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Original Research Article

Bioassay Guided Partition of Dandang Gendis (*Clinacanthus nutans* L.) Monitored by Glucose Consumption Assay on Myoblast Cells and GLUT4 Molecular Docking Analysis

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ABSTRACT

Diabetes mellitus (DM) is a prevalent global metabolic disorder, with an increasing incidence in Indonesia. *Clinacanthus nutans* L. has been traditionally used for its hypoglycemic properties, yet its precise mechanism of action remains unclear. This study aimed to assess the effects of *C. nutans* partitions on glucose consumption and to predict their potential interaction with GLUT4 through in silico analysis. A bioassay-guided partition approach was employed to partition *C. nutans*. Insulin resistance was induced in C2C12 myoblasts using 0.75 mM palmitic acid, followed by treatment with *C. nutans* partitions at concentrations of 500, 250, and 125 µg/mL. Glucose consumption was quantified using the GOD/PAP assay, with statistical significance at $p<0.05$. Moreover, molecular docking analysis was conducted to evaluate the binding affinity of *C. nutans* bioactive compounds to GLUT4. Results indicated that the insoluble partition of *C. nutans* significantly enhanced glucose uptake in insulin-resistant C2C12 cells at 500 µg/mL ($p = 0.0021$) and 250 µg/mL ($p = 0.0198$) compared to untreated controls. However, molecular docking analysis did not reveal direct binding interactions between *C. nutans* compounds and GLUT4. In conclusion, the insoluble partition of *C. nutans* effectively improves glucose consumption in insulin-resistant C2C12 cells, though its mechanism of action does not appear to involve direct GLUT4 modulation. These findings suggest that *C. nutans* holds promise as a complementary therapy for insulin resistance, aligning with Sustainable Development Goal (SDG) 3 by contributing to alternative treatments for type 2 diabetes mellitus (T2DM) and SDG 9 by fostering pharmaceutical innovation and sustainable plant-based therapies.

Keywords: *Clinacanthus nutans*, Diabetes, Glucose Transporter Type 4 (GLUT4), Bioassay guided partition, Glucose uptake.

Introduction

Diabetes mellitus (DM) is a major global health threat and one of the leading causes of mortality. Its prevalence continues to rise annually, with an estimated 9.3% of the global population affected in 2019, a figure projected to increase to 10.9% by 2045.[1] Furthermore, studies suggest that glucose-lowering treatments for type 2 diabetes (T2D), including synthetic medications and insulin therapy, may pose more risks than benefits, particularly for elderly individuals.[2] Consequently, the development of safer and more effective antihyperglycemic therapies remains an urgent priority.

Three key proteins—IRS-1, Akt, and GLUT4—play essential roles in the pathophysiology of diabetes mellitus (DM). When insulin binds to its receptor, it activates insulin receptor substrate-1 (IRS-1), a signaling mediator that transmits the insulin signal into the cell. IRS-1 becomes active upon phosphorylation at its tyrosine-612 residue. This activation leads to the phosphorylation of Akt, a cytosolic kinase, at its serine-473 residue.

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Subsequently, activated Akt facilitates the translocation of GLUT4, which is a glucose transporter protein, to the cell membrane. GLUT4 is responsible for mediating glucose uptake from the bloodstream into the cytosol.[3-5] Dandang Gendis (*Clinacanthus nutans*) is an indigenous plant in Indonesia that has been traditionally used as a herbal remedy for lowering blood glucose levels. Several studies have demonstrated its antihyperglycemic properties. The extract of *C. nutans* has been shown to significantly reduce blood glucose levels in Wistar rats with diabetes when administered at a dose of 75 mg/kg body weight.[6] Its ethanolic extract contains bioactive compounds, including flavonoids, saponins, and tannins, which are recognized for their antidiabetic potential.[7] Flavonoids regulate blood glucose levels by enhancing insulin secretion, promoting β-cell proliferation in the pancreas, reducing insulin resistance, and alleviating inflammation in muscle cells.[8] Saponins contribute to glucose homeostasis by restoring insulin signalling, stimulating insulin release from β-cells, inhibiting gluconeogenesis, and suppressing α-glucosidase activity.[9] Tannins enhance glucose uptake by activating key mediators in the insulin signaling pathway, such as phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinase (p38 MAPK), while also promoting GLUT4 translocation.[10]

A study using a diabetic rat model investigated the effects of a four-week intervention with methanol extract of *Clinacanthus nutans* (CNME). The results demonstrated a significant reduction in total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and atherogenic index (AI) levels in serum. In addition, CNME enhanced vascular antioxidant capacity, reduced inflammation in blood vessels, and decreased intima-media thickness (IMT) of the aorta. These effects were comparable to those observed with metformin treatment, indicating CNME's potential in mitigating atherosclerotic risk in type 2 diabetic (T2D) rats.[11] Furthermore, *C.*

nutans extract was found to lower malondialdehyde (MDA) serum levels, as well as the pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6). Particularly, administration of *C. nutans* extract at doses of 75 and 150 mg/kg body weight increased the size of the pancreatic islets of Langerhans in diabetic rats.[12]

Bioassay-guided partition is a widely used method for isolating bioactive compounds from complex extracts, utilizing biological activity assays and thin-layer chromatography (TLC) for continuous monitoring.[13] This approach has been instrumental in drug discovery. For instance, bioassay-guided fractionation has successfully been applied to isolate bioactive constituents from *Physalis angulata* (locally known as *ciplukan*) and *Kappaphycus alvarezii* (red algae).[14-15] In the present study, bioassay-guided partition was employed to separate and evaluate the biological activity of two partitions derived from *C. nutans*. In addition, molecular docking analysis was conducted to predict the potential binding affinity of *C. nutans* bioactive compounds with GLUT4. This study contributes to Sustainable Development Goal (SDG) 3 by promoting accessible healthcare solutions and to SDG 9 by advancing research and innovation in herbal medicine.

