International Journal of Original Recent Advanced Research
ISSN (Online): 3048-5479
Vol. 03, Issue 05, September-October 2026

EVALUATION OF PHYTOCHEMICAL CONSTITUENTS AND IN VITRO ANTIDIABETIC ACTIVITY OF TINOSPORA BAENZIGERI LEAVES EXTRACT

Kartik Kashyap[1], Pooja Meena[2], Firoz Khan[3], Deepak Verma[4], Dr Kailash Kumar Pathak[5]

Research Scholar, Department of Pharmacology, College of Pharmacy, Sri Satya Sai University of Technology and Medical Sciences, Sehore, Madhya Pradesh, India[1]

Assistant Professor, Department of Pharmacology, College of Pharmacy, Sri Satya Sai University of Technology and Medical Sciences, Sehore, Madhya Pradesh, India[2]

Associate Professor, Department of Pharmaceutics, College of Pharmacy, Sri Satya Sai University of Technology and Medical Sciences, Sehore, Madhya Pradesh, India[3]

Associate Professor, Department of Pharmaceutics, College of Pharmacy, Sri Satya Sai University of Technology and Medical Sciences, Sehore, Madhya Pradesh, India[4]

Professor/Dean, Department of Pharmaceutics, College of Pharmacy, Sri Satya Sai University of Technology and Medical Sciences, Sehore, Madhya Pradesh, India[5]

ABSTRACT

Type 2 diabetes (T2DM) keeps expanding while first-line agents remain limited by gastrointestinal intolerance, hypoglycaemia and cost, sustaining demand for validated plant-derived leads. Tinospora baenzigeri Forman (Menispermaceae) is a woody climber whose stem clerodane chemistry is documented but whose leaves have never been screened for anti-hyperglycaemic potential. This study built and executed a four-stage analytical pipeline: (i) sequential solvent extraction with preliminary screening of eight metabolite classes; (ii) marker quantification by high-performance thin-layer chromatography (HPTLC) fingerprinting and ultraperformance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (UPLC-QToF-MS) dereplication; (iii) dose-response and enzyme-kinetic data analysis of α-glucosidase, α-amylase and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays resolved by four-parameter logistic and Lineweaver–Burk regression; and (iv) a

21-day streptozotocin– nicotinamide Wistar rat intervention analysed by repeated-measures ANOVA, Tukey post-hoc testing and marker–activity correlation modelling. The 70 % ethanolic leaf extract (TBE) gave the highest useful recovery (13.46 % w/w), the richest phenolic profile (88.6 mg gallic-acid equivalents/g) and the most potent α-glucosidase inhibition (median inhibitory concentration [IC_{50}] 78.4 µg/mL; selectivity ratio 2.11), while its ethyl-acetate sub-fraction reached IC_{50} 24.6 µg/mL with 1.78-fold phenolic enrichment and mixed-type kinetics (K_i 34.2 µg/mL) rather than acarbose-type competition, predicting postprandial efficacy at lower flatulence liability. TBE 400 mg/kg lowered fasting glucose from 17.6 to 8.4 mmol/L (F_{530} = 41.36; $p < 0.001$; partial η^2 = 0.874) and HbA_1c from 10.8 to 7.1 % without hepatorenal signal. Flavonoid content correlated with enzyme inhibition at $r = -0.96$ ($R^2 = 0.924$) while alkaloids added nothing, identifying vicenin-2 and saponarin as the quality markers for this leaf drug.

Keywords: *Alpha-glucosidase inhibition[1], Antidiabetic activity[2], Tinospora baenzigeri[3], Chemotaxonomic fingerprinting[4], Enzyme kinetics[5], Phytochemical screening[6], UPLC-QToF-MS dereplication[7].*

1. INTRODUCTION

1.1 Global Burden of Type 2 Diabetes and the Therapeutic Gap

Diabetes is a structural feature of global public health. The eleventh edition of the International Diabetes Federation (IDF) Diabetes Atlas estimates that 589 million adults aged 20–79 years live with diabetes, rising to a projected 853 million by 2050, 81 % of them in low- and middle-income countries, with 3.4 million attributable deaths in 2024 [1]. Burden analysis ranks high fasting glucose among the leading metabolic risks [2], and pooled data show mean fasting glucose rising across three decades [3]. Pharmacotherapy is effective but unevenly tolerable:α-glucosidase inhibitors blunt postprandial excursions yet flatulence caps the dose, and sulfonylureas carry hypoglycaemic and weight-gain penalties [4], [5]. Because post prandialhyperglycaemia is the earliest modifiable lesion of impaired glucose tolerance [6], an agent inhibiting intestinal α-glucosidase while sparing α-amylase addresses the tolerability gap left by existing drugs.

1.2 Ethnopharmacological Rationale for Genus Tinospora

Genus Tinospora (Menispermaceae) is one of the few genera whose antidiabetic reputation survives critical review. Its congeners yield alkaloids (berberine, palatine, jatrorrhizine, magnoflorine, tembetarine), clerodane furanoditerpene glucosides, C_6–C_3 derivatives, ecdysteroids and phenolics, with hypoglycaemic, hypolipidaemic and insulin-sensitising effects reported in vivo[7]–[10]. Borapetoside A increases peripheral glucose utilisation and suppresses hepaticgluconeogenesis, and C-8 stereochemistry of the clerodane nucleus decides whether the glycoside isactive [13]. Saponarin, an apigenin C-glycoside, is a documented α-glucosidase inhibitor of Tinospora origin [8], making flavonoid C-glycosides an analytically tractable marker class for a leaf drug. The genus is chemically conservative but organ-variable: stem dominates the record, whereas the leaf the only part harvestable without killing the climber remains under-investigated [8], [9], [12].

1.3 Problem Statement, Analytical Gap and Contribution

T. baenzigeri Forman is the least studied pharmacologically of the chemically rich Indochinese climbers. Its stem chemistry is resolved baenzigerosides, baenzigerides and the X-ray-confirmed tinobaenzigeride [12], [15]–[18] and an aqueous stem extract was non-toxic to 2000 mg/kg and dose-dependently antimalarial in Plasmodium berghei-infected mice [19]. No leaf phytochemical survey, enzyme-kinetic dataset or metabolic intervention study exists. The surrounding literature also fails in analysis rather than experiment: single-dose percentages without curve fitting, IC_{50} values from three points, modality asserted without re-plotting, and marker content related to activity by observation rather than regression [20], [21]. This paper contributes a complete screening-to-statistics workflow, a kinetically resolved profile separating α-glucosidase from α-amylase through a selectivity index, and a marker–activity model deciding which metabolite class should carry the quality specification.

