


1. Author registered to A and V Publication (4-8-2021)
  - Verification link for new registration
2. Article submitted to Research Journal of Pharmacy and Technology (7-8-2021)
  - Certificate of conflict of interest
  - Copyright transfer form
3. First revision (10-8-2021)
  - Article revision letter for authors
4. Plagiarism check (15-8-2021)
  - Plagiarism check result
5. Second revision: Minor revision (19-11-2021)
  - Article revision letter for authors
  - Correction list from author
6. Article accepted for publication (3-1-2022)
  - Question from author regarding progress of paper
  - Decision letter to authors
7. Article published (28-9-2022)
  - Notification letter
  - Final paper for publication

1. Author registered to A and V Publication (4-8-2021)

- Verification link for new registration

• Verification link for new registration in A & V Publication 2 Yahoo/Sent ☆

---

 **anvpublications@anvpublication.org** Wed, Aug 4, 2021 at 7:37 PM ☆  
To: purwantielly@gmail.com


Dear Elly Purwanti,

Thanks for registering in A and V Publication. The Verification link for your new registration is as follows. Please click (or copy and paste on address bar) on the link below to verify your account:

[http://anvpublication.org/Verify\\_Subscribed\\_User.aspx?subscriberID=purwantielly@gmail.com&sUbScRiBeD\\_uSer\\_OTPInk=DTbn6516](http://anvpublication.org/Verify_Subscribed_User.aspx?subscriberID=purwantielly@gmail.com&sUbScRiBeD_uSer_OTPInk=DTbn6516)

Please do not share your OTP link with any one.

**This is auto generated mail. Please do not reply.**



2. Article submitted to Research Journal of Pharmacy and Technology (7-8-2021)  
- Certificate of conflict of interest



**Certificate of Conflict of Interest:**

The article entitled **Unfolding Biomechanism of *Dolichos lablab* Bean as A Dietary Supplement in Type 2 Diabetes Mellitus Management through Computational Simulation** is herewith submitted for publication in **Research Journal of Pharmacy and Technology** (Name of Journal).

It has not been published before, and it is not under consideration for publication in any other journal (s).

I/We certify that I/We have obtained written permission for the use of text, tables, and/or illustrations from any copyrighted source(s), and I/We declare no conflict of interest.

Signature of author(s)

Name(s) and designation

**Dr. Elly Purwanti**

Name(s) of Institution/Organization

**Department of Educational Biology,  
Faculty of Teacher Training and Education,  
University of Muhammadiyah Malang**

- Copyright transfer form



**Transfer of copyright agreement:**

The article entitled *Unfolding Biomechanism of Dolichos lablab Bean as A Dietary Supplement in Type 2 Diabetes Mellitus Management through Computational Simulation* is herewith submitted for publication in Research Journal of Pharmacy and Technology. It has not been published before, and it is not under consideration for publication in any other journal (s). It contains no matter that is scandalous, obscene, libelous, or otherwise contrary to law. I/We agree that copies made under these circumstances will continue to carry the copyright notice that appeared in the original published work. I/We certify that I/We have obtained written permission for the use of text, tables, and/or illustrations from any copyrighted source(s), and I/We agree to supply such written permission(s) to Research Journal of Pharmacy and Technology upon request.

When the article is accepted for publication, I/We, as author/authors, hereby agree to transfer(s), assign(s), or otherwise convey(s) all copyright ownership, including any and all rights incidental thereto, exclusively to the Journal, in the event that such work is published by the Journal. The Journal shall own the work, including 1) copyright; 2) the right to grant permission to republish the article in whole or in part, with or without fee; 3) the right to produce preprints or reprints and translate into languages other than English for sale or free distribution; and 4) the right to republish the work in a collection of articles in any other mechanical or electronic format.

The article will be published under the terms of the latest Creative Commons Attribution-NonCommercial- ShareAlike License, unless the journal notifies the author otherwise in writing.

Signature of author(s)

Name(s) and designation:

Dr. Elly Purwanti

Name(s) of Institution/Organization:

Department of Educational Biology,  
Faculty of Teacher Training and Education,  
University of Muhammadiyah Malang



### 3. First revision (10-8-2021)

#### - Article revision letter for authors

● Regarding resubmission of submitted manuscript. Yahoo/Inbox ☆

---

 ● **anvpublishments@anvpublication.org**  
To: purwantielly@gmail.com; feri.eko.hermanto@hotmail.com; wahyuprihanta@gmail.com; purwantielly@gmail.com Tue, Aug 10, 2021 at 12:36 PM ☆

**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),

Thanks for submission of paper at enlisted journals of 'A & V Publications'. In the manuscript, reviewers has found some revisions. Therefore, we are requested you to kindly go through the comments of the reviewers and revise your article as needed.

**Note:- Please resubmit the revised article after corrections with in 7 Days.**

Please Login to your account from which you have submitted the article for more details. ([Click Here to Login Now.](#))

Thank you.

