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Original Article

Antihyperlipidemic Constituents of Indonesian Medicinal Plant, *Pterospermum javanicum* Jungh.

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Abstract

Background: Indonesia, an archipelagic nation, is home to the second-highest biodiversity in the world, which includes Bajur (*Pterospermum javanicum* Jungh.) leaves, which has been used to treat lipid metabolic disorders. To the best of our knowledge, there is no report on *P. javanicum* Jungh regarding its antihyperlipidemic potency and its chemical constituents. This study aims to evaluate the antihyperlipidemic activity of the *P. javanicum* methanolic leaves extract, its fractions and its constituents.

Methods: The *P. javanicum* leaves extraction was performed using maceration and liquid-liquid extraction method. The major constituent of the ethyl acetate fraction was isolated using column chromatography and further purification using high performance liquid chromatography (HPLC) and its structure was elucidated through nuclear magnetic resonance (NMR). The antihyperlipidemic activities of isolated compounds were evaluated using computational studies by mean binding affinity with lipase (1LPB) enzymes, in which their potency was compared against the standard drugs, orlistat. Antihyperlipidemic activity of *P. javanicum* extracts were screened using lipase enzyme inhibition assay. The antioxidant activity was evaluated using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging reaction.

Results: The *in vitro* study showed the ethyl acetate fraction significantly inhibited the lipase enzyme and free radicals with an IC_{50} value of 1.84 ± 0.09 µg/mL and 76.67 ± 1.37 µg/mL, respectively. Two flavonoids, kaempferol-3-*O*-rhamnoside **1** and quercetin-3-*O*-rhamnoside **2** were successfully isolated and elucidated with stronger binding affinity with 1LPB enzymes than orlistat.

Conclusion: The study confirmed *P. javanicum* leaves potency as source for antihyperlipidemic and antioxidant agents.

Introduction

Hyperlipidemia is a metabolic disease characterized by an increase in triglycerides (TG), low-density lipoprotein (LDL), and a decrease in high density lipoprotein (HDL) levels. The leading cause of hyperlipidemia is unhealthy lifestyle including high fat diet, smoking, and lows of physical activity.[1] Lipid dysregulation is also associated with various conditions, including obesity and hyperlipidemia. Lipase enzyme is enzyme that produced by pancreas that is plays a vital role in fat absorption by regulating hydrolysis of triglycerides to monoglycerides and free fatty acids.[2] Activation of lipase enzyme can increase lipid absorption and lead to several conditions, such as obesity and hyperlipidemia.[3]

Hyperlipidemia is correlated with several cardiovascular diseases, such as coronary artery disease (CAD), myocardial infarction, and ischemic stroke as the ailment caused by accumulation of myocardial lipids, which decreases coronary blood flow and capillary elasticity.[4] Current hyperlipidemia therapy evolves several strategy including the use of statins, highly recommended medicine in preventing elevation of LDL and TG levels by inhibiting HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase with several side effects including myalgia, myopathy, myositis, rhabdomyolysis, joint pain, and abdominal pain being reported.[5,6] In addition, orlistat

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is a drug that is commonly used in clinical settings to treat lipid dysregulation by the mechanism of lipase enzyme inhibition, with gastrointestinal and hepatic impairment as side effects.[7] Therefore, the need for new drug entities in lipid management is necessary.

Mankind have been used medicinal plant to survive against several ailments inducing metabolic diseases. In recent years, there has been an increasing in development of plants derived drugs used in treatment of hyperlipidemia.[8] *Pterospermum* genera are known to have medicinal properties by the Asian, including the indigenous people Indonesia, who have prepared the plants as traditional medicament.[9] The roots of *P. heterophyllum* Hance and *P. lanceifolium* Roxb. plants are known for its anti-inflammation and anti-rheumatoid properties.[10,11] The roots of *P. semisagittatum* Buch. plants are widely used by the Tripura tribe in Bangladesh to treat fever and general pain.[12] Species within the genus *Pterospermum* were reported to exhibit diverse bioactivities, including antihyperlipidemic activity. A study on the flower of *P. acerifolium* L. Willd. reported ethanol extract to possess antidiabetic activity by reducing blood sugar levels in rats induced by streptozotocin-nicotinamide.[13] Another study reported the ethanol extract from the bark of *P. acerifolium* to display antidiabetic and antihyperlipidemic activity by reducing cholesterol and TG levels in rats model.[14]

The aim of this study is to evaluate the antihyperlipidemic potency of *P. javanicum* leaves as shown in Figure 1 by *in vitro* and *in silico* studies. Methanolic extract, hexane fraction, and ethyl acetate fraction of *P. javanicum* leaves were evaluated by lipase inhibition assay compared to orlistat as a standard drug. The antioxidant activity was evaluated using DPPH scavenging reaction. Isolation and structure elucidation of *P. javanicum* leaves by using HPLC and NMR instruments. *In silico* studies of isolated compounds from *P. javanicum* leaves were carried out to evaluate their antihyperlipidemic activities based on their ligand-enzymes interactions.

