RESEARCH PAPER

Analytical phytochemistry of selected medicinal plant extracts supporting polyherbal formulation for metabolic syndrome

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Abstract

Metabolic syndrome (MS) is a multifactorial disorder characterized by obesity, insulin resistance, Diabetes mellitus, dyslipidemia, and hypertension, demanding multiple therapeutic strategies to target pathological pathways. Addressing the limitations of single-target pharmacotherapy, this study establishes a systematic phytochemical framework for a rationally designed polyherbal approach using five medicinal plants: *Tinospora cordifolia(TC), Momordica charantia(MC), Trigonella foenum-graecum (TG), Tecomella undulata(TU),* and *Acorus calamus(AC).* Authenticated medicinal plants were extracted using 65% methanol in a Soxhlet system and subjected to integrated qualitative, quantitative, physicochemical, and spectral characterisation. Screening of individual plant extract confirmed the presence of major secondary metabolites and its quantitative estimation revealed substantial levels of alkaloids (5.62%), flavonoids (4.07%), phenols (6.53%), tannins (8.83%), and saponins (5.02%). Further, HPTLC of TC extract revealed specifically apigenin (0.155%) and quercetin (0.133%), while TG extract mainly contained mangiferin (4.392%). Gallic acid (6.752%) was a major constituent in the TU plant extract, whereas small fraction of apigenin (0.055%) was identified in the MC extract. AC extract revealed the presence of kaempferol (0.079%) and quercetin (0.054%). Notably, β-sitosterol was detected in all five plant extracts at different proportions. FT-IR spectral analysis identified multiple functional groups in the extract primarily amines, alcohols, fluoro compounds, phenols, alkanes, alkenes, and conjugated acid groups. Our research provides evidence for a synergistic, multi-target herbal approach and establishes a basis for creating formulations for the assessment of MS.

Keywords: Metabolic syndrome; Polyherbal formulation; Phytochemical profiling; HPTLC; FTIR;

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Introduction

Metabolic syndrome (MS) is a complex and fast expanding global public health challenge characterized by a constellation of interrelated metabolic abnormalities, including central (abdominal) obesity, insulin resistance, dyslipidemia, and hypertension, which synergistically escalate the risk of developing diabetes mellitus and cardiovascular disease (CVD)[1,2]. Ended the past two decades, the global prevalence of MS has increased substantially, through recent estimates indicating that approximately 1.54 billion adults worldwide are affected, and rates continue to rise across regions, age groups, and socioeconomic settings[3]. This clustering of cardiometabolic risk factors not only predisposes individuals to T2DM and CVD but also contributes to high morbidity and mortality globally[1,2]. Despite this growing burden, current therapeutic strategies that predominantly target individual risk factors often fall short of addressing the multifactorial pathophysiology of MS, highlighting an urgent need for multi-targeted and integrative interventions[4,5].

Conventional allopathic treatments for components of MS—such as hyperglycaemia, dyslipidemia, and hypertension—are effective in isolated pathways but often exhibit limitations including adverse effects, poor tolerability, hepatotoxicity, and inability to address the syndrome's multifactorial pathogenesis simultaneously[6,7]. These constraints have stimulated scientific interest in herbal and polyherbal formulations derived from Ayurveda and other traditional medicine systems, which may offer multi-targeted effects through their rich and diverse phytochemical profiles[8,9]. Among these, bitter-tasting plants have attracted attention for their potential to modulate metabolic regulation, in part by interacting with gut bitter-taste receptors expressed on enteroendocrine cells, thereby influencing hormones such as glucagon-like peptide-1 (GLP-1) that are central to glucose homeostasis[10]. Experimental evidence suggests that bitter plant extracts such as *Momordica charantia* (bitter melon) can act as GLP-1 secretagogues, improving insulin sensitivity and contributing to glucose regulation, while bitter phytochemicals have been shown to engage bitter taste receptors and modulate glucose and lipid metabolism, antioxidant defences, and inflammatory responses relevant to metabolic control [10,11,12].