**Editor**


**This is auto generated mail. Please do not reply.**



[Reply](#), [Reply All](#) or [Forward](#)

● You have new comments for your submitted article. Yahoo/Inbox ☆

---

 ● **anvpublishments@anvpublication.org**  
To: purwantielly@gmail.com; feri.eko.hermanto@hotmail.com; wahyuprihanta@gmail.com; purwantielly@gmail.com Tue, Aug 10, 2021 at 12:36 PM ☆

**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),

Thanks for submission of your article. You have following comments from Resubmission of Article for your submitted article titled "**Unfolding Biomechanism of Dolichos lablab Bean as A Dietary Supplement in Type 2 Diabetes Mellitus Management through Computational Simulation**"  
Date: DD-Aug-YYYY, 11:06 AM  
**COMMENTS:**  
Dear Author, Pl Resubmit the article after making following corrections.. Ur article is long. please reduce the length of article up to 8-10 pages. Thanks Editor

Please Login to account from which you have submitted the article for more details. ([Click Here to Login Now.](#))

Thank you.

**Editor**

4. Plagiarism check (15-8-2021)
  - Plagiarism check result

# Plagiarism\_Check\_1.docx

*by*

- Revision letter to editor

---

**Submission date:** 15-Aug-2021 07:54PM (UTC+0700)

**Submission ID:** 1631567701

**File name:** Plagiarism\_Check\_1.docx (60.4K)

**Word count:** 2243

**Character count:** 13786

## 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  $\alpha$ -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:**  $\alpha$ -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 <sup>1</sup>. Further, it is estimated that around 642 million people will suffer from DM in 2040 <sup>2</sup>. 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 <sup>3</sup>. A few nutritional compositions have been suggested for people with DM, including legumes consumption <sup>4</sup>. 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 <sup>5</sup>. Previous studies have reported DL's efficacy in regulating glycaemic levels, despite the precise mechanism still unresolved <sup>6,7</sup>.

Since metabolic disease like DM involved many proteins for its progression, targeting specific proteins becomes the promising way to develop anti-diabetic drugs <sup>8</sup>. Altering glucose metabolism and insulin signalling can turn into an effective way of controlling DM <sup>8,9</sup>. Thus, targeting  $\alpha$ -amylase, DPP4, and PTP1B have favourable results in preventing DM advancement. As previously described,  $\alpha$ -amylase plays a role in starch metabolism and contributes to plasma glucose enhancement <sup>10</sup>. On the other hand, DPP4 and PTP1B orchestrate insulin signalling, mainly involved in insulin sensitization, secretion, and post-prandial blood glucose levels <sup>11,12</sup>. Some studies have been employed to inhibit those proteins from achieving average blood glucose concentration and insulin performance <sup>13-15</sup>. 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 <sup>16</sup>. 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 <sup>17</sup>. 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 <sup>18</sup>. 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 proteins 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  $\alpha$ -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 <sup>18</sup>.

### Binding Energy Calculations

AutoDock Vina integrated into PyRx software was employed for molecular docking simulations <sup>19,20</sup>. 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 <sup>21</sup>, 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  $24.91 \pm 0.08\%$  of the total contents, while  $7.03 \pm 0.02\%$  and  $0.36 \pm 0.01\%$  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  $\alpha$ -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  $\alpha$ -Amylase. Those compounds could interact with the  $\alpha$ -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  $\alpha$ -Amylase, which has been able to bind with three different key residues (figure 1 A-G and K-Q).

Different from the  $\alpha$ -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 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, Illicic 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<sup>5</sup>. Nevertheless, DL has been used in different regions of the world as human food and animal feed<sup>23</sup>. Consisting of adequate main macronutrients needed for daily food intake, DL has promising potential as nutritional therapy for several metabolic diseases including DM<sup>5</sup>. Diet management has been suggested for diabetic patients to maintain plasma glycemic levels<sup>24,25</sup>. Consuming high fiber and protein content can increase insulin response and prevent plasma glycemic augmentation<sup>24,26-28</sup>. Also, low-fat nutritional sources help fulfill energy requirement and prevent cardiovascular risk<sup>24</sup>. 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<sup>29</sup>. 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<sup>30</sup>. Other dominant polyphenol compound, rutin, also identified<sup>30</sup>. Phenolic acid has been proved to exhibits an anti-diabetic nature, particularly by inhibiting  $\alpha$ -amylase<sup>31,32</sup>. 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<sup>33,34</sup>. An enzyme called  $\alpha$ -amylase plays a vital role in glucose metabolism from dietary intake<sup>35</sup>. Targeting its catalytic sites could lead to inhibition of the catalytic activity of  $\alpha$ -amylase then prevent uprisings in glycemic levels<sup>10,14</sup>. In the present study, ursolic acid, oleanolic acid, isoquercetin, psilostachyin B, Rutin, 9-cis-retinal, and icaraside B1 from DL extract could bind with some key residues of  $\alpha$ -amylase in the catalytic sites as mentioned in the earlier experiments<sup>14,36</sup>. Therefore, the interaction of those compounds with  $\alpha$ -amylase implies plasma glucose regulation.