Figure 1. (A) *Pterospermum javanicum* Jungh tree grow at Tumpeng Village, Wonosari District, Bondowoso Regency, Jawa Timur – Indonesia. **(B)** Leaves appearance of *P. javanicum*. **(C)** Contrast back leaves appearance of *P. javanicum*. (source: Ari Satia Nugraha)

Methods

Materials

Plant sample, *Pterospermum javanicum* Jungh. leaves were collected from Meru Betiri National Park, Jember, East Java, Indonesia, and transported to Drug Utilization and Discovery Research Group (DUDRG), Faculty of Pharmacy, University of Jember under accession number code PJ. A voucher sample was sent to Purwodadi Botanical Garden, Pasuruan, East Java, Indonesia for taxonomic identification. This species was also reported under its synonyms, *P. javanicum* var. montanum Koord. & Valeton and *Pterospermum subinaequale* Miq. The leaves were dried under sun shade prior grinding into powder. Lipase porcine pancreas, 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, quercetin, ascorbic acid and *p*-nitrophenyl butyrate (*p*-NPB) were purchased from Sigma-Aldrich. Triton X-100, acetic acid, and acetone were purchased from Merck. The standard drug, orlistat was purchased from Roche S. P. A. Milan. Solvents, *n*-hexane, dichloromethane, and ethyl acetate were purchased from Merck.

Extraction and Fractionation

The *P. javanicum* Jungh. leaf powder (200 g) was loaded into Erlenmeyer and soaked with methanol (3 × 2L) and stirred for 24 hours at room temperature. The filtrate was collected through a Buchner filtration. The pooled filtrate was then dried using a rotary evaporator to dryness to produce crude leaf extract (30.5 g). Crude leaf extract was further partitioned by sequential liquid-liquid extraction to produce *n*-hexane (3.3 g), dichloromethane (0.9 g), and ethyl acetate (0.8 g) fractions.

Total Phenolic and Flavonoid Content Analysis

The total phenolic and flavonoid contents were determined following the research design published by Ratnasaridewi[15] and Denni.[16] Gallic acid was used as the standard for total phenolic content, while quercetin was used for total flavonoid content. For the determination of total phenolics, 1 mL of Na_2CO_3 (2% *w/v*) was added to four separate test tubes. Subsequently, 50 µL of the sample solution was introduced into each tube and incubated for 2 minutes. Folin-Ciocalteu reagent (50% *v/v*, 50 µL) was then added to each tube and incubated for another 30 minutes prior to absorbance measurement at 750 nm. Total phenolic content was expressed as gallic acid equivalents (GAE) using a standard curve based on gallic acid.

As in total flavonoid content, 1 mL of sample solution and 200 µL $AlCl_3$ (10% *w/v*) were mixed into same tube (3 replications). Subsequently, 200 µL of CH_3COOK

1 M and 3 mL of water were added to each tube then mixed prior to final incubation (30 minutes). The final mixture absorbance was analyzed at 433 nm, and the total flavonoid value was calculated as quercetin equivalents based on a quercetin standard curve.

Antioxidant Activity

Antioxidant activities of *P. javanicum* leaf extract and its fraction was determined using microplate DPPH assay following Ratnadewi's research design[15]. Ascorbic acid and methanol were used as a positive and negative control, respectively. Sample solution (75 µL) with five replications of methanolic extracts of 0.25, 0.5, 1.0, 1.5, and 2.0 µg GAE/mL; hexane extracts of 3.0, 5.0, 7.0, 9.0, and 11.0 µg GAE/mL; and ethyl acetate extracts 0.25, 0.5, 1.0, 1.5, 2.0 µg GAE/mL were pipetted into separate tubes and serially diluted. Subsequently, 658 µL ethanol 80% and 300 µL DPPH (90 µM) was added to the first three tubes of sample solution, while the last tubes were reserved for background absorbance correction (Abs blank). The sample was then incubated for 30 minutes in the absence of light prior to absorbance measurement at 515 nm. Lower absorbances (i.e loss of purple color) indicated higher free radicals dampening activities. Antioxidant activities were calculated using equation (1) and the results were then graphed against logarithmic sample solution to obtain the common antioxidant activity value, IC_{50} mean ± SD. One-Way ANOVA test used to assess significant differences in antioxidant activity data, with a significance threshold of $\alpha = 0.05$ using GraphPad Prims 10 software.

$$\text{Free radical scavenging activity} = \left[\frac{Abs_{blank} - Abs_{test}}{Abs_{blank}}\right] \times 100\% \quad (1)$$

Lipase Inhibition Assay

The lipase inhibition was conducted following the research design proposed by Padilla-Cambreros with minor modifications.[17] For the substrate, 1.61 mg *p*-NPB was dissolved in 10 mL Triton X-100 4% (in acetic acid buffer) and heated at 60 °C for ± 2 minutes. Acetic acid buffer 1 M pH 5.61 (1 mL) was added into the substrate solution and incubated at room temperature for 5 minutes. Distilled water (8 mL) was added to the mixture prior to storing at 20-30 °C.

