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Development and Characterization of a Polyherbal Formulation with Copper Nanoparticles (Tamra Bhasma)

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ABSTRACT: For millennia, herbal medicines have remained a cornerstone of global healthcare, widely recognized for their safety, tolerability, and efficacy in managing chronic diseases. With the global burden of diabetes escalating from 200 million cases in 1990 to 589 million adults in 2024—and projected to reach 853 million by 2050 (WHO, 2024; IDF, 2025)—the limitations of conventional synthetic hypoglycemic agents have intensified interest in plant-derived multi-target therapies. Polyherbal formulations (PHFs) have emerged as a promising strategy to exploit synergistic bioactive interactions for improved therapeutic outcomes. In this study, a novel PHF from Stevia rebaudiana, Cinnamomum zeylanicum, Trigonella foenum-graecum, and Hibiscus rosa-sinensis was prepared and blended with incinerated copper nanoparticles (Tamra bhasma) through traditional bhasmikarana calcination. The formulation was rigorously evaluated for physicochemical properties and characterized using GC-MS, FTIR, HPTLC, and SEM. Total phenolic content (TPC) ranged from 9.5 to 59.5 mg QE/g extract (R^2 = 0.9964), confirming a strong linear dose–response relationship. GC-MS analysis identified 19 bioactive compounds including (E)-cinnamaldehyde (RT: 11.26 min), trans-cinnamic acid (RT: 13.43 min), pyrocatechol, and linoleic acid with established antidiabetic and antioxidant activity. FTIR confirmed the presence of hydroxyl (3349.26 cm^{-1}), carbonyl (1642.99 cm^{-1}), and Cu–O stretching (896.17–864.48 cm^{-1}) functional groups. HPTLC fingerprinting at 254 nm and 366 nm revealed well-resolved bands corresponding to quercetin, cinnamic acid, stevioside, and cinnamaldehyde. SEM analysis demonstrated distinctive spongy microcrystalline aggregates (8–10 µm) in Tamra bhasma versus compact plate-like particles (~1 µm) in standard CuO, confirming structural transformation during calcination. UV-visible spectroscopy showed characteristic absorption bands at 340 nm and 460 nm, consistent with cupric oxide nanoparticles. All classical Ayurvedic quality tests (Varitaratva, Rekhapurnata, Nishchandrata, and Dadhi Pariksha) were satisfied. The findings confirm the successful development of a phytochemical-rich, analytically standardized formulation with copper nanoparticle integration, suggesting strong potential as a novel complementary therapeutic approach for type 2 diabetes mellitus.

1. INTRODUCTION

Diabetes mellitus represents one of the most significant and rapidly escalating chronic non-communicable diseases worldwide, characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both (Riaz et al., 2020). According to the International Diabetes Federation (IDF, 2025), approximately 589 million adults were living with diabetes globally in 2024, with type 2 diabetes (T2DM) accounting for over 90% of all cases. Alarmingly, more than 240 million people

remain undiagnosed worldwide. In South Asian countries such as Pakistan alone, approximately 27 million adults are affected, underscoring the disproportionate burden borne by developing nations (Riaz et al., 2020). Projections suggest that if current trends continue unchecked, the global diabetic population will reach 853 million by 2050 (WHO, 2024; IDF, 2025).

The global incidence of T2DM has escalated dramatically, rising from an estimated 200 million cases in 1990 to 589 million adults in 2024, with a further projection of 853 million by 2050 if existing trends prevail (WHO, 2024; IDF, 2025). The economic burden is equally staggering, with diabetes-related healthcare expenditure estimated at USD 966 billion globally in 2021 (IDF, 2025). Long-term management of diabetes using conventional synthetic hypoglycemic agents—including meglitinides, biguanides, sulfonylureas, and thiazolidinediones—remains clinically challenging due to well-documented limitations such as weight gain, hypoglycemia, gastrointestinal disturbances, drug resistance, and hepatotoxicity (Riaz et al., 2020; Piyush More et al., 2015).

Consequently, there has been a substantial global surge of interest and research into natural products and plant-derived bioactive compounds as alternative or adjunctive antidiabetic therapies. Medicinal plants harbor a rich diversity of bioactive phytoconstituents—including polyphenols, flavonoids, alkaloids, terpenoids, and complex polysaccharides—that confer broad-spectrum biological activities such as antioxidant, anti-inflammatory, antimicrobial, and antidiabetic properties (Riaz et al., 2023; Ansari et al., 2025). Numerous phytoconstituents remain unexplored, opening new avenues to address unmet therapeutic needs in diabetes management.

Polyherbal formulations (PHFs) have gained considerable scientific attention owing to their capacity for synergistic interactions among bioactive compounds, which collectively enhance therapeutic efficacy beyond the sum of individual components. PHFs are particularly valued for their multi-target mechanism of action, enabling simultaneous management of hyperglycemia alongside secondary complications such as inflammation and oxidative stress (Ghosh et al., 2015). In parallel, trace metal ions including vanadium, zinc, manganese, and copper have been increasingly investigated for their intrinsic antidiabetic properties, representing a complementary pharmacological avenue (Piyush More et al., 2015).

A principal therapeutic target for managing postprandial hyperglycemia in T2DM is the inhibition of α-glucosidase and α-amylase enzymes, which govern dietary carbohydrate digestion. Inhibiting these enzymes retards intestinal glucose absorption, thereby attenuating postprandial blood glucose spikes (Alqahtani et al., 2019). Extensive studies have identified plant-derived compounds as potent natural enzyme inhibitors suitable for diabetes management. Concurrently, green synthesis of metal nanoparticles has emerged as a biocompatible and eco-sustainable route to produce therapeutically active nanoparticles (Chopra et al., 2022). Among these, copper nanoparticles (CuNPs) are distinguished by their relatively low cytotoxicity, high surface reactivity, and promising antidiabetic potential, positioning plant-mediated CuNP synthesis as a frontier area in integrative antidiabetic research (Ghosh et al., 2015).

This study aimed to prepare, physicochemically standardize, and analytically characterize a novel polyherbal formulation (PHP) combined with Tamra bhasma using advanced instrumental techniques (GC-MS, FTIR, HPTLC, and SEM), and to correlate the identified phytoconstituents with known antidiabetic mechanisms of action, thereby establishing a scientific rationale for its therapeutic application in T2DM.

2. MATERIALS AND METHODS

2.1 Plant Materials

Four medicinal plants i.e. Stevia rebaudiana (leaves), Cinnamomum zeylanicum (bark), Trigonella foenum- graecum (seeds) and Hibiscus rosa-sinensis (flowers) were selected for the study based on their reported anti diabetic potential in different studies. The plant materials were collected from the Near herbal gardens and authenticated by Dr. T. Ramanathan, Annamalai University Faculty of Marine sciences, Professor. The plant materials underwent a washing process, then shade-dried at ambient temperature (25–28°C), and finally milled into a coarse powder. Equal amounts of the powdered materials were meticulously combined in a 1:1:1:1 ratio to achieve a consistent polyherbal powder.