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

Augmenting insulin sensitization can be reached by altering PTP1B activity<sup>40,41</sup>. Recently, allosteric and catalytic sites blocking of PTP1B have been reported. Directing LEU192, ASN193, PHE196, GLU276, PHE280, and TRP291 generate allosteric inhibition<sup>42</sup>, while ARG47, ASP48, PHE182, SER216, ALA217, GLY218, ILE219, GLY220, ARG221, and GLN266 perform catalytic inhibition<sup>43</sup>. 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)<sup>44,45</sup>. With the suitable PP, a compound will achieve an adequate absorption, distribution, efficacy, metabolism and excretion (ADME) and prevent adverse drug reactions<sup>44,46</sup>. 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 sp<sup>3</sup> hybridization, and number of rotatable bond, respectively<sup>21</sup>. 19-Norandrostenedione, 19-Nortestosterone, Icaraside B1, Ilicic Acid, and Psilostachyin B were the most compatible compound with those described properties. Thus, those compounds have high probability to have excellent bioavailability, flexibility, and affinity to the target proteins. In advance, 19-Nortestosterone, 19-Norandrostenedione, Icaraside 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 serve as suitable dietary interventions for diabetes therapy with good nutritional contents and numerous biologically active compounds. Several compounds, mainly 19-Norandrostenedione, 19-Nortestosterone, Icaraside B1, Ilicic Acid, and Psilostachyin B are highly probable to act as glucose metabolism modulator and insulin signaling repairment agent through inhibiting  $\alpha$ -amylase, DPP4, and PTP1B, respectively.

# Plagiarism\_Check\_1.docx

## ORIGINALITY REPORT

3%

SIMILARITY INDEX

2%

INTERNET SOURCES

2%

PUBLICATIONS

%

STUDENT PAPERS

## PRIMARY SOURCES

1	<a href="http://www.tandfonline.com">www.tandfonline.com</a> Internet Source	1%
2	Xiaohong Cao, Huanbin Wen, Cuijuan Li, Zhenxin Gu. "Differences in functional properties and biochemical characteristics of congenetic rice proteins", Journal of Cereal Science, 2009 Publication	1%
3	<a href="http://link.springer.com">link.springer.com</a> Internet Source	1%
4	<a href="http://hdl.handle.net">hdl.handle.net</a> Internet Source	<1%
5	<a href="http://www.ncbi.nlm.nih.gov">www.ncbi.nlm.nih.gov</a> Internet Source	<1%
6	Krishnasamy, Gopinath, and Karthikeyan Muthusamy. "A computational study on role of 6-(hydroxymethyl)-3-[3,4,5-trihydroxy-6-[(3,4,5-trihydroxyoxan-2-yl)oxymethyl]oxan-2-yl]oxyoxane-2,4,5-triol in the regulation of	<1%

# blood glucose level", Journal of Biomolecular Structure and Dynamics, 2015.

Publication

---

---

Exclude quotes      On

Exclude matches      Off

Exclude bibliography      On



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

- Article revision letter for authors

• Resubmission of submitted manuscript after minor correction. 2

Yahoo/Sent ☆



• **anvpublications@anvpublication.org**

To: purwantielly@gmail.com; feri.eko.hermanto@hotmail.com; wahyuprihanta@gmail.com; purwantielly@gmail.com



Fri, Nov 19, 2021 at 4:36 PM ☆

### 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),

Thanks for submission of paper at enlisted journals of 'A & V Publications'. In the manuscript, it is compulsory to cite references of the related articles from journals of A&V Publications. Therefore, we are requested you to kindly cite / refers at least ten published manuscript from the enlisted journals. The link of published articles is embedded at the bottom of this mail, which you would to click and select any ten manuscripts as applicable to the submitted manuscript.

After citing the related articles, please resubmit the revised manuscript at article submission portal within 7 days.

[Click here to view the simalar works as yours.](#)

Please Login to account from which you have submitted the article, for more details. ([Click Here to Login Now.](#))

Thanks for your kind submission of manuscript.

**Editor**

**This is auto generated mail. Please do not reply.**



- Correction list from author

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

### 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	<i>Dolichos lablab</i>
1	Right	1	3	...intervention <sup>3,4,4,5</sup>	...intervention <sup>3-5</sup>
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 too small.	Please resize and place in larger size and space, if possible.
5	Left	Figure 2 and 3	Figure 2 and 3	The figure is too small.	Please resize and place in larger size and space, if possible.

6. Article accepted for publication (3-1-2022)

- Question from author regarding progress of paper

• Question on Progress of Paper ID 2187190136788882 4 Yahoo/Inbox ☆


 • **Elly Purwanti** <purwantielly@gmail.com>  
To: editor.rjpt@gmail.com

Mon, Jan 3, 2022 at 1:44 PM ☆

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

• Regarding submission of final version of article. 2 Yahoo/Sent ☆

 • **anvpublications@anvpublication.org**  
To: purwantielly@gmail.com; ferieko.hermanto@hotmail.com; wahyuprihanta@gmail.com,  
purwantielly@gmail.com

Mon, Jan 3, 2022 at 6:54 PM ☆

**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.


**Note:- Please submit the final version of article with in 7 Days.**

Please Login to account from which you have submitted the article for more details. (To login now click : [http://www.anvpublication.org/Subscribed\\_User\\_Login.aspx](http://www.anvpublication.org/Subscribed_User_Login.aspx))

Thank you.