The present investigation focuses on five medicinal plants with established ethnobotanical backgrounds and reported individual anti-diabetic, anti-lipidemic, antioxidant, and hepatoprotective activities: *Tinospora cordifolia* (Guduchi), *Momordica charantia* (Bitter melon), *Trigonella foenum-graecum* (Fenugreek), *Tecomella undulata* (Rohitaka), and *Acorus calamus* (Vekhand)[13–17]. This paper reports in detail the extraction procedures, qualitative and quantitative phytochemical screening, physicochemical standardization, HPTLC fingerprinting, and FTIR spectral characterization of the collected plant extracts, forming the foundation for subsequent polyherbal formulation and in vivo evaluation for MS[18,19].

Materials and methods

Collection and authentication of plant materials

Dried stems of *Tinospora cordifolia* were gathered from the Sahyadri region in Kolhapur, Maharashtra, whereas plant samples of *Momordica charantia, Trigonella foenum-graecum, Tecomella undulata,* and *Acorus calamus* were obtained from verified locations. Every material was dried in the shade, ground, and kept in airtight containers. Dr. S. S. Khot performed the identification and authentication. Compounds of analytical grade were obtained from E. Merck, SD Fine, and CDH Chemicals. Rationale for Solvent Selection: Hydroalcoholic (65% Methanol)

A hydroalcoholic solvent (65% methanol in water) remained employed for its ability to extract a range of polar bioactive compounds. This mix of solvents is completely miscible, preserving adequate polarity for phenolic compounds, flavonoids, glycosides, alkaloids, tannins, and saponins, while enabling the co-extraction of polar glycosides. The process was conducted using Soxhlet continuous extraction with 150 g of washed, shade-dried, and ground plant material, circulating the solvent at 40°C for 4 hours, until clear siphoning signaled was complete. Extract was subsequently filtered through Whatman No. 42 paper, concentrated with a rotary evaporator at 50°C, and preserved in airtight containers shielded from light and heat.

Qualitative phytochemical screening

Qualitative phytochemical screening for secondary metabolites was performed on hydroalcoholic extracts. Alkaloids were identified employing Mayer's reagent; a cream-colored precipitate signified their presence. Flavonoids displayed a bright yellow hue with sodium hydroxide, which became colourless when acid was added (Lead acetate test). Glycosides were evaluated using Modified Borntrager's and Legal's tests post-hydrolysis. Ferric chloride indicated phenols by producing a bluish-black hue. Saponins created enduring foam during the Froth test. Phytosterol exhibited a golden yellow colour when treated with concentrated sulphuric acid (Salkowski's test). Tannins formed a blue-black precipitate with ferric chloride, whereas fixed oils, fats, proteins, and amino acids were detected using specific tests.

Quantitative estimation of phytoconstituents

Total alkaloids were assessed by combining 5 g of extract with 8 mL of 20% acetic acid and 32 mL of ethanol, letting it sit for 4 hours prior to filtration. The filtrate was concentrated, and concentrated ammonium hydroxide was introduced until precipitation took place. The precipitate was filtered, dried, and weighed, with results reported as percentage alkaloid concentration. For total flavonoids, 1 mL of extract was combined with 4 mL of de-ionized water and 0.3 mL of 10% sodium nitrate, and incubated for 5 minutes. Subsequently, 0.3 mL of 10% aluminium chloride and 2 mL of 1M sodium hydroxide were added, and the OD was recorded at 510 nm, with results represented as mg/g of extract using rutin as a standard. Tannins were measured by combining 0.5 mL of Folin-Ciocalteu reagent with water and sodium carbonate, bringing the total volume to 10 mL, and then measuring absorbance at 700 nm, with results reported as mg tannic acid equivalents per gram of dry sample. Saponins remained obtained from a 5 g powdered sample using 20% aqueous ethanol, filtered, and treated to eliminate non-saponin lipids, evaporating the residual layer to a constant weight[7].

