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Original Article

Evaluation of Glucose Uptake and Gene Expression Modulation of GLUT-4 and PPARγ by Methanolic Leaf Extract of *Lipoblepharis urticifolia* (Blume) Orchard in 3T3-L1 Mouse Pre Adipocyte Cell Lines

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ABSTRACT

Background: *Lipoblepharis urticifolia* (Blume) Orchard. (syn.*Wedelia urticifolia*), a member of the Asteraceae family, is recognized in traditional medicine but its antidiabetic properties remain unexplored. This study evaluates the antidiabetic potential of its methanolic leaf extract using *in vitro* models. **Materials and Methods:** The extract was assessed for alpha-glucosidase inhibition, glucose uptake in 3T3-L1 pre adipocyte cells, cytotoxicity by MTT assay, and gene expression of GLUT-4 and PPARγ by qRT-PCR normalized to housekeeping gene (β-Actin). **Results:** The extract showed strong, concentration-dependent alpha-glucosidase inhibition (up to 90.89±0.30% at 100 µg/mL; IC_{50}: 1.06 µg/mL) and low cytotoxicity in 3T3-L1 adipocyte cell lines. It enhanced glucose uptake maximally at 50 µg/mL and upregulated GLUT-4 expression (3.66-fold at 50 µg/mL), with modest increases in PPARγ at higher doses. **Conclusion:** This investigation pioneers the exploration of the antidiabetic activity of *L. urticifolia* methanolic leaf extract, highlighting its promise as a natural antidiabetic agent and offering valuable insights into its potential mechanisms of action.

Keywords: 3T3-L1, cytotoxicity, Glucose uptake, GLUT-4, *Lipoblepharis urticifolia*, PPARγ, Quantitative Real time PCR.

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels due to issues with insulin production, its efficacy, or a combination thereof.[1] The global burden of diabetes is rising, leading to increased morbidity and mortality worldwide.[2] Although current antidiabetic therapies are effective, they are often linked to adverse effects and limited accessibility, especially in developing regions.[3] Consequently, there is increasing attention on discovering safer, cost-effective, and potent antidiabetic compounds derived from natural sources, especially medicinal plants traditionally used for treatment.[4]

The Asteraceae family includes many species with ethnomedicinal uses, several of which have demonstrated antidiabetic properties.[5] *Lipoblepharis urticifolia* (Blume) Orchard. (syn.*Wedelia urticifolia*) is a lesser-known member of this family, traditionally recognized for its therapeutic benefits.[6] However, there are no scientific studies evaluating its antidiabetic potential.

Methanolic extraction is commonly used to isolate bioactive phytochemicals, such as polyphenols and flavonoids, which are known to modulate carbohydrate metabolism and improve insulin sensitivity.[7] Investigating the antidiabetic activity of methanolic leaf extract of *L. urticifolia* may provide valuable information on its pharmacological potential and mechanisms of action. This study aims to systematically assess the antidiabetic efficacy of methanolic leaf extract of *Lipoblepharis urticifolia in vitro*, focusing on alpha-glucosidase inhibition, glucose uptake in pre adipocyte cell lines (3T3 L1), cytotoxicity, and the expression of critical genes in glucose metabolism, such as GLUT 4 and PPARγ.

MATERIALS AND METHODS

Plant collection and authentication

Leaves of the plant specimen *Lipoblepharis urticifolia* (syn.*Wedelia urticifolia*) were collected from Vazhathope in the Idukki district of Kerala during the month of June 2025. The identification

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was performed by a senior scientist from the Forest Botany Department at KFRI, with a voucher specimen archived under accession number 17701.

Extraction

The leaves collected were shade-dried, coarsely powdered, passed through a 100-mesh (particle size ~150 µm) sieve. Extraction was performed by ultrasonication method using methanol as the solvent. Ultrasonic-assisted extraction of the mixture was performed using a bath-type sonicator (Johnson Plastonics). The equipment was operated at a frequency of 50 kHz. Sonication was performed at room temperature for a total of 30 min, using multiple cycles of 2 min each.

