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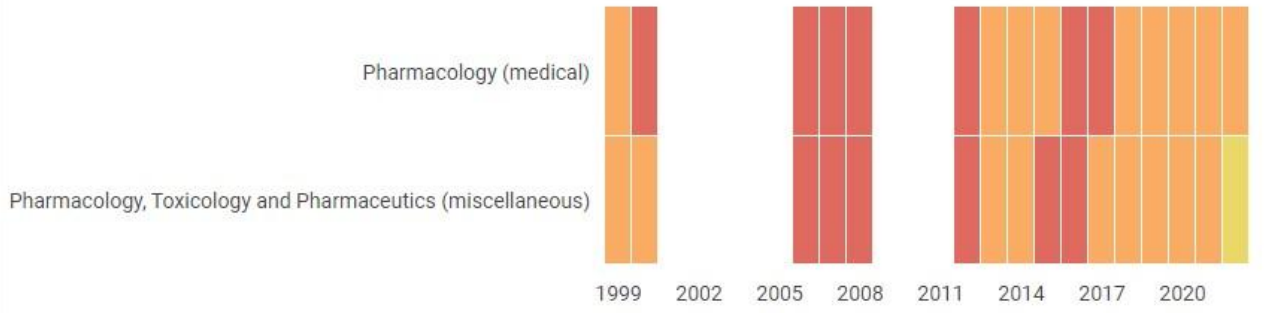

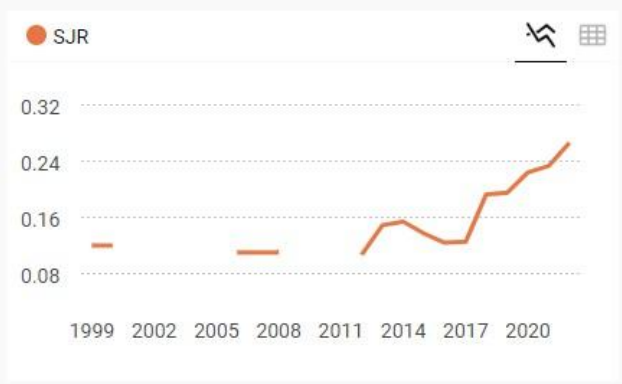
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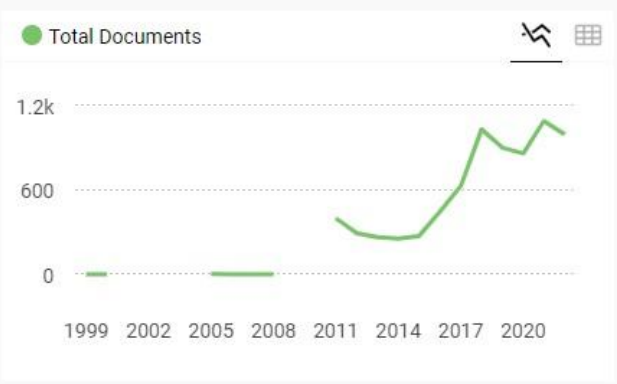
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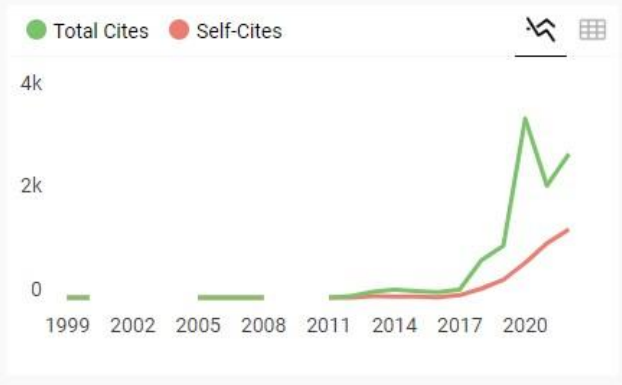
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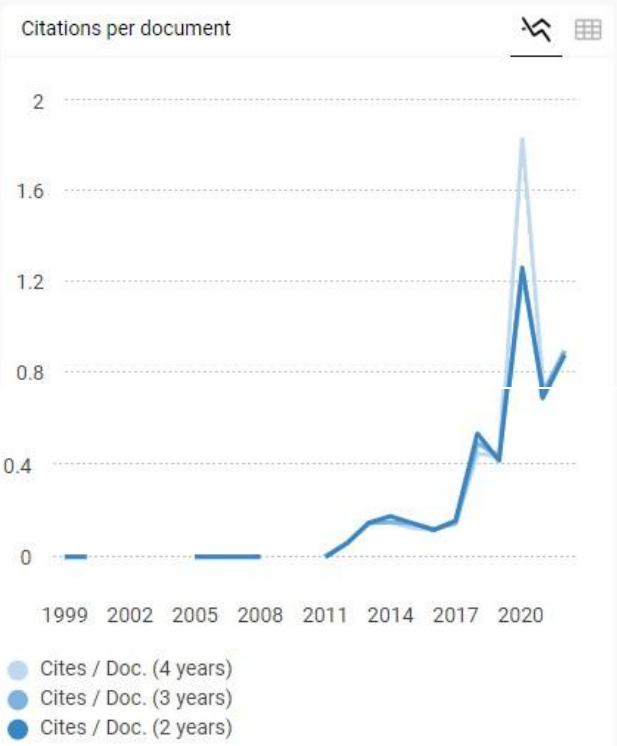