In the experimental phase, 425 µL *p*-NPB substrate was pipetted into separate tubes and incubated at 37 °C for 5 minutes. Sample solutions (50 µL) with concentration of 1.0, 2.0, 3.0, 4.0, and 5.0 GAE/mL were added into each tube. As positive control, orlistat with the same concentration and volume as sample solution were also added into different tubes. Subsequentially, lipase enzyme (100 µL) was mixed into each tube and incubated at 37 °C for 60 minutes. Acetone (1 mL) was added to stop the reaction prior to absorbance measurement at 412 nm. Lipase inhibition value was determined using equation (2) and the result were then graphed against sample solution to obtain common lipase inhibition value, IC_{50} mean ± SD. Data on all lipase inhibition activities were subsequently analyzed for significant differences using a One-Way ANOVA test, with a significance level set at *P*-value = 0.05 using GraphPad Prism 10 software.

$$\text{Lipase inhibition activity} = \left[\frac{Abs_{blank} - Abs_{test}}{Abs_{blank}}\right] \times 100\% \quad (2)$$

Isolation and Structure Elucidation

Based on the results of the lipase inhibition assay and antioxidant assay, the ethyl acetate extract of the *P. javanicum* leaf extract was selected for further investigation. Phytochemical study initiated by employing the ethyl acetate crude extract (0.8 g) into a Shimadzu reverse phase HPLC system using Phenomenex Gemini column (19 × 150 mm, 5 µm 100Å) with gradient solvent elution from 0 to 20% of acetonitrile (0.1% TFA) within 2 minutes followed by steadily increasing to 30% within 22 minutes. The chromatogram was recorded using Shimadzu diode array detector SPD-M20A with single wavelength selection at λ 254 nm. The flowrate was adjusted to 15 mL/mins and 21 batch of sample were injected. The procedure produced two pure isolates at t*R* 14.68, 18.37 minutes respectively. Collected isolates were then dissolved in sufficient CD_3OD and subjected into NMR Modules for 1D-NMR and 2D-NMR analysis. Analysis conducted at 500 MHz for proton and 125 MHz for carbon. The obtained spectral data were then processed and analyzed using Mnova software v14.1.

Computational Studies of Isolated Compounds

A computational study by structure-based docking was performed to confirm the efficacy of *P. javanicum* phytochemicals as antihyperlipidemic agents. The study was conducted using AutoDock Vina v1.2.3 on pancreatic lipase (RCSB PDB ID: 1lpb) with orlistat as the positive control. Prior to docking, molecular energy minimization and format conversion to pdbqt were performed using Avogadro v1.2.0 and OpenBabel v3.1.1, respectively. The resulting ligand-protein interactions and binding affinities were then observed and compared against the standard ligand.

Results

Total Phenolic and Flavonoid Content, Antioxidant, and Antihyperlipidemic Activity

The total phenolic and flavonoid content of *P. javanicum* leaf extracts is illustrated in Figure 2. The total phenolic content is expressed as mg gallic acid equivalent per gram of extract, while mg quercetin equivalent per gram of extract is used as the standard for total flavonoid content.

According to this experiment, the ethyl acetate extract constituted the highest phenolic content compared to hexane and methanol extract with concentration value of 38.85 ± 0.27, 25.82 ± 0.14 and 28.84 ± 1.31 mg gallic acid equivalent/g, respectively. This pattern was similar to total flavonoid evaluation of hexane, ethyl acetate and

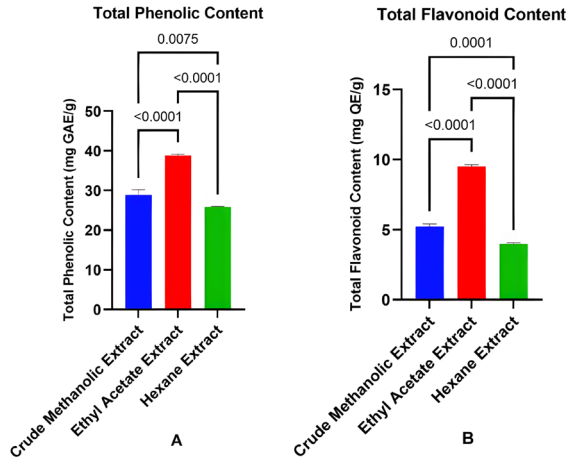

Figure 2. (A) Total phenolic and **(B)** flavonoid content of various *P. javanicum* extracts with statistical comparison (One-way ANOVA and Tukey's test). Data show mean ± SD, n = 3, values of $P < 0.01$ and $P < 0.0001$ were considered statistically different

methanol extract with value of 3.97 ± 0.08, 9.49 ± 0.14, and 5.20 ± 0.2 mg quercetin equivalent/g, respectively.

In this study, the antioxidant activity was evaluated using a DPPH radical scavenging assay and summarized in Figure 3. The antioxidant activity of crude methanol, ethyl acetate and hexane extracts indicated IC_{50} value of 23.99 ± 0.44, 76.67 ± 1.37, and 198.22 ± 3.98 µg/mL (p-value < 0.05, positive control, ascorbic acid gave IC_{50} value of 13.86 ± 0.10 µg/mL). The methanolic extracts possessed a higher IC_{50} value, followed by the ethyl acetate extracts.

