Research Article

Active compounds of fingerroot (*Boesenbergia pandurata*) for obesity treatment: *in silico* approaches

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Submitted : 04-01-2022, *Revised* : 05-03-2022, *Accepted* : 01-06-2022

ABSTRACT: *Obesity has become increasingly prevalent worldwide each year. Several studies have proven that herbs are effective in preventing obesity. The research delved into the active compounds of Boesenbergia pandurata to reveal their mechanisms of action by utilizing bioinformatics approaches. The research methods included active compounds selection, QSAR analysis, networking analysis, molecular docking, and ADME prediction. The QSAR analysis predicted that the active compounds were correlated with some theoretical activities with more than 0.5 probability, namely vasoprotective, anti-hypercholesterolemic, anti-inflammatory, free radical scavenging, and as a lipid metabolism regulator and TNF expression inhibitor. Furthermore, the results of the networking analysis showed that five compounds (pinocembrin, cardamonin, flavokawain B, flavokawain C, and tectochrysin) had direct interactions with RPS6KB1. Pinocembrin exhibited the highest binding affinityof -7.26 kcal/mol, although not as strong as that of the control ligand (FS9). The ADME prediction indicated that the five compounds were non-toxic and had excellent absorption. It can be concluded that the active compounds of B. pandurata have the ability to improve metabolic syndrome, especially obesity, in silico through several mechanisms, such as suppression of pro-inflammatory cytokine, regulation of lipid metabolism, and those associated with antioxidants.*

Keywords: *herb; in silico; metabolic syndrome; obesity*

1. Introduction

Obesity has been recognized as a non-communicable disease. It is defined as abnormal and excessive fat accumulation that may have serious impacts on health. Obesity and overweight have become increasingly prevalent worldwide [1]. According to the World Population Review in 2022, Nauru (61%) has the highest obesity rate, followed by Cook Islands (55.9%), Palau (55.3%), Marshall Islands (52.9%), and Tuvalu (51.6%). In Indonesia, with a population of 276,361,783, 6.9% of the adults are currently obese. Obesity rates vary substantially across nations, believed to stem from widely differing lifestyles and diets. Despite the absence of a specific link between a country's obesity rate and its economic standing, wealthier nations have more resources available for introducing programs, campaigns, and measures to raise awareness and educate their citizens about what they consume.

On June 9, 2021, WHO released facts and statistics about obesity in the world. Since 1975, the number of overweight children aged five years and under had tripled to 39 million in 2020. Obesity and overweight are associated with more fatalities than underweight. Except for sections of Sub-Saharan Africa and Asia, there is a higher share of people who are overweight worldwide than underweight. In the same fact sheet, it is said that obesity and overweight may be attributed to shifts in daily diets and physical activities.

Overweight and obesity are closely linked to metabolic syndrome (MS), which, according toepidemiological research, affects 20–45% of the population. The most common manifestation of MS is abdominal fat. By 2035, the prevalence of MS is predicted to have risen to almost 53% [2]. The key components of MS include abdominal obesity, imbalance in glucose, lipid, and cholesterol metabolisms regulated by nuclear peroxisome proliferator-activated receptors (PPARs), insulin resistance (IR) with or without glucose intolerance, atherogenic dyslipidemia, elevated blood pressure, and pro-inflammatory states [3]. The size of adipocytes in both subcutaneous and omental fat increases as body fat mass increases. Adipocyte hypertrophy occurs in both the omental and subcutaneous compartments, whereas hyperplasia occurs exclusively in the subcutaneous fat tissue. Fat accumulation in the subcutaneous tissue eventually outnumbers fat formation in the visceral fat compartment. Waist circumference (WC) and body mass index (BMI) have been proposed as the most reliable surrogate measures of visceral adiposity in young people and as strong indicators of IR hepatic steatosis. Overweight people with identical BMIs, on the other hand, have significantly varying visceral adipose tissue (VAT) levels. For instance, in obese male populations, those with high VAT had considerably higher levels of soluble tumor necrosis factor-alpha receptor 2 (TNFR2) than those with low VAT and lean controls [4]. Even though VAT and subcutaneous abdominal adipose tissue are both linked to metabolic risk factors, VAT is more strongly associated with a poor metabolic profile. Obesity is generally attributed to unhealthy eating, and a healthy plant-based diet may thus be beneficial in lowering inflammatory markers [5-6]. Besides, several studies have shown that many plants effectively prevent obesity [7], e.g., onion, *Aloe vera*, cumin, garcinia gummi-gutta, roselle, yerba mate, Asian lobelia, mango, and black pepper [8]. Natural products can act as anti-obesity agents through different mechanisms, including augmentation of energy expenditure, appetite suppression, and inhibitions of lipase enzyme, adipocyte differentiation, and lipid metabolism [9].

Boesenbergia pandurata, commonly known as fingerroot or temu kunci in Indonesia, belongs to the family *Zingiberaceae* that grows in vast areas in Southeast Asian countries, including Indonesia, Thailand, and Malaysia. Empirically, the rhizome of *B. panduratahas* been widely used as a spice in many different foods and an ingredient in folk medicine to treat inflammation, diarrhea, skin disorders, and other diseases [10-12]. The research investigated the active compounds of *B.*

pandurata to reveal their mechanisms of action in the pathology of metabolic syndrome, specifically obesity, by utilizing a bioinformatics approach. It ultimately sought to figure out measures to deal with the increasing obese cases worldwide.

