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Efficacy of *Beta Vulgaris L.* Ethanol Extract Treating Diabetic: An in Silico and in Vivo Study in Rat Model

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Abstract | The purpose of the study was to examined the antidiabetic efficacy of *Beta vulgaris* L. ethanol extract using in vivo and in silico methodologies. Phytochemical analysis was conducted through qualitative screening with specific tests for key bioactive compounds: Mayer's, Dragendorff's, and Bouchardat's reagents for alkaloids; hydrochloric acid with magnesium powder for flavonoids; and the foam test for saponins. The Microwave-Assisted Extraction (MAE) method was used to extract compounds from the beetroot tubers. Furthermore, diabetic rats were induced via a single intraperitoneal injection of streptozotocin at a dose of 65 mg/kg body weight (BW), which was administered 15 min after the administration of nicotinamide at 110 mg/kg body weight. Diabetic rats were subsequently administered varying doses of *Beta vulgaris* L. ethanol extract (100, 200, and 400 mg/kg BW) for 21 days, following which blood samples were obtained to evaluate hematological parameters, and statistical analysis was conducted using ANOVA followed by Tukey's Multiple Comparison Test to determine significance ($p < 0.05$). Phytochemical screening showed that the betroot extract contained flavonoids, alkaloids, saponin, tannins, and triterpenoids. The in vivo results indicated that all treatment doses significantly decreased blood glucose levels ($p< 0.05$) compared with the untreated control group, exhibiting a dose-dependent manner. Furthermore, hematological parameters demonstrated significant improvement ($p < 0.05$) compared to those in the untreated control group. Moreover, in silico analysis showed that the key compounds in *Beta vulgaris* L. demonstrated stable binding affinities with glucokinase,while toxicity examination also affirmed the safety of the identified compounds. In conclusion, *Beta vulgaris* L. ethanol extract has potential as an antidiabetic medication.

Keywords | Antidiabetic, *Beta vulgaris L.*, Extract, Hematology, In silico, In vivo

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both, leading to metabolic irregularities in carbohydrates, lipids, and proteins (Ojo *et al.*, 2023). Diabetes mellitus is a widespread condition that affects a substantial portion of the global population, causing high morbidity and mortality rates (Ansari *et al.*, 2023). The International Diabetes Fed-

eration (IDF) claimed that there were approximately 537 million adults (20-79 years) living with diabetes in 2021, which has been projected to increase to 643 million adults by 2030 and 783 million by 2045 (Alrasheedi, 2024). The management of diabetes has traditionally relied on lifestyle changes and pharmacotherapy, but the limitations and side effects of conventional antidiabetic drugs have spurred interest in alternative therapies, such as medicinal plants with hypoglycemic properties (Ansari *et al*., 2023). Research has highlighted the potential of phytochemicals found in plants, such as beetroots, offering promise in the prevention and control of diabetes (Oboh *et al*., 2021).

Beta vulgaris L., commonly known as beetroot, has traditionally been used for its medicinal properties. Beetroots are rich in various secondary metabolites including tannins, saponins, alkaloids, flavonoids, glycosides, steroids, and terpenoids. Additionally, it contains essential minerals such as iron (Fe), magnesium (Mg), copper (Cu), sodium (Na), potassium (K), manganese (Mn), calcium (Ca), and zinc (Zn). In addition, the betalain pigment (Sentkowska and Pyrzyńska, 2023). Flavonoid compounds in beetroot demonstrate significant antidiabetic properties by inhibiting gluconeogenesis, increasing the AMP-activated protein kinase (AMPK) ratio, thus enhancing glucose uptake in skeletal muscle and adipose tissue, and promoting insulin secretion. Furthermore, betacyanin in beetroot demonstrated strong antioxidant properties, likely due to the phenolic hydroxy groups in its structure and the abundance of unsaturated bonds on the benzene ring (Butera *et al*., 2002; Chen *et al*., 2021; Costa *et al*., 2017). This structure has the potential to inhibit the reactive oxygen species (ROS) pathways associated with cardiovascular diseases caused by diabetic complications.

Recent studies have explored the pharmacological potential of *Beta vulgaris* L. extracts in various diabetes models. For instance, one study (Stander *et al*., 2021) demonstrated that beetroot juice supplementation significantly improved glycemic control and insulin sensitivity in individuals with type 2 diabetes. Similarly, an investigation (Nimse and Pal, 2015) highlighted that the antioxidative capacity of *Beta vulgaris* L., which can mitigate oxidative stress, is a key factor in the pathogenesis of diabetes. Additionally, a study Domínguez *et al*. (2018) reported that dietary nitrate from beetroots improved muscle function and glucose uptake, suggesting a beneficial role in glucose homeostasis by enhancing erythropoiesis and normalizing hemoglobin levels. Recent studies have explored the antidiabetic potential of *Beta vulgaris* L. (beetroot) and its related plants. *B. vulgaris* L. extracts significantly inhibited α-amylase and α-glucosidase enzymes, which are key targets in diabetes management (Üst *et al*., 2024; Ojo *et al*., 2024). Flavonoids isolated from *B. vulgaris*, including vitexin and acacetin derivatives, have shown promising antidiabetic activity (Mohammed *et al*., 2019). *Beta vulgaris* L. has demonstrated potential in reducing blood glucose levels and enhancing insulin sensitivity, which is attributed to its bioactive compounds, including flavonoids, betacyanins, and saponins. Nevertheless, excessive consumption of beetroot may pose health risks, such as an increased risk of kidney stone formation due to its high oxalate content (Ugrinović, 2012), as well as potential interference with mineral absorption. These safety concerns warrant further investigation to determine the appropriate consumption levels.

This comprehension gap is a significant shortcoming of the present study. The empirical data from various studies support the effectiveness of *Beta vulgaris* L. ethanol extract in rectifying glucose levels and hematological profiles. Research has shown that *Beta vulgaris* L. extract has protective effects against doxorubicin-induced cardiotoxicity, indicating its potential for managing hematological parameters (Nugraha *et al*., 2023). However, its acceptability and incorporation into traditional medical practice are hindered by a lack of holistic understanding of its mechanism of action, including specific pathways and molecular interactions involved in the antidiabetic action and glucose homeostasis of *Beta vulgaris* L.

