INFORMATICS JOURNALS

Short Communication

Journal of Natural Remedies
DOI: 10.18311/jnr/2026/52535

eISSN: 2320-3358 | Vol 26(8), 2026 | pp. 2179-2184

Standardisation and *In Vitro* Glucose Uptake Study of *Thiripalathi Kashayam* in L6 Cell Lines - A *Siddha* Polyherbal Decoction

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Abstract

Background: Type 2 Diabetes mellitus is one among the threatening non-communicable diseases that poses a serious health threat if not adequately controlled. Although several conventional medicines are currently available, natural remedies are considered more cost-effective, better tolerated, and safer for long-term use. **Aim:** The aim of this study is to scientifically validate and prove the authenticity of the traditional *Siddha* anti-diabetic formulation *Thiripalathi Kashayam*. **Materials and Methods:** The formulation was subjected to physicochemical standardisation, high-performance thin-layer chromatography, and an *in vitro* glucose uptake study in order to assess its quality, safety, and preliminary therapeutic potency. **Results:** *Thiripalathi Kashayam* (TPCK) showed optimal pH, high extractive values, and clear High Performance Thin Layer Chromatography (HPTLC) peaks indicating major bioactive compounds. It significantly enhanced glucose uptake in L6 myotubes in a dose-dependent manner (48.19% at 100 µg/mL, $R^2 = 0.996$). **Conclusion:** Findings from these studies indicate that *Thiripalathi Kashayam* complies with established standards of quality and purity, and it contains bioactive constituents responsible for its pharmacological actions. Furthermore, the *in vitro* glucose uptake assay demonstrated that the formulation significantly enhances glucose uptake in a dose-dependent manner. These results provide preliminary evidence supporting its antidiabetic potential and justify its traditional use, while also underscoring the need for *in vivo* and clinical studies to substantiate its therapeutic efficacy.

Major Findings: All other physicochemical parameters were within acceptable limits, indicating that TPCK was free from contamination or adulteration and properly manufactured. HPTLC revealed 11 peaks (R_f 0.02–0.60), with peaks 11 and 7 showing major intensities and areas, indicating multiple active constituents. Glucose uptake showed a strong positive linear correlation with TPCK concentration ($R^2 = 0.996$, $p < 0.01$). Thus, TPCK appears to be a promising modulator of glucose uptake.

Keywords: HPTLC, Glucose Uptake, L6 Cell Line, Physicochemical, *Siddha*

(Article Chronicle: Article Received on: 11.10.2025; Revised on: 24.06.2026; Accepted on: 11.07.2026)

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1. Introduction

Diabetes mellitus is an advancing disease, and with disease progression, self-care management may not be adequate; pharmacotherapy then becomes inevitable. According to the 2025 edition of the International Diabetes Federation (IDF) Diabetes Atlas, diabetes affects 11.1% of adults aged 20–79 years, meaning roughly one in nine individuals worldwide is living with the condition. Projections suggest that by 2050, the burden will rise further, with nearly one in eight adults—around 853 million people—expected to have diabetes, representing a 46% increase from current estimates[1]. The World Health Organisation (WHO) highlights that natural plants are rich sources of bioactive compounds, many of which possess therapeutic properties or serve as primary precursors for the semi-synthesis of pharmaceutical agents. Owing to these attributes, medicinal plants are in great demand within the pharmaceutical industry for their valuable bioactive constituents[2]. Glucose transport through GLUTS marks the initial stage of glucose metabolism. In skeletal muscle, both insulin and muscle contraction enhance the movement of GLUT4 transporters from intracellular storage sites to the plasma membrane. Impaired responsiveness to this insulin-driven process is a key pathological characteristic of Diabetes mellitus. L6 cells serve as an effective *in vitro* model for studying glucose uptake as they possess a functional insulin signalling pathway, express the insulin-responsive GLUT4 transporter, and have been widely employed to investigate mechanisms of muscle glucose utilisation[3]. *Thiripalathi Kashayam* is a traditional polyherbal decoction mentioned in *Siddha* literature for the treatment of diabetes. It is composed of herbs with well-established antidiabetic potential and therapeutic value. Despite its long-standing use, scientific validation of this formulation has been lacking. Hence, the current study was undertaken to investigate the anti-diabetic efficacy of *Thiripalathi Kashayam* through glucose uptake assays, along with physicochemical standardisation of the preparation, to provide evidence-based justification for its traditional application.

