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# Molecular Mechanism of DLBS3233 Bioactive Fraction in Type-2 Diabetes Mellitus: Network Pharmacology and Docking Study

(Mekanisme Molekul Pecahan Bioaktif DLBS3233 dalam Diabetes Mellitus Jenis-2: Farmakologi Rangkaian dan Kajian Dok)

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ABSTRACT

Although several antidiabetic agents are currently available, they commonly have undesirable effects and are not fully effective in reducing blood glucose. Therefore, since a long time ago, some Indonesians have preferred herbal medicine to cure and prevent diseases such as *Lagerstroemia speciosa* (L.) Pers. and *Cinnamomum burmanni.* (Nees & T.Nees) Blume, plants from Indonesia, are believed to be able to treat type 2 diabetes mellitus (T2DM). Both herbs are present in DLBS3233 bioactive fraction, a standardized herbal extract that acts as an insulin sensitizer like thiazolidinediones (TZDs). However, the molecular mechanisms, the bioactive compounds, and the target proteins involved remain unclear. To understand more about the potential molecular mechanism of DLBS3233 in treating T2DM, this network pharmacology study is conducted for the first time. Quercetin, kaempferol, and ellagic acid were discovered to have antidiabetic effects in this study as selected compounds of DLBS3233, primarily on eight core target proteins, including AKT1, EGFR, GSK3B, IL6, PTK2, RELA, SRC, and VEGFA. We also found that they exhibited ligand-receptor binding activity comparable to pioglitazone in the molecular docking study. Concisely, as a reference for furthering the development of this bioactive fraction, this study provides novel information on DLBS3233 in T2DM treatment that was not shown in prior studies.

Keywords: *Cinnamomum burmanni.* Blume; DLBS3233; *Lagerstroemia speciosa*; network pharmacology; type 2 diabetes mellitus

ABSTRAK

Walaupun beberapa agen antidiabetik kini tersedia, agen tersebut biasanya mempunyai kesan yang tidak diingini dan tidak berkesan sepenuhnya dalam mengurangkan glukosa darah. Oleh itu, sejak dahulu lagi, sebilangan masyarakat Indonesia lebih menggemari jamu bagi mengubati dan mencegah penyakit seperti *Lagerstroemia speciosa* (L.) Pers. dan *Cinnamomum burmanni.* (Nees & T.Nees) Blume, tumbuhan dari Indonesia, dipercayai mampu merawat diabetes mellitus jenis 2 (DMJ2). Kedua-dua herba hadir dalam pecahan bioaktif DLBS3233, ekstrak herba piawai yang bertindak sebagai pemeka insulin seperti *thiazolidinediones* (TZDs). Walau bagaimanapun, mekanisme molekul, sebatian bioaktif dan protein sasaran yang terlibat masih tidak jelas. Untuk memahami lebih lanjut mengenai potensi mekanisme molekul DLBS3233 dalam merawat DMJ2, kajian farmakologi rangkaian ini dijalankan buat kali pertama. Kuersetin, kaempferol dan asid elagik didapati mempunyai kesan antidiabetis dalam kajian ini sebagai sebatian terpilih DLBS3233, terutamanya pada lapan protein sasaran teras, termasuk AKT1, EGFR, GSK3B, IL6, PTK2, RELA, SRC, dan VEGFA. Kami juga mendapati bahawa mereka menunjukkan aktiviti mengikat reseptor ligan yang setanding dengan pioglitazone dalam kajian dok molekul. Secara ringkas, sebagai rujukan untuk melanjutkan pembangunan pecahan bioaktif ini, kajian ini menyediakan maklumat baru mengenai DLBS3233 dalam rawatan DMJ2 yang tidak didedahkan dalam kajian terdahulu.

Kata kunci: *Cinnamomum burmanni.* Blume; diabetes mellitus jenis 2; DLBS3233; farmakologi rangkaian; *Lagerstroemia speciosa*

## Introduction

Metabolic syndrome (MetS) is a syndrome marked by the presence of a group of factors, such as high blood pressure and high levels of blood sugar, that are strongly associated with an increased risk of cardiovascular disease and type 2 diabetes mellitus (T2DM) (Kassi et al. 2011). The International Diabetes Federation (IDF) reported that the prevalence of T2DM worldwide will increase from 415 million in 2015 to 642 million by 2040 (Nurcahyanti et al. 2018). Indonesia is 4$^{th}$ the rank country with the highest prevalence of DM (Priyadi et al. 2021). T2DM management has focused on applying lifestyle modification and antidiabetic agents. Some antidiabetic agents are known to have various adverse effects and are not fully effective in reducing blood glucose (Ko et al. 2017; Perkumpulan Endokrinologi Indonesia 2021; Serbis et al. 2021).

To avoid side effects, Indonesians prefer herbal medicine to cure and prevent T2DM, such as *Lagerstroemia speciosa* (L.) Pers. and *Cinnamomum burmanni* (Nees & T.Nees) Blume. Previous studies have shown that both herbs in DLBS3233 bioactive fraction are safe and effective in decreasing blood glucose levels (Manaf, Tjandrawinata & Malinda 2016; Wiweko & Susanto 2017). DLBS3233, a standardized herbal extract, acts as an insulin sensitizer via modulation of peroxisome proliferator-activated receptor gamma (PPARγ) (Tandrasasmita et al. 2011). However, the molecular mechanisms, the bioactive compounds, and the target proteins involved remain unclear. Hence, this study was conducted using a network pharmacology approach to predict their bioactive compounds and target proteins to investigate the potential molecular mechanism of DLBS3233 in T2DM treatment and provide a new reference for drug discovery and clinical application.