The antihyperlipidemic activity of *P. javanicum* leaf extracts was evaluated in an *in vitro* experiment using lipase inhibitory assay. Our study revealed that crude methanol, ethyl acetate and hexane extracts exerted moderate to strong activity with IC_{50} value of 19.61 ± 0.81, 1.84 ± 0.09, 34.15 ± 0.88 µg/mL, respectively (P value < 0.05, positive control, orlistat gave IC_{50} value of 14.00 ± 2.00 µg/mL). The ethyl acetate extract displayed superior lipase

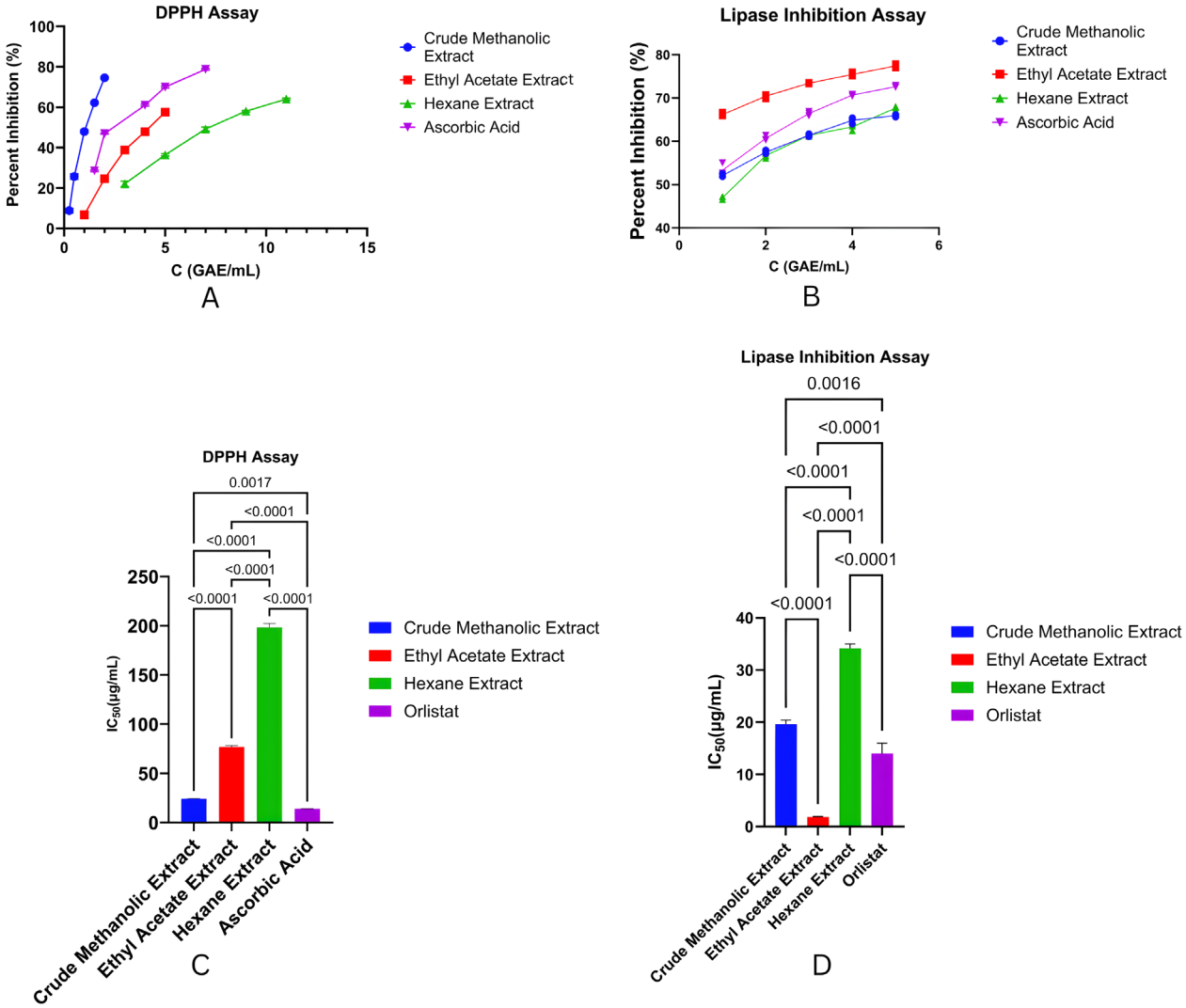

Figure 3. (A) Antioxidant evaluation using DPPH assay, **(B)** antihyperlipidemic evaluation using lipase inhibition assay, **(C)** IC_{50} value for antioxidant activity using DPPH assay, and **(D)** IC_{50} value for antihyperlipidemic activity using lipase inhibition assay of various *P. javanicum* extracts with statistical comparison (One-way ANOVA and Tukey's test)

inhibition activities, as evidenced by its lower IC_{50} values compared to hexane and methanolic extracts.

Isolation and Structure Elucidation Isolated Compound from the Ethyl Acetate Fraction of P. javanicum Leaves

The ethyl acetate extract of *P. javanicum* leaves was further separated using HPLC with a reverse phase column and gradient mobile phase system. The structure elucidation of two isolated compounds was obtained using NMR. Two flavonoid compounds, were isolated and identified as kaempferol-3-*O*-rhamnoside **1** and quercetin-3-*O*-rhamnoside **2**, respectively, as shown in Figure 4.