**Editor**

**This is auto generated mail. Please do not reply.**



8. Article published (28-9-2022)

- Notification letter

• Regarding Publication of your Article.

Yahoo/Inbox ☆



• [anvpublications@anvpublication.org](mailto:anvpublications@anvpublication.org)

To: purwantielly@gmail.com; feri.eko.hermanto@hotmail.com; wahyuprihanta@gmail.com; purwantielly@gmail.com



Wed, Sep 28, 2022 at 7:22 PM



### Research Journal of Pharmacy and Technology

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**

Dear Author(s),  
Regards,

Thanks for submission of paper at enlisted journals of 'A & V Publications'. It is our great pleasure to inform you that your manuscript titled: **"UNFOLDING BIOMECHANISM OF DOLICHOS LABLAB BEAN AS A DIETARY SUPPLEMENT IN TYPE 2 DIABETES MELLITUS MANAGEMENT THROUGH COMPUTATIONAL SIMULATION"** has been published in **VOLUME - 15, ISSUE - 7** of **Research Journal of Pharmacy and Technology**. with published paper ID : 2022-15-7-64.

You can now download the e-Certificate for individual authors by logging in to your registered account from which you have submitted the article. You can also download the e-Certificate by clicking on the following links:

[Click Here to Download e-Certificate for Elly Purwanti](#)

[Click Here to Download e-Certificate for Feri Eko Hermanto](#)

[Click Here to Download e-Certificate for Wahyu Prihanta](#)

[Click Here to Download e-Certificate for Tutut Indria Permana](#)

Please Login to account from which you have submitted the article for more details. ([Click Here to Login Now](#))

We hope the submission of manuscript in future too.  
Thank you.

Editor

**This is auto generated mail. Please do not reply.**



**RESEARCH ARTICLE**

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

Elly Purwanti<sup>1\*</sup>, Feri E. Hermanto<sup>2</sup>, Wahyu Prihanta<sup>1</sup>, Tutut I. Permana<sup>1</sup>

<sup>1</sup>Department of Educational Biology, Faculty of Teacher Training and Education, University of Muhammadiyah Malang, East Java, Indonesia 65144.

<sup>2</sup>Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, Malang, East Java, Indonesia 65145.

\*Corresponding Author E-mail: [purwantielly@gmail.com](mailto:purwantielly@gmail.com)

**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  $\alpha$ -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:**  $\alpha$ -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<sup>1</sup>. Further, it is estimated that around 642 million people will suffer from DM in 2040<sup>2</sup>. 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<sup>3,4,5</sup>. A few nutritional compositions, particularly natural products<sup>6-8</sup>, have been suggested for people with DM, including legumes consumption<sup>9</sup>. 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<sup>10</sup>. Previous studies have reported DL's efficacy in regulating glycaemic levels, despite the precise mechanism still unresolved<sup>11,12</sup>.

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



Altering glucose metabolism and insulin signalling can turn into an effective way of controlling DM<sup>13,14</sup>. As previously described,  $\alpha$ -amylase plays a role in starch metabolism and contributes to plasma glucose enhancement<sup>15</sup>. On the other hand, DPP4 and PTP1B orchestrate insulin signalling, mainly involved in insulin sensitization, secretion, and post-prandial blood glucose levels<sup>16,17</sup>. Some studies have been employed to inhibit those proteins for achieving average blood glucose concentration and improving insulin performance<sup>18-20</sup>. Thus, targeting  $\alpha$ -amylase, DPP4, and PTP1B have favourable results in preventing DM advancement<sup>21-25</sup>. 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<sup>26</sup>. 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<sup>27</sup>. 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<sup>28</sup>. 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  $\alpha$ -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<sup>28</sup>.

### Binding Energy Calculations:

AutoDock Vina integrated into PyRx software was employed for molecular docking simulations<sup>29,30</sup>. 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 webservice<sup>31</sup>, 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 properties. step then continued for the screening of anti-diabetic

**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:**

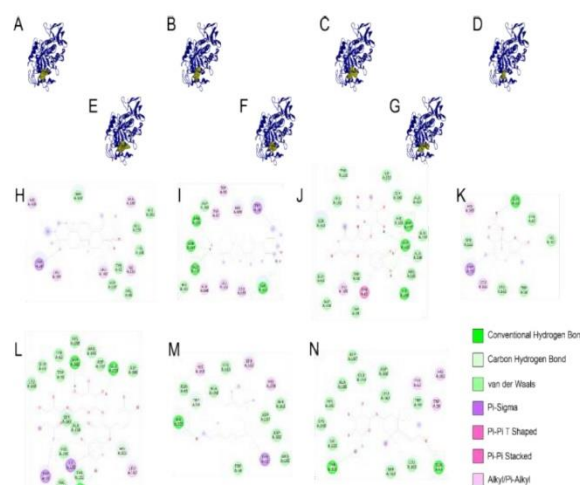
Eighteen compounds could interact with a minimum of one of the target proteins at low binding energy. Ursolic acid, rutin, and 19-Nortestosterone are the compounds with the lowest binding energy for  $\alpha$ -Amylase, DPP4, and PTP1B, respectively (table 3). Unfortunately, not all of the screened compounds 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)		
	$\alpha$ -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