## Materials and Methods

This study used a notebook with system model specifications: Dell with Intel(R) Core(TM) i5-10210U CPU @ 1.60GHz 2.11GHz processor; Random Access Memory (RAM) 8 gigabyte, Windows 10. We conducted this study in three stages. The first stage was pre-processing, including data collection and screening. All data in this research was collected from May until June 2022. The second stage was network analysis. In this step, we constructed networks and then analyzed them. Furthermore, we carried out molecular docking validation in the post-analysis stage. The workflow of this study (Tjandrawinata et al. 2022) is shown in Figure 1.

### Pre-processing stage

*Compounds data collection*

Data on the compounds of DLBS3233 were primarily collected from three natural product databases, including the KNApSAcK Core System (http://www.knapsackfamily.com/knapsack_core/top.php) (Afendi et al. 2012), Chemical Entities of Biological Interest (ChEBI, https://www.ebi.ac.uk/chebi/) (Hastings et al. 2016), and Indian Medicinal Plants, Phytochemistry and Therapeutics (IMPPAT, https://cb.imsc.res.in/imppat/) (Mohanraj et al. 2018). Additionally, we collected data from the literature of the previous study on *L. speciosa* and *C. burmanni* phytochemicals (Li et al. 2019).

*Bioactive compounds selection*

The bioactive compounds of DLBS3233 were selected in two steps. First, we chose based on water solubility properties since DLBS3233 is a bioactive fraction in the water phase. We employed a simple method for estimating the aqueous solubility (ESOL – Estimated SOLubility) of a compound directly from its structure (Delaney 2004). In this study, we merely selected the compounds that have characteristics of highly soluble, very soluble, or soluble in water according to their information from SwissADME (http://www.swissadme.ch/index.php) (Daina, Michielin & Zoete 2017).

Once the water-soluble compounds had been selected, we filtered them again based on their pharmacokinetic properties. In this step, we used two ways from different databases. We screened by two important parameters, including oral bioavailability (OB) ≥30% and drug-likeness (DL) ≥0.18 in the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, https://tcmsp-e.com/tcmsp.php). OB, which measures the proportion of an oral dose that enters the body unchanged, is a crucial pharmacokinetic parameter in the screening of active substances for medications that are taken orally. DL is associated with elements that affect the absorption, distribution, metabolism, and excretion of compounds, as well as their pharmacodynamics and pharmacokinetics (Feng et al. 2018; Jia et al. 2021; Zhang et al. 2019; Zhou et al. 2022).

For the compounds data that are not available in TCMSP, we used SwissADME to assess the drug-likeness using each canonical Simplified Molecular Input Line Entry System (canonical SMILES) obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/).

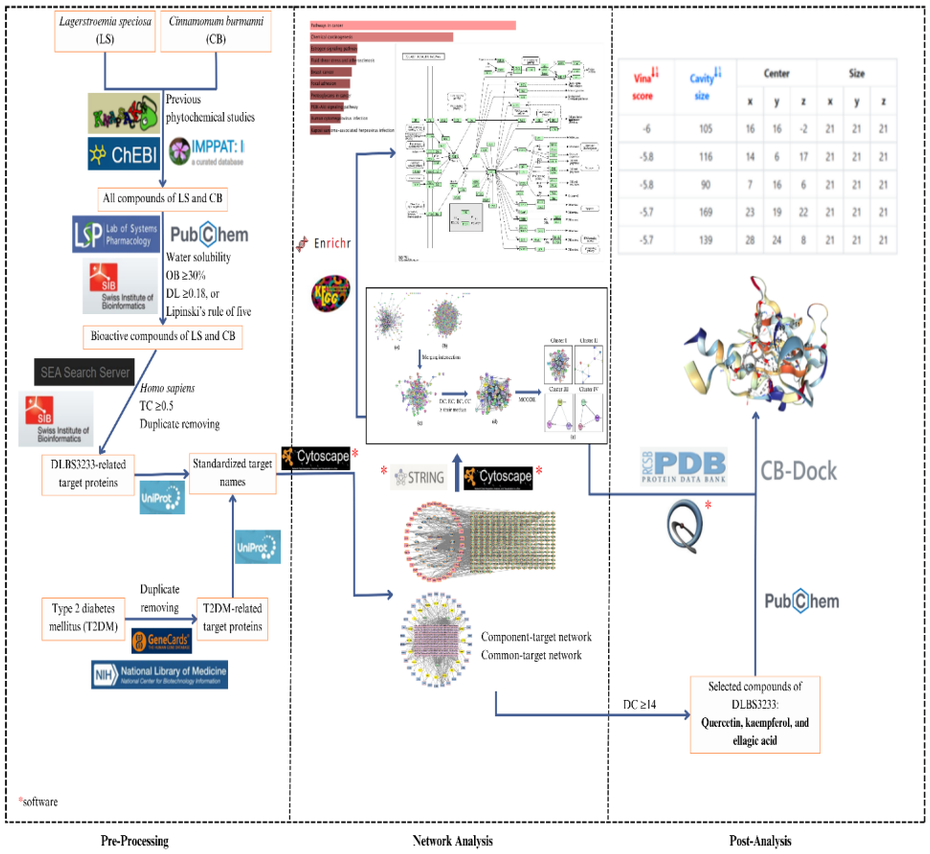



FIGURE 1. The workflow of this study

For the compounds that passed Lipinski's rule of five, we determined them as bioactive compounds. A high probability of being an oral drug (or the drug-likeness) is indicated by molecular weight (MW) <500 Da, MLOGP ≤4.15, number of H-bond acceptors ≤10, and number of H-bond donors ≤5 (Daina, Michielin & Zoete 2017; Lipinski et al. 2001; Ranjith & Ravikumar 2019).