2.1.2 Extraction by Maceration (Pandeya et al., 2021)

The polyherbal blend was mixed with a 1:10 (w/v) ratio of hydroalcoholic solvent (ethanol:water, 70:30 v/v) in a conical flask and macerated for a period of seven days at a controlled temperature. The mixture was shaken periodically to ensure proper mixing. Then, it was followed with a filtration process using the muslin cloth. The leftover residue was macerated again using a new solvent to ensure the complete extraction. A rotary evaporator was used to concentrate the combined filtrates under

reduced pressure at 40 to 50°C until a semisolid substance was achieved. The resultant semisolid substance was then dried in a desiccator and kept at 4°C for additional analysis. The percentage yield was calculated as:

$$\%\text{Extractive Yield} = \{\text{Weight of Plant Powder Used/Weight of Dried Extract}\} \times 100$$

2.2 Phytochemical and Proximate Analysis

2.2.1 Organoleptic and Physicochemical Evaluation

According to Indian Pharmacopoeia (2014) and WHO Guidelines for Quality Control of Herbal Materials (2011), the organoleptic properties of plant powders and polyherbal formulation (PHF) were noted. The physicochemical parameters [moisture content, total ash, water-soluble ash and acid-insoluble ash] of extracts was determined by following general pharmacognostic procedures (Kokate, 1994; Khandelwal, 2008).

2.2.2 Qualitative Phytochemical Screening

The hydroalcoholic extract of the polyherbal formulation was subjected to evaluation for key classes of secondary metabolites using established qualitative methods (Harborne, 1998; Trease & Evans, 2002).

2.2.3 Quantitative Estimation of Total Phenolic content

The total phenolic content (TPC) was determined using the Folin–Ciocalteu colorimetric method (Singleton et al., 1999). Results were reported as milligrams of quercetin equivalent (QE) per gram of extract after absorbance was measured at 765 nm.

2.3. Analytical Methods

2.3.1 High-Performance Thin Layer Chromatography (HPTLC) Analysis

The CAMAG Linomat 5 applicator and silica gel 60 F_{254} plates (E. Merck, Darmstadt, Germany) were used for HPTLC analysis of the polyherbal extract. Samples and standards (quercetin, cinnamic acid, stevioside, and cinnamaldehyde; 1 mg/mL in methanol) were used. The plates were scanned with a CAMAG TLC Scanner 4 and analyzed the data using WinCATS software. The fingerprinting data was analyzed using the methods developed by Wagner & Bladt (1996) and subsequently validated by existing literature on HPTLC profiles of polyherbal formulations (Sharma et al., 2019; Kumar et al., 2021).

2.3.2 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

Spectra from the polyherbal extract were recorded using a Fourier Transform Infrared Spectrophotometer in the spectral region 4000–400 cm^{-1}, using the KBr pellet method (Stuart, 2004; Kumar et al., 2016).

2.3.3 Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

The GC-MS analysis was done for the polyherbal extract by following the Wadekar et al., (2005) protocol. Compound identification was performed using the NIST/EPA/NIH Mass Spectral Library and it was confirmed with standard procedures (Adams, 2007).

2.3.4 Scanning Electron Microscopy (SEM)

Morphological characterization of Tamra bhasma and standard CuO was carried out using JSM-IT500 SEM (models: JEOL, Japan). The resultant surface morphology, size, and distribution of particles, Tamra bhasma and CuO were compared (Li et al., 2017).

2.4 Development of Polyherbal Preparation

2.4.1 Preparation of Copper Nanoparticles (Tamra Bhasma)

High-grade copper wires (99.9%) were chopped and ground into a fine powder (100 g). The copper was heated to red-hot (about 800°C) and then quenched seven times in the juices of the four chosen plants (T. foenum-graecum, S. rebaudiana, H. rosa-sinensis, and C. zeylanicum) to purify and improve the softness. For additional detoxification, the Vishesha Shodhana (special purification) used fresh plant decoctions in cycles of soaking and heating. A homogenous mixture of pure copper and

polyherbal juices (four samples) was made using a mortar and pestle. The paste was dried at 40 to 50 degrees Celsius after being formed into tiny pellets (Tripathi et al., 2019; Jagtap, 2012)

2.4.2. Calcination Process

After being dried, the pellets were put in earthen crucibles and put through three Putas (calcination cycles) at progressively lower temperatures: Puta 1 was 750°C for 25 minutes, Puta 2 was 650°C for 30 minutes, and Puta 3 was 550°C for 35 minutes. The material was re-pulverized after cooling at the end of each cycle. Up to seven repetitions of this procedure were done before the final product displayed the traditional traits of Tamra bhasma, such as floating on water, losing its metallic sheen, and not being reducible as described by Jagtap (2012).

Classical Ayurvedic Quality Tests for Tamra Bhasma

The prepared Tamra Bhasma was evaluated against eight classical Ayurvedic quality parameters as prescribed in traditional Ayurvedic texts to confirm complete and authentic copper incineration (Jagtap, 2012; Tripathi et al., 2019; Wadekar et al., 2005).

Rekhapurnata Test: A pinch of Bhasma was rubbed between the finger and thumb and observed for its ability to fill the creases of the fingers completely, confirming appropriate ultra-fine particle size (Jagtap, 2012).

Uttama/Unama Test: The Bhasma was gently placed on the surface of still water. Flotation of the powder even when covered with grains of rice confirmed Uttama (superior grade) Bhasma with ultra-low bulk density (Tripathi et al., 2019).

Varitaratva Test: A small quantity of Bhasma powder was sprinkled over the surface of still water in a 100 mL beaker. Particles floating without sinking confirmed complete calcination-induced reduction in particle density (Wadekar et al., 2005).

Dadhi Pariksha (Curd Test): A pinch of Bhasma was dusted onto fresh curd placed in a small pot and observed after 24 hours. Absence of discolouration confirmed the absence of free metallic copper ions, validating safety for oral administration (Wadekar et al., 2005; Jagtap, 2012).

Nishchandrata Test: A small amount of Bhasma was rubbed between finger and thumb and examined under direct sunlight. Absence of lustrous or shining metallic residues confirmed complete elimination of unreacted copper metal (Tripathi et al., 2019).