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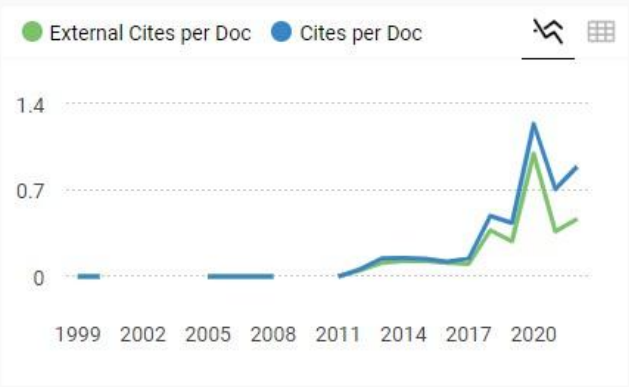
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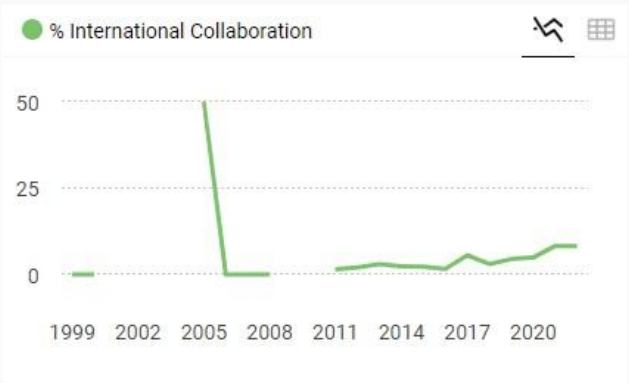
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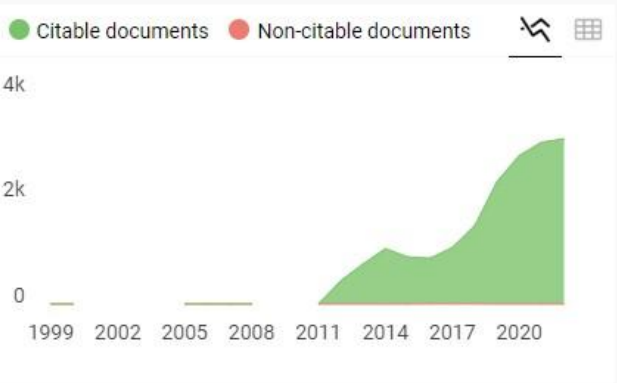
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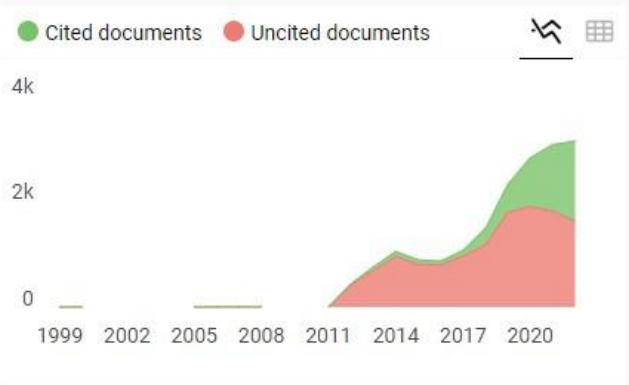
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RESEARCH ARTICLE

