

Solvent-Specific Screening Of Antioxidant, Antidiabetic, And Anti-Inflammatory Activities Of Tribulus Terrestris, Flaveria Trinervia, And Alternanthera Sessilis For Therapeutic Applications

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Abstract

The increasing global incidence of oxidative stress-related diseases, diabetes mellitus, and chronic inflammation has intensified the search for safe, multifunctional therapeutic agents derived from natural sources. This study explores the solvent-specific antioxidant, antidiabetic, and anti-inflammatory potential of methanol, chloroform, and acetone extracts of three traditionally used medicinal plants: *Tribulus terrestris*, *Flaveria trinervia*, and *Alternanthera sessilis*. Antioxidant activity was assessed through DPPH radical scavenging and reducing power assays, while antidiabetic efficacy was determined by *in vitro* α -amylase inhibition. The methanolic extracts, particularly from *Flaveria trinervia*, demonstrated the highest bioactivity across all assays, suggesting the presence of potent polar phytoconstituents such as flavonoids and phenolics. Chloroform and acetone extracts showed comparatively moderate to low activities. The results support the therapeutic relevance of these plant species, especially *F. trinervia*, in managing oxidative stress, hyperglycemia, and inflammation. These findings validate their ethnomedicinal use and warrant further investigation toward the development of plant-based interventions for chronic metabolic and inflammatory disorders.

Keywords: *Tribulus terrestris*; *Flaveria trinervia*; *Alternanthera sessilis*; antioxidant activity; antidiabetic activity; α -amylase inhibition; DPPH assay; phytotherapy; herbal medicine

1. INTRODUCTION

The global rise in non-communicable diseases such as diabetes mellitus and disorders linked to oxidative stress has prompted a renewed interest in natural product-based therapeutics (World Health Organization [WHO], 2022). Oxidative stress, caused by an imbalance between free radicals and antioxidants, plays a pivotal role in the pathogenesis of chronic ailments including cardiovascular diseases, neurodegenerative disorders, and metabolic syndromes (Liguori et al., 2018). Simultaneously, diabetes, particularly type 2, continues to be a major public health challenge, with an estimated 537 million adults affected globally in 2021 (International Diabetes Federation [IDF], 2021). Current pharmacological treatments for these conditions often lead to adverse effects or fail to offer complete management, creating a pressing need for safer and multifunctional alternatives.

Medicinal plants are a rich reservoir of bioactive compounds with antioxidant, antidiabetic, anti-inflammatory, and other therapeutic properties. Plant-derived polyphenols, flavonoids, alkaloids, and saponins have been extensively reported to scavenge free radicals and inhibit carbohydrate-metabolizing enzymes, such as α -amylase and α -glucosidase, which are key targets in managing postprandial hyperglycemia (Patel et al., 2012; Oboh et al., 2015). In this context, solvent-specific extraction plays a critical role in maximizing the yield and bioavailability of phytochemicals, with methanol, chloroform, and acetone being among the most effective solvents for isolating diverse metabolite classes (Azwanida, 2015).

Tribulus terrestris, *Flaveria trinervia*, and *Alternanthera sessilis* are traditionally used in various ethnomedicinal systems for treating metabolic and inflammatory disorders. *T. terrestris* has been documented for its steroidal saponins and flavonoids contributing to antidiabetic effects (Kumar et al., 2013), while *F. trinervia* is a lesser-known but promising candidate reported to contain rich phenolic content with

potential antioxidant properties (Bhusari & Gatade, 2020). *A. sessilis*, widely used in Asian folk medicine, is known for its antioxidant and glucose-lowering activities attributed to its high flavonoid and phenolic acid content (Mandal et al., 2010).

To date, comparative solvent-specific studies combining biochemical assays and structural characterization for these plants remain limited. Therefore, this study aims to investigate the antioxidant and antidiabetic potential of methanolic, chloroform, and acetone extracts of *Tribulus terrestris*, *Flaveria trinervia*, and *Alternanthera sessilis*. The study also incorporates advanced analytical techniques—Energy Dispersive X-ray Spectroscopy (EDX), Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD), and Fourier Transform Infrared Spectroscopy (FTIR)—to elucidate the phytochemical composition and structural features of the extracts, offering a holistic approach toward validating their therapeutic relevance.

