknowledge the Ministry of Research, Technology, and Higher Indonesia, the Republic of Indonesia, for funding this research under Grant no. 229/SP2H/LT/DRPM/2019.

CONFLICT OF INTEREST

None.

REFERENCES

Acosta MAT, Singer BD. Pathogenesis of COVID-19-induced ARDS: implications for an aging population. Eur Respir J, 2020; 56(3):2002049.

Ahkam AH, Hermanto FE, Alamsyah A, Aliyyah IH, Fatchiyah F. Virtual prediction of antiviral potential of ginger (*Zingiber officinale*) bioactive compounds against spike and MPro of SARS-CoV2 protein. Berkala Penelitian Hayati J Biol Res, 2020; 25:52–7.

Baba J, Yokota, T, Takahashi N. Brassinolide-related new bioactive steroids from *Dolichos lablab* seed. Agric Biol Chem, 1983; 47:659–61.

Bahtiar A, Vichitphan K, Han J. Leguminous plants in the Indonesian archipelago: traditional uses and secondary metabolites. Nat Prod Commun, 2017; 12:461–72.

Bosch BJ, Martina, BEE, Van Der Zee R, Lepault J, Haijema, BJ, Versluis C, Heck AJR, De Groot R, Osterhaus ADME, Rottier PJM. Severe acute respiratory syndrome coronavirus (SARS-CoV) infection inhibition using spike protein heptad repeat-derived peptides. Proc Natl Acad Sci U S A, 2004; 101:8455–60.

Catanzaro M, Fagiani F, Racchi M, Corsini E, Govoni S, Lanni C. Immune response in COVID-19: addressing a pharmacological challenge by targeting pathways triggered by SARS-CoV-2. Signal Transduct Target Ther, 2020; 5(1):84.

Chagas CM, Moss S, Alisaraie L. Drug metabolites and their effects on the development of adverse reactions: revisiting lipinski's rule of five. Int J Pharm, 2018; 549:133–49.

Conti P, Ronconi G, Caraffa A, Gallenga C, Ross R, Frydas I, Kritas S. Induction of pro-inflammatory cytokines (IL-1 and IL-6) and lung inflammation by coronavirus-19 (COVI-19 or SARS-CoV-2): anti-inflammatory strategies. J Biol Regul Homeost Agents, 2020; 34:327–31.

Dai W, Zhang B, Su H, Li J, Zhao Y, Xie X, Jin Z, Liu F, Li C, Li Y, Bai F, Wang H, Cheng X, Cen X, Hu S, Yang X, Wang J, Liu X, Xiao G, Jiang H, Rao Z, Zhang LK, Xu Y, Yang H, Liu H. Structure-based design of antiviral drug candidates targeting the SARS-CoV-2 main protease. Science. 2020; eabb4489. <AQ5

Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep, 2017; 7:42717.

Dallakyan S, Olson AJ. Small-molecule library screening by docking with PyRx. Methods Mol Biol, 2015; 1263:243–50.

DeDiego ML, Nieto TJL, Regla NJA, Jimenez GJM, Fernandez DR, Fett C, Castaño RC, Perlman S, Enjuanes L. Inhibition of NF-κB-Mediated Inflammation in severe acute respiratory syndrome coronavirus-infected mice increases survival. J Virol, 2014; 88:913–24.

Di Matteo G, Spano M, Grosso M, Salvo A, Ingallina C, Russo M, Ritieni A, Mannina L. Food and COVID-19: preventive/co-therapeutic strategies explored by current clinical trials and in silico studies. Foods, 2020; 9(8):1036.

Gilmore TD, Herscovitch M. Inhibitors of NF- κ B signaling: 785 and counting. Oncogene, 2006; 25:6887–99.

Gupta SC, Sundaram C, Reuter S, Aggarwal BB. Inhibiting NF-κB activation by small molecules as a therapeutic strategy. Biochim Biophys Acta, 2010; 1799:775–87.

Gyebi GA, Ogunro OB, Adegunloye AP, Ogunyemi OM, Afolabi SO. 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; 1–1

Habib HM, Theuri SW, Kheadr E, Mohamed FE. DNA and BSA damage inhibitory activities, and anti-acetylcholinesterase, anti-porcine α-amylase and antioxidant properties of *Dolichos lablab* beans. Food Funct, 2017a; 8:881–7.

Habib HM, Theuri SW, Kheadr EE, Mohamed FE. Functional, bioactive, biochemical, and physicochemical properties of the *Dolichos lablab* bean. Food Funct, 2017b; 8:872–80.

Hayden MS, West AP, Ghosh S. NF- κ B and the immune response. Oncogene, 2006; 25:6758–80.

Heck TG, Ludwig MS, Frizzo MN, Rasia FAA, Homem BPI. Suppressed anti-inflammatory heat shock response in high-risk COVID-19 patients: lessons from basic research (inclusive bats), light on conceivable therapies. Clin Sci (Lond), 2020; 134:1991–2017.

Hilgenfeld R. From SARS to MERS: crystallographic studies on coronaviral proteases enable antiviral drug design. FEBS J, 2014; 281:4085–96.

Hosseini-Zare MS, Thilagavathi R, Selvam C. Targeting severe acute respiratory syndrome-coronavirus (SARS-CoV-1) with structurally diverse inhibitors: a comprehensive review. RSC Adv. 2020; 10:28287–99.

Jha AK, Kumar R, Goenka MK, Dayal VM. Emerging treatment and prevention strategies against COVID-19: a brief update. J Dig Endosc, 2020; 11:69–72.

Jin Z, Du X, Xu Y, Deng Y, Liu M, Zhao Y, Zhang B, Li X, Zhang L, Peng C, Duan Y, Yu J, Wang L, Yang K, Liu F, Jiang R, Yang, Xinglou, You T, Liu, Xiaoce, Yang, Xiuna, Bai F, Liu H, Liu, Xiang, Guddat LW, Xu W, Xiao G, Qin C, Shi Z, Jiang H, Rao Z, Yang H. Structure of M pro from SARS-CoV-2 and discovery of its inhibitors. Nature, 2020; 582:289–93.

Joshi RS, Jagdale SS, Bansode SB, Shankar SS, Tellis MB, Pandya VK, Chugh A, Giri AP, Kulkarni MJ. Discovery of potential multi-target-directed ligands by targeting host-specific SARS-CoV-2 structurally conserved main protease. J Biomol Struct Dyn, 2020; 1–1

Kobayashi T, Tanuma S, Kino K, Miyazawa H. New scaffolds of inhibitors targeting the DNA binding of NF-κB. Integr Mol Med, 2016; 3(5):769–73.

Lin S, Zhao Y, Zhou D, Zhou F, Xu F. Coronavirus disease 2019 (COVID-19): cytokine storms, hyper-inflammatory phenotypes, and acute respiratory distress syndrome. Genes Dis, 2020. <AQ6

Lipinski CA. Lead- and drug-like compounds: the rule-of-five revolution. Drug Discov Today Technol, 2004; 1:337–41.

Liu S, Xiao G, Chen Y, He Y, Niu J, Escalante CR, Xiong H, Farmar J, Debnath AK, Tien P, Jiang S. Interaction between heptad repeat 1 and 2 regions in spike protein of SARS-associated coronavirus: implications for virus fusogenic mechanism and identification of fusion inhibitors. Lancet, 2004; 363:938–47.

McKee DL, Sternberg A, Stange U, Laufer S, Naujokat C. Candidate drugs against SARS-CoV-2 and COVID-19. Pharmacol Res, 2020; 157:104859.

Minde JJ, Venkataramana PB, Matemu AO. Dolichos Lablab-an underutilized crop with future potentials for food and nutrition security: a review. Crit Rev Food Sci Nutr, 2020; 1–1

Müller CW, Rey FA, Sodeoka M, Verdine GL, Harrison SC. Structure of the NF-kappa B p50 homodimer bound to DNA. Nature, 1995; 373:311–7.