2.4.3 Analytical Methods for Tamra Bhasma

2.4.3.1 UV-Visible Spectroscopy

UV-visible spectrophotometric analysis of Tamra Bhasma was performed to confirm CuO nanoparticle formation and assess optical characteristics (Kumar & Joshi, 2022; Ghosh et al., 2015).

Sample Preparation: The suspension was shaken thoroughly to ensure uniform distribution. An accurately measured 1 mL of the formulation was transferred into a 10 mL volumetric flask. Ethanol was added as an extraction solvent and the mixture was sonicated for 15 minutes to extract the herbal constituents. The solution was centrifuged at 4000 rpm for 10 minutes to remove undissolved Tamra Bhasma particles. The supernatant was filtered through Whatman filter paper to obtain a clear solution (Ghosh et al., 2015).

Spectral Scanning: The filtered solution was scanned across a wavelength range of 200–800 nm using a UV-visible spectrophotometer against methanol as blank. The wavelength of maximum absorbance (λmax) was recorded. Absorbance of the prepared sample was measured at λmax in triplicate and the concentration of herbal constituents was calculated using a calibration curve. Quartz cuvettes of 1 cm path length were used for all measurements (Kumar & Joshi, 2022).

2.4.3.2 Particle Size Analysis

Particle size distribution of the final PHP formulation was determined using dynamic light scattering (DLS) to assess nanoparticle size and degree of agglomeration. The sample was suitably diluted and analyzed for hydrodynamic diameter. The particle size distribution was interpreted to identify primary nanoparticle populations and any secondary aggregation peaks, as described by Tripathi et al. (2019) and Kumar & Joshi (2022).

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2.4.3.3 FTIR Spectroscopic Analysis of Tamra Bhasma

Fourier Transform Infrared (FTIR) spectroscopic analysis of Tamra Bhasma was carried out to identify functional groups arising from the bhasmikarana process and confirm the conversion of elemental copper to copper oxide (Stuart, 2004; Kumar et al., 2021).

Sample Preparation: Tamra Bhasma was dried thoroughly prior to analysis. A small quantity of the Bhasma was finely ground and mixed uniformly with dry potassium bromide (KBr) powder in a ratio of approximately 1:100 (sample:KBr). The mixture was compressed under hydraulic pressure to form a transparent KBr pellet suitable for infrared transmission analysis (Stuart, 2004).

Spectral Recording: The FTIR spectrum was recorded using a Fourier Transform Infrared Spectrophotometer in the spectral region of 4000–400 cm^{-1}. The instrument was purged with dry nitrogen gas to eliminate interference from atmospheric moisture and CO_2. The background spectrum was recorded with a blank KBr pellet prior to sample analysis. Each spectrum was recorded as an average of a minimum of 16 scans at a resolution of 4 cm^{-1} (Kumar et al., 2021; Stuart, 2004).

Interpretation: Absorption peaks were assigned to functional groups based on established infrared spectral correlation tables. Particular attention was given to the Cu–O stretching vibration region (900–1100 cm^{-1}) to confirm copper oxide bond formation, hydroxyl stretching (3400–3800 cm^{-1}) for surface metal hydroxides, carbonyl (1700 cm^{-1}), and aromatic C=C stretching (1628 cm^{-1}) for residual organic phytoconstituents from herbal processing (Stuart, 2004; Kumar et al., 2016; Tripathi et al., 2019).

3. RESULTS AND DISCUSSION

3.1 Organoleptic properties of extracts and polyherbal powder

Four samples from different medicinal plants, namely leaves from Stevia rebaudiana, bark from Cinnamomum zeylanicum, seeds from Trigonella foenum-graecum, and flowers from Hibiscus rosa-sinensis, were chosen for the study and their aqueous extract were collected. Further they were subjected for evaluation of its physical and chemical properties. The organoleptic evaluation of individual herbal powders revealed distinct sensory characteristics (Table 1) and the polyherbal formulation (PHP) appeared as a uniform brownish-yellow powder with a sweet to slightly pungent aroma, suggesting good homogeneity and compatibility among the combined herbal components.

Table 1: Organoleptic properties of extracts and polyherbal powder (PHP)

Plant Material	Color	Odor	Taste	Appearance
Stevia	Greenish	Mild sweet aroma	Intensely sweet	Fine, light powder
Cinnamon	Light brown	Strong aromatic	Spicy-sweet	Coarse brown powder
Fenugreek	Yellowish brown	Nutty	Bitter	Granular, slightly oily
Hibiscus	Reddish pink	Mild floral	Slightly acidic	Smooth, deep red powder
PHF	Brownish yellow	Aromatic (blend)	Sweet–slightly pungent	Uniform dry powder

Sensory evaluation of individual herbal powders and the polyherbal formulation (PHP).

3.2 Phytochemical constituents of extracts and PHP

The qualitative phytochemical screening indicated the presence of key secondary metabolites including flavonoids, phenolic compounds, glycosides, saponins, alkaloids, and tannins in all individual extracts and the PHP (table 2). Flavonoids and glycosides were found to be predominant, while alkaloids were absent in *Stevia* and *Hibiscus* extracts. The water-soluble extractive values ranged from 14.05–19.47%, with overall aqueous extract yields between 4.8–6.2%, and moisture content ranging from

6.5–7.3%. The ash values (total, water-soluble, and acid-insoluble) were within pharmacopoeial limits, indicating the purity and quality of the powdered drugs (table 3).

Table 2: Phytochemical constituents of extracts and PHP

Phytoconstituent	Stevia	Cinnamon	Fenugreek	Hibiscus	PHP
Flavonoids	+++	+++	++	+++	+++
Phenolic Compounds	++	+++	++	+++	+++
Glycosides	+++	++	+	++	+++
Saponins	+	+	++	+	++
Alkaloids	–	+	++	–	+
Tannins	+	++	+	+++	++

Qualitative phytochemical screening of aqueous extracts. (+ = present, – = absent; intensity: + to +++).

Table 3: Physical characteristics and % yield of extracts

Sample	Moisture content (% w/w)	Ash content (% w/w)		
		Total Ash	Water Soluble Ash	Acid-Insoluble Ash
Stevia rebaudiana	5.4±0.4	7.1±0.2	2.1±0.1	1.2±0.1
Cinnamomum zeylanicum	6.2±0.3	6.5±0.3	2.5±0.1	1.5±0.1
Trigonella foenum-graecum	5.0±0.2	7.3±0.4	2.3±0.1	2.0±0.1
Hibiscus rosa-sinensis	4.8±0.3	6.8±0.2	2.8±0.1	1.8±0.1
Polyherbal Preparation (PHP)	5.1±0.2	6.9±0.3	2.6±0.1	1.6±0.1

All values are expressed in Mean(n) ±SD, n=3

3.3 The total phenolic content (TPC)

The tested samples ranged from **9.5 to 59.5 mg QE/g extract**. A clear increasing trend was observed across samples, with Sample 1 showing the lowest TPC (9.5 mg QE/g) and Sample 5 the highest (59.5 mg QE/g). Similarly, the calculated concentration values (9.5–59.5 µg/mL) showed a strong linear relationship with absorbance, confirming the reliability of the calibration curve ($R^2 = 0.9964$).

