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Phytoconstituents Analysis and Anti-Diabetic Potential of *Sembung* Leaf Extract (*Blumea balsamifera* L.) through Inhibition of NF-KB p65, GLP-1, and DPP-4 Proteins with *In-Silico* Approaches

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Traditional herbal remedies have an important role in human health. Empirically, *Blumea balsamifera* is often used as a traditional beverage to alleviate fever symptoms, lower cholesterol levels, and maintain body immunity. The purpose of this study was to discover the phytoconstituent profile that contributes to the anti-diabetic properties of *B. balsamifera* leaf extract (BBLE) using in silico approaches.LCMS/MS was used to identify the constituent profile of BBLE, and the ability of these compounds against diabetes-related proteins was analyzed computationally.Three proteins related to diabetes are NF-KB p65, GLP-1, and DPP-4. A total of 18 compounds were successfully identified through LCMS/MS, including 4 compounds known to be flavonoid derivatives and can be used as markers of BBLE. Pheophorbide A and 1,1-Cyclopentanediacetic acid were reported for the first time to inhibit the NF-KB p65, GLP-1, and DPP-4 proteins in docking simulation studies. Based on these findings, it can be confirmed that the bioactive compounds in BBLE show strong inhibitory potential against anti-diabetic proteins.

Keywords: Antidiabetic activity; Blumea balsamifera; In Silico; LCMS/MS; Phytoconstituent.

Diabetes mellitus is a widespread metabolic condition in which glucose levels in the blood rise due to beta cells' inability of producing enough insulin (Type-1) or ineffective

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release of insulin (Type-2). Both are caused by insulin resistance, production issues, and alter the way proteins, lipids, and carbohydrates are synthesized[1]. Diabetes raises the risk of amputation, renal failure, stroke, cardiovascular disease, and lifelong blindness.Diabetes is influenced by genetic and environmental variables, and the incidence rate varies among ethnic groups and specific communities[2]. According to estimates, diabetes affected about 463 million people globally in 2019[3].

Interestingly, urban areas have a higher incidence rate of 10.8% compared to rural areas at 7.2%. Similarly, high-income nations have a greater frequency of 10.4% than nations with low incomes at 4% [3]. Despite the fact that extensive research has been conducted to control diabetes and a number of oral medications, gene therapies, and stem cell therapies have been successfully implemented, the development of new diabetes medications through the search for chemical compounds with specific bioactive features that help manage glucose homeostasis and improve insulin sensitivity is necessary[4].

Antioxidants have an important function in the treatment of disorders like diabetes mellitus [5]. This is because the formation of free radicals in cells due to oxidative stress exposure has been linked to the occurrence of diabetes, especially Type-2[6]. Natural compounds have attracted considerable attention over the past few decades for their biological and pharmacological properties as sources of antioxidants[7]. Natural compounds are widely known to provide anti-inflammatory, anti-cancer, anti-bacterial, anti-fungal, and anti-viral activities[8]. Since they have been scientifically proven to exhibit hypoglycemic activity, these active compounds are of great interest for exploration in the development of new diabetes medications.

Indonesia is a center of mega-biodiversity for medicinal plants in the Asia region, and their traditional use is employed by many ethnic community societies across various regions in Indonesia to support their health needs[9]. The Sembung plant (*Blumea balsamifera*) is one of the plants used in traditional medicine practices as an anti-hypercholesterol[10], anti-bacterial[11], anti-cancer[12], anti-neuroinflammation[13], gastroprotectant[14], and as a source of natural antioxidants[15]. Empirically, in the Bali Province, *B. balsamifera* can be processed into a health drink known as "Loloh," which is considered capable of maintaining immune function. Previous reports revealed that the extract of *B. balsamifera* (BBLE) contains a profile of secondary metabolites such as flavonoids, saponins, phenols, tannins, and steroids[16]. Apart from Indonesia, several regions around the world also utilize plants from the Blumea species as traditional medicine. For example, in China, dried preparations of *Blumea riparia* have been standardized and used to treat irregular menstruation, postpartum hemorrhage, infertility, and vulva wounds. These plants are also commercialized because their phenolic compounds, flavonoids, acetylenes, and sesquiterpenes are considered to play a crucial role in health[17,18].

However, so far, reports on the metabolite profile of BBLE aimed at anti-diabetic purposes are very limited. Phytochemical screening of *Blumea spp.* is crucial, given their wide abundance in the wild, and the morphological similarity of the *B. balsamifera* species with other *Blumea spp.* such as *B. megacephala* and *B. riparia*[19]. As a result, the aim of this research is to identify the secondary metabolites of BBLE using High Performance Liquid Chromatography/Mass Spectrometry (HPLC/MS) investigations and discover its anti-diabetic mechanism by in *silico analysis*. This study is valuable for developing new biologically active molecules, especially for controlling diabetes through the inhibition of related proteins.

MATERIALS AND METHODS

Preparation of crude extract

Fresh *Sembung* (*B. balsamifera*) leaves were collected from a plantation in Luwus Village, Tabanan Regency, Bali Province. Types of *B. balsamifera* L. (DC.) leaf samples were determined at the Bali Botanical Gardens, National Research and Innovation Agency (BRIN) with Sample Registration Number B. 206/IPH.7/AP/VIII/2020. The same kind of extract had been used in our previous publication[16]. *Sembung* leaves were then cleaned of organic material or contaminants using running water several times. The samples were then air-dried at room temperature to reduce moisture content and subsequently dried for 24 hours at

50! using an oven, resulting in dried samples or simplicia for further processing. The simplicia were then ground using a blender and sieved through a 20 mesh screen to obtain a powdered sample.