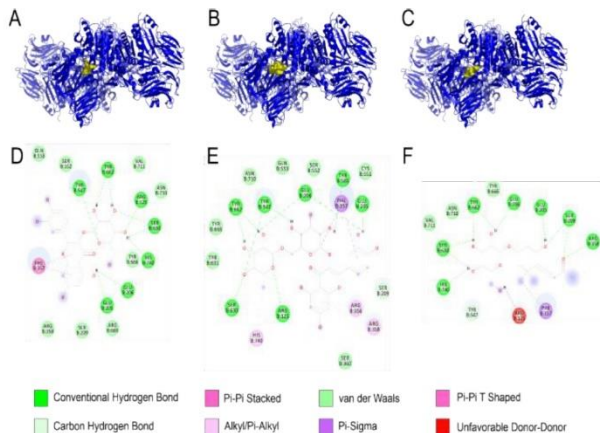
Ursolic Acid, Oleanolic Acid, Isoquercetin, Psilostachyin B, Rutin, 9-cis-Retinal, and Icariside B1 were the compounds that been able to bind directly to the active sites of  $\alpha$ -Amylase. Those compounds could

interact with the  $\alpha$ -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  $\alpha$ -Amylase with three different interaction at the key residues (figure 1).



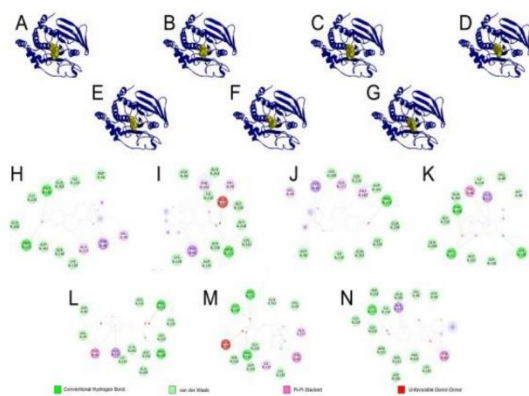
**Figure 1. Structural orientation and residues interaction of  $\alpha$ -amylase along with ursolic acid (A, H), oleanolic acid (B, I), isoquercetin (C, J), psilotachyin B (D, K), rutin (E, L), 9cis-retinal (F, M), and icariside B1 (G, N).**

Different from the  $\alpha$ -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 icaricide 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).

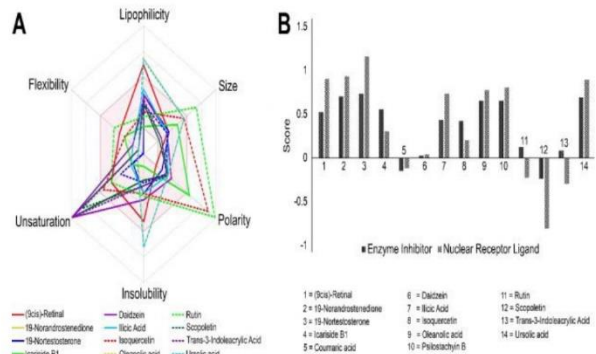


**Figure 3.** Structural orientation and residues interaction of PTP1B along with 19-Nortestosterone (A, H), ilicic acid (B, I), 19-Norandrostenedione (C, J), scopoletin (D, K), coumaric acid (E, L), trans-3-indoleacrylic acid (F, M), and daidzein (G, N).

**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<sup>10</sup>. Nevertheless, DL has been used in different regions of the world as human food and animal feed<sup>32</sup>. Consisting of adequate main macronutrients needed for daily food intake, DL has promising potential as nutritional therapy for several metabolic diseases including DM<sup>10</sup>. Diet management has been suggested for diabetic patients to maintain plasma glycemic levels<sup>33,34</sup>. Consuming high fiber and protein content can increase insulin response and prevent plasma glycemic augmentation<sup>33,35-37</sup>. Also, low-fat nutritional sources help fulfill energy requirement and prevent cardiovascular risk<sup>33</sup>. 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<sup>38</sup>. 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<sup>39</sup>. Other dominant polyphenol compound, rutin, also identified<sup>39</sup>. Phenolic acid has been proved to exhibits an anti-diabetic nature, particularly by inhibiting  $\alpha$ -amylase<sup>21,23,24,40-42</sup>. 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<sup>21,24,43,44</sup>. An enzyme called  $\alpha$ -amylase plays a vital role in glucose metabolism from dietary intake<sup>45</sup>. Targeting its catalytic sites could lead to inhibition of the catalytic activity of  $\alpha$ -amylase then prevent uprising glycemic levels<sup>15,19</sup>. 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  $\alpha$ -amylase in the catalytic sites as mentioned in the earlier experiments<sup>19,46</sup>. Therefore, the interaction of those compounds with  $\alpha$ -amylase implies plasma glucose regulation.