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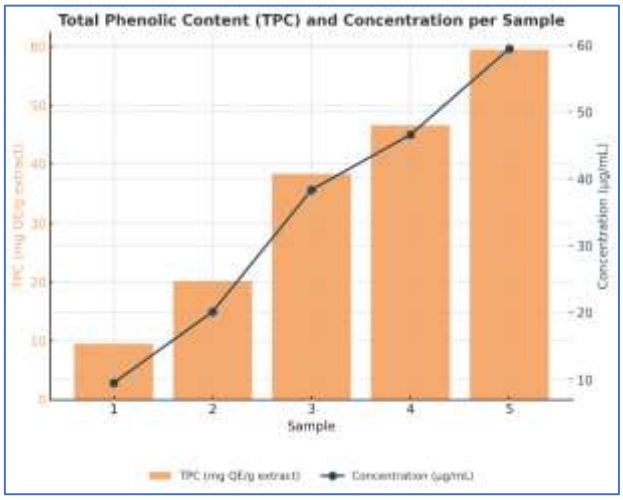

Fig: 1 Total Phenolic Content (TPC) and Concentration per Sample
(Orange colour bars = TPC (mg QE/g extract; Blue line = Concentration (µg/mL)

3.4 The HPTLC fingerprinting of the PHP

The displayed distinct, well-resolved bands under UV light at 254 nm and 366 nm, confirming the presence of multiple phytoconstituents (fig 2). The chromatographic profiles corresponded to flavonoid standards such as quercetin, cinnamic acid, stevioside, and cinnamaldehyde, confirming their presence in the extracts. The uniform distribution and sharp resolution of bands validated the purity, reproducibility, and chemical consistency of the formulation under UV light at 254 nm and 366 nm. Distinct, well-resolved bands were observed at both wavelengths, indicating the presence of multiple phytoconstituents. The darker quenching zones at 254 nm represent conjugated aromatic compounds, while the bright blue–green fluorescent bands at 366 nm confirm the presence of flavonoids and phenolic derivatives.

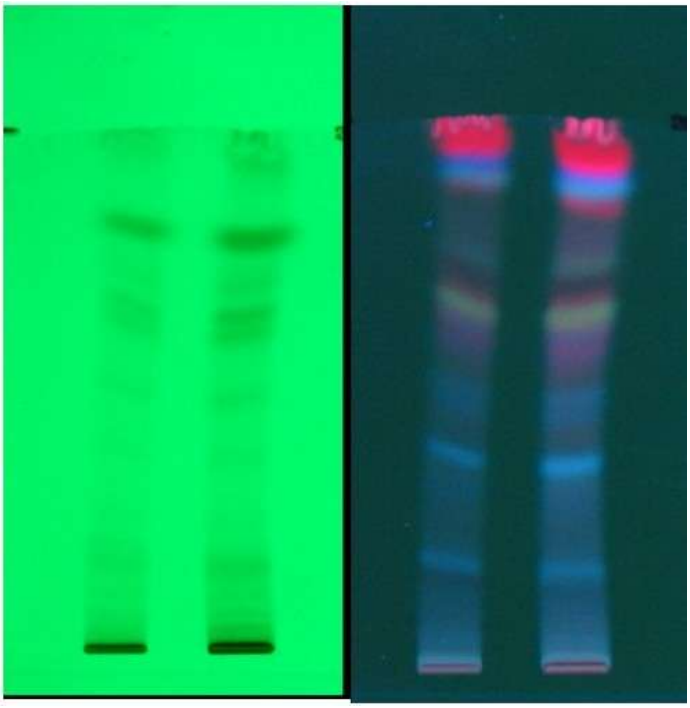

Fig 2: HPTLC fingerprinting profile of the polyherbal formulation (PHP)

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3.5 The Fourier Transform Infrared (FTIR) spectrum

The polyherbal extract was recorded in the range of 4000–400 cm^{-1} to determine the presence of functional groups associated with the phytoconstituents (Fig. 3). The broad peak at 3349.26 cm^{-1} corresponds to O–H stretching vibration, indicating the presence of alcohols or phenolic compounds, which are common in polyphenolic and flavonoid-rich plant extracts. The peaks at 2929.06 cm^{-1} and 2844.15 cm^{-1} represent C–H stretching vibrations of alkenes and aldehydes, respectively. The band at 2112.12 cm^{-1} may be attributed to aromatic C–H bending, signifying the presence of aromatic compounds. The distinct band at 1642.99 cm^{-1} corresponds to C=C stretching of alkenes (mono-substituted), while the strong peak at 1530.75 cm^{-1} is due to N–O stretching vibrations, suggesting the occurrence of nitro groups possibly from secondary metabolites. The bands at 1444.09 cm^{-1} and 1375.69 cm^{-1} are assigned to C–H bending vibrations of alkanes, indicating the presence of methylene groups. A medium-intensity peak at 1319.15 cm^{-1} is attributed to O–H bending of phenols, while the absorption bands at 1248.56 cm^{-1} and 1156.25 cm^{-1} correspond to C–O stretching of esters and aliphatic ethers, respectively. The low-frequency peaks at 896.17 cm^{-1} and 864.48 cm^{-1} confirm metal–oxygen (Cu–O) stretching vibrations typical of cupric oxide. These findings confirm both the presence of CuO and several additional organic functional group peaks, indicating surface complexation with phytochemical residues derived from the herbal ingredients used in the traditional incineration process.

