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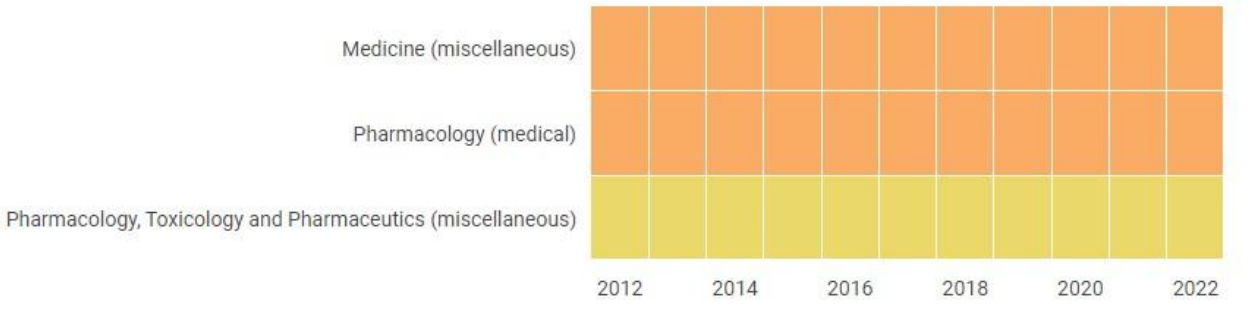

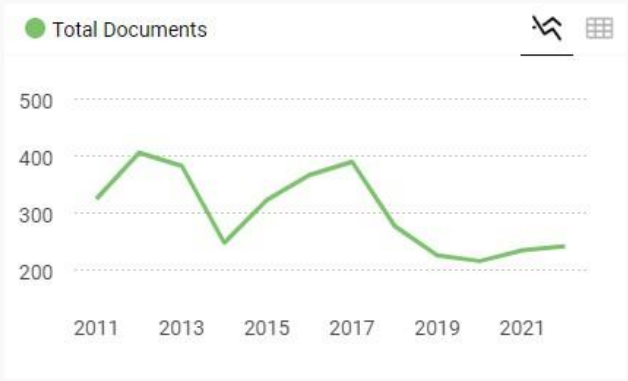

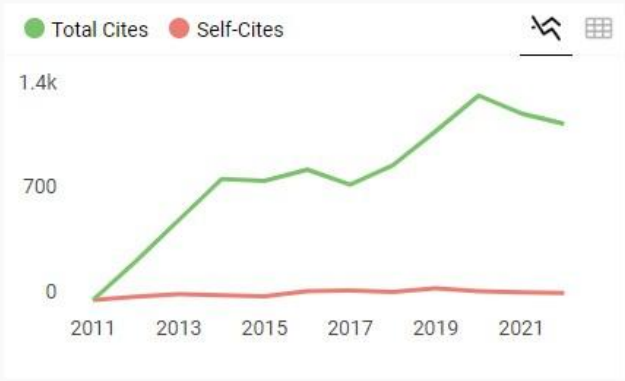

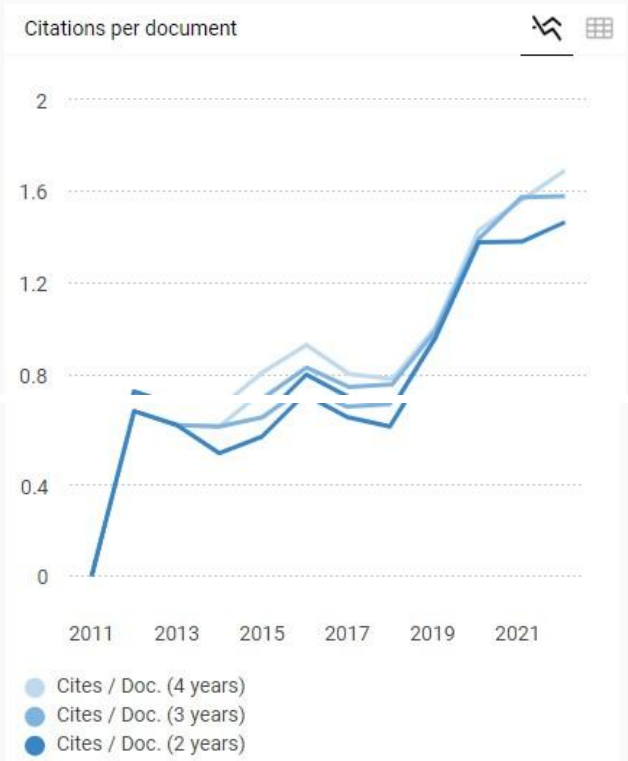

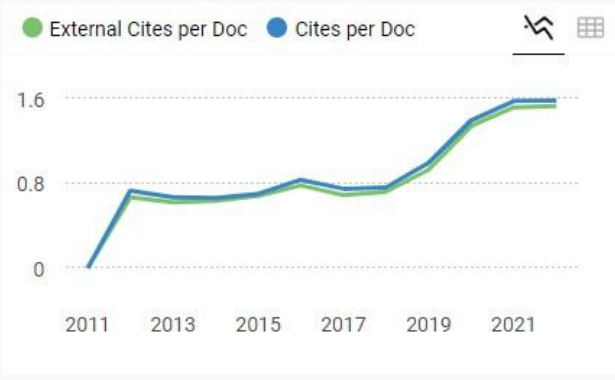

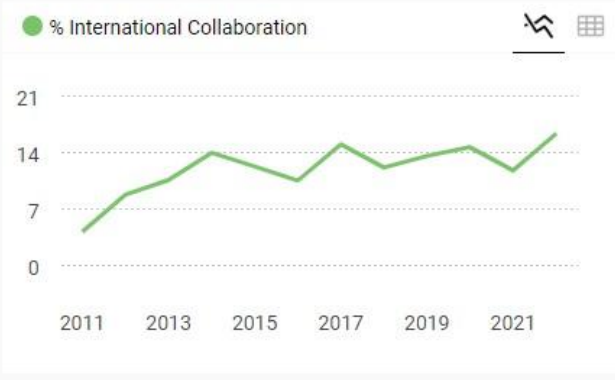

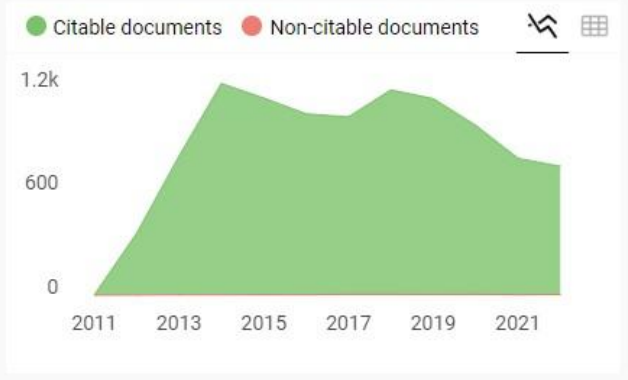

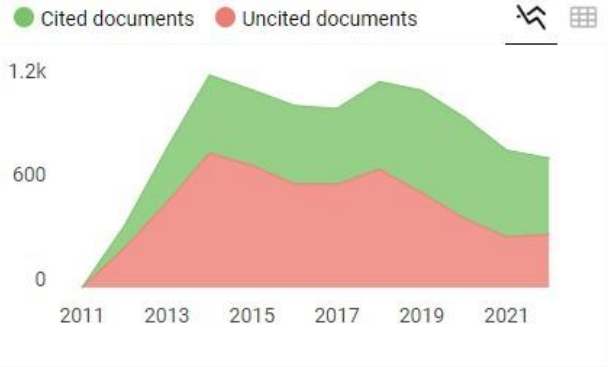

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Exploring public health benefits of *Dolichos lablab* as a dietary supplement during the COVID-19 outbreak: A computational study

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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

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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 entrys (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. 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).

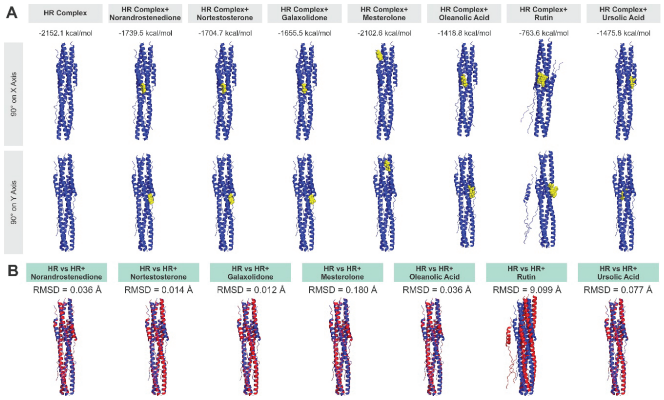

Figure 1. Structural orientation and binding energy of the HR complex after being bound with phytochemical ligands (A) and structural alignment of HR complex before and after being bound with phytochemical ligands (B). The blue ribbons in Figure A represent the HR protein complex, while the yellow spheres 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.