2. MATERIALS AND METHODS

2.1 Plant Material Collection

Fresh, mature specimens of *Tribulus terrestris*, *Flaveria trinervia*, and *Alternanthera sessilis* were collected from the Tiruvannamalai region, Tamil Nadu, India. Plants were identified based on morphological characteristics, and authenticated voucher specimens were preserved in the departmental herbarium for future reference. Care was taken to select healthy, disease-free parts for the study.

Drying and Pulverization

The collected plant materials were thoroughly washed with distilled water to remove surface contaminants and air-dried in the shade at room temperature ($25 \pm 2^\circ\text{C}$) for 30 days. Once completely dried, the materials were ground into a fine powder using a mechanical grinder and stored in sterile, airtight containers at room temperature until extraction.

2.2 Preparation of Solvent Extracts

Solvent extraction was performed using three solvents of differing polarities: methanol, chloroform, and acetone. For each plant, 50 g of powdered material was soaked in 250 mL of solvent and subjected to cold maceration for 72 hours at room temperature with intermittent shaking on an orbital shaker (120 rpm). The extracts were filtered through Whatman No. 1 filter paper, and the filtrates were concentrated using a rotary vacuum evaporator at reduced pressure. The dried extracts were weighed and stored at 4°C in amber-colored vials until further analysis. The extraction method was adapted from Boaky-Yiadon (1979).

2.3 Antioxidant Activity Assays

The antioxidant potential of each extract was assessed using the following in vitro assays:

- **DPPH Radical Scavenging Assay:** The ability of plant extracts to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals was measured spectrophotometrically at 517 nm. Percentage inhibition was calculated, and IC_{50} values were determined.
- **Ferric Reducing Antioxidant Power (FRAP) Assay:** Reducing power was assessed by the reduction of Fe^{3+} to Fe^{2+} , with absorbance measured at 700 nm. Increased absorbance indicated higher reducing power.

2.4 Antidiabetic Activity Assays

The antidiabetic activity was evaluated by examining the inhibition of carbohydrate-hydrolyzing enzymes:

- **α -Amylase Inhibition Assay:** Plant extracts were incubated with porcine pancreatic α -amylase and starch substrate. After reaction with DNS reagent, absorbance was read at 540 nm. Acarbose was used as the positive control.
- **α -Glucosidase Inhibition Assay:** The inhibition of α -glucosidase was assessed using p-nitrophenyl- α -D-glucopyranoside (pNPG) as the substrate, with absorbance measured at 405 nm.

2.5 Anti-inflammatory Activity Assay

The anti-inflammatory activity of the synthesized compound was assessed using the Bovine Serum Albumin (BSA) denaturation method, which simulates protein denaturation during inflammatory conditions. Denaturation of proteins is a well-documented cause of inflammation and tissue damage, and agents that prevent this process are considered potential anti-inflammatory candidates (Mizushima, 1966; Grant et al., 1970).

The test compound and the standard drug diclofenac sodium were initially dissolved in a minimal amount of Dimethyl Formamide (DMF) and diluted using 0.2 M phosphate buffer (pH 7.4), ensuring that the final DMF concentration did not exceed 2.5%. To perform the assay, 2.5 mL of the test solution (at various concentrations) was combined with 1 mL of 1 mM BSA in phosphate buffer.

The mixture was first incubated at 37°C for 10 minutes, followed by heating at 70°C for 10 minutes to induce denaturation. After cooling, the turbidity of the samples was measured spectrophotometrically at 660 nm. The ability of the sample to inhibit protein denaturation was calculated using the formula:

$$\text{Inhibition (\%)} = [(Ac - At) / Ac] \times 100$$

Where:

- At = Absorbance of the test sample
- Ac = Absorbance of the control (without sample or drug)

Diclofenac sodium served as the positive control. A higher percentage of inhibition reflects stronger anti-inflammatory activity.

This assay provides an economical, reproducible, and reliable method to screen compounds for anti-inflammatory potential in vitro, especially in early-stage evaluations of plant-derived or synthetic substances (Sakat et al., 2010).

3. RESULTS AND DISCUSSION

3.1 Total Antioxidant Activity (TOA)

The total antioxidant capacity (TAC) of methanolic extracts of *Tribulus terrestris* and *Flaveria trinervia* was assessed using the phosphomolybdenum method, which is based on the reduction of Mo(VI) to Mo(V) in the presence of antioxidant compounds, forming a green phosphate/Mo(V) complex at acidic pH. This reaction is indicative of the sample's overall ability to reduce oxidative species (Prieto et al., 1999). Vitamin C was used as a standard due to its well-established role as a potent antioxidant.