Insulin sensitization also the primary outcome in diabetes therapy<sup>4</sup>. As the proteins involved in the insulin signaling process, DPP4 and PTP1B frequently used as the target for increasing insulin sensitivity<sup>17,25,47</sup>. ARG125, GLU205, TYR547, SER630, ASP708, ASN710, and HIS740 have been reported as catalytic residues in DPP4<sup>20</sup>. Interaction in those residues could alter the biomechanism of DPP4, driving to the enhancement of glucose-dependent insulin secretion<sup>48</sup>. Also, addressing DPP4 for diabetes therapy has gained more attention and gave promising recovery effects<sup>49</sup>. 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<sup>50,51</sup>. Recently, allosteric and catalytic sites blocking of PTP1B have been reported. Directing LEU192, ASN193, PHE196, GLU276, PHE280, and TRP291 generate allosteric inhibition<sup>52</sup>, while ARG47, ASP48, PHE182, SER216, ALA217, GLY218, ILE219, GLY220, ARG221, and GLN266 perform catalytic inhibition<sup>53</sup>. 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)<sup>54,55</sup>. With the suitable PP, a compound will achieve an adequate absorption, distribution, efficacy, metabolism, and excretion (ADME) and prevent adverse drug reactions<sup>54,56</sup>. 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 sp<sup>3</sup> hybridization, and number of rotatable bond, respectively<sup>31</sup>. 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 serve 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 repairment agent through inhibiting  $\alpha$ -amylase, DPP4, and PTP1B, correspondingly.

### CONFLICT OF INTEREST:

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

### ACKNOWLEDGMENTS:

The authors thank to Ministry of Research, Technology, and Higher Education, the Republic of Indonesia for funding this research (Grant no. 229/SP2H/LT/DRPM/2019).

### REFERENCES:

1. Khan MAB, Hashim MJ, King JK, Govender RD, Mustafa H, Al Kaabi J. Epidemiology of Type 2 Diabetes – Global Burden of Disease and Forecasted Trends. *Journal of Epidemiology and Global Health*. 2020;10(1):107–111.
2. Ogurtsova K, da Rocha Fernandes JD, Huang Y, Linnenkamp U, Guariguata L, Cho NH, Cavan D, Shaw JE, Makaroff LE. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Research and Clinical Practice*. 2017;128:40–50.
3. Guess ND. Dietary Interventions for the Prevention of Type 2 Diabetes in High-Risk Groups: Current State of Evidence and Future Research Needs. *Nutrients*. 2018;10(9).
4. Dewangan V, Pandey H. Pathophysiology and Management of Diabetes: A Review. *Research Journal of Pharmacology and Pharmacodynamics*. 2017;9(4):219–222.
5. Parsa P, Ahmadiania-Tabesh R, Mohammadi Y. Assessment of the risk of Coronary Heart Disease in Diabetes Patients Type-II. *Asian Journal of Nursing Education and Research*. 2019;9(2):267–270.
6. Khan MY, Aziz I, Bihari B, Kumar H, Roy M, Verma VK. A Review- Phytomedicines Used in Treatment of Diabetes. *Asian Journal of Pharmaceutical Research*. 2014;4(3):135–154.
7. Pandeya SN, Kumar R, Kumar A, Pathak AK. Antidiabetics Review on Natural Products. *Research Journal of Pharmacy and Technology*. 2010;3(2):300–318.
8. Preethi PJ. Herbal Medicine for Diabetes Mellitus: A Review. *Asian Journal of Pharmaceutical Research*. 2013;3(2):57–70.
9. Bielefeld D, Grafenauer S, Rangan A. The Effects of Legume Consumption on Markers of Glycaemic Control in Individuals