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]
[1]Department of Educational Biology, Faculty of Teacher Training and Education,
University of Muhammadiyah Malang, East Java, Indonesia 65144.
[2]Department of Biology, Faculty of Mathematics and Natural Sciences,
Universitas Brawijaya, Malang, East Java, Indonesia 65145.
*Corresponding Author E-mail: **purwantielly@ymail.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 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.

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Health management has been applied to halt DM's progression, including lifestyle changes and dietary intervention[3-5]. 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	$C_{26}H_{42}O_4$	418.308	0.54	936,248.27	8380
L-Histidine	$C_6H_9N_3O_2$	155.06944	0.778	2,000,058.30	6274
DL-Arginine	$C_6H_{14}N_4O_2$	174.11162	0.784	10,687,018.08	232
Trigonelline	$C_7H_7NO_2$	137.04752	0.853	114,012,627.64	5570
Betaine	$C_5H_{11}NO_2$	117.07902	0.854	5,520,343.02	247
N3,N4-Dimethyl-L-arginine	$C_8H_{18}N_4O_2$	202.14284	1.258	7,730,350.73	169148
N6-Methyladenine	$C_6H_7N_5$	149.06996	1.277	3,306,074.91	67955
Pipecolic acid	$C_6H_{11}NO_2$	129.07887	1.289	541,352,086.71	849
Adenine	$C_5H_5N_5$	135.05433	1.314	36,842,152.69	190
Nicotinic acid	$C_6H_5NO_2$	123.0321	1.328	6,123,811.35	938
2-Hydroxyphenylalanine	$C_9H_{11}NO_3$	164.04732	1.348	7,003,574.34	91482
δ-Valerolactam	C_5H_9NO	99.06864	1.459	5,682,585.39	12665
4-Piperidone	C_5H_9NO	99.06864	1.61	10,433,494.32	33721
L-(+)-Arginine	$C_6H_{14}N_4O_2$	174.11162	1.714	1,196,106.39	6322
Senkyunolide H	$C_{12}H_{16}O_4$	206.09422	1.738	11,410,856.53	13965088
N-Acetyldopamine	$C_{10}H_{13}NO_3$	195.08943	1.996	11,282,218.02	100526
trans-3-Indoleacrylic acid	$C_{11}H_9NO_2$	187.06311	2.019	360,015,558.16	5375048
4-Indolecarbaldehyde	C_9H_7NO	145.05263	2.023	7,753,170.99	333703
Ferulic acid	$C_{10}H_{10}O_4$	194.05786	2.194	18,955,718.90	445858
8-Hydroxyquinoline	C_9H_7NO	145.05263	2.445	42,324,020.88	1923
4-Hydroxybenzaldehyde	$C_7H_6O_2$	122.03679	2.49	22,922,938.63	126
Sinapinic acid	$C_{11}H_{12}O_5$	224.06836	2.578	16,788,350.39	637775
Pyrogallol	$C_6H_6O_3$	126.03161	2.686	7,197,375.67	1057
Caffeine	$C_8H_{10}N_4O_2$	194.08026	2.765	130,944,470.60	2519
4-Coumaric acid	$C_9H_8O_3$	164.04722	3.255	12,099,306.61	637542
Isovanillic acid	$C_8H_8O_4$	168.04215	3.264	2,838,264.12	12575
Icariside B	$C_{19}H_{30}O_8$	386.19365	3.398	4,745,053.93	45783010
Jasmonic acid	$C_{12}H_{18}O_3$	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	$C_{15}H_{20}O_3$	230.13053	4.611	7,148,969.45	540288
Psilostachyin B	$C_{15}H_{18}O_4$	262.12035	4.615	2,093,036.74	5320768
Maltol	$C_6H_6O_3$	126.03165	4.69	2,094,936.46	8369
Butyl benzoate	$C_{11}H_{14}O_2$	178.09931	4.766	2,374,368.41	8698
Scopoletin	$C_{10}H_8O_4$	192.04213	4.91	38,999,675.85	5280460
Rutin	$C_{27}H_{30}O_{16}$	610.15325	4.919	461,606.60	5280805
D-(+)-Camphor	$C_{10}H_{16}O$	152.12008	4.926	5,934,546.27	159055
Isoquercetin	$C_{21}H_{20}O_{12}$	464.09508	5.093	773,077.83	5280804
Citral	$C_{10}H_{16}O$	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	$C_{15}H_{20}O_3$	248.14108	5.673	2,132,451.00	23928145
Ilicic Acid	$C_{15}H_{24}O_3$	274.15422	6.365	332,753.11	496073
Ageratriol	$C_{15}H_{24}O_3$	234.1619	6.369	3,667,818.31	181557
Daidzein	$C_{15}H_{10}O_4$	254.05779	6.37	2,651,879.82	5281708
9S,13R-12-Oxophytodienoic acid	$C_{18}H_{28}O_3$	292.20354	7.329	3,829,088.87	14037063
Oleanolic acid	$C_{30}H_{48}O_3$	456.35965	7.585	8,995,648.05	10494
9-Oxo-10(E),12(E)-octadecadienoic acid	$C_{18}H_{30}O_3$	294.21916	7.723	3,519,971.30	5283011
19-Nortestosterone	$C_{18}H_{26}O_2$	274.19298	7.934	153,999.92	9904
Ursolic acid	$C_{30}H_{48}O_3$	456.35964	8.122	44,150,341.55	64945
OPEO	$C_{16}H_{26}O_2$	250.19298	8.456	307,176.99	201055
Dimethomorph	$C_{21}H_{22}ClNO_4$	387.12352	9.059	186,440.71	5889665
19-Norandrostenedione	$C_{18}H_{24}O_2$	272.1772	9.812	105,991.24	92834
α-Eleostearic acid	$C_{18}H_{30}O_2$	278.22432	10.443	3,022,006.29	5282820
(+/-)12(13)-DiHOME	$C_{18}H_{34}O_4$	296.23476	10.465	6,551,886.55	5282961
Benzoic Acid	$C_{15}H_{22}O_3$	250.15666	10.575	866,671.61	15007
1-Tetradecylamine	$C_{14}H_{31}N$	213.24547	11.03	2,455,695.81	16217
Methyl palmitate	$C_{17}H_{34}O_2$	287.28197	11.037	8,756,300.57	8181
Diazinon	$C_{12}H_{21}N_2O_3PS$	304.10073	11.727	262,096.73	3017
Tributyl phosphate	$C_{12}H_{27}O_4P$	266.16435	11.908	597,823.49	31357
Nootkatone	$C_{15}H_{22}O$	218.16689	12.628	253,913.08	1268142
Galaxolidone	$C_{18}H_{24}O_2$	272.1772	12.967	500,319.44	69131857
Dibutyl phthalate	$C_{16}H_{22}O_4$	278.15139	13.031	76,116,574.22	3026
Bis(2-ethylhexyl) amine	$C_{16}H_{35}N$	241.27667	13.62	436,429.49	7791
Mesterolone	$C_{20}H_{32}O_2$	304.23998	13.835	1,250,518.08	15020