Kaempferol-3-*O*-rhamnoside 1, was obtained as a pale yellow amorphous solid; 4.8 mg (0.24 mg/g dried sample); ^{1}H NMR (500 MHz, CD_3OD, δ, ppm): 7.82 (2H, dd, J=8.5, 2.0 Hz, H-2'/6'), 6.96 (2H, dd, J=8.5, 2.0, H-3'/5'), 6.42 (1H, d, J=2.0 Hz, H-8), 6.23 (1H, d, J=2 Hz, H-6), 5.40 (1H, s, H-1''), 4.24 (1H, m, H-2''), 3.80 (1H, m, H-4''), 3.74 (1H, m, H-5''), 3.36 (1H, m, H-3''), 0.95 (3H, d, J=5 Hz, H-6''); ^{13}C-NMR (125 MHz, CD_3OD, δ, ppm): 178.2 (C-4), 164.5 (C-7), 161.7 (C-5), 157.2 (C-2), 157.9 (C-9), 134.9 (C-3), 130.5 (C-2'/6'), 121.1 (C-1'), 115.2 (C-3'/5'), 102.1 (C-1''), 104.5 (C-10), 98.5 (C-6), 93.3 (C-8), 71.1 (C-2''), 71.8 (C-4''), 70.7 (C-3''), 70.7 (C-5''), 16.3 (C-6''). Compound **1** was obtained as a pale yellow amorphous solid. The ^{1}H-NMR data of compound **1** showed kaempferol-3-*O*-rhamnoside has six proton signals in aromatic system. NMR spectral showed two proton signals at δH 7.82 ppm (2H, dd, J=8.5, 2.0 Hz, H-2' and H-6') and two proton signals at 6.96 ppm (2H, dd, J=8.5, 2.0 Hz, H-3' and H-5') as symmetric pattern. The aromatic proton at H-2' showed *meta*-coupled with H-6', and H-3' with H-5' in B ring. The trihydroxyl substitutions appeared at C-5 (161.68 ppm), C-7 (164.52 ppm), and C-4' (157.97 ppm) in flavone skeleton system. The sugar moiety signal appeared in the HMBC spectrum due to correlation between an anomeric proton signal at 5.40 ppm (1H, s, H-1'') and an anomeric carbon signal at 134.9 ppm (C-3). In addition, the ^{1}H-NMR spectrum displayed a methyl signal at 0.95 ppm (3H, d, J=5 Hz, H-6''), while the ^{13}C-NMR showed a carbon signal at 16.3 ppm (C-6''), which indicated that the sugar moiety was rhamnose. Based on the data analysis above and comparing the spectral data with related literature, the isolated compound was identified as kaempferol-3-*O*-rhamnoside.[18]

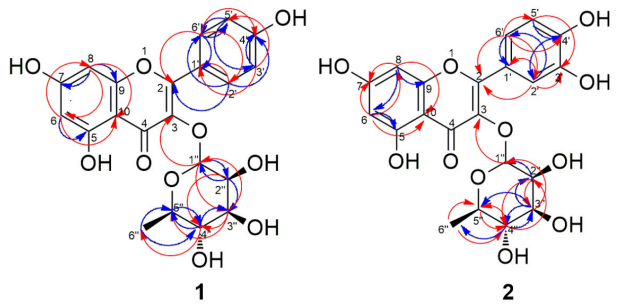

Figure 4. HMBC correlation in the 2D-NMR experiments for the isolated compounds, **(1)** kaempferol-3-*O*-rhamnoside and **(2)** quercetin-3-*O*-rhamnoside

Quercetin-3-*O*-rhamnoside 2, was obtained as a pale yellow amorphous solid; 5.2 mg (0.26 mg/g dried sample); ^{1}H NMR (500 MHz, CD_3OD, δ, ppm): 7.37 (1H, d, J=8.5 Hz, H-5'), 7.32 (1H, dd, J=8.5, 2.0 Hz, H-6'), 6.93 (1H, d, J=2.0 Hz, H-2'), 6.40 (1H, d, J=2 Hz, H-8), 6.23 (1H, d, J=2 Hz, H-6), 5.37 (1H, s, H-1''), 4.24 (1H, m, H-2''), 3.77 (1H, m, H-3''), 3.43 (1H, m, H-5''), 3.36 (1H, m, H-4''), 0.96 (3H, d, J=5.0 Hz, H-6''); ^{13}C-NMR (125 MHz, CD_3OD, δ, ppm): 178.3 (C4), 164.5 (C7), 161.8 (C5), 157.1 (C9), 148.4 (C2), 145.0 (C3'), 134.9 (C3), 121.6 (C1'), 121.5 (C6'), 115.5 (C5'), 114.9 (C2'), 104.5 (C10), 102.1 (C1''), 98.4 (C6), 93.3 (C8), 71.8 (C4''), 70.7 (C2''), 70.6 (C3''), 70.5 (C5''), 58.0 (C'4), 16.3 (C6''). Compound **2** was obtained as a pale yellow amorphous solid. The ^{1}H-NMR data of compound **2** showed quercetin-3-*O*-rhamnoside has five aromatic proton signals at δH 6.23 ppm (1H, d, J=2.0Hz, H-6), 6.40 ppm (1H, dd, J=2.0 Hz, H-8), 6.93 ppm (1H, d, J=2.0 Hz, H-2'), 7.37 ppm (1H, d, J=8.5 Hz, H-5'), and 7.32 ppm (1H, dd, J=8.5, 2.0 Hz, H-6'). The ^{1}H-NMR data also showed *meta*-coupled aromatic proton signals at 6.23 ppm (1H, d, J=2.0 Hz, H6) and 6.40 ppm (1H, dd, J=2.0 Hz, H-8) in ring A, 6.93 ppm (1H, d, J=8.5 Hz, H-2') and 7.32 ppm (1H, dd, J=8.5, 2.0 Hz H-6') in B ring, which indicated a flavone skeleton system. In addition, the protons from sugar moiety appeared at 5.37 ppm (1H, s, H-1'') which correlate with carbon signals at 134.9 ppm (C3) in the HMBC spectrum. The sugar was identified as a rhamnose, which was supported by proton and carbon signals. The four proton signals appeared at 3.36–5.40 ppm for oxygenated methines and 0.95 ppm (3H, d, J=5 Hz, H-6'') for one methyl group. The ^{13}C-NMR data displayed the 6'' carbon signals from the rhamnosyl group that appeared at 102.1, 70.7, 70.6, 71.8, 70.5, and 16.3 ppm. Based on the observed data and the related literature, the isolated compound was confirmed as quercetin-3-*O*-rhamnoside.[19]