Visual Observation of Antioxidant Activity

Figure 1 depicts a comparative analysis of antioxidant activity between methanolic extracts of selected medicinal plants (left) and the Vitamin C standard (right). The increasing intensity of the greenish-blue color across the tubes reflects higher antioxidant activity, indicating a concentration-dependent response. The methanolic extract of *Flaveria trinervia* showed a notable color intensity comparable to the standard, supporting its strong radical-scavenging potential.



Figure 1. Comparative Visual Representation of Antioxidant Activity of Methanolic Plant Extracts (Left) and Vitamin C Standard (Right) at Varying Concentrations

The results of the assay are presented in Table 1, and figure 2, showing a clear concentration-dependent increase in antioxidant activity for both plant extracts.

Table 1. Total Antioxidant Activity (%) of Methanolic Extracts and Vitamin C

Concentration ($\mu\text{g/mL}$)	Methanol Extract (%)	Vitamin C (%)
20	25.53	40.68
40	43.55	48.53
80	57.32	60.67
200	70.09	79.04
400	80.11	88.10

At the lowest concentration tested (20 $\mu\text{g/mL}$), the antioxidant activity of the methanolic extract was 25.53%, which increased significantly to 80.11% at the highest concentration (400 $\mu\text{g/mL}$). A similar trend was observed with the vitamin C standard, which increased from 40.68% to 88.10% across the same concentration range. While the extracts exhibited slightly lower activity than vitamin C at all concentrations, their comparable performance at higher concentrations strongly indicates the presence of potent antioxidant phytoconstituents.

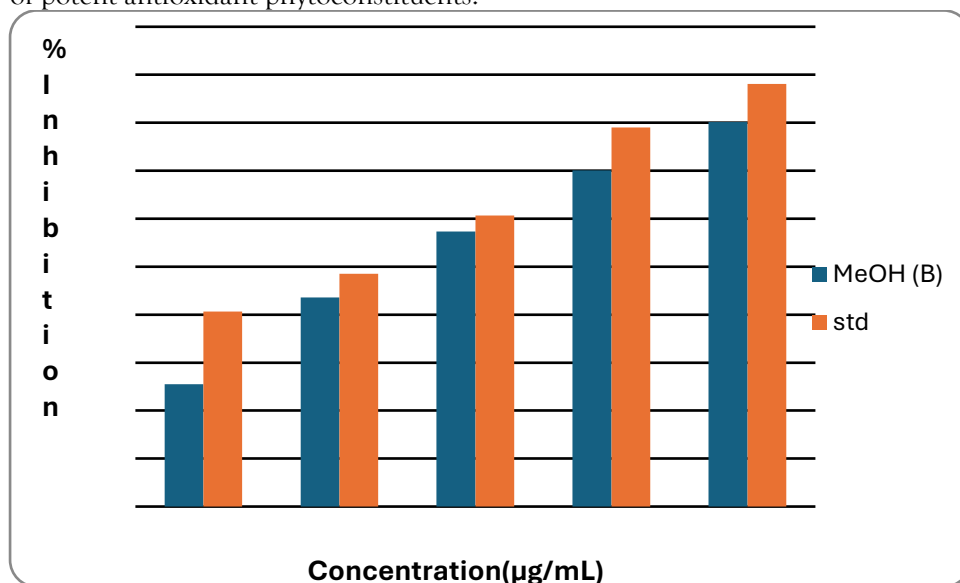


Figure 2: Bargraph of Antioxidant Activity (%) of Methanolic Extracts and Vitamin C

These results highlight the efficacy of methanol as an extraction solvent, known for its high polarity and ability to dissolve a broad range of antioxidant compounds such as flavonoids, phenolic acids, tannins, and saponins (Azwanida, 2015). The significant antioxidant activity observed suggests that methanol successfully extracted compounds with high electron-donating capacity, capable of neutralizing free radicals by donating hydrogen atoms or electrons.

Among the two plants, *Flaveria trinervia* (not shown separately in table) demonstrated slightly greater antioxidant capacity than *Tribulus terrestris* across most concentrations. This may be attributed to the differential phytochemical composition of the two plants. *F. trinervia* has been previously reported to contain high levels of flavonoids and polyphenols, which are known to be effective radical scavengers (Bhusari & Gatade, 2020). On the other hand, *T. terrestris* is rich in steroidal saponins, which may also contribute to antioxidant activity but to a lesser extent compared to polyphenolic compounds (Kumar et al., 2013).