A total of 250 grams of sembung leaf powder preparation was weighed and moistened using 70% ethanol solvent, then left for 4 hours in a glass container covered with sterile gauze and wrapped in plastic. The maceration process was carried out for 24 hours, and during this process, stirring was performed to evenly extract the metabolites. The macerate was then separated by filtration method using sterile flannel cloth. The filtration process was performed three times with the same type and amount of solvent.The collected macerate was evaporated using a vacuum rotary evaporator at 40! and 100 rpm until a viscous extract was produced.This viscous extract of *sembung*, referred to as BBLE in this study, was adjusted according to the standards of the Indonesian Herbal Pharmacopoeia of 2017, which includes a yield of not less than 10.6%, flavonoid/ quercetin identity compound (+), moisture content of no more than 14%, and total ash of no more than 6.7%[10,20].

Identification of the phytochemical from BBLE

The chemical profile of BBLE was identified and quantified using the pure compound as a reference by High Performance Liquid Chromatography (HPLC) (LC: ACQUITY UPLC® H-Class System, Waters, USA) and mass spectrometry (MS) (Xevo G2-S QTof, Waters, USA). Briefly, an AC18 column measures 1.8 ìm 2.1 × 100 mm at 50°C (column) and 25°C (ambient temperature). HPLC analysis used a mobile phase of water + 5 mM ammonium formate and acetonitrile + 0.05% formic acid, with a flow rate of 0.2 mL/min running for 23 minutes (mobile phase) and an injection volume of 5 ìL. Mass spectrometer (MS) investigations were carried out using electrospray ionization in positive mode with a mass range of 50–1200 m/z and source and dissolution temperatures of 100 and 350°C. The conical gas and dissolution flows were 0 L/h and 793 L/h, respectively, with collision energies ranging from 4 to 60 eV. MassLynx software version 4.1 was used for data collection, analysis, and instrument control[21–23].

***In silico* analysis**

Molecular docking analysis

The phytochemical profile, consisting of 18 compounds detected by BBLE extract analysis with HPLC/MS, was used in molecular docking studies. The PubChem database was utilized to obtain compound names, PubChem IDs, molecular weights, and structures of the obtained compounds[24]. ADMET profiling was carried out for assessing the pharmacokinetic characteristics of the compounds utilizing online pkCSM and Swiss ADME [25–27].The 3D structures of each compound were downloaded in .sdf format, and the respective proteins were obtained from the RCSB PDB website. NF-kB p65, Glucagon-like peptide (GLP-1), and Dipeptidyl peptidase-4 (DPP-IV) were identified as potential target receptors. Analysis was performed using PyRx 0.8 software with specific coordinates corresponding to the active site of each protein. The strength of the bond between the ligand and protein was measured based on binding energy and RMSD from the molecular docking results. The smaller/negative the binding energy value, the more stable the bond between the ligand and protein.

Ligand-Protein Interaction Analysis

The types of chemical bond interactions formed in the ligand-protein complex were further analyzed using Discovery Studio software. This analysis aims to identify the position of the active site and the amino acids that bond with the compounds through hydrogen bonding.

Druglikeness and Toxicity Analysis

The druglikeness analysis of compounds in *sembung* leaves was conducted using the websitehttp://www.swissadme.ch/index.php by copying the Simplified Molecular Input Line Entry System (SMILES) of each compound. Subsequently, the toxicity (LD50) of the compounds was assessed using the websitehttps://pubchem.ncbi.nlm.nih.gov/compound/.

Bioactivity Analysis with PASS Online Server

The bioactivity analysis of compounds contained in *sembung* leaves was performed by copying the Simplified Molecular Input Line Entry System (SMILES) notation of each compound onto the website https://www.way2drug.com/PassOnline/predict.php. The Pa (probability of

activity) and Pi (probability of inactivity) values were determined for each ligand. Finally, only activities involved in diabetes were considered[28].

RESULTS AND DISCUSSION

Chemical profiling

The phytochemical profiling results of BBLE using HPLC/MS revealed the presence of 18 chemical compounds at various retention times (RT) (Table 1). The compound 2,3-Dihydroxypropyl (9Z,12Z,15Z) – 9,12,15-octadecatrienoate ($C_{21}H_{36}O_4$) with an RT of 11.61 had the highest peak area percentage of 20.89%. Similarly, the compound 2-Hexyl-3,5-dipentylpyridine ($C_{21}H_{37}N$) with an RT of 11.80 had a peak area percentage of 20.89%. The BBLE chromatogram in this study is shown in Figure 1 below.

The chemical 2,3-Dihydroxypropyl, also called Octadecatrienoic Acid, is an a link in the production of arachidonic acid and an important component of essential oils in plants [29]. Reports indicate that conjugated Octadecatrienoic Acid can cause a decrease in the viability of LNCaP and PV-3 (human prostate cancer cells) cells, depending on the concentration, but is not toxic to normal human prostate epithelial cells RWPE-1, which are normal epithelial cells[30].