Although numerous studies have highlighted the antidiabetic potential of *Beta vulgaris* L., few studies have integrated both in vivo and in silico approaches to elucidate its mechanisms. In this study, we used in vivo diabetic rat models to observe the physiological effects of *Beta vulgaris* L. ethanol extract on blood glucose levels and hematological parameters. In parallel, in silico molecular docking was used to investigate the interactions between key phytochemicals and glucokinase, a critical enzyme in glucose regulation. This combined approach aims to provide both empirical evidence and mechanistic insights, offering a comprehensive understanding of the antidiabetic effects of *Beta vulgaris* L. and informing future research on its dosage and safety.

To fill this gap, the study examined the effectiveness of *Beta vulgaris* L. in an animal model of non-obese type 2 diabetes induced by nicotinamide (NA) and streptozotocin (STZ). The use of STZ-NA to induce diabetes in rats is a well-established model that closely mimics human type 2 diabetes (Yan, 2022). A comprehensive method combining computer simulations (in silico) with experiments on living organisms (in vivo) was also carried out. The in silico component involves computer analysis and simulation tools to predict how phytochemicals in *Beta vulgaris* L. tuber interact with biological pathways related to glucose metabolism and diabetes management. Performing these tests is essential for assessing the safety, efficacy, and optimal dosage of *Beta vulgaris* L. tuber extract in biological systems, laying the groundwork for future clinical trials. In this study, we used in vivo and in silico approaches to evaluate the antidiabetic

effects of *a Beta vulgaris* L. ethanol extract. Diabetic rats were divided into control and treatment groups with extract doses of 100, 200, and 400 mg/kg. Blood glucose and hematological parameters were measured over a 21 days. In silico molecular docking was used to analyze the interactions between the bioactive compounds and glucokinase. This combined approach validated the computational predictions with in vivo results and assessed the efficacy of the extract against standard therapy.

MATERIALS AND METHODS

Materials

The tools used included laboratory glassware, set of surgical instruments, aluminum foil, stirring rods, a glucometer (EasyTouch®GC), Glucotest strips (EasyTouch®GCU), animal cages, cotton, parchment paper, pH paper, filter paper, mortar and pestle, an electric balance, oral probes, dropper pipettes, an EDTA tube rack, syringes, cloth gloves, ethylenediaminetetraacetic acid EDTA tubes, Hematology Analyzer XN 450 (Sysmex), and a microwave oven (Samsung). The microwave oven was modified with the hydro-distillation apparatus located at the Laboratory of Biology, Faculty of Pharmacy, Universitas Sumatera Utara. The chemical used were Ethanol (96%, BrataChem), citric acid (99%, BrataChem), ascorbic acid (99%, BrataChem), CMC-Na (0.5%, Merck), streptozotocin (STZ, ≥98%, Cayman Chemical), nicotinamide (≥98%, Chemindo), Mayer's reagent, Dragendorff's reagent, Bouchardat's reagent (Sigma-Aldrich), hydrochloric acid (37%, Merck), magnesium powder (99%, Merck), ferric chloride (FeCl3, 5%, Merck), and Liebermann-Burchard reagent.

Phytochemical Constituent Analysis

The qualitative analysis of phytochemicals in the *Beta vulgaris* L. ethanol extract followed established protocols, as described by Banu and Cathrine (2015) and Harborne (1998). For alkaloid screening, Mayer's, Dragendorff's, and Bouchardat reagents were used, with positive results indicated by the formation of white, orange, and brown precipitates, respectively. Flavonoid detection was conducted by treating the extract with concentrated hydrochloric acid and magnesium powder, resulting in a pink or red color indicating the presence of flavonoids. Saponin testing involved a foam test, in which persistent foam lasting more than 10 min indicated the presence of saponins. Tannin was identified by mixing the extract with ferric chloride (FeCl3), with a blue-black or greenish color indicating tannins. Finally, steroids and triterpenoids were detected using the Liebermann-Burchard reagent, with a color change to blue or green, confirming their presence.

Preparation and Extraction

The sample used in this study was beetroot obtained from Gajah Village, Simpang Empat District, Karo Regency, North Sumatra Province. The beetroot tubers were cleaned with water, chopped into small pieces, dried in a drying cabinet at 50 °C, and then ground into powder. Fifty grams of beetroot powder was placed in a round-bottom flask and dissolved in 300 ml of 96% ethanol (1:6 g/ml). Additionally, 0.5% citric acid and 0.1% ascorbic acid were added relative to the amount of the solvent used (Neagu and Barbu, 2014). The mixture was stirred and allowed to stand for 20 min, and the pH was measured using a pH indicator. The extraction process was carried out using the micwave-assisted extraction (MAE) method with a microwave set for 15 min, 180 W power, and three cycles of irradiation. The filtrate was separated from the residue using a filter paper, and the residue was re-extracted with 150 ml of fresh solvent, with the acid amount adjusted to the solvent volume. The filtrates from the extractions were combined and concentrated using a rotary vacuum evaporator at a pressure of 100 mbar, temperature of 50 °C, and speed of 60 rpm. Microwave-Assisted Extraction (MAE) was utilized for the efficient extraction of bioactive compounds from *Beta vulgaris* L. by leveraging microwave energy to disrupt plant cell walls and enhance the release of phytochemicals. A power level of 180 W was selected, as supported by the literature (Mustapa *et al*., 2015; Lovrić *et al*., 2017), to ensure adequate energy for extraction without degrading sensitive compounds, such as flavonoids and betacyanins. The extraction lasted 15 min and was divided into three 5-minute cycles with cooling intervals to prevent overheating and maintain compound stability.

Sample Size Determination

The sample size for the in vivo study was calculated using Federer's formula, which is commonly used to estimate the appropriate sample sizes in animal experiments to ensure reliable results.

$$Federer\ Formula = (n-1)\ (t-1) \geq 15$$

Description: n is the total sample size; t is the number of treatment groups (in this study, t=6).

Each group consisted of 5 rats, totaling 30 rats. Groups included a normal control, a negative control or untretated group, a positive control (metformin 0.45 mg/kg), and three treatment groups receiving *Beta vulgaris* L. extract at 100, 200, and 400 mg/kg BW. This sample size ensured sufficient statistical power and adhered to the ethical guidelines.

