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Research article

Evaluating the antidiabetic and antioxidative efficacy of binahong leaf extract (ble): a pre-clinical study

Hetty Andriani[1], Jekson Martiar Siahaan*[2], Hendrika Andriana Silitonga[3], Endy Juli Anto[4], Binarwan Halim[5]

[1] Department of Biomedical Sciences, Faculty of Medicine, Universitas Methodist Indonesia, Medan, Indonesia
[2] Department of Physiology, Faculty of Medicine, Institut Kesehatan Deli Husada, Deli Tua, Indonesia
[3] Department of Histology, Faculty of Medicine, Universitas Methodist Indonesia, Medan, Indonesia
[4] Department of Parasitology, Faculty of Medicine, Universitas Methodist Indonesia, Medan, Indonesia
[5] Department of Sexology and Andrology, Faculty of Medicine, Universitas Methodist Indonesia, Medan, Indonesia

Corresponding author: Jekson Martiar Siahaan, ✉ jekson.siahaan.sked@gmail.com, **Orcid Id**: https://orcid.org/0000-0001-8015-8649

Department of Physiology, Faculty of Medicine, Institut Kesehatan Deli Husada, Deli Tua, Indonesia

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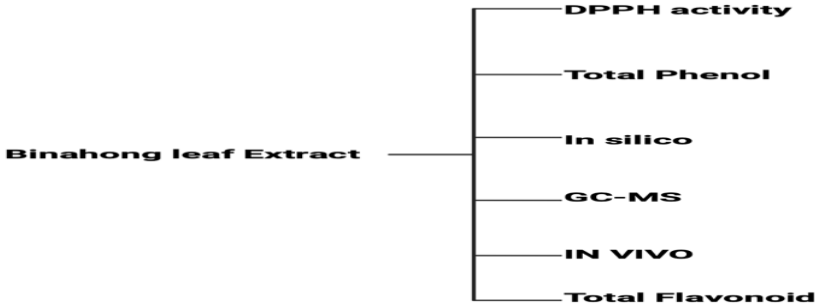
ABSTRACT

Diabetes mellitus is a chronic condition characterized by hyperglycemia and associated with oxidative stress, leading to various complications. Natural plant extracts, rich in bioactive compounds, have been explored for their antidiabetic and antioxidant properties. This study investigates the therapeutic potential of Binahong leaf extract (BLE) in managing diabetes and oxidative stress. The hypoglycemic effect of BLE was evaluated in a 30-day study, where diabetic-induced rats were treated with varying doses of BLE (100, 150, and 300 mg/kg). The effects on blood glucose levels, lipid profiles, and the activities of antioxidative enzymes (superoxide dismutase [SOD], glutathione peroxidase [GPx], and catalase) were measured and compared to normal and diabetic controls. The total phenolic and flavonoid contents of BLE were also quantified to correlate with the observed effects. BLE treatment resulted in a dose-dependent decrease in blood glucose levels, with the highest dose restoring levels close to normal. Significant reductions in total cholesterol and triglycerides were observed, along with an increase in HDL cholesterol at the highest dose. BLE also significantly increased the activities of SOD, GPx, and catalase, indicating enhanced antioxidative defenses. The presence of high levels of phenolic and flavonoid compounds in BLE correlated with the observed biological activities. The findings suggest that BLE possesses considerable antidiabetic and antioxidative properties, likely due to its rich content of phenolic and flavonoid compounds. The extract demonstrates potential as a natural therapeutic agent for managing diabetes and associated oxidative stress. However, further clinical studies are warranted to confirm these effects in human populations and to establish comprehensive safety and efficacy profiles.



Keywords: Binahong Leave Extract (BLE), Antioxidant, Diabetes, Flavonoid, Phenol, Docking Simulation.

INTRODUCTION

Around 537 million people worldwide currently live with diabetes, a number projected to escalate to 783 million by the year 2045. Obesity, which often coincides with diabetes as part of the metabolic syndrome, affects over a billion people globally. The incidence of obesity is climbing, contributing to a variety of cardiometabolic diseases, including diabetes. The interplay between obesity and diabetes involves numerous metabolic complications such as impaired glucose tolerance, elevated levels of blood lipids, oxidative stress, inflammation, hypertension, insulin resistance, and increased abdominal fat. Concurrently, over a billion individuals are grappling with obesity, a primary contributor to metabolic syndrome and a known risk factor for type-II diabetes mellitus (TIIDM). The pharmaceutical industry is actively developing new medications to lower the occurrence of diabetes and associated health concerns. Treatment strategies for metabolic syndrome encompass various aspects and face obstacles related to cost and accessibility. Access to treatment, especially medication, is inconsistent and usually expensive, with richer countries generally having better treatment options [1-3]. It is noteworthy that most diabetic adults live in lower- to middle-income countries. Investigating treatment alternatives derived from natural sources could offer more cost-effective and accessible management for metabolic syndrome. The metabolic complications of obesity and diabetes are manifold, encompassing impaired glucose tolerance and heightened blood lipid levels, which exacerbate the risk of cardiovascular diseases. Despite advancements in pharmacotherapy, disparities in access to medications persist, with cost and availability posing significant barriers, particularly in lower-income nations where the majority of affected individuals reside. This inequity underscores the urgency of exploring alternative therapies, including natural products, which may offer more accessible treatment options [4,5]. The functional food sector, propelled by emerging research, posits a link between diet and metabolic health, suggesting a potential role for dietary interventions in disease management. The pathophysiology of TIIDM, characterized by insulin resistance and hypercholesterolemia, is influenced by genetic and lifestyle factors, with obesity increasing the risk sevenfold. The condition is compounded by the body's maladaptive response to excess fat, particularly when distributed around the abdomen, which impairs glucose uptake and increases plma free fatty acid levels, further contributing to insulin resistance. This dysfunctional metabolic state is also linked to increased creatinine and bilirubin levels, indicative of renal and hepatic stress [6-8]. The traditional use of the Madeira vine (*Anredera cordifolia*) in Indonesian folk medicine, particularly known as binahong, has been documented for various ailments, including diabetes. Recent studies have illuminated its pharmacological potential, attributing its therapeutic effects to its antioxidant, lipid-lowering, and anti-inflammatory properties. Given its historical and cultural significance in traditional medicine, and its growing empirical support, binahong presents as a viable candidate for further investigation as a functional food with antidiabetic and cardioprotective benefits [9,10]. This study aims to delineate the antidiabetic and antioxidative efficacy of BLE, providing a scientific basis for its traditional use and paving the way for its integration into contemporary therapeutic strategies.