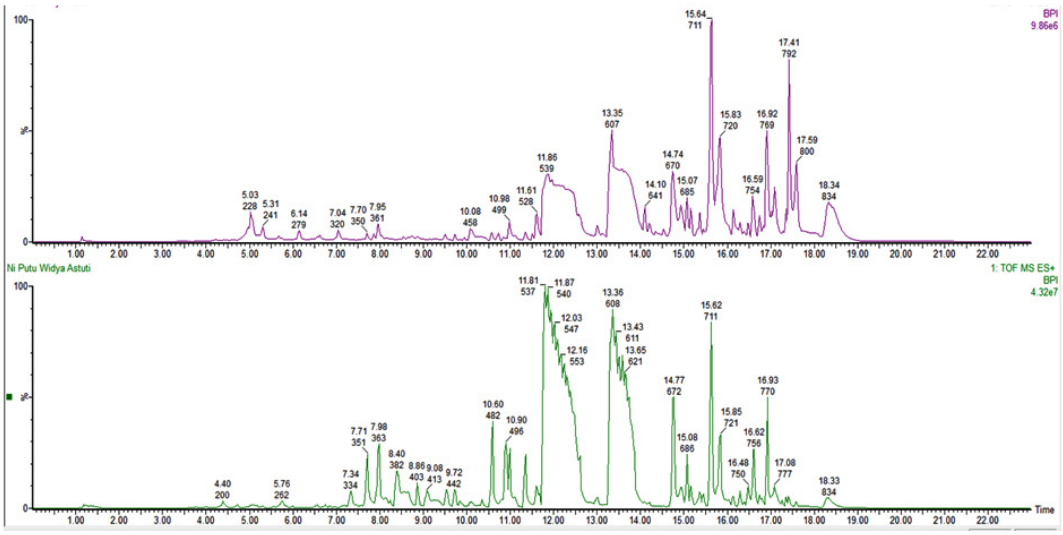

Fig. 1. Chromatogram of peaks from *B. balsamifera* leaf extract (BBLE) analyzed using LCMS/MS

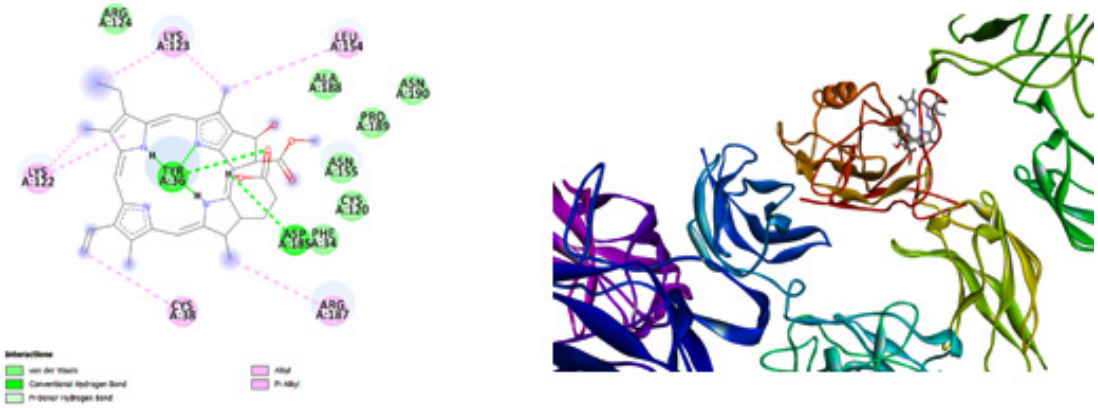

Fig. 2. 2D Interaction and 3D Visualization of the Pheophorbide A Compound with the NF KB-p65 protein (1LE5)

Table 1. Phytoconstituent content of *B. balsamifera* leaf extract (BBLE) characterized by LCMS/MS