*DLBS3233-related target proteins prediction*

The SwissTargetPrediction platform (http://www.swisstargetprediction.ch/) (Daina, Michielin & Zoete 2019) and Similarity Ensemble Approach (SEA, https://sea.bkslab.org/) (Keiser et al. 2007) were used to predict target proteins associated with DLBS3233 using input canonical SMILES that were collected from PubChem for each bioactive compound of *L. speciosa* and *C. burmanni*. The parameters we used to screen target proteins were *Homo sapiens* (human) and Tanimoto Coefficient (TC), or the probability of drug similarity is more than or equal to 0.5 (Rahman et al. 2022). A similarity threshold for TC is different in several studies. However, the typical range used is 0.5-0.85. As a note, the higher the threshold, the fewer predicted target proteins are (Gottlieb et al. 2012). Target proteins were predicted from both databases and both bioactive compounds were merged. Then, we removed duplicate targets. The UniProt database (https://beta.uniprot.org/) should be used to standardize those target protein names (The UniProt Consortium 2021).

*T2DM-related target proteins collection and screening*

Target proteins associated with T2DM were obtained from the GeneCards database (https://www.genecards.org) (Stelzer et al. 2016) and the National Center for Biotechnology Information Gene (NCBI Gene, https://www.ncbi.nlm.nih.gov/gene/) (Li et al. 2019; Zhou et al.

2022) using the keyword of 'type 2 diabetes mellitus'. Since the target protein search results were too many, the results obtained from GeneCards were limited to targets with a relevance value of more than or equal to 10.00 (Jia et al. 2021). Target proteins were merged from both databases, duplicate targets were eliminated, and the names of the remaining target proteins were then standardized using the UniProt database.

## NETWORK ANALYSIS STAGE

### *Component-target network and common-target network construction*

A component-target network was constructed using Cytoscape v3.9.1 software (https://cytoscape.org/) (Shannon et al. 2003) employing DLBS3233-related target proteins and the bioactive compounds of DLBS3233. Target proteins associated with DLBS3233 and the bioactive compounds of DLBS3233 were shown as nodes, and their interactions were shown as edges.

Using Cytoscape v3.9.1, a common-target network was created using the intersection of DLBS3233-related target proteins and T2DM-related target proteins. The key proteins, or the nodes with degrees greater than or equal to the median degree, could be identified by analyzing this common-target network. A compound is crucial if it targets more key target proteins (Li et al. 2019).

### *Protein-protein interaction (PPI) network construction and cluster analysis*

Utilizing the stringApp, a Cytoscape plug-in, PPI networks of DLBS3233-related target proteins and T2DM-related target proteins were constructed. As a limitation, the parameters of *Homo sapiens* (human) organisms with a medium confidence level of 0.400 were chosen (Zhang et al. 2019). The intersection was created by merging both PPI networks in Cytoscape. The important proteins were then identified by analyzing the merging intersection using a Cytoscape plug-in, CytoNCA. When the screening criteria of degree centrality (DC), eigenvector centrality (EC), betweenness centrality (BC), and closeness centrality (CC) are greater than or equal to their median were not met, those target proteins should be omitted. The rest of the target proteins were established as the candidate target proteins (Li et al. 2019).

In this study for cluster analysis, we used a Cytoscape plug-in, molecular complex detection (MCODE). This clustering algorithm can detect regions with a high degree of connectivity in networks of large protein-protein interactions that may represent molecular complexes (Ahmed, Bhattacharyya & Kalita 2015; Bader & Hogue 2003). This cluster analysis could determine the core targets or essential proteins (Li et al. 2019).

### *Enrichment analysis*

DLBS3233 and T2DM-related essential proteins were subsequently investigated to obtain information on biological processes, molecular functions, cellular components, and signaling pathways regarding potential antidiabetic activity using Enrichr (https://amp.pharm.mssm.edu/Enrichr/) (Chen et al. 2013) and Kyoto Encyclopedia of Genes and Genomes (KEGG) PATHWAY Database (https://www.genome.jp/kegg/pathway.html) (Kanehisa 2000) with *P-value* ≤0.05 (Jia et al. 2021; Li et al. 2019; Shahid et al. 2021; Zhang et al. 2019).