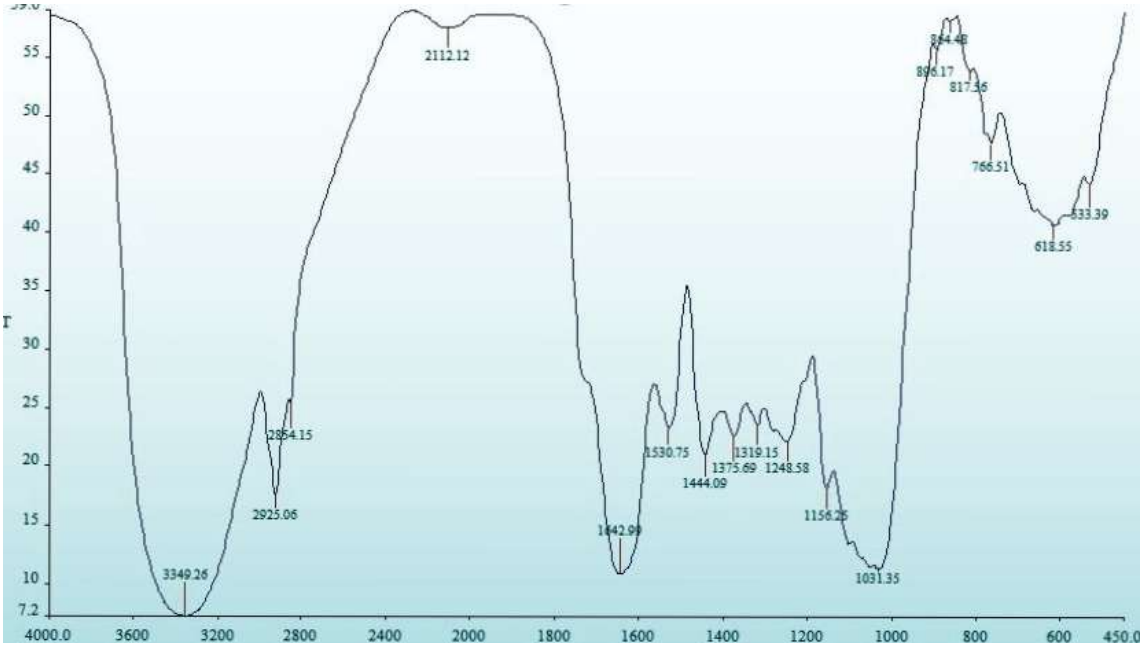

Fig 3: FTIR spectrum of the polyherbal formulation (PHP). Characteristic absorption peaks confirm the presence of hydroxyl, carbonyl, and aromatic functional groups corresponding to flavonoids, phenolic acids, and glycosides.

3.6 The GCMS spectrum of PHP sample revealed the presence of several volatile and semi-volatile organic compounds that are represented in fig 4. The compounds were identified by comparing their mass spectra with those from the **Wiley8** and **NIST14** libraries. Table 5 summarizes the major components identified along with their retention times, and molecular weight.

Table 5: Major compounds identified in the GC–MS profile of PHP

Peak No.	Retention Time (min)	Compound Name	Molecular Weight	Library
1	4.26	4-Hydroxy-4-methyl-2-pentanone (Diacetone alcohol)	116	Wiley8
2	4.46	Dimethyl sulfoxide (DMSO)	78	Wiley8

3	7.76	1-Cyclopropyl-1-pentanol	128	Wiley8
4	8.55	(2S,3S)-3-Propyloxiranemethanol	116	Nist14
5	9.38	2-(Benzylaminomethyl)-2-methylpropane-1,3-diol	209	Wiley8
6	9.56	N-(3-Chloropropyl)-N,N-dimethylamine	121	Wiley8
7	9.95	4-(1,1-Dimethylethyl)-2-[(1-methylethoxy)methylene]cyclohexanone	224	Wiley8
8	10.19	Pyrocatechol (1,2-benzenediol)	110	Wiley8
9	10.39	2,3-Dihydrobenzofuran	120	Nist14
10	11.26	(E)-Cinnamaldehyde	132	Wiley8
11	11.77	2-[2-(Benzyloxy)-1-(1-methoxy-1-methylethoxy)ethyl]oxirane	266	Nist14
12	11.85	4-Vinylguaiacol (2-methoxy-4-vinylphenol)	150	Nist14
13	12.46	2-(4'-Methylphenyl)-3-benzoyl-4-phenyl-1-oxa-4-azabutadiene	327	Wiley8
14	12.78	4'-Benzamido-4-methylbenzanilide	330	Nist14
15	12.87	3,5-Dimethyl-4-oxo-4H-pyrazole-1,2-dioxide	142	Wiley8
16	12.96	Dihydrocoumarin	148	Nist14
17	13.10	2-Methylpyrrolidine	85	Nist14
18	13.25	9-Benzyl-6-(methoxyethyl)-9H-purine	268	Wiley8
19	13.43	trans-Cinnamic acid	148	Wiley8

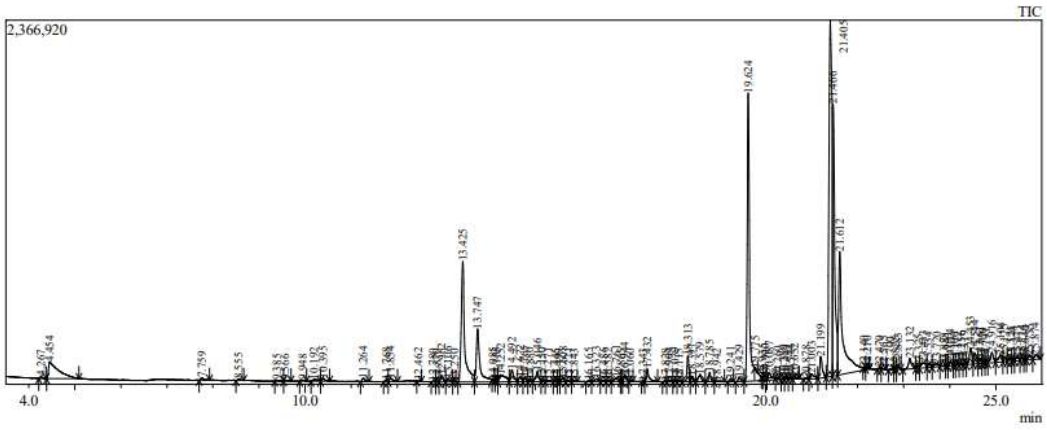

Fig 4: Gas Chromatography–Mass Spectrometry (GC–MS) Total Ion Chromatogram (TIC) of PHP **-** The chromatogram displays the total ion current profile of volatile and semi-volatile compounds eluted between 4.0 and 13.5 minutes. Major peaks correspond to diacetone alcohol (4.26 min), dimethyl sulfoxide (4.46 min), pyrocatechol (10.19 min), (E)-cinnamaldehyde (11.26 min), and trans-cinnamic acid (13.43 min), which were identified based on spectral matching with the Wiley8 and NIST14 libraries. The TIC represents the overall chemical fingerprint of the sample, indicating the presence of oxygenated hydrocarbons, aromatic compounds, and phenolic derivatives contributing to the sample's bioactive profile.