Animal Test Preparation and Treatment Design

Thirty male Wistar rats (10-12 weeks old, 150-200 g) were obtained from the Rat Breeding Centre, Pharmacology Laboratory, University Sumatera Utara. The rats were kept under controlled conditions with a 12-hour light/dark cycle and were given a standardized diet and tap water. They were acclimatized for one week before the study began. The

in vivo study was conducted using six groups of diabetic rats, with five rats per group.

- Normal Control Group: This group received no treatment and served as the baseline for comparison.
- Negative Control Group: Rats in this group were administered 0.5% CMC-Na (Carboxymethyl Cellulose, vehicle control), which was used as a solvent for the plant extract, to assess the effects of the vehicle without any active compound.
- Positive Control Group: This group was treated with metformin (0.45 mg/kg body weight), a standard antidiabetic drug, to provide a benchmark for evaluating the efficacy of the *Beta vulgaris* L. extract.
- Low-Dose Treatment Group: Rats were administered *Beta vulgaris* L. ethanol extract at a dose of 100 mg/kg body weight.
- Medium-Dose Treatment Group: This group received 200 mg/kg body weight of the extract.
- High-dose Treatment Group: Rats were administered the extract at a dose of 400 mg/kg body weight.

The vehicle control (0.5% CMC-Na) was used to assess solvent effects, while different doses (100, 200, and 400 mg/kg BW) were used to evaluate the dose-dependent response. The positive control (metformin) provided a comparison of the efficacy. The test animals were administered 110 mg/kg BW nicotinamide intraperitoneally (i.p.), followed by 65 mg/kg BW streptozotocin i.p. after 15 min. For three days, the rats were provided with sufficient food and water without treatment. Treatment was initiated once the rats were confirmed to have diabetes with blood glucose levels > 200 mg/dL. Treatmnet design illustration can be seen in Figure 1.

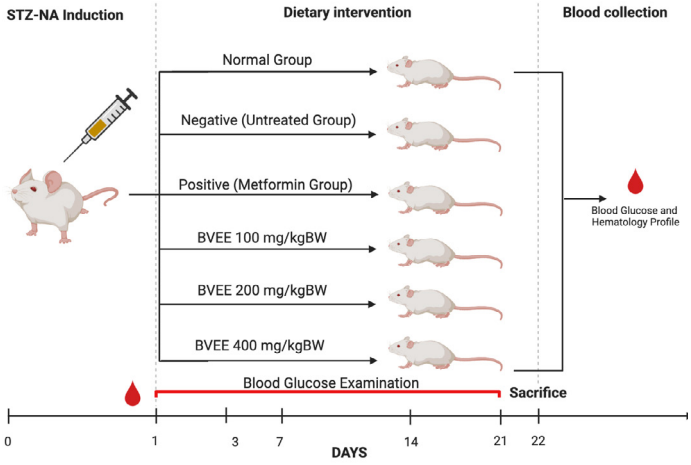
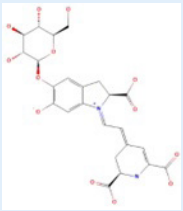
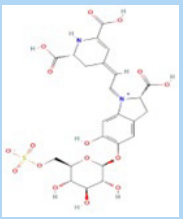
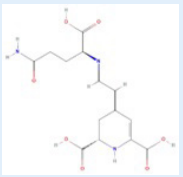
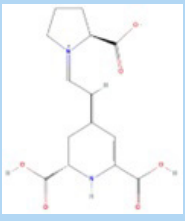


Figure 1: Treatment design illustration.

Blood Sugar Measurement and Hematology Analyzed Testing

Blood glucose levels were measured using a glucometer (EasyTouch® GC) equipped with a glucose strip (EasyTouch® GCU). Measurements were performed under fasting conditions to ensure consistency and reduce variability. Rats were fasted for 12 h overnight with access to water prior to blood sampling. Fasting blood glucose levels were measured at baseline (day 0) and on days 7, 14, and 21 of the treatment period. To obtain blood samples, the tail vein of each rat was punctured using a sterile lancet, and a drop of blood was placed on the test strip for analysis. All measurements were conducted in the morning to minimize diurnal variations in the blood glucose levels. The fasting condition and standardized timing of the measurements helped to ensure the reliability and comparability of the results across all treatment groups (Abarnadevika *et al*., 2022). After 21 days of treatment, the rats were sacrificed using inhalation anesthesia with chloroform in a closed chamber until they were unconscious. The rats were then dissected, and 2 ml of blood was collected from the heart and placed in an EDTA tube for complete blood hematology measurement using a Hematology Analyzer XN 450.

Table 1: Ligand name.

Ligand	Formula	Chemical Structure
Isobetanin	$C_{24}H_{26}N_2O_{13}$	
Prebetanin	$C_{24}H_{26}N_2O_{16}S$	
Vulgaxanthin I	$C_{14}H_{17}N_3O_7$	
Indicaxanthin	$C_{14}H_{16}N_2O_6$	

In silico Tools

The equipment comprised an Lenovo Laptop equipped with a Windows 11 operating system, 64-bit architecture, 4 GB RAM, 256 GB SSD, and a 14-inch display. This study utilizes a range of software tools for diverse objectives. The software and databases are as follows: Windows 11 64-bit operating system, Chimera 1.16 for molecular structure vis-

ualization, Protein Data Bank for protein structure data access, PubChem for chemical compound information access, and SwissDock for protein-ligand docking simulations.

Preparation of Ligands and Proteins

The Glucokinase receptore gene was obtained from the Protein Data Bank website (*). PDB file format. Subsequently, the UCSF Chimera 1.16 tool was used to prepare the sample by eliminating residues. The test compounds were generated using the UCSF Chimera 1.16. This was achieved by inputting the PubChem CID of the ligand, which was acquired earlier using the PubChem online service and stored in mol2 format. Molecular docking involves interactions between proteins and either test chemicals or natural ligands. The Swiss Dock platform was used to execute the docking procedure. Moreover, the ligand names, molecular formulas, and chemical structures of the compounds analyzed in this study are presented in Table 1.

Preparation of Compound for In Silico Toxicity Prediction

Each compound was prepared to obtain Canonical SMILES using the PubChem website (https://pubchem.ncbi.nlm.nih.gov/) (Glüge *et al*., 2023).