## POST-ANALYSIS STAGE

This molecular docking approach was designed to confirm the network pharmacology study. The selected compounds discovered by network pharmacology were employed as small molecule ligands to carry out molecular docking with potential target proteins. We downloaded the 2D structure of the ligand in SDF format from the PubChem database. To download the PDB format, the 3D structure of receptor protein was searched in the UniProt database, which was linked directly to the RCSB Protein Data Bank (PDB, https://www.rcsb.org/) (Berman et al 2000; Rahardjo, Ramdani & Tjandrawinata 2020; Ramdani, Yanuar & Tjandrawinata 2019). Eventually, we acquired the receptor protein structure after eliminating the original ligands and water molecules using UCSF Chimera v.1.16 software (https://www.cgl.ucsf.edu/chimera/download.html) (Pettersen et al 2004).

We utilized the server CB-Dock (http://clab.labshare.cn/cb-dock/php/) to predict potential target protein binding regions and calculate the centers and sizes to attain the best pose with the lowest binding energy. CB-Dock displayed an interactive 3D visualization of the binding modes and ordered the binding modes based on the Vina score (Liu et al. 2020). According to the molecular docking principle, the most stable ligand structure is represented by the energy value with the lowest Vina score (Abbas et al. 2018). It could be assumed that there is a high ligand-receptor binding activity if the minimum binding energy is less than −5.0 (Lin et al. 2021; Zhou et al. 2022).

## Results

### Pre-processing stage

DLBS3233 is a bioactive fraction of two herbs including *L. speciosa* and *C. burmanii*. We collected a total of 63 and 121 compounds for *L. speciosa* and *C. burmanni*, respectively (Tables S1 & S2), from prior phytochemical studies and three natural product databases, including IMPPAT, KNApSAcK Core System, and ChEBI.

Following the filtration of the water-soluble compounds with 'OB ≥30% and DL ≥0.18' criteria from TCMSP or the parameter of Lipinski's rule of five from SwissADME, a total of 28 and 80 bioactive compounds of *L. speciosa* and *C. burmanni* were selected, respectively (Tables S3 & S4). Rutin (Al-Dhubiab 2012; Rao & Gan 2014), 2,3-(S)-hexahydroxydiphenoyl-alpha/beta-D-glucose (Bai et al. 2008), procyanidin B1 and B2, (-)-epicatechin (Arozal, Louisa & Soetikno 2020), proanthocyanidin (Verdini et al. 2020), o-methoxycinnamaldehyde, 2-hydroxycinnamaldehyde, cinnamaldehyde, and cis-cinnamaldehyde (Tisnadjaja et al. 2020), were included together since they are reported to have potential as antidiabetic agents in several prior studies though they did not meet the inclusion criteria.

### Network analysis stage

*Component-target network and common-target network construction*

From 28 bioactive compounds of *L. speciosa*, we collected 442 target proteins (Table S5). Meanwhile, we obtained 493 target proteins from 80 bioactive compounds of *C. burmanni* (Table S6). After merging all target proteins related to *L. speciosa* and *C. burmanni*, and removing duplicates, we had 229 DLBS3233-related target proteins (Table S7). A DLBS3233 component-target network containing 286 nodes and 935 edges was subsequently constructed using Cytoscape v3.9.1 (Figure 2(a)).

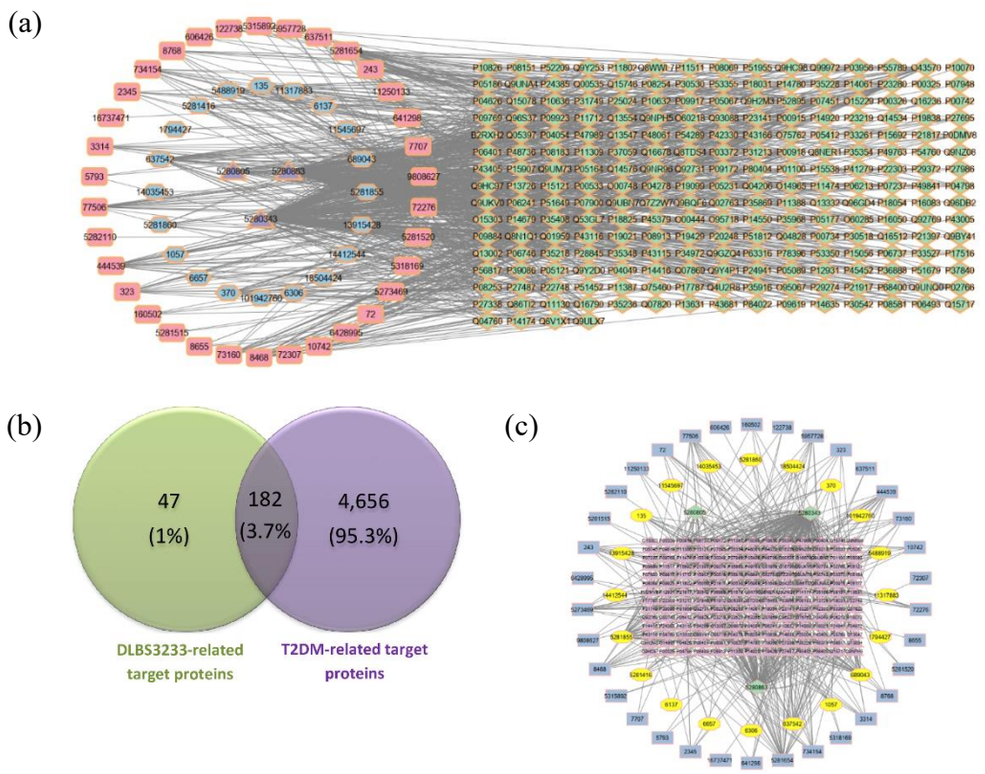



FIGURE 2. (a) DLBS3233 component-target network; containing 286 nodes and 935 edges; target proteins are represented as green nodes; bioactive compounds of *L. speciosa*, *C. burmanni*, and their intersection are represented as blue, pink, and purple nodes, respectively; (b) venn diagram, including 182 target proteins associated with DLBS3233 and T2DM; (c) the common target network, containing 239 nodes and 649 edges; target proteins are represented as purple nodes; bioactive compounds of *L. speciosa*, *C. burmanni*, and their intersection are represented as yellow, blue, and green nodes, respectively