Computational Study of Isolated Compounds

In the computational studies, the binding pocket was identified based on a reported study, which characterizes the binding site for methoxyundecylphosphinic acid (MUP).[20] Molecular docking was performed using AutoDock Vina (version 1.2.5) with the Vina scoring function. The docking grid was centered at (x: 10, y: 23, z: 50) with dimensions of 12×18×15 Å, covering the catalytic cavity where MUP binds. An exhaustiveness value of 100 was set. Docking validation was conducted by redocking MUP into the lipase binding pocket to evaluate the protocol's accuracy. The docking results yielded a binding score of -9.15 kcal/mol with a root-mean-square deviation (RMSD) of 1.8661 Å, indicating high docking reliability.

The findings of a computational study based on ligand structural docking are presented in Table 1 as ligand binding affinity, where as its interaction are visualized in Figure 5 and Figure 6. The result indicates that ligands

Table 1. Binding energy and amino acid residue resulted from docking experiment

Compound	Binding affinity (kJ/mol)	Key amino acid residues
Orlistat	-6.2	H-bond: Gly76, His151, Arg256 Hydrophobic: Ile78, Tyr114, Ala178, Pro180, Phe215, Ala259, Ala260
Kaempferol-3-*O*-rhamnoside (**1**)	-8.1	H-bond: Asp79, Ser152 Hydrophobic: Phe215, Ala259
Quercetin-3-*O*-rhamnoside (**2**)	-8.2	H-bond: Asp79, Ser152 Hydrophobic: Phe77, Tyr114, Phe215, Ala259, Ala260

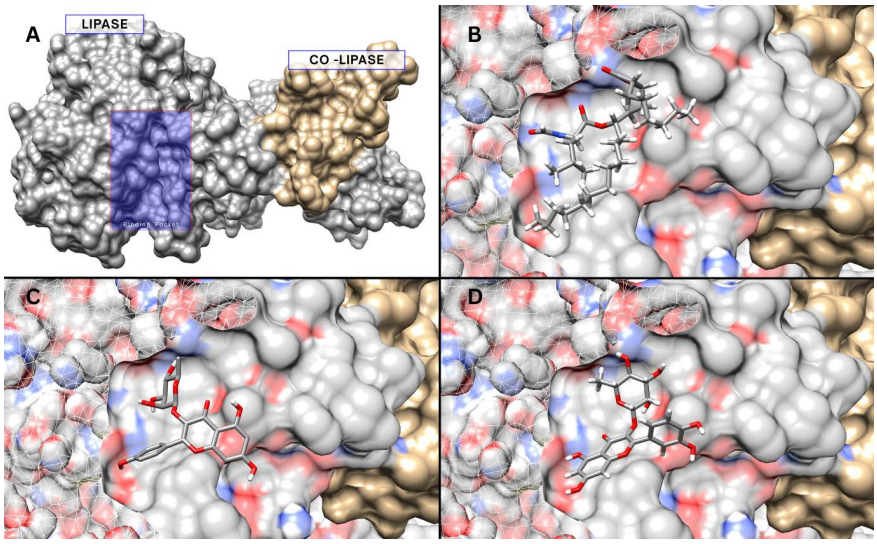

Figure 5. (A) Binding pocket of ligands into lipase enzyme (1LPB). **(B)** position of orlistat, **(C)** compound **1**, **(D)** compound **2** in the binding pocket