Materials and Methods

This research has received recommendations from the Ethics Committee of Faculty of Medicine, Public Health, and Nursing, Gadjah Mada University (UGM) with protocol no.: KE/1047/07/2024.

Materials

Clinacanthus nutans L. was collected from Bantul Region, Yogyakarta (-7.8415480, 110.3351337), in January 2024 and was taxonomically identified at the Department of Pharmaceutical Biology, Faculty of Pharmacy, Gadjah Mada University, under voucher specimen number 1870/UN1/FA.2/BF/PT.01.06/2024. The study utilized the following materials: Dulbecco's Modified Eagle Medium (DMEM, Gibco, Lot: 2512444), Fetal Bovine Serum (FBS, Sigma, Lot: 001671877), Horse Serum (HS, Sigma, Lot: 22B441), antibiotics (Elabscience, Lot: GY1142RN8913), Bovine Serum Albumin (BSA, Elabscience, Lot: DI02260R2362), and the GOD/PAP kit (DSI, Lot: 60159945).

Methods

Cell culture and differentiation

The C2C12 cell line (ATCC-CRL-1772) was obtained from the Department of Pharmacology and Therapy, Faculty of Medicine, Public Health, and Nursing, Gadjah Mada University. The cells were used at passage 30. C2C12 myoblasts were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS) at 37°C, 5% CO_2. Cells were then differentiated using DMEM containing 2% horse serum (HS) under the same incubation conditions (37°C, 5% CO_2). The cells' morphology was observed using Inverted Light Microscope (CKX41, Olympus, Japan), with 100x magnification. Experiments were conducted after 3–4 days of differentiation. Both the growth and differentiation media were refreshed every 1–2 days.

Bioassay guided partition of C. nutans

Approximately 300 grams of dried *C. nutans* leaf powder was extracted through maceration using 1 litre of methanol, following a previously described protocol.[14] This process yielded two distinct extracts: a methanol extract and a chloroform extract. The biological activity of each extract was evaluated using a glucose consumption assay, while the extraction efficiency was monitored through thin-layer chromatography (TLC), employing a benzene:ethyl acetate (3:1) mobile phase.

Two grams of the active methanol extract was dissolved in benzene and centrifuged (PLC-05, Gemmy, Taiwan) at maximum speed for 10 minutes. The supernatant (soluble partition) was collected, while the pellet (insoluble partition) was re-dissolved in benzene and centrifuged again. This process was repeated until the supernatant appeared as clear as possible. As with the extraction process, the success of the partitioning was monitored using thin-layer chromatography (TLC) with a benzene:ethyl acetate (3:1) mobile phase. In addition, the biological activity of each partition was assessed using a glucose consumption assay.

Induction of insulin resistance, treatment, and glucose consumption assay

C2C12 cells were seeded at a density of 3×10^3 cells per well in a 96-well plate. Upon full differentiation, the cells were divided into four groups: palmitic acid control (KPA) and treatment groups (P1, P2, and P3). To induce insulin resistance, the culture medium was replaced with 100 µL of serum-free DMEM containing 0.75 mM palmitic acid (PA). After 16 hours of PA incubation, the cells exhibited insulin resistance and were prepared for subsequent treatment.

To identify the active extract, treatment was administered by replacing the PA-containing medium with 100 µL of serum-free DMEM containing either 500 µg/mL (P1), 250 µg/mL (P2), or 125 µg/mL (P3) of the polar or non-polar extract. The cells were then incubated for 24 hours. Similarly, to determine the active partition, insulin-resistant cells were treated by replacing the PA-containing medium with 100 µL of serum-free DMEM containing 500 µg/mL (P1), 250 µg/mL (P2), or 125 µg/mL (P3) of the soluble or insoluble partition. The incubation period was maintained for 24 hours.

After 24 hours of treatment incubation, the cells were exposed to 100 µL of 100 nM insulin to activate the insulin signaling pathway. Following a 1-hour incubation, the glucose concentration in the culture medium was assessed using the GOD/PAP assay (DSI, catalog number 125009983021). Glucose concentration was measured at 490 nm (iMark Microplate Absorbance Reader, BioRad, USA) and quantified. Statistical analysis was performed using one-way ANOVA in GraphPad Prism 9, with significance set at $p < 0.05$. All experiments were conducted in triplicate, and data were collected three times per replication.

Molecular docking of GLUT4

The three-dimensional structures of GLUT4 (PDB ID: 7WSM) and isomaltose, a homolog of α-glucosidase (PDB ID: 3AJ7),[16] were retrieved from the Protein Data Bank (PDB). The phytochemical composition of *C. nutans*[17] was screened using Lipinski's Rule of Five as the selection criterion, yielding six compounds: four flavonoids, one steroid, and one phenolic acid. Protein preparation was performed using AutoDockTools 1.5.7, where the GLUT4 structure was preprocessed by removing water molecules, adding Gasteiger charges, and incorporating polar hydrogen atoms. To validate the docking protocol, a RMSD (root-mean-square deviation) was calculated using PyMOL, with the method considered valid if the RMSD value was <2 Å.[18] Once docking system validity was confirmed, the interactions between proteins and compounds were visualized and analyzed using BioVia Discovery Studio 2021. Binding affinity values were determined using AutoDock Vina.