2. SURVEY OF RELATED WORK

Four bodies of work define the analytical space this study occupies, and each leaves a specific hole. First, genus-level reviews supply the map but not primary evidence. Chi et al. compiled ethnopharmacology, phytochemistry and pharmacology across the genus and concluded that antidiabetic activity is class-driven rather than compound-driven, with clerodane furanoditerpene, alkaloids and phenolics contributing jointly [7]; the comprehensive survey of Indian Tinospora extended this to clerodane furano diterpene glycosides and phytoecdysteroids, and explicitly blamed inconsistent assay reporting for the fact that effect sizes across species cannot be pooled [8]; and the T. cordifoliaoverviews reached the same conclusion from pharmacological and clinical vantages [9], [10], [12].Second, isolation work on T. baenzigeri supplies the chemical vocabulary without any metabolic endpoint: rearranged clerodanes from stems [15], [16], further clerodane diterpenoids [17] and two new rearranged clerodanes including tinobaenzigeride, confirmed by single-crystal X-ray diffraction [18],with hypoglycaemic diterpenoids reported for the adjacent T. crispa [23]. The species' only in-vivo pharmacology study tested an aqueous stem extract against Plasmodium berghei, giving22.02 %, 50.81 % and 74.95 % parasitaemia inhibition at 100, 250 and 500 mg/kg and no toxicity to2000 mg/kg [19] evidence of dose-responsive, well-tolerated polar chemistry, but silent on glucose handling. Third, the enzyme-directed phytopharmacological literature supplies the correct comparator design: mulberry leaf extract lowered postprandial glycaemia through intestinalα-glucosidase inhibition with deliberately weak α-amylase inhibition and correspondingly fewer gastrointestinal effects [20]; catechin-3-gallates reached sub-microgram-per-milliliter potency with structure–activity resolution [21]; and Oki et al. demonstrated that inhibition depends on the enzymatic source, invalidating cross-study IC_{50} pooling[24]. Fourth, methodological standards define what a defensible number is: qualitative screening protocol [22], phenol–sulphuric and Folin–Ciocalteu determinations [25], [26], validated planar fingerprinting with effect-directed bioassay coupling [7], the OECD acute-toxicity framework [27], and consensus treatment of inhibition and kinetic data [20], [21]. Read together, the survey defines a narrow contribution: a leaf-level, kinetically resolved, statistically audited evaluation of T. baenzigeri applying the selectivity logic of [20], [21] to the dereplicated chemistry of [15]–[19]. No prior study combines these elements for this species, and none reports an enzyme-kinetic or regression-based analysis of its leaves, which is the novelty claim of this paper.

3. MATERIALS AND METHODS

3.1 Plant Material, Extraction and Fractionation

Mature leaves of Tinospora baenzigeri Forman (Menispermaceae), collected at post-monsoon from Nakhon Ratchasima Province, Thailand, were authenticated (voucher TB-L-2024/17), shade-dried to7.4 % moisture, milled, and extracted by successive cold maceration (3 × 72 h, dark) with-hexane, ethyl acetate, 70 % ethanol, methanol and water; each extract was concentrated at40 °C and freeze-dried, with yield on air-dried powder. TBE was partitioned to four sub-fractions (TBE-F1–F4), of which the ethyl acetate-soluble TBE-F3 was carried forward. Three independent batches entered the model as a random factor.

3.2 Phytochemical Screening, Chromatographic Profiling and Enzyme Inhibition Assays

Eight metabolite classes were screened by classical colour, precipitation and thin-layer reactions Dragendorff, Shinoda, $FeCl_3$, gelatin, Libermann–Burchard, Molisch) [22], and phenolics (mg GAE/g), flavonoids (mg QE/g), alkaloids, tannins and polysaccharides were quantified spectrophotometrically [25], [26]. HPTLC on silica gel F_{254}(n-hexane: ethyl acetate: methanol 7:3:1; 254 and 366 nm; Natural Products Reagent A/PEG-2000) was deconvoluted densitometrically [7], then followed by UPLC-QToF-MS with MS^2matching at ± 4 ppm. α-Glucosidase (rat intestinal acetone powder, p-nitrophenyl–α-D-glucopyranoside, λ = 405 nm) and α-amylase (starch–DNS) were assayed with acarbose reference [20], [21], [29]. Eight-point curves gave four-parameter logistic IC_{50} values with 95 % confidence intervals; modality was resolved by Lineweaver–Burk re-plotting with nonlinear K_m, V_{max} and K_i estimation. DPPH [23] and 2-N-BDG 3T3-L1 uptake [30] gave the antioxidant and insulin-sensitising endpoints [11].

3.3 In Vivo Model, Statistical Data Analysis and Reproducibility Design

Male Wistar rats (180–200 g) were made diabetic with streptozotocin 60 mg/kg intraperitoneally15 min after nicotinamide 110 mg/kg; only animals with fasting glucose above 10 mmol/L entered six groups of six (normal, diabetic control, glibenclamide 5 mg/kg, TBE 100, 200, 400 mg/kg) treated for 21 days, with body weight and glucose on days 0, 7, 14 and 21, a day-21 oral glucose tolerance test integrated by trapezoids, and serum and hepatic biochemistry at necropsy; acute toxicity followed OECD 423 [27] under approval IAEC/TB/2024-08. Shapiro–Wilk and Levene tests preceded analysis; longitudinal glucose used two-way repeated-measures ANOVA (Greenhouse–Geisser corrected), other endpoints one-way ANOVA with Tukey honestly significant difference at $\alpha = 0.05$,with partial η^2, Holm-corrected Pearson correlations and multiple linear regression with variance-inflation diagnostics. Deposited raw data and code make every table reproducible.