The visualized component-target network demonstrated that 229 potential target proteins were associated with 23 (of 28) and 37 (of 80) bioactive compounds of *L. speciosa* and *C. burmanni*, respectively. Moreover, this network showed that there were three bioactive compounds as the result of the intersection between *L. speciosa* and *C. burmanni*, including rutin (PubChem ID 5280805), quercetin (PubChem ID 5280343), and kaempferol (PubChem ID 5280863).

In this study, we collected a total of 4,838 target proteins related to T2DM from two human genomic databases after deleting duplicates (Table S8). After the screening, we obtained 900 targets from NCBI genes and 4,756 (of 13,395) targets from GeneCards. A total of 182 from 4,838 target proteins were associated with 229 DLBS3233-related target proteins (Table S9; Figure 2(b)). The interaction between 182 target proteins and bioactive compounds of DLBS3233 is shown in Table S10 and visualized in Figure 2(c).

Subsequently, we used the degree centrality (DC) measure to analyze the common-target network (Table S11). The importance of nodes is reflected in the degree of centrality. The more connections a molecule has, the more significant it is, as indicated by a higher DC. By the requirement that the DC be greater than or equal to 14, we established quercetin (PubChem ID 5280343), kaempferol (PubChem ID 5280863), and ellagic acid (PubChem ID 5281855) as selected compounds of DLBS3233.

*Protein-protein interaction (PPI) network and cluster analysis*

All target proteins resulting from merging the DLBS3233-T2DM PPI network are shown in Table S12. The visualized PPI networks can be seen in Figure 3. A total of 182 target proteins associated with DLBS3233 and T2DM were further analyzed using CytoNCA, resulting in 65 candidate target proteins (Table S13). Furthermore, we conducted clustering, which resulted in four clusters that were then analyzed. We eventually obtained 44 core target proteins (Tables S14 & S15).

*Enrichment analysis*

A total of 44 core target proteins were obtained after analyzing the clusters of the core-target PPI network using MCODE. All core target proteins were subsequently input into Enrichr for enrichment analysis. We merely selected biological processes (BPs), molecular functions (MFs), cellular components (CCs), and signaling pathways having *P-value* ≤0.05. Eventually, we chose 1,112 BPs, 128 MFs, 46 CCs, and 148 KEGG pathways, as shown in Tables S16, S17, S18, and S19, respectively. The top ten BPs, MFs, CCs, and signaling pathways were ordered based on *P-value* (from the smallest to the largest), which can be seen in Table S20.

## POST-ANALYSIS STAGE

We analyzed 44 core target proteins based on the amount of involvement in biological processes and signaling pathways associated with T2DM. Core target proteins with an amount of involvement more than or equal to their average could be assumed as the most potential target proteins. We selected eight target proteins (Table S21) to perform molecular docking to validate the network pharmacology study. The top five poses for each potential target protein and selected compound of DLBS3233 or pioglitazone using CB-Dock based on the Vina score can be seen in Tables S22 and S23, respectively. The best docking models are shown in Table 1 and Table S24.