MATERIALS AND METHODS

Materials

96% ethanol, ethyl acetate, and chloroform from Merck, heparin branded as inviclot, Elisa Kits for SOD, GPx, Catalase, TC, TG, and HDLc from E-Lab Science, Quercetin from Sigma, ABTS, potassium persulfate, and distilled water. Laboratory apparatus included standard glassware, a 96-well microtitration plate, microtubes, a Mingyi rotary evaporator, a Peak UV-Vis Spectrophotometer, and a DIATEK Elisa microplate reader.

Animals

We used rats that weighed between 150-180 grams provided by the Faculty of Pharmacy at Universitas Methodist Indonesia. These animals were approved for use by the Animal Research Ethics Committees (AREC) at Universitas Methodist Indonesia, under the approval number 01281/UMI/2022.

Plant Collection and Extract Preparation

We macerated 300 grams of powdered dried Binahong leaves in an ethanol-water solution (70:30 v/v) at 25°C with constant shaking for 24 hours, filtering the mixture afterwards. This process was repeated twice more, totaling three extractions. The extracts were then centrifuged at 3500 rpm for 10 minutes at room temperature. The resulting supernatant was concentrated using a rotary evaporator at 38°C to obtain a Binahong Leave Extract (BLE).

Phytochemical Constituent Analysis

The phytochemicals were analyzed with a GC-MS system combining a 7890A gas chromatograph and a mass spectrophotometer with an HP-5 MS column and a Triple-Axis detector. We used helium as the carrier gas with a flow rate of 1.0 ml/min. The system conditions included temperatures for the ion-source and interface, a set pressure, an outlet time, and a split mode injection with a certain ratio. The column temperature was initially held, then incrementally increased to specific temperatures over a set time. The total runtime for the compounds was 47.5 minutes. The proportion of each compound was quantified by peak area comparison using the MS solution software.

Acute Toxicity Study

The acute toxicity of the BLE was evaluated following the OECD guideline No.423. Fasted rats were administered varying doses of the extract orally. The rats were monitored for immediate behavioral or neurological reactions for an hour, with additional observations over 6 hours, and at 24 hours for any acute toxicity symptoms.

Antioxidant activity of NT by DPPH scavanging activity

The assessment of the antioxidant activity of the extracts encompassed the amalgamation of approximately 1.0 mL of a 0.1 M solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) with 0.9 mL of a 50 mM Tris-HCl buffer (pH 7.4). Subsequently, a volume of 0.1 mL was added to the combination, which could either consist of the sample extract or deionized water serving as a reference. The resulting solution underwent complete homogenization and was thereafter incubated at room temperature for a duration of 30 minutes. Following the incubation period, the absorbance was quantified utilizing a UV-Vis spectrophotometer set at a specific wavelength of 517 nm. The calculation of the DPPH scavenging activity was performed using the following formula:

$$\%\ \text{inhibition} : \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{Absorbance\ of\ control}\ x\ 100\%$$

Total Phenolic Compound

The determination of total phenolic content (TPC) was performed using the Folin–Ciocalteu technique. In this experiment, a 20 µL aliquot of the desiccated sample-extract solution, which had been produced in a methanol solution at a weight-to-volume ratio of 1:10, was combined with 1.58 mL of distilled water and 100 µL of the Folin-Ciocalteu reagent. Subsequently, a 5% sodium carbonate solution with a volume of 300 µL was introduced into the mixture. Following this, the resulting solution was exposed to a controlled environment with limited light, while being kept at a constant temperature of 25 °C, for a duration of 2 hours. The measurement of absorbance was conducted at a specific wavelength of 765 nm. Additionally, a control sample was created by employing distilled water in accordance with the identical approach. The determination of the overall phenolic content (TPC) was performed by employing gallic acid equivalents (GAE) and quantified in milligrams per gram of dry extract. The calibration curve was generated by employing solutions of Gallic acid with varying concentrations (5, 10, 20, 40, and 80 mg/L). The calculated coefficient of determination (R2) was found to be 0.9871.

Total Flavonoid Compound

The determination of total flavonoid content (TFC) was conducted using a simplified methodology. The experimental procedure involved combining 1 mL of the NT with 300 µL of a 5% NaNO2 solution and 300 µL of a 10% aluminum chloride solution. The resulting combination was then subjected to incubation at a temperature of 25°C for a duration of 5 minutes. Subsequently, a 2 mL aliquot of a 1 N sodium hydroxide solution was introduced into the amalgamation. The solution was diluted with water in order to achieve a final volume of 10 mL. Subsequently, it was exposed to agitation using a vortex mixer to ensure thorough homogenization. The measurement of absorbance was conducted at a specific wavelength of 510 nm. A calibration curve was established in order to ascertain the content of quercetin, yielding a coefficient of determination (R2) value of 0.974. The determination of the total flavonoid content (TFC) in the sample was conducted by quantifying it in milligrams of equivalents (CE) per gram of sample, utilizing the dry weight.

Induction of Diabetes Mellitus

Experimental diabetes was induced by a single intraperitoneal injection of STZ (60 mg/kg body weight; dissolved in 0.1 M ice-cold citrate buffer (pH 4.5)) in the overnight-fasted animals. Hyperglycemia was confirmed by the elevated blood glucose level and was estimated on day 0 and 72 h after STZ injection. The rats with blood glucose levels of more than 200 mg/dL were used for the study.

Experimental Design

The animals were divided into five groups (n = 6) and were treated daily as follows

Group I: Normal control (receives 0.5% CMC)
Group II: Diabetic control.