Muzaffer U, Paul VI, Rajendra PN. Molecular docking of selected phytoconstituents with signaling molecules of Ultraviolet-B induced oxidative damage. In Silico Pharmacol, 2017; 5(1):17.

Naeem M, Shabbir A, Ansari AA, Aftab T, Khan MMA, Uddin M. Hyacinth bean (*Lablab purpureus* L.) – an underutilised crop with future potential. Sci Hortic, 2020; 272:109551.

O'Boyle NM, Banck M, James CA, Morley C, Vandermeersch T, Hutchison GR. Open babel: an open chemical toolbox. J Cheminform, 2011; 3:33.

Ou X, Liu Y, Lei X, Li P, Mi D, Ren L, Guo L, Guo R, Chen T, Hu J, Xiang Z, Mu Z, Chen X, Chen J, Hu K, Jin Q, Wang J, Qian Z. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat Commun, 2020; 11:1620.

Panyod S, Ho CT, Sheen LY. Dietary therapy and herbal medicine for COVID-19 prevention: a review and perspective. J Tradit Complement Med, 2020; 10:420–7.

Procacci P, Macchiagodena M, Pagliai M, Guarnieri G, Iannone F. Interaction of hydroxychloroquine with SARS-CoV2 functional proteins using all-atoms non-equilibrium alchemical simulations. Chem Commun (Camb.), 2020; 56: 8854–6.

Ragab D, Salah Eldin H, Taeimah M, Khattab R, Salem R. The COVID-19 cytokine storm; What We Know So Far. Front Immunol, 2020; 11:1446.

Rahman SA, Akhter MS. Antibacterial and cytotoxic activity of seeds of white hyacinth bean (*Lablab purpureus* L. sweet 'white') -. J Adv Biotechnol Exp Ther, 2018; 1:49–54.

Ritchie, DW, Kemp, GJ. Protein docking using spherical polar Fourier correlations. Proteins, 2000; 39:178–94.

Schmitz ML, Kracht M, Saul VV. The intricate interplay between RNA viruses and NF-κB. Biochim Biophys Acta, 2014; 1843(11):2754–64.

Soy M, Keser G, Atagündüz P, Tabak F, Atagündüz I, Kayhan S. Cytokine storm in COVID-19: pathogenesis and overview of anti-inflammatory agents used in treatment. Clin Rheumatol, 2020; 39:2085–94.

Su H, Yao S, Zhao W, Li M, Liu J, Shang W, Xie H, Ke C, Hu H, Gao M, Yu K, Liu H, Shen J, Tang W, Zhang L, Xiao G, Ni L, Wang D, Zuo J, Jiang H, Bai F, Wu Y, Ye Y, Xu Y. Anti-SARS-CoV-2 activities *in vitro* of shuanghuanglian preparations and bioactive ingredients. Acta Pharmacol Sin, 2020; 41:1167–77.

Tahir QM, Alqahtani SM, Alamri MA, Chen, LL. Structural basis of SARS-CoV-2 3CLpro and anti-COVID-19 drug discovery from medicinal plants. J Pharm Anal, 2020; 10:313–9.

Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. J Comput Chem, 2010; 31:455–461.

Walls AC, Park, YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell, 2020; 181:281–92.

Xia S, Liu M, Wang C, Xu W, Lan Q, Feng S, Qi F, Bao L, Du L, Liu S, Qin C, Sun F, Shi Z, Zhu Y, Jiang S, Lu L. Inhibition of SARS-CoV-2 (previously 2019-nCoV) infection by a highly potent pan-coronavirus fusion inhibitor targeting its spike protein that harbors a high capacity to mediate membrane fusion. Cell Res, 2020; 30:343–55.

Yin J, Seo CS, Hwang IH, Lee MW, Song KH. Anti-obesity activities of chikusetsusaponin IVa and *Dolichos lablab* L. Seeds. Nutrients, 2018; 10:1221.

Yoshikawa M, Murakami T, Komatsu H, Matsuda H. Medicinal foodstuffs. XII. Saponin constituents with adjuvant activity from hyacinth bean, the seeds of Dolichos lablab L. (1): structures of lablabosides A, B, and C. Chem Pharm Bull (Tokyo), 1998; 46: 812–6.

Zhang L, Lin D, Sun X, Curth U, Drosten C, Sauerhering L, Becker S, Rox K, Hilgenfeld R. Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors. Science, 2020a; 368:409–12.

Zhang W, Zhao Y, Zhang F, Wang Q, Li T, Liu Z, Wang J, Qin Y, Zhang X, Yan X, Zeng X, Zhang S. The use of anti-inflammatory drugs in the treatment of people with severe coronavirus disease 2019 (COVID-19): the Perspectives of clinical immunologists from China. Clin Immunol, 2020b; 214:108393.

Author Queries

AQ1 Kindly check and confirm if the edits made to the title are ok.

AQ2 Please provide the expansion for the Abbreviations "LC-HRMS, DARS, CID."

AQ3 Please check and confirm whether all section heads have been set correctly.

AQ4 Please clarify that the reference citation "Zhang et al., 2020" refers to "Zhang et al. (2020a)" or "Zhang et al. (2020b)" throughout the article.

AQ5 Please provide volume number and issue number for the reference " Dai et al. (2020), Gyebi et al. (2020), Joshi et al. (2020), Minde et al. (2020)."

AQ6 Please provide volume number, issue number and page range for the reference "Lin et al. (2020)."

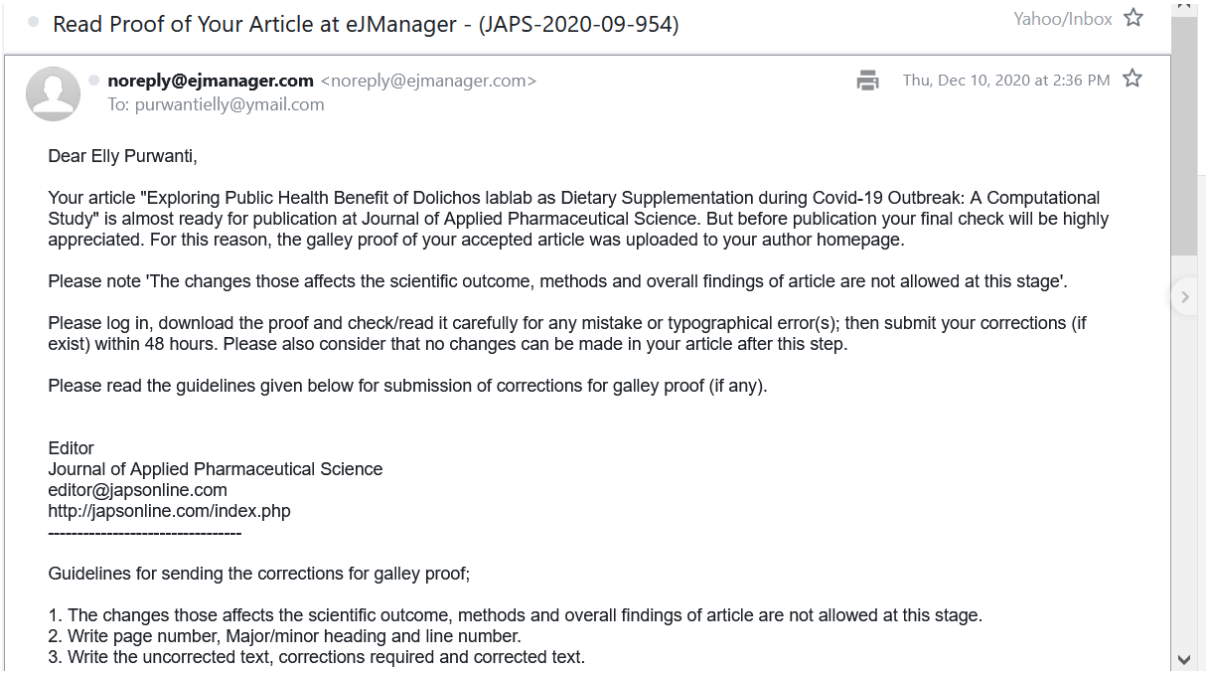
AQ7 Kindly check and confirm if the edits made to the title are ok.