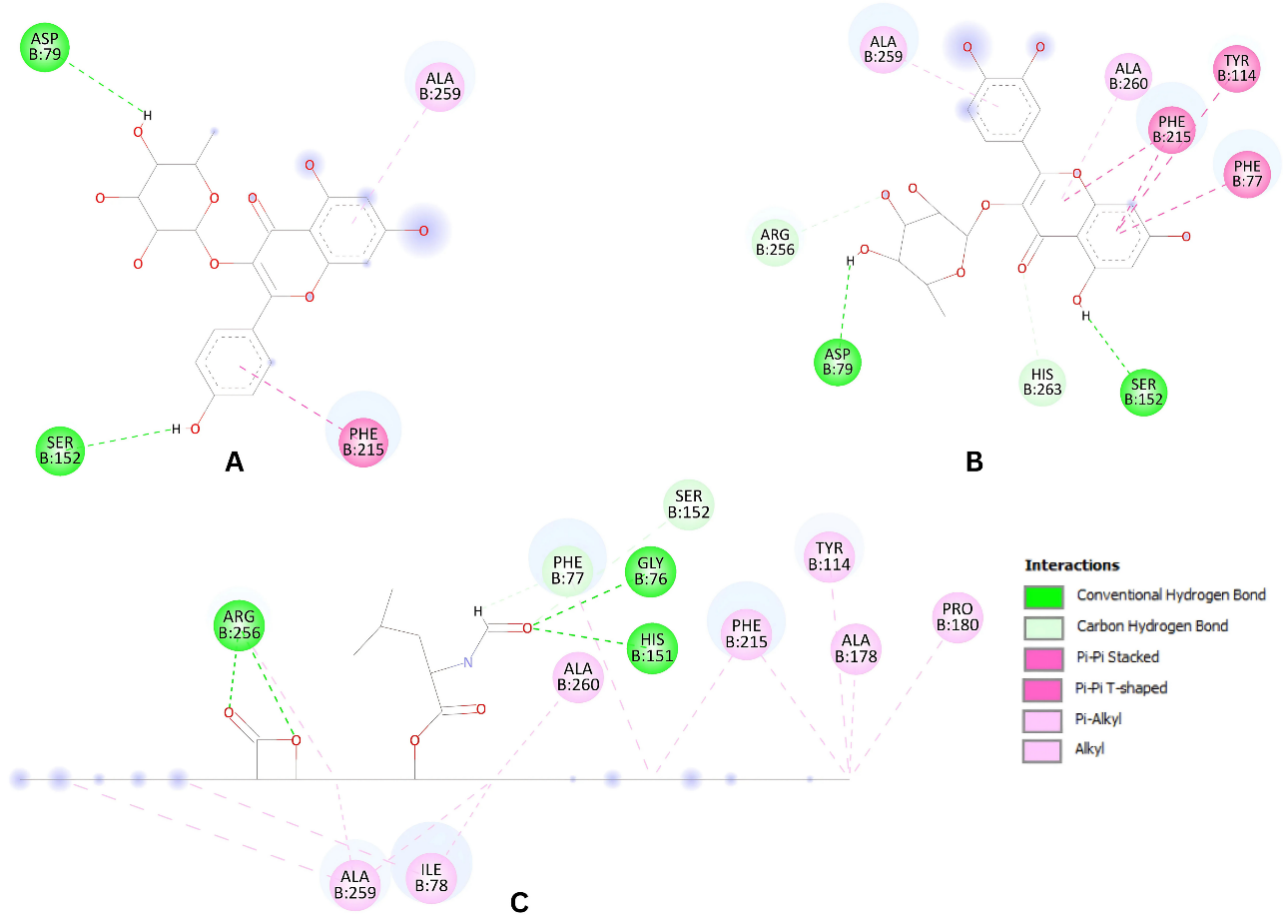

Figure 6. Interaction between amino acid residues of lipase enzyme (1LPB) and **(A)** compound **1**, **(B)** Compound **2**, and **(C)** orlistat

1 and 2, isolated from *P. javanicum*, demonstrated a remarkably similar binding mode to the target enzyme. Hydrogen bonds with Asp79 and Ser152 residues were observed in both flavonoids through hydroxy group of glycone side chain and B ring. The hydrophobic bonds (pi-alkyl and pi-pi stacked) with Phe215 and Ala259 residues were also observed via the A and B rings of both tested ligands. Specifically for ligand (B), additional interactions were observed: a pi-alkyl between the C ring and Ala260; pi-pi stacked between the A ring with Phe77 and Tyr114; and carbon hydrogen bonds with His263 and Arg256.

Discussion

The widespread distribution of the *Pterospermum* genus in Asian region is closely associated with the high use of its plants genus as herbal medicines. The *Pterospermum* genus and *P. javanicum* species has been used by the community to treat various diseases, including as an antidiabetic, antihyperlipidemic, and other metabolic diseases.[13,21] However, there are no scientific research was conducted to prove the role of *P. javanicum* species against metabolic disease in both *in vitro* and *in silico* studies. Here we have demonstrated the *P. javanicum* extracts and their isolated compound by *in vitro* and *in silico* study against hyperlipidemia.