Group III: Diabetic animals treated with glibenclamide (5 mg/kg, p.o)

Group IV: Diabetic animals treated with BLE (100 mg/kg, p.o)

Group V: Diabetic animals treated with BLE (150 mg/kg, p.o)

Group VI: Diabetic animals treated with BLE (300 mg/kg, p.o)

The study was carried out for a period of 30 days, and the hypoglycemic potential of the extract was evaluated by estimating the serum glucose levels at regular intervals of 10, 20 and 30 days.

Serum preparation

Blood samples were collected in a dry test tube and allowed to coagulate at ambient temperature for 30 min. The serum was separated by centrifugation at 2000 r/min for 10 min. We measured the levels of SOD, GPx, Catalase.

Biochemical Assays

Serum total cholesterol (TC), triglycerides (TG) and high-density lipoprotein cholesterol (HDLc) were estimated using the standard protocol and as per the procedures mentioned in commercial kits (Merck Laboratories, Maharashtra, India).

In silico Tools

The computational analyses were performed on an HP Laptop equipped with Windows 11 OS, 64-bit system, 4 GB RAM, 256 GB SSD, and a 14-inch screen. The study utilized several software applications for various tasks. This included Windows 11 64-bit for the operating environment, Chimera 1.16 for molecular visualization, the Protein Data Bank for protein structural data, PubChem for chemical compound details, and SwissDock for protein-ligand docking simulations.

Preparation of ligands and proteins

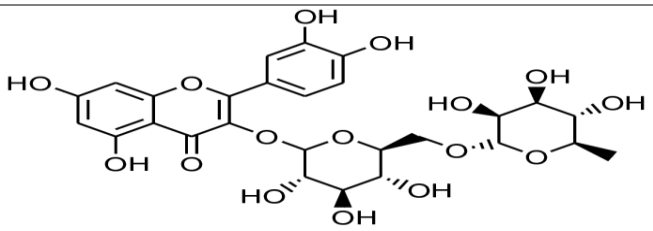
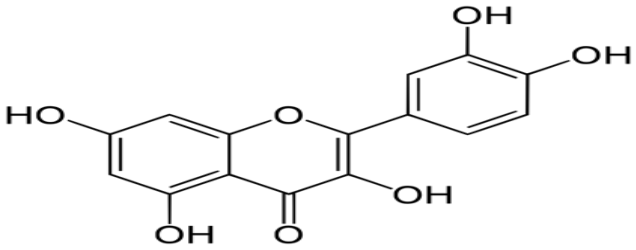
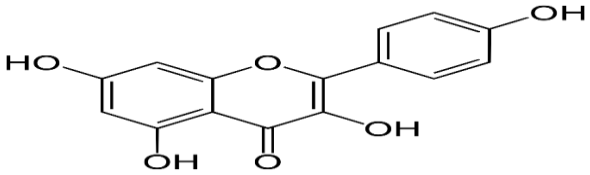
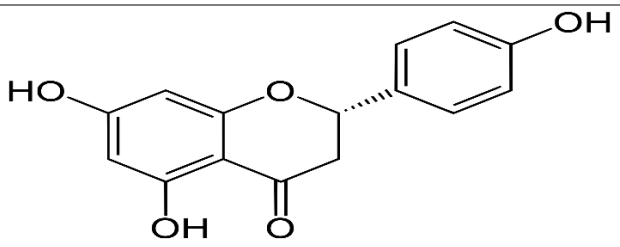
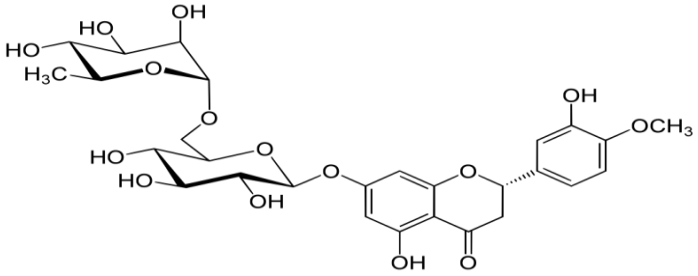
Ligands and proteins were prepped for the study with the insulin receptor 1IRK sourced from the Protein Data Bank. The 3D structure was prepared using Chimera 1.16 by removing extraneous

residues. Test compounds were constructed in UCSF Chimera 1.16 by entering the ligand's PubChem CID, which was previously obtained from PubChem's online resource and saved in mol2 file format. Molecular docking, the process that predicts the preferred orientation of a ligand to a protein to form a stable complex, was performed using SwissDock. The effectiveness of the docking was measured by calculating the Gibbs free energy (ΔG) of the interactions, and Table 1 delineates the distinct characteristics of these ligands.

Rendering of docking outcomes

The visualization process was carried out using the USCF Chimera 1.16. The protein data and docking results were entered into pdb file format. Visualization illustrates the specific type of bond interaction established, together with the amino acid that serves as the binding site. The visualization results are saved in *. png file format.

Table 1: Ligand name

Name	Formula	Chemical Structure
Rutin	610.5	
Quercetin	302.23	
Kaempferol	286.24	
Naringenin	272.25	
Hesperidin	302.28	

Statistical analysis

In vivo results were analyzed using ANOVA with Tukey's Multiple Comparison Test. P values for significance were set at 0.05. Values for all measurements are expressed as the mean ± SD. Histogram data were constructed by using GraphPad Prism Software 9.0.