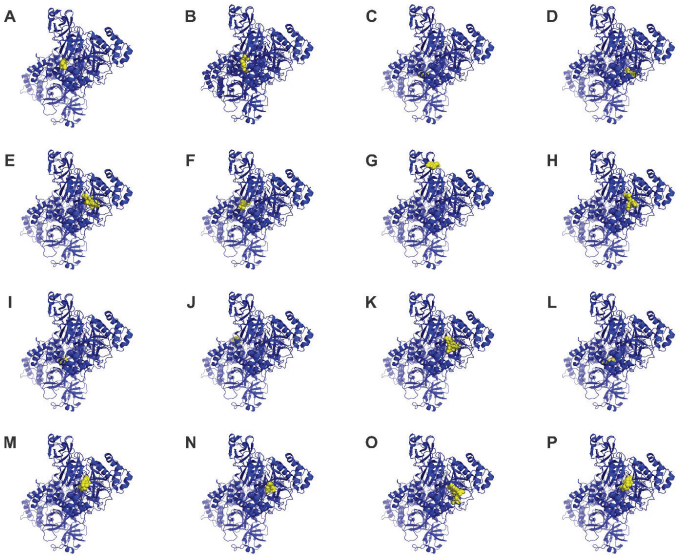

Figure 2. Structural visualization of MPro after being bound with its inhibitor and phytochemical ligands: HCQ (A), (9cis)-retinal (B), 19-norandrostenedione (C), 19-nortestosterone (D), 3-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-buten-1-ylidene)cyclohexyl β-D-glucopyranoside (E), benzoic acid (F), daidzein (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), suggesting their potentials as NF-κB inhibitors

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

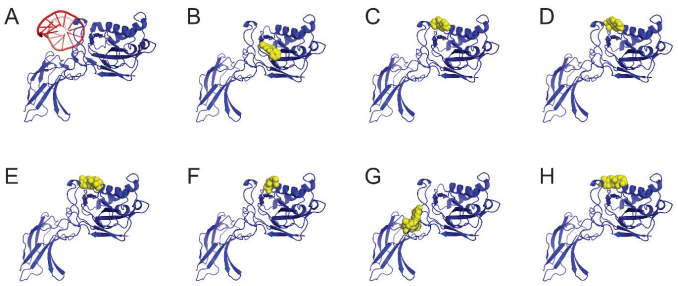

Figure 3. Structural visualization of NF-κB after being bound with DNA (A), NI241 (B), 19-nortestosterone (C), mesterolone (D), oleanolic acid (E), psilostachyin B (F), rutin (G), and ursolic acid (H).

(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.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

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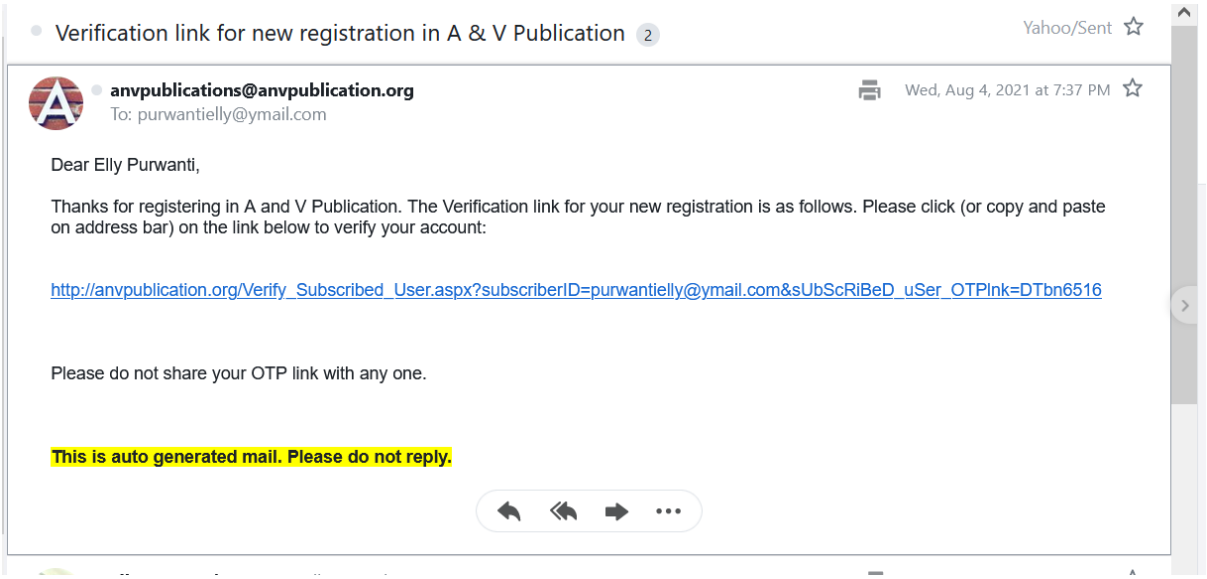

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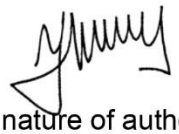

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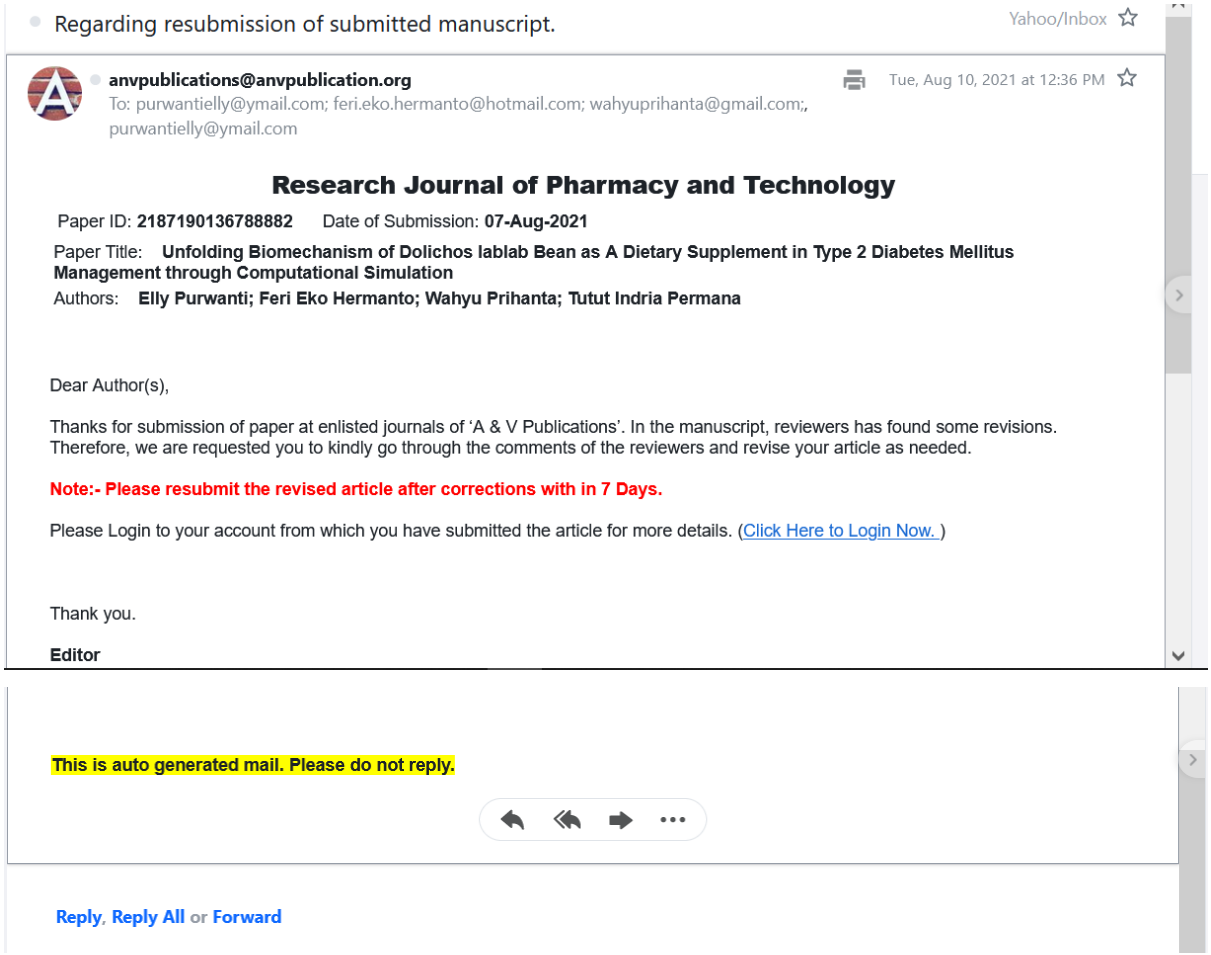

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Unfolding Biomechanism of Dolichos lablab Bean as A Dietary Supplement in Type 2 Diabetes Mellitus Management through Computational Simulation

ABSTRACT:

Dietary intervention, particularly legumes consumption, plays a significant role in promoting health status in diabetes mellitus management. As poorly known legumes, *Dolichos lablab* (DL) is possibly to be one of the dietary options for diabetes intervention. However, the predictive or precise mechanism of DL's anti-diabetic activity remains inconclusive. This study aimed to determine the nutritional and phytochemical content in addition to anti-diabetic properties of DL. Total protein, crude fat, crude fibers, and gross energy were evaluated, while anti-diabetic properties were predicted using molecular docking according to identified compound from Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) analysis. Screened compound from molecular docking then passe to physicochemical properties and bioactivity prediction using Swiss-ADME and molinspiration, separately. The result showed that DL has high protein fiber and gross energy content with a lower fat percentage. Additionally, DL has numerous phenolic acid and flavonoid compounds according to LC-HRMS analysis. From the docking analysis, fourteen compounds have substantial probability to give the beneficial effect of glucose metabolism regulator and insulin signaling repairers through inhibition of α-amylase, DPP4, and PTP1B. Finally, from the physicochemical properties and bioactivity estimations, 19-Norandrostenedione, 19-Nortestosterone, Icariside B1, Ilicic Acid, and Psilostachyin B have excellent pharmacokinetic properties along with considerable biological activity as enzyme inhibitors and nuclear receptor ligands. In conclusion, nutritional evaluation and molecular docking analysis revealed that DL might serve as a suitable dietary intervention for diabetes mellitus management.