Toxicity Prediction of Compound with pK-CSM Tools

Prediction of compound toxicity using pK-CSM tools via http://biosig.unimelb.edu.au/pkcsm/prediction is done by entering Canonical SMILES, then pressing ADMET to obtain absorption analysis results distribution (VDss, Fraction unbound, BBB permeability, and CNS permeability), metabolism, and toxicity (Ayipo *et al*., 2023).

Toxicity Prediction of Compound with Pro-Tox II

Prediction of compound toxicity with Pro-Tox II is accessed via https://tox-new.charite.de/protox_II/, then press Tox Prediction and enter Canonical SMILES, tick all toxicity parameters, and then Start Tox-Prediction to obtain the results of the toxicity analysis of the compound (LD50, Hepatotoxicity, Carcinogenicity, Immunotoxicity, Mutagenicity, Cytotoxicity, AhR, AR, AR-LBD, Aromatase, ER, ER-LBD, PPAR-Gamma, nrf2/ARE, HSE, MMP, Phosphoprotein tumor suppressor, and ATAD 5) (Abhishek *et al*., 2023).

Statistical Analysis

The in vivo results, including the blood glucose concentration and hematology profile, were analyzed using ANOVA with Tukey's Multiple Comparison Test. P-values for significance were set at $P < 0.05$. Values for all measurements are expressed as the mean ± SD. The histogram data were constructed using GraphPad Prism Software 9.0.

RESULTS AND DISCUSSION

Phytochemical Constituent Analysis

Qualitative analysis of the compounds in the extracts of beetroot tubers was performed using the standard procedures shown in Table 2.

Table 2: Phytochemical screening result.

Content	Reagent	Dried Sample	Ethanol extract
Flavonoids	HCL(c), Mg powder, Amyl alcohol	+	+
Alkaloids	Mayer, Bouchardat, Dragendorf	+	+
Saponins	Foam test	+	+
Tannins	FeCl3	+	+
Steroids/ triterpenoid	Liberman Burchard	+	+

The phytochemical screening results in Table 2 indicate that both dried beetroot powder and its ethanol extract contain flavonoids, alkaloids, saponins, tannins, and steroids/triterpenoids, all of which contribute to their antidiabetic potential. Flavonoids are known for their antioxidant properties, ability to reduce oxidative stress, and ability to improve insulin sensitivity (Guo *et al*., 2020). Alkaloids help regulate glucose metabolism by enhancing insulin secretion or by improving its action (Eddouks and Amssayef, 2023). Saponins exhibit hypoglycemic effects by inhibiting enzymes involved in carbohydrate digestion and reducing postprandial blood glucose levels (Choudhary *et al*., 2021). Tannins can also improve insulin sensitivity and protect pancreatic β-cells (Papuc *et al*., 2021). These compounds make beetroot a promising candidate for further research on diabetes.

Microwave-Assisted Extraction (MAE) was chosen in this study for its ability to rapidly heat the solvent and plant matrix, thereby enhancing the release of bioactive compounds. While MAE offers several advantages, including shorter extraction times and reduced solvent usage, it is important to compare its efficiency with that of conventional methods, such as maceration and Soxhlet extraction (El Maaiden *et al*., 2022). Studies have shown that MAE typically yields a higher concentration of flavonoids and phenolic compounds than maceration or Soxhlet extraction. For instance, Mustapa *et al*. (2015) reported that the yield of total phenolics using MAE was higher than that obtained using other extraction methods. Similarly, Lovrić *et al*. (2017) demonstrated that MAE has higher flavonoid content. Moreover, Kapoore *et al*., (2018) stated that MAE has a high extraction yield and requires less extraction time. In the context of *Beta vulgaris* L., the use of MAE in this study was guided by the reported advantages. The rapid heating and efficient cell wall disruption provided by microwave

Table 3: Average glucose level results.

Groups	Average Glucose Levels ± SD				
	Before induction of STZ and nicotinamide 0th day	After induction of STZ and nicotinamide 3rd day	7th day	14th day	21th day
Normal	84.4±6.34	86.8±6.30[b]	83.2±6.14*	82.0±3.53*	87.8±6.83*
Negative control	89.8±8.40	493.0±58.39[b]	496.8±57.89 [b]	505.8±60.50	502.0±55.29
Positive control	84.2±8.22	527.8±50.18[b]	393.4.0±49.95*[b]	246.0±46.30*[b]	99.6±11.08*
BVEE 100mg/kgbw	89.0±8.33	515.8±70.27[b]	429.0±40.83* [ab]	318.6±37.77*[a b]	204.2±18.64*[ab]
BVEE 200mg/kgbw	86.4±7.30	508.0±61.32[b]	420.0±60.19* [ab]	300.0±50.60* [ab]	165.0±41.12*[ab]
BVEE 400mg/kgbw	94.2±7.53	497.6±58.20[b]	417.0±50.28* [ab]	256.0±48.26*[b]	116.6±23.49*[ab]

Description: Values are presented as the mean ± SD; n=5. *P<0.05; significantly different from the negative control. (a) P<0.05; significantly different from the positive control. (b)P<0.05; significantly different from the normal control.

energy are particularly beneficial for extracting sensitive phytochemicals, such as betacyanins and flavonoids, which may degrade under prolonged heat exposure in conventional methods. Thus, MAE not only enhances the yield of these bioactive compounds, but also preserves their stability, supporting the choice of this extraction technique in our study.

Antidiabetic Activities

An antidiabetic test was conducted using ethanol extract from beet root tuber, which was divided into three different doses (100,200,400 mg/kgBW), along with a normal control, a negative control (CMC-Na), and a positive control (metformin) in rats. Blood sugar levels were measured with a glucometer in male Wistar rats that had been induced with streptozotocin-nicotinamide as a diabetic agent. Treatment commenced when the blood glucose levels exceeded 200 mg/dL, as shown in Table 3.