Following the sonication process, the solution was passed through Whatman No. 1 filter paper to remove plant residues from the solvent extract. The filtrate that resulted was subsequently concentrated under decreased pressure using a rotary evaporator (D LAB, RE 100-S) and then air-dried at ambient temperature to obtain the crude extract. The final extract was preserved in a sealed container at 4°C until required for subsequent analysis

Inhibition of α-Glucosidase

The α-glucosidase inhibition assay followed the procedure described by Shai *et al.*[8] This method is based on the enzymatic breakdown of 4-nitrophenyl-α-D-glucopyranoside (pNPG) by α-glucosidase, resulting in the release of p-Nitrophenol (PNP), which produces a yellow coloration measurable at 400 nm. Briefly, 400 µL of α-glucosidase solution (0.067 U/mL) was combined with varying concentrations of the test compound and preincubated for 30 min at 37°C. The reaction was initiated by adding 200 µL of 3.0 mM pNPG, prepared in 0.1 M sodium phosphate buffer with a pH of 6.9, and the mixture was incubated again at 37°C for another 30 min. To stop the reaction, 2 mL of 0.1 M Sodium Carbonate (Na_2CO_3) was added. The formation of p-nitrophenol was determined by measuring the absorbance at 400 nm with a spectrophotometer. Acarbose (1 mg/mL stock solution) was used as the reference inhibitor under the same experimental conditions. The percentage inhibition was calculated using the following formula:

$$\text{Percentage of Inhibition (\%)} = [(A_{Blank} - A_{sample}) / (A_{Blank} - A_{Control})] \times 100$$

Where,

A_{Blank} refers to the absorbance measured for the blank sample.

A_{sample}, absorbance for test sample.

$A_{Control}$ corresponds to the absorbance of the control sample.

Cell Culture

The 3T3-L1 mouse pre adipocyte cell line was utilized to evaluate toxicity, glucose uptake, and the expression of GLUT4 and PPARγ genes via qRT-PCR analysis. Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum and 1% penicillin-streptomycin. Incubation took place at 37°C under humidified conditions with 5% CO_2 (New Brunswick). For experiments, cells were seeded into 12-well plates and allowed to grow for 48 hr until reaching a semi-confluent state. Gene expression study of GLUT4 and PPARγ was performed using cells growing in 25 cm^2 tissue culture flasks, kept at 37°C in a 5% CO_2 humidified atmosphere (Galaxy® 170, Eppendorf, Germany).

MTT Cytotoxicity Assay

The cytotoxic effects of the *L. urticifolia* methanolic extract were determined using the MTT assay on 3T3 L1 pre adipocyte cell lines, as described by Mosmann.[9] After seeding 10,000 cells per well in 96-well plates, the cells were incubated for 24 hr. The methanolic extract was produced in DMSO (10 mg/mL), sterilized using a 0.2 µm filter, and diluted with DMEM to achieve the appropriate concentrations. These were added to the wells, while untreated wells served as controls. All tests were performed in triplicate. After another 24-hr incubation, remove the medium and replace with 100 µL of MTT solution (0.5 mg/mL in PBS). The plates were maintained at 37°C for 2 hr to encourage the formation of formazan crystals. Following incubation, the medium was discarded, and 100 µL of undiluted DMSO was introduced to each well to solubilize the formazan. The absorbance was then recorded at 570 nm with a microplate reader. The following formula was used to determine cell viability.

$$\text{Percentage of cell viability} = \frac{\text{Average absorbance treated}}{\text{Average absorbance of control}} \times 100$$

Glucose Uptake Assay

The glucose uptake activity in 3T3 L1 pre adipocyte cell lines was evaluated using the procedures provided by Gupta *et al.*[10] Cells were seeded in 12-well plates and incubated at 37°C in a CO_2 environment for 48 hr to achieve a semi-confluent monolayer. The culture medium was then replaced with serum-free DMEM containing 0.2% BSA, and the cells were further incubated at 37°C for 18 hr to induce serum starvation. The media was then removed and the cells were washed with PBS at pH 7.4. The cells were exposed to 1000 µg/mL glucose, alone or in combination with the test compound at concentrations of 12.5, 25 and 50 µg/mL, for 1 hr. Glucose uptake was assessed by measuring the change in glucose concentration in the medium, calculated as the difference between the initial and final levels using Anthrone method at 630 nm. The final glucose concentration was quantified using a glucose calibration curve. Uptake values for the treated groups were then compared to those observed in the untreated control group.