KEYWORDS: α-amylase; *Dolichos lablab*; diabetes mellitus; DPP4; PTP1B.

INTRODUCTION :

Diabetes mellitus (DM) has recognized as a global health problem with increasing cases in forthcoming years. A total of 6.28% of the global populations were affected by DM, contributing to the ninth cause of mortality worldwide [1]. Further, it is estimated that around 642 million people will suffer from DM in 2040 [2]. With those conditions, health management and prevention play a vital role in delaying DM development day by day.

Health management has been applied to halt DM's progression, including lifestyle changes and dietary intervention [3]. A few nutritional compositions have been suggested for people with DM, including legumes consumption [4]. One of the high potential legumes for dietary intake in DM conditions is *Dolichos lablab* (DL). With the high content of fibres and other nutritional compositions, DL has a good potential as a dietary supplement for DM [5]. Previous studies have reported DL's efficacy in regulating glycaemic levels, despite the precise mechanism still unresolved [6,7].

Since metabolic disease like DM involved many proteins for its progression, targeting specific proteins becomes the promising way to develop anti-diabetic drugs [8]. Altering glucose metabolism and insulin signalling can turn into an effective way of controlling DM [8,9]. Thus, targeting α-amylase, DPP4, and PTP1B have favourable results in preventing DM advancement. As previously described, α-amylase plays a role in starch metabolism and contributes to plasma glucose enhancement [10]. On the other hand, DPP4 and PTP1B orchestrate insulin signalling, mainly involved in insulin sensitization, secretion, and post-prandial blood glucose levels [11,12]. Some studies have been employed to inhibit those proteins from achieving average blood glucose concentration and insulin performance [13–15]. Nevertheless, there were no studies for understanding the role of bioactive compounds in DL to inhibit those proteins and regulate DM conditions. Therefore, this study aimed to determine the nutritional content and the bioactive compounds in DL as DM complementary treatment.

MATERIALS AND METHODS:

Plant Samples and Extraction

Sample was obtained from Madura Island, Indonesia. Detailed characteristics of the sample as explained in previous work [16]. Bean was ground prior to the extraction process. Extraction was carried out by soaking in 96% ethanol in a 1:3 ratio (weight/volume) for 24 hours. After submerging, the solvent was evaporated and freeze-dried to obtain DL extract.

Total Protein, Crude Fat, Crude Fiber, and Gross Energy Determination

Determination of total protein, crude fat, and crude fiber was performed according to a previously described method [17]. Gross energy was measured using IKA C2000 Calorimeter System (IKA Works, Germany) following the manufacturer's protocol.

Bioactive Metabolites Identification

Thermo Scientific Dionex Ultimate 3,000 RSLCnano Liquid Chromatography (LC) linked with Thermo Scientific Q Exactive High Resolution Mass Spectrometry (HRMS) was employed to identify the presence of bioactive compound in DL extract. Detailed protocols for chromatography as mentioned in earlier work [18]. Total ionic chromatograms then analyzed using Compound Discoverer and matched with mzCloud in the MS/MS Library. Compound with match score higher or equal with 80 then selected for molecular docking simulations as the ligands.

Data Mining of Protein and Ligand Structures

Three-dimensional (3D) structures of protein were retrieved from Protein Data Bank (PDB; https://www.rcsb.org/), while 3D ligand structures were obtained from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). In detail, the structures of protein used in this study are α-amylase (PDB ID: 1HNY), DPP4 (PDB ID: 5Y7K), and PTP1B (PDB ID: 1BZC). The list of phytochemicals and their identity (PubChem CID) as shown in table 2 [18].

Binding Energy Calculations

AutoDock Vina integrated into PyRx software was employed for molecular docking simulations [19,20]. Water molecules and the previous-attached ligand in each 3D protein structure were removed before the docking process. Protein structures were set as a rigid molecule, while the phytochemicals as the ligands were set as a flexible molecule. Blind docking was applied with a maximum grid setting for searching binding sites.

Data Analysis

Complex with binding energy lower or equal with -7 kcal/mol was directed into further analysis for amino acid-ligand interaction. Interacted residues in each complex and visualization were analyzed using Discovery Studio 2019 to determine the chemistry of formed interaction.

Drug-Likeness Evaluation and Structure-Activity Relationship (SAR)

The drug-likeness characteristics was determined by Swiss-ADME webserver [21], while Molinspiration (https://www.molinspiration.com/cgi-bin/properties) was used to determine SAR of selected compounds based on the molecular docking process.

RESULT:

Nutritional Values of DL

The protein was the higher constituent from the analyzed nutritional contents, followed by crude fibers, while fat content has the smallest portion. Protein constitutes 24.91±0.08% of the total contents, while 7.03±0.02% and 0.36±0.01% consist of crude fiber and fat, respectively. Also, gross energy measurement showed that DL has a high energy source for daily energy uptake (table 1).

Bioactive Compounds in DL

Phenolic acid and flavonoid were the most abundance compound in DL (table 2). Pipecolic acid, trans-3-Indoleacrylic acid, caffeine, choline, and trigonelline were major constituent according to peak area. Some amino acids like arginine and histidine also found since DL is a legumes species. In addition, a common isoflavones in legumes, daidzein, also identified in DL extract. From the screening revealed that DL has diverse phytochemical compounds which have a potential to contribute in biological mechanism, particularly for health purposes. The identified compounds from this step then continued for the screening of anti-diabetic properties.

Potential Mechanism of Phytochemicals from DL in Diabetic Pathway

Eighteen compounds could interact with a minimum of one of the target proteins at low binding energy. Ursolic acid, rutin, and 19-Nortestosterone are the compounds with the lowest binding energy for α-Amylase, DPP4, and PTP1B, respectively (table 3). Unfortunately, not all of the screened compounds have good potential for protein target inhibitors. Protein-ligand structure analysis revealed that only 14 compounds could interact directly with several essential residues in each targeted protein.

Ursolic acid, oleanolic acid, isoquercetin, psilostachyin B, Rutin, 9-cis-retinal, and icariside B1 were the compounds that been able to bind directly to the active sites of α-Amylase. Those compounds could interact with the α-Amylase mostly at HIS305 by hydrophobic or hydrogen bond interaction. Some compounds also bind with other key residues in the active sites, including ASP197, GLU233, and ASP300. Rutin and oleanolic acid are the compounds with the most binding sites in the active sites of α-Amylase, which has been able to bind with three different key residues (figure 1 A-G and K-Q).

Different from the α-Amylase, DPP4 has higher selectivity to bind with the analyzed compounds. There were three compounds bound to DPP4 at the active sites, i.e., isoquercetin, rutin, and icariside B1. GLU205, GLU206, TYR547, SER630, HIS740 were the active sites of DPP4, which interacted with all of those three compounds. Interestingly, isoquercetin and rutin have similar binding sites with one additional interaction of catalytic residues at ARG125 (figure 1 H-J and R-T).

Seven compounds could bind with the PTP1B at its catalytic sites. 19-nortestosterone, ilicic acid, 19-norandrostenedione, scopoletin, coumaric acid, trans-3-indoleacrylic acid, and daidzein were the compounds that have interaction with the catalytic sites of PTP1B. Scopoletin and trans-3-indoleacrylic acid were the compounds that could interact with more catalytic residues. On the other hand, daidzein was the compound that has less interaction with catalytic residues. In general, PHE182, ALA217, and ARG221 are the most preferred residues of those compounds (figure 2).

Drug Likeness Characteristics of Screened Phytochemicals

Drug-likeness properties and SAR were predicted using Swiss-ADME webserver and molinspiration, respectively. Six criterias including lipophility, molecular size, polarity, insolubility, unsaturation, and flexibility were employed to predict the drug-likeness properties of each screened compound. The pink areas represent the most favorable criterias with high similarity as the drug. Accordingly, 19-Norandrostenedione, 19-Nortestosterone, Icariside B1, Ilicic Acid, and Psilostachyin B were compounds with the most resemblance with drug (figure 3A). Further, SAR prediction discovered that nine out of fourteen compounds have potential as both enzyme inhibitors and nuclear receptor ligands (figure 3B).