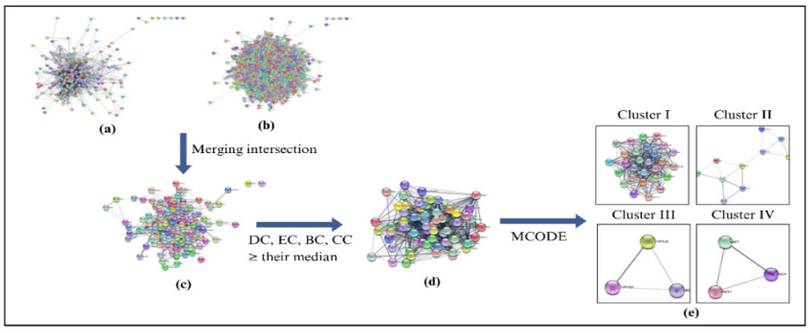
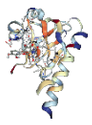
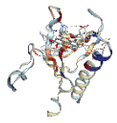
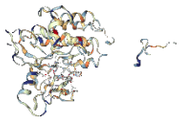
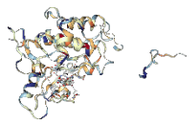
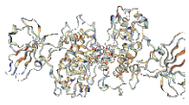
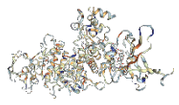
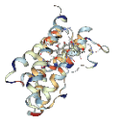
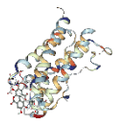
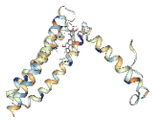
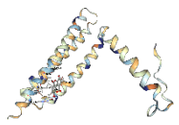
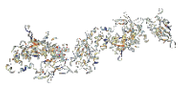
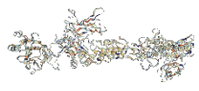
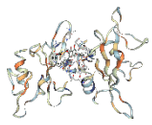
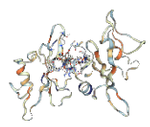
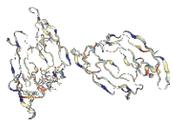
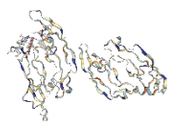
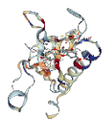
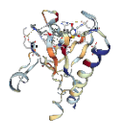
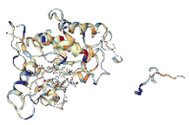
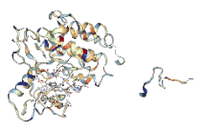
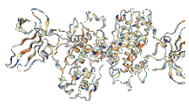
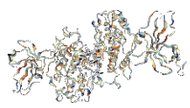
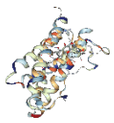
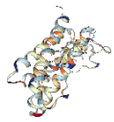
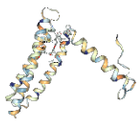
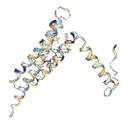
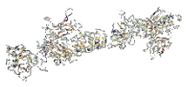
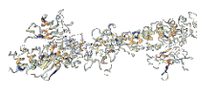
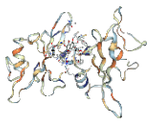
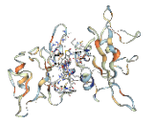
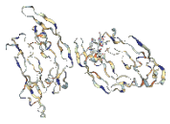
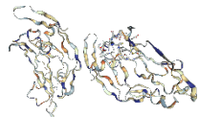



FIGURE 3. (a) DLBS3233-related targets PPI network (229 nodes and 1,922 edges); (b) T2DM-related targets PPI network (4,809 nodes and 216,186 edges); (c) the merging intersection of the PPI network (182 nodes and 1,671 edges); (d) the core PPI network was obtained by the screening parameter of DC ≥14.0, EC ≥0.038753505, BC ≥52.9146275, and CC ≥0.23598436 (65 nodes and 819 edges); (e) clusters of core-target PPI network using MCODE; cluster I (28 nodes and 310 edges); cluster II (10 nodes and 15 edges); cluster III (3 nodes and 3 edges); and cluster IV (3 nodes and 3 edges)

TABLE 1. The best docking model for each potential target protein and selected compound of DLBS3233 or pioglitazone using CB-Dock

| Compound | Target protein | Docking model | Vina score | Compound | Target protein | Docking model | Vina score |
|---|---|---|---|---|---|---|---|
| Quercetin | AKT1 |  | -6 | Ellagic acid | AKT1 |  | -6.3 |
| Quercetin | EGFR |  | -7.9 | Ellagic acid | EGFR |  | -8 |
| Quercetin | GSK3B |  | -8.4 | Ellagic acid | GSK3B |  | -8.4 |
| Quercetin | IL6 |  | -6.5 | Ellagic acid | IL6 |  | -7.1 |
| Quercetin | PTK2 |  | -5.9 | Ellagic acid | PTK2 |  | -5.9 |
| Quercetin | RELA |  | -9.2 | Ellagic acid | RELA |  | -8.4 |
| Quercetin | SRC |  | -8.1 | Ellagic acid | SRC |  | -7.8 |
| Quercetin | VEGFA |  | -7.4 | Ellagic acid | VEGFA |  | -6.7 |
| Kaempferol | AKT1 |  | -6.2 | Pioglitazone | AKT1 |  | -6.2 |
| Kaempferol | EGFR |  | -7.6 | Pioglitazone | EGFR |  | -7.5 |
| Kaempferol | GSK3B |  | -8.5 | Pioglitazone | GSK3B |  | -8.6 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Kaempferol | IL6 |  | -6.3 | Pioglitazone | IL6 |  | -6.3 |
| Kaempferol | PTK2 |  | -5.9 | Pioglitazone | PTK2 |  | -6.6 |
| Kaempferol | RELA |  | -9.1 | Pioglitazone | RELA |  | -9.2 |
| Kaempferol | SRC |  | -7.8 | Pioglitazone | SRC |  | -7.8 |
| Kaempferol | VEGFA |  | -7.3 | Pioglitazone | VEGFA |  | -6.9 |

## DISCUSSION

Previous studies found that DLBS3233 has a mode of action like thiazolidinediones (TZDs) in reducing insulin resistance and improving insulin signaling and sensitivity. Hence, the study conducted by Nailufar, Tandrasasmita and Tjandrawinata (2011) and Tandrasasmita et al. (2011) concluded that DLBS3233 possibly contains the bioactive compound(s) that may act as a direct ligand for peroxisome proliferator-activated receptor gamma (PPARγ) and delta (PPARδ). Their study also demonstrated genes involved in insulin signal transduction, such as PI3 kinase, Akt, PPARγ, PPARδ, GLUT4, adiponectin, and resistin. Although the DLBS3233 mechanism of action in T2DM treatment has been known, the bioactive compounds responsible for the antidiabetic activity and how they regulate the expression of genes involved in insulin signal transduction are not yet known.