Citroflex A-4	$C_{20}H_{34}O_8$	402.22486	14.317	552,076.62	10222764
1-Linoleoyl glycerol	$C_{21}H_{38}O_4$	354.27628	15.062	859,472.95	5283469
Oleoyl ethanolamide	$C_{20}H_{39}NO_2$	325.29772	15.646	2,237,329.66	5283454
Palmitoyl ethanolamide	$C_{18}H_{37}NO_2$	299.28206	15.739	4,281,993.75	4671
Monoolein	$C_{21}H_{40}O_4$	356.29213	16.57	491,056.13	5283468
Oleamide	$C_{18}H_{35}NO$	281.27153	17.112	3,894,904.96	5283387
Hexadecanamide	$C_{16}H_{33}NO$	255.25594	17.826	1,591,948.23	69421
Eicosapentaenoic acid ethyl ester	$C_{22}H_{34}O_2$	330.25536	18.249	3,519,978.76	9831415
(9cis)-Retinal	$C_{20}H_{28}O$	284.2134	18.25	23,205,473.84	6436082
Bis(2-ethylhexyl)adipate	$C_{22}H_{42}O_4$	370.30776	19.249	648,409.29	7641
Phthalic acid	$C_8H_6O_4$	166.02602	19.249	468,285.38	1017
Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.27613	19.257	66,697,492.22	8343
Stearamide	$C_{18}H_{37}NO$	283.28713	20.144	1,184,511.61	31292
Choline	$C_5H_{13}NO$	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).

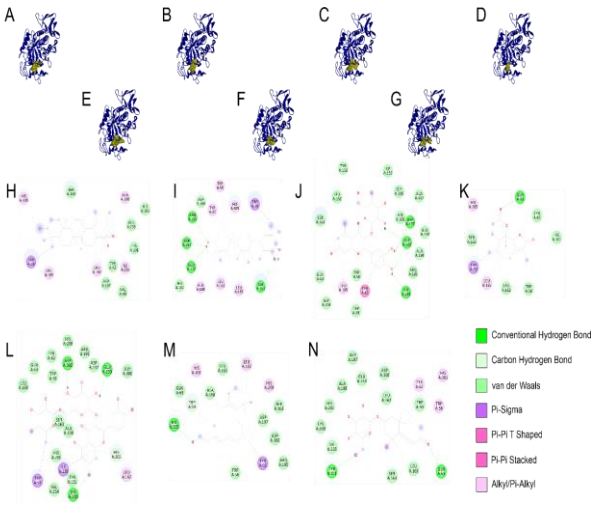

Figure 1. Structural orientation and residues interaction of α-amylase along with ursolic acid (A, H), oleanolic acid (B, I), isoquercetin (C, J), psilotachyin B (D, K), rutin (E, L), 9cis-retinal (F, M), and icariside B1 (G, N).

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).

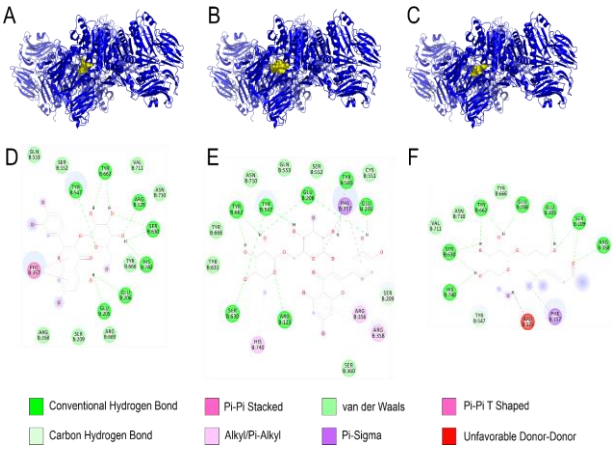

Figure 2. The visualization of structural orientation and residues interaction of DPP4 along with isoquercetin (A, D), rutin (B, E), and icariside B (C, F).

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).

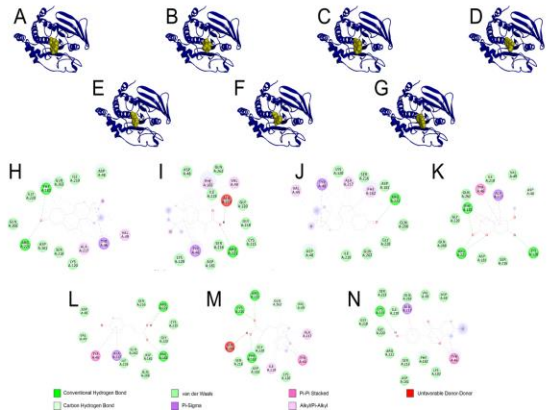

Figure 3. Structural orientation and residues interaction of PTP1B along with 19-Nortestosterone (A, H), ilicic acid (B, I), 19-Norandrostenedione (C, J), scopoletin (D, K), coumaric acid (E, L), trans-3-indoleacrylic acid (F, M), and daidzein (G, N).

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).

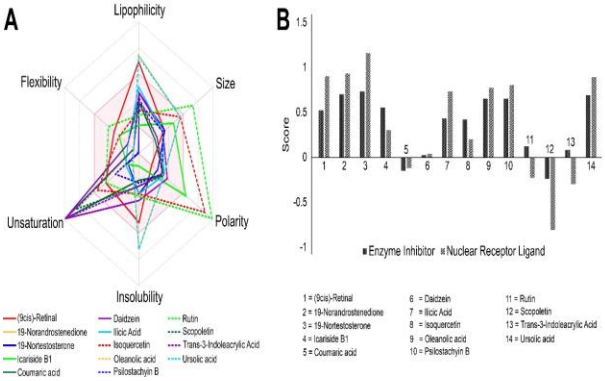

Figure 4. Drug-likeness properties of each screened compound according to bioavailability radar from Swiss-ADME (A) and SAR prediction using Molinspiration (B).