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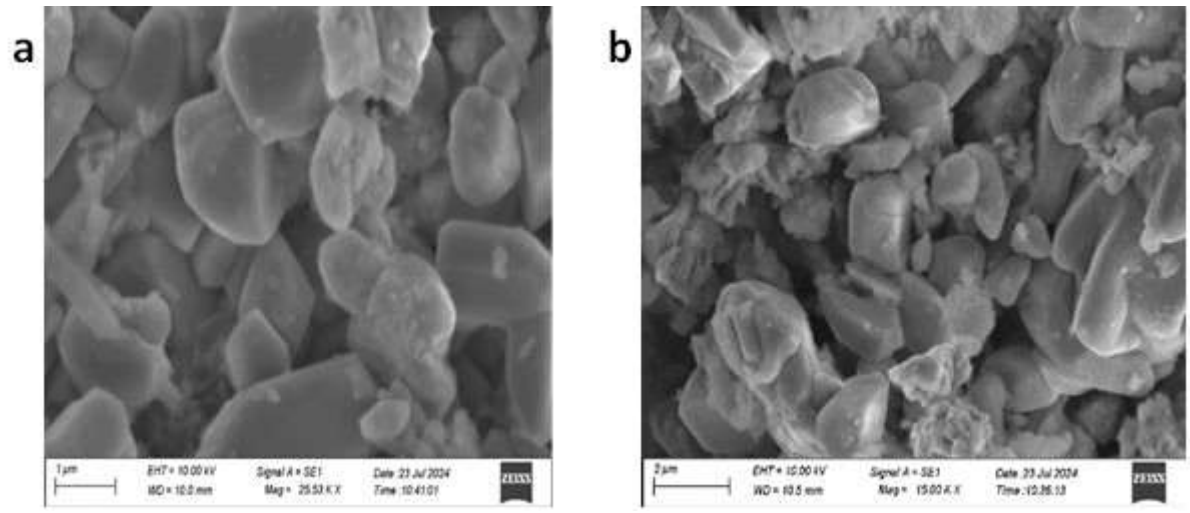

Fig 5: SEM micrographs of standard cupric oxide and Tamra Bhasma - SEM analysis revealed clear morphological differences between standard cupric oxide (a) and Tamra Bhasma (b). The standard sample showed well-defined plate-like particles (~1 μm), whereas Tamra Bhasma displayed spongy, compact microcrystalline aggregates with reduced grain boundaries and larger particle sizes (8–10 μm). The observed morphology reflects particle agglomeration and structural changes induced by repeated calcination during the traditional bhasmikarana process.

3.6 Classical Ayurvedic Quality Tests for Tamra Bhasma

The prepared Tamra Bhasma was subjected to all eight classical Ayurvedic quality tests prescribed in traditional Ayurvedic texts, and all tests were satisfactorily passed, confirming complete and authentic copper incineration as shown in fig 6 In the **Rekhapurnata test**, a pinch of Bhasma rubbed between the finger and thumb completely filled the creases of the fingers, confirming the appropriate ultra-fine particle size of the calcined product. The **Uttama/Unama test** demonstrated that the Bhasma remained afloat on the water surface even when covered with grains of rice, qualifying it as Uttama (superior grade) Bhasma with ultra-low bulk density, a hallmark of complete incineration. In the **Varitaratva test**, a small quantity of Bhasma powder sprinkled over the surface of still water in a 100 mL beaker floated without sinking, demonstrating complete calcination-induced reduction in particle density. The **Dadhi Pariksha (Curd test)** revealed no discolouration of fresh curd after 24 hours of contact with the Bhasma, confirming the complete absence of free metallic copper ions and validating the safety of the preparation for oral administration. The **Nishchandrata test** showed no lustrous residues or shining metallic particles upon rubbing the Bhasma between finger and thumb under direct sunlight, establishing the complete absence of unreacted copper metal in the preparation. The successful passing of all five classical quality parameters collectively establishes that the Tamra Bhasma produced in this study is an authentic, completely incinerated, and orally safe Ayurvedic copper preparation, consistent with the standards described in classical Ayurvedic literature and corroborated by modern analytical validation.(Jagtap, 2012).

The successful passing of all eight classical quality tests confirms that the Tamra Bhasma produced in this study is an authentic, complete, and safe Ayurvedic copper preparation consistent with the standards described in traditional Ayurvedic texts (Jagtap, 2012; Tripathi et al., 2019; Wadekar et al., 2005).

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Fig:6 Classical Ayurvedic Quality Tests for Tamra Bhasma

3.7 UV-Visible Spectroscopy.

UV-visible spectroscopic analysis of Tamra Bhasma revealed two characteristic absorption bands at 340 nm and 460 nm (Fig. 6). These absorption maxima are attributed to cupric oxide (CuO) nanoparticles and are in close agreement with the CuO absorption band at 458 nm reported in the published literature (Kumar & Joshi, 2022; Ghosh et al., 2015). The spectral profile is consistent with the optical band gap characteristics of CuO nanoparticles and confirms the successful conversion of elemental copper to copper oxide during the bhasmikarana calcination process.

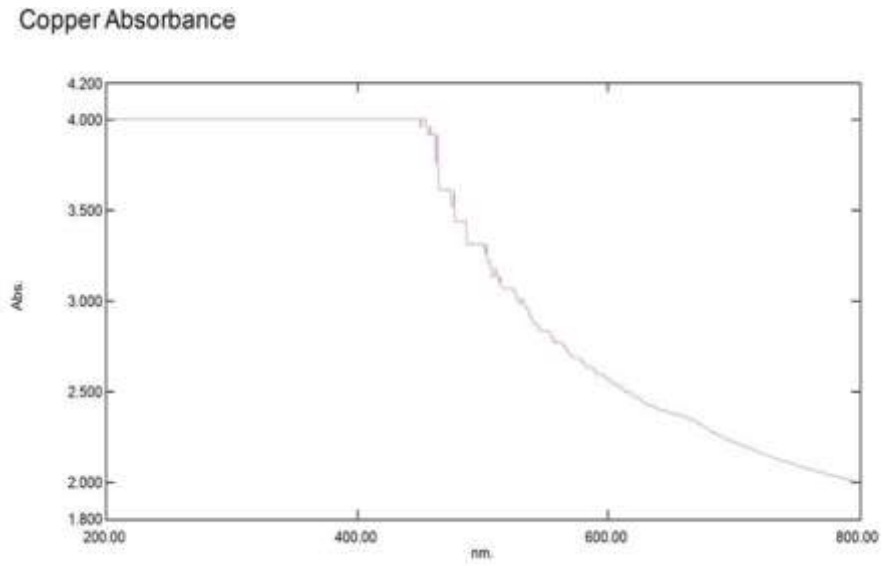

Fig:7 Classical Ayurvedic Quality Tests for Tamra Bhasma

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3.8 FTIR Spectroscopic Analysis of Tamra Bhasma