Retention Time	m/z Result (M+H) Precursor Ion	Product Ion	Compound Prediction	%IFIT	Peak Area Area	%
7,71	361,0927	346,0688	3',4',5-Trihydroxy-6,7,8-trimethoxyflavone ($C_{18}H_{16}O_8$)	99	3702571	2.08
7,85	132,0817	105,0707, 91,0553	3-Methyl-1H-indol(C_9H_9N)	97	5378427	3.02
8,86	375,1090	342,1925 233,1551	3',5-Dihydroxy-3,4',6,7-tetramethoxyflavone($C_{19}H_{18}O_8$)	100	4368431	2.45
10,59	389,1252	353,2695, 261, 2227, 243,2121	5-Hydroxy-3',4',6,7,8-pentamethoxyflavone($C_{20}H_{20}O_8$)	99	4655888	2.61
11,34	359,1133	343,0828, 316, 2856, 283,0616	3,3',4',7-Tetramethylquercetin ($C_{19}H_{18}O_7$)	99	3238175	1.82
11,61	353,2695	335,2593, 261, 2227, 243,2121	2,3-Dihydroxypropyl (9Z,12Z,15Z)-9,12, 15-octadecatrienoate ($C_{21}H_{36}O_4$)	99	37204892	20.89
11,80	304,3012	212,2384	2-Hexyl-3,5-dipentylpyridine ($C_{21}H_{37}N$)	99	37204892	20.89
12,06	408,2378	318,3161, 304, 3003, 231,1388	2-(2,6-Dimethyl-1-piperidinyl)-2-oxoethyl 3, 4,5-triethoxybenzoate($C_{22}H_{33}NO_6$)	90		17.58
13.36	332,3322	240,2699	N,N-Dimethylpregnan-3-amine($C_{23}H_{41}N$)	100	31302042	4.31
14.10	607,2572	91,0554	(3S)-3-[(2E)-3-Carboxy-2-buten-1-yl]-7-hydroxy-4-methoxy-1,1,8,8,9-pentamethyl-11-(3-methyl-2-buten-1-yl)-6-oxo-3,6,8,9-tetrahydro-1H-difuro[3,2-b:3',4'-h]xanthene – 3-carboxylic acid ($C_{34}H_{38}O_{10}$)	71	7671267	4.31
14,74	360,3631	304,3004	n-benzyloctadecylamine($C_{25}H_{45}N$)	100	7671762	1.63
15,07	609,2714	360,3630	2-[4-(Diphenylmethyl)-1-piperazinyl]ethyl methyl 2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate ($C_{35}H_{36}N_4O_6$)	99	2894592	1.63
15.14	625,2670	609,2716	(1S,5S)-1,5-Anhydro-2,3-di-O-benzyl-4-deoxy-5-(2-methoxy-2-oxoethyl)-1-[(6R,9R)-6-methyl-4,11-dioxo-3,5,10,12-tetraoxapentadec-14-en-7-yn-9-yl]-L-threo-pentitol($C_{34}H_{40}O_{11}$)	99	2895590	6.80
15,62	593,2755	368,4253	Pheophorbide A($C_{35}H_{36}N_4O_5$)	99	12109066	2.30
16,13	593,2759	535,2703, 332, 3317, 304,3007	Bis[2-(4-butoxyphenoxy)ethyl] (4-hydroxybenzylidene)malonate($C_{34}H_{40}O_9$)	95	4089161	2.30
16,28	611,4679	458,4736,	1,1-Cyclopentanediacetic acid($C_{39}H_{62}O_5$)	86	4089110	2.29
16,62	637,3012	-	N'^1,N'^9-Bis[(4-biphenylyloxy)acetyl] nonanedihydrazide($C_{37}H_{40}N_4O_6$)	99	4076512	3.11
16,92	621,3037	486,5033	N-{[(3S)-2-(L-Tyrosyl)-1,2,3, 4-tetrahydro-3-isochinolinyl]methyl}-L-phenylalanyl-L-phenylalanin($C_{37}H_{40}N_4O_5$)	99	5534532	2.08

The compound 2-Hexyl-3,5-dipentylpyridine was also previously found in mango peel waste extract analyzed using LC-MS with a retention time (RT) of 19.266 minutes and a molecular weight of 303.29137 g/mol[31]. This compound is capable of inhibiting the ACE2 receptor, which is a membrane protein on alveolar cells acting as an entry point for viruses into the human body[32–34]. The compound 2-Hexyl-3,5-dipentylpyridine was successfully identified in

Table 2. Samples of ligand compounds from BBLE accessed from the PubChem database and SMILES notation

No.	Compounds	PubChem ID	SMILE
1	3',4',5'-Trihidroxy-6,7,8-trimethoxyflavone	6453535	COC1=C(C=CC(=C1)C2=C(C(=O)C3=C(C(=C(C=C3O2)OC)OC)O)O)O
2	3-Methyl-1H-indole	6736	CC1=CNC2=CC=CC=C12
3	3',5-Dihidroxy-3,4',6,7-tetramethoxyflavone	5459184	COC1=C(C=CC(=C1)C2=C(C(=O)C3=C(O2)C(=C(C=C3O)OC)OC)OC)O
4	5-Hidroxy-3',4',6,7,8-pentamethoxyflavone	183466	COC1=C(C=C(C=C1)C2=CC(=O)C3=C(O2)C(=C(C(=C3OC)OC)OC)OC)O
5	3,3',4',7-tetramethylquercetin	5352005	COC1=C(C=C(C=C1)C2=C(C(=O)C3=C(C=C(C=C3O2)OC)O)OC)OC
6	2,3-Dihydroxypropyl)9Z,12Z,15Z)-9, 12,15-octadecatrienoate	5367328	CCC=CCC=CCC=CCCCCCCCC(=O)OCC(CO)O
7	2-Hexyl-3,5-dipentylpyridine	6430301	CCCCCCC1=C(C=C(C=N1)CCCCC)CCCCC
8	2-(2,6-Dimethyl-1-piperidinyl)-2-oxoethyl 3,4,5-triethoxybenzoate		
9	N,N-Dimethylpregnan-3-amine	22214757	CCC1CCC2C1(CCC3C2CCC4C3(CCC(C4)N(C)C)C)C
10	(3S)-3-[(2E)-3-Carboxy-2-buten-1-yl]-7-hydroxy-4-methoxy-1,1,8,8,9-pentamethyl-11-(3-methyl-2-buten-1-yl)-6-oxo-3,6,8,9-tetrahydro-1H-difuro[3,2-b:3',4'-h)xanthene-3-carboxylic acid	162879284	CC1C(C2=C(O1)C(=C(C3=C2OC4=C5C(=C(C=C4C3=O)OC)C(OC5(C)C)(CC=C(C)C(=O)O)C(=O)O)O)CC=C(C)C)(C)C
11	n-benzyloctadecylamine	88404	CCCCCCCCCCCCCCCCCCCNCC1=CC=CC=C1
12	2-[4-(Diphenylmethyl)-1-piperazinyl] ethyl methyl 2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate	128529	CC1=C(C(=C(C(=N1)C)C(=O)OCCN2CCN(CC2)C(C3=CC=CC=C3)C4=CC=CC=C4)C5=CC(=CC=C5)[N+](=O)[O-])C(=O)OC
13	(1S,5S)-1,5-Anhydro-2,3-di-O-benzyl-4-deoxy-5-(2-methoxy-2-oxoethyl)-1-[(6R,9R)-6-methyl-4, 11-dioxo- 3,5,10,12-tetraoxapentadec-14-en-7-yn-9-yl]-L-threo-pentitol	10675413	CCOC(=O)OC(C)C#CC(C1C(C(CC(O1)CC(=O)OC)OCC2=CC=CC=C2)OCC3=CC=CC=C3)OC(=O)OCC=C
14	Pheophorbide A	253193	CCC1=C(C2=NC1=CC3=C(C4=C(C(C(=C5C(C(C(=CC6=NC(=C2)C(=C6C)C=C)N5)C)CCC(=O)O)C4=N3)C(=O)OC)O)C)C
15	Bis[2-(4-butoxyphenoxy)ethyl] (4-hydroxybenzylidene)malonate	43836111	CCCCOC1=CC=C(C=C1)OCCOC(=O)C(=CC2=CC=C(C=C2)O)C(=O)OCCOC3=CC=C(C=C3)OCCCC
16	1,1-Cyclopentanediacetic acid	473107	CC(=C)C1CCC2(C1C3CCC4C5(CCC(C(C5CCC4(C3(CC2)C)C)(C)C)O)C)COC(=O)CC6(CCCC6)CC(=O)O
17	N'1,N'9-Bis[(4-biphenylyloxy) acetyl]nonanedihydrazide	66554672	CC1=C(N=C(C(=N1)C)COC2=C(C=C(C=C2)C=CC(=O)CC(=O)C=CC3=CC(=C(C=C3)OCC4=NC(=C(N=C4C)C)C)OC)OC)C
18	N-{[(3S)-2-(L-Tyrosyl)-1,2,3,4-tetrahydro -3-isochinolinyl]methyl}-L-phenylalanyl-L-phenylalanin	5311481	C1C(N(CC2=CC=CC=C21)C(=O)C(CC3=CC=C(C=C3)O)N)CNC(CC4=CC=CC=C4)C(=O)NC(CC5=CC=CC=C5)C(=O)O