AQ8 Please provide the in-text citation for Figure 3.

How to cite this article:
Purwanti E, Hermanto FE, Souhaly JW, Prihanta W, Permana TI. Exploring public health benefits of *Dolichos lablab* as a dietary supplement during the COVID-19 outbreak: A computational study. J Appl Pharm Sci, XXXX; XX(XX):XX–XX. <AQ7>

11. Galley proof sent to author (10-12-2020)

- Notification letter



Read Proof of Your Article at eJManager - (JAPS-2020-09-954)

Yahoo/Inbox

noreply@ejmanager.com <noreply@ejmanager.com>
To: purwantielly@ymail.com

Thu, Dec 10, 2020 at 2:36 PM

Dear Elly Purwanti,

Your article "Exploring Public Health Benefit of Dolichos lablab as Dietary Supplementation during Covid-19 Outbreak: A Computational Study" is almost ready for publication at Journal of Applied Pharmaceutical Science. But before publication your final check will be highly appreciated. For this reason, the galley proof of your accepted article was uploaded to your author homepage.

Please note 'The changes those affects the scientific outcome, methods and overall findings of article are not allowed at this stage'.

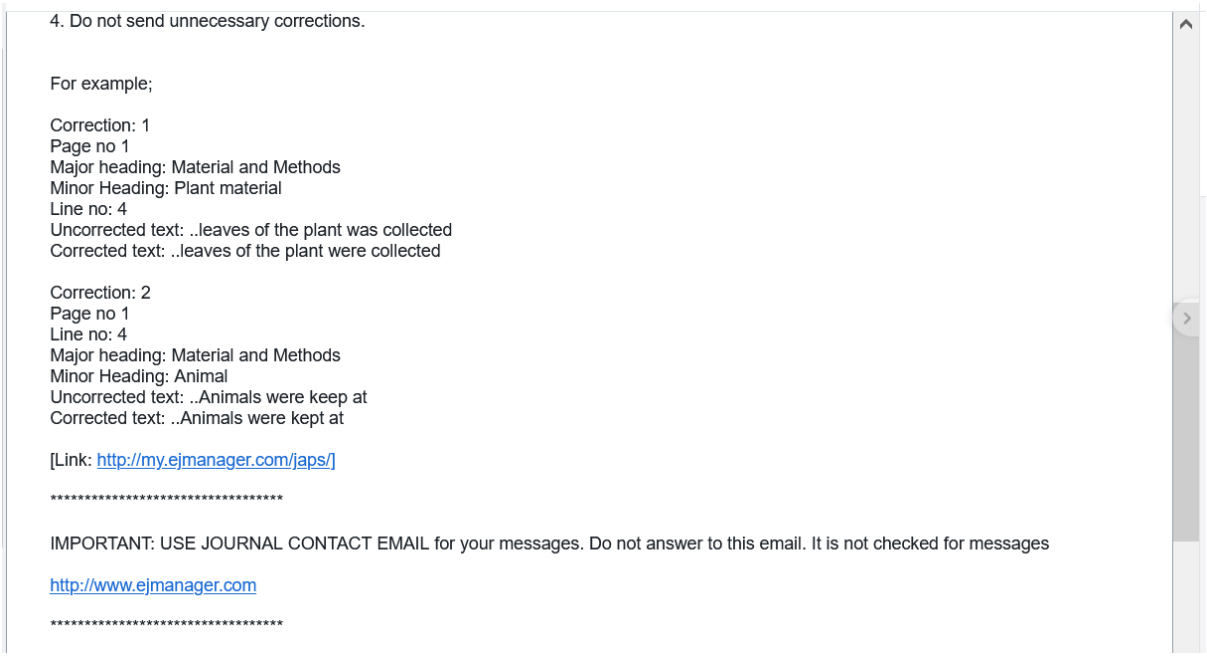
Please log in, download the proof and check/read it carefully for any mistake or typographical error(s); then submit your corrections (if exist) within 48 hours. Please also consider that no changes can be made in your article after this step.

Please read the guidelines given below for submission of corrections for galley proof (if any).

Editor
Journal of Applied Pharmaceutical Science
editor@japsonline.com
http://japsonline.com/index.php

Guidelines for sending the corrections for galley proof;

1. The changes those affects the scientific outcome, methods and overall findings of article are not allowed at this stage.
2. Write page number, Major/minor heading and line number.
3. Write the uncorrected text, corrections required and corrected text.



4. Do not send unnecessary corrections.

For example;

Correction: 1
Page no 1
Major heading: Material and Methods
Minor Heading: Plant material
Line no: 4
Uncorrected text: ..leaves of the plant was collected
Corrected text: ..leaves of the plant were collected

Correction: 2
Page no 1
Line no: 4
Major heading: Material and Methods
Minor Heading: Animal
Uncorrected text: ..Animals were keep at
Corrected text: ..Animals were kept at

[Link: http://my.ejmanager.com/japs/]

****

IMPORTANT: USE JOURNAL CONTACT EMAIL for your messages. Do not answer to this email. It is not checked for messages

http://www.ejmanager.com

****

12. Galley proof sent to author (15-12-2020)

- Notification letter

Read Proof of Your Article at eJManager - (JAPS-2020-09-954)

Yahoo/Inbox

noreply@ejmanager.com <noreply@ejmanager.com>
To: purwantielly@ymail.com

Tue, Dec 15, 2020 at 1:47 PM

Dear Elly Purwanti,

Your article "Exploring Public Health Benefit of Dolichos lablab as Dietary Supplementation during Covid-19 Outbreak: A Computational Study" is almost ready for publication at Journal of Applied Pharmaceutical Science. But before publication your final check will be highly appreciated. For this reason, the galley proof of your accepted article was uploaded to your author homepage.

Please note 'The changes those affects the scientific outcome, methods and overall findings of article are not allowed at this stage'.

Please log in, download the proof and check/read it carefully for any mistake or typographical error(s); then submit your corrections (if exist) within 48 hours. Please also consider that no changes can be made in your article after this step.

Please read the guidelines given below for submission of corrections for galley proof (if any).

Editor
Journal of Applied Pharmaceutical Science
editor@japsonline.com
http://japsonline.com/index.php

Guidelines for sending the corrections for galley proof;

1. The changes those affects the scientific outcome, methods and overall findings of article are not allowed at this stage.
2. Write page number, Major/minor heading and line number.
3. Write the uncorrected text, corrections required and corrected text.
4. Do not send unnecessary corrections.

For example;

Correction: 1
Page no 1
Major heading: Material and Methods
Minor Heading: Plant material
Line no: 4
Uncorrected text: ..leaves of the plant was collected
Corrected text: ..leaves of the plant were collected

Correction: 2
Page no 1
Line no: 4
Major heading: Material and Methods
Minor Heading: Animal
Uncorrected text: ..Animals were keep at
Corrected text: ..Animals were kept at

[Link: http://my.ejmanager.com/japs/]

****

IMPORTANT: USE JOURNAL CONTACT EMAIL for your messages. Do not answer to this email. It is not checked for messages

http://www.ejmanager.com

****

13. Article published (2-6-2021)

- Notification letter

Your Article has been Published!

Dear Elly Purwanti, Your article **"Exploring public health benefits of Dolichos lablab as a dietary supplement during the COVID-19 outbreak: A computational study"** has been published by **"Journal of Applied Pharmaceutical Science** and its final volume/issue numbers were assigned as **2021, 11(2): 135-140"**, and we are very grateful. Thanks for choosing our Journal for publication. Click here to see your article.