Physicochemical screening of plants extracts

Physicochemical analyses of powdered dried stems were conducted in accordance with WHO guidelines (2011), evaluating moisture level, total ash, acid-insoluble ash, water-soluble ash, water-insoluble ash, and pH. For chromatographic evaluation, thin layer chromatography (TLC) was performed utilizing pre-coated silica gel plates. A hydroalcoholic extract was formulated and created with a defined mobile phase, subsequently undergoing UV analysis. A value of 0.56 for R_f was established for the main active area following the development and air-drying of the plate.

High-Performance thin layer chromatography (HPTLC)

HPTLC fingerprinting employed CAMAG equipment such as Linomat V and TLC Scanner 3, using 100 µL Hamilton syringes for the manual application of samples. Silica gel 60 F254S is a stationary phase, while Toluene, Ethyl acetate, Methanol, Water, and Formic acid constituted the mobile phase. Plates were immersed for 20 minutes, and detection took place at 356 nm and 520 nm. Samples (100 mg) were treated with methanol, resulting in a final concentration of 10,000 ppm, whereas standards were created at 100 ppm.

FTIR spectroscopy

FTIR analysis of the hydroalcoholic extracts was performed using an ABB MB 3000-PH FT-IR Spectrometer with Horizon software. KBr pellet technique was employed: 10 mg of dried extract was encapsulated in 100 mg of KBr and pressed into a translucent disc. Spectra were recorded over the scan range of 400 – 4000 cm^{-1} at a resolution of 4 cm^{-1}.[11]

Results and Discussion

Extraction yield and physical characterization

Alcoholic extraction (65% methanol) of the five medicinal plants produced dark green to brown semisolid/solid extracts with characteristic appearances. TC yielded a dark brown semisolid extract with a percentage yield of 10%. MC produced a dark green semisolid (12.66 %), TG a brownish semisolid (30 %), TU a brown solid (31 %) and AC a dark brown semisolid (30 %). The uniform semisolid nature and intense coloration of the extracts indicate efficient extraction of soluble secondary metabolites, forming the basis for subsequent phytochemical evaluation.

Qualitative analysis

Qualitative screening of the alcoholic extracts revealed a consistent presence of major bioactive phytochemical classes, notably alkaloids, flavonoids, glycosides, saponins, phytosterol, phenols, and tannins. The extract of TC was representative of this shared profile, which was similarly observed MC, TG, TU, and AC. These phytochemical groups were widely recognized for their antioxidant, antidiabetic, anti-inflammatory, hypolipidemic, and hepatoprotective activities, aligning with the ethnopharmacological relevance of the selected plants and supporting their suitability for polyherbal intervention in metabolic syndrome (Table 1). Notably, fixed oils and proteins were not identified, indicating selective enrichment of secondary metabolites of pharmacological interest in the extracts. The presence of diverse phytochemical

classes particularly flavonoids, alkaloids, phenols, tannins, and saponins consistent with the reported pharmacological activities of these plants including antioxidant, antidiabetic, and anti-inflammatory actions. The absence of fixed oils and proteins indicates a selective extractability profile under hydroalcoholic conditions (Table 1)

Table 1: Qualitative phytochemical studies of plants extracts

Chemical constituent	***TC***	***MC***	***TG***	***TU***	***AC***
Alkaloids	+ve	+ve	+ve	+ve	+ve
Flavonoids	+ve	+ve	+ve	+ve	+ve
Glycosides	+ve	+ve	+ve	+ve	+ve
Saponins	+ve	+ve	+ve	+ve	+ve
Phytosterol	+ve	+ve	+ve	+ve	+ve
Phenols	+ve	+ve	+ve	+ve	+ve
Fixed Oils and Fats	-ve	+ve	+ve	+ve	+ve
Tannins	+ve	+ve	+ve	+ve	+ve
Proteins and Amino Acids	-ve	-ve	-ve	-ve	-ve
Terpenoids	+ve	+ve	+ve	+ve	+ve