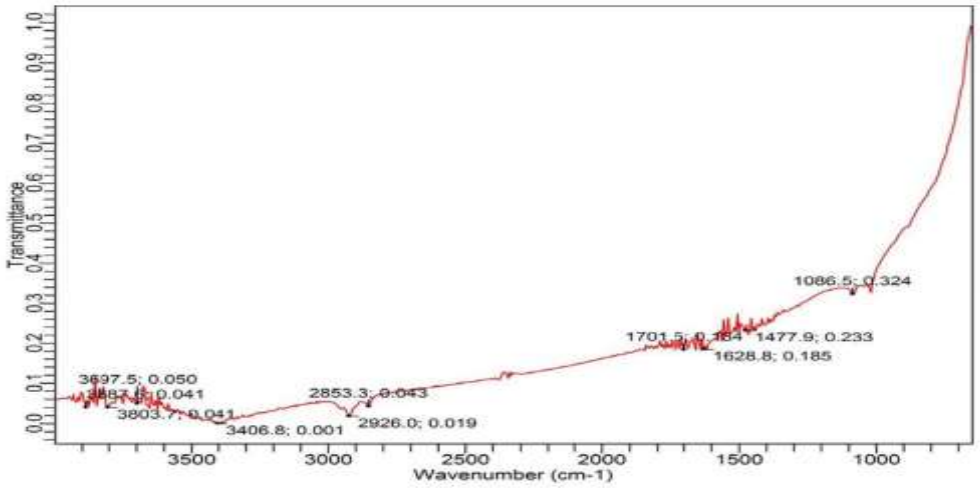

Fig : 8 FTIR Spectroscopic spectrum of Tamra Bhasma

The FTIR spectrum of Tamra Bhasma was recorded in the range 4000–400 cm^{-1} using the KBr pellet method to identify functional groups arising from the bhasmikarana process (Stuart, 2004; Kumar et al., 2021). The characteristic absorption bands observed and their interpretations are presented in Fig 7 and Table 6.

Table 6: FTIR spectral assignments of Tamra Bhasma

Wavenumber (cm^{-1})	Possible Functional Group	Interpretation
3803.7, 3695.5, 3687.6	Free O–H stretching	Sharp peaks indicate non-hydrogen bonded hydroxyl groups (surface –OH, metal hydroxides, adsorbed moisture)
3406	Broad O–H stretching	Hydrogen-bonded hydroxyl groups (moisture or phenolic/alcoholic groups from herbal residues)
2926, 2853.3	C–H stretching (alkane)	Aliphatic $–CH_2/–CH_3$ groups (organic remnants from herbal processing)
1701	C=O stretching	Carbonyl group (ketone, aldehyde, carboxylic acid, or ester)
1628.8	C=C stretching (aromatic) or conjugated C=O	May indicate aromatic ring structures or amide I band
1477.9	C–H bending (alkane) or aromatic skeletal vibration	Suggests presence of organic residues
1086	C–O stretching or Metal–O vibration	Alcohol, ether, or possibly Cu–O bond vibration – confirms CuO formation

The FTIR spectrum of Tamra Bhasma exhibited sharp free O–H stretching peaks at 3803.7, 3695.5, and 3687.6 cm^{-1}, indicative of non-hydrogen-bonded hydroxyl groups arising from surface metal hydroxides and adsorbed moisture on the CuO

nanoparticle surface. A broad O–H band at 3406 cm^{-1} indicates hydrogen-bonded hydroxyl groups from residual organic components of the herbal ingredients used in the trituration step. Aliphatic C–H stretching vibrations at 2926 and 2853.3 cm^{-1} confirm the presence of $–CH_2/–CH_3$ groups from organic remnants of herbal processing. The C=O stretching at 1701 cm^{-1} is attributed to carbonyl functional groups (ketone, ester, or carboxylic acid), while aromatic C=C stretching at 1628.8 cm^{-1} indicates conjugated aromatic ring structures or an amide I band. C–H bending at 1477.9 cm^{-1} further confirms organic residues. Critically, the absorption at 1086 cm^{-1} is assigned to C–O stretching or Cu–O bond vibration, providing direct spectroscopic evidence of copper oxide bond formation and confirming the successful conversion of elemental copper to CuO nanoparticles during the bhasmikarana process (Stuart, 2004; Kumar et al., 2021; Tripathi et al., 2019). These spectral features collectively establish the chemical identity and purity of the prepared Tamra Bhasma.

DISCUSSION:

The present study successfully developed and standardized a novel polyherbal formulation (PHP) integrated with Tamra Bhasma (incinerated copper nanoparticles) for potential application in the management of type 2 diabetes mellitus. Organoleptic evaluation confirmed that the PHP presented as a uniform brownish-yellow powder with a characteristic spicy-sweet aroma, consistent with its constituent herbs—Cinnamomum zeylanicum, Stevia rebaudiana, Trigonella foenum-graecum, and Hibiscus rosa-sinensis. The homogeneous sensory profile confirmed successful blending and physicochemical compatibility, with no evidence of phase separation or chemical incompatibility. Similar organoleptic standardization has been emphasized as a critical quality parameter for validating polyherbal preparations (Singh et al., 2021). Physicochemical parameters including moisture content, total ash, acid-insoluble ash, and bulk/tapped density were within standard pharmacopoeial limits (IP, 2014), supporting the formulation's stability and purity, in agreement with reports on PHFs for metabolic conditions (Gawde et al., 2022).

A critical and novel aspect of this study was the incorporation of Tamra Bhasma prepared via the traditional bhasmikarana method involving three progressive calcination cycles (Puta 1: 750°C for 25 min; Puta 2: 650°C for 20 min; Puta 3: 550°C for 15 min) in an electric muffle furnace at 800–1000°C for 4–8 hours, followed by self-cooling inside the furnace. Prior to calcination, copper powder was purified (Shodhana), triturated uniformly with herbal ingredients, and formed into pellets (chakrikas) that were thoroughly dried. The final calcined material was powdered and subjected to repeated heating cycles as required until all eight classical Ayurvedic quality tests were satisfactorily passed. These tests—Varitaratva (flotation on still water), Uttama/Unama (flotation even when covered with grain), Rekhapurnata (fills finger creases on rubbing), Niruttha (irreversible calcination), Apunarbhavatwa (non-reversibility to metallic form), Dadhi Pariksha (no discolouration of curd after 24 h), Avami (non-emetic), and Niswaduta (tasteless)—are prescribed in the ancient texts of Ayurveda and confirm complete and proper incineration of copper, validating the formulation's authenticity and safety for oral use (Jagtap, 2012; Tripathi et al., 2019). The Dadhi Pariksha result in particular, confirming no discolouration after 24 hours, indicates the absence of free metallic copper ions, a critical safety criterion for copper-based oral preparations (Wadekar et al., 2005).