In the current study, total phenolic and flavonoid levels in *P. javanicum* leaves extract were evaluated. Various extracts of *P. javanicum* leaves and their isolated compounds **(1-2)** were evaluated for antihyperlipidemic and antioxidant activities through both *in vitro* and *in silico* studies. In an *in vitro* study, the antioxidant activity of crude extract from *P. javanicum* leaves were evaluated using DPPH scavenging assay. The methanolic extract of *P. javanicum* leaves showed the highest IC_{50} values followed by the ethyl acetate and hexane extract. It indicates that the methanolic extract of *P. javanicum* leaves possesses high antioxidant properties. The antioxidant potency in several extracts of *P. javanicum* leaves may be due to the presence of phenolic and flavonoid compounds. Phenolic and flavonoid compounds are known to have antioxidant properties against various reactive oxygen species (ROS) and nitrogen species.[22] The hydroxyl group in the benzene ring of phenolic and flavonoid compounds is correlated with their antioxidant properties. These hydroxyl group can donate their hydrogen atom to free radicals and preventing cellular damage. The quantity and arrangement of these hydroxyl groups significantly affect the antioxidant activity of these compounds.[23,24] In previous study, leaf extracts of other species in *Pterospermum* genera including, *P. acerifolium* and *P. suberifolium,* have been reported to contain phenolics and flavonoids, both of which have demonstrated antioxidant activity in a DPPH radical scavenging assay.[25,26] In antihyperlipidemic evaluation using lipase inhibition assay, the ethyl acetate extract of *P. javanicum* leaves indicated superior activities with lower IC_{50} values compared to the methanolic and hexane extracts, as shown in Figure 3. It seems that high levels of phenolic and flavonoid compounds might be responsible for the antioxidant activity and lipid regulation. The significant presence of phenolics and flavonoids has been shown to possess antioxidant activity and is correlated with the pathophysiology of hyperlipidemia.[27] This finding suggests that the observed lipase inhibition activities of the ethyl acetate extract could be attributed to the presence of a wide variety of phytoconstituents, particularly phenolics and flavonoids, as mentioned in previous study.[28] In addition to inhibiting the lipase enzyme, this group of secondary metabolites has been proposed to inhibit HMG-CoA reductase, reduce stearoyl-CoA desaturase 1 (SCD1) gene expression, increase cholesterol excretion into bile acids, reduce bile acid reabsorption, and alter gut microbiota, thereby improving the overall blood lipid profile.[29-31]

Two flavonoids compounds, kaempferol-3-*O*-rhamnoside and quercetin-3-*O*-rhamnoside were sucessfully isolated from ethyl acetate extracts and elucidated using NMR instrument. Nevertheless previous study reported kaempferol **1** and quercetin **2** as constituents of ethyl acetate extract *P. acerifolium* flower.[13] The isolated compounds from ethyl acetate extracts of *P. javanicum* were evaluated for antihyperlipidemic activity by *in silico* study. *In silico* study ilustrates that hydrogen bonding and hydrophobic forces dominates the interaction between tested ligands and enzyme's binding pocket. The stability of ligand-enzyme interaction was assessed using computational molecular binding affinity. Both tested ligands exhibited stronger binding affinity (-8.1 and -8.2 kJ/mol) compared to standard ligand (-6.2 kJ/mol) as shown in Figure 6. This might be due to favourable hydrophobic stabilization through pi-alkyl and pi-pi stacking observed in the both tested ligands with key residues located in enzyme's lid domain (Phe77, Asp79, Phe215). Both tested ligands also interacted with critical residues, Ser152 and His263, which are integral components of the enzyme's catalytic triad (Ser152-Asp176-His263).[28]

Based on previous structure-activity relationship studies, it has been demonstrated that hydroxylation, glycosylation, presence of a ketone group, and the π-bonded carbon within the C ring of the flavonoid-based ligands significantly contribute to the mechanism of lipase inhibition. Li et al reported that hydroxylation A and B rings of flavonoid compound significantly enhances their lipase inhibitory activity.[32] This enhancement is likely due to introduction of functional groups that promote the formation of hydrogen bonds between ligand and key critical residues. The presence of glycosidic side chains has been shown to improve solubility, thereby maximizing their efficacy.[33] Additionally, the ketone group and π-conjugated carbon on the C ring contribute to the molecular planarity, thereby enhancing its ligand rigidity and overall solubility.[34] These structural features, combined with ligand-enzyme complex interactions, strongly support the assumption that

flavonoid-based ligands, isolated from the ethyl acetate fraction of *P. javanicum*, possess significant potential as antihyperlipidemic agents through lipase inhibition.

Conclusion

In conclusion, the *in vitro* study indicated that the ethyl acetate extract exhibited the highest lipase inhibition activity, while the methanol extract demonstrated the strongest antioxidant activity, as indicated by its IC_{50} value. Based on these findings, phytochemical isolation was performed on the ethyl acetate extract fraction, yielding two flavonoid-based compounds referred to as kaempferol-3-*O*-rhamnoside **1** and quercetin-3-*O*-rhamnoside **2**. Computational ligand structural docking data indicated that both flavonoid-based ligands exhibit a stronger binding affinity with the lipase enzyme's binding pocket compared to the standard drug, orlistat. These results suggest that *P. javanicum* holds significant potential as an antihyperlipidemic agent, likely through a mechanism involving lipase inhibition. Nevertheless, further *in vitro* and *in vivo* studies are essential to validate this reported *in silico* activity and further uncover the potential of *P. javanicum*.

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Competing Interests

The authors declare no conflict of interest.

Consent for Publication

Not applicable.

Data Availability Statement

All the data are included in this manuscript.

Ethical Approval

Not applicable.

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