2. Method

2.1. Collection of active compounds

Samples of the active compounds of *Boesenbergia pandurata* were retrieved from KNApSAcK(http://www.knapsackfamily.com/KNApSAcK/) and Dr. Duke (https://phytochem.nal.usda.gov/phytochem/search)databases. In addition, the 3D structure and SMILE information were collected from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/).

2.2. QSAR analysis of compounds for metabolic syndrome treatment

The biological activity of each compound was predicted online using the quantitative-structure activity relationship (QSAR) analysis in the WAY-DRUG PASS (Prediction of Activity Spectra for Substances) webserver(http://www.pharmaexpert.ru/passonline/predict.php). The terms observed in this process included TNF expression inhibitor, lipid metabolism regulator, phospholipase A2 inhibitor, phospholipase inhibitor, cholesterol synthesis inhibitor, cholesterol oxidase inhibitor, insulin promoter, lipoprotein lipase inhibitor, glycogen synthase kinase-3 beta(GSK-3β) inhibitor, protein-tyrosine phosphatase 1B inhibitor, dipeptidyl peptidase (DPP)-IV inhibitor, sodium-dependent glucose cotransporter 2 (SGLT2) inhibitor, peroxisome proliferator-activated receptor agonist, alpha-glucosidase inhibitor, glucose oxidase inhibitor, angiogenesis stimulant, and anti-inflammatory, anti-diabetic, antihypertensive, anti-hypercholesterolemic, cardio-protectant, andvasoprotective activities. In the analysis, PASS produced a Pa score (probability to be active) for each predicted activity of each compound. A Pa score represents the accuracy of the biological activity prediction in a range of 0 to 1, with a higher Pa score indicating a more accurate prediction in the *in vivo/in vivo* experiment[13]. In this experiment, a 0.5 threshold was set for each term.

2.3. Networking analysis

The networking analysis was performed using the STITCH (http://stitch.embl.de/cgi/network.pl) and STRING (https://string-db.org//) web servers to explain the roles of each compound in obesity treatment, particularly lipid metabolism regulator and its correlation with TNF protein. The selected compounds were inputted to the search column, including 5,7-dimethoxyflavone, tectochrysin, 5,7,4'-trimethoxyflavone, flavokawain B, flavokawain A, galangin 3,7-dimethyl ether, cardamomin, 2',6'-dihydroxy-4'-methoxychalcone, flavokawain C, (2S)-pinocembrin, (-)-pinocembrin, 5,6-dehydrokawain, geranyl-2,4-dihydroxy-6-phenethylbenzoate. These compounds were selected because they form interaction with the protein targets. Proteins related to the regulation of lipid metabolism, namely PS6KB1, SREBF1, and tumor necrosis factor-alpha (TNF-α), were inputted to the search column as well. In addition, the gene ontology (GO) comprising a Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway, molecular functions, and biological processes was generated. Only compounds with an interaction score (combined score) > 0.5 were used in further analysis. To deepen the analysis and develop the most complex pathway possible, Cytoscape v3.8.2 software with GOlorize and BinGO plugins was used. The hypergeometric testused a multiple testing correction, i.e., Benjamini-Hochberg False Discovery Rate (FDR) at 0.05.

2.4. Molecular docking analysis and interaction visualization

The docking analysis aimed to examine the molecular interaction between selected active compounds and ribosomal protein S6 Kinase B1

(RPSKB1) as a crucial molecular component in developing insulin resistance due to nutrient overload. Pinocembrin, cardamonin, flavokawain B, flavokawain C, tectochrysin were the compounds set as the ligands. In this experiment, the control was FS9, an inhibitor control retrieved from the PDB database (ID 3WF7). The docking analysis was conducted using PyRx 0.8 with the grid center at X: 11.229, Y: -9.730, and Z: 11.370, number of points X: 45, Y: 43, Z: 38, and spacing at 0.374 Å. The docking results were visualized using Discovery Studio R17 and PyMOL programs.

2.5. Absorption, Distribution, Metabolism, and Excretion (ADME) for drug-likeness prediction

To predict the bioavailability of a compound as a requirement for drug formulation, the ADME component was examined using the SwissADME webserver(www.swissadme.ch). Lipinski's rule of five was used to determine the pharmacokinetic function of the active compounds in the human body. A molecule is considered to have the ability to operate as a medication if it meets Lipinski's rule: molecular mass of less than 500 Dalton, high lipophilicity (given as LogP less than 5), less than five hydrogen donor bonds, less than ten hydrogen acceptor bonds, and molar refraction of 40–130. ProTox (https://tox-new.charite.de/protox II/_) was used to predict compound toxicity and LD_{50}. The LD_{50}, or median lethal dosage, is the level at which 50% of the test subject dies after being exposed to the treatment substance. Acute toxicity refers to the negative effects of a chemical due to single or multiple exposures in a short duration (e.g., less than 24 hours). The 2D similarity analysis (SMILE input) and identification of hazardous pieces in 38,000 distinct compounds with known oral LD_{50} values recorded in rats were used to predict acute oral toxicity.