2. Materials and Methods

The raw materials required for preparing TPCK were procured from accredited country drug stores in Chennai and Nagercoil. Authentication of the plant materials was carried out by a botanist at the National Institute of Siddha, Chennai. Following authentication, the raw drugs underwent purification, and the formulation was subsequently prepared in the Gunapadam laboratory of the National Institute of Siddha.

2.1 Preparation of Study Drug - *Thiripalathi Kashayam* (TPCK)

Table 1. Ingredients of *Thiripalathi Kashayam*[4]

S. No.	Raw drugs	Parts used	Quantity
1	*Aavarampattai* (*Cassia auriculata* (L.))	Stem bark	4 g
2	*Kadalazhinjilppattai* (*Salacia reticulata* Wight)	Stem bark	4 g
3	*Nellimulli (Emblica officinalis* L.)	Dry unripe fruit	4 g
4	*Thanrikkai thol* (*Terminalia bellerica* Roxb)	Epicarp of unripe fruit	4 g
5	*Manjal Kadukkai thol* (*Terminalia chebula* Retz)	Epicarp of unripe fruit	3 g

2.2 Preparation of *Thiripalathi Kashayam*

The said ingredients (Table 1) were ground into coarse powder, and the decoction was prepared by boiling the ingredients, weighing 20 g in 335 ml, and reducing it to 45 ml[4].

2.3 Study Setting

The study was conducted at the Siddha Central Research Unit, Chennai, and the Centre for Research on Molecular and Applied Sciences, Trivandrum.

2.4 Soxhlet extraction

A 100 g portion of the sample was weighed, wrapped in muslin cloth, and placed in the chamber of a Soxhlet extractor. Distilled water was used as the extraction solvent and filled into the round-bottom flask. The apparatus was assembled with continuous circulation of cooling water through the condenser, and the solvent was heated to initiate reflux, allowing uninterrupted extraction for the required duration. After completion, the aqueous extract was collected in sterile Petri dishes, dried in a hot-air oven, and the resulting residue was

carefully scraped, pooled, and preserved for subsequent analysis.

2.5 Physicochemical Parameters

All the physicochemical parameters were carried out as per the standard test procedures[5].

2.6 High-Performance Thin Layer Chromatography

For thin-layer chromatographic analysis, one gram of the extract was dissolved in 10 mL of ethanol, subjected to sonication for 15 min, and filtered to obtain the test solution. The sample was applied onto silica gel plates using a Camag ATS4 applicator, and chromatographic separation was carried out with a mobile phase consisting of toluene, ethyl acetate, and formic acid in the ratio of 9:1:0.5 (v/v/v), allowing the solvent front to migrate up to 9 cm. Developed plates were visualised under ultraviolet light at 254 nm and 366 nm with a Camag Visualizer, followed by derivatisation with 5% vanillin–sulphuric acid reagent and heating at 105°C until the appearance of coloured bands. Densitometric scanning was performed using a Camag Scanner 4 at 254 nm (D2 lamp, absorption mode) to generate fingerprint profiles, after which the plates were rescanned at 520 nm (W lamp, absorption mode) and documented under visible light for further analysis[6].

2.7 *In Vitro* Glucose Uptake Assay

Phenotypic assays are generally high-throughput cellular assays that measure the levels of various proteins or identify molecules with the ability to alter the phenotype of the cell. One such phenotypic assay is the glucose uptake assay[7].