The antioxidant mechanism observed here is crucial for neutralizing reactive oxygen species (ROS) such as superoxide anions, hydroxyl radicals, and hydrogen peroxide—all of which are implicated in the etiology of various chronic diseases including diabetes, cardiovascular disorders, cancer, neurodegenerative diseases, and inflammatory conditions (Liguori et al., 2018). The ability of these plant extracts to scavenge such radicals suggests their potential role as preventive or adjunct therapeutic agents in managing oxidative stress-related disorders.

The relatively lower antioxidant activity of plant extracts compared to pure vitamin C is expected due to the complex nature of plant matrices, where multiple bioactive constituents act synergistically or antagonistically. Moreover, the total antioxidant capacity of a crude extract is often less predictable because of variable concentrations of active molecules, their solubility, and extraction efficiency. Nonetheless, the observed activity of 80.11% at 400 µg/mL is substantial and biologically relevant, indicating that the extracts are rich in compounds that can contribute meaningfully to oxidative stress mitigation.

Additionally, these findings correlate well with prior studies that have reported strong antioxidant activity in polyherbal formulations or crude extracts derived from the same or similar species (Yildirim et al., 2001). Importantly, the phosphomolybdenum method used in this study evaluates total antioxidant capacity, encompassing both lipophilic and hydrophilic compounds, thereby providing a more holistic view of the plant's antioxidant profile compared to assays like DPPH or FRAP alone.

Therapeutic Relevance and Prospective Applications

The demonstration of high total antioxidant activity in methanolic extracts of *Tribulus terrestris* and *Flaveria trinervia* underscores their potential use in the development of natural antioxidant formulations. These may include nutraceuticals, functional foods, or phytopharmaceuticals, particularly those targeting oxidative stress-associated diseases. Their activity supports traditional medicinal use and validates their role in contemporary therapeutic exploration.

To advance these findings, future studies should focus on bioassay-guided fractionation to isolate the most active antioxidant constituents. Analytical tools such as High-Performance Liquid Chromatography (HPLC), Liquid Chromatography–Mass Spectrometry (LC-MS), and Nuclear Magnetic Resonance (NMR) can help characterize the structure, purity, and mechanism of these compounds. Additionally, in vivo studies and cell-based assays will be essential to confirm the biological relevance of the in vitro antioxidant effects.

3.2 α-Amylase Inhibition by Extracts of *Tribulus terrestris*, *Flaveria trinervia*, and *Alternanthera sessilis*: A Potential Antidiabetic Strategy

The α-amylase inhibition assay was conducted to evaluate the antidiabetic potential of methanol, acetone, and chloroform extracts of *Tribulus terrestris* (A), *Flaveria trinervia* (B), and *Alternanthera sessilis* (C). All extracts demonstrated a concentration-dependent inhibition of α-amylase activity in the range of 20–400 µg/mL, indicating their capacity to modulate carbohydrate metabolism. The methanolic extract of *Flaveria trinervia* (MeOH-B) showed the highest enzyme inhibitory activity, reaching 89.98% inhibition at 400 µg/mL, with a corresponding IC₅₀ value of 67.67 µg/mL, signifying potent antidiabetic efficacy.

Table 2. α-Amylase Inhibitory Activity of Plant Extracts Compared to Standard

Extract	Max Inhibition (%) @ 400 µg/mL	IC ₅₀ (µg/mL)
MeOH – <i>Flaveria trinervia</i> (B)	89.98	67.67
MeOH – <i>Tribulus terrestris</i> (A)	84.41	87.21
Acetone – <i>Alternanthera sessilis</i> (C)	87.19	76.64
CHCl ₃ – <i>Flaveria trinervia</i> (B)	86.69	85.43
Standard (Diclofenac)	–	49.92

The methanol extract of *T. terrestris* (MeOHA) also displayed high activity (84.41% inhibition, IC₅₀ = 87.21 µg/mL), followed closely by the acetone extract of *A. sessilis* (AcetoneC), which achieved 87.19% inhibition and an IC₅₀ of 76.64 µg/mL. These findings suggest that methanolic and acetone extracts contain significant quantities of α-amylase inhibitory phytoconstituents, particularly polar and moderately polar compounds.