3. Results and discussion

3.1. Active compounds exploration and QSAR analysis

The active compounds of *Boesenbergia pandurata* selected from the databases (as listed in Table 1) show potential as metabolic syndrome treatment. The QSAR analysis revealed that the top terms with more than 0.5 probability of activity were vasoprotector (0.510), anti-hypercholesterolemic (0.530), lipid metabolism regulator (0.461), free radical scavenging (0.689), TNF expression inhibitor (0.501), and anti-inflammatory (0.634) (Figure 1).

A vasoprotector, also known as a vasoprotective, is a compound that relieves and prevents cardiovascular risk factors, including hypertension and hypercholesterolemia that affect the blood vessels. Obesity is associated with cardiovascular diseases. At least up to 60% of overweight patients have hypertension. Because weight loss and diet are effective in controlling blood pressure, a vasoprotector is thus one of the parameters evaluated in this study to factor in the correlation between obesity and hypertension in metabolic syndrome. Lipid metabolism regulator, TNF expression inhibitor, and anti-inflammatory were the three terms observed with strong correlations with obesity cases. Obesity promotes macrophage infiltration and pro-inflammatory cytokines, notably in adipose tissue. Elevated production of TNF-α is the first indicator of increased pro-inflammatory cytokines in obesity. TNF-α is closely linked to the degree of adiposity and insulin resistance because it is produced and released by adipose tissue. In the case of obesity, the elevated expression of transmembrane TNF-α in adipose tissue can be controlled without affecting TNF-α converting enzyme (TACE) levels. TACE is involved in the breakdown of TNF-αmolecules. TNF-α is a powerful inhibitor of adipocyte differentiation that plays a role in adipogenesis and lipogenesis by suppressing the expression of genes involved in the absorption and storage of non-esterified fatty acids and glucose. Expression of TNF-α likewise affects interleukin (IL)-6 and monocyte chemoattractant protein (MCP)-1 [14]. It also protects obese model mice with reduced TNF-α expression from insulin

Table 1. Active compounds of *Boesenbergia pandurata*

No	Metabolite	Molecular formula	ID compound	SMILE canonical
1	5,7-Dimethoxyflavone	$C_{17}H_{14}O_4$	88881	COC1=CC2=C(C(=C1)OC)C(=O)C=C(O2)C3=CC=CC=C3
2	Galangin 3,5,7-trimethyl ether	$C_{18}H_{16}O_5$	117900	COC1=CC2=C(C(=C1)OC)C(=O)C(=C(O2)C3=CC=CC=C3)OC
3	Tectochrysin	$C_{16}H_{12}O_4$	5281954	COC1=CC(=C2C(=C1)OC(=CC2=O)C3=CC=CC=C3)O
4	5,7,4'-Trimethoxyflavone	$C_{18}H_{16}O_5$	79730	COC1=CC=C(C=C1)C2=CC(=O)C3=C(O2)C=C(C=C3OC)OC
5	5,7,3',4'-Tetramethoxyflavone	$C_{19}H_{18}O_6$	631170	COC1=C(C=C(C=C1)C2=CC(=O)C3=C(O2)C=C(C=C3OC)OC)OC
6	Galangin 3,7-dimethyl ether	$C_{17}H_{14}O_5$	5748697	COC1=CC(=C2C(=C1)OC(=C(C2=O)OC)C3=CC=CC=C3)O
7	3,5,7,3',4'-Pentamethoxyflavone	$C_{20}H_{20}O_7$	97332	COC1=C(C=C(C=C1)C2=C(C(=O)C3=C(O2)C=C(C=C3OC)OC)OC)OC
8	2',6'-Dihydroxy-4'-methoxychalcone	$C_{16}H_{14}O_4$	5316793	COC1=CC(=C(C(=C1)O)C(=O)C=CC2=CC=CC=C2)O
9	Cardamomin	$C_{16}H_{14}O_4$	641785	COC1=CC(=CC(=C1C(=O)C=CC2=CC=CC=C2)O)O
10	Flavokawain B	$C_{17}H_{16}O_4$	5356121	COC1=CC(=C(C(=C1)OC)C(=O)C=CC2=CC=CC=C2)O
11	Flavokawain C	$C_{17}H_{16}O_5$	6293081	COC1=CC(=C(C(=C1)OC)C(=O)C=CC2=CC=C(C=C2)O)O
12	Flavokawain A	$C_{18}H_{18}O_5$	5355469	COC1=CC=C(C=C1)C=CC(=O)C2=C(C=C(C=C2OC)OC)O
13	Boesenbergin A	$C_{26}H_{28}O_4$	6313827	CC(=CCCC1(C=CC2=C(C=C(C(=C2O1)C(=O)C=CC3=CC=CC=C3)O)OC)C)C
14	Boesenbergin B	$C_{26}H_{28}O_4$	23643133	CC(=CCCC1(C=CC2=C(C(=C(C=C2O1)OC)C(=O)C=CC3=CC=CC=C3)O)C)C
15	Rubranine	$C_{25}H_{26}O_4$	42607681	CC1(C2CCC3(CC2C4=C(O3)C=C(C(=C4O1)C(=O)C=CC5=CC=CC=C5)O)C)C
16	(-)-Alpinetin	$C_{16}H_{14}O_4$	154279	COC1=CC(=CC2=C1C(=O)CC(O2)C3=CC=CC=C3)O
17	(2S)-Pinocembrin	$C_{16}H_{14}O_4$	68071	C1C(OC2=CC(=CC(=C2C1=O)O)O)C3=CC=CC=C3
18	(-)-7,4'-Dihydroxy-5-methoxyflavanone	$C_{16}H_{14}O_5$	188424	COC1=CC(=CC2=C1C(=O)CC(O2)C3=CC=C(C=C3)O)O
19	(-)-4-Hydroxypanduratin A	$C_{25}H_{28}O_4$	25023022	CC1=CCC(C(C1CC=C(C)C)C(=O)C2=C(C=C(C=C2O)O)O)C3=CC=CC=C3
20	5,6-Dehydrokawain	$C_{14}H_{12}O_3$	5273621	COC1=CC(=O)OC(=C1)C=CC2=CC=CC=C2
21	(-)-6-Geranylpinocembrin	$C_{25}H_{28}O_4$	129864322	CC(=CCCC(=CC(=O)C1=C(C2=C(C=C1O)OC(CC2=O)C3=CC=CC=C3)O)C)C
22	(-)-Panduratin A	$C_{26}H_{30}O_4$	6483648	CC1=CCC(C(C1CC=C(C)C)C(=O)C2=C(C=C(C=C2O)OC)O)C3=CC=CC=C3
23	(-)-Pinocembrin	$C_{15}H_{12}O_4$	667544	C1C(OC2=CC(=CC(=C2C1=O)O)O)C3=CC=CC=C3
24	(-)-(2R)-8-Geranylpinostrobin	$C_{26}H_{30}O_4$	23656470	CC(=CCCC(=CCC1=C(C=C(C2=C1OC(CC2=O)C3=CC=CC=C3)O)OC)C)C
25	(2S)-6-Geranylpinostrobin	$C_{26}H_{30}O_4$	44444914	CC(=CCCC(=CCC1=C(C=C2C(=C1O)C(=O)CC(O2)C3=CC=CC=C3)OC)C)C
26	Geranyl-2,4-dihydroxy-6-phenethylbenzoate	$C_{25}H_{30}O_4$	23656469	CC(=CCCC(=CCOC(=O)C1=C(C=C(C=C1O)O)CCC2=CC=CC=C2)C)C