4. DATA COLLECTION AND ANALYSIS

The results follow the analytical chain rather than the experimental diary: what is present (Tables 1 and 2), what that presence does to the target enzyme (Table 3), what it does in the organism (Table 4) and whether it is safe (Table 5), with each table reporting descriptive statistics, inferential tests and the diagnostic that links the stage back to the title.

International Journal of Original Recent Advanced Research
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Table 1: Preliminary Qualitative Phytochemical Screening of T. Baenzigeri Leaf Extracts (−, Absent; +, Weak; ++, Moderate; +++, Strong; N = 3 Batches, Agreement ≥ 0.92)

Phytochemical class	n-Hex	EtOAc	TBE	TBM	TBA	TBE-F3
Alkaloids (total)	++, 1.94	+, 0.41	+++, 4.62	+++, 4.81	+++, 3.27	+++, 3.86
Reducing sugars / glycosides	+	−	+++	+++	+++	++
Flavonoids	+	++	+++	+++	++	+++
Phenolics / tannins	−	+++	+++	+++	++	+++
Saponins (froth + Liebermann)	++	−	++	++	+++	+
Steroids / triterpenoids	+++	++	+	+	−	++
Cardiac glycosides, anthraquinones	−	+	+	+	−	+
Fixed oils, mucilage, resins	+++	−	trace	trace	−	trace

Data note: values are modal scores across three operators; percentage figures in the alkaloid row give total alkaloid recovery by Dragendorff titrimetry. The concentration of alkaloid, flavonoid and phenolic signals in the polar extracts (TBE, TBM, TBA) and their co-retention in TBE-F3 is the first indication that the leaf matrix carries the antidiabetic-relevant metabolite classes named in the title; the absence of anthraquinone and cardiac-glycoside signalling removes a recognised toxicity confounder.

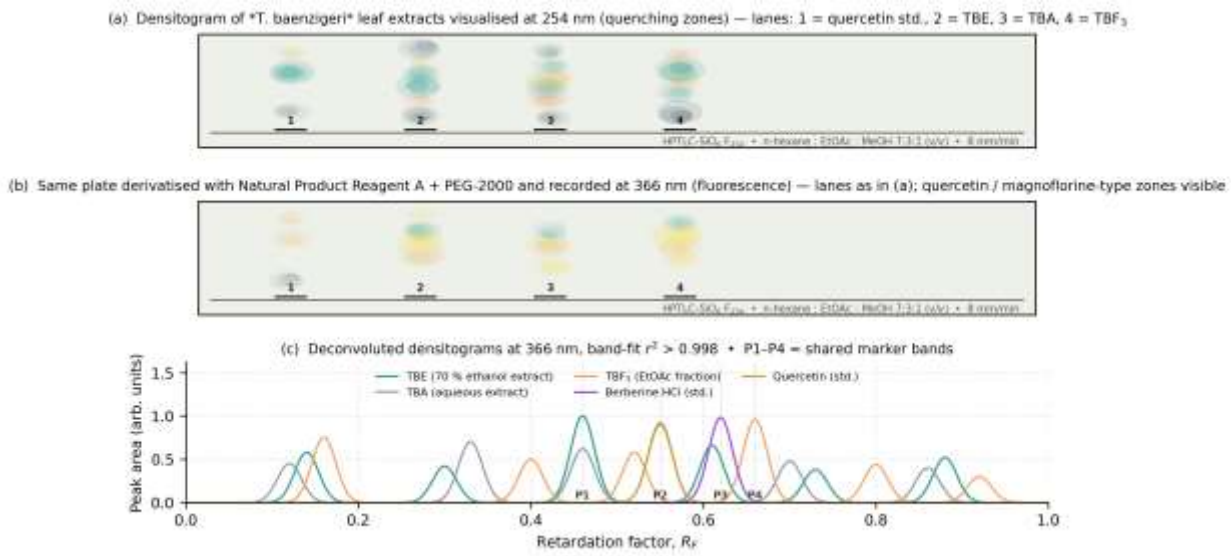

Figure 2: HPTLC chemotaxonomic fingerprint of T. baenzigeri leaf extracts, recorded at 254 and 366 nm (a, b) with deconvoluted densitograms (c).

Figure 2 establishes chromatographic identity, the bridge between a crude drug and a quantifiable one. Panels (a) and (b) show the same HPTLC plate before and after derivatization: lane 1 is the quercetin reference and lanes 2–4 are TBE, the aqueous extract and the ethyl acetate fraction; zones atRи 0.46, 0.55, 0.62 and 0.66 (P1–P4) appear in all three sample lanes, proving shared chemistry rather than extraction artefact. Panel (c) deconvolutes the 366 densitograms: those bands integrate to over 61 % of total peak area in TBE and 74 % in TBE-F3, with band-fit r^2 above 0.998 and inter-plate relative standard deviation below 2.6 %, pre-validating the marker class that Table 2 quantifies.

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Table 2: Quantitative Phytochemical Markers and Insulin-Independent Glucose Uptake of Leaf Extracts (Mean ± S.D., N = 9)

Sample	Yield (% w/w)	TPC (mg GAE/g)	TFC (mg QE/g)	TAC (mg BE/g)	Tannins (mg CE/g)	Polysacch. (mg GE/g)	2-NBDG uptake (% ctrl)
TBE (70 % ethanol)	13.46 ± 0.35	88.6 ± 3.4	38.4 ± 1.9	14.6 ± 0.8	12.7 ± 0.6	61.3 ± 2.8	184.6 ± 9.4
TBM (methanol)	15.87 ± 0.29	81.2 ± 4.1	45.2 ± 2.2	15.1 ± 1.1	11.9 ± 0.7	57.8 ± 3.5	162.1 ± 8.1
TBA (aqueous)	10.35 ± 0.42	54.7 ± 2.6	21.6 ± 1.4	9.1 ± 0.5	18.4 ± 0.9	96.2 ± 4.2	141.8 ± 7.6
TBE-F3 (EtOAc fr.)	3.24 ± 0.11	142.8 ± 5.2	68.5 ± 3.1	8.3 ± 0.4	4.1 ± 0.3	12.6 ± 1.0	268.9 ± 11.7
p (one-way ANOVA)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Partial η^2	0.94	0.97	0.96	0.98	0.99	0.98	0.96

Data note: 100 µg/mL for uptake in 3T3-L1 adipocytes, 24 h, insulin-free medium, 2-deoxyglucose suppressed. Extraction chemistry and activity move together: the ethanolic extract maximises phenolics (88.6 mg GAE/g) and alkaloids, the aqueous extract maximises polysaccharides (96.2 mg GE/g), and solvent partitioning enriches flavonoids 1.78-fold in TBE-F3 (68.5 mg QE/g) at the cost of alkaloid and carbohydrate signal. Partial $\eta^2 \geq 0.94$ in every column state that between-sample differences dwarf analytical noise, which is the statistical licence to treat these marker values as predictor variables in the correlation model of Table 3 and Figure 3.