This study is the first to investigate the molecular mechanism of DLBS3233 for T2DM using a network pharmacology approach. We successfully predicted the selected compounds of DLBS3233 bioactive fraction of *L. speciosa* and *C. burmanni*, namely quercetin, kaempferol, and ellagic acid. Moreover, this research found that eight target proteins play a key role in antidiabetic activity, including AKT1, EGFR, GSK3B, IL6, PTK2, RELA, SRC, and VEGFA. Based on the prior study results and the guidelines for T2DM management, we used the primary mechanism of action of TZDs as a classification to facilitate the explanation of the DLBS3233 molecular mechanism (Table 2) (Haq et al. 2021; Ko et al. 2017; Nurcahyanti et al. 2018; Perkumpulan Endokrinologi Indonesia 2021; Serbis et al. 2021).

Interestingly, the finding of this study did not show that DLBS3233 bioactive compounds have PPARγ and PPARδ target proteins. However, they are involved in the signaling pathway associated with T2DM through PPARγ and PPARδ (KEGG:05200). Therefore, it suggests that DLBS3233 indirectly increases the genetic expression of PPARs, resulting in the synthesis of new glucose transporter type 4 (GLUT-4) (Wiweko & Susanto 2017). PPARγ regulates the PI3 kinase enzyme which is vital in mediating insulin's biological actions through insulin receptor substrate (IRS) 1 and 2. According to Nailufar, Tandrasasmita and Tjandrawinata (2011) and Tandrasasmita et al. (2011), DLBS3233 increased PI3 kinase expression even more than pioglitazone. This finding was confirmed by an increase in Akt, a PI3 kinase downstream effector.

TABLE 2. The main mechanisms of action of DLBS3233 which are similar to TZDs

| Antidiabetic activity | | |
|---|---|---|
| Increased insulin sensitivity | Decreased insulin resistance | Decreased hepatic glucose production (gluconeogenesis) |
| a. The activation of phosphatidylinositol-3 kinase (PI3K) as a broad activator of insulin action<br>b. The activation of Akt effector of PI3K<br>c. The modulation of PPARγ and PPARδ | a. Increased GLUT4 as glucose-transport protein to increase glucose uptake into cells | a. The upregulation of adiponectin<br>b. The downregulation of resistin |

When insulin is bound to the receptor, the receptor phosphorylates and activates IRS-1, which enables IRS-1 to activate several signaling pathways, including the PI3K pathway (Figure 4). PI3K enzyme catalyzes the conversion of phosphatidylinositol (4,5)-bisphosphate (PI(4,5)P2 or PIP2) to phosphatidylinositol (3,4,5)-trisphosphate (PI(3,4,5)P3 or PIP3). PIP3 is subsequently bound to Akt, signaling for PDK1 to phosphorylate Akt (protein kinase B or PKB). Once phosphorylated, Akt becomes active and phosphorylates other targets that stimulate GLUT4 translocation from the cytoplasm into the membrane (Permadi et al. 2021; Pessin & Saltiel 2000).

In this study, those mechanisms were demonstrated in the mTOR signaling pathway (KEGG:04150), PIK3-AKT signaling pathway (KEGG:04151), insulin signaling pathway (KEGG:04910), thyroid hormone signaling pathway (KEGG:04919), insulin resistance (KEGG:04931; Figure 4), and diabetic cardiomyopathy (KEGG:05415). Those pathways indicate that DLBS3233 is a glucose transport stimulant since it upregulates GLUT4 expression, resulting in an enhancement of glucose uptake by cells in the insulin-resistant 3T3-Swiss-Albino adipocytes, as shown in the prior study (Tjandrawinata, Suastika & Nofiarny 2012).

PPARγ also regulates adiponectin, a protein hormone almost exclusively produced in adipocytes, that may act as an insulin-secretion enhancer and insulin sensitizer (Fasshauer & Paschke 2003; Jonas et al. 2017; Permadi et al. 2021). Adiponectin enhances insulin inhibition of hepatic gluconeogenesis (KEGG:04068); thus, its levels are reduced in patients with obese insulin resistance and T2DM (Nailufar, Tandrasasmita & Tjandrawinata 2011). Adiponectin modulates insulin sensitivity by stimulating glucose utilization and fatty acid oxidation via phosphorylation and activation of AMPK in muscle and liver (KEGG:04152) (Okamoto et al. 2006).

While resistin, a peptide with an opposite mechanism to adiponectin in regulating glucose and lipid metabolism, was elevated in obese patients. Because of its pro-inflammatory properties, high serum resistin levels have been linked to the development of insulin resistance and T2DM, atherosclerosis, and cardiovascular diseases (Fasshauer & Paschke 2003; Jonas et al. 2017; Permadi et al. 2021). In previous studies, DLBS3233 showed upregulating adiponectin and downregulating resistin (Nailufar, Tandrasasmita & Tjandrawinata 2011). But whether it acts directly or indirectly on the gene encoding for the hormones adiponectin (ADIPOQ) and resistin (RETN) is unknown. From this network pharmacology study, we found that DLBS3233 is involved in the adipocytokine signaling pathway (KEGG:04920) through genes of AKT1, PPARA, and RELA. Thus, it suggests that DLBS3233 may affect adiponectin and resistin protein levels indirectly.