Quantitative PCR

Cell Culture and Treatment Protocol

The 3T3-L1 mouse fibroblast cell line was sourced from the National Centre for Cell Sciences (NCCS), Pune, India. The cells were cultured in Dulbecco's Modified Eagles Medium (DMEM-Himedia), supplemented with 10% heat inactivated Fetal Bovine Serum (FBS) and 1% antibiotic cocktail containing Penicillin (100 U/mL), Streptomycin (0.1 mg/mL), and Amphotericin B (0.25 µg/mL). The cell containing TC flasks (25 cm^2) were incubated at 37°C at 5% CO_2 environment with humidity in a cell culture incubator (Galaxy® 170,Eppendorf, Germany). When the cells reached approximately 80% confluency, they were exposed to the extract at concentrations of 12.5, 25, and 50 µg/mL for 24 hr. Control cells were not subjected to extract treatment and were kept under identical conditions.

RNA Isolation

Total RNA was isolated utilizing Trizol reagent, succeeded by chloroform extraction and column purification. DNase treatment was performed to remove genomic DNA. RNA purity and integrity were assessed via spectrophotometry (A260/280, A260/230) and agarose gel electrophoresis. The RNA yield and quality were assessed using a microdrop reader (Multiscan SkyHigh, ThermoScientific).

cDNA Synthesis

For cDNA synthesis, 500 ng of RNA was added to a 10 µL reaction mixture containing 5 µL of 2× RT Easy Mix (G Bioscience), 0.5 µl of random primer (50 µM), 1 µL of RNA sample, and 3.5 µL of RNase-free double-distilled water. The assembled mixture underwent reverse transcription in a thermal cycler at 42°C for 20 min, followed by heating at 85°C for 5 min to stop the reaction. The synthesized cDNA was then stored at -20°C until further use in qPCR analysis.

Quantitative PCR (qPCR) was carried out with SYBR Green Master Mix utilizing the Bio-Rad CFX Connect system. The specific primers employed for the reactions were as follows:

GLUT-4: F: GTAACTTCATTGTCGGCATGG, R: AGCTGAGATCTGGTCAAACG

PPARγ: F: GTACTGTCGGTTTCAGAAGTGCC, R:ATCTCCGCCAACAGCTTCTCCT

β-actin: F: GATTACTGCTCTGGCTCCTAG, R: GACTCATCGTACTCCTGCTTG

A quantitative PCR was performed in a 10 µL reaction volume, comprising 5 µL of 2× SYBR Green qPCR Mix (G Biosciences), 1 µL each of forward and reverse primers (10 µM), 1 µL of template DNA, and 2 µL of nuclease-free water. The amplification reaction was performed utilizing a CFX Connect Real-Time device (Bio-Rad). The qPCR amplification process began with an initial denaturation at 95°C for 3 min, followed by 39 cycles consisting of denaturation at 95°C for 10 sec and gene-specific annealing for 45 sec. Optimized annealing temperatures for each target gene were as follows: 53°C for β-actin, 55°C for GLUT-4, and 60°C for PPARγ.

Statistical Analysis

The results are represented as mean ± SD differences between the groups, determined through one-way ANOVA and Tukey's multiple comparison test for *post-hoc* analyses. The level of significance for all statistical tests was set at $p<0.05$.

ETHICAL STATEMENT

All experiments were conducted using commercially available immortalized cell lines. No human or animal subjects were involved, and therefore, institutional ethical approval was not required.

RESULTS

α-Glucosidase Inhibition Assay

The Methanolic leaf extract of *Lipoblepharis urticifolia* (MLU) demonstrated potent, dose-dependent α-glucosidase inhibitory activity, with percentage inhibition significantly increasing from 77.51 ± 0.18% at 6.25 µg/mL to 90.89 ± 0.30% at 100 µg/mL. Notably, MLU exhibited substantially higher inhibition at lower concentrations compared to the standard drug acarbose, which showed only 19.25 ± 0.14% inhibition at 6.25 µg/mL and achieved comparable inhibition to MLU only at the highest concentrations tested (92.70 ± 0.33% at 50 µg/mL and 93.83 ± 0.16% at 100 µg/mL) (Table 1). Due to consistently high inhibition and the inability to generate a standard dose-response curve, the assay was repeated at lower concentrations (0.78, 1.56, and 3.12 µg/mL). To determine the IC_{50} value, the linear regression equation ($y = 12.11x + 37.19$) obtained from the dose-response data was used, where y is the percentage of α-glucosidase inhibition and x is the extract concentration. By setting y to 50% and solving for x, the extract concentration required for 50% inhibition (IC_{50}) was calculated to be 1.06 µg/mL (Figure 1).