In Table 3, it was shown that the normal control group, blood sugar levels stayed relatively consistent, starting at 84.4±6.34 mg/dL on Day 0 and slightly changing throughout the experiment period, ending at 87.8±6.83 mg/dL on Day 21. In the positive control group, treated with metformin, it was clearly shown a sharp increase in blood sugar levels from 84.2±8.22 mg/dL on Day 0 to 527.8±50.18 mg/dL on Day 3. However, it then showed a notable decrease, with levels dropping to 393.4±49.95 mg/dL on Day 7, followed by 246.0±46.30 mg/dL on Day 14, and reaching 99.6±11.08 mg/dL by Day 21.

On the other hand, the negative control group, which received CMC-Na, showed a significant rise in blood sugar levels, from 89.8±8.40 mg/dL on Day 0 to 493.0±58.39 mg/dL on Day 3, and further increasing to 502.0±55.29 mg/dL by Day 21. Meanwhile, the groups treated with BVEE showed a dose-dependent reduction in blood sugar levels. The group receiving 100 mg/kg bw experienced an initial rise in blood sugar levels, increasing from 89.0±8.33 mg/dL on Day 0 to 515.8±70.27 mg/dL by Day 3. However, over time, their blood sugar levels significantly decreased, reaching 429.0±40.83 mg/dL by Day 7, 318.6±37.77 mg/dL by Day 14, and eventually dropping to 204.2±18.64 mg/dL by Day 21.

Similarly, the 200 mg/kg bw group began with a blood sugar level of 86.4±7.30 mg/dL, which peaked at 508.0±61.32 mg/dL on Day 3. This group also saw a gradual decrease in blood sugar levels, falling to 420.0±60.19 mg/dL by Day 7, 300.0±50.60 mg/dL by Day 14, and 165.0±41.12 mg/dL by Day 21. Finally, the highest dose group, receiving 400 mg/kg bw, showed a comparable pattern. Their blood sugar levels rose to 497.6±58.20 mg/dL on Day 3 but then steadily declined to 417.0±50.28 mg/dL by Day 7, 256.0±48.26 mg/dL by Day 14, and finally 116.6±23.49 mg/dL by Day 21. This consistent decrease across the different dosages of BVEE indicated a dose-dependent effect on lowering blood sugar levels in diabetic rats Figure 2.

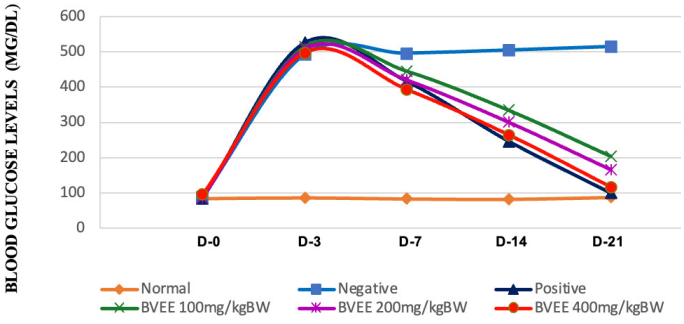


Figure 2: Reduction of blood glucose levels profile.

The non-obese type 2 diabetes model, induced by streptozotocin (STZ) and nicotinamide (NAD), is commonly used to evaluate antidiabetic substances because of their opposing effects on β cells; STZ is cytotoxic, while NAD is cytoprotective (Szkudelski, 2012; Yan, 2022). Nicotinamide mitigates STZ-induced β-cell damage, allowing some β-cells to remain functional and release insulin in response to glucose (Masiello *et al.*, 1998; Yan, 2022). Figure 2 shows a significant difference (p<0.05) between the normal and negative control groups, indicating that STZ (65 mg/kg)

and NAD (110 mg/kg) increased the blood glucose levels in rats. STZ toxicity occurs via GLUT2-mediated entry into β-cells, forming reactive methyl carbonium ions that lead to DNA methylation and PARP-1 activation, depleting NAD+, and causing energy deficits. Nicotinamide helps maintain β-cell function by replenishing NADH, preventing ATP and NAD+ depletion through PARP-1 inhibition, and enhancing insulin production (Szkudelski, 2012).

The positive control group treated with metformin showed a significant difference ($p<0.05$) compared to the negative control group. The mechanism of action of metformin involves the activation of the AMPK pathway to increase glucose uptake in muscle cells, as recent research suggests. While it enhances glucose uptake in peripheral tissues, such as muscles (Banerjee *et al*., 2016; Rahelić and Šakić, 2023), it also reduces blood glucose levels by increasing glucose clearance and lactate production, independent of reductions in endogenous glucose production (Sarabhai *et al*., 2023). Additionally, metformin promotes weight loss by upregulating the anorectic cytokine GDF15, which is involved in sustaining full AMPK activation (Aguilar-Recarte *et al*., 2023). Furthermore, studies have shown that metformin can affect glucose uptake in the skeletal muscle, liver, and adipose tissue, indicating its potential to regulate protein expression and translocation to improve glucose metabolism (Kohler *et al*., 2023).

The administration of BVEE at doses of 100 mg/kg BW and 200 mg/kg BW showed significant differences compared with BVEE at 400 mg/kg BW and the positive control on days 7, 14, and 21 ($p<0.05$). The differences in antidiabetic activity at each extract dose can be attributed to several factors, including individual organ variability, differing physiological conditions, and pancreatic damage in male White rats, which disrupt the metabolic system. This results in active substances not being fully dissolved in the rats' bodies and not being maximally absorbed by the receptors (Li *et al*., 2022). The dose-dependent effects of *Beta vulgaris* ethanol extract (BVEE) may fluctuate owing to metabolic variations that affect the absorption and metabolism of phytochemicals (Rathaur and Sr, 2020). Moreover, genetic heterogeneity among rat strains can influence their sensitivity to hypoglycemic effects (Woods *et al*., 2012).

The statistical test results for the reduction in blood glucose levels (BGL) with various doses of beetroot ethanol extract (BVEE) at 100 mg/kgBW, 200 mg/kgBW, and 400 mg/kg W showed a significant difference ($p<0.05$) compared with the negative control group. This indicates that BVEE administration can lower the blood glucose levels in rats. Administration of BVEE at a dose of 400 mg/kg BW was significantly different from that of the positive control on the 7th day of treatment ($p<0.05$). However, on days 14th and 21st days, the analysis did not show a significant difference, indicating that the effect of the extract at a dose of 400 mg/kg BW was comparable to that of the positive control metformin at 45 mg/kg BW.