Results and Discussion

Culture and differentiation of C2C12 cells

Prior to differentiation, C2C12 cells remained in the myoblast stage, exhibiting a spindle-like morphology. These myoblasts required approximately 96 hours of culture to reach 70–80% confluence. Once confluence was achieved, the growth medium was replaced with the differentiation medium to induce the transition into myotubes. The differentiated myotubes displayed an elongated, tubular morphology and expressed various proteins characteristic of human muscle cells, including GLUT4. This protein is commonly used to investigate the insulin signalling pathway in response to exercise, pharmacological agents, natural compounds, and glucose uptake mechanisms.[19] Morphological changes indicative of differentiation became evident as early as two days after the introduction of the differentiation medium. The complete differentiation process required an additional 96 hours, as illustrated in **Figure 1**.

Bioassay guided partition of C. nutans

Thin-layer chromatography (TLC) analysis revealed distinct chromatographic profiles between the methanol and chloroform extracts of *C. nutans*. Both solvents are capable of eluting semi-polar natural compounds;[13] however, methanol preferentially extracts more polar compounds, whereas chloroform is more effective at dissolving

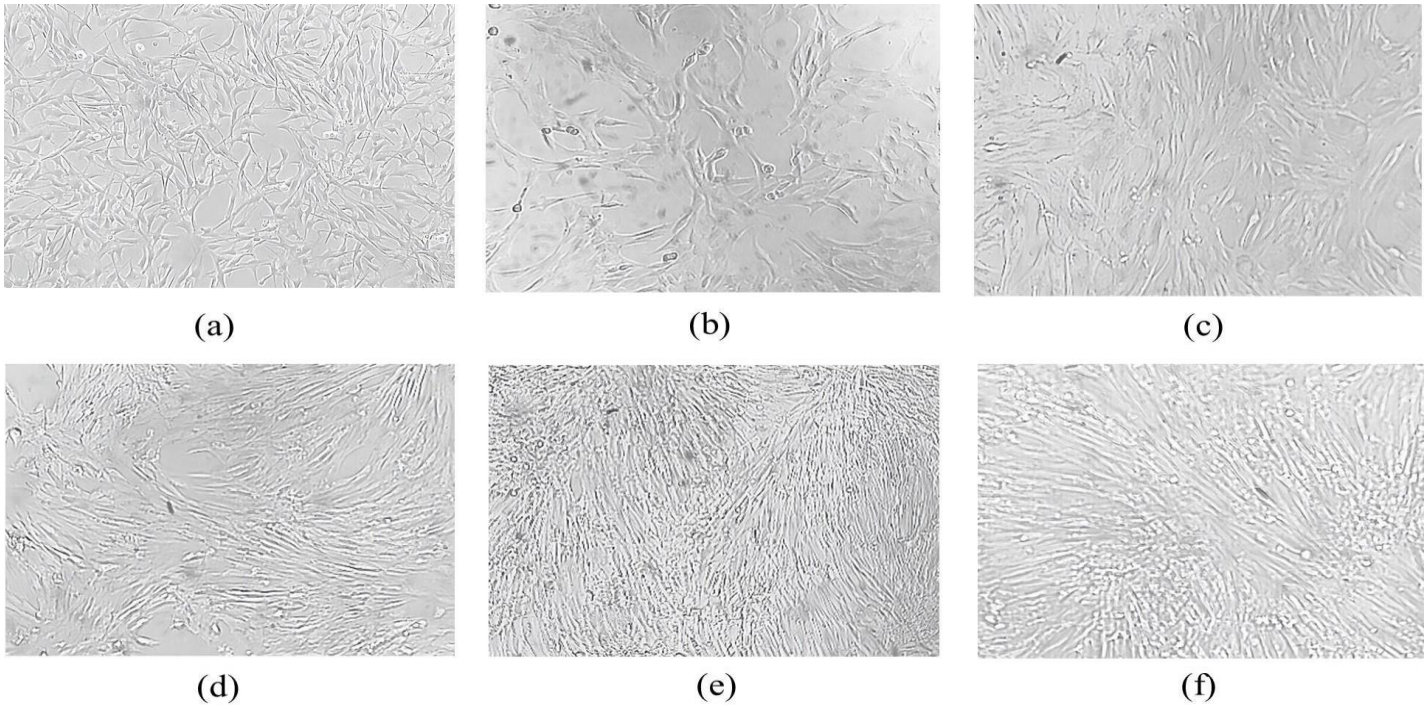

Figure 1: Differentiation process of C2C12 cells. Cells were viewed under 100x magnification. **(A):** myoblast, confluency of 70–80%, **(B):** myotube (H0), **(C):** myotube (H+1), **(D):** myotube (H+2), **(E):** myotube (H+3); **(F):** myotube (H+4).

non-polar compounds. As shown in **Figure 2**, the chloroform (I) and methanol (II) extracts exhibited prominent peaks at Rf 0.75 and Rf 0.7, respectively, with comparable intensity under UV light at 254 nm and 366 nm. In addition, multiple peaks were observed in both extracts, suggesting the presence of similar chemical constituents. However, the methanolic extract displayed lighter peak intensities in some regions.