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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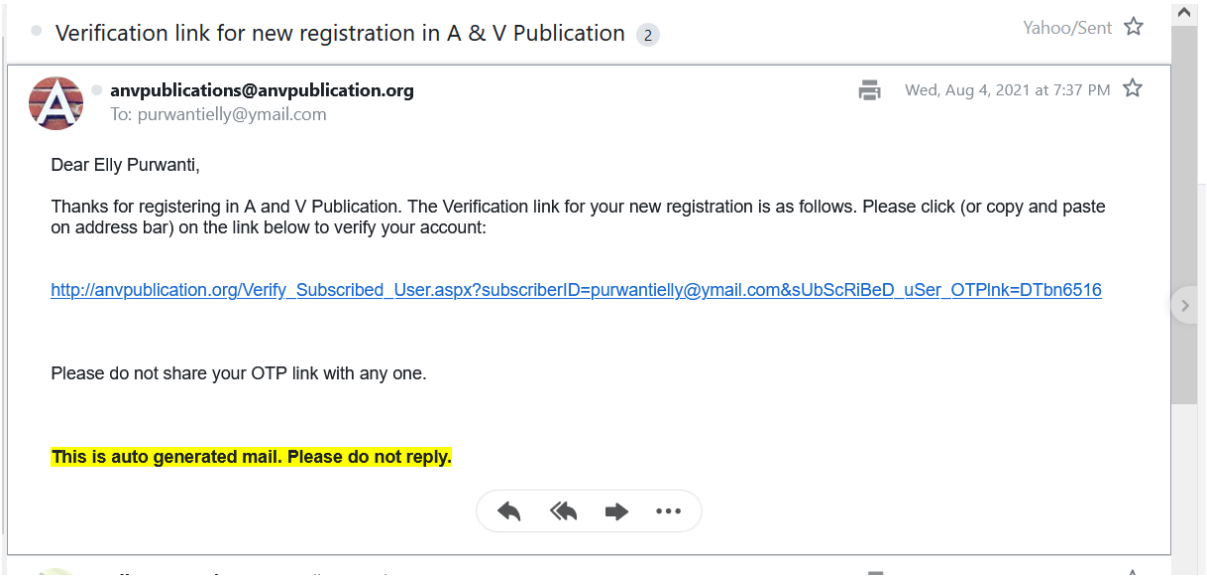

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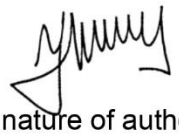

Signature of author(s)

Name(s) and designation:

Dr. Elly Purwanti

Name(s) of Institution/Organization:

Department of Educational Biology,

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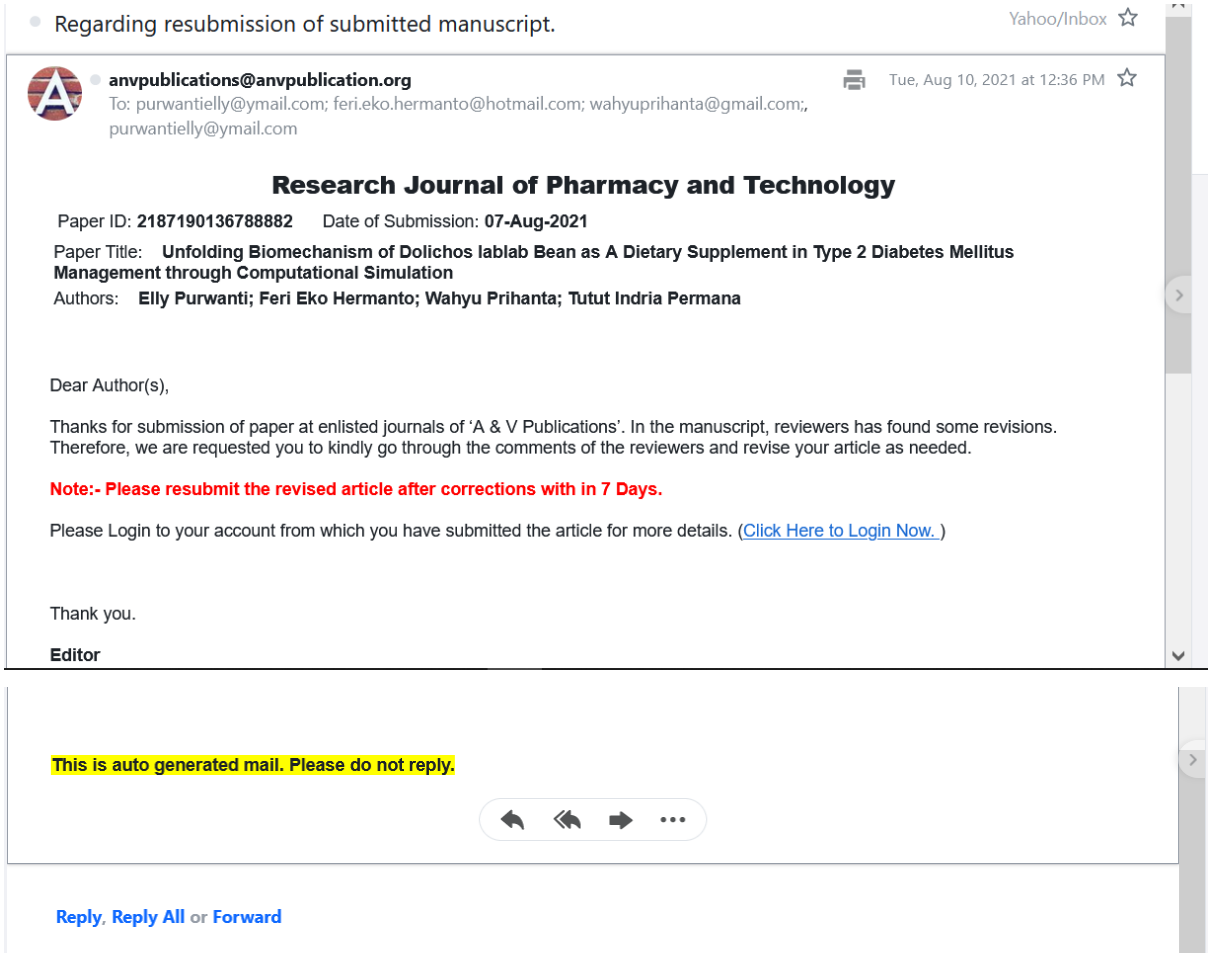