Previous preclinical research has provided an optimistic outlook on the potential of bioactive food components to prevent and treat metabolic disorders via various molecular mechanisms (Abedimanesh *et al*., 2021; Gupta and Prakash, 2014). Several studies have demonstrated the significant antidiabetic effects of *Beta vulgaris* through various mechanisms, including inhibition of α-amylase and α-glucosidase enzymes and reduction in blood glucose levels (Sik Haam *et al*., 2022; Bouchmaa *et al*., 2022). In this study, metformin was used as a positive control, and its primary mechanism involved activation of the AMP-activated protein kinase (AMPK) pathway, which enhances glucose uptake in muscle cells and inhibits hepatic gluconeogenesis (Rena *et al*., 2017). Similarly, *Beta vulgaris* ethanol extract (BVEE) activates insulin-related pathways, including AMPK and IRS-1/PI3K/AKT, improving glucose metabolism and mimicking the action (Wu *et al*., 2022). *Beta vulgaris* enhances glucose-stimulated insulin secretion by increasing acetylcholine and GLP-1 levels while also promoting glucose uptake through elevated GLUT4 transporters (Kabir *et al*., 2015). Moreover, betanin, the primary natural pigment in red beetroots, has demonstrated anti-diabetic effects (Hadipour *et al*., 2020). Betanin regenerates the remaining pancreatic beta cells and increases the amount of insulin-immunoreactive beta cells, thereby stimulating insulin secretion and improving the level of blood glucose in experimental animals (Abedimanesh *et al*., 2021; Dhananjayan *et al*., 2017; Han *et al*., 2014). The hypoglycemic effects of betalain compounds in beetroots are believed to stem from their ability to suppress glycogenolysis and gluconeogenesis. (Abedimanesh *et al*., 2021; Madadi *et al*., 2020).

Furthermore, the high content of polyphenol compounds and betacyanin pigments in beetroot can effectively improve blood glucose levels and reduce the risk of diabetic complications. This is supported by a previous study (Lugo-Radillo *et al*., 2012), which reported that the antidiabetic activity in rats treated with 9.6 mg of betacyanin for 40 days resulted in a 50.94% reduction in glucose levels. Betacyanin acts as an antidiabetic agent by regenerating pancreatic beta cells, increasing insulin production and sensitivity, and serving as an antioxidant to prevent excessive oxidation and damage to β cells. It also reduces the production of AGE compounds in the blood vessels, thereby potentially preventing complications in patients with diabetes (Lu *et al*., 2009; Rahimi *et al*., 2019). Both metformin and BVEE affect glucokinase, an enzyme critical for glucose sensing in pancreatic β-cells. However, BVEE provides unique effects through its rich content of antioxidants,

Table 4: Hematological parameter results.

Para-meters	Treatment Groups (Average values ± SD)					
	Negative Control	Positive Control	BVEE 100mg/kgbw	BVEE 200mg/kgbw	BVEE 400mg/kgbw	Normal
RBC (10^{12}/L)	6.24±0.24	9.58±0.59	9.25±0.48*	9.23±0.50*	9.46±0.68*	10.12±1.62*
HGB (g/dL)	8.92±1.7	13.78±1.25	12.90±1.58*	13.50±2.54*	14.52±1.65*	14.41±1.85*
HCT (%)	31.2±0.59	45.0±7.80	46.3±3.56*	44.46±4.66*	48.79±2.55*	50.66±2.82*
MCV (fL)	40.02±2.75	48.12±3.05	48.76±1.90*	48.62±1.75*	49.28±1.09*	50.58±2.63*
MCH (pg)	10.53±1.32	14.22±0.56	14.01±0.52*	12.34±0.32*	14.34±3.13*	14.64±0.38*
MCHC(g/dL)	26.33±0.76	30.06±0.33	28.9±0.82*	29.44±0.68*	31.75±0.42*	30.64±3.85*
PLT (10^9/L)	401.2±129.2	890±130.02	876.1±105.16*	895.0±173.21*	900.5±144.5*	868±123.46*
WBC (10^9/L)	1.58±0.44	5.98±0.51	5.44±0.70*	5.5±0.67*	6.1±0.54*	6.3±0.81*
NEU (%)	34.5±2.06	14.0±1.00	14.6±1.00*	15.2±0.46*	15.5±0.78*	14.8±1.92*
LYMP (%)	45.6±1.19	70.5±2.78	66.7±11.48*	72.1±2.57*	75.6±3.55*	71.0±14.16*
MON (%)	6.6±0.12	3.62±0.67	4.0±0.10	5.1±0.64	5.0±1.23	4.8±0.86
EOS (%)	2.4±0.52	2.7±0.33	2.8±0.11	3.0±0.15	3.3±0.56	3.6±0.89
BAS (%)	2.7±0.12	3.0±4.39	3.1±0.29	3.5±3.35	3.3±2.54	2.8±0.21

Description: All values are mean±SD (number of experiments, n= 5); * represents significant difference compared to the negative control group.

particularly betacyanins, which help mitigate oxidative stress, a feature that is not directly targeted by metformin (Rahimi *et al*., 2019). Furthermore, BVEE saponins inhibit α-glucosidase**,** delay carbohydrate absorption, and reduce postprandial glucose levels, whereas metformin primarily decreases hepatic gluconeogenesis.

Hematological Analysis

The hematological analysis presented in Table 4 shows the results of key blood parameters measured across different treatment groups, including the negative control, positive control, and various dosages of BVEE (100 mg/kg, 200 mg/kg, and 400 mg/kgBW).

Treatment with BVEE resulted in a significant increase in hemoglobin and hematocrit levels compared to the negative control group ($p < 0.05$). The RBC count also increased by 46%, indicating enhanced erythropoiesis compared to that in the negative control group ($p < 0.05$). The hematological test results indicated that beetroot extract influenced blood parameters in rats. There was a noticeable change in the red blood cell and platelet profiles, with the rats receiving the extract showing higher values than the normal rats. This finding is consistent with Nugraha's study, which demonstrated that beetroot extract supplementation can enhance platelet and red blood cell profiles (Nugraha *et al*., 2020, 2021), as well as increase hemoglobin and hematocrit, suggesting enhanced red blood cell production, likely from iron-rich compounds and betanin's role in erythropoiesis (Hadipour *et al*., 2020). These changes imply a better oxygen transport and health status. Stable WBC counts indicated no effect on immune function, supporting the safety of the extract. No signs of anemia or immunosuppression were observed. Slight platelet changes at higher doses may require further study to determine potential coagulation risks. Overall, the hematological results suggest improved blood health without toxicity.

