Indian Journal of Traditional Knowledge
Vol 25(2), February 2026, pp 125-137
DOI: 10.56042/ijtk.v25i2.17247

HPTLC characterization of *Tinospora cordifolia* (Guduchi/Giloy) mother tincture and a randomized clinical comparison with individualized homeopathic medicines in adults with type 2 diabetes mellitus

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Received 18 February 2025; revised 29 January 2026; accepted 09 February 2026

Diabetes mellitus (DM) is a chronic metabolic disorder and remains a major contributor to the global burden of disease. The present study focused on the standardization of *Tinospora cordifolia* (TC) mother tincture (Ø), a medicinal plant widely used in Ayurveda for its antidiabetic potential and known to contain alkaloids such as berberine and palmatine. Standardization was carried out using high-performance thin layer chromatography (HPTLC), along with other established quality-control procedures, and the clinical effects of TCØ were compared with those of individualized homeopathic medicines (IHMs) in adults with type II DM. Pharmacognostic (microscopic), physicochemical (including ash and extractive values), and pharmacological evaluations were performed, supported by thin layer chromatography (TLC), UV spectrophotometry, and HPTLC, to assess the purity, consistency, and overall quality of the preparation. The clinical component was an open-label, randomized (1:1), two-arm pragmatic trial conducted over 3 months in 60 participants with type II DM. Patients received either TCØ (n = 30) or IHMs (n = 30), in addition to standardized dietary advice and lifestyle modification. Primary outcome measures included fasting blood sugar (FBS), post-prandial blood sugar (PPBS), and HbA1c%, while secondary outcomes comprised lipid profile, urea, and creatinine. All outcomes were assessed at baseline and at the end of 3 months, and between-group differences were analysed using unpaired t-tests with Bonferroni-adjusted significance ($p<0.0167$). TCØ demonstrated superior efficacy: HbA1c% (mean difference -1.3±0.5, 95% CI -2.3 to -0.4, $p=0.006$), FBS (-38.4±13.7, 95% CI -66.0 to -10.9, $p=0.007$), PPBS (-70.8±22.9, 95% CI -116.7 to -24.9, $p=0.003$), favouring TC over IHMs. Secondary outcomes showed trends that did not reach statistical significance ($p>0.0167$), and non-inferiority could not be demonstrated ($p>0.773$). Among the individualized homeopathic medicines prescribed, *Carcinosinum*, *Causticum*, and *Crataegus oxyacantha* were most frequently used. No adverse events were reported in either study arm. Overall, these findings are consistent with earlier trials of *Tinospora cordifolia* that have reported improvements in glycemic parameters when used as an add-on intervention, and they support the need for larger, well-designed blinded studies.

Keywords: High-performance thin-layer chromatography (HPTLC), Homeopathy, *Tinospora cordifolia* mother tincture, Type 2 diabetes mellitus

IPC Code: Int Cl.[26]: A61K 36/00

Tinospora cordifolia (TC), belonging to the Menispermaceae family, is a heart-leaved moonseed plant and is popular by many names in India, including Guduchi, Giloy, and Amrita. Its therapeutic efficacy has earned it widespread recognition in Ayurveda and traditional systems of medicine[1]. According to the Indian Ayurvedic medical system, TC is a notable herb with a wide range of pharmacological properties, including antispasmodic, antiallergic, immunostimulatory, antidiabetic, anticancer, antineoplastic, & anti-oxidative[2,3]. The components of TC, namely berberine, palmatosides, and palmetine, exhibit potent anti-diabetic properties. The anti-diabetic action of TC is mediated through an

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insulin-dependent mechanism via activating phosphatidyl inositol-OH kinase and insulin receptor tyrosine kinase, as evidenced by the suppression of glucose consumption efficiency. These inhibitors did not, however, totally stop the efficiency of glucose consumption. TC mostly acts via the insulin pathway, with a small amount also coming from other pathways such as peroxisome proliferator-activated receptors, c-Jun N-terminal kinase, activated protein kinase, and Ras-Raf-MEK-ERK[4]. According to a report published by the WHO in November 2017, it is estimated that one in ten medicinal products available in low- and middle-income nations are either falsified or of substandard quality. This situation poses a significant risk to global health and has detrimental implications for national economies. The hazards associated with substandard pharmaceuticals can precipitate various adverse health outcomes[5]. Drug standardization involves an exhaustive assessment of homeopathic medications concerning their pharmacognostic, physicochemical, and pharmacological attributes to examine the diverse qualitative and quantitative characteristics of these substances. The physicochemical evaluation of both the raw material and the prepared mother tincture included assessment of moisture content, ash values, extractive values, the presence of active constituents in the raw drug, and organoleptic characteristics. In addition, specific phytochemical tests, thin layer chromatography (TLC), and ultraviolet (UV) spectrophotometry were carried out to evaluate the quality of the mother tincture. Together, these parameters provide a reference framework that can be used for comparison with future commercial samples[6]. High-performance thin layer chromatography (HPTLC) is a well-established analytical technique known for its practicality, reliability, and adaptability in herbal drug evaluation. Beyond aiding identification, it offers a reproducible means of verifying authenticity and detecting possible adulteration. HPTLC is also useful for monitoring different stages of production, including cultivation, harvesting, extraction, and stability assessment[7]. According to the monograph issued by the Central Council for Research in Homoeopathy (CCRH), HPTLC profiling of this drug had not been reported previously[8].