DISCUSSION:

Natively grown in Africa and Indian subcontinent, DL has been labeled as underutilized crops due to its limited global market potential and unpopular nutritional sources [5]. Nevertheless, DL has been used in different regions of the world as human food and animal feed [23]. Consisting of adequate main macronutrients needed for daily food intake, DL has promising potential as nutritional therapy for several metabolic diseases including DM [5]. Diet management has been suggested for diabetic patients to maintain plasma glycemic levels [24,25]. Consuming high fiber and protein content can increase insulin response and prevent plasma glycemic augmentation [24,26–28]. Also, low-fat nutritional sources help fulfill energy requirement and prevent cardiovascular risk [24]. With the high protein, fibers, and low-fat composition, DL has worthy potential for dietary intervention in diabetes management. Phenolic acid is major secondary metabolite founded in DL, particularly in raw beans [29]. Some phenolic acids including ferulic acid and coumaric acid make several major phenolic acid in DL, and those compounds were identified and confirmed at present study [30]. Other dominant polyphenol compound, rutin, also identified [30]. Phenolic acid has been proved to exhibits an anti-diabetic nature, particularly by inhibiting α-amylase [31,32]. Therefore, this result discover a wide potential of DL as anti-diabetic agent.

Regulating glucose metabolism and insulin performance are the key factors in diabetes management [33,34]. An enzyme called α-amylase plays a vital role in glucose metabolism from dietary intake [35]. Targeting its catalytic sites could lead to inhibition of the catalytic activity of α-amylase then prevent uprising glycemic levels [10,14]. In the present study, ursolic acid, oleanolic acid, isoquercetin, psilostachyin B, Rutin, 9-cis-retinal, and icariside B1 from DL extract could bind with some key residues of α-amylase in the catalytic sites as mentioned in the earlier experiments [14,36]. Therefore, the interaction of those compounds with α-amylase implies plasma glucose regulation.

Insulin sensitization also the primary outcome in diabetes therapy. As the proteins involved in the insulin signaling process, DPP4 and PTP1B frequently used as the target for increasing insulin sensitivity [12,37]. ARG125, GLU205, TYR547, SER630, ASP708, ASN710, and HIS740 have been reported as catalytic residues in DPP4 [15]. Interaction in those residues could alter the biomechanism of DPP4, driving to the enhancement of glucose-dependent insulin secretion [38]. Also, addressing DPP4 for diabetes therapy has gained more attention and gave promising recovery effects [39]. Thus, blocking DPP4 by isoquercetin, rutin, and icariside B1 from DL has immense opportunity to improve the health of diabetic patients.

Augmenting insulin sensitization can be reached by altering PTP1B activity [40,41]. Recently, allosteric and catalytic sites blocking of PTP1B have been reported. Directing LEU192, ASN193, PHE196, GLU276, PHE280, and TRP291 generate allosteric inhibition [42], while ARG47, ASP48, PHE182, SER216, ALA217, GLY218, ILE219, GLY220, ARG221, and GLN266 perform catalytic inhibition [43]. With some compounds interacting at the catalytic sites, particularly PHE182, ALA217, and ARG221, DL may serve as a catalytic inhibitor for PTP1B and ameliorates insulin-signaling impairments.

The drug-likeness and drug promiscuity of a compound strongly associate with its physicochemical properties (PP) [44,45]. With the suitable PP, a compound will achieves an adequate absorption, distribution, efficacy, metabolism and excretion (ADME) and prevent adverse drug reactions [44,46]. Lipophilicity, molecular size, polarity, solubility, saturation, and flexibility were determined based on XLOGP3, molecular weight, total polar surface area (TPSA) value, log S, fraction of carbons in the sp3 hybridization, and number of rotatable bond, respectively[21]. 19-Norandrostenedione, 19-Nortestosterone, Icariside B1, Ilicic Acid, and Psilostachyin B were the most compatible compound with those described properties. Thus, those compounds has high probability to have excellent bioavailability, flexibility, and affinity to the target proteins. In advance, 19-Nortestosterone, 19-Norandrostenedione, Icariside B1, and Psilostachyin B also have a reasonable probability of giving biological activity as an enzyme inhibitor and nuclear receptor ligands. Consequently, those compounds seem to have great potential for modulating glucose metabolism and insulin signaling fault in diabetes mellitus patients and good diet therapy for complementary medicine.

CONCLUSION:

DL may serves as suitable dietary interventions for diabetes therapy with good nutritional contents and numerous biologically active compounds. Several compounds, mainly 19-Norandrostenedione, 19-Nortestosterone, Icariside B1, Ilicic Acid, and Psilostachyin B highly probable to act as glucose metabolism modulator and insulin signaling repairmen agent through inhibiting α-amylase, DPP4, and PTP1B, respectively.

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5. Second revision: Minor revision (19-11-2021)

- Article revision letter for authors

Resubmission of submitted manuscript after minor correction. 2 Yahoo/Sent

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Research Journal of Pharmacy and Technology

Paper ID: **2187190136788882** Date of Submission: **07-Aug-2021**

Paper Title: **Unfolding Biomechanism of Dolichos lablab Bean as A Dietary Supplement in Type 2 Diabetes Mellitus Management through Computational Simulation**

Authors: **Elly Purwanti; Feri Eko Hermanto; Wahyu Prihanta; Tutut Indria Permana**

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Journal's name	Research Journal of Pharmacy and Technology
Article title	**Unfolding Biomechanism of *Dolichos lablab* Bean as A Dietary Supplement in Type 2 Diabetes Mellitus Management through Computational Simulation**
Article Number (original Submitted manuscript No)	**2187190136788882**
Only Corresponding authors name	Elly Purwanti
I would like to recheck the corrections: corrections to be done within week very carefully	Yes ~~/No~~

List of corrections

Page number	Column (Left / Right)	Paragraph number from top/ Name of Paragraph	Line number from top of paragraph	Delete this text (Error)	Replace deleted text with (correction)
1		Title	1	Dolichos lablab	*Dolichos lablab*
1	Right	1	3	…intervention[3,4,4,5]	…intervention[3-5]
3	Left	1 (above table 2)	1 and 2	The identified compounds from this properties step	The identified compounds from this step then

				then continued for the screening of anti-diabetic.	continued for the screening of anti-diabetic properties. (or better move this sentence below the table 2).
4	Right	Figure 1	Figure 1	The figure is to small.	Please resize and place in larger size and space, if possible.
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6. Article accepted for publication (3-1-2022)

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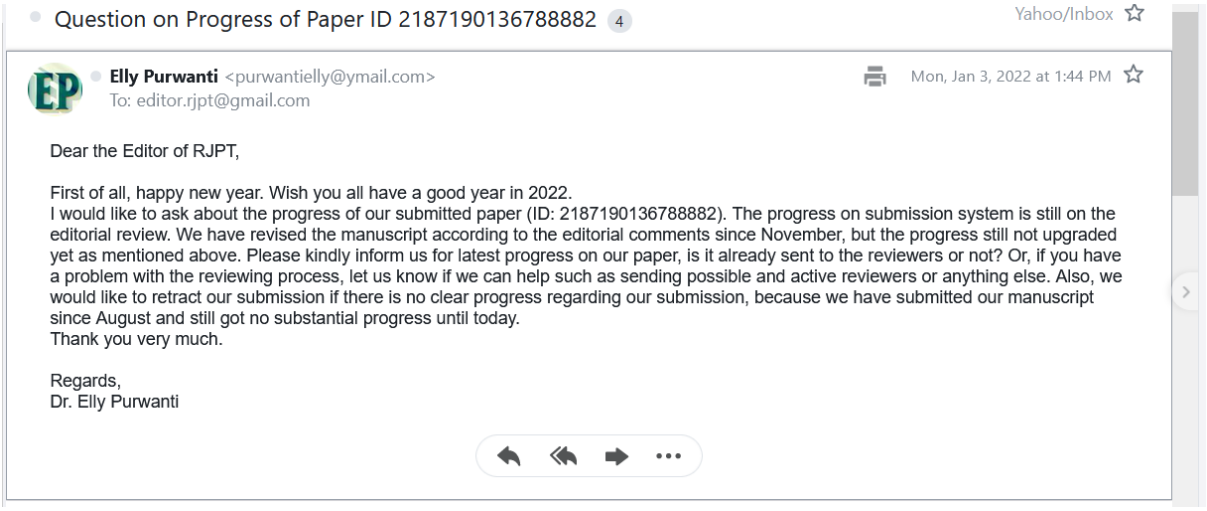