the extract of the tuber of *Merremia mammosa* with bioactivities as an antiviral, antioxidant, anti-inflammatory, and anti-tuberculosis agent[35].

In addition to those three compounds, there are phytochemical compounds identified in BBLE that belong to the flavonoid group, such as 3',4',5-Trihydroxy-6,7,8-trimethoxyflavone (TTF); 3',5-Dihydroxy-3,4',6,7-tetramethoxyflavone (TMF); 5-Hydroxy-3',4',6,7,8-pentamethoxyflavone (PMF); and 3,3',4',7-Tetramethylquercetin (TMQ). Compounds from the flavonoid group have been reported as standardization markers for BBLE phytochemistry and are known for their role as sources of natural antioxidants with various important bioactivities[10,36]. Similar research revealed that the compound 3,5,42 -trihydroxy-6,7,32 -trimethoxyflavone (TTF) isolated from *Achillea fragrantissima* extract could prevent cell damage due to oxidative stress and inhibit protein phosphorylation that signals cells, including the mitogen-activated protein kinase (MAPK) family[37]. Compounds from this flavonoid group were also found in *Loranthus acutifolius* extract with antityrosinase bioactivity[38].

The compound 3',5-Dihydroxy-3,4',6,7-tetramethoxyflavone (TMF), also known as Casticin, is a bioactive compound that can be found in various parts of plants. Casticin's therapeutic properties include anti-tumor, anti-inflammation, neuroprotective activity, and natural analgesic[39].

Table 3. Molecular Docking analysis of the compounds contained in BBLE against NF protein KB-p65 (1LE5)

No.	Compounds	Protein	Binding Affinity (Kcal/mol)	RMSD (Å)
1	3',4',5'-Trihidroxy-6,7,8-trimethoxyflavone	NF KB-p65	-6.7	0
2	3-Methyl-1H-indole	(1LE5)	-4.8	0
3	3',5-Dihidroxy-3,4',6,7-tetramethoxyflavone		-6.4	0
4	5-Hidroxy-3',4',6,7,8-pentamethoxyflavone		-5.9	0
5	3,3',4',7-tetramethylquercetin		-6.3	0
6	2,3-Dihydroxypropyl)9Z,12Z,15Z)-9,12, 15-octadecatrienoate		-5.1	0
7	2-Hexyl-3,5-dipentylpyridine		-4.8	0
8	2-(2,6-Dimethyl-1-piperidinyl)-2-oxoethyl 3, 4,5-triethoxybenzoate			
9	N,N-Dimethylpregnan-3-amine		-6.9	0
10	(3S)-3-[(2E)-3-Carboxy-2-buten-1-yl]-7-hydroxy-4-methoxy-1,1,8,8,9-pentamethyl-11-(3-methyl-2-buten-1-yl)-6-oxo-3,6,8,9-tetrahydro-1H-difuro[3,2-b:3',4'-h)xanthene-3-carboxylic acid			
11	n-benzyloctadecylamine		-3.7	0
12	2-[4-(Diphenylmethyl)-1-piperazinyl]ethyl methyl 2, 6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate		-7.5	0
13	(1S,5S)-1,5-Anhydro-2,3-di-O-benzyl-4-deoxy-5-(2-methoxy-2-oxoethyl)-1-[(6R,9R)-6-methyl-4, 11-dioxo- 3,5,10,12-tetraoxapentadec-14-en-7-yn-9-yl]-L-threo-pentitol			
14	Pheophorbide A		-8.1	0
15	Bis[2-(4-butoxyphenoxy)ethyl] (4-hydroxybenzylidene)malonate		-5.9	0
16	1,1-Cyclopentanediacetic acid		-7.1	0
17	N'1,N'9-Bis[(4-biphenylyloxy) acetyl]nonanedihydrazide		-6.8	
18	N-{[(3S)-2-(L-Tyrosyl)-1,2,3,4-tetrahydro-3-isochinolinyl]methyl}-L-phenylalanyl-L-phenylalanin		-6.0	0