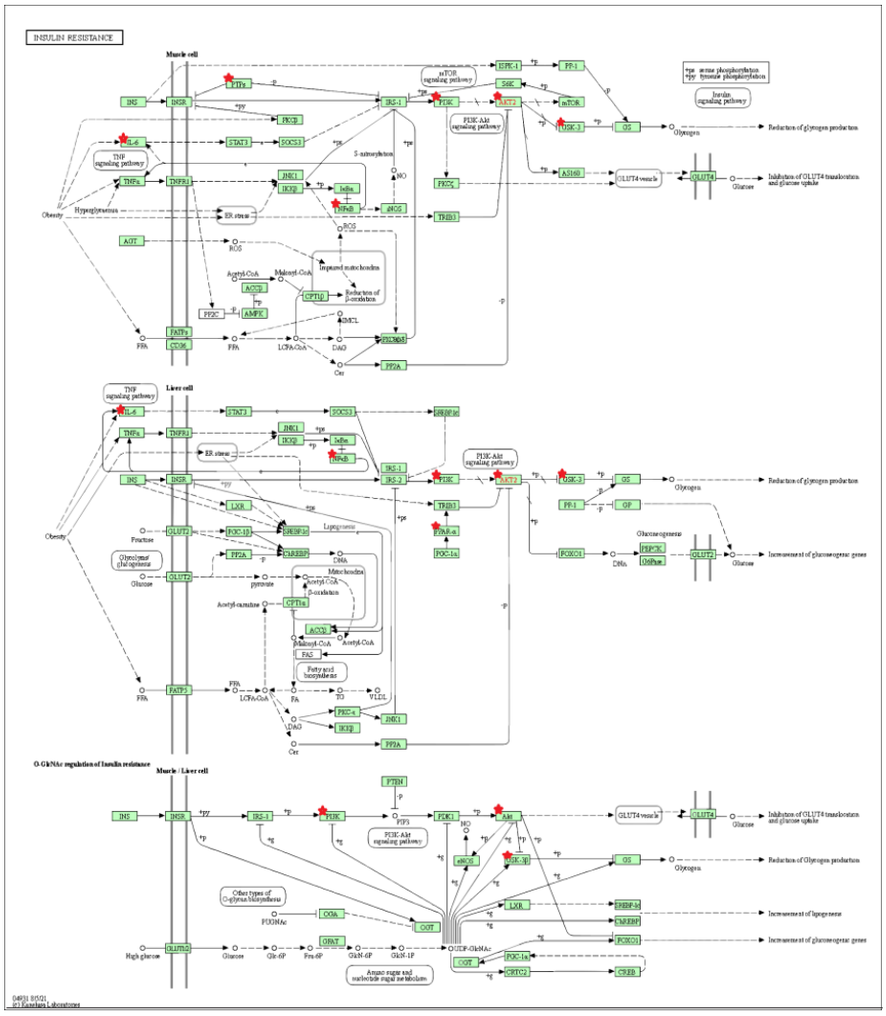



FIGURE 4. The signaling pathways of PIK3-AKT and insulin resistance; red stars indicate DLBS3233-related target proteins involved in those pathways

PPARδ was known to regulate aspects of lipid homeostasis by modulating cholesterol metabolism. As mentioned before, DLBS3233 affects PPARδ. Thus, it could be said that DLBS3233 modifies lipid metabolism via PPARδ. The statement was in line with this study that showed DLBS3233-related target proteins involved in the signaling pathway regarding the regulation of lipolysis in adipocytes (KEGG:04923) and nonalcoholic fatty liver disease (KEGG:04932). Elevated high-density lipoprotein cholesterol (HDL-C) levels and reduced triglyceride levels were correlated with higher adiponectin levels. Conversely, elevated triglycerides and serum apolipoprotein B (Apo B) levels were related to higher plasma resistin levels (Maximus et al. 2020). In brief, DLBS3233 may improve lipid profiles and regulate blood glucose and insulin levels (Tjokroprawiro, Murtiwi & Tjandrawinata 2016).

The molecular docking study has validated the results of this network pharmacology study. We discovered that all potential target proteins have high binding activities with all selected compounds of DLBS3233. It was apparent from their Vina score of less than -5. Moreover, we found that the binding energy of selected compounds of DLBS3233 was as low as pioglitazone. It suggests that quercetin, kaempferol, and ellagic acid might have stable ligand structures to

T2DM-related potential target proteins and be comparable to pioglitazone. The mechanism of action of DLBS3233 found in this network pharmacology study is in line with previous *in vitro* and *in vivo* tests. In addition, the results of this study showed the bioactive compound of DLBS3233 and their potentially promising target proteins for the prevention and treatment of T2DM, as well as explained the molecular mechanisms, which are unknown yet.

## Conclusions

Quercetin, kaempferol, and ellagic acid have been successfully identified as selected compounds of DLBS3233 with antidiabetic activity, notably on eight core target proteins, including AKT1, EGFR, GSK3B, IL6, PTK2, RELA, SRC, and VEGFA. We also found that they exhibited ligand-receptor binding activity comparable to pioglitazone in the molecular docking study. Briefly, as a reference for furthering the development of DLBS3233 bioactive fraction, this developed network pharmacology study provides novel information on DLBS3233 in T2DM treatment that was not revealed in prior studies.

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