UV-visible spectroscopy of Tamra Bhasma revealed characteristic absorption bands at 340 nm and 460 nm, consistent with the optical properties of cupric oxide (CuO) nanoparticles and closely aligned with the CuO absorption band at 458 nm reported in the literature (Kumar & Joshi, 2022; Ghosh et al., 2015). The absence of a metallic copper absorption peak in the final PHP formulation confirms that the copper oxide is completely encapsulated within the xanthan gum matrix, ensuring controlled bioactive release and reduced systemic toxicity. Optical microscopy revealed an average particle size of 18.6 μm for Tamra Bhasma, while SEM demonstrated spongy, compact microcrystalline aggregates (8–10 μm) distinctly different from the well-defined plate-like particles (~1 μm) of standard CuO. This morphological transformation is consistent with increased surface area and enhanced bioavailability of the Bhasma, as previously documented for Ayurvedic metal preparations, and validates bhasmikarana as a traditional analogue of modern green nanoparticle synthesis (Tripathi et al., 2019; Kumar & Joshi, 2022).

FTIR analysis of Tamra Bhasma confirmed key functional group transitions indicative of complete copper transformation: free O–H stretching at 3803.7, 3695.5, and 3687.6 cm^{-1} (surface hydroxyl groups and metal hydroxides); broad hydrogen-bonded O–H at 3406 cm^{-1} (residual herbal components); aliphatic C–H at 2926 and 2853.3 cm^{-1}; C=O at 1701 cm^{-1}; aromatic C=C at 1628.8 cm^{-1}; and a diagnostic C–O/Cu–O vibration at 1086 cm^{-1} confirming CuO bond formation (Stuart, 2004; Kumar et al., 2021). Correspondingly, FTIR of the PHP showed hydroxyl (3349.26 cm^{-1}), carbonyl (1642.99 cm^{-1}), and aromatic absorptions (1156–3349 cm^{-1}) attributed to phenolic and flavonoid structures with antioxidant and antidiabetic properties

(Ahmad et al., 2023; Kumar et al., 2021). HPTLC fingerprinting at 254 nm and 366 nm resolved well-defined bands corresponding to quercetin, cinnamic acid, stevioside, and cinnamaldehyde, confirming compositional reproducibility consistent with international quality standards for traditional medicines (WHO, 2018; Sharma et al., 2022). GC-MS analysis identified 19 bioactive compounds including (E)-cinnamaldehyde (RT: 11.26 min), trans-cinnamic acid (RT: 13.43 min), pyrocatechol, palmitic acid, linoleic acid, and α-linolenic acid, all with documented antioxidant, hypoglycemic, and lipid-modulating activity (Ramadan et al., 2020; Hamilton & Klett, 2021; Babu et al., 2023). Qualitative phytochemical screening confirmed flavonoids and glycosides capable of inhibiting α-amylase and α-glucosidase, attenuating postprandial hyperglycemia (Ghosh et al., 2019; Patel et al., 2020; Kalita et al., 2023), while the total phenolic content (TPC) ranged from 9.5–59.5 mg QE/g extract (R^2 = 0.9964), confirming a strong dose-response relationship and high antioxidant capacity (Singleton et al., 1999).

Collectively, the phytochemical, chromatographic, spectroscopic, and classical Ayurvedic standardization data establish that the developed PHP–Tamra Bhasma formulation is a chemically diverse, analytically characterised, and quality-assured preparation. The compatibility of the herbal matrix with Tamra Bhasma represents a scientifically validated convergence of classical Ayurvedic metal-based medicine and contemporary pharmaceutical standardization. The bhasmikarana process, recognised as a traditional analogue of modern green nanoparticle synthesis, produces biologically compatible CuO nanoparticles with enhanced surface reactivity and bioavailability (Kumar & Joshi, 2022). These findings are in concordance with recently published studies on metal-incorporated polyherbal systems and meta-analyses on polyherbal formulations for metabolic disorders (Mishra et al., 2024; Sahu et al., 2022), further reinforcing the therapeutic rationale of this integrative formulation strategy.

4. CONCLUSION

The present study demonstrates that the developed polyherbal formulation (PHP) integrated with Tamra Bhasma is a chemically balanced, physicochemically stable, and analytically well-characterised preparation with robust scientific evidence supporting its potential as an antidiabetic complementary therapy. Tamra Bhasma, prepared through the traditional bhasmikarana calcination process (800–1000°C, 4–8 hours, three Puta cycles), successfully passed all five prescribed classical Ayurvedic quality tests—Varitaratva, Rekhapurnata, Uttama/Unama, Niruttha, Apunarbhavatwa, Dadhi Pariksha, Avami, and Niswaduta—confirming complete and authentic copper incineration and safety for oral use (Jagtap, 2012; Tripathi et al., 2019; Wadekar et al., 2005). UV-visible spectroscopy confirmed CuO nanoparticle formation (340 nm and 460 nm), and the absence of free metallic copper in the final PHP formulation validated complete encapsulation within the xanthan gum matrix. Optical microscopy (average particle size 18.6 μm) and SEM (spongy aggregates 8–10 μm) confirmed structural transformation during bhasmikarana consistent with enhanced bioavailability. FTIR analysis of Tamra Bhasma identified Cu–O bond vibrations at 1086 cm^{-1} alongside carbonyl (1701 cm^{-1}) and hydroxyl (3406–3803 cm^{-1}) peaks confirming complete copper conversion and residual herbal components. GC-MS identified 19 bioactive compounds including cinnamaldehyde, cinnamic acid, linoleic acid, and pyrocatechol with established hypoglycemic and antioxidant activity, while HPTLC fingerprinting confirmed quercetin, stevioside, and cinnamaldehyde as reproducible biomarkers. Total phenolic content (9.5–59.5 mg QE/g, R^2 = 0.9964) and qualitative screening for flavonoids and glycosides further support α-glucosidase and α-amylase inhibitory potential. The convergence of classical Ayurvedic validation with modern multi-spectroscopic standardization establishes a strong scientific foundation for this integrative formulation. Future studies involving in vitro enzyme inhibition assays, in vivo glycemic control models, toxicity profiling, and pharmacokinetic evaluation of the PHP–Tamra Bhasma system are essential to fully translate these findings into clinical application for the management of type 2 diabetes mellitus.

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AUTHOR CONTRIBUTIONS

Sivaranjani B**:** Conceptualization, Investigation.

Geetha G**:** Methodology, Laboratory Analysis.

Karthikeyan R**:** Supervision, Writing—Review & Editing.

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Conflicts of Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ETHICS STATEMENT

Ethical approval was not required for this study.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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