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycaemia. It results from impaired insulin secretion, reduced peripheral insulin sensitivity, or a combination of both. Sustained hyperglycaemia, together with associated metabolic disturbances, can damage multiple organ systems over time and lead to serious, potentially life-threatening complications. These include microvascular complications such as retinopathy, nephropathy, and neuropathy, as well as macrovascular disease, which is associated with an approximately two- to four-fold increase in cardiovascular risk[9]. The WHO reports that non communicable diseases (NCDs) caused 74% of fatalities worldwide in 2019. Of these, diabetes caused 1.6 million deaths, making it the ninth largest cause of death worldwide[10]. Once considered exclusive to affluent Western nations, type 2 diabetes-which comprises 90% of all diabetes cases-has spread globally and now ranks as a top cause of disability and death, particularly among younger populations. In numerous emerging economies, including China and India, diabetes has escalated into a full-blown pandemic[11]. One systematic review and meta-analysis of complementary and alternative medicine (CAM) showed that herbal medicine, acupuncture, homeopathy, and spiritual healing were the common CAMs used[12]. Inhomeopathy, evidence of diabetes remains seriously compromised despite ever-growing research. Therefore, this trial was undertaken to standardize the mother tincture (MT, Ø) of TC by evaluating the HPTLC values. At the same time, the study intends to find out the effectiveness of TCØ in comparison with IHMs in the treatment of type II DM.

Materials and Methods

Preparation of TCMT

Fresh stem of the drug TC was collected at the factory premises of the Hahnemann Publishing Company, Titagarh, West Bengal. The raw drug was air-dried and verified macroscopically and microscopically under the guidance of a botanist. The macroscopic identification of the plant was performed in consultation with the monograph of TC in Homoeopathic Pharmacopoeia of India (HPI), volumes II and X. For microscopic identification, the stem of the plant was taken and introduced into section cutting, and it was mounted in water. Standardization of the drug substance was done by determination of moisture content, ash,

water-soluble ash, and acid-insoluble ash. MT was prepared from the drug substances by maceration by mixing TC coarse powder 100 mg, purified water 557 mL, and strong alcohol 480 mL, to make 1000 mL of the MT with drug strength of 1/10. The basic parameters for testing the standards of an MT were weight/ml, determination of total solids, pH estimation, alcohol content, UV-vis spectroscopy, TLC, HPTLC evaluation, and phytochemical screening.

Study design

The basic experiment part comprised of pharmacognostic, physicochemical, and pharmacological evaluation. The clinical component was an open-label, randomized (1:1), two parallel arm pragmatic trial.

Study setting

This study was conducted in the institution's outpatient and inpatient departments.

Participants

The inclusion criteria were Participants had a confirmed diagnosis of type 2 diabetes mellitus, were aged 18-65 years, included all sexes (including transgender individuals), and provided voluntary written informed consent. Patients were excluded if they had type 1 diabetes mellitus; were receiving standard antidiabetic medications; had diagnosed unstable psychiatric illness, other uncontrolled or life-threatening conditions impairing quality of life or affecting vital organs, organ failure; a history of substance abuse or dependence; self-reported immunocompromise; homeopathic treatment for any chronic condition within the past 6 months; were pregnant, postpartum, or lactating; or were participating concurrently in another clinical trial.

Intervention

TCMT group

It was administered orally in personalized dosage (10-20 drops, once to three times daily in half a cup of normal water) on an empty stomach along with dietary recommendations and lifestyle modifications. Duration of treatment: 3 months.

IHMs group

The intervention involved administering individualized homeopathic remedies in centesimal (C) or fifty-millesimal (LM) potencies, selected based on case-specific indications. For centesimal potencies, each dose consisted of one drop of the remedy (preserved in 90% v/v ethanol) diluted in 5 mL distilled water. Dosage and repetition were tailored to individual patient needs. For fifty-millesimal potencies, one medicated globule (no. 10 size, poppy seed equivalent) was dissolved in 90 mL distilled water with 2 drops of 90% v/v ethanol. The vial was marked for 16 doses; each 5 mL dose was prepared by administering 10 uniform succussions to the vial, then diluting 5 mL of the succussed solution in 45 mL water in a clean cup, stirring thoroughly, administering 5 mL orally, and discarding the remainder. Dietary advice and lifestyle modifications were provided alongside the remedies. Treatment duration was 3 months.

Outcomes

Primary: Blood HbA1c%, FBS, and PPBS.

Secondary: Blood lipid profile, urea, and creatinine.

Timeline: All the laboratory values of the patient were measured at baseline and after 3 months of intervention.

Sample size

Absence of reference data for TC therapy precluded formal power calculations for non-inferiority testing. Consequently, a predetermined non-inferiority margin (Δ) of 1.0% HbA1c was assumed based on clinical judgment and minimal clinically important differences reported in analogous homeopathy trials. With 30 participants allocated to each arm (total $n = 120$), the study was estimated to have 80% power to assess non-inferiority at a one-sided α level of 0.025, assuming a standard deviation of 1.5% and allowing for 20% attrition. The primary non-inferiority analysis was conducted using the intention-to-treat (ITT) population, and additional sensitivity analyses were performed under per-protocol (PP) conditions.

Randomization

Randomization was carried out using a permuted block design to achieve balanced allocation between the two study arms while reducing the risk of predictability. The random sequence was generated by an independent third party who was not involved in the conduct of the trial, using the Stat Trek online random number generator[13]. Variable block sizes were used, with block lengths randomly drawn from the set {4, 6, 8, 10}, to further minimize the possibility of foreseeing group assignment. A 1:1 allocation ratio was maintained, resulting in equal

numbers of participants in each arm (n = 30) for the final analysis. The allocation sequence was stored in a secure, password-protected electronic file, and group assignments were applied in sequential order at the time of participant enrolment. Post-hoc assessment of baseline characteristics confirmed that key prognostic variables, including age, sex, body mass index, and baseline HbA1c, were comparably distributed between groups (all p>0.05).

Blinding

The study was designed as an open-label trial, and neither participants nor investigators were blinded to treatment allocation. Blinding was not considered feasible because individualized homeopathic prescribing depends on detailed case-taking, assessment of symptom totality, and tailored remedy selection, all of which are difficult to mask without affecting the integrity of the intervention. Participants were informed of their group assignment at enrolment, and investigators remained unblinded to allow appropriate clinical decision-making, dose adjustments, and ongoing safety monitoring.