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Elly Purwanti <purwantielly@ymail.com>
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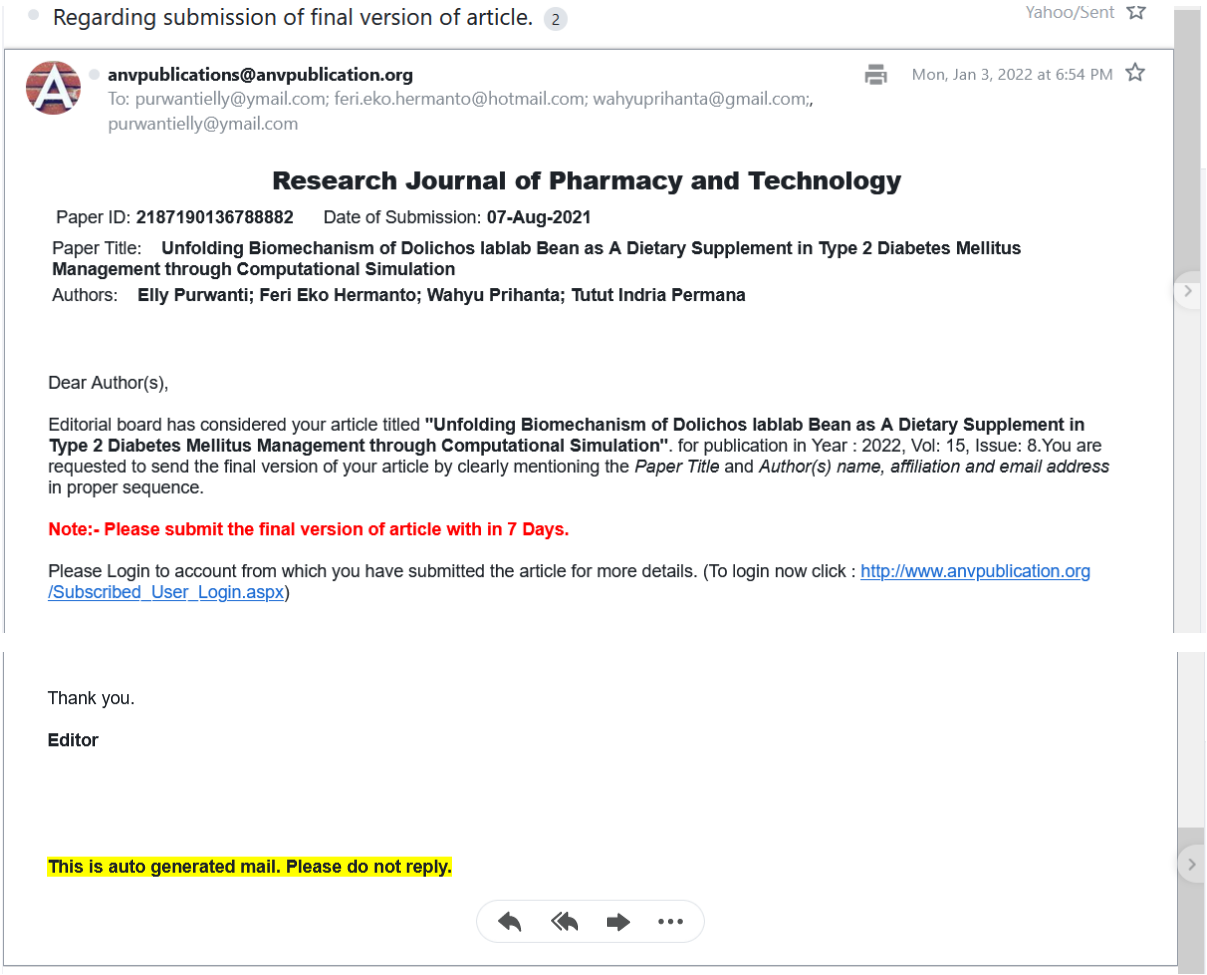
Dear the Editor of RJPT,

First of all, happy new year. Wish you all have a good year in 2022.
I would like to ask about the progress of our submitted paper (ID: 2187190136788882). The progress on submission system is still on the editorial review. We have revised the manuscript according to the editorial comments since November, but the progress still not upgraded yet as mentioned above. Please kindly inform us for latest progress on our paper, is it already sent to the reviewers or not? Or, if you have a problem with the reviewing process, let us know if we can help such as sending possible and active reviewers or anything else. Also, we would like to retract our submission if there is no clear progress regarding our submission, because we have submitted our manuscript since August and still got no substantial progress until today.
Thank you very much.

Regards,
Dr. Elly Purwanti

- Decision letter to authors



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Research Journal of Pharmacy and Technology

Paper ID: **2187190136788882** Date of Submission: **07-Aug-2021**

Paper Title: **Unfolding Biomechanism of Dolichos lablab Bean as A Dietary Supplement in Type 2 Diabetes Mellitus Management through Computational Simulation**

Authors: **Elly Purwanti; Feri Eko Hermanto; Wahyu Prihanta; Tutut Indria Permana**

Dear Author(s),

Editorial board has considered your article titled **"Unfolding Biomechanism of Dolichos lablab Bean as A Dietary Supplement in Type 2 Diabetes Mellitus Management through Computational Simulation"**. for publication in Year : 2022, Vol: 15, Issue: 8.You are requested to send the final version of your article by clearly mentioning the *Paper Title* and *Author(s) name, affiliation and email address* in proper sequence.

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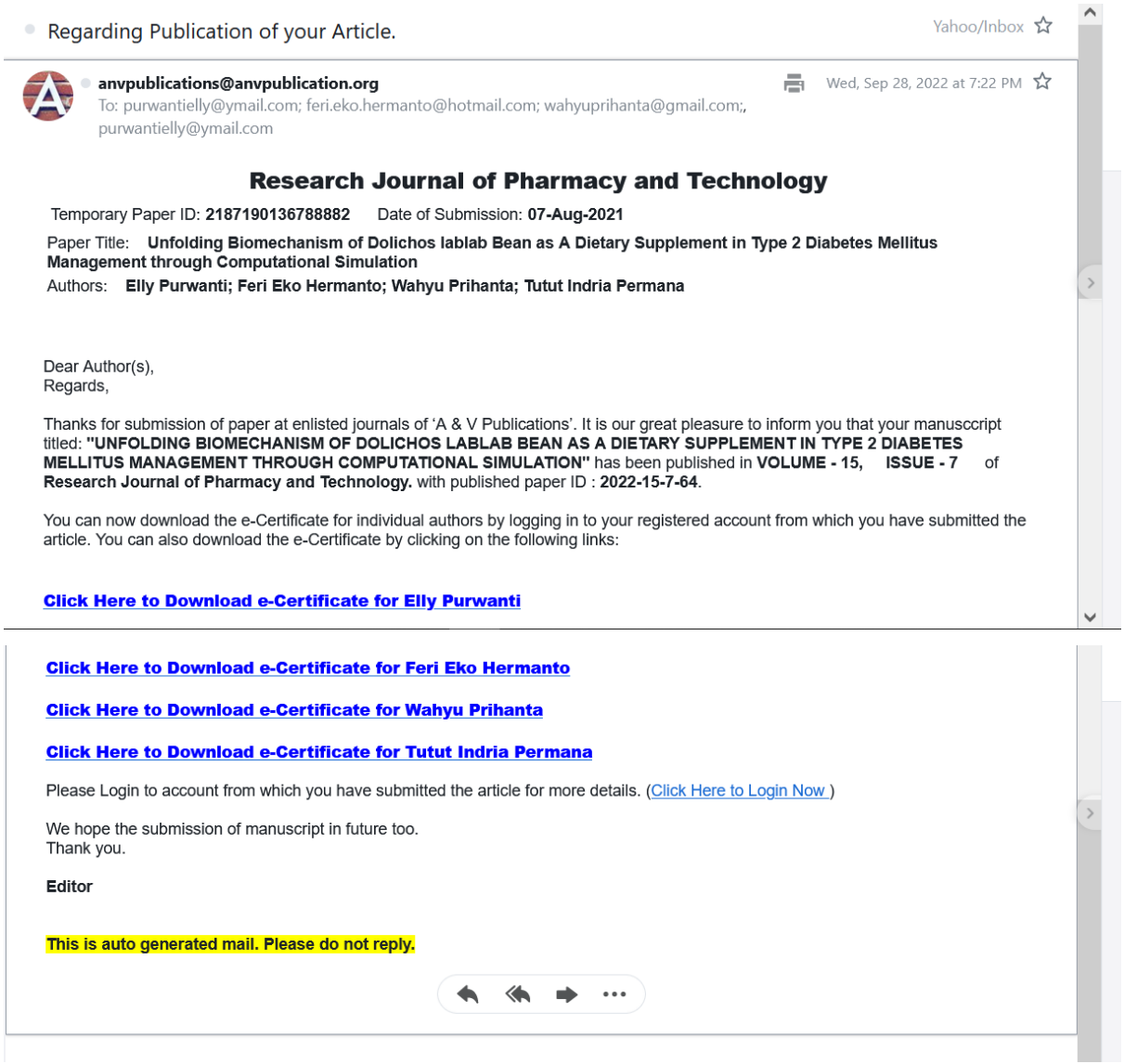

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Temporary Paper ID: **2187190136788882** Date of Submission: **07-Aug-2021**

Paper Title: **Unfolding Biomechanism of Dolichos lablab Bean as A Dietary Supplement in Type 2 Diabetes Mellitus Management through Computational Simulation**

Authors: **Elly Purwanti; Feri Eko Hermanto; Wahyu Prihanta; Tutut Indria Permana**

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RESEARCH ARTICLE

Unfolding Biomechanism of Dolichos lablab Bean as A Dietary Supplement in Type 2 Diabetes Mellitus Management through Computational Simulation

Elly Purwanti[1]*, Feri E. Hermanto[2], Wahyu Prihanta[1], Tutut I. Permana[1]
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ABSTRACT:
Dietary intervention, particularly legumes consumption, plays a significant role in promoting health status in diabetes mellitus management. As poorly known legumes, *Dolichos lablab* (DL) is possibly to be one of the dietary options for diabetes intervention. However, the predictive or precise mechanism of DL's anti-diabetic activity remains inconclusive. This study aimed to determine the nutritional and phytochemical content in addition to anti-diabetic properties of DL. Total protein, crude fat, crude fibers, and gross energy were evaluated, while anti-diabetic properties were predicted using molecular docking according to identified compound from Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) analysis. Screened compound from molecular docking then passed to physicochemical properties and bioactivity prediction using Swiss-ADME and molinspiration, separately. The result showed that DL has high protein fiber and gross energy content with a lower fat percentage. Additionally, DL has numerous phenolic acid and flavonoid compounds according to LC-HRMS analysis. From the docking analysis, fourteen compounds have substantial probability to give the beneficial effect of glucose metabolism regulator and insulin signaling repairers through inhibition of α-amylase, DPP4, and PTP1B. Finally, from the physicochemical properties and bioactivity estimations, 19-Norandrostenedione, 19-Nortestosterone, Icariside B1, Ilicic Acid, and Psilostachyin B have excellent pharmacokinetic properties along with considerable biological activity as enzyme inhibitors and nuclear receptor ligands. In conclusion, nutritional evaluation and molecular docking analysis revealed that DL might serve as a suitable dietary intervention for diabetes mellitus management.