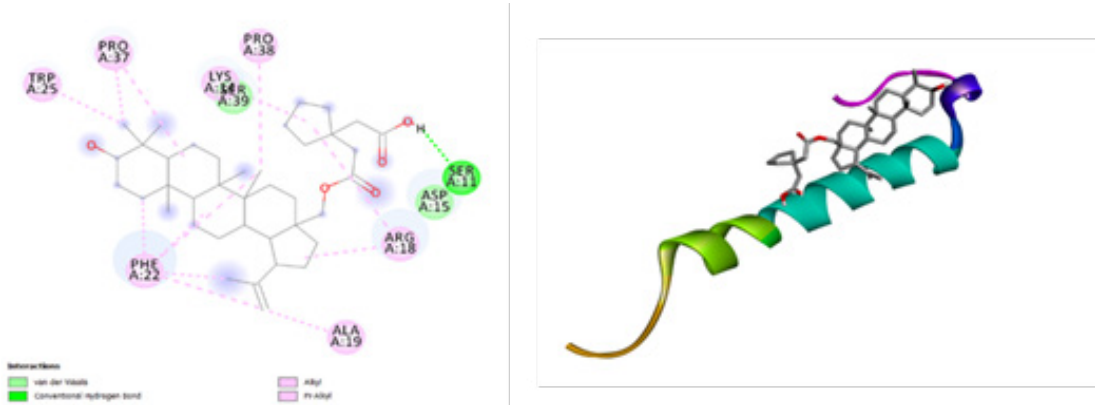

Fig. 3. 2D Interaction and 3D Visualization of Compound 1,1-Cyclopentanediacetic acid contained in BBLE with GLP 1 protein (5NIQ)

Table 4. Molecular Docking analysis of the compounds contained in BBLE against GLP 1 (5NIQ) protein

No.	Compounds	Protein	Binding Affinity (Kcal/mol)	RMSD (Å)
1	3',4',5'-Trihidroxy-6,7,8-trimethoxyflavone	GLP 1 (5NIQ)	-5.6	0
2	3-Methyl-1H-indole		-4.8	0
3	3',5-Dihidroxy-3,4',6,7-tetramethoxyflavone		-5.5	0
4	5-Hidroxy-3',4',6,7,8-pentamethoxyflavone		-5.3	0
5	3,3',4',7-tetramethylquercetin		-5.6	0
6	2,3-Dihydroxypropyl)9Z,12Z,15Z)-9,12,15-octadecatrienoate		-4.6	0
7	2-Hexyl-3,5-dipentylpyridine		-4.7	0
8	2-(2,6-Dimethyl-1-piperidinyl)-2-oxoethyl 3,4,5-triethoxybenzoate			
9	N,N-Dimethylpregnan-3-amine		-6.8	0
10	(3S)-3-[(2E)-3-Carboxy-2-buten-1-yl]-7-hydroxy-4-methoxy-1,1,8,8,9-pentamethyl-11-(3-methyl-2-buten-1-yl)-6-oxo-3,6,8,9-tetrahydro-1H-difuro[3,2-b:3',4'-h)xanthene-3-carboxylic acid			
11	n-benzyloctadecylamine		-3.7	0
12	2-[4-(Diphenylmethyl)-1-piperazinyl]ethyl methyl 2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate		-6.7	0
13	(1S,5S)-1,5-Anhydro-2,3-di-O-benzyl-4-deoxy-5-(2-methoxy-2-oxoethyl)-1-[(6R,9R)-6-methyl-4,11-dioxo- 3,5,10,12-tetraoxapentadec-14-en-7-yn-9-yl]-L-threo-pentitol			
14	Pheophorbide A		-6.3	0
15	Bis[2-(4-butoxyphenoxy)ethyl] (4-hydroxybenzylidene)malonate		-4.8	0
16	1,1-Cyclopentanediacetic acid		-7.2	0
17	N'1,N'9-Bis[(4-biphenylyloxy) acetyl]nonanedihydrazide		-6.6	0
18	N-{[(3S)-2-(L-Tyrosyl)-1,2,3,4-tetrahydro-3-isochinolinyl]methyl}-L-phenylalanyl-L-phenylalanin		-6.7	0

Casticin isolated from *Larrea tridentata* has shown antibacterial activity against *Mycobacterium tuberculosis*, including sensitive and multidrug-resistant strains[40]. The compound 5' – Hydroxy -6, 7,8,3',4'-pentamethoxyflavone (PMF), isolated from the mandarin orange *Citrus reticulata*, has anti-inflammatory properties and modulates immune function. Recent studies also report the effect of this compound in preventing psoriasis, a chronic and benign proliferative skin disease, through the regulation of several gene expressions related to immunity and inflammation[41].