Table 1: Alpha-Glucosidase Inhibitory Activity of Methanolic Leaf Extract of *L. urticifolia* (MLU) compared to Acarbose.

Concentration (µg/mL)	%Inhibition (mean ± SD) MLU	%Inhibition (mean ± SD) Acarbose
6.25	77.51 ± 0.18^a	19.25 ± 0.14^a
12.5	84.13 ± 0.28^b	37.08 ± 0.35^b
25	89.10 ± 0.17^c	70.79 ± 0.44^c
50	89.82 ± 0.17^d	92.70 ± 0.33^d
100	90.89 ± 0.30^e	93.83 ± 0.16^e

One-way ANOVA shows significant differences between group means ($p < 0.05$). Tukey's *post hoc* test: All groups are significantly different from each other.

MTT Cytotoxicity Assay

The MTT assay showed that the methanolic leaf extract of *L. urticifolia* did not prove toxic to 3T3 L1 adipocyte cells at any of the tested concentrations (6.25, 12.5, 25, 50, and 100 µg/mL), (Figure 2). Cell viability stayed high at lower concentrations (6.25 and 12.5 µg/mL), and it only dropped down slightly as the concentration went higher: 25, 50, and 100 µg/mL. Even at the highest concentration (100 µg/mL), most of the cells stayed alive, which shows that 3T3 L1 cells generally tolerate the extract well. These results indicate that the methanolic leaf extract of *L. urticifolia* exhibits reduced cytotoxicity and may be regarded as safe for subsequent biological studies within the evaluated concentration range.

Glucose Uptake Assay

The *in vitro* glucose uptake assay conducted on mouse adipocyte 3T3-L1 cells demonstrated that the methanolic leaf extract of *L. urticifolia* (MLU) significantly enhanced glucose uptake in a concentration-dependent manner (Table 2). At concentrations of 12.5, 25, and 50 µg/mL, the MLU extract induced glucose uptake of 53.66 ± 0.35%, 59.19 ± 0.20%, and 66.29 ± 0.46%, respectively. These values, while slightly lower than those observed for insulin (56.42 ± 0.21%, 67.52 ± 0.12%, and 88.09 ± 0.20% at the corresponding concentrations), indicate that the extract is capable of stimulating glucose uptake comparably to the standard. The results suggest that the MLU extract possesses notable glucose uptake-promoting activity, supporting its potential as a candidate for further investigation in the management of diabetes.

Table 2: *In vitro* Glucose uptake Assay in Mouse Pre Adipocyte 3T3-L1 cell lines.

Concentration (µg/mL)	Insulin (%Glucose Uptake) mean ± SD	MLU (% Glucose Uptake) mean ± SD
12.5	56.42 ± 0.21[a]	53.66 ± 0.35[a]
25	67.52 ± 0.12[b]	59.19 ± 0.20[b]
50	88.09 ± 0.20[c]	66.29 ± 0.45[c]

Values are Mean ± SD (n = 3). Different superscripts (a, b, c) indicate statistically significant differences ($p < 0.05$, one-way ANOVA, Tukey's multiple comparison test). MLU: Methanolic Leaf Extract of *Lipoblepharis urticifolia.*

Quantitative (Real Time) PCR- Gene Expression Analysis of GLUT4 and PPARγ

Quantitative real-time PCR analysis revealed a dose-dependent increase in the expression of both GLUT4 and PPARγ genes following treatment with Methanolic leaf extract of *Lipoblepharis urticifolia* (MLU) when normalized to β-actin.

A significant decrease in ΔCt values was observed with increasing concentrations of MLU, indicating elevated expression of the GLUT4 gene (Table 3). The fold change in GLUT4 expression increased progressively from 1.33 ± 0.13 at 25 µg/mL to 3.70 ± 0.39 at 100 µg/mL compared to control (1.00 ± 0.00), with the highest concentration showing a statistically significant upregulation ($p < 0.05$).

PPARγ expression exhibited a concentration-dependent increase, with higher MLU concentrations leading to greater gene expression. The ΔCt value decreased from 2.25 ± 0.03 to 1.98 ± 0.05 at 100 µg/mL, corresponding to a fold change increase from 1.00 ± 0.00 (control) to 1.21 ± 0.06 at the highest dose (Table 3). Although the upregulation of PPARγ was more modest compared to GLUT4, statistical analysis confirmed significant differences at higher concentrations ($p < 0.05$).