KEYWORDS: α-amylase, *Dolichos lablab*, diabetes mellitus, DPP4; PTP1B.

INTRODUCTION:

Diabetes mellitus (DM) has recognized as a global health problem with increasing cases in forthcoming years. A total of 6.28% of the global populations were affected by DM, contributing to the ninth cause of mortality worldwide[1]. Further, it is estimated that around 642 million people will suffer from DM in 2040[2]. With those conditions, health management and prevention play a vital role in delaying DM development day by day.

Health management has been applied to halt DM's progression, including lifestyle changes and dietary intervention[3,4,4,5]. A few nutritional compositions, particularly natural products[6–8], have been suggested for people with DM, including legumes consumption[9]. One of the high potential legumes for dietary intake in DM conditions is *Dolichos lablab* (DL). With the high content of fibres and other nutritional compositions, DL has a good potential as a dietary supplement for DM[10]. Previous studies have reported DL's efficacy in regulating glycaemic levels, despite the precise mechanism still unresolved[11,12].

Since metabolic disease like DM involved many proteins for its progression, targeting specific proteins becomes the promising way to develop anti-diabetic drugs[13].

Altering glucose metabolism and insulin signalling can turn into an effective way of controlling DM[13,14]. As previously described, α-amylase plays a role in starch metabolism and contributes to plasma glucose enhancement[15]. On the other hand, DPP4 and PTP1B orchestrate insulin signalling, mainly involved in insulin sensitization, secretion, and post-prandial blood glucose levels[16,17]. Some studies have been employed to inhibit those proteins for achieving average blood glucose concentration and improving insulin performance[18–20]. Thus, targeting α-amylase, DPP4, and PTP1B have favourable results in preventing DM advancement[21–25]. Nevertheless, there were no studies for understanding the role of bioactive compounds in DL to inhibit those proteins and regulate DM conditions. Therefore, this study will discover the potential bioactivity of DL as dietary intervention for DM based on nutritional and phytochemical contents using computational approach.

MATERIALS AND METHODS:

Plant Samples and Extraction:

Sample was obtained from Madura Island, Indonesia. Detailed characteristics of the sample as explained in previous work[26]. Bean was ground prior to the extraction process. Extraction was carried out by soaking in 96% ethanol in a 1:3 ratio (weight/volume) for 24 hours. After submerging, the solvent was evaporated and freeze-dried to obtain DL extract.

Total Protein, Crude Fat, Crude Fiber, and Gross Energy Determination:

Determination of total protein, crude fat, and crude fiber was performed according to a previously described method[27]. Gross energy was measured using IKA C2000 Calorimeter System (IKA Works, Germany) following the manufacturer's protocol.

Bioactive Metabolites Identification:

Thermo Scientific Dionex Ultimate 3,000 RSLCnano Liquid Chromatography (LC) linked with Thermo Scientific Q Exactive High Resolution Mass Spectrometry (HRMS) was employed to identify the presence of bioactive compound in DL extract. Detailed protocols for chromatography as mentioned in earlier work [28]. Total ionic chromatograms then analyzed using Compound Discoverer and matched with mzCloud in the MS/MS Library. Compound with match score higher or equal with 80 then selected for molecular docking simulations as the ligands.

Data Mining of Protein and Ligand Structures:

Three-dimensional (3D) structures of protein were retrieved from Protein Data Bank (PDB; https://www.rcsb.org/), while 3D ligand structures were obtained from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). In detail, the structures of protein used in this study are α-amylase (PDB ID: 1HNY), DPP4 (PDB ID: 5Y7K), and PTP1B (PDB ID: 1BZC). The list of phytochemicals and their identity (PubChem CID) as shown in table 2[28].

Binding Energy Calculations:

AutoDock Vina integrated into PyRx software was employed for molecular docking simulations[29,30]. Water molecules and the previous-attached ligand in each 3D protein structure were removed prior to the docking process. Protein structures were set as a rigid molecule, while the phytochemicals as the ligands were set as a flexible molecule. Blind docking was applied with a maximum grid setting for searching binding sites.

Data Analysis:

Complex with binding energy lower or equal with -7 kcal/mol was directed into further investigation for amino acid-ligand interaction. Interacted residues in each complex and visualization were analyzed using Discovery Studio 2019 to determine the chemistry of formed interaction.

Drug-Likeness Evaluation and Structure-Activity Relationship (SAR):

The drug-likeness characteristics was determined by Swiss-ADME webserver[31], while Molinspiration (https://www.molinspiration.com/cgi-bin/properties) was used to determine SAR of selected compounds based on the molecular docking result.

RESULT:

Nutritional Values of DL:

The protein was the higher constituent from the analyzed nutritional contents, followed by crude fibers, while fat content has the smallest portion. Protein constitutes 24.91±0.08% of the total contents, while crude fiber and fat have 7.03±0.02% and 0.36±0.01%, respectively. Also, gross energy measurement showed that DL has a high energy source for daily energy uptake (table 1).

Table 1. Nutritional value of DL.

Protein (%)	Fat (%)	Crude Fiber (%)	Gross Energy (kcal/g)
24.91±0.08	0.36±0.01	7.03±0.02	3.86±0.007

Bioactive Compounds in DL:

Phenolic acid and flavonoid were the most abundance compound in DL (table 2). Pipecolic acid, trans-3-Indoleacrylic acid, caffeine, choline, and trigonelline were major constituent according to peak area. Some amino acids like arginine and histidine also found since DL is a legumes species. In addition, a common isoflavones in legumes, daidzein, also identified in DL extract. From the screening revealed that DL has diverse phytochemical compounds with possible potential to contribute in biological mechanism, particularly for

health purposes. The identified compounds from this step then continued for the screening of anti-diabetic properties.

Table 2: Identified bioactive compounds from ethanolic extract of DL using LC-HRMS