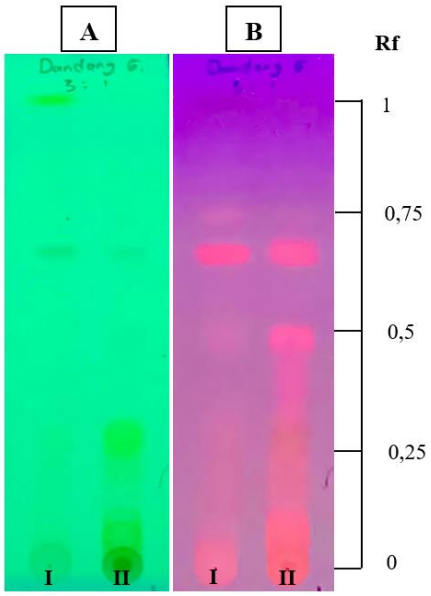

Figure 2: Thin-layer chromatography of *C. nutans*'s extracts. **(A):** Visualisation under UV 254 nm, **(B):** Visualisation under UV 366 nm. **(I):** Chloroform extract, **(II):** methanol extract.

No significant increase in glucose uptake was observed in the chloroform extract of *C. nutans* or in the palmitic acid control group (**Figure 3**). Interestingly, lower concentrations of the methanol extract (250 µg/mL and 125 µg/mL) did not show a significant difference from the control group. However, treatment with 500 µg/mL of the methanol extract resulted in a statistically significant enhancement of glucose consumption in insulin-resistant C2C12 cells in comparison to the palmitic acid control group (p value = 0.0299).

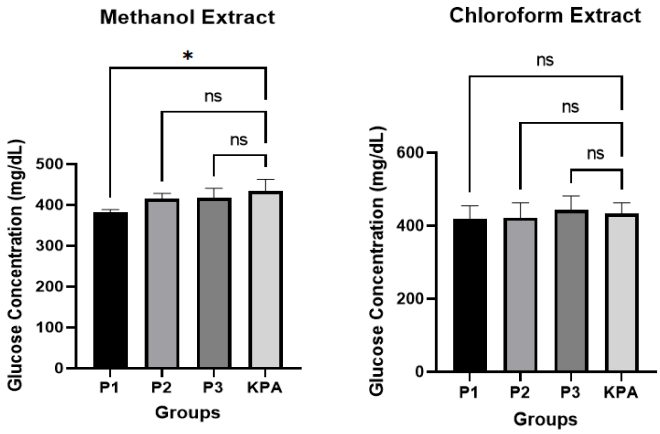

Figure 3: The difference in glucose consumption of each group of treatment using *C. nutans*'s extracts. **(P1):** 500 µg/mL, **(P2):** 250 µg/mL, **(P3):** 125 µg/mL were the insulin-resistant cells treated with *C. nutans*' extracts. **(KPA):** the control group for insulin-resistant cells. This group was only given palmitic acid. * represent P-value < 0.05, while (ns) represents not significant.

To enhance purification, the methanolic extract of *C. nutans* was further partitioned via centrifugation. Thin-layer chromatography (TLC) analysis (**Figure 4**) confirmed the success of the partitioning process, as no identical or duplicated spots were observed between the two partitions. This partition resulted in a soluble partition and an insoluble partition. The soluble partition primarily contained non-polar compounds, as evidenced by its complete elution in the non-polar mobile phase. In contrast, the insoluble partition consisted of semi-polar to polar compounds.

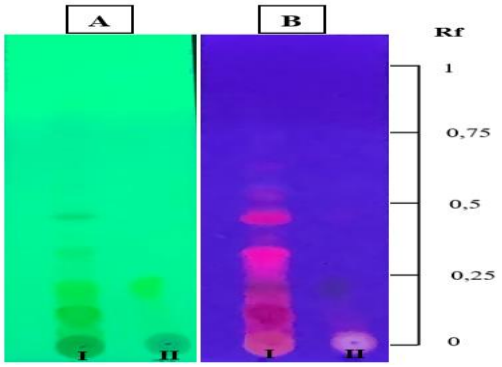

Figure 4: Thin-layer chromatography of *C. nutans*'s partitions. **(A):** Visualisation under UV 254 nm, **(B):** Visualisation under UV 366 nm. **(I):** The soluble partition, **(II):** The insoluble partition.

While the soluble partition did not exhibit a significant effect on glucose consumption, the insoluble partition of *C. nutans* demonstrated a significant enhancement to uptake glucose in insulin-resistant C2C12 cells in comparison to the palmitic acid control group (**Figure 5**). Among the three tested concentrations, the 500 µg/mL and 250 µg/mL treatments of the insoluble partition yielded statistically significant increases in glucose consumption, with p values of 0.0021 and 0.0198, respectively.

Molecular docking of GLUT4

Among the 29 compounds identified in the leaves of *C. nutans*,[16] six compounds met Lipinski's Rule of Five criteria (**Table 1**). These six compounds were soluble in methanol but insoluble in benzene, indicating their likely presence in the insoluble partition of *C. nutans*. Their three-dimensional (3D) structures were retrieved from the Protein Data Bank (PDB) using the following IDs: gallic acid (PDB ID: 4Z5X), caffeic acid (PDB ID: 1KOU), kaempferol (PDB ID: 6M8B), catechin (PDB ID: 4C94), luteolin (PDB ID: 4QYA), and quercetin (PDB ID: 1H1I).