- with and without Diabetes Mellitus: A Systematic Literature Review of Randomised Controlled Trials. *Nutrients*. 2020;12(7).
10. Minde JJ, Venkataramana PB, Matemu AO. Dolichos Lablab-an underutilized crop with future potentials for food and nutrition security: a review. *Critical Reviews in Food Science and Nutrition*. 2020;1–13.
  11. Balekari U, Reedy CS, Krishnaveni K. Antihyperglycemic and antihyperlipidaemic activities of Dolichos lablab seed extract on streptozotocin-nicotinamide induced diabetic rats. *Proceedings of International Conference on Pharmacognosy, Phytochemistry & Natural Products*. 2013;4(2):170.
  12. Singhal P, Kaushik G, Mathur P. Antidiabetic Potential of Commonly Consumed Legumes: A Review. *Critical Reviews in Food Science and Nutrition*. 2014;54(5):655–672.
  13. Nguyen NDT, Le LT. Targeted proteins for diabetes drug design. *Advances in Natural Sciences: Nanoscience and Nanotechnology*. 2012;3(1):013001.
  14. Guo S. Insulin Signaling, Resistance, and the Metabolic Syndrome: Insights from Mouse Models to Disease Mechanisms. *The Journal of endocrinology*. 2014;220(2):T1–T23.
  15. Sales PM, Souza PM, Simeoni LA, Silveira D.  $\alpha$ -Amylase inhibitors: a review of raw material and isolated compounds from plant source. *Journal of Pharmacy & Pharmaceutical Sciences: A Publication of the Canadian Society for Pharmaceutical Sciences, Societe Canadienne Des Sciences Pharmaceutiques*. 2012;15(1):141–183.
  16. Eleftheriou P, Geronikaki A, Petrou A. PTP1b Inhibition, A Promising Approach for the Treatment of Diabetes Type II. *Current Topics in Medicinal Chemistry*. 2019;19(4):246–263.
  17. Röhrborn D, Wronkowitz N, Eckel J. DPP4 in Diabetes. *Frontiers in Immunology*. 2015;6.
  18. Figueiredo A, Leal EC, Carvalho E. Protein tyrosine phosphatase 1B inhibition as a potential therapeutic target for chronic wounds in diabetes. *Pharmacological Research*. 2020;159:104977.
  19. Rasouli H, Hosseini-Ghazvini SM-B, Adibi H, Khodarahmi R. Differential  $\alpha$ -amylase/ $\alpha$ -glucosidase inhibitory activities of plant-derived phenolic compounds: a virtual screening perspective for the treatment of obesity and diabetes. *Food & Function*. 2017;8(5):1942–1954.
  20. Selvaraj G, Kaliamurthi S, Cakmak ZE, Cakmak T. Computational screening of dipeptidyl peptidase IV inhibitors from microalgal metabolites by pharmacophore modeling and molecular docking. *Physiological Research*. 2016;64(4):291–299.
  21. Karthikeyan P, Prakash MVD, Sendurapandi PD, Periandavan K. Assessment of the antidiabetic potential of Gymnemic acid as  $\alpha$ -amylase and  $\alpha$ -Glucosidase inhibitor using invitro and insilico tools. *Research Journal of Pharmacy and Technology*. 2021;14(9):4755–4759.
  22. Nagini DV, Krishna MSR, Karthikeyan S. Identification of Novel Dipeptidyl Peptidase -IV Inhibitors from *Ferula asafoetida* through GC-MS and Molecular Docking Studies. *Research Journal of Pharmacy and Technology*. 2020;13(11):5072–5076.
  23. Karthik VP, Punnagai, Suresh P, David DC. In Vitro Hydrogen Peroxide Scavenging Activity and Alpha Amylase Inhibitory Activity of *Croton tiglium* extract. *Research Journal of Pharmacy and Technology*. 2019;12(6):3045–3047.
  24. Khamis M, Talib F, Rosli NS, Dharmaraj S, Mohd KS, Srenivasan S, Latif ZA, Utharkar MRS. In vitro  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition and increased glucose uptake of *Morinda citrifolia* fruit and scopoletin. *Research Journal of Pharmacy and Technology*. 2015;8(2):189–193.
  25. Chandramore K. Review on Dipeptidyl Peptidase IV Inhibitors as a Newer Target for Diabetes Mellitus Treatment. *Asian Journal of Pharmaceutical Research*. 2017;7(4):230–238.
  26. Purwanti E, Prihanta W, Fauzi A. The Diversity of Seed Size and Nutrient Content of Lablab Bean from Three Locations in Indonesia. *International Journal of Advanced Engineering, Management and Science*. 2019;5(6):395–402.
  27. Thiex N. Evaluation of Analytical Methods for the Determination of Moisture, Crude Protein, Crude Fat, and Crude Fiber in Distillers Dried Grains with Solubles. *Journal of AOAC INTERNATIONAL*. 2009;92(1):61–73.
  28. 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. *Journal of Applied Pharmaceutical Science*. 2021;11(2):135–140.
  29. Dallakyan S, Olson AJ. Small-molecule library screening by docking with PyRx. *Methods in Molecular Biology (Clifton, N.J.)*. 2015;1263:243–250.
  30. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. *Journal of computational chemistry*. 2010;31(2):455–461.
  31. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*. 2017;7:42717.
  32. Maass BL, Knox MR, Venkatesha SC, Angessa TT, Ramme S, Pengelly BC. Lablab purpureus—A Crop Lost for Africa? *Tropical Plant Biology*. 2010;3(3):123–135.
  33. American Diabetes Association. 5. Lifestyle Management: Standards of Medical Care in Diabetes-2019. *Diabetes Care*. 2019;42(Suppl 1):S46–S60.
  34. Sami W, Ansari T, Butt NS, Hamid MRA. Effect of diet on type 2 diabetes mellitus: A review. *International Journal of Health Sciences*. 2017;11(2):65–71.
  35. McRae MP. Dietary Fiber Intake and Type 2 Diabetes Mellitus: An Umbrella Review of Meta-analyses. *Journal of Chiropractic Medicine*. 2018;17(1):44–53.
  36. Riccardi G, Rivellese AA. Effects of dietary fiber and carbohydrate on glucose and lipoprotein metabolism in diabetic patients. *Diabetes Care*. 1991;14(12):1115–1125.
  37. Yang J, Park HJ, Hwang W, Kim TH, Kim H, Oh J, Cho MS. Changes in the glucose and insulin responses according to high-protein snacks for diabetic patients. *Nutrition Research and Practice*. 2021;15(1):54–65.
  38. Maheshu V, Priyadarsini DT, Sasikumar JM. Effects of processing conditions on the stability of polyphenolic contents and antioxidant capacity of Dolichos lablab L. *Journal of Food Science and Technology*. 2013;50(4):731–738.
  39. Habib HM, Theuri SW, Kheadr EE, Mohamed FE. Functional, bioactive, biochemical, and physicochemical properties of the Dolichos lablab bean. *Food & Function*. 2017;8(2):872–880.
  40. Pieczykolan A, Pietrzak W, Gawlik-Dziki U, Nowak R. Antioxidant, Anti-Inflammatory, and Anti-Diabetic Activity of Phenolic Acids Fractions Obtained from *Aerva lanata* (L.) Juss. *Molecules (Basel, Switzerland)*. 2021;26(12):3486.
  41. Vinayagam R, Jayachandran M, Xu B. Antidiabetic Effects of Simple Phenolic Acids: A Comprehensive Review. *Phytotherapy research: PTR*. 2016;30(2):184–199.
  42. Lindawati NY, Puspitasari D, Murtiswiwi L, Rahmanita TA. Correlation of Flavonoid content on Antidiabetic activity in red beans (*Phaseolus vulgaris* L.) and its Processed Products. *Research Journal of Pharmacy and Technology*. 2021;14(3):1293–1297.
  43. Aronoff SL, Berkowitz K, Shreiner B, Want L. Glucose Metabolism and Regulation: Beyond Insulin and Glucagon. *Diabetes Spectrum*. 2004;17(3):183–190.
  44. Bonadonna RC. Alterations of Glucose Metabolism in Type 2 Diabetes Mellitus. An Overview. *Reviews in Endocrine and Metabolic Disorders*. 2004;5(2):89–97.
  45. Tundis R, Loizzo MR, Menichini F. Natural products as alpha-amylase and alpha-glucosidase inhibitors and their hypoglycaemic potential in the treatment of diabetes: an update. *Mini Reviews in Medicinal Chemistry*. 2010;10(4):315–331.
  46. Nyambe-Silavwe H, Villa-Rodriguez JA, Ifie I, Holmes M, Aydin E, Jensen JM, Williamson G. Inhibition of human  $\alpha$ -amylase by dietary polyphenols. *Journal of Functional Foods*. 2015;19:723–732.
  47. Zou W. PTP1B, A Potential Target of Type 2 Diabetes Mellitus. *Molecular Biology: Open Access*. 2016;5(4):1–6.