Physicochemical characterization

Physicochemical evaluation of the all plant extracts demonstrated obtained values consistent with acceptable quality parameters for herbal raw materials. The lower moisture content (7.1%) indicates adequate drying and reduced risk of microbial growth or degradation. The total ash value (10%) reflects the inherent inorganic mineral content of the plant material, while the very low acid-insoluble ash (0.20 g) confirms minimal impurity with siliceous matter such as sand or soil. The mildly acidic pH (6.1) is consistent with the abundance of phenolic and tannin constituents identified in the extract (Table 2). The mildly acidic pH of 6.1 is consistent with the presence of phenolic and tannin components. Collectively, these parameters support the purity, stability, and suitability of the plant material for further phytochemical analysis and formulation development.

Table 2: Physicochemical characteristics of *plants extracts*

Parameters	**TC**	**MC**	**TG**	**TU**	**AC**
Total ash values (%)	10	14	10	11	15
Acid insoluble ash (gm)	0.20	0.18	0.20	0.09	0.20
Water soluble ash value (%)	3	2	3	1	1
Water insoluble ash value (%)	10	12.5	10	10	14
Moisture content (%)	7.1	5.5	7.1	6.8	7.14
PH	6.1	5.8	6.1	5.7	5.5

Quantitative profiling

Quantitative estimation demonstrated a rich abundance of key pphytoconstituents in the alcoholic extract of plants (Table 3). The higher proportions of tannins and phenols indicate strong antioxidant potential, while the appreciable alkaloid content supports reported antidiabetic and anti-inflammatory activities. Although they are present in comparatively lower amounts, flavonoids contribute important modulatory effects on glucose and lipid metabolism. Collectively, this quantitative profile underscores the phytochemical richness of the extract and its relevance to metabolic regulation.

Table 3: Quantitative phytochemical studies of plants extracts (mg/gm)

Parameters	**TC**	**MC**	**TG**	**TU**	**AC**
Alkaloid	5.50	6.20	4.62	5.62	5.62
Flavonoid	5.07	5.07	5.07	4.07	4.07
Phenol	4.53	5.53	5.53	6.53	6.53
Tannin	7.83	6.83	7.83	8.83	8.83
Saponin	3.02	5.02	4.02	5.02	5.02

In the present study, TLC analysis of plant extracts revealed the presence of multiple phytochemical compounds with varying R_f values. The appearance of distinct spots under visible light or UV illumination indicated the separation of different secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids, glycosides, and phenolic compounds. The obtained R_f standards were compared with standard references and previously reported literature to assist in the characterization of bioactive constituents present in the extracts (Table 4).

Table 4: TLC studies of plants extracts

Plants	***R_f* value**	**Ref. value**	**Interpretation**
TC	0.58 0.56	0.1-0.9 0.4-0.7	Flavonoids, Phytosterol
MC	0.56	0.2-0.8	Flavonoids
TG	0.40 0.80	0.2-0.8 0.4-0.7	Glycosides, Phytosterol
TU	0.50	0.1-0.9	Flavonoids
AC	0.56 0.40	0.1-0.9 0.4-0.7	Flavonoids Phytosterol

HPTLC and FTIR profiling

All plant extracts contained β-sitosterol, indicating it as a shared phytosterol component. The TC extract revealed the presence of quercetin (0.155%) and apigenin (0.133%) through HPTLC analysis, and FTIR profiling confirmed the presence of β-Sitosterol and quercetin in the same extract. Apigenin (0.055%) was detected in the MC extract via HPTLC, and notably, FTIR additionally identified quinic acid and cucurbitacin.. According to HPTLC, mangiferin was found to be most abundant in TG (4.392%), aligning with its unique glycoside profile, while quercetin and diosgenin identified through FTIR analysis. The TU extract's HPTLC results showed the highest concentration of gallic acid (6.752%), suggesting its elevated phenolic content related antioxidant potential and FTIR profiling of the same extract established the presence of flavonoid chemicals. HPTLC of AC revealed a significantly high β-sitosterol content