Table 1: Screening results of the *C. nutans*'s compounds based on Lipinski's Rule of five

No.	Compound Group	Compound	Lipinski's Rule of 5				
			MW (<500 Da)	**H-bond Acceptors (≤10)**	**H-bond Donors (≤5)**	**LogP (<5)**	**Violation**
1.	Steroid	Gallic acid	170.12	5	4	0.7	0
2.	Phenolic acid	Caffeic acid	180.16	4	3	1.2	0
3.	Flavonoid	Kaempferol	286.24	6	4	1.9	0
4.		Catechin	290.27	6	5	0.4	0
5.		Luteolin	286.24	6	4	1.4	0
6.		Quercetin	302.23	7	5	1.5	0

The visualization of receptor-ligand interactions can be interpreted based on two key factors: the type of bonds formed and their respective distances. Various types of interactions can occur between a receptor and a ligand, including hydrogen bonds, Van der Waals forces, and unfavorable bumps. Among these, hydrogen bonds possess the highest bond energy. Bond strength is also influenced by bond distance. Shorter bond distances generally result in stronger interactions, whereas bond lengths exceeding 3 Å typically correspond to weak binding affinity.[20] On the other hand, unfavorable bumps are a type of bonds which could occur due to the unfavorable probability when water interacts with proteins. This interaction can lead to increased enthalpy and entropy.[21] Based on the three-dimensional (3D) visualization using BioVia Discovery Studio 2021, none of the six tested compounds exhibited direct binding interactions with GLUT4. However, kaempferol and caffeic acid demonstrated binding affinity toward another key receptor in the insulin signaling pathway, i.e., α-glucosidase/isomaltose (Table 2). Since the 3D structure of α-glucosidase remains unresolved, isomaltose was used as a homologous model for molecular docking analysis.[16] The docking analysis revealed that kaempferol formed hydrogen bonds with several residues of isomaltose, including proline at position 8 (distance: 4.28 Å), threonine at position 10 (distance: 4.20 Å), and glutamic acid at position 11 (distance: 3.71 Å). In addition, kaempferol exhibited unfavorable bumps with serine residues at positions 4 and 5, with bond distances ranging from 1.24 Å to 3.90 Å. Caffeic acid also demonstrated binding interactions with isomaltose, forming three distinct types of bonds. A hydrogen bond was observed with the aspartic acid residue at position 519, with a bond distance of 4.97 Å.

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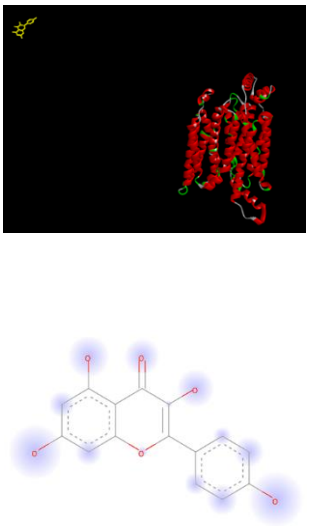
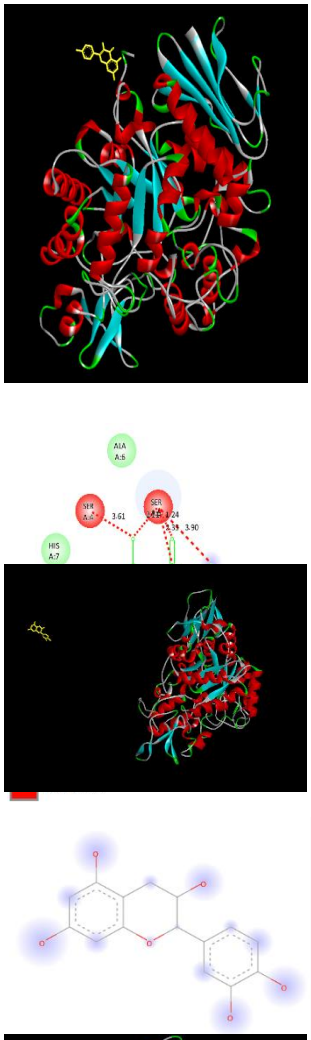
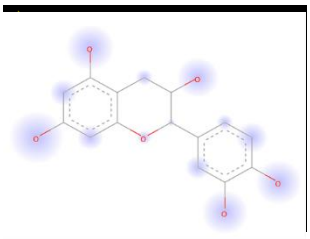
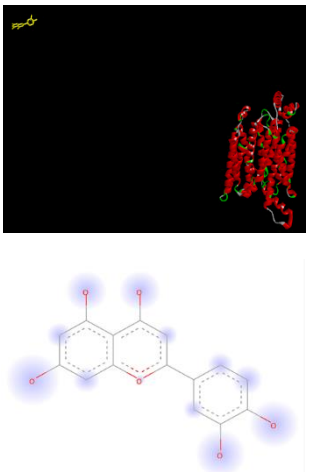
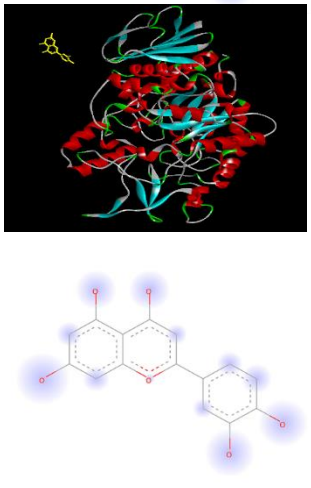
Table 2: 3D and 2D visualisations of interactions between the six compounds of C. *nutans* with GLUT4 and isomaltose proteins

Compound	Molecular Docking			
	Affinity (kcal/mol)	Interaction (with GLUT4)	Affinity (kcal/mol)	Interaction (with Isomaltose)
Kaempferol	-9.4		-5.6	
Catechin	-9.5		-5.3	
Luteolin	-9.6		-5.6	