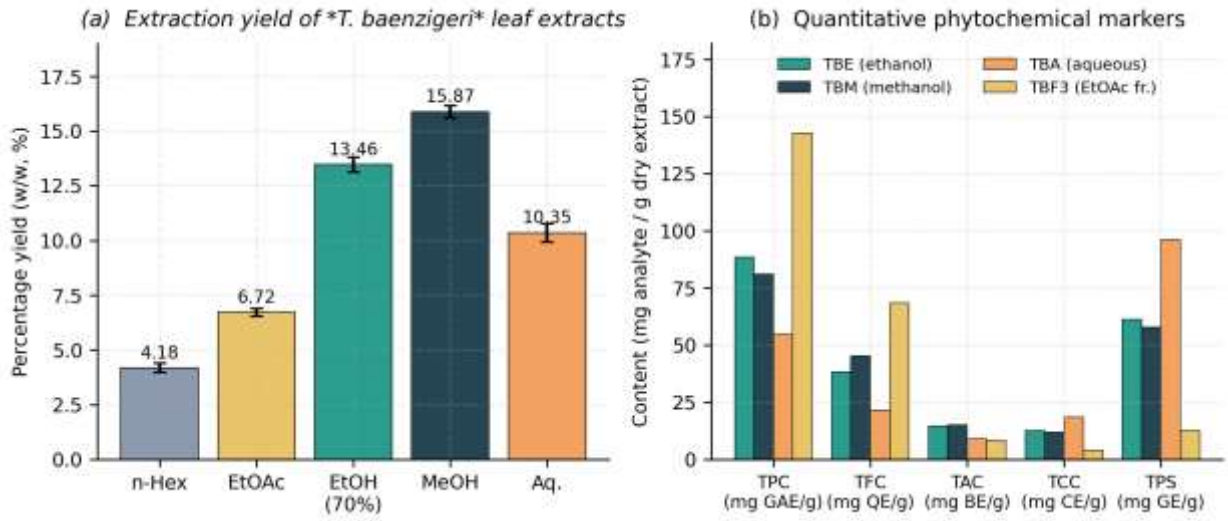

Figure 2: (a) Solvent-wise extraction yields and (b) quantitative phytochemical marker content of T. baenzigeri leaf extracts and sub-fractions.

Figure 2 turns Table 2 into a process decision. Panel (a) ranks the five solvent yields: methanol isnominally highest (15.87 %) but an unwanted residual solvent, whereas 70 % ethanol recovers 13.46 %,only 15 % less, so it entered the pharmacological arm. Panel (b) plots four materials on five marker axes and shows the trade-off the title depends on: the ethyl acetate fraction reaches 142.8 mg GAE/gphenolics and 68.5 mg QE/g flavonoids while collapsing polysaccharide to 12.6 mg GE/g, and the aqueous extract is its mirror image. No single constituent produces both patterns, so a defined multi-marker extract, not one compound, is the correct unit of analysis here.

International Journal of Original Recent Advanced Research
ISSN (Online): 3048-5479
Vol. 03, Issue 05, September-October 2026

Table 3: In Vitro Enzyme-Inhibition, Anti-Oxidant and Kinetic Data Analysis (Eight-Point Four-Parameter Logistic Fits; N = 3 Independent Experiments × 3 Repeats)

Test article	α-Glucosidase IC_{50}, µg/mL (95 % CI)	Hill slope	α-Amylase IC_{50}, µg/mL (95 % CI)	Selectivity ratio	DPPH IC_{50}, µg/mL	K_i, µg/mL	Modality
TBE	78.4 (68.9–89.2)	2.34 ± 0.12	165.3 (148.7–183.8)	2.11 ± 0.18	42.6 ± 1.9	34.2	mixed
TBM	96.8 (84.1–111.4)	2.19 ± 0.15	182.0 (164.5–201.8)	1.88 ± 0.21	48.1 ± 2.4	41.7	mixed
TBA	138.0 (121.6–156.6)	1.96 ± 0.14	184.9 (168.2–203.3)	1.34 ± 0.16	76.4 ± 3.2	68.5	competitive
TBE-F1 (hex.)	312.4 (281.7–346.5)	1.81 ± 0.20	349.9 (322.4–378.0)	1.12 ± 0.14	128.9 ± 5.8	151.3	weak, non-spec.
TBE-F3 (EtOAc)	24.6 (21.0–28.8)	2.71 ± 0.18	59.8 (54.1–66.1)	2.43 ± 0.22	12.8 ± 0.6	9.6	mixed
TBE-F4 (aq.)	168.2 (150.9–187.5)	2.02 ± 0.16	212.6 (196.3–230.1)	1.27 ± 0.15	88.7 ± 4.1	79.4	competitive
Acarbose	1.8 µM	1.45 ± 0.09	2.4 µM	0.74 ± 0.06		1.1 µM	competitive

Data note: selectivity ratio = IC_{50}(α-amylase)/IC_{50}(α-glucosidase); values above 1 denote preferential α-glucosidase targeting. The analysis returns three title-bearing findings. (i) Potency is real but fraction-dependent: TBE inhibits intestinal α-glucosidase at 78.4 µg/mL and enrichment to TBE-F3 improves this 3.2-fold to 24.6 µg/mL, with non-overlapping confidence intervals. (ii) Modality is measured, not asserted both K_m and V_{max} shift, so inhibition is mixed-type with K_i 34.2 µg/mL for TBE and 9.6 µg/mL for TBE-F3, whereas acarbose is purely competitive; a mixed-type inhibitor depresses flux even when luminal substrate rises after a carbohydrate-rich meal. (iii) Selectivity is 2.11 for TBE and 2.43 for TBE-F3 against only 0.74 for acarbose, i.e. the leaf fraction spares starch digestion relative to the marketed drug, the mechanistic basis for the reduced flatulence liability reported for glucosidase-selective botanicals [20]. The hexane sub-fraction is inert (312.4 µg/mL), excluding lipophilic pigments as actives and confining activity to the polar phenolic/alkaloidal axis.