L6 rat myoblasts were obtained from the National Centre for Cell Sciences (NCCS), Pune, and maintained in Dulbecco's Modified Eagle's Medium (DMEM; Sigma Aldrich, USA) supplemented with 10% fetal bovine serum, L-glutamine, sodium bicarbonate (Merck, Germany), and an antibiotic mixture containing penicillin (100 U/mL), streptomycin (100 µg/mL), and amphotericin B (2.5 µg/mL). Cells were cultured in 25 cm^2 flasks at 37°C in a humidified atmosphere with 5% CO_2 (NBS Eppendorf, Germany). For experimentation, cells were detached with 0.025% trypsin–EDTA (0.5 mM, Invitrogen) in PBS, seeded into 24-well plates, and grown to approximately 80% confluence. The cultures were then incubated in glucose-free DMEM for 24 h before exposure to the test extract, which was prepared from a 1 mg/mL stock and added at final concentrations of 25, 50, and 100 µg/mL. Cells were further incubated for 24 h in DMEM supplemented with 300 mM glucose, while high-glucose untreated wells served as controls. Following treatment, cells were harvested by centrifugation at 6000 rpm for 10 min, resuspended in 200 µL of lysis buffer (1 M Tris-HCl, 0.25 M EDTA, 2 M NaCl, 0.5% Triton X-100), and incubated at 4°C for 30 min. Glucose uptake was then quantified using a high-sensitivity glucose oxidase assay kit. Glucose concentration (mg/dL) was calculated as (Absorbance of test/Absorbance of standard) × 100, and the percentage uptake was expressed as [(OD test – OD control)/OD test] × 100. Data were analysed using SPSS version 29, with regression analysis performed to determine dose–response relationships; a p-value of < 0.05 was considered statistically significant[8].

3. Results

3.1 Physicochemical Analysis of TPCK

Table 2. The physicochemical analysis of TPCK

Experiment	Mean ± SD	CV%
Loss on drying	9.03 ± 0.22	2.43
Total Ash	5.64 ± 0.09	1.63
Water-soluble ash	1.43 ± 0.08	5.44
Acid-insoluble ash	1.80 ± 0.04	2.36
Alcohol soluble extractive	33.46 ± 0.25	0.74
Water soluble extractive	33.50	-
PH (10%of solution)	4.10	-