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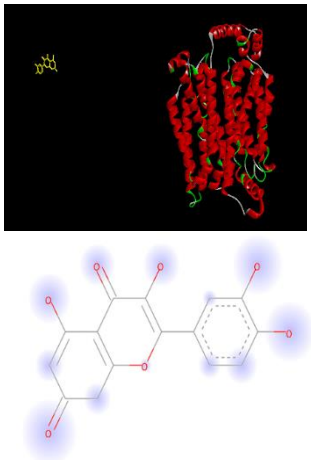
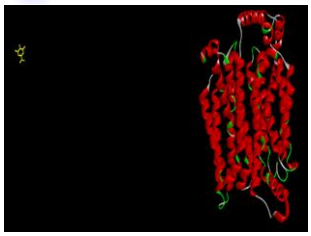
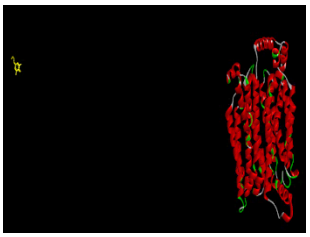
Quercetin	-9.4		-5.6
Gallic acid	-6.3		-3.7
Caffeic acid	-9.2		-5.6

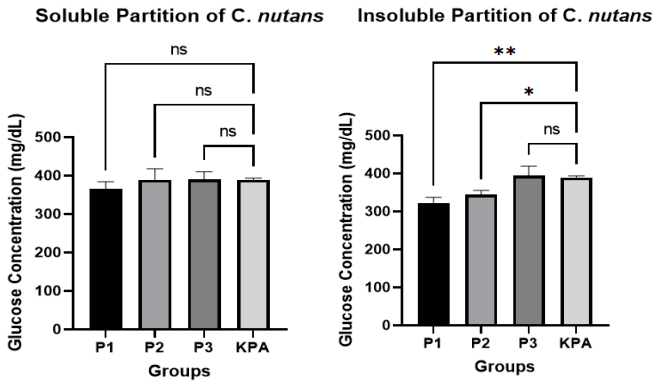


Figure 5: The difference in glucose consumption of each group of treatment using *C. nutans*'s partitions. **(P1):** 500 µg/mL, **(P2):** 250 µg/mL, **(P3):** 125 µg/mL were the insulin-resistant cells treated with *C. nutans*' partitions. **(KPA):** the control group for insulin-resistant cells. This group was only given palmitic acid. * represent P-value < 0.05, ** represent P-value <0.01, while (ns) represents not significant.

In addition, several unfavorable bumps were detected with multiple amino acid residues, including asparagine (2.40 Å to 4.25 Å), leucine (2.53 Å to 5.73 Å), and threonine (2.66 Å to 4.02 Å). Moreover, caffeic acid formed a π-lone pair interaction, a type of non-covalent bond that stabilizes interactions between a lone pair of electrons and a π-system. This bond has been suggested to contribute to the structural stability of both DNA and proteins.[22]

The effect of C. nutans's extracts and partitions on C2C12-insulin resistant cells

Several studies have explored the antidiabetic potential of *C. nutans* through in vitro and in vivo models. However, none have utilized insulin-resistant C2C12 cells as a diabetic model. One in vitro study we identified did not employ any cell lines but instead used a dialysis tube as a model system.[7] In addition, most existing studies have relied on in vivo models, typically assessing antidiabetic activity through α-glucosidase inhibition assays or blood glucose level measurements.[23] Our study is the first to successfully separate bioactive compounds from *C. nutans* using bioassay-guided partition while employing insulin-resistant C2C12 cells as a diabetic model. Furthermore, we provide novel insights into the potential mechanism of action of *C. nutans* by predicting its interaction with the GLUT4 receptor through molecular docking analysis.

This present study further reinforces findings from previous research demonstrating the potential of *C. nutans* leaf extract in improving insulin resistance.[24-25] *C. nutans* has been reported to exhibit both antihyperglycemic and antioxidant properties, which play a crucial role in mitigating insulin resistance.[23-28] Insulin resistance is a precursor to type 2 diabetes and occurs when cells fail to respond effectively to insulin, leading to persistently elevated blood glucose levels. Several factors contribute to insulin resistance, including obesity,[29] oxidative stress,[30] and inflammation. Saturated fatty acids, such as palmitic acid, are known to promote inflammation and exacerbate insulin resistance in peripheral tissues.[31] One of the main characterizations of insulin resistance is the reduced synthesis and translocation of GLUT4, a glucose transporter predominantly found in adipocytes and muscle cells.[32] Several other plant extracts, including bay leaves, black seed, and *Artemisia annua*, have been shown to enhance GLUT4 translocation. [33]

Based on our molecular docking analysis, we propose that *C. nutans* does not directly affect GLUT4 but instead influences another key protein in the insulin signaling pathway, i.e., α-glucosidase. α-Glucosidase is an intestinal enzyme responsible for carbohydrate digestion, and its inhibition can slow glucose absorption, thereby preventing postprandial hyperglycemia. Consequently, α-glucosidase inhibitors serve as a well-established therapeutic strategy for managing diabetes.[34] Kaempferol and caffeic acid, identified in *C. nutans*, could be further isolated and explored as lead compounds for developing safer and more effective antidiabetic drugs, specifically targeting α-glucosidase inhibition. Previous studies have demonstrated that both kaempferol and caffeic acid exhibit potent α-glucosidase inhibitory activity.[35-38]

This present study has several limitations. First, we lack direct evidence that *C. nutans* enhances GLUT4 translocation, as we did not perform ELISA or Western blot analysis to confirm its expression or membrane translocation. Second, although previous studies have established that quercetin is a potent α-glucosidase inhibitor,[39-40] our molecular docking analysis did not support these findings. We hypothesize that this discrepancy arises from our use of an α-glucosidase homologous protein as the receptor in the docking simulations. To address this, we recommend re-docking using different α-glucosidase homologous proteins for further validation. Despite these limitations, we propose a novel hypothesis suggesting that α-glucosidase inhibition may indirectly influence GLUT4 translocation and enhance glucose uptake in cells.