Name	Formula	Molecular Weight	Retention Time (min.)	Area (max.)	PubChem CID
Octyl decyl phthalate	C26 H42 O4	418.308	0.54	936,248.27	8380
L-Histidine	C6 H9 N3 O2	155.06944	0.778	2,000,058.30	6274
DL-Arginine	C6 H14 N4 O2	174.11162	0.784	10,687,018.08	232
Trigonelline	C7 H7 N O2	137.04752	0.853	114,012,627.64	5570
Betaine	C5 H11 N O2	117.07902	0.854	5,520,343.02	247
N3,N4-Dimethyl-L-arginine	C8 H18 N4 O2	202.14284	1.258	7,730,350.73	169148
N6-Methyladenine	C6 H7 N5	149.06996	1.277	3,306,074.91	67955
Pipecolic acid	C6 H11 N O2	129.07887	1.289	541,352,086.71	849
Adenine	C5 H5 N5	135.05433	1.314	36,842,152.69	190
Nicotinic acid	C6 H5 N O2	123.0321	1.328	6,123,811.35	938
2-Hydroxyphenylalanine	C9 H11 N O3	164.04732	1.348	7,003,574.34	91482
δ-Valerolactam	C5 H9 N O	99.06864	1.459	5,682,585.39	12665
4-Piperidone	C5 H9 N O	99.06864	1.61	10,433,494.32	33721
L-(+)-Arginine	C6 H14 N4 O2	174.11162	1.714	1,196,106.39	6322
Senkyunolide H	C12 H16 O4	206.09422	1.738	11,410,856.53	13965088
N-Acetyldopamine	C10 H13 N O3	195.08943	1.996	11,282,218.02	100526
trans-3-Indoleacrylic acid	C11 H9 N O2	187.06311	2.019	360,015,558.16	5375048
4-Indolecarbaldehyde	C9 H7 N O	145.05263	2.023	7,753,170.99	333703
Ferulic acid	C10 H10 O4	194.05786	2.194	18,955,718.90	445858
8-Hydroxyquinoline	C9 H7 N O	145.05263	2.445	42,324,020.88	1923
4-Hydroxybenzaldehyde	C7 H6 O2	122.03679	2.49	22,922,938.63	126
Sinapinic acid	C11 H12 O5	224.06836	2.578	16,788,350.39	637775
Pyrogallol	C6 H6 O3	126.03161	2.686	7,197,375.67	1057
Caffeine	C8 H10 N4 O2	194.08026	2.765	130,944,470.60	2519
4-Coumaric acid	C9 H8 O3	164.04722	3.255	12,099,306.61	637542
Isovanillic acid	C8 H8 O4	168.04215	3.264	2,838,264.12	12575
Icariside B	C19 H30 O8	386.19365	3.398	4,745,053.93	45783010
Jasmonic acid	C12 H18 O3	210.12553	3.823	14,410,134.05	5281166
7-Methyl-3-methylene-6-(3-oxobutyl)-3,3a,4,7,8,8a-hexahydro-2H-cyclohepta[b]furan-2-one	C15 H20 O3	230.13053	4.611	7,148,969.45	540288
Psilostachyin B	C15 H18 O4	262.12035	4.615	2,093,036.74	5320768
Maltol	C6 H6 O3	126.03165	4.69	2,094,936.46	8369
Butyl benzoate	C11 H14 O2	178.09931	4.766	2,374,368.41	8698
Scopoletin	C10 H8 O4	192.04213	4.91	38,999,675.85	5280460
Rutin	C27 H30 O16	610.15325	4.919	461,606.60	5280805
D-(+)-Camphor	C10 H16 O	152.12008	4.926	5,934,546.27	159055
Isoquercetin	C21 H20 O12	464.09508	5.093	773,077.83	5280804
Citral	C10 H16 O	152.12007	5.191	23,207,422.31	638011
(3aR,8R,8aR,9aR)-8-Hydroxy-8a-methyl-3,5-bis(methylene)decahydronaphtho[2,3-b]furan-2(3H)-one	C15 H20 O3	248.14108	5.673	2,132,451.00	23928145
Ilicic Acid	C15 H24 O3	274.15422	6.365	332,753.11	496073
Ageratriol	C15 H24 O3	234.1619	6.369	3,667,818.31	181557
Daidzein	C15 H10 O4	254.05779	6.37	2,651,879.82	5281708
9S,13R-12-Oxophytodienoic acid	C18 H28 O3	292.20354	7.329	3,829,088.87	14037063
Oleanolic acid	C30 H48 O3	456.35965	7.585	8,995,648.05	10494
9-Oxo-10(E),12(E)-octadecadienoic acid	C18 H30 O3	294.21916	7.723	3,519,971.30	5283011
19-Nortestosterone	C18 H26 O2	274.19298	7.934	153,999.92	9904
Ursolic acid	C30 H48 O3	456.35964	8.122	44,150,341.55	64945
OPEO	C16 H26 O2	250.19298	8.456	307,176.99	201055
Dimethomorph	C21 H22 Cl N O4	387.12352	9.059	186,440.71	5889665
19-Norandrostenedione	C18 H24 O2	272.1772	9.812	105,991.24	92834
α-Eleostearic acid	C18 H30 O2	278.22432	10.443	3,022,006.29	5282820
(+/-)12(13)-DiHOME	C18 H34 O4	296.23476	10.465	6,551,886.55	5282961
Benzoic Acid	C15 H22 O3	250.15666	10.575	866,671.61	15007
1-Tetradecylamine	C14 H31 N	213.24547	11.03	2,455,695.81	16217
Methyl palmitate	C17 H34 O2	287.28197	11.037	8,756,300.57	8181
Diazinon	C12 H21 N2 O3 P S	304.10073	11.727	262,096.73	3017
Tributyl phosphate	C12 H27 O4 P	266.16435	11.908	597,823.49	31357

Nootkatone	C15 H22 O	218.16689	12.628	253,913.08	1268142
Galaxolidone	C18 H24 O2	272.1772	12.967	500,319.44	69131857
Dibutyl phthalate	C16 H22 O4	278.15139	13.031	76,116,574.22	3026
Bis(2-ethylhexyl) amine	C16 H35 N	241.27667	13.62	436,429.49	7791
Mesterolone	C20 H32 O2	304.23998	13.835	1,250,518.08	15020
Citroflex A-4	C20 H34 O8	402.22486	14.317	552,076.62	10222764
1-Linoleoyl glycerol	C21 H38 O4	354.27628	15.062	859,472.95	5283469
Oleoyl ethanolamide	C20 H39 N O2	325.29772	15.646	2,237,329.66	5283454
Palmitoyl ethanolamide	C18 H37 N O2	299.28206	15.739	4,281,993.75	4671
Monoolein	C21 H40 O4	356.29213	16.57	491,056.13	5283468
Oleamide	C18 H35 N O	281.27153	17.112	3,894,904.96	5283387
Hexadecanamide	C16 H33 N O	255.25594	17.826	1,591,948.23	69421
Eicosapentaenoic acid ethyl ester	C22 H34 O2	330.25536	18.249	3,519,978.76	9831415
(9cis)-Retinal	C20 H28 O	284.2134	18.25	23,205,473.84	6436082
Bis(2-ethylhexyl)adipate	C22 H42 O4	370.30776	19.249	648,409.29	7641
Phthalic acid	C8 H6 O4	166.02602	19.249	468,285.38	1017
Bis(2-ethylhexyl) phthalate	C24 H38 O4	390.27613	19.257	66,697,492.22	8343
Stearamide	C18 H37 N O	283.28713	20.144	1,184,511.61	31292
Choline	C5 H13 N O	103.09976	25.18	128,907,165.10	305

Potential Mechanism of Phytochemicals from DL in Diabetic Pathway:

Eighteen compounds could interact with a minimum of one of the target proteins at low binding energy. Ursolic acid, rutin, and 19-Nortestosterone are the compounds with the lowest binding energy for α-Amylase, DPP4, and PTP1B, respectively (table 3). Unfortunately, not all of the screened compounds have good potential for protein target inhibitors. Protein-ligand structure analysis revealed that only 14 compounds could interact directly with several essential residues in each targeted protein (figure 1-3).

Table 3. Selected compounds based on binding affinity lower than or equal to –7 kcal/mol.

Compound	Binding Energy (kcal/mol)		
	α-Amylase	DPP4	PTP1B
(3aR,8R,8aR,9aR)-8-Hydroxy-8a-methyl-3,5-bis(methylene)decahydronaphtho[2,3-b]furan-2(3H)-one	-7.9	-8.4	-6.6
(9cis)-Retinal	-7.0	-8.0	-6.4
19-Norandrostenedione	-8.4	-8.6	-7.1
19-Nortestosterone	-8.0	-8.9	-9.3
Icariside B1	-7.4	-7.8	-6.8
Coumaric acid	-6.0	-6.2	-7.2
Daidzein	-8.1	-7.7	-7.8
Galaxolidone	-8.2	-8.2	-6.4
Ilicic Acid	-7.7	-8.3	-7.1
Isoquercetin	-8.2	-8.1	-7.2
Mesterolone	-8.6	-8.6	-6.8
Nootkatone	-7.5	-7.6	-6.2
Oleanolic acid	-9.5	-8.9	-8.5
Psilostachyin B	-7.8	-8.8	-7.1
Rutin	-8.8	-9.1	-7.6
Scopoletin	-5.8	-6.7	-7.2
Trans-3-Indoleacrylic Acid	-6.5	-7.0	-7.4
Ursolic acid	-10.1	-8.9	-7.9

Ursolic Acid, Oleanolic Acid, Isoquercetin, Psilostachyin B, Rutin, 9-cis-Retinal, and Icariside B1 were the compounds that been able to bind directly to the active sites of α-Amylase. Those compounds could interact with the α-Amylase mostly at HIS305 by hydrophobic or hydrogen bond interaction. Some compounds also bind with other key residues in the active sites, including ASP197, GLU233, and ASP300. Rutin and oleanolic acid are the compounds with the most binding sites in the active sites of α-Amylase with three different interaction at the key residues (figure 1).

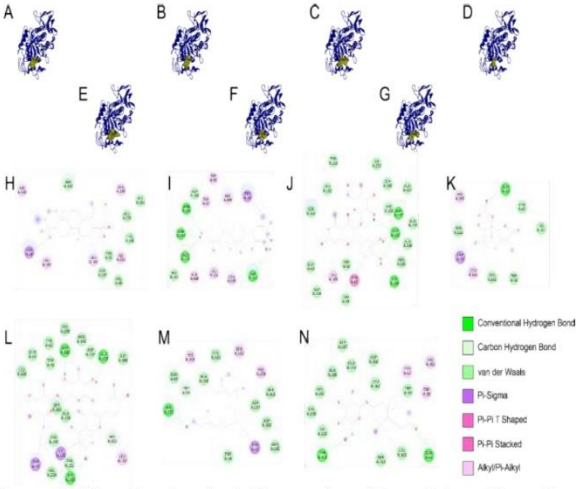

Figure 1. Structural orientation and residues interaction of α-amylase along with ursolic acid (A, H), oleanolic acid (B, I), isoquercetin (C, J), psilotachyin B (D, K), rutin (E, L), 9cis-retinal (F, M), and icariside B1 (G, N).

Different from the α-Amylase, DPP4 has higher selectivity to bind with the analyzed compounds. There were three compounds bound to DPP4 at the active sites, i.e., Isoquercetin, Rutin, and Icariside B1. GLU205, GLU206, TYR547, SER630, HIS740 were the active sites of DPP4, which interacted with all of those three compounds. Interestingly, Isoquercetin and Rutin have similar binding sites with one additional interaction of catalytic residues at ARG125 (figure 2).