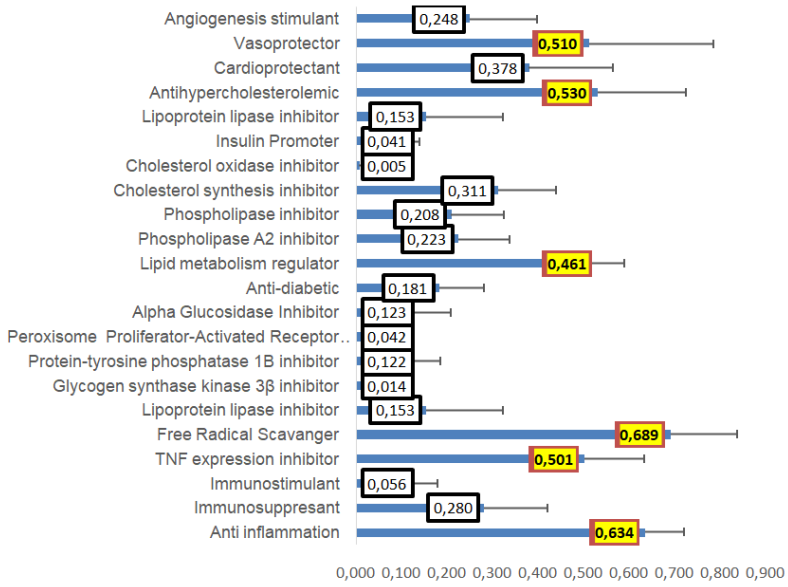

Figure 1. Biological activities of the active compounds of *Boesenbergi apandurata* analyzed using the QSAR approach

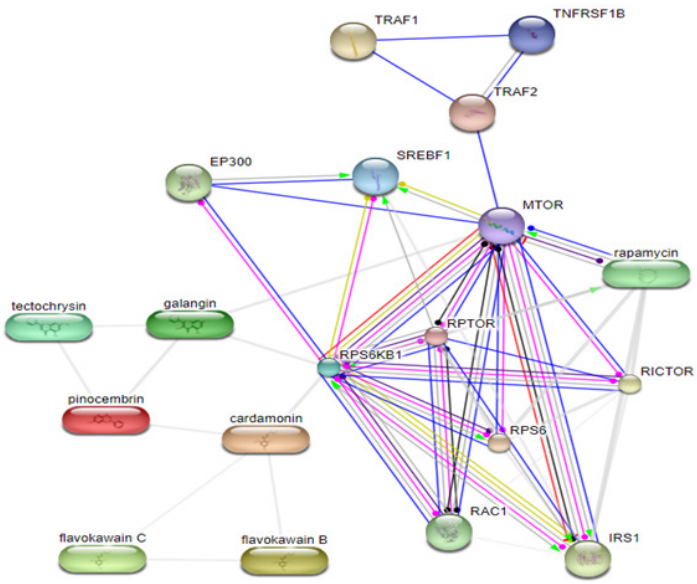

Figure 2. Networking analysis of interactions between active compounds and several proteins

resistance, as demonstrated by the amino acid Ser307 in the insulin receptor substrate 1 (IRS-1) protein, which has been identified as the location where the inhibitory action of TNF-α takes place. Also, it has been found that TNF-α administration in mice results in increased expression of protein-tyrosine phosphatase (PTP) 1B. PTP1B is a negative regulator of insulin signaling [15]. In the QSAR study, the active compounds of *B. pandurata* showed strong potential as a TNF-α expression inhibitor, indicating their prospect for use in therapy for obesity.