(7.526%) including quercetin (0.054%) and Kaempferol (0.079%), consistent with reports that its rhizome is rich in saponins and triterpenoids, further FTIR profiling confirmed the presence of flavonoids. These profiles show the distribution of significant bioactive markers among the selected plants and their complementary phytochemical contributions to a possible polyherbal composition. (Table 5)

Table 5: HPTLC studies of plant extracts

Plants	Mangiferin %	Apigenin %	Gallic Acid %	Quercetin %	β-sitosterol %	Kaempferol %
TC	–	0.133	–	0.155	0.940	–
MC	–	0.055	–	–	–	–
TG	4.392	–	–	–	1.074	–
TU	–	–	6.752	–	0.795	–
AC	–	–	–	0.054	7.526	0.079

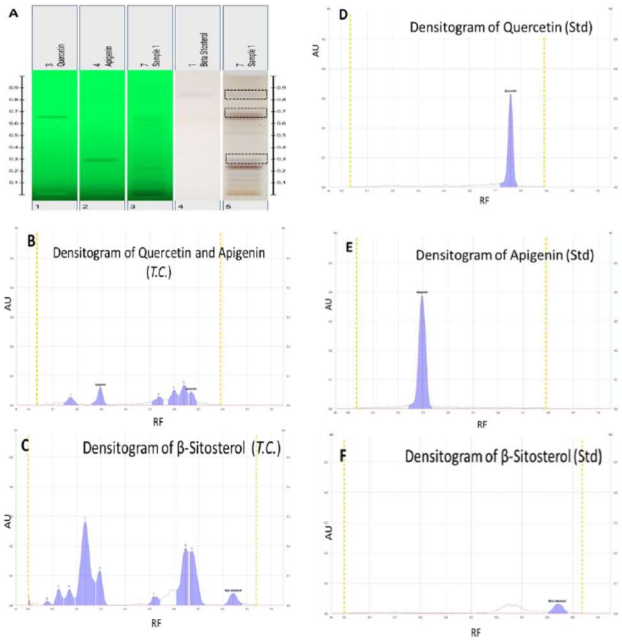

Fig.1. HPTLC of TC. Chromatogram analysis of TC showing bands corresponding to Quercetin and Apigenin (A). Densitogram showing the peaks corresponding to Quercetin and Apigenin (B), β-sitosterol (C) observed in TC. The peaks are shown for standard compounds; Quercetin (D), Apigenin(E), and β-sitosterol (F).

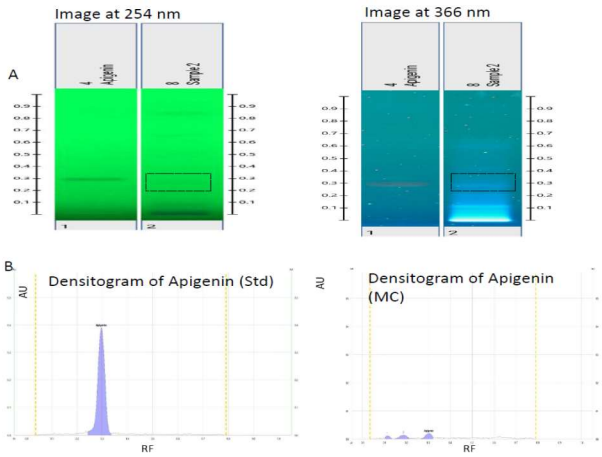

Fig.2. HPTLC of MC. Chromatogram analysis of MC showing bands corresponding to Apigenin at 254 nm and 366 nm (A). Densitogram showing the peaks corresponding to Apigenin observed in MC with the standard compound Apigenin (B).