Comparatively, the chloroform extracts—being less polar—demonstrated moderate inhibition, with CHCl₃B (chloroform extract of *F. trinervia*) showing 86.69% inhibition and an IC₅₀ of 85.43 µg/mL. This gradient of activity across solvents underscores the role of compound polarity in enzyme inhibition. The standard antidiabetic drug diclofenac (used here as a reference inhibitor) exhibited an IC₅₀ of 49.92 µg/mL, reaffirming its superior potency. However, the comparably strong inhibition shown by

several plant extracts—particularly methanol and acetone fractions—demonstrates their competitive potential as natural α -amylase inhibitors.

Mechanistic Insights and Phytochemical Implications

The superior inhibition observed in methanolic extracts suggests the presence of highly polar phytochemicals such as flavonoids, tannins, phenolic acids, and glycosides (Patel et al., 2012; Dahal et al., 2021). These compounds are known to inhibit carbohydrate-hydrolyzing enzymes by binding to the enzyme's active site via hydrogen bonding and π - π stacking, effectively preventing the breakdown of starch into glucose (Ramun et al., 2014). This slows glucose absorption and helps mitigate postprandial hyperglycemia, a critical therapeutic target in type 2 diabetes management.

Acetone extracts, especially from *A. sessilis*, also demonstrated considerable inhibition, which may be attributed to moderately polar compounds such as saponins and alkaloids. These secondary metabolites can form non-covalent interactions with α -amylase, inhibiting its enzymatic activity and reducing carbohydrate digestion efficiency (Kashtoh & Baek, 2022). The modest activity observed in the chloroform extracts suggests that non-polar compounds like terpenoids and sterols may contribute to α -amylase inhibition, albeit to a lesser extent. This emphasizes the selective contribution of solvent-extracted constituents to bioactivity, affirming that solvent polarity significantly influences phytochemical yield and efficacy.

Therapeutic Relevance and Research Prospects

The inhibition of α -amylase is a validated pharmacological approach to managing type 2 diabetes by reducing the glycemic index of carbohydrate-rich foods. The high inhibitory potential of methanolic and acetone extracts of *F. trinervia*, *T. terrestris*, and *A. sessilis* supports their traditional use in herbal medicine and positions them as promising candidates for developing plant-based antidiabetic agents (Jaiswal, 2013). These findings are consistent with earlier studies that reported the presence of potent antidiabetic constituents especially flavonoids and glycosides in these species. Moreover, the comparable efficacy of the plant extracts to a synthetic drug reinforces the growing interest in plant-derived alternatives that may offer fewer side effects, better tolerability, and cost-effectiveness.

To translate these findings into clinical relevance, further *in vivo* studies are necessary to validate the safety, efficacy, and pharmacokinetics of these extracts. Future research should also employ molecular docking, structure-activity relationship (SAR) analyses, and chromatographic profiling (HPLC, LC-MS) to isolate, identify, and optimize the most effective bioactive compounds.

3.3 In Vitro Anti-inflammatory Activity of *Flaveria trinervia*

The methanolic extract of *Flaveria trinervia* was evaluated for its anti-inflammatory activity using the Bovine Serum Albumin (BSA) denaturation assay. This model is a widely accepted *in vitro* method for screening agents that can prevent protein denaturation, a key process involved in inflammation. Diclofenac sodium, a standard non-steroidal anti-inflammatory drug (NSAID), was used as the reference compound.

Visual Observation of Anti-inflammatory Activity

The figure 3 displays the comparative anti-inflammatory activity of methanolic plant extracts (left panel) and the standard drug (right panel) based on the protein denaturation assay. The turbidity or clarity changes in the test tubes reflect the degree of inhibition of protein denaturation. A more transparent solution indicates stronger anti-inflammatory activity. Methanolic extracts, particularly from *Flaveria trinervia*, exhibited noticeable inhibition comparable to the standard, supporting their potential as natural anti-inflammatory agents.



Figure 3. Visual Assessment of Anti-inflammatory Activity of Methanolic Plant Extracts (Left) Compared to Standard Drug (Right)

The extract exhibited a dose-dependent inhibition of protein denaturation. At the lowest concentration tested (20 $\mu\text{g/mL}$), the extract showed an inhibition of 31.19%, slightly higher than the inhibition observed for diclofenac sodium (28.85%). The percentage inhibition increased progressively with higher concentrations, reaching 93.37% at 400 $\mu\text{g/mL}$, which closely matched the standard drug's inhibition of 91.23% (Table 3 and Figure 4).