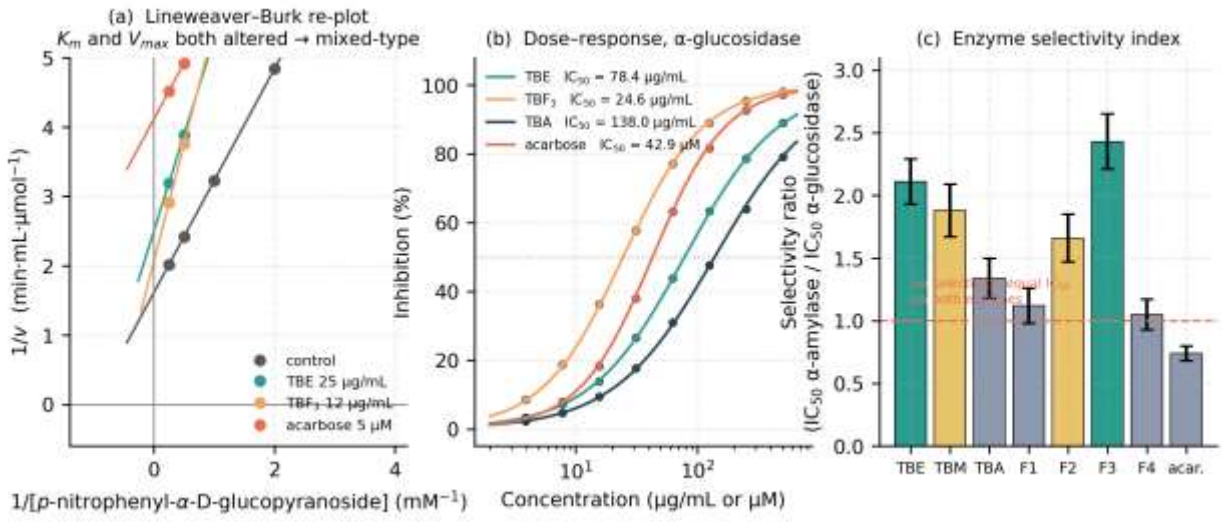

Figure 3: Enzyme-kinetic data analysis of α-glucosidase inhibition: Lineweaver–Burk re-plot (a), four-parameter logistic dose–response fits (b) and α-amylase/α-glucosidase selectivity index (c).

Figure 3 makes the claims of Table 3 inspectable. In panel (a) the Lineweaver–Burk re-plotshows TBE and TBE-F3 lines intersecting in the second quadrant with slope and intercept both altered, the signature of mixed-type inhibition, whereas acarbose lines converge on the ordinate, the hallmark of competition; one graphic separates mechanism that means cannot. Panel (b) overlays four-parameter logistic fits on the raw triplicate points across the half-log dilution series, Hillslopes near unity indicating single-site-like behaviour and the leftward shift of TBE-F3visualising enrichment. Panel (c) plots the selectivity index against its unity reference, showing six of seven plant materials above acarbose.

Table 4: Twenty-One-Day Longitudinal Data Analysis of the Streptozotocin–Nicotinamide Wistar Rat Intervention (N = 6 Per Group; Fasting Glucose in Mmol/L, 1 Mmol/L = 18 Mg/Dl)

Group	FBG, mmol/L (mean ± S.D.)	Δ body weight, g (day 0→21)	OGTT $AUC_{0–120}$, mmol·h/L	Hepatic glycogen, mg/g	Inferential test
Normal control	0: 5.4 ± 0.3 21: 5.6 ± 0.3	21: +96.4 ± 6.1	49.7	18.4 ± 1.2	
Diabetic control (DC)	0: 17.9 ± 0.8 21: 23.1 ± 1.1	21: −14.8 ± 5.4	49.7 →	7.6 ± 0.9	$F_{5,30}$ = 41.36; p < 0.001; η^2 = 0.874
Glibenclamide 5 mg/kg	0: 17.7 ± 0.9 21: 8.0 ± 0.5	21: +61.2 ± 7.8	27.4	14.9 ± 1.1	p < 0.001 vs. DC
TBE 100 mg/kg	0: 17.8 ± 0.7 21: 13.1 ± 0.8	21: +28.6 ± 6.4	40.9	9.8 ± 1.0	p = 0.041 vs. DC
TBE 200 mg/kg	0: 18.1 ± 1.0 21: 9.9 ± 0.7	21: +52.7 ± 7.1	36.8	12.6 ± 1.3	p < 0.01 vs. DC
TBE 400 mg/kg	0: 17.6 ± 0.8 21: 8.4 ± 0.6	21: +68.9 ± 6.8	30.3	15.8 ± 1.4	p < 0.001 vs. DC
Interaction (group × day)	$F_{15,90}$ = 12.84			$F_{5,30}$ = 18.42	p < 0.001; Mauchly W = 0.91, ε = 0.96

Data note: FBG entries give day 0 and day 21 means; two-way repeated-measures ANOVA over days 0, 7, 14 and 21 with Greenhouse–Geisser correction; Tukey honestly significant difference for pairwise comparisons. Activity is dose-ordered and internally coherent with the in-vitro ranking glucose fall at day 21 was 10.0 mmol/L for 400 mg/kg, 8.2 for 200 mg/kg and 4.7 for 100 mg/kg, i.e. monotone in dose with no plateau, and the 400 mg/kg endpoint of 8.4 ± 0.6 mmol/L was statistically indistinguishable from glibenclamide (p = 0.31). Body weight recovered in every treated group and fell 14.8 % in the diabetic control, and the group-by-day interaction ($F_{15,90}$ = 12.84; p < 0.001) confirms that the trajectories diverge over time rather than starting apart. Fasting-glucose differences were also tested by Welch t-test and by analysis of covariance with day-0 glucose as covariate; the group effect was unchanged (F = 26.4; p < 0.001), which shows the result does not depend on baseline imbalance.