3.2 HPTLC Fingerprinting of TPCK

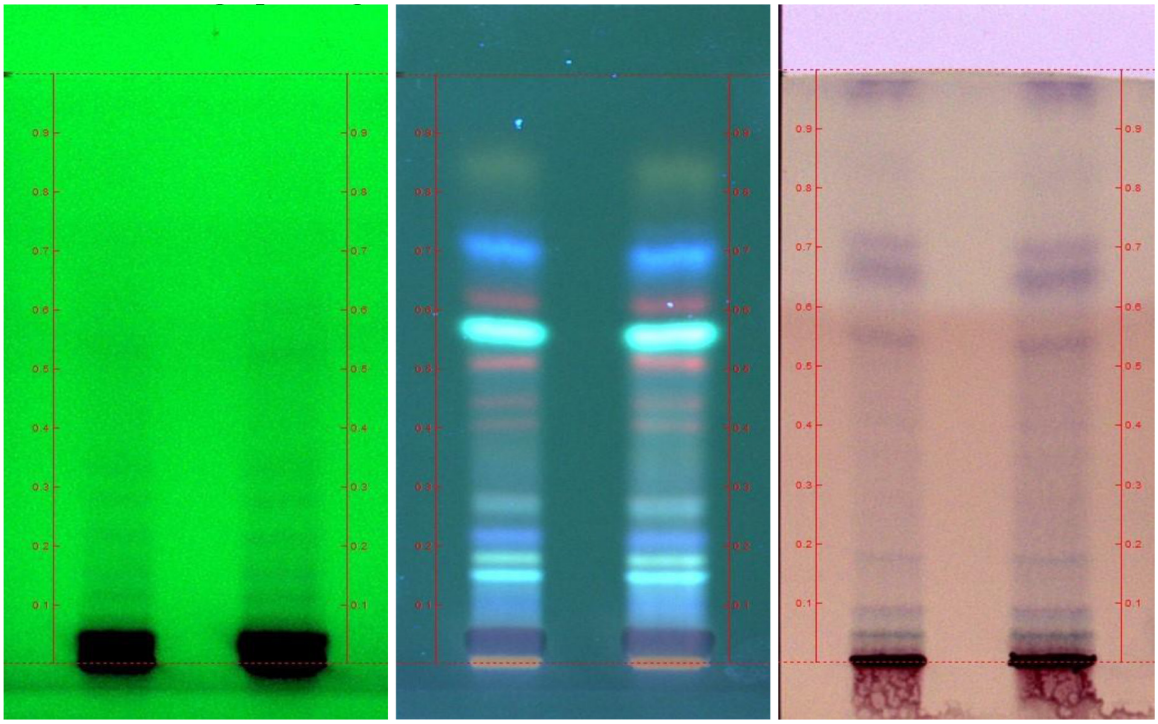


Figure 1. HPTLC Photo documentation of TPCK.

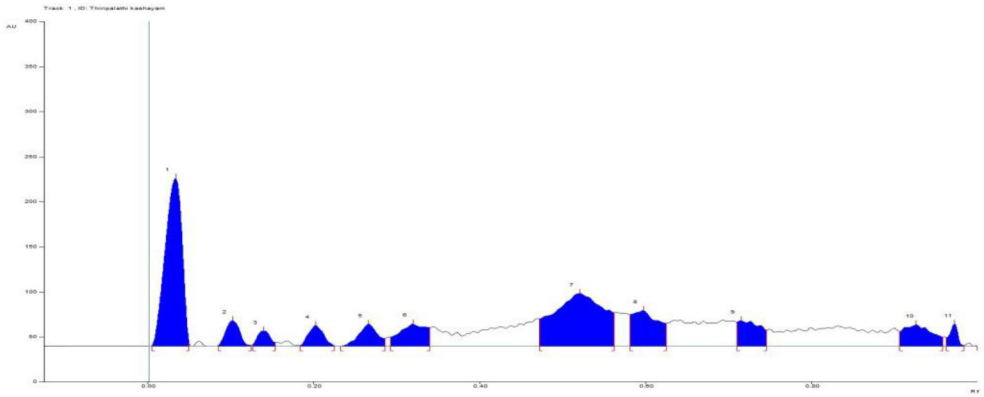


Figure 2. HPTLC Densitometric scan at 254 nm.

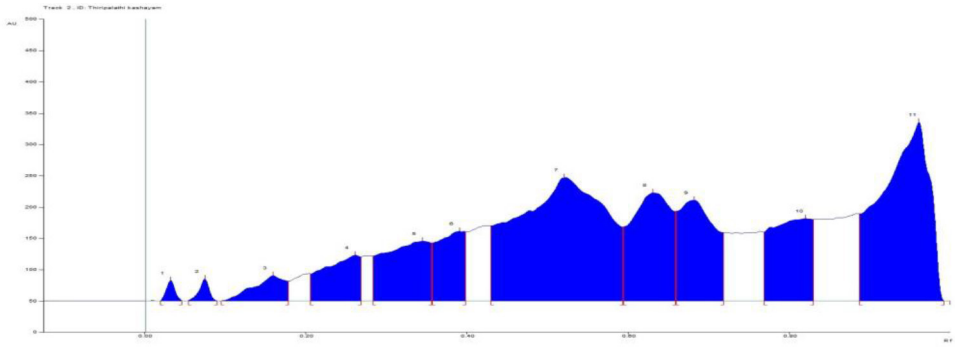


Figure 3. HPTLC Densitometric scan at 520 nm.

3.3 Glucose Uptake Activity of TPCK

Table 3. The percentage of Glucose uptake activity of TPCK

Concentration of sample(µg/mL)	Absorbance	Glucose (mg/dl)	% Glucose uptake
Control	0.1061	34.0719	0.0000
25	0.1216	39.0495	12.7467
50	0.1456	46.7566	27.1291
100	0.2048	65.7675	48.1934

4. Discussion

The physicochemical analysis of TPCK (Table 2) revealed a pH value of 4.10. A pH of 4 or higher is considered optimal for absorption. As this is an acidic pH, absorption is more in the stomach[9]. Elevated levels of both alcohol- and water-soluble extractives suggest that the formulation contains a substantial number of bioactive compounds. This indicates that the extraction process effectively preserved both polar and non-polar constituents, reflecting the potential therapeutic efficacy of the formulation[10]. All other parameters were within range, which suggests TPCK is free from contamination or adulteration and has been prepared through an appropriate manufacturing process.