Allocation concealment

Allocation concealment was carefully implemented to minimize the risk of selection bias during participant recruitment and enrolment. Trial recruiters, who were independent of both the randomization authority and the treating physicians, were not aware of the randomization sequence or block structure. Group assignments were accessed only through sequentially numbered, sealed opaque envelopes prepared by an independent third party. Each envelope was tamper-evident, numbered in order, and opened only after eligibility had been confirmed and written informed consent obtained. This envelope-based procedure prevented recruiters from anticipating forthcoming allocations or influencing the order of enrolment. Compliance with the allocation process was verified after trial completion by cross-checking enrolment records against the master randomization list, which showed complete concordance and no protocol deviations. The effectiveness of allocation concealment was further supported by comparable baseline characteristics between groups and the absence of temporal imbalances.

Statistical methods

All randomized participants were included in the analysis in accordance with the intention-to-treat (ITT) principle, with missing data imputed via multiple linear regression predictions incorporating baseline predictors. Normality was assessed using Shapiro-Wilk tests, Q-Q plots, and histograms; no significant deviations were observed (all p>0.05), justifying parametric analyses. Baseline socio-demographic characteristics were summarized using means ± standard deviations (SD) for continuous variables and frequencies (percentages) for categorical variables. Intra-group changes from baseline were reported as mean differences with 95% confidence intervals (CIs). Between-group differences were expressed as mean differences, 95% CIs, and one-sided p values. To control family-wise type I error across the three primary outcomes (HbA1c, fasting glucose, postprandial glucose), Bonferroni correction was applied, yielding an adjusted α=0.0167 (0.05/3) per comparison. Non-inferiority of TC therapy versus IHMs was tested at α=0.05 (one-sided) using a fixed-margin approach with Δ=-1.0% HbA1c. Non-inferiority was declared if the lower bound of the 90% CI for the mean difference (TC - IHM) excluded the non-inferiority margin (*i.e.,* LCL > -1.0%). Equivalence was corroborated via one-sided *t*-tests with pooled variance estimates:

$$SE = \text{sqrt}\left(\left((n_1 - 1)s_1^2 + (n_2 - 1)s_2^2\right) / (n_1 + n_2 - 2)\right)$$

Where, n_1, n_2 represent group sizes, and s_1^2, and s_2^2 denote variance. No interim analyses or subgroup evaluations were pre-specified. All analyses were conducted using SPSS v23.0 and StatGraphics Centurion v18.

Adverse events reporting

Participants were instructed by investigators to maintain daily logs documenting all treatment-emergent adverse events (TEAEs), serious adverse events (SAEs), unexpected adverse events (UAEs), adverse drug reactions (ADRs), and suspected adverse reactions (SARs) throughout the trial, with events graded by severity (mild/ moderate/ severe) and causality (definite/ probable/ possible/ unlikely) using WHO-UMC criteria[14]. Un-coded IHMs designated as "rescue remedies" were permitted solely by trial homeopaths for acute intercurrent illnesses, limited to low-potency, short-acting remedies selected based on acute symptom totality and unrelated to primary glycemic outcomes, thereby minimizing potential confounding of trial-specific IHM effects[15,16].

Trial reporting

The study followed the RedHot guidelines and the CONSORT extension for non-inferiority trials (Supplementary Table S1 & S2)[17,18].

Results

Identification of the drug substance

***Macroscopic description of the stem* (*part used*)**

The stem is long, cylindrical, soft-wooded, greenish to greenish yellow, studded with warty tubercles due to the presence of lenticels, while the skin surface is longitudinally fissured. The wood appears white, soft, and porous; freshly cut surfaces rapidly develop a yellow tint upon air exposure. Long, thread-like aerial roots emerge from the branches. Dried stems measure 1.5-2.5 cm in diameter, exhibit no characteristic odour, and possess a distinctly bitter taste. The fracture is short and smooth, revealing a radial structure with prominent medullary rays traversing the porous tissue. Organoleptic tests were performed: taste bitter, colour light grey, odorless, and coarse on touch (Table 1).

Microscopic description of the stem

The transverse section of the stem reveals an outermost cork layer differentiating into an outer zone of thick-walled, brownish, compressed cells and an inner zone of 3-4 rows of thin-walled, colorless, tangentially elongated cells; lenticels interrupt the cork at intervals. This is followed by a secondary cortex of 5+ rows, with outer cells smaller than inner ones. The cortex features an outer zone of 3-5 rows of irregularly arranged, tangentially elongated sclereids, succeeded inwardly by polygonal cortical cells richly filled with simple ovoid, irregular ovoid-elliptical, or occasionally compound (2-4 components) starch grains, alongside scattered secretory cells. The vascular region comprises 10-12+ wedge-shaped xylem strands externally delimited by semicircular phloem patches, alternating with broad medullary rays. Phloem contains sieve tubes, companion cells, and polygonal to tangentially elongated parenchyma, some bearing calcium oxalate crystals. Cambium consists of 1-2 layers of tangentially elongated cells per bundle. Xylem includes lignified vessels (cylindrical, thick-walled, bordered-pitted), tracheids, parenchyma, and fibers; larger vessels often contain tyloses and transverse septa. Medullary rays, 15-20+ cells wide, exhibit rounded to ovoid cells with faint concentric striations and central hilum, traversing the vascular tissue. The pith is narrow, composed of large thin-walled cells predominantly filled with starch grains.

Standardization of the MT

Several analytic tests were run; appearance, color, odor, sediments, weight/ml, total solids, alcohol content, pH value, λmax (Fig. 1), and TLC values at 254 and 366 nm (Fig. 2-4). Subsequently, several phytochemical tests were performed: alkaloids, carbohydrates, tannins, steroids, flavonoids, saponin glycosides, amino acids, cardiac glycosides, anthraquinone glycosides, proteins, and iodine.

HPTLC

Details of HPTLC fingerprints of the sample were obtained under UV light, and the bands were reported (Table 2, Fig. 2-4).