3.2. Interactions between the active compounds and ribosomal protein S6 kinase (RPS6KB1) and TNF-alpha

The results of the networking analysis showed that five compounds, namely pinocembrin, cardamonin, flavokawain B, flavokawain C, and tectochrysin, had direct interactions with RPS6KB1 (Figure 2), which acts as the downstream of the mammalian target of

rapamycin (mTOR) signaling that responds to growth factors and nutrients to induce cell proliferation. Although the pathway is not defined yet, it is likely that these five compounds bind to the RPS6KB1 when administered to the cells system. Furthermore, hypoinsulinemia, glucose intolerance, and reduced beta cells have been observed in S6K1-deficient mice. S6K1 is responsible for controlling glucose release and keeping it at normal levels. Furthermore, S6K1 is a key regulator of insulin resistance in conditions of excess nutrients [16]. Research has shown that S6K-deficient mice exhibit a protective ability from the pathological effects of dietary lipids and suggested that the S6K pathway in skeletal muscle acts as the target in metabolic diseases treatments [17]. Furthermore, it is known that RPS6KB1 has transcriptional regulations (yellow lines in Figure 2) and posttranslational modifications (purple lines) with sterol regulatory element-binding transcription factor 1 (SREBF1), which is a protein that regulates cholesterol production, biosynthesis, and lipid homeostasis. In addition, the interaction between the TNF receptor and SREBF1 is mediated by mTOR, i.e., a catalytic subunit that plays a role in the regulation of growth and catabolic processes andresponse to nutrients in the body [18]. Interaction scores of nodes 1 and nodes 2 based on the network analysis is shown in Table 2.

Gene ontology data in the search tool for interactions of chemicals (STITCH) showed that proteins involved in the interaction networks were associated with TNF binding, mTOR signaling, insulin response, and several pathways related to the mTOR signaling pathway, nonalcoholic fatty liver diseases (NAFLDs), and type 2 diabetes mellitus (Figures 3-5).

mTOR is a major regulator of adipogenesis and lipid metabolism, including lipogenesis and lipolysis. It is also known that mTORC2 is a key regulator of lipid metabolism, lipogenesis in the liver, lipolysis in white adipose tissue, and adipogenesis. In other words, inhibition of mTORC2 has the potential to treat obesity and NAFLDs [19]. Protein-protein interaction analysis was then performed to predict proteins that could be targeted by pinocembrin, galangin 3,7-dimethyl ether, cardamonin, flavokawain B, flavokawain C, and tectochrysin. Proteins from SEA Target and STITCH were used to draw the interactions. Proteins involved in a particular pathway were each visualized in colors representing their respective biological processes. The data showed

Table 2. Interaction scores of nodes 1 and 2 based on the network analysis

Node1	Node2	Combined score
RPS6	mTOR	0.995
SREBF1	RPS6KB1	0.891
RPS6KB1	Cardamonin	0.800
MTOR	TRAF2	0.743
MTOR	Galangin	0.734
RPS6KB1	Galangin	0.700
SREBF1	IRS1	0.663
Pinocembrin	Tectochrysin	0.651
Cardamonin	FlavokawainB	0.609
Galangin	Tectochrysin	0.578
Pinocembrin	Cardamonin	0.577
FlavokawainB	FlavokawainC	0.529
Cardamonin	FlavokawainC	0.496

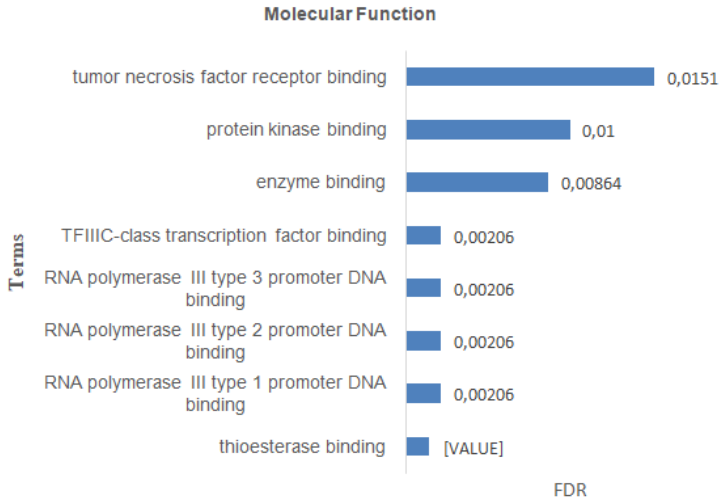

Figure 3. Molecular function terms of the proteins in the protein-active compound interaction networks obtained from STITCH