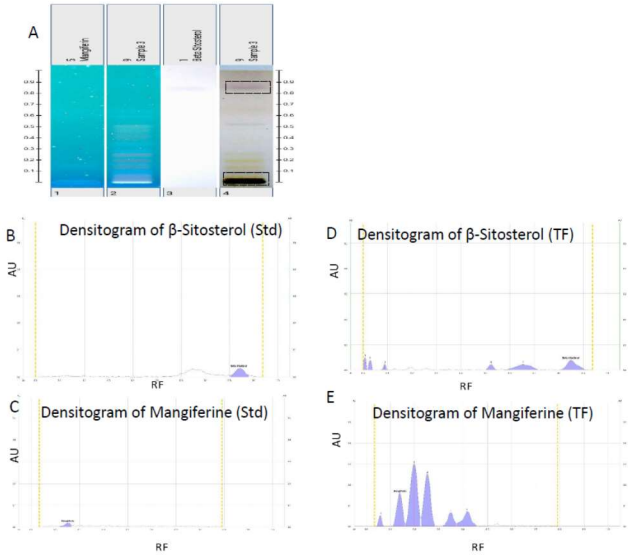

Fig.3. HPTLC of TG. Chromatogram analysis of TG showing bands corresponding to β-sitosterol and Mangiferine (A). Densitogram showing the peaks corresponding to β-sitosterol (D), and Mangiferine (E) observed in TC. The peaks are shown for standard compounds; β-sitosterol (B) and Mangiferine (C).

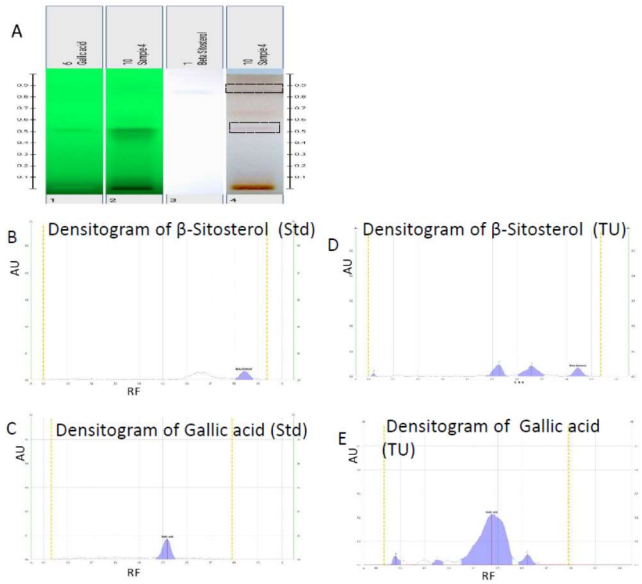

Fig.4. HPTLC of TU. Chromatogram analysis of TU showing bands corresponding to β-sitosterol and Gallic acid (A). Densitogram showing the peaks corresponding to β-sitosterol (D), and Gallic acid (E) observed in TU. The peaks are shown for standard compounds; β-sitosterol (B) and Gallic acid (C).