We believe that your colleagues would find it very interesting and useful, and we will send your article to our members.

Also it would be very useful and pleasing for your friends to know that your article is published, and it is a good idea to inform your friends/colleagues in order to increase your article's citation, Please do.

Thanks again,

eJManager Team

- Final paper for publication

Journal of Applied Pharmaceutical Science Vol. 0(00), pp 001-006, December, 2020
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2018.8801
ISSN 2231-3354

Exploring public health benefits of *Dolichos lablab* as a dietary supplement during the COVID-19 outbreak: A computational study

Elly Purwanti[1*], Feri Eko Hermanto[2], Jantje Wiliem Souhaly, Wahyu Prihanta, Tutut Indria Permana

[1]Department of Educational Biology, Faculty of Teacher Training and Education, University of Muhammadiyah Malang, East Java, Indonesia.
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, Malang, East Java, Indonesia.

ARTICLE INFO

Received on: 04/09/2020
Accepted on: 23/11/2020
Available online: XX

Key words:
COVID-19, *Dolichos lablab*, heptad repeat, main Protease, NF-κB.

ABSTRACT

The emerging case of coronavirus disease-19 (COVID-19) caused by the severe acute respiratory syndrome-coronavirus (SARS-CoV-2) virus has become a global health issue. Since there is no available developed vaccine, health-promoting foods play a vital role in maintaining the immune system against the disease. *Dolichos lablab* (DL), an unutilized highly nutritional legume, has an excellent potential to cope with this pandemic with various health benefit phytochemicals. This study appraised the possibility of phytochemical content from DL to prevent virus infection and hyperinflammation in COVID-19 *in silico*. DL's phytochemicals from liquid chromatography–high-resolution mass spectrometry analysis were docked with several SARS-CoV-2 proteins, including main protease and HR. Also, NF-κB docking was executed to pursue anti-inflammatory properties. The drug-likeness properties of screened phytochemicals were then evaluated using SwissADME. According to the results, there were 16 phytochemicals with a high affinity to targeted proteins. Among those, five phytochemicals consistently gave a low binding affinity to all targeted proteins. Those five phytochemicals' physicochemical properties, except for rutin and (9cis)-retinal, also coped with small-molecule bioavailability, permeability, and flexibility according to the SwissADME calculations. In conclusion, DL has a high probability of complementing the medical effort as dietary supplementation to modulate the immune system and prevent viral infection.

INTRODUCTION

The outbreak of coronavirus disease-19 (COVID-19) has now become a significant global health issue. Until the November 2020 update, more than 50 million people have become infected, and over a million have died due to this pandemic (https://covid19.who.int/). This number is still growing day by day, describing the war against COVID-19 as continued. Although several companies already announced that they have proposed vaccine candidates entering the final phase of clinical trials, complementary medicine and dietary intervention still needed to prevent the severity of infected people and prevent healthy people from getting infected (Di Matteo *et al*., 2020; Panyod *et al*., 2020). Therefore, exploring the proper diet for patients or healthy people to help against COVID-19 infection becomes essential.

Thwarting severe acute respiratory syndrome-coronavirus (SARS-CoV-2) attachment and replication have become the main target for combating COVID-19 (Jha *et al*., 2020; McKee *et al*., 2020). Several proteins from SARS-CoV-2 have been modeled and appropriately studied as a target to decrease the number of positive cases (Dai *et al*., 2020; Xia *et al*., 2020; Zhang *et al*., 2020a). Spike protein is the primary key for SARS-CoV-2 to enter the host's cell, consisting of unique parts called heptad repeat 1 (HR1) and HR2 inside the receptor binding domain (RBD) for performing membrane fusion after attachment (Bosch *et al*., 2004; Walls *et al*., 2020; Xia *et al*., 2020). With this critical role, HR1 and HR2 have been proposed as the main targets to evade viral entry and infection (Xia *et al*., 2020). Another protein called main protease (MPro) has also become the right candidate due to its vital role in the viral replication and transcription (Hilgenfeld, 2014). Therefore, several studies also used MPro as a target to inhibit

*Corresponding Author
Elly Purwanti, Department of Educational Biology, Faculty of Teacher Training and Education, University of Muhammadiyah Malang, East Java, Indonesia. E-mail: purwantielly @ ymail.com

the severity of the COVID-19 infection (Ahkam *et al*., 2020; Dai *et al*., 2020; Jin *et al*., 2020; Joshi *et al*., 2020).

Another perspective to support a patient's survival is suppressing massive inflammation in lung tissue (Heck *et al*., 2020; Zhang *et al*., 2020b). This inflammation, known as cytokine storms, occurs through the deregulation of the immune response, leading to the disturbance of tissue homeostasis and severe organ damage (Ragab *et al*., 2020; Soy *et al*., 2020). Subsequently, injury in the lung tissue gives rise to breathing difficulties and speeds up the patient's death (Acosta and Singer, 2020; Lin *et al*., 2020). NF-κB, a kind of transcription factor which controls several cytokines involved in cytokine storms like interleukin- (IL-) 1 and IL-6, has a good starting point to diminish the hyperinflammation (Catanzaro *et al*., 2020; Conti *et al*., 2020; Soy *et al*., 2020). Previously, suppressing NF-κB could increase the survival rate after coronavirus infection (DeDiego *et al*., 2014). Thus, targeting this transcription factor has a reasonable probability of improving patient survival.

Legumes have been a good source of nutrition for years. However, masses of legume species are still underutilized as nutritious food. One of the rarely used legumes is *Dolichos lablab* (DL) (Minde *et al*., 2020). DL not only has a high nutritional content but also has innumerable natural compounds with numerous biological activities. Several bioactive compounds were reported to be contained in DL, including gallic acid, 4-hydroxy-3-methoxybenoic acid, p-coumaric acid, ferulic acid, cinnamic acid, catechin, and rutin. Also, saturated and unsaturated fatty acids, terpenoids, and steroids were found as a constituent inside DL beans (Baba *et al*., 1983; Bahtiar *et al*., 2017; Habib *et al*., 2017b; Yoshikawa *et al*., 1998). Previously, DL was explored for its antioxidant, antidiabetic, antimicrobial, and even anti-inflammatory properties (Habib *et al*., 2017a; Naeem *et al*., 2020; Rahman and Akhter, 2018; Yin *et al*., 2018). With those various health benefits, DL is a promising candidate for dietary supplementation to avoid COVID-19 infection.

MATERIAL AND METHOD

Phytochemical content screening

Thermo Scientific Dionex Ultimate 3,000 RSLCnano liquid chromatography (LC) coupled with Thermo Scientific Q Exactive Mass Spectrometry (MS) was run to identify the phytochemical content inside the methanolic extract of DL. Hypersil GOLD aQ 50 × 1 mm × 1.9 µ particle size was installed in the LC instrument as stationary phase, while the mobile phase consists of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile). The LC was operated under the following conditions: flow rate of 40 µl/minutes, 30 minutes run time, and 30°C column temperature. The obtained data were analyzed using Compound Discoverer with mzCloud in the MS/MS Library. Compound with mzCloud best matched a score higher than 80, and then directed for further analysis.

Ligand and protein structures retrieval

The compounds from liquid chromatography–high-resolution mass spectrometry (LC–HRMS) analysis were used as the ligand. The three-dimensional (3D) structure of the ligands was assessed through the PubChem database (Supplementary File 1). The protein's 3D structures were retrieved from Protein Data Bank (https://www.rcsb.org/), i.e., MPro (PDB ID: 6M2N), HR complex (6LXT), and NF-κB (1SVC) according to previous studies (Muzaffer *et al*., 2017; Su *et al*., 2020; Xia *et al*., 2020).