The comparative analysis (Table 3 and Figure 3) demonstrates that MLU notably increased GLUT4 expression in a dose-dependent fashion, while PPARγ also showed a mild but significant upregulation. These findings suggest that MLU may enhance glucose uptake and improve insulin sensitivity by upregulating GLUT4 and PPARγ gene expression, providing a molecular basis for its observed antidiabetic effects.

DISCUSSION

The findings of this investigation highlight the promising antidiabetic properties of the methanolic leaf extract of *L. urticifolia* (Asteraceae). In our study, the extract showed a clear, concentration-dependent inhibition of α-glucosidase, with a notably low IC_{50} of 1.06 µg/mL (Figure 1). This level of activity is comparable to that reported for other well-studied members of the Asteraceae family, including *Taraxacum officinale* and *Artemisia absinthium*, which have also demonstrated considerable α-glucosidase inhibitory and antidiabetic activities.[11,12] Consistent with these observations, studies on *W. trilobata* have shown that both methanolic and ethanolic extracts can inhibit α-glucosidase

Table 3: Comparative Analysis of GLUT-4 and PPARγ Gene Expression after Methanolic Leaf Extract of *Lipoblepharis urticifolia* (MLU) Treatment.

Sample (MLU)	ΔCt GLUT-4 (Mean ± SD)	Fold Change GLUT-4 (Mean ± SD)	ΔCt PPARγ (Mean ± SD)	Fold Change PPARγ (Mean ± SD)
Control	8.30 ± 0.84[a]	1.00 ± 0.00[a]	2.25 ± 0.03[a]	1.00 ± 0.00[a]
12.5 µg/mL	7.89 ± 0.15[a]	1.33 ± 0.13[a]	2.08 ± 0.06[b]	1.13 ± 0.05[b]
25 µg/mL	7.75 ± 0.10[a]	1.47 ± 0.10[a]	2.01 ± 0.06[b]	1.18 ± 0.05[b]
50 µg/mL	6.42 ± 0.16[b]	3.66 ± 0.15[b]	1.98 ± 0.05[b]	1.21 ± 0.04[b]

Values are Mean ± SD (n = 3), $p < 0.05$ (one-way ANOVA, Tukey's multiple comparison test). Different superscripts (a, b) indicate statistically significant differences.

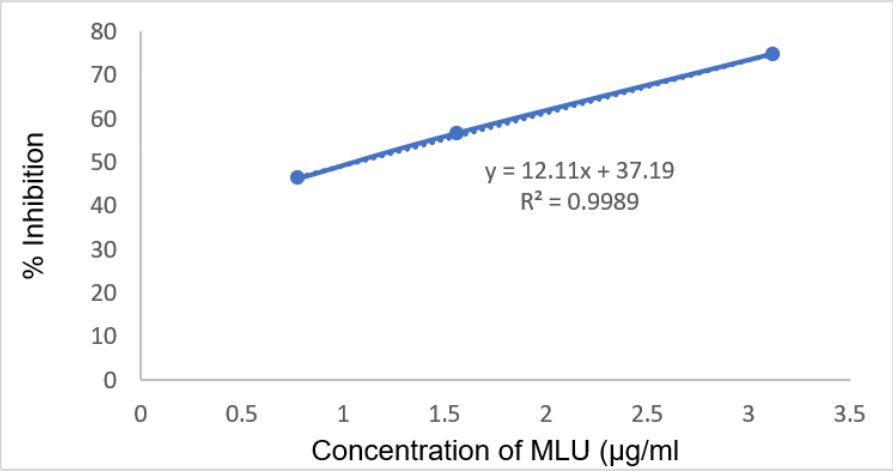


Figure 1: IC_{50} Value of Methanolic Leaf extract of *L. urticifolia* (MLU) for Alpha-Glucosidase Inhibition Assay.