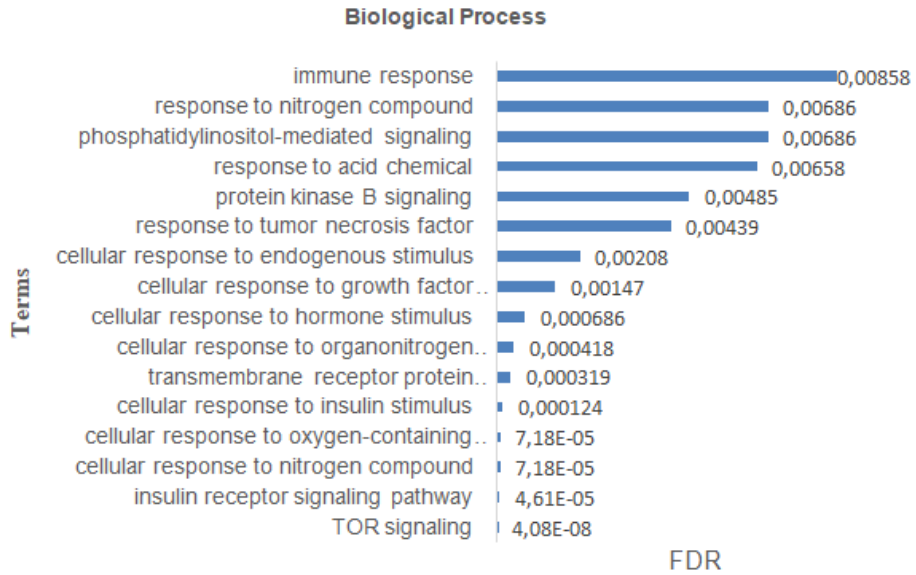

Figure 4. Biological functions of the proteins involved in the protein-active compound interaction networks obtained from STITCH

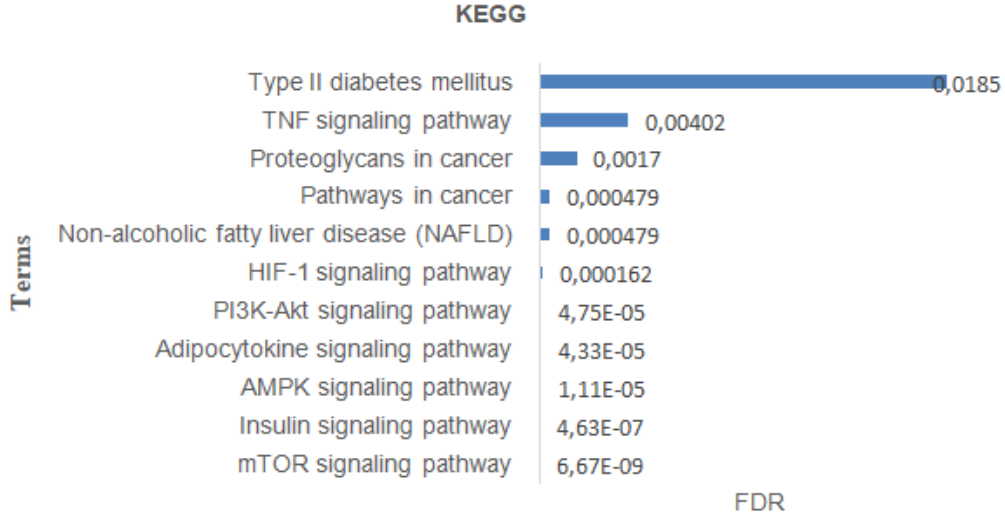

Figure 5. KEGG pathways of the proteins involved in the protein-active compound interaction networks obtained from STITCH

that the target proteins regulated inflammatory responses, responses to stress, and lipid biosynthetic and metabolic processes related to obesity (Figure 6). Obesity is a chronic low-grade inflammatory and metabolic disease. Unbalanced products of cytokines can be a factor in obesity, and chronic inflammation contributes to lipid storage function [20].

3.3. Molecular docking analysis

After obtaining the pathways that might occur when the active compounds administered to human cells activate RPS6KBI, virtual screening was conducted to estimate the interaction's intensity between the active compounds and RPS6KB1. Pinocembrin exhibited the highest binding affinity of -7.26 kcal/mol, although not as strong asthat of the control ligand (FS9). Tectochrysin had the second-highest affinity, i.e.,-6.98 kcal/mol (Table 3, Figure 7). The affinity linkages of the other three compounds, i.e. , cardamonin, flavokawain B, and flavokawain C, were nearly identical. Visualization of the molecular interactions occurring in the protein-ligand complexes: (A) S6KB1-FS9, (B) S6KB1-Pinocembrin, (C) S6KB1-Tectochrysin, (D) S6KB1-Flavokawain C, (E) S6KB1-Flavokawain B, (F) S6KB1-Cardamonin, and (G) S6KB1-Galanginis shown in Figure 8.