Participant flow

Of 145 patients screened against pre-specified inclusion and exclusion criteria, 85 were excluded for various reasons (detailed in CONSORT flow diagram), while 60 met eligibility requirements and were enrolled. Baseline socio-demographic characteristics and primary/secondary outcome measures were recorded, following which participants were randomized 1:1 to receive either TCØ or IHMs. During the 3-month follow-up, 8 participants from the

Table 1 — Standardization of mother tincture of TC

Analytic tests	CCRH references	Sample values
1. Appearance	Clear, non-viscous	Clear, non-viscous
2. Color	Brown	Brown
3. Odor	Alcoholic, with a characteristic smell	Alcoholic, with a characteristic smell
4. Sediments	Absent	Absent
5. Weight/mL	0.923 at room temp.	0.934 at room temp.
6. Total solids (% w/w)	1.21	1.01
7. Alcohol content (% w/w)	46-49	48
8. pH value at room temperature	5.8-6.0	6.14
9. λmax (nm)	202, 277, and 321	270
10. TLC values at 254 nm	4 spots appear at Rf 0.3, 0.4, 0.5, 0.6	7 spots appear at Rf 0.051, 0.248, 0.291, 0.503, 0.569, 0.627, 0.686
11. TLC values at 366 nm	4 spots appear at Rf 0.3, 0.4, 0.5, 0.6	4 spots appear at Rf 0.051, 0.248, 0.291, 0.503, 0.569, 0.627, 0.686

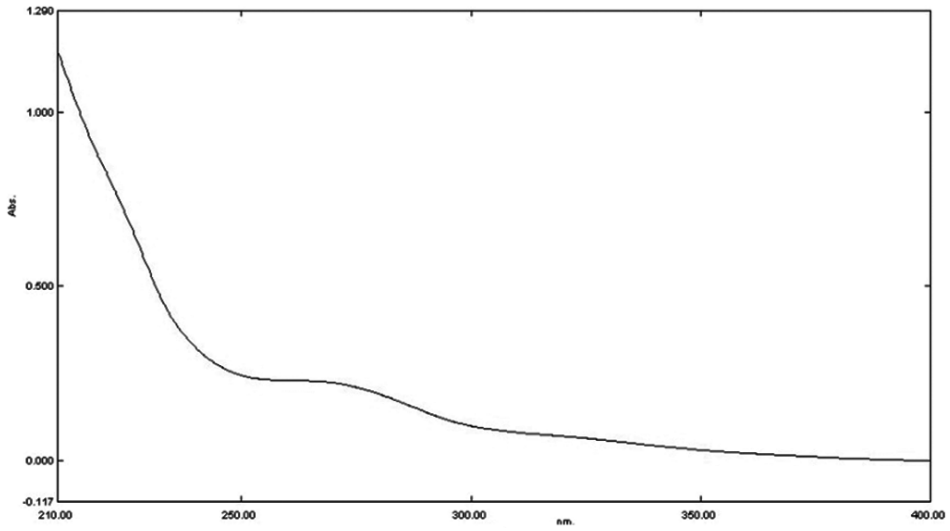

Fig. 1 — λ_{max} graph of the prepared sample

Table 2 — HPTLC bands

ID	Rf	Area	Volume
1_1	0.909	11256	781.43
1_2	0.855	12060	636.53
1_3	0.779	14673	446.64
1_4	0.632	16281	838.04
1_5	0.517	19095	425.32
1_6	0.435	17085	497.68
1_7	0.121	11859	767.9
1_8	0.072	7839	2231.3
1_9	0.029	6834	2105.2
2_1	0.911	13134	638.75
2_2	0.851	13134	309.2
2_3	0.771	14129	936.82
2_4	0.615	15124	870.86
2_5	0.487	13134	595.58
2_6	0.094	11542	497.42
2_7	0.027	6169	1306
3_1	0.91	19055	1535
3_2	0.833	8880	803.95
3_3	0.78	7400	908.08
3_4	0.749	10730	1994
3_5	0.601	11285	3293.3
3_6	0.504	8325	1051.8
3_7	0.466	14060	1784.6
3_8	0.135	9805	529.48
3_9	0.08	8695	1659.2
3_10	0.029	6290	1781.9

IHMs group and 2 participants from the TCØ group discontinued the study. All 60 participants were included in the final analysis (Fig. 5).

Recruitment

The study was carried out for 16 months (November 2018 to February 2020), where an individual subject was enrolled for 3 months.

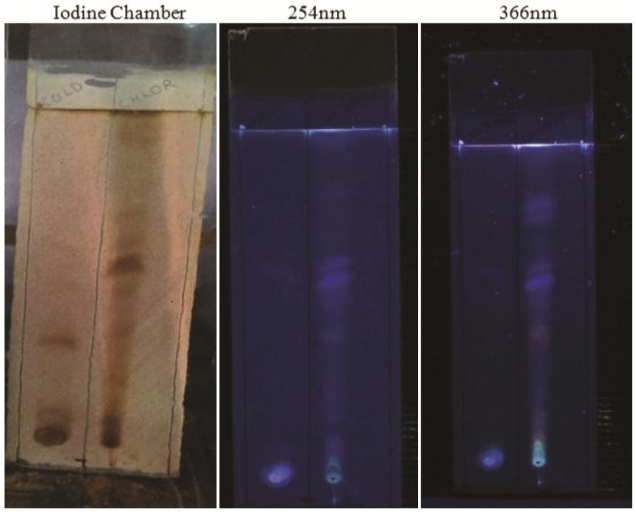

Fig. 2 — TLC plates viewed in iodine chamber and UV light

Baseline confounders

The distribution of the sociodemographic characteristics has been presented using descriptive statistics (Table 3).

Number analyzed

The data from all the randomized participants (*n*=60) were entered into the final analysis.

Estimation of outcomes

Primary outcomes

Between-group differences in all the primary outcomes revealed statistically significant differences (all p<0.0167) favoring TC against IHMs; HbA1c%: mean difference -1.3±0.5, 95% CI -2.3 to -0.4, p=0.006, FBS: mean difference -38.4±13.7, 95%

CI -66.0 to -10.9, p=0.007, and PPBS: mean difference: -70.8±22.9, 95% of CI -116.7 to -24.9, p=0.003 (Table 4). Still, non-inferiority was not demonstrated; HbA1c%: mean difference -1.3, SE = 0.5, lower 95% confidence limit -2.1, *t* = -0.755, p= 0.773; FBS: mean difference -38.4, SE = 13.7, lower 95% confidence limit -61.4, *t* = -2.723, p= 0.996; PPBS: mean difference -70.8, SE = 22.9, lower 95% confidence limit -109.2, *t* = -3.0, p= 0.998 (Fig. 6).