Molecular docking

Water molecules and native ligand from the 3D protein structures were removed using Discovery Studio 16. Energy minimization of the ligand structures was prepared using Open Babel integrated into PyRx 8.0 (O'Boyle *et al*., 2011). All compounds from LC-HRMS were screened using molecular docking to predict its interaction against protein targets. Protein–ligand docking was carried out using AutoDock Vina integrated into PyRx 8.0 (Dallakyan and Olson, 2015; Trott and Olson, 2010) with a maximum grid-size setting. HEX 8.0 was run for protein–protein docking using the default setting and operated under Shape + DARS correlation type (Ritchie and Kemp, 2000). As a comparison, hydroxychloroquine [HCQ, compound identity number (CID): 3,652] (Procacci *et al*., 2020) and 4,6-dichloro-N-phenyl-1,3,5-triazine-2-amine (NI241, CID: 167,66) (Kobayashi *et al*., 2016) were employed as a control inhibitor for MPro and NF- κB, respectively.

Data analysis

The protein–ligand complex, which has a binding energy lower than −7 kcal/mol, was directed for further analysis. Interacted residues in each protein–ligand complex and structure conformation were analyzed and visualized using Discovery Studio. The protein structure alignment was executed using PyMOL 2.3.2 with the RMSD value determined as a structural difference among aligned proteins. The alignment was achieved by setting the HR1–HR2 complex as a reference structure.

Prediction of drug-likeness properties

Drug-likeness properties were analyzed according to Lipinski's Rule of 5 (LRO5) (Lipinski, 2004). Drug resemblance properties were determined using the SwissADME physicochemical properties (Daina *et al*., 2017), including molecular weight (MW), LogP value, the number of H-bond donors, H-bond acceptor, rotatable bond, and total polar surface area (TPSA).

RESULT AND DISCUSSION

According to the binding affinity, 16 compounds were identified to have biological activities against SARS-CoV-2 infection and inflammation; five of them have a binding energy lower than −7 kcal/mol consistently in every target protein. 19-Nortestosterone, mesterolone, oleanolic acid, rutin, and ursolic acid are the compounds that have a low binding energy in MPro, HR1, and NF-κB. MPro is the protein that can interact with more compounds tested with a binding energy lower than or equal to −7 kcal/mol. Based on the binding affinity lower than or equal to −7 kcal/mol, seven compounds could interact with HR1, while six compounds were docked with NF-κB (Table 1).

19-Norandrostenedione, 19-nortestosterone, galaxolidone, mesterolone, oleanolic acid, rutin, and ursolic acid are the compounds that have good affinity to HR1. Attachment of these compounds could alter the HR1–HR2 binding motif represented

Table 1. Phytochemical with binding energy less than or equal to −7 kcal/mol during screening process using molecular docking.

Compound	Binding energy (kcal/mol)		
	HR-1	MPro	NF-κB
(9cis)-Retinal	−6.2	−7.6	−5.9
19-Norandrostenedione	−7.3	−7.6	−6.6
19-Nortestosterone	−7.6	−7.2	−7.3
3-Hydroxy-3,5,5-trimethyl-4-(3-oxo-1-buten-1-ylidene)cyclohexyl β-D-glucopyranoside	−6.8	−7.5	−6.3
Benzoic acid	−6.4	−7.1	−5.4
Daidzein	−6.3	−7.4	−6.1
Dimethomorph	−6.1	−8.6	−6.3
Galaxolidone	−7.4	−7.8	−6.3
Ilicic acid	−6.5	−7.3	−5.8
Isoquercetin	−6.7	−8.8	−6.8
Mesterolone	−7.1	−7.5	−7.3
Oleanolic acid	−7.8	−9.1	−8.2
Psilostachyin B	−6.2	−7.7	−7.1
Rutin	−7.5	−9.1	−7.3
Ursolic acid	−8.1	−9.5	−7.9
Hydroxychloroquine (MPro Inhibitor)	–	−6.5	–
NI241 (NF-κB inhibitor)	–	–	−5.4

by declining the HR complex's binding energy after being bonded with those compounds compared to the HR complex without ligand (Fig. 1a). Among the seven compounds that have an excellent affinity to HR1, rutin could modify the HR1–HR2 interaction. This was described by the RMSD value of the HR complex with rutin inside compared to the HR complex alone, which has a greater value than other complexes (Fig. 1b). Interaction of HR1–HR2 to form the helix bundle is the crucial step for SARS-CoV-2 membrane fusion (Liu *et al*., 2004; Ou *et al*., 2020). Altering the helix bundle formation has been studied to prevent viral entry s (Xia *et al*., 2020), suggesting that 19-norandrostenedione, 19-nortestosterone, galaxolidone, mesterolone, oleanolic acid, ursolic acid, and in particular rutin have an excellent potency to inhibit viral infection.

MPro is the target protein with plenty of interacted compounds. Compared to HCQ as control, all the compounds have a lower binding energy. Each compound has its favorable binding region, presented by structural visualization (Figure. 2) or interacted residues between the ligand molecule and MPro (Supplementary File 2). Among those compounds, only daidzein and (9cis)-retinal have an interaction with catalytic residues of MPro at HIS:41. HCQ did not show any interaction with the catalytic residues (Supplementary File 2). HIS:41 and CYS:145 have been known as essential residues during MPro enzymatic activity (Tahir ul Qamar *et al*., 2020). Inhibiting these residues has a promising effect on preventing virus replication and prevent viral spreading throughout the tissues (Ahkam *et al*., 2020; Gyebi *et al*., 2020; Hosseini-Zare *et al*., 2020; Tahir ul Qamar *et al*., 2020).

NF-κB is the transcription factor of several proteins related to infection response, including cytokines related to defense mechanisms (Hayden *et al*., 2006; Schmitz *et al*., 2014).

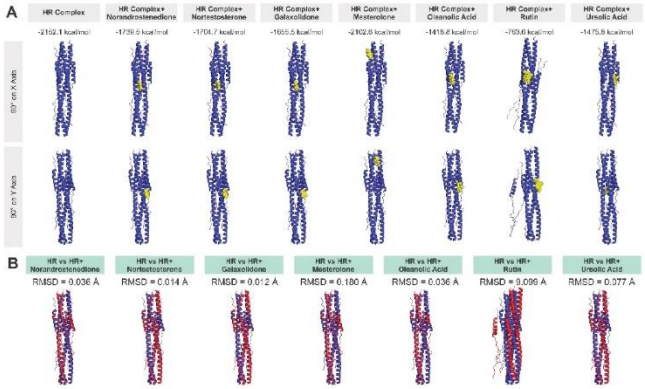


Figure 1. Structural orientation and binding energy of the HR complex after being bound with phytochemical ligands (A) and structural alignment of HR complex before and after being bound with phytochemical ligands (B). The blue ribbons in Figure A represent the HR protein complex, while the yellow spheres describe the phytochemical ligand. In Figure B, the blue ribbons represent the initial form of the HR complex without the ligand, while the red ribbons represent the HR complex structure after being bound with the phytochemical ligands.

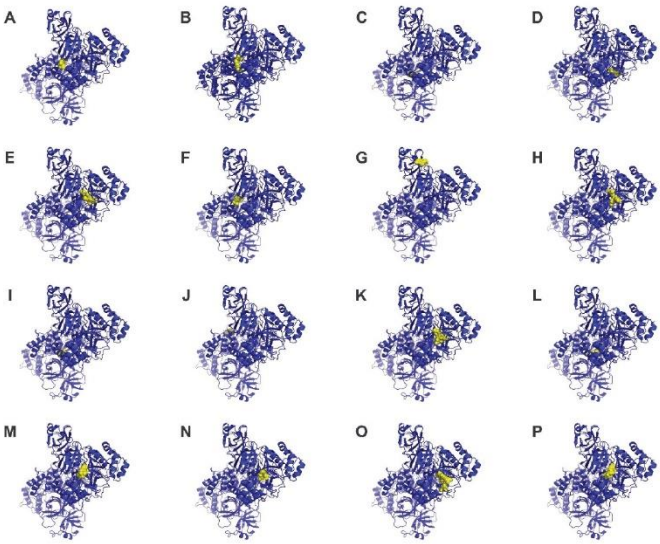


Figure 2. Structural visualization of MPro after being bound with its inhibitor and phytochemical ligands: HCQ (A), (9cis)-retinal (B), 19-norandrostenedione (C), 19-nortestosterone (D), 3-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-buten-1-ylidene)cyclohexyl β-D-glucopyranoside (E), benzoic acid (F), daidzein (G), dimethomorph (H), galaxolidone (I), ilicic acid (J), isoquercetin (K), mesterolone (L), oleanolic acid (M), psilostachyin B (N), rutin (O), and ursolic acid (P).