Phytochemical contents of BLE by GC-MS

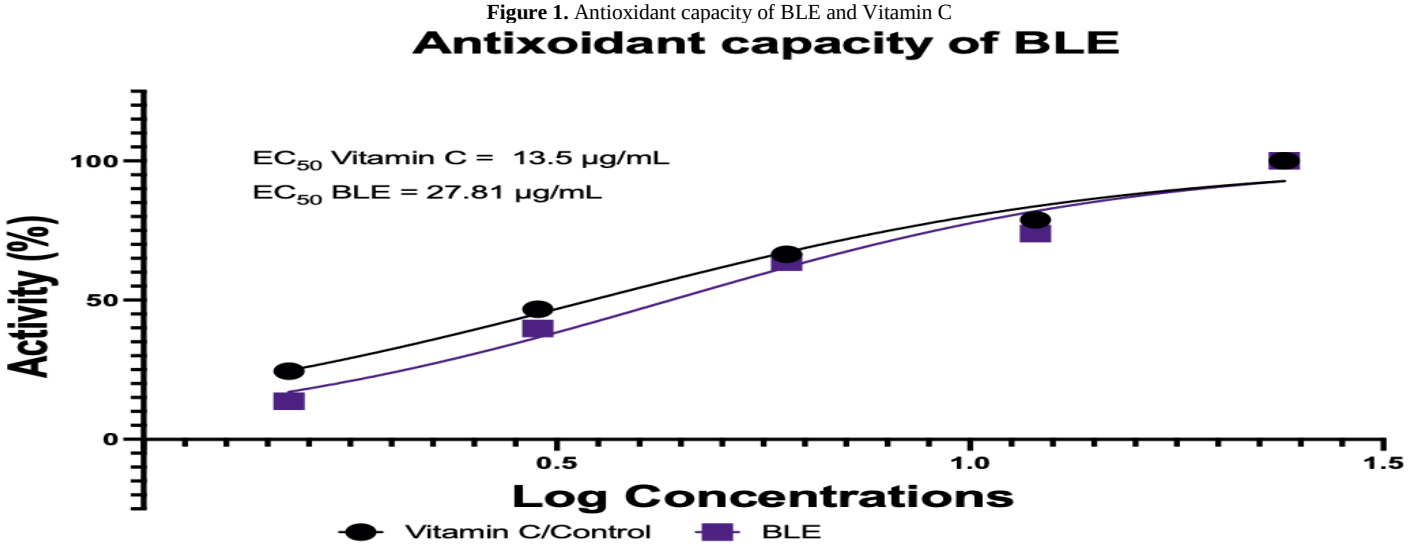
GC-MS examination of the BLE disclosed five distinct phytochemicals. Table 2 details their retention times, molecular structures, weights, and relative concentrations within the BLE. This analysis pinpointed a range of active phytochemicals, including rutin, quercetin, kaempferol, naringenin, and hesperidin.

RESULT

Table 2: Bioactive compounds found in BLE

Chemical Name	Molecular Weight (g/mol)	Molecular Formula	Retention Time (Min)	Relative area (%)
Rutin	610.5	C27H30O16	5.38	13.44
Quercetin	302.23	C15H10O7	6.71	13.58
Kaempferol	286.24	C15H10O6	8.19	8.53
Naringenin	272.25	C15H12O5	12.41	1.45
Hesperidin	302.28	C16H14O6	14.18	0.91

Figure 1. Antioxidant capacity of BLE and Vitamin C



The analysis of BLE using GC-MS revealed a spectrum of bioactive compounds, each with distinct molecular properties. Rutin was prominent, displaying a molecular weight of 610.5 g/mol and a molecular formula of C27H30O16; it emerged early in the GC-MS run with a retention time of 5.38 minutes and constituted 13.44% of the relative area in the chromatogram. Following closely was quercetin, with a molecular weight of 302.23 g/mol and a formula of C15H10O7, which appeared at a retention time of 6.71 minutes and represented 13.58% of the relative area, suggesting a significant presence. Kaempferol, another component, had a molecular weight of 286.24 g/mol and a formula of C15H10O6, and was identified at 8.19 minutes with 8.53% of the relative area. Naringenin, slightly lighter, had a molecular weight of 272.25 g/mol and a formula of C15H12O5; it had a longer retention time of 12.41 minutes, accounting for 1.45% of the relative area, indicating a smaller quantity. Lastly, hesperidin, with a molecular weight of 302.28 g/mol and a formula of C16H14O6, was the least prevalent, having a retention time of 14.18 minutes and making up just 0.91% of the relative area. These findings underscore the diversity of phytochemicals present in BLE, each potentially contributing to its overall bioactivity.

Antioxidant activity of BLE

The DPPH assay revealed that BLE has significant antioxidant capabilities, as evidenced by an IC50 of 27.81 µg/mL. In comparison, BLE showed a marked antioxidant effect, yet vitamin C, used as the control standard, showed superior effectiveness with an IC50 of 13.15 µg/mL. This comparison highlights vitamin C as a more efficient DPPH radical neutralizer than BLE. Nonetheless, the antioxidant properties displayed by BLE are considerable and indicate its potential role in bolstering the body's antioxidant defense. Further research could shed light on the distinct antioxidants in BLE and the specifics of their antioxidant activities (Figure 1).

Total Phenol and Flavonoid Content

The total phenolic and flavonoid contents of BLE were measured using specific analytical methods. The Folin-Ciocalteu procedure was applied to determine the total phenolic content (TPC), while the total flavonoid content (TFC) was assessed through the aluminium chloride colorimetric method. For phenolic content, a linear equation y = 0.008x + 0.01 was used, showing an almost perfect correlation with an R^2 value of 0.999. These results were expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g). Flavonoid levels were similarly determined by a standard calibration curve of quercetin with the linear equation y = 0.010x + 0.05, resulting in an R^2 of 0.988, indicating a high degree of correlation. Flavonoid content was reported in milligrams of quercetin equivalent per gram of extract (mg QE/g). The study documented the highest values of TPC and TFC in the plant extract as 250.81 ± 11.81 mg GAE/g and 19.76 ± 0.98 mg QE/g, respectively, which reflects the content in the tested BLE.

Acute Toxicity Study

During the acute toxicity evaluation, oral doses of the extract ranging from 100 to 2000 mg/kg were administered and observed not to induce any adverse effects on the general condition, motor skills, or behavior of the subjects, indicating the extract's safety. The animals maintained normal body weight and showed no difference in food

intake when compared to the control group that received a placebo treatment. Based on these observations, subsequent experimental dosages were set at 100 and 200 mg/kg for the Binahong leaf extract. absence of acute toxic effects at such a wide dose range suggests a high margin of safety for the Binahong leaf extract, making it a potentially viable candidate for use in further pharmacological studies. The normalcy in body weight and food consumption patterns post-administration of the extract mirrors a lack of disruption to metabolic functions, which is often a concern with new therapeutic agents. These positive indicators support the continued exploration of the extract's therapeutic potential and its underlying mechanisms of action. Further investigations could also examine long-term effects, potential therapeutic efficacy, and the pharmacokinetics of the active constituents within the extract to fully establish its viability as a medicinal resource.