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Unfolding Biomechanism of Dolichos lablab Bean as A Dietary Supplement in Type 2 Diabetes Mellitus Management through Computational Simulation

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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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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Article Number (original Submitted manuscript No)	**2187190136788882**
Only Corresponding authors name	Elly Purwanti
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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

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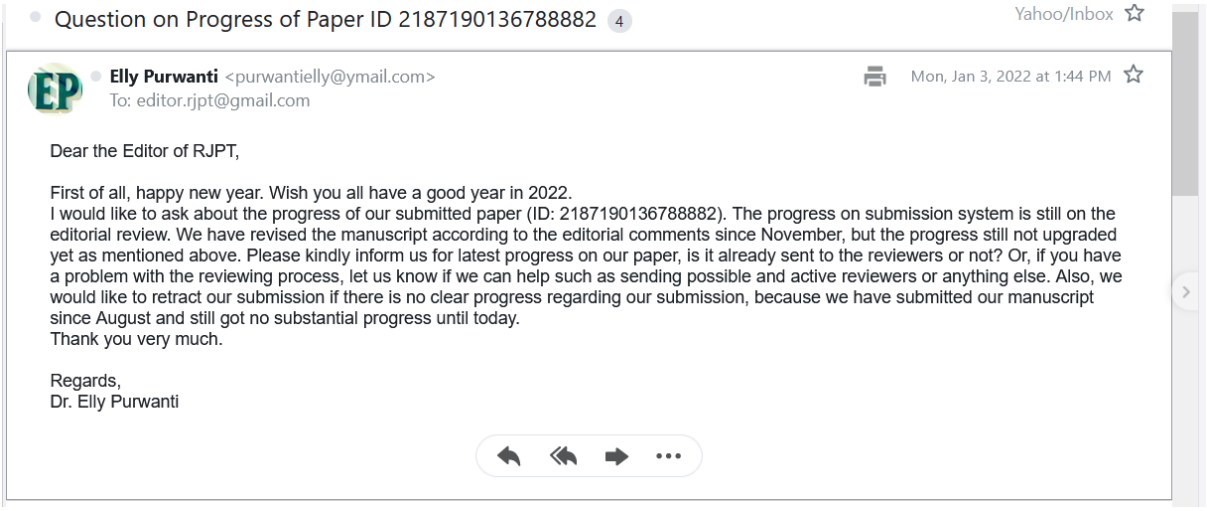

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Elly Purwanti <purwantielly@ymail.com>
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Dear the Editor of RJPT,