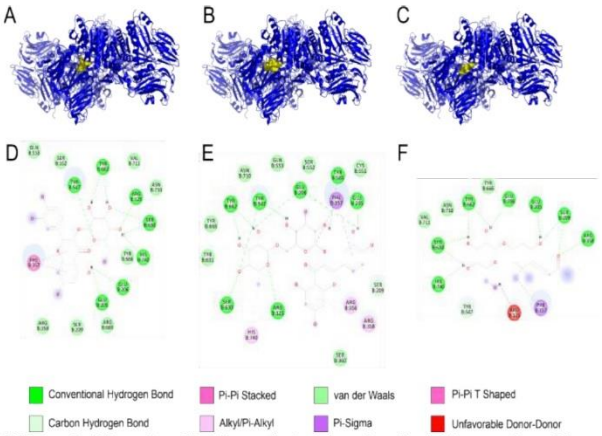

Figure 2. The visualization of structural orientation and residues interaction of DPP4 along with isoquercetin (A, D), rutin (B, E), and icariside B (C, F).

Seven compounds could bind with the PTP1B at its catalytic sites. 19-Nortestosterone, Ilicic Acid, 19-Norandrostenedione, Scopoletin, Coumaric Acid, Trans-3-Indoleacrylic Acid, and Daidzein were the compounds that have interaction with the catalytic sites of PTP1B. Remarkably, Scopoletin and Trans-3-Indoleacrylic Acid were the compounds that could interact with more catalytic residues. In contrast, Daidzein was the compound that has less interaction with catalytic residues. In general, PHE182, ALA217, and ARG221 are the most preferred residues of those compounds (figure 3).

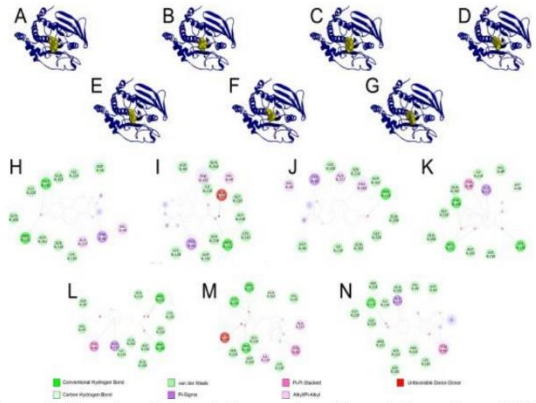

Figure 3. Structural orientation and residues interaction of PTP1B along with 19-Nortestosterone (A, H), ilicic acid (B, I), 19-Norandrostenedione (C, J), scopoletin (D, K), coumaric acid (E, L), trans-3-indoleacrylic acid (F, M), and daidzein (G, N).

Drug Likeness Characteristics of Screened Phytochemicals:

Drug-likeness properties and SAR were predicted using Swiss-ADME webserver and molinspiration, respectively. Six criteria, including lipophilicity, molecular size, polarity, insolubility, unsaturation, and flexibility, were employed to predict the drug-likeness properties of each screened compound. The pink areas represent the most favorable criterias with high similarity as the drug. Accordingly, 19-Norandrostenedione, 19-Nortestosterone, Icariside B1, Ilicic Acid, and Psilostachyin B were the compounds with the most resemblance with drug (figure 4A). Further, SAR prediction discovered that nine out of fourteen compounds have potential as both enzyme inhibitors and nuclear receptor ligands (figure 4B).

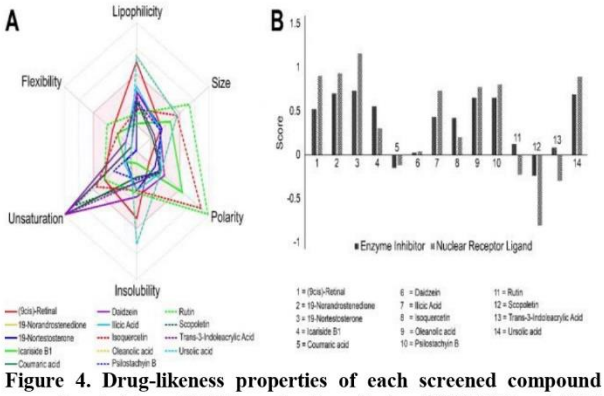

Figure 4. Drug-likeness properties of each screened compound according to bioavailability radar from Swiss-ADME (A) and SAR prediction using Molinspiration (B).

DISCUSSION:

Natively grown in Africa and Indian subcontinent, DL has been labeled as underutilized crops due to its limited global market potential and unpopular nutritional sources [10]. Nevertheless, DL has been used in different regions of the world as human food and animal feed[32]. Consisting of adequate main macronutrients needed for daily food intake, DL has promising potential as nutritional therapy for several metabolic diseases including DM[10]. Diet management has been suggested for diabetic patients to maintain plasma glycemic levels[33,34]. Consuming high fiber and protein content can increase insulin response and prevent plasma glycemic augmentation[33,35–37]. Also, low-fat nutritional sources help fulfill energy requirement and prevent cardiovascular risk[33]. With the high protein, fibers, and low-fat composition, DL has worthy potential for dietary intervention in diabetes management.

Phenolic acid is major secondary metabolite founded in DL, particularly in raw beans[38]. Some phenolic acids including ferulic acid and coumaric acid make several major phenolic acid in DL, and those compounds were identified and confirmed at present study[39]. Other dominant polyphenol compound, rutin, also identified[39]. Phenolic acid has been proved to exhibits an anti-diabetic nature, particularly by inhibiting α-amylase [21,23,24,40–42]. Therefore, this result discover a wide potential of DL as anti-diabetic agent.

Regulating glucose metabolism and insulin performance are the key factors in diabetes management[21,24,43,44]. An enzyme called α-amylase plays a vital role in glucose metabolism from dietary intake [45]. Targeting its catalytic sites could lead to inhibition of the catalytic activity of α-amylase then prevent uprising glycemic levels[15,19]. In the present study, Ursolic Acid, Oleanolic Acid, Isoquercetin, Psilostachyin B, Rutin, 9-cis-Retinal, and Icariside B1 from DL extract could bind with some key residues of α-amylase in the catalytic sites as mentioned in the earlier experiments[19,46]. Therefore, the interaction of those compounds with α-amylase implies plasma glucose regulation.

Insulin sensitization also the primary outcome in diabetes therapy[4]. As the proteins involved in the insulin signaling process, DPP4 and PTP1B frequently used as the target for increasing insulin sensitivity[17,25,47]. ARG125, GLU205, TYR547, SER630, ASP708, ASN710, and HIS740 have been reported as catalytic residues in DPP4[20]. Interaction in those residues could alter the biomechanism of DPP4, driving to the enhancement of glucose-dependent insulin secretion[48]. Also, addressing DPP4 for diabetes therapy has gained more attention and gave promising recovery effects[49]. Thus, blocking DPP4 by Isoquercetin, Rutin, and Icariside B1 from DL has immense opportunity to improve the health of diabetic patients.

Augmenting insulin sensitization can be reached by altering PTP1B activity[50,51]. Recently, allosteric and catalytic sites blocking of PTP1B have been reported. Directing LEU192, ASN193, PHE196, GLU276, PHE280, and TRP291 generate allosteric inhibition[52], while ARG47, ASP48, PHE182, SER216, ALA217, GLY218, ILE219, GLY220, ARG221, and GLN266 perform catalytic inhibition[53]. With some compounds interacting at the catalytic sites, particularly PHE182, ALA217, and ARG221, DL may serve as a catalytic inhibitor for PTP1B and ameliorates insulin-signaling impairments.

The drug-likeness and drug promiscuity of a compound strongly associate with its physicochemical properties (PP)[54,55]. With the suitable PP, a compound will achieves an adequate absorption, distribution, efficacy, metabolism, and excretion (ADME) and prevent adverse drug reactions[54,56]. Lipophilicity, molecular size, polarity, solubility, saturation, and flexibility were determined based on XLOGP3, molecular weight, total polar surface area (TPSA) value, log S, fraction of carbons in the sp3 hybridization, and number of rotatable bond, respectively[31]. 19-Norandrostenedione, 19-Nortestosterone, Icariside B1, Ilicic Acid, and Psilostachyin B were the most compatible compound with those described properties. Thus, those compounds has high probability to have excellent bioavailability, flexibility, and affinity to the target proteins. In advance, 19-Nortestosterone, 19-Norandrostenedione, Icariside B1, and Psilostachyin B also have a reasonable probability of giving biological activity as an enzyme inhibitor and nuclear receptor ligands. Consequently, those compounds seem to have great potential for modulating glucose metabolism and insulin signaling fault in diabetes mellitus patients and good diet therapy for complementary medicine.

CONCLUSION:

DL may serves as suitable dietary interventions for diabetes therapy with good nutritional contents and numerous biologically active compounds. Several compounds, mainly 19-Norandrostenedione, 19-Nortestosterone, Icariside B1, Ilicic Acid, and Psilostachyin B highly probable to act as glucose metabolism modulator and insulin signalling repairmen agent through inhibiting α-amylase, DPP4, and PTP1B, correspondingly.

CONFLICT OF INTEREST:

The authors declare no potential conflicts of interest concerning this research.

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