Effect of BLE on glucose level at day 0, 10, 20 30

The data provided from Table 3 illustrates the effect of Binahong leaf extract (BLE) on glucose levels over a 30-day period. The normal control group (NC), which did not receive the diabetic induction, maintained consistent glucose levels throughout the study, demonstrating the stability of glucose metabolism under normal conditions. In contrast, the diabetic control group (DC) exhibited significantly higher glucose levels initially, which continued to rise by day 30, suggesting the persistence of hyperglycemia due to the diabetic condition. The groups receiving Binahong leaf extract showed a dose-dependent decrease in glucose levels. The BLE100 group, which received the lowest dose of the extract, experienced a gradual reduction in glucose levels, starting from an elevated baseline similar to the DC group and achieving a significant decrease by day 30. More pronounced effects were observed in the BLE150 and BLE300 groups, with the BLE300 group showing the most substantial reduction in glucose levels. Starting from an elevated baseline, the BLE300 group's glucose levels decreased sharply by day 10, continued to decline at day 20, and reached the lowest levels among all groups by day 30. The results suggest that Binahong leaf extract has a potent hypoglycemic effect in a dose-dependent manner, with higher doses correlating with greater reductions in blood glucose levels. These findings support the potential therapeutic benefit of BLE in managing hyperglycemia associated with diabetes.

Table 3. Effect of BLE on glucose level at day 0, 10, 20, 30

Groups	Glucose Level (mg/dL)			
	0	**10**	**20**	**30**
NC	128.82 ± 12.91	127.29 ± 19.21	125.82 ± 16.29	125.17 ± 11.18
DC	290.98 ± 15.99	278.92 ± 10.87	280.28 ± 12.91	317.13 ± 14.21
BLE100	280.13 ± 16.82	230.29 ± 11.86	200.21 ± 12.29	180.18 ± 12.41
BLE150	290.92 ± 18.15	245.28 ± 18.15	180.28 ± 15.28	150.29 ± 11.28
BLE300	312.82 ± 17.28	180.28 ± 12.29	158.27 ± 12.92	130.28 ± 13.28

Information: NC (Normal control), DC (Diabetic control), BLE (Binahong leave extract 100), BLE (Binahong leave extract 150), BLE (Binahong leave extract 300).

Effect of BLE on Serum total cholesterol (TC), triglycerides (TG) and high-density lipoprotein cholesterol (HDLc)

The data from **Figure 2** reveal the influence of Binahong leaf extract (BLE) on serum lipid profiles, including total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDLc). For total cholesterol (TC), the normal control group (NC) had the lowest levels, indicating a healthy baseline. The diabetic control (DC) group showed significantly elevated cholesterol levels. Treatment with BLE resulted in a dose-dependent decrease in TC, with BLE300 showing the greatest effect, significantly lowering TC closer to NC levels. Triglyceride (TG) levels were also highest in the DC group, suggesting dyslipidemia associated with diabetes. BLE treatments at 100, 150, and 300 mg/kg dosages all significantly reduced TG levels, with BLE300 leading to the most substantial reduction, approaching the levels seen in the NC group. Regarding HDLc, the NC group had higher levels compared to the diabetic control, which is consistent with protective lipid profiles. BLE treatments did not significantly alter HDLc levels at any dose compared to DC, as indicated by the 'ns' (not significant) marker, except for BLE300, which showed a significant increase in HDLc, though not reaching NC levels. the findings suggest that BLE has a beneficial effect on lipid metabolism in a diabetic model, particularly at higher doses, with the potential to reduce risk factors associated with cardiovascular diseases in diabetes. The **** ($p<0.001$) indicates a highly significant difference between the groups compared, confirming the efficacy of BLE in modulating lipid profiles.

Effect of BLE on Oxidative Stress Markers (SOD, GPx, Catalase)

The results from **Figure 2** demonstrate the impact of Binahong leaf extract (BLE) on oxidative stress markers, including superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase in the study groups. For SOD activity, the normal control group (NC) displayed the highest activity, followed closely by the BLE300 group. The diabetic control (DC) group had significantly lower SOD activity compared to NC. Both BLE100 and BLE150 showed increased SOD activity compared to DC, but less than NC and BLE300. GPx levels were highest in the NC group, with the BLE300 group showing a comparable level of GPx activity. The DC group had the lowest GPx activity. However, the BLE100 and BLE150 groups demonstrated increased GPx levels compared to DC, suggesting a dose-dependent improvement but still lower than NC and BLE300. The catalase activity followed a similar pattern, with NC showing the

highest levels and DC the lowest. The BLE300 group nearly matched the NC group's catalase activity, indicating a substantial increase. Both BLE100 and BLE150 exhibited improved catalase activity over DC but did not reach the levels of NC or BLE300. In all markers, BLE300 consistently showed the closest activity levels to the NC group, indicating that at higher doses, the BLE has a considerable antioxidant effect. The **** ($p<0.001$) indicates a highly significant difference between the groups compared, while ns indicates no significant difference. These results underscore the potential of BLE as a supportive antioxidant therapy, especially at higher dosages, in the context of diabetes-induced oxidative stress.