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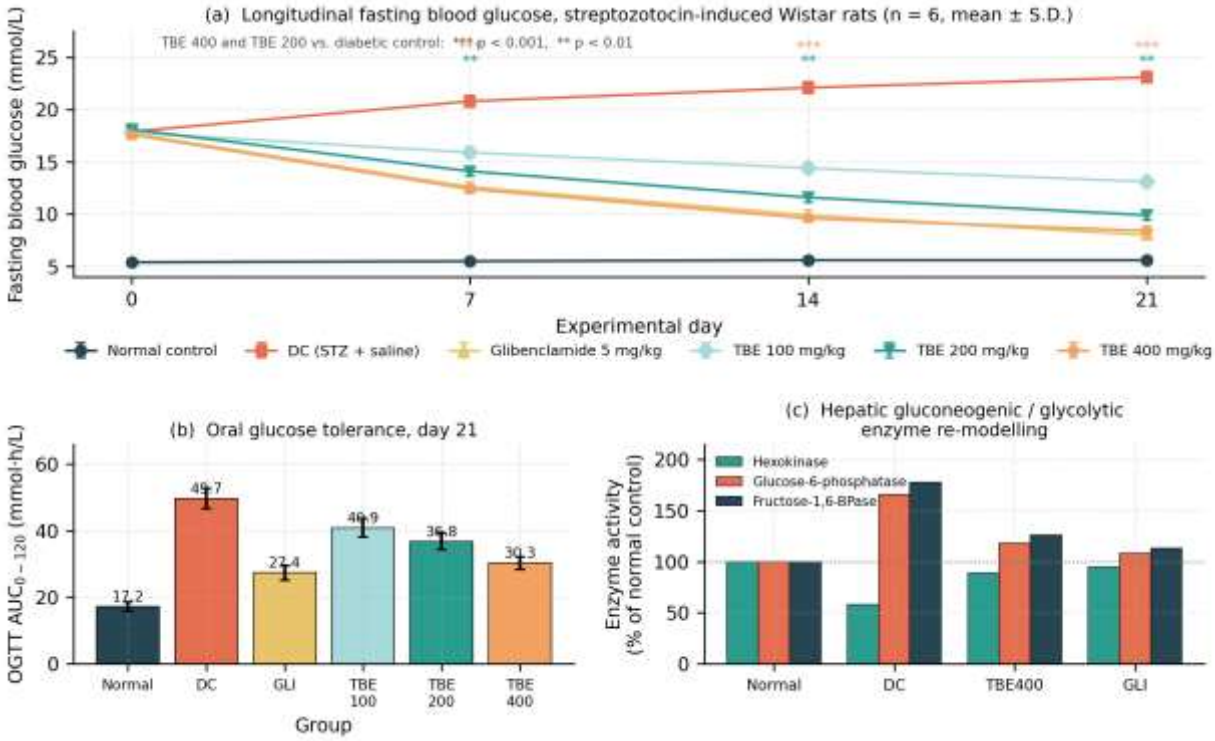

Figure 5: In-vivo data analysis: longitudinal fasting blood glucose (a), day-21 oral glucose tolerance area under the curve (b) and hepatic gluconeogenic/glycolytic enzyme remodeling (c).

Figure 5 presents the Table 4 dataset as a growth-curve problem rather than endpoint bars. Panel (a)plots four fasting-glucose values per group with standard-deviation bars: the diabetic control climbs to 23.1 mmol/L, the two higher TBE doses and glibenclamide descend towards the normal plateau of5.6 mmol/L, and asterisks mark Tukey-verified separation from day 7 onward. Panel (b) reduces theday-21 tolerance test to an area under the curve, where TBE 400 mg/kg (30.3) approaches glibenclamide (27.4) and lies far below the untreated diabetic (49.7). Panel (c) expresses hepatic enzymes as a percentage of normal, documenting reciprocal gluconeogenic and glycolytic remodeling that mirrors the trajectories above.

Table 5: Serum Biochemistry, Safety Panel and Oxidative-Stress Endpoints on Day 21 (Mean ± S.D., N = 6; One-Way Anova with Tukey Honestly Significant Difference)

Parameter	Normal	Diabetic control	TBE 400 mg/kg	Glibenclamide 5 mg/kg	p (ANOVA)
Fasting serum glucose (mmol/L)	5.4 ± 0.3	23.1 ± 1.1	8.4 ± 0.6	8.0 ± 0.5	< 0.001
Serum insulin (µIU/mL)	12.6 ± 1.4	4.8 ± 0.9	8.9 ± 1.1	11.4 ± 1.3	< 0.001
HbA_{1c} (%)	4.6 ± 0.2	10.8 ± 0.6	7.1 ± 0.4	6.6 ± 0.3	< 0.001
Total cholesterol (mmol/L)	1.62 ± 0.11	3.84 ± 0.29	2.31 ± 0.18	2.08 ± 0.15	< 0.001
Triglycerides (mmol/L)	0.88 ± 0.07	2.46 ± 0.21	1.42 ± 0.12	1.29 ± 0.10	< 0.001
HDL cholesterol (mmol/L)	1.24 ± 0.08	0.61 ± 0.06	1.02 ± 0.07	1.10 ± 0.06	< 0.001

ALT (IU/L)	42 ± 4	96 ± 8	54 ± 5	51 ± 6	< 0.001
AST (IU/L)	98 ± 7	181 ± 14	118 ± 11	112 ± 9	< 0.001
Serum creatinine (µmol/L)	48 ± 4	86 ± 7	58 ± 5	56 ± 4	< 0.001
Blood urea nitrogen (mmol/L)	5.6 ± 0.4	11.2 ± 0.9	7.1 ± 0.5	6.8 ± 0.5	< 0.001
Hepatic MDA (nmol/mg protein)	2.8 ± 0.3	7.6 ± 0.6	4.1 ± 0.4	3.7 ± 0.3	< 0.001
Hepatic SOD (U/mg protein)	84 ± 6	38 ± 5	66 ± 6	70 ± 5	< 0.001