The compound 3,3',4',7-Tetramethylquercetin (TMQ) is one of the parent compounds expected to be found in the phenolic hydroxyl groups within quercetin. The bioactivity demonstrated by TMQ includes acting as an anti-prostate cancer agent through the activation of apoptosis in PC-3 cells[42]. On the other hand, this quercetin derivative is capable of multi-drug resistance as well as human breast cancer cells (MCF-7) by inhibiting the activity of TrxR, which activates cell death through apoptosis[43]. TMQ has also been reported in extracts from *Cissus quadrangularis* extracted with various solvents[44].

Table 5. Molecular docking analysis of the compound contained in BBLE with the DPP-IV protein (5YP1)

No	Compounds	Protein	Binding Affinity (Kcal/mol)	RMSD (Å)
1	3',4',5'-Trihidroxy-6,7,8-trimethoxyflavone	DPP IV	-8.3	0
2	3-Methyl-1H-indole	(5YP1)	-6.2	0
3	3',5-Dihidroxy-3,4',6,7-tetramethoxyflavone		-8.2	0
4	5-Hidroxy-3',4',6,7,8-pentamethoxyflavone		-7.9	0
5	3,3',4',7-tetramethylquercetin		-8.1	0
6	2,3-Dihydroxypropyl)9Z,12Z,15Z)-9,12,15-octadecatrienoate		-5.4	0
7	2-Hexyl-3,5-dipentylpyridine		-5.4	0
8	2-(2,6-Dimethyl-1-piperidinyl)-2-oxoethyl 3,4,5-triethoxybenzoate			0
9	N,N-Dimethylpregnan-3-amine		-8.0	0
10	(3S)-3-[(2E)-3-Carboxy-2-buten-1-yl]-7-hydroxy-4-methoxy-1,1,8,8,9-pentamethyl-11-(3-methyl-2-buten-1-yl)-6-oxo-3,6,8,9-tetrahydro-1H-difuro[3,2-b:3',4'-h)xanthene-3-carboxylic acid			
11	n-benzyloctadecylamine		-5.4	0
12	2-[4-(Diphenylmethyl)-1-piperazinyl] ethyl methyl 2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate		-8.7	0
13	(1S,5S)-1,5-Anhydro-2,3-di-O-benzyl-4-deoxy-5-(2-methoxy-2-oxoethyl)-1-[(6R,9R)-6-methyl-4, 11-dioxo- 3,5,10,12-tetraoxapentadec-14-en-7-yn-9-yl]-L-threo-pentitol			
14	Pheophorbide A		-9.1	0
15	Bis[2-(4-butoxyphenoxy)ethyl] (4-hydroxybenzylidene)malonate		-7.5	0
16	1,1-Cyclopentanediacetic acid		-10.5	0
17	N'1,N'9-Bis[(4-biphenylyloxy) acetyl]nonanedihydrazide		-9.5	0
18	N-{[(3S)-2-(L-Tyrosyl)-1,2,3,4-tetrahydro-3-isochinolinyl]methyl}-L-phenylalanyl-L-phenylalanin		-9.3	0

Ligand Compounds

Phytochemical compounds acting as ligands must meet inclusion criteria that fulfill both pharmacological and pharmacodynamic criteria. Based on their similarity as drug candidate materials, each compound successfully identified using HPLC/MS is used as a ligand compound in this study (Table 2).

The results of the molecular docking analysis of each BBLE compound against the target protein NF KB-p65 (1LE5) show that the best binding energy was obtained by the compound Pheophorbide-A, with a binding energy value of -8.1 kcal/mol and an RMSD of 0 Å. Pheophorbide A is a breakdown product of chlorophyll A, reported to be used as a photosensitizer and utilized in photodynamic therapy to reduce tumor growth[45,46]. The inhibition of Pheophorbide A detected in BBLE against the target protein NF KB-p65 (1LE5) is the first report of its kind (Table 3).

In several cell-based and animal experimental systems, it has been proven that NF-KB activation has a role in the early pathobiology of diabetes[47]. NF-KB is activated by increased oxidative stress, which in diabetes patients is caused by high glucose levels and advanced glycation end products[48]. Inhibition of this protein can offer new opportunities in the treatment process of diabetes played by BBLE through the compound Pheophorbide-A. The 2D interaction and 3D visualization of the Pheophorbide A compound against the NF KB-p65 protein (ILE5) are shown in Figure 2 below

The Glucagon-like peptide-1 (GLP-1) protein is one of the important incretin hormones for preventing postprandial hyperglycemia[49]. The results of the molecular docking analysis of the compounds contained in BBLE against the GLP1 protein show that the best binding energy was obtained by the compound Cyclopentanediacetic acid with a binding energy value of -7.2 kcal/mol and an RMSD of 0 Å (Table 4).