Effect of rutin, quercetin, kaempferol, naringenin on Insulin receptor via Molecular Docking Simulation

The docking affinity scores presented in Table 4 suggest a significant interaction between various bioactive compounds and the insulin receptor, which is a key protein in the regulation of glucose homeostasis. The strength of these interactions is indicated by the Gibbs free energy of binding (ΔG), where a more negative value reflects a stronger binding affinity. Rutin and Hesperidin exhibit the strongest binding affinities with ΔG values of -7 kcal/mol each, which could be indicative of their potential to act as insulin mimetics or enhancers of insulin action. Quercetin and Naringenin also show favorable binding affinities with ΔG values of -4.8 and -4 kcal/mol, respectively, while Kaempferol presents the least affinity at -2.9 kcal/mol among the compounds listed The interactions with specific amino acid residues, such as ARG1000, LEU1002, and GLU1012, are crucial as these are often involved in the activation and signaling cascades of the insulin receptor. The modifications in these residues upon ligand binding could potentially affect the receptor's conformation and subsequent insulin signaling pathway, impacting glucose uptake and metabolism. These findings align with the pharmacological understanding that natural compounds can interact with protein targets in a manner similar to pharmaceutical agents, influencing metabolic pathways and potentially ameliorating conditions like diabetes (Williamson, 2017; Patel et al., 2017). The docking results are shown in Table 4 and Figure 3 for visualization interaction between ligand and receptor

Figure 2. The effect of BLE on Total Cholestrol (A), Tryglicerides (B), and HD: (C) expresssion. (NC (Normal control), DC (Diabetic control), BLE (Binahong leave extract 100), BLE (Binahong leave extract 150), BLE (Binahong leave extract 300), the data were presented in mean ± standard error of the mean (SEM). (*: p<0,05, **: p<0,01, ***: p<0.001, P>0.05 ns/not significance).

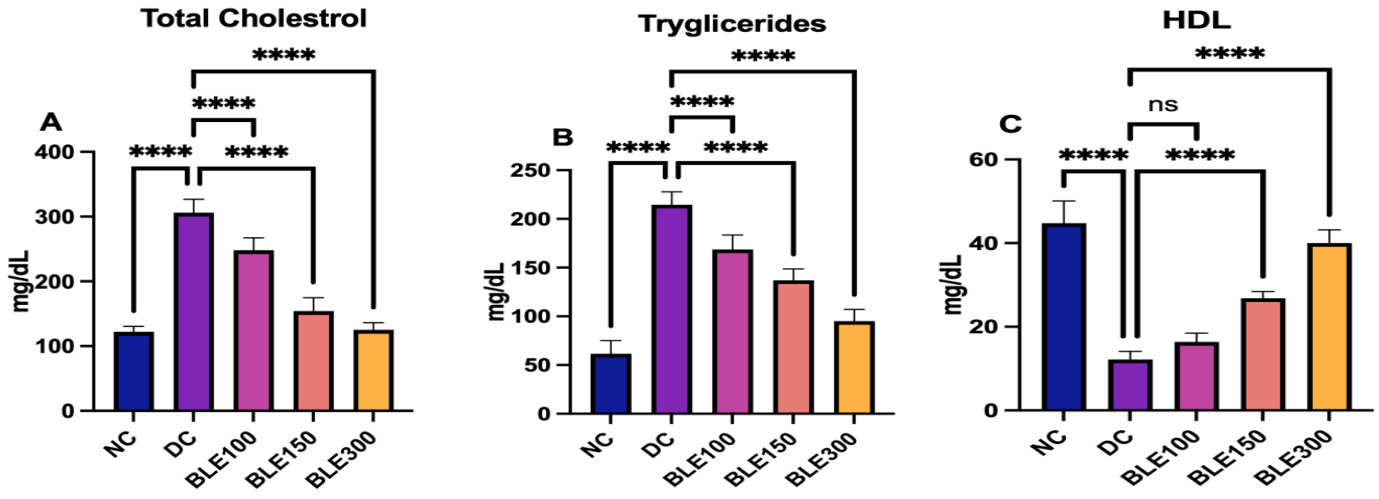


Figure 2. The effect of BLE on SOD (A), GPX (B), and Catalase (C) expresssion. (NC (Normal control), DC (Diabetic control), BLE (Binahong leave extract 100), BLE (Binahong leave extract 150), BLE (Binahong leave extract 300), the data were presented in mean ± standard error of the mean (SEM). (*: p<0,05, **: p<0,01, ***: p<0.001, P>0.05 ns/not significance).