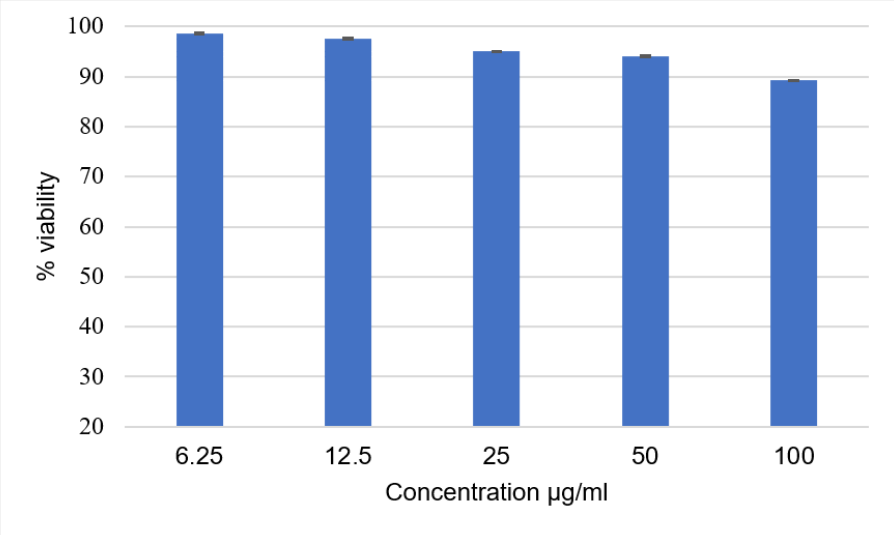


Figure 2: Cytotoxic Effects of Methanolic Leaf Extract of *L. urticifolia* on 3T3 L1 Pre Adipocyte cell lines Using the MTT Assay.

to a degree similar to standard agents like acarbose, potentially delaying postprandial glucose uptake.[13] *Wedelia trilobata* and *L. urticifolia* both showed similar abilities to block α-glucosidase, which suggests that α-glucosidase inhibition is a widespread antidiabetic mechanism among Asteraceae plants. It is plausible that this effect is largely attributable to the rich content of phenolic and flavonoid compounds in these species, as these phytochemicals are widely recognized for their enzyme inhibitory capabilities.[14]

Beyond enzyme inhibition, the extract significantly promoted glucose uptake in 3T3-L1 adipocytes, further supporting its antidiabetic potential (Table 2). This effect echoes findings with other Asteraceae species, such as *Silybum marianum* and*Eclipta prostrata*, which have been reported to enhance cellular glucose uptake and improve insulin responsiveness.[15,16] Although the extent of glucose uptake observed with *L. urticifolia* was somewhat less than with insulin, the results suggest that the extract may exert an insulin-mimetic or insulin-sensitizing effect, a property that has been described for other phytoconstituents from Asteraceae family.[17]

A notable aspect of this study is the robust, dose-dependent upregulation of GLUT4 gene expression observed following treatment with the extract (Table 3). Considering that GLUT4 serves as the primary insulin-sensitive glucose transporter in adipose and muscle tissues, its increased expression indicates an enhanced capacity for glucose uptake at the cellular level. Since diminished GLUT4 expression and impaired translocation are key features of insulin resistance and type 2 diabetes, agents that can restore or enhance GLUT4 expression are of significant therapeutic interest.[18] Our findings align with previous reports on *Cichorium intybus* and *Sonchus oleraceus*, where upregulation of GLUT4 has been linked to better glycemic control and improved insulin action.[19,20] The ability of plant-derived compounds particularly polyphenols, flavonoids, and terpenoids to stimulate