This study is the first to report the inhibition of the GLP-1 protein by the active compound 1,1-Cyclopentanediacetic acid in an in silico manner. There are not many reports explaining the bioactivity of this compound against degenerative diseases like diabetes. However, similar compounds that are derivatives of diacetic acid have been reported to have bioactivity as anti-inflammatory agents. Interestingly, the compound regulated several pro-inflammatory cytokines in microglial BV-2 cells induced with lipopolysaccharide (LPS)[50]. On a molecular level, the compound 1,1-Cyclopentanediacetic acid contained in BBLE, associated with anti-diabetic effects through the activation of GLP1 protein and its analogs, can be linked to the pancreatic GLP1R signaling that activates insulin production[51,52]. Pre-

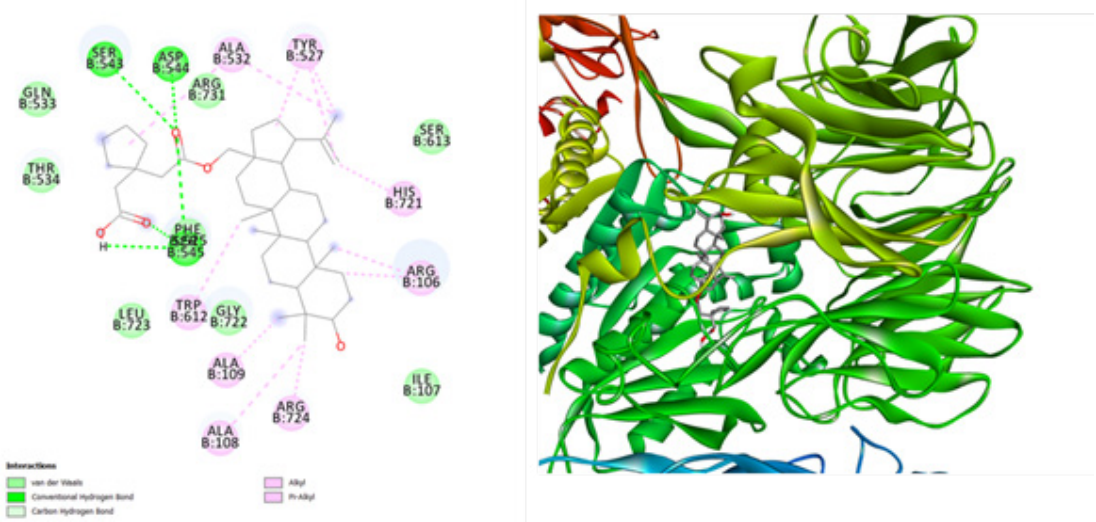

Fig. 4. 2D Interaction and 3D Visualization of Compound 1,1-Cyclopentanediacetic acid with DPP IV protein (5YPI)

clinical scale research is still needed to prove the activation of BBLE compounds on the expression of GLP-1 and GLP1R genes in the ileum of diabetic rats. The 2D interaction and 3D visualization of the compound 1,1-Cyclopentanediacetic acid binding with the GLP-1 protein are displayed in Figure 3 below.

The molecular docking analysis of the compound contained in BBLE against the DPP-IV protein (5YP1) shows the highest binding energy by the compound 1,1-Cyclopentanediacetic acid with a binding energy value of -10.5 kcal/mol and an RMSD of 0 Å (Table 5). DPPIV inhibitors have been proven to benefit various organs, including renal and cardiovascular health[53]. DPP-IV inhibitors are widely contained in incretin-based oral hypoglycemic drugs intended for diabetes patients, which have been commercially available for nearly a decade. Several types of DPP-IV inhibitors, such as Litagliptin and Saxagliptin, are capable of controlling glycemia and reducing the risk of renal and cardiovascular complications in diabetic patients and have been approved by the US FDA [54–56].

This study reveals that the compound 1,1-Cyclopentanediacetic acid from BBLE has good potential as a DPP-IV inhibitor in silico. The 2D interactions and 3D visualization of the compound 1,1-Cyclopentanediacetic acid with the DPP-IV protein are displayed in Figure 4. Several related studies have confirmed the role of herbal plant extracts and their constituents as DPP-IV inhibitors. Procyanidin compounds isolated from *Vitis vinifera* seed extracts were able to reduce DPP-IV levels and down-regulate its gene expression in the human intestinal cell model Caco-2.In vivo, procyanidin compounds also decrease the regulation of DPP-IV in the intestines of obese Wistar rats [57]. Similarly, the compound Emodin from the extract of *Rheum palmatum* L. shows inhibition against the DPP-IV enzyme in vitro and a dose-dependent decrease in DPP-IV levels in the plasma of Balb/c mice [58]. However, in vivo scale research is still needed to determine the effectiveness of the BBLE compound in reducing DPP-IV levels in diabetic rats, thereby clarifying the results from in silico studies.

CONCLUSION

In this study, LCMS/MS and molecular docking methods were used to analyze the phytoconstituent profile of BBLE with potential anti-diabetic properties. A total of 18 active compounds were identified in BBLE, including several that are flavonoid derivatives. Docking studies showed that Pheophorbide A could inhibit the NF KB-p65 protein (1LE5), and the compound 1,1-Cyclopentanediacetic acid inhibited the GLP 1 (5NIQ) and DPP-IV (5YP1) proteins. This study reveals the anti-diabetic effects of BBLE and further research is needed to determine the effectiveness of BBLE's active compounds and to identify their molecular mechanisms of action on diabetic animal models.

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Conflict of Interest

The authors reported no declarations of interest.

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