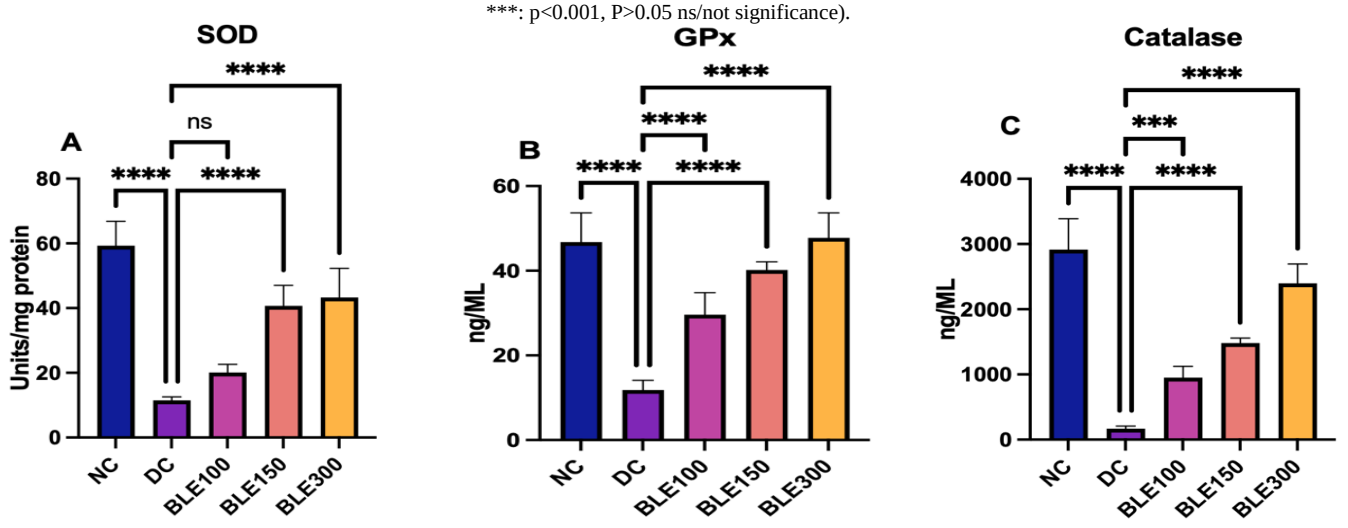
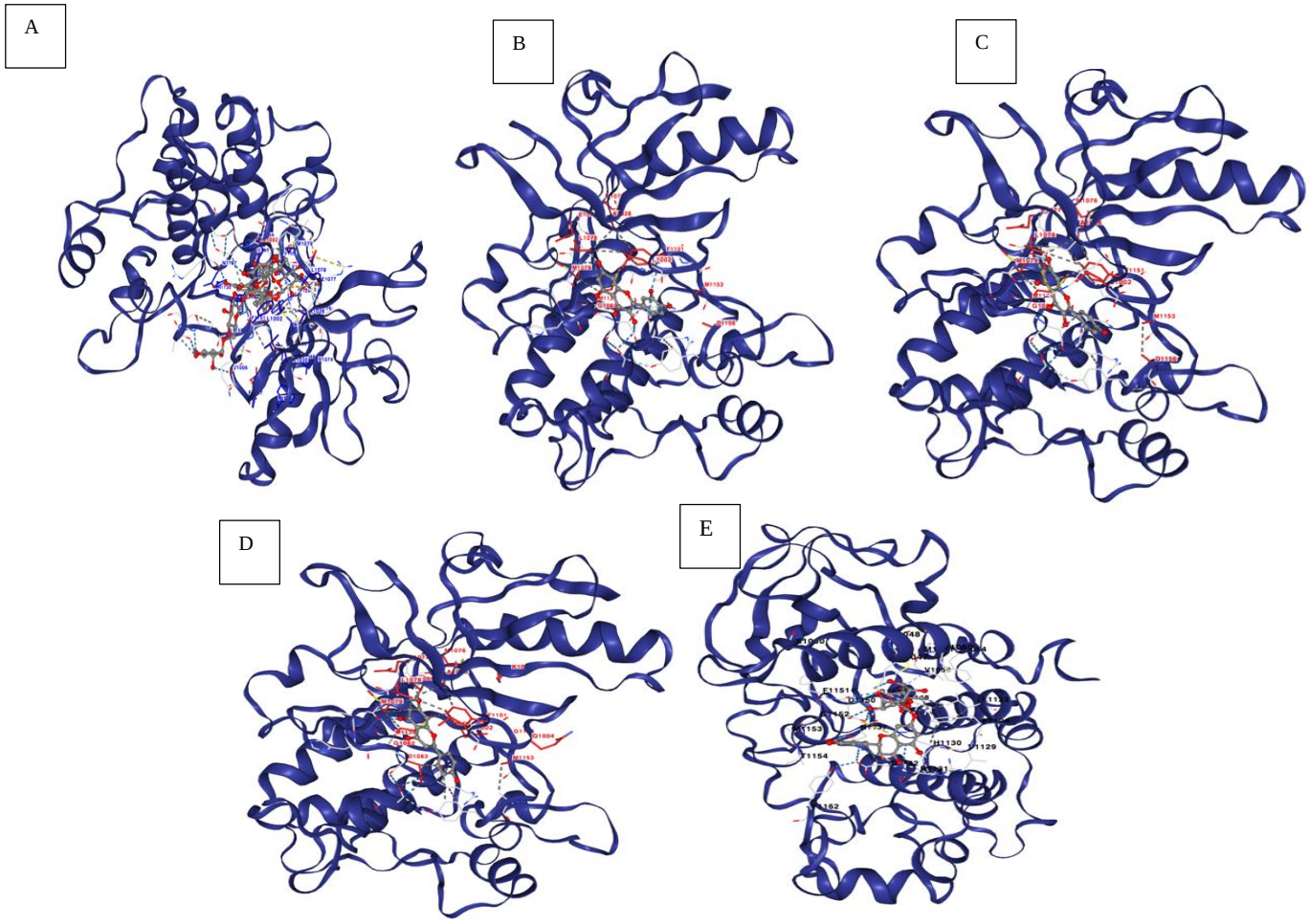


Table 4: Docking affinity scores - kcal/mol

Ligand	Protein	ΔG (kkal/ mol)	Amino Acid Residue
Rutin	Insulin reseptor	-7	ARG1000 LEU1002 GLU1012 ALA1028 MET1051 VAL1059 VAL1060 ARG1061 LEU1062 MET1076 GLU1077 LEU1078 MET1079 ALA1080 HIS1081 GLY1082 ASP1083 ARG1136 ASN1137 MET1139 LYS1147 ILE1148 GLY1149 ASP1150 PHE1151 GLY1152
Quercetin	Insulin reseptor	-4.8	LEU1002 ALA1028 GLU1077 LEU1078 MET1079 ALA1080 HIS1081 GLY1082 ASP1083 SER1086 ARG1136 ASN1137 MET1139 GLY1149 ASP1150 PHE1151 GLY1152 MET1153 TYR1158
Kaempferol	Insulin reseptor	-2.9	ARG1000 LEU1002 GLU1012 ALA1028 GLU1077 LEU1078 MET1079 ALA1080 GLY1082 ASP1083 SER1086 ARG1136 ASN1137 MET1139 ASP1150 PHE1151 MET1153 ASP1156 TYR1158
Naringenin	Insulin reseptor	-4	LEU1002 ALA1028 VAL1060 GLU1077 LEU1078 MET1079 ALA1080 HIS1081 GLY1082 ASP1083SER1086 ARG1136 MET1139 PHE1151 MET1153 ASP1156 TYR1158
Hesperidin	Insulin reseptor	-7.8	GLN1004 GLY1005 SER1006 PHE1007 LYS1030 GLU1043 ASN1046 GLU1047 ALA1048 VAL1050 MET1051 PHE1054 VAL1059 VAL1060 LEU1123 LYS1126 LYS1127 PHE1128 VAL1129 HIS1130 ARG1131 ASP1132 ARG1136 ASN1137 ILE1148 GLY1149 ASP1150 PHE1151 GLY1152 MET1153 THR1154 ARG1155 TYR1162 GLY1169 LEU1170 LEU1171 PRO1172 PHE1186