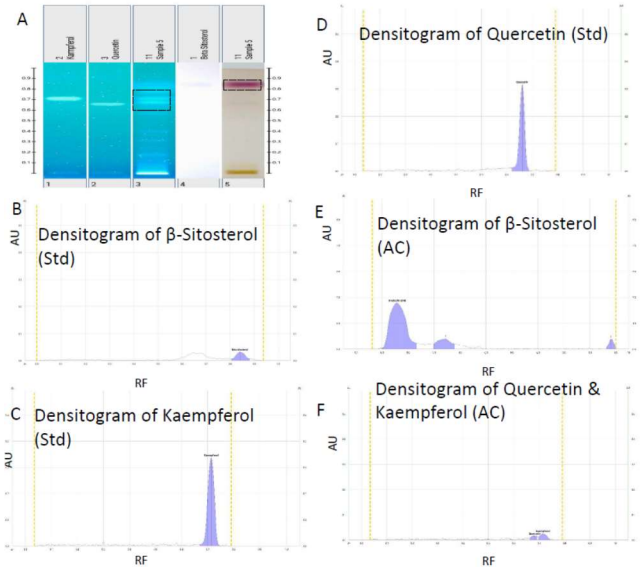

Fig.5. HPTLC of AC. Chromatogram analysis of AC showing bands corresponding to β-sitosterol, Quercetin and Kaempferol (A). Densitogram showing the peaks corresponding to β-sitosterol (E) Quercetin and Kaempferol (C) observed in AC. The peaks are shown for standard compounds to β-sitosterol (B) Quercetin (C) and Kaempferol (D).

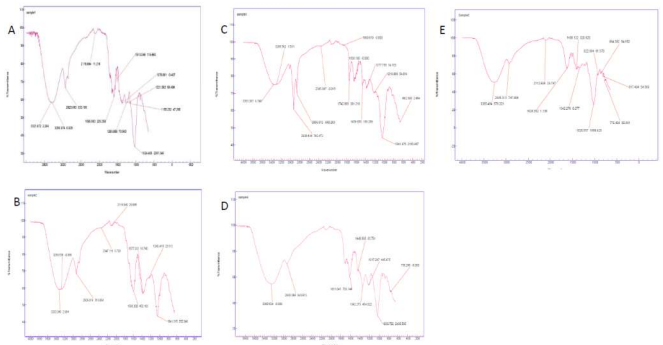

Fig. 6: - IR Spectral series of Plants. A) *Tinospora cordifolia* B) *Memordica charantia Mizo* C) *Trigonellafoenum-graecum* D) *Tecomel undulate* E) *Acorus Calamus.*

Conclusion

This work provides a scientific basis for developing a polyherbal formulation strategy against metabolic syndrome by confirming the presence of diverse and complementary bioactive phytoconstituents across all five medicinal plants. The overall analytical evidence supports the quality, consistency, and phytochemical richness of the extracts, authenticating their suitability for further formulation development. The collective phytochemical profiles indicate the potential for a synergistic, multi-target therapeutic approach. These findings justify advancing to formulation optimization and subsequent pharmacological evaluation to assess efficacy in metabolic syndrome. HPTLC and FTIR profiling revealed that all five alcoholic extracts possess unique yet overlapping phytochemical signatures, with β-sitosterol identified as a common bioactive marker across all five plants. Notably, the extracts demonstrated rich and diverse profiles of therapeutically relevant phytoconstituents such as quercetin and apigenin in TC, apigenin, quinic acid, and cucurbitacin in MC, high levels of mangiferin along with quercetin and diosgenin in TG, highest gallic acid content with abundant flavonoids in TU, and significantly high β-sitosterol with kaempferol in AC. These bioactive compounds are well-documented for their multi-target actions against the core components of MS, including diabetes mellitus, insulin resistance, oxidative stress, dyslipidemia, inflammation, and obesity. The complementary phytochemical distribution observed in this study strongly justifies the potential of a polyherbal combination for synergistic therapeutic effects. Such formulations could offer broader and more balanced coverage of metabolic pathways compared to single-plant interventions, making it clinically promising as an adjunctive approach in MS management.

Future research should focus on standardization of the polyherbal formulation, bioavailability, and clinical trials to validate its efficacy and safety in human subjects with MS. Overall, the phytochemical profiling of these five plants establishes a scientifically grounded base for their combined use in management of MS, offering a likely avenue for developing effective herbal therapeutics with high clinical translational value.

Conflict of interest

The authors declare no conflict of interest.

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Declaration of Competing 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.

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