The HPTLC study (Table 3, Figures 1-3) shows the Rf values of TPCK at 254nm and 520nm using ethanol and derivatisation with vanillin sulphuric acid. At 254 nm, multiple peaks across the Rf range (0.02-0.89) indicate the various UV-absorbing constituents in TPCK. At 520 nm, the peaks are more prominent in the mid–high Rf range, suggesting the presence of coloured or conjugated compounds, likely flavonoids, triterpenoids, and phenolic compounds. There are 11 peaks observed in the polyherbal decoction values, ranging from 0.02-0.6. Peak 11 has the highest intensity (285.3 AU) and largest percentage of area (21.39%), suggesting a dominant constituent. Peak 7 Rf (0.52)is also significant with 196.8 AU and 28.71 area, confirming multiple major components in the mid polar rage. The major peak indicates the predominant bioactive component, which could be correlated with pharmacological activity. This HPTLC profile may serve as a reference model for future batch preparations, as it represents a newly developed polyherbal formulation.

Thiripalathi Kashayam (TPCK) demonstrated a dose-dependent increase in glucose uptake in L6 myotubes, rising from 12.75% at 25 µg/mL to a peak of 48.19% at 100 µg/mL. Although lower than standard oral hypoglycemic drugs, this effect indicates significant potential to enhance peripheral glucose utilisation. Regression analysis showed a strong positive linear relationship ($R^2 = 0.996$, $p < 0.01$), confirming that 99.6% of the variation in glucose uptake was explained by TPCK concentration. These results establish TPCK as a promising modulator of glucose uptake.

Metformin, the primary biguanide used in managing type 2 diabetes, is known to boost glucose uptake mainly through activation of the AMP-Activated Protein Kinase (AMPK) pathway, which promotes the translocation of GLUT4 transporters in skeletal muscle. Studies with L6 cells consistently demonstrate that metformin can increase glucose uptake by about 60–75% at concentrations of 50–100 µM. Pioglitazone, a typical Thiazolidinedione (TZD), works by activating peroxisome Proliferator-Activated Receptor Gamma (PPAR-γ), thereby improving insulin sensitivity and raising GLUT4 expression[11]. In similar L6 cell experiments, pioglitazone has been reported to cause a 55–70% increase in glucose uptake, depending on dose and incubation time[12,13]. Compared to these standards, TPCK at 100 µg/mL achieved roughly two-thirds of the glucose uptake effectiveness of metformin or pioglitazone. This suggests its potential insulin-mimetic or insulin-sensitising properties. The activity of TPCK may be due to its high content of polyphenolic compounds, including gallic acid, ellagic acid, and chebulinic acid, all of which are reported to affect glucose metabolism through modulation of GLUT4 expression and AMPK signalling.

5. Conclusion

Establishing proper standardisation is essential to ensure the quality, safety, and efficacy of traditional medicines before their use, thereby minimising the risks of toxicity or contamination. The standardised TPCK formulation demonstrated improved palatability, extended shelf life, and promising preliminary antidiabetic activity, which may contribute to its wider acceptance and therapeutic potential.

6. Acknowledgement

This work is a part of the PhD thesis of The T. N. Dr. M.G.R. Medical University, Chennai, Tamil Nadu, India. The authors thank *Siddha* Central Research Unit, Chennai, and the Centre for Research on Molecular and Applied Sciences, Trivandrum, for their assistance in conducting the study.

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Author Contribution

Conceptualisation, Methodology, Original draft preparation: JS; Visualization and Supervision: TL, MR; Validation and Formal analysis: SK, PS. All authors have read and agreed to the published version of the manuscript.