Beetroots are a rich source of various essential nutrients including K, Ca, Mg, Na, Fe, Zn, phosphorus, Cu, and Mn (Al-Harbi *et al*., 2021; Bhupinder and Bahadur, 2014). Owing to its nutritional value, it may serve as a functional food to protect against oxidative stress, which is a factor in chronic metabolic diseases such as type 2 diabetes and cardiovascular disease (Al-Harbi *et al*., 2021; Chhikara *et al*., 2019).

Given these known benefits, it is crucial to further understand the molecular mechanisms underlying the bioactive compounds in beetroot, particularly the pigments betacyanin and betaxanthin, along with their derivatives, such as betanin, isobetanin, prebetanin neobetanin, vulgaxanthin I, vulgagxanthin II, and indicaxanthin. These compounds are known for their potent antioxidant properties, which contribute to the scavenging of free radicals and the reduction of oxidative stress in the body (Al-Numair *et al*., 2012; Nugraha *et al*., 2024). Therefore, by employing computational methods, we can simulate and predict the interactions of bioactive compounds in beetroots with specific molecular targets involved in metabolic diseases. In silico studies allow for a deeper exploration of the potential efficacy of the compounds, offering insights into their binding affinities, pharmacokinetics, and possible pathways for therapeutic action (Shaikh *et al*., 2007).

Table 5: Docking affinity scores on Glucokinase receptor.

Ligand	Protein	ΔG (kcal/ mol)	Amino Acid Residue
Isobetanin	Glucokinase	-8,6	Chain A: ASP78 LEU79 GLY80 GLY81 THR82 ASN83 PHE84 ARG85 LYS102 MET107 TYR108 SER109 ILE110 THR149 SER151 LYS169 ASP205 THR209 ILE225 GLY227 THR228 GLY229 CYS230 GLY294 GLY295 LYS296 TYR297 MET298 GLY299 GLU300 ARG327 GLY328 ALA329 PHE330 GLU331 THR332 ARG333 PHE334 SER336 ASP409 GLY410 SER411 VAL412 LYS414 LEU415 SER441 GLU442 GLU443 GLY444 SER445 GLY446
Prebetanin	Glucokinase	-8,8	Chain A: ASP78 LEU79 GLY80 GLY81 THR82 ASN83 PHE84 ARG85 MET107 THR149 SER151 LYS169 GLY170 ASP205 THR209 ILE225 VAL226 GLY227 THR228 GLY229 CYS230 VAL277 SER280 SER281 ALA282 GLY295 LYS296 TYR297 MET298 GLY299 GLU300 ARG327 GLY328 ALA329 PHE330 GLU331 THR332 ARG333 ASP409 GLY410 SER411 VAL412 LYS414 LEU415 GLU443 GLY444 SER445 GLY446
Vulgaxanthin-I	Glucokinase	-7.5	Chain A: ASP78 LEU79 GLY80 GLY81 THR82 ASN83 ARG85 LYS102 MET107 THR149 SER151 LYS169 ASP205 THR209 ILE225 VAL226 GLY227 THR228 GLY229 CYS230 GLY295 LYS296 THR332 ASP409 GLY410 SER411 VAL412 LYS414 LEU415 SER441 GLU442 GLU443 GLY444 SER445 GLY446
Indicaxanthin	Glucokinase	-7,5	Chain A: ASP78 LEU79 GLY80 GLY81 THR82 ASN83 ARG85 MET107 THR149 SER151 LYS169 ASP205 THR209 ILE225 VAL226 GLY227 THR228 GLY229 CYS230 ASP409 GLY410 SER411 LYS414 LEU415 SER441 GLU442 GLU443 GLY444 SER445 GLY446

Docking Visualization

In this study, the antidiabetic molecular docking of *Beta vulgaris* L tuber extract was evaluated. The docking Affinity Score visualization results for isobetanin, prebetanin, vulgaxanthin-I, and indicaxanthin are summarized in Table 5.

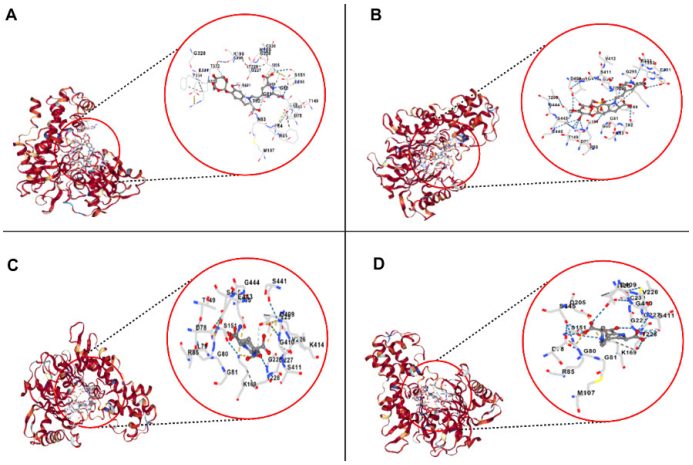


Figure 3: Docking visualization Glucokinase with; **A:** Isobetanin; **B:** Prebetanin; **C:** Vulgaxanthin I; **D:** Indicaxanthin.

The molecular docking results shown in Figure 3 and Table 5 indicate that prebetanin exhibits the most potent interaction with the active sites of glucokinase proteins. Prebetanin demonstrates the highest binding affinity with Glucokinase protein (8,8 kcal/mol), suggesting it may effectively activate this enzyme, which plays a crucial role in regulating glucose in pancreatic cells (Raj *et al*., 2013). Additionally, Isobetanin shows promising antidiabetic potential, with affinity scores of -8.6 kcal/mol. Considering their interactions with both proteins, isobetanin may have a similar impact on glucose regulation and insulin sensitivity. Vulgaxanthin I and Indicaxanthin exhibit relatively weaker binding affinities compared to betanin and isobetanin, but still demonstrate moderate binding strengths, implying that they may have antidiabetic properties, though not as potent as Prebetanin and Isobetanin. Overall, this study provides valuable insights into the molecular mechanisms and potential therapeutic uses of *Beta vulgaris* tuber extract, particularly in the treatment of type 2 diabetes. Further research is required to examine the pharmacodynamic effects and therapeutic implications of these novel binding interactions with B. Vulgaris compounds and similar drugs, especially those that exhibit antidiabetic properties.