The visualization of the interactions and the amino acids involved showed that six compounds, namely cardamonin, flavokawain B, flavokawain C, tectochrysin, pinocembrin, and galangin had the potential to inhibit the activity of RPS6KB1, thus affecting SREBF1 and suppressing cholesterol biosynthesis. Also, the amino acids involved in the docking of the five key junction compounds to RPS6KB1 had several major binding sites similar to FS9, including LEU97, GLY98, LYS99, GLY100, TYR102, GLY103, LYS104, VAL105, LYS123, VAL124, LEU125, TYR174, MET225, and THR235. In addition, amino acids in the docking complexes

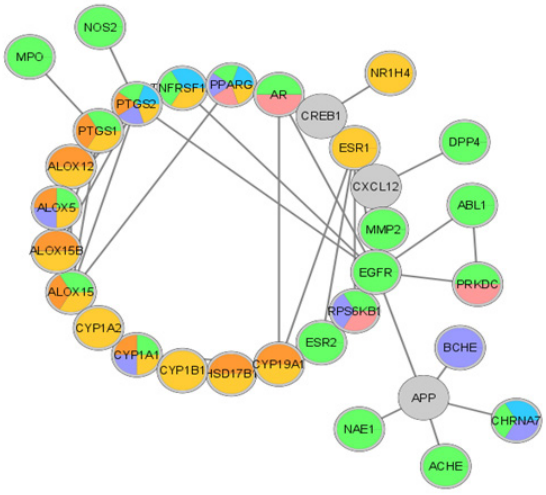

Figure 6. Target proteins of the active compounds of *Boesenbergia pandurata*

is shown in Table 4.

3.4. Absorption, Distribution, Metabolism, and Excretion (ADME) Analysis

The ADME analysis used Lipinski's rule of five (Ro5), which isa prediction tool used to decide how a compound meets the pharmacological requirements for an oral drug that enters the circulation and can have an active effect. Its parameters determine whether or not a molecule can be adequately absorbed by the body. The ADME prediction analysis revealed that all

Table 3. Binding affinity score of the active compounds and S6KB1

Receptor	Ligand	Binding Energy (kcal/mol)	RMSD
Cortikosteroid	Cardamonin	-6.23	16.38
	Flavokawain B	-6.28	16.72
	Flavokawain C	-6.21	16.68
	Tectochrysin	-6.98	18.17
	Pinocembrin	-7.26	19.44
	Galangin 3,7-dimethyl ether	-6.77	18.98
	FS9	**-8.05**	**0.74**

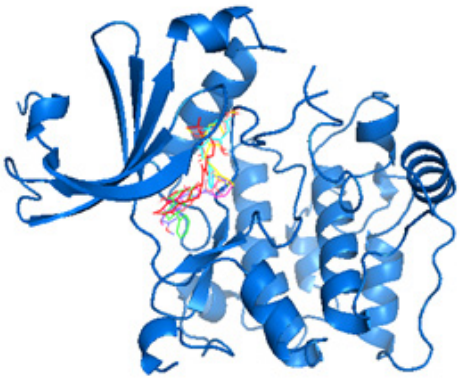

Figure 7. Visualization of ligand-protein complexes produced from docking S6KB1 (blue, ribbon) to pinocembrin (green, stick), cardamonin (cyan, stick), tectochrysin (magenta, stick), flavokawain B (yellow, stick), flavokawain C (salmon, stick), galangin (purple, stick), and FS9 (red, stick)

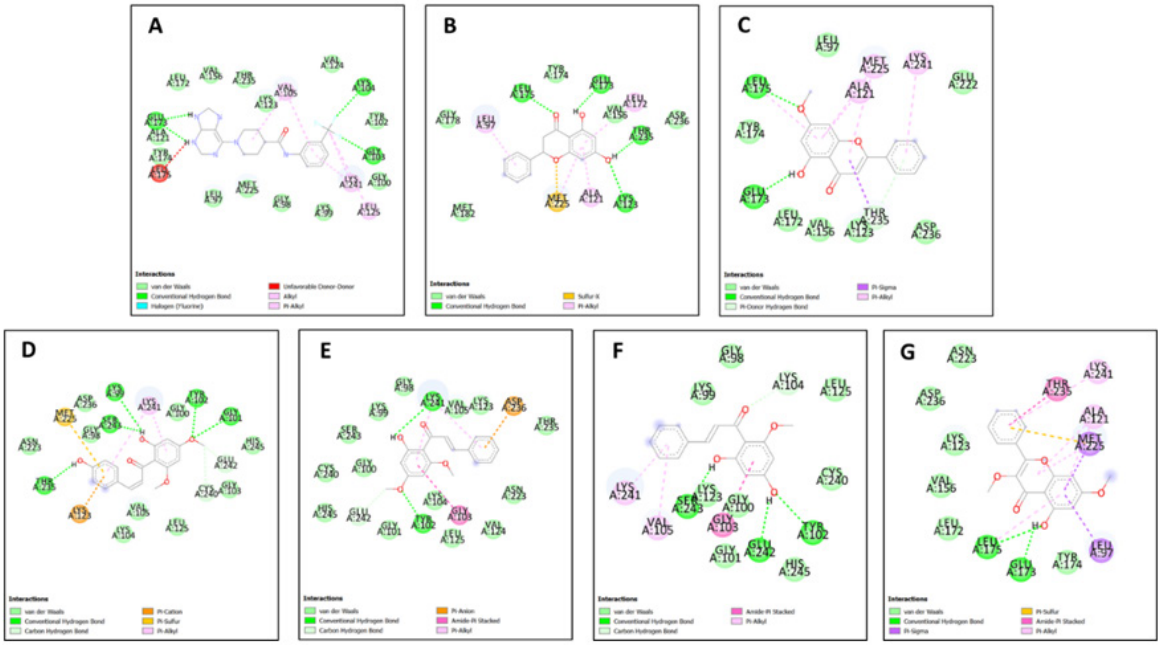

Figure 8. Visualization of the molecular interactions occurring in the protein-ligand complexes: (A) S6KB1-FS9, (B) S6KB1-Pinocembrin, (C) S6KB1-Tectochrysin, (D) S6KB1-Flavokawain C, (E) S6KB1-Flavokawain B, (F) S6KB1-Cardamonin, and (G) S6KB1-Galangin