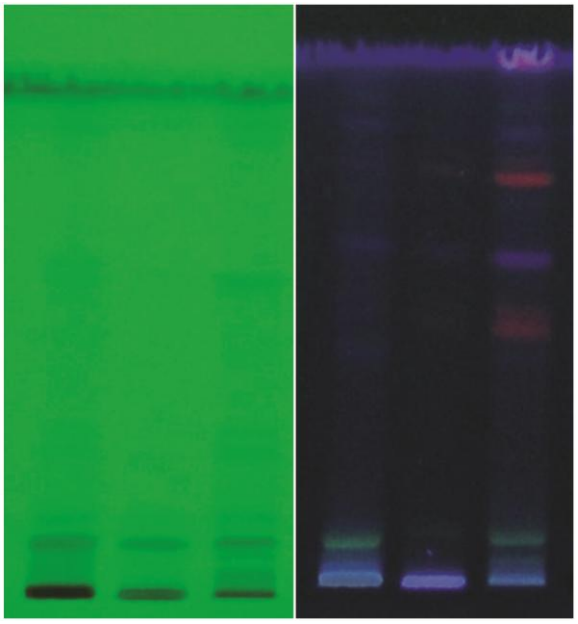

Fig. 3 — HPTLC plates viewed under UV light

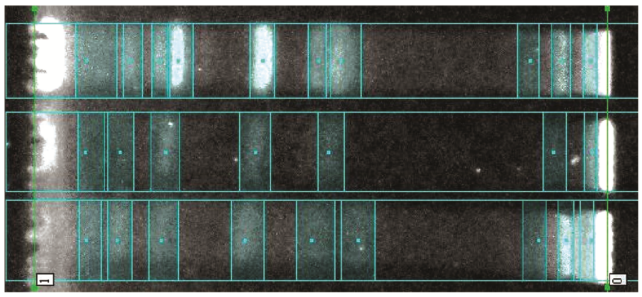

Fig. 4 — HPTLC plates with bands

Secondary outcomes

All the secondary outcomes showed non-significant trends (all p>0.0167); total cholesterol (p=0.557), triglyceride (p=0.480), HDLc (p=0.5), LDLc (p=0.710), VLDLc (p=0.495), urea (p=0.057), and creatinine (p=0.736). Non-inferiority was not demonstrated in any of the outcomes: total cholesterol (p=0.684), triglyceride (p=0.737), HDLc (p=0.126), LDLc (p=0.305), VLDLc (p=0.649), except urea (p=0.004), and creatinine (p<0.0167) (Table 4, Fig. 6).

Medicines used

Twenty-four different medicines were prescribed at baseline in the IHMs group: *Carcinosinum*, *Causticum*, *Crataegus oxycantha*, *Mezereum*, *Natrum muriaticum*, and *Staphysagria macrosperma* were mostly prescribed (each *n*=2).

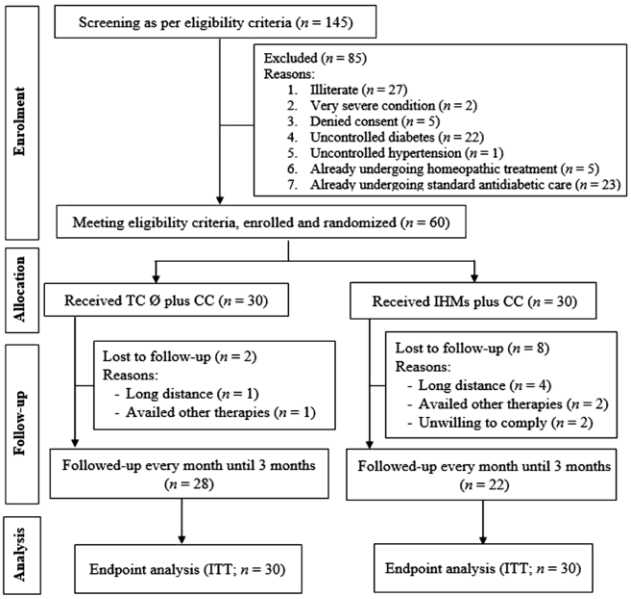

Fig. 5 — CONSORT study flow diagram

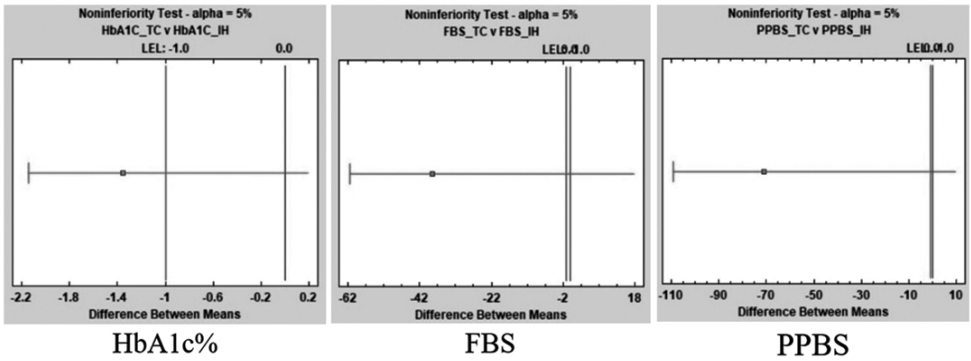

Fig. 6 — Non-inferiority statistics of HbA1c%, FBS, and PPBS

Table 3 — Distribution of the socio-demographic characteristics at baseline (n = 60)