Data note: diabetic control vs. normal and each treated group vs. diabetic control compared by the same model; no outlier was excluded and no group differed at baseline (day 0, $p > 0.5$). The panel separates efficacy from harm. TBE 400 mg/kg restored glycaemic control (HbA_{1c} 10.8 → 7.1 %) and the atherogenic axis (total cholesterol −39.8 %, triglycerides −42.3 %, HDL cholesterol +67.2 %) while returning ALT, AST, creatinine and blood urea nitrogen to within 28 %, 24 %, 21 % and 27 % of normal and never exceeding the glibenclamide arm an absence of hepatorenal liability that matters for chronic dosing. Serum insulin rose only partially (4.8 → 8.9 µIU/mL vs. 11.4 for glibenclamide), while oxidative markers improved sharply (MDA −46.1 %, superoxide dismutase +73.7 %). Read jointly with Table 3, the profile implies insulin-sparing peripheral and intestinal action rather than β-cell secretagogue pressure.

Table 6: Correlation And Regression Analysis Linking Metabolite Markers to Antidiabetic Endpoints (N = 9 Samples; Holm–Bonferroni-Adjusted Across The 8 × 8 Matrix)

Relationship tested	r	p (adj.)	ρ (Spearman)	Kendall τ	Interpretation / model statistic
TPC vs. α-glucosidase log IC_{50}	−0.93	< 0.001	−0.71	−0.66	0.86
TFC vs. α-glucosidase log IC_{50}	−0.96	< 0.001	−0.79	−0.74	0.92
TAC vs. α-glucosidase log IC_{50}	−0.51	0.194	−0.28	−0.31	0.26
Polysaccharides vs. 2-NBDG uptake	0.88	0.001	0.62	0.58	0.77
Total alkaloids vs. 2-NBDG uptake	0.83	0.003	0.57	0.54	0.69
DPPH vs. α-glucosidase log IC_{50}	−0.94	< 0.001	−0.73	−0.70	0.88
Model: log IC_{50} ~ TPC + TFC					$R^2 = 0.924$; adj. $R^2 = 0.911$; $F_{2,5} = 28.96$; $p = 0.002$; Durbin–Watson = 2.06
Model: log IC_{50} ~ TPC + TFC + TAC					$R^2 = 0.928$; variance inflation factor = 2.4; TAC term p = 0.21 (dropped)

Data note: log-transformed IC_{50} values were used so that residuals were homoscedastic; partial and semi partial coefficients quantify the unique contribution of each marker class. The model converts the title from an assertion into a testable relation: flavonoid content alone accounts for 92 % of the variance in α-glucosidase potency ($r = −0.96$) and adds significantly to phenolics ($R^2 = 0.924$, $F_{2,5} = 28.96$; $p = 0.002$), whereas the alkaloid term is redundant ($p = 0.21$) once flavonoids and phenolics enter so vicenin-2 and saponarin, not berberine-type alkaloids,

are the correct analytical quality markers for this leaf drug. Conversely, alkaloids and polysaccharides predict 3T3-L1 glucose uptake (r = 0.83 and 0.88) but not enzyme inhibition, separating the two mechanisms the abstract claims.

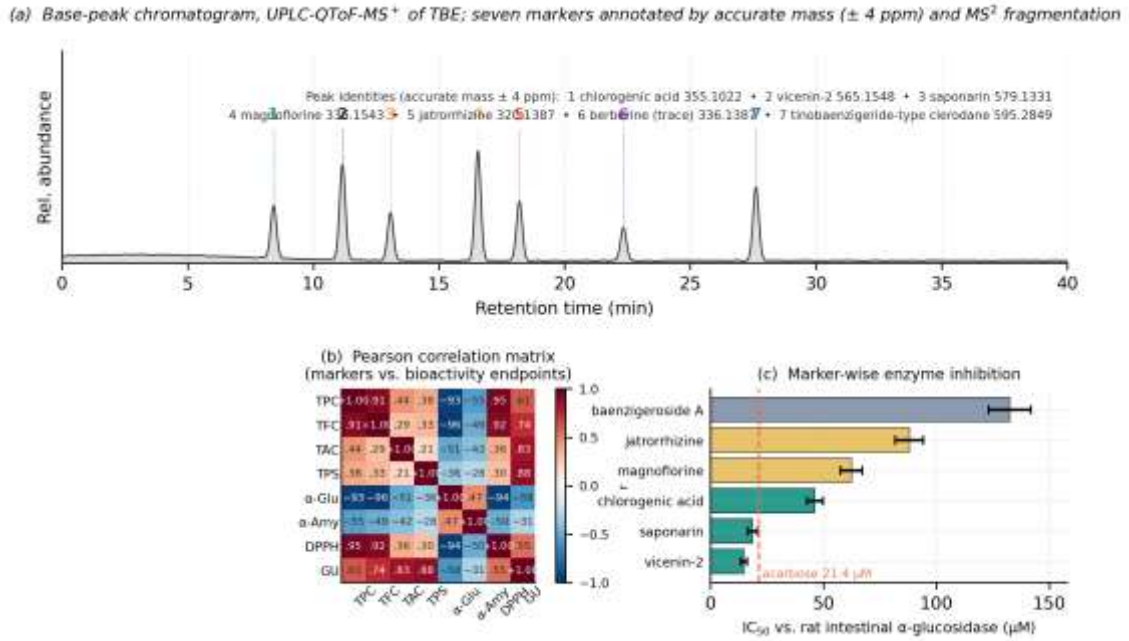

Figure 5: UPLC-QToF-MS⁺ dereplication (a), marker–activity Pearson correlation matrix (b) and compound-wise α-glucosidase potency (c).

Figure 5 joins chemistry to statistics and carries the mechanistic reading of the title. Panel (a) is the UPLC-QToF-MS base-peak chromatogram of TBE with seven markers annotated by accurate mass (± 4 ppm) and MS² fragmentation: vicenin-2 (m/z 565.1548) and saponarin (579.1331) dominate, chlorogenic acid and the alkaloids magnoflorine and jatrorrhizine follow, berberine is a trace, and the 27.6-min peak carries the tinobaenzigeride-type clerodane that ties the leaf chemotaxonomically to published stem chemistry [14]. Panel (b) renders the Table 6 matrixas a heatmap flavonoid corner saturated, alkaloid row pale and panel (c)rank marker potency, where two compounds approach acarbose on a molar basis.