At this critical condition, cytokine storms are the main factors that contribute to lung damage due to the overexpression of proinflammatory cytokines (Lin *et al*., 2020; Soy *et al*., 2020). Thus, targeting NF-κB as the main transcription factor for suppressing those cytokines' hyperexpression could play a vital role in augmenting patient survival (Catanzaro *et al*., 2020). From the docking result, six compounds could interact with NF-κB at -7 kcal/mol or lower. Although the ligands did not bind with the vital residues involved in NF-κB DNA-binding site, several interacted amino acids take place adjacent to these binding sites (Figure 3 and Supplementary File 2), suggesting their potentials as NF-

Table 2. Physicochemical properties of screened phytochemicals according to SwissADME measurement.

Compound	Mol. weight (g/mol)	LogP value	H-bond donor	H-bond acceptor	Rotatable bonds	TPSA
(9cis)-Retinal	464.38	2.11	8	12	4	210.51 Å²
19-Norandrostenedione	272.38	2.6	0	2	0	34.14 Å²
19-Nortestosterone	274.4	2.79	1	2	0	37.30 Å²
3-Hydroxy-3,5,5-trimethyl-4-(3-oxo-1-buten-1-ylidene)cyclohexyl β-D-glucopyranoside	386.44	2.21	5	8	4	136.68 Å²
Benzoic acid	250.33	2.74	2	3	3	57.53 Å²
Daidzein	254.24	1.77	2	4	1	70.67 Å²
Dimethomorph	387.86	3.67	0	4	6	48.00 Å²
Galaxolidone	272.38	3.17	0	2	0	26.30 Å²
Ilicic acid	252.35	2.26	2	3	2	57.53 Å²
Mesterolone	304.47	3.09	1	2	0	37.30 Å²
Oleanolic acid	456.7	3.92	2	3	1	57.53 Å²
Psilostachyin B	262.3	2.17	0	4	0	52.60 Å²
Quercetin	284.44	3.76	0	1	5	17.07 Å²
Rutin	610.52	2.43	10	16	6	269.43 Å²
Ursolic acid	456.7	4.01	2	3	1	57.53 Å²

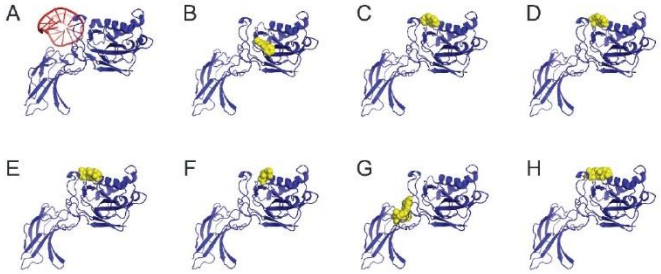


Figure 3. Structural visualization of NF-κB after being bound with DNA (A), NI241 (B), 19-nortestosterone (C), mesterolone (D), oleanolic acid (E), psilostachyin B (F), rutin (G), and ursolic acid (H).

κB inhibitors (Müller *et al*., 1995). Targeting DNA-binding sites of NF-κB has been employed to preclude chronic inflammation (Gilmore and Herscovitch, 2006; Gupta *et al*., 2010). Therefore, the presence of those compounds in the NF-κB DNA-binding domain could reduce hyperinflammation by altering the proinflammatory cytokines' transcription process.

Several criteria have been developed by Lipinski for a small molecule to have good oral bioavailability, permeability, and flexibility (Lipinski, 2004). The oral bioavailability of small molecules is determined by several criteria, including MW, LogP value, the number of hydrogen bond donors, and acceptor less than 500 g/mol, 5, 5, and 10, respectively (Lipinski, 2004). Also, a molecule with a TPSA value equal or less than 140 Å will carry out good permeability, while the number of rotatable bonds less than 10 represents molecule flexibility (Chagas *et al*., 2018). Hence, SwissADME was employed to do the calculations related to LRO5. All of the analyzed compounds, except for rutin and (9cis)-retinal, have no violations of the MWmolecular weight, LogP value, H-bond donor, H-bond acceptor, rotatable bonds, and TPSA criteria. Rutin had a low oral bioavailability and permeability with 610.52 g/mol of MW, 10 of H-bond donor, 16 of H-bond acceptor, and 269.43 Å² TPSA value. (9cis)-Retinal is also not better than rutin in terms of oral bioavailability and permeability with 8 of H-bond donor, 12 of H-bond acceptor, and 210.51 Å² TPSA value (Table 2).

CONCLUSION

There are seven herbal compounds from DL, which have good potential as a preventive or complementary agent for COVID-19 treatment based on the constant binding energy lower or equal to -7 kcal/mol with MPro or HR1. However, daidzein has better potency as an MPro inhibitor, while rutin showed a worthy effect to prevent viral-host fusion by modifying the HR complex structure orientation. As anti-inflammatory candidates, 19-nortestosterone, mesterolone, oleanolic acid, and ursolic acid have a satisfactory result as NF-κB inhibitors. Lastly, all compounds with a binding energy lower than or equal to −7 kcal/mol, except for (9cis)-retinal and rutin, have good oral bioavailability, permeability, and flexibility.

ACKNOWLEDGMENTS

The authors acknowledge the Ministry of Research, Technology, and Higher Indonesia, the Republic of Indonesia, for funding this research under Grant no. 229/SP2H/LT/DRPM/2019.

CONFLICT OF INTEREST

None.

REFERENCES

Acosta MAT, Singer BD. Pathogenesis of COVID-19-induced ARDS: implications for an aging population. Eur Respir J, 2020; 56(3):2002049.

Ahkam AH, Hermanto FE, Alamsyah A, Aliyyah IH, Fatchiyah F. Virtual prediction of antiviral potential of ginger (*Zingiber officinale*) bioactive compounds against spike and MPro of SARS-CoV2 protein. Berkala Penelitian Hayati J Biol Res, 2020; 25:52–7.

Baba J, Yokota, T, Takahashi N. Brassinolide-related new bioactive steroids from *Dolichos lablab* seed. Agric Biol Chem, 1983; 47:659–61.

Bahtiar A, Vichitphan K, Han J. Leguminous plants in the Indonesian archipelago: traditional uses and secondary metabolites. Nat Prod Commun, 2017; 12:461–72.

Bosch BJ, Martina, BEE, Van Der Zee R, Lepault J, Haijema, BJ, Versluis C, Heck AJR, De Groot R, Osterhaus ADME, Rottier PJM. Severe acute respiratory syndrome coronavirus (SARS-CoV) infection inhibition using spike protein heptad repeat-derived peptides. Proc Natl Acad Sci U S A, 2004; 101:8455–60.

Catanzaro M, Fagiani F, Racchi M, Corsini E, Govoni S, Lanni C. Immune response in COVID-19: addressing a pharmacological challenge by targeting pathways triggered by SARS-CoV-2. Signal Transduct Target Ther, 2020; 5(1):84.

Chagas CM, Moss S, Alisaraie L. Drug metabolites and their effects on the development of adverse reactions: revisiting lipinski's rule of five. Int J Pharm, 2018; 549:133–49.