Features	TCMT group (n = 30)	IHMs group (n = 30)	P
Age (yrs) [a]	46.0±11.5	47.3±9.0	0.645 (ns)
Body mass index [a]	23.9±3.4	23.8±3.3	0.910 (ns)
Duration of suffering (months) [a]	38.2±47.1	49.3±43.8	0.349 (ns)
Blood pressure (mm of Hg) [a]			
- Systolic	123.1±15.0	122.9±13.6	0.957 (ns)
- Diastolic	80.1±7.2	80.9±6.1	0.671 (ns)
Sex [b]			
- Male	11 (36.7)	12 (40)	1.000 (ns)
- Female	19 (63.3)	18 (60)	
Treatment taken [b]			
- Standard care	13 (43.3)	11 (36.7)	0.474 (ns)
- Homeopathy	1 (3.3)	3 (10)	
- Both	13 (43.3)	8 (26.7)	
- None	3 (10)	8 (26.7)	
Co-morbidities [b]			
- Rheumatic complaints	5 (16.7)	8 (26.7)	0.802 (ns)
- Hypertension	4 (13.3)	3 (10)	
- Liver complaints	2 (6.7)	2 (6.7)	
- Miscellaneous	7 (23.3)	10 (33.3)	
- None	12 (40)	7 (23.3)	
Residence [b]			
- Rural	17 (56.7)	12 (40)	0.573 (ns)
- Semi-urban	7 (23.3)	8 (26.7)	
- Urban	6 (20)	10 (33.3)	
Marital status [b]			
- Married	29 (96.7)	29 (96.7)	0.472 (ns)
- Single/others	1 (3.3)	1 (3.3)	
Educational status [b]			
- Primary	27 (90)	20 (66.7)	0.200 (ns)
- Secondary	2 (6.7)	4 (13.3)	
- Higher than secondary	1 (3.3)	6 (20)	
Employment status [b]			
- Business	7 (23.3)	6 (20)	0.078 (ns)
- Service and retired	2 (6.7)	10 (33.3)	
- Dependent and others	21 (70)	14 (46.7)	
Family income status [b]			
- Poor	8 (26.7)	13 (43.3)	0.422 (ns)
- Middle	16 (53.3)	10 (33.3)	
- Affluent	6 (20)	7 (23.3)	

TC: *Tinospora cordifolia* mother tincture; IHMs: Individualized homeopathic medicines; [a] Continuous variables are presented as mean ± standard deviation; [b] Categorical variables are reported as absolute numbers with corresponding percentages.; ns: not significant

Adverse events

No serious adverse event from either of the groups was reported throughout the study.

Discussion

The standardization of TC was conducted by the protocols established in HPI Vol. II & X, in addition to the monograph provided by the CCRH. The macroscopical and microscopical characteristics of the plant were observed to align with the descriptions delineated in the HPI documentation. The analysed sample conformed to the parameters outlined by both HPI and CCRH concerning the fundamental characteristics of colour, odour, appearance, sedimentation, weight per mL, total solids, and alcohol content. The measured pH value was slightly higher than expected and minor variation was also observed in the λ_{max} peak, although both remained within acceptable limits. These differences may reflect seasonal or geographical factors affecting the raw material. Thin-layer chromatography (TLC) revealed seven distinct spots in the analyzed sample, compared with four spots reported in the HPI and CCRH monographs. An extensive HPTLC investigation was conducted, and the results obtained have not been previously recorded in the HPI, thereby enhancing the understanding of the quantitative analysis of this pharmacological agent.

Table 4 — Comparison of the primary and secondary outcome measures after 3 months (n = 60)

Outcome measures	Baseline: Mean ± SD	After 3 months: Mean ± SD	Intra-group changes: Mean ± SD (95% CI)
HbA1C%			
- TCMT group	8.1±1.5	7.4±1.4	0.7±1.0 (0.3, 1.1)
- IHMs group	8.4±1.8	8.7±2.2	-0.3±1.0 (-0.6, 0.1)
Mean difference ± SE	-0.3±0.4	-1.3±0.5	
95% CI	-1.3, 0.5	-2.3, -0.4	
P	0.388 (ns)	0.006**	
FBS			
- TCMT group	159.8±41.5	137.2±40.4	22.6±24.9 (13.3, 31.9)
- IHMs group	161.1±55.7	175.6±63.6	-14.5±52.1 (-33.9, 5.0)
Mean difference ± SE	-1.3±12.7	-38.4±13.7	
95% CI	-26.8, 24.0	-66.0, -10.9	
P	0.915 (ns)	0.007**	
PPBS			
- TCMT group	237.1±64.1	208.2±57.4	28.7±39.3 (14.2, 43.5)
- IHMs group	249.1±93.7	279.0±111.8	-29.9±72.8 (-57.1, -2.7)
Mean difference ± SE	-12.0±20.7	-70.8±22.9	
95% CI	-53.5, 29.4	-116.7, -24.9	
P	0.563 (ns)	0.003**	
Total cholesterol			
- TCMT group	182.5±35.2	179.1±31.5	3.4±11.0 (-0.7, 7.5)
- IHMs group	176.9±38.6	184.5±39.4	-7.6±16.0 (-13.6, -1.7)
Mean difference ± SE	5.6±9.5	-5.4±9.2	
95% CI	-13.5, 24.7	-23.8, 13.0	
P	0.557 (ns)	0.557 (ns)	
Triglyceride			
- TCMT group	147.1±44.1	150.6±42.7	-3.4±26.8 (-13.4, 6.6)
- IHMs group	147.4±54.8	160.6±64.3	-13.2±28.8 (-24.0, -2.5)
Mean difference ± SE	-0.2±12.8	-10.0±14.1	
95% CI	-25.9, 25.5	-38.2, 18.2	
P	0.987 (ns)	0.480 (ns)	
HDLc			
- TCMT group	46.6±7.3	48.2±7.9	-1.6±2.7 (-2.6, -0.6)
- IHMs group	44.3±7.9	46.8±8.2	-2.5±6.3 (-4.9, -0.1)
Mean difference ± SE	2.3±1.9	1.4±2.1	
95% CI	-1.6, 6.2	-2.7, 5.6	
P	0.250 (ns)	0.500 (ns)	
LDLc			
- TCMT group	109.1±28.5	106.1±24.6	2.9±10.6 (-1.0, 6.9)
- IHMs group	100.4±29.4	103.5±30.1	-3.1±12.7 (-7.8, 1.6)
Mean difference ± SE	8.7±7.5	2.6±7.1	
95% CI	-6.2, 23.7	-11.5, 16.9	
P	0.248 (ns)	0.710 (ns)	
VLDLc			
- TCMT group	28.2±6.3	30.3±5.5	-2.1±5.0 (-4.0, -0.2)
- IHMs group	27.5±11.6	32.6±17.3	-5.1±12.8 (-9.9, -0.3)
Mean difference ± SE	0.7±2.4	-2.3±3.3	
95% CI	-4.1, 5.5	-8.9, 4.4	
P	0.774 (ns)	0.495 (ns)	
Urea			
- TCMT group	25.9±5.5	28.7±4.0	-2.8±6.0 (-5.0, -0.5)
- IHMs group	24.3±6.3	26.3±5.3	-2.0±5.6 (-4.1, 0.1)
Mean difference ± SE	1.6±1.5	2.4±1.2	
95% CI	-1.5, 4.6	-0.1, 4.8	
P	0.303 (ns)	0.057 (ns)	
Creatinine			
- TCMT group	0.9±0.2	0.9±0.1	0.01±0.01 (-0.04, 0.06)
- IHMs group	0.8±0.1	0.9±0.1	-0.03±0.1 (-0.09, 0.02)
Mean difference ± SE	0.03±0.03	-0.01±0.03	
95% CI	-0.04, 0.1	-0.1, 0.04	
P	0.380 (ns)	0.736 (ns)	