Figure 3. Docking Visualization. A= Rutin – Insulin Receptor; B= Quercetin – Insulin receptor; C= Kaempferol – Insulin Receptor; D= Naringenin – Insulin Receptor; E = Hesperidin – Insulin Receptor



DISCUSSION

The identification of bioactive compounds in Binahong leaf extract (BLE) as listed in Table 2 provides a compelling basis for its potential antidiabetic effects. Each of the compounds has a profile that suggests possible mechanisms through which they could exert beneficial effects in the management of diabetes[11,12]. Rutin has been documented to improve blood glucose control and enhance the antioxidant defense system, which is often compromised in diabetic patients. Its substantial relative area in BLE indicates that it may be a significant contributor to the antidiabetic effects of the extract. Quercetin is reported to not only have antioxidant properties but also to positively affect insulin secretion and improve pancreatic β-cell function, which are critical in the pathophysiology of diabetes. Kaempferol has been associated with the inhibition of carbohydrate digestion and absorption enzymes, which may result in lower postprandial blood glucose levels. Naringenin has shown potential in modulating glucose metabolism, reducing insulin resistance, and exhibiting anti-inflammatory effects[13,14]. Hesperidin has

demonstrated the ability to improve lipid metabolism and enhance glucose uptake by cells, thereby contributing to its antidiabetic propertie. The antioxidant potential of Binahong leaf extract (BLE) as demonstrated by the DPPH assay is a promising avenue for therapeutic exploration. With BLE exhibiting an IC50 of 27.81 µg/mL, it shows a noteworthy capacity to neutralize free radicals, albeit not as potently as Vitamin C, which has an IC50 of 13.15 µg/mL. Despite this, the relatively strong antioxidant activity of BLE suggests its role in mitigating oxidative stress, which is implicated in the pathophysiology of various chronic diseases. The substantial total phenolic content (TPC) and total flavonoid content (TFC) within BLE are likely to contribute to this effect[15,16]. Phenolic compounds are recognized for their redox properties, which allow them to act as antioxidants, reducing agents, and singlet oxygen quenchers. Flavonoids, on the other hand, possess a range of biological activities, including radical scavenging, which can be directly correlated with their chemical structure. The high correlation coefficients (R^2) close to unity for both phenolic and flavonoid contents indicate the reliability of the methods used and the consistent presence of these compounds in BLE. The TPC was expressed as gallic acid equivalents, and TFC as quercetin equivalents, further confirming the presence of these bioactive substances in significant amounts. These findings are consistent with Prior, Wu, and Schaich, who emphasize the importance of standardized methods for determining antioxidant capacity to ensure the comparability of results. The functional implications of the rich phenolic and flavonoid content in BLE extend beyond their antioxidant activity[17,18]. These compounds have been identified in dietary sources as critical to human health and have been associated with a reduced risk of chronic diseases. This is particularly relevant given the growing interest in natural products and their bioactive compounds for disease prevention and health promotion. The results presented in Table 3 from the study underscore the potential antihyperglycemic effect of Binahong leaf extract (BLE) across different dosages (100, 150, and 300 mg/kg) over a 30-day period. The normal control (NC) group maintained stable glucose levels throughout the study, which aligns with expectations for non-diabetic subjects. In contrast, the diabetic control (DC) group exhibited a significant elevation in glucose levels at the outset, which continued to increase by day 30, indicative of uncontrolled diabetes. The BLE-treated groups exhibited a dose-dependent reduction in glucose levels. The BLE100 group showed a notable decrease, but the most pronounced reduction was seen with the BLE300 group. By day 30, the BLE300 group had glucose levels that approached those of the NC group, demonstrating the efficacy of the extract at higher doses. The findings align with previous research indicating the efficacy of plant extracts in modulating blood glucose levels. For instance, the hypoglycemic effects of flavonoids like rutin and quercetin—both present in BLE—have been well-documented. Furthermore, studies have shown that such bioactive compounds can exert their effects through various mechanisms, including enhancing insulin secretion, improving insulin sensitivity, and inhibiting carbohydrate-hydrolyzing enzymes[19,20]. These results provide a strong rationale for considering BLE as a supplementary treatment for hyperglycemia. However, it is important to acknowledge that translating these findings from experimental models to human subjects necessitates clinical trials to verify efficacy and safety. In the context of total cholesterol, BLE has demonstrated a dose-dependent reduction in levels. The BLE300 group, in particular, showed a significant decrease in cholesterol, approaching the levels of the normal control (NC), which suggests a strong potential for BLE in managing hypercholesterolemia. This is in line with previous research indicating that plant extracts containing flavonoids can positively influence lipid profiles. Regarding triglycerides, all BLE-treated groups showed a substantial reduction, with the highest dose group (BLE300) once again exhibiting the most significant effect. This result supports the use of BLE as a potential natural remedy for hypertriglyceridemia, which is a known risk factor for cardiovascular disease. However, the effect of BLE on HDLc levels was not significant except for the BLE300 group, which showed an improvement. The role of HDLc in cardiovascular health is well-established, as it assists in the removal of cholesterol from the bloodstream. These results underscore the therapeutic potential of BLE in correcting dyslipidemia, a common comorbidity in diabetes. Dyslipidemia is characterized by elevated levels of TC and TG, alongside low HDLc, and is a significant risk factor for the development of atherosclerosis. The findings of this study suggest that BLE, particularly at higher dosages, could offer a multipronged approach to managing this condition by lowering TC and TG while potentially increasing HDLc levels[21,22].

CONCLUSION

In conclusion, Binahong leaf extract presents a promising natural therapeutic option for managing diabetes and improving overall metabolic health. Its multifaceted action on blood glucose levels, lipid metabolism, and antioxidative defenses offers a comprehensive approach to combating the complexities of diabetes. However, it is essential to acknowledge that while the preclinical data are encouraging, clinical trials are necessary to confirm these benefits in human populations and to fully understand the mechanisms of action, optimal dosages, and long-term safety profiles of BLE.

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