Insilico Toxicity Prediction

The in silico toxicity predictions supported the potential safety of the main compound in the beetroot extract. Ensuring the safety of beetroot extracts and their main components is crucial. The results of in silico toxicity prediction are presented in Tables 6 and 7.

The in silico toxicity prediction models for the main compounds of *Beta vulgaris* L. (isobetanin, prebetanin, vulgaxanthin-I, and indicaxanthin) suggested a generally favorable safety profile, with the absence of AMES toxicity, indicating a low likelihood of mutagenic effects. This aligns

Table 6: Prediction of toxicity (pKCSM) *Beta vulgaris* main compounds.

Property	Model Name	Predicted Value				Unit
		Isobetanin	Prebetanin	Vulgaxanthin I	Indicaxanthin	
Toxicity	AMES toxicity	No	No	No	No	Categorical (Yes/No)
Toxicity	Max. tolerated dose (human)	0.678	0.507	0.797	0.954	Numeric (log mg/kg/day)
Toxicity	hERG I inhibitor	No	No	No	No	Categorical (Yes/No)
Toxicity	hERG II inhibitor	No	No	No	No	Categorical (Yes/No)
Toxicity	Oral Rat Acute Toxicity (LD50)	2.471	2.481	2.328	2.167	Numeric (mol/kg)
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	3.652	2.986	2.353	2.324	Numeric (log mg/kgbw/day)
Toxicity	Hepatotoxicity	Yes	No	Yes	Yes	Categorical (Yes/No)
Toxicity	Skin Sensitisation	No	No	No	No	Categorical (Yes/No)
Toxicity	T.Pyriformis toxicity	0.285	0.285	0.285	0.285	Numeric (log ug/L)
Toxicity	Minnow toxicity	8.496	9.931	4.619	3.89	Numeric (log mM)

Table 7: Toxicity class of *Beta vulgaris* L. Main compound (protox online).

No	Parameters	Isobetanin	Prebetanin	Vulgaxanthin-I	Indicaxanthin
1.	Predicted LD 50	305 mg/kg	305 mg/kg	1059 mg/kg	1000 mg/kg
2.	Predicted toxicity class	Class 4	Class 4	Class 4	Class 4
3.	Average similarity	55.23%	52.4%	46.72%	47.42%
4.	Prediction accuracy	67.38%	67.38%	54.26%	54.26%

with the expected safety profile for similar natural compounds. The prediction results showed that none of these compounds inhibited hERG I or II, which are crucial factors because hERG inhibition can lead to cardiotoxicity. The non-inhibition of hERG channels by all four compounds is a positive indicator of their safety in terms of potential cardiac effects. The predicted oral rat acute toxicity (LD50) values for isobetanin, prebetanin, vulgaxanthin-I, and indicaxanthin were 2.471, 2.481, 2.328, and 2.167 mol/kg, respectively, suggesting moderate acute toxicity across these compounds. Chronic toxicity (LOAEL) values further indicated moderate toxicity, with isobetanin showing the highest value and indicaxanthin showing the lowest, consistent with the expected toxicity profiles of natural compounds. However, the models predict hepatotoxicity for isobetanin, vulgaxanthin-I, and indicaxanthin, suggesting a potential risk of liver damage, in contrast to prebetanin, which shows no predicted hepatotoxicity. This highlights the need for further investigation, particularly of isobetanin and indicaxanthin, to understand their potential impact on liver health. The absence of skin sensitization potential across all four compounds was a positive outcome, reducing concerns regarding topical applications. T. pyriformis toxicity values were consistent across the compounds, indicating a similar level of environmental toxicity in this model. Minnow toxicity values showed variability, with isobetanin and prebetanin displaying higher values than vulgaxanthin-I and indicaxanthin, suggesting potential differences in environmental impacts. Overall, the in silico toxicity prediction indicated that *Beta vulgaris L.* compounds exhibited a generally safe profile with specific considerations for hERG inhibition and skin sensitization, which is consistent with the existing literature on the safety of natural compounds and provides a basis for further in vivo and clinical studies to confirm these predictions.

In comparison, betalains, pigments that give beetroots their bright color, have been linked to some potential toxic effects when consumed in large amounts. These pigments can interfere with the absorption of minerals, such as iron, and may act as pro-oxidants in certain situations (Baião *et al*., 2017). Although beetroots are known for their health benefits, there are concerns regarding their possible toxicity. Some studies have suggested that eating beetroots could increase the risk of kidney stones or other kidney problems, especially in people who already have kidney issues (Mirmiran *et al*., 2020). In addition, while the health benefits of beetroot are well documented, more research is needed to fully understand the potential toxicity and determine the safety of beetroot consumption, particularly with long-term or high-dose use.

CONCLUSIONS AND RECOMMENDATIONS

The results of the phytochemical screening showed that the ethanol extract of beetroot tubers contained flavonoids, alkaloids, saponins, tannins, and steroids/triterpenoids. In animal tests, *Beta vulgaris L.* ethanol extract showed potential as a treatment for diabetes, with certain doses significantly reducing blood glucose levels and improving

hematological profiles compared to metformin ($p<0.05$), particularly at 400 mg/kg body weight. Additionally, an in silico study revealed the toxicity profiles of the main compounds in beetroot and predicted their molecular interactions, indicating potential therapeutic uses. However, the toxicity class of these compounds suggests that careful consideration of dosage is necessary for practical applications.

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NOVELTY STATEMENT

The novelty of this study was highlighted through in vivo and in silico evaluations of the effects of beetroot extract on the treatment of type 2 diabetes in rats.

AUTHOR'S CONTRIBUTIONS

All authors contributed equally to the manuscript.

Conflict of Interest

The authors declare that they have no conflicts of interest related to this research.

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