TC: *Tinospora cordifolia* mother tincture; IHMs: Individualized homeopathic medicines; HbA1C: Glycosylated hemoglobin; FBS: fasting blood sugar; PPBS: post-prandial blood sugar; SD: standard deviation; CI: confidence interval; ns: not significant

This open-label, randomized, pragmatic trial was carried out on 60 participants with type II DM treated with either TCØ (*n*=30) or IHMs (*n*=30). FBS, PPBS, and HbA1c% were used as the primary outcome measures; blood lipid profile, plasma urea, and creatinine were the secondary outcome measures, and all were measured at baseline and after 3 months of intervention. Ten patients had dropped out (TC: 2, IHMs: 8). Intention-to-treat (ITT) sample (*n*=60) was analyzed. The between-group differences in HbA1c%, FBS, and PPBS all favored TC over IHMs. The secondary outcomes showed non-significant trends in all the outcomes; however, non-inferiority was not demonstrated in any of the primary outcomes. *Carcinosinum*, *Causticum*, *Crataegus oxycantha*, *Mezerium*, *Natrum muriaticum*, and *Staphysagria macrosperma* were the most frequently prescribed remedies.

In a previous study by Banerjee *et al*.[19], IHMs were found to be more effective than placebos in intervening with the development of diabetes from pre-diabetes. One online observational study by Varanasi *et al.*[20] found that the clinical experience of homeopathic practitioners might help diabetic patients benefit from homeopathic treatment in terms of lowering complications and enhancing the quality of life. Furthermore, it stated that in the management of diabetes mellitus, homeopathy might be a useful therapy to traditional treatment[20]. Another study by Ghosh *et al.*[21] found that the patients with prediabetes showed a promising but nonsignificant trend for combining MTs and IHMs over IHMs alone. Another study by Guha *et al.*[22] showed that IHMs significantly outperformed placebos in FBS and diabetes symptoms checklist-revised (DSC-R) scores, but not in the OGTT, in the treatment of prediabetes. Unlike prediabetes trials where IHMs outperformed placebo in FBS but not OGTT, this pragmatic design highlights TCØ's edge in established diabetes, consistent with its Glut-4 mediated insulin-mimetic effects in myotubes. However, the open-label format and short duration contrast blinded, longer herbal trials reporting dyslipidemia improvements, underscoring the need for head-to-head homeopathic comparisons[23,24].

This study demonstrated several methodological strengths that enhance its credibility within pragmatic homeopathy research. The prospective, randomized parallel-arm design with permuted block randomization and allocation concealment by blinded recruiters minimized selection bias, while ITT analysis, including all 60 enrolled participants, preserved trial integrity despite dropouts. The study achieved comprehensive standardization of TCØ using pharmacognostic and physicochemical evaluation, TLC, UV spectrophotometry, and HPTLC profiling, thereby providing a clear reference framework consistent with HPI and CCRH guidelines. In addition to confirming established parameters, the analysis generated new quantitative information, including the identification of seven TLC spots and detailed HPTLC band patterns. The primary clinical outcomes (HbA1c, FBS, and PPBS) demonstrated statistically significant between-group differences in favour of TCMT (all $p \leq 0.007$ after Bonferroni correction). These findings were accompanied by consistent, though non-significant, trends in secondary biochemical measures and the absence of any serious adverse events, supporting the overall safety of the intervention. Baseline characteristics were comparable across sociodemographic variables, comorbidities, and outcome measures, strengthening causal interpretation. The study was further supported by appropriate ethical approvals (IEC clearance and CTRI registration), adherence to the Declaration of Helsinki[25], and transparent reporting in accordance with RedHot and CONSORT non-inferiority guidelines.