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

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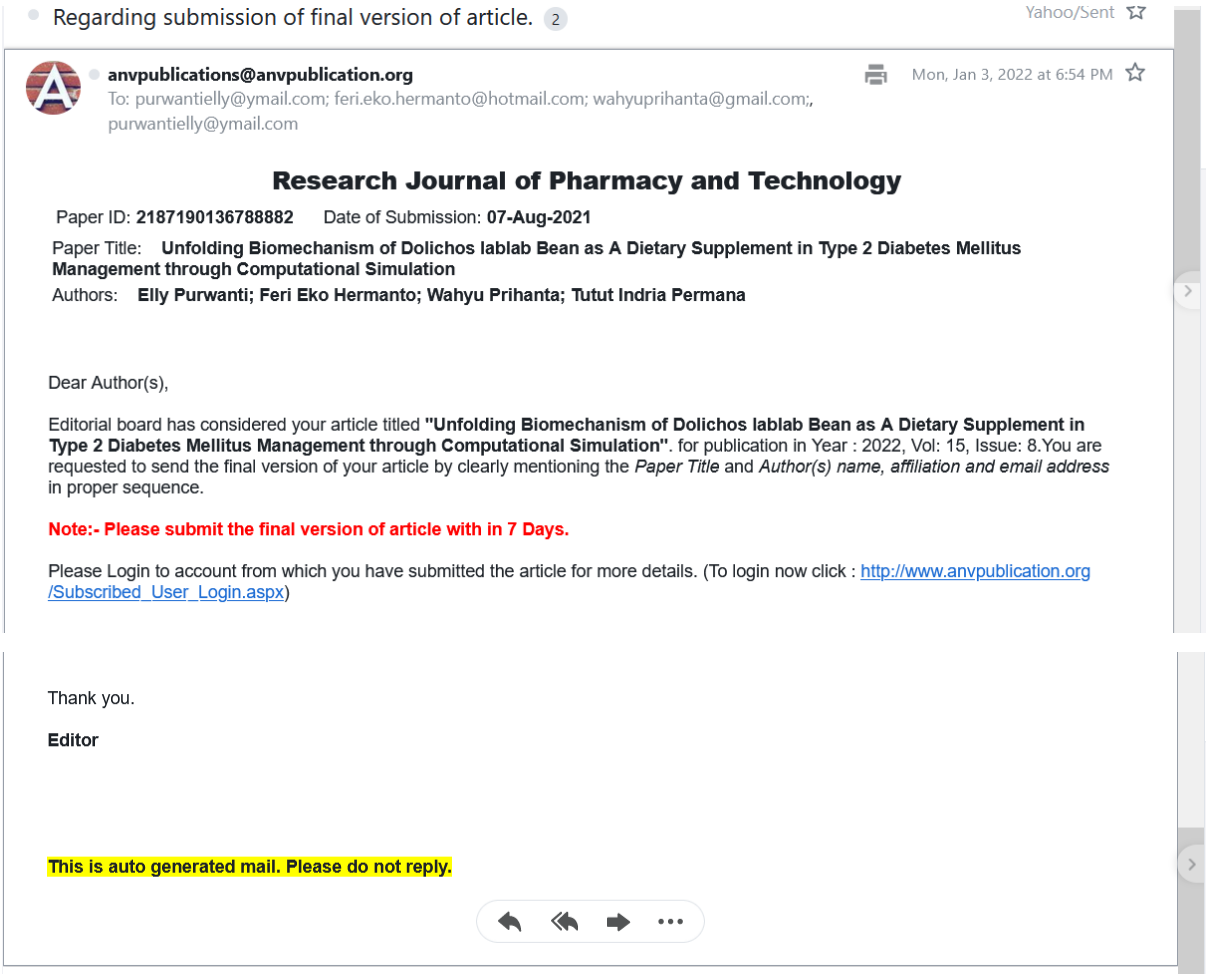

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Research Journal of Pharmacy and Technology

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

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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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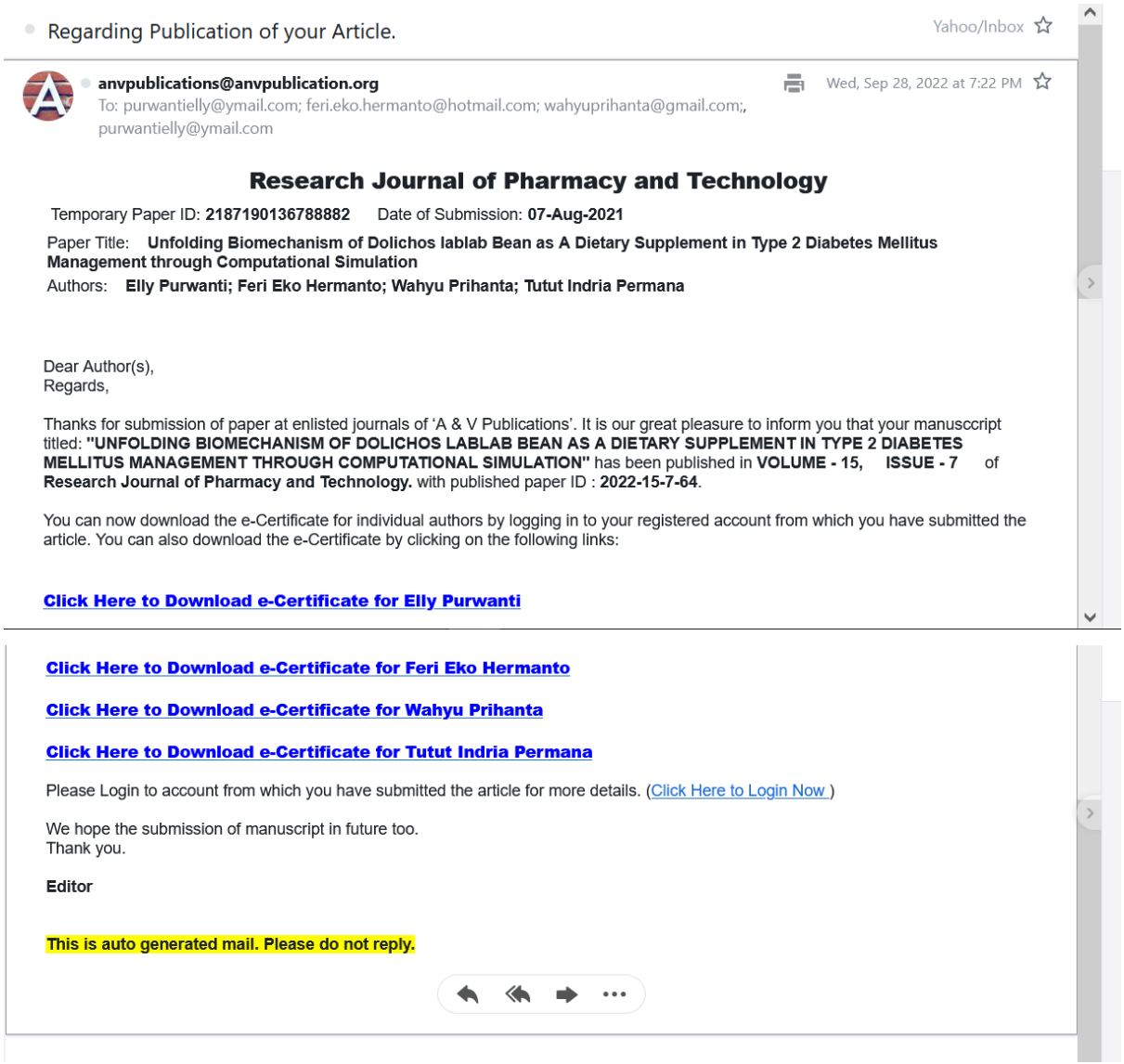