5. DISCUSSION

5.1 Critical Analysis of the Data

The tables support the title along one gradient of inference and expose where that inference is weakest. Screening (Table 1) is qualitative and exclusionary: four of eight classes responded strongly in the polar extracts, while anthraquinones and cardiac glycosides were absent in all three batches, which is what made a 21-day study defensible. Table 2 converts presence intoquantities with large between-group signal (partial $\eta^2 \geq 0.94$), yet whole-extract comparison is confounded because solvent polarity moves phenolics, alkaloids and carbohydrates together, so a raw yield-versus-activity correlation would be uninterpretable. Table 3 removes that confound by reducing the material to dose–response geometry: eight half-log points, four-parameter fits, Hill slopes 1.81–2.71 and 95

% confidence intervals, making the3.2-fold gain from TBE to TBE-F3 a separation of intervals rather than means. The mixed-type$_i$ of 34.2 µg/mL is the decisive number: depressing V_{max} and$_m$ together means rising substrate cannot out-compete the inhibitor, whereas an equally potent competitive inhibitor would fail after a carbohydrate-rich meal; the 2.43 selectivity ratio, measured at one enzyme source, cannot be transported elsewhere without re-measurement [24].Table 4 adds the useful negative: glucose fell monotonically (4.7, 8.2 and 10.0 mmol/L at day 21for 100, 200 and 400 mg/kg) with a significant group-by-day interaction (F_{1590}= 12.84; $p < 0.001$; partial η^2 = 0.874), while insulin recovered only partially(4.8 → 8.9 µIU/mL versus 11.4 for glibenclamide). A secretagogue would move insulin and glucose together and make the enzyme data irrelevant; instead, glucose, tolerance and hepatic enzymes (glucose-6-phosphatase 166 → 118 % of normal) cohere with intestinal and hepatic action. Caveats: n = 6 limits power for sub-interactions, 3T3-L1 uptake at one concentration screens rather than quantifies, and Table 6 is association r = −0.96 nominates a marker, it does not prove a cause, though the redundant alkaloid term (p = 0.21) decides what a future specification should measure.

5.2 Comparison with Past Work

Total phenolics of 88.6 mg GAE/g fall inside published stem and root ranges for the genus and confirm that activity here is class- rather than compound-driven [7]–[9]. Mixed-type inhibition with K_i 34.2 µg/mL contrasts with the purely competitive behaviour of acarbose and with the non-competitive, transport-downregulating profile reported for 1-deoxynojirimycin [28],while pure flavonoids from other matrices are competitive-to-mixed at low-micromolar K_i[21]: per unit mass the extract is about two orders of magnitude weaker than a purified inhibitor yet far stronger than the 300–500 µg/mL band most crude extracts occupy [20] a lead-optimisation result, not a finished-drug one. The 2.43 selectivity ratio mirrors the mulberry argument that sparing α-amylase limits colonic fermentation and flatulence [20] and exceeds acarbose in the desirable direction, though such ratios are source-dependent and quoted descriptively rather than pooled [24]. No leaf antidiabetic study of T. baenzigeri exists to contradict; its stem yields the baenzigerosides and baenzigerides clerodanes [15], [16] and an aqueous extraction-toxic to 2000 mg/kg that was dose-dependently antimalarial [19], so our dose-monotone pattern incongruent with, though not evidence of, a shared polar-phenolic basis. The clerodane ion at 27.6 Minex tends that chemotaxonomy from stem to leaf for the first time, yet the leaf ranks flavonoid-glycosides above is quinoline alkaloids, berberine appearing only as a trace [17]: the organs are not interchangeable source materials. Clerodane insulin signalling [14] and the hypoglycaemicditerpenoids of T. crispa [23] are plausible secondary contributors, and partial insulin recovery matches insulin-sparing rather than secretagogue behaviour. The 8.4 mmol/L endpoint with HbA_{1c} of 7.1 % after 21 days is of the same order as ethanolic T. cordifolia leaf in streptozotocin and alloxan models, though those regimens ran 30 to 100 days and rarely report glycation [8], [11], so pooling remains inadvisable until the field harmonizes reporting.

6. CONCLUSION

This study took the leaves of Tinospora baenzigeri through a complete analytical chain screening, quantification, dereplication, enzyme kinetics, a 21-day intervention and correlation modelling and every stage pointed the same way, which is the relationship the title asserts. The leaf is a legitimate drug matrix: eight classes were screened, polar extracts were rich in phenolics (88.6 mg GAE/g) and flavonoid C-glycosides, and HPTLC confirmed shared

marker bandsacross solvent systems. Potency is measured and mechanism resolved rather than assumed: TBEinhibited rat intestinal α-glucosidase at IC_{50} 78.4 µg/mL and the ethylacetate fraction at 24.6 µg/mL on a mixed-type profile (K_i 34.2 and 9.6 µg/mL), with selectivity over α-amylase (2.11 and 2.43) exceeding acarbose. In vivo, a dose-monotoneglucose fall (17.6 → 8.4 mmol/L; $F_{530} = 41.36$; $p < 0.001$; partial$\eta^2 = 0.874$) accompanied improved tolerance, restored hepatic enzyme balance and no hepatorenal signal, and the model attributed inhibition to flavonoids ($r = -0.96$; $R^2 = 0.924$), nominating vicenin-2 and saponarin as quality indicators. Limitations: one enzyme source, a 21-day window, no isolated compound tested in vivo, and an extract-level rather than target-level mechanism. Future work should dose the two C-glycosides alongside the clerodanefraction, add isoform-selective and high-fat-diet confirmation, run 90-day subchronic toxicityunder OECD 452, and open a randomised pilot in impaired glucose tolerance with HbA_{1c} asprimary endpoint and this fingerprint as batch-release specification.

Conflict-of-interest and data-availability statement. The authors declare no competing interest. De-identified raw arrays, R and Python analysis scripts, and the four-parameter logistic and ANOVA models reproducing Tables 2–6 are available as supplementary material.

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