Table 4. Amino acids involved in the docking complexes

Complex	Interaction*
S6KB1-FS9	Van der Waals: ALA121, TYR174, LEU97, MET225, GLY98, LYS99, GLY100, TYR102, VAL124, LYS123, THR235, LEU172
S6KB1-Pinocembrin	Van der Waals: GLY178, MET182, TYR174, VAL156, ASP236
S6KB1-Tectochrysin	Van der Waals:LEU97, TYR174, LEU172, VAL156, LYS123, THR235, ASP236, GLU222 Hydrogen bond: LEU175, GLU173 Alkyl:MET225, ALA121, LYS241
S6KB1-Flavokawain C	Van der Waals: ASN223, GLY98, ASP236, GLY100, HIS245, GLU242, GLY103, CY240, LEU125, LYS104, VAL105 Hydrogen bond: SER243, LYS99, TYR102, GLY101, THR235 Alkyl: LYS241
S6KB1-Flavokawain B	Van der Waals: SER243, CYS240, HIS245, GLU242, GLY101, LYS99, GLY98, VAL105, LYS123, THR235, ASN223, VAL124, LEU125 Hydrogen bond: LYS241, TYR102 Alkyl:GLY103
S6KB1-Cardamonin	Van der Waals:GLY98, LYS99, LYS104, LEU125, LYS123, GLY100, GLY101, HIS245, CYS240 Hydrogen bond: SER243, GLU242, TYR102 Alkyl: LYS241, VAL105, GLY103
S6KB1-Galangin 3,7-dimethyl ether	Van der Waals: ASN223, ASP236, LYS123, VAL156, LEU172, TYR174 Hydrogen bond: LEU175, GLU173 Alkyl: LYS241, ALA121

*Blue letters indicate binding sites observed in complex and control (FS9)

Table 5. Predicted drug-likeness of the active compounds of *Boesenbergia pandurata*

Molecule	MW	Rotatable bonds	H-bond acceptors	H-bond donors	Molar refracity	Consensus Log P	GI absorption	Lipinski'srule of 5	Predicted LD_{50} (mg/kg)	Predicted toxicity class
Tectochrysin	**268.26**	**2**	**4**	**1**	**76.44**	**2.95**	**High**	**Yes**	**3919**	**5**
Galangin 3,7-dimethyl ether	298.29	3	5	1	82.93	2.87	High	Yes	3919	5
Cardamomin	270.28	4	*4*	2	76.79	2.6	High	*Yes*	3000	5
Flavokawain B	284.31	5	4	1	81.26	3.06	High	Yes	3000	5
Flavokawain C	300.31	5	*5*	2	83.28	2.68	High	*Yes*	3000	5
(-)-Pinocembrin	256.25	1	4	2	69.55	2.26	High	Yes	2000	4

the compounds used for the network analysis and molecular docking had high or excellent absorption in the gastrointestinal intestine (GI) tract and passedthe Ro5. Predicted drug-likeness of the active compounds of *Boesenbergia panduratais* shown in Table 5.

Toxicity class is a non-toxic class of substances; the higher class a compound is categorized into, the less hazardous it is (Class 6 Classification). However, if these substances are taken in excess of the permissible limit, they will be hazardous. The LD_{50}, or median lethal dosage, is the level at which 50% of the test subject dies after being exposed to the treatment chemical. Acute toxicity refers to the negative effects of a chemical as a result of single or multiple exposures in a short duration (e.g., less than 24 hours). Structure similarity analysis and identification of toxic fragments in 38,000 distinct compounds with known oral LD_{50} values observed in rats were used to predict acute

oral toxicity. The Globally Harmonized System of Classification and Labeling of Chemicals (GHS) was used for the compound categorization. Based on these parameters, it was found that all the compounds used for the network analysis and molecular docking were non-toxic.

4. Conclusion

The active compounds of *Boesenbergia pandurata* have several biological activities with the greatest potential: vasoprotective (0.510), anti-inflammatory (0.634), anti-hypercholesterolemic (0.530), free radical scavenging (0.689), and as a TNF expression inhibitor (0.501) and lipid metabolism regulator (0.461). Also, RPS6KB1 has been found as the main protein target of the compounds. This protein is the downstream of mTOR that plays a role in insulin resistance, imports long-chain fatty acids into the cells, and regulates glucagon secretion, glucose metabolism, and responses to nutrients. It can be concluded that the active compounds of *B. pandurata* have the ability to improve metabolic syndrome, especially obesity, *in silico.*

Acknowledgement

The authors are thankful to the Research and Community Services Institute (LPPM) of Universitas Pembangunan Nasional Veteran Jakarta for supporting this research.

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