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RESEARCH ARTICLE

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]
[1]Department of Educational Biology, Faculty of Teacher Training and Education,
University of Muhammadiyah Malang, East Java, Indonesia 65144.
[2]Department of Biology, Faculty of Mathematics and Natural Sciences,
Universitas Brawijaya, Malang, East Java, Indonesia 65145.
*Corresponding Author E-mail: **purwantielly@ymail.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 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.

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MATERIALS AND METHODS:

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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).

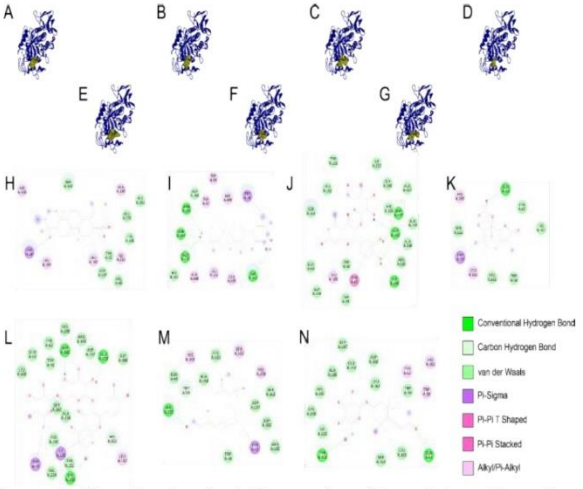

Figure 1. Structural orientation and residues interaction of α-amylase along with ursolic acid (A, H), oleanolic acid (B, I), isoquercetin (C, J), psilotachyin B (D, K), rutin (E, L), 9cis-retinal (F, M), and icariside B1 (G, N).

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).

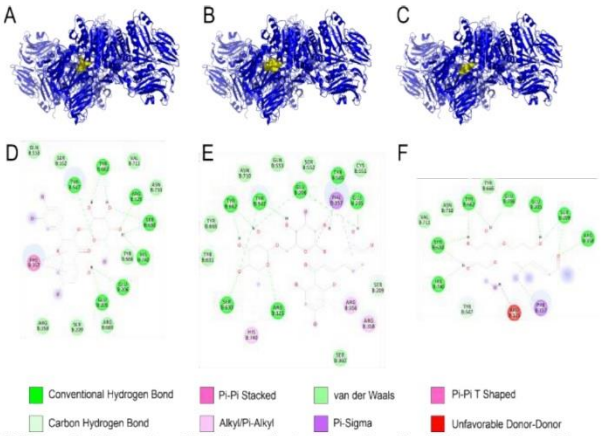

Figure 2. The visualization of structural orientation and residues interaction of DPP4 along with isoquercetin (A, D), rutin (B, E), and icariside B (C, F).

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).

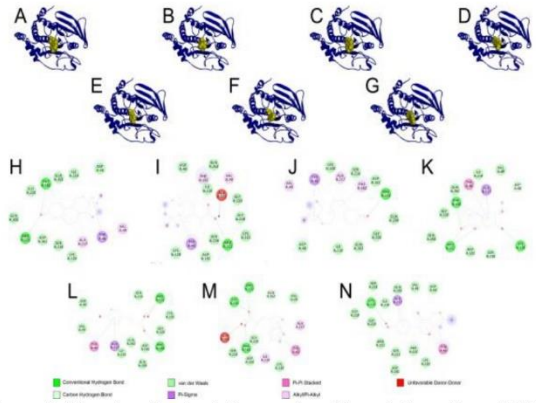

Figure 3. Structural orientation and residues interaction of PTP1B along with 19-Nortestosterone (A, H), ilicic acid (B, I), 19-Norandrostenedione (C, J), scopoletin (D, K), coumaric acid (E, L), trans-3-indoleacrylic acid (F, M), and daidzein (G, N).

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).

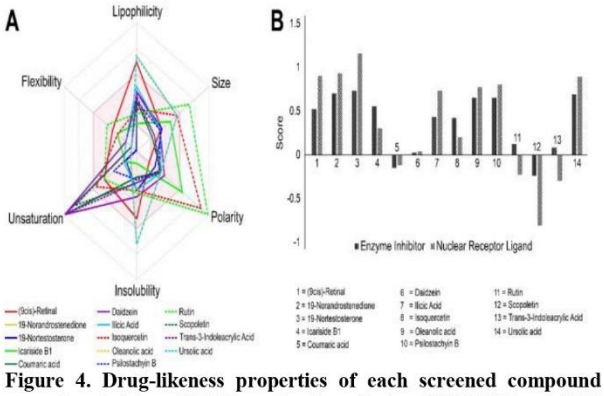

Figure 4. Drug-likeness properties of each screened compound according to bioavailability radar from Swiss-ADME (A) and SAR prediction using Molinspiration (B).

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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