Conclusion

The findings of this study indicate that the insoluble partition from the methanol extract of *C. nutans* demonstrated stronger glucose-lowering activity compared to the soluble partition, particularly at concentrations of 500 and 250 µg/mL. However, molecular docking analysis suggests that the mechanism of action of these bioactive compounds does not involve direct binding to GLUT4. This study contributes to Sustainable Development Goal (SDG) 3 (Good Health and Well-Being) by advancing herbal-based strategies for managing type 2 diabetes mellitus (T2DM). In addition, this study also aligns with SDG 9 (Industry, Innovation, and Infrastructure) by supporting innovation in herbal medicine and sustainable pharmaceutical research.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. IDF (*International Diabetes Federation*). IDF Diabetes Atlas 10th edition. [Online]. 2021 [cited 2024 Dec 20]. Available from: www.diabetesatlas.org.
2. Vijan S, Sussman JB, Yudkin JS, Hayward RA. Effect of Patients' Risks and Preferences on Health Gains With Plasma Glucose Level Lowering in Type 2 Diabetes Mellitus. JAMA Intern Med. 2014; 174(8): 1227-1234.
3. Satoh T. Molecular mechanisms for the regulation of insulin-stimulated glucose uptake by small guanosine triphosphatases in skeletal muscle and adipocytes. Int. J. Mol. Sci. 2014; 15(10): 18677-18692.
4. Esposito DL, Li Y, Cama A, Quon MJ. Tyr612 and Tyr632 in Human