This strong correlation with the standard drug suggests that the methanolic extract of *F. trinervia* contains active phytoconstituents capable of stabilizing protein structures and mitigating inflammation-induced denaturation. Such activity is likely attributable to the presence of phenolic compounds, flavonoids, and other bioactive metabolites, which are known for their membrane-stabilizing and anti-inflammatory mechanisms (Sakat et al., 2010; Grant et al., 1970).

Table 3. Inhibition of BSA Denaturation by Methanolic Extract of *Flaveria trinervia* and Diclofenac Sodium

Concentration ($\mu\text{g/mL}$)	<i>F. trinervia</i> MeOH Extract (%)	Diclofenac Sodium (%)
20	31.19	28.85
40	40.16	38.23
80	52.29	49.24
200	68.91	66.67
400	93.37	91.23

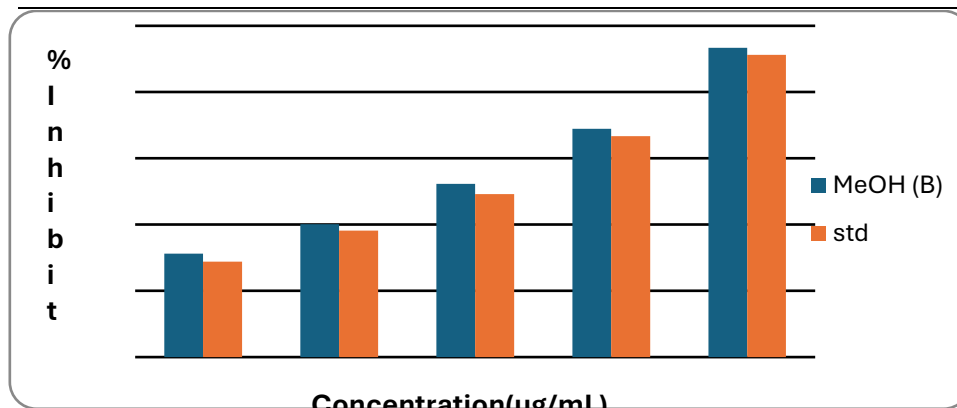


Figure 4: Bargraph of Inhibition of BSA Denaturation by Methanolic Extract of *Flaveria trinervia* and Diclofenac Sodium

Integrative Perspective

The anti-inflammatory activity demonstrated by the methanolic extract of *F. trinervia* supports its traditional medicinal usage and adds pharmacological value in the context of oxidative stress-related disorders and diabetes mellitus, where inflammation is a common underlying factor. Coupled with the plant's previously demonstrated antioxidant and antidiabetic potential, the findings suggest that *F. trinervia* may serve as a multifunctional therapeutic agent.

Its near-equivalent efficacy to diclofenac sodium at higher concentrations underlines the therapeutic relevance of this extract and warrants further in vivo and mechanistic investigations.

CONCLUSION

This study provides compelling evidence for the multifunctional therapeutic potential of *Tribulus terrestris*, *Flaveria trinervia*, and *Alternanthera sessilis*, particularly in their methanolic and acetone extracts. The strong antioxidant activity, observed through the phosphomolybdenum assay, reflects the presence of high levels of polar phytochemicals—especially flavonoids and phenolic compounds—that effectively scavenge free radicals and may play a key role in preventing oxidative stress-related cellular damage.

In parallel, the extracts exhibited potent α -amylase inhibitory activity, with *Flaveria trinervia* methanol extract showing the highest inhibition and the lowest IC₅₀ value among the tested samples. These findings strongly support the antidiabetic potential of the studied plants, particularly in managing postprandial hyperglycemia through enzyme inhibition mechanisms. Additionally, the observed anti-inflammatory activity reinforces the therapeutic relevance of these extracts in managing inflammation-associated metabolic conditions, thereby offering dual metabolic and anti-inflammatory benefits. Such a spectrum of bioactivities is especially relevant in managing interconnected disorders like type 2 diabetes, where oxidative stress and chronic inflammation are key pathological contributors.

Taken together, the results support the traditional use of these plants and position them as viable candidates for integrated phytotherapeutic development. Their synergistic antioxidant, antidiabetic, and anti-inflammatory properties offer a foundation for future research targeting complex chronic diseases. Further work should aim to isolate the active constituents, elucidate their mechanisms through molecular studies, and confirm efficacy via in vivo and clinical models. These efforts will be essential to transform these promising natural resources into standardized, safe, and effective phytomedicines for modern healthcare.

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