Conti P, Ronconi G, Caraffa A, Gallenga C, Ross R, Frydas I, Kritas S. Induction of pro-inflammatory cytokines (IL-1 and IL-6) and lung inflammation by coronavirus-19 (COVI-19 or SARS-CoV-2): anti-inflammatory strategies. J Biol Regul Homeost Agents, 2020; 34:327–31.

Dai W, Zhang B, Su H, Li J, Zhao Y, Xie X, Jin Z, Liu F, Li C, Li Y, Bai F, Wang H, Cheng X, Cen X, Hu S, Yang X, Wang J, Liu X, Xiao G, Jiang H, Rao Z, Zhang LK, Xu Y, Yang H, Liu H. Structure-based design of antiviral drug candidates targeting the SARS-CoV-2 main protease. Science, 2020; 368(6497):1331–5

Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep, 2017; 7:42717.

Dallakyan S, Olson AJ. Small-molecule library screening by docking with PyRx. Methods Mol Biol, 2015; 1263:243–50.

DeDiego ML, Nieto TJL, Regla NJA, Jimenez GJM, Fernandez DR, Fett C, Castaño RC, Perlman S, Enjuanes L. Inhibition of NF-κB-Mediated Inflammation in severe acute respiratory syndrome coronavirus-infected mice increases survival. J Virol, 2014; 88:913–24.

Di Matteo G, Spano M, Grosso M, Salvo A, Ingallina C, Russo M, Ritieni A, Mannina L. Food and COVID-19: preventive/co-therapeutic strategies explored by current clinical trials and in silico studies. Foods, 2020; 9(8):1036.

Gilmore TD, Herscovitch M. Inhibitors of NF- κ B signaling: 785 and counting. Oncogene, 2006; 25:6887–99.

Gupta SC, Sundaram C, Reuter S, Aggarwal BB. Inhibiting NF-κB activation by small molecules as a therapeutic strategy. Biochim Biophys Acta, 2010; 1799:775–87.

Gyebi GA, Ogunro OB, Adegunloye AP, Ogunyemi OM, Afolabi SO. 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; 1–13.

Habib HM, Theuri SW, Kheadr E, Mohamed FE. DNA and BSA damage inhibitory activities, and anti-acetylcholinesterase, anti-porcine α-amylase and antioxidant properties of *Dolichos lablab* beans. Food Funct, 2017a; 8:881–7.

Habib HM, Theuri SW, Kheadr EE, Mohamed FE. Functional, bioactive, biochemical, and physicochemical properties of the *Dolichos lablab* bean. Food Funct, 2017b; 8:872–80.

Hayden MS, West AP, Ghosh S. NF- κ B and the immune response. Oncogene, 2006; 25:6758–80.

Heck TG, Ludwig MS, Frizzo MN, Rasia FAA, Homem BPI. Suppressed anti-inflammatory heat shock response in high-risk COVID-19 patients: lessons from basic research (inclusive bats), light on conceivable therapies. Clin Sci (Lond), 2020; 134:1991–2017.

Hilgenfeld R. From SARS to MERS: crystallographic studies on coronaviral proteases enable antiviral drug design. FEBS J, 2014; 281:4085–96.

Hosseini-Zare MS, Thilagavathi R, Selvam C. Targeting severe acute respiratory syndrome-coronavirus (SARS-CoV-1) with structurally diverse inhibitors: a comprehensive review. RSC Adv. 2020; 10:28287–99.

Jha AK, Kumar R, Goenka MK, Dayal VM. Emerging treatment and prevention strategies against COVID-19: a brief update. J Dig Endosc, 2020; 11:69–72.

Jin Z, Du X, Xu Y, Deng Y, Liu M, Zhao Y, Zhang B, Li X, Zhang L, Peng C, Duan Y, Yu J, Wang L, Yang K, Liu F, Jiang R, Yang, Xinglou, You T, Liu, Xiaoce, Yang, Xiuna, Bai F, Liu H, Liu, Xiang, Guddat LW, Xu W, Xiao G, Qin C, Shi Z, Jiang H, Rao Z, Yang H. Structure of M pro from SARS-CoV-2 and discovery of its inhibitors. Nature, 2020; 582:289–93.

Joshi RS, Jagdale SS, Bansode SB, Shankar SS, Tellis MB, Pandya VK, Chugh A, Giri AP, Kulkarni MJ. Discovery of potential multi-target-directed ligands by targeting host-specific SARS-CoV-2 structurally conserved main protease. J Biomol Struct Dyn, 2020; 1–16.

Kobayashi T, Tanuma S, Kino K, Miyazawa H. New scaffolds of inhibitors targeting the DNA binding of NF-κB. Integr Mol Med, 2016; 3(5):769–73.

Lin S, Zhao Y, Zhou D, Zhou F, Xu F. Coronavirus disease 2019 (COVID-19): cytokine storms, hyper-inflammatory phenotypes, and acute respiratory distress syndrome. Genes Dis, 2020.

Lipinski CA. Lead- and drug-like compounds: the rule-of-five revolution. Drug Discov Today Technol, 2004; 1:337–41.

Liu S, Xiao G, Chen Y, He Y, Niu J, Escalante CR, Xiong H, Farmar J, Debnath AK, Tien P, Jiang S. Interaction between heptad repeat 1 and 2 regions in spike protein of SARS-associated coronavirus: implications for virus fusogenic mechanism and identification of fusion inhibitors. Lancet, 2004; 363:938–47.

McKee DL, Sternberg A, Stange U, Laufer S, Naujokat C. Candidate drugs against SARS-CoV-2 and COVID-19. Pharmacol Res, 2020; 157:104859.

Minde JJ, Venkataramana PB, Matemu AO. Dolichos Lablab-an underutilized crop with future potentials for food and nutrition security: a review. Crit Rev Food Sci Nutr, 2020; 1–13.

Müller CW, Rey FA, Sodeoka M, Verdine GL, Harrison SC. Structure of the NF-kappa B p50 homodimer bound to DNA. Nature, 1995; 373:311–7.

Muzaffer U, Paul VI, Rajendra PN. Molecular docking of selected phytoconstituents with signaling molecules of Ultraviolet-B induced oxidative damage. In Silico Pharmacol, 2017; 5(1):17.

Naeem M, Shabbir A, Ansari AA, Aftab T, Khan MMA, Uddin M. Hyacinth bean (*Lablab purpureus* L.) – an underutilised crop with future potential. Sci Hortic, 2020; 272:109551.

O'Boyle NM, Banck M, James CA, Morley C, Vandermeersch T, Hutchison GR. Open babel: an open chemical toolbox. J Cheminform, 2011; 3:33.

Ou X, Liu Y, Lei X, Li P, Mi D, Ren L, Guo L, Guo R, Chen T, Hu J, Xiang Z, Mu Z, Chen X, Chen J, Hu K, Jin Q, Wang J, Qian Z. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat Commun, 2020; 11:1620.

Panyod S, Ho CT, Sheen LY. Dietary therapy and herbal medicine for COVID-19 prevention: a review and perspective. J Tradit Complement Med, 2020; 10:420–7.

Procacci P, Macchiagodena M, Pagliai M, Guarnieri G, Iannone F. Interaction of hydroxychloroquine with SARS-CoV2 functional proteins using all-atoms non-equilibrium alchemical simulations. Chem Commun (Camb.), 2020; 56: 8854–6.

Ragab D, Salah Eldin H, Taeimah M, Khattab R, Salem R. The COVID-19 cytokine storm; What We Know So Far. Front Immunol, 2020; 11:1446.

Rahman SA, Akhter MS. Antibacterial and cytotoxic activity of seeds of white hyacinth bean (*Lablab purpureus* L. sweet 'white') -. J Adv Biotechnol Exp Ther, 2018; 1:49–54.

Ritchie, DW, Kemp, GJ. Protein docking using spherical polar Fourier correlations. Proteins, 2000; 39:178–94.