Insulin Receptor Substrate-1 Are Important for Full Activation of Insulin-Stimulated Phosphatidylinositol 3-Kinase Activity and Translocation of GLUT4 in Adipose Cells. Endo. 2001; 142(7): 2833–2840.
5. Huang X, Liu G, Guo J, Su Z. The PI3K/AKT pathway in obesity and type 2 diabetes. Int. J. Biol. Sci. 2018; 14(11): 1483-1496.
6. Dewinta NR, Mukono IS, Mustika A. The Effect of Dandang Gendis (*Clinacanthus nutans*) Extract Administration on Blood Glucose Levels in Wistar Rats with Diabetes Mellitus Model. J. Med. Vet. 2020; 3(1): 76-81.
7. Abdullah N, Kasim KF. In-Vitro Antidiabetic Activity of *Clinacanthus nutans* Extracts. Int. J. Pharm. Phytochem. Res. 2017; 9(6): 846-852.
8. Vinayagam R, Xu B. Antidiabetic properties of dietary flavonoids: a cellular mechanism review. Nutr. Metab. 2015; 12(1): 60-79.
9. El Barky AR, Hussein SA, Eldeen AAEA, Hafez YA, Mohamed TM. Saponins and their potential role in diabetes mellitus. Diab. Manag. 2017; 7(1): 148-158.
10. Kumari M, Jain S. Tannins: An Antinutrient with Positive Effect to Manage Diabetes. Res. J. Recent Sci. 2012; 1(12): 1-8.
11. Azemia AK, Mokhtara SF, Sharif SE, Rasoola AHG. *Clinacanthus nutans* attenuates atherosclerosis progression in rats with type 2 diabetes by reducing vascular oxidative stress and inflammation. Pharm. Biol. 2021; 59 (1): 1430-1438.
12. Mustika A, Fatimah N, Safitri I, Susanti N, Noor NS. *Clinacanthus nutans* L Extracts Reduce the Serum Tumor Necrosis Factor-α, Malondialdehyde, and Interleukin-6 Levels and Improve the Langerhans Islet Area in Diabetic Rat Models.
Clin. Med. Insights Endocrinol. Diabetes..2023; 16: 1–7.
13. Wahyuningsih MSH, Rakhmawati R. Penemuan dan Pengembangan Senyawa Obat dari Bahan Alam. (1st ed.). Yogyakarta: Gadjah Mada University Press; 2024. 112 p.
14. Wahyuningsih MSH, Ketut SSW, Regita AP, Nugrahaningsih DAA, Yuniyanti MM. *Bioassay Guided Fractionation* of Ciplukan (*Physalis angulata* L.) Monitored by Glucose Consumption Assay and Thin Layer Chromatography on Myoblast Cells. Trad. Med. J. 2023; 28(1): 22-30.
15. Yulianti E, Sunarti, Wahyuningsih MSH. The effect of *Kappaphycus alvarezii* active fraction on oxidative stress and inflammation in streptozotocin and nicotinamide-induced diabetic rats. BMC Complement. Med. Ther. 2022; 22 (1): 1-8.
16. Fan M, Feng Q, He M, Yang W, Peng Z, Huang Y, Wang G. Synthesis, a-glucosidase inhibition and molecular docking studies of natural product 2-(2-phenyethyl) chromone analogues. Arab. J. Chem. 2022; 15: 104301-104314.
17. Sabindo NH, Yatim RM, Thirumulu KP. Phytochemical composition of *Clinacanthus nutans* based on factors of environment, genetics and postharvest processes: A review. Biomed. 2024; 14(2): 1-11.
18. Anggraeni VJ, Purwaniati, Budiana W, Nurdin T. Molecular Docking Compounds in Methanol Extract of Mango Leaves (*Mangifera indica* L.) as Anti-Inflammatory Agent. J. Kim. Ris. 2022; 7(1): 57-65.
19. Wong CY, Al-Salami H, Dass CR. C2C12 cell model: its role in understanding of insulin resistance at the molecular level and pharmaceutical development at the preclinical stage. J. Pharm. Pharmacol. 2020; 72: 1667-1693.
20. Pratama AA, Rifai Y, Marzuki A. Molecular Docking of the Compound 5,5'-Dibromomethylsesamin. Pharmacy and Pharmacology Magazine. 2017; 21 (3):67-69.
21. Pantsar T, Poso A. Binding Affinity via Docking: Fact and Fiction.
39. Li YQ, Zhou FC, Gao F, Bian JS, Shan F. Comparative evaluation of quercetin, isoquercetin and rutin as inhibitors of alpha-glucosidase. J Agric Food Chem. 2009; 57(24): 11463-11468.
Mol. 2018; 23: 1899.
22. Novotný J, Bazzi S, Marek R, Kozelka J. Lone-pair–π interactions: analysis of the physical origin and biological implications. Phys. Chem. Chem. Phys. 2016; 18: 19472-19481.
23. Susanti, N. Potential of Clinacanthus Nutans as an Alternative Therapeutic Agent for Diabetes Mellitus. In: Yueniwati Y, Tchoyoson LCC, Hamid H, Arsana PM, Nakagoshi N. Proceedings of the International Conference of Medical and Life Science (ICoMELISA 2021). Netherlands: Atlantic Press; 2023. 27-36 p.
24. Sarega N, Imam MU, Esa NM, Zawawi N, Ismail M. Effects of phenolic-rich extracts of *Clinacanthus nutans* on high fat and high cholesterol diet-induced insulin resistance. BMC Complement. Altern. Med. 2016; 16(88): 1-11.
25. Sarega N, Imam MU, Ooi DJ, Chan KW, Esa NM, Zawawi N, Ismail M. Phenolic rich extract from *Clinacanthus nutans* attenuates hyperlipidemia-associated oxidative stress in rats. Oxid Med Cell Longev. 2016; 2016: 1-16.
26. Arullappan S, Rajamanickam P, Thevar N, Kodimani CC. In vitro screening of cytotoxic, antimicrobial and antioxidant activities of *Clinacanthus nutans* (Acanthaceae) leaf extracts. Trop J. Pharm Res. 2014; 13(9): 1455-1461.
27. Wong FC, Yong AL, Ting EPS, Khoo SC, Ong HC, Chai TT. Antioxidant, metal chelating, anti-glucosidase activities and phytochemical analysis of selected tropical medicinal plants. Iran J Pharm Res. 2014; 13(4): 1409-1415.
28. Yong YK, Tan JJ, Teh SS, Mah SH, Ee GCL, Chiong HS, Ahmad Z. *Clinacanthus nutans* extracts are antioxidant with antiproliferative effect on cultured human cancer cell lines. eCAM. 2013; 2013: 1-8.
29. CDC (Centers for Disease Controls and Prevention). About Insulin Resistance and Type 2 Diabetes. [Online]. 2024 [cited 2024 Dec 28]. Available from: https://www.cdc.gov/diabetes/about/insulin-resistance-type-2-diabetes.html.
30. Pitocco D, Tesauro M, Alessandro R, Ghirlanda G, Cardillo C. Oxidative stress in diabetes: Implications for vascular and other complications. Int. J. Mol. Sci. 2013; 14: 21525-21550.
31. Amine H, Benomar Y, Taouis M. Palmitic acid promotes resistin-induced insulin resistance and inflammation in SH-SY5Y human neuroblastoma. Sci. Rep. 2021; 11(5427): 1-12.
32. Holloszy J. Regulation by exercise of skeletal muscle content of mitochondria and GLUT4. J. Physiol. Pharmacol. 2008; 59(7): 5-18.
33. Adawiyah R, Shabrina A, Yuliani S, Nurkhasanah. Potential Plants with Antidiabetic Activity in GLUT4 Translocation: A Narrative Review. JIFFK. 2023; 1(1): 43-52.
34. Van De Laar FA, Lucassen PL, Akkermans RP, Van De Lisdonk EH, Rutten GE, Van Weel C. α-Glucosidase inhibitors for patients with type 2 diabetes: Results from a cochrane systematic review and meta-analysis. Diabetes Care. 2005; 28: 154-163.
35. Peng X, Zhang G, Liao Y, Gong D. Inhibitory kinetics and mechanism of kaempferol on α-glucosidase. Food Chem. 2016; 190: 207-215.
36. Habtemariam S. α-Glucosidase Inhibitory Activity of Kaempferol-3-O-rutinoside. Nat. Prod. Commun. 2011; 6(2): 201-203.
37. Amin S, Ullah B, Ali M, Khan H, Rauf A, Khan SA, Sobarzo-Sánchez E. In Vitro α-glucosidase Inhibition and Computational Studies of Kaempferol Derivatives from *Dryopteris cycanida*. Curr Top Med Chem. 2020; 20(9): 731-737.
38. Oboh G, Agunloye OM, Adefegha SA, Akinyemi AJ, Ademiluyi AO. Caffeic and chlorogenic acids inhibit key enzymes linked to type 2 diabetes (in vitro): a comparative study. J Basic Clin Physiol Pharmacol. 2015; 26(2): 165-170.
40. Liu H, Wang Y, Tong J, Li J, Ding H. Quercetin analogs as α-glucosidase inhibitors with antidiabetic activity. Food Biosci. 2024; 58:103713.