Several methodological limitations should be considered when interpreting the findings of this study. First, the relatively small sample size (60 participants, 30 per arm) reduced statistical power, particularly for demonstrating non-inferiority and detecting modest effects in secondary outcomes. A formal a priori power calculation was not feasible because of the absence of previous clinical data on TCØ in this setting. In addition, the open-label design, with no blinding of participants or investigators, may have introduced performance and detection bias, especially given the individualized nature of homeopathic prescribing and reliance on patient-reported adherence to dietary and lifestyle advice. Attrition differed between groups, with more withdrawals in the IHMs arm (8 vs. 2 in the TCØ arm), which may reflect differences in tolerability or treatment expectations. Although intention-to-treat analysis with linear regression–based imputation was used to address missing data, some degree of attrition bias cannot be fully excluded. The relatively short follow-up period of three months allowed assessment of short-term changes in FBS, PPBS, and HbA1c but

did not permit evaluation of longer-term glycemic control, durability of effects, or diabetes-related complications such as retinopathy or nephropathy. Potential confounding from co-interventions also warrants consideration. In the IHMs arm, uncoded remedies were permitted as rescue medications for acute conditions, which could have influenced outcomes despite their short-acting intent. Moreover, both groups received dietary and lifestyle advice that was not blinded, standardized, or formally monitored. The reliance on biochemical surrogate outcomes, including HbA1c and lipid parameters, without accompanying patient-reported outcomes such as quality of life or symptom burden, further limits a comprehensive assessment of diabetes management. Finally, the single-center pragmatic design restricts generalizability, and minor variability observed in TCØ standardization parameters (such as pH and λ_{max}) suggests that seasonal or sourcing effects may be relevant. These factors highlight the need for larger, blinded, multicenter studies incorporating longer follow-up and advanced analytical profiling (e.g., GC-MS or LC-MS) to confirm and extend the present findings.

The findings suggest that TCØ was associated with greater short-term improvement in glycemic control than individualized homeopathic medicines in adults with type II diabetes. Mean HbA1c decreased by 0.7% in the TCØ group, compared with an increase of 0.3% in the IHMs group, corresponding to a between-group difference of −1.3% (95% CI −2.3 to −0.4). While these results should be interpreted cautiously, they indicate that a standardized TCØ preparation may offer a more consistent therapeutic option than highly individualized prescribing approaches. The observed effects are broadly in line with earlier experimental and clinical evidence suggesting insulin-mimetic actions of *Tinospora cordifolia*, potentially mediated through PI3K and PPAR-related pathways, and support its possible role as an adjunct in diabetes management without concurrent conventional drug therapy. Secondary outcomes, including lipid parameters and renal markers, showed non-significant but directionally favourable trends, suggesting potential broader metabolic effects that merit further investigation. These findings may be particularly relevant in resource-limited settings, where affordable and well-tolerated botanical interventions could contribute to reducing the overall burden of diabetes. From a clinical perspective, the results support consideration of TCØ alongside lifestyle modification in individuals with mild to moderate type II diabetes, especially in light of the higher dropout rate observed in the IHMs ar m (8 vs. 2 participants), which may reflect differences in tolerability or treatment acceptability. For homeopathy practice, they highlight standardization's value in pragmatic trials, challenging critiques of reproducibility while suggesting IHMs may suit prediabetes or adjunctive use per prior studies.

Future research should focus on larger, multicenter, double-blind superiority trials with adequate sample sizes (exceeding 200 participants per arm) and formally defined non-inferiority margins informed by pilot data on TCØ. Such studies would be better positioned to confirm the observed glycemic benefits and to evaluate longer-term clinical outcomes, including microvascular complications, over follow-up periods of 12-24 months. In parallel, more advanced analytical approaches, including GC–MS, LC-MS, and NMR, should be employed to quantify key bioactive alkaloids such as berberine and palmatine across production batches. This would help address the minor variability observed in parameters like pH and λ_{max}, which may be influenced by seasonal or sourcing-related factors. Incorporate patient-centered measures (e.g., EQ-5D, diabetes-specific QoL scales) alongside biomarkers, with blinded co-intervention protocols and adherence monitoring via diaries or biomarkers. Platform trials comparing TCØ to placebos, IHMs, or allopathic add-ons in diverse populations (e.g., varying BMI, comorbidities) could elucidate moderators, while mechanistic studies in myocytes validate Glut-4 upregulation. Pragmatic effectiveness trials in primary care would bridge efficacy to real-world utility.

Conclusion

HPTLC evaluation of TCØ revealed new information on its analytical parameters and recommended updates to HPI and CCRH monographs to improve the acceptability of homeopathic medicines worldwide. TCØ appeared superior to IHMs in achieving better glycemic control over 3 months. Future research, including Gas Chromatography-Mass Spectrometry (GC-MS), Liquid Chromatography coupled to Mass Spectrometry (LC-MS), and Nuclear Magnetic Resonance (NMR), may be undertaken to obtain a deeper knowledge of the constituents of the drugs.

Supplementary Data

Supplementary data associated with this article is available in the electronic form at https://nopr.niscpr.res.in/jinfo/ijtk/IJTK_25(2)(2026)125-137_SupplData.pdf

Acknowledgments

The authors express gratitude to the late Prof. (Dr.) Dilip Panakkada, MD (Hom), former Head, Department of Pharmacy, National Institute of Homoeopathy, and to the institutional academic and hospital leadership for granting permission to conduct this trial. We sincerely thank the postgraduate trainees, support staff, pharmacists, and participating patients for their invaluable contributions to the study.

Author Contributions

RG: Conceptualization, methodology, validation, investigation, resources, writing – review and editing, supervision, project administration, SS, AM: Software, validation, investigation, data curation, writing – original draft, review, and editing, MK, SS: Conceptualization, methodology, software, formal analysis, investigation, resources, data curation, writing – original draft, review and editing, visualization, project administration

Conflict of Interest

There are no conflicts of interest to declare.

Ethics Statement

The study protocol was approved by the Institutional Ethical Committee (IEC) [Ref. No. 5-23/NIH/PG/Ethical Comm. 2008/Vol 5/2953; dated September 27, 2018] and was registered prospectively in the Clinical Trials Registry – India CTRI/2018/03/012671with a secondary identifier UTN: U1111-1210-5392. The study abided by Helsinki's declaration for the ethical conduct of clinical trials involving human participants and stuck to the International Conference on Harmonization guidelines for good clinical practice.

Informed Consent

Before enrolment, each participant was given a patient information sheet detailing the objectives, methods, risks, and benefits of participating, as well as confidentiality issues, and written informed consent was obtained thereafter.

Data Availability

All the information gathered or analyzed throughout the investigation is included in this article. For any further queries, the first and corresponding author can be contacted.

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