Schmitz ML, Kracht M, Saul VV. The intricate interplay between RNA viruses and NF-κB. Biochim Biophys Acta, 2014; 1843(11):2754–64.

Soy M, Keser G, Atagündüz P, Tabak F, Atagündüz I, Kayhan S. Cytokine storm in COVID-19: pathogenesis and overview of anti-inflammatory agents used in treatment. Clin Rheumatol, 2020; 39:2085–94.

Su H, Yao S, Zhao W, Li M, Liu J, Shang W, Xie H, Ke C, Hu H, Gao M, Yu K, Liu H, Shen J, Tang W, Zhang L, Xiao G, Ni L, Wang D, Zuo J, Jiang H, Bai F, Wu Y, Ye Y, Xu Y. Anti-SARS-CoV-2 activities *in vitro* of shuanghuanglian preparations and bioactive ingredients. Acta Pharmacol Sin, 2020; 41:1167–77.

Tahir QM, Alqahtani SM, Alamri MA, Chen, LL. Structural basis of SARS-CoV-2 3CLpro and anti-COVID-19 drug discovery from medicinal plants. J Pharm Anal, 2020; 10:313–9.

Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. J Comput Chem, 2010; 31:455–461.

Walls AC, Park, YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell, 2020; 181:281–92.

Xia S, Liu M, Wang C, Xu W, Lan Q, Feng S, Qi F, Bao L, Du L, Liu S, Qin C, Sun F, Shi Z, Zhu Y, Jiang S, Lu L. Inhibition of SARS-CoV-2 (previously 2019-nCoV) infection by a highly potent pan-coronavirus fusion inhibitor targeting its spike protein that harbors a high capacity to mediate membrane fusion. Cell Res, 2020; 30:343–55.

Yin J, Seo CS, Hwang IH, Lee MW, Song KH. Anti-obesity activities of chikusetsusaponin IVa and *Dolichos lablab* L. Seeds. Nutrients, 2018; 10:1221.

Yoshikawa M, Murakami T, Komatsu H, Matsuda H. Medicinal foodstuffs. XII. Saponin constituents with adjuvant activity from hyacinth bean, the seeds of Dolichos lablab L. (1): structures of lablabosides A, B, and C. Chem Pharm Bull (Tokyo), 1998; 46: 812–6.

Zhang L, Lin D, Sun X, Curth U, Drosten C, Sauerhering L, Becker S, Rox K, Hilgenfeld R. Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors. Science, 2020a; 368:409–12.

Zhang W, Zhao Y, Zhang F, Wang Q, Li T, Liu Z, Wang J, Qin Y, Zhang X, Yan X, Zeng X, Zhang S. The use of anti-inflammatory drugs in the treatment of people with severe coronavirus disease 2019 (COVID-19): the Perspectives of clinical immunologists from China. Clin Immunol, 2020b; 214:108393.

How to cite this article:
Purwanti E, Hermanto FE, Souhaly JW, Prihanta W, Permana TI. Exploring public health benefits of *Dolichos lablab* as a dietary supplement during the COVID-19 outbreak: A computational study. J Appl Pharm Sci, XXXX; XX(XX):XX–XX.

Figure 2. Visualization of MPro docked with its inhibitor and phytochemical ligand: HCQ (A), (9cis)-Retinal (B), 19-Norandrostenedione (C), 19-Nortestosterone (D), 3-Hydroxy-3,5,5-trimethyl-4-(3-oxo-1-buten-1-ylidene)cyclohexyl β-D-glucopyranoside (E), Benzoic Acid (F), Daidzein (G), Dimethomorph (H), Galaxolidone (I), Ilicic Acid (J), Isoquercetin (K), Mesterolone (L), Oleanolic Acid (M), Psilotachyin B (N), Rutin (O), and Ursolic Acid (P).

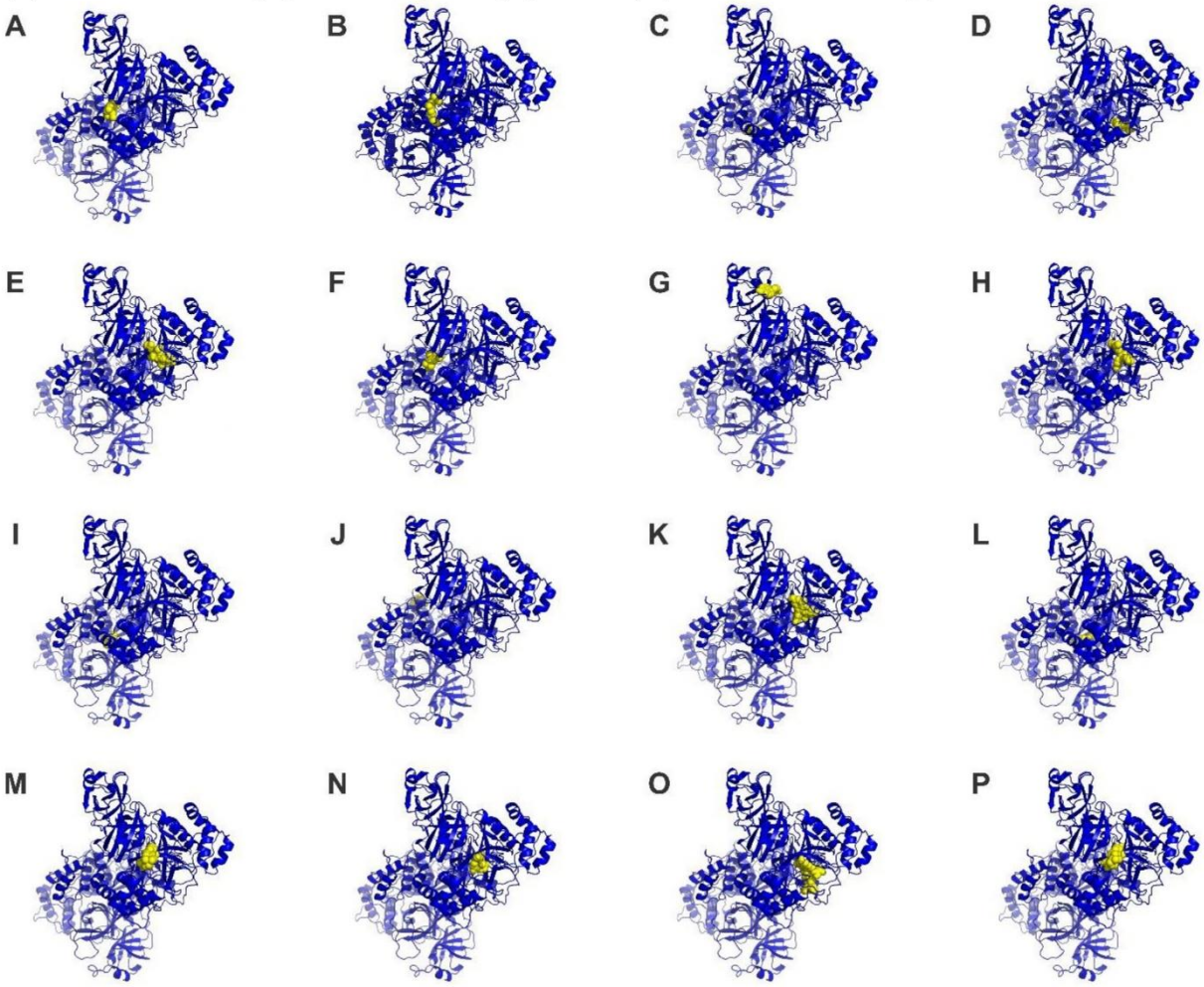


Figure 3. Structural visualization of NF- ϰB binds with DNA (A), NI241 (B), 19-Nortestosterone (C), Mesterolone (D), Oleanolic Acid (E), Psilotachyin B (F), Rutin (G), and Ursolic Acid (H).