48. Vardarli I, Nauck MA, Köthe LD, Deacon CF, Holst JJ, Schweizer A, Foley JE. Inhibition of DPP-4 with Vildagliptin Improved Insulin Secretion in Response to Oral as well as “Isoglycemic” Intravenous Glucose without Numerically Changing the Incretin Effect in Patients with Type 2 Diabetes. *The Journal of Clinical Endocrinology & Metabolism*. 2011;96(4):945–954.
49. Wani JH, John-Kalarickal J, Fonseca VA. Dipeptidyl peptidase-4 as a new target of action for type 2 diabetes mellitus: a systematic review. *Cardiology Clinics*. 2008;26(4):639–648.
50. Gum RJ, Gaede LL, Koterski SL, Heindel M, Clampit JE, Zinker BA, Trevillyan JM, Ulrich RG, Jirousek MR, Rondonone CM. Reduction of Protein Tyrosine Phosphatase 1B Increases Insulin-Dependent Signaling in ob/ob Mice. *Diabetes*. 2003;52(1):21–28.
51. Krishnan N, Bonham CA, Rus IA, Shrestha OK, Gauss CM, Haque A, Tocilj A, Joshua-Tor L, Tonks NK. Harnessing insulin- and leptin-induced oxidation of PTP1B for therapeutic development. *Nature Communications*. 2018;9(1):283.
52. Wiesmann C, Barr KJ, Kung J, Zhu J, Erlanson DA, Shen W, Fahr BJ, Zhong M, Taylor L, Randal M, McDowell RS, Hansen SK. Allosteric inhibition of protein tyrosine phosphatase 1B. *Nature Structural & Molecular Biology*. 2004;11(8):730–737.
53. Groves MR, Yao Z-J, Roller PP, Burke Terrence R, Barford D. Structural Basis for Inhibition of the Protein Tyrosine Phosphatase 1B by Phosphotyrosine Peptide Mimetics. *Biochemistry*. 1998;37(51):17773–17783.
54. Lagorce D, Douguet D, Miteva MA, Villoutreix BO. Computational analysis of calculated physicochemical and ADMET properties of protein-protein interaction inhibitors. *Scientific Reports*. 2017;7(1):46277.
55. Tarscsay Á, Keserü GM. Contributions of Molecular Properties to Drug Promiscuity. *Journal of Medicinal Chemistry*. 2013;56(5):1789–1795.
56. Chagas CM, Moss S, Alisaraie L. Drug metabolites and their effects on the development of adverse reactions: Revisiting Lipinski’s Rule of Five. *International Journal of Pharmaceutics*. 2018;549(1–2):133–149.