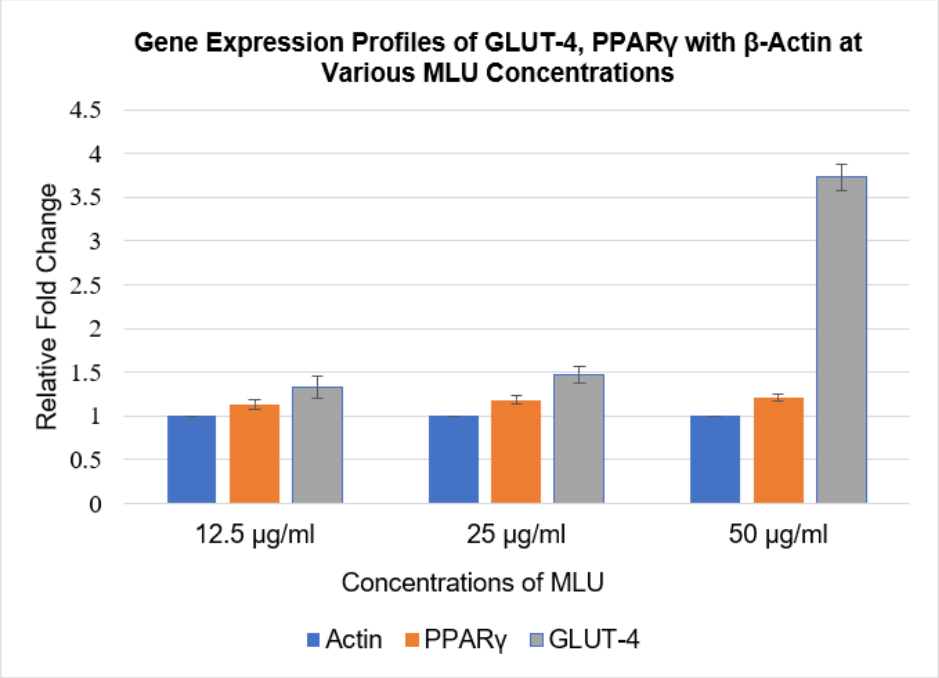


Figure 3: Comparative Expression of GLUT-4, PPARγ with reference gene (β-Actin) at Different Concentrations of Methanolic Leaf Extract of *L. urticifolia* (MLU).

GLUT4 expression is often ascribed to activation of signaling pathways such as PI3K/Akt, which ultimately facilitate GLUT4 transcription and translocation to the cell membrane.[21]

The study also documented a modest but significant increase in PPARγ expression after extract treatment (Table 3). PPARγ is a pivotal regulator of adipogenesis, lipid metabolism, and insulin sensitivity.[22] Upon activation, it induces the expression of genes involved in glucose transport and storage, including GLUT4, and promotes the maturation of preadipocytes into insulin-responsive adipocytes.[23] This result is consistent with earlier findings for*Artemisia dracunculus* and *Eclipta prostrata*, both of which have exhibited stimulatory effects on PPARγ and have been associated with improved glucose uptake and reduced hyperglycemia in diabetic models.[24,25] The observed elevation of PPARγ in our study suggests that the antidiabetic effects of *L. urticifolia* may, at least partly, be mediated through this pathway and warrants further mechanistic exploration.

Overall, the data suggest that the methanolic leaf extract of *L. urticifolia* exerts its antidiabetic action through a combination of direct enzyme inhibition and the modulation of molecular pathways related to glucose uptake and insulin sensitivity. These findings provide a rationale for further *in vivo* studies and support the continued exploration of Asteraceae species as sources of novel antidiabetic agents.

CONCLUSION

This research highlights that the methanolic leaf extract of *Lipoblepharis urticifolia* displays considerable antidiabetic effects *in vitro*. The extract showed potent α-glucosidase inhibitory activity, indicating its capacity to reduce postprandial hyperglycemia. It also significantly enhanced glucose uptake in 3T3-L1 Pre Adipocyte cells in a concentration-dependent manner, supporting its insulin-mimetic effect. Furthermore, gene expression analysis revealed marked upregulation of GLUT4 and PPARγ, key regulators of glucose transport and insulin sensitivity, respectively. These findings suggest that *L. urticifolia* leaf extract acts through multiple mechanisms relevant to diabetes management, including enzyme inhibition and modulation of gene expression involved in glucose metabolism. To the best of my understanding, this is the first report to highlight the antidiabetic properties of *Lipoblepharis urticifolia*, providing a new scientific basis for its traditional use and underscoring the need for further studies to identify active components and confirm its efficacy *in vivo*.

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infrastructure and facilities that enabled the successful completion of this work.

ABBREVIATIONS

GLUT 4: Glucose Transporter Type 4; **PPARγ:** Peroxisome Proliferator-Activated Receptor Gamma; **3T3-L1:** Mouse embryonic Adipocyte cell line; **MLU:** Methanolic Leaf Extract of *Lipoblepharis urticifolia*; **IC_{50}:** Half maximal Inhibitory concentration; **NCCS:** National Centre for Cell Science; **MTT:** 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **DMSO:** Dimethyl sulfoxide; **DMEM:** Dulbecco's Modified Eagle Medium; **BSA:** Bovine Serum Albumin; **PNP:** p-nitrophenol**; pNPG**: para-Nitrophenyl-β-D-glucopyranoside; **qPCR:** Quantitative Polymerase Chain Reaction; **KFRI:** Kerala Forest Research Institute; **PBS:** Phosphate Buffered Saline; **RNA:** Ribonucleic Acid**; cDNA:** Complementary Deoxyribonucleic Acid; **Ct:** Cycle threshold; **SD:** Standard Deviation; **ANOVA:** Analysis